Effect of Body Size and Zebra Mussel Attachment on Exuviae Emergence-Site Selection in Five Dragonfly Species (*Hagenius brevistylus, Didymops transvera, Macromia illinoiensis, Dromogomphus spinosus, Epitheca*)

Adam Kamps, Corrinne Scieszka, Brianna Westmoreland

University of Michigan Biological Station
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Abstract

Emergence-site selection in dragonflies is an important factor for their survival during metamorphosis because of possible predation and other factors that may end the dragonfly life cycle during this vulnerable process. This study examined the emergence-site selection of exuviae in five dragonfly species from a lake in Northern Michigan to identify whether or not body size or presence of zebra mussels has a significant impact on site selection. We found that as length increased in proportion to body area, dragonflies were able to travel farther away from the shore. Individual species showed differing trends, when it came to body size relating to distance travelled from the shore. Understanding trends in emergence-site selection is important to understanding how dragonfly larvae have adapted to their individual environments in order to increase their chances of surviving to adulthood.

Dragonflies (*Anisoptera*) are vulnerable during emergence, a process in which they transition from a larval form to a winged adult through the shedding of larval skin, or exuviae (Corbet, 1962). These exuviae are valuable indicators of how dragonfly larvae have adapted to local environments, as many factors can influence their site-selection, such as local predation (Zebsa et al., 2014), flooding (Worthen, 2010), temperature (Trottier, 1973) or intra and interspecific competition (Cordero, 1995). As a result of different environmental factors, a previous study has shown that certain species of dragonflies often choose emergence sites that are near water, while others can travel as far as 45 meters away from the shore (Worthen 2010).

In addition, zebra mussels were recently introduced to the Great Lakes Region around 1985 or 1986, and have been having numerous impacts on species with which they coexist (Herbert et al., 1989; Griffiths et al., 1991). In fact, the presence of zebra mussels has been shown to impact dragonfly larvae by reducing their foraging success (Fincke and Tylczak, 2011) and reducing their mobility, including their ability to adjust themselves when knocked over (Fincke et al., 2009). In fact, some exuviae have been found with three or more times their own weight in zebra mussels and the accumulated sand and debris that accrues between the mussels (Fincke et al. 2009).

With so many factors that influence exuvia site selection, we investigated specific factors that influence emergence-site selection within and between five different species of dragonfly larvae that populated the area around Douglas Lake in Northern Michigan. We hypothesized that dragonfly larvae will want to remove themselves as far as they can from shore in order to seek cover from predators. Certain features like longer legs, or larger, more muscular bodies would be favored to help these larvae travel further into the forest, and higher up trees. If dragonfly larvae truly want to remove themselves as far as they can from the shore, than these morphological

features will be limiting factors in their travel. Therefore, we predicted that exuviae with larger bodies will have travelled farther.

Our second hypothesis regarded dragonfly larvae that choose selection sites closer to shore. We predicted that dragonfly larvae that chose emergence sites closer to shore would exhibit clustering or grouping behavior in order to decrease their chances of individual predation. We made this hypothesis based on the assumption that exuviae located closer to shore, rather than deeper into the woods, would potentially be more exposed, and therefore more visible, to potential predators.

Lastly, we sought to examine how the presence of invasive zebra mussels (*Dreissena polymorpha*) at Douglas Lake affected emergence-site selection of dragonfly larvae. We hypothesized that exuvia found with zebra mussels attached would be located closer to shore and closer to the ground on trees relative to those found without zebra mussels, due to the fact that zebra mussels might hinder the mobility of the larvae.

Methods

The location of our study was Douglas Lake in Northern Michigan, an area in which we identified five dragonfly species, *Hagenius brevistylus, Didymops transversa, Epitheca cynosura, Dromogomphus spinosus,* and *Macromia illinoiensis*. We collected exuviae from three different sites around the lake to control for differing environmental factors, such as wind exposure, as wind has been found to to influence emergence-site selection in dragonfly exuviae (Khelifa et al., 2013).

At each site, we walked along through the forest, or canoed along the edge of the forest, until we found an exuvia. From there we would spread out as far as 21 meters into the forest, to find other exuviae nearby. We collected the exuviae from trees and measured their distance from

the water, their distance above the ground, the species of the tree they were located on, the DBH of the tree, and whether or not zebra mussels were attached. After these data were collected, the species of each exuvia were identified using the *Aquatic Insects of Wisconsin* by William L. Hilsenhoff, and its length and width were measured. In addition, back-leg length was measured in order to determine whether longer legs correlated to a farther distance traveled, either from the shore or up the trees.

We split the size measurements up by species and compared the size measurements of the exuviae to the distances that they were able to travel using linear regressions. A kruskal-wallis test was used to compare the means distances travelled by dragonflies with zebra mussels attached to their exuvia and absent. The same test was used to compare the mean total distances travelled by dragonflies that clustered and those that were alone on trees. A chi-squared test was used to see if there was a relationship between tree species and the amount of times that ecdysis took place on that tree.

Figure 1. The three sample sites on the South Fishtail of Douglas Lake

Results

Back Leg Length

There was no significant relationship between the back-leg length of *Dromogomphus spinosus* and the total distance that they travelled (distance from shore + distance up tree); however, it is worth mentioning that the relationship was nearly significant (F= 3.906, df= 20, p-value= .06207, figure 2). There was also no significant relationship between the back-leg length of *Macromia illinoiensis* and the distance that it travelled.

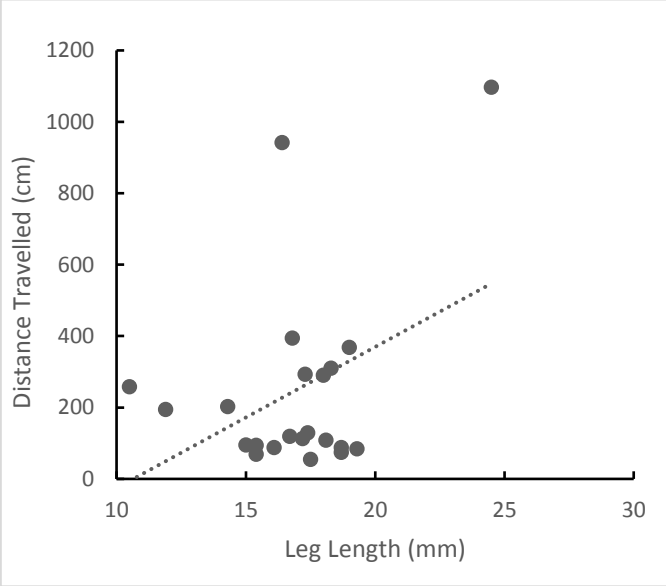

Figure 2. The relationship between the back leg length of *Dromogomphus spinosus* and the total distance that they travelled from shore.

Body Length

There was no significant relationship between the body length of *Dromogomphus spinosus* exuviae and the distance that they travelled. However, as the length of *Macromia illinoiensis* exuviae increased, the total distance travelled decreased. For every millimeter that body length increased, the total distance travelled decreased by 309.3 centimeters. This relationship was tested with regression analysis and the relationship was statistically significant (F= 5.375, df= 15, p-value= 0.03489, figure 3).

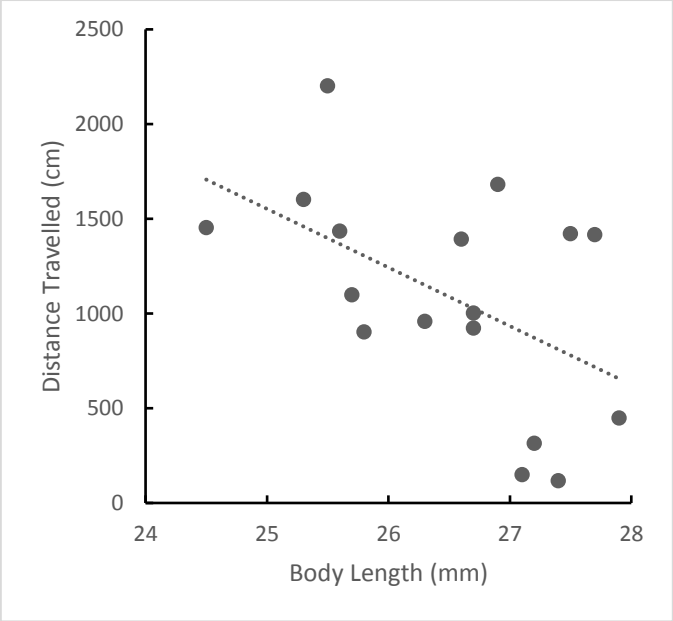

Figure 3. The relationship between the body length of *Macromia illinoiensis* and the total distance that they travelled from shore.

Body Width

There was no significant relationship between the width of *Macromia illinoiensis* and the distance travelled by them. However, as the width of the *Dromogomphus spinosus* bodies increased, so did the distance that they travelled. For every millimeter that body width increased, distance travelled from shore increased by 36.47 centimeters. This relationship was tested with regression analysis and the relationship was statistically significant (F= 10.13, df=21, p-value= 0.004478, figure 4).

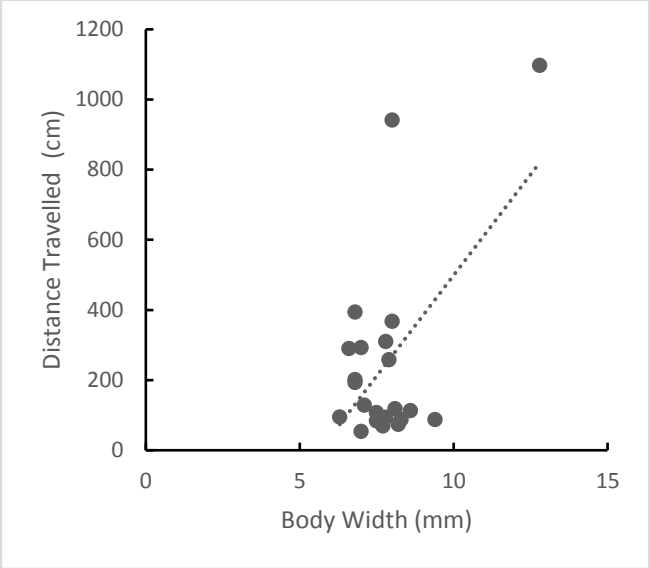

Figure 4. The relationship between the body width of *Dromogomphus spinosus* and the total distance that they travelled from shore.

Ratio of Leg Length to Body Area

As leg length increased in comparison to body area, total distance travelled also increased. The relationship was tested with regression analysis and the relationship was statistically significant (F= 5.403, df= 44, p-value= 0.02478, figure 5).

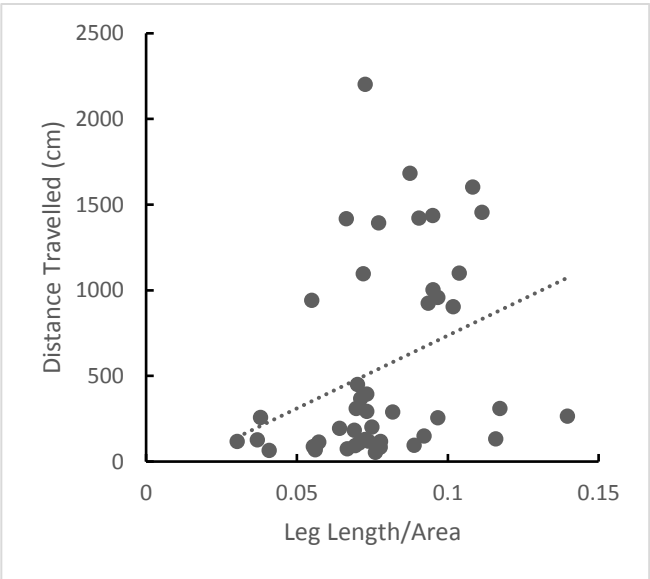

Figure 5. The relationship between the leg length/body area to the total distance travelled by exuviae

Exuviae Proximity

There was no significant difference between the mean distance travelled by dragonflies that shared trees and dragonflies that were

alone, when the means were compared with a Wilcoxen sign-ranked test (W = 304, p-value = 0.2155, figure 6)

Zebra Mussel Attatchment

There was no significant difference between the mean distance travelled by dragonflies that had zebra mussels attatched to their exuviae and those that were unhindered (W= 31, p-value= 0.9656, figure 7). There was also no significant difference between the mean distance travelled by *Macromia illinoiensis* that had zebra mussels attatched to their exuviae and those that were unhindered (W= 39, p-value= 0.5054).

Tree Climbed

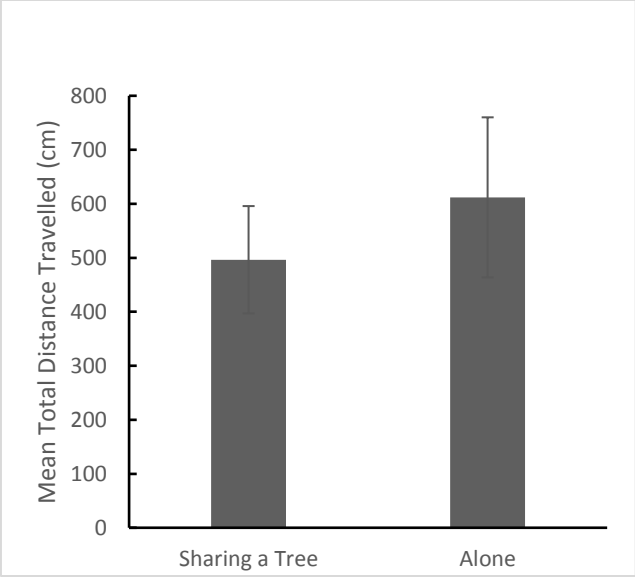

Figure 6. Mean values of total distance travelled by whether or not the exuviae were sharing a tree

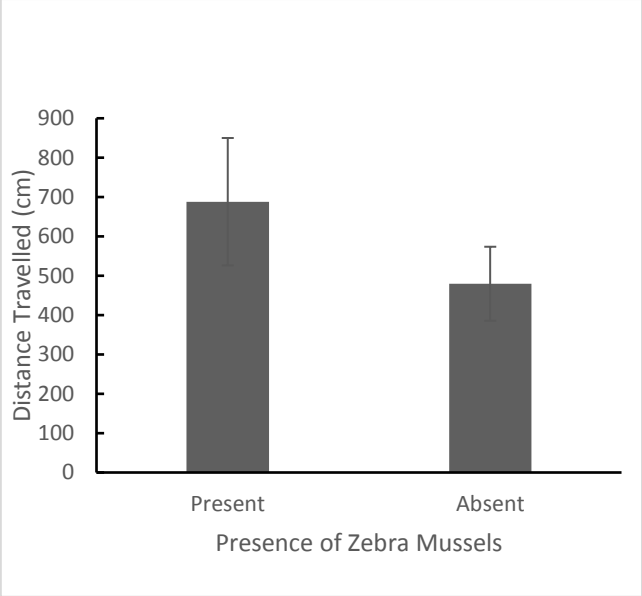

Figure 7. Mean values of total distance travelled by whether or not the zebra mussels were present on the exuviae

There was no significant relationship between tree species, and the amount of times that ecdysis occurred on that tree (X-squared= 24, df= 20, p-value= 0.2424, figure 8).

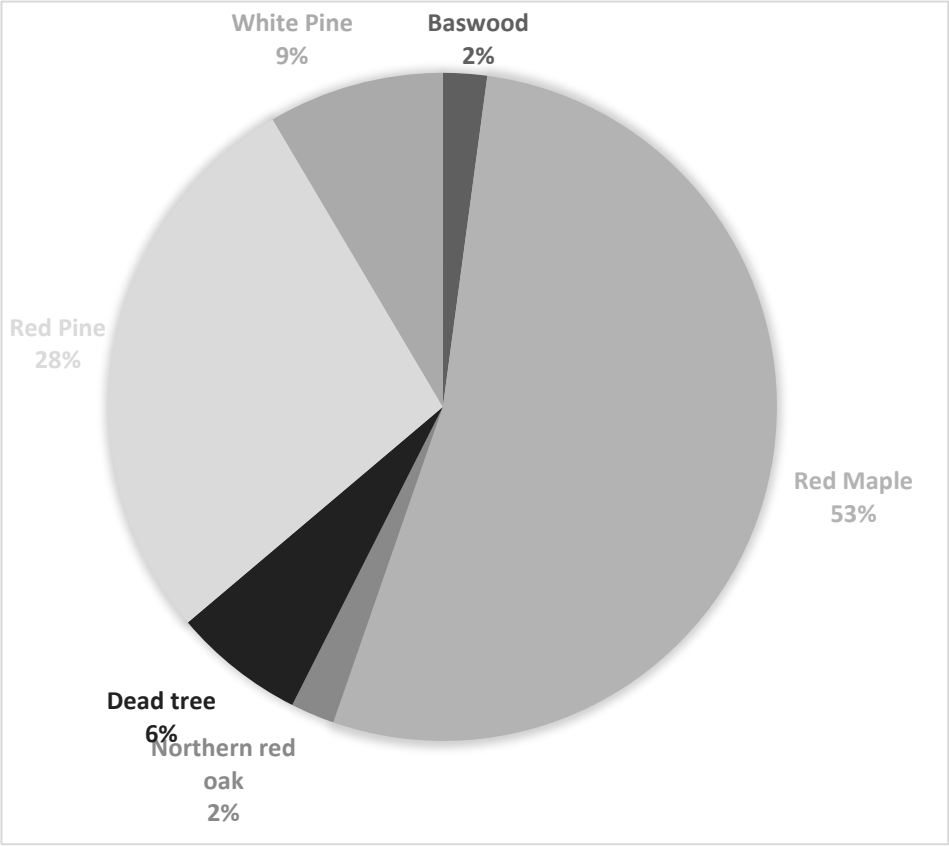

Figure 8. Proportion of trees that were climbed before ecdysis

Discussion

D. spinosus and *M. illinoiensis* were the most frequently identified species throughout our study and therefore, we only had enough of these two species to perform statistical tests. Even though we had enough of these two species to conduct tests, there was still a very small sample size. In order to improve our study, we preferably would have collected a larger sample size that was more inclusive of different species of dragonfly.

In regard to our hypothesis that back leg length would correlate to a greater distance traveled, this did not appear to differ significantly within species. Both within *D. spinosus* and *M. illinoiensis*, there was no significant correlation between distance traveled and back leg length. These data suggest that any increase in leg length within a species is not significant enough to cause any difference in total distance traveled in emergence-site selection.

Our hypothesis for the relationship between body length and distance traveled was also not supported for *D. spinosus,* with our data showing that there was no significant relationship between the body length and distance. However, there was a significant relationship for *M. illinoiensis* that showed the distance traveled decreasing as body length increased. This was opposite of our hypothesis, which suggests that a smaller body weight is actually advantageous to travelling farther from the water. This could be because extra body weight is more costly to dragonfly larvae in terms of weight, than it is beneficial in terms of muscle.

There was no significant difference between the width of *M. illinoiensis* and the total distance that it was able to remove itself from shore. However, as body width increased for *D. spinosus*, the distance that these dragonflies travelled also tended to increase. In so far as width can be used as a proxy for strength, this finding supports our hypothesis that dragonflies want to get as far away from shore as possible before going through ecdysis, in order to avoid predation.

Previous studies have shown that predation does in fact have an impact on the emergence-site selection of various dragonfly larvae (Zebsa et al., 2014).

As leg length increased in comparison to body area, total distance travelled also increased. This could be because stronger, more muscular legs can carry smaller bodies further than they could carry bigger bodies, which suggests that leg length is much more essential to traveling greater distances than body size.

There was no significant difference between the mean total distances travelled by dragonflies that clustered into the same trees and those that were alone. If exuviae truly would benefit by clustering, they would still have to overcome the enormous problem of being able to tell which trees already had exuviae on them. Most of the trees that we found that did have more than one exuviae also happened to be in positions that were easily accessible in comparison to other trees. It is more likely that exuviae end up on the same trees because they are more accessible than because they have been selected for to cluster together. Previous studies have confirmed that dragonfly larvae exhibit clustering behavior, however this was attributed to preferable environmental conditions such as vegetation patterns, water flow, and sunlight rather than predatory influences (Chwala et al., 1996). However, our data also showed that in regard to tree species, there was no preference exhibited by dragonfly larvae for which species of tree that they prefered for emergence.

Our data did not support our hypothesis that zebra mussel attachment would negatively impact the distance traveled by dragonfly exuviae. It is important to note that the maximum number of zebra mussels attached to any one of our exuvia samples of 3, while most only had one or two attached. Therefore, it is possible that the negative impact of zebra mussels may occur before larvae leave the water. If the larvae were so hindered in their mobility that they were

unable to leave the water in order to emerge, then they would not have been included within our samples at all. It is possible that our results could have been improved by collecting a larger sample size in order to include exuviae that had a larger number of zebra mussels attached, as previous studies at Douglas Lake have found that exuviae can hold up to three times their weight in zebra mussels and the debris that accumulates between them (Fincke et al., 2009).

If this study were to be redone, there should be a larger number of sample sizes and exuvia species. The study should also be done early in the summer because dragonfly exuviae typically emerge in between late June and early July, and we collected our data in late July. This left a period of time for exuviae to fall off of trees due to wind or other factors, which could have affected our results. Overall, dragonfly exuviae are important indicators of how dragonflies are able to adapt to local environments, as many different factors affect their emergence site selection (Zebsa et al., 2014; Worthen, 2010; Trottier, 1973; Cordero, 1995). Therefore, studying their emergence-site selection is important to understanding the evolutionary process of how dragonfly larvae have adapted to increase their chances of surviving to adulthood.

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