Substrate Preference in Immature Anisoptera of Lake Douglas

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Abstract. Animals that are subject to predation must evolve strategies to avoid predators or go extinct. Those that lack the physiology to flee at high speed or employ physical defenses usually rely on methods such as burrowing or using camouflage. Dragonfly naiads, unlike their brightly-colored adult counterparts, are dully-colored and usually blend with the sandy lake bottom on which they live. Because they have a cryptic coloration, they show certain preferences and aversions to different substrate types which benefit or inhibit their crypsis. In this experiment, it was found that a group of naiads in the family Libellulidae showed preference for certain substrates over others during different times of day. Overall, Dragonfly naiads preferred cryptic substrate over the non-cryptic substrate, regardless of day or night conditions. Further studies are necessary to determine the cause of crypsis in dragonfly naiads.

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Introduction

Dragonflies are an integral part of aquatic and terrestrial ecosystems. As adults, they are predators of various flying insects as well as prey for animals such as birds, small mammals, and amphibians. Juvenile dragonflies, also known as nymphs or naiads, share the adults' role as both predator and prey. Unlike their terrestrial adult counterparts, dragonfly naiads hatch and live in the water until metamorphosis (Borror and White, 1970). They are benthic invertebrates that feed on other aquatic arthropods and in turn are eaten by things such as fish, birds, and other naiads. These generalized carnivores are also known to be cannibalistic in certain situations (Merritt and Cummins, 1978). After hatching, naiads almost immediately molt into a new instar and go through 10-15 instars before metamorphosing into the adult form. Naiads crawl, but can also use their rectums as propellers, within the limnetic zone as they search for food. With gills located in the anus, naiads bring in and push out water to breathe, which also allows them to have short bursts of speed (Merritt and Cummins, 1978).

Dragonfly naiads are relatively soft-bodied, slow-swimming, and have few defenses. Due to their vulnerability, naiads rely on cryptic behaviors to hide from predators, seeking shelter beneath objects such as wood, rock, and vegetation. They have been known to conceal themselves by burrowing, hiding amongst fine sediments, and taking shelter in detritus and vegetation. This experiment is intended to test the naiads' preferences for different substrates, and to determine whether or not the time of day will affect their choices. Due to predation pressures in the daytime hours, naiads are believed to emerge from shelters at night to hunt (Merritt and Cummins, 1978). Similar experiments have been conducted with juvenile toads (Heinen, 1985), as well as turbots and flounders (Kristensen et al., 2014).

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We conducted an experiment to test two hypotheses: 1) we predicted that substrates which allowed for cryptic behavior would be preferred in the day trials; for this experiment, cryptic substrates were gravel, vegetation, and black material, and 2) we predicted that during night trials, naiads would show less preference for cryptic substrates.

Material & Methods

This experiment was conducted using naiads from Lake Douglas at a research facility at the University of Michigan Biological Station (UMBS). Lake Douglas is a freshwater lake in the northernmost part of the southern peninsula of Michigan (Heinen and Vande Kopple, 2003). It is one of Michigan's many glacial lakes and a kettle hole lake. Its deepest point is 24 meters, and it feeds into the headwaters of the nearby Maple River in Pellston, Michigan. Samples were taken from the shallows of South Fishtail Bay at depths no deeper than one meter.

Libellulidae naiads were pulled from the water using eight separately stationed cinder blocks attached to buoys. Blocks were left in the water for five to seven days and then retrieved to remove the specimens, which concealed themselves in the crevices.

The naiads were moved into a 10 gallon holding tank filled with sand and oxygenated well water, which allowed the naiads to acclimate to any changes between the lake and the artificial aquaria. Insects are sensitive to changes in the physical environment because their high surface area to volume ratio (Schowalter, 2011). The aquaria temperatures were maintained within one degree Celsius of source lake reading. The oxygen supply was halted before testing but resumed after naiads were removed to maintain oxygenated water (Schowalter, 2011). After two to three hours of acclimation, the naiads were subjected to testing via placement into three groups of test tanks, all 10 gallons in size.

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The first set of test tanks had a substrate layer of gravel on one side, and sand on the other (Figure 1). The second set had a sandy bottom, with one half 'vegetated' with plastic aquarium plants. The third set of tanks had bottom and sides covered in white material, and the other half covered in black; construction paper was used to achieve this effect. The materials were placed on the exterior of the tanks to affect only the color and not the content of the water.

Each naiad was moved from the acclimation tank into each test tank, then left for one hour to move freely and settle. At the end of the hour, we recorded which side of the tank was chosen as well as if the naiad was in a corner or not. The lights were then switched off to simulate an hour of night. Again, after one hour in the tanks, the naiad location was recorded. Photoreceptors have been known to allow the naiads to maintain information about day and night lengths, which influence their diapause phases (Schowalter, 2011). Because of this, night simulations were commenced as close to sundown as possible for the trials. Following the first set of day/night trials, each naiad was released back into Douglas Lake. The insects were released in close proximity to their capture sites. Another set of naiads was selected from the acclimation tank and the tests were repeated. Unfortunately, after the release of the first cohort, the traps' rate of catch dramatically decreased. To compensate for having fewer naiads available, subsequent trials were conducted using a set of naiads repeatedly. One sample binomial tests and chi-square test of homogeneity were deemed appropriate for this study and were used to analyze the results.

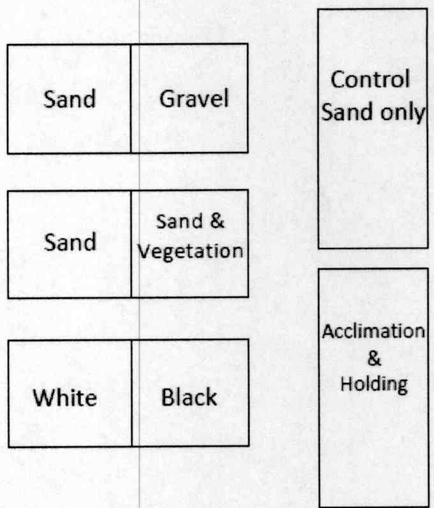

Figure 1. Visual representation of experimental setup of aquaria with substrates

These tests were determined to be suitable because of the categorical nature of variables and the small sample size (Heckard and Utts, 2010).

Results

The naiads appeared to show a preference for cryptic surfaces regardless of the time of day. During the simulated days, the naiads preferred the vegetated, black, gravel substrates (p-values 0.007, 0.012, and 0.018, respectively). For the night trails, the vegetated and the gravel were favored by the insects (p-values 0.001, 0.05 respectively). There was no significant difference in the distribution of naiads in the black and white aquariums during the simulated nights (p-value=0.078). See Figure 2 for a graphical representation of results. Naiads were often found in the corners of the aquaria. This behavior was shown to be significant, especially in the black and white aquaria, during the simulated night, where the naiads were found in the corners more often than in other treatments (p-value=0.027). In contrast, this behavior was absent from the vegetated/non-vegetated treatment, with only one naiad choosing the corner during the day and none during the night (p-value<0.001; Figure 3).

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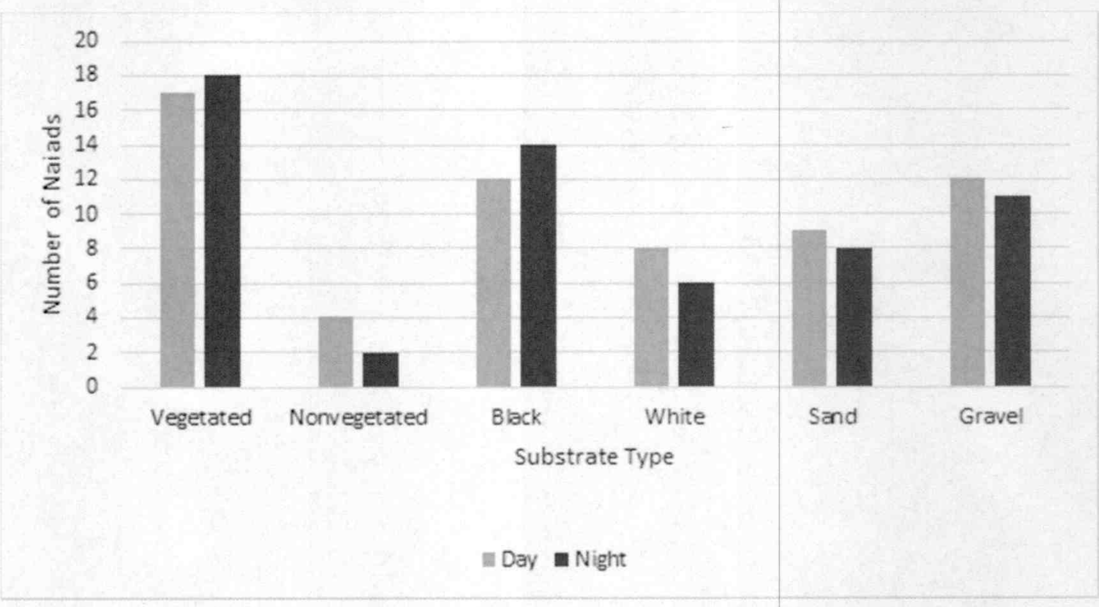

Figure 2. Bar graph representing the number of naiads found in each substrate type during day and night conditions.

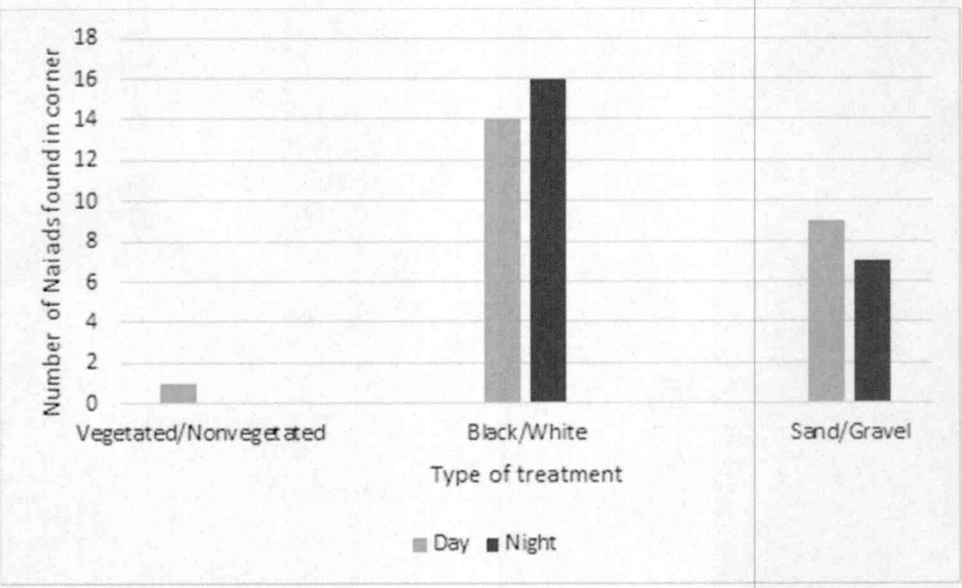

Figure 3. Number of naiads found in corner of tank as a function of treatment type.

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Discussion and Conclusion

Overall, the substrates that provided more crypsis were preferred by the naiads during the day and night conditions. This supported one of our two hypotheses; the more cryptic substrate was favored by the naiads during the day. A potential explanation for this is antipredator strategy employed by the naiads to better conceal themselves during periods when predators can see the best. However, the naiads also showed preference for the more cryptic substrate during the simulated nights. This behavior refuted our second hypothesis that the naiads would show less preference for the more cryptic substrates during the night. This could mean that preference for cryptic substrates may not be an antipredator strategy. The naiads are predators themselves and perhaps more prey are available during the night in substrates that also happen to provide more crypsis.

Some behaviors observed in the holding tank, while not relevant to substrate preference, offered insight into naiad social behavior. Clustering behavior was observed, wherein the naiads clung to one another and created a clump of 2-5 naiads in one place. This is possibly due to the cold temperatures in the tank, making the naiads cluster together in order to conserve heat. This behavior was not seen in the testing tanks because naiads were tested individually. However, the results noted that individuals were often found in the corners of their testing tanks. This may be for a couple reasons. It's possible that the corners were the warmest portions of the aquaria, despite the water temperatures being regulated to lake temperature. It's also possible that the insects, upon sensing the solid walls, mistook the walls for protective cover, such as rocks.

There are some errors to consider when analyzing these results. In the latter half of the experiments, naiads were not released back into the lake because the traps were not catching enough naiads to replicate trials. Instead, the same naiads were used to run multiple trials and were rotated throughout the tanks. While there was no cannibalism, the stress from hunger may have affected the insects' behavior (Wäckers, 1994). It should also be noted that only 21 individual naiads were tested in this experiment, making the sample size relatively small. In future experiments, it may be of benefit to take on larger sample sizes to decrease the risk of error (Erb, 1990).

In future research, it may be beneficial to test if naiad substrate preference changes in the presence of predators or prey (Lima and Dill, 1990).The laboratory conditions placed the naiads in an artificial environment devoid of both food and threats, which may have had an effect on behavior. Predators may be simulated by placing fish into the aquaria (either living or artificial), and prey could be simulated by placing some of the naiads' natural prey such as mayfly or mosquito larvae into the aquaria (Schaffner and Anholt, 1998). Live predator trial experiments similar to this have been conducted with juvenile toads and snakes (Heinen, 1993). In that work, predators were shown to influence substrate choice and a similar result could be expected in predator trials with naiads. There are at least two different species of naiads in Lake Douglas; our experiment tested only the light-green, sand-colored naiads but we observed a black colored species in the lake as well. These color differences may be a factor in substrate choice as well (Heinen, 1994). Predators may have different rates of success on the different colored naiads as a function of differing substrates.

Another experiment that could expand on the results observed in this experiment would be recording naiad behavior in plastic wading pools with different substrates available. This would remove the corners from the experiments and more than two substrates could be tested in each arena. More naiads would be expected to congregate in the preferred substrates. Experiments could also be done to test if other factors have an effect on naiad substrate preference. Temperature variability and extremes would be an important factor to test as it can yield implications for climate change and predator-prey interactions (Smolinský and Gvoždík, 2014). Seasonal adaptations in behavior have also been observed in insects and could offer another area of research for dragonfly naiads (Tauber et al., 1986). Different species of naiads may also be tested in future experiments. These trials used only a single species from a single location, and it is possible that naiads from different geographical regions and families of Anisoptera will have varying behavior. Naiads of the same species but at different instars and sizes may also have differing behaviors. Blue dasher naiads (*Pachydiplax longipennis*) can have up to a threefold increase in size between hatching and reaching adulthood (Anholt et al., 1991). This great difference in size between the stages of the nymph's life may have an effect on its substrate preference and cryptic behavior, since the size of its prey as well as its noticeability will change as it grows.

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