Microhabitat Preferences of Larval Odonates:

Ladona Julia and *Epitheca spinigera*

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Abstract

Microhabitat preference of larval odonates is an important factor in the coexistence of the two species, *Ladona julia* and *Epitheca spinigera,* studied during the summer of 2002 at UNDERC- the University of Notre Dame's Environmental Research Center in the Upper Peninsula of Michigan. Laboratory microhabitat experiments were used to determine these species' microhabitat preferences and results were compared to a similar study by Dunlap (2001). Microhabitat preferences will show how the *Ladona julia* and *Epitheca spinigera* species coexist in close proximity to each other. *L.julia*'s pattern of preference was: Lg sticks/mud > Sm sticks/mud > Sm sticks/rocks > Lg sticks/rocks. *E. spingera* preferred vegetation because of their morphological ability to cling to it. More specifically, they preferred dead leaves to green grass vegetation. Microhabitat selection at time 0 was different than after 24hrs because in those 24hrs the larvae had a chance to explore and settle in their true preferred microhabitat.

Introduction

Odonates are an important component of littoral communities where they make up a dominant portion of the predacious invertebrate biomass. They have been known to reach densities of 1000/m^2 and occupy a diverse range of habitats. Eighty-eight odonate species have been documented at UNDERC, located in the Ottawa National Forest in the Upper Peninsula of Michigan (Dunlap 2001). The larval stage of odonates shows a great diversity in adaptations necessary for interspecific competition for space and food in relatively confined habitats (Corbet 1999). Odonates fall into 3 behavioral groups on the basis of habitat preference: burrowers, sprawlers and climbers. **Burrowers** bury themselves in sediment, cover themselves in it or hide among debris. A few species are believed to remain in a single burrow throughout their lives, enlarging it with each successive instar. **Sprawlers** are ambush hunters that lie on the surface of sediment or amid drifted bark and leaves and wait, with legs outspread, until prey wanders within reach. They have little color pattern and are hidden in a coat of ooze that surrounds their hairy bodies. **Climbers** are active hunters that rely on vision. They live among large stable objects like stones, sticks or upright plants that they grasp tightly but do not attempt to cover themselves with. These are more active larval odonates that climb in green vegetation or cling to stems of reeds or hanging roots (Needham 2000).

In his research on the *Odonata of the Western Upper Peninsula of Michigan*, Michael Dunlap (2001) studied adult and larval odonates at UNDERC at 13 bog, lake and stream sites. He determined the general microhabitat preferences of *L. julia* and *C. shurtleffi* larvae through laboratory microhabitat preference and field colonization experiments. He allowed larvae to choose between mud, leaf, twig and sand substrates and found that *L. julia* preferred mud while C. *shurtleffi* preferred leaves. These differences in preferred microhabitats tend to segregate the

two species in space. The four microhabitat substrates Dunlap used were chosen to be distinct and sharply contrasting so were therefore somewhat course. This study will take Dunlap's microhabitat experiment and use a narrower range of similar microhabitats such as different types of vegetation (grass, ferns, leaves) and varying sizes of sticks to precisely determine the microhabitats of *Ladona julia* and *Epitheca spinigera*, two species that prefer very similar microhabitats.

Ladona julia, the most abundant odonate in the region, is a shallow burrower that inhabits decaying organic matter at the bottom of bog ponds and swampy bays (Walker and Corbet 1975). *Epitheca spinigera* is a sprawler that crawls over loose trash and timber at the water's edge and climbs projecting roots or sticks to transform (Needham 1929). Both species are found closely associated in lakes and ponds with muddy or litter covered bottoms and it is the difference in their microhabitat usage that allows them to coexist in close proximity to each other. Microhabitat preferences of *E. spinigera* will be compared to the preferences of Dunlap's *C. shurtleffi* because that similar species is also a sprawler that inhabits bogs and boggy lakes and prefers muddy or litter-covered lake and pond bottoms (Dunlap 2001).

The aim of this study is to duplicate Dunlap's experiment and see how a narrower range of microhabitats can further segregate two odonate species in close proximity to each other. The microhabitat the odonates settle in when first placed in a microhabitat arena, Time 0, will be recorded to determine whether initial choice of microhabitat is based on a preference or is purely random. Initial microhabitat selection by the odonates will be compared to the final microhabitat selection to see if the final selection is based on the initial selection. If the odonate has no reason to move from it's hiding place in search of food then the final selection might be solely based on

the initial desire of the odonate to hide itself rather than an actual preference for a specific microhabitat.

Materials and Methods

Study Site and Organisms

Larvae were collected from Morris Lake (N46° 15.22' W89° 31.18') at the University of Notre Dame Environmental Research Center (UNDERC) located in Gogebic County, Michigan. Morris Lake, fairly shallow with a surface area of 4.9 ha, is composed of one main body with a prominent point jutting from the north bank. Morris has several beaver lodges and is surrounded by alder and a few evergreen stands. The littoral zone is vegetated with submerged macrophytes, mainly *Elodea,* and patches of lily pads growing from a muddy lake bottom. The fish population consists of minnows, stunted northern pike (*Esox lucius*) and a few yellow perch (*Perca flavescens*) (Internet 1).

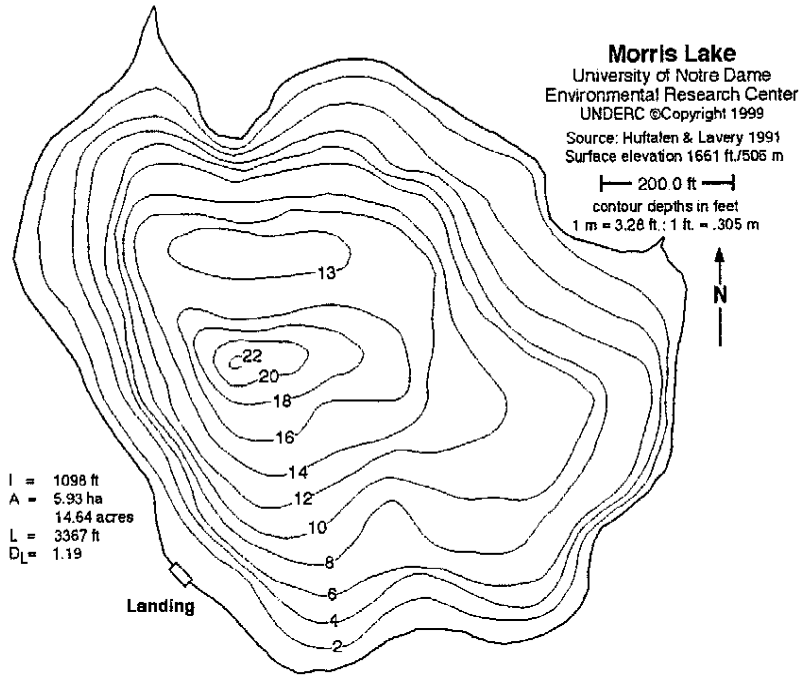
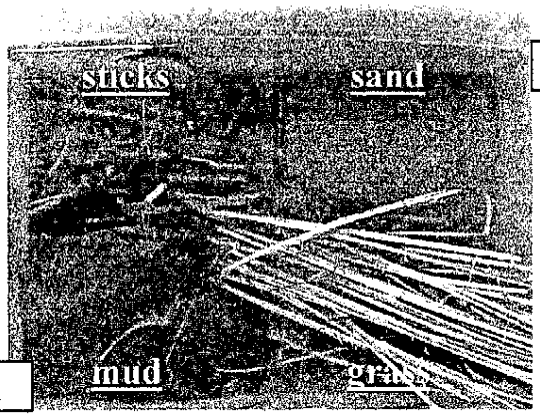


Figure 1—Bathymetric map of Morris Lake, University of Notre Dame Environmental Research Center.

Microhabitat Experiments

Methods followed those used by Michael Dunlap (2001). Larvae were collected and held without food for 24 hours prior to use. The experimental arenas, obtained from Michael Dunlap, were 40.6 x 27.9 x 15.24cm plastic tubs containing a 1cm layer of sand and filled to 10cm with untreated well water. Each arena was divided into 4 equal quadrants with aluminum flashing into which mud, leaf, twig and sand substrates were added for the general run of both species.



a.

h.

Substrates used in three microhabitat experiments

	Quad I	Quad II	Quad III	Quad IV
Gen run	sand	grass	mud	sticks
L. julia	Lg sticks / mud	Lg sticks / rocks	Sm sticks/ mud	Sm sticks/ rocks
E.spinigera	leaves	ferns	grass	mud

Figure 2—(a.) picture of experimental arena for the general run of both *L. julia* and *E. spinigera.* (b.) Chart of all substrates used in the three microhabitat experiments.

The experiment was repeated for *L. julia* with substrates: large sticks/mud, small sticks/mud, large sticks/rocks and small sticks/rocks. A 1cm layer of mud was added to coat the top of the 1cm sand layer then sticks or rocks (diameter 8mm-82mm) were added to the mud in each quadrant. Large sticks (7-15mm) and small sticks (1-6mm) were added to each quadrant so that the total diameter of all sticks was 50mm. The experiment was repeated for *E. spinigera* with substrates: 1cm mud, 5 ferns (*Dryopteris intermedia)*, 5 leaves (*Quercus rubra*) and a handful of grass (*Elodea canadensis).*

The mud was collected from Morris Lake (N46° 15.22' W89° 31.18') and kept in sealed containers in a warm, sunny location for several days prior to use and the top centimeters were skimmed off to remove larvae that did not die of anorexia. Leaves were collected from the forest floor and soaked in lake water for 3 days before use. Collected twigs were waterlogged and submerged. For *E. spinigera*, one larva was placed in the center of each arena and observed until it settled in one of the four quadrants then a second larva was added to each arena and observed till settling. Since *L. julia* is larger and more active than *E. spinigera* only a single larva was added to each arena. Larvae were left in the tubs for 24 hours then the divider was replaced and substrates were searched for larvae. Ten replicates at a time were run for each species. G-tests for Goodness were calculated to determine if habitat selection differed from random.

Results

a. **General Microhabitat Experiment with *L. julia* and *E. spinigera***

Species	**Substrates**	**Time** (0, 24hrs)	G_{corr}	**df**	**p**	**# reps**	**Differ from random**
L. julia	mud, grass, sticks, sand	0	10.117	3	**0.025**	24	Yes
"	"	24 hrs	14.254	3	**0.005**	24	Yes
E. spinigera	mud, grass, sticks, sand	0	9.487	3	**0.025**	34	Yes
"	"	24 hrs	5.650	3	**0.5**	30	Yes

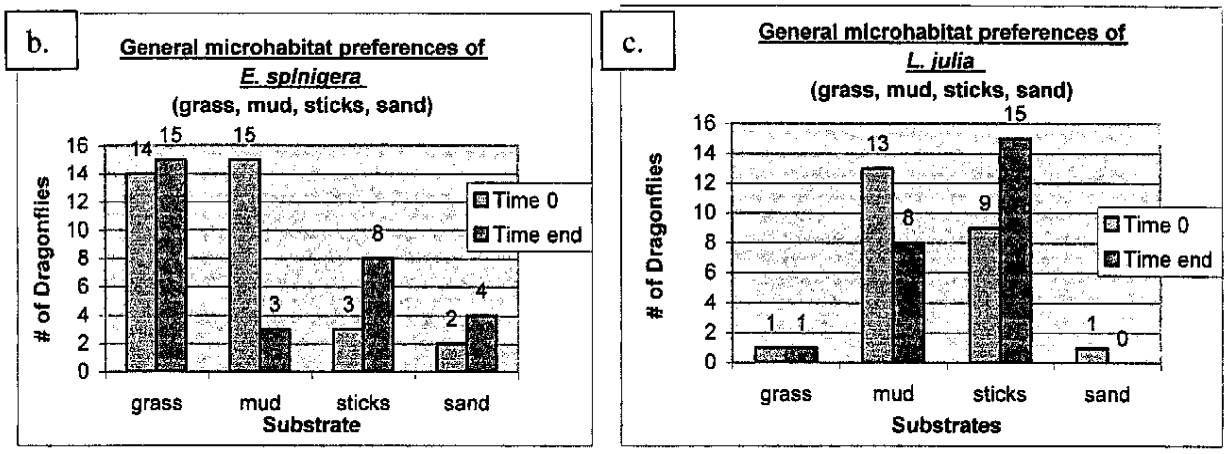


Figure 3—(a.) Microhabitat selection at times 0 and 24hrs for both species differs from random with p values ranging from 0.005-0.5. (b.) *E. spinigera* preferred mud and grass equally at time 0 but preferred grass almost 2:1 to sticks after 24hrs. (c.) *L. julia* preferred mud (1.4:1 to sticks) at time 0 but preferred sticks almost 2:1 to mud after 24hrs.

Microhabitat Experiments specific to *L. julia* and *E. spinigera* general preferences

	Species	Substrates	Time (0,end)	G_{corr}	df	p	# reps	Differ from random
4a-L	*L. julia*	mud/LS, mud/SS rocks/LS,rocks/SS	0	0.560	3	~~0.95~~	36	No
	"	"	end	2.541	3	**0.5**	36	Yes
	- *L. julia*	mud (LS+SS) rocks (LS+SS)	0	0.056	1	~~0.95~~	36	No
	"	"	end	2.038	1	**0.5**	36	Yes
4a-E	*E. spinigera*	mud, ferns leaves, grass	0	6.762	3	**0.5**	70	Yes
	"	"	end	15.391	3	**0.005**	70	Yes

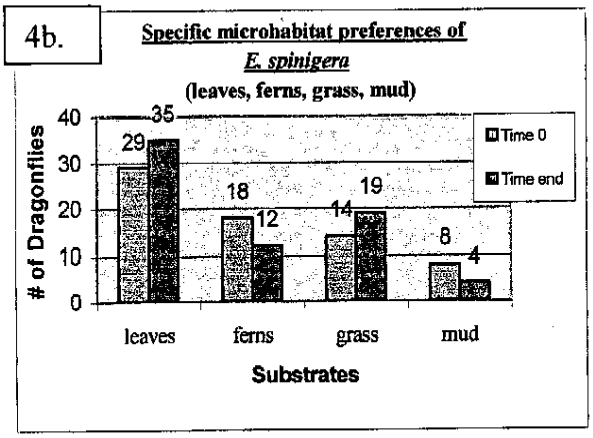
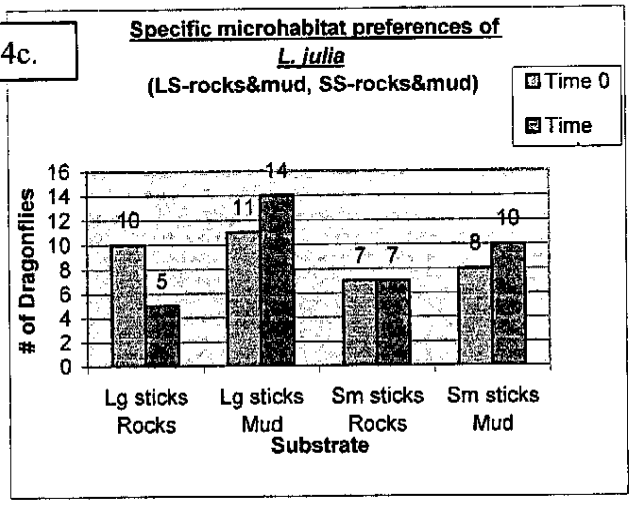
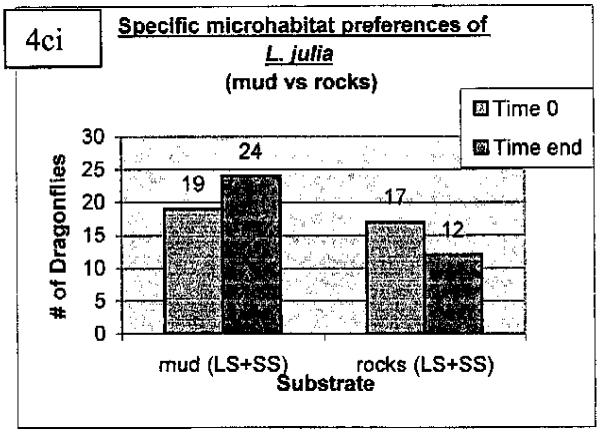
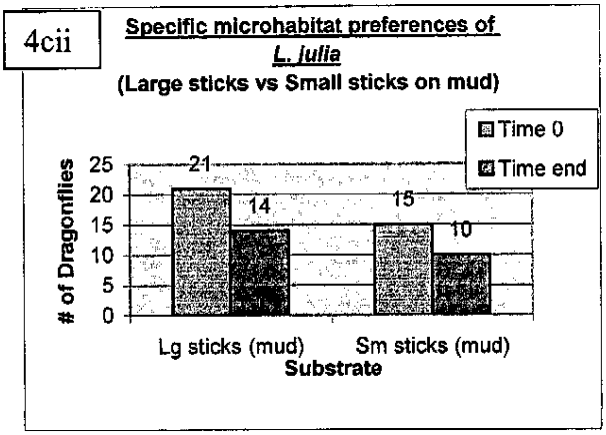


Figure 4—(a-E.) Microhabitat selection by *E.spinigera* at times 0 and 24 hrs differs from random with p values of 0.5 and 0.005. For *L.julia* microhabitat selection is random at time 0 and differs from random at 24 hrs with p values from 0.01-0.5. (b.) *E. spinigera* preferred leaves at both times 0 (1.6:1 to ferns) and 24hrs (2:1 to grass). (c.) Microhabitat selection by *L. julia* was random at time 0. At 24hrs *L. julia* large sticks on mud with small sticks on mud a close 2nd.

Figure 4—(a-L.) Combining large and small sticks and looking at mud vs rocks, selection is random at time 0 and differs from random at time 24hrs with a p value of 0.5.
(ci.) At time 24hrs mud was preferred 2:1 to rocks.
(cii.) At both times 0 and 24hrs large sticks on mud were preferred 1.4:1 to small sticks on mud.







Both species showed strong microhabitat preferences, especially after 24hrs. *E. spinigera* had a strong preference for mud and grass at time 0 but preferred grass after 24hrs ($G_{corr,\ 24hrs}$=5.650, df=3, p<0.5) (Figure 3 a,b). In the 2nd more specific microhabitat preference

experiment, *E. spinigera* preferred leaves at both times 0 (1.6:1 to ferns) and 24hrs (2:1 to grass) ($G_{corr,24hrs}$=15.391, df=3, p<0.005) (Figure 4 a,b). Usually in the grass or leaves they were found pressed between blades of grass or upside down between leaves.

L. julia preferred mud at time 0 ($G_{corr,0}$=10.117, df=3, p<0.025) but strongly preferred sticks after 24hrs ($G_{corr,24hrs}$=14.254, df=3, p<0.005) (Figure 3 a,b). The 2nd more specific microhabitat preference experiment indicated that selection is random at time 0 but differs from random at 24 hrs with *L. julia* preferring large sticks on mud with small sticks on mud a close 2nd ($G_{corr,24hrs}$=2.541, df=3, p<0.5) In combining preferences for large and small sticks and looking just at mud vs rocks, selection is random at time 0 and differs from random at 24hrs with a preference of mud 2:1 to rocks ($G_{corr,24hrs}$=2.038, df=1, p<0.5) (Figure 4a,cii). At both times 0 and 24hrs *L. julia* preferred large sticks on mud 1.4:1 to small sticks on mud. Upon addition to an arena *L. julia* larvae swam to the bottom and immediately tried to burrow into the mud. Usually they were found dug in the mud under a stick, which provided extra cover. Both species concealed themselves upon addition to the arenas and very rarely were any found in the open.

Discussion

As Dunlap 2001 showed, this study also demonstrated that *Ladona julia* and *Epitheca spinigera* have strong microhabitat preferences and it is these preferences that allow the two species to segregate in space even though they live in close proximity to each another. At time 0 of the general microhabitat experiment 54% of *L. julia* burrowed into the mud and 38% were found in the sticks. After 24hrs only 33% burrowed in the mud and 63% of *L. julia* were under sticks (Figure 3c). This seems to indicate that *L. julia* has a preference for mud in the short term and sticks in the long term. This short-term preference could have been due to the stress of being

in the open—the muddy substrate was the easiest to see and it was the first substance most of the larvae encountered so they burrowed into it to get out of the open. After becoming acclimated to the arena though, the larvae explored their surroundings and 63% ended up in the sticks. When burrowing under sticks a larva would keep the stick in close contact with its back possibly using it for camouflage or for a more stable cover than the mud provided. In his laboratory microhabitat experiment, Dunlap (2001) found 82% of *L. julia* in the mud after 24hrs. This difference in results could be a result of the criteria the two experiments used to qualify the final position of the larvae. To be considered "in sticks" for this study the larva only had to be under sticks and not actually climbing *in* them as it seems was the criteria for the Dunlap (2001) study. It is not clear whether Dunlap considered larvae burrowed in the mud under the sticks as preferring sticks or mud. All larvae in this study "preferring sticks" were found burrowed in the mud under sticks and rarely ever actually climbing in them. This agrees with the classification of *L. julia* as a burrower but indicates that it is a burrower that may prefer to burrow under debris that offers more cover than just mud.

To determine more specific preferences of *L. julia* the experiment was repeated with substrates being large sticks/mud, small sticks/mud, large sticks/rocks, and small sticks/rocks. Microhabitat selection at time 0 for this experiment was random ($p>0.95$) but differed from random at 24 hrs with ($p<0.5$). The randomness of the initial (T=0) microhabitat preference seems to indicate that the range of microhabitats was narrow enough that the larvae could not distinguish, by sight, the differences between them. After 24hrs microhabitat selection differed from random ($p<0.5$) with 67% of larvae preferring small and large sticks on mud (figure 4ci). This preference would only be seen after a larva had had enough time to settle and explore its new environment because the presences of the rocks would not have been apparent to a newly

inserted larva—hence why the original preference was random. *L. julia* preferred a mud substrate to rocks because the rocks did not allow them to sufficiently burrow into the mud. In the cases where the larvae were found on the rocky substrate they would pull sticks onto themselves or wedge themselves in between a stick and a rock. This was observed more often with the smaller sticks (1-6mm diameter), because they were easier to move than the larger sticks, and is suggestive in the trend (figure 4c) that shows larvae on rocks preferring small sticks to large sticks (7:5). This also suggests why large sticks (mud+rocks) were preferred to small sticks (mud+rocks) at T=0 ($p<0.5$) and why this number for large sticks/rocks dropped by half at 24hrs. At T=0 the larvae could see the larger sticks better and that they offered more shelter so these were chosen. Large sticks on rocks were not ideal because (1) larvae couldn't burrow into the mud and (2) larvae couldn't compensate for not being able to burrow by pulling sticks around them because the sticks were too large or heavy to be moved. The trend for the larvae that chose mud shows that they also chose large sticks (14:10). If they could burrow into the mud then it wasn't necessary to have small sticks to pull around them so burrowing in mud under large sticks was preferred because it offered the most cover of all the combinations. The order of preference is: Lg sticks/mud > sm sticks/mud > sm sticks/rocks > lg sticks/rocks.

At time= 0, of the general microhabitat experiment, 41% of *E. spinigera* swam directly to the grass while 44% covered themselves in mud ($p<0.5$). After 24hrs 50% were found in the grass and 27% in sticks. This is consistent with Dunlap's (2001) microhabitat lab experiment that found 78% of *C. shurtleffi* in the leaves. Grass was used in this study's general run instead of leaves because the aquatic grass was the most abundant vegetation at the study site where the larvae were collected. *E. spinigera's* preference of mud, grass and sticks supports their classification as sprawlers that lie on the surface of sediment or amid drifted bark and leaves and

wait with legs outspread (Needham 2000).

The preferred habitat in the general experiment was grass so the experiment was run again with mud and three types of vegetation: leaves, ferns and grass. Microhabitat selection by *E.spinigera* at times 0 and 24hrs differed from random with p values of 0.5 and 0.005 respectively (Figure 4a). At T=0, 43% of the larvae preferred leaves 1.6: 1 to ferns and 50% preferred leaves 2:1to grass after 24hrs (Figure 4b). In the general run *E. spinigera* larvae preferred grass because their leg morphology , adapted at grasping substrate (Corbet 1999), allowed them to grasp and hang onto the grass. This strong grasping behavior was noticed in handling the larvae. Often the *E. spinigera* would cling to the experimenter's finger when they were being handled. It seems that *E. spinigera* chooses their microhabitat based on texture and form of the substrate with preference given to vegetation. In the general habitat experiment they preferred the grass because it was the only vegetation present. In the narrower, more defined experiment they preferred leaves over the ferns and grass. The leaves were dead and water logged and so resembled the dead leaf litter at the bottom of the lake where *E. spinigera* is usually found. Also *E. spinigera* might have chosen the brown leaves because they were better camouflaged against them so it would be harder for a predator to see them. These results indicate that *E. spinigera* has a general preference for vegetation, because of its texture, but when given the choice between green bundles of grass and piles of dead leaves they chose leaves because they provided better camouflage and because they were closer to the substrate at the bottom of a lake where *E. spinigera* normally live.

This study has shown that both *L.julia* and *E. spinigera* have distinct microhabitat preferences and exact substrate preferences can be determined using a narrow range of similar microhabitats. *L.julia*'s pattern of preference was: Lg sticks/mud > Sm sticks/mud > Sm

sticks/rocks > Lg sticks/rocks. Mud was preferred most strongly over rocks and while on mud large sticks were preferred over small sticks because they offered the most cover. If on rocks though, where a larva couldn't burrow, small sticks were preferred over large sticks because they could be moved and held closer to the larva's body imitating a "burrowed" situation. *E. spingera* preferred vegetation because of their morphological ability to cling to it. They prefer dead leaves over green grass indicating that although some may be found in their natural environment climbing in the grass, it is more likely they spend more time among the dead leaf litter at the bottom of the lake. These features of the two species' microhabitat preferences explain how they are able to coexist while living in close relation to each other. In future experiments it would be interesting to see the effects of different substrates or even a combination of substrates... like the stick size vs mud and rocks experiment. For *E. spinigera* it would be interesting to see the effects color of the leaf substrates has on microhabitat preference. This could determine whether it is the texture of the leaf or the camouflaging property that the larvae prefer.

Odonates are of great economic importance. No known species of dragonfly is a pest and larval dragonflies function as population regulators in aquatic ecosystems. Unsuccessful attempts have been made to use adults to control mosquito populations. Although this attempt has proved unsuccessful, further behavioral research could lead to discoveries in how to use dragonflies to reduce populations of pest or disease-causing mosquitoes (Arnett. 2000). Determining larval microhabitat preferences would be a step in reducing these mosquito populations. If the researchers knew which species of larval odonate were found in the vicinity of the mosquitoes then they could limit their study to only adults that would be emerging near the mosquitoes and, subsequently, eating them.

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