

Is Fish Predation on *Enallagma* Selective Pressure?

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Abstract

Attempts to explain female color polymorphism in various species of damselfly—particularly *Enallagma*—have failed to yield convincing data. Increased risk of fish predation on the more conspicuous female *Enallagma* morph is a worthwhile hypothesis to test because *Enallagma* are found co-existing with species of fish that are able to detect differences in UV reflection, such as rock bass (*Ambloplites rupestris*). Rock bass caught in Douglas Lake, Michigan were tested for the ability to differentiate between a conspicuous blue male *Enallagma carunculatum* damselfly, a male *E. carunculatum* whose UV reflectance had been diminished with sunscreen (in order to mimic the UV reflectance of a female heteromorph) and male *Ishnura*, whose color morphology is similar to the female *E. carunculatum* heteromorph. Attempts to mimic a natural setting failed at obtaining data due to no reactivity from the fish being tested. Rock bass were responsive to a line and hook test. No trend in the reaction time of the rock bass to each of the morphs tested was observed. This does not sufficiently accept the null hypothesis, and more testing should be done on how much UV reflection has an effect on fish prey choice, and also how that relates to polymorphic damselflies.

Introduction

The selective mechanism from which female polymorphisms in the Odonate genus *Enallagma* sprung is becoming somewhat of a controversy in evolutionary biology. Many females within the *Enallagma* family appear in two different color morphologies: the greenish, more cryptic “heteromorph” and the blue “andromorph” (Sherrat, 2001). This color dimorphism

is not specific to *Enallagma*, but found in other genus of odonate, as well. The question is, *why* are these varying color morphisms, seen in species such as *Enallagma*, maintained?

Many hypotheses have been proposed as to how this morphology is maintained—how does a female *Enallagma* benefit from being green, how does she benefit from being blue? If one morph were more fit than the other, you would expect one morph color to eventually die out, and the other to become fixed within a population. Both morphs, however, are found in nature. The frequency of each type differs, possibly because the andromorph color is controlled by a recessive allele, and not necessarily due to selective pressures (Finke, 1994). The two most popular hypotheses are the “male mimicry hypothesis” and “frequency dependent” hypothesis. The former hypothesis is the idea that that female andromorphs resemble males and therefore get harassed less, providing the andromorph a sexually selected advantage—except with regards to predation, because the andromorph is more conspicuous (Robertson, 1985). Sexual harassment has been shown to decrease fitness in damselflies (Sirot and Brockman, 2001). The latter hypothesis favors the idea that male preference for each morph is frequency dependent; whichever morph is in abundance is the morph that becomes mated with more often, thus maintaining both phenotypes in the population (Van Gossum et al, 2001). A more specific hypothesis modeled after the frequency-dependent hypothesis was proposed by Miller and Finke (1999). They proposed that males are able to detect which morph is the most frequent in the population, and therefore mate more often with that particular morph. This creates a negative feedback on that particular morph due to increased harassment from males, leading to an increase in the opposite morph population size during the next reproductive season.

While both hypotheses seem plausible, testing has been done on both, and there has not been enough data to overwhelmingly support either (Finke, 1994). We are proposing that female

color dimorphism in *Enallagma* may not have an intraspecific cause, but may be a result of predation. A study using humans as “model predators” found that the andromorph in *Enallagma cyathigerum* was spotted more often than the green heteromorph (VanGossum, 1996). Male *Enallagma*, when analyzed for reflectance, reflected the brightest, female andromorphs reflected the next brightest, and female heteromorphs were fairly dull in coloration (T.D. Shultz unpublished data, 2007) If female andromorphs are easier to see than the heteromorphs, they would be more likely to be eaten by predators who can detect changes in color. Of the known predators of damselflies—other Odonates, amphibians and fish—fish are known to cue especially to UV reflectance (Rocco *et al*, 2001, Letourneau, 2004). Other Odonates—dragonflies, in particular—*can* detect color, but are thought to respond mostly to the motion of their prey (Corbet, 1999). While human “model predators” are a far cry from blue gill, it is likely that rock bass (*Ambloplites rupestris*, of the Centrachidae family) can detect differences in UV refraction, as does a closely related species, the slimy sculpin (Letourneau, 2004).

Fish predation is also likely in *Enallagma* females because of their reproductive behavior. In order to protect their eggs, female *Enallagma hageni* oviposit beneath the surface of the water (Finke, 1985). While studies have shown that some species of *Enallagma* are aware of the presence of fish and respond to it, the oviposition duration of female *Enallagma ebrium* was similar in the presence of fish predators to oviposition duration in waters without fish (McGuffin *et al*, 2005). Attacks on damselflies in tandem by pumpkinseed—which are Centrachidae--were observed at a man-made lake in Germany (Gohmert and Martens, 2005).

Enallagma carunculatum are a species common to the Douglas Lake area of northern Michigan. We are testing the detection of rock bass—a species of fish that is easy to catch in Douglas Lake—to each female morph. We suspect their preference is a function of their ability

to detect UV reflection. Because blue males and andromorphs are more reflective, we propose that rock bass will be quicker to respond to males and andromorphs relative to the response time to a female heteromorph, or a damselfly with the UV reflectance blocked so that it mimics a heteromorph.

Materials and Methods

Natural History Observation Site

Observation of *E. carunculatum* in the field was done on 8/4/2007 at 13:30 for 45 minutes. A lily pad patch in North Fishtail Bay of Douglas Lake was observed. Number of tandem formations was noted, as well as frequency of males in the area.

Experimental Trials

Five 1m by 1m enclosures were set up in South Fishtail Bay in Douglas Lake, Michigan for testing. Enclosures were fashioned using netting, which was held up using 8 reinforcement bars per each enclosure. Bars were fastened to netting using floss. Water depth was 34 cm at testing site. To mimic the a natural setting, vegetation from the lake as well as naturally occurring sources of cover—dead logs and rocks—were placed inside each enclosure. For all of the following tests, *Enallagma* were caught using butterfly nets the day prior and refrigerated until use.

Ten rock bass (*Ambloplites rupestris*, a species of Centrachidae found in Douglas Lake) were caught 3 days prior to the test and kept in aerated 10 gallon tanks. Feeding was ceased. Five rock bass were then transplanted into the enclosure—one fish for each enclosure—and placed in the center under a mesh bucket. Fish were left to acclimate to the enclosure setting for 48 hours. *Enallagma carunculatum* were caught using insect nets the day prior to testing in

South Fishtail Bay of Douglas Lake. Individual *E. carunculatum* were put in bags and kept in a refrigerator until testing began. Testing began at 20:30 on 8/2/2007.

For each trial, time to react was measured using stopwatches. Trials lasted ten minutes each. At the start of the test, the mesh bucket under which the rock bass was under was removed, and 5 minutes were observed in order for the fish to acclimate. After five minutes, either a control male *E. carunculatum* was lowered into the enclosure at the corner, or an experimental male who had been covered in UV block sunscreen (in order to mimic the reflectance of the female heteromorph). Damselflies were glued to reeds with duco-cement. Rock bass were given ten minutes to react to the damselfly being tested. The corner at which the damselfly was being lowered into was varied for each test. Each fish was tested twice, for an N of 10.

The same test was tried within an artificial stream set up in the boat well of Lakeside lab at the University of Michigan Biological Station. One rock bass was placed within the stream setup and given 5 minutes to acclimate. The tests was administered in the same way as the above test in the enclosure. 5 tests were tried for each of the control males and the experimental UV blocked males.

Aerated tanks 10 gallon tanks were set up as well to do the same test. Two tanks were set up in Lakeside lab—one for holding purposes, one for experimental purposes. Rock bass were given 5 minutes to acclimate to the experimental tanks, and then tested for damselfly detection in the same manner as above. 5 tests were tried for each of the control males and the experimental UV blocked males.

The last test performed was with the use of a fishing rod. A large population of rock bass was observed in the boat well. Three types of damselfly were tested for reaction time from the rock bass during this test: control *E. carunculatum* males, UV blocked *E. carunculatum* males,

and male *Ishnura* damselflies whose blue thorax had been colored in with a black sharpie marker in order block all blue UV reflection. At the time of this test—late July—green heteromorphs of *E. carunculatum* were extremely hard to find, and the green color seen on male *Ishnura* resembles that of an *E. carunculatum* heteromorph (T. Shultz, unpublished data). Damselflies were attached to a fish hook and lowered 2m into the boat well. Stopwatches were used to record the time to react from rock bass. The location of where the hook was lowered was varied each time. After being lowered into the water, the hook was kept still and not jerked. If a rock bass made an attempt to eat the damselfly but was not hooked, that was counted as a reaction. If the rock bass was caught on the hook, that was counted as a reaction, and it was taken out of the sample pool and released at a later date. Thirty trials of the control, UV block male, and *Ishnura* male were recorded.

Results

Natural History

Observation on 8/4 lasted 30 minutes. Water depth ranged between 1-3 meters, and a high density of small fish was observed in deeper areas. Density of male *E. carunculatum* appeared to be high. 5 tandem pairs were counted during observation time. Females observed were all blue andromorphs and seen only in tandem. One andromorph was observed ovipositing on vegetation just below the surface of the water (fig 2). Oviposition began approximately 20 minutes after her tandem formation was first observed. The male mate of this female seemed to exhibit guarding behavior of this vegetation. This male was also attacked by another male after 22 minutes. Other tandem pairs were observed flying around in attempts to look for suitable vegetation to copulate upon.

Experimental Trials

Tests performed in the outdoor experimental enclosure located in South Fishtail Bay on 8/2/2007 were inconclusive. 10 trials were conducted—5 with the control male *E. carunculatum* and 5 with the experimental male—in the enclosure at 20:30. No reaction from each of the rock bass tested was observed for all tests throughout the ten minute duration of each.

The artificial stream experiment yielded no results—rock bass did not react to trials. Tests performed in aerated tanks yielded no measurable results as well.

The average time to react to a control male *E. carunculatum*, UV blocked male and Ishnnura male varied between groups during the timed line and hook test (table 1). Because the data was found to be non-parametric, a Kruskal-Wallis test was done and revealed no significance ($F=0.8$, $p<0.2$). A box plot of each of the test groups shows the differing median reaction time of the rock bass to each variable damselfly (figure 3).

UV reflectance was analyzed by T. Shultz. Wavelengths for reflectance of each of the morphs of *E. carunculatum* are shown in Figure 4. Blue males were shown to reflect the most UV, while green heteromorph females were shown to be the most inconspicuous.

Discussion

Unfortunately, the experiment that would have most mimicked a natural setting—the fish enclosure in South Fishtail Bay—yielded no measurable results. One cannot, however, assume the null hypothesis to be true based on this failed experiment alone. Our experimental fish were removed from the boat well, which, although in the same lake as South Fishtail Bay, differs in many important ways with respect fish behavior. Shade and cover have been found to be an important factor in habitat selection among Centrachidae (the family of fish which includes sunfish, pumpkinseed, rock bass and smallmouth bass) as well as water temperature (Probst et al. 1984, Sechnick et al. 1986, Todd and Rabeni, 1989, Reynolds 1976). The range of temperature

smallmouth bass prefer is particularly slim (Reynolds 1976). We attempted to provide ample cover in our enclosures by placing reeds and water logs inside. Except for those attempts, however, our enclosure was in a very well lit area of the lake. The boat well in Lakeside Lab is well lit at some parts, but because it is surrounded by a building, ample shade is always available. Few rock bass or blue gill were observed in the area the enclosure was set up in—perhaps due the lack of natural cover, or an unfavorable temperature. Any or all of these changes could be reasons why the rock bass tested failed to react in the experimental enclosure—they felt like they were in an unfavorable environment.

Another reason rock bass were chosen as the experimental fish was due to the fact they have a large gape size, enabling them to eat something about the size of an *Enallagma* damselfly (Hambright 1991). Because rock bass are gape limited, they tend to ingest prey that is small relative to their mouth size (Tonn and Paszkowski 1986). It's possible that our method of gluing the damselfly to a reed made it appear larger than it actually is, discouraging ingestion attempts from the rock bass. Rock bass are physically able to eat damselflies; our line and hook test showed this, and *Lestes* damselflies, which are typically slightly larger than *Enallagma*, have been shown to be preyed upon by Centrachidae (Stoks and McPeck, 2003). For whatever reason, they chose not to during our tests. This could help explain why the line and hook test generated a response over the other- the fishing line is thin and inconspicuous, the hook is barely visible with prey attached to it. A reed, however, is highly visible and provides a thicker background. Observations of *E. carunculatum* in the field showed that females underwater perch on top of vegetation rather than alongside it—the orientation that our damselflies were placed at on the reed may not have been conducive to detecting contrasting UV spectra.

While no trend was observed regarding the time to react of the boat well rock bass to each of the tested damselfly morphs, this does not conclusively support that rock bass cannot more quickly differentiate the UV reflective morph from a more inconspicuous one. This data coupled with another, differently designed experiment *could* support that hypothesis, and there is good reason to test it. While we know that fish can detect some levels of UV (Rocco *et al*, 2001; Letourneau, 2004) it has also been shown that some fish can detect motion and direction, and this is important in their foraging behavior as well as predator avoidance (Ingle, 1967). The reason our data shows no trend toward any of the damselfly morphologies tested could be for this reason—motion may simply be more important than UV contrast cues. There has been little work on the importance of UV contrast versus motion detection in the fish eye, especially in the Centrachidae family, and more support for this is needed.

*Is motion of greater importance than UV contrast in the detection of *Enallagma* damselflies by their fish predators?* It is true that *E. hageni* females move underwater by climbing down vegetation during oviposition, and are buoyant underwater because of air between their tracheal airsacs and wings (Fincke, 1985). Perhaps just this movement is all the fish relies on when preying upon a damselfly. Also, air as a medium for UV reflectance differs from water. Figures 3 and 4 show the female andromorph of a tandem pair of *E. carunculatum* above water and then underwater. It appears that her color changes, although this has not been measured directly. It's possible that andromorphs are indeed, more conspicuous in air, but not so much compared to the heteromorph while underwater. This would support our null hypothesis, leaving the question of female polymorphisms in non-territorial damselflies still unanswered. It is likely that detection of damselfly prey by fish is a combination of UV reflectance and motion, but more studies are needed to determine which factor is of more importance. Given that the

predatory fish that are likely to eat damselflies—i.e. Centrachidae such as rock bass—have a very narrow range of tolerable conditions in which they will behave normally, testing and observations done in truly natural settings would be best. An experiment that would be worth attempting would be one in which the damselfly being tested could be tethered and placed in a natural setting—a site such as the North Fishtail Bay lily pad patch would be ideal-- in a position that mimicked oviposition observed for reaction from fish in the area. In the event that someone with a very large budget got behind a project such as this one, underwater cameras that could be placed in vegetation that is where female oviposition is known to have occurred would be a good way to observe the fate of female damselflies underwater. No one has been able to show that either the andromorph or the heteromorph in damselfly populations differ in fitness (Fincke 1994); results from this sort of experiment would either support the hypothesis that there is no difference in survivorship between each morph--or disprove it—which would be novel. Also, this could show whether fish cue to UV reflectance as well. If the frequency of andromorphs preyed upon during peak hours of sunlight differs as the sunlight decreases, UV reflectance may play a role in damselfly detection by fish. If not, this would support the idea that motion is a bigger cue for fish predators.

It is possible that the question as to how female polymorphisms in damselflies are maintained can never sufficiently be answered by one hypothesis. Based on the data collected during this study, as well as past studies done of female polymorphism in *Enallagma*, it may be that this color polymorphism is maintained not by one definite cause, but by many factors. Perhaps decreased harassment by males in conjunction with increased predation is the mechanism, or maybe morph frequencies are negative frequency dependent with the added

pressure of fish predation. More studies are needed, and this study should be helpful in troubleshooting for new studies on fish predation and damselflies.

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Tables and Figures

Figure 1. A tandem pair of *E. carunculatum* in North Fishtail Bay.



Figure 2. A female *E. carunculatum* beneath the water in North Fishtail Bay.



Figure 3. A box plot showing the mean reaction time of rock bass to each morph tested during the line and hook test.

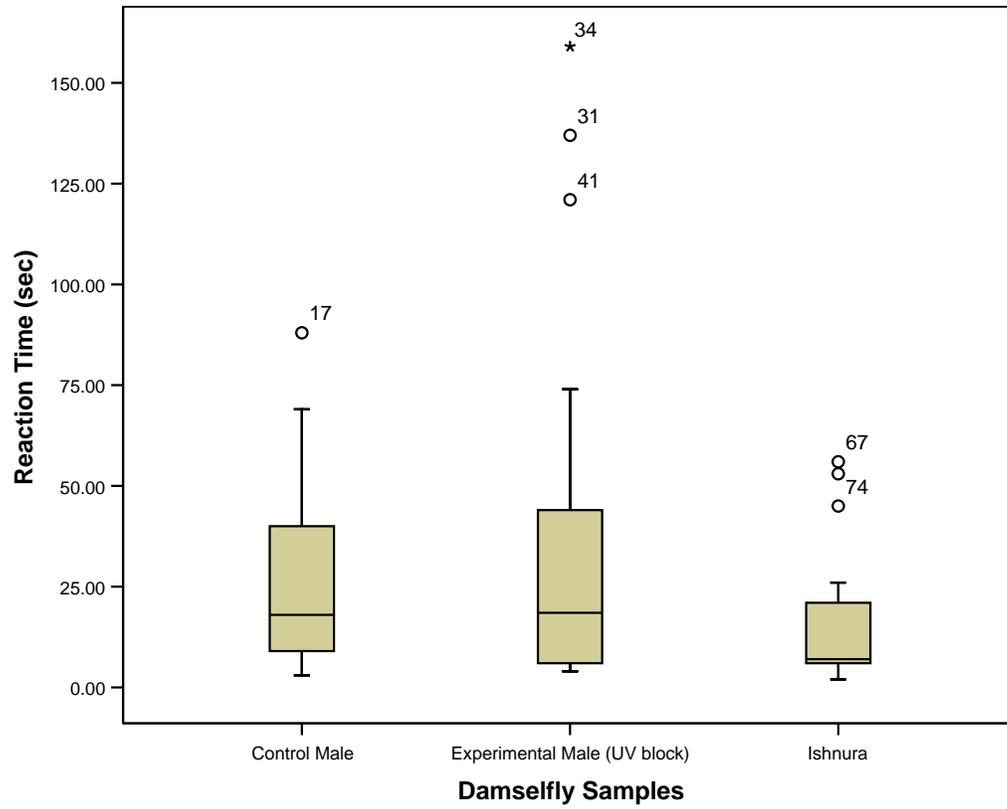


Figure 4. UV reflectance wavelengths for the blue male, blue andromorph and green heteromorph of *E. carunculatum* (T. Shultz, unpublished data).

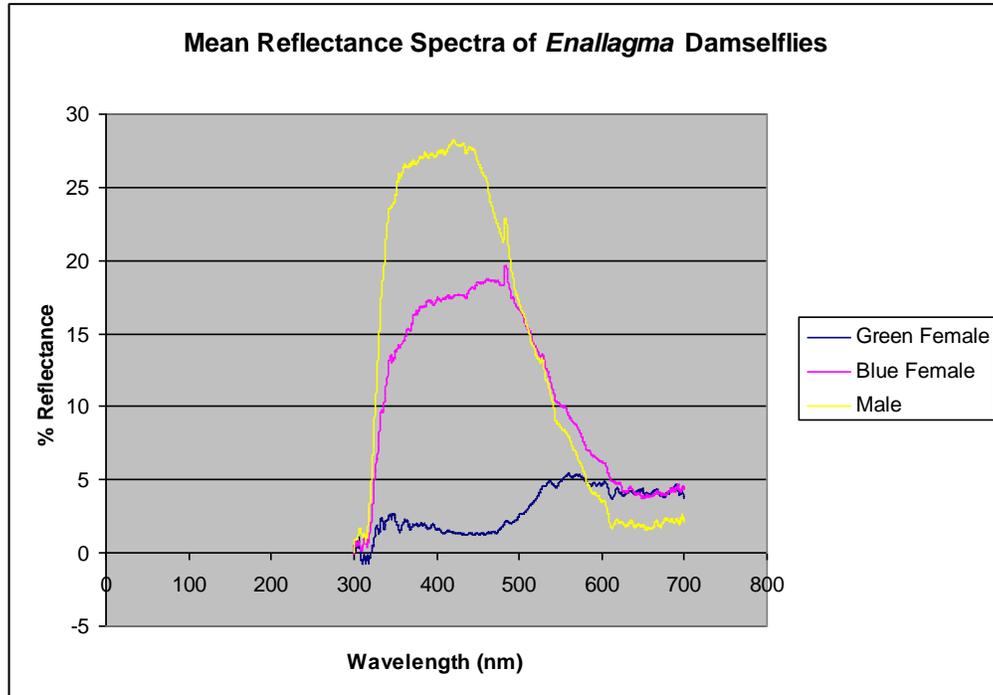


Table 1. Mean reaction time of rock bass to each morph tested during the line and hook test.

Group	Mean	Std. Deviation
Control	25.7647	5.18165
UV block male	32.8235	12.52181
Ishnura	16.9412	4.3084