Dragonfly Naiad Preference for Color and Luster in the Presence of a Predator

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Abstract

Horizontally polarized light attracts aquatic insects. Consequently, aquatic insects, including odonate (dragonfly) naiads, are drawn to water and shiny surfaces, which both polarize light. Aquatic insects' main predators—insectivorous fish—are also drawn to horizontally polarized light. Generally, movement and behavior of insects depends on population dynamics and predator presence. I investigated the effect of predator presence on odonate naiad behavior by placing insects in tanks with colored shiny and matte tiles. I recorded naiad position with and without insectivorous fish present. I hypothesized that the insects would move toward the shiny tiles without a predator present and toward the matte tiles with a predator present, and that they would generally prefer darker colors. During the trials, I observed no preference in colors within the different odonate size classes, although there was a significant preference when comparing all of the naiads together. Overall, the greatest number of odonates moved to the green and black tiles. In tanks including fish, odonates showed no significant color preferences, although green and black were again most popular. The odonates also showed no preference for a particular luster—shiny or matte. The insects placed themselves in three areas of the tank—on the tiles, on the edges of the tiles, or above the tiles on the walls of the tank—instead of remaining on the tiles as I had hoped. Further study is needed to determine the effect size (instar level) has on changes in naiad behavior toward color. Future studies should also attempt to elucidate more precisely the effect predators have on naiad movement.

Introduction

Many aquatic insects have complex life cycles in which they spend part of their life in the water and part on land. As adults, these insects lay their eggs in water, which they recognize by horizontally polarized reflected light (Blaho *et al.* 2014). Over 300 species of aquatic insects have an orientation to polarized light (Horvath *et al.* 2009), and because many surfaces besides water reflect this type of light, this attraction can be detrimental to the insects. For example, Blaho *et al.* demonstrated that shiny car bodies attract insects, which causes some of them to lay their eggs on the cars. Consequently, the eggs become dehydrated and die (2014). In addition, there exists a phenomenon known as “polarization captivity effect” in which some insects are so attracted to the light that they are incapable of moving, eventually resulting in their death (Horvath *et al.* 2009).

One well-known aquatic insect is the dragonfly (order Odonata, suborder Anisoptera). Dragonflies often swarm around shiny car surfaces, specifically red and black shiny surfaces because of the strong polarization patterns (Kriska *et al.* 2006, Horvath *et al.* 2009). Although odonates have an exceptional ability to detect color due to a large number of opsins, it is light polarization that attracts the insects and not the color of the surfaces (Kriska *et al.* 2006). Smooth, dark surfaces produce more polarized light than lighter surfaces (Horvath *et al.* 2009), and aquatic insects approach these darker colors more often than lighter colors like white and yellow (Kriska *et al.* 2006).

Fish are also attracted to horizontally polarized light, as they use the polarization patterns as orientation cues (Horvath *et al.* 2009). Since many fish feed on aquatic insects such as odonates (Montgomery and Kasun 1994), it seems insects would be more vulnerable to predation when on or near shiny surfaces. Fish predation results in the exclusion of some odonate species from certain areas (McPeek 1990) as well as changes in the movement habits of the insects, such as reduced foraging (Strobbe *et al.* 2011). Some aquatic insects use chemical cues in addition to visual cues to sense predators, meaning their presence can be detected even if they are camouflaged or hard to see (Petranka and Fakhoury 1991).

An insect's movement depends on presence of other insects as well. Odonates are cannibals (Johnson *et al.* 1985). Cannibalism is rare if the insects are in the same instar, but becomes more common as the age/size gap grows (Hopper *et al.* 1996). Populations of small naiads are often less dense when larger naiads are present, and population numbers of these insects are often related to the spacing of naiads (Baker 1987). In addition, smaller naiads move around less in the presence of larger naiads than they do when by themselves (Crowley *et al.* 1987).

In previous studies, aquatic insects were compared primarily as adults, since their attraction to horizontally polarized light is most prevalent in the egg-laying stage of their lives (Wildermuth 1998). However, the feeding of predators on naiads can affect the population dynamics of adults greatly and can thereby change future populations (Wesner 2010). This experiment studies odonate naiads rather than adults in an effort to determine whether attraction to polarized light is present throughout the dragonfly life cycle, or only in the adult stage. I look at the movement of odonate naiads and their attraction to color/light in the presence and absence of a predator fish. Although studies similar to this have been done in the field (Kriska *et al.* 2006), they have not been conducted in a controlled lab environment, nor have they compared insect preference in the presence or absence of a predator. I hypothesize that insects will be attracted to shiny surfaces when fish are absent and matte surfaces when fish are present, as sitting on a matte surface could be a camouflage tactic. I also hypothesize that the insects will be more attracted to darker colors as these polarize more light.

METHODS

Experimental habitat

I placed eight plexiglass squares in the bottom of each of four 20-gallon aquariums to completely cover the bottom surface of each tank. Four squares were spray painted a matte luster and the other four squares were left shiny. Four different colors of plexiglass were used: red, green, black, and white. Each color/luster combination was represented in the tank, with the placement of each square in the tank determined using a random number generator (Figure 1). The squares were glued to the bottom of the tank using silicone, and the edges were caulked with silicone to prevent the nestling of the odonates between the edge of the tiles and the tank. In the two tanks used for the fish trials, I used silicone to glue 1/8-inch clear mesh sheets to the inside of the tank at 12 cm above the base of the tank. I added Velcro® strips and thin fishing wire in the center between the two sheets to hold them together (Figure 2). This allowed me to put a fish above the mesh so that the insects below the mesh would be able to see the fish and sense its movement above them as well as detect any chemical cues without the fish being able to eat them.

Study organisms

I collected odonate naiads from Tenderfoot Creek, Morris Lake, and Bay Lake at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan using D-frame aquatic sweep nets on shore areas with vegetation. I then sorted the insects into groups based on size. The groups were "tiny" (5-7 mm in length), "small" (8-9 mm), "medium" (10-11 mm), "large" (12-14 mm), and "huge" (>15 mm) (Figure 3). This kept odonates of similar instars in the same group, as there is a close correspondence between age of the naiads and size class (Buskirk 1992). Size sorting also prevented larger odonates from eating smaller ones and allowed for the study of whether odonates change their tile preference based on life stage, since they may have to remain more camouflaged when they are younger because of the increased risk of predation.

Trials

For each trial, I randomly placed five odonates of the same size group in the four tanks. In the two tanks containing mesh, I added a juvenile bluegill (*Lepomis macrochirus*) above the mesh, as well as an air stone to provide oxygen for the fish. After two hours, I recorded the color and luster of the tiles where the odonates were situated, as well as their position on the tiles–i.e., on the tile itself, on the edge of the tile on the silicone, climbing up the side of the tank, or crawling on the mesh. I ran 50 trials without fish and 20 trials with fish.

Analysis

I analyzed my data in R (R Core Team) using chi-square tests for independence (McHugh 2013). My α was set at 0.05 for each of the tests. For comparisons within trial groups for color and luster, the distribution of naiads was expected to be equal on each tile. For comparisons between the groups with and without fish, the “without fish” trials were considered to be the control.

RESULTS

The odonates were not randomly distributed on the tiles when both edge and on tile placements were compared without fish (x^2 = 26.957, df = 7, *p-value* = 0.0003392). More insects went to matte tiles for every color except red. Overall, most insects went to black and the fewest to white (Figure 4). They were also not randomly distributed when only on-tile insects were compared without fish (x^2 = 14.506, df = 7, *p-value* = 0.04288). In this scenario, most insects went to green and the least to white. One and a half times as many insects went to matte for green, and about equal numbers of insects went to matte or shiny for the rest of the tile colors

(Figure 5). Insects preferred certain colors of tiles in the tanks without fish (x^2 = 11.988, df = 3, *p-value* = 0.007426);the most popular color was green with about 38% of the insects. The least popular color was white with about 11% of the insects moving to those squares. About 25% of insects went to red or black tiles. There was no preference for color in the tanks with fish (x^2 = 2.0345, df = 3, *p-value* = 0.5653), in which green was half as popular as it had been in the trials without fish, and white was almost twice as popular, with red remaining approximately the same and black increasing by about 10% (Figure 6). There was no preference for matte versus shiny tiles in the insects in the tanks without fish (x^2 = 0.60494, df = 1, *p-value* = 0.4367) or in the tanks with fish (x^2 = 0.31034, df = 1, *p-value* = 0.5775). When observed separately by size class, each of the insect groups was distributed randomly on the colors for the trials without fish (tiny - x^2 = 7.4545, df = 3, *p-value* = 0.05874, small - x^2 = 5.1481, df = 3, *p-value* = 0.1613, medium - x^2 = 5, df = 3, *p-value* = 0.1718, large - x^2 = 1, df = 3, *p-value* = 0.8013, huge - x^2 = 7, df = 3, *p-value* = 0.0719).

Of the odonates in the trials without fish, 61.6% of the insects positioned themselves on the edge of the tiles, 32.4% positioned themselves on the tiles directly, and 6.0% were climbing up the side of the tank. Of the odonates in the trials with fish, 36% were on the edge, 29% were directly on the tiles, and 35% were climbing up the tank or on the mesh in the middle of the tank. There was no significant difference in placements of the insects in tanks without fish and the placements of the insects in tanks with fish (x^2 = 1.5116, df = 2, *p-value* = 0.4696), although almost 6 times as many insects were climbing in the trials with fish, and nearly twice as many were on the edge in the trials without fish (Figure 7).

DISCUSSION

Since my data consisted of counts of each insect on the different tiles with the assumption that they would be randomly distributed, I used chi-square tests for independence. I compared the preference for tiles, color, and luster using only the insects that were directly on the tiles. I excluded those that were on the edge because it is likely that the insects on the edges of the tiles simply preferred the tactile sensation of the silicone rather than a feature of that particular tile. This lowered the sample size of the "without fish" trials from 250 insects to 81 insects, which decreased the power of the chi-square test. In a similar way, only 29 of the 100 insects tested with fish were actually located on the tiles, which may explain why no significance was found for color distribution in this group.

The odonates showed a patterned attraction toward colors of tiles and tiles in general; however, there was no pattern for luster preference. The preference in tiles therefore could be completely due to preference for color. Most insects were located on the black and green tiles for the trials without fish, which makes sense as these are darker colors that polarize more light, which attracts more insects. This finding supports my hypothesis and the results of Kriska *et al.*, even though that study found red (in addition to black) to be more commonly attractive since yellow, a light color, was used instead of green (2006). Factors other than color could serve to explain the non-random distribution of odonates. It could be that the odonates simply preferred a certain section of the tank—evidenced by how many odonates moved to the corners of the tanks—even though the tiles were randomly distributed. This theory is supported by the fact that there was no significant pattern within any of the size groups, although the small sample size of insects that were actually on the tiles could have affected this result. The lack of significance within specific size groups could also mean that there were significant differences in the color affinity of each size group and therefore that color attraction changes with age, although more studies would have to be conducted to determine this.

Notably, there was no color preference in the trials with fish, although McPeek has shown naiad movement and behavior can be affected by fish presence (1990). This could mean that the insects were more focused on avoiding the predator rather than which color they went to in the tank. Still, more insects were located on the black and green tiles than any other tiles in the presence of fish, even though this difference was not statistically significant.

The lack of preference for a certain luster refutes my hypothesis and supports the findings of Blaho *et al.*, where matte car paint was shown to emit the same amount of polarized light pollution as shiny car paint (2014). This lack of preference could also be due to the fact that insects may have preferred the slightly rougher matte tiles because they enjoyed the tactile sensation of the tiles, not the actual luster of the tiles. In addition, naiads have fewer opsins than adults, meaning naiads see fewer light wavelengths than adults (Futahashi 2016). Thus, the preference may have been due to chance rather than reflected light preference.

Even though position was not significantly different in the trials with fish and without fish, there seemed to be many more insects climbing in the trials with fish. This seems counterintuitive, as climbing up onto the mesh puts the naiads much closer to the predator. Some naiads even crawled on top of the mesh, directly exposing themselves to the fish. This means that the naiads are either not afraid of predation, or they care more about the tactile sensation of the mesh than the presence of the fish. It could also mean they are not sensing the fish well enough to know there is danger. This could be due to their lack of reliance on chemical signals (Futahashi 2016). It could also mean the fish may not have eaten any odonates previously, as chemosensory recognition of a predator may depend on the predator's diet (Chivers *et al.* 1995).

In repeating this experiment, I would not add mesh to the tanks, because it gave the odonates a tempting surface to explore and prevented them from sitting on the tiles. Although the mesh did keep the fish in the top half of the tank, the fish did not eat any of the odonates that climbed on top of the mesh. Therefore, the complex installation process was both onerous and unnecessary. I would consider using a larger fish in the future, as many odonates, especially those in the "huge" size class, did not seem to fear the juvenile bluegill. It is possible that these odonates were simply too big for the bluegill to eat.

This study explored the behavior of odonate naiads toward colors and reflected light, both in the presence and absence of predators. It informs future studies on differences in attraction to light based on instars of these insects. My results support the findings of previous studies concerning aquatic insect color attraction and provide a basis for experiments focused on obtaining more knowledge about odonate naiad behavior.

FIGURES

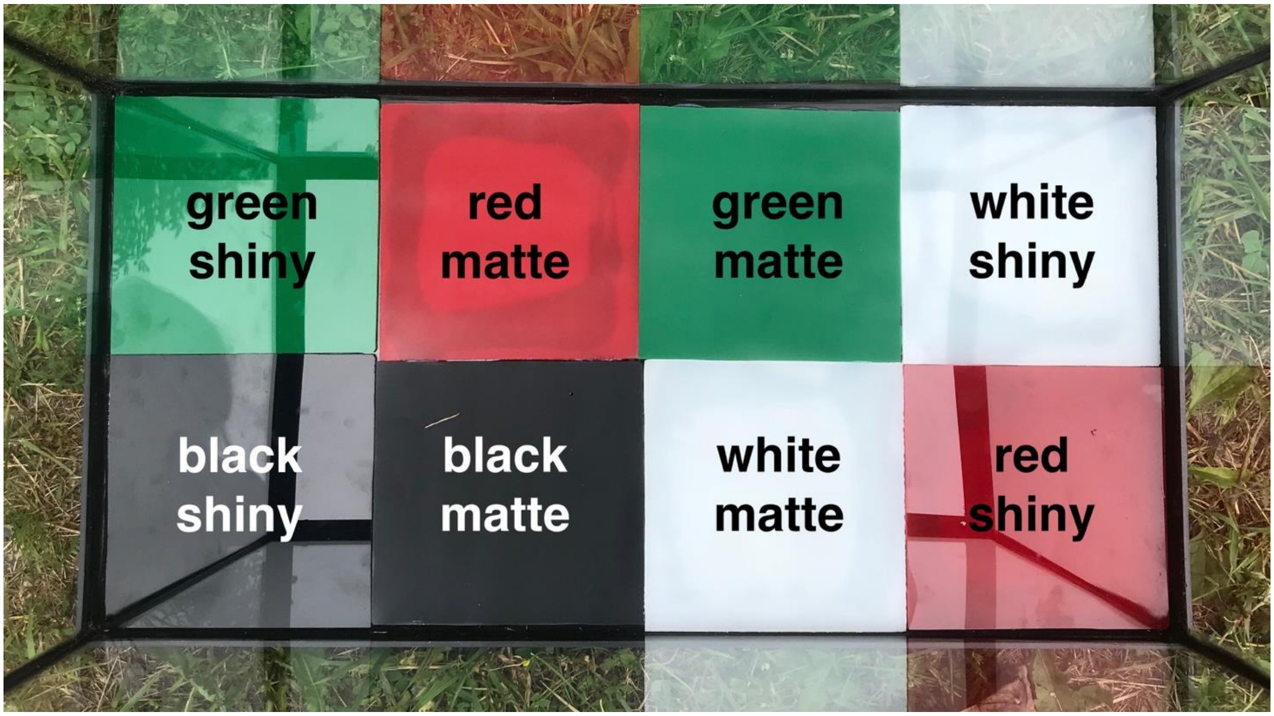

Figure 1. Tank setup with different colored shiny and matte plexiglass squares.

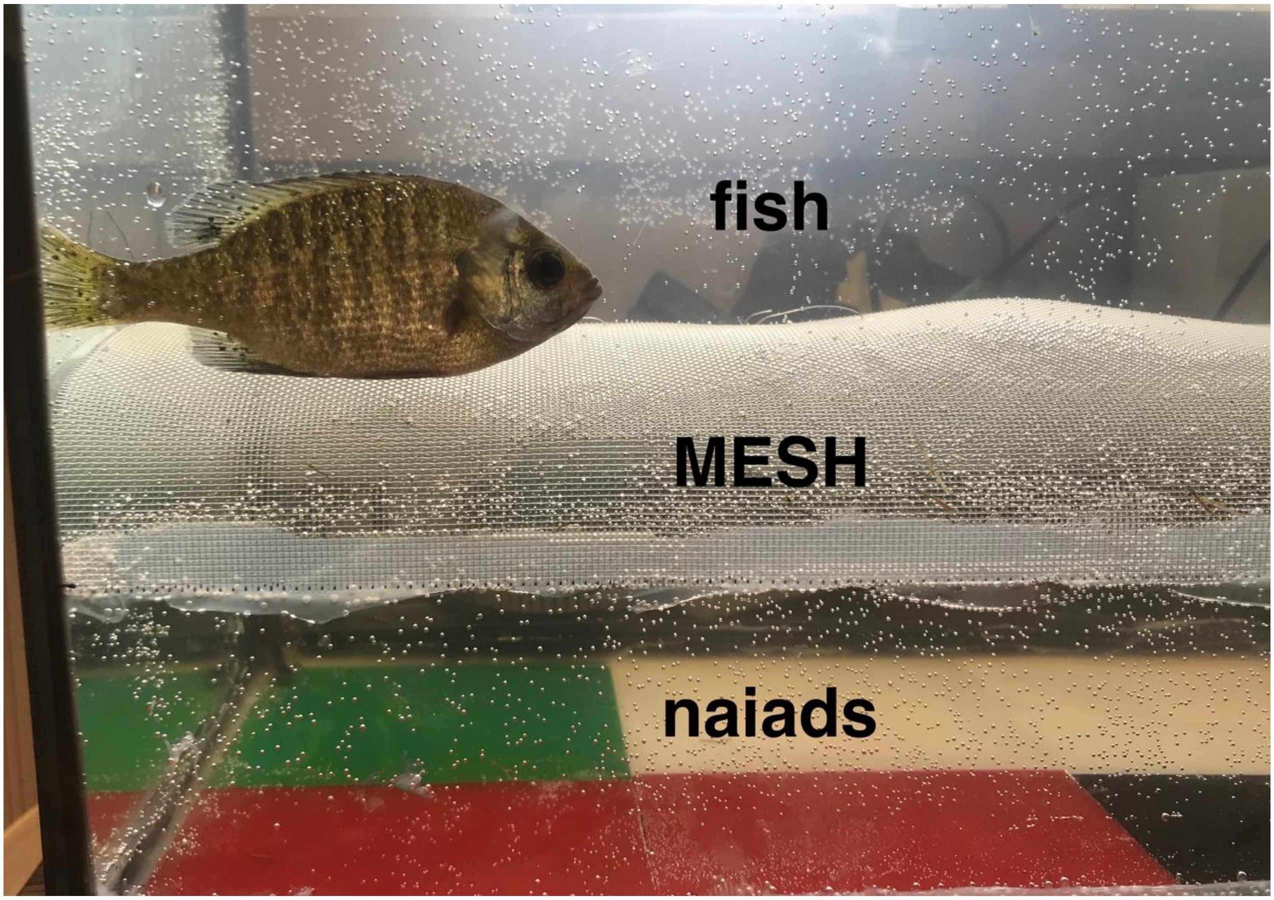

Figure 2. Tank setup for trials with fish.

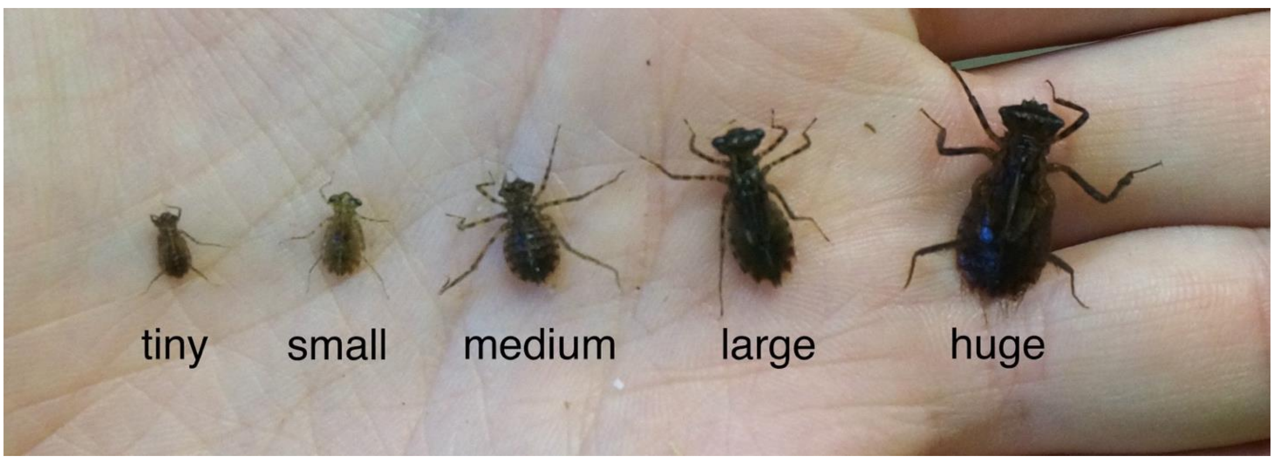

Figure 3. Odonate naiad size classes. Tiny: 5-7 mm, Small: 8-9 mm, Medium: 10-11 mm, Large: 12-14 mm, Huge: 15+ mm.

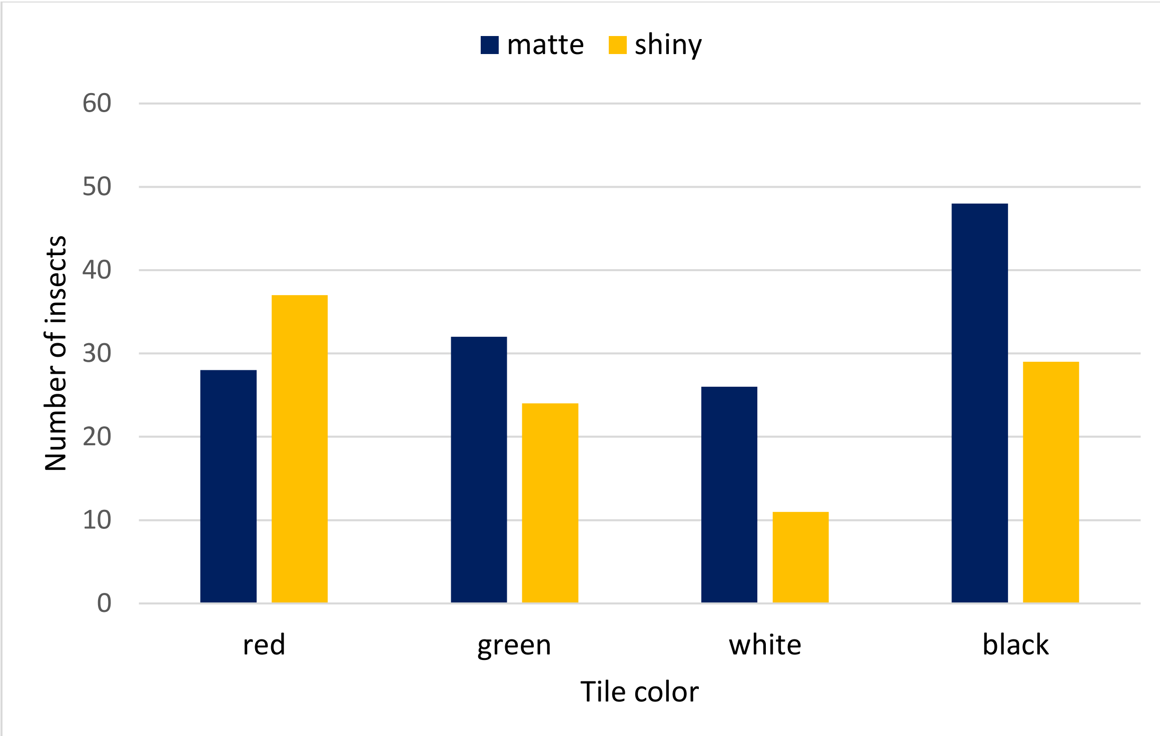

Figure 4. Distribution of odonate naiads on different tiles while including insects that sat on the edges of the tiles as well as directly on the tiles without fish present. There was a significant preference in certain tiles (x^2 = 26.957, df = 7, *p-value* = 0.0003392). About half as many insects were located on shiny white tiles as were located on matte white tiles, and fewer than 2/3 of insects on black matte tiles were located on black shiny tiles. Overall the most insects were located on black matte tiles, and the fewest insects were located on the white shiny tiles.

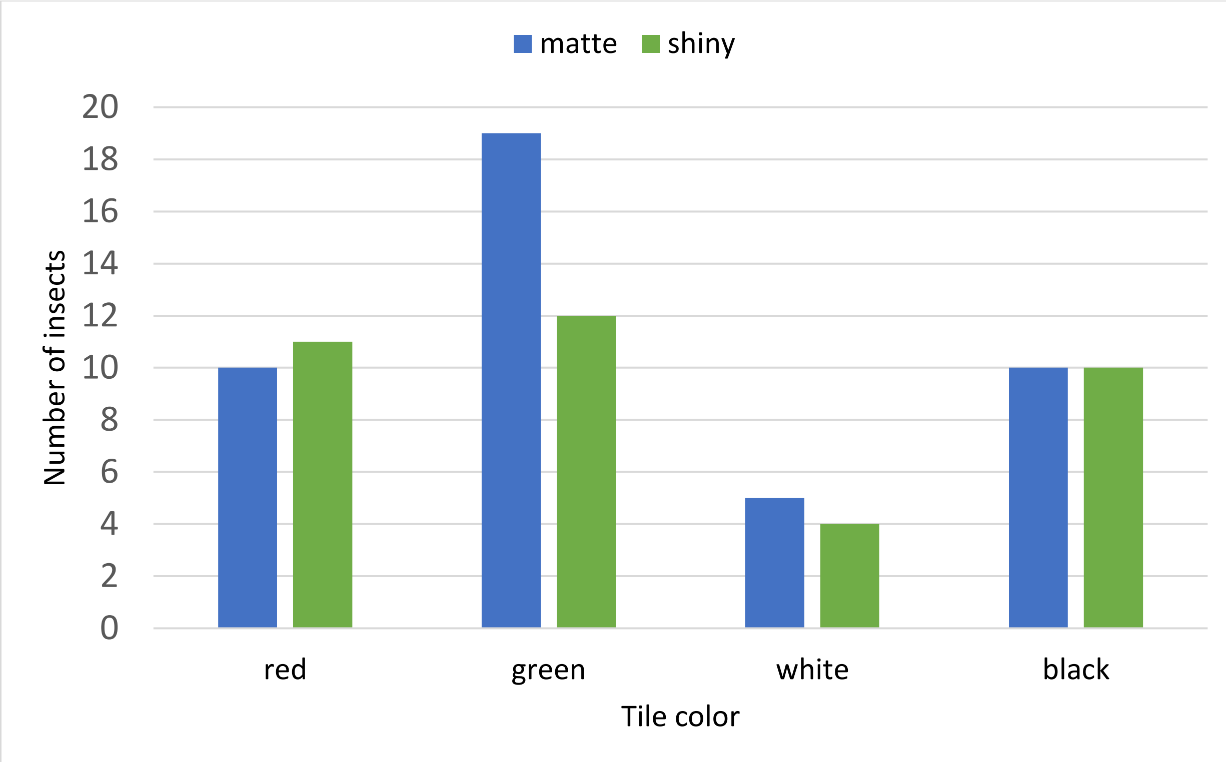

Figure 5. Distribution of odonate naiads on different tiles only including insects that sat directly on the tiles without fish present. There was a significant preference in certain tiles (x^2 = 14.506, df = 7, *p-value* = 0.04288), although less so than the test using all insects showed due to the smaller sample size. There were approximately the same number of insects on matte and shiny for all colors except green, for which there were approximately 1.6 times as many insects on the matte tiles than the shiny tiles.

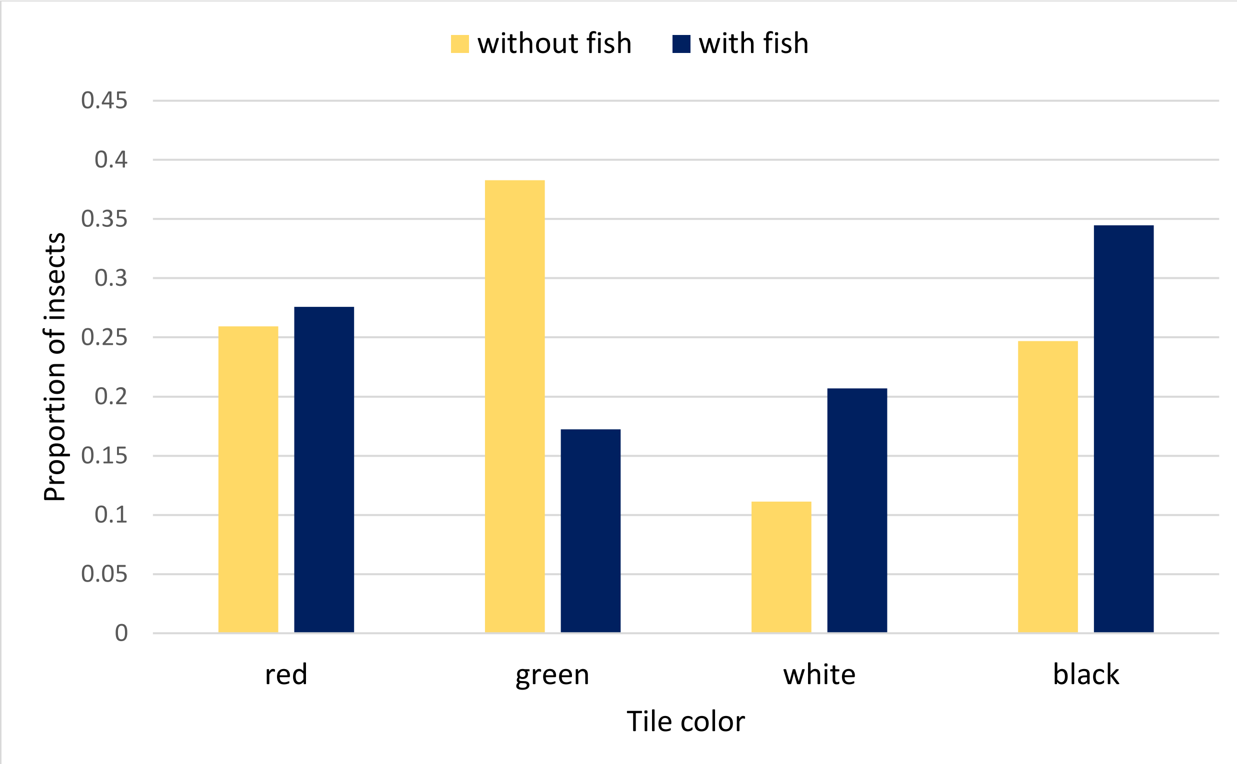

Figure 6. Distribution of insects on tile colors with and without fish present. There was a significant preference in color for the insects on the tiles in the tanks without fish (x^2 = 11.988, df = 3, *p-value* = 0.007426). The most popular color for these insects was green, and the least popular color was white, with similar numbers in between for red and black. There was no preference for color in the tanks with fish (x^2 = 2.0345, df = 3, *p-value* = 0.5653), in which green was half as popular as it had been in the trials without fish, and white was almost twice as popular, with red remaining approximately the same and black slightly increasing.

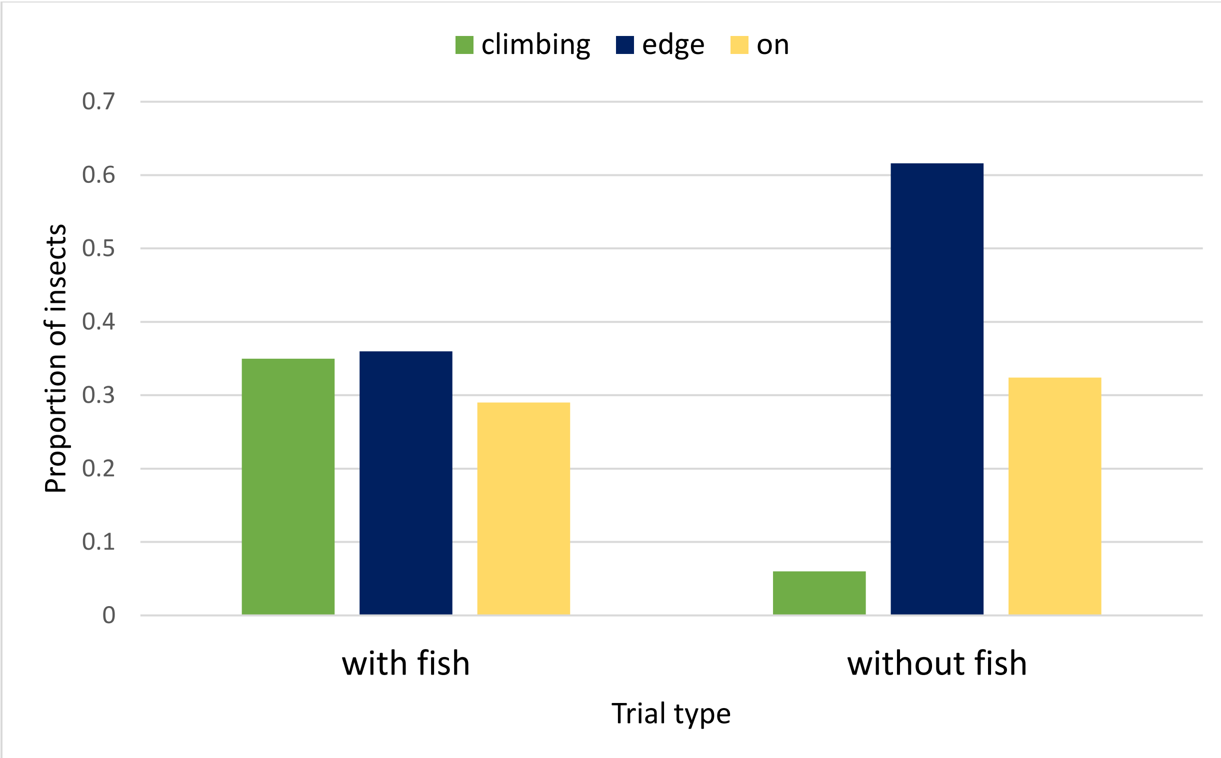

Figure 7. Proportion of insects with fish present and without fish present at each position in the tank. There was no significant difference in the proportions of insects at each position in the trails with fish as compared to the control trials without fish (x^2 = 1.5116, df = 2, *p-value* = 0.4696). Still, there were many more insects climbing with the fish present, most likely because there was the mesh for them to climb onto, whereas many more were located on the edge for the trials without fish, with about half as many directly on the tile and about 1/10 climbing.

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