

Effects of Environmental Pond Drying Cues on *Asynarchus nigriculus* Cannibalism Rates

Maya Tainatongo and Hamish Grieg

Rocky Mountain Biological Laboratory

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Abstract:

Mexican Cut Preserve in the Elk Mountains is home to a unique system of ponds with a high diversity of aquatic life. Many species of caddisflies inhabit the various temporary, semi-permanent, and permanent ponds, including *Asynarchus nigriculus*. *A. nigriculus* is an aggressive caddisfly species that occasionally engages in cannibalism. The factors that cause *A. nigriculus* to go from a detritivore diet to cannibalism are not fully known. However, there is evidence that pond drying events may prompt a shift to cannibalistic behaviors to provide *A. nigriculus* individuals with the nutrients necessary to develop quickly. My research tested whether some of the environmental factors associated with pond drying events induce cannibalism. Specifically, I evaluated increased presence of other caddisfly species, increased conductivity, and greater temperature fluctuations as potential cues for cannibalistic behavior. First, species density tests were used to determine how the presence of another species, *Agrypnia deflata*, affects cannibalism rates in *A. nigriculus*. Here, one treatment contained both *A. nigriculus* and *A. deflata*, while the other two treatments contained a population of only *A. nigriculus* and a population of *A. deflata*. Second, I used a 2x2 factorial design experiment to evaluate temperature and conductivity as cues for cannibalism. Thus, I used these two experiments to gather data on several abiotic and biotic environmental factors that could alter cannibalism rates in the species *A. nigriculus*.

Introduction:

Many caddisfly species have been studied in-depth at the Mexican Cut Preserve in the Elk Mountains of Colorado. One species, *Asynarchus nigriculus*, is known for its predatory and cannibalistic behaviors (Wissinger et al., 1996). Because they live in temporary ponds, they must

develop quickly to emerge as aerial-dispersing adults before ponds dry. *A. nigriculus* is primarily a detritivorous species that feeds on low quality resources, high in recalcitrant carbon with little nitrogen and phosphorus, making their diets inopportune for fast development (Wissinger et al., 2004B). Thus, cannibalism may facilitate rapid development by providing an additional boost of protein and energy (Wissinger et al., 2004B). However, the factors that prompt *A. nigriculus* to shift from detrital resources to cannibalism are not fully known.

Previous work at the Mexican Cut Preserve measured the effects of caddisfly density and reduced water levels on cannibalism rates (Lund et al., 2016). This work determined that the number of aggressive interactions and the duration of these interactions were higher in drying treatments than non-drying treatments (Lund et al., 2016). Drying events can change water temperature, salinity, and competition in the ponds as water volume decreases, but, it is unclear which triggered the behavioral shift in *A. nigriculus*, or whether the increased aggressive behaviors, such as cannibalism, led to lower survival of the caddisflies within the tank (Lund et al., 2016).

Identifying the mechanism linking pond drying to increased cannibalistic behavior is pressing given that recent work indicates climate change is altering pond hydroperiods, including decreasing the time it takes for temporary ponds to fully dry (Lee et al., 2015). Therefore, these environmental changes in pond hydroperiod duration might alter the rates and seasonal timing of cannibalism in species like *A. nigriculus*. In order to investigate the environmental factors (abiotic and biotic) that could influence cannibalism rates in *A. nigriculus*, I performed two experiments: one testing the influence of species density and the second testing physicochemical environmental cues of imminent drying. I hypothesized that the presence of other species, increased temperature, and increased conductivity would each lead to higher rates of *A.*

nigriculus cannibalism due to more stress being put onto the individuals. First, previous research indicated that caddisfly species that inhabit temporary ponds such as *A. nigriculus* are more likely to be aggressive towards other caddisfly species and more cannibalistic within the species, whereas caddisfly species that inhabit permanent pools are more likely to be aggressive and predatory (Wissinger et al., 2004A). For example, my focal species *A. nigriculus* is beginning to co-occur in semi-permanent ponds (ponds that dry during summer some years but remain inundated in others) with *Agrypnia deflata*, which generally inhabits permanent ponds. As climate change makes hydroperiods more variable from year to year, there is a greater chance that these two caddisfly species will interact (Lee et al., 2015). No studies have been performed so far that investigate interactions between these two species. Finally, there have not been any studies into the environmental factors that may lead to varied rates of *A. nigriculus* cannibalism, such as temperature or conductivity

Methods:

Presence of other species

To determine how the presence of *A. deflata* affects the cannibalism rate of *A. nigriculus*, *A. nigriculus* from Pond L8 and *A. deflata* from Pond L1 were placed in shoe-box sized aquaria (Figure 1). I used three treatments in the *A. nigriculus* versus *A. deflata* experiment: one treatment with eight *Asynarchus*, one with eight *Agrypnia*, and one with eight *Agrypnia* and eight *Asynarchus*. These specific numbers were based on the mean density of 400 larvae/m² for high densities of *A. nigriculus* along the shoreline of the ponds presented by Wissinger (Wissinger et al., 1996). Each of the three treatments were replicated six times making an overall total of 18 bins. The treatments were housed inside a weather port set up at Mexican Cut Preserve to protect

the experiment from outside weather conditions and interference from animals. To account for variable temperatures inside the weather port, treatments were split up into blocks and randomly placed in four quadrants of the port so that there was at least one replicate of each treatment in each quadrant. Each container contained 20g of sedge from Pond L1, 15ml of pond substrate from Pond L1, and 2.5l of water from Pond L8. The caddisflies remained in the containers for five days to interact, which is consistent with the duration of other cannibalism and predation experiments with these species (Wissinger et al 1996; 2004). On the fifth day, the number of *A. nigriculus* and *A. deflata* left in each container were recorded and the surviving individuals were returned to permanent mesocosm tanks set up near the Mexican Cut Preserve weather port to complete the remainder of their development.

Cues of imminent drying

Cues that imply forthcoming drying events may also be major drivers of cannibalism rates in *A. nigriculus*. To test two of these variables, conductivity, and temperature, I set up a 2x2 factorial design experiment on July 7, 2022. These specific variables were chosen because temporary ponds will often increase in conductivity and mean temperature as they dry due to less water volume being held in the pond. As water evaporates, solutes concentrate and increase conductivity, and the remaining body of water heats up more quickly during the day. There were two treatments that tested temperature, and nested within these were two more treatments that tested high and low conductivity. To alter temperature, a shade cloth was placed over one treatment set of 10 aquaria while the other treatment set of 10 aquaria were left in the sun where the treatments received full sun at 12:30pm. The goal of the shade cloth was to reduce mean temperature and dampen diurnal temperature fluctuations in those treatments.

The two treatments of high and low conductivity were created in shoebox sized aquaria by adding different amounts of NaCl to the twenty replicates. A concentrated NaCl solution ($\sim 1833 \mu\text{S}$) was mixed with water from Pond L8 and 25-100mL of this salt water was added to high conductivity treatments to alter conductivity. The conductivity in all tanks started between 3-5 μS , which was the ambient conductivity of the nearby pond L8. Conductivity was then increased to 10-13 μS in ten randomly selected containers on July 11, 2022. Conductivity in these same containers were increased to 24-28 μS on July 12, 2022, and then increased further to 46-50 μS on July 14, 2022. The remaining ten tanks were left at a conductivity of around 5 μS . Conductivity in all treatments was measured and recorded each day before and after salt water was added to the high conductivity treatments. Conductivity measurements were taken from temporary ponds at Mexican Cut Preserve throughout the experiment to ensure that treatment conductivities were rising at the same rate as pond conductivity. Each of the twenty treatment aquaria in this experiment contained ten *A. nigriculus* individuals from pond L19, along with 60mL of sedge detritus and 140mL of pond substrate from Pond L1. Sedge was cleaned of other insects before being placed in the experimental aquaria. Aquaria contained 2.5L of water from Pond L8. Hobo temperature loggers were also added to seven treatment aquaria with three data loggers in the sun temperature treatment and four data loggers in the shade temperature treatment (Figure 2). On July 20, 2022, the experiment was taken down and final conductivity and caddisfly numbers were recorded. Hobos were taken back to the lab and data was downloaded and stored. Remaining caddisflies from each treatment were placed into a labeled ethanol container with one container for each of the twenty treatments. In the lab, caddisfly larvae and pupae dry weights were measured. This was done by removing larvae and pupae from their cases

and then placing them in pre-weighed weigh boats. Larvae and Pupae were measured separately for each treatment tank so growth rates could be analyzed at each life stage.

Results:

Presence of other species

Experimental data from the research conducted at the Mexican Cut Preserve indicates that the presence of *A. deflata* reduced the survival of *A. nigrliculus* ($F_{(1,16)}$ ratio=125, $p<0.005$) (Figure 3), however, the presence of *A. nigrliculus* did not have an effect on the survival of *A. deflata* ($F_{(1,16)}$ ratio=1, $p=0.341$) (Figure 4). We observed an overall mean of 4.75 surviving *A. nigrliculus* per treatment and a mean of 7.917 *A. deflata* per treatment (Figure 5). When looking only at the treatments with both *A. nigrliculus* and *A. deflata*, *A. nigrliculus* had a mean survival of 1.833 individuals per treatment, whereas *A. deflata* had a mean survival of 8 individuals per treatment (Figure 5).

Cues of imminent drying

There is evidence that higher temperatures positively influenced the mean larval body mass of *A. nigrliculus* ($F_{(1,16)}=14.18$, $p=.002$) (Figure 6). However, conductivity did not have an effect on mean larval body mass ($F_{(1,16)}=.03$, $p=0.866$) (Figure 7). Mean pupal body mass was also not affected by changing conductivity ($F_{(1,9)}=.001$, $p=.972$), nor temperature changes ($F_{(1,9)}=.402$, $p=.542$) (Figures 8 and 9). Overall, *A. nigrliculus* cannibalism and survival rates were not affected by fluctuations in either temperature ($F_{(1,16)}=2.299$, $p=.149$) or conductivity ($F_{(1,16)}=.092$, $p=.7656$) (Figures 10 and 11).

Hobo data loggers showed that the average daily temperatures of the sun and shade treatments did not differ significantly (Figure 12). However, when the difference between the

average maximum and minimum daily temperatures for the two treatments were compared, it was determined that daily temperatures fluctuated over a broader range in the sun treatment than the shade treatment (Figure 13).

Discussion:

There is evidence of decreased *A. nigriculus* survival in the presence of *A. deflata*. This is most likely caused by increased rates of intraguild predation and not increased rates of cannibalism because survival rates of *A. nigriculus* remained high in the treatments with only *A. nigriculus*. The presence of *A. nigriculus* did not affect the survival rate of *A. deflata*. *A. deflata* begins larval development earlier than *A. nigriculus*, so size differences between the two species could explain why the smaller *A. nigriculus* was preyed on by the larger *A. deflata*.

As pond drying timelines are altered by climate change, caddisfly species like *A. nigriculus* and *A. deflata* have a higher likelihood of encountering each other (Wissinger et al., 1996). This can lead to more instances of intraguild predation, but not necessarily higher rates of cannibalism. Higher rates of intraguild predation between competing caddisfly species can greatly alter predator and prey population numbers and overall pond species diversity.

When experimenting with cues that imply pond drying, such as temperature and conductivity, higher temperatures led to higher mean larval body mass temperature increases, caddisfly activity may have increased due to higher metabolic rates, thus causing the caddisflies to eat and grow more (Clarke et al., 2004). Whereas conductivity, whether it was high or low, did not affect mean larval body mass. Conductivity and temperature also did not affect the mean body mass of pupae. However, caddisflies often decrease in body mass while pupating, which could explain why there was no obvious trend across environmental treatments. In terms of

cannibalisms rates, *A. nigriculus* survival rates were not affected by changes in either water temperature or conductivity. Because of this, my research suggests that these specific environmental drying cues did not affect *A. nigriculus* cannibalism rates.

While cannibalism rates of *A. nigriculus* were not significantly altered by any of our treatments, we did find that biotic interactions are stronger than abiotic factors in determining *A. nigriculus* survival. Specifically, the presence of permanent pond species, like *A. deflata*, affected survival of *A. nigriculus* more so than any abiotic factors examined in the study. Thus, as climate change alters pond hydroperiods and prompts novel invertebrate community compositions, species interactions may be more important than abiotic factors in determining which species can and cannot persist in changing habitats (Shepard, 2021).

Literature Cited

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Tables and Figures

Mexican Cut Preserve- *A. nigriculus* Experiment

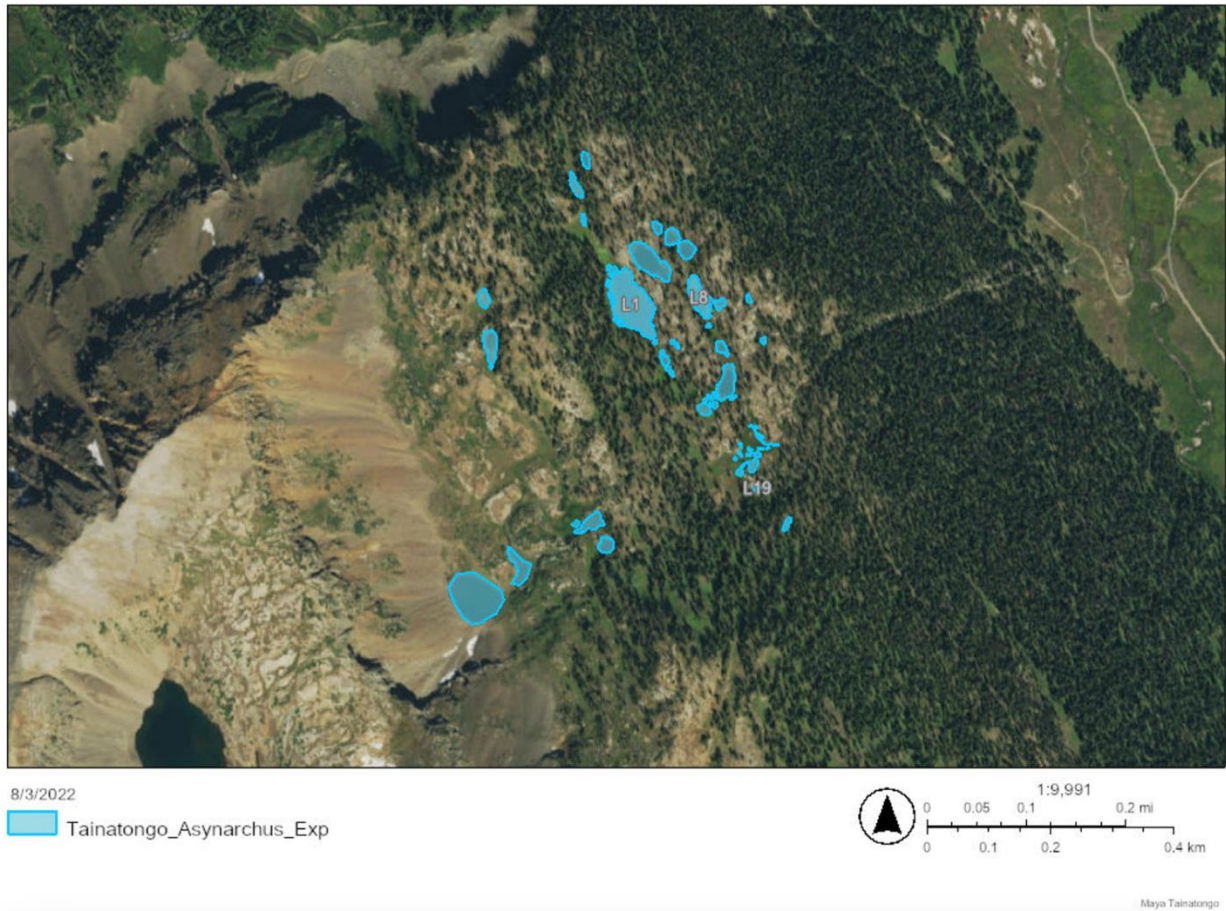


Figure 1: Mexican Cut Preserve Pond locations. Ponds used in this experiment include Ponds L1, L8, and L19.

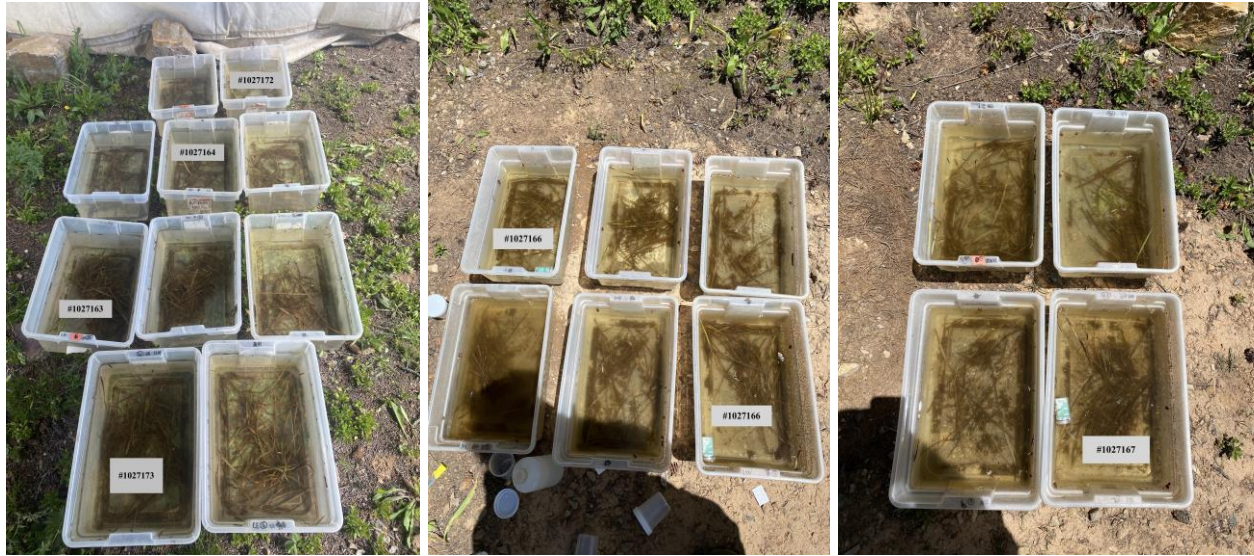
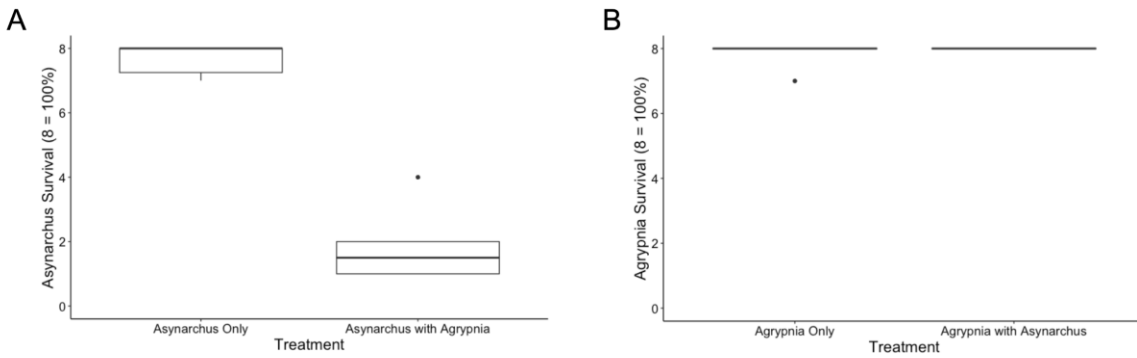


Figure 2: Placement of Hobo data loggers in the sun (right two images) and shade (left image) treatments.



Figures 3 and 4: The effect of *A. deflata* on cannibalism and survival rates of *A. nigriculus*.

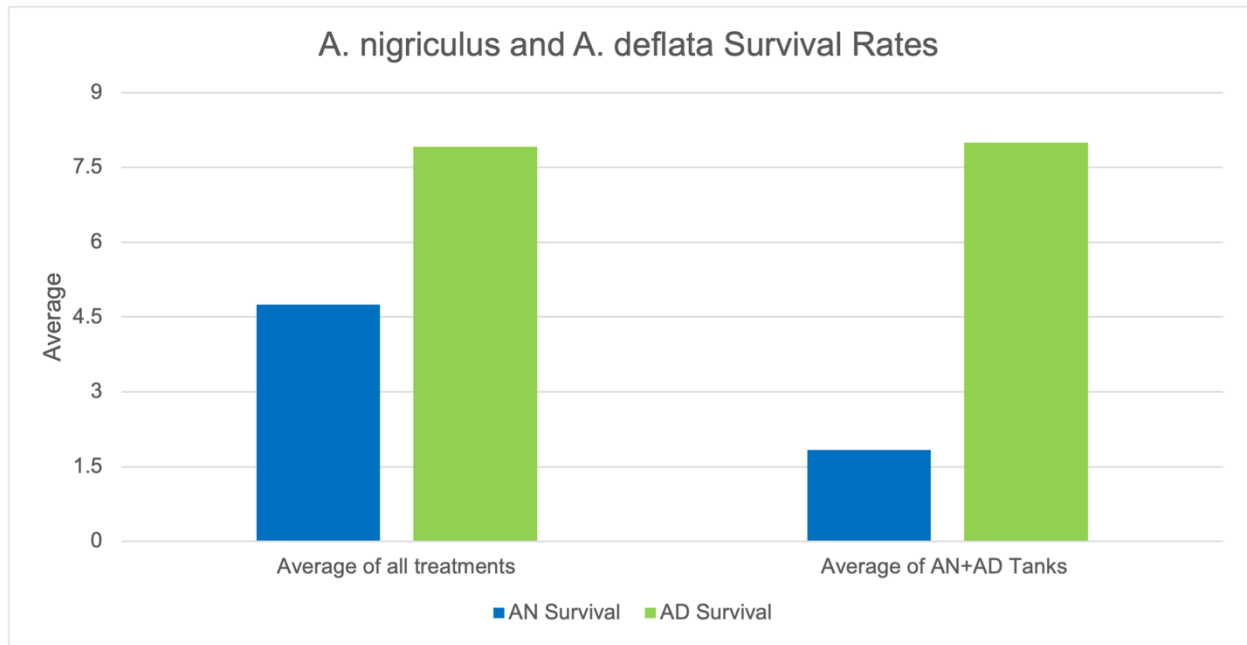
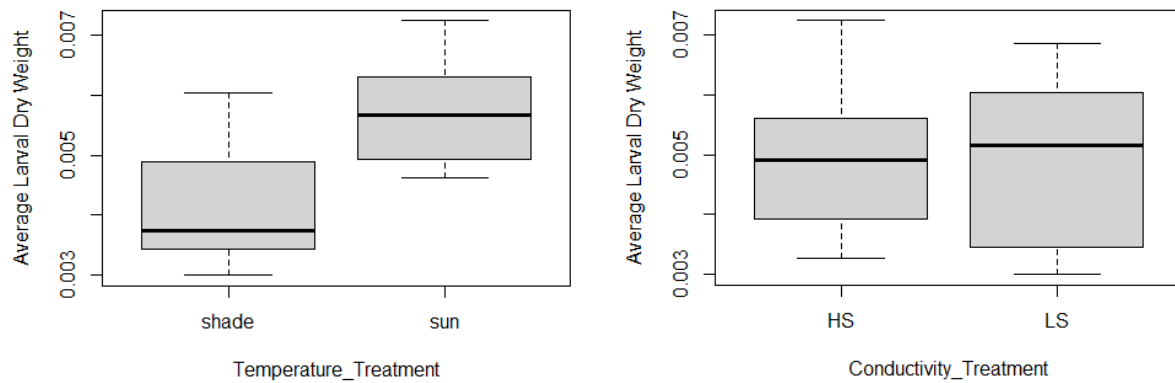
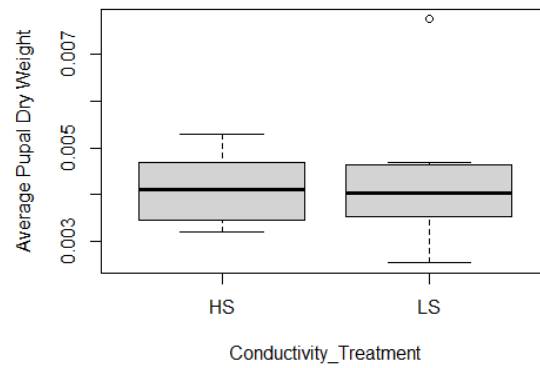
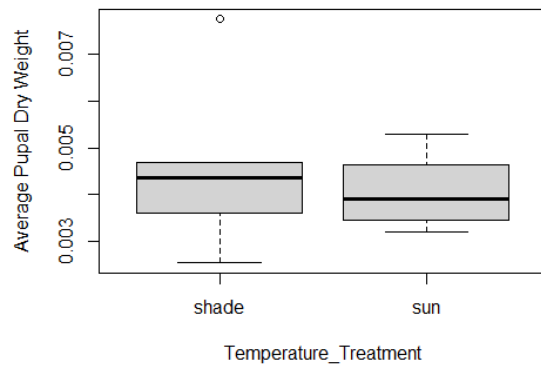


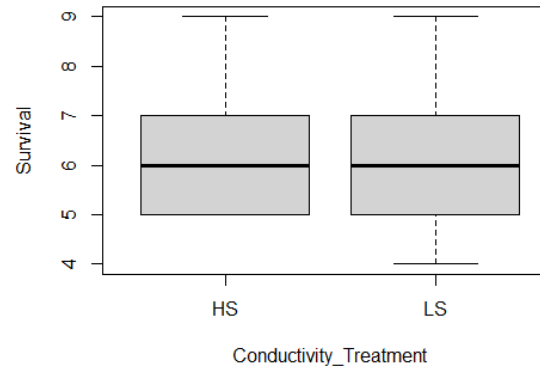
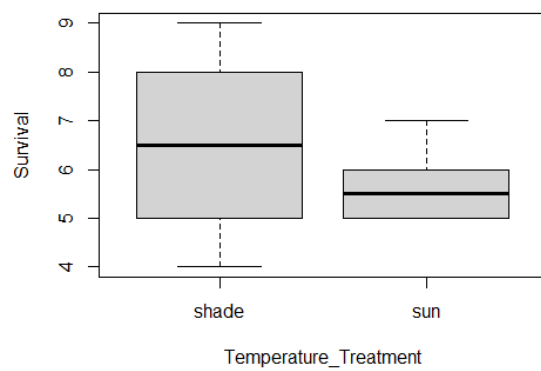
Figure 5: Average survival rates of *A. nigrificus* and *A. deflata* across all treatments (left) and average survival rates of *A. nigrificus* and *A. deflata* in the treatments with both *A. nigrificus* and *A. deflata*.



Figures 6 and 7: The effect of temperature and conductivity on average *A. nigrificus* larval dry weights.



Figures 8 and 9: The effect of temperature and conductivity on average *A. nigrificus* pupal dry weights.



Figures 10 and 11: The effect of temperature and conductivity on *A. nigrificus* survival rates.

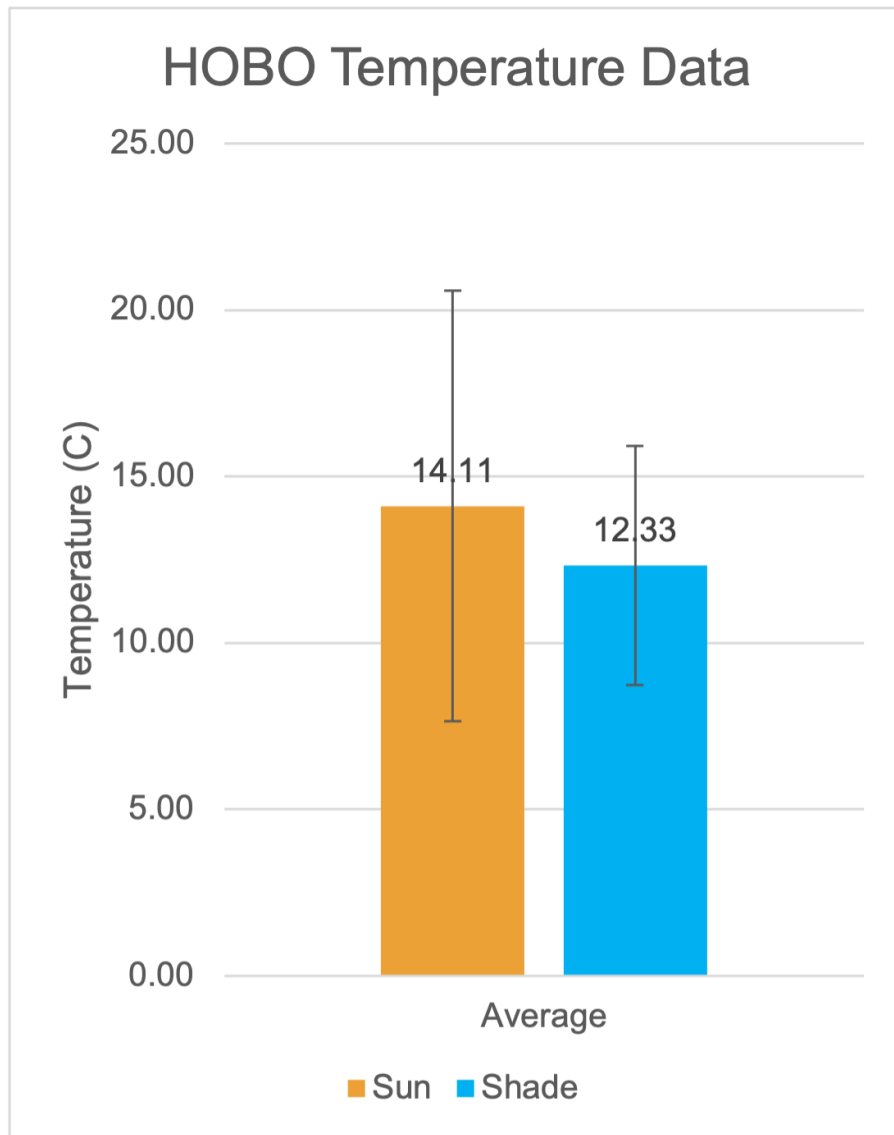


Figure 12: Average daily temperature data for both sun and shade temperature treatments.

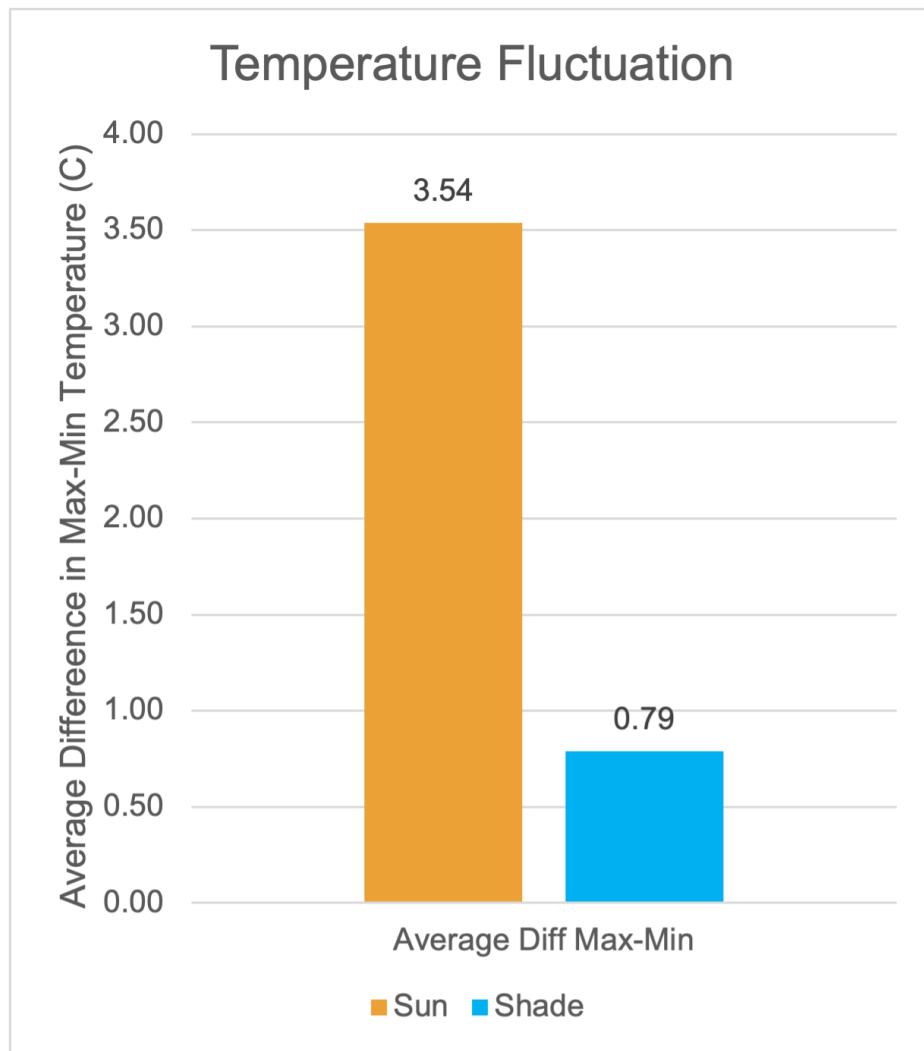


Figure 13: Amount of fluctuation between average minimum and maximum daily temperatures in the sun and shade treatments.