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Rates of Water Loss and Absorption in the Eggs of Stick Insect *Eurycantha calcarata*

Garret Jolma
Department of Biological Sciences
University of Montana
32 Campus Drive
Missoula, MT 59812

Faculty Adviser: Dr. Art Woods

Abstract

The thorny devil stick insect *Eurycantha calcarata* (Phasmatodea: Lonchodidae) of New Guinea has eggs that take three months or more to develop—incredibly long for an insect. Long development times can be a challenge for eggs because of their finite resources, including nutrients, energy to support development, and water. These experiments investigated the physiological mechanisms underlying long development times in stick insect eggs. The first experiment examined rates of water loss and survival of eggs held in different experimental humidities (0, 75, or 100% relative humidity). Eggs dried quickly in the 0% humidity "dry" container and more slowly in the 75% humidity container. The eggs did not dry out in the 100% "saturated" container and maintained their original mass throughout the experiment. While none of the dry treatment eggs hatched, one of the 75% RH treatment eggs did, and all but one of the saturated treatment eggs hatched. To see if the eggs could reabsorb water, a fresh batch of eggs were dried until they reached 90% of their original mass. Then they were transferred into a 100% humidity or wet cotton treatment. In both cases, the eggs gained some mass, but never returned to their original mass. These experiments show that the eggs require a high humidity to survive, and that they cannot absorb water from their environment. For thorny devil stick insect eggs, conserving water is of the utmost importance.

Keywords: dehydration, thorny devil stick insect, eggs

1. Introduction

The thorny devil stick insect *Eurycantha calcarata* (Phasmatodea: Lonchodidae) lives in the forests of New Guinea. Adults live in tree trunk cavities during the day, then move into the canopy to feed at night. Like closely related stick insects,[1] *E. calcarata* females (Figure 1) crawl to the forest floor and deposit eggs one by one into the soil. The eggs (Figure 2) take three months or more to develop.[2]

Figure 1: An adult female *E. calcarata*.[3] Females, which are larger than males, can get up to 14 cm in length.

Figure 2: An egg of *E. calcarata*.[4] Eggs are typically 8 mm by 4 mm, and weigh 80-100 mg.

Long development times can be a challenge for eggs because of their finite resources, including nutrients, energy for development, and water. The diapausing eggs of at least one species of stick insect (*Extatosoma tiaratum*) have been shown to absorb water from the environment[6], and it would be of great benefit for the eggs of the thorny devil stick insect to be able to do the same. These experiments investigated water loss in the eggs of *Eurycantha calcarata*.

2. Methods

2.1 Dehydration

To observe how quickly the stick insect eggs lost water in varying conditions, 60 eggs were divided evenly into three 24-well plates. Those plates went into three different plastic containers, each with a different relative humidity (RH): The 100% humidity treatment had a pool of water in the bottom. The 75% humidity treatment had a saturated salt-water mixture in the bottom. The 0% humidity treatment had Drierite desiccant in the bottom to absorb any water in the air. All three experimental treatments were held at room temperature (22±1 °C).

While the experiment ideally needed 60 eggs at once, that was more than the stick insect colony could provide at once. The experiment started with 17 eggs, equally split between the three treatments, and more were added to each treatment as they became available. The laying date and the code for the female that laid each egg were also recorded.

Eggs were weighed every three days on a microbalance (Sartorius MC5, ±1 µg). After some time, that interval increased to a week, and then a month, and then two months.

2.2 Rehydration

To see if the stick insect eggs could reabsorb water from their environment once they had lost it, 30 eggs were placed in a 0% humidity container with Drierite desiccant in the bottom. The eggs were then weighed once a week to track water loss. Once an egg reached 90% of its initial mass, it was transferred to one of two rehydration treatments and assigned a new egg ID to match the new 12-well plate it was put in.

The two rehydration treatments differed slightly in construction: The high humidity treatment had 100% humidity thanks to a pool of water in the bottom of the plastic container (identical to the 100% RH treatment in the dehydration experiment). The wet substrate treatment also had 100% humidity due to a pool of water in the bottom of the container, but also had wet cotton in each of the wells of the 12-well plate to simulate wet soil.

With 15 eggs in a 12-well plate, a few were placed between wells; but all had consistent egg IDs. Each egg was then weighed regularly to observe any changes in mass. This experiment was also run at room temperature (22±1 °C).

3. Analysis

The masses of the eggs in the dehydration and rehydration experiments were logged in a lab notebook and backed up in a Google Sheets spreadsheet. Line graphs of these masses were later made in Python.

4. Results

4.1 Dehydration

Figure 3 shows the results of the saturated 100% RH treatment. Blue lines represent eggs that hatched; orange lines represent those that did not. Eggs in this treatment lost negligible mass throughout their development time.

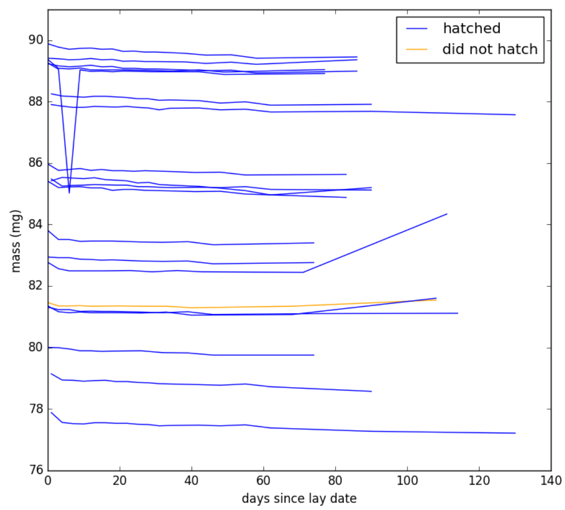

Figure 3: The masses of 20 eggs in the saturated humidity treatment. These eggs lost relatively little mass.

All but one of the eggs in the saturated treatment went on to hatch. Any hatchlings found from these experiments were captured, labeled, and placed in the lab freezer for future morphological measurements.

Figure 4 shows the results of the 75% RH treatment. Eggs lost mass slowly over time. Only one egg in this treatment hatched.

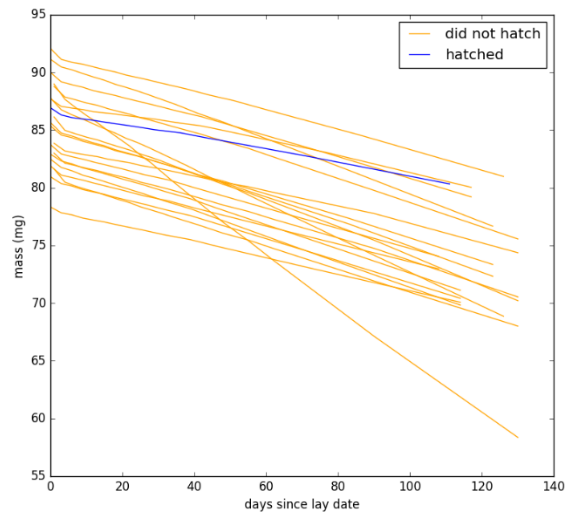

Figure 4: The masses of 20 eggs in the 75% RH treatment. These eggs lost mass, but at a slow rate.

Finally, Figure 5 shows the results of the 0% RH treatment. Eggs lost mass quickly in this treatment. None of the eggs in this treatment hatched.

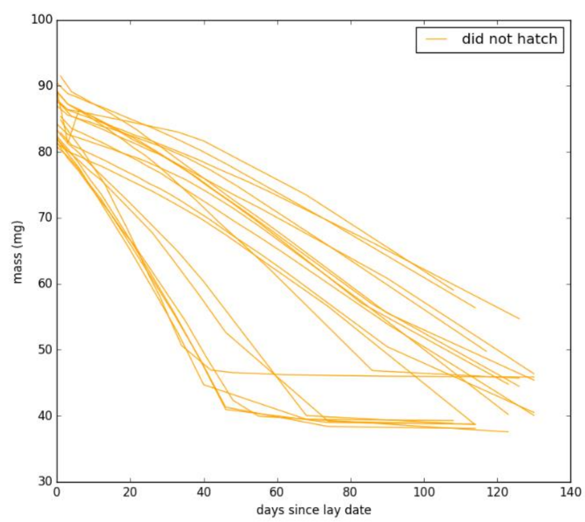

Figure 5: The masses of 20 eggs in the dry treatment. These eggs lost mass quickly.

Some of the eggs in the dry treatment lost mass at such a rate that they reached their dry mass of about 40 mg. This is evident in the line graph of this treatment, where the lines with the most negative slope suddenly change behavior and exhibit a near-zero slope.

4.2 Rehydration

Figure 6 shows the results of the rehydration experiment. Purple lines represent eggs placed in the humidity-only treatment; brown lines represent the humidity and wet soil treatment. Solid lines of either color represent eggs that hatched; dashed lines of either color represent those that did not hatch.

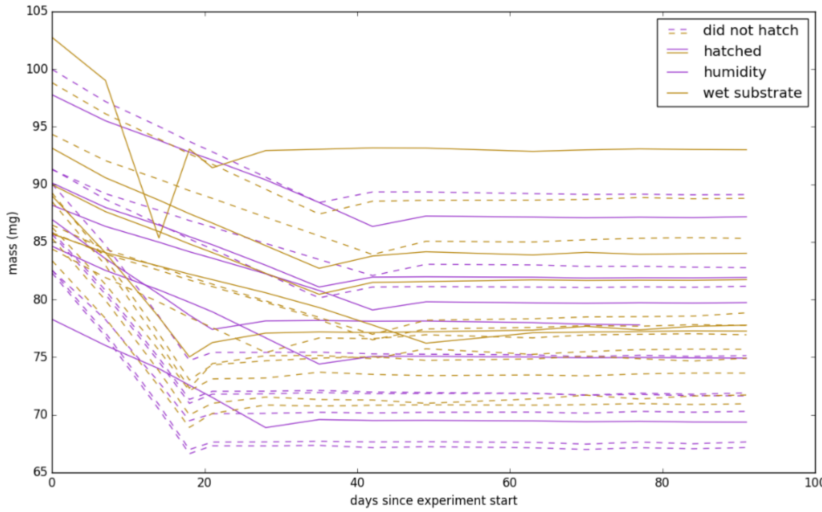

Figure 6: The mass of 30 eggs that were dehydrated and then placed into rehydration treatments. Note the small uptick in mass after placement into the rehydration treatment and the relatively level slope of each line after that.

The small uptick in mass after each egg was transferred into a rehydration treatment likely represents the eggshell's surface becoming rehydrated with water vapor again. However, the eggs did not continue to absorb water, suggesting that the embryos inside cannot absorb water from their surroundings.

5. Discussion

The dehydration experiments show that the lower the ambient humidity, the quicker the rate of water loss. The eggs in the 100% RH treatment lost almost no mass because the air around them was already saturated with water vapor.

While all but one of the 100% RH eggs survived to hatching, only one of the 75% RH eggs hatched. Further, it appears that the single egg from the 75% RH treatment that hatched had a lower rate of water loss compared to the other eggs in the same treatment, as shown in Figure 4. This likely allowed the egg to lose less water proportional to its original mass. Together, these observations suggest that there may be a narrow range of survivable humidities for these eggs, with the lower bound of this range being higher than 75% RH. However, because mass loss is related to the relative humidity of the treatment and time spent in that treatment, perhaps it is not the range of humidities that determines survivability, but percentage mass lost. Future studies could dehydrate eggs to varying percentages of their original mass and observe which eggs survive.

The search for high-humidity environments may be the reason why these stick insects lay their eggs in the soil. The interstitial spaces there are near 100% RH, providing an optimal microhabitat for minimizing water loss.

Insect eggs have been known to increase their eggshell conductance in response to internal factors such as tissue oxygenation,[5] and the eggs of the walking stick *Extatosoma tiaratum* have been shown to absorb water vapor from the air,[6] so it is not unthinkable that the eggs of *Eurycantha calcarata* could also absorb water from the air in response to internal signals. However, this does not seem to be the case. The eggs of *E. calcarata*, and those of most terrestrial species,[7] cannot absorb water from their environment; the only water available is the water that was included with the egg upon oviposition.

Of the 30 eggs involved in the rehydration experiment, 19 did not survive to hatching. The fact that these eggs did not absorb water is not a surprise since their embryos may have died before the attempted rehydration. However, the 11 eggs that hatched also did not absorb water.

The attempted rehydration of the eggs shows that, for these eggs, water is a finite resource. The eggs are laid with a certain amount of water, which they may lose during development. As shown, the eggs cannot absorb water from their environment. This means the only water available to an egg is what was included with it upon oviposition; water is a finite resource. Perhaps this drive to conserve water, as well as the large size of the eggs[8] and the ecology of their oviposition[9], drives these stick insects' long egg development times; low eggshell conductance minimizes water loss, but also leads to low oxygen flux, limiting the speed at which the egg can develop.[10]

6. Acknowledgments

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7. References

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