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Rates of Water Loss and Absorption in Stick Insect Eggs

Garret Jolma - Organismal Biology, Ecology, and Evolution

About the insect

The thorny devil stick insect (*Eurycantha calcarata*) 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. To lay their eggs, the females crawl to the forest floor and deposit eggs one by one into the soil [1]. The eggs take four 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 and water. In these experiments, I investigated how water is lost and regained in stick insect eggs.

An adult female thorny devil stick insect.

A thorny devil stick insect egg.

Methods

Dehydration experiment: I placed 60 eggs total into three 24-well plates. The plates went into plastic containers of differing humidities:

- The saturated treatment had a pool of water in the bottom for 100% humidity.
- The intermediate treatment had a saturated salt-water pool in the bottom for 75% humidity.
- The dry treatment had Drierite salt in the bottom to absorb water and create 0% humidity.

Eggs were weighed every three days on a microbalance (Sartorius MC5, ± 1 µg); the time interval between recordings increased as the experiment progressed.

Rehydration experiment: I placed 30 eggs into a 0% humidity container with Drierite salt 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:

- The high humidity treatment had 100% humidity from a pool of water in the bottom of the container.
- The wet substrate treatment 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, so that it simulated wet soil.

Each egg was then weighed regularly.

Dehydration of eggs

100% humidity

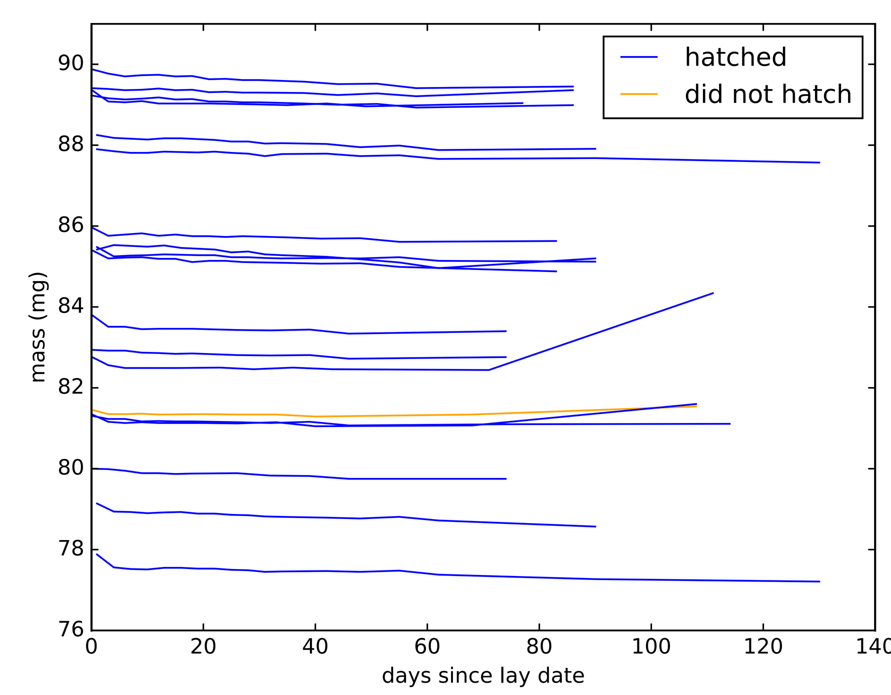

Figure 1: The masses of the eggs in the 100% humidity treatment. These eggs kept their mass throughout the experiment. All but one of these eggs hatched.

75% humidity

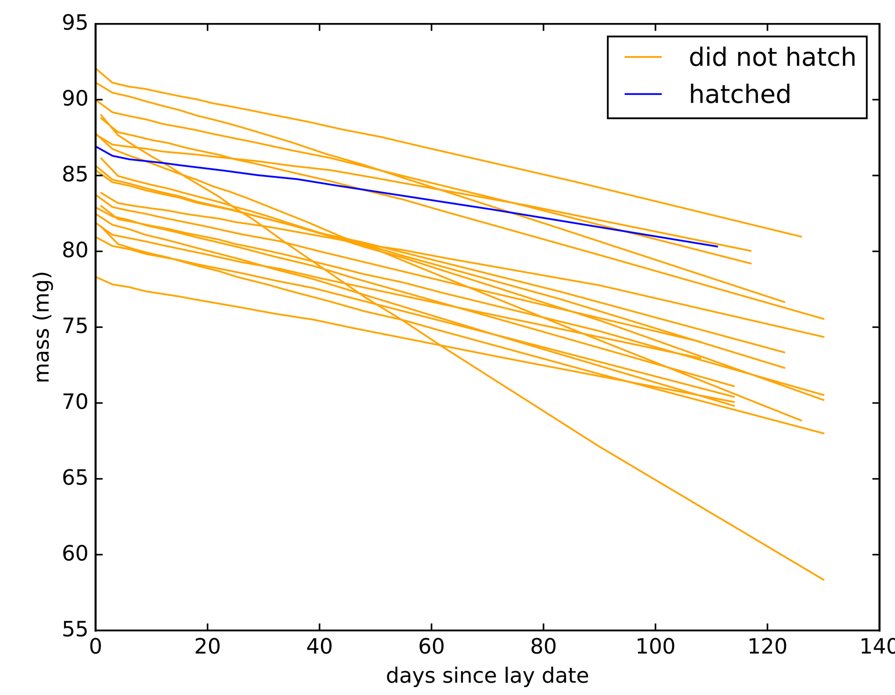

Figure 2: The masses of the eggs in the 75% humidity treatment. These eggs lost water slowly over time. Only one egg hatched; this implies that the lower bound of the range of habitable humidities is above 75% for the average egg.

0% humidity

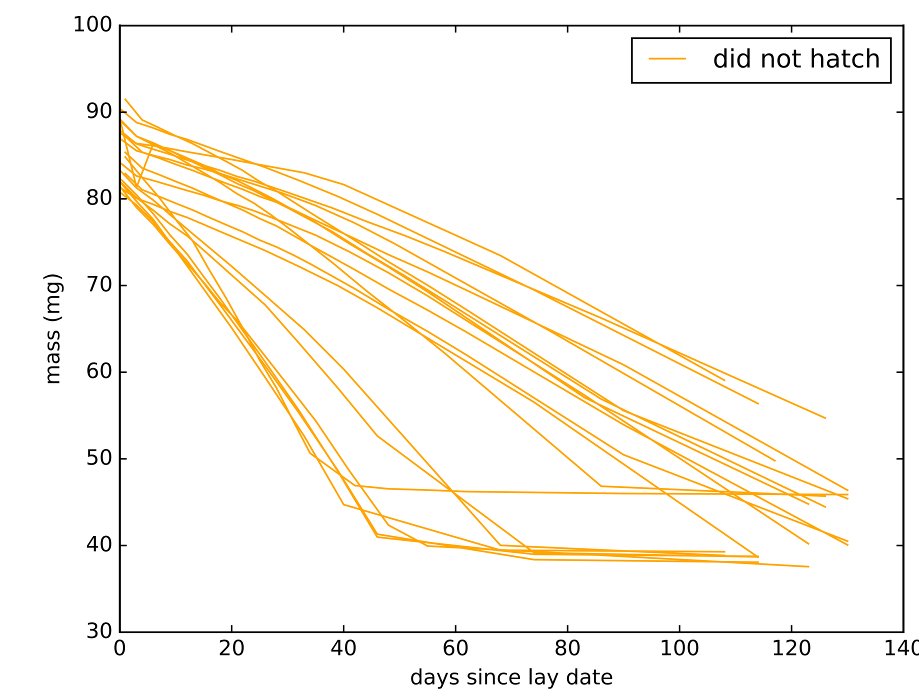

Figure 3: The masses of the eggs in the 0% humidity treatment. These eggs lost water quickly; many of the eggs reached dry mass—their rate of water loss decreased significantly because they had no more water to lose.

Rehydration of eggs?

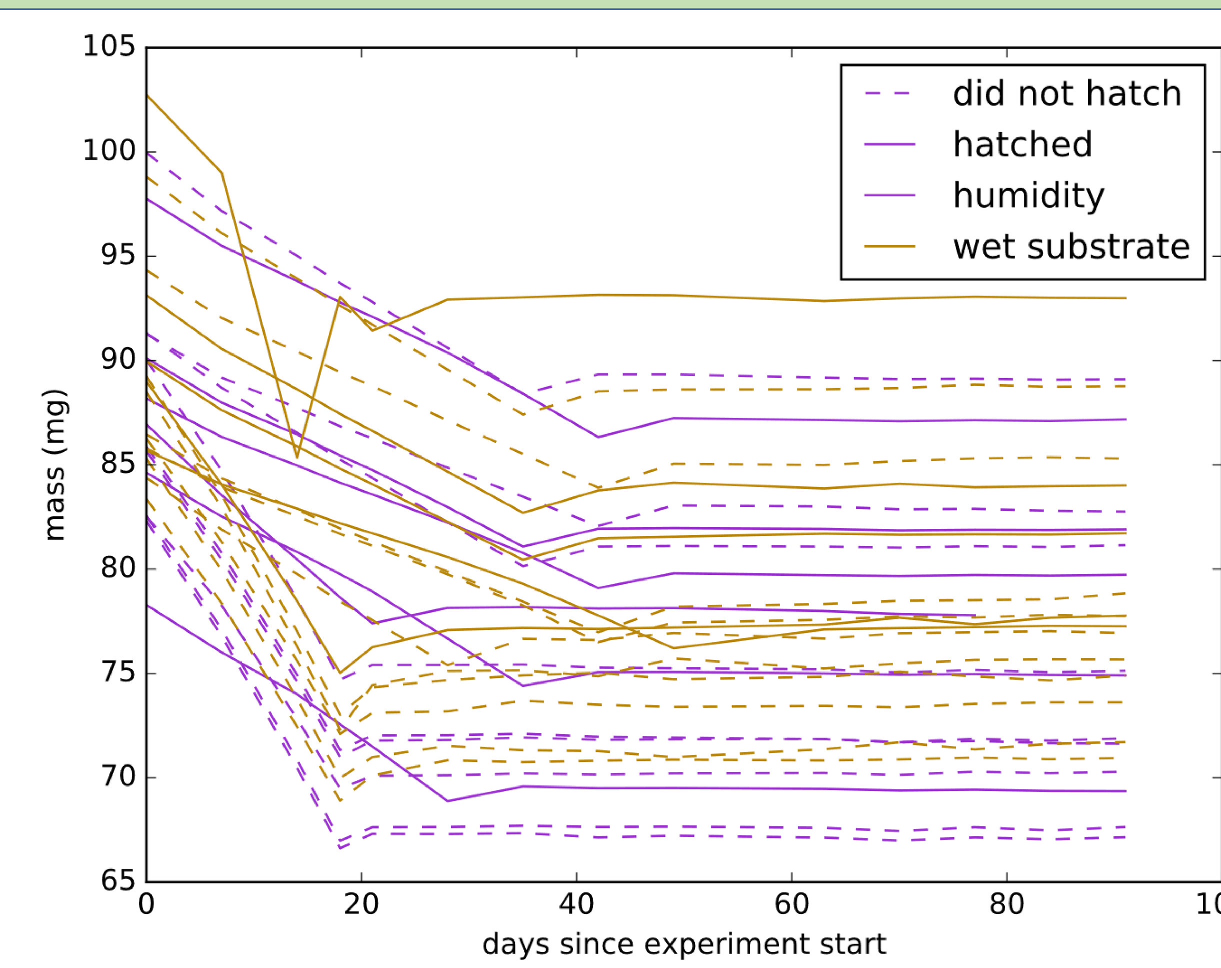

Figure 4: Although all eggs showed slight increases in mass immediately after being moved into the rehydration treatments, this likely represents rewetting of the eggshell. There were no long-term gains in mass that would indicate continued water uptake by the embryo.

Dashed line = egg did not hatch. Solid line = egg did hatch.

Purple line = egg placed in 100% humidity treatment.

Brown line = egg placed in humidity + wet substrate treatment.

Conclusion & Implications

Together, these experiments show:

- The lower the humidity, the higher the rate of water loss in stick insect eggs.
- These eggs can only survive in a narrow range of humidities, with the lower bound of habitability being >75% relative humidity.
- Once lost, water cannot be reabsorbed by the eggs, making water a valuable, finite resource.

This research speaks to the water loss – oxygen flux trade-off that all insect eggs must deal with. Perhaps this drive to conserve water also drives these stick insects' long egg development times; low eggshell conductance leads to low oxygen flux, limiting the speed at which the egg can develop.

In addition, the need for high humidity may suggest a reason why *E. calcarata* lay their eggs in the soil; the interstitial spaces there have high humidities (and protection from predators).

Acknowledgments & Citations

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[1] Hsiung, C.-C. (2006). Aspects of the biology of the Melanesian stick-insect Eurycantha calcarata Lucas (Cheleutoptera: Phasmatidae) . *Journal of Natural History*, *21*(5), 1241–1258. https://doi.org/10.1080/00222938700770761