

A pilot study comparing maternal immunity, egg yolk immunity, and offspring wound healing in Side-blotched lizards (*Uta stansburiana*) following an immune challenge

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Introduction

- Maternal allocation of physiological factors before birth can drastically alter offspring phenotype or quality.¹
- In vertebrates, mothers can invest antibodies or immune factors via egg yolk or placenta to provide protection from pathogens.²
- Infection during reproduction can alter maternal investment into offspring.³
- Maternal investment of immune components can reduce the physiological costs of an infection.⁴
- It is unclear if this occurs in reptilian species.



Research Questions

1. Do immune challenged females alter egg size or egg yolk investment of bactericidal factors?
2. Are offspring from immune challenged females larger? Do they have better wound healing ability?

Hypotheses

Experiment 1:

- Offspring of immune challenged females will have larger egg size and more egg yolk investment than those of non-immune challenged females.

Experiment 2:

- Offspring of immune challenged females will be larger and have quicker wound healing ability than the offspring on non-immune challenged females.

Methods and Design

Field Capture and Housing

- Reproductive female lizards were captured from the St. George area and then processed and housed at Utah State until oviposition

Immune Challenge and Assay

- Females were injected with lipopolysaccharide (LPS) to mimic a bacterial infection, saline (PBS), or no injection (NI) 24 hours after being housed
- Blood samples were collected 72 hours after treatment
- Yolk samples and female plasma samples were assayed *ex vivo* to measure bactericidal ability following exposure to *E. coli*⁵

Wound Analysis

- ImageJ was used to analyze the area of the open wound from photographs of hatchlings
- Percent healed was determined by comparing wound area on day 1 to day 7

Statistical Analyses

• Mixed effects Analysis of Variance

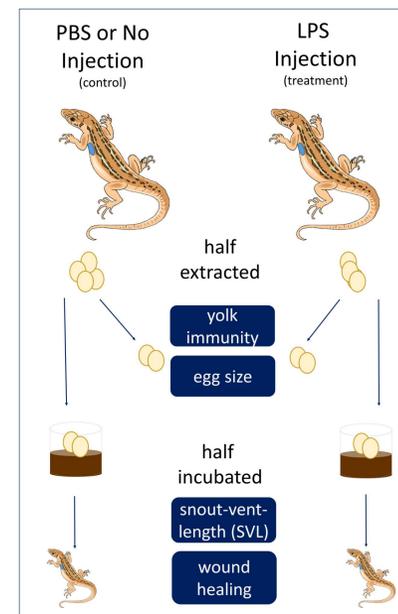
Experiment 1:

Dependent Variable: yolk immunity, egg size
Independent Variable: Treatment
Random Effect (Intercept): Maternal ID and Site

Experiment 2:

Dependent Variable: hatchling immunity and size
Independent Variable: Treatment
Random Effect (Intercept): Maternal ID

- Determine whether hypothesized model is significantly different ($p < 0.05$) from null model



Timeline of Wounding and Measurements of Hatchlings

Day	Measurements
Day 0 (hatch date)	Take photo, measure mass and SVL
Day 1	Administer 1 mm wound
Day 3	Take photo, measure mass and SVL
Day 5	Take photo, measure mass and SVL
Day 7	Take photo, measure mass and SVL

Experiment 1 –Egg yolk immunity (Fig. 1) and egg mass (Fig. 2) do not differ across maternal treatments

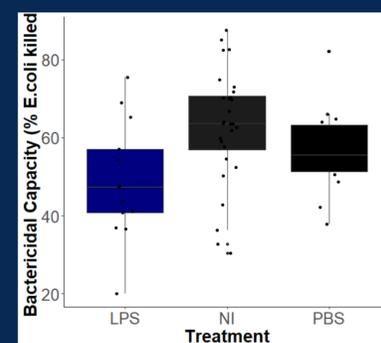


Figure 1: When controlling for maternal ID and site, egg yolk BKA does not differ among treatment groups and is not significantly different from the null model. (ANOVA between hypothesized and null model: $p = 0.13$)

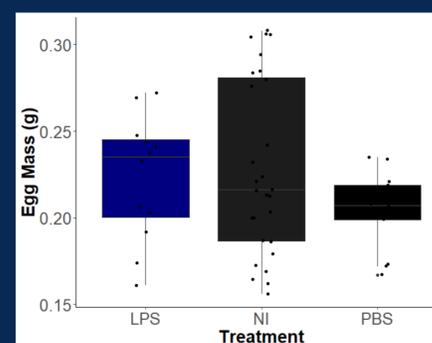


Figure 2: When controlling for maternal ID and site, egg mass does not differ among treatment groups and is not significantly different from the null model. (ANOVA between hypothesized and null model: $p = 0.48$)

Experiment 2 –Offspring wound healing ability (Fig. 3) and size (Fig. 4) do not differ across maternal treatments

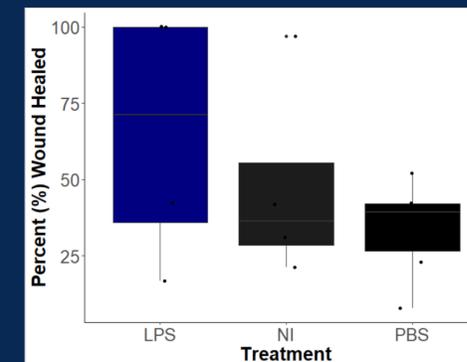


Figure 3: When controlling for maternal ID, hatchling immunity does not differ among treatment groups and is not significantly different from the null model. (ANOVA between hypothesized and null model: $p = 0.47$)

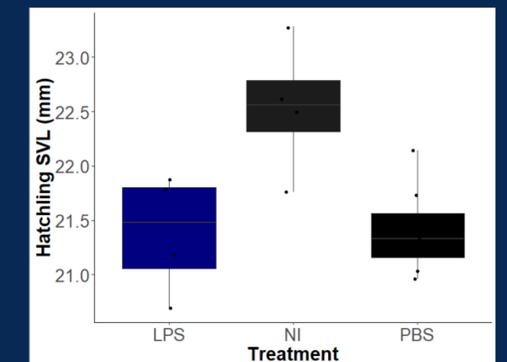


Figure 4: When controlling for maternal ID, hatchling SVL differs between LPS and NI treatment, but model fit is not significantly different (ANOVA between hypothesized and null model: $p = 0.01$, but model fit (BIC) is the same)



Conclusion

- We saw no statistical difference in egg and hatchling characteristics across maternal treatment groups, apart from hatchling size differing between the LPS and NI group.
- Differences in hatchling size between LPS and NI group could be due to low sample sizes, since model fit did not differ between the hypothesized and null model.
- Increasing the sample sizes of eggs and hatchlings may provide more statistical power and help us explore trends that began to emerge.
- It is possible that the timing of treatment might not have been sufficient to alter maternal investment into eggs and hatchlings.
- It is possible that lizards invest only pathogen-specific antibodies as opposed to investing innate immune components into the egg.
- Future studies should include physiological assays that measure both innate and adaptive immune factors.

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