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Examining egg surface morphology and microbial content of Sceloporus virgatus eggshells

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INTRODUCTION:

- Beneficial microorganisms are commonly found on eggshells in birds and reptiles (Lombardo et al. 1996; Ruiz-De-Castañeda et al. 2011; Singh et al 2014; Van Hoek 2014) and are thought to be transmitted from females to their offspring via the reproductive tract.
- The cloaca, an opening at the end of the digestive tract in reptiles and birds used for both excrement removal and reproduction, provides a sample of the diverse array of microbial life that could potentially be transmitted to offspring.
- *Sceloporus virgatus* female lizards have been shown to have decreased cloacal microbial diversity during the reproductive season, potentially allowing a small number of species of beneficial microbiota to become more abundant to protect the egg (Martin et al 2010; Cox 2015).
- Past data from the Weiss lab indicate that eggs dissected out of female Striped Plateau Lizards, *S. virgatus* have reduced hatching success which could be due to a lack of beneficial microorganisms on the eggs that do not pass through the cloaca.

QUESTIONS:

- What morphological features are visible on laid and dissected eggshells? Are there changes in eggshell morphology from 0 to 3 weeks of incubation?
- What microbial growth exists in the bacterial loads of *S. virgatus* eggshells at 0 weeks and 3 weeks of incubation?

METHODS:

- Collected 6 female lizards from Southwestern Research Station in Arizona
- Dissected out eggs of 3 females & induced laying in 3 females
- Swabbed eggs at 0 and 3 week incubation times
- Plated swabate onto Brain and Heart Infusion, Tryptic Soy Broth, and Enterococcosel media to observe any microbial growth on media
- Fixed eggshells in solution of 2% PFA, 2% GTA, 2% DMSO, and 1x PBS (pH 7.2) before drying for SEM
- Imaged eggshells using SEM to find microorganisms, fungi, and determine eggshell morphology

RESULTS 1: EGGSHELL MORPHOLOGY

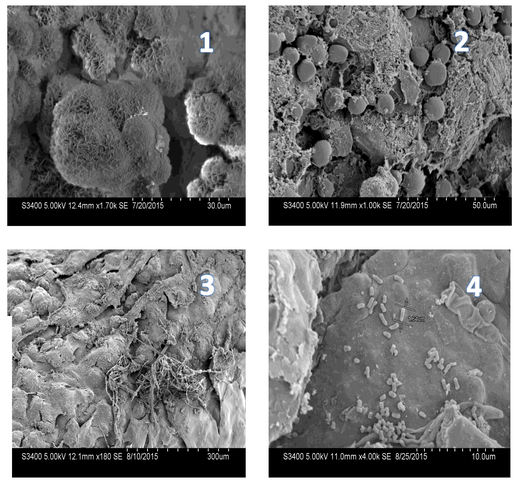
At both 0 and 3 weeks of incubation eggshells displayed a crest and trough morphology (Deeming and Ferguson 1991) with raised polygons surrounded by a smoother surface. The smoother surface cracked in some places revealing the fibrous material of the shell

0 week incubation:

- Some eggshells possess crystalline spheres (1)
- Other spheres (potentially pores; Deeming and Ferguson 1991) average diameter 7.0 ± 1.5um (2)
- Covered with a layer of calcite-like material

3 week incubation:

- Eggshell surface smoother, more crystalline sphere coverage
- Fungal structures present on some eggs (3)
- Microorganisms (D'Alba et al 2014) on some eggshells (4)



Appearance of eggshell after 3 weeks (without magnification)

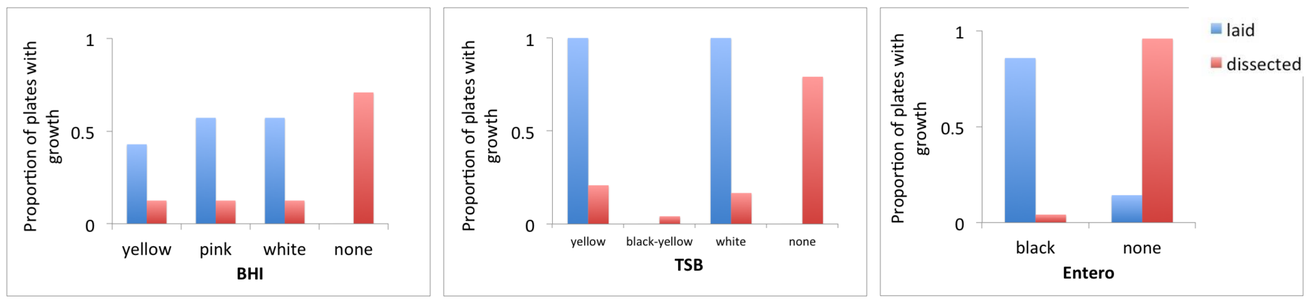
- Characteristics: red striations, black growth, green growth, and orange growth
- 14.3% of laid eggs had black or green growth while 56.4% of dissected eggs had black or green growth
- 57.1% of laid eggs had red striations while 21.7% of dissected eggs had red striations

Red Striations | Green growth | Orange growth | Black growth

RESULTS 2: MICROBIAL AND FUNGAL PLATE GROWTH

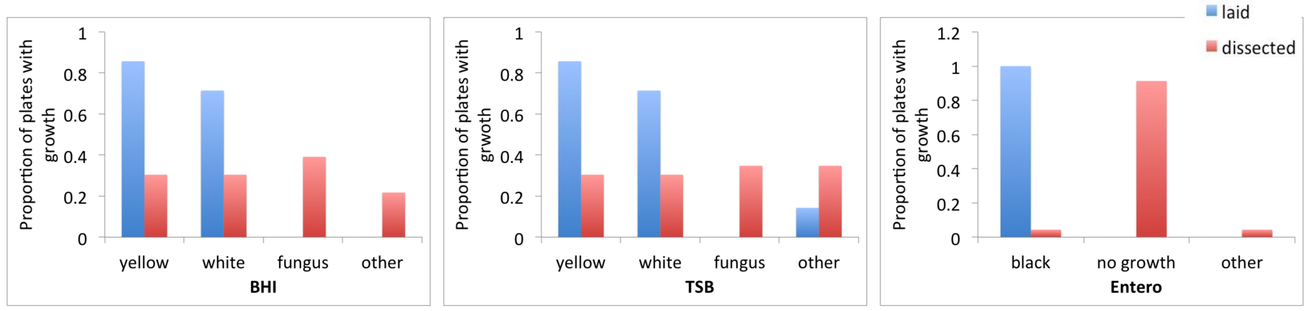
0 weeks of incubation:

- Microbial loads on BHI agar include yellow, pink, and white mucus-like growth, and no growth. Bacterial loads on TSB agar included yellow and white mucus-like growth, black and yellow growth, and no growth. Bacterial loads on Entero agar were identified as black growth and no growth.
- Bars on graphs do not add up to 1 for each type because many plates contained several types of growth
- Laid eggs sample size=7, dissected eggs sample size=23



3 weeks of incubation:

- Microbial and fungal loads on BHI and TSB agar include yellow and white mucus-like growth, fuzzy growth identified as fungus, and several unique growths grouped as "other". Bacterial loads on Entero agar include black growth, other growth, and no growth.
- Bars on graphs do not add up to 1 because many plates contained several types of growth.



DISCUSSION OF RESULTS:

- Eggshell morphology changed from 0 to 3 weeks of incubation, with eggshells having a smoother coating with more crystalline spheres and less visible pore-like spheres.
- Microbial life was not found under SEM on any 0 week eggshells. This is likely because it was more difficult to find bacteria within the calcite-like covering, or there were a larger quantity of microorganisms on the eggshells after 3 weeks of incubation.
- The majority of laid eggs at 0 weeks of incubation had visible microbial growth on at least one type of agar, while a lesser proportion of dissected eggs showed microbial growth on media. This indicates that there was either less microbial life on the dissected eggs, or the species present on the dissected eggs did not grow on the media selected.
- There was no apparent fungal growth from laid eggs after 3 weeks incubation, which indicates that there may have been less protective bacteria on the dissected eggs, or the laid eggs possess antifungal agents.
- The microbial communities on laid eggs differed from those present on dissected eggs. The laid eggs appear to have little to no fungal growth compared to the dissected eggs which may indicate the presence of beneficial protective microorganisms on laid eggs.

FUTURE QUESTIONS:

- What identifiable microbial species are present on the laid and dissected eggs? Do the microbial communities differ between laid and dissected eggs?
- Do laid and/or dissected eggs possess antifungal agents?
- Are there changes to the eggshell morphology between 3 and 6 week incubation?

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