

Introduction and Research Question

Freshwater turtles face many diseases including shell rot, also known as ulcerative shell disease. Shell rot is a bacterial or fungal infection causing lesions, dryness, ulcers, and cracking. Previous research has associated shell rot with gram-negative bacteria including *Streptococcus* spp., *Citrobacter freundii*, and *Pseudomonas*, and fungus including *Fusarium* spp in freshwater turtles including *Trachemis scripta elegans* (red-eared sliders). However, we observed shell rot in a different species, *Pelusios castaneus* (African side-necked turtles). This study aims to survey for these and other related pathogens that may cause infection and shell rot in African side-necked turtles. These turtles commonly share tanks with fish such as *Amphilophus labiatus* (red devil cichlids), which could influence the microbiome. We plan to take a topical swab from the fish. We will culture the microbes and use morphology, DNA extraction, and amplification and sequencing of the 16S rRNA gene through Lauer PCR to identify them. We expect to find the listed pathogens or similar ones linked to shell rot present in our samples. The occurrence of these pathogens would show a potential cause for the shell rot discovered on the African side-necked turtles.

Objective: To survey for bacterial and fungal pathogens that may cause infections and shell rot in African side-necked turtles.

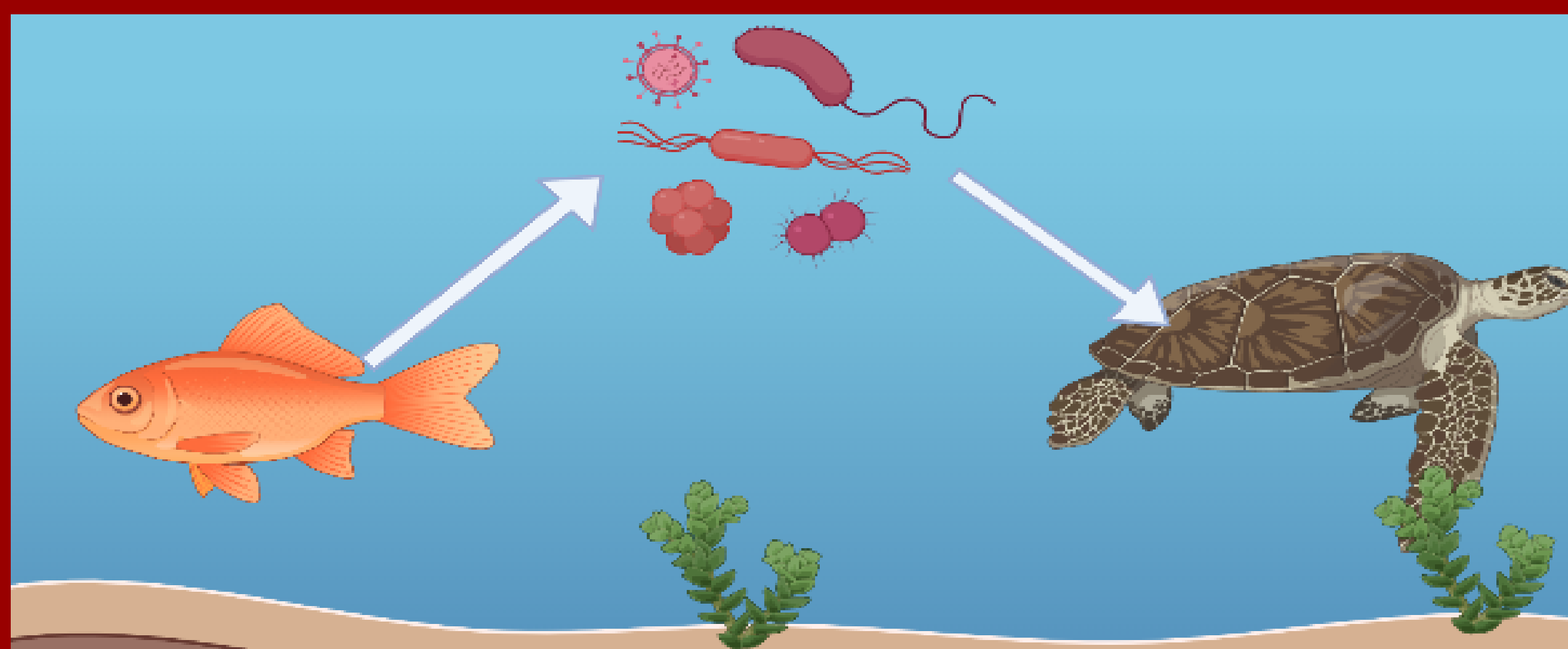


Figure 1. Figure representing hypothesized findings: bacteria and fungi from the fish, also in the water, can make its way onto the turtles in shared tank (Credited with Biorender.com, 2023).

Background

Ulcerative Shell Disease

- Ulcerative shell disease, also known as shell rot, is a common dermatological issue in freshwater turtles and is detected by white spots, lesions on the carapace and plastron, and yellow to tan shell discoloration.
- Some potential links to the emergence of shell rot could be improper husbandry mechanisms, poor nutrition, feces contamination, and environmental factors such as improper temperatures, high humidity, and not enough dry places to bask.

Fish Swabbing and Microbiome

- Because the turtles of interest were inhabiting the same tank as fish (*Amphilophus labiatus*) at the time the turtles developed shell rot, fish microbiome may be an important factor in this study.
- Phylum Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were found in both the gut and on the skin of fish and thus may be present in samples.
- The safest and least invasive method to assess fish microbiome is skin mucus swabbing.
- Other methods could include fin clipping and biopsy.



Figure 2. Red Devil Cichlid (*Amphilophus labiatus*) (Wortman, 2023).



Figure 3. Side-neck turtle (*Pelusios castaneus*) (Wortman, 2023).

Methods and Materials

Red Devil Cichlid Swab Collection:

- Six Red Devil Cichlids will be caught using nets.
- Sterile swabs will be used to swab each fish five times back and forth from the nose to the caudal fin and on the sides.
- Swabs will be cultured and isolated on TSA agar.
- Morphology will be observed to aid in identification.

DNA Extraction and Identification:

- DNA will be extracted using the Freeze-Thaw method.
- The 16S rRNA gene of the DNA will be amplified using Lauer PCR for identification of potential pathogens.
- Gel electrophoresis will be done to solidify that the PCR worked.
- Results will be sent to Eurofins for specific identification of potential pathogens.



Figure 4. An example of microbes found in the freshwater tank of interest (Wortman, 2023).



Figure 5. An example of microbes cultured on an agar plate (Piddock, 2022).

Expected Results

We expect to find gram-negative bacteria linked to shell rot from the literature present in our samples. We expect to see similarities between samples as displayed by Shannon's diversity and Morisita's index values.

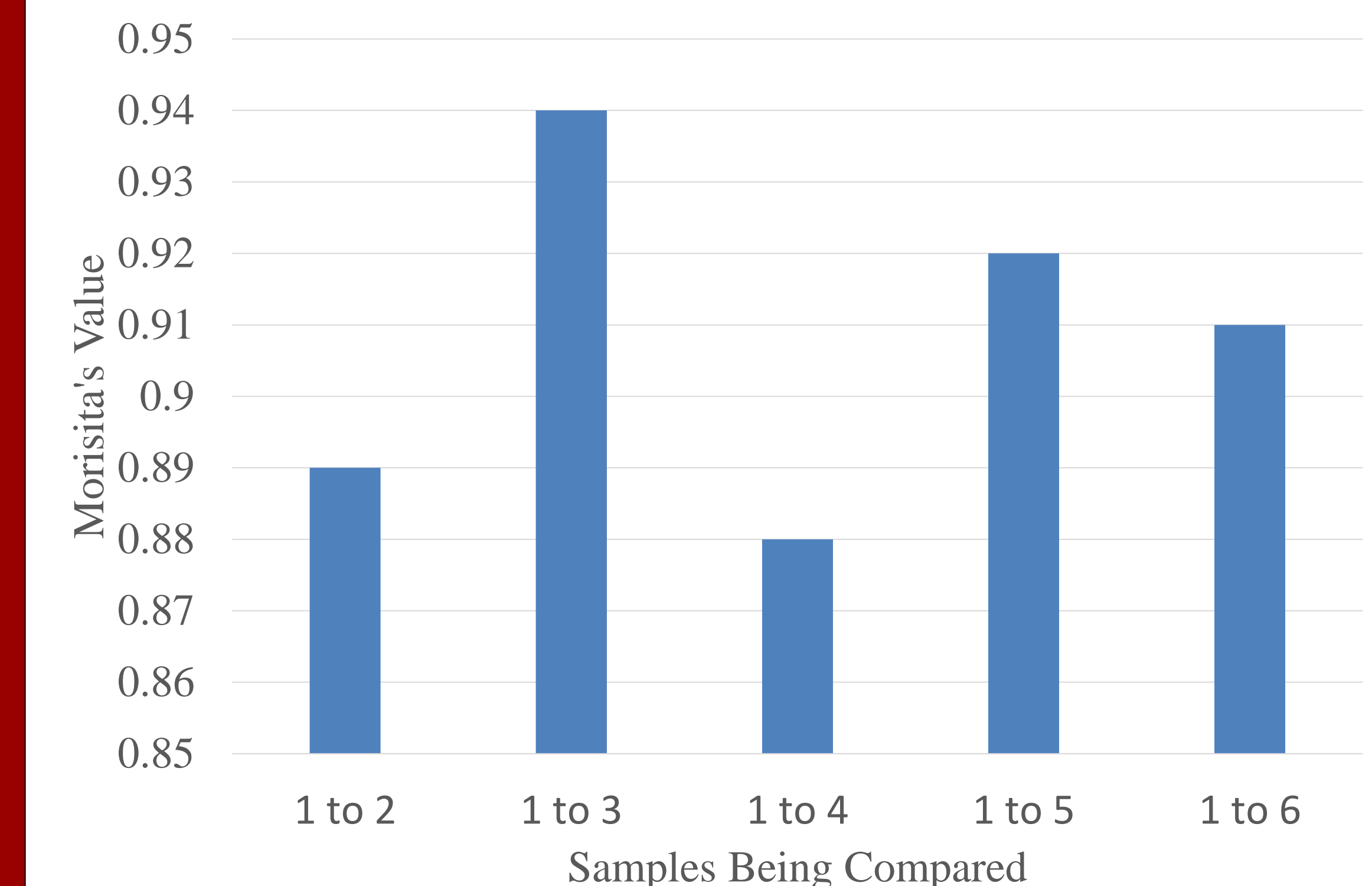


Figure 6. Sample Morisita's Index Values.

Future Work

1. Establish records of shell rot in African side-necked turtles
2. Add to knowledge of microbiomes in freshwater turtle tanks
3. Husbandry implications in determining which species can cohabitate in tanks

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