Effect of Carotenoid Dietary Supplementation on the Cutaneous Microbiome in Captive Golden Dart Frogs (*Phyllobates terribilis*)

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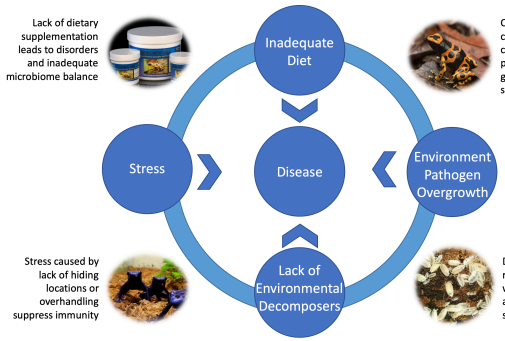
Background & Rationale

Captive frogs tend to have relatively diminished cutaneous microbiome abundance/diversity, and higher susceptibility to pathogenic bacteria and fungi, as compared to wild conspecifics with richer microbiome - an advantage that may be explained by wild frogs' exposure to microbial-rich environments and superior nutrition. Modifications in husbandry, including dietary optimization, have proven beneficial in several species (e.g., Antwis, *et al.* 2014 demonstrated that dietary carotenoid supplementation increases cutaneous microbiome diversity in captive tree frogs). Poison dart frogs constitute another group of popular pet frogs, mainly due to their vibrant colors, inexpensive maintenance, cleanliness, and non-toxicity of captive individuals (wild frogs derive skin toxins from arthropod pray). Dart frogs are typically non-demanding with regard to care, but species vary in disease susceptibility. *Phyllobates terribilis* (**Golden Dart Frog**) appears to have a higher incidence of skin lesions and infections in captivity as compared to other poison dart frogs, causing higher mortality in this species. A greater diversity and abundance of the cutaneous microbiome may inhibit growth of pathogenic bacteria and fungi, and avoid potential colonization of cutaneous pathogens. This study focused on the relationship between dietary carotenoid supplementation and the diversity & abundance of the cutaneous microbiome in *P. terribilis* frogs.



Hypothesis & Objectives

Hypothesis: Dietary supplementation with carotenoid powder (through powdering of feeder insects) drives the skin microbiome of captive Golden Dart Frogs (*Phyllobates terribilis*) towards greater diversity and abundance. **Objectives**: 1) Measure standard frog health parameters of study subjects (dietary supplement vs. control groups). 2) Characterize the cutaneous microbiomes and identify differentially represented taxa between the groups before supplementation and at the end of 4 wks.

Design & Methods

A total of nineteen (19) clinically healthy young *P. terribilis* (approximately 12 weeks of age). Dietary supplement group: n_1 = 9 frogs were fed a flightless fruit fly diet with carotenoid supplementation [Repashy SuperPig] via feeder insect powdering for 4 wks. Control group: n_2 =10 frogs were fed a flightless fruit fly diet without carotenoid supplementation. Skin swabs were obtained weekly for 4 weeks. Samples from each of the two groups were pooled and analyzed (controls vs. supplemented). The frog microbiomes were established using next generation DNA sequencing (Illumina, MiDOG). Comparative data will also be obtained using MinION technology by Oxford Nanopore Technologies.

1. Tadpole Rearing → 2. Homogenizing Sample → 3. Supplement → 4. Sample Collection → 5. Metagenomics

Control of diet before metamorphosis → Subjects living together before study → Separation into groups → Collection of skin swabs → Metagenomics analyses (MiDOG, Irvine CA)

Results

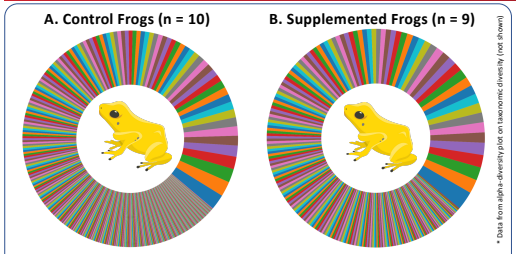


Figure 1 – Comparison btw. bacterial composition in controls (A) vs. supplemented frogs (B) after 4 wks. Colors = different species (size of each piece = abundance). Observed no. of bacterial species was 637 in controls vs. 325 in supplemented frogs*

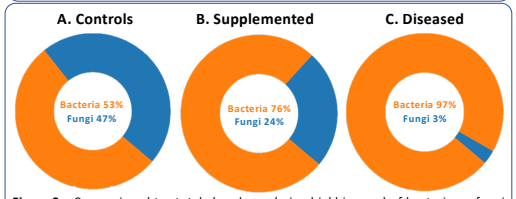


Figure 2 – Comparison btw. total abundance (microbial biomass) of bacteria vs. fungi in controls (A) vs. supplemented frogs (B), and clinical case w. *Red Leg Syndrome* (C). Interestingly, both supplementation and skin disease lower total abundance of fungi.

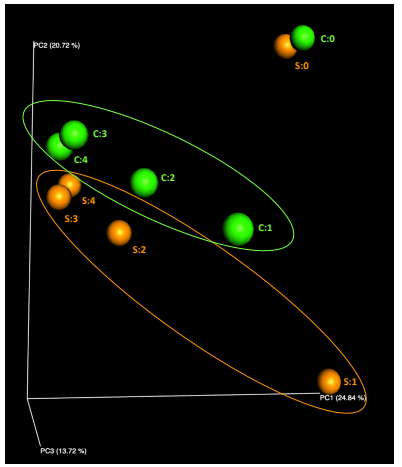
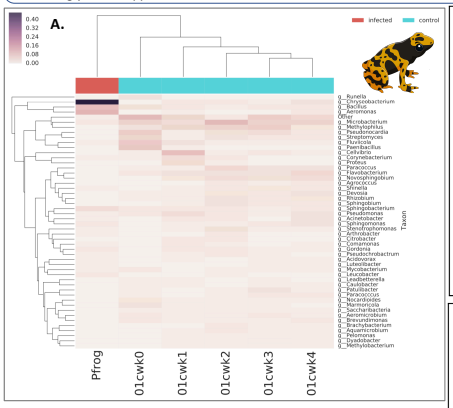
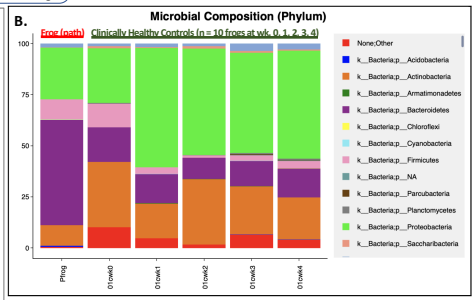


Figure 3 – Beta diversity plot reflects *microbial structure diversity* in time series for controls (C, green) vs. supplemented (S, orange) frogs. The cutaneous microbiome was analyzed at wk. 0, 1, 2, 3, 4. Supplemented frogs had *lower* skin microbiome structural diversity.





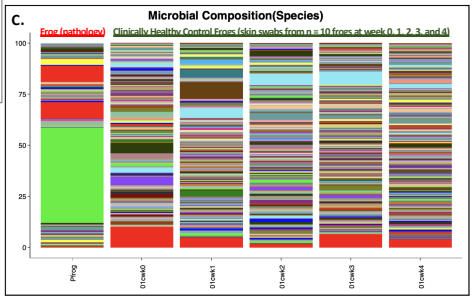


Figure 4 – Clinical Case: Red Leg Syndrome (Bacterial Dermatosepticemia). Heatmap (A) illustrates *bacterial taxonomic abundance and composition* in a critically ill frog (red) vs. clinically healthy controls over time (turquoise). Unlike control frogs, the microbiome in the diseased frog is dominated by Chryseobacterium, Bacillus, Aeromonas (species from these genera have also been reported in connection with Red Leg Syndrome in captive frogs). Composition plots illustrate the *taxonomic microbiome distribution* at the phylum (B) and species (C) levels. Distinct differences are noted in skin microbiomes from the frog with pathology versus controls: Bacteroidetes dominates at the phylum level, whereas *Chryseobacterium wanjuense* is highly abundant (46%) at the species level, accounting for less diversity.

Discussion & Conclusion

Pilot study data and clinical case findings were presented as follows: Fig.1,2,3: Comparisons btw. *controls* (pooled sample, n = 10 frogs) vs. *supplemented* frogs (pooled sample, n = 9 frogs). Fig.4,5: Clinical case w. bacterial dermatosepticemia based on microbiome & clinical signs.

Dietary supplementation with carotenoid powder over 4 wks. was associated with less taxonomic (Fig.1) and structural (Fig.3) microbial diversity, as compared to controls. The emerging opportunistic, multi-drug resistant, zoonotic pathogen *Stenotrophomonas maltophilia* was detected in supplemented frogs (1.4% relative microbial abundance), but not in controls. Total microbial biomass (bacteria vs. fungi) in supplemented frogs showed increase in bacteria and decrease in fungi. A similar, but more pronounced shift was noted in the sick frog (Fig.2). The skin microbiome in a frog with necrotizing skin lesions on hind legs and diminished response to external stimuli was dominated by pathogens reported w. Red Leg Syndrome, e.g., Chryseobacterium spp.(Fig.4) - which also contains multi-drug resistant zoonotic species. Beta diversity plot supported distinct differences between microbial structure diversity in controls over time vs. the diseased frog (Fig.5).

Poison dart frogs have become increasingly popular pets in the U.S. Dart frog breeders and other aficionados recognize that Golden Dart Frogs (*P. terribilis*) are more prone to skin infections compared to other captive frog species. This study contributes new data that may help in tailoring recommendations to Golden Dart Frog breeders about the role of carotenoid dietary supplementation in skin health. Data generated from this pilot project may help pave the way to future studies on the use of dietary supplements in captive frog populations in context of conservation and reintroduction projects.

Acknowledgements & References

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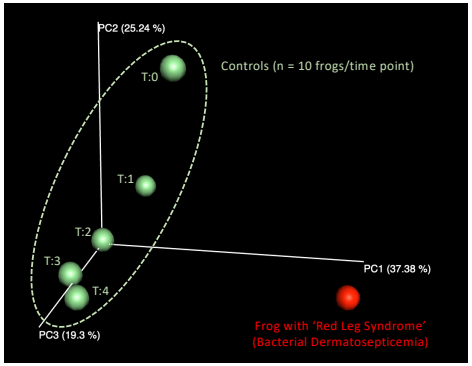

Figure 5 – Clinical Case: Frog with Red Leg Syndrome. Beta diversity plot (Principal Coordinate Analysis (PCoA) based on Bray Curtis dissimilarity) is included to illustrate the observed differences in *microbial structure diversity* between the diseased frog (red) vs. time series (wk.0-4) for controls (green).