Project Overview and Objectives

Host-microbe-parasite systems consist of complex interactions which are poorly understood, representing an area of research that requires attention for a better knowledge of the disease dynamics (Lively et al. 2014). Besides, pesticides can affect hosts, microbes, and parasites independently, but the mechanisms by which pesticides affect these interactions synergistically still need to be explored and understood. In this context, our proposal aims to understand how symbiotic microbiomes, parasites, and pesticides affect the immune response of Cuban tree frogs (*Osteopilus septentrionalis*) by looking at differential gene expression. This proposal, which is part of a larger project that aims to understand the different paths of the pesticide-microbiome-parasite interactions with each other and the immune system, labeled in Figure 1, will focus on the blood RNA samples. The RNA-seq analysis will provide information about how the pesticide treatment, symbiotic microbes, and parasites are impacting gene expression and the immune response.

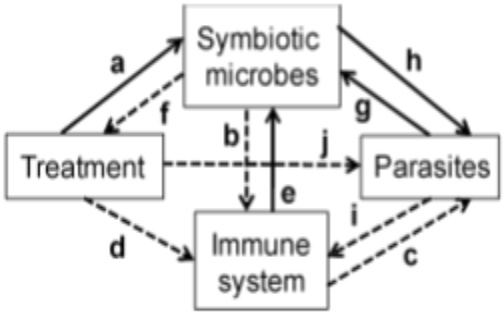

Fig. 1. Potential directional interactions (indicated with arrows) among treatment, symbiotic microbes, immune system, and parasites described in the proposed research. Solid lines indicate the pathways for which we have addressed with previous research (*paths a-h, g*) or will not be addressed (*path e*). Dotted lines indicate the pathways for which we intend on also pursuing the proposed work.

For a better understanding of the importance of the host-associated microbiota and pesticides in the development of the immune system and disease risk, Cuban tree frog tadpoles were exposed to a treatment of Imidacloprid (pesticide) or solvent control for 7 days, crossed with either sterile water or pond water, for the entirety of the tadpole stage. A subset of the tadpoles was sampled for their gut microbiome determination, and the rest of the individuals were kept until complete metamorphosis. Adult frogs were then exposed to *Aplectana* spp. nematodes, consisting in the parasitized treatment, or to a non-parasitized condition. After three weeks, adults were necropsied, their worms were counted, and a blood sample was taken for RNA extractions. After, 32 RNA samples were sequenced using Illumina technology, with four individuals from each of the eight possible treatment combinations (Figure 2).

Workflow

To understand how genes may be differentially expressed in the different treatment groups, we will follow a de novo RNA-seq protocol since there is not an available genome and annotation file for the Cuban tree frog. This process begins on the Xanadu cluster, with quality control and trimming of samples, followed by assembling transcriptomes. Next, coding regions will be identified, aiming to identify and remove the clustering of redundant transcripts, and the

assembly will be evaluated, annotated, and indexed. Once indexed, we will generate counts of each gene, for each sample, and analyze their expression in R software version 4.0.4 (R Core Team 2021). For this step, we identified different methods for differentially expressed gene recognition, including Gfold, NOISeq, and DESeq2. However, the R packages or Xanadu modules that will be used for every step were not chosen yet.

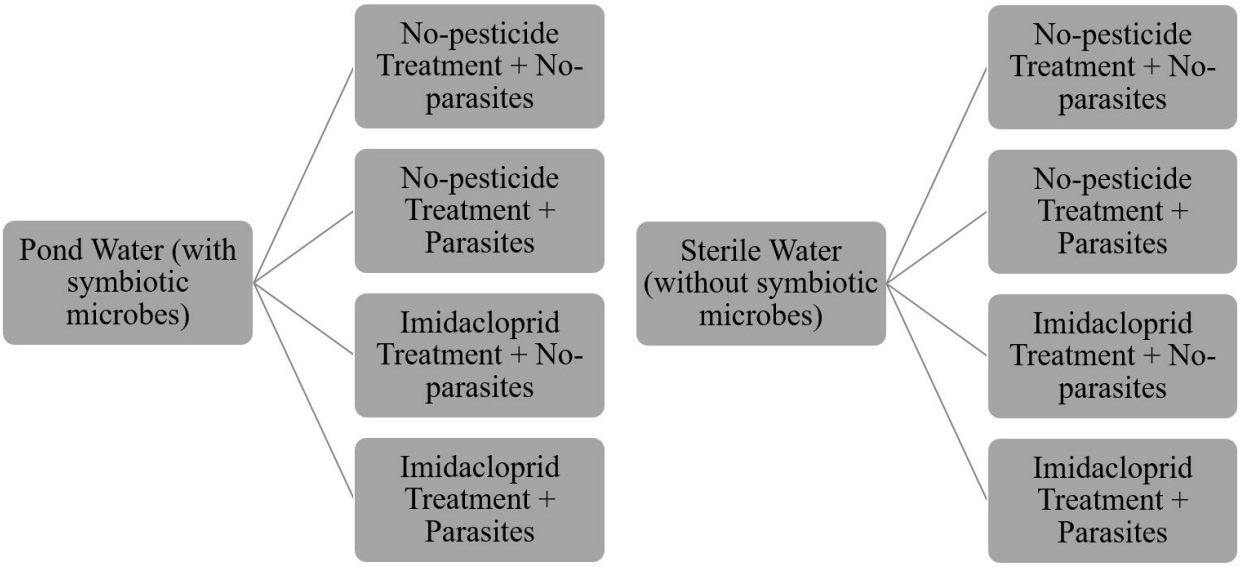

Fig. 2. Conditions for comparison of effects of a pesticide (imidacloprid), symbiotic microbes (present in pond water), and parasites in the immune system of Cuban tree frogs (*Osteopilus septentrionalis*).

References

Lively CM, de Roode JC, Duffy MA, Graham AL, Koskella B (2014) Interesting open questions in disease ecology and evolution. The American Naturalist (184): S1-S8.

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. https://www.R-project.org/. Accessed 08 Mar 2021.