DEVELOPMENT OF A MOLECULAR GENETIC METHOD FOR CHARACTERIZING AMPHIBIAN DIETS

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Figure 1. Alternative reproductive tactics in *Eurycea* cf. *wilderae*; *searching* male (left) and *guarding* male (right). Arrows point to cirri and the mental gland.

Introduction

We are developing a DNA metabarcoding protocol for characterizing amphibian diets from fecal samples. To test this protocol, we will prepare COI amplicon libraries from feces collected from Blue Ridge two-lined salamanders (*Eurycea* cf. *wilderae*). These salamanders have alternative male reproductive tactics[1], and we will test the following hypothesis:

Diets of *guarding* males will have greater relative frequency of aquatic insect prey than those of *searching* males.

Methods

- 27 fecal samples from *Eurycea* cf. *wilderae*
  - 12 from gravid adult females
  - 10 from *searching* males
  - 5 from *guarding* males
- DNA extraction with Qiagen QIAamp PowerFecal kit
- PCR amplification of COI mtDNA locus[2]
- Sequencing on Illumina MiSeq
- Compare sequences to BOLD[3] database in QIIME 2
- Compare reproductive forms using chi-square tests in R
- **Preliminary tests using fecal samples from captive frogs (Fig. 3)**

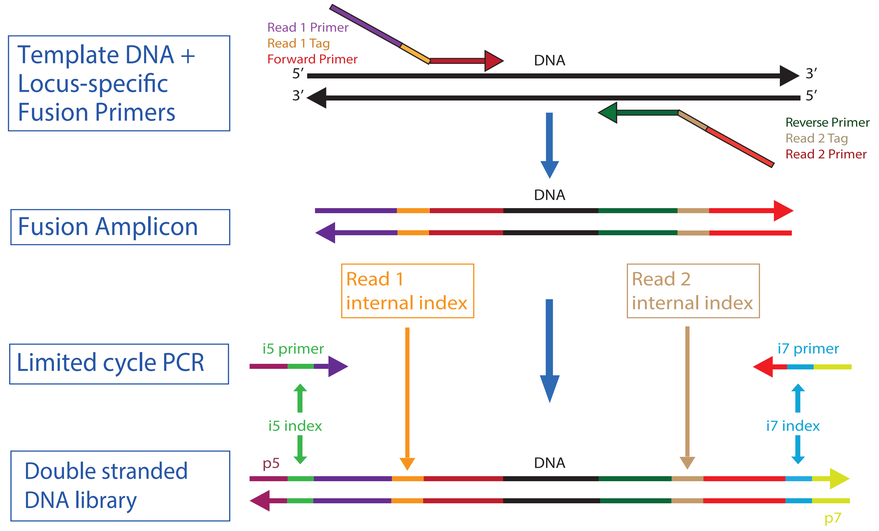

Figure 2. Amplicon library preparation method from Glenn *et al.* (In Prep).

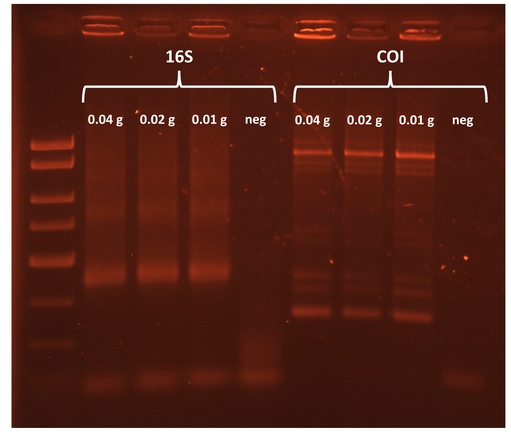

Figure 3. 16S and CO1 amplicons from test extractions and PCRs of frog fecal samples.

Results & Discussion

Our initial results suggest the possibility of extracting and amplifying DNA from small fecal samples (0.01 g). We are still testing this protocol, but if successful, it will provide a useful tool for scientists studying the diets of amphibians. DNA metabarcoding is a relatively new molecular approach, and we believe that our project is justified in its use and will widen the scope of its potential applications.

References: [1] Pierson *et al.* In Press, [2] Jusino *et al.* 2018, [3] Ratnasingham & Hebert 2007; **Permits:** LHU IACUC (01501), TWRA (3840), NCWRC (15-SC00977, 18-SC01274), and the Cherokee, Nantahala, and Pisgah NF. **Acknowledgments:** We thank the North Carolina Herpetological Society for funding and J. Deitloff, B. Fitzpatrick, T. Kieran, and G. McAllister for their assistance.