Seasonal Fluctuations of Salamander eDNA in Central Kentucky Streams

Ronald B. Sams, Cierla McGuire, Malinda Stull and Ben Brammell

Department of Natural Sciences, Asbury University, Wilmore, KY 40390

Introduction

Environmental DNA (eDNA) provides an effective, non-invasive method to document the presence of aquatic species in an efficient manner by confirming the presence of organisms through the capture and detection of DNA released into the water column particles (Ficetola et al. 2008, Lodge et al. 2012). Salamander species native to Kentucky have varying lifecycles, with the majority including an aquatic phase. Streamside salamanders (Ambystoma barbouri) can only be found in aquatic systems during their breeding season and short larval stage, while cave salamanders (Eurycea lucifuga) have an extended juvenile aquatic phase.

Methods

**Filtration** – One liter volumes from each site were filtered. Samples were thawed overnight and filtered in the lab using a 47 mm magnetic filter apparatus (VWR) and 4.7 cm fine particle filters (VWR). Filters were dried, cut in half, and one-half of each filter used in the extraction.

**Extraction** – Extraction was conducted using a modified version of the procedure described by Goldberg et al. (2011) using a DNeasy extraction kit (Qiagen). The dried filter was placed into 12-15 pieces and placed in 1.5 ml tubes with 365 µl ATL buffer and 40 µl proteinase K. The tube was incubated overnight at 56°C and then extracted according to the DNeasy extraction protocol.

PCR – All end-point PCR reactions were run using Life Technologies Environmental Master Mix 2.0 and iDT primer/probe assays.

**Assay Design**

Initial primers designed based on published sequences proved unsatisfactory. Previously published primers that amplify cyt b in a wide variety of amphibian species (Zhang and Wake 2009) were then used to amplify and sequence a 769 BP segment of cyt b in E. lucifuga (Access #: KT873718). Both primers and RT probe were then designed based on this sequence (Fig. 3). Primers were designed using iDT’s primer design software and checked for specificity using MEGA6.

**Species Specificity Testing**

Primers were designed to amplify a 105 BP region of cytochrome b from A. barbouri specimens collected in Fayette and Jessamine County, KY (Bi and Bogart 2010).

**Results**

**eDNA Results – Highbridge and McGee**

A. barbouri eDNA (Fig. 8) was only detected once at the Highbridge site (9/19) and once at the McGee site (2220) and may represent incidental presence of adults in these systems as neither dates correspond with the presence of larvae. No A. barbouri larvae were observed in either of these sites at any time during the project. E. lucifuga was not detected at Highbridge at any date, a finding consistent with field observations (Fig. 9). E. lucifuga eDNA was present at the McGee site in each sample analyzed beginning on 3/22 (Fig. 9). E. lucifuga larvae were not observed but were likely present in this system at this time.

**Conclusions**

- eDNA primers effectively and specifically identify both A. barbouri and E. lucifuga and not sympatric salamander species.
- eDNA data from Stoney Run indicates fluctuations in eDNA levels corresponding to the life history of A. barbouri with the possible exception of the 7/31 occurrence. These results correlate with field observations of A. barbouri’s life history at this site.
- The completion of the project should provide additional insight into seasonal fluctuations in salamander eDNA at the following tasks as completed:
  - The addition of quantitative, real time PCR which would greatly enhance the resolution of these data.
  - The analysis of the remaining samples collected biweekly at each site between 2/9 and 6/22/2015.
  - The inclusion of the following additional salamander species: Eurycea cirrigera, Eurycea cinerea, Desmognathus fuscus, and P. montanus
- The identification of collected larvae via tissue extraction and development of specific primers.

**Acknowledgements**

The authors would like to sincerely thank John MacGregor (KYDFPR) for his invaluable assistance in generously providing information regarding sample sites, salamander life cycles, and tissue samples. Thanks are also extended to Rebekah Blackburn (Asbury University) for her assistance in the laboratory.

**Bibliography**
