The search for a rare salamander: The use of eDNA in detection of Eurycea junaluska in the Great Smoky Mountains National Park

Ben F. Brammell1, Sara A. Brewer1, Elizabeth K. Strasko2, Jarrett R. Johnson2, Madeline Cox1

1Department of Science and Health, Asbury University, Wilmore, KY 40390
2Department of Biology, Western Kentucky University, Bowling Green, KY 42101

Introduction

Environmental DNA (eDNA) utilizes DNA that is released from aquatic organisms into the environment to detect their presence and provides an effective, non-invasive method to determine organism presence or absence in an efficient manner1,2,3. We developed species-specific oligos to detect a rare species of semiaquatic salamander, Eurycea Junaluska using eDNA.

Methods

Inhibition testing

All samples were run with an internal positive control (TaxMan™ Enzymatic Internal Positive Control) to assess potential qPCR inhibition.

Results

eDNA quantification

Extracted DNA was quantified using a StepOnePlus™ Real-Time PCR system. Each run contained tissue-extracted target species DNA (1.0 μg/mL) as a positive control and also included a non-template negative control. Each 20.0 μL reaction contained the following: TaqMan™ EMM 2.0 (2.0 μL), nuclease-free water (IDT™) (2.0 μL), eDNA extract (7.0 μL), and assay (1.0 μL). Thermocycler conditions were as follows: 50°C for ten minutes, and 55 cycles of 95°C for 15 seconds and 60°C for one minute. Samples will be run in triplicate but only one replicate per sample has been completed at this time.

In Silico Testing

Water samples were collected periodically from fifty sites throughout the Great Smoky Mountains National Park over a period of two weeks in July 2023 (Figures 4 and 5). One liter water samples were collected using sterile filter funnels and vacuum filtration. Filters were field preserved in ATL buffer in 1.5 μL tubes on ice. All collection equipment in contact with the sterile hood known to previously have no contact with target salamander DNA.

In Vitro Testing

End-point reactions (40 cycles, annealing temp. of 60°C) with target DNA and six closely related sympatric species demonstrated little to no amplification of seven non-target species, including three Eurycea species.

Conclusions

- Primers designed for E. junaluska were species-specific among the thirty-one sympatric GSMPN species tested in silico and seven tested in vitro.
- Although all amplification probabilities predicted by the model fall below the recommended 0.55 threshold, E. wildei (0.52) and E. jelenakosha (0.51) are close. Further in vitro qPCR testing will be completed with these species.
- Initial field eDNA results indicate a relatively low percentage of positive records.

Acknowledgements


Bibliography


Figure 1. Eurycea Junaluska (the Junaluska salamander) photo by Todd Person.

Figure 2. Eurycea Junaluska range, from Ryan and Seaver (2008).