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 manner. We developed species-specific oligos to detect two semiaquatic salamander species.

Methods

In Situ Testing

Water samples were collected periodically from four eastern Kentucky streams located in Robinson Forest (Little Millseat, Falling Rock, Clemons Fork, and Cubie Fork) over an approximately one-year period. Approximately 10 samples were collected from each stream, 36 samples total.

Water eDNA Extraction

Environmental DNA extraction was performed using a modified version of an established protocol. The extraction was conducted using a DNeasy Blood & Tissue Kit (Qiagen). eDNA quantification was performed using a StepOnePlus Real-Time PCR system. Standard curves were generated using synthetic DNA (gBlock IDT) to both enable data reporting in copy number and assess lowest observed limits of detection and quantification.

Inhibition testing

All samples were run with an internal positive control (TagMan Exogenous Internal Positive Control) to assess potential PCR inhibition.

Results

Specificity testing in Viro Testing

Both our P. ruber and G. porphyriticus assays exhibited varying numbers of mismatches across their four respective subspecies (or northern red ruber subspecies included here). In brief, modeling results indicate only 9/19 P. ruber subspecies tested would be detected using our assay (prob. of 0.61 or greater). Of the 12 G. porphyriticus subspecies tested all except one have an amplification probability indicating amplification (results not shown).

Conclusions

• Primers designed for G. porphyriticus and P. ruber were species-specific among the 12 sympatric species tested in silico and six tested in vitro.
• eDNA results for G. porphyriticus and P. ruber indicate a relatively low percentage of positive results, lower than that observed for a sympatric salamander species (E. cirrigera) in these streams. These data are consistent with the trophic status of these species.
• Previous studies in these streams have reported greater salamander abundance in Little Millseat relative to other streams, consistent with our observations.
• Species-specific analysis indicates assays are broadly, but not universally, effective across subspecies, emphasizing the importance of phylogenetic history in the implementation of eDNA studies.

Bibliography


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