**Amphibian species detection via environmental DNA and conventional methods in Southern Ontario vernal pools**

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Amphibians are a globally declining taxa which warrants the need for effective species monitoring and ongoing conservation. Conventional monitoring efforts have included visual surveys, auditory surveys, and trapping which vary in level of invasiveness and can be unreliable for detection of the diverse amphibian life history strategies such as cryptic species or those with low population densities. Environmental deoxyribonucleic acid (eDNA) barcoding is an emerging, non-invasive, method for species monitoring by detecting unique sequences of nucleic acid, specific to a target species, which persists in the environment after being shed from the organism. Collection of environmental samples (water, soil air) and detection of these species-specific sequences removes the need for direct observations of taxa. Our research aims to determine if eDNA barcoding is a sensitive method of detection for six amphibian species (wood frog, spotted salamander, green frog, northern leopard frog, American toad, and spring peeper) in vernal pools in Southern Ontario compared to conventional detection methods (auditory and visual surveys). We hypothesize that eDNA barcoding will provide equal or greater detection of the target amphibians, compared to conventional survey methods. From April to July 2019, conventional surveys and eDNA collections were conducted in collaboration with rare Charitable Research Reserve, Cambridge, ON. Conventional detection methods included collection of daily auditory data using acoustic song meters and weekly to biweekly auditory and visual surveys according to the amphibian marsh monitoring protocol. Audio data was analyzed using advanced cluster analysis in Kaleidoscope Pro and remaining conventional data was tallied. Alongside conventional surveys, duplicate 1 L water samples containing eDNA were collected at multiple sampling locations around the periphery of three vernal pools. After water collection, eDNA was concentrated by filtration, extracted, and quality controlled to account for inhibition of reactions from compounds collected in environmental samples and integrity of the eDNA. Species-specific eDNA barcoding assays were optimized for species detection using quantitative polymerase chain reaction (qPCR) and detections of target species in eDNA samples is currently underway. Each survey method (eDNA/auditory/visual) will be assessed for overall ability to detect species presence over a spatiotemporal scale and inform surveys techniques ideal for amphibian detection. Results will provide evidence needed to evaluate the ability of eDNA detection methods to enhance ongoing amphibian monitoring by end users in academia, government, and private industry.
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