Examining the persistence of environmental DNA in caves

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Introduction

Cave and other subterranean habitats are particularly difficult to study and survey using traditional approaches, as many areas are hard to access. A promising new method of detecting and monitoring cave organisms is through Environmental DNA, or eDNA, which is DNA left behind by organisms in water, soil, or air from shed cells, feces, gametes, etc.

In surface habitats, eDNA degrades within a few days to a few weeks due to biotic and abiotic factors, such as UV radiation and warm temperatures. However, little is known about how long eDNA persists in subterranean habitats where sunlight does not reach and cooler, stable temperatures exist.

In this study, we set up an experiment at Shelta Cave to determine how long eDNA persists in an environment void of sunlight and with stable conditions.

Methods

• We established three arrays of seven mesocosms filled with cave water along a transect from the entrance to dark zone at Shelta Cave in north Huntsville, Alabama
• Each mesocosm was inoculated with 10g of American Lobster (*Homarus americanus*) slurry
• 250 mL water samples were collected from each mesocosm on days 0, 15, 28, 41, and 68 post-inoculation
• Water samples were vacuum-filtered through 0.45μM cellulose nitrate filters
• DNA was extracted from each filter using the Qiagen Dneasy® kit
• We designed and tested a primer-probe qPCR assay to amplify a 107-bp fragment of the *H. americanus* CO1 locus
• We screened 6 qPCR replicates for each water sample along with positive and negative controls

Discussion

• With the lack of UV radiation, stable cool temperatures and limited microbial activity, eDNA may persist for weeks to months or longer in subterranean habitats
• Long persistence times must be considered when inferring eDNA species detection in such habitats, particularly for species of conservation concern
• We will continue to collect water samples every 6 weeks until DNA can no longer be detected
• We plan to set up another greenhouse experiment to compare the results to how quickly DNA degrades when exposed to sunlight

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