Using Environmental DNA and Citizen Science to Monitor Stream Salamanders in Great Smoky Mountains National Park

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Citizen Science

- Educate public on local scientific research and conservation issues through personal involvement
- Valuable to researchers for conducting large-scale or long-term field sampling

Great Smoky Mountains Institute at Tremont

- Connecting people and nature: residential school programs, summer camps, volunteers
Stream Salamander Monitoring

- Semi-aquatic salamanders are highly abundant and diverse in stream ecosystems in the Smokies.
- Long-term monitoring can help assess threats to these streams including:
  - Acidification
  - Invasive species: feral hogs, hemlock wooly adelgid
  - Amphibian diseases: ranavirus, chytrid fungus
  - Global climate change
Monitoring Methods

• **Direct monitoring:** Capture, identification, and measurement of salamanders
  – Can compare capture rates over time
  – Labor intensive
  – Challenges in detection and identification of certain species

• **Indirect monitoring:** “Salamander CSI” – collection and analysis of genetic material
  – Relatively simple sample collection
  – May detect rarely captured species
  – More precise identification of difficult species
  – Uncertain potential for evaluating relative abundance of species
Comparison of Methods

Direct Monitoring:
Leaf Litter Bags
• Artificial salamander habitats
• 21 bags spaced along stream transect
• Allows for direct capture and identification
• Standardized comparison of capture rates over time

Indirect Monitoring:
Environmental DNA (eDNA)
• Water samples collected along same transect as leaf bags
• Water filtered in the laboratory
• DNA extracted from filtered samples for genetic analysis
eDNA Genetic Analysis

- DNA extracted from filtered sample (Qiagen DNEasy Blood and Tissue Kit)
- Specific region of salamander DNA (mitochondrial locus 12S) isolated with primers (iTru fusion)
- Isolated DNA sequence amplified with Polymerase Chain Reaction
- Salamander species identified from sequences (QIIME program, GenBank reference library supplemented with locally-collected tissue)
With training, volunteers can collect eDNA samples with relative ease.

Researchers working with citizen scientists could survey wide areas, or over long periods of time.
Comparison of Methods

• Monthly survey: June 2015 – April 2016
  – Direct monitoring: Leaf litter bags
  – Indirect monitoring: eDNA sampling
  – Purpose: Compare species identification and relative abundance as determined by both methods

• Pigpen Branch:
  – First order, low elevation (1,640 ft) stream
  – Site of established long-term monitoring project (since 2000) by Tremont citizen scientists
Both surveys identified the same five species of salamanders:

- Blue-ridge Two Line
  \[\textit{Eurycea wilderae}\]

- Black-bellied
  \[\textit{Desmognathus quadramaculatus}\]

- Seal
  \[\textit{Desmognathus monticola}\]

- Black-chinned Red
  \[\textit{Pseudotriton ruber}\]

- Spring
  \[\textit{Gyrinophilus porphyriticus}\]
Blue-ridge Two Line Salamander
(*Eurycea wilderae*)

![Graph showing relative abundance of Blue-ridge Two Line Salamander over time, with data points for eDNA and Leaf Bags.](image)
Black-bellied Salamander
(Desmognathus quadramaculatus)
Seal Salamander
(*Desmognathus monticola*)

![Graph showing relative abundance of Seal Salamander from May 2015 to May 2016. The graph compares eDNA and Leaf Bags.](image)
Black-chinned Red Salamander
*Pseudotriton ruber*

![Graph showing relative abundance of Black-chinned Red Salamander from May 2015 to May 2016. The graph plots relative abundance on the y-axis and months from May 2015 to May 2016 on the x-axis. Two lines are shown: one for eDNA and one for Leaf Bags. Peaks are observed in September 2015, December 2015, and January 2016 for eDNA, with no significant peaks for Leaf Bags.](image)
Spring Salamander
(*Gyrinophilus porphyriticus*)

![Graph showing relative abundance of Spring Salamander from May 2015 to May 2016. The graph includes two lines: one for eDNA and one for Leaf Bags. The eDNA line shows peaks in December 2015 and March 2016, while the Leaf Bags line shows a peak in March 2016.]
Conclusion

- eDNA sampling identified the same species confirmed through direct surveys
- Measuring relative abundance:
  - Some limitations
  - Evidence of seasonal trends observed
  - May be valuable for monitoring with baseline data
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