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Introduction

The Carolina Heelsplitter (*Lasmigona decorata*) is a critically endangered freshwater mussel endemic to North and South Carolina (Fig 1).

- Of the 11 known extant populations, 10 are in decline and 9 are comprised of less than 20 individuals.
- The remaining populations are highly fragmented and isolated from one another due to impoundments.
- There are many factors that are suspected to be responsible for the decline including habitat degradation and fragmentation, water quality deterioration, and potential competition from invasive species.[1]

While general habitat characteristics have been established for the species within current populations (Fig 2), little is known about specific habitat requirements, thresholds within those requirements, or the extent to which identified threats are impacting the species.[1]

Understanding habitat requirements for the Carolina Heelsplitter is essential for determining factors driving their decline and for guiding future management and restoration efforts. Habitat models that predict occupancy for the Carolina Heelsplitter as a function of habitat characteristics and host fish distribution could be used to:

- Identify suitable habitat for the Carolina Heelsplitter.[2]
- Identify environmental variables that are important for species persistence.
- Define objectives for habitat restoration.
- Identity release sites for propagated mussels.[2]

Figure 1: An adult Carolina Heelsplitter.

Figure 2: Typical habitat within extant populations. Location: Flat Creek, SC.

Objectives

1. Investigate occupancy patterns of the Carolina Heelsplitter in relation to environmental variables hypothesized to influence occurrence using an established eDNA protocol.

2. Investigate occupancy patterns of the Bluehead Chub (a known host fish) in relation to environmental variables using an established eDNA protocol.

3. Synthesize information on Carolina Heelsplitter and Bluehead Chub distribution and in-stream biotic integrity to develop decision support tools and predictive maps for management and restoration efforts.

Methods

eDNA protocol validation:

- Positive control samples from propagation tanks at the Orangeburg National Fish Hatchery and stream sites with known occupancy.
- Negative control samples from stream sites in the Clemson Experimental Forest.

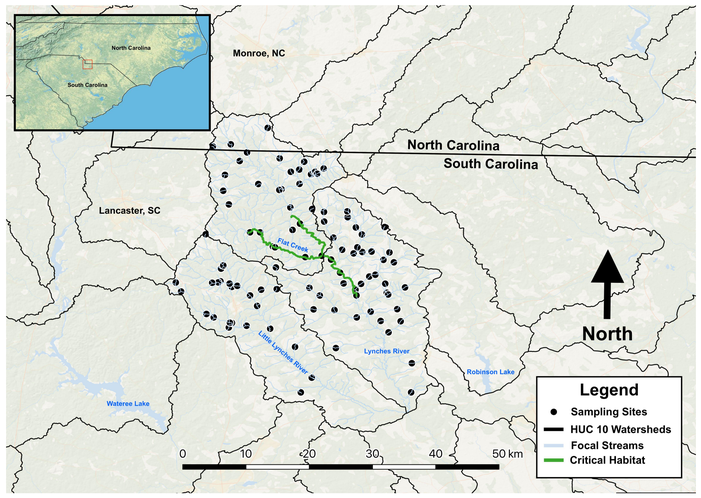

Figure 3: Study Area showing sampling locations. Includes three HUC 10 watersheds within the Lynches River sub-basin of the Pee Dee River basin. Locations determined through a GRTS sampling design stratified by stream order.

eDNA sampling:

- Upper Lynches River drainage (part of the greater Pee Dee River basin).
- 100 sampling locations (Fig. 3).
- Two 1 L samples of stream water at each site to estimate detection probability.
- One 1 L negative control sample at each site to monitor for contamination.[4]
- Habitat attributes including water chemistry, channel morphology, and substrate composition measured along a 100 m reach above each sample location.
- Land use attributes and riparian structure quantified for each site using GIS.
- Water samples filtered using an electric vacuum pump (Fig 4) and stored in ethanol (Fig 5).

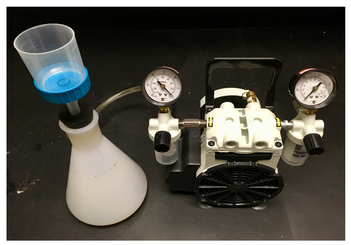

Figure 4: Sample filtration assembly showing pump, vacuum flask, filter housing, and funnel.

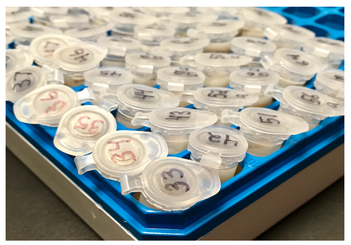

Figure 5: Filtered samples ready to be shipped to The Wilds for analysis.

Data Analysis

- Samples processed using DNeasy Blood and Tissue Kit to isolate eDNA and Zymo Spin Kit to remove potential inhibition.
- Triplicate samples run in a multiplex assay with separate dyes for Carolina Heelsplitter, Bluehead Chub, and internal positive control (Fig 7).
- Ct thresholds established using gblock specific standards (IDT) at 5 concentrations (Fig 6).
- Samples which amplify the IPC and target DNA in at least two triplicates will be considered positive, indicating species presence within the sampling reach.[5]

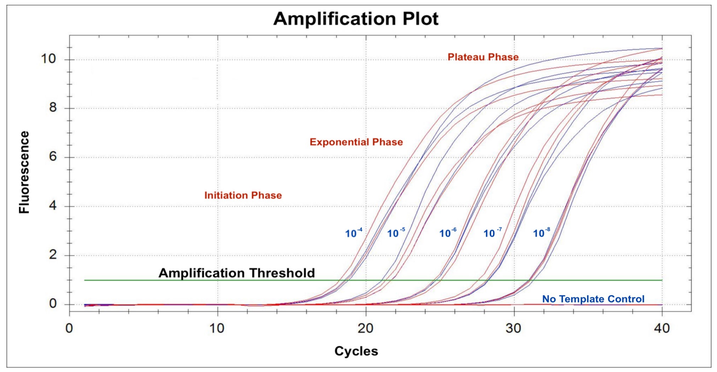

Figure 6: An example of an amplification plot showing the gblock standards at 5 concentrations and a no template control. The amplification threshold for the qPCR plate is established based on average C_t values.

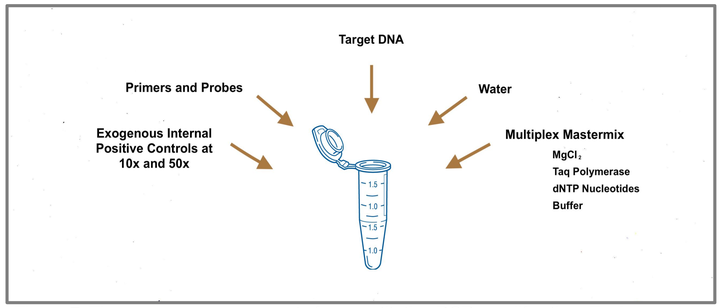

Figure 7: The necessary components for quantitative PCR amplification. Each sample is pipetted into 3 wells on the qPCR plate to be run in triplicate along with gblock standards and a no template control.

References

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