

Spatiotemporal Prevalence of a Parasitic Dinoflagellate, *Hematodinium perezii*, in the blue crab, *Callinectes sapidus*, and the Water Column of the Charleston Harbor Estuary, SC, USA.

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Introduction

Blue Crabs

- Blue crabs are a commercially, recreationally, and ecologically important species.
- Blue crabs support one of South Carolina's oldest and largest fisheries, however, landings have been steadily decreasing, reaching a 50-year low in 2021.
- Declines in blue crab abundance may be correlated with coastal development, environmental change, and disease.

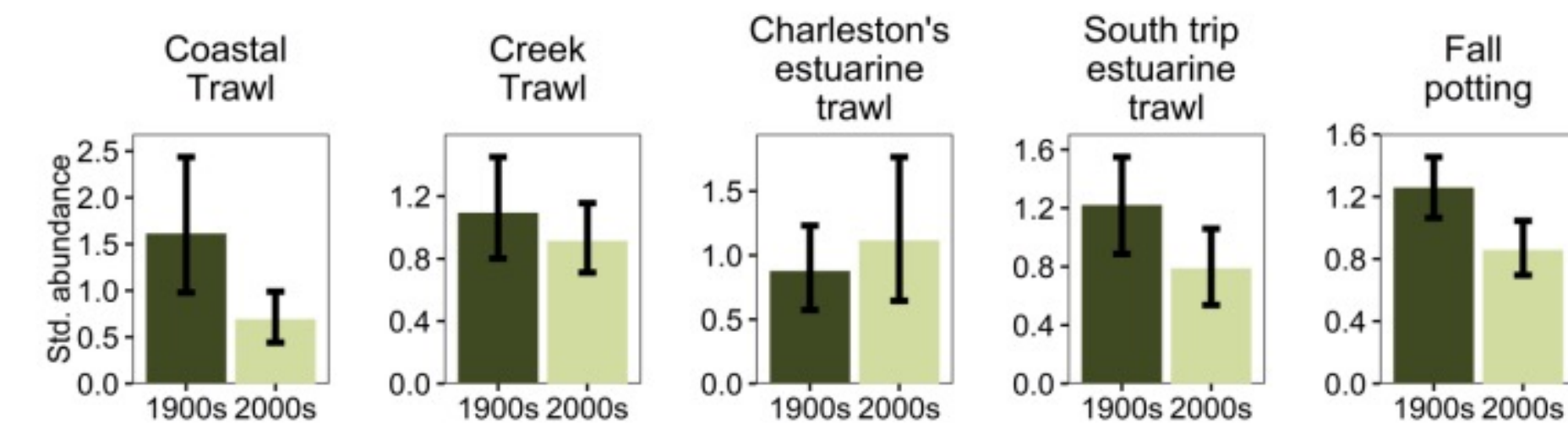


Figure 1. Comparison of mean standardized blue crab abundance (± confidence limits) from five SCDNR fisheries-independent surveys between samples collected through 1999 and since 2000 (SCDNR, 2023).

Hematodinium perezii

- H. perezii* is a parasitic dinoflagellate in the order syndiniales.
- Reported to parasitize over 40 species of crustaceans worldwide.
- Resides and multiplies in the hemolymph of crustacean hosts, leading to mortality due to malfunction of hepatopancreas, degradation of muscle, and respiratory dysfunction.

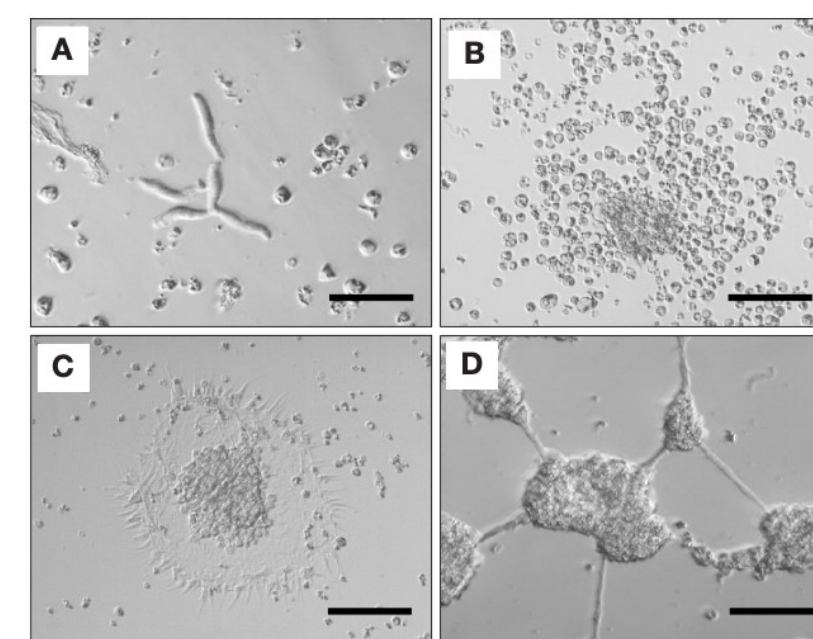


Figure 2. In vitro life stages of *Hematodinium* from *Callinectes sapidus*. (A) Vermiform plasmodia (B) Trophont (C) Arachnoid trophont. (D) Arachnoid trophont/clump colony. (Stentiford & Shields, 2005).

H. perezii in blue crabs

- High prevalence in blue crabs has seasonal, sex, and size related relationships.
 - Juveniles & Females
 - Salinity (26-30 ppt)
 - Temperature (>20 °C)
- Transmission is thought to be waterborne via the dinospore life stage.
- Blue crabs experience a mortality rate of 86-100% over 40 days when infected with *H. perezii* (Messick & Shields, 2000; Shields & Squyars, 2000).

Relevance

- Few *Hematodinium* studies have been conducted in South Carolina.
- South Carolina may exhibit different trends than the thoroughly studied Mid-Atlantic.
 - Blue crabs in South Carolina have an earlier spawning season (Mar.-May).
 - South Carolina is dominated by salt marsh systems compared to submerged aquatic vegetation.
- The environment is shifting to warmer and drier conditions, which may affect spatiotemporal prevalence of both *H. perezii* in blue crabs.

Objectives

1. What is the spatiotemporal prevalence of *H. perezii* based on blue crab hemolymph in South Carolina?

Spatial Prevalence

- Six surveyed estuaries during Fall Crab Potting

Temporal Prevalence

- Wando and Ashley Rivers

2. What is the prevalence of *H. perezii* within the water column of the Charleston Harbor Estuary?

Methods: Blue Crab Hemolymph

Sampling of Blue Crabs

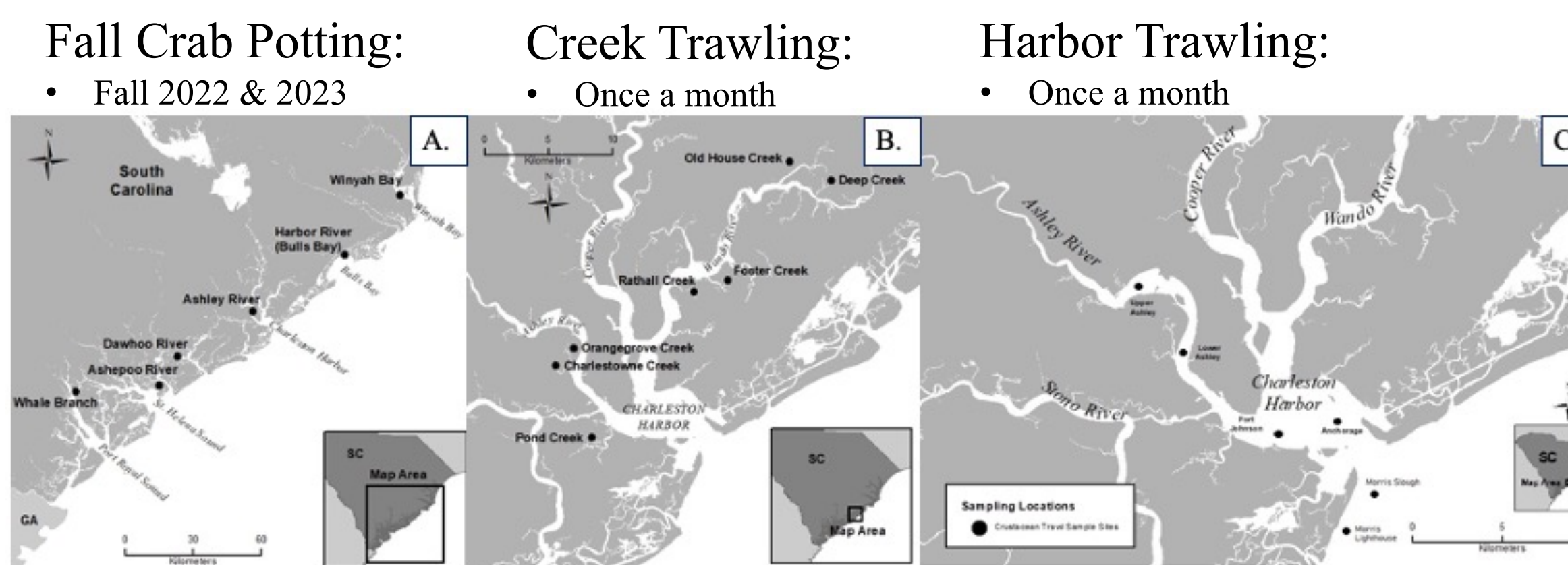


Figure 3. Locations of blue crab hemolymph collection during A) Fall Crab Potting (n=168), B) Creek Trawling (n=89), and C) Harbor Trawling (n=46).

Collection of Hemolymph



Figure 4. Hemolymph extraction from a blue crab

- 100 µl of hemolymph is drawn from juncture of the basis and ischium of the 5th swimming leg.
- Collected hemolymph is preserved in 95% ethanol (1:10 dilution).
- Crab size, width, maturity and molt sign are recorded for each collection.

Methods: Charleston Harbor eDNA

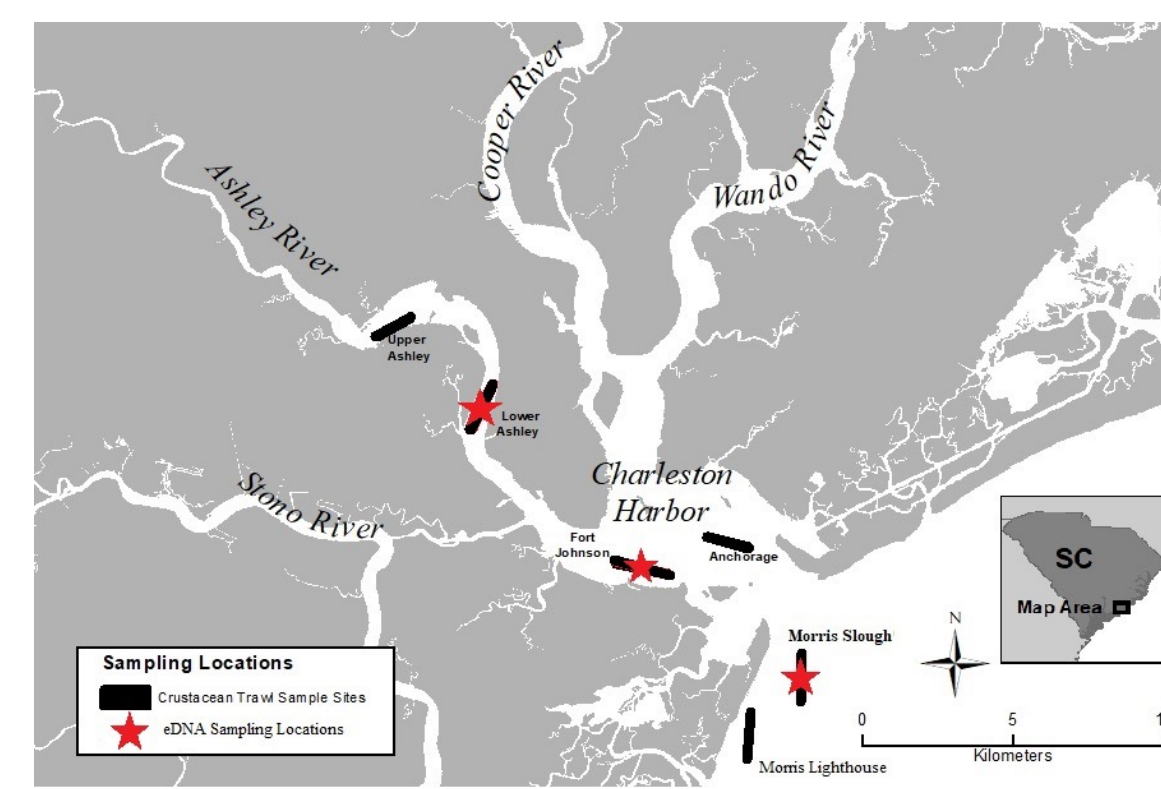


Figure 5. Sampling sites in the Charleston Harbor Estuary. Black lines indicate all trawl sampling sites. Red stars indicate eDNA sampling localities.

- eDNA water is collected at three sites within the Charleston Harbor Estuary.
- Water is collected once a month for 12 months (April 2023– March 2024).
- Two replicates and one control are collected at each site.
 - 108 filters will be collected in total.



Figure 6. eDNA collection methods A) Smith Root eDNA water sampler and B) Two filter water collection method

- A Smith Root eDNA water sampler collects water onto a 5 µl pore-sized filter.
- Up to 2L of water is collected from slightly above the benthos (< 1 m).
- Temperature, salinity, and dissolved oxygen are recorded at each site.

Methods: DNA Extraction & Amplification

DNA Extraction

- Blue crab hemolymph: Qiagen Blood and Tissue Kits
- eDNA filters: Qiagen DNeasy PowerSoil Kits

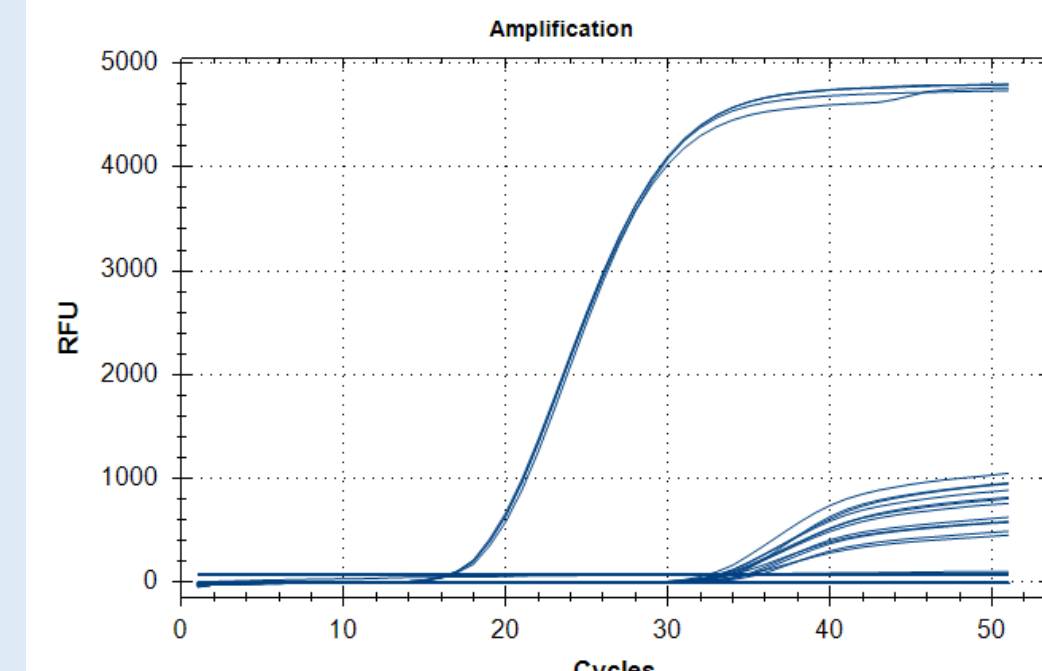


Figure 7. Example qPCR curve of eDNA detection. First curve indicates positive control samples from an infected blue crab. All other curves indicate presence/absence from environmental samples.

H. perezii Detection

- Presence/absence is determined using quantitative polymerase chain reaction (qPCR).
- A TaqMan qPCR assay on the internal transcriber (ITS2) spacer region of ribosomal RNA is used.
- Each sample is run in 8 technical replicates.

Preliminary Results

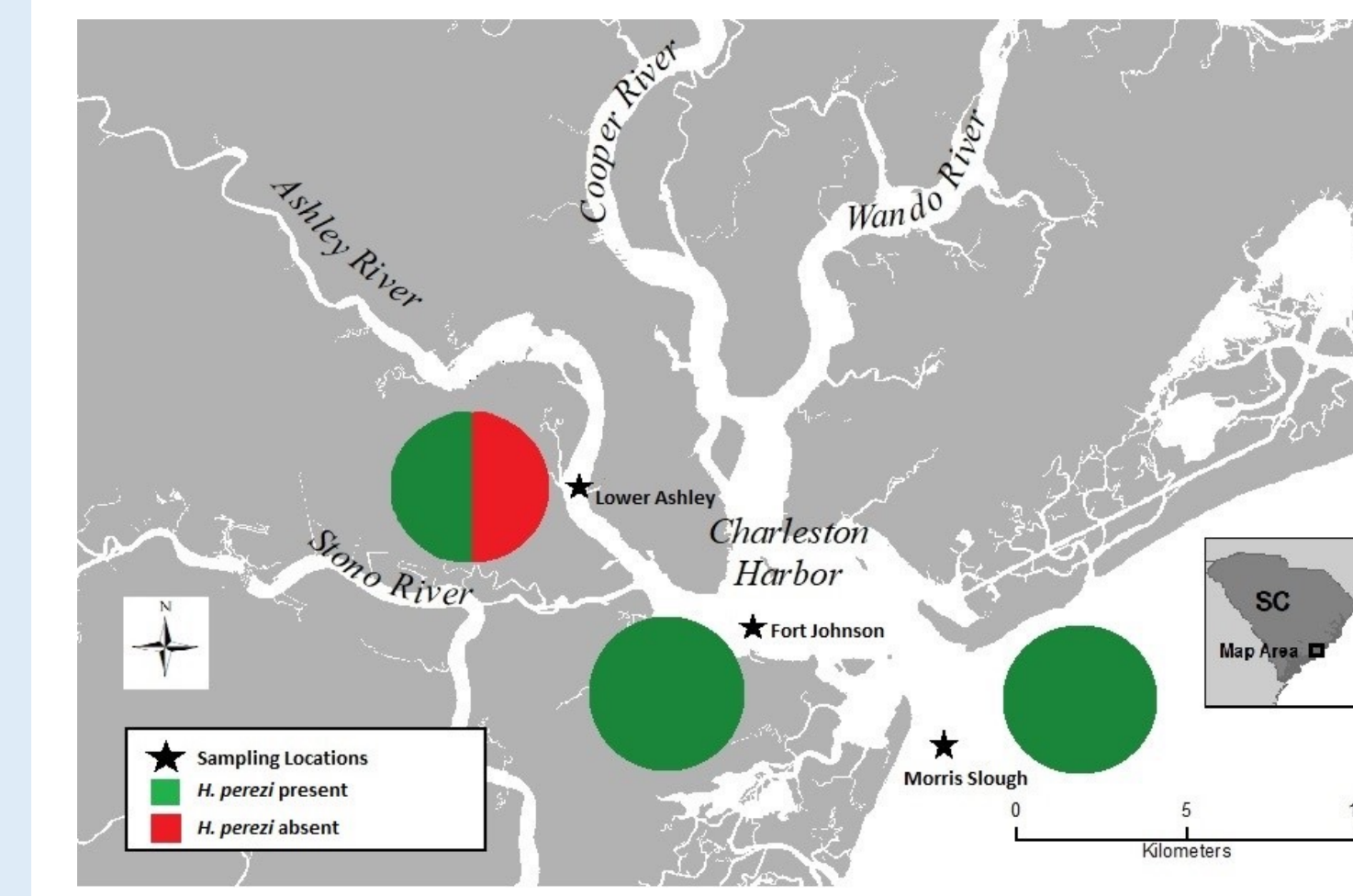


Figure 8. *H. perezii* prevalence across three Charleston Harbor Sampling sites (April – September 2023).

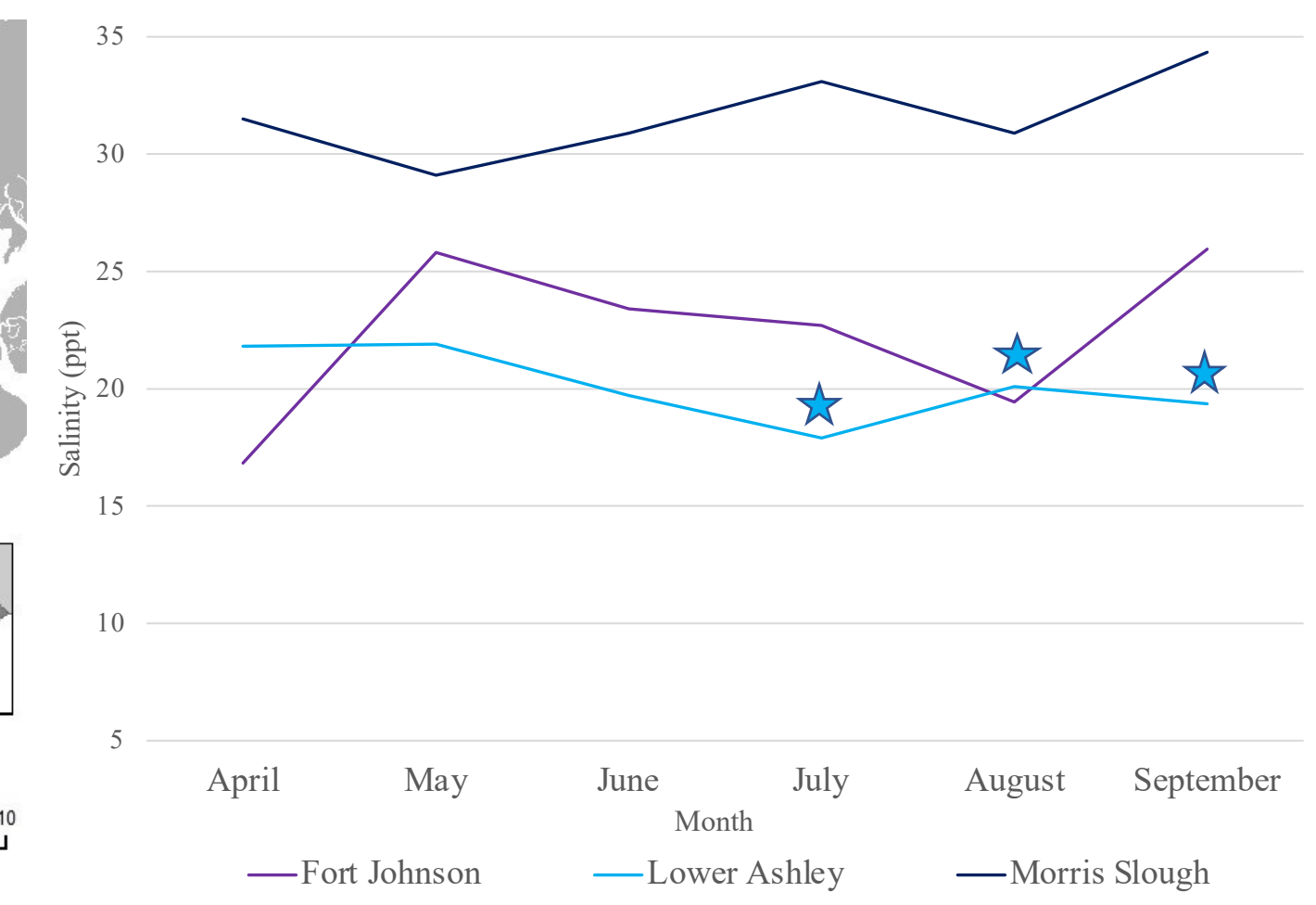


Figure 9. Bottom salinity measurements from three Charleston Harbor Sampling sites (April – September 2023). Stars indicate absence of *H. perezii* seen in Lower Ashley sites.

eDNA results

- H. perezii* presence has been detected at Fort Johnson and Morris Slough sites for each sampling day from April-September 2023.
- H. perezii* has been detected at the Lower Ashley site in April, May, and June but not July, August and September.
- Salinity may be contributing to presence/absence of *H. perezii*.

Hemolymph results

- Hemolymph is currently being extracted and processed.
 - 303 samples have been collected for analysis with collection continuing.

Expected Results

Objective 1: Blue crab hemolymph

- High prevalence in high salinity and low freshwater input estuaries
- High prevalence during late fall months

Objective 2: Charleston Harbor eDNA

- High prevalence in high salinity sites
- High prevalence late summer, early fall

Implications

- South Carolina's blue crab fishery is significantly less regulated than neighboring states, leading to increased fishing pressure in South Carolina.
 - There is a need for new management methods.
- Restrictions may be put in place for the blue crab fishery in certain areas or specific times of year.

Disease Control

- Possible measures of control include prevention of harmful fishing practices.
 - Disassembling crab catch at sea
 - Baiting with potentially infected crabs
- Inclusion of disease data into fishery models could help with site specific management decisions for at-risk populations.

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