SOUTH CAROLINA STATE REPORT
SCDNR Marine Resources Division

Gulf and South Atlantic Regional Panel on Aquatic Invasive Species

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eDNA zebra mussel, *Dreissena polymorpha*, tool optimization and testing.

Zebra mussels (*Dreissena polymorpha*) are freshwater molluscs that are native to eastern Europe and western Asia and were first found in North America in the Great Lakes in 1988 (Hebert *et al.*, 1989). Since that time, they have rapidly spread through large parts of North America (https://nas.er.usgs.gov/UserImages/current_zm_quag_map.pdf), becoming one of the nation's most widespread and abundant freshwater animals. Concerns over the introduction of zebra mussels (and the congeneric quagga mussel, *D. rostriformis*) relate to their ability to cause significant economic and ecological impacts as a result of their biofouling capabilities and ability to disrupt ecosystem through significant phytoplankton consumption, respectively.

Given the overlapping invasive range of zebra and quagga mussels, and the potential negative ecological and economic impacts posed by both species, SCDNR researchers set about optimizing and testing existing eDNA tools (Sepulveda *et al.*, 2020) for the detection of both species in the southeastern United States. The optimization and testing of an active in-house (SCDNR Population Genetics Research Laboratory) panel of species-specific markers for zebra and quagga mussels for use in the highly diverse aquatic landscape of the southeastern U.S. would support the development of a proactive aquatic survey for these invasive species, as well as a rapid evaluation of the distributional extent of an invasion once detected.

While native South Carolina (SC) molluscs are not closely related to these invasive species, it is an important step to ensure that any field-collected sample results are properly interpreted by ensuring that the eDNA assay for zebra and quagga mussels does not amplify when tested against native species. Staff from the SCDNR Population Genetics Research Laboratory tested a pool of glochidia (larval mussels) samples that were collected for an unrelated research project. These samples came from two different creek systems in SC and included glochidia that were collected from 52 individual fish of five species: green sunfish, *Lepomis cyanellus*; margined madtom, *Noturus insignis*; pirate perch, *Aphredoderus sayanus*; redbreast sunfish, *Lepomis auritus*; and tessellated darter, *Etheostoma olmstedi*). All glochidia from a fish were pooled into a single sample and included 5 – 88 glochidia among individual fishes, and a total of 1,191 glochidia. These

samples were amplified at a COI metabarcoding region for species identification and included several species from the genera *Villosa* and *Elliptio* (Poelmann *et al.*, 2024). These glochidia samples did not amplify when tested with the zebra and quagga mussel assay.

Screening archived samples for northern snakehead (*Channa argus*) and bullseye snakehead (*Channa marulius*).

An archived series of eDNA extractions from water samples collected from blackwater systems across South Carolina and Georgia were screened using both the snakehead and zebra/quagga mussel tools to provide a baseline dataset for these species across the region. These eDNA samples were previously collected in 2015 – 2016 as part of an eDNA study for blackbanded sunfish, *Enneacanthus chaetodon*, in which 60 blackwater sites throughout the sandhills region of SC and Georgia were sampled (see Figure 1 below). Utilizing the optimized eDNA tool protocols for both snakehead and invasive mussels, a sample was tested from each of these 60 sites with 8 technical replicates per sample and including both positive and negative controls. All samples tested negative with the snakehead and zebra/quagga mussel assays.

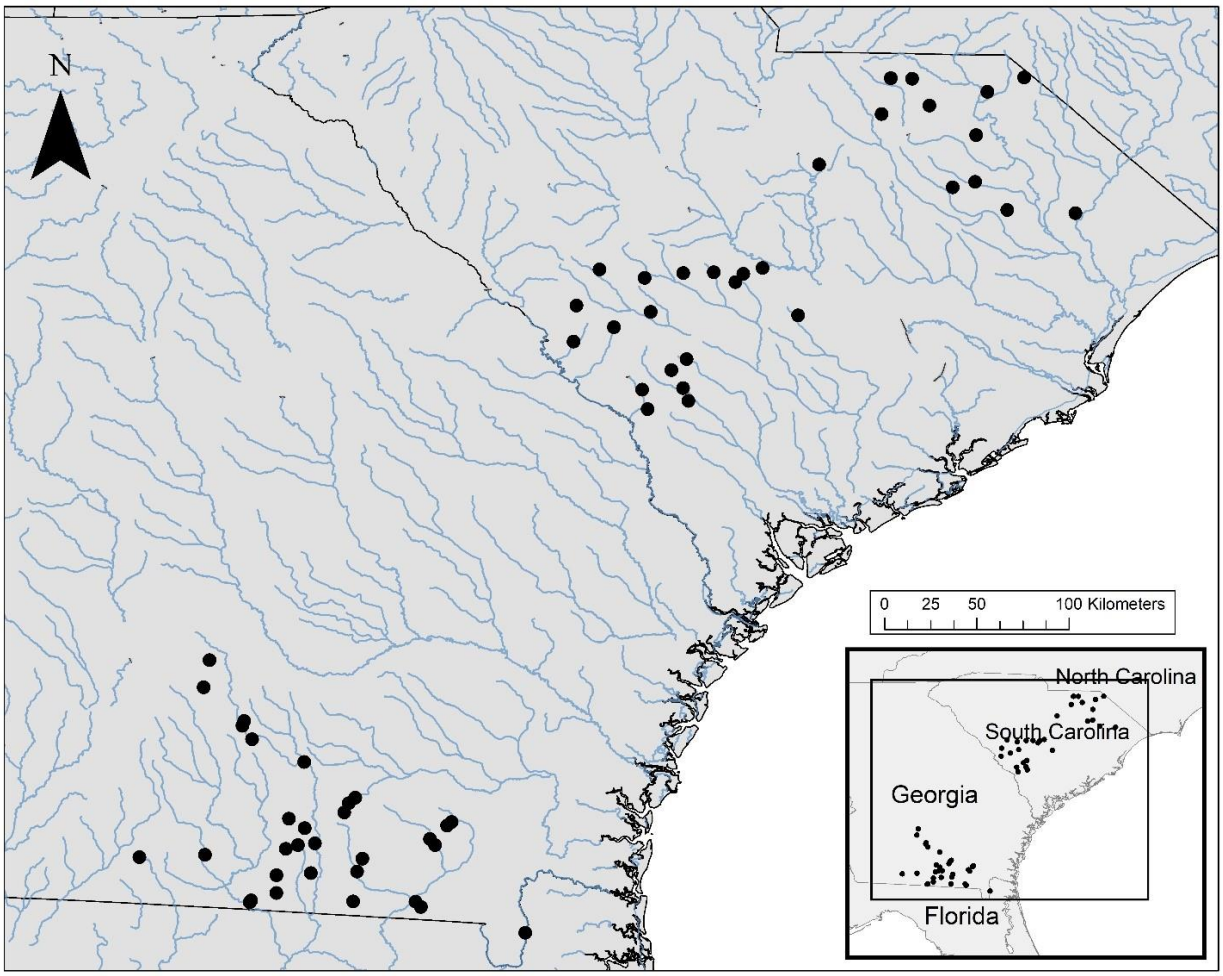

Figure 1. The 60 sampling locations for eDNA collections, originally targeting blackbanded sunfish that were used to screen for the presence of snakehead and zebra/quagga mussels.

Observations of the Caribbean blue land crab, *Cardisoma guanhumi*.

The MRRI Crustacean Research and Monitoring Section continued working on a project to better understand the distribution of the non-native blue land crab, *Cardisoma guanhumi*, in South Carolina. The blue land crab is semi-terrestrial and native along the Atlantic coast of the Americas, from Brazil to southern Florida, and throughout the Caribbean, Gulf of Mexico, and the Bahamas. It is not known whether the species arrived here through natural expansion or human-mediated sources or what/if any impact the blue land crab may have on ecosystems in SC. Adult blue land crabs live in terrestrial habitats, sometimes as far as five miles from the nearest coastal waterway and dig burrows up to six feet deep. They are considered pests in some areas due to their extensive burrowing. Adults can vary in color from blue/purple to ash-gray while juveniles are typically orange/dark-brown.

SCDNR staff issued a call on social media in July 2023 for the public to report any sightings to the public sightings database created last year, but which remains active, found here: https://survey123.arcgis.com/share/73155cf36b124961a366a8b116147a54?open=menu

These reports enabled staff to recently publish a note describing our improved understanding of the non-native distribution of the blue land crab, largely based upon this citizen-science approach:

Scott, E.U., Kendrick, M.R., Kingsley-Smith, P.R., James, M., Lemeris, J., Weeks, E., & Daniel Sasson, D.A. (2023). Using public sightings to document the widespread distribution of the non-endemic blue land crab, *Cardisoma guanhumi*, in South Carolina. *Southeastern Naturalist* 22:498–503.

Quantifying interactions between Atlantic blue crab (*Callinectes sapidus*) and nuisance blue catfish (*Ictalurus furcatus*) in South Carolina.

Blue catfish, *Ictalurus furcatus*, are native to the Mississippi, Missouri, Ohio, and Rio Grande River basins, but have been introduced into numerous systems across the United States as a means of enhancing local fisheries. Blue catfish are fast growing and long-lived (Hilling *et al.*, 2018), and are generalist, omnivorous predators that can survive prolonged periods of limited prey availability (Nepal *et al.*, 2021). They can also tolerate a wide range of salinities (Fabrizio *et al*., 2018; Nepal & Fabrizio, 2019). These characteristics have led to blue catfish threatening native species throughout their introduced range through predation and competition (Schloesser *et al.*, 2011; Schmitt *et al*., 2019, 2021).

The Atlantic blue crab, *Callinectes sapidus*, utilizes a range of habitats through its life cycle. Larval stages are spent in high salinity oceanic waters (Epifanio, 2019), before being brought into estuarine systems via tidal transport and settling in lower salinity waters (Mense & Wenner, 1989, Archambault *et al.*, 1990). Juveniles grow and develop in these lower salinity waters, before moving into more saline waters to spawn (Fischler & Walburg, 1962). This utilization of lower salinity waters during juvenile life stages has led to interactions between native Atlantic blue crabs and blue catfish outside of their native range. In the James River, a tributary of Chesapeake Bay in Virginia, blue catfish, especially those in intermediate size classes (301-500 mm fork length), were estimated to consume more than two million Atlantic blue crabs annually, with juvenile crabs thought to be most impacted (Fabrizio *et al.*, 2021). Due to this documented interaction, staff from the MRRI Crustacean Research and Monitoring Section have been collecting blue catfish to quantify their interactions with Atlantic blue crabs via gut contents analysis.

Blue catfish are being collected monthly from the Ashley River, a tributary of the Charleston Harbor, leveraging existing monitoring surveys (*i.e.,* SCDNR Inshore Fisheries Research Section Electrofishing Survey, SCDNR Estuarine Trawl Survey), as well as through targeted efforts (*i.e.,* targeted electrofishing, hook-and-line sampling). Blue catfish stomachs are removed, and the contents sorted into categories. Any food item suspected to be from an Atlantic blue crab is retained for later taxonomic identification via genetic approaches. Since November 2023, the stomach contents of 55 blue catfish have been examined, of which four have contained possible Atlantic blue crab pieces. Thus far, the most common food items have been fish, crustaceans (*i.e.,* non-portunid crabs, shrimp), detritus, and unidentifiable materials. Monthly collections of blue catfish will continue through September 2024, and patterns of gut contents will be investigated in relation to both season and blue catfish size.

Assessing potential transmission pathways for the transmission of white spot syndrome virus (WSSV) to native crustaceans.

White spot syndrome virus (WSSV) is highly pathogenic (Escobedo-Bonilla *et al.,* 2008), infects many crustacean species, and was recently associated with both wild and farmed red swamp crayfish, *Procambarus clarkii* in Louisiana. Since Louisiana exports live *P. clarkii* to SC, the potential introduction of WSSV via these importations raises concerns over the threats to commercially- and recreationally-important native crustacean species, such as the white shrimp, *Penaeus setiferus*, and Atlantic blue crab, *Callinectes sapidus*, both of which are known to be susceptible to WSSV.

Researchers from SCDNR conducted experimental trials to compare pathways of transmission of WSSV from *P. clarkii* to *P. setiferus* and to assess the effects of salinity on WSSV transmission. Pathways of WSSV transmission examined comprised both ingestion of infected crayfish tissue by white shrimp and indirect cohabitation (*i.e.,* physically separated but sharing the same water) of the two species. To investigate the effect of salinity on WSSV transmission, WSSV-infected crayfish tissue was fed to white shrimp held under low (5 psu) and high (25 psu) salinity conditions. Positive infection was determined using real-time PCR assays of gill tissue. Rates of transmission were significantly higher through ingestion as compared to indirect cohabitation, with transmission via ingestion occurring at a rate of 41.7%. The high salinity treatment produced a transmission rate of 25% and a mortality rate of 16.6%, while the low salinity treatment produced a transmission rate of 12.5% and a mortality rate of 12.5%; however, the differences in transmission rate and mortality between salinity treatments were not statistically significant.

Related to WSSV and some recent testing of both wild caught and commodity frozen seafood, the following manuscript was also published:

Sasson, D. A., Allen, J. M., Walker, M. J., Huber, J. H., Rothman, G. K., Kingsley-Smith, P. R., Darden, T. L. & Kendrick, M. R. (2024). Prevalence of white spot syndrome virus in wild-caught and commodity decapod crustaceans in coastal South Carolina, USA. *Journal of Crustacean Biology* 44(1). https://doi.org/10.1093/jcbiol/ruae002.

Research described in this paper found extremely low levels of the virus in wild-caught decapods but high levels of WSSV in commodity crayfish (50%) and imported frozen shrimp (60%). While additional work is needed to understand the environmental conditions that affect the transmission potential of WSSV, these results suggest that care must be taken with commodity crustaceans to prevent introductions of WSSV and subsequent harm to natural ecosystems.

Evaluating differences in symbiont communities of native and non-native crayfish species.

While the introduction of non-native crayfish can present a significant hazard for some environments, crayfish can also be host to symbiotic organisms which may pose significant risks for native species and community biodiversity (Bojko *et. al.,* 2021). Although these risks are poorly understood, the potential for non-native crayfish to be vectors of introductions for these symbionts warrants further study (Patoka *et al.*, 2016). To assess how crayfish may be vectors of other non-native species, researchers evaluated symbiont communities of both the native crayfish, *Procambarus troglodytes*, and the non-native crayfish, *P. clarkii*, with collections being made between June 2023 and February 2024. Endo- and ectosymbiont communities were recorded for both species (Table 1 below), and efforts to resolve identifications of specimens are ongoing.

Table 1. Symbionts associated with non-native *P. clarkii* and native *P. troglodytes* crayfish hosts. All symbionts were identified to the lowest possible taxonomic resolution. Letters represent the tissues in which each symbiont was found: E = External; G = Gills; IM = Intestinal mesentery; CS = Cardiac stomach; H = Heart; M = Muscle; A = Antennal gland; He = Hepatopancreas.

ECTOSYMBIONTS	***Procambarus clarkii***	***Procambarus troglodytes***
Family Branchiobdellidae		
Cambarincola mesochoreus	E	
Cambarincola sp.		E
Family Entocytheridae	E	E
Ankylocythere ancyla	E	E
Unicirocythere simondsi	E	
Class Bdelloidae	G	
Class Monogononta	G	G
Order Sessilida	G	
ENDOSYMBIONTS	***Procambarus clarkii***	***Procambarus troglodytes***
Family Polymorphidae		
Ibirhynchus dimorpha	IM	IM
Undetermined sp. A	IM	
Class Digenea		
Gorgodorya sp.	CS	G
Undetermined sp. A	IM	
Undetermined sp. B	H	
Phylum Nematoda	IM	IM
Family Ichthyophonidae		
Psorspermium haecklii	M, A, G, He	M

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