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SCDNR Marine Resources Division

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Optimizing eDNA detection tool for invasive northern snakehead (*Channa argus*) and bullseye snakehead (*Channa marulius*).

The bullseye snakehead, *Channa marulius*, has been documented to occur in southern Florida (Benson *et al.*, 2018) where it is known to compete with a variety of bass species and to consume native reptiles, amphibians, and smaller fishes (USGS, 2019). Northern snakehead are more prevalent across the Atlantic Coast than bullseye snakehead (Fuller *et al.*, 2020), posing a more probable threat to native species. Freshwater ecosystems on the Atlantic Coast are extremely rich in biodiversity and have a high number of native species that would be at risk to an invasion of snakehead species. Although not currently documented in South Carolina, both *C. marulius* and *C. argus* are found – to varying degrees – in Florida, Georgia, and North Carolina. Typically when first documented in a new area, however, invasive snakehead have already established a persistent population (Odenkirk & Owens, 2007).

The SCDNR Population Genetics Research Laboratory has completed the development of a panel of species-specific markers for snakehead species to support the rapid evaluation of the distributional extent of an invasion once detected. Data sources from Serrao *et al.* (2014), Simmons *et al.* (2016), Roy *et al.* (2018), and Hunter *et al.* (2019) are being used to optimize an efficient suite of eDNA tools. Benchtop tests were conducted with all identified tools with DNA from *C. marulius* and *C. argus*, from its sister family Osphronemidae (Gouramis), and from a diversity of freshwater fishes available in the SCDNR Genetics Tissue Collection. An understanding of distribution is extremely beneficial in identifying potential pathways of movement for snakehead into freshwater ecosystems. Once potential pathways are identified, biologists can make more informed management decisions on how to maximize containment of a snakehead invasion and design possible eradication strategies. Providing timely and accurate data is the most effective way to inform management to reduce the risk of invasive snakehead species across the region.

Table 2. Target (*) and non-target (†) snakehead species tested via eDNA primers and probes.

Species	Common Name	# Individuals Tested
Channa argus	Northern snakehead*	13
Channa aurantimaculata	Orange-spotted snakehead†	1
Channa maculata	Blotched snakehead†	1
Channa marulius	Bullseye snakehead*	1
Channa micropeltes	Giant snakehead†	8

Three pairs of previously designed and tested primer/probe combinations specific to bullseye snakehead (Hunter *et al.* 2019) and Northern snakehead (Simmons *et al.* 2015, Roy *et al.* 2018) were ordered for optimization in the SCDNR Population Genetics Research Laboratory. Positive control tissue for these snakehead species and other snakehead species for non-specific amplification testing was requested from the Florida Museum of Natural History, the Georgia Department of Natural Resources, and the North Carolina Museum of Natural Sciences. Each primer and probe combination was amplified by qPCR following published protocols, testing both target and non-target snakehead species (**Table 1** above).

Table 2. Non-target freshwater species tested via eDNA primers and probes.

Family	Species	Common Name
Acipenseridae	*Acipenser brevirostrum*	Shortnose sturgeon
	Acipenser oxyrhynchus	Atlantic sturgeon
Anguillidae	*Anguilla rostrata*	American eel
Centrarchidae	*Centrarchus macropterus*	Flier
	Enneacanthus chaetodon	Black banded sunfish
	Lepomis macrochirus	Bluegill sunfish
	Micropterus sp. *cf M. cataractae*	Bartram's redeye bass
	Micropterus cataractae	Shoal bass
	Micropterus dolomieu	Smallmouth bass
	Micropterus floridanus	Florida bass
	Micropterus henshalli	Alabama bass
	Micropterus salmoides	Largemouth bass
	Micropterus punctulatus	Spotted bass
	Moxostoma robustum	Robust redhorse
Cyprinidae	*Nocomis leptocephalus*	Bluehead chub
	Notropis lutipinnis	Yellowfin shiner
Gobiidae	*Lentipes concolor*	Freshwater goby
Helostomatidae	*Helostoma temminckii*	Kissing gourami
Ictaluridae	*Ameiurus catus*	White catfish
	Ictalurus furcatus	Blue catfish
Lepisosteidae	*Lepisosteus osseus*	Longnose gar
Moronidae	*Morone chrysops*	White bass
	Morone saxatilis	Striped bass
Osphronemidae	*Trichogaster lalia*	Flame dwarf gourami
	Trichopodus leerii	Pearl gourami
	Trichopodus trichopterus	Blue gourami (Three spot)
	Trichopodus trichopterus	Opaline gourami (Three spot)
	Trichopodus trichopterus	Gold gourami (Three spot)

All three combinations successfully amplified the target species and did not amplify the other snakehead species. Further testing was performed to ensure that there is not amplification in non-target species, such as four species of gourami that have been obtained from local pet stores and other native freshwater species for which the Population Genetics team already have tissues archived (**Table 2** above). All three primer and probe combinations failed to amplify any of the non-target species.

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

Since tool development is complete, an archived series of eDNA extractions from 60 sites in blackwater systems across South Carolina and Georgia that were previously collected as part of an eDNA study for blackbanded sunfish (*Enneacanthus chaetodon*) is currently being screened for the presence of invasive snakehead. All contamination controls and protocols will be implemented, and samples will be evaluated with multiple technical replicates and positive/negative DNA controls.

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 South Carolina (SC), the potential presence of WSSV in these specimens raises concerns over WSSV threats to commercially- and recreationally-important native crustacean species in SC, such as the white shrimp (*Penaeus setiferus*) and blue crab (*Callinectes sapidus*) that are known to be susceptible to WSSV.

Researchers were able to conduct two consecutive trials to assess the effects of salinity on transmission of WSSV from *P. clarkii* to *P. setiferus.* Preliminary work suggested that transmission of the virus via the ingestion of previously infected tissue had the highest potential for positive infection over indirect or direct contact with infected individuals. Using this vector, trials were conducted by feeding individual white shrimp (n = 48) previously infected *P. clarkii* muscle and gill tissue under two controlled salinity treatments including high (25 psu) and low (5 psu) conditions. Positive infection was determined using qPCR assays of gill tissue from experimentally fed shrimp. Results are still being analyzed to determine whether these conditions influenced both mortality and positive transmission of the virus.

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

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. Prevalence of white spot syndrome virus in wild-caught and commodity decapod crustaceans in coastal South Carolina, USA. *Journal of Crustacean Biology*.

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.

Occurrence patterns of non-native portunid crabs (family Portunidae).

Commercial and recreational crabbers have increasingly reported the occurrence of invasive portunid crabs in South Carolina. This includes the Indo-Pacific swimming crab, *Charybdis hellerii* and the bocourt swimming crab, *Callinectes bocourti*. To manage any potential ecological and fisheries impacts, researchers with SCDNR MRRI's Shellfish Research Section are interested in understanding the distribution and occurrence of these invasive portunid species. Portunid crabs are often difficult to identify at the juvenile stage leading to a lack of life history information for many portunid species in this age class. Therefore, researchers at the MRRI are using a combination of morphological and genetic approaches to facilitate greater taxonomic resolution for juvenile portunid species. Specimens were collected and retained from the SCDNR Estuarine Trawl Survey, which includes 26 statewide sampling locations. Sampling for this project has been completed, resulting in the collection of over 700 juvenile portunids from the *Callinectes, Arenaeus,* and *Achelous* genera. To date, 175 of 180 samples sent for sequencing were successfully sequenced. The 5 that failed were due to DNA degradation or contamination. Researchers at the MRRI are currently using these genetic results in combination with morphological characteristics to refine the taxonomic identification of these juvenile portunid species.

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

The Crustacean Research and Monitoring Section (CRMS) continued working on a project to better understand the distribution of the non-native blue land crab, *Cardisoma guanhumi*, in South Carolina. SCDNR staff issued a call on social media in July for the public to report any sightings to the public sightings database created last year, found here:

https://survey123.arcgis.com/share/73155cf36b124961a366a8b116147a54?open=menu

To date, there have been 28 confirmed reports of blue land crabs in South Carolina in 2023, ranging from Myrtle Beach in the north to Beaufort in the south. 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 South Carolina. 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. CRMS staff hope to gain a better understanding of the distribution and extent of this species in coastal South Carolina through data collected by the public reporting website and have been collaborating with researchers in North Carolina who have also received reports of this species in recent months.

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