# *SOUTH CAROLINA STATE REPORT (SCDNR Marine Resources Division)*

***Gulf and South Atlantic Regional Panel on Invasive Species***
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*Lead*: Dr. Peter Kingsley-Smith, MRRI Assistant Director
SCDNR Marine Resource Research Institute (MRRI)
217 Fort Johnson Road, Charleston, SC USA 29412
Tel. No.: 843-953-9840 (office); 843-655-8763 (cell)
E-mail: kingsleysmithp@dnr.sc.gov

*Collaborators*: Dr. Michael Kendrick (Assistant Marine Scientist), Dr. Tanya Darden (MRRI Director), Dr. Daniel Sasson (Assistant Marine Scientist), Matthew Walker (Wildlife Biologist III) Kim Sitta (Wildlife Biologist II), Meagan Roy (Wildlife Biologist II) & Greg Rothman (Wildlife Biologist I).

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## Investigating hybridization between the invasive red swamp crayfish (*Procambarus clarkii*) and its sister species the eastern red swamp crayfish (*Procambarus troglodytes*).

Researchers at the SCDNR MRRI have developed molecular tools to test whether hybridization is occurring within wild populations of the *Scapulicambarus* subgenus of crayfish. The red swamp crayfish, *Procambarus clarkii* (invasive to South Carolina) is nested within the subgenus *Scapulicambarus*, which it shares with only four other species, including the native eastern red swamp crayfish, *Procambarus troglodytes*, which Busack (1989) showed to be the species most closely related to *P. clarkii*. The eastern red swamp crayfish, *P. troglodytes* is the most abundant native crayfish species in South Carolina, where much of its range overlaps with known locations of invasive *P. clarkii*. Hybridization is common among crayfish species, however, the majority of the research to assess hybridization of non-native crayfish with native species has focused on the genus *Faxonius* (Perry *et al.*, 2001; Arcella *et al.*, 2014), with little data currently available for hybridization within the genus *Procambarus*.

Baited minnow traps and dip netting were the primary techniques used to locate *P. clarkii* and *P. troglodytes*. Microsatellite markers were used to genotype *P. clarkii*, *P. troglodytes* and any potential hybrids collected in the field. The resulting genotypes were subjected to the model-based Bayesian clustering methods implemented in STRUCTURE to estimate and visualize potential shared ancestry that would be expected if hybridization is occurring between these two species. Of the 17 loci that consistently amplify in *P. clarkii*, 6 also amplified and binned reliably in *P. troglodytes* (**Table 1** below).

A total of 259 samples, 127 *P. clarkii* and 132 *P. troglodytes*, were included in the final STRUCTURE analysis estimating shared ancestry between the two species. **No individuals included in these analyses were identified as hybrids and as such there is no indication of recent or rampant hybridization between *P. clarkii* and *P. troglodytes* at any of the sampling locations.** Loci Pcl-12 and Pcl-64 were particularly informative as hybridization indicators as the 2 to 3 alleles that occur in *P. troglodytes* are ubiquitous in *P. troglodytes* and completely absent in

*P. clarkii*. If hybridization were occurring, introgression of alleles at these loci between these species would be expected.

**Table 1.**
Microsatellite loci and number of alleles found in samples for *P. troglodytes* (Ptr) and *P. clarkii* (Pcl).

| Locus Name | Number of Alleles (Ptr) | Number of Alleles (Plc) |
|---|---|---|
| Pcl-12 | 2 | 23 |
| Pcl-34 | 5 | 8 |
| Pcl-70 | 24 | 22 |
| Pcl-53 | 34 | 29 |
| Pcl-64 | 3 | 20 |
| Pcl-79 | 39 | 15 |

## 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. As such, sampling locations for this study focused on brackish water habitats where *P. setiferus* and *C. sapidus* are known to occur.

Specimens were tested using molecular qPCR assays modified and optimized from Blaylock *et al.* (2019) by the SCDNR Population Genetics Research Laboratory. **Screening of 60 wild-caught specimens of white shrimp (*Penaeus setiferus*), brown shrimp (*P. aztecus*), and blue crab (*C. sapidus*) resulted in no wild-caught estuarine specimens testing positive for WSSV.** These methods were also used to screen several tissue types obtained from *P. clarkii*, including gill, muscle, and pleopods for the presence/absence of the virus. **Based on sampling of 14 locations in the Charleston area, no wild-caught crayfish have tested positive for WSSV.**

In 2022, to investigate an additional pathway of potential WSSV transmission, store-bought specimens of *P. clarkii* (n = 75) and *P. troglodytes* (n = 30) were also screened using the optimized qPCR assay, along with positive control samples, negative control samples, and no DNA template controls. All specimens of *P. troglodytes* tested negative for WSSV; **however, nearly half of the store-bought *P. clarkii* specimens tested positive for WSSV**. In addition, store-bought specimens of *P. setiferus* (n = 90) and Pacific whiteleg shrimp, *Litopenaeus vannamei* were screened for WSSV. **The virus was not detected in any of the store-bought *P. setiferus* samples, but WSSV was detected in 17 of the 30 store-bought *L. vannamei* specimens.**

Researchers are preparing for experimental trials to investigate the mechanisms of WSSV transmission from *P. clarkii* to estuarine crustaceans. These trials will focus on the relationships between environmental conditions and WSSV transmission dynamics, including the rate of initial transmission, infection intensity, and mortality rate. Trials will utilize a combination of

environmentally relevant salinities and temperatures to assess transmission from experimentally inoculated *P. clarkii* to uninfected *P. setiferus.* These trials are scheduled for August-September 2022. Staff recently presented results from qPCR screening alongside the experimental design of future trials in the form of a poster at a recent research symposium hosted by the South Carolina Sea Grant Consortium in Charleston, SC in May, 2022 (Rothman *et al.*, 2022).

## 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 begun 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 (family Channidae) 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 2** above). **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 3** below). **All three primer and probe combinations failed to amplify any of the non-target species.**

**Table 3.**
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) |

## Optimizing an eDNA detection tool for zebra mussels (*Dreissena polymorpha*).

Zebra mussels (*Dreissena polymorpha*) are freshwater molluscs native to eastern Europe and western Asia that 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 ecosystems 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, the SCDNR Population Genetics Research Laboratory is working to optimize and evaluate existing eDNA tools (Sepulveda *et al.*, 2020) for the detection of both species in the southeastern U. S. The optimization and testing of a panel of species-specific markers for zebra and quagga mussels for use in the highly diverse aquatic landscape of the southeastern U. S. will support proactive aquatic surveys for these invasive species, as well as the rapid evaluation of the distributional extent of an invasion once detected. As a complement to eDNA tools, an SOP for field implementation that provides a Decision Tree to allow for the establishment of criteria and actions that can quickly be put into place in the event of a positive detection is also being developed.

**Initial steps for the project have included identifying native mollusc species that will be tested with the zebra mussel and quagga mussel probes to ensure that no non-target amplification occurs with native species.** Although local native mollusc species are not closely related to these possible invasive species, it is an important step to ensure that any field-collected sample results are properly interpretated. Tissue samples or extracted DNA from several native species will be procured to test against these eDNA probes.

## Screening archived samples for northern snakehead (*Channa argus*), bullseye snakehead (*Channa marulius*), and zebra mussels (*Dreissena polymorpha*).

Once 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*) will be screened for the presence of invasive snakehead and zebra mussels to provide a baseline dataset for these species across the region. All contamination controls and protocols will be implemented, and samples will be evaluated with multiple technical replicates and positive/negative DNA controls.

## Asian tiger shrimp (*Penaeus monodon*) reporting in the GSARP region.

Researchers with the SCDNR MRRI's Shellfish Research Section remain interested in understanding the invasion of the South Atlantic Bight and Gulf of Mexico by the Asian tiger shrimp, *Penaeus monodon*. **The SCDNR received no reports of *P. monodon* in the current reporting period.** Although reports have declined in recent years, it is likely that a high proportion of the *P. monodon* collected are being kept for consumption instead. In addition, researchers at the MRRI have continued their collaboration with researchers at Auburn University (Justin Krol and Ash Bullard) to explore the viral diseases present in *P. monodon*. SCDNR staff provided whole specimen and/or gill tissue samples and collection information for 4 additional archived *P. monodon* specimens for this effort.

## Surveying the distribution of Island apple snails (*Pomacea maculata*).

SCDNR and USGS continue to receive reports of Island apple snails from the public. The majority of these recent observations have been in areas where Island apple snails were previously documented by SCDNR MRRI's Shellfish Research Section staff; however, the SCDNR received

**one observation of Island apple snails near the Santee Cooper resort on Lake Marion, SC, which would represent a new location for these snails**. Staff plan to conduct surveys in this area to verify these observations. SCDNR staff have also been in contact with and provided information to US Fish and Wildlife staff conducting research on the link between gastropods (including the non-native Island apple snail) and limpkins in South Carolina.

## Assessing abundance trends for 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 helleri* and the bocourt swimming crab, *Callinectes bocourti*. To manage any potential ecological and fisheries impacts, researchers with the SCDNR MRRI's Shellfish Research Section are interested in understanding the distribution and occurrence of these invasive portunid species. **Staff collected one adult male *C. bocourti* from a commercial crabber in Beaufort County during the current reporting period.** A tissue sample was collected from the specimen and sent off for genetic analysis. Staff will continue to accept specimens from commercial and recreational crabbers.

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. Genetic samples have been sent for analysis and the genetically verified identifications will be used to develop a guide to increase the accuracy of native and non-native portunid identifications in the field.

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