***SOUTH CAROLINA STATE REPORT (SCDNR Marine Resources Division)***
***Gulf and South Atlantic Regional Panel on Invasive Species,***
***GoToMeeting Virtual Gathering, April 21st 2021***

*Lead*: Dr. Peter Kingsley-Smith, Senior Marine Scientist
SCDNR Marine Resource Research Institute
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 Assistant Director; Dr. Larry Bowman, Assistant Marine Scientist; Matthew Walker, Wildlife Biologist II; Elizabeth Gooding, Wildlife Biologist II; Jeanette Huber, Wildlife Biologist I.

[Research activities reported here were supported through funding from the State and Interstate Aquatic Nuisance Species Management Plan Program (#F19AS00117 & #F20AS00034) and from the USFWS State Wildlife Grants Program.]

## Investigating recent geographical spread and new invasions by non-native crayfish species (*Procambarus clarkii*).

To better understand the distribution of the invasive red swamp crayfish *Procambarus clarkii* and to predict its potential impacts on native species, SCDNR biologists surveyed stream and wetland habitats across the Savannah River (n=53) and Pee Dee River (n=15) basins (Figure 1). *Procambarus clarkii* was collected at only one sampling location in the Savannah River basin. The 15 locations sampled in the Pee Dee River basin were previously known to have populations of *P. clarkii*, with new efforts resulting in an additional 1,142 *P. clarkii* specimens from these locations.

## Assessing the invasive red swamp crayfish *Procambarus clarkii* as a potential vector for the transmission of white spot syndrome virus (WSSV) to native crustaceans.

White spot syndrome virus (WSSV) infects many crustacean species, is highly pathogenic (Escobedo-Bonilla *et al.*, 2008), and was recently associated with both wild and farmed red swamp crayfish, *Procambarus clarkii* in Louisiana. Since Louisiana exports a considerable number of live *P. clarkii* to South Carolina, the potential presence of WSSV in these specimens raises concerns that this virus could infect native crustacean species in South Carolina, such as the commercially- and recreationally-important white shrimp (*Penaeus setiferus*) and blue crab (*Callinectes sapidus*) that are known to be susceptible to the effects of WSSV.

To determine if *P. clarkii* is a vector for the WSSV, this species was tested using molecular qPCR assays recently modified and optimized from Blaylock *et al.* (2019) by colleagues in the SCDNR Population Genetics Research Section. These qPCR methods were used to screen samples of several tissue types including gill, muscle, and pleopods, obtained from *P. clarkii*. The results for this study are in presence/absence form for all individuals that are screened. Since WSSV can have significant impacts on estuarine crustaceans, sampling locations will focus on brackish water habitats and habitats near brackish water where *P. setiferus* and *C. sapidus* would have a higher likelihood of being exposed if the virus is present. To date, 208 *P. clarkii* and 158 *P. troglodytes* have been screened using the optimized qPCR assay along with positive control samples, negative control samples, and no DNA template controls. Despite positive WSSV results for store-bought crayfish, sampling of 14 locations in the Charleston area has so far resulted in no records of wild-caught crayfish testing positive for WSSV.

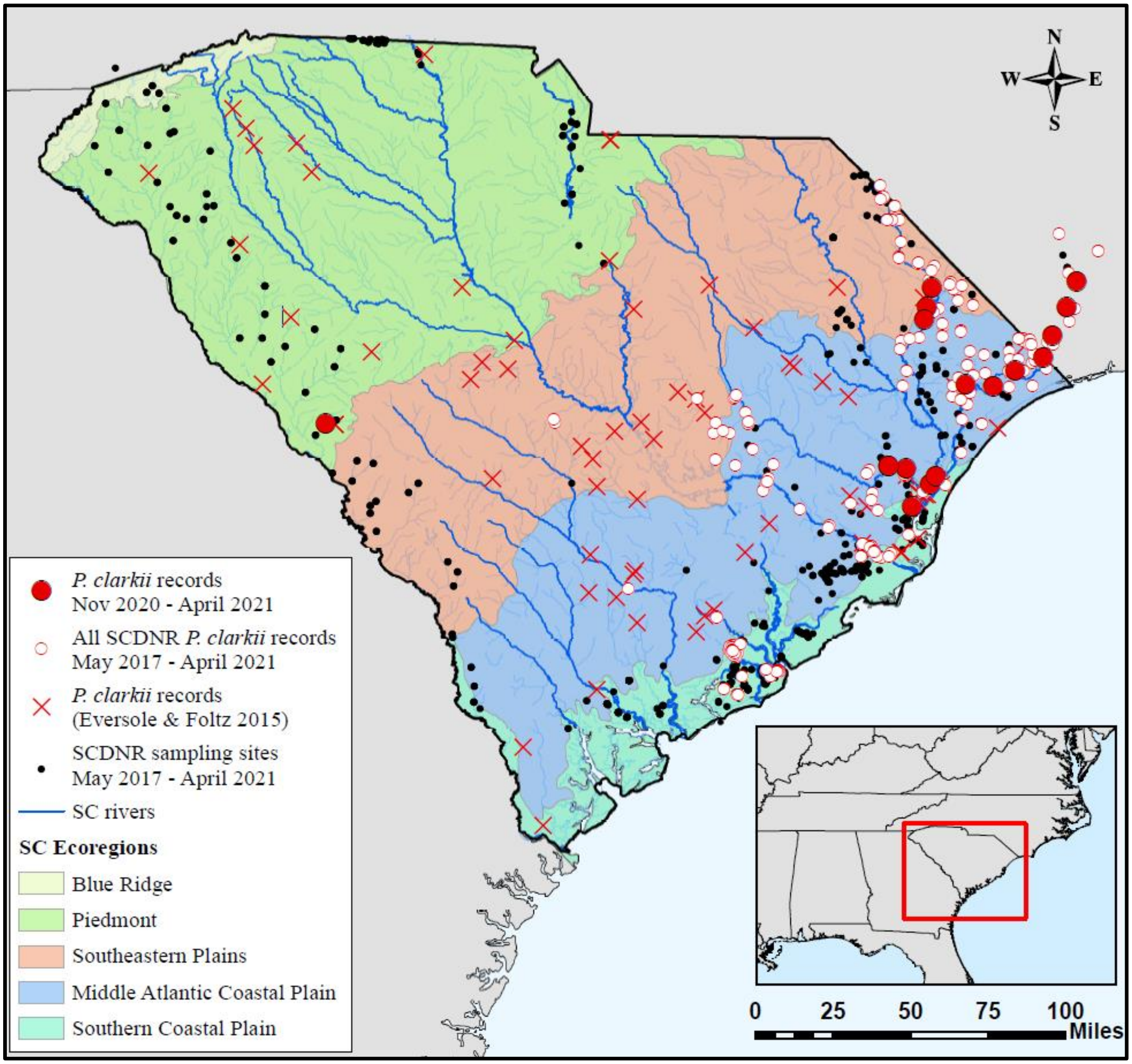

**Figure 1.** All records of *Procambarus clarkii* in the SCDNR MRRI collection are shown as white dots; *P. clarkii* collected by SCDNR staff from November 2020 to April 2021 are shown as red dots; records of *P. clarkii* reported in Eversole & Foltz (2015) are shown as red crosses.

## Potential hybridization of the invasive red swamp crayfish (*Procambarus clarkii*) with its sister species the eastern red swamp crayfish (*Procambarus troglodytes*).

Researchers at the SCDNR MRRI are developing molecular tools to test whether hybridization is occurring within wild populations of the *Scapulicambarus* subgenus of crayfish. The red swamp crayfish, *Procambarus clarkii* is nested within the subgenus *Scapulicambarus* which it shares with only four other species. Within this subgenus, the eastern red swamp crayfish, *Procambarus troglodytes* was shown by Busack (1989) 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*.

A subset of sites sampled to investigate the presence of WSSV are used to also assess hybridization. Baited minnow traps and dip netting were the primary techniques used to locate *P. clarkii* and *P. troglodytes*. Microsatellite markers will be used to genotype both *P. clarkii*, *P. troglodytes* and any potential hybrids collected in the field. The resulting genotypes will be subjected to the model-based Bayesian clustering methods implemented in STRUCTURE in order to estimate and visualize potential shared ancestry that would be expected if hybridization is occurring between these two species. So far 17 microsatellite markers have been optimized for amplification in *P. clarkii* and the optimization of the multiplex panel is complete. The first attempt at genotyping both *P. clarkii* and *P. troglodytes* for this project is complete and we are currently screening for reliable amplification in both species. Allele binning analyses are underway and once complete all data will be scored before re-running amplifications that failed. Markers that amplify in both species will be used for the final hybridization analyses.

## Hybridization, gene flow, and introgression between native redeye bass (*Micropterus coosae*) and introduced Alabama bass (*M. henshalli*).

The redeye bass (*Micropterus coosae*) in the Savannah River Basin is one of three priority species included in the National Fish and Wildlife Foundation's Native Black Bass Initiative and is a species of highest concern in SCDNR's State Wildlife Action Plan (SWAP). This listing is primarily due to the effects of hybridization with the Alabama bass (*M. henshalli*), which was introduced into the reservoir systems in the Savannah River Basin in the 1980s. Since then, hybridization between the two species in the reservoirs has been documented in the field and confirmed by genetic analysis.

Researchers at the SCDNR have developed a microsatellite-based genetic tool to investigate hybridization in black bass populations in the Savannah River basin. Their variability make microsatellites an effective tool to investigate genetic structure and hybridization among populations. In addition to 10 polymorphic microsatellites developed for redeye bass (Wong 2011), microsatellites originally developed for largemouth bass (Seyoum *et al.*, 2013) have been used for other black bass species to assess hybridization impacting endemic shoal bass in the Chipola River (Tringali *et al.*, 2015). Hierarchical STRUCTURE analysis suggests that black bass genotyped at 17 loci with sets of reference samples of regional congeneric species can be assigned to clusters with high confidence. Individuals are assigned as "pure" species if over 98% genetic ancestry results from a single species, "near pure" species if 90-98% genetic ancestry occurs from a single species, and "hybrid" if less than 90% genetic ancestry occurred from a single species.

Project results to date include: 1) evidence of widespread invasion by Alabama bass (*M. henshalli*) and active hybridization throughout the redeye bass range; 2) documentation of 25 tributary sites where pure/near-pure red eye bass are located without incident of hybridization; and 3) documentation for the first time of hybridization occurring on nests in running waters (nearly 60% of sampled streams contained hybrids). The SCDNR Population Genetics Research Section is continuing to process unknown field samples to increase sample sizes for informative analyses of gene flow patterns within species, as well as hybridization rate patterns across the landscape.

### Optimization of an eDNA tool for the detection of 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 (*C. argus*) are more prevalent across the Atlantic Coast than the *C. marulius* (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). Since *C. argus* are found in states and watersheds closely surrounding South Carolina, it is important to be able to rapidly assess waters once a detection of any snakehead species occurs.

The SCDNR Population Genetics Research Section has begun the development of a panel of species-specific markers for snakehead species. An optimized and functional panel will support the rapid evaluation of the distributional extent of an invasion once detected. This research team is using existing genetic sequence data and published eDNA tools to design and optimize a suite of eDNA tools for detection of both *C. marulius* and *C. argus*. Serrao *et al.* (2014), Simmons *et al.* (2016), Roy *et al.* (2018), and Hunter *et al.* (2019) all provide data sources which will be used to optimize an efficient suite of eDNA tools. Benchtop tests will be 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.

### Zebra mussel, *Dreissena polymorpha* observed in South Carolina pet stores

On March 1, 2021, U.S. Geological Survey (scientists) were alerted that zebra mussels (*Dreissena polymorpha*) had been found attached and inside aquarium moss balls. Within three days of this first report, similarly contaminated products were found in aquarium supply stores in more than 25 states across the U.S. In a rapid, coordinated effort in South Carolina, led by Ross Self and Lynn Quattro of the SCDNR Freshwater Fisheries Section, staff visited pet stores and aquarium supply stores across the state to both inform store owners as to the issue and inspect a number of different moss balls products (if still present in the store). Information was also provided on appropriate steps for decontamination and disposal. Information was also distributed through SCDNR's social media platforms.

As part of this coordinated inspection effort, Crustacean Research and Monitoring Section staff collected moss balls from three pet stores in the Charleston County on March 4, 2021 and observed zebra mussels on four of eight specimens examined. The invasive mussels were observed on at least some of the moss balls examined from all three stores visited. All site visit information conducted by the SCDNR (both presence and absence of invasive zebra mussels) was gathered using the ArcGIS Survey123 platform, and an export of all these data is being shared with USGS.

## Update on reports of Asian tiger shrimp (*Penaeus monodon*) in the GSARP region.

**Table 1.** Number of Asian tiger shrimp *P. monodon* reported to the USGS Non-indigenous Aquatic Species database (https://nas.er.usgs.gov/) by state (columns) and by year (rows).

| ***Year / State*** | **NC** | **SC** | **GA** | **FL** | **PR** | **AL** | **MS** | **LA** | **TX** | **All states** |
|---|---|---|---|---|---|---|---|---|---|---|
| **2005** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| **2006** | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | **6** |
| **2007** | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | **4** |
| **2008** | 8 | 6 | 4 | 2 | 0 | 1 | 0 | 0 | 0 | **21** |
| **2009** | 14 | 15 | 3 | 1 | 0 | 5 | 3 | 4 | 0 | **45** |
| **2010** | 2 | 20 | 1 | 2 | 0 | 0 | 0 | 7 | 0 | **32** |
| **2011** | 329 | 144 | 3 | 25 | 0 | 28 | 16 | 128 | 5 | **678** |
| **2012** | 21 | 64 | 55 | 41 | 1 | 3 | 14 | 8 | 1 | **208** |
| **2013** | 4 | 100 | 193 | 30 | 0 | 0 | 6 | 0 | 0 | **333** |
| **2014** | 1 | 63 | 0 | 5 | 0 | 5 | 5 | 1 | 0 | **80** |
| **2015** | 4 | 16 | 5 | 7 | 0 | 41 | 17 | 9 | 3 | **102** |
| **2016** | 0 | 2 | 0 | 3 | 0 | 23 | 5 | 1 | 0 | **34** |
| **2017** | 0 | 11 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | **17** |
| **2018** | 3 | 11 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | **19** |
| **2019** | 1 | 15 | 0 | 2 | 0 | 0 | 1 | 2 | 0 | **21** |
| **2020** | 4 | 8 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | **14** |
| **2021** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| **All years** | **397** | **476** | **264** | **131** | **1** | **107** | **69** | **161** | **9** | **1615** |

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 total number of *P. monodon* reported in SC is currently 476, with a size range of 58 to 330 mm TL. 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 initiated a collaboration with researchers at Auburn University (Justin Krol and Ash Bullard) to explore the viral diseases present in *P. monodon*, initially making use of archived specimens housed at the MRRI.

## Literature Cited

Arcella, T.E., Perry, W.L., Lodge, D.M., & Feder, J.L. (2014). The role of hybridization in a species invasion and extirpation of resident fauna: hybrid vigor and breakdown in the rusty crayfish, *Orconectes rusticus*. *Journal of Crustacean Biology* 34(2):157-164.

Benson, A.J., Schofield, P.J., & Gestring, K.B. (2018). Introduction and dispersal of non-native bullseye snakehead *Channa marulius* (Hamilton, 1822) in the canal system of southeastern Florida, USA. *BioInvasions Records* 7(4):451-457.

Blaylock, R.B., Curran, S.S., & Lotz, J.M. (2019). White spot syndrome virus (WSSV) in cultured juvenile blue crabs *Callinectes sapidus*: oral versus injection exposure, and feeding frequency effects. *Diseases of Aquatic Organisms* 133:147-156. https://doi.org/10.3354/dao03334

Busack, C.A. (1989). Biochemical systematics of crayfishes of the genus *Procambarus*, subgenus *Scapulicambarus* (Decapoda: Cambaridae). *Journal of the North American Benthological Society* 8(2):180-186.

Escobedo-Bonilla, C.M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M.B., & Nauwynck, H.J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *Journal of Fish Diseases* 31(1):1-18.

Eversole, A.G., & Foltz, J.W. (2015). Distribution and Conservation Status of the Crayfish Fauna of South Carolina, USA. *Freshwater Crayfish.* 21(1):33-41.

Fuller, P.L., Benson, A.J., Nunez, G., Fusaro, A., & Neilson, M. (2020). *Channa argus* (Cantor, 1842): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, https://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=2265, Revision Date: 12/31/2019, Peer Review Date: 9/22/2015, Access Date: 6/2/2020.

Hunter, M.E., Schofield, P.J., Meigs-Friend, G., Brown, M.E., & Ferrante, J.A. (2019). Environmental DNA (eDNA) detection of nonnative bullseye snakehead in southern Florida. *American Fisheries Society Symposium* 89:115-135.

Odenkirk, J., & Owens, S. (2007). Expansion of a Northern snakehead population in the Potomac River System. *Transactions of the American Fisheries Society*. 136(6):1633-1639.

Perry, W.L., Feder, J.L., & Lodge, D.M. (2001). Implications of hybridization between introduced and resident *Orconectes* crayfishes. *Conservation Biology* 15(6):1656-1666.

Roy, M., Belliveau, V., Mandrak, N.E., & Gagne, N. (2018). Development of environmental DNA (eDNA) methods for detecting high-risk freshwater fishes in live trade in Canada. *Biological Invasion*s 20:299-314.

Serrao, N., Steinke, D., & Hanner, R.H. (2014). Calibrating snakehead diversity with DNA barcodes: expanding taxonomic coverage to enable identification of potential and established invasive species. *PLOS One* 9(6):e99546.

Seyoum, S., Barthel, B.L., Tringali, M.D., Davis, M.C., Schmitt, S.L., Bellotti, P.S., & Porak W.F. (2013). Isolation and characterization of eighteen microsatellite loci for the largemouth bass, *Micropterus salmoides*, and cross amplification in congeneric species. *Conservation Genetics Resources* 5(3):697-701.

Tringali, M., Strickland, A., Krause, R., Seyoum, S., Brathel, B., Alvarez, A., & Puchulutegui, C. (2015). Conservation status of shoal bass in the Chipola River, Florida: The threat of hybridization with native and nonnative congeners. *American Fisheries Society Symposium* 82:523-536.

USGS. United States Geological Survey. 2019. Nonindigenous Aquatic Species – Bullseye Snakehead (*Channa marulius*).

Wong, L. (2011). Chapter 3: Population structure of redeye bass (*Micropterus coosae*) in Alabama. *In*: DNA Barcoding and related molecular markers for fish species authentication, phylogenetic assessment and population studies. Ph.D. Dissertation, Auburn University, Alabama, pp. 72-96.