SOUTH CAROLINA STATE REPORT (SCDNR Marine Resources Division)
Gulf and South Atlantic Regional Panel on Invasive Species,
GoToMeeting Virtual Gathering, December 17th 2020

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. This report also includes brief information on some additional invasive species observations.

INVASIVE SPECIES RESEARCH PROJECT UPDATES

1) Monitoring the distribution of the island apple snail, *Pomacea maculata* and its potential to invade the estuarine habitats of the Ashley River.

Recent research conducted at the SCDNR MRRI to investigate the salinity tolerance of newly hatched *P. maculata* demonstrated their ability to tolerate salinities as high as 8 psu (Underwood *et al.*, 2019), representative of upstream estuarine habitats in South Carolina. Furthermore, there is a well-established population of *P. maculata* in a suburban neighborhood in West Ashley (specifically within the stormwater retention ponds at the Village Green residential development), located less than 3 km from the Ashley River (Gooding *et al.*, 2018). SCDNR researchers continue to survey these populations on an annual basis. Since the initial survey in 2018, the survey has been expanded to include 30 additional retention ponds outside of the Village Green neighborhood, as well as more natural areas, to monitor the spread of *P. maculata*. Island apple snails have been observed in five additional ponds in Village Green (for a total of 26 ponds with island apple snails), eight newly surveyed ponds in the Shadowmoss Plantation neighborhood nearby, and along Church Creek, which connects the two West Ashley neighborhoods and ultimately leads into the Ashley River. During the current reporting period, a total of 58 retention ponds and their surrounding wetlands and creeks were surveyed, of which 55 ponds were sampled in both the summer and fall of 2020. The three additional ponds were added to the fall survey following a new observation of island apple snails in a retention pond on the southeastern edge of the survey area. Island apple snails were observed in two of the three newly surveyed retention ponds, in addition to a drainage ditch and further down Church Creek. These results suggest that *P. maculata* has additional dispersal capabilities within this area of West Ashley.

2) 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 predict potential impacts to native species, SCDNR biologists surveyed stream and wetland habitats in the Southern and Middle Atlantic Coastal Plains and Southeastern Plains ecoregions in both the Pee Dee basin and in the Charleston Area (***Figure 1***). Since April 2020, SCDNR biologists have surveyed 66 locations, collecting *P. clarkii* at 32 of these locations. A total of 9 species of native crayfish were collected during this time period, but 88% of all collected specimens were identified as *P. clarkii*.

3) 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 that will allow testing of the hypothesis that hybridization is occurring within wild populations of the *Scapulicambarus* subgenus of crayfish. The genus *Procambarus* is a diverse genus with at least 177 species. The red swamp crayfish, *P. 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,* including some locations near Charleston, SC. Hybridization is common among crayfish species; however, the majority of the research to assess the role of non-native crayfish hybridizing with native species has focused on the genus *Faxonius* (Perry *et al.*, 2001; Arcella *et al*., 2014), with little evidence currently available for wild hybridization within the genus *Procambarus*.

During the current reporting period, SCDNR biologists sampled a total of 14 aquatic habitats in the Charleston area, a subset of which are being used to assess potential hybridization (***Figure 2***). Baited minnow traps and dip netting were the primary techniques used to locate *P. clarkii* and *P. troglodytes*. Microsatellite markers that are being developed for *P. clarkii* 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 nearing completion. Screening of samples from *P. troglodytes* for reliable amplification will begin shortly. Markers that amplify in both species will be used for the final hybridization analyses.

4) Is *Procambarus clarkii* a vector for the white spot syndrome virus (WSSV)?

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, *P. clarkii* in Louisiana. Since Louisiana exports a considerable number of live *P. clarkii* to SC, the potential presence of WSSV in these specimens raises concerns that this virus could infect native crustacean species in SC, such as the commercially- and recreationally-important white shrimp (*Penaeus setiferus*) and blue crab (*Callinectes sapidus*) that inhabit brackish waters and that are known to be susceptible to the effects of WSSV.

To determine if *P. clarkii* is a vector for the WSSV, this species will be 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 will be used to screen samples of several tissue types including gill, muscle, and pleopods, obtained from *P. clarkii.* The sampling for this project will mirror the sampling conducted for the hybridization project discussed above, with both *P. clarkii* and *P. troglodytes* collected from locations across the Charleston area (as shown in ***Figure 2***). The results for this study will be in presence/absence form for all individuals that are screened. Since WSSV can also 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. Progress on this project has been impacted by restrictions on fieldwork and laboratory access due to the pandemic.

5) 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.

6) 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 often 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.

Environmental DNA (eDNA) is a useful tool in identifying organismal presence within an area without sampling the organism directly, providing a substantial benefit for rare species, for those that occur in low densities, or for those that are logistically difficult to detect (Simmons *et al.*, 2016). Tools utilizing eDNA have become increasingly popular for monitoring threatened, endangered, or invasive aquatic species. The use of eDNA can be useful in both identifying potential pathways of invasion and identifying invasive snakehead species while at low densities before they establish a population.

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. The SCDNR Population Genetics Research Section will conduct benchtop tests 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.

7) 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	3	7	0	0	0	0	1	0	0	**11**
All years	**396**	**475**	**264**	**129**	**1**	**107**	**69**	**161**	**9**	**1611**

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 475, 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.

NEW INVASIVE SPECIES OBSERVATIONS

1) Range expansion of the cloudy periwinkle, *Littoraria nebulosa* (Lamarck, 1822)

In July 2020, researchers at the SCDNR MRRI began receiving reports of the cloudy periwinkle, *Littoraria nebulosa* in SC from Chris Marsh, Executive Director, Spring Island Trust/Lowcountry Institute. Prior to this report, the geographic range for this species was generally thought to be restricted to Florida. After communicating with Susan Hewitt at the American Museum of Natural History, SCDNR staff learned that new records for this species were documented for Georgia and South Carolina, demonstrating a substantial expansion in the known range for this species. New records in 2020 on the website iNaturalist.org lists records for this species near Jacksonville, FL, Jekyll Island, GA, and Sapelo Island, GA. SCDNR staff conducted multiple surveys for the presence of this species in SC and documented locations for this species throughout much of the state, including new locations (listed from south to north and indicating dates of surveys) at Hunting Island, SC (August 27th), Botany Bay (October 15th), Folly Beach, SC (August 2nd), Capers Island (September 3rd) and Cedar Island (September 25th). The Cedar Island record at the mouth of the Santee River represents the most northern recorded location for this species.

2) Observation of blue land crab *Cardisoma guanhumi* in South Carolina

SCDNR received a call about an individual who had observed a blue land crab (*Cardisoma guanhumi*) on his property in Bohicket, SC. The reporting individual is known to be familiar with native crustaceans such that his report was deemed trustworthy. SCDNR staff investigated the observation but were unable to provide confirmation that this species was present at the location. SCDNR staff will continue to follow up on this report in the hopes of observing the specimen.

3) Indo-Pacific swimming crab, *Charybdis hellerii.*

SCDNR staff helped to identify a crab that was collected by a commercial shrimper off Brunswick Island, GA as the Indo-Pacific swimming crab (*Charybdis hellerii*; superfamily Portunoidea). With a broad Indo-Pacific native geographic range, *C. hellerii* was introduced to the eastern Mediterranean Sea, beginning in Israel in 1920s (Galil *et al.*, 2002). In the Western Atlantic, it was first reported in Folly Creek, near Charleston, SC in 1986 in association with an SCDNR clam farm. This specimen remains housed within SCDNR's Southeast Regional Taxonomic Center (SERTC) collection. This specimen pre-dates the first published report for this species on the East Coast (LeMaitre, 1995). Since then *C. hellerii* has been reported across 62° of latitude and now ranges from south of Cape Hatteras, North Carolina to Florianopolis, Brazil (Fofonoff *et al.*, 2018).

4) Additional records of non-native *Callinectes*

Records of non-native *Callinectes* collected in commercial crab pots include the bocourt swimming crab *(Callinectes bocourti*) collected in Battery Creek (September 2nd) and Archers Creek (September 25th), both in Beaufort County, SC in the Port Royal Sound area, and the rugose swimming crab (*Callinectes exaperatus*) collected in Archers Creek (September 30th).

5) Invasive flatworms

The SCDNR MRRI recently received two reports of non-native flatworms in South Carolina. The first, reported from Summerville, was identified by Dr. Leigh Winsor, Adjunct Senior Research Fellow at James Cook University in Australia, as *Caenoplana* sp. This species was likely introduced from Papua New Guinea (Winsor, 1997) and has been reported in the United States from Texas and Florida. Three individual specimens were collected and preserved in the SCDNR Southeast Regional Taxonomic Center's Taxonomic Collection at the MRRI.

A new terrestrial hammerhead flatworm was reported from Santee State Park to Chris Page, SCDNR ANS Program Manager. The beige flatworm was identified as the land planarian, *Bipalium kewense* based on the description of the individual; however, no photos were captured. It was estimated to be approximately 6" long and was found on the railing of a boat. The reporting individual, who was visiting from Georgia, was familiar with these flatworms after seeing fliers in Georgia calling for reports of this invasive species. *Bipalium kewense* is native to the Indo-China region, and has been reported in the wild from southern California, Louisiana, Florida, Georgia, North Carolina, South Carolina, and Texas. This species is commonly found in greenhouses across the United States is likely spreading to new areas through the distribution of potted plants.

6) Oriental river prawn, *Macrobrachium nipponense*

On September 10, 2020 the SCDNR received a call from a local aquaculturist that he had collected an unusual specimen of a freshwater prawn in the genus *Macrobrachium* from one of his land-based ponds adjacent to Toogoodoo Creek, SC. The gentleman believed the specimen to be *M. rosenbergii,* but requested that someone collect the specimen and identify it. Upon receipt of the specimen, several SCDNR researchers independently examined the specimen and were unable to determine its identity either as a native SC species of *Macrobrachium*, or *M. rosenbergii*, or any of the other species in this genus that have previously been reported from the southern part of the U.S. East Coast. To determine the identity of this specimen, the SCDNR Population Genetics Research Section extracted and amplified DNA from a tissue sample (pleopod) of the specimen, sent the DNA out for sequencing and then blasted the sequences against records in GenBank (these efforts were led by SCDNR Assistant Marine Scientist, Dr. Larry Bowman). Results from the sequencing revealed the following:

Amplification of COI fragments:

1) LCO-HCO (generic invertebrate primer): *Macrobrachium nipponense* (Oriental River Prawn) 87% coverage; 98.84% identity; e-value 0.0
2) Crust1-HCO (prawn specific primer 1): *Macrobrachium nipponense* (Oriental River Prawn) 93% coverage; 98.60% identity; e-value 0.0
3) Crust2-HCO (prawn specific primer 2): *Macrobrachium nipponense* (Oriental River Prawn) 87% coverage; 98.00% identity; e-value 0.0

[Coverage is how much of the COI fragment is covered; these values are high for such generic primers; % identity is how much of a sequence perfectly matches a sequence in the database; e-values are similar to significance measures or p-values; e-values all at 0.0 means any mismatches in our sequences were not "mismatched" bases but rather holes or inconclusively called bases ("N" as opposed to ATCG).]

Upon the determination of the specimen collected from South Carolina as *M. nipponense*, further literature and database searches yielded no established records of this species in the U.S. The species is commonly cultivated in China and is known to be invasive in Iraq, the Ukraine and Russia, but no confirmed reports exist for this species in the U.S. An internet search for this species, however, yielded a ‘hit’ on the website ncfishes.com (The Fishes of North Carolina). Dr. Kingsley-Smith contacted this website to learn more about why *M. nipponense* is included among its gallery of aquatic invertebrates. In the response to this inquiry, Dr. Kingsley-Smith was informed that members of this group first collected *M. nipponense* in North Carolina in 2014 and have since been working to establish its distribution. According to a respondent from the ncfishes.com group "*they appear to be very common in estuarine waters and coastal rivers from North Carolina to Florida… and we believe the vector for introduction is ballast water, as all of our collections have all been made near shipping ports, and we have been unable to locate any aquaculture in the US working with this species. To give you an idea of how widespread they are, I've made roughly 60 collections in NC from Morehead City to Wilmington, and every last one has been nipponense.*"

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.

Fofonoff, P.W., Ruiz, G.M., Steves, B., Simkanin, C., & Carlton, J.T. (2018). National Exotic Marine and Estuarine Species Information System. http://invasions.si.edu/nemesis/

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.

Galil, B., Froglia, C., & Pierre, N. (2002). CIESM atlas of exotic species in the Mediterranean: Vol. 2: Crustaceans: Decapods and Stomatopods. Brand, F. (ed.). Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée (CIESM).

Gooding, E.L., Fowler, A.E., Knott, D.M., Dillon, R.T. Jr., Brown, T., Kendrick, M.R. & Kingsley-Smith, P.R. (2018). Life history and phenological characteristics of invasive island apple snails, *Pomacea maculata* (Perry 1810), in stormwater retention ponds in coastal South Carolina, USA.

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.

Lemaitre, R. (1995). *Charybdis hellerii* (Milne Edwards, 1867), a nonindigenous portunid crab (Crustacea: Decapoda: Brachyura) discovered in the Indian River lagoon system of Florida. *Proceedings of the Biological Society of Washington* 108(4):643-648.

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.

Simmons, M., Tucker, A., Chadderton, W.L., Jerde, C.L., & Mahon, A.R. (2016). Active and passive environmental DNA surveillance of aquatic invasive species. *Canadian Journal of Fisheries and Aquatic Sciences* 73(1):76-83.

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.

Underwood, E.B., Darden, T.L., Knott, D.M. & P.R. Kingsley-Smith (2019). Determining the salinity tolerance of invasive island apple snail *Pomacea maculata* (Perry, 1810) hatchlings to assess the invasion probability in estuarine habitats in South Carolina, USA. *Journal of Shellfish Research* 38(1):177-182.

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

Winsor, L. (1997). The biodiversity of terrestrial flatworms (Tricladida: Terricola) in Queensland: A preliminary report. *Memoirs of the Museum of Victoria* 56(2):575-579.

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.

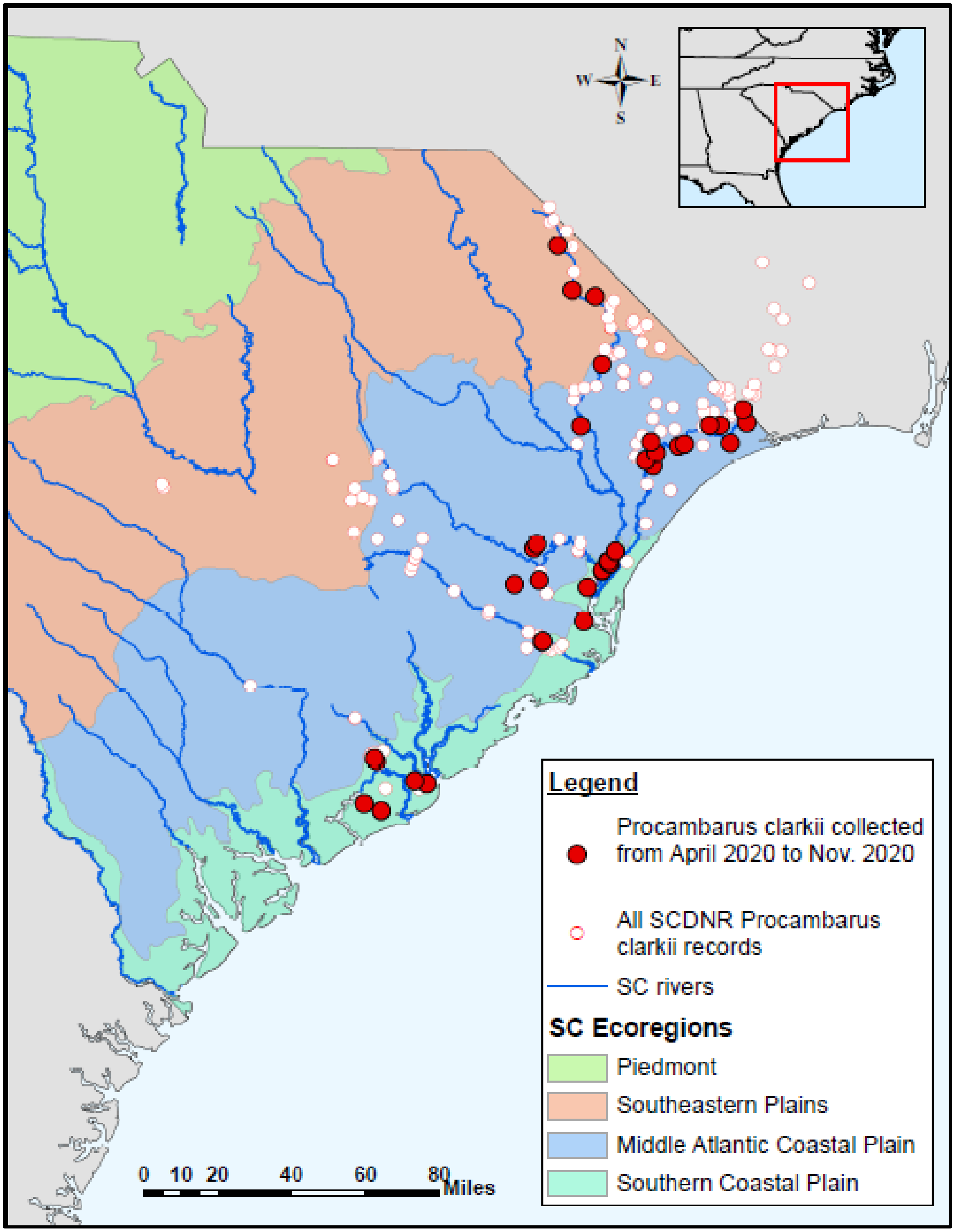

Figure 1 All collection records of *Procambarus clarkii* in the SCDNR MRRI collection are shown in white. *Procambarus clarkii* collected from April 2020 to November 2020 by SCDNR staff are shown in red.

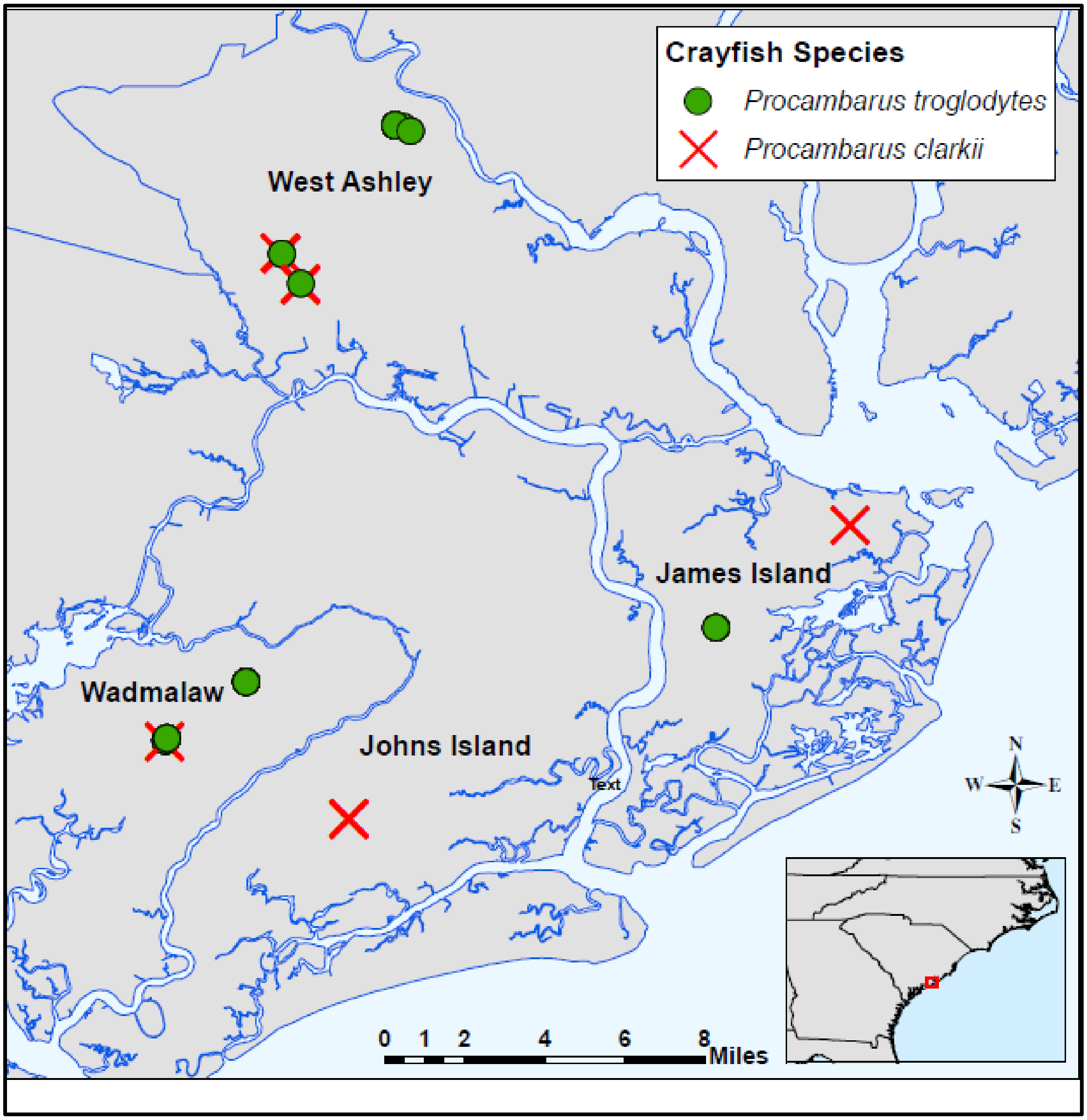

Figure 2. Specimen collection sites of the invasive *Procambarus clarkii* and its native sister species *P. troglodytes* around the Charleston, SC area.