SCDNR MARINE RESOURCES RESEARCH INSTITUTE STATE REPORT.

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Asian tiger shrimp (*Penaeus monodon*) invasion of the South Atlantic Bight.

Researchers at the South Carolina Department of Natural Resources' (SCDNR) Marine Resources Research Institute remain committed to conducting research to improve our understanding of the recent invasion of the South Atlantic Bight and Gulf of Mexico by the Asian tiger shrimp, *Penaeus monodon*. While levels of reporting and concern over this species among the general public have declined in recent years, since their peak in 2011, (see Table 1 below), SCDNR Wildlife Biologist I Elizabeth Gooding (goodinge@dnr.sc.gov) remains the point of contact for reports and specimen collections of this invasive species from South Carolina. The total number of *P. monodon* reported from SC is 429 specimens, with a size range of 61 to 330 mm total length.

Table 1. Number of Asian tiger shrimp *P. monodon* reported to the USGS Aquatic Nuisance Species database by state and by year between 2005 and 2016.

	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	0	0	2	0	0	3	0	0	**5**
All years	**389**	**429**	**264**	**116**	**1**	**84**	**64**	**158**	**9**	**1514**

When *P. monodon* specimens are donated to the MRRI, in addition to measuring and sexing the individuals, the first three pairs of pleopods are collected. These samples are currently being held in a tissue repository in Beaufort, NC which is maintained by the NOAA National Centers for Coastal Ocean Science Laboratory.

At the MRRI, our current research focuses on the collection of *P. monodon* across a range of habitats, from shallow coastal juvenile nursery habitats in estuaries and tidal creeks to sandy or muddy-sand offshore habitats of mature adults, as well as on investigations of their diet, including ontogenetic shifts, with a recent shift to focusing on stable isotope techniques (as opposed to microscope-based identification of gut contents). Muscle tissue samples from 59 *P. monodon* and 103 potential prey items were taken for stable isotope analyses. Prey items included several small finfish species, as well as white shrimp, snapping shrimp, seabob shrimp, grass shrimp, mud crabs, and polychaetes. Tissues were prepared and analyzed in Dr. Jay Brandes' laboratory at the Skidaway Institute of Oceanography in Savannah, Georgia. All tissue

samples have been processed but have not yet been analyzed. Results should help to address concerns that *P. monodon* may compete for food resources with native shrimp species and may even consume some of them directly.

MRRI researchers are keen to learn the status of efforts by USGS researchers to use genetic approaches to identify the geographic origins of *P. monodon* living along the Gulf and Atlantic coasts of the US, as well as the population structure of this shrimp in its introduced range.

Update on the Island apple snail, *Pomacea maculata*.

In 2008, the Island apple snail, *Pomacea maculata* (previously *P. insularum*), was reported from a stormwater pond in Socastee, nearby Myrtle Beach, South Carolina. Native to South America, *P. maculata* was likely introduced to the US via the aquarium trade. In 2010 and 2011, *P. maculata* shells were subsequently reported from a location in Mount Pleasant, South Carolina, and in 2013, *P. maculata* was also reported from a pond in West Ashley, South Carolina.

On a bi-weekly basis from May 2015 through May 2016, MRRI researchers visited two ponds in West Ashley with an established *P. maculata* population to determine seasonal patterns of abundance and size-frequency distribution of juvenile and adult *P. maculata*, as well as egg mass production, and the relationships with physical parameters associated with these ponds. All *P. maculata* observed were removed from the ponds and all accessible egg masses were destroyed. Since the beginning of the sampling period, 1382 live *P. maculata* have been removed from these two ponds and 3644 egg masses have been observed and, in most cases, destroyed. (Some egg masses, such as those in culverts connecting the ponds, are not accessible and therefore could not be destroyed.)

In addition to the bi-weekly sampling of the West Ashley study site, MRRI researchers completed additional surveys to determine the extent of *P. maculata* spread. (More details of these spread surveys were provided at the GSARP meeting in Orange Beach, Alabama in April 2016.). In addition to the bi-weekly surveys and determinations of spread beyond the original ponds in these three locations, SCDNR researchers completed a survey of 100 stormwater ponds across the five eastern South Carolina counties between May and September 2015. A stratified randomized sampling design was used to determine the abundance and distribution of *P. maculata* and other native snails throughout the state. During the sampling of ponds in the southern part of the state, an additional invasive freshwater snail, *Melanoides tuberculata,* was discovered. Researches then expanded the survey on Hilton Head Island, South Carolina in November and December 2015, and three additional invasive freshwater snail species were discovered. Two of these snail species, *Bellamya japonica* and *Biomphalaria havanensis*, had previously been observed in South Carolina; however, these were the first and only reports of *M. tuberculata* and *Pyrgophorus parvulus* in South Carolina. In June 2016, the sites with positive detections of *M. tuberculata* and/or *P. parvulus*, were revisited and more intensively surveyed. A 0.25 m^2 quadrat was placed on the substrate at three randomly selected locations along each of two 10 m transects at each site, for a total of six replicate quadrats per site. All snails present within the surface 2.5 cm depth of sediment and foliage within each quadrat sample were collected. Various substrates within each quadrat sample, including branches, leaves, and manmade materials, were carefully examined. All snails collected within each quadrat sample were brought back to the MRRI, identified and measured. In total, 8388 *M. tuberculata* specimens were collected, and 1009 *P. parvulus* specimens were collected.

Two peer-reviewed manuscripts have been submitted to *BioInvasion Records*:

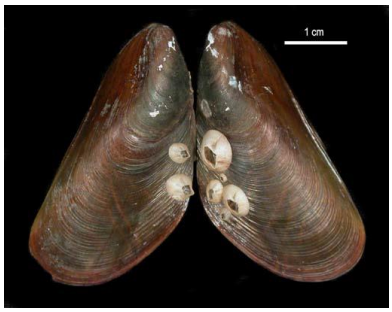
- Gooding E, Dillon Jr. R, Brown T, Kingsley-Smith P, Knott D, and Fowler A. 2016. Northern range expansion of the freshwater snail *Pyrgophorus parvulus* (Guilding 1828) and first confirmed report from South Carolina, USA.
- Gooding E, Dillon Jr. R, Brown T, Kingsley-Smith P, Knott D, and Fowler A. 2016. First record of the freshwater Malaysian trumpet snail, *Melanoides tuberculata* (Müller, 1774), in South Carolina, USA.

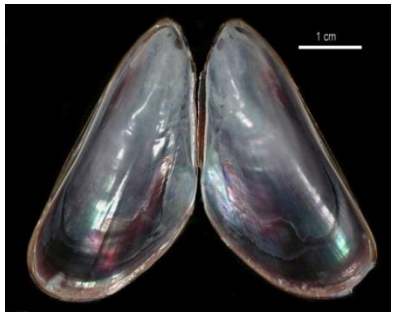
In the spring of 2016, College of Charleston Graduate Program in Marine Biology M.S. student Elizabeth Underwood began her thesis research on *P. maculata*, under the supervision of Dr. Peter Kingsley-Smith. Her research will include determining the population genetic structure of *P. maculata* in South Carolina and Georgia using a suite of microsatellite markers, calculating the frequency of the rat lungworm parasite (*Angiostrongylus cantonensis*) in *P. maculata* in South Carolina, and determining the salinity and temperature tolerances of newly hatched *P. maculata* under laboratory conditions to predict potential habitat for invasion.

Lung and mantle tissue samples have been removed from 100 snails collected from a West Ashley retention pond and 100 snails collected from a Myrtle Beach retention pond and preserved in ethanol for future genetic analyses (see Figure 1 below). Forty-five snails were also collected from a retention pond in Mount Pleasant in July and September 2016. Three sites in Georgia were also sampled in August 2016 with the help of GADNR Wildlife Biologist Eamonn Leonard to obtain samples for the population genetics study. Fifty snails were collected from one pond in Pooler, 90 snails from two ponds in St. Marys, and 48 snails from two ponds in Kingsland, GA. Lung and mantle tissue samples were taken from all of these snails and placed in ethanol at the Skidaway Institute of Oceanography and transported back to the MRRI. DNA has been isolated from lung tissues samples from 39 individuals collected from the West Ashley retention pond and 39 individuals collected from the Myrtle Beach retention pond and screened for the rat lungworm parasite using real-time quantitative PCR. Thus far, none of the *P. maculata* have tested positive for the parasite *A. cantonensis*.

Left: Isolating lung and mantle tissue from *P. maculata* for the parasite study using published qPCR techniques (Qvarnstrom et al., 2010) with positive controls (extracted parasite DNA) obtained from collaborators at CDC.

Charrua mussel, *Mytella charruana*, appears to be expanding northward in South Carolina.





The charrua mussel, *Mytella charruana*, (shown *right*), a mussel in the same family (Mytilidae) as the invasive Asian green mussel, *Perna viridis* as well as several species native to South Carolina (e.g., the ribbed mussel *Geukensia demissa*), has the potential to heavily foul structures that are submerged in seawater. Native to Central and South America, *M. charruana* first appeared in the United States in 1986 at a power plant in Jacksonville, FL where they clogged a seawater intake pipe. It is believed that their arrival was most likely via transport in ballast water or in the fouling community on hulls of vessels arriving from South America. Although cold winter temperatures eliminated that population in 1987, *M. charruana* has since returned to the Indian River Lagoon, FL where it has increased in number since its discovery in 2004, suggesting that a reproducing population has become established there. A large population of *M. charruana* was discovered in 2006 in Liberty County, Georgia, near Savannah.

Until very recently (September 2016), few living *M. charruana* had previously been found in South Carolina. In 2008 a single specimen was reported from Hunting Island and two specimens were found in the Ashepoo River. In 2013, several more specimens were found in the Ashepoo River, which was the most northerly record of its occurrence on the US east coast at that time. Despite the meager number of reports of living *M. charruana* in South Carolina, in 2008 large numbers of shell valves from recently dead mussels were found in the hull scrapings from the bottom of a large barge (shown *above left*) was engaged in interstate transport between Jacksonville, FL and Norfolk, VA. Thousands of adult *M. charruana* valves, along with a similar number of the invasive *P. viridis*, were found on the ground following routine hull maintenance at a shipyard on Yonges Island, SC (shown *right*).

Unfortunately, federal regulations that aim to reduce the risk of introductions through the international shipping trade fail to address the problem of the further dispersal of invasive species through this kind of interstate commerce, once a founding population has become established. The arrival of recreational vessels from areas where the mussel is already established provides an additional potential vector of introduction.

It seems certain that the movement of large numbers of reproductive *M. charruana* through South Carolina provides a substantial, perhaps continual, supply of larval propagules capable of settling and extending the range of the species into suitable habitats along the Intracoastal Waterway. Such propagule pressure may explain what appears to be a recent population explosion of *Mytella charruana* in Charleston Harbor.

During the first two weeks of September, 2016, owners of hull cleaning services in Charleston reported that they have seen substantial aggregations of *M. charruana* on recreational boats docked at four different marinas in Charleston. A small sample from one boat (three images shown *below*) included individual mussels ranging in size from 4.5 mm to 38.5 mm shell length. These business owners expressed their concern about the significant increase in the amount of time it takes to clean hulls that are fouled by *M. charruana*. Since the businesses generally charge by the job, not by the hour, this is already having an effect on the profitability of hull cleaning services in the Charleston Harbor area.

Since the 2004 resurgence of introduced populations of *M. charruana* in the southeastern US, a number of investigations have sought to determine physiological tolerances of the species. Studies have been conducted on lethal low temperatures of submerged and exposed mussels, on the synergistic effects of salinity and temperature, on byssal thread production at low temperatures, and on the effect of food availability on sex ratio and sex reversal. Information from such studies may provide the means, in the future, to predict the potential spread of this species and to determine which factors may be important in limiting it.