FINAL PERFORMANCE REPORT

South Carolina State Wildlife Grant SC-T-F16AF01121

South Carolina Department of Natural Resources

December 31st 2016 – December 31st 2019

Project Title: Integration of field surveys and GIS analysis to document horseshoe crab nesting beaches and egg densities available to federally threatened and highest priority migratory and resident shorebirds in the ACE Basin.

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Objectives: To document the spatial variability in horseshoe crab nesting beaches and available horseshoe crab egg densities to resident and migratory shorebirds in order to prioritize areas within the ACE Basin for conservation of nesting beaches and available prey for shorebirds.

Accomplishments: This research study found extensive evidence that horseshoe crabs use salt marsh habitat for spawning in South Carolina (hereafter SC) and that the eggs laid in this habitat will develop to the trilobite stage. Further, research findings presented here support previous studies that suggest that salt marshes may be suboptimal on an individual basis in that eggs develop more slowly, or are less likely to reach the trilobite stage. Given the extensive acreage of salt marsh habitat in SC, however, these habitats could still support a significant component of horseshoe crab embryos at the population level. Our research also shows that shoreline characteristics and orientation are potentially important for determining the number of adult horseshoe crabs coming ashore to spawn. A new public reporting system was developed with support from this grant and provided further evidence to demonstrate that horseshoe crabs spawn throughout coastal SC. A total of 686 horseshoe crabs were tagged in SC, and most of those tags were deployed within the first year of the project period. The continuation of long-term tag-recapture efforts was partially supported by this project, which helped to demonstrate that a small proportion of tagged horseshoe crabs made substantial migrations between SC and other states to the south (Florida) and north (Massachusetts and Connecticut).

Significant deviations:

The objectives of the project were largely completed, but there were some significant deviations from the original proposal. The most significant deviation was the expansion of the project beyond the ACE Basin and across the coast of SC. For objective 1, field surveys of horseshoe crab eggs were completed in a manner that differed slightly from the original proposal. Instead of quantifying densities of horseshoe crab eggs and the associated sediment characteristics, horseshoe crab eggs were collected from different habitats and individually assigned into developmental categories. This required the implementation of protocols for staging horseshoe crab eggs and statistical analyses for comparing the relative abundances of egg stages between habitats. Objective 2 was largely completed as originally proposed, whereas for objective 3, GIS analyses were restricted to mapping the presence of spawning horseshoe crabs, as reported to SCDNR by members of the public. This project revealed that the dispersed nature of horseshoe crab spawning activity negated the validity of restricting sampling to only beach habitats and made associating spawning abundances with egg densities unfeasible.

Abstract/Summary Paragraph

This project provided insight into the spatial variability of horseshoe crab spawning activity and an ability to compare embryonic development between habitat types (i.e., beaches vs. salt marshes). Although salt marshes are widely thought to be sub-optimal for embryonic development of horseshoe crabs, eggs were seen to successfully develop to the trilobite stage in this habitat. Spawning and fertilization were successful, but a lower percentage of trilobites was observed in salt marshes compared to beaches. In another component of the study, relationships between shoreline migration characteristics (i.e., erosional vs. accretional) and geographic orientation on levels of the abundances of spawning horseshoe crabs on

beaches were investigated. Higher densities of horseshoe crabs spawned on North-facing, accretional shorelines compared to south-facing, erosional beaches. The public reported horseshoe crabs spawning throughout the state of South Carolina. The continuation of long-term tag-recapture efforts partially supported by this project helped to demonstrate that a small proportion of tagged horseshoe crabs made substantial migrations between SC and other states to the south (Florida) and north (Massachusetts and Connecticut).

Project 1: Viability of horseshoe crab eggs laid in salt marsh and sandy beach habitats

Introduction

In South Carolina, horseshoe crabs typically spawn from April to June; however, the timing of spawning varies with latitude (Smith et al. 2017). Increasing water temperatures stimulate adult horseshoe crabs to migrate from deeper water to the shallow water along the shore to spawn (Shuster 1982; Smith el al. 2017). Each season, spawning females bury themselves in the sediment and deposit between 14,500 eggs (Leschen et al. 2006) and 88,000 eggs (Shuster et al. 1982) at 5-20 cm deep (Cohen and Brockmann 1983; Loveland and Botton 1992; Brockmann 2003; Shuster 1982). These eggs are externally fertilized by attached or satellite males that are congregated around the spawning female (Cohen and Brockmann 1983, Penn and Brockmann 1994).

Sandy beaches are generally considered the preferred spawning habitat for horseshoe crabs because the sediment is porous and well oxygenated, allowing for the circulation of gases and water necessary for embryonic development (Shuster 1982; Botton et al. 1988; Loveland et al. 1996). In addition, habitats that provide spawning adults with some protection from wave energy are preferred over higher energy habitats (Jackson et al. 2002; Jackson et al. 2005; Smith et al. 2002).

Horseshoe crab embryos develop in sediments through four embryonic molts before hatching into the juvenile stage (Shuster 1982; Botton et al. 1988; Loveland et al. 1996). Morphological characteristics associated with these molt stages, as well as patterns of intra-molt development, have been used as the basis for categorizing horseshoe crab embryos into a series of developmental stages (Sekiguchi et al. 1988; Shuster and Sekiguchi 2003; Botton et al. 2010). It takes approximately 2-4 weeks for horseshoe crab eggs to hatch into trilobites (Botton et al. 1992), but abiotic conditions such temperature, salinity, moisture, and oxygen availability can have significant effects on developmental rates (Penn and Brockmann 1994; Jackson et al. 2008; Vasquez et al. 2015).

Figure 1. Horseshoe crab eggs collected from salt marsh sediments.

In addition to sandy beaches, horseshoe crabs spawn in a variety of other sediments, such as peat, and mud, sediment types that are characteristic of salt marsh habitat (Sekiguchi et al. 1977, Beekey and Mattei 2008; see Figure 1). The low oxygen availability in salt marsh sediments, however, is often considered sub-optimal for horseshoe crab embryonic growth and development (Botton et al. 1998) as highly hypoxic conditions (<2% oxygen saturation) prevent development into the first embryonic molt (Funch et al. 2016). These findings suggest that complete development to the trilobite stage is unlikely to occur with insufficient oxygen availability.

In South Carolina, U.S.A., the estuarine shoreline is dominated by expansive salt marshes comprised of dense mud and smooth cordgrass, *Spartina alterniflora*. These marshes are generally characterized by low oxygen content (Bradley and Morris 1990). Nevertheless, the use of salt marshes for spawning by horseshoe crabs would suggest that this habitat supports the viable development of horseshoe crabs.

The objective of this project was to compare the viability of horseshoe crab eggs laid in natural beach and salt marsh sediments. Clusters of horseshoe crab embryos were collected from both habitats to test the following predictions: 1) more unfertilized/early development eggs will be found in marsh habitats than in beach habitats; and 2) more eggs will develop into trilobites in beach habitats compared to marsh habitats.

Methods

Sample collection and developmental staging

A broad spatial survey of egg developmental stages across beach and marsh habitats was conducted together with three targeted comparisons of paired beach and marsh sites. These surveys of horseshoe crab eggs were conducted in April, May, and June 2019 across coastal South Carolina (Figure 2). Sites were chosen haphazardly based on previous reports of documented spawning activity by horseshoe crabs provided to SCDNR via multiple reporting channels (e.g. public reporting outlets, personal observations, and collaborations with suppliers of horseshoe crabs to the biomedical industry). At each survey location, hand trowels were used to locate clusters of eggs within beach or marsh sediments. Once egg clusters were located in an area, at least 100 eggs from 1 or more clusters were preserved in 95% ethanol.

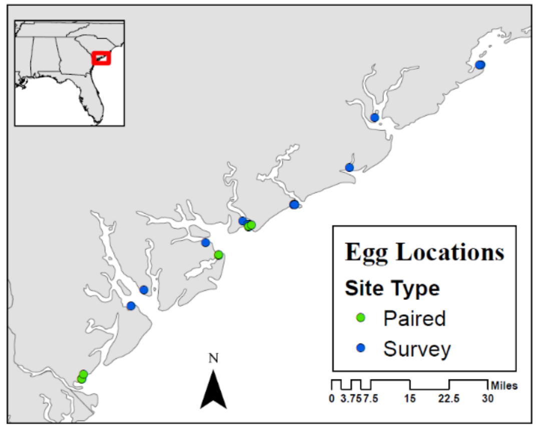

Figure 2. Locations of sampling sites, where horseshoe crab eggs were collected for analysis of developmental stage.

In addition to the broad spatial survey sites, three locations known to support horseshoe crab spawning (Turtle Island, Harbor Island, and Edisto Island) were chosen for paired comparisons of horseshoe crab development at beach and marsh locations. At each paired beach and marsh site, hand trowels were used to locate egg clusters. Once an egg cluster was located, an approximately 5cm x 5cm x 20cm area was excavated and all eggs from that cluster were retained and preserved in 95% ethanol. For each paired beach and marsh location, 7-11 replicate egg clusters were collected from sediments and individually retained.

For both survey and paired locations, at least 50 but not more than 75 eggs from each sample were categorized under 200x stereoscopic magnification into one of 7 morphologically-based developmental stages (Figure 3, Table 1; Sekiguchi et al. 1988; Shuster and Sekiguchi 2003; Botton et al. 2010).

Statistical Analysis

All analyses were conducted using ‘R’ statistical computing platform (R Core Team 2018). All statistical analyses were restricted to data collected in June 2019. For both survey and paired locations, sampling was designed to assess the relative abundance of each developmental stage. As such, data from both sampling regimes were used in generalized linear models with quasi-binomial distributions to determine how the relative abundance of eggs in given developmental stages differed between habitat types. Pairwise comparisons were computed using the ‘emmeans’ package (Lenth 2018), using estimated marginal means, to compare differences in relative abundance of egg stages. Additional GLM models with binomial distributions were developed using location as a random effect for paired sites, where higher levels of replication within each habitat was available, to compare the relative abundance of trilobites and early-staged embryos across habitats. Multivariate analyses of the relative abundance of eggs across developmental stages and habitats were conducted using the ‘vegan’ package (Oksanen et al. 2018) for ordinations using both unconstrained (non-metric multidimensional scale (NMDS)) and constrained (distance-based redundancy analysis) approaches and permutational analysis of variance (PERMANOVA) using location as a strata for permuting data.

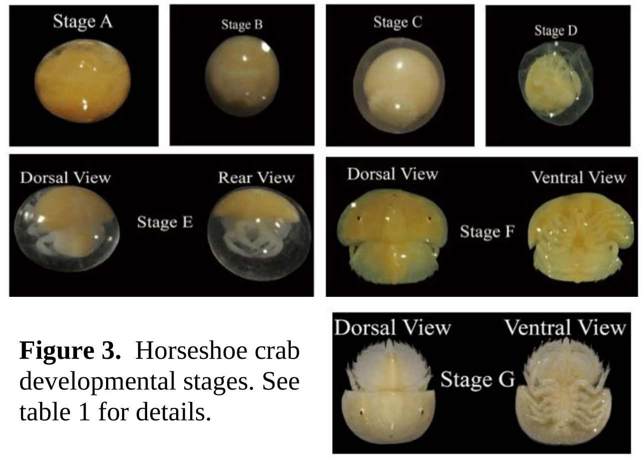

Figure 3. Horseshoe crab developmental stages. See table 1 for details.

Table 1. Description of embryonic stages in early horseshoe crab development.

Stage	Description
A	Unfertilized or early stage of fertilization. Typically greenish-blue, greenish-gray, or pink. Egg surface is smooth, and may have a dent in the surface. Has a large volume of yolk.
B	Limb bud tissue is just starting to develop, but is not defined yet. More tissues are beginning to appear in the egg, but it is not yet apparent what they are. (Consistent with stages 15-17, and prior to first embryonic molt stage; Botton et al. 2010)
C	Early limb bud development clearly defined. Legs are short and close to the body. Following the first embryonic molt.
D	Limb buds are elongated, starting to unfold from body, and becoming more defined. Following second embryonic molt stage.
E	Embryo easily seen, even with unaided eye. Segmentation along dorsal surface is evident. Book gills forming. Fully segmented legs. Eyes are visible and embryo is still in egg membrane. Following the third embryonic molt.
F	Trilobite (first instar). Generally hatched from egg membrane (although not always). Telson is attached to the fan-shaped opisthosoma edge. Following fourth embryonic molt stage.
G	Advanced trilobite. Telson is separated from the fan-shaped opisthosoma edge and beginning to grow past the opisthosoma.

Results

Analysis of monthly data for all beach (n = 42) and marsh (n = 33) sites shows that early-staged embryos were most abundant early in the spawning season in April and May (Figure 4). To better compare viability across beach and marsh habitats, all additional analyses were restricted to samples collected in June. For survey sites in June, horseshoe crab eggs were collected from beach (n = 3) and salt marsh sites (n = 3). Horseshoe crab egg clusters were collected from both beach and marsh locations at Edisto Island (Beach: n=10; Marsh: n=11), Harbor Island (Beach: n=11; Marsh: n=10), and Turtle Island (Beach: n=11; Marsh: n=7). Eggs were successfully staged into 7 embryonic development stages (Figure 4; Table 1) and all developmental stages were found in both beach and marsh habitats (Figure 5).

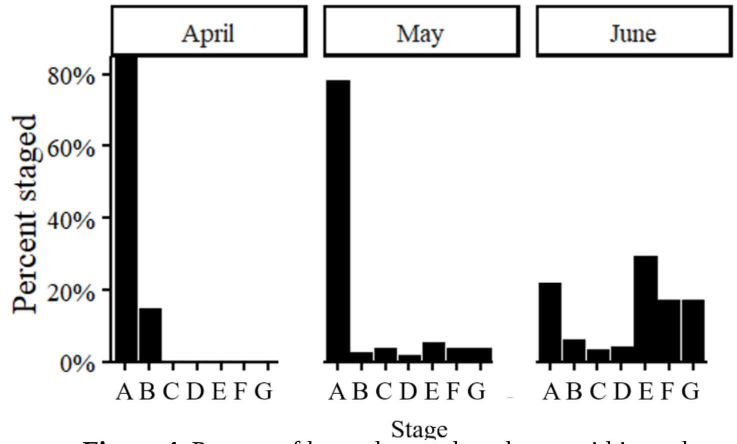

Figure 4. Percent of horseshoe crab embryos within each developmental stage for each of the months April, May, and June from all sites.

Generalized linear models using data from both survey and paired sampling locations showed significant differences in the percent of eggs among developmental stages (*P* = 0.006), but no differences between habitats (*P* = 0.992), as shown in Figure 5. Post-hoc analyses showed that stage A was more abundant than stage B (*P* = 0.040), stage C (*P* = 0.037), and stage D (*P* = 0.038). Furthermore, stage E was more abundant than stage B (*P* = 0.038), stage C (*P* = 0.036) and stage D (*P* = 0.036).

Multivariate analyses were performed using unconstrained and constrained ordinations of developmental stages from samples collected at all locations (Figure 6). While significant differences in egg composition were documented between habitats (*P* = 0.003) in these multivariate analyses, only 6% of variability was explained, indicating that most of the variability in developmental stages was not associated with differences in habitats.

Since eggs at paired sampling locations were collected on the same day, paired sites allowed for a more direct comparison of egg development between beach and marsh habitats. In the analysis of data from paired locations, generalized linear models showed that the percent of early stage embryos (stages A and B) did not differ between habitats (*P* = 0.85, data not shown). The percent of trilobites (stages F and G), however, was greater in beach (43%) than in marsh habitats (24%; *P* = 0.057; Figure 7), location was not significant (*P*=0.11), but a marginally significant interaction was present (*P* = 0.074).

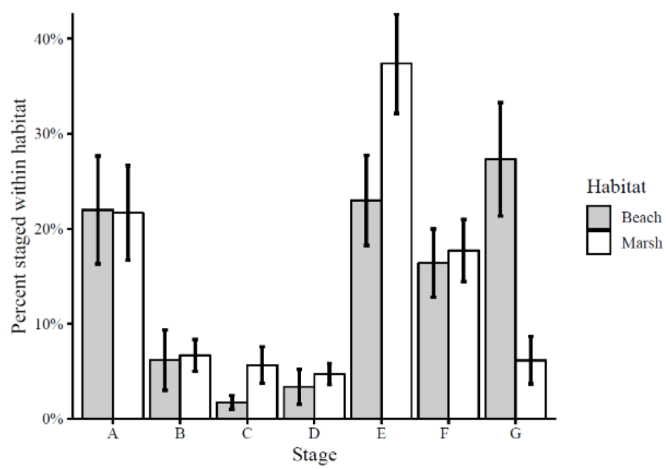

Figure 5. Mean percent (±SE) of each developmental stage from each habitat.

Discussion

This study documented the presence of horseshoe crab eggs and developing embryos across both beach and marsh habitats. The lack of significance in generalized linear models and low explanatory power of multivariate approaches demonstrate that very little variability in overall composition of horseshoe crab egg developmental stages is attributed to differences in beach and marsh habitats.

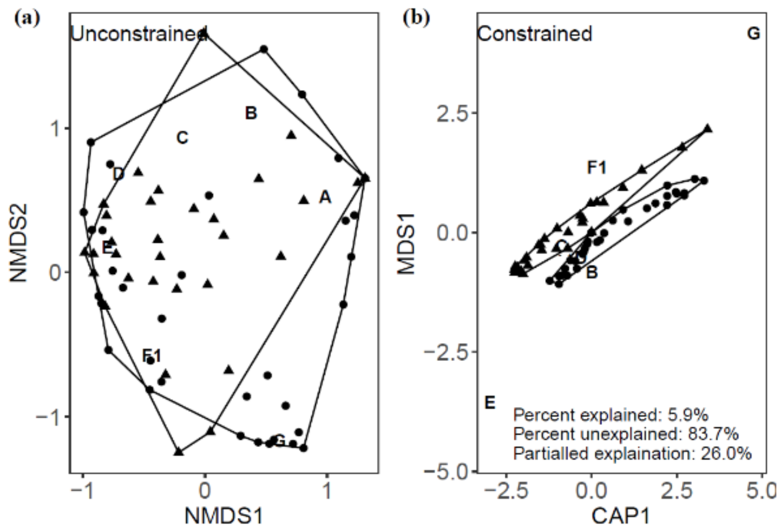

Figure 6. Multivariate analyses showing unconstrained (a) and constrained (b) ordinations of developmental stages.

Despite these overall findings, important differences in development across habitats were highlighted in these analyses. Early-stage embryos, defined as pre-1st embryonic molt, did not show significant differences between marsh and beach habitats. Late-stage embryos, defined as post-4th embryonic molt (also commonly referred to as trilobites), however, were collected in significantly greater abundance in beach habitats as compared to marsh habitats. This finding was driven by large differences in trilobite abundance at two of the three paired sampling locations (Edisto and Turtle Islands), which was not observed at the third location (Harbor Island).

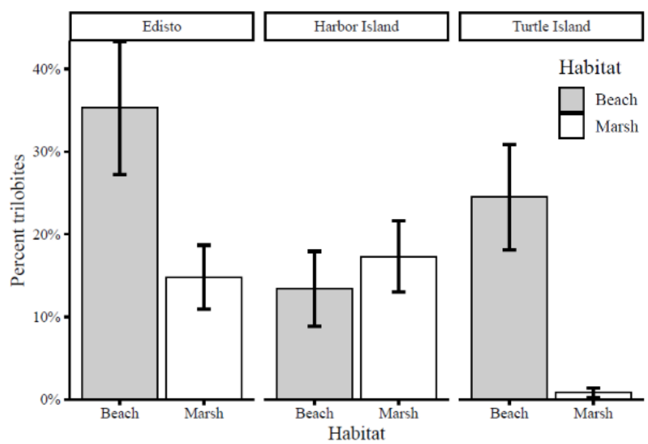

Figure 7. Mean percent (± SE) of horseshoe crab eggs that developed to trilobites (Stages F and G) in the beach and marsh at the three paired sampling locations with GLM showing significantly greater percent of trilobites found in beaches as compared to marshes.

Horseshoe crab embryos are tolerant of low oxygen availability prior to their first embryonic molt, but require greater oxygen availability in later developmental stages (Funch et al. 2016). The low oxygen levels that generally characterize salt marsh habitats (Bradley and Morris 1990) and the tolerance of horseshoe crab embryos of such conditions prior to their first embryonic molt may have contributed to our findings of no difference in early developmental stages, but a reduced number of late-stage trilobites were in marsh as compared to beach habitats. It should be considered that the snap-shot approach used in this study may not accurately reflect the total survival of horseshoe crab embryos to trilobite or juvenile stages. Reduced oxygen availability or other abiotic conditions that differ between beach and marsh habitats may serve to slow embryo development (Jackson et al. 2008, Vasquez et al. 2015), but may not ultimately prevent development to later stages. Additional research is required to determine if abiotic conditions in salt marshes represent developmental thresholds, or whether those conditions only serve to slow reproductive development.

While the findings from the three sampling locations support previous studies that have described marsh habitat as sub-optimal for horseshoe crab embryonic development (Botton et al. 1998, Smith et al. 2017), the present study found substantial evidence that horseshoe crabs spawn in salt marsh sediments and that a large proportion of embryos collected from salt marsh sediments reach the trilobite stage. South Carolina has among the highest acreage of coastal marshes along the U.S. eastern seaboard with >350k acres of estuarine marsh (Tiner and Rorer 1993). Given the large amount of salt marsh habitat, and the extensive use of this habitat by horseshoe crabs as documented by the presence of eggs in many of the surveyed areas, salt marshes could represent significant spawning habitat in South Carolina.

Much of the work that is planned for the FY2020-FY2021 funding cycle of the State Wildlife Grant Program will address the questions raised by this analysis of horseshoe crab embryonic development across beach and marsh habitats.

Project 2: Shoreline migration characteristics and orientation at nesting beaches in South Carolina

Introduction

Horseshoe crabs play an important ecological role in South Carolina ecosystems. Many species of migratory shorebirds, such as the federally threatened red knot (*Calidris canutus rufa*), rely on the eggs that horseshoe crabs lay on beaches during their mass spawning events (Castro and Myers 1993, Botton et al. 1994). Little is known, however, about how beach habitat characteristics and temporal patterns of horseshoe crabs relate to the spawning behavior of horseshoe crabs in South Carolina. The aim of this project was to investigate the roles of shoreline orientation, lunar cycle and erosional dynamics on the abundance of spawning horseshoe crabs observed on coastal beaches.

Figure 8. Locations of horseshoe crab spawning survey beaches and relative abundances of spawning horseshoe crabs observed on each beach.

Methods

In order to better understand the preferred spawning habitats of horseshoe crabs, transect surveys were conducted within the ACE Basin of South Carolina in the spring of 2017. Specific shorelines within the ACE Basin to be surveyed were selected based on shoreline migration characteristics and orientation. Prevailing shoreline erosional/accretion trajectory was determined using AMBUR (*Analyzing Moving Boundaries Using R*) data so that beaches could be classified as either erosional or accretional (Jackson et al. 2012). Shorelines were further classified into groups based on orientation, i.e., whether they were predominantly North- or South-facing. As a result, surveyed shorelines were divided into four types: North-facing erosional, North-facing accretional, South-facing erosional, and South-facing accretional. Three replicates of each type of shoreline were selected, yielding a total of 12 shorelines that were surveyed throughout the season (Figure 8). Four nesting beaches per night were surveyed by SCDNR biologists and volunteers for the presence of horseshoe crabs. Surveys occurred on three consecutive nights beginning on the nights of the full and new moons in April, May, and June 2017 so that each of the 12 beaches were surveyed within a moon phase. Horseshoe crabs were enumerated and categorized as single males, single females, mating pairs, or satellite males. Transects ranged from 50m to 750m in length, due to variation in available beach habitat, with an average width of 4.6m. Transects were analyzed for horseshoe crab abundances by calculating spawning crab densities as number of crabs m^{-2}.

Results

There were significantly more crabs spawning on new moons rather than full moons, which was possibly facilitated by the higher spring tides during new moon phases (Figure 9A). North-facing shorelines had a significantly higher density of spawning crabs than South-facing shorelines (Figure 9B; North range: 0 to 1.15 crabs m^{-2}, South range: 0 to 0.03 crabs m^{-2}; Figure 9B). Accretional shorelines had significantly higher spawning densities than erosional shorelines (accretional range: 0 to 1.15 crabs m^{-2}; erosional range: 0 to 0.6 crabs m^{-2}; Figure 9C).

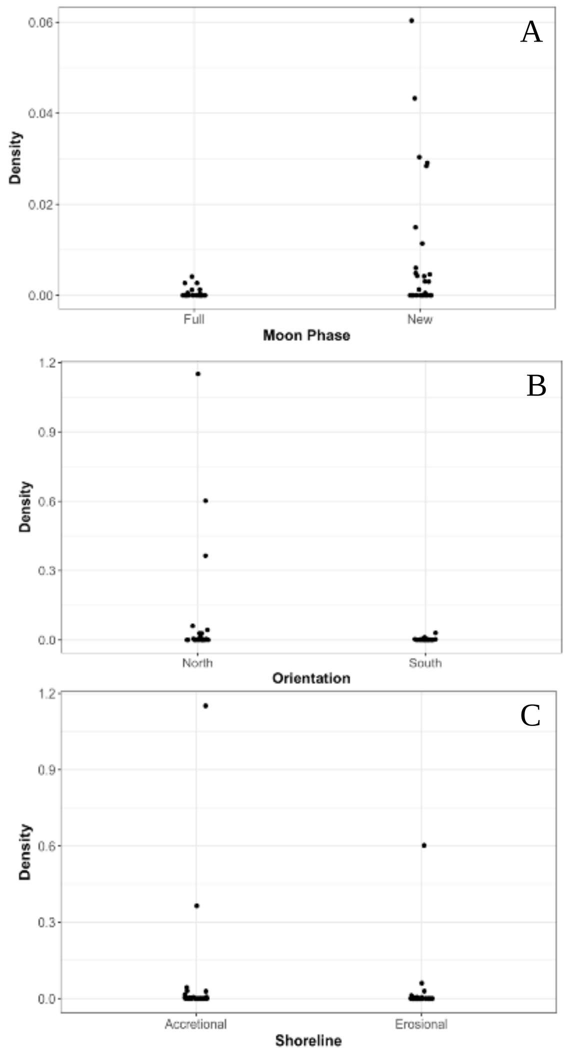

Figure 9. Data from transects assessing spawning horseshoe crab densities across 12 sites as they relate to relevant beach characteristics including: A) moon phase (full vs. new moon); B) orientation (North- vs. South-facing); and C) shoreline migration pattern (accretional vs. erosional beaches).

During the spring of 2018, information was collected on spawning behavior of horseshoe crabs at representative beaches in the ACE Basin and Charleston Harbor, South Carolina. On 5 separate dates in 2018, female horseshoe crabs that were coming onto the beach were observed to bury themselves into the sand in an apparent attempt to initiate spawning. The path along which the horseshoe crab moved was marked to facilitate the excavation of all egg clutches. After a female unburied herself from the sand, she was weighed and measured across the prosoma (prosomal width) and between the two compound eyes (interocular distance). All of the sand in the area in which the animal was observed to bury was excavated and all eggs were retained for further analysis. A total of 23 females were observed burying in the sand from April, 16 to May, 17. Excavation of the sand revealed that in six of those cases, no evidence that eggs had been extruded could be found. Two of these observations were made on April 16, one on May 14, one on May 15, one on May 16, and one on May 17, thus spanning a substantial portion of the typical horseshoe crab season in SC. Our data show that over 25% of females sampled did not lay eggs.

Discussion

Data obtained from these transect surveys indicated some patterns with respect to spawning. The significantly higher density of spawning crabs on North-facing shorelines, as compared to South-facing shorelines might be due to the protection that North-facing shorelines provide from prevailing southwest winds during the spawning season. Book gills of horseshoe crabs allow them to detect interstitial oxygen content in potential nesting sediments. Significantly higher spawning densities on accretional shorelines than erosional shorelines could be related to the limited interstitial oxygen content on erosional, as compared to accretional beach sediments (Crabtree and Page 1974). As such, horseshoe crabs may be choosing beaches that have higher interstitial oxygen content. Further research investigating the response of spawning horseshoe crabs to interstitial oxygen content is needed for a better understanding of their habitat preferences.

The finding of females coming onto beaches but not laying eggs was at first surprising, given the energy expenditure and risk associated with burying in the sediment. Female horseshoe crabs are, however, known to come onto beaches and leave without laying eggs. Female horseshoe crabs, for example, will leave the beach if they do not encounter the males with which they wish to reproduce with polyandrous females preferring to mate with multiple satellite males while monadrous females prefer not to mate with satellite males (Johnson and Brockmann 2012). It is unclear if this phenomenon was associated with the relatively low levels of spawning observed by SCDNR staff and reported across the state of South Carolina in 2018 or if this is a regular occurrence for horseshoe crabs in South Carolina.

Project 3: Public reports of spawning horseshoe crabs

Project overview and outcomes

In order to encourage engagement by the public in citizen science activities and to begin to develop a map of locations that are important for spawning horseshoe crabs, a public reporting outreach effort was initiated. Information on locations where horseshoe crabs were sighted in South Carolina in 2019 was collected using a public online reporting database (SurveyMonkey). The report requested the following information: date/time of sighting, GPS coordinates, location description, photo of horseshoe crabs, and approximate number of horseshoe crabs. The reporting form was live April 3 - July 30, 2019 and a state-wide effort to increase awareness of the project was initiated by SCDNR's Coastal Reserves and Outreach Section through a social media campaign on Facebook, Instagram, and Twitter and through partner email listservs.

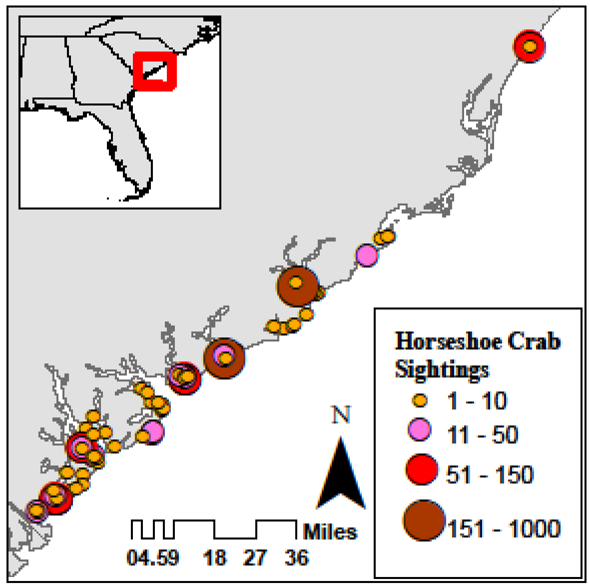

Figure 10. Approximate number of horseshoe crabs at each of the locations as reported through the public reporting systems from Turtle Island to Murrells Inlet, SC, April 3-July 30, 2019.

Each horseshoe crab spawning report was individually examined for quality and accuracy. The QA/QC process ensured that all reported location descriptions matched given latitude and longitude coordinates and that these locations were plausible horseshoe crab spawning areas. Additionally, when available, photographs were assessed to confirm the reported observations. Reports were included in analysis if they occurred in South Carolina during 2019 and if they included the location or GPS coordinates and a photo or approximate number of horseshoe crabs in the spawning aggregation. If a detailed description of the location was provided, but GPS points were not, GPS points were assigned by staff using Google Earth.

Of the 122 reports received, 108 reports were used in analysis and map development (Figure 10). Reports spanned from Turtle Island, South Carolina (32.0663ºN, -80.894ºW) to Murrells Inlet, South Carolina (33.533ºN, 79.029ºW), with most sightings reported south of Mt. Pleasant, South Carolina (32.832ºN, 79.828ºW). The approximate number of horseshoe crab sightings per report ranged from 1 to 1,000 horseshoe crabs ($\bar{x} \approx 33.55$), with the two largest sightings reported at Shutes Folly Island ($n \approx 300$) and Deveaux Bank ($n \approx 1{,}000$).

Project 4: Tag recapture analysis of USFWS tags in South Carolina

Project overview and outcomes

Since May 1999, the SCDNR has participated in the United States Fish and Wildlife Service (USFWS) Horseshoe Crab Tagging Program. Efforts have focused on tagging horseshoe crabs in South Carolina during the new and full moon spring high tides. For each individual horseshoe crab, prosoma width, sex and, for females, evidence of previous spawning (presence of mating scar) were recorded. A one-inch, circular USFWS identification tag was attached to each horseshoe crab by drilling a 2.8 mm hole through the posterior portion of the left prosoma point of the carapace and then pushing the plastic tag fastener into the hole. After tagging, all horseshoe crabs were released back into ocean. Beaches where horseshoe crabs were tagged were scouted by staff for resightings throughout the spawning season and year after year. At the end of each year's spawning season, annual data from SCDNR tag recapture efforts were submitted to USFWS for inclusion in their U.S. east coast-wide horseshoe crab tag recapture database.

All SC tag recapture data (May 1999 - October 2019) were collated from the USFWS coast-wide database, including all horseshoe crabs tagged in SC, all tagged horseshoe crabs recaptured in SC, and all horseshoe crabs tagged in SC and recaptured elsewhere (Table 2). Of the 10,743 horseshoe crabs tagged in SC, about 15% have been resighted in SC and 0.5% have been resighted in nine other states. Georgia had the most out-of-state resights (n = 27) and New Jersey had the second most resights (n = 8) of horseshoe crabs tagged in SC. Although most horseshoe crabs resighted in SC were tagged in SC (n = 1,606), 4% were tagged in 8 other states, ranging from Massachusetts to Florida (Table 3), with most of those tagged horseshoe crabs from Georgia (n = 54).

For the purposes of this project, SC tag recapture data from December 31, 2016 to October 4, 2019 were analyzed. During this period, a total of 686 horseshoe crabs were tagged in SC, and almost half (46%) of those tags were deployed the first year of the project. Each year, more than half of the horseshoe crabs that were tagged were male. Over the three years, the prosoma width of tagged horseshoe crabs ranged from 195-342 mm ($\bar{x}$ = 254 mm), with females being larger on average than males.

Between December 31, 2016 and October 4, 2019, there were 50 sightings of 30 individual tagged horseshoe crabs in SC waters from Hilton Head Island to Bulls Island, SC. Although most horseshoe crabs tagged during this period were male, only 40% of the resights were males. Horseshoe crabs were resighted from 1 to 756 days ($\bar{x}$ = 77.06 days) since tagging. During this period, only one live horseshoe crab was resighted more than a year after being tagged. This animal was tagged at Coffin Point Beach and resighted at that same beach 382 days later. In addition, two horseshoe crabs were resighted in Georgia, one 20 days and the other 125 days after being tagged in SC. The farthest interstate migration was approximately 124 miles, from Coffin Point Beach, SC to Cumberland Island, GA, whereas the farthest intrastate migration was approximately 63 miles, from Coffin Point Beach, SC to Bull Island, SC.

Table 2. Tag and resight information for horseshoe crabs (HSCs) tagged in South Carolina (SC) from May 1999 to October 2019.

	Females	Males	Undetermined	Total
HSCs tagged in SC	3742	6972	29	10743
HSCs tagged in SC resighted in SC	481	1117	8	1606
HSCs tagged in SC resighted elsewhere:				
MA	-	5	-	5
CT	2	2	-	4
NY	2	3	-	5
NJ	4	4	-	8
DE	3	-	-	3
MD	1	-	-	1
VA	-	1	-	1
GA	3	24	-	27
FL	3	2	-	5

Table 3. Resights in South Carolina of horseshoe crabs tagged from Massachusetts to Florida from May 1999 to October 2019.

Tagging Location	Females	Males	Undetermined	Total
MA	-	2	-	2
CT	-	1	-	1
NJ	-	3	-	3
DE	1	1	-	2
MD	-	1	-	1
VA	-	1	-	1
SC	481	1117	8	1606
GA	5	46	3	54
FL	-	2	-	2
All States	487	1174	11	1672

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Estimated Federal Cost: $102,664.13

Recommendations: Close the grant.