Inland Bays Volunteer Horseshoe Crab Spawning Survey Annual Report for 2017

Andrew McGowan and Dennis Bartow

Delaware Center for the Inland Bays
39375 Inlet Rd
Rehoboth Beach, DE 19971

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The Delaware Center for the Inland Bays is a non-profit organization and a National Estuary Program. It was created to promote the wise use and enhancement of the Inland Bays watershed by conducting public outreach and education, developing and implementing restoration projects, encouraging scientific inquiry and sponsoring needed research, and establishing a long-term process for the protection and preservation of the Inland Bays watershed.

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EXECUTIVE SUMMARY

The tenth year of the Inland Bays volunteer horseshoe crab survey, managed by the Delaware Center for the Inland Bays, was completed in 2017. It was the third consecutive year in which horseshoe crabs were monitored in the Inland Bays over the full course of the spawning season using the same protocol as the Delaware Bay survey, a change made in 2015 in order to make data comparable with that of other regional programs. Six beaches in the three Inland Bays were surveyed by teams of volunteers two nights prior to, the night of, and two nights following the full and new moons between May 8th and June 25nd, 2017, for a total of 53 individual spawning surveys.

Activity on a given night ranged between zero and 2,336 horseshoe crabs at a single beach. The Coastal Kayak beach in Little Assawoman Bay had the lowest average number of crabs per square meter of beach, while Tower Road beach in Rehoboth Bay had the highest. These results are consistent with previous years, which generally show that Coastal Kayak has few crabs, while Tower Road or James Farm have the highest average crab counts. A total of 20,201 crabs were counted in 2017 at all beaches, yielding a spawning index of 0.53 females/m^2, and a sex ratio of 6.1 males for every female. These results approximate previous years, with a similar spawning index to 2016, and a higher sex ratio than 2016 but lower than 2015. Coastal Kayak had the lowest average sex ratio for 2017, while Ellis Point had the highest. Temporally, the peak spawning activity occurred during the third lunar period in 2017 (June 7th-11th), which was similar to 2016 and one lunar period later than 2015.

INTRODUCTION

Previous increases in the harvest of Atlantic horseshoe crabs (*Limulus polyphemus*) for bait and medical uses, along with loss of spawning habitats, raised questions about this species status throughout the Mid-Atlantic (Botton and Ropes 1987; Berkson and Shuster 1999; Widener and Barlow 1999; Lathrop et al. 2006). In response, a fisheries management plan and subsequent addendums were established to control bait harvest in recent years (ASMFC 1998; ASMFC 2012). However, due to the importance of horseshoe crabs to the medical field, as well as the numerous migrating bird species that rely heavily on the eggs of horseshoe crabs (Myers 1986; Tsipoura and Burger 1999; Smith et al. 2002a), changes in horseshoe crab abundance could have far ranging implications for humans and numerous other species. Therefore, it is important to monitor horseshoe crab populations in order to assess both the annual variability and any long-term changes in spawning populations.

To address these questions locally, the Center for the Inland Bays (CIB) established a long-term citizen science monitoring program to track horseshoe crab populations within the Delaware Inland Bays (Rehoboth Bay, Indian River and Bay, and Little Assawoman Bay). This effort began in 2007; in 2015, the survey protocol was changed in order to match that used in the Delaware Bay survey program. 2017 was the third year in which horseshoe crabs were monitored throughout the spawning period following the updated protocol. Previously, all beaches were monitored using an 8-meter pull rope to survey random points along the beach. Beginning in 2015, the survey was changed to randomly sample 100 1-m^2 quadrats along each beach. This change standardized the number of observations between beaches, making it easier to directly compare crab numbers among different beaches. The protocol change also facilitates comparisons between the Inland Bays and Delaware Bay and allows for the

potential inclusion of Inland Bays data into the Atlantic States Marine Fisheries Commission's horseshoe crab stock assessments.

The goals of this ongoing study are to assess current population levels and sex ratios within the Inland Bays, and to track changes in these over time. To accomplish these goals, horseshoe crabs were systematically counted at six beach sites during the spring and early summer of 2017, which corresponds to the spawning period of the crabs.

Previous annual survey reports can be found online at https://www.inlandbays.org/projects-and-issues/all/horseshoe-crab-survey/.

METHODS and MATERIALS

Survey Sites

Six different sandy beaches distributed throughout the Delaware Inland Bays (Figure 1) were surveyed between May 8th and June 25th, 2017, on dates that coincide with the 12 primary spawning surveys conducted in the Delaware Bay by Delaware's Department of Natural Resources and Environmental Control (DNREC). These surveys usually fall in May and June. In previous years five beaches were surveyed, but in 2017, Ellis Point was added as a site covering a large stretch of sandy shoreline in Indian River Bay. This site had previously been surveyed using the older protocol but was dropped due to access issues. Those issues have been resolved and the site has been brought back into the survey program.

Because horseshoe crabs appear to prefer beaches dominated by coarse sandy sediments and avoid beaches that have a high amount of peaty sediments or are adjacent to exposed peat banks (Botton et al. 1988; Smith et al. 2002a), all of the beaches selected for this study were sandy beaches. These beaches were also selected because they were easily and safely accessible for volunteers.

Figure 1. The six Inland Bays survey sites for 2017.

Survey Protocol

The spawning surveys were conducted two days prior to, the night of, and two nights following the new or full moon. Surveys were conducted at the highest of the lunar high tides during these periods, occurring at night (when the moon exerts the greatest pull on the tidal levels).

Each beach is surveyed by a team of volunteers who have been trained in the survey protocol and how to determine the sex of horseshoe crabs. Teams begin surveys at the point when the nightly high tide begins to recede. A coin flip is used to randomly select one end of the beach from which to begin the survey. Starting at that end, the team extends a pull rope (marked at one-meter intervals) at the high tide line towards the opposite end of the beach. The length of the pull rope is dependent upon the length of the beach and is designed to allow systematic placement of 100 $1m^2$

quadrats along the beach. The length of the rope is determined by dividing the overall length of the beach by 50. The James Farm, Coastal Kayak, and Tower Road sites each use a 4-meter pull rope. The Bay Colony and Ellis Point sites use a 6-meter rope. The Peninsula site does not use a pull rope, because the length of the beach is only 100 meters, and therefore all quadrats along the beach are counted.

In addition to randomizing the direction of travel, the placement of the quadrats within each rope pull is randomized for a single night. Two quadrats are sampled per rope pull, for a total of 100 quadrats. The same two randomized locations along the pull rope are used for the duration of the night. Once the pull rope has been extended, the $1m^2$ quadrat is placed at the first random quadrat location for that given night. The quadrat is positioned so that one side is even with the line of crabs, and the opposite side extends toward the bay. All crabs with at least half of their body inside the quadrat are sexed and counted. Upon completion of the first quadrat, the team moves the quadrat to the second randomly selected location and repeats the counting process. Once the two quadrats have been counted for the first rope pull, the rope is extended along the next section of beach, and the same two random quadrat locations are sampled. This is repeated until 100 quadrats have been sampled. The 'horseshoe crab line' that is followed is not a straight line, and it may be above or below the water line; however, it is never more than 1 meter away from the high tide line.

At all sites, salinity samples were collected each night in sealed 50-mL tubes. These samples were measured later using a Fisherbrand Traceable Salinity Probe Model #S98200. Air and water temperature measurements were made during the survey with a thermometer.

Weather conditions occasionally necessitate cancellation of a survey due to concern for safety of the volunteers. A total of 19 surveys were cancelled in 2017 (Figure 2) because of storms or issues with scheduling enough volunteers. A total of ten surveys were missed during the second and third moon cycle (usually corresponding to the largest crab counts), compared to three missed counts in 2016 during this same time frame. A quality control report for the 2017 season is presented in Appendix A.

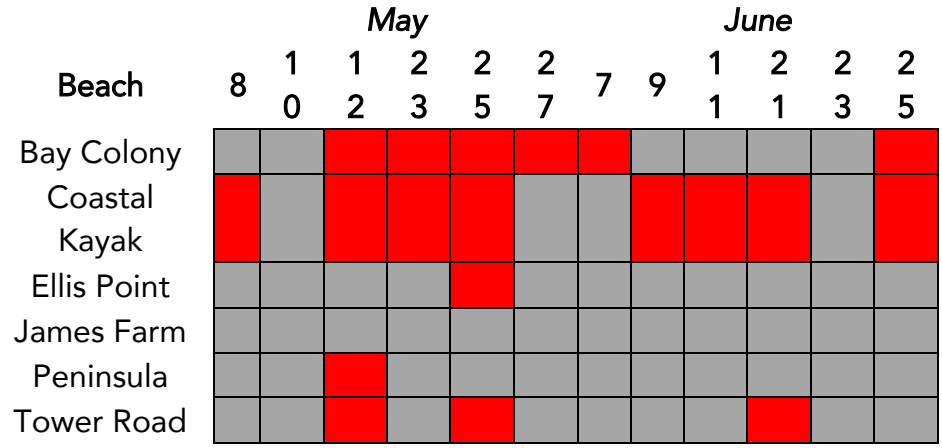

Figure 2. Survey cancellations are indicated in red, while completed surveys are highlighted grey.

Data Analysis

Average spawning densities per $1m^2$ were calculated for each beach by dividing the total number of crabs per night by 100 (the number of quadrats), and averaging each night to obtain one spawning density per beach. A female spawning index was calculated for each beach by dividing the number of females each night by 100 (number of quadrats), then averaging the nightly values together. The index of female spawning activity is a standardized measure of the relative density of spawning females on a beach for a season, and can be compared with female spawning indices from other regions. Cumulative spawning densities and indices for a given year are calculated by averaging each nightly density or index for each beach together to get one density or index for a given year. The average nightly crab count for each year for each beach was calculated by averaging all each nightly total of given beach for a given year.

Sex ratios for each beach are calculated by summing the total number of males counted and dividing by the total number of females counted. To derive an Inland Bays sex ratio, the total number of males counted from all beaches for a given year is divided by the total number of females counted for a given year.

Correlations between total crab abundance and water temperature and salinity were examined using Kendall's tau correlation test ($\alpha = 0.05$).

Determination of the temporal peak of spawning activity is determined by summing the number of crabs counted on a given night across all surveyed beaches and dividing by the number of surveys occurring on that night to get an average number of crabs counted per beach per night. The peak is attributed to a lunar period (defined as the five days around a full or new moon during which the survey occurs; the sample day of the full or new moon, the 2 days before and the 2 days after).

Tagging Study

In addition to the spawning survey, 1,250 Horseshoe crabs were tagged using U.S. Fish and Wildlife Service tags as part of the U.S.F.W.S. Cooperative Horseshoe Tagging Program (https://www.fws.gov/northeast/marylandfisheries/native-species/horseshoe-crab.html). Crabs from all six survey beaches were tagged on multiple nights coinciding with the spawning survey schedule using a 5/32" drill bit. Table 1 lists the number of crabs tagged per beach during 2017. Resighted crabs can be reported by surveyors or by members of the public, and reports are sent directly to the US Fish and Wildlife Service, which sends the reported resights to the Delaware Center for the Inland Bays upon request.

The results of the Inland Bays tagging efforts from previous years are presented in a paper titled "Horseshoe crab (*Limulus polyphemus*) movements following tagging in the Delaware Inland Bays, U.S.A." (McGowan 2018).

Table 1. The number of crabs tagged per beach in 2017.

Beach	# Tagged
James Farm	675
Tower Road	250
Bay Colony	125
Peninsula	100
Ellis Point	100
Coastal Kayak	0

RESULTS

In 2017 a total of 20,201 horseshoe crabs were counted over the season on all six beaches. The average crabs per night for each beach was in general lower in 2017 than it was in 2016, with the exception of Tower Road (Figure 3). Of the five beaches surveyed, Tower Road had the highest spawning density (6.3 per m^2) and female spawning index (1.06 m^2) while Coastal Kayak had the lowest spawning density (0.06) and female spawning index (0.02 m^2, Table 2). The cumulative female spawning density was 0.53 females/m^2, slightly greater than 2016 (0.51 females/m^2) and 2015 (0.39 females/m^2).

Table 2. Spawning density and index results for 2017.

Location	Spawning Density	Spawning Index
Bay Colony	1.69	0.22
Coastal Kayak	0.06	0.02
Ellis Point	6.3	0.72
James Farm	5.2	0.75
Peninsula	0.27	0.06
Tower Road	6.33	1.06

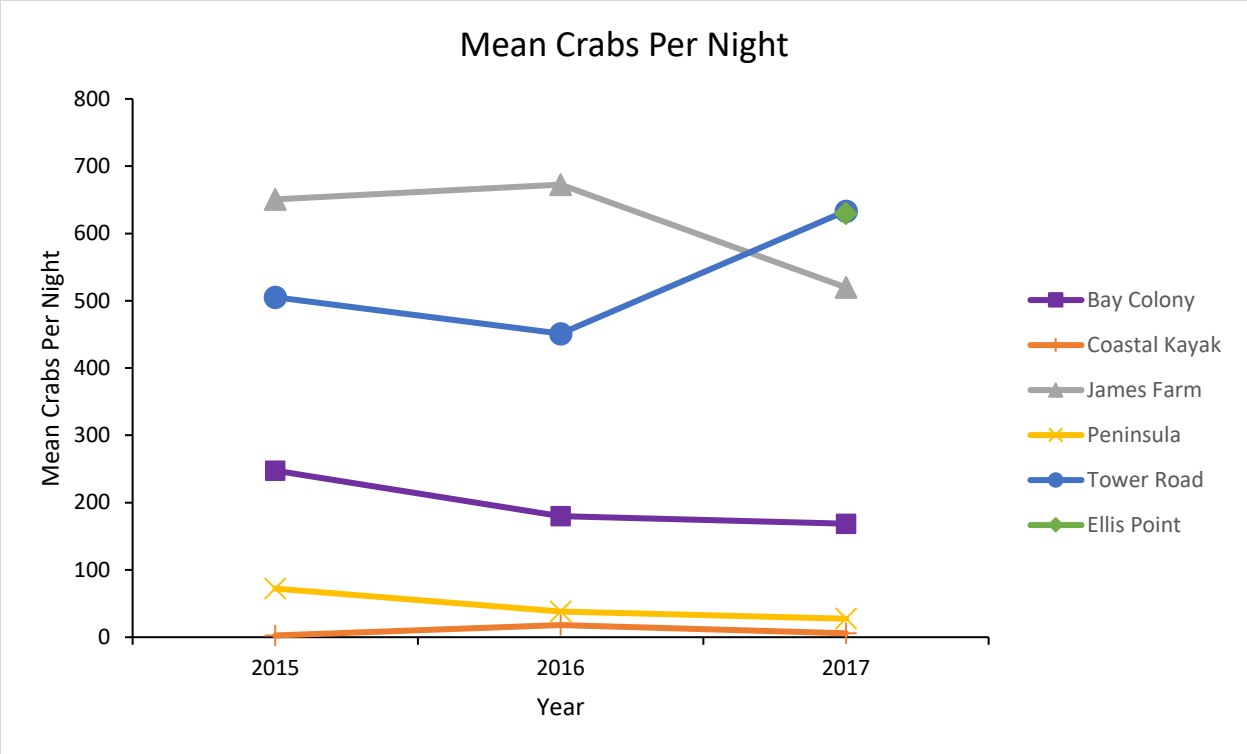

Figure 3. Average crabs per night for each beach over the three years since the protocol change in 2015. Ellis Point data began in 2017.

The cumulative sex ratio for 2017 was 6.1 males for every female, greater than the sex ratio 4.8 seen during 2016, but less than the 6.9 seen during 2015 (Table 3; McGowan and Bartow 2017; McGowan and Bartow 2018). Ellis Point had the highest average male:female sex ratio (7.8), while Coastal Kayak had the lowest ratio (2.8, Table 3). While Ellis Point is a new beach, Coastal Kayak has consistently had the lowest sex ratio.

Table 3. Sex ratio per beach per year.

Year	Bay Colony	Coastal Kayak	Ellis Point	James Farm	Peninsula	Tower Road	Cumulative
2015	10.4	3.6	N/A	6.7	5.7	6.8	7.0
2016	5.4	5.0	N/A	4.6	4.0	5.1	4.8
2017	6.8	2.8	7.8	5.9	3.9	5.0	6.1

In general, as the total number of crabs increased, the male:female ratio also increased (Figure 4) though this relationship did not appear to be as strong as in previous years due in part to some low sex ratios on nights with high crab activity.

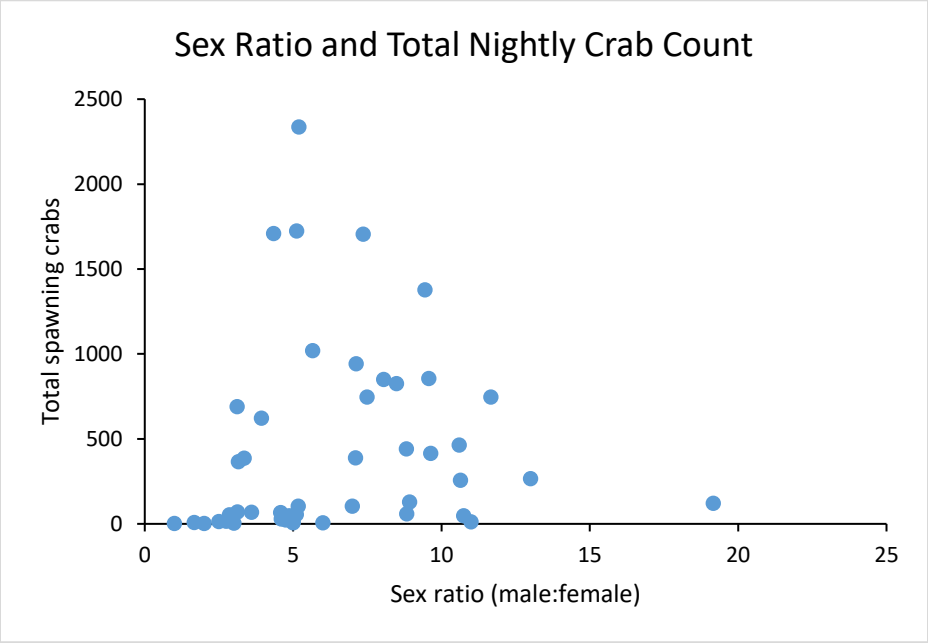

Figure 4. Sex ratio compared to total crabs for a given night per site during 2017.

Horseshoe crab activity was positively correlated with water temperature ($p < 0.001$, tau = 0.36) and marginally significantly with salinity ($p = 0.07$, tau = 0.17). Water temperature and salinity during each sampling event are presented in Tables 4 and 5.

Table 4. Water temperature measurements (°C) from each sampling event. Blanks indicate no samples were collected that night due to no survey taking place.

Date	Bay Colony	Coastal Kayak	Ellis Point	James Farm	Peninsula	Tower Road
5/8/2017	13.5		16	16	15	15
5/10/2017	6	18	17.9	18	15	17
5/12/2017			14.5	13		
5/23/2017			18.9	17	17	18
5/25/2017				19	18	
5/27/2017		19	18.5	18	19.5	20
6/7/2017		20	18	19	16	20
6/9/2017	18		19.4	21	19	22
6/11/2017	23		23.2	26	25	24

6/21/2017	24.5		26	25	25.5	
6/23/2017	25	26	21.7	25	25	25
6/25/2017			25.4	24	25	25

Table 5. Salinity measurements (ppt) from each sampling event. Blanks indicate no sampling event occurred or a failure by the survey team to collect the sample. Failure to collect sample indicated with a *.

Date	Bay Colony	Coastal Kayak	Ellis Point	James Farm	Peninsula	Tower Road
5/8/2017	25.9		25	28.9	27.9	*
5/10/2017	27.6	26.4	27.8	30.8	26.8	28.5
5/12/2017			29.1	29.8		
5/23/2017			27.6	28.2	27.8	27.7
5/25/2017				28	27	
5/27/2017		24.2	27.8	28.6	25.6	27.4
6/7/2017		*	29.1	29.5	27.3	28.2
6/9/2017	25.6		25.4	28.2	28.3	28.6
6/11/2017	28.4		27.6	27.6	25.6	29.2
6/21/2017	*		25.9	30	25.4	
6/23/2017	*	26.7	27.2	29.3	26.1	28.2
6/25/2017			28.3	30.1	28.5	28.5

Temporally, spawning activity peaked during the third lunar period of 2017 (June 7th-11th, Figure 5).

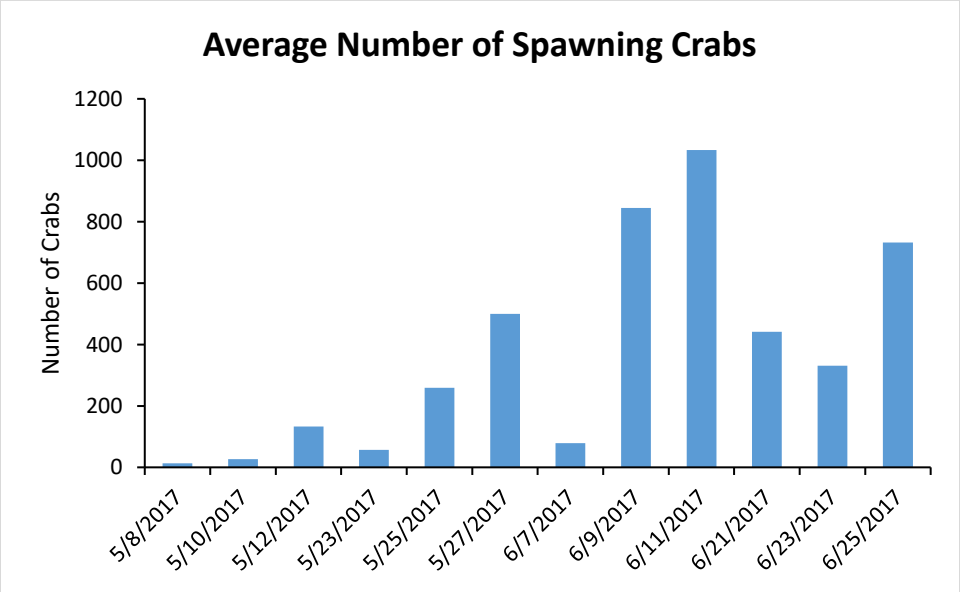

Figure 5. The temporal distribution of spawning activity during 2017.

DISCUSSION

The cumulative spawning index for the Inland Bays was lower than that of the Delaware Bay survey (0.53 vs 0.71; Zimmerman et al. 2017), but this is likely influenced by the inclusion of two poor horseshoe crab beaches (Coastal Kayak and Peninsula). Tower Road, which had the highest spawning index of our six beaches (1.06) was better than 75% of the actively surveyed Delaware Bay beaches (Zimmerman et al. 2017). This suggests that although total area of spawning habitat in the Inland Bays is smaller than in Delaware Bay, some of the habitat that does exist is high quality and important to the regional population. This stresses the need to protect and restore sandy shorelines within the Inland Bays.

Average nightly counts for each beach except Tower Road were lower in 2017 than 2016. While this may seem like a decrease in spawning numbers, poor weather caused ten surveys to be cancelled during the second and third lunar periods of 2017, when crab numbers are typically highest, while only three surveys were cancelled in 2016 during this time period. This may explain some of the decreases, particularly for Bay Colony and Coastal Kayak, but does not explain the decrease at James Farm or Peninsula which had fewer or the same cancellations from 2016 to 2017 over this critical time period. The variability associated with cancellations between years emphasizes the need for a long term data record (>10 years) before trends in spawning activity are appropriate. Over the long term, the influence of the number of cancellations per beach should diminish as some years are likely to have similar cancellation numbers, allowing a more accurate determination of true spawning trends. Similarly, comparisons between beaches within a given year should account for the number of surveys performed at each beach for that year, with particular attention paid to the second and third lunar periods.

As in previous years, the James Farm and Tower Road beaches had highest levels of activity, while the Peninsula and Coastal Kayak sites had low levels of activity. This

pattern may be due to the physical and geographical characteristics of the beaches. Coastal Kayak is a considerable distance from both the Indian River Inlet (15 km in linear distance) or Ocean City Inlet (16.5 km), and crabs reaching Coastal Kayak must travel through one of two shallow mucky canals. This site is also subject to fairly high wave energy and dramatic fluctuations in salinity following rain events. The Peninsula beach has firm sandy substrate along the shoreline, but approximately 10 m from the shoreline the substrate changes to a mucky soft bottom (mud/peat). This type of sediment is known to be utilized significantly less by horseshoe crabs than sandy sediments (Botton et al. 1988). Crabs using the Peninsula beach to spawn must travel over the less preferred substrate as well as navigate their way between two curved rock jetties. In contrast, James Farm beach has a gradually sloped, well protected shoreline with much lower wave energy, which is preferred by spawning crabs (Smith et al. 2002b; Lathrop et al., 2006). Tower Road also has similar conditions under calmer weather.

The Bay Colony site, which typically has high numbers and densities of spawning crabs, had numerous surveys cancelled in 2017 due to unsafe conditions from unusually stormy weather and high water levels. The addition of Ellis Point to the survey provided another location in Indian River, and the numbers of spawning crabs at this location was on par with James Farm and Tower Road. Ellis Point surveys will continue in the future.

Coastal Kayak has continually had low spawning activity such that the overall spawning density and index in the Inland Bays is artificially lower than what it actually present in Indian River and Rehoboth Bays. Going forward, alternative locations within Little Assawoman Bay will be explored, and if spawning numbers are still low at other locations, then efforts may be refocused to an additional site in Rehoboth Bay (which currently only has one survey site), with surveys occurring in Little Assawoman once every two years to ensure numbers of still low in this bay.

The male to female sex ratio of 6.1 was higher than that of 4.8 reported last year for this survey (McGowan and Bartow 2018). The spawning index was very similar to last year, further reflecting an increase in the proportion of males (McGowan and Bartow 2018). The sex ratio recorded by this survey was higher than the sex ratio of 5.2 reported by the Delaware Bay survey (Zimmerman et al. 2017), though that survey also

saw an increase in the bay-wide sex ratio from 2016 to 2017 (Zimmerman et al. 2017). The Inland Bays survey has had a greater sex ratio than the Delaware Bay survey in each of the last three years since the survey protocol was updated to match the protocol of the Delaware Bay survey. The reason for this is not fully understood and may reflect either an interregional difference in horseshoe crab populations, or a bias in observers. In either case, the availability of males does not appear to be limited within the Inland Bays as sex ratios are consistently greater than four males for every female. Future survey years will help uncover if the increased sex ratio in the Inland Bays is a consistent pattern over the long term. While more males for each female would in theory increase the proportion of fertilized eggs, too few females could reduce the overall number of eggs being deposited onto the beaches, likely reducing the population over the long term. Crabs within the Inland Bays belong to the Delaware Bay regional population (Swan 2005; McGowan 2018), and long-term trends from the Delaware Bay survey have not detected a significant change in female spawning activity or male spawning activity (Zimmerman et al. 2017). This suggests that the regional population is stable and that female activity is both stable and sufficient to support the species.

Temporally, the peak spawning period was the third lunar period of the survey (June 7th-11th). This was similar to the period seen in 2016, and one period later then in 2015, but was the same to what was seen in Delaware Bay in 2017 (Zimmerman et al. 2017). Also similar to the Delaware Bay survey, spawning activity was associated with water temperatures. As the water temperature increased, activity increased.

2017 marked the third year that the protocol used in the Inland Bays survey matched the Delaware Bay survey protocol. This switch was made to facilitate comparisons between the surveys. Because of this change in protocol, results cannot be compared directly to those from surveys conducted prior to 2015. Additional years with matching protocols are needed before trend analyses become appropriate. Despite this, we believe the change in protocols will provide more meaningful data on horseshoe crab populations and spawning activity in the Bays over the long term.

Perhaps the most important finding thus far is that while much less spawning habitat is available in the Inland Bays compared to the Delaware Bay, the spawning densities present during the 2015, 2016, and 2017 surveys suggest that some of the available

spawning habitat within the Inland Bays is of similar quality to Delaware Bay habitat. This reinforces the importance of protecting natural sandy shorelines in the Inland Bays and provides support for the potential inclusion of Inland Bays spawning surveys into future horseshoe crab stock assessments.

ACKNOWLEDGEMENTS

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Appendix A. Quality Control Report

SUMMARY

Data recorded during the 2017 horseshoe crab survey was tested according to the quality control measures outlined in the program's EPA approved QAPP effective February of 2016. These measures included:

- a random sampling for accuracy of 10% of the data entered from field sheets to electronic formats
- at least one horseshoe crab survey was performed under direct supervision from the Program manager or trained staff member per beach
- two random recounts of two quadrants once per survey for each beach for each night performed by the trained team leader for that beach.

In summary, no inaccuracies were found to be present in the random sampling of data sheets. All teams demonstrated proper protocol and data recording during their supervisory survey.

Coastal Kayak was determined to be no longer safe for volunteers due to objects in the water which make falls increasingly likely. Given safety concerns, Coastal Kayak will be dropped from the 2018 survey. Fenwick Island, a stretch of sandy shoreline north of Coastal Kayak will be added to the 2018 survey.

ISSUES AND CORRECTIVE ACTIONS

No corrective actions were needed.

Coastal Kayak will be dropped from the 2018 survey, and Fenwick Island State Park will take its place during 2018.

RECOMMENDATIONS

Fenwick Island State Park may serve as a suitable replacement for Coastal Kayak. The site is long enough, within Little Assawoman Bay, has a sandy shoreline without major obstructions, is located on publicly accessible land, and is convenient to travelers from Bethany, South Bethany, and Fenwick.