

Inland Bays Volunteer Horseshoe Crab Spawning Survey Annual Report for 2018



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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

2018 marked the eleventh year of the Inland Bays Volunteer Horseshoe Crab survey. It was the fourth 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. Six beaches were sampled two nights prior to, the night of, and two nights following the full and new moons between April 27th and June 30th, for a total of 70 individual spawning surveys.

Activity for a given night ranged between zero crabs, and 1,432 Horseshoe Crabs at a single beach. Fenwick Island State Park beach in Little Assawoman Bay had the lowest average number of crabs per square meter of beach, while Tower Rd beach in Rehoboth Bay had the highest. A total of 16,491 crabs were counted in 2018 resulting in a sex ratio of 5.7 males for every female, and a cumulative spawning density of 2.35 crabs per square meter. Fenwick Island State Park had the lowest average sex ratio for 2018, while James Farm had the highest.

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. 2018 was the fourth 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-m² 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 2018, 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

Six different sandy beaches distributed throughout the Delaware Inland Bays (Figure 1) were surveyed between April 27th and June 30th, 2018, on dates that coincide with the 15 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 Coastal Kayak Beach (Little Assawoman Bay) was surveyed, but due to safety concerns and sparse crab numbers, the site was dropped and Fenwick Island State Park Bayside Beach (Little Assawoman Bay) was picked up.

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 accessible for volunteers.



Figure 1. The six Inland Bays survey sites for 2018.

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 1m² quadrats along the beach. The length of the rope is determined by dividing the overall length of the beach by 50. The James Farm, Ellis Point, and Tower Road sites each use a 4-meter pull rope. The Bay Colony site uses a 6-meter rope. The Peninsula and Fenwick State Park sites do not use pull ropes, because the lengths of the beaches are only 100 meters, and therefore all quadrats along the beaches 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 1m² 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 20 surveys were cancelled in 2018 (Figure 2) because of storms or issues with scheduling enough volunteers. A total of 11 surveys were missed during the third and fourth moon cycle (in other years the second and third lunar period), which typically corresponds to the nights with the largest crab counts. In comparison, ten surveys were missed during this same period in 2017, and

three were missed in 2016. A quality control report for the 2018 season is presented in Appendix A.

Beach	April			May						June				
	2 7	2 9	1 3	1 5	1 7	2 7	2 9	3 1	1 1	1 3	1 5	2 6	2 8	3 0
Bay Colony														
Ellis Point														
Fenwick State Park														
James Farm														
Peninsula														
Tower Rd														

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

Data Analysis

Average spawning densities per 1m² 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) which had at least two beaches surveyed.

Tagging Study

In addition to the spawning survey, 2,498 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 five of the 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 2018. 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 2018.

Beach	# Tagged
James Farm	1,185
Tower Road	400

Ellis Point	400
Bay Colony	300
Peninsula	213
Fenwick State Park	0

RESULTS

In 2018 a total of 16,491 Horseshoe Crabs were counted on all six beaches. The average crabs per night for each beach was in general lower in 2018 than it was in 2017, with the exception of Peninsula (Figure 3). The cumulative spawning density was 2.35, less than the 3.81 seen in 2017 (McGowan and Bartow 2020), while the female spawning index was 0.35, less than the .53 seen in 2017 (McGowan and Bartow 2020). Tower Road had the highest spawning density and female spawning index, while Fenwick State Park had the lowest spawning density and female spawning index (Table 2).

Table 2. Spawning density and index results for 2018.

Location	Spawning Density	Spawning Index
Bay Colony	1.14	0.20
Ellis Point	2.84	0.39
Fenwick Island	0.04	0.01
James Farm	3.71	0.47
Peninsula	0.79	0.13
Tower Road	4.58	0.77

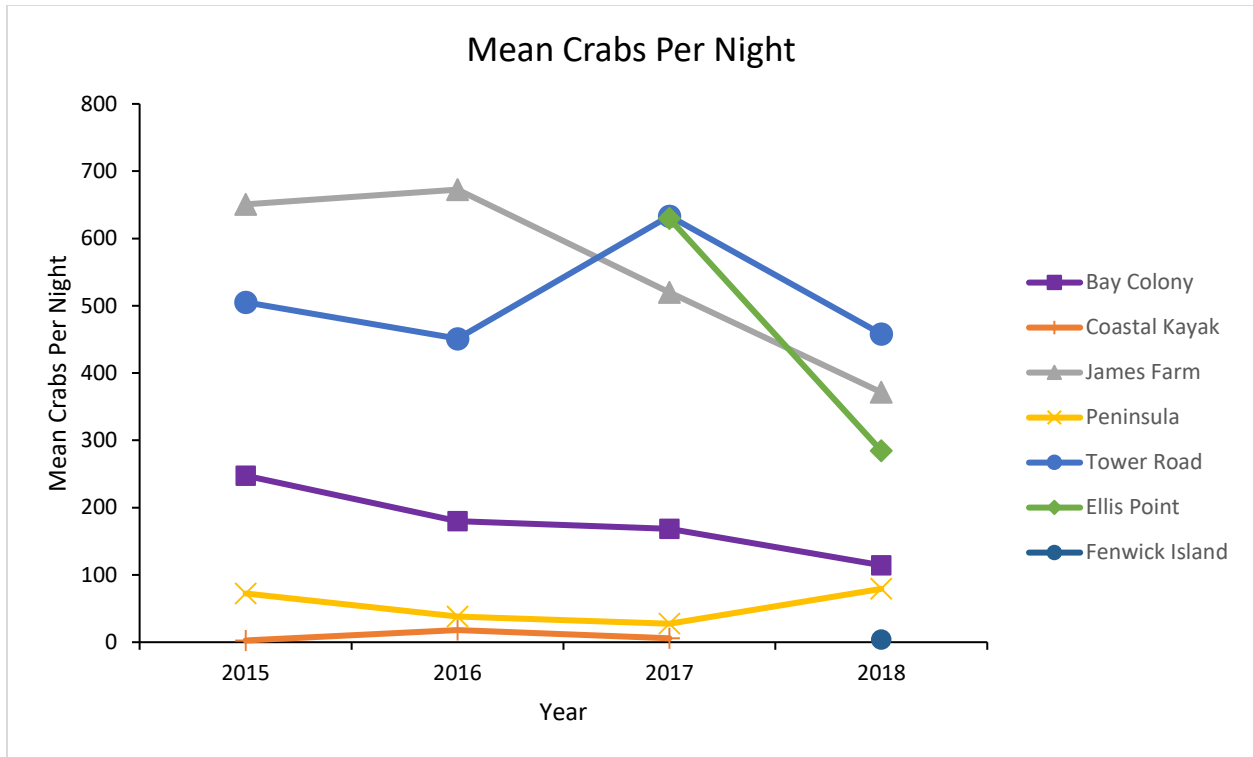


Figure 3. Average crabs per night for each beach over the three years since the protocol change in 2015. Fenwick Island data began in 2018.

The cumulative sex ratio in 2018 was 5.7 males for every female (Table 3). This was slightly lower than 2017, but almost identical to the sex ratio seen in Delaware Bay during the same spawning period (5.6; McGowan and Bartow 2020; Zimmerman et al. 2019). James Farm had the highest average male:female sex ratio (6.8), while Fenwick Island State Park had the lowest ratio (1.9, Table 3). In general, as total crab abundance increased, sex ratio also increased (Figure 4).

Table 3. Sex ratio per beach per year.

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

2018	4.7	6.2	1.9	6.8	5.0	4.9	5.7
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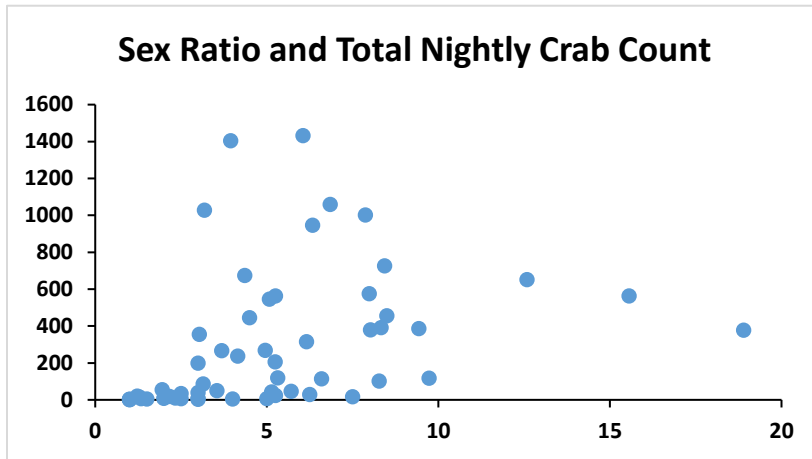


Figure 4. Sex ratio compared to total crabs for a given night per site during 2017.

Horseshoe Crab activity was positively correlated with water temperature ($p < 0.001$, $\tau = 0.34$), but was not correlated with salinity ($p = 0.42$, $\tau = 0.06$). Water temperature and salinity during each sampling event are presented in Tables 4-5.

Table 4. Water temperature measurements ($^{\circ}\text{C}$) from each sampling event. Blanks indicate no sampling event occurred.

Date	Bay Colony	Ellis Point	Fenwick Island	James Farm	Peninsula	Tower Road
4/27/2018	18	17	19	18	16	15
4/29/2018	11	17	14	11	14	13
5/1/2018	15	16	17.5	16	15	16
5/13/2018	17	17	18	18	18	19
5/15/2018				22		
5/17/2018		18		18	18	16
5/27/2018						
5/29/2018	22	22	22	22	24	22
5/31/2018		22		22	23	23
6/11/2018		18	20.5	18	18	
6/13/2018	24	23	23	24	24	25
6/15/2018	22	22		23	21	21
6/26/2018	25	25	20	27	25	25
6/28/2018	25	27	27.5	26	26	26

6/30/2018		26		27	28	28
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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	Ellis Point	Fenwick Island	James Farm	Peninsula	Tower Road
4/27/2018	27.5	28.8	21.6	28.9	25.8	29.2
4/29/2018	27.2	25.5	20.4	29.8	*	29.9
5/1/2018	26.6	26.8	18.9	28.5	28.2	28.5
5/13/2018	27.3	29.2	21.6	29.2	21.8	30.1
5/15/2018				27.5		
5/17/2018		25.6		26.9	26.6	29.2
5/27/2018						
5/29/2018	25.2	24.5	11.4	26.6	26.4	29
5/31/2018		24.5		27.6	19.4	28.9
6/11/2018		20.9	9.2	26.8	19.1	
6/13/2018	20.4	20.5	7.1	24.8	19.7	28.9
6/15/2018	21.6	20.8		*	26.9	24.3
6/26/2018	27	28	12.4	28.5	24.8	28.5
6/28/2018	25.1	24	12.8	27.9	26.4	*
6/30/2018		26.5		28.8	28.4	29.2

Temporally, spawning peaked during the fourth lunar period (June 11th -15th; Figure 5).

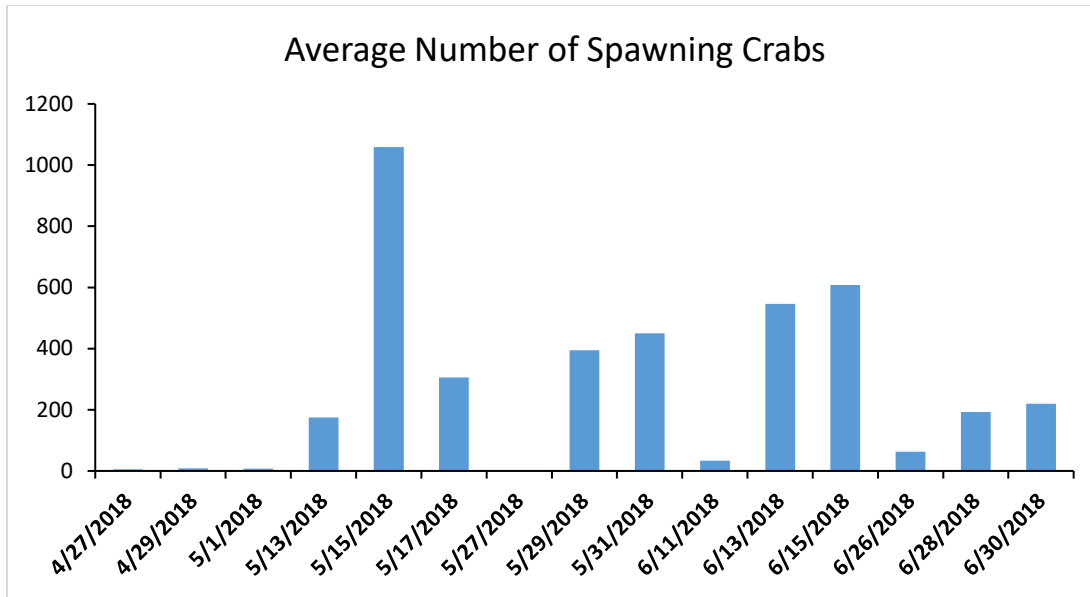


Figure 5. The temporal distribution of spawning activity during 2017. Note that on May 15th only one beach was surveyed, the temporal peak of the survey was June 15th.

DISCUSSION

2018 was the fourth year of the Inland Bays Horseshoe Crab survey in which the protocol matched that of the Delaware Bay survey. Overall, crab counts were lower in 2018. The female spawning index was lower compared to 2017 (0.35 in 2018 compared to .53 in 2017), and was the lowest recorded thus far by the survey since the protocol switch in 2015. One possible explanation for the reduced crab numbers in 2018 was the unusually cold winter and spring. During early January 2018, vast areas of the Inland Bays waters were frozen, and on January 9th 2018, hundreds of dead horseshoe crabs washed up on the ocean beach just north of the inlet. It is unclear how many crabs in total were killed during this cold water period, but a winter kill is unusual (pers. obs.) and may have affected the spring spawning numbers. In addition to the crab kill, the survey began in late April, and encompassed an additional lunar cycle, totaling 15 survey days as opposed to the typical 12. These three survey days in late April corresponded with low water temperatures (Table 4) which typically do not see large amounts of crab activity, and therefore may have lowered the female spawning index.

The average nightly crab count per beach was also, in general, lower in 2018 than in 2017. Cancellations due to weather during the critical lunar periods (ordinarily the second and third, but this year the third and fourth due to the earlier start date) were similar between the years (11 in 2018, ten in 2017), and therefore were likely not the reason for the reduced crab averages. The winter kill may have influenced the lower nightly crab counts. It is important to note that regionally, there has been no significant trends in Delaware Bay male spawning or female spawning activity (Zimmerman et al. 2019). Many more years of data are needed for the Inland Bays before accurate and meaningful trends in spawning activity can be calculated, and until then, average counts across years should be used anecdotally and not interpreted beyond that.

As in previous years, the James Farm and Tower Road beaches had high levels of spawning, on par with high quality beaches found in the Delaware Bay. James Farm beach has a gradually sloped, well protected shoreline with low wave energy, which is preferred by spawning crabs (Smith et al. 2002b; Lathrop et al., 2006), and Tower Road also has similar conditions under calmer weather. Coastal Kayak, which typically had the lowest crab activity in the Inland Bays survey was dropped, and Fenwick Island State Park Bayside was included. This site is located just north of Coastal Kayak in the Little Assawoman Bay. Echoing what was seen at Coastal Kayak, Fenwick Island State Park Bayside had the lowest crab activity, and further illustrates that Little Assawoman Bay is not heavily utilized by spawning horseshoe crabs. This may be due to the geographical characteristics of the bay. The sandy shorelines of Little Assawoman Bay are a considerable distance from both the Indian River Inlet (15 km in linear distance) or Ocean City Inlet (16.5 km), and crabs reaching this bay must travel through one of two shallow mucky canals. The distance from the sandy shorelines of Little Assawoman to either inlet may limit the number of crabs that make it to the bay to spawn.

The male to female sex ratio of 5.7 was less than that of 6.1 reported last year for this survey (McGowan and Bartow 2020), but very similar to the sex ratio reported by the Delaware bay survey (5.6; Zimmerman et al. 2019). This similarity to the Delaware bay survey is unusual in that the previous three years the Inland Bays has had a greater sex ratio. The previous three years may have been a coincidence, but more years of data are needed in order to determine if these varying sex ratios between the Delaware Bay and the Inland Bays represent an interregional difference in horseshoe crab

populations, or if the pattern seen over the first three years (2015-2017) happened by chance. In either case, the purpose of recording sex ratios is to ensure that there are sufficient males to fertilize female eggs, and the sex ratios seen by this survey since 2015 have exceeded a minimum target of 2 males for every female (ASMFC 2019).

James Farm had the highest sex ratio while Fenwick Island State Park Bayside had the lowest sex ratio. Typically, Tower Road and James Farm have the greatest spawning densities and correspondingly the highest sex ratios, while the Little Assawoman sites (Coastal Kayak in previous years and now Fenwick State Park) have the lowest spawning densities and correspondingly lowest sex ratios.

Temporally, spawning activity peaked in the fourth lunar period (June 11th – 15th). This peak in spawning time was one lunar period later than what was seen in the Delaware Bay survey (Zimmerman et al. 2019). The actual date of peak spawning time was similar to the peak of 2017 (McGowan and Bartow 2020) but due to the earlier survey start date (April), this corresponded to the fourth lunar period in 2018 rather than the third lunar period.

2018 marked the fourth 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 be beneficial to horseshoe crab monitoring in the Bays over the long term. Perhaps the biggest 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, 2017, and 2018 surveys suggest that some of the available spawning habitat within the Inland Bays is of similar quality to Delaware Bay habitat, which 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.

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Appendix A. Quality Control Report

SUMMARY

Data recorded during the 2018 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 observed teams demonstrated proper protocol and data recording during their supervisory survey. Teams did fail to collect salinity samples for several surveys.

ISSUES AND CORRECTIVE ACTIONS

The program manager failed to supervise Peninsula at least once during the 2018 season due to scheduling conflicts and weather cancellations. In 2019, the manager will attend a survey at this location.

Team leaders will be reminded of the importance of collecting salinity samples and the need to check to ensure they have completely filled out their data sheets in order to prevent missing water samples.

RECOMMENDATIONS

Drop the Little Assawoman Bay from the survey, and include a new site in Rehoboth Bay for the 2019 survey. The LAB site will be monitored every five years in order to continue to include it in trend reports. The Quality Assurance Project Plan has been updated to reflect this change.