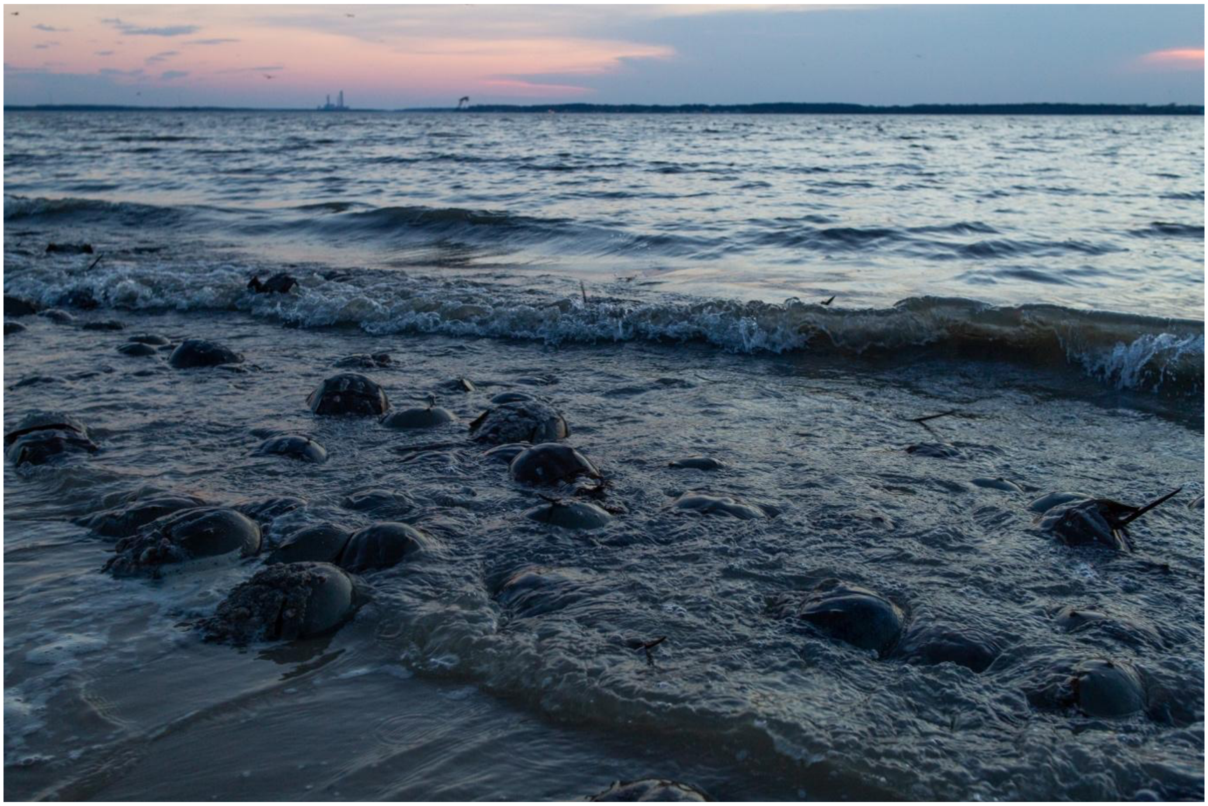
Inland Bays Volunteer Horseshoe Crab Spawning Survey
Five-Year Trend Report: 2015-2019



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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

2019 was the twelfth consecutive year of the Inland Bays volunteer horseshoe crab survey and the fifth where protocols matched those followed in surveys conducted in the Delaware Bay. In a change from prior years, only five locations were surveyed. Due to consistently low spawning populations, the sixth survey site, Fenwick Island State Park, will only be surveyed every five years. The five beaches were scheduled to each be surveyed 12 times, with surveys coinciding with full and new moons during May and June.

Twelve surveys were canceled due to inclement weather with a total of 48 surveys completed. Sampling occurred in conjunction with tidal progression on the evening two nights before, the astronomical peak itself, and the second night following the full and new moons. A total of 32,547 crabs were counted at all sites in 2019, with an overall sex ratio of 7.8 males for every female. The cumulative spawning density was 6.78 crabs per square meter, and the female spawning density was 0.76 crabs per square meter.

Over the last five years, volunteers and CIB staff have completed more than 250 surveys and counted nearly 100,000 crabs. Since the survey's inception in 2002, a total of 13,318 horseshoe crabs have been tagged, which has lead to greater knowledge regarding the movement and spawning site fidelity of horseshoe crabs in the larger Delaware Bay region.

The Inland Bays sex ratio has consistently exceeded the minimum target goal of 2:1, an important spawning population indicator. Additionally, results from other regional surveys, along with the data gathered in the last five years indicate that spawning horseshoe crab populations are likely somewhat stable within the Inland Bays. However, additional survey seasons are needed before long-term spawning population trends can be analyzed in any statistically significant manner.

INTRODUCTION

Historic 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 to 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 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 modified to match that used in the Delaware Bay survey program. 2019 was the fifth year in which horseshoe crabs were monitored throughout the spawning period following the updated protocol. Previously, all beaches were monitored using an eight-meter pull rope to survey random points along the beach. Beginning in 2015, the survey was changed to randomly sample 100 one-meter^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 spawning population levels and sex ratios within the Inland Bays and to track changes in these over times. To accomplish these goals, horseshoe crabs were systematically counted and sexed at five beach sites during the spring and early summer of 2019, which corresponds to the spawning period of the crabs. Survey reports from previous years can be found online at https://www.inlandbays.org/projects-and-issues/all/horseshoe-crab-survey/.

METHODS and MATERIALS

Five different sandy beaches distributed throughout Rehoboth and Indian River Bays (Figure 1) were surveyed between May 2nd and June 19th, 2019, on dates that coincide with the 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, with occasional surveys occurring in late April. Up until 2018, a survey was also conducted in Little Assawoman Bay, initially at the Coastal Kayak Beach (2015-2017) and later at Fenwick State Park in 2018 (Figure 1). However, both locations consistently saw low crab numbers, likely due to the substantial distance necessary for the crabs to travel from either the Ocean City or Indian River Inlet. Tthe Fenwick Island State Park site is approximately 12.5 miles from Ocean City Inlet and 9.5 miles from the Indian River Inlet. Due to consistently low numbers of spawning crabs, Fenwick Island State Park will no longer be surveyed annually, but rather will be monitored every five years and included in future trend reports (McGowan and Bartow 2020).

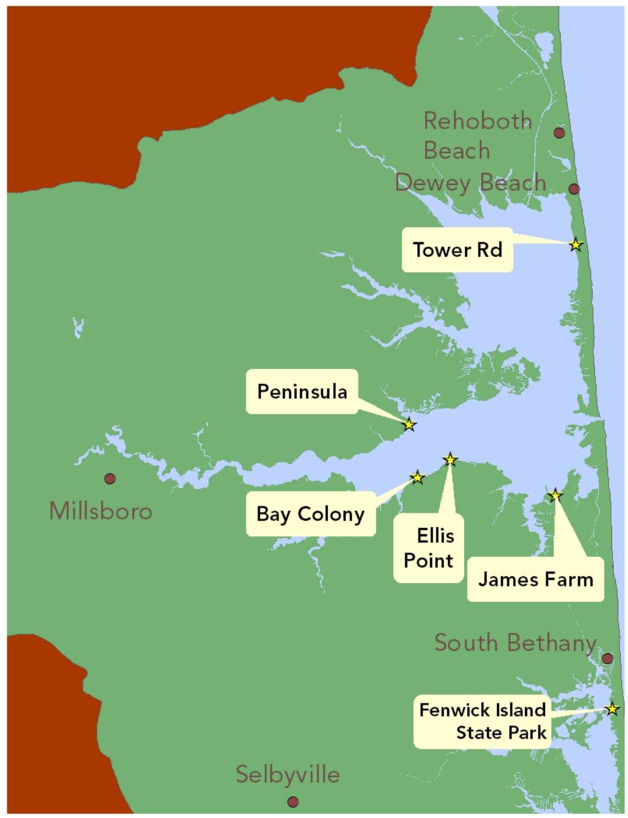

Figure 1. Map of HSC survey sites. Fenwick Island S.P. (located bottom right) will no longer be measured annually but will be surveyed once every five years.

Of the five beaches surveyed in 2019, four (Bay Colony, James Farm, Peninsula, & Tower Road) have been consistently surveyed since the methodology change in 2015. Ellis Point was added to the survey in 2017, making 2019 its third year of inclusion in the 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 for the survey because they were easily accessible to volunteers.

<u>Survey Protocol</u>

The spawning surveys were conducted in conjunction with the new and full moon cycles, occurring on May 2^{nd}, 4^{th}, 6^{th}, 16^{th},18^{th}, 20^{th}, and June 1^{st}, 3^{rd}, 5^{th}, 15^{th}, 17^{th}, and 19^{th}. 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 on how to determine the sex of horseshoe crabs. Teams begin the survey at the onset of the tidal change as the evening high tide begins to ebb. 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, Ellis Point, and Tower Road sites each use a 4-meter pull rope. The Bay Colony site uses 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 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 $1m^2$ 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 the 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 straight, and it may be above or below the waterline; however, it is never more than one 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 the safety of the volunteers. However, weather was generally favorable in the 2019 study, with most cancelations occurring at the very beginning and end of the survey season. A total of 12 surveys were canceled in 2019 (Figure 2) due to storms or volunteer scheduling issues. Of these, seven were missed during the third and fourth moon cycle, which typically corresponds to the nights with the largest crab counts. Two surveys were canceled on 6/5, which was later identified as the peak spawning evening for 2019 (Figure 4). Comparatively, eleven surveys were missed during this period in 2018, ten in 2017, and three were missed in 2016. A Quality Control report for the 2019 season is presented in Appendix A.

Tagging Study

In conjunction with the spawning survey, 3,000 horseshoe crabs were tagged as part of the U.S. Fish and Wildlife Service Cooperative Horseshoe Tagging Program (https://www.fws.gov/northeast/marylandfisheries/native-species/horseshoe-crab.html) in 2019. 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. Sex, approximate age (young, mature, old), and width of the prosoma were recorded, along with latitude and longitude, date, and the person responsible for tagging the crab. Resighted crabs are reported by survey participants or by members of the public, and reports are sent directly to the U.S. Fish and Wildlife Service, which sends the reported resights to the Delaware Center for the Inland Bays upon request.

Beach	5/2	5/4	5/6	5/16	5/18	5/20	6/1	6/3	6/5	6/15	6/17	6/19
Bay Colony	X										X	
Ellis Point	X										X	
James Farm	X										X	
Peninsula	X								X		X	
Tower Road	X								X		X	

Figure 2. 2020 survey dates. Cancelations due to weather are indicated in red with a white X; completed surveys are highlighted green.

<u>Data Analysis</u>

Average spawning densities per square meter 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 each nightly total of a 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 (**α** = 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 specific lunar period, defined as the five days around a full or new moon during which the survey occurs.

2019 RESULTS

Total Crabs & Spawning Density

During the 2019 survey season, a total of 32,547 horseshoe crabs were counted across all five beaches. This was a substantial increase from 2018, just shy of doubling the total number of spawning crabs observed (McGowan and Bartow 2020). 2019 was also the largest number of crabs counted since the current survey methods were implemented in 2015. This occurred despite decreases in both the number of survey locations and total surveys completed.

The cumulative spawning density was 6.78 crabs per square meter (Table 2), far greater than the 2018 average of 2.35 (McGowan and Bartow 2020). The female spawning index (average number of female crabs per square meter) also increased from 0.35 in 2018 to 0.76 in 2019 (McGowan and Bartow 2020). In 2019, Ellis Point had the highest spawning density, while James Farm had the highest female spawning index (Table 2). Of all five sites, Peninsula had both the lowest spawning density and lowest female spawning index of all the beaches surveyed.

Table 1. Spawning density, female spawning index, and sex ratio for the five locations surveyed in 2019.

Location	Spawning Density	Female Spawning Index	M:F Sex Ratio
Bay Colony	6.40	0.64	8.9
Ellis Point	9.71	1.01	8.6
James Farm	9.65	1.16	7.3
Peninsula	1.13	0.16	5.9
Tower Road	6.41	0.79	7.1
Cumulative	6.78	0.76	7.6

Table 2. Historic horseshoe crab numbers, mean annual spawning density, mean annual female spawning index, and mean annual sex ratio

Year	Total Crabs	Spawning Density	Female Spawning Index	M:F Sex Ratio
2015	15,439	3.15	0.39	7
2016	14,527	2.72	0.51	4.8
2017	20,201	3.81	0.53	6.1
2018	16,491	2.35	0.35	5.7
2019	32,547	6.78	0.76	7.6

<u>Sex Ratio</u>

The cumulative sex ratio in 2019 was 7.6 males for every female, an increase from 5.7 the year prior (Table 3) (McGowan and Bartow 2020). This was also higher than the sex ratio that was seen in Delaware Bay during their concurrent 2019 spawning period, which at 5.38 remained relatively constant to the 2018 results (Swan et al. 2020).

In the Inland Bays, Bay Colony had the highest average sex ratio at 8.9 while Peninsula had the lowest at 5.9. In 2019, all five beaches saw an increase in both cumulative spawning density and cumulative female spawning index (Table 4). Additionally, all five beaches saw an increase in sex ratio compared to 2018. Other than Bay Colony, all beaches surveyed observed the highest recorded sex ratio since the current survey methods were adopted in 2015 (Table 3). In previous years, as total crab abundance increased, the sex ratio generally tended to do so as well (McGowan and Bartow 2020). However, no such correlation was present in the 2019 survey data.

Table 3. Historic sex ratios by survey location

Year	Bay Colony	Ellis Point	James Farm	Peninsula	Tower Road	Cumulative
2015	10.4	N/A	6.7	5.7	6.8	7.0
2016	5.4	N/A	4.6	4.0	5.1	4.8
2017	6.8	7.8	5.9	3.9	5.0	6.1
2018	4.7	6.2	6.8	5.0	4.9	5.7
2019	8.9	8.6	7.3	5.9	7.1	7.6

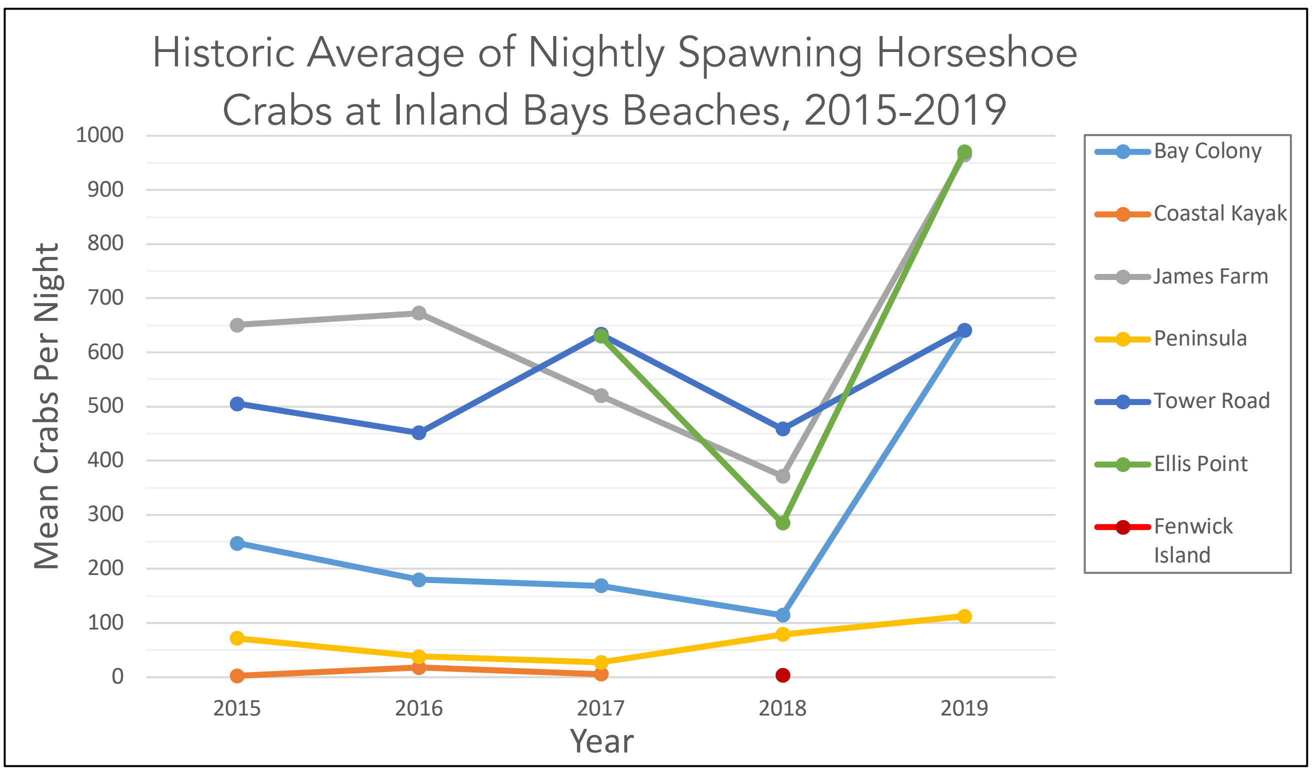

Figure 3. Mean nightly horseshoe crabs by location, 2015 to 2019. Neither Coastal Kayak nor Fenwick Island State Park was sampled in 2019. While still an active survey site, Fenwick Island State Park will only be surveyed every five years.

Peak Spawning Period

Horseshoe crab spawning peaked temporally during the third lunar period, with 18,507 crabs recorded over the three survey nights (June 1st-5th; Figure 4). Of the three nights, June 5th recorded the highest average number of spawning crabs despite two surveys (Tower Road & Peninsula) being canceled due to adverse weather conditions. On that night alone, 7091 crabs were counted across the three beaches surveyed. Due to unsafe weather conditions, no surveys occurred on May 2nd or June 17th.

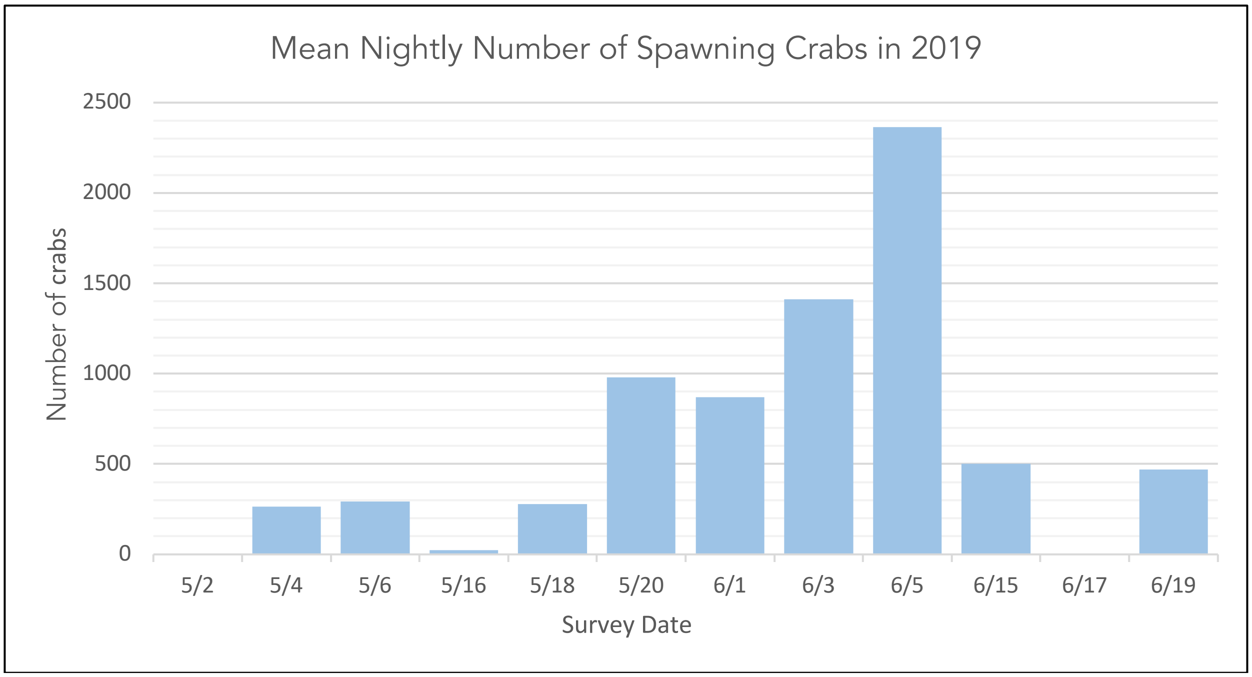

Figure 4. The average nightly number of spawning crabs across the five beaches surveyed in 2019

Horseshoe crab activity was positively correlated with water temperature ($p < 0.001$, tau = 0.159) and salinity ($p < 0.05$, tau = 0.215). Water temperature and salinity during each 2019 sampling event are presented below in Tables 4-5.

*Table 4. Water temperature measurements (°C) from each sampling even. Survey cancelations are marked in red and a failure to collect a sample is indicated with a *.*

Date	Bay Colony	Ellis Point	James Farm	Peninsula	Tower Road
5/2/19					
5/4/19	20	20	20	20	18
5/6/19	17	17	17	18	16
5/16/19	18	18	18	19	18
5/18/19	18	20	20	*	19
5/20/19	22	23	22	23	23
6/1/19	24	25	23	25	22
6/3/19	21	21	18	23	18
6/5/19	23	23	22		
6/15/19	23	22	23	24	22
6/17/19					
6/19/19	25	25	24	27	25

*Table 5. Salinity measurements (ppt) from each sampling event. Survey cancelations are marked in red and a failure to collect a sample is indicated with a *.*

Date	Bay Colony	Ellis Point	James Farm	Peninsula	Tower Road
5/2/19					
5/4/19	20.3	23.1	25.5	24.4	25.8
5/6/19	22.1	24.1	25.3	21.4	24.9
5/16/19	22.3	22.4	24.7	24.3	23.7
5/18/19	23.8	24.7	26.2	25.8	*
5/20/19	24.2	23.8	*	22.6	26
6/1/19	24	24.2	26.2	25.4	25.7
6/3/19	23.5	24.9	27.7	26.2	24.9
6/5/19	23.7	23.6	26.3		
6/15/19	24.1	25.1	27.4	23.6	26.8
6/17/19					
6/19/19	*	26.6	27.8	24.9	26.1

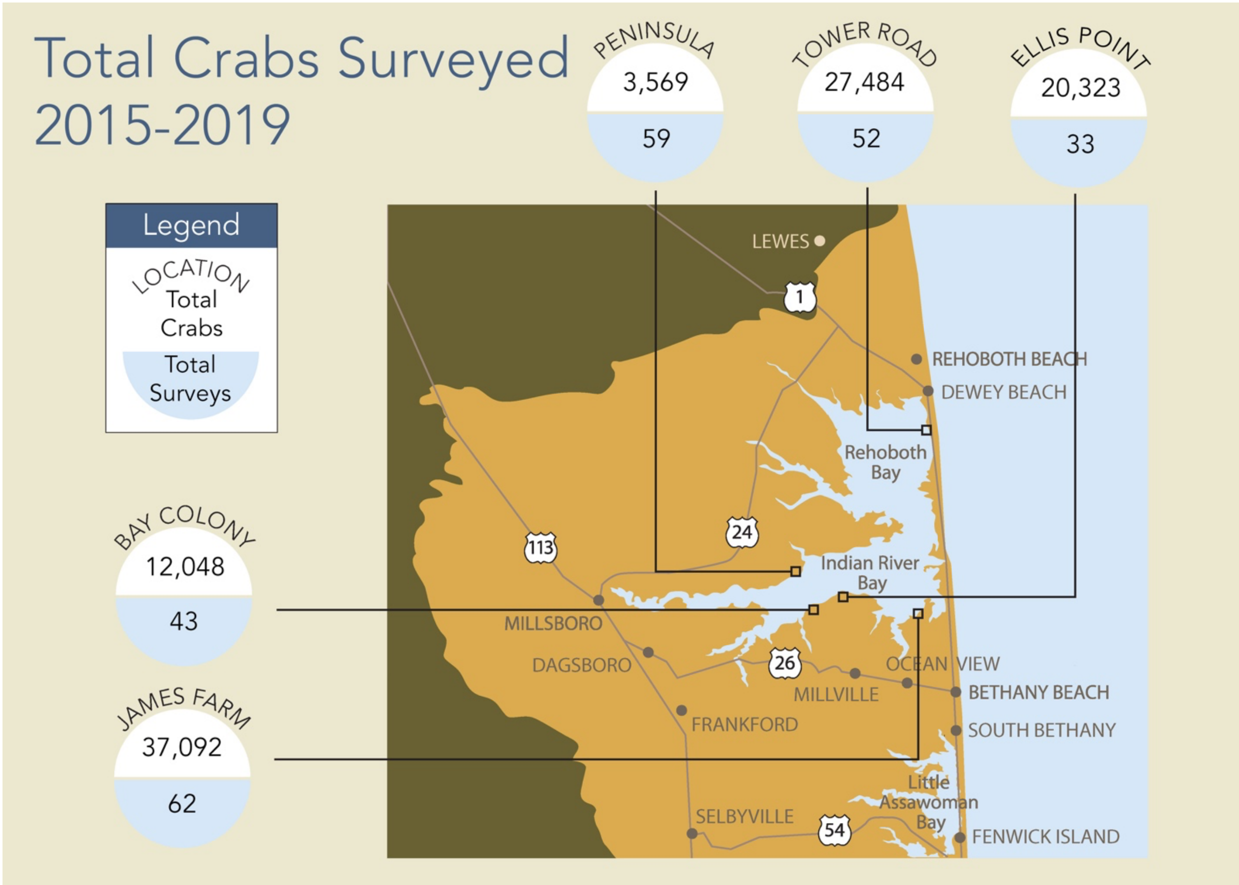

Figure 5. Total surveys and cumulative crabs observed per beach 2015-2019.

DISCUSSION

The 2019 Inland Bays horseshoe crab survey was distinguished by substantial increases in crabs across survey locations in the Inland Bays. Total crabs surveyed increased from 16,491 in 2018 (Bartow & McGowan, 2020) to 32,547 in 2019, making this the largest number of crabs counted since the survey methods were standardized to match those of Delaware Bay's protocol in 2015. This upturn occurred despite a decrease in total surveys completed. Furthermore, this increase in crab counts occurred across all five locations surveyed, though the increase was not equally proportionate across all locations (Figure 3).

When considering long-term temporal population dynamics, more survey seasons are necessary to distinguish any quantifiable trends of spawning density and abundance.

However, the amount of data collected over the last five years in the current methodology, as well as the information collected under prior protocols, are substantive enough to provide initial answers to a number of the questions regarding spawning crabs in the Inland Bays set forth at this survey's inception. Both the results of the 2019 survey, as well as these broader trends and will be discussed below.

<u>2019 Spawning Density:</u>

The overall increase in crabs counted was reflected in an increase in the cumulative spawning density, which rose from 2.35 in 2018 to 6.78 in 2019. The female spawning index rose to 0.76, up from 0.35 in 2018 as well. Both the cumulative spawning density and the female spawning index were at the highest seen since over the past five years.

The increase of the cumulative spawning density between 2018 and 2019 could be partially explained by the decrease in sampling attempts early in the season. Because 2018 included an additional lunar cycle, several early surveys occurred with low recorded water temperatures and few crabs, lowering the cumulative spawning density (McGowan and Bartow 2020). However, early surveys like this did not occur in years previous to 2018.

Cancelations in 2018 often occurred during critical lunar periods when crab spawning was at its temporal peak, which may account for some of the change as well. While cancelations did occur in 2019, most of them occurred in the first and fourth lunar cycles, which are typically less significant than others. In 2019, only two cancelations occurred during the temporal peak spawning period. Historic cancelations by spawning period are discussed further on page 23.

Another potential explanation for the bays-wide increase in spawning crabs observed during 2019 is the removal of a survey location in Little Assawoman Bay (LAB), which has had historically low numbers. Table 6 compares cumulative historic spawning density with and without the LAB sites. While dropping LAB sites from the data analysis in previous years does result in an increase in spawning density, it alone does not explain the increase seen in 2019. Therefore, the increase in numbers of crabs observed in 2019 was likely a true increase in the number of spawning crabs and not a relic of changing beaches or sampling frequencies.

Table 6. Comparative annual spawning densities with and without LAB sites

Year	Cumulative Spawning Density	Density without LAB sites
2015	3.15	3.46
2016	2.72	2.96
2017	3.81	3.99
2018	2.35	2.69
2019	6.78	6.78

James Farm and Ellis point had the highest average spawning densities in 2019, a slight shift from previous years, in which Tower Road consistently was among the most productive beaches. However, Tower Road's spawning density was likely depressed due to a survey cancelation, at this site only, on June 5th, which lines up with the peak spawning date for the three successful surveys that evening. At all three beaches surveyed on June 5th - Bay Colony, Ellis Point, and James Farm - a relatively proportionate increase in mean crabs was observed from 2018 to 2019, and while the two which canceled on that evening, Tower Road and Peninsula, also observed increases, but at a subdued rate.

Similar increases in crab counts were observed in the 2019 Delaware Bay survey, which also documented its highest number of crabs ever recorded under their current protocol (Swan et al. 2020). Without a long-term data record, it remains unclear if the increases seen in our survey were unusual or represent normal fluctuations in spawning numbers. But the record counts reported by the Delaware Bay survey (which has a longer period of record) suggest this past year was a substantial increase in crab numbers beyond typical interannual fluctuations. Several factors, ranging from ideal weather scenarios to improved management measures, may have played a role in this increase.

Comparisons to Regional Historic Spawning Densities

While the increase in spawning horseshoe crabs in 2019 is notable, additional survey years are needed in the Inland Bays before statistically significant trends can be determined regarding spawning population dynamics. In the meantime, spawning density data can still be assessed, but should not be used for trend determination.

One notable way to assess the population data from the last five years is to compare with other regional spawning surveys. The ASMFC guidelines delineate the Delaware Bay region to span between Barnegat Bay to the north and Chesapeake Bay to the south (ASMFC, 2019). Thus, comparisons between the Inland Bays survey and those conducted in immediate spatial neighbors, Delaware Bay and Maryland's Coastal Bays, may provide insight into overall regional spawning trends.

Maryland's Coastal Bays Spawning Densities

The Maryland Coastal Bays Program (MCBP) has surveyed spawning horseshoe crab populations since 2002 in Sinepuxent, Isle of Wight, and Assawoman Bays (Toulan and Doctor, 2020). In contrast to the Inland Bays survey, the 2019 Maryland Coastal Bays survey recorded a decrease in both the number of horseshoe crabs counted and the overall catch-per-unit-effort (CPUE), their primary standardizing method (Toulan and Doctor, 2020). This followed two years of a much higher CPUE in 2017 and 2018.

Table 7. Annual MCBP Horseshoe Crab Survey Results, 2015-2019 (Toulan and Doctor, 2020).

Year	Surveys Conducted	Total Crabs Observed	CPUE
2015	30	14,238	474.60
2016	31	9,095	293.39
2017	29	14,554	501.52
2018	41	22,140	540.00
2019	44	6,663	151.43

Unfortunately, differences in sampling methods and chronology make the direct comparison of spawning populations difficult. The Maryland Coastal Bays survey season runs nearly twice the length of its Inland Bays counterpart and relies on catch-per-unit-effort (CPUE) as its primary standardizing method. Future analysis and collaboration with MCBP may make such direct comparative analyses possible, but currently there is no definitive way to conclude whether the differences in annual survey results are due to varying methodology or to regional population trends.

Delaware Bay Spawning Densities

The Delaware Bay horseshoe crab survey has been conducted since 1999 and surveys approximately 25 beaches around the bay in both New Jersey and Delaware. This survey is considered to be a premier method of estimating the spawning population of horseshoe crabs and is the basis for the methodology change adopted by the CIB in 2015.

The consistency in methodology allows for the direct comparison of numerous aspects of the two surveys. While there are important variables, such as survey scale, that are worth considering when directly comparing the two embayments, parameters such as spawning density and sex ratio have the potential to provide insight into regional spawning trends and management decisions.

Over the last five years, the Delaware Bay survey has consistently found higher cumulative densities of spawning crabs than in the Inland Bays (Zimmerman et al. 2019, Table 8). This trend continued in 2019, when both surveys observed a nearly proportional increase in crab densities. Additionally, the Female Spawning Index for the Delaware Bay has also been historically higher, though the data for 2019 are not yet available (Zimmerman et al. 2019). This is also reflected in the annual cumulative sex ratios (Table 11).

Table 8. Delaware & Inland Bays Cumulative Spawning Densities: 2015-2019.

Cumulative Spawning Density	Inland Bays	Delaware Bay
2015	3.15	3.41
2016	2.72	5.00
2017	3.81	4.39
2018	2.35	4.86
2019	6.78	9.27

Table 9. Delaware & Inland Bays Cumulative Female Spawning Indices: 2015-2019.

Female Spawning Index	Inland Bays	Delaware Bay
2015	0.39	0.66
2016	0.51	0.9
2017	0.53	0.71
2018	0.35	0.74
2019	0.76	

In 2019, the highest Delaware Bay spawning densities recorded were both at the Pickering survey site. On May 20th, an average of 45.21 crabs per square meter was reported, and on June 1st an average of 41.77 crabs per square meter was reported (Swan et. al., 2020). In the Inland Bays, the highest spawning densities were both recorded on June 5th. Ellis Point recorded a density of 25.95 crabs per square meter and James Farm recorded a density of 30.78 crabs per square meter.

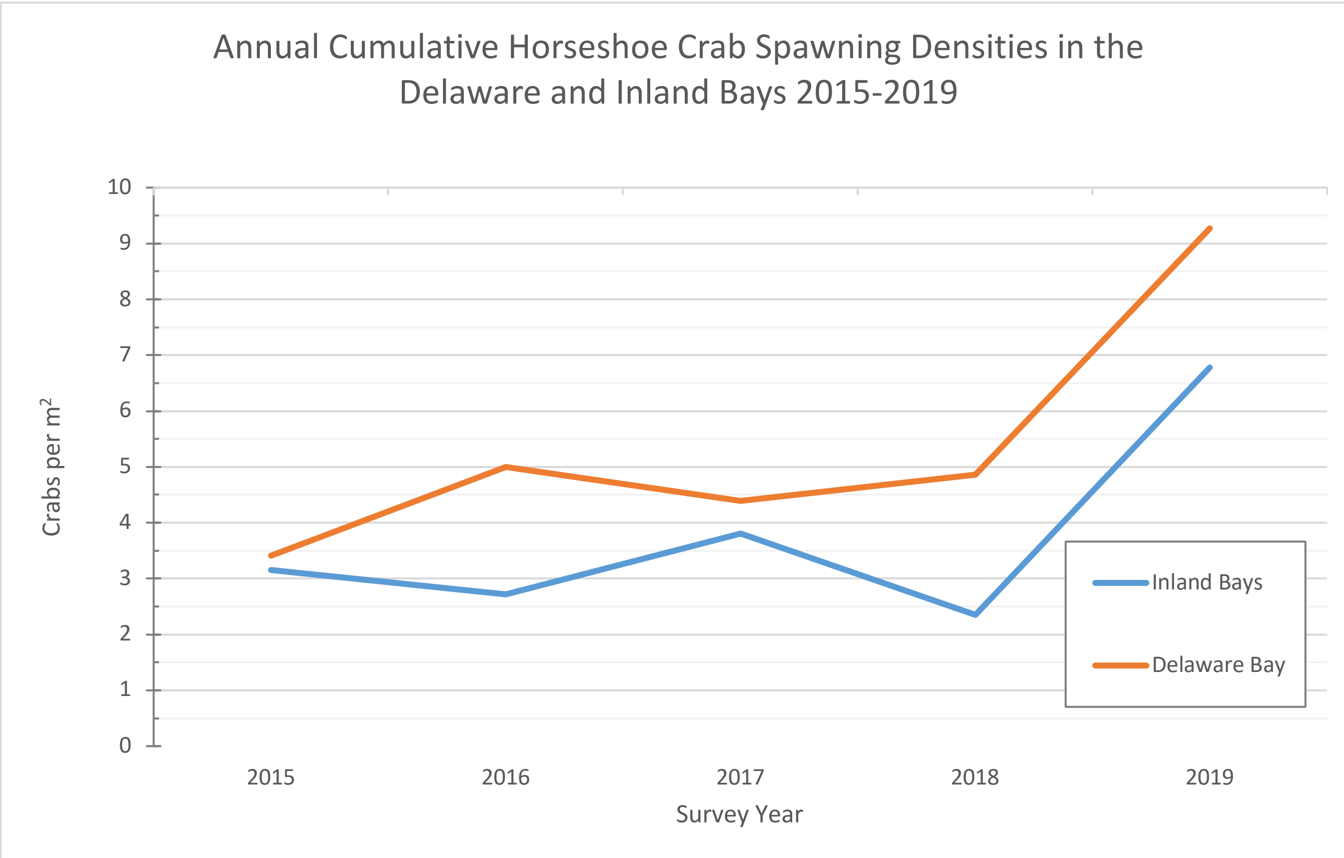

Figure 6. The historic cumulative averages of spawning crabs counted in the Delaware Bay and Inland Bays Horseshoe Crab Surveys, 2015-2019.

The Delaware Bay Survey also groups each individual survey into four categories (zero, low, moderate, or high) based on the relative density of crabs observed (Zimmerman et al. 2019). "No crabs" indicates that zero crabs were found on given survey. Low activity indicates an average of less than 5 crabs per square meter, moderate activity equals 5 to 10 crabs per square meter, and high activity equals greater than 10 crabs per square meter. These data are analyzed in percentages because the number of surveys and survey locations may vary annually due to a variety of factors.

Based on these classifications, Inland Bays beaches have had a historically lower percentage of moderate and high-density beaches relative to the Delaware Bay (Table 10). Generally speaking, both embayments have had a similar number of low and no crab survey percentages. The discrepancy in the high and moderate categories may be in part due to the consistently higher percentage of annual canceled surveys in the Inland Bays but does not explain away the discrepancy entirely. Furthermore, while the canceled surveys in the Inland Bays may potentially have skewed these data, it is impossible to determine the horseshoe crab densities on these evenings and thus any presumptions are purely speculation.

Table 10. Annual percentages of horseshoe crab density classifications in the Inland and Delaware Bays (Zimmerman et al. 2019).

	Location	No Crabs	Low (<5)	Moderate (5-10)	High (>10)	No Survey
2015	New Jersey	10	55	9	19	7
	Delaware	14	56	10	13	7
	Inland Bays	9.5	51	4	8	27
2016	New Jersey	2	58	15	18	7
	Delaware	13.5	56	11.5	17	3
	Inland Bays	9.5	60.5	6.5	8	14.5
2017	New Jersey	4	63	6	15	11
	Delaware	17	47	15	14	6
	Inland Bays	5	50.5	11	8	25.5
2018	New Jersey	3	49	13	17	17
	Delaware	3	44	8	24	22
	Inland Bays	3	51	11	7	28
2019	New Jersey	0	37	17	27	20
	Delaware	3	33	17	29	18
	Inland Bays	1.5	28	8	21.5	20

When looking at both of these density comparisons, it is clear that on average, the spawning densities observed in the Delaware Bay are consistently larger than those in the Inland Bays. This is unsurprising, particularly since Delaware Bay hosts the largest concentrations of spawning horseshoe crabs anywhere on the eastern seaboard. Notably, the comparison supports the idea that multiple Inland Bays spawning locations have equivalent densities to the high productivity sites found in Delaware Bay. While the sites may be fewer in number, their spawning densities are clearly comparable.

The difference between the two embayments may also be due to the relatively low sample size in the Inland Bays. On average, four of the sites in the Inland Bays - Bay Colony, Ellis Point, James Farm, and Tower Road - have had relatively high numbers of spawning crabs, while the Peninsula and both LAB sites have extremely low numbers. While Delaware and Inland Bays both have low spawning productivity sites, the smaller sample size in the Inland Bays may be contributing to their disproportionate weight. The difference in scale may also be limiting the ability for direct comparison. In 2019, 234 surveys were completed were completed in Delaware Bay, more than four times the number in completed in the Inland Bays

The Delaware Bay Survey has found no statistically significant trends in either male or female horseshoe crab spawning activity since the survey's inception (Zimmerman et al. 2019). While there are not enough seasons of survey data in the Inland Bays to make similar determinations, the intermixing between the two populations may allow for some circumstantial conclusions regarding the stability of the Inland Bays spawning population to be reached. Furthermore, it is clear that while the Inland Bays are smaller, and thus support less spawning habitat overall, the quality of habitat is still consistent with that of the Delaware Bay.

Variability in Spawning Density Between Sites in the Inland Bays:

One of the initial goals of this survey was to examine any potential variability in spawning populations between different survey sites. Within the Inland Bays, there has been consistent annual variation in spawning density among different survey sites over the last five years (Figure 3). Certain sites, such as the LAB locations and the Peninsula, regularly record far lower numbers of spawning crabs than sites such as James Farm and Ellis Point. Distance from the Indian River Inlet is likely the cause of the low crab numbers in Little Assawoman Bay, but this does not explain the variability among the other survey locations.

While horseshoe crabs may show some site fidelity during a given spawning season (Brousseau et. al. 2004), there does not appear to be any site loyalty over the course of successive years or generations (Smith el. Al. 2010, McGowan 2018). There is some evidence that spawning crabs return to their natal estuary, but it is unlikely they return to the same beach itself (Shuster 1985). Thus, it is unlikely that the variations in spawning densities in the Inland Bays are caused by generational preferences due to site fidelity.

However, when selecting spawning locations, horseshoe crabs appear to prioritize beaches primarily consisting of coarse sandy sediments. Additionally, spawning crabs appear to actively avoid beaches with higher levels of peaty sediments or that are adjacent to exposed peat banks (Botton et al. 1988; Smith et al. 2002a). The Peninsula is known to have peak banks directly offshore the beach, which may account for the site's consistently low horseshoe crab spawning numbers.
Wave action may also play a role in introducing variability among survey sites. Wave action is used by horseshoe crabs to help them accurately orient towards spawning beaches, with Smith et al. (2002a) finding an inverse correlation between female spawning activity and wave heights of less than 0.3 meters. However, when waves become too strong, they can deter crabs from attempting to spawn (Smith 2011).

The Tower Road survey site faces due west and is often subjected to intense storm activity and wave energy during the survey season. Conversely, James Farm is well protected from all but the hardest north or north east winds, which are fairly uncommon for the Inland Bays in spring. Generally speaking, Bay Colony, the Peninsula, and Ellis Point are intermediates with regards to wave action. Accounting for the peat ant typical wave conditions, the relationships between the sites are clearer. When calm, Tower Road beach is heavily utilized like the beaches at Bay Colony, Ellis Point, and James Farm. When wave energy increases, particularly from the west, Tower Road is used less. However, with the exception of the Peninsula, all of these beaches have relatively similar spawning crab densities.

Inland Bays spawning survey does take measurements of wave height, classifying them as either greater than or less than one foot. Since the methodology change in 2015, 269 surveys have recorded a wave height of less than one foot, and 20 surveys have recorded a wave height of greater than one foot. The average spawning density for surveys with less than one-foot waves was 3.51 and the average spawning density for surveys with waves greater than one foot was 4.26. However, based on the subjective nature of this measurement and the disproportionate number of surveys with less than one foot of wave height, it would be difficult to make any substantive conclusions regarding the correlation.

Sediment size and type may also play a role in determining preferential spawning beaches. Additionally, some studies indicate that females horseshoe crabs avoid laying eggs in eroded beaches, particularly those with lower sediment pore oxygen levels (Smith et. al. 2017). Further analyses and study of the sediments at each of the Inland Bays survey sites would be necessary for this to be determined.

Sex Ratio:

Sex ratio is an important metric when considering the health of a spawning horseshoe crab population. A higher ratio of male to female crabs helps ensure genetic diversity and high fecundity (ASMFC, 2019). Additionally, a high proportion of males increases the likelihood that a clutch of eggs will be properly fertilized (Mattei et al., 2010). The baseline target goal for an optimal sex ratio is 2:1 (ASMFC 2019).

The cumulative male to female sex ratio observed in the Inland Bays was 7.6 in 2019, an increase from 5.7 in 2018. While this was also the highest recorded sex ratio since the protocol shift in 2015, it was a less dramatic change than those seen in other indices (Table 11).

The 2019 ratio also deviated again from that of the Delaware Bay Survey, which was 5.38 (Swan et al. 2020). This was similar to the 2015-2017 surveys, where the sex ratio in

Table 11. Comparative annual sex ratios for the Inland and Delaware Bays.

Year	Inland Bays Annual Sex Ratio	Delaware Bay Annual Sex Ratio
2015	6.99	4.47
2016	4.81	4.60
2017	6.10	5.20
2018	5.70	5.54
2019	7.60	5.38

Table 12. Comparative annual sex ratios for the Inland and Maryland Coastal Bays.

Year	Inland Bays Annual Sex Ratio	Maryland Coastal Bays Sex Ratio
2015	6.99	3.64
2016	4.81	3.58
2017	6.10	4.45
2018	5.70	3.23
2019	7.60	7.85

the Inland Bays was higher than that found in the Delaware Bay. In 2018, sex ratios between the Delaware and Inland Bays were similar, however the gap widened again in 2019.

Based on comparisons between both the spawning index data, as well as the sex ratio, it is possible that the differences between the two embayments are explained in part by a higher number of female crabs in the Delaware Bay. Additional years of data collection are needed to determine whether this discrepancy is coincidental or represents spatial differences in horseshoe crab population dynamics.

For most of the last five years, the Maryland Coastal Bays survey has also found a consistently lower sex ratio than the Inland Bays. However, in 2019, the Maryland Coastal Bays sex ratio increased dramatically to 7.85 (Table 12, Toulan and Doctor, 2020). This may in part be due to the dramatically reduced number of crabs observed in Maryland's Coastal Bays in 2019. Unfortunately, it is unclear as to whether the variations in methodology between the Maryland Coastal Bays and the Inland Bays surveys influence the ability for direct comparison.

Over the last five years, the Inland Bays sex ratio has exceeded the minimum target goal of 2:1 (ASMFC 2019). Additionally, while results from years when a different survey protocol was used in the Inland Bays are not directly comparable to the current protocols, that survey, which ran from 2008-2014, also consistently recorded a cumulative sex ratio which met this benchmark (McGowan, 2016). This is an encouraging sign, particularly when combined with the knowledge that both male and female crab abundance has remained stable within the region (Bi et al. 2020, Zimmerman et al. 2019). Taken together, the healthy sex ratio and stable overall abundance appear to indicate a stable status for horseshoe crabs in the Delaware Bay region.

<u>Historic Survey Cancelations:</u>

The timing and number of survey cancelations, both due to weather and volunteer issues can vary dramatically from year to year. This may potentially have substantial implications for analyzing and comparing annual results. This is particularly true for surveys during the second and third lunar periods, the times when traditionally peak spawning in the Inland Bays has occurred.

Different regional surveys use a variety of methods to attempt to counteract this problem. In Maryland's Coastal Bays, the survey is measured by count per unit effort, then those numbers are compared on an annual basis. This allows for a relative number to be derived regardless of the precise number of surveys completed (Toulan and Doctor, 2020). The Delaware Bay survey uses raw densities to calculate the overall estimated number of spawning crabs after normalizing for survey cancelations.

Other than observing when the cancelations took place and accounting for them in the annual report, the Inland Bays survey takes no specific measures to account for these variations. In the future, the Inland Bays survey should work with the Delaware Bay survey to establish a modeling program in order to best account the inevitable cancelations throughout the survey season.

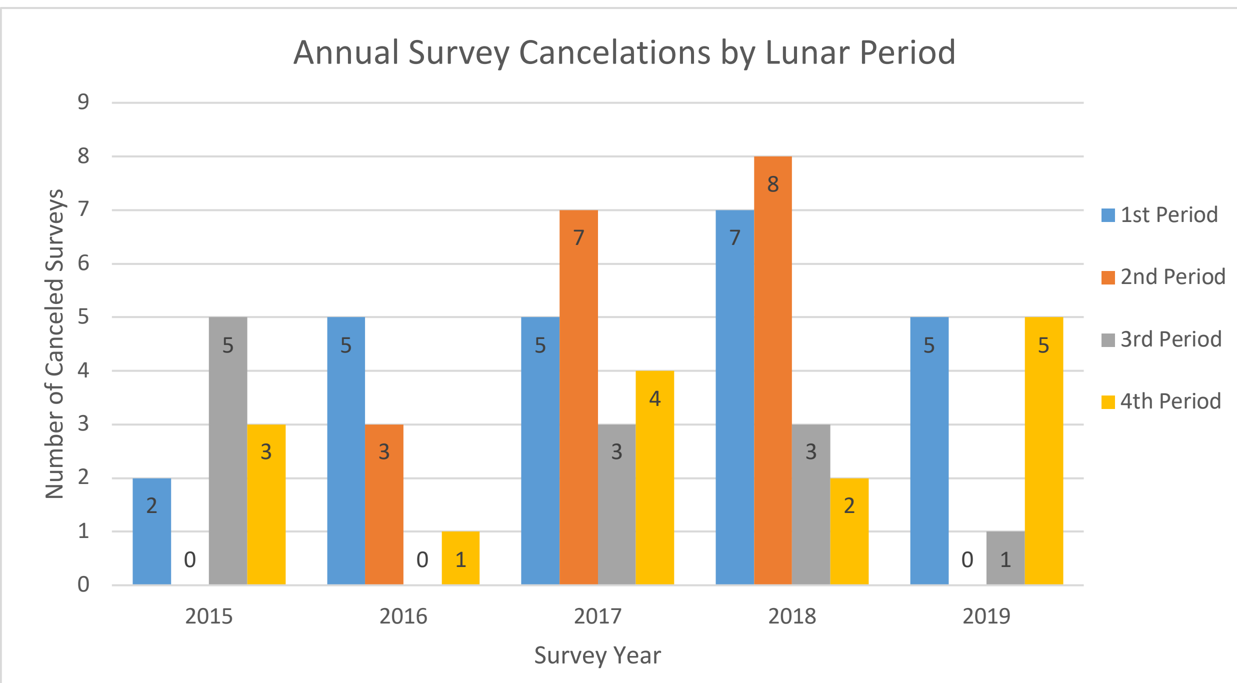

Figure 7. Annual survey cancelations sorted by lunar survey period

Over the last five years, the first lunar period has seen the most survey cancelations, with a total of 24 canceled surveys occurring during the first cycle. The second lunar period was the second highest, with a total of 18 cancelations. The fourth lunar period saw a total of 15 cancelations and the third lunar period saw 11. Thus, the largest portion of survey cancelations are occurring early in the season, generally before temperatures have reached the threshold to induce peak spawning. However, the substantial number of survey cancelations in the second period may potentially be obscuring enough spawning data to impact temporal spawning results.

Historic Peak Spawning Periods:

Over the last five years, peak spawning has predominantly occurred in the 3rd lunar cycle during the first and second weeks of June. The only deviation from this was in 2015, when average water temperatures were substantially warmer during the second period than in subsequent years (McGowan et. al. 2017). However, based on the data collected thus far, no noticeable trend in the peak spawning period can be determined. Additional years of phenological data are needed before any temporal movements regarding peak density can be observed.

Table 13. Historic peak spawning dates and lunar cycles in the Inland Bays

Year	Date	Lunar Cycle
2015	20-May	2
2016	6-Jun	3
2017	9-Jun	3
2018	13-Jun	3
2019	5-Jun	3

Another goal of the Inland Bays survey is to determine if there is any variation in peak spawning activity among the various sites, however survey cancelations during peak spawning evens can complicate this process. As observed in Figure 7, cancelations occurred in nearly all survey periods, potentially skewing or obstructing the precise peak survey time. Thus, when comparing beaches, lunar cycles were used to determine the peaks rather than specific evenings.

Table 14. Historic peak spawning lunar cycles for each of the five current survey sites in the Inland Bays

Year	Bay Colony	Ellis Point	James Farm	The Peninsula	Tower Road	Overall
2015	2		2	2	2	2
2016	3		4	4	3	3
2017	3	3	4	4	3	3
2018	2	3	3	2	3	3
2019	3	3	3	3	3	3

When the historic peak spawning cycles are separated by survey site, there does appear to be some temporal variation during most years. This may be due to a number of potential factors, including spatial differences in temperature or weather-related phenomenon, such as wave direction and intensity.

Impacts of Climate Change on Horseshoe Crab Spawning Events:

Water temperature plays a critical role in the determination of the timing of horseshoe crab spawning events. Similar to other regional survey sites, spawning in the Inland Bays is usually catalyzed once water temperatures reach 15 degrees Celsius. It is therefore possible that changes in water temperature in response to global climate change may alter spawning times. Additionally, the timing of spawning may also affect the survival of egg, larvae and juvenile stages (Smith et. al. 2017). However, any evidence of climate change impacting these aspects of spawning in the Inland Bays cannot be observed on the current timescale of the survey. Many more years of phenologic data are needed before any possible correlations may be observed.

Sea level rise is another potential concern for spawning crab populations in the Inland Bays. Many of the beaches included in the survey are narrow, and it is unclear as to if the beaches will migrate proportionately. This emphasizes the need to protect not only the sandy beaches in the Inland Bays, but also the buffers in the vicinity. This particularly true for the highly developed shorelines of the Inland Bays watershed.

Tagging Survey:

Between 2002 and 2019, CIB staff and volunteers have tagged 13,318 horseshoe crabs as part of the U.S.F.W.S Cooperative Horseshoe Tagging Program. Detailed results of the Inland Bays tagging efforts are presented in a paper titled "Horseshoe crab (*Limulus polyphemus*) movements following tagging in the Delaware Inland Bays, the U.S.A." (McGowan 2018). This paper tracks 1123 resighted crabs initially tagged in the Inland Bays between 2002-2016.

These tagging efforts indicate that there is substantial evidence of mixing between populations of horseshoe crabs within the Delaware Bay region, particularly between the Inland Bays and Delaware Bay itself (McGowan 2018). Mixing between the two embayments was so prevalent that after a year, a crab tagged in the Inland Bays was more likely to be found in the Delaware Bay than it was in the location it was initially captured (McGowan 2018).

Additionally, all but two resighted crabs were found between Barnegat Bay, NJ to the north, and the Chincoteague Bay Inlet, VA to the south. This supports the spatial boundaries used by the ASMFC guidelines which delineate the Delaware Bay region between Barnegat Bay to the north and the Chesapeake Bay to the south (ASMFC, 2019).

The interconnectivity between these regional embayments strengthens the need to conserve spawning habitat throughout all the coastal bays in the region, since substantial loss of habitat in any of these systems could potentially impact the Delaware Bay population as a whole. Furthermore, this supports utilizing spawning surveys (if statistically sound) from all coastal embayments when regional management decisions are made (McGowan 2018).

Looking Forward:

While there may be a desire after the last five years to draw major conclusions regarding the spawning population of horseshoe crabs in the Inland Bays, additional years of surveying under the current protocol are necessary before long-term trends can be appropriately analyzed. However, it is clear that the change in survey methodology made in 2015 was a valuable investment. The benefits of directly comparing results with Delaware Bay and potentially other locations using this methodology will only continue to develop as time goes on. This standardization has already produced a number of these benefits even in the first five years of its use.

Yet some inferences regarding the regional health of the Inland Bays' horseshoe crab populations may already be gleaned by examining other regional surveys. Over the last 20 years, the Delaware Bay survey has found no noticeable trend in horseshoe crab spawning populations (Zimmerman et. al. 2019). The Virginia Tech trawl survey has found no noticeable trend in immature horseshoe crabs in the coastal Delaware Bay region but has observed an apparent increase in mature crabs overall (Bi et. al. 2020). The ASMFC 2019 Horseshoe Crab Benchmark Stock Assessment found the Delaware

Bay Region to have a neutral status determination overall (ASMFC 2019). Based on these analyses, along with survey data collected since 2015, it is most likely that spawning populations in the Inland Bays are relatively stable, with no definitive trends indicating an increase or decrease in horseshoe crabs.

The long-term goal of the Inland Bays survey is to analyze spawning horseshoe crab population levels independent from other regional metrics, however, this will not be possible until additional survey seasons are completed. However, it is important to note that the crabs which spawn in the Inland Bays are also surveyed as part of the larger Delaware Bay stock, and thus conclusions drawn at Delaware Bay scale reflect crabs observed in the Inland Bays.

Comparing the data from this study to that collected from Delaware Bay, it is clear that a number of Inland Bays survey locations observe crab densities nearly equivalent to those of some of the higher productivity areas in Delaware Bay. This suggests that much of the habitat available to spawning crabs in the Inland Bays is of similar quality and importance to that of their northern neighbor. While more years of this survey are needed to substantiate these findings, this conclusion is further supported by the CIB tagging efforts, which show that there is substantial interchange between the Delaware and Inland Bays spawning crab populations. Furthermore, it highlights the vital importance of protecting natural sandy shorelines in Rehoboth and Indian River Bays, as well as supporting the eventual inclusion of the Inland Bays spawning surveys into future horseshoe crab stock assessments.

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Appendix A. Quality Control Report

SUMMARY

Data recorded during the 2019 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:

- 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 datasheets. 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

Team Leaders will be reminded of the importance to collect temperature and salinity data at all surveys when possible and the need to check their data sheets at the end of each survey to prevent missing water/temperature samples.

RECOMMENDATIONS

A new site in Rehoboth Bay should be included for the 2020 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. Research into spawning population models based on this survey, along with the potential sediment sampling of survey beaches should both be considered for future seasons.