

Inland Bays Volunteer Horseshoe Crab Survey: Annual Report for 2020



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EXECUTIVE SUMMARY

2020 was the thirteenth consecutive year of the Inland Bays volunteer horseshoe crab survey and the sixth where protocols matched those followed in surveys conducted in the Delaware Bay. Due to the ongoing COVID-19 pandemic, all surveys were completed by CIB staff and interns. Five beaches were scheduled to each be surveyed 12 times, coinciding with full and new moons during May and June.

Four surveys were canceled due to staff logistics and inclement weather, resulting in a total of 56 completed surveys. Sampling occurred in conjunction with the night time high tide two nights before, the astronomical peak itself, and the second night following the full and new moons. A total of 16,198 crabs were counted at all sites in 2020, with an overall sex ratio of 5.67 males for every female. The cumulative spawning density was 2.93 crabs per square meter, and the female spawning density was 0.44 crabs per square meter.

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 (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. 2020 was the sixth 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-meter² 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 time. To accomplish these goals, horseshoe crabs were systematically counted and sexed at five beach sites during the spring and early summer of 2020, 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 5th and June 23rd, 2020, 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 crabs numbers, likely due to the substantial distance necessary for the crabs to travel from either the Ocean City or Indian River Inlet. The 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, Little Assawoman Bay will no longer be surveyed annually, but rather will be monitored every five years and included in future trend reports (McGowan and Bartow 2020).

Of the five beaches surveyed in 2020, 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. A sixth survey site, located in Rehoboth Bay, was initially set to be added in 2020, but complications arising from the ongoing COVID-19 pandemic made this addition unfeasible.

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 the beaches



Figure 1. Map of sites surveyed in 2020 horseshoe crab survey.

selected for this study were sandy beaches. These beaches were also selected for the survey because they were easily accessible to volunteers.

Survey Protocol

The spawning surveys were conducted in conjunction with the new and full moon cycles, occurring on May 5th, 7th, 9th, 20th, 22nd, 24th, and June 3rd, 5th, 7th, 19th, 21st, and 23rd. 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).

In prior years, each beach was surveyed by a team of volunteers who have been trained in the survey protocol and on how to determine the sex of horseshoe crabs. However, due to concerns regarding COVID-19, the CIB made the decision to not use volunteers and to complete all surveys using teams of two interns at each site. Prior to the start of the season, all staff and interns were required to attend a virtual training explaining and depicting the survey methods. Additionally, each survey team was again taught the methods during an in-person training session at the start of the season. Each in-person training was taught by a staff member who had previously completed the survey and could ensure the protocols were implemented accurately.

The methodology used to properly determine each quadrat had to be augmented to allow for physical distancing between the two team members completing the survey. As in previous years, 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.

In prior survey seasons, each team would then extend 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 designed to allow systematic placement of 100 1m² quadrats along the beach. 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.

However, the use of the rope was not feasible while maintaining social distancing. Instead, individual meters would be counted using a roll method. To perform this method, teams rolled the quadrat on its side along the survey line, skipping over quadrat numbers to get to the random numbers for each night. By rolling the quadrat end over end, the teams could recreate the rope pull method used to allow for

systematic placement of each square meter. This method was used for all 56 surveys completed in 2020, and does not impact the comparability between the 2020 survey season and any previous season.

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. 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 2020 study, with most cancelations occurring at the very beginning of the survey season. A total of four surveys were canceled in 2020 (Figure 2) due to storms or staff scheduling issues. Of these, one was missed during the third and fourth moon cycle, which typically corresponds to the nights with the largest crab counts. A Quality Control report for the 2020 season is presented in Appendix A.

Beach	5/5	5/7	5/9	5/20	5/22	5/24	6/3	6/5	6/7	6/19	6/21	6/23
<i>Bay Colony</i>	x											
<i>Ellis Point</i>	x											
<i>James Farm</i>												
<i>Peninsula</i>	x											
<i>Tower Road</i>										x		

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

Tagging Study

In past years, in conjunction with the spawning survey, 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>).

However, due to personnel issues arising from the COVID-19 pandemic, no horseshoe crabs were tagged during the 2020 survey season. 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.

Data Analysis

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.

Spawning densities from each survey event are also categorized into five categories based on the proportion of horseshoe crabs. These categories (zero, low, moderate, or high) are identical to those used by the Delaware Bay survey. "No crabs" indicates that zero crabs were found on a 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 (Zimmerman et al. 2019).

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 specific lunar period, defined as the five days around a full or new moon during which the survey occurs.

RESULTS

Total Crabs & Spawning Density

In total, the 2020 survey observed 16,388 spawning horseshoe crabs during 56 surveys at five beaches. This was an overall decline from the 2019 survey observations, but somewhat similar to the results found in 2018 (Table 2). Of the 16,388 horseshoe crabs observed, 13,919 were male and 2,469 were female.

The cumulative spawning density for the 2020 survey was 2.93 crabs per square meter (Table 1). The 2020 cumulative female spawning index (average number of female crabs per square meter) was 0.44 crabs per square meter. Both metrics saw a decline relatively proportional to the total crabs observed.

Of all five survey locations, Tower Road had the highest spawning density, while Ellis point had the highest female spawning index. Of all five sites, Peninsula had both the lowest spawning density and lowest female spawning index of all the beaches surveyed. All five survey sites saw a decline in spawning density, female spawning index, and mean nightly spawning crabs (Table 1), (Figure 3). These declines were not uniform but varied based on parameter and location.

Table 1. Spawning density, female spawning index, and sex ratio for the five locations surveyed in 2020

Location	Spawning Density	Female Spawning Index	M:F Sex Ratio
Bay Colony	1.85	0.25	6.3
Ellis Point	4.60	0.78	4.9
James Farm	2.27	0.39	4.8
Peninsula	0.20	0.05	2.8
Tower Road	5.77	0.73	6.9
Cumulative	2.93	0.44	5.7

Table 2. Historic spawning horseshoe crab survey totals, mean annual spawning density, mean 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
2020	16,388	2.93	0.44	5.7

Percentages of Horseshoe Crab Densities

When each survey is categorized by density, the 2020 survey season shows a decline in the number of high density spawning events, with 5% of surveys finding an average spawning density of more than ten crabs per square meter. Additionally, the number of low density surveys (those with less than 5 crabs per square meter) increased to more than 69%, as well as surveys with no crabs, which increased to 14.4%. The number of moderate surveys, which corresponds to an average of 5-10 spawning crabs per square meter, was similar to past years at 10.7%.

Table 3. Annual percentages of horseshoe crab density classifications in the Inland Bays.

Year	No Crabs	Low (<5)	Moderate (5-10)	High (>10)	No Survey
2015	9.5%	51.0%	4.0%	8.0%	27%
2016	9.5%	60.5%	6.5%	8.0%	14.5%
2017	5.0%	50.5%	11.0%	8.0%	25.5%
2018	3.0%	51.0%	11.0%	7.0%	28%
2019	1.5%	28.0%	8.0%	21.5%	20%
2020	14.4%	69.6%	10.7%	5.0%	6.7%

Sex Ratio

The 2020 cumulative sex ratio was 5.7, a moderate decline from the year prior (Table 2), (Garmoe et al. 2020). Of the five sites surveyed in 2020, Tower Road had the highest

sex ratio at 6.9 and the Peninsula had the lowest at 2.8 males for every female (Table 1). All five sites saw a decrease in sex ratio from 2019. In previous years, as total crab abundance increased, the sex ratio generally tended to do so as well (McGowan and Bartow 2020). However, this correlation was not observed in 2019 and no such correlation is present in the 2020 survey data.

Table 4. 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
2020	6.3	4.9	4.8	2.8	6.9	5.7

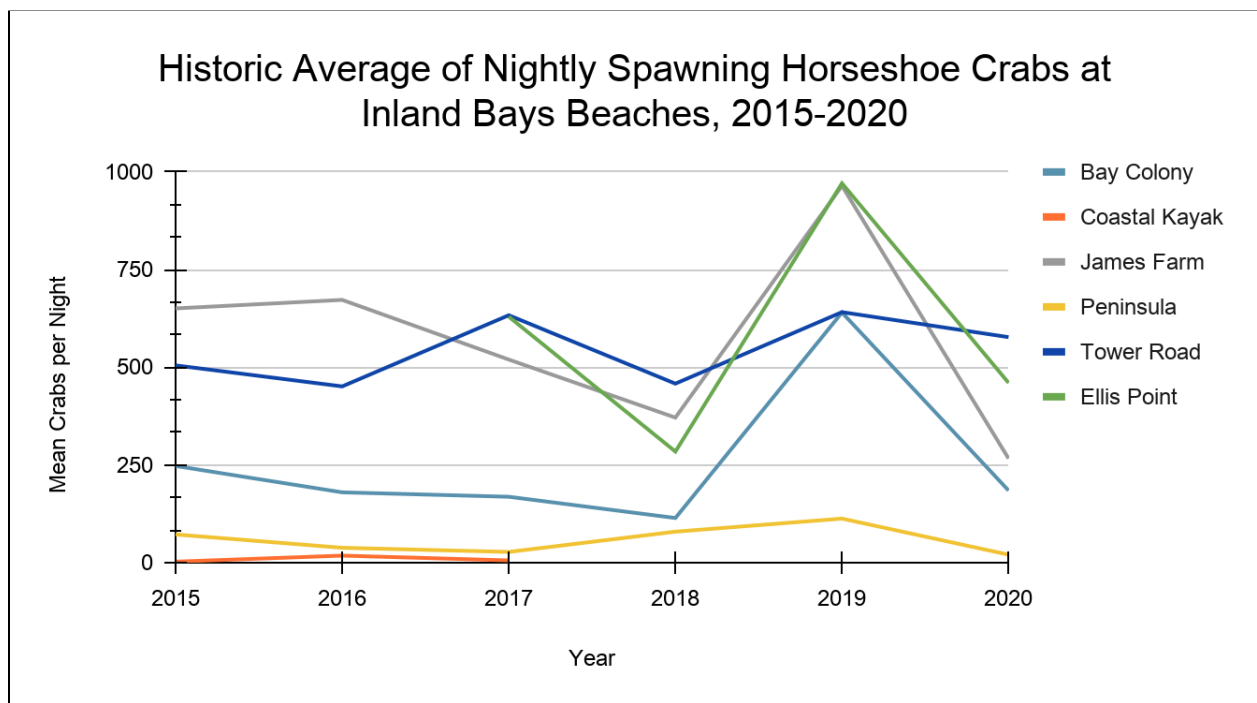


Figure 3. Mean nightly horseshoe crabs by location, 2015 to 2020.

Peak Spawning Period

Horseshoe crab spawning peaked temporally during the third lunar period, with 9,305 crabs recorded over the three survey nights (June 3rd-7th; Figure 4). Of the three nights, June 3rd recorded the highest average number of spawning crabs. Unlike prior years, only one cancellation occurred in the final three spawning periods, compared to six in 2019 and 13 in 2018 (Garmoe et al., 2020).

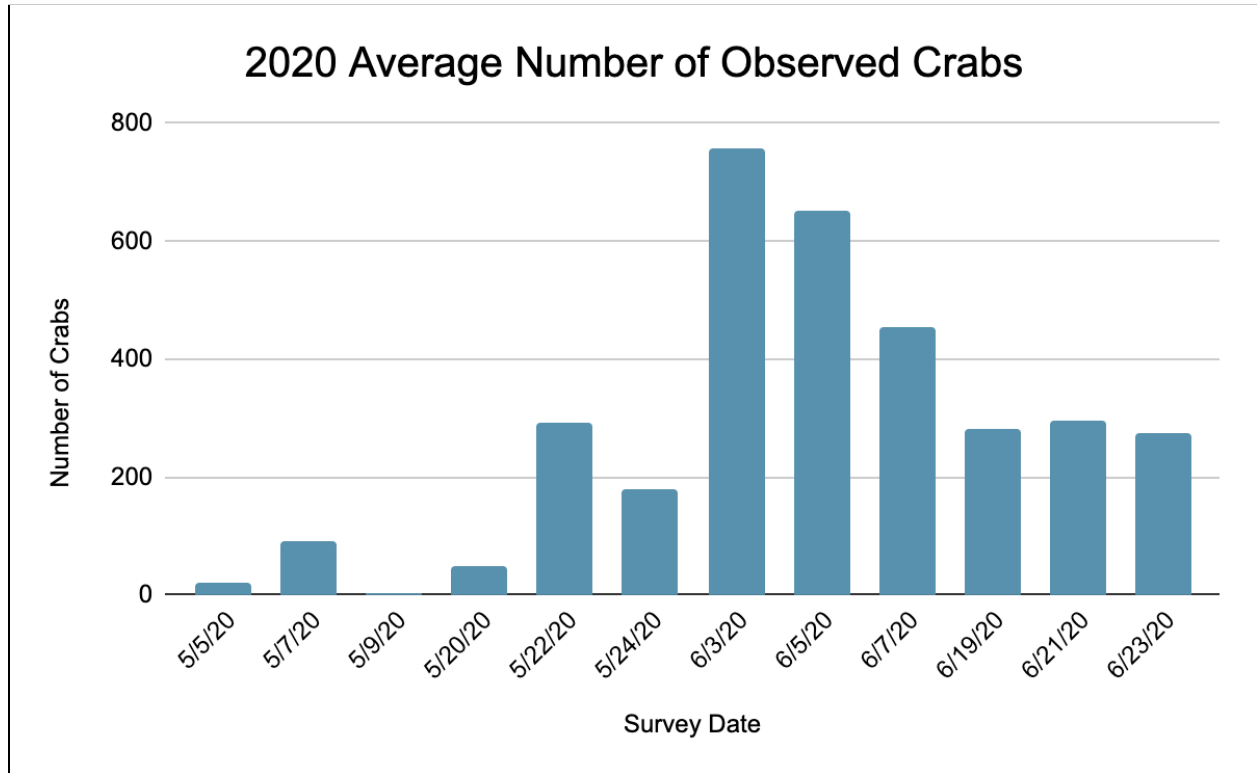


Figure 4. The average nightly number of spawning crabs across the five beaches surveyed in 2020

Horseshoe crab activity was positively correlated with water temperature ($p < 0.001$, $\tau = 0.384$) and salinity ($p < 0.01$, $\tau = 0.293$). Water temperature and salinity during each 2020 sampling event are presented below in Tables 4-5.

Table 5. Water temperature measurements (°C) from each sampling event. Survey cancellations are marked in red. Failure to collect a sample is indicated with an asterisk.

Date	Bay Colony	Ellis Point	James Farm	Peninsula	Tower Road
5/5/20			15.8		15
5/7/20	15	15	14.7	13.5	15
5/9/20	9	10*		10.5	8.5
5/20/20	14	12.5	11	14.5	14
5/22/20	18	18	15	18	17.5
5/24/20	15	15	14	16	16
6/3/20	24	21*		22	25
6/5/20	20	25.5*		23	24
6/7/20	22	22.5*		22.5	23
6/19/20	22	23.5	23	24.5	
6/21/20	25	25	24	26	25
6/23/20	25	26	22	27.5	26.5

Table 6. Salinity measurements (ppt) from each sampling event. Survey cancellations are marked in red. Failure to collect a sample is indicated with an asterisk.

Date	Bay Colony	Ellis Point	James Farm	Peninsula	Tower Road
5/5/20			25.2		24.1
5/7/20	23.6	23.5	26.5	27.1	24.4
5/9/20	20.3	21.2	25.6	25.7	24.6
5/20/20	27.5	28.1	28.9	24.5	26.7
5/22/20	25.8	27.1	28.9	25.8	27.2
5/24/20	26.4	27.2	27.6	26.6	26.8
6/3/20	25.5	26.2	27.9	28.3	28.9
6/5/20	26.1	27.2	29.5	26.5	28.9
6/7/20	27.2	28.6	27.2	27.4	28.1
6/19/20	27.2	27.1	28.5	28.3	
6/21/20	27.5	27.5	28.3	28.3	29.4
6/23/20	28.1	*	29.4	28.4*	

DISCUSSION

Overall Numbers and Spawning Density

2020 was the sixth year in which the protocols used in the Inland Bays matched those of the Delaware Bay survey. Overall, horseshoe crab observations declined from 2019, but were still near the approximate median of spawning populations recorded over the last six years. This held true for a number of specific metrics, such as the female spawning index, which declined in 2020, but was still within 0.1 crabs per square meter of every other year recorded since 2015 (Table 2). Similarly, the cumulative spawning density also fell, but was within an average of one crab per square meter as every other year since 2015 (Table 2).

Results of CIB tagging efforts observed that, in the years following their tagging, horseshoe crabs were more likely to be re-spotted in Delaware Bay or other regional embayments rather than returning to the Inland Bays to spawn (McGowan, 2018). When considered alongside evidence that horseshoe crabs exhibit very little annual site fidelity (Smith et al. 2010), the variations observed in the Inland Bays may be due in part, to annual fluctuations in site selection amongst the embayments in the larger Delaware region.

Water Temperature:

When analyzing the results from the 2018 survey, McGowan & Bartow (2020) theorized that a decline in spawning horseshoe crabs was due to an unusually cold winter and spring. While no such climate evidence is immediately noticeable this year, a lower than average early-season water temperature may have also impacted the results of the 2020 spawning survey. The average water temperature for the first two lunar periods was the lowest since the 2015 change in survey methods. On the eastern seaboard, horseshoe crab spawning generally peaks once water temperatures are between 15 °C and 20 °C (Cheng et al. 2016). In the Inland Bays, peak spawning usually does not occur until the higher end of this spectrum is reached (Table 6).

A cooler start to the season may have impacted horseshoe crab spawning for 2020. Additionally, an unusually warm 2019 season may have led to the record number of spawning crabs observed during that season as well. Additional years of surveys will be necessary to determine if cooler water temperatures impact overall spawning levels or only relevant to the onset and peak of spawning events.

Table 7. Average water temperature measurements (°C) for each lunar period. Peak spawning events are marked in yellow. Lunar periods adjusted for seasons with five segments to coincide by date.

Year	Lunar Period 1	Lunar Period 2	Lunar Period 3	Lunar Period 4
2020	12.91	15.23	22.88	24.64
2019	18.30	20.32	22.15	25.82
2018	15.47	18.09	22.40	21.77
2017	14.99	18.45	20.91	24.86
2016	13.28	16.83	22.73	23.00
2015	15.54	20.65	20.33	26.08

Sex Ratio

Sex ratio is an important metric when considering the genetic health of a spawning horseshoe crab population. A higher ratio of male to female crabs during spawning events 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 operational sex ratio is 2:1 (ASMFC 2019).

As in prior years, the spawning populations observed in the Inland Bays again met this bar across all five sites surveyed. However, the sex ratio reported for the Peninsula was the lowest cumulative score of any location over the last six years. All other sites saw a modest decrease in sex ratio, but all were still well above the 2:1 threshold (Table 3). Notably, the cumulative spawning density has been historically far more variable than the female spawning index, which indicates that variation in sex ratios (and total crabs observed) has been driven predominately by fluctuations in the male spawning population rather than the females (Table 2).

Impacts of the COVID-19 Pandemic

A potential impact of COVID-19 was a complete shift in the individuals conducting the surveys. Other than for training purposes, no individual who conducted a survey in 2020 had done so in 2019 or any year prior. Unfortunately, the personnel issues arising from COVID-19 kept in-person quality control site visits from occurring. Thus, corrective actions on the part of the project manager never occurred, so error on the part of the surveyors is in-theory, a not unlikely possibility in the 2020 survey. This may be particularly true for aspects such as determining the sex of the crabs, which involves more specialized knowledge than the act of counting the crabs themselves.

However, one notable difference regarding the 2020 survey was the lack of cancellations due to weather. Of all the surveys, only a single one at Tower Road was canceled due to weather (the other three cancellations were due to logistical issues at the start of the season). This is a substantial drop from prior years, when ten or more cancellations were not uncommon. Unfortunately, a persistent issue with this survey is the current inability to quantify the influence this may have on the overall results. Working with the Delaware Bay survey team and population modeling experts to determine a solution to this issue should be a high priority for the Inland Bays survey in future years.

Regional Comparisons

In prior annual reports, the results found in the Inland Bays would be compared to those of the Delaware Bay survey to see if any noticeable factors or trends were present between the two surveys. However, due to the COVID-19 pandemic, the Delaware Bay survey did not occur in 2020, and thus no such comparisons were possible. Additionally, while the annual Horseshoe Crab survey did occur in Maryland's Coastal Bays, the differentiation between survey methods make direct comparison difficult between the two embayments. However, in contrast to observations in the Inland Bays, the survey conducted in Maryland's Coastal Bays recorded an increase in spawning horseshoe crabs relative to 2019 (Toulan and Doctor, 2020).

Management Implications

The 2020 survey season observed a significant decline in the overall number of spawning horseshoe crabs present in the Inland Bays. This decline, while substantial relative to the 2019 survey results, is still consistent with results observed over the last six years under the current survey methodology. Furthermore, while it was impossible to compare these results to Delaware Bay in 2020, it is clear that spawning habitat in the Inland Bays share a similar capacity to that found in its northern neighbor. While the number of high-density spawning surveys declined in 2020, data from prior years indicate that the habitat in the Inland Bays has the ability to support horseshoe crab densities on par with those found in Delaware Bay.

This underscores the importance of this survey, and supports the eventual inclusion of Inland Bay's spawning data into future ASMFC stock assessments. Moreover, the results of this survey continue to highlight the importance of restoring and conserving natural sandy coastlines in the Inland Bays.

ACKNOWLEDGEMENTS

We would like to thank all of our incredible volunteers who were patient and understanding as we determined that prioritizing their safety meant keeping them from assisting with the 2020 survey. Even as he was unable to personally count and tag horseshoe crabs Dennis Bartow continues to be the blue-blood of this survey and deserves special recognition for his diligent work and efforts.

We would also like to extend our deepest gratitude to the nine CIB interns who helped us make this survey season possible: Amanda K Pappas, Amanda Williams, Bella Leishear, Bryanna Lisiewski, Carly Collins, Emily Kaiser, Gabriella Fritz, Justin Guider, Samantha Pringle, and Sydney Messick. Without their commitment to numerous late nights in the midst of finals, jobs, and the onset of a pandemic, this survey simply would not have occurred.

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Appendix A. Quality Control Report

Summary

Data recorded during the 2020 horseshoe crab survey were tested according to the quality control measures outlined in the program's EPA approved QAPP effective February of 2016. A random sampling for accuracy of 10% of the data entered from field sheets to electronic formats was enacted for the 2020 data. No inaccuracies were found to be present in the random sampling of data sheets.

Due to personnel issues stemming from the COVID-19

, in-field quality control measures, such as site visits and quadrat recounts were not completed. All teams demonstrated proper protocol and data recording methods during the initial supervisory survey, but the implications of this are unclear. Teams did fail to collect both salinity and temperature measurements on occasion.

Issues and Corrective Actions

Due to the longer timeframe between surveys completed by volunteers, Team Leaders will be retrained in all survey protocols, particularly those which may still be augmented due to the COVID-19 pandemic. Team Leaders will be reminded of the importance to collect temperature and salinity data at all surveys 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 2021 survey. The LAB site will be monitored every five years in order to continue to include it in trend reports. Research into spawning population models based on this survey, along with the potential sediment sampling of survey beaches should be considered for future seasons.