



Assessment of Spawning Horseshoe Crabs (*Limulus polyphemus*) at Cape Cod National Seashore, 2008-2009

Natural Resource Technical Report NPS/CACO/NRTR—2012/573



ON THE COVER

Spawning horseshoe crabs at Hog Island (top left and right) and Marsh 2-3 (bottom left), Pleasant Bay, Cape Cod National Seashore. Horseshoe crabs tagged with US Fish and Wildlife button tags (bottom right). Photographs by: Mary-Jane James-Pirri.

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Executive Summary

The objectives of this study were to re-survey spawning horseshoe crabs at Cape Cod National Seashore (CACO), compare data on spawning indices and sex ratios to a previous study conducted in 2001 to 2002, and to evaluate the influence of the ban on horseshoe crab harvesting within CACO (instituted in 2001) on these parameters. A second objective was to use both traditional tagging and acoustic telemetry to document movement patterns of horseshoe crabs in Pleasant Bay. Lastly, horseshoe crab egg counts and juvenile horseshoe crab abundance were estimated at selected spawning beaches in CACO and at Monomoy National Wildlife Refuge (NWR).

Horseshoe crab spawning surveys were conducted at CACO in Pleasant Bay and Nauset Estuary in 2008 and 2009. Crabs were also tagged with US Fish and Wildlife (USFWS) button tags to track movement patterns in Pleasant Bay. Sediment samples from spawning beaches were collected to evaluate horseshoe crab egg (eggs, embryos, and trilobite larvae combined) and juvenile crab abundance. Concurrently with this effort, 55 crabs were fitted with acoustic transmitters to track subtidal movement in Pleasant Bay from 2008 to 2010. Results from that study have been previously published in James-Pirri (2010) and are not presented in this report. This study compliments previous work conducted on the spawning dynamics and movement patterns of horseshoe crabs in Cape Cod National Seashore (James-Pirri et al. 2002, James-Pirri et al. 2005).

Horseshoe crab spawning indices (number of females per unit area) in Pleasant Bay and Nauset Estuary in 2008-2009 ranged from 0.01 to 0.98 females 25 m^{-2} and were statistically similar to those observed in the 2000-2002 study. Spawning sex ratios in Pleasant Bay were 1 female to 8 males and were more male-biased than those observed in 2000-2002. Spawning sex ratios in Nauset Estuary were 1 female to 1.3 males in 2008-2009 and were not different from those observed during the earlier study. Single females (a female with no male in amplexus or nearby satellite males) were observed for the first time in CACO during the current study. Two to 4% of females at survey sites had no mate and single females were observed in 22 to 25% of the surveys. Seventy-two percent of the spawning clusters in Pleasant Bay were paired clusters (one female to one male), whereas 97% of the spawning clusters in Nauset Estuary were pairs. Over 2000 horseshoe crabs were tagged with US Fish and Wildlife Service (USFWS) button tags in Pleasant Bay from 2008-2009. The overall live recapture rate was 12% as of August 2011. Twenty-four percent of live recaptures were re-sighted in subsequent years. Eighty-eight percent of the live recaptures were found within 2.5 km of the spawning beach where they were originally tagged. All of the live recaptures, except for one, were re-sighted within Pleasant Bay.

Horseshoe crab egg abundance in beach sediments ranged from 0 to 67 eggs (eggs, embryos, and trilobite larvae combined) per core. Egg abundance in 2008-2009 was lower than in 2000-2002 in all three embayments (Pleasant Bay, Nauset Estuary, and Monomoy NWR). Juvenile horseshoe crab abundance ranged from 0 to a maximum of 11 juveniles per 0.25 m^2 (observed at Barley Point, Pleasant Bay). Juvenile density varied greatly, with highest mean densities observed at Barley Point and Pochet Inlet washover sites in Pleasant Bay. Very few juvenile crabs were observed at Monomoy NWR and Nauset Estuary. Juvenile horseshoe crabs ranged in size from 6 to 60 mm prosomal width and represented age classes of year 2, year 3, year 4, and older juvenile crabs.

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Prologue

This document summarizes data associated with spawning surveys and tag-recapture data for horseshoe crabs in Pleasant Bay and Nauset Estuary, Cape Cod National Seashore from 2008-2009. This study complemented spawning surveys that were conducted during these same years in other areas of Cape Cod (Wellfleet Bay, Monomoy National Wildlife Refuge, and Buzzards Bay) and regionally (Massachusetts, Rhode Island, and Connecticut). The network of regional spawning surveys, currently in their fourth year [2008-2011]), is informally organized by the Southern New England Horseshoe Crab Technical Committee. This committee is composed of members of academia (Sacred Heart University, University of New Hampshire, University of Rhode Island, Worcester Polytechnic Institute), state (Massachusetts Division of Marine Fisheries), federal (US Fish and Wildlife Service), conservation organizations (Massachusetts Audubon Society), and citizen stakeholders (Horseshoe Crab Conservation Association). Data from spawning surveys are provided to both state and federal managers to assist with the fisheries management of this species. This study complements earlier work in Cape Cod National Seashore from 2000-2002 (James-Pirri et al. 2002, James-Pirri et al. 2005), and results from the current study were compared to these earlier data in this report. Additionally, in conjunction with the spawning surveys a behavior study using acoustic telemetry to track subtidal movements of horseshoe crabs in Pleasant Bay was conducted from 2008 to 2010. These data and results have been previously published in a special issue of *Current Zoology* (James-Pirri 2010) and are not presented in this document.

Introduction

The horseshoe crab is an integral part of the marine ecosystem. They are predators of estuarine benthic infauna (e.g., bivalves, worms) and are prey for loggerhead sea turtles and sharks (Botton 1984, Walls et al. 2002). The reproductive biology and behavior of horseshoe crabs is unique among marine arthropods. Horseshoe crabs come ashore to spawn along protected sandy beaches around the new and full moons in May and June. Males intercept females as they approach spawning beaches, clasping onto a female with a pair of modified pedipalps or claws (Brockman 1990, 2003a). A spawning pair (a female in amplexus with an attached male) may have several satellite males that crowd around pair forming large spawning clusters along the water's edge (Brockmann 1990, 2003a). Fertilization is external and eggs are laid in excavated nests, with females often nesting several times during one tide (Shuster and Botton 1985, Brockmann 2003b). Horseshoe crab eggs are an important food resource for migrating shorebirds in Delaware Bay. While several lines of evidence support the intrinsic and critical link between the viability of migratory shorebird populations and egg availability in that region (Castro and Meyers 1993, Clark 1996, Botton et al. 2003, Karpanty et al. 2006, Mizrahi and Peters 2009), the reliance of shorebirds on horseshoe crab eggs in New England is not as well understood. Trilobite larvae emerge from nests in four to six weeks and settle on adjacent mudflats after a brief planktonic period (Botton and Loveland 2003). Post-settlement larvae and juveniles can be found in intertidal flats and shallow subtidal areas, but may migrate to deeper water as they grow (Rudloe 1981, Shuster 1982, Anderson and Shuster 2003, Carmichael et al. 2003, Burton et al. 2009). Since little research has been conducted on the ecology of juvenile horseshoe crabs, this life history stage is not well understood.

A charismatic species, the horseshoe crab has remained virtually unchanged for 400-500 million years (Shuster and Anderson 2003). On Cape Cod, as elsewhere throughout their range, horseshoe crabs are harvested as bait for conch and eel fisheries and are used by the biomedical industry. The biomedical industry produces *Limulus* amebocyte lysate or LAL from their blood. LAL is the pharmacological standard used to detect fever-causing bacteria (pyrogenic endotoxins) in all injectable drugs (e.g., vaccines) and implantable medical devices. LAL is also used for the diagnosis of diseases such as spinal meningitis. In addition, new applications, including the detection of bacterially contaminated meat, fish, and dairy products, continue to be found (Novitsky 1984, 2009, Berkson and Shuster 1999, Walls et al. 2002). Currently, there is no synthetic equivalent to LAL. After bleeding, horseshoe crabs are returned to the water where they were collected, usually within 72 hours (Walls et al. 2002). Early estimates of mortality due to biomedical bleeding were 15% (Rudloe 1983); however, recent studies that have included handling stress place the mortality closer to 30% (Hurton and Berkson 2004; Leschen and Correia 2010).

Horseshoe crab harvesting and management on Cape Cod is an extremely contentious issue, one that polarizes not only fishing and environmental groups, but the general public as well. Historically, as many as million horseshoe crabs were destroyed each year as part of local shellfish predator control programs. Many Massachusetts towns had a three-cent bounty on horseshoe crab tails and in the early 1960s, the town of Chatham paid as much as \$1500 in bounty (Germano 2003). As recently as 2000, regulations requiring fishermen to kill horseshoe crabs encountered while shellfishing or risk a fine were still on the books of eight Massachusetts

towns (Germano 2003). Horseshoe crabs have been commercially harvested for both bait and biomedical purposes from the waters of Cape Cod including Pleasant Bay, which is partially within the boundaries of Cape Cod National Seashore (CACO), for over three decades. In Pleasant Bay, the majority of crabs are harvested for biomedical purposes (Rutecki 2002, Rutecki et al. 2004). A small bait fishery has recently emerged in Town Cove, which is within the Nauset Estuary system, where the harvest has been < 3000 crabs annually since 2007, with no fishery reported prior to 2005 (V. Malkoski, personal communication). Both the bait and biomedical fisheries preferentially target female crabs as they are larger and yield a greater volume of bait and blood (Rutecki et al. 2004).

Commercial landings of horseshoe crabs showed a dramatic increase in harvest from 1990-1996 in the mid-Atlantic region. Concerns about the sustainability of the fishery prompted the Atlantic States Marine Fisheries Commission (ASMFC) in 1998 to require all states with horseshoe crab fisheries to initiate basic monitoring, regulatory, and reporting procedures (ASMFC 1998, 1999). This prompted the Department of the Interior (DOI) and the National Park Service (NPS) to review the status of horseshoe crabs at CACO, and in 1999 the park was made aware of the illegal harvest of horseshoe crabs from park waters (Pleasant Bay) for bait and for biomedical purposes. In 2000, CACO implemented active protection of horseshoe crabs due to the DOI Solicitor's review determining that the horseshoe crab was neither "shellfish" nor "fish" under state game laws, and was therefore considered a protected wildlife species by the NPS. In response to this action, Associates of Cape Cod (an LAL producing biomedical facility in Falmouth, MA) and a horseshoe crab biomedical harvester initiated legal action against the DOI to block the prohibition of harvest from federal waters on Cape Cod. During the litigation (spring and summer of 2000) the harvester was permitted, as per court order, to harvest horseshoe crabs for biomedical purposes within CACO boundaries. However in 2001, the courts ruled in favor of the DOI, stating that the Seashore was correct in enforcing the prohibition for the taking of wildlife. Therefore as of 2001, the harvest of horseshoe crabs from all federal Seashore lands, including those in Pleasant Bay, was prohibited in perpetuity. In 2006, the non-federal waters of Pleasant Bay were closed by the Commonwealth of Massachusetts to bait harvest after a dramatic increase in commercial landings (194 crabs in 2001 to nearly 40,000 crabs in 2006) drew concerns from fishery managers about the sustainability of the Pleasant Bay fishery (Commonwealth of Massachusetts 2006). As of 2011, the bay remains closed to bait harvest but biomedical harvesting is allowed in the non-federal areas of Pleasant Bay. There is only one LAL producing facility in New England (Associates of Cape Cod) and horseshoe crabs in Pleasant Bay are an important source for this facility. Unfortunately statistics on the proportion of the biomedical harvest attributed to Pleasant Bay are not available from the Commonwealth of Massachusetts due to confidentiality issues.

In response to the ASMFC's request for monitoring, the NPS contracted with a University of Rhode Island Cooperator (Dr. M. J. James-Pirri) to collect information on horseshoe crabs within CACO in 2000. This resulted in the first standardized and systematic spawning surveys and tagging program for horseshoe crabs in Southern New England. The research was coordinated with other agencies (US Fish and Wildlife Service [USFWS] and Massachusetts Audubon) on Cape Cod and resulted in a Cape-wide standardized baseline dataset for horseshoe crab population characteristics (e.g., spawning indices, spawning sex ratios, size data, egg abundance, and movement patterns) (James-Pirri et al. 2002, 2005, Kurz and James-Pirri 2002). The objectives of the current study were to re-survey some of the same spawning survey sites within

CACO (Pleasant Bay and Nauset Estuary) to document the influence of the 2001 closure of federal waters to harvest on horseshoe crab spawning indices and spawning sex ratios. A second objective of the current research was to document movement patterns of horseshoe crabs using both traditional tagging and acoustic telemetry to gather detailed information on subtidal movement patterns and habitat use, including spawning site fidelity, of horseshoe crabs in Pleasant Bay (James-Pirri 2010). Finally, abundance of horseshoe crab eggs and juvenile horseshoe crabs was determined at selected spawning beaches in Pleasant Bay, Nauset Estuary (both within CACO), and at Monomoy National Wildlife Refuge (NWR) and egg count data were compared to the 2000-2002 study (juvenile horseshoe crabs were not surveyed in 2000-2002).

Methods

Survey Sites

Sites surveyed for spawning horseshoe crabs in Pleasant Bay were: Marsh 2-3, Hog Island, and Barley Point (Figure 1). A washover area in Pochet Inlet (north Pleasant Bay) was periodically visited to document spawning activity in this area. Nauset Beach in Nauset Estuary was also surveyed (Figure 1). Three of the spawning beaches surveyed in 2000-2002 were re-surveyed in this study: Marsh 2-3, Hog Island, and Nauset Beach (James-Pirri et al. 2002, 2005). The Pleasant Bay Northville site, surveyed in 2000-2002, was no longer in existence due to the North Inlet breach on Nauset Beach that occurred during the April 2007 Nor'easter (Adams and Giese 2008). The Barley Point site (property of the Parra family) was added for the current study.

Spawning Surveys

Spawning horseshoe crabs were surveyed using a modification of the Delaware Bay spawning survey method (Smith et al. 2002). This method was identical to the method used during the 2000-2002 study (James-Pirri et al. 2002, 2005). The modification for the Cape Cod region involved increasing the size of the sample quadrat from 1 m² (1 m x 1 m) to 25 m² (5 m X 5 m), since spawning densities on Cape Cod were much lower than those observed in Delaware Bay (James-Pirri et al. 2005).

At each survey site a coin flip determined the starting point of the survey (e.g., the north or south end of the beach) and a random number table was used to locate the position of the first quadrat within the initial 10 m of beach. After the first 25-m² quadrat was located, all subsequent quadrats were placed immediately adjacent to each other, resulting in a continuous survey transect delineated by individual replicate quadrats. Each quadrat was located adjacent to the swash zone, with the quadrat extended into the water. Over the course of the survey the leading edge of the quadrat moved slightly down the beach slope to coincide with the receding tide.

Spawning surveys were conducted simultaneously at each survey site on three days during new and full moon high tides, with surveys taking place throughout a 5-day period (2 days prior [day 1], the day of [day 3], and 2 days after [day 5]) around the new or full moons in May and June (Table 1). Limited surveys were also conducted during the first moon period in July; however, spawning had mostly ceased by this time and these data were not included in analyses because not all sites were consistently surveyed during this moon period. Surveys commenced at peak high tide and were conducted during both nighttime and daytime tides, if logistically possible. Marsh 2-3 could not be surveyed at night due to safety issues (e.g., walking across a flooded, ditched marsh at night). Every effort was made to conduct surveys on the same days of the moon period (e.g., day 1, 3, and 5) to avoid having the day of the survey as a confounding factor. Environmental conditions (e.g., weather, wave height, water temperature) were recorded prior to the start of each survey. All horseshoe crabs within each quadrat were counted, and the number and size of spawning clusters (females with attached males and/or satellite males in distinct spawning groups), single males, and single females were recorded. A horseshoe crab was counted if more than half of its body was inside the quadrat when it the quadrat was first laid down. Care was taken not to double count crabs that moved from adjacent, previously counted quadrats.

Spawning indices, defined as the number of female crabs in a 25-m² quadrat, were calculated for each survey. Mean spawning indices, weighted by the number of quadrats sampled (a proxy for beach length), were calculated by moon period for each survey site and year. Sex ratios (number of females to males) were calculated for each survey based on the total number of males and females observed.

Since spawning indices did not adhere to the assumptions of Analysis of Variance (ANOVA), analyses were conducted using the permutation procedures of PERMANOVA+ for PRIMER (Primer-E Ltd, Plymouth, UK, Clarke and Warwick 2001, McArdle and Anderson 2001, Anderson et al. 2008). PERMANOVA+ is an add-on package that extends the resemblance-based methods of PRIMER to allow the analysis of multivariate (or univariate) data in the context of more complex sampling designs. Like other programs in the PRIMER software package, PERMANOVA is a permutation procedure and a robust analysis tool for ecological data because, unlike traditional multivariate analyses, it makes no explicit assumptions regarding the distribution of the original variables (McArdle and Anderson 2001, Anderson et al. 2008). PERMANOVA+ was used to compare the 2008-2009 spawning indices to data from 2000-2002 to determine trends in spawning indices through time at each site. To evaluate if the harvest closure influenced spawning indices, a before/after control/impact model was used with the PERMANOVA+ model containing the following terms: survey site, study period, and the survey site-study period interaction. Nauset Beach site represented the control site as the Nauset Estuary system (e.g., Town Cove) has only had recent, small fishery (< 3000 crabs annually with no reported fishery prior to 2005) (V. Malkoski, personal communication), and Marsh 2-3 was the impact site. The 2000-2002 study period represented the before time, and the 2008-2009 study period represented the after time. The effect of the closure was considered significant if the interaction term was statistically significant. To determine if spawning indices at individual survey sites changed between the study periods, permutation tests were conducted using a macro in Microsoft Excel to compare the original test statistic (difference between the mean spawning indices per site) to the permuted distribution of test statistics (500 permutations), and a probability value was calculated based on a 2-tailed distribution of the permuted test statistics. Linear regression was used to evaluate the relationship between water temperature, and a ranked ANOVA was used to evaluate the relationship between spawning indices and wave height. Spawning sex ratios among survey sites and among study periods were compared using Chi-Square tests. If a significant Chi-Square Test was found, Freeman-Tukey Deviates (Sokal and Rohlf 1995) were calculated to determine which sex occurred at a higher or lower frequency than expected.

Table 1. Dates of full and new moons. Horseshoe crab spawning surveys were conducted two days before, the day of, and two days after the full or new moon.

Year	Full Moon ¹	New Moon ¹
2000 ²	May 18, June 16	May 4, June 2, July 1
2001 ²	May 7, June 22, July 5	May 22, June 21
2002 ²	May 26, June 24	May 5, June 10, July 10
2008	May 19, June 18	May 5, June 3, July 2
2009	May 9, June 7, July 7	May 23, June 22

¹ July survey dates were not included in analyses, as spawning had mostly ceased by this moon period.

² Dates for surveys conducted in 2000 to 2002 are shown for comparison (James-Pirri et al. 2005).

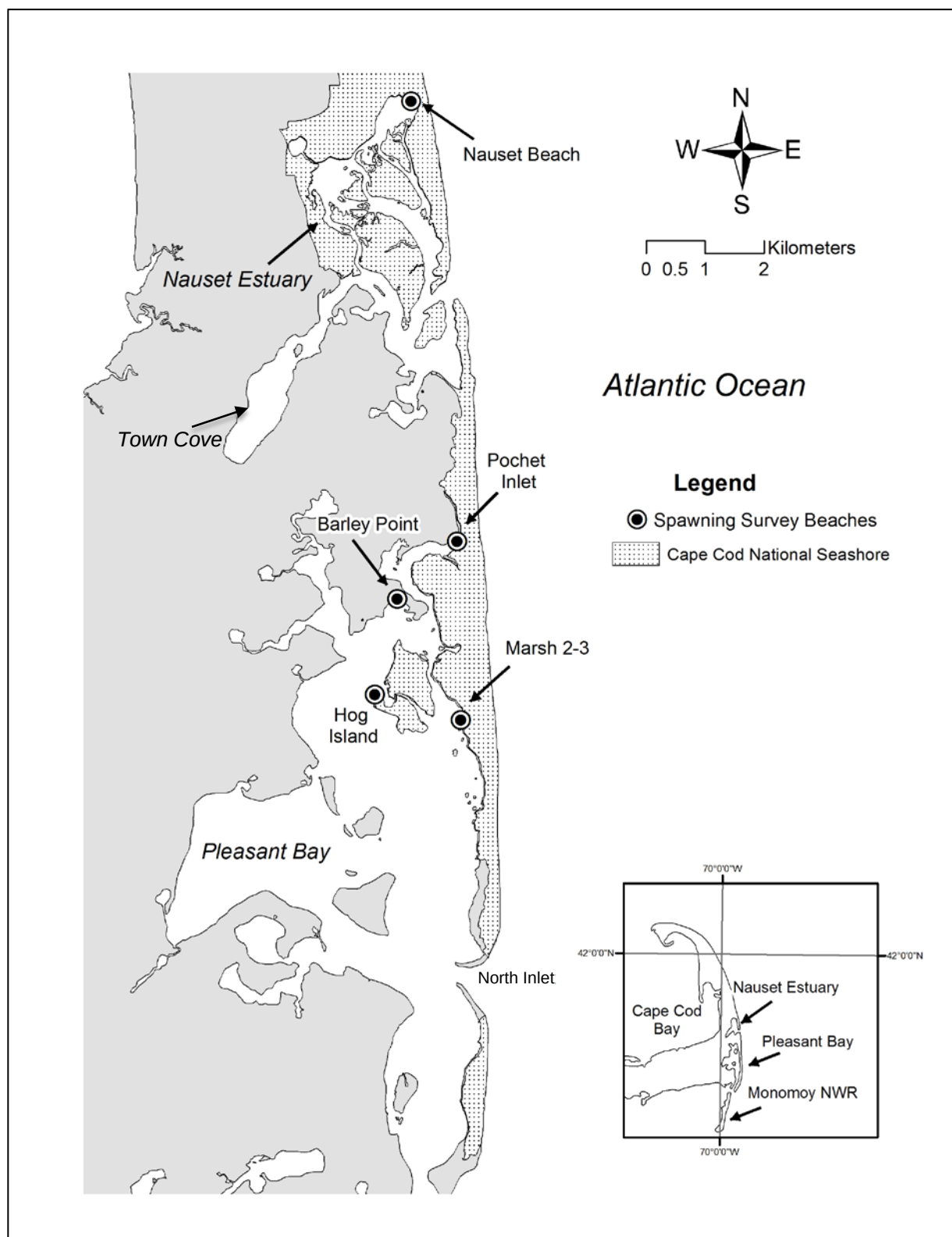


Figure 1. Map of horseshoe crab spawning survey sites and tagging locations (horseshoe crabs were not tagged in Nauset Estuary).

Tagging

Spawning adult male and female horseshoe crabs were tagged with USFWS button tags as part of the Horseshoe Crab Cooperative Tagging Program coordinated by the Maryland Fisheries Resources Office (MFRO) (USFWS 2011). A smaller version of the original button tag used in Delaware Bay (Figure 2) was used on Cape Cod, as horseshoe crabs are smaller in New England than their mid-Atlantic counterparts (James-Pirri et al. 2005). All button tags carried a unique identification number, toll free phone number (1-888- LIMULUS), and the words “Report” and “Release”. Signage, provided by MFRO, indicating the presence of tagged crabs, was placed at major boat landings on Pleasant Bay to encourage the public to report tagged horseshoe crabs. MFRO sent pewter reward pins and a certificate of participation to individuals who reported tagged crabs. Several interns also searched for tagged crabs in 2008 and 2009 while working on other components of this study (e.g., spawning surveys). Tags were attached to the lower point of the prosoma by drilling a 2.8-mm hole and securely inserting the tag stem into the hole. There is no mortality associated with this tagging procedure (Mattei et al. 2011). Information recorded for each crab included prosomal width, sex, tagging location, and tagging date. Crabs were tagged at spawning beaches in Pleasant Bay (crabs were not tagged in Nauset Estuary) (Figure 1) from May to July in 2008 and 2009. Tagging data were sent to MFRO and information on re-sighted individuals (location and date of re-sight, disposition of crab [dead or alive]) was received from MFRO in the fall of each year. Since horseshoe crabs reach a terminal molt upon maturity (Shuster 1955, Smith et al. 2009), the tags are retained for a long time. Horseshoe crabs tagged with the USFWS button tags have been reported alive as long as nine years after tagging (Swan 2005, S. Eyler, personal communication). Sizes of tagged male and female horseshoe crabs were compared between the study periods using ANOVA.

Egg Abundance

Sediment cores (10 cm diameter, 20 cm deep) were collected from all survey sites as well as from North Monomoy Island, Monomoy NWR (Figure 1) during early to mid-July to estimate horseshoe crab egg abundance (the summed counts of eggs, embryos, and trilobite larvae). At each survey site, approximately 20, 1-m² quadrats were randomly located in the mid-tide zone and a composite core consisting of five cores (one in the center of the quadrat and one at each of the four corners) were collected from each section (after James-Pirri et al. 2005). Each of the five cores comprising the composite core were divided into shallow sediment (top 5 cm) and deep sediment (bottom 5 to 20 cm) to evaluate if eggs were present in shallow sediments where they would be available to foraging shorebirds. A composite core allowed for a larger spatial coverage of the sample while minimizing sorting time. The shallow and deep sediment sections from the five cores were placed in separate buckets, thoroughly mixed, and a sub-sample representing the same volume (10 cm diameter by 5 cm deep for shallow sediment and 10 cm diameter by 15 cm for deep sediment) was removed, and placed in plastic bags for latter sorting. Sediment was sifted through a 1-mm sieve and all eggs, embryos, and trilobite larvae were enumerated. Mean counts of horseshoe crab eggs (eggs, embryos, and trilobite larvae combined) were calculated for each site for shallow and deep sediments. Mean egg counts were compared between study periods for each site using permutation tests.



Figure 2. Pewter reward pin (left) sent by MFRO to individuals reporting tagged horseshoe crabs, original button tag used in Delaware Bay (middle), and smaller button tag (right) used in this study (quarter and ruler included for size reference).

Juvenile Horseshoe Crab Abundance

Juvenile horseshoe crab abundance was estimated using 0.25-m² quadrats (0.5 m by 0.5 m) at the Barley Point survey site during low tide every 7 to 10 days from 16 May to 3 July 2009.

Approximately 20 to 30 quadrats were randomly located along two transects, with one transect adjacent to the swash zone (lower intertidal zone) and the other 10 m from the swash zone in the upper intertidal zone. These locations were selected based upon observations of juvenile horseshoe crabs in the lower to upper intertidal zone. In each quadrat the top 3 cm of sediment was removed and sieved through 3-mm mesh. All juvenile horseshoe crabs were measured (prosomal width) and then released. Age classes for juvenile horseshoe crabs were estimated after Sekiguchi et al. (1988).

During mid-July (10-14 July 2009) four additional spawning beaches (Nauset Beach, Hog Island, Pochet washover, and North Monomoy Island at Monomoy NWR) were surveyed for juvenile horseshoe crabs using the above methods. These surveys only lasted one day each and were conducted in an effort to identify other areas where juvenile crabs may be present.

Results

Spawning Indices

Spawning indices ranged from 0 to 2.8 female crabs 25 m⁻² per moon period (maximum value from Hog Island during daytime May 2009 full moon surveys). Spawning indices were higher during day surveys for both Pleasant Bay and Nauset Estuary survey sites (Figure 3). Similar to the 2000-2002 study, peak spawning usually occurred during May and spawning was finished by late June (Figures 4 to 7). There were no significant differences (permutation tests, $p > 0.05$) in the spawning indices between the two study periods for any of the survey sites (Table 2).

The full model (survey site, study period, survey site-study period interaction) PERMANOVA analysis was used to determine if spawning indices differed between study periods. The model was not significant (PERMANOVA, interaction term, Pseudo- $F = 1.354$, $p = 0.2577$) indicating that horseshoe crab spawning indices in Nauset Estuary and Pleasant Bay showed similar trends between the two study periods.

There was a weak, but significant positive relationship between water temperature and spawning indices (linear regression, $r^2 = 0.056$, $p = 0.0029$) (Figure 8). In 2008, increased spawning indices were associated with wave heights greater than 12 inches (30 cm) (ranked ANOVA, $p = 0.0401$, Least Squares Means, $p < 0.05$) (Figure 9), but this was due to one survey at Barley Point with a high spawning index (2.4 females 25 m⁻² on 21 May 2008). If this datum was omitted, the mean spawning index would be 0.21 females 25 m⁻² in 2008 and was not significantly different from indices at other wave heights (ranked ANOVA, $p = 0.1583$) (Figure 9). In 2009, there was no difference in spawning indices with wave height (ranked ANOVA, $p = 0.7415$).

A storm event in 2008 at the northern end of South Nauset Beach adjacent to the upper reach of Pochet Inlet created a sand washover area. The washover area is just south of the entrance to the sand road from South Nauset Beach parking lot. Horseshoe crabs spawn on the newly created sand flats (Figure 10), but this area was only surveyed occasionally because of restricted access due to nesting by the federally endangered piping plover (*Charadrius melodus*).

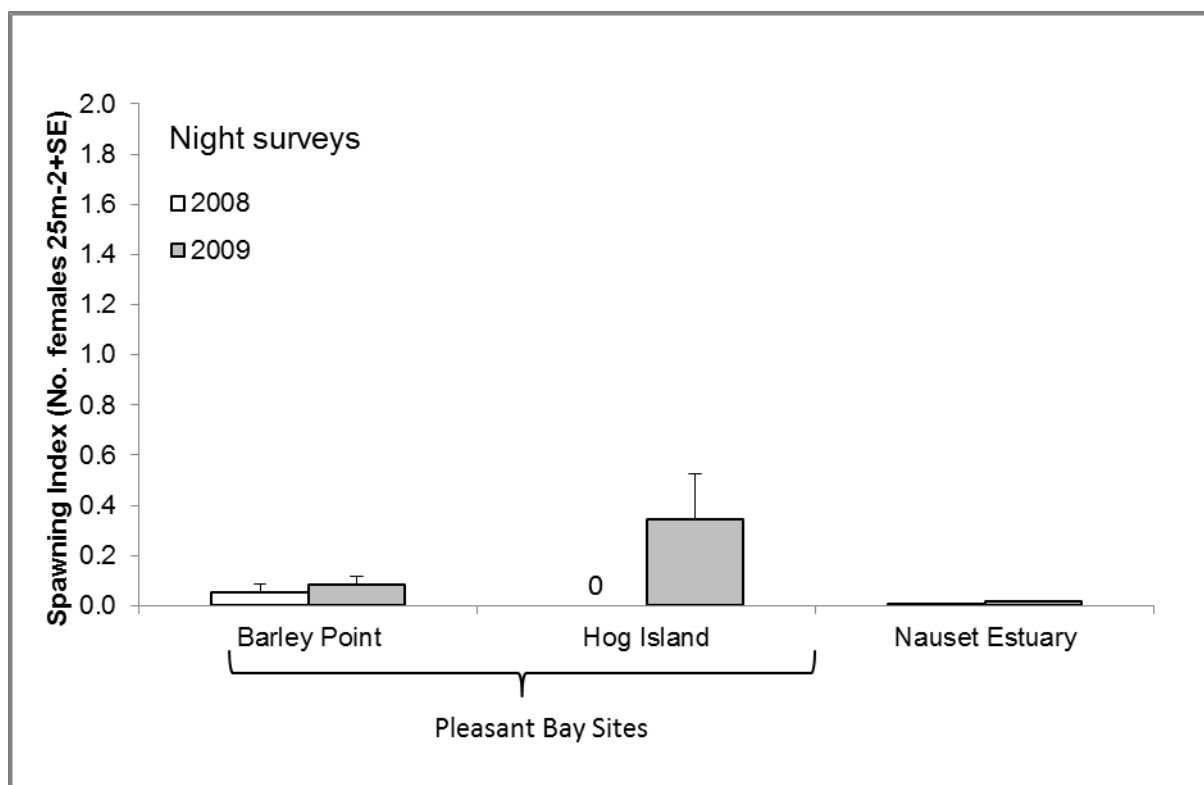
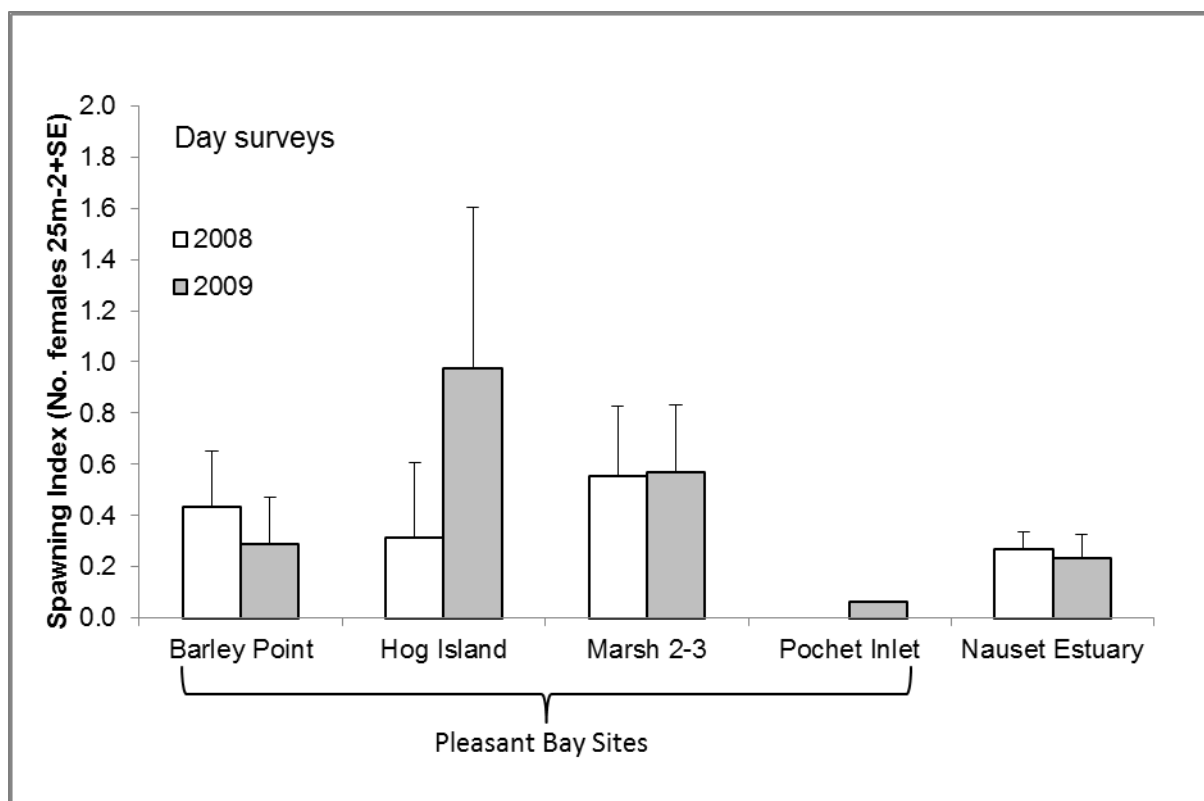


Figure 3. Mean weighted spawning indices (number of female crabs $25 \text{ m}^{-2} + \text{SE}$) for day (top graph) and night (bottom graph) surveys in Pleasant Bay and Nauset Estuary 2008 and 2009. Note: Pochet Inlet was only surveyed in 2009 during the day.

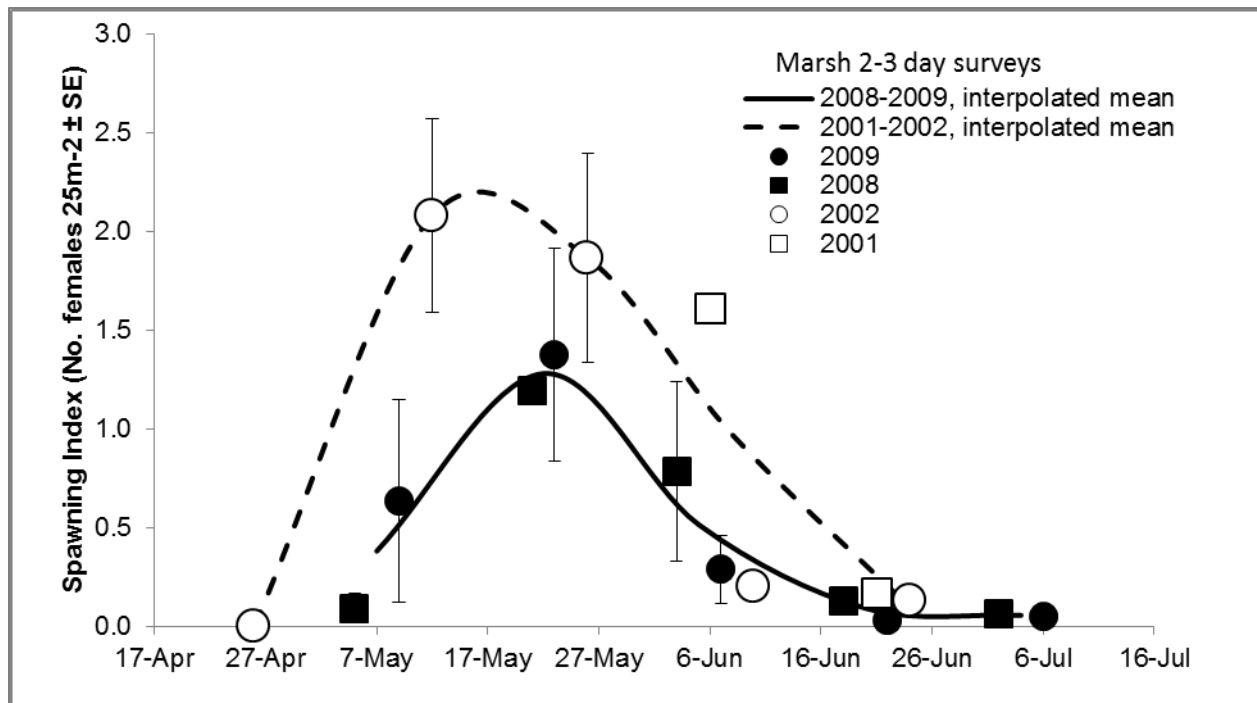


Figure 4. Mean weighted spawning indices (number of female crabs 25 m⁻² ± SE) by moon period at Marsh 2-3. Lines are interpolated means for each study period. Note: Marsh 2-3 was only surveyed during the day.

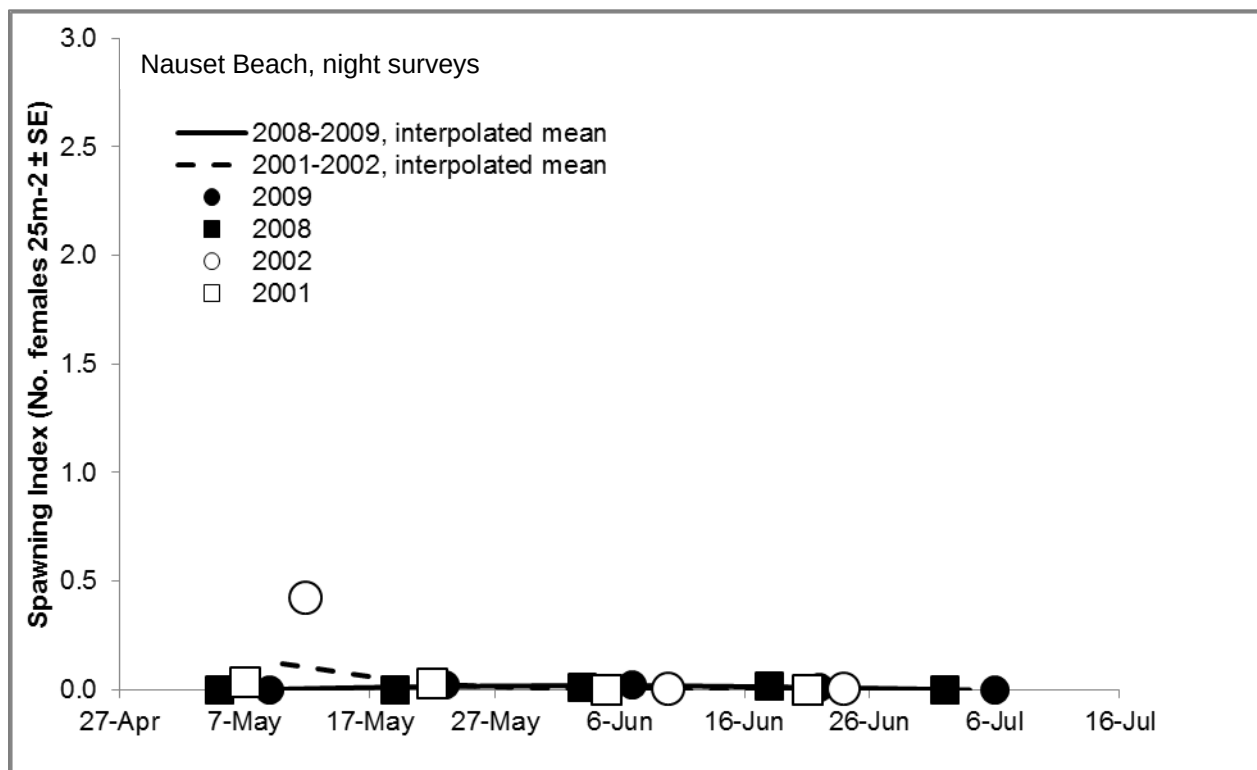
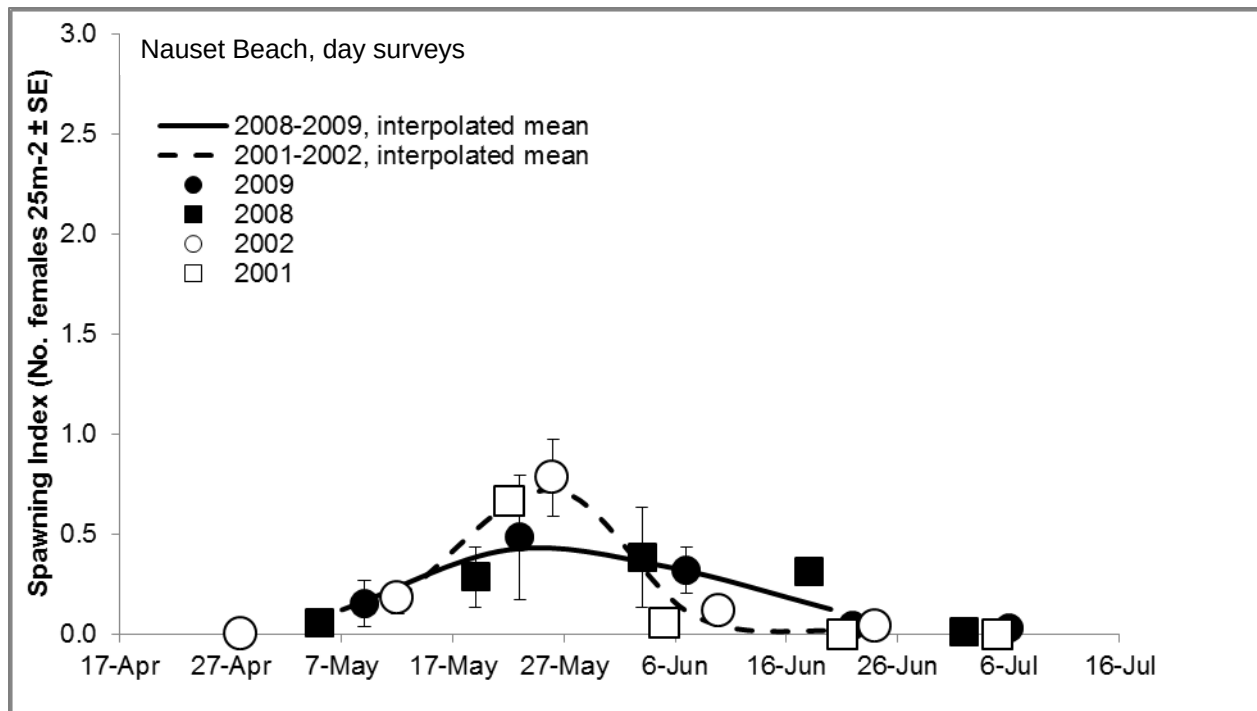


Figure 5. Mean weighted spawning indices (number of female crabs 25 m² $\pm$ SE) by moon period for day surveys (top graph) and night surveys (bottom graph) at Nauset Beach in Nauset Estuary. Lines are interpolated means for each study period.

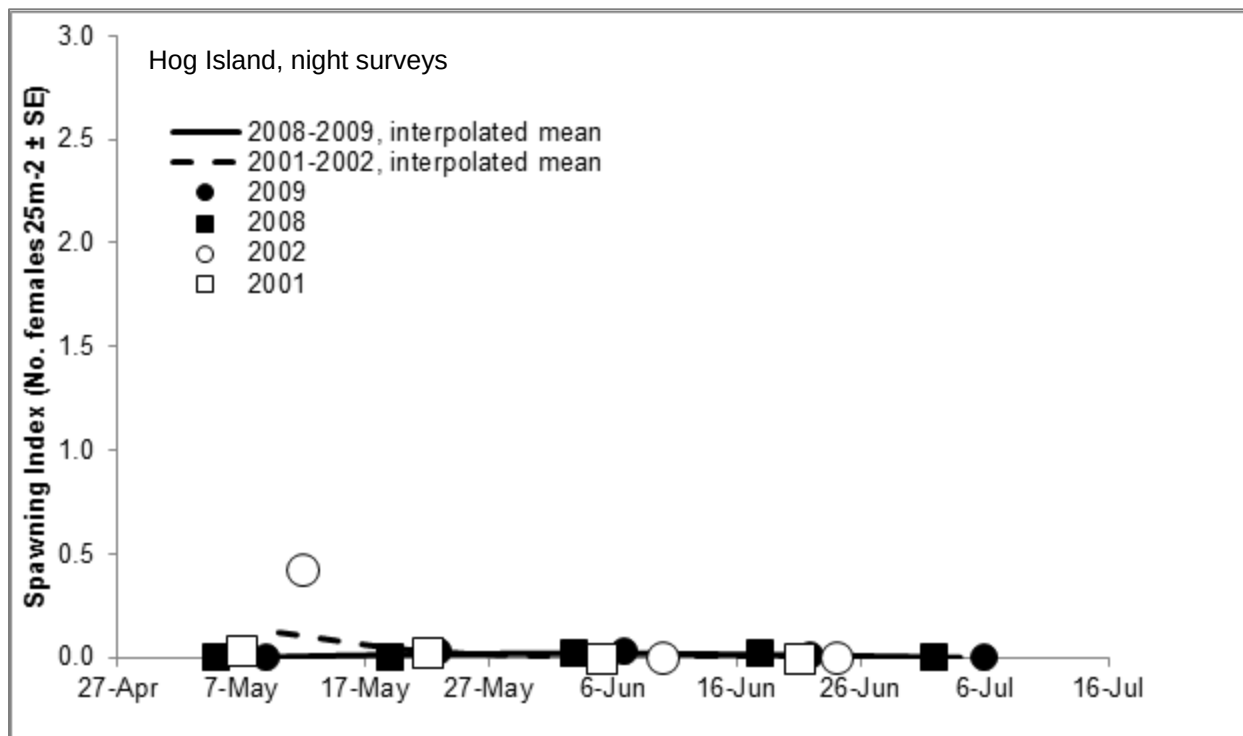
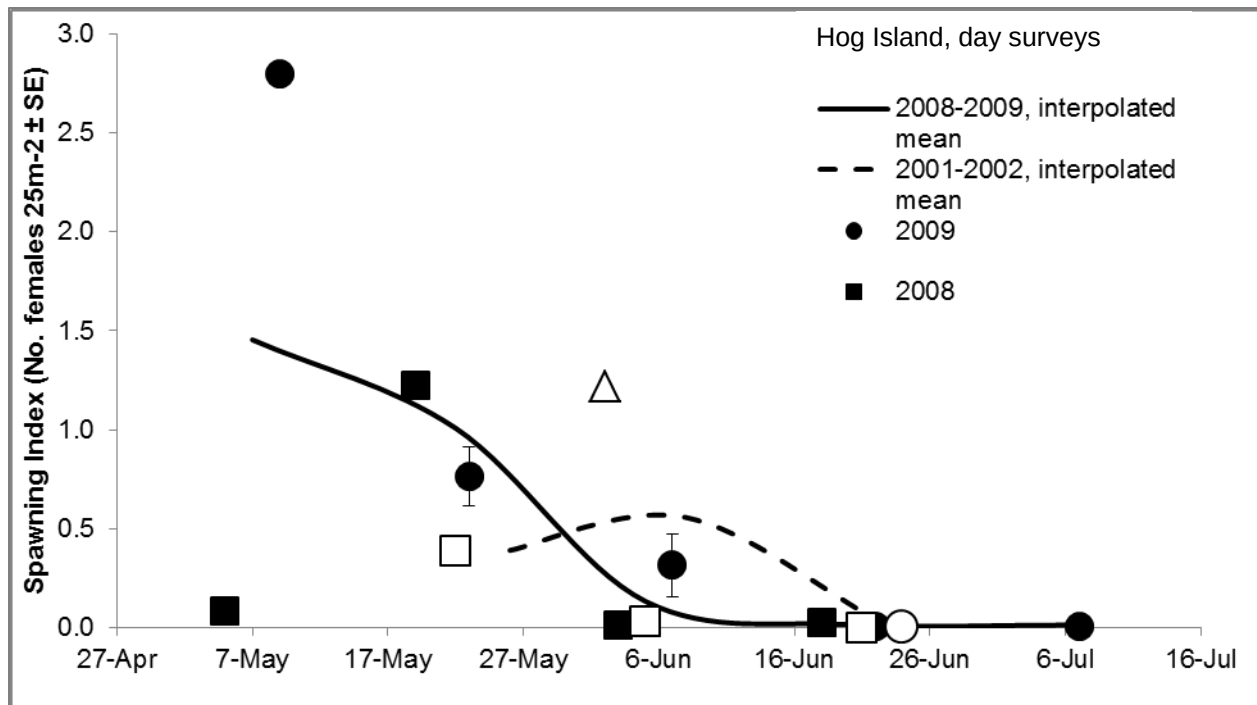


Figure 6. Mean weighted spawning indices (number of female crabs 25 m⁻² ± SE) by moon period for day (top graph) and night (bottom graph) surveys at Hog Island, Pleasant Bay. Lines are interpolated means for each study period.

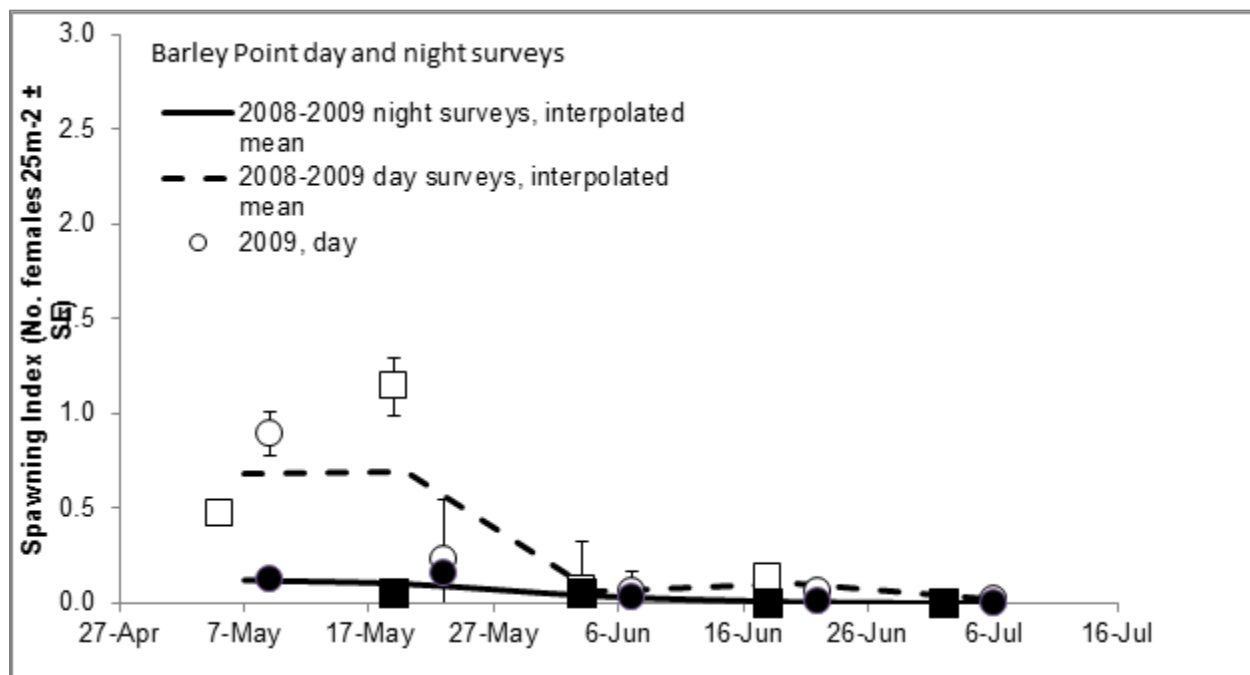


Figure 7. Mean weighted spawning indices (number of female crabs 25 m⁻² ± SE) by moon period for day and night surveys at Barley Point, Pleasant Bay. Lines are interpolated means for day and night surveys (2008 and 2009 data combined).

Table 2. Mean annual weighted spawning indices (number females 25 m⁻²) ± standard error (number of moon periods surveyed¹) for each study period at survey sites and p-value from permutation tests comparing spawning indices between study periods.

Location	Spawning Index No. Females 25 m ⁻²		p-value
	2001-2002	2008-2009	
Barley Point, Pleasant Bay, day surveys	Not surveyed	0.38 ± 0.15 (8)	n/a
Barley Point, Pleasant Bay, night surveys	Not surveyed	0.06 ± 0.02 (8)	n/a
Hog Island, Pleasant Bay, day surveys	0.33 ± 0.23 (5)	0.65 ± 0.34 (8)	0.570
Hog Island, Pleasant Bay, night surveys	0 (2)	0.15 ± 0.11 (4)	0.262
Marsh 2-3, Pleasant Bay, day surveys ²	1.01 ± 0.38 (6)	0.57 ± 0.18 (8)	0.258
Nauset Beach, Nauset Estuary, day surveys	0.23 ± 0.12 (7)	0.26 ± 0.06 (8)	0.970
Nauset Beach, Nauset Estuary, night surveys	0.07 ± 0.06 (7)	0.01 ± 0.003 (8)	0.578

¹ Includes only surveys conducted in May and June.

² Marsh 2-3 was only surveyed during the day.

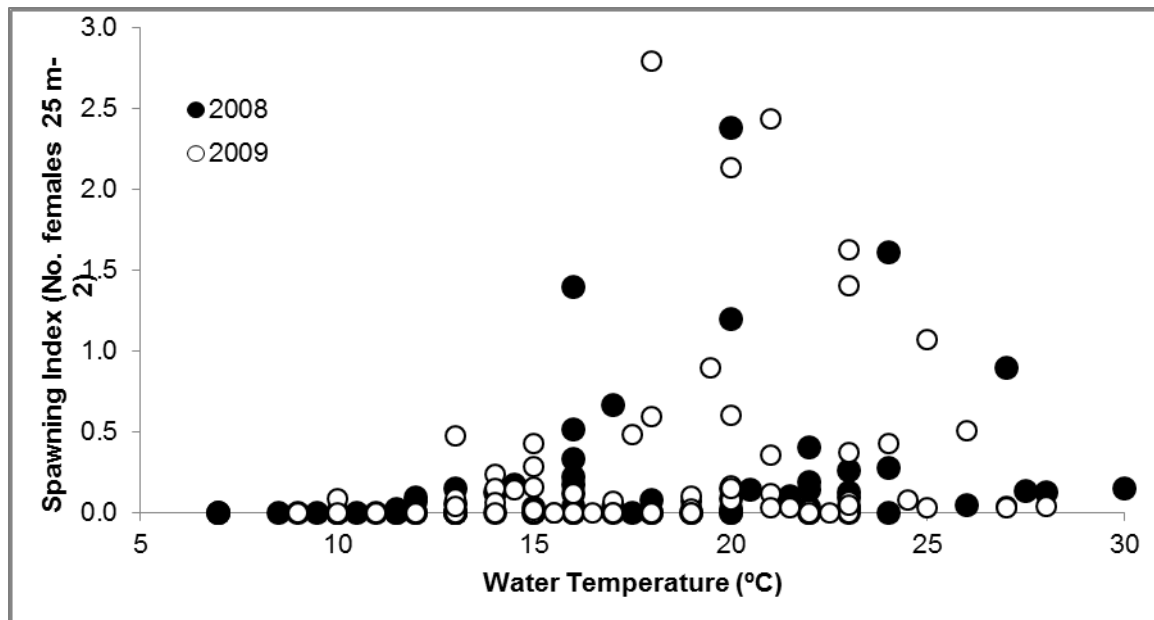


Figure 8. Relationship between spawning index and water temperature (°C) for all spawning surveys.

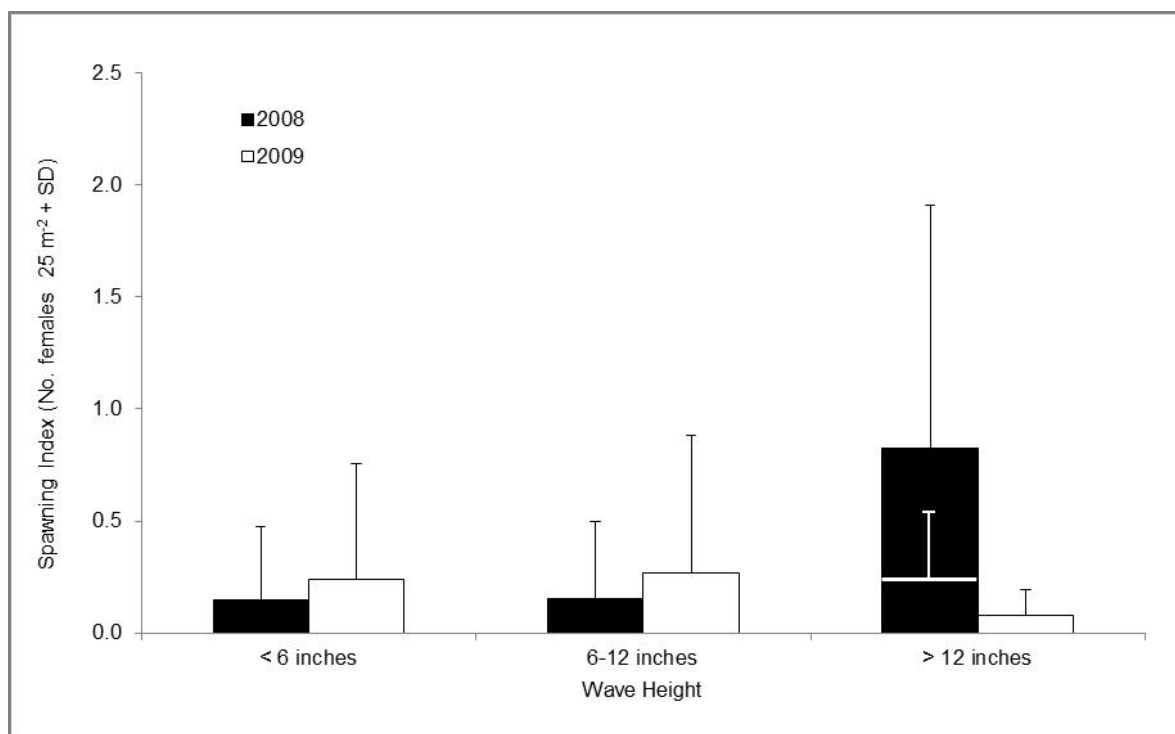


Figure 9. Mean spawning index and wave height for all spawning surveys. White lines on >12 inch wave height in 2008 indicate mean (horizontal line) and SD (vertical line) when survey with high spawning index on 21 May 2008 was deleted.

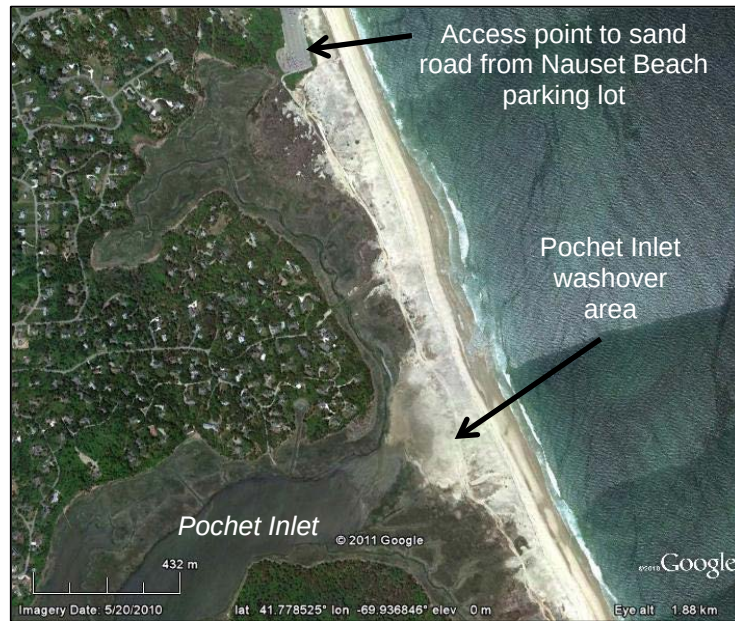


Figure 10. Location of Pochet Inlet washover area (top photo) and spawning horseshoe crabs in the washover (photograph courtesy of Elizabeth Hogan). Crab in center has an attached acoustic transmitter tag, refer to James-Pirri (2010) for details of the acoustic telemetry study

Spawning Sex Ratios and Cluster Size

Two of the three survey sites in Pleasant Bay (Barley Point and Marsh 2-3) had extremely male-biased spawning sex ratios, ranging from 1 female to 8.5-14.0 males (equivalent to 11-7% female) (Table 3). Hog Island had slightly less male-biased spawning sex ratios (1 female to 3.9-5.3 males, equivalent 21-16% female), but was still higher in comparison to other areas of Cape Cod (Table 4). A comparison of sex ratios between Pleasant Bay and Nauset Estuary showed that sex ratios in Pleasant Bay were significantly more male biased (Chi-Square Test, $\chi^2 = 636.55$, $df=1$, $p<0.0001$), with Freeman-Tukey Deviates indicating that there were significantly fewer females and significantly more males at survey sites in Pleasant Bay than in Nauset Estuary during the 2008-2009 study period.

Overall, the spawning sex ratios observed in Pleasant Bay during the 2008-2009 study were significantly higher (i.e., more male biased) than those observed during the 2000-2002 study (Chi-Square Test, $\chi^2 = 31.19$, $df=1$, $p<0.0001$). Freeman-Tukey Deviates indicated that there were significantly fewer females at survey sites during the 2008-2009 study period compared to the 2000-2002 study period. In contrast, Nauset Estuary (a population that only recently has been subjected to harvesting) had a sex ratio of 1 female to 1.3 males (equivalent to 43% female) for the 2008-2009 study period, which was not significantly different from the sex ratio (1 female to 1.6 males, Table 4) observed during the 2000-2002 study (Chi-Square Test, $\chi^2 = 1.56$, $df=1$, $p=0.2122$). Horseshoe crabs in Pleasant Bay continued to exhibit the previously documented male biased spawning sex ratios during the 2008-2009 study (James-Pirri et al. 2005), whereas other areas of Cape Cod (Cape Cod Bay, Monomoy NWR, and Nauset Estuary) had much lower sex ratios (Table 4, Figure 11, sex ratios converted to percent of spawning crabs that were female).

In Pleasant Bay, 1% of the females observed during spawning surveys were single females (a female with no male in amplexus or nearby satellite males) (Table 3). Single females were recorded in 22% of the spawning surveys in Pleasant Bay. In Nauset Estuary, 3% of females were single (Table 3) and were observed in 25% of the surveys. Single females were observed at all survey locations in CACO in 2008-2009, but were not observed during the 2000-2002 study (James-Pirri et al. 2002) (Table 3). A high number of single males (males that were not in amplexus with a female or in a spawning cluster as satellite males) were also observed during spawning surveys. In Pleasant Bay the percent of crabs that were single males ranged from 53% (1339 single males at Hog Island in 2009) to 82% (2210 single males at Marsh 2-3 in 2008) (Table 3). Comparatively, Nauset Estuary only had 13-16% single males (61 and 37 single males in 2009 and 2008, respectively) (Table 3).

The vast majority (72%) of spawning clusters (a female with a male in amplexus or with satellite males) in Pleasant Bay were paired spawning groups (one female and one male), although clusters greater than one female to 12 satellite males were occasionally observed, and clusters of up to one female to 30 males were recorded (Figure 12). In Nauset Estuary, 97% of the spawning clusters were pairs and clusters larger than one female to three males were not observed (Figure 12). The frequency distribution of the spawning clusters was significantly different (Kolmogorov-Smirnov Test, $p<0.0001$) between Pleasant Bay and Nauset Estuary.

Table 3. Spawning sex ratios, number of crabs, number of single females, and number of single males by survey site for 2008 and 2009 (May and June surveys only).

Year and Site ¹	No. female ² (F) to No. males ² (M)	No. Single females	No. Single males	Sex ratio (F: M) ²	Percent female ²
2008					
Barely Point, PB	181 to 1540	2	1029	1 : 8.5	11%
Hog Island, PB	109 to 420	0	295	1 : 3.9	21%
Marsh 2-3, PB	180 to 2520	0	2210	1 : 14.0	7%
Pleasant Bay (all sites)	470 to 4480	2	3534	1 : 9.5	9%
Nauset Beach	101 to 136	4	37	1 : 1.3	43%
2009					
Barely Point, PB	146 to 1550	4	1373	1 : 10.6	9%
Hog Island, PB	403 to 2139	6	1339	1 : 5.3	16%
Marsh 2/3, PB	277 to 2449	2	1938	1 : 8.8	10%
Pleasant Bay (all sites)	826 to 6138	12	4650	1 : 7.4	12%
Nauset Beach	209 to 272	6	61	1 : 1.3	43%

¹ PB: Pleasant Bay

² Includes single females and single males.

Table 4. Comparison of spawning sex ratios from historic (1950s), previous studies (2000-2002), and the current study (2008-2009) for Cape Cod.

Location	Historic 1950s ¹	2000-2002 ¹	2008-2009
Cape Cod Bay	1 to 2.4	1 to 2.9	1 to 1.7 ²
Monomoy National Wildlife Refuge	-	1 to 1.9	1 to 1.8 ³
Nauset Estuary	-	1 to 1.6	1 to 1.3
Pleasant Bay	1 to 2.5	1 to 5.8	1 to 8.2

¹ Data source: Carl Shuster, Jr., unpublished data from 1953 field notes, and as summarized in Shuster (1950, 1979), and James-Pirri et al. (2005).

² Data source: Massachusetts Audubon Wellfleet Bay Wildlife Sanctuary, unpublished data.

³ Data source: US Fish and Wildlife Service, Monomoy National Wildlife Refuge, unpublished data.



Figure 11. Percent of spawning crabs that were female, based on spawning sex ratios, in Pleasant Bay. Shaded box indicates percent of crabs that were female at other Cape Cod locations (based on sex ratios presented in Table 4).

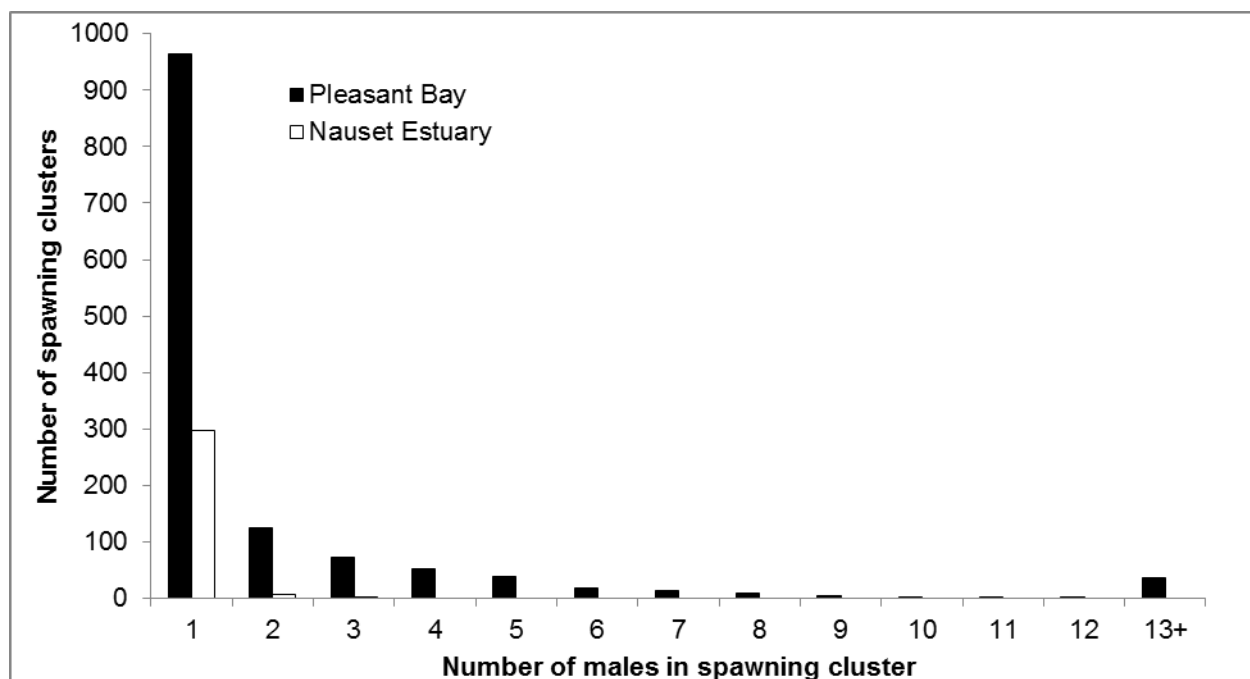


Figure 12. Spawning cluster sizes (females with attached and/or satellite males) for Pleasant Bay and Nauset Estuary during 2008-2009 surveys.

Tag-Recapture Data ¹

Over 2000 horseshoe crabs were tagged in Pleasant Bay with USFWS button tags in 2008 (242 females and 519 males) and 2009 (273 females and 993 males). The overall live recapture rate was 12% (240 live recaptures, overall recapture rate of 16% as of August 2011). Fifty-seven crabs (24% of live recaptures) tagged in previous spawning seasons were re-sighted in subsequent years (52 crabs after one year at large, 4 crabs after two years at large, and 1 crab after three years at large [1163 days]). Specific recapture locations were known for 223 of the recaptures. For the four years that recaptured crabs have currently been reported (2008-2011), 88% of the live recaptures were found within 2.5 km of the spawning beach where they were tagged (Figure 13). The majority (70%) of individuals found within 2.5 km of the tagging location were recaptured in the same year that they were tagged and 17% were recaptured after more than one year at large (Figure 13). All live recaptures were re-sighted within Pleasant Bay with the exception of one female that was tagged in June 2008 on Hog Island and was recaptured by a trawler in Nantucket Sound off of Monomoy NWR, in July of the same year.

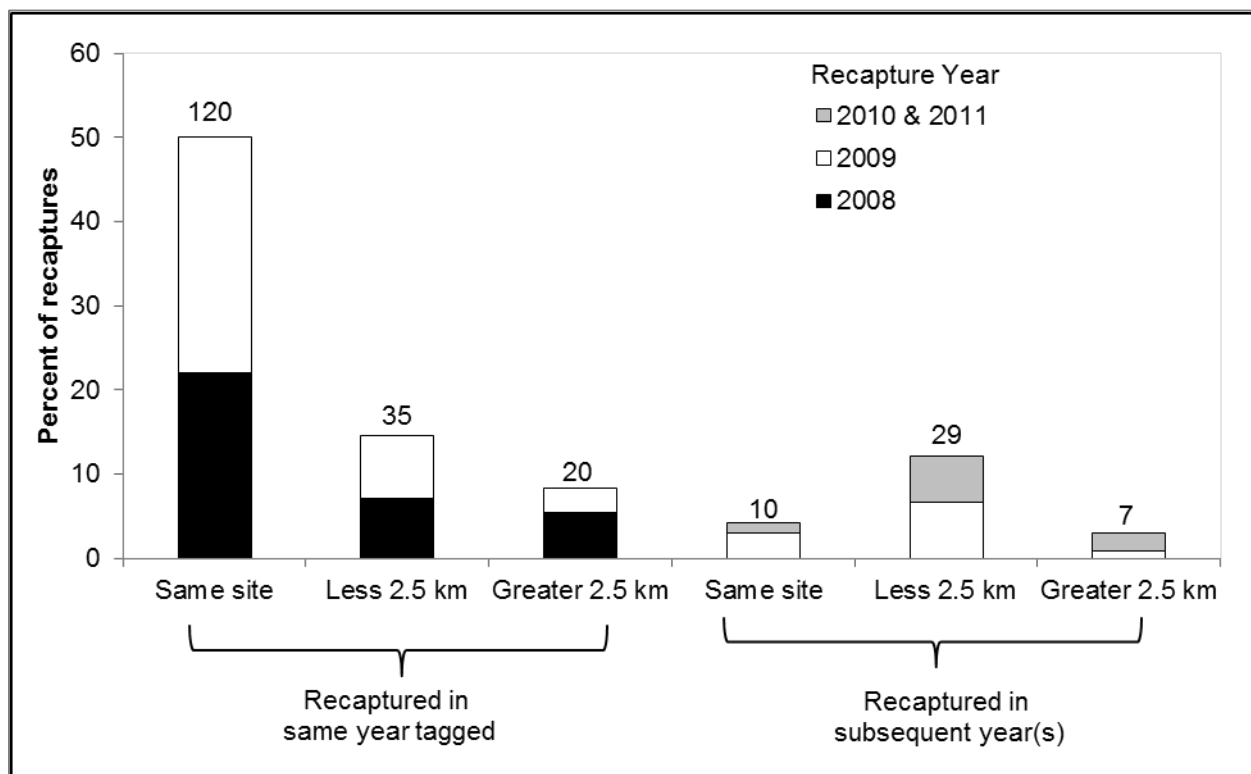


Figure 13. Percent of live recaptured horseshoe crabs, by recapture year, in relation to original tagging location. Total number of recaptured crabs is indicated above bars.

¹ Recapture data presented in this document differ slightly from those reported in James-Pirri (2010). Data herein represent recaptures through 17 August 2011 whereas James-Pirri (2010) presented recapture data through 30 August 2010.

Size Data

Mean prosomal width size for female and male crabs was 230 ± 19.9 mm ($n=508$) and 181.8 ± 13.7 mm ($n=1512$), respectively (data from 2008 and 2009 combined). Both female and male crabs were significantly smaller in 2009 (females: 227.4 mm; males: 180.2 mm) than in 2008 (females: 233.5 mm; males: 184.8 mm) (ANOVA, $df=1$, $F=12.36$, $p=0.0005$ for females; ANOVA, $df=1$, $F=39.51$, $p<0.0001$ for males) (Figure 14).

Previous work in Pleasant Bay (2000-2002) reported mean prosomal widths of 228.2 ± 21.1 mm ($n=1175$) for females and 180.0 ± 14.6 mm ($n=1891$) for males (James-Pirri et al. 2005). There was no significant difference in the mean prosomal widths for female crabs between the two study periods (ANOVA, $df=1$, $F=3.33$, $p=0.0681$). There was a difference in the mean prosomal width for male crabs (ANOVA, $df=1$, $F=12.84$, $p=0.0003$), with males in 2008-2009 being slightly larger than those in 2000-2002.

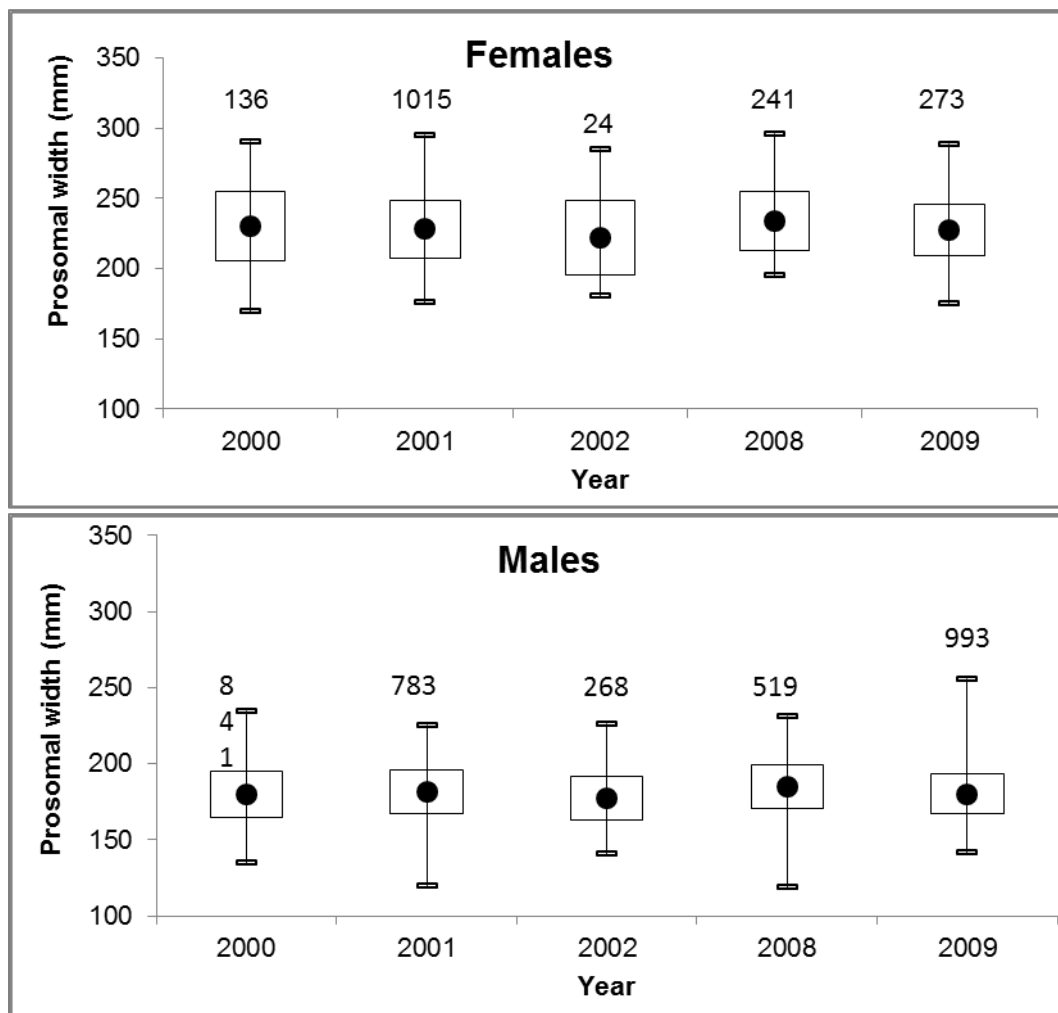


Figure 14. Prosomal width (mm) of female (top graph) and male (bottom graph) horseshoe crabs tagged in Pleasant Bay. Number of crabs measured indicated above bars. Box plots show mean (circle), standard deviation (box), and minimum and maximum prosomal width (vertical lines). Data from 2000-2002 shown for comparison.

Egg Abundance

Four of the spawning beaches (Hog Island, Marsh 2-3, Nauset Beach, and North Monomoy Island, Monomoy NWR) that were sampled for horseshoe crab eggs (eggs, embryos, and trilobites combined) in 2000-2001 were re-sampled in this study. Two additional sites (Barley Point and Pochet Inlet washover) were also sampled in 2008-2009. As observed in the earlier study, more horseshoe crab eggs were observed in the deeper beach sediments (>5 cm depth) than in the surface sediments (<5 cm depth) (Table 5).

Total egg abundance (top and bottom cores combined) was numerically lower in this study than during the previous study (Figure 15). However, horseshoe crab egg counts were only significantly lower for Nauset Beach (permutation test, $p < 0.0001$) and Marsh 2-3 (permutation test, $p = 0.004$) in 2008-2009. There were no statistical differences in egg counts for either Hog Island or North Monomoy Island (permutation tests, $p = 0.614$ and $p = 0.058$, respectively). In 2008, horseshoe crab eggs were found at all sites sampled. In 2009, no eggs were found at Hog Island (Pleasant Bay), Nauset Beach (Nauset Estuary), or North Monomoy Island (Monomoy NWR) (Table 5).

Juvenile Horseshoe Crab Abundance and Size

Juvenile horseshoe crab abundance ranged from 0 to 11 crabs 0.25 m^{-2} (observed at Barley Point). Juvenile density varied greatly, with the highest mean densities observed at Barley Point and Pochet Inlet washover sites in Pleasant Bay with very few juvenile crabs observed at either Monomoy NWR or Nauset Estuary (Figure 16).

Juvenile horseshoe crabs ranged in size from 6 to 60 mm (Figure 17). There were two distinct peaks in the size frequency distribution that likely represented different age classes, one at 6 to ~20 mm (year 2 age class) and the other at ~20 to ~35 mm prosomal width (year 3 age class) (after Sekiguchi et al. 1988). There were two individuals in the 50 to 60 mm range that represented older age class(es) (year 4 age class and older).

Table 5. Mean count of horseshoe crabs eggs (eggs, embryos, and trilobite larvae combined) per composite core ($\pm$ SE) and replicate sample size at selected spawning beaches. Composite cores were 10 cm in diameter (approximately 83 cm²). The top 0–5 cm of the core represents shallow sediments and the bottom 5–20 cm of the core represents deep sediments. Data from the 2000-2002 study are shown for comparison.

Site, core portion	Mean count of eggs, embryos, and trilobites combined $\pm$ SE (sample size)			
	2001 ¹	2002 ¹	2008	2009
Barley Point, Pleasant Bay (CACO)				
Shallow	n/a	n/a	0.3 $\pm$ 0.1 (20)	0.2 $\pm$ 0.1 (20)
Deep	n/a	n/a	54.3 $\pm$ 31.2 (20)	0.1 $\pm$ 0.1 (20)
Hog Island, Pleasant Bay (CACO)				
Shallow	0.7 $\pm$ 0.4 (12)	0.4 $\pm$ 0.4 (10)	3.0 $\pm$ 2.2 (20)	0 (20)
Deep	78.0 $\pm$ 33.9 (12)	0.1 $\pm$ 0.1 (10)	53.1 $\pm$ 30.6 (20)	0 (20)
Marsh 2-3, Pleasant Bay (CACO)				
Shallow	0.3 $\pm$ 0.3 (10)	26.3 $\pm$ 13.0 (10)	0.8 $\pm$ 0.4 (20)	0.2 $\pm$ 0.1 (20)
Deep	0 (10)	455.2 $\pm$ 192.1 (10)	3.1 $\pm$ 1.4 (20)	0.1 $\pm$ 0.1 (20)
Pochet Inlet washover, Pleasant Bay (CACO)				
Shallow	n/a	n/a	n/a	4.1 $\pm$ 2.0 (19)
Deep	n/a	n/a	n/a	66.9 $\pm$ 39.7 (19)
Nauset Beach, Nauset Estuary (CACO)				
Shallow	29.2 $\pm$ 16.9 (10)	74.4 $\pm$ 42.1 (10)	0.1 $\pm$ 0.05 (20)	0 (20)
Deep	359.9 $\pm$ 110.3 (10)	160.3 $\pm$ 54.3 (10)	0 (20)	0 (20)
North Monomoy Island, Monomoy NWR				
Shallow	3.9 $\pm$ 1.9 (10)	5.1 $\pm$ 4.2 (15)	2.1 $\pm$ 0.8 (20)	0 (20)
Deep	4.4 $\pm$ 2.6 (10)	42.1 $\pm$ 41.7 (15)	28.3 $\pm$ 22.1 (20)	0 (20)

¹ Data from James-Pirri et al. (2005).

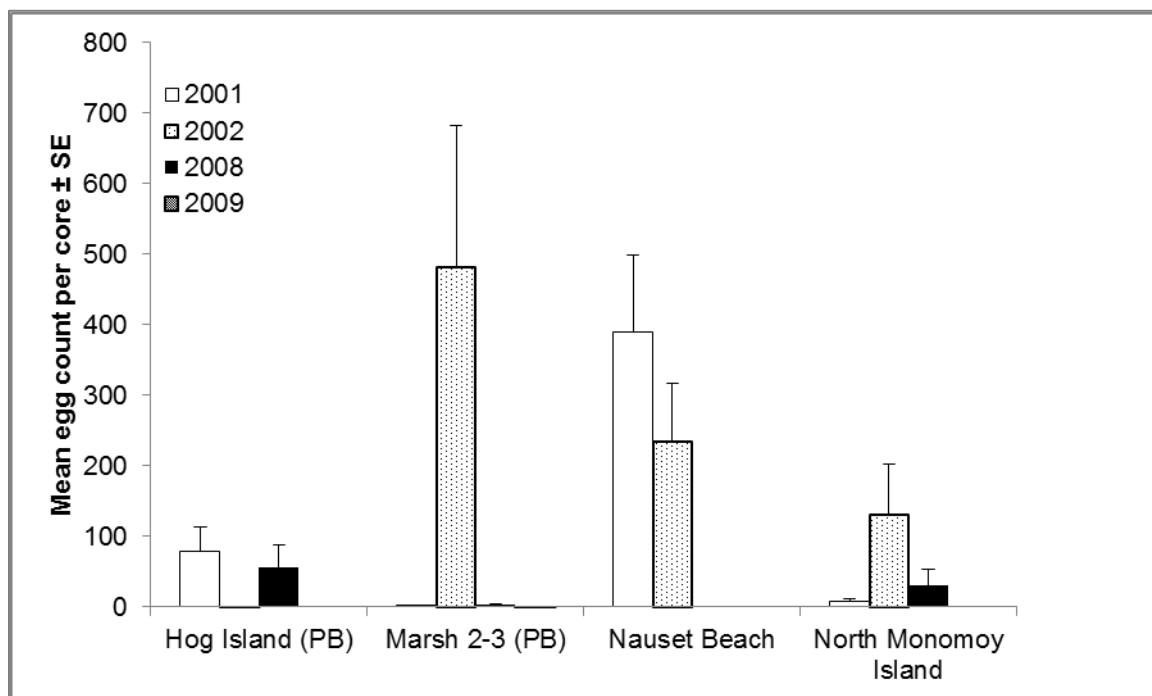


Figure 15. Mean count of eggs (top and bottom portions combined) (+ SE) per core for spawning beaches sampled during both study periods. PB: Pleasant Bay.

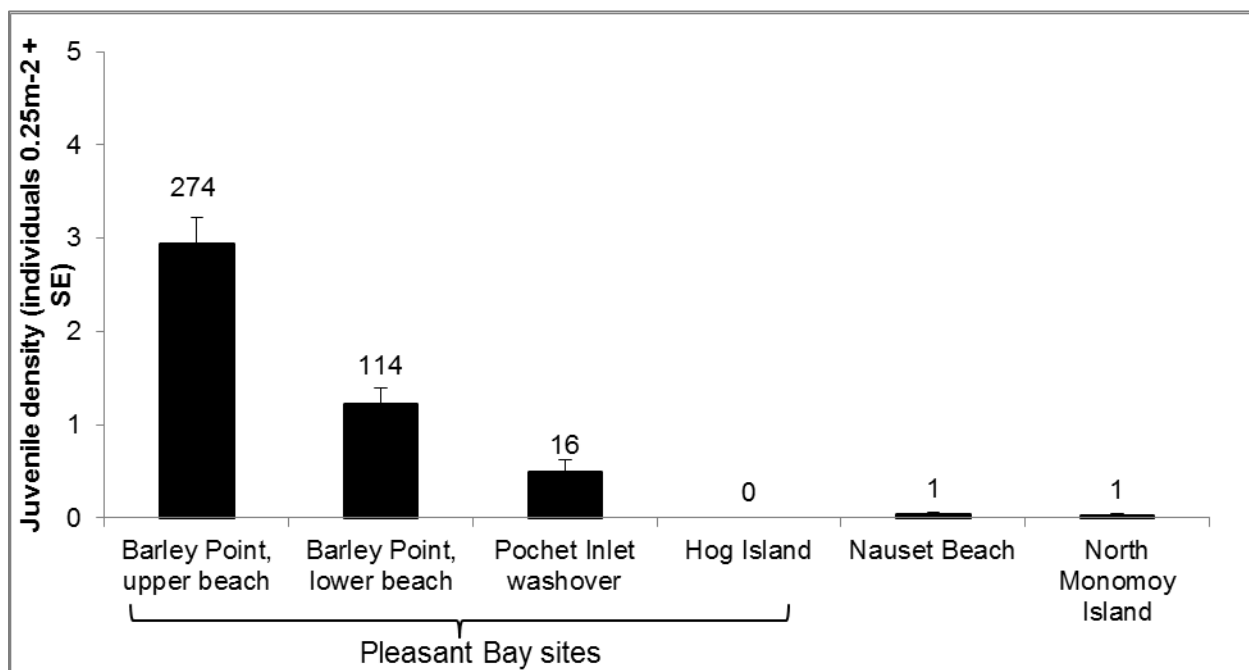


Figure 16. Mean density of juvenile horseshoe crabs (all <75 mm prosomal width) sampled from May to July, 2009. Total number of individuals observed is indicated above bars.

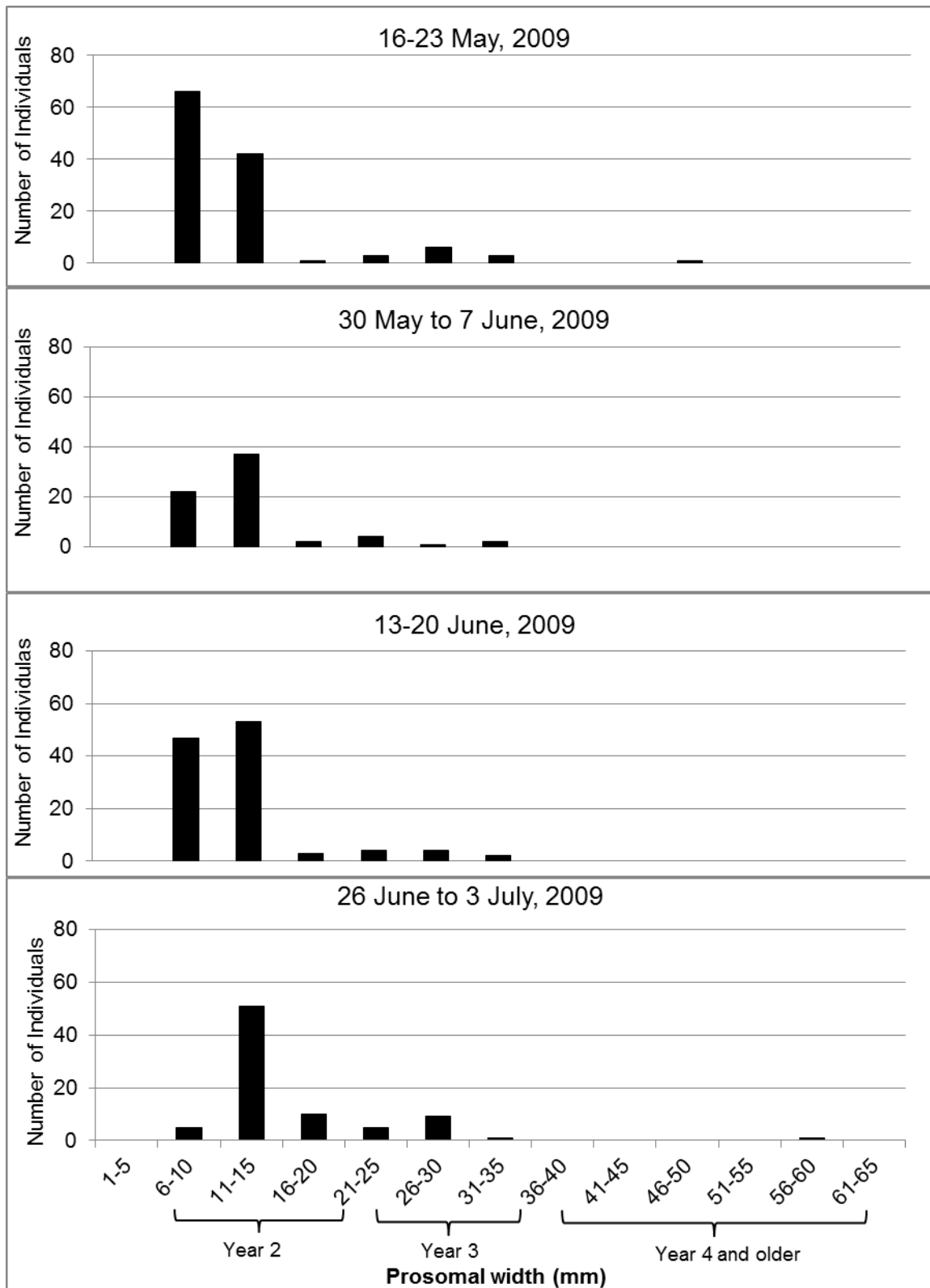


Figure 17. Size frequency distribution of juvenile horseshoe crabs sampled at Barley Point, Pleasant Bay, MA, May to July 2009. Approximate prosomal widths for year classes are indicated by brackets (after Sekiguchi et al. 1988).

Discussion

Horseshoe crab spawning indices in Pleasant Bay and Nauset Estuary in 2008-2009 were not significantly different from those observed in 2000-2002 indicating that the harvest closure had no influence on the density of spawning females in Pleasant Bay. However, differences in spawning sex ratios were observed. Spawning sex ratios were more male biased in Pleasant Bay in 2008-2009 than in 2000-2002. In 2000-2002, there was approximately 1 female to 6 males, whereas in this study the ratio increased to 1 female to 8 males. This represents a decrease in percent of females on spawning beaches from 15% to 11% over the past decade, and a decline from 25% females over the past 60 years in Pleasant Bay (historic data Carl Shuster's 1953 field notes [C. Shuster personal communication], Shuster 1950, 1979, James-Pirri et al. 2005). In Nauset Estuary, similar to other areas of Cape Cod surveyed during the 2000-2002 study, sex ratios remained unchanged over the past decade with approximately 40% of the crabs on spawning beaches being female. While horseshoe crab spawning behavior plays a role in the bias of sex ratios towards males (e.g., males staging in shallows off spawning beaches to intercept females [Brockman 1990]), the sex ratios observed in Pleasant Bay over the past decade were extreme. Sex ratios in other areas of Cape Cod are 1:1 to 1:3 (female to male) (James-Pirri et al. 2005, Commonwealth of Massachusetts 2008, 2009) and were 1:2 to 1:5 (female to male) in Delaware Bay and elsewhere throughout their range (Rudloe 1980, Swan et al. 1996, Thompson 1998, Smith et al. 2002, 2009, ASMFC 2010, Mattei et al. 2010). Compounding the issue of extreme male-biased sex ratios in Pleasant Bay was the presence of single females observed for the first time on spawning beaches in 2008. The presence of single females was not unique to Pleasant Bay, as they were also observed on other Massachusetts spawning beaches in 2008 and 2009. Data collected concurrently with this study observed single females in 20% to 30% of surveys conducted throughout Massachusetts (Commonwealth of Massachusetts 2009). In Connecticut, approximately 18% of females on spawning beaches were single (Mattei et al. 2010). Since eggs deposited by single females are not fertilized and will not develop, the presence of single females on spawning beaches represents a serious problem for local horseshoe crab populations and is disconcerting for state fishery managers (Commonwealth of Massachusetts 2009).

The ban on horseshoe crab harvest within federal waters in Pleasant Bay could have had an immediate effect of increasing the number of spawning adults on beaches, potential leading to an increase in spawning indices and decrease in male-biased sex ratios (by increasing the number of spawning females). Protected crabs would not have been collected by biomedical harvesters and therefore not subjected to direct mortality (a 30% mortality associated with bleeding and handling was recently estimated by Leschen and Correia 2010) or any potential behavioral modifications that may result from the biomedical bleeding process. However, since CACO's boundary only encompasses the northeastern portion of Pleasant Bay, the protected area within the Seashore may not be enough to compensate for the harvest allowed in the non-federal areas of Pleasant Bay. After spawning on beaches at high tide, horseshoe crabs follow the ebbing tide back into the bay and in between new and full moon spawning periods they move throughout Pleasant Bay (James-Pirri 2010). Thus, crabs that spawn in protected areas of the Seashore can be harvested once they leave the park's boundary. There was considerable variability in the spawning indices as noted in this study and the earlier study (James-Pirri et al. 2005) and this could have masked small changes in spawning indices. However, a difference in spawning sex

ratios was observed and indicated that there were fewer females on spawning beaches in Pleasant Bay in this study than in 2000-2002. Spawning sex ratio may be more sensitive to changes in abundance and may indicate that the Pleasant Bay spawning population is in decline.

Alternatively, other factors may be influencing the number of spawning crabs on beaches in Pleasant Bay. Spawning habitat degradation and loss may have contributed to the lack of a rebound in spawning indices after the harvest closure. In 1998, 8% of the Pleasant Bay shoreline was armored with about 133 erosion control structures such as bulkheads and revetments. In 2008, the estimated number of erosion control structures increased to 165 (Pleasant Bay Resource Alliance 2008). These structures alter the Bay's natural erosion and nourishment processes, and can result in the conversion of a sandy beach to a stony shoreline (Pleasant Bay Resource Alliance 2008). This was apparent at some of the spawning beaches in this study, for example, the Barley Point beach was once an entirely sandy beach, whereas it is now a sand and cobble beach (E. Parra, personal communication).

As noted in previous studies, spawning indices in Southern New England are one to two orders of magnitude lower (0.0003 to 0.0390 females m^{-2} , this study) than those observed in Delaware Bay (0.8000 females m^{-2}) (Smith et al. 2002, James-Pirri et al. 2005, Mattei et al. 2010). Similar to the 2000-2002 study (James-Pirri et al. 2005), spawning indices were higher during the day than at night, especially in Pleasant Bay. Mean high water in Pleasant Bay is approximately 1.5m NVGD. Nighttime high tides often inundate beaches to bluffs that border the beaches or extend far into the marshes on the back barrier beach, completely submerging the spawning beaches associated with these areas. The North Inlet breach, caused by the April 2007 Patriot's Day Nor'easter, further increased the tidal range by 0.2 m (at Meeting House Pond) to 0.4 m (at Chatham Pier) in Pleasant Bay (Kelley and Ramsey 2008). The extensive flooding of spawning habitat during nighttime high tide may make the daytime tides, which usually have a lower tidal range, more acceptable for spawning crabs.

The frequency distribution of spawning cluster sizes was different between Pleasant Bay and Nauset Estuary. The distribution of spawning clusters in Pleasant Bay more closely resembled that of Delaware Bay, where 30-60% of the females were found with two or more males, whereas the distribution in Nauset Estuary mirrors that observed for the Connecticut coast (Mattei et al. 2010). Mattei et al. (2010) hypothesized that *Limulus* subpopulations with locally low population densities will exhibit paired spawning clusters (one female to one male, e.g., predominantly monogamous mating behavior) and those at higher densities (as in Delaware Bay) will tend to have larger spawning clusters (e.g., polygynandrous behavior). These authors further hypothesized that locations where single females were present combined with low levels of polygynandrous behavior were evidence that horseshoe crabs in these populations were having difficulty finding mates and were not able to maximize their reproductive effort (Mattei et al. 2010). The paradox in Pleasant Bay is the presence of a comparatively high amount of polygynandrous behavior in a low-density *Limulus* population. It is possible that relatively low proportion of females on spawning beaches in Pleasant Bay has intensified male competition for mates, resulting in a higher proportion of larger spawning clusters.

The recapture results of the USFWS button tagged crabs from this study mirror the results previously reported for Pleasant Bay providing further evidence of a localized population. Previous work in Pleasant Bay reported a 10% live recapture rate of tagged crabs and similarly

observed that the majority of recaptures occurred within a short distance (2 km) of the original tagging location (James-Pirri et al. 2005). A high percentage of crabs were recaptured at the same beach where they were originally tagged, indicating fidelity to spawning beaches within the same season. The acoustic telemetry study (James-Pirri 2010) showed that female horseshoe crabs tend to return to the northern area of Pleasant Bay (where many spawning beaches are located) in subsequent spawning seasons, suggesting a spawning fidelity to this general area of the bay. During the same time-period as this study, tagging was also conducted in other areas of Cape Cod (e.g., Monomoy NWR and Cape Cod Bay). For example, Monomoy NWR has consistently tagged approximately 500 crabs per year since 2000. No individuals tagged in these other areas have ever been reported as being recaptured inside Pleasant Bay (James-Pirri et al. 2005, M. Williams and S. Eyler, US Fish and Wildlife Service, personal communication). Additionally, data from the acoustic telemetry study indicated that horseshoe crabs did not leave Pleasant Bay after spawning, but overwintered in the deeper portions of the bay and then returned to spawning beaches in the following spring (James-Pirri 2010). These data show that there is limited movement of adults between Pleasant Bay and other embayments on Cape Cod and support the hypothesis that horseshoe crabs in Pleasant Bay are likely a non-migratory population.

A decline in egg abundance in 2008-2009 was observed in three separate embayments (Pleasant Bay, Nauset Estuary, and Monomoy NWR). Since the pattern was similar among the embayments, the lower egg counts were likely a result of large-scale environmental factors (e.g., weather events) rather than embayment-specific factors (e.g., changes in harvest regulations). It was noted in 2009, that a severe storm in May eroded portions of the spawning beach at Barley Point and may have destroyed horseshoe crab nests (E. Parra, personal communication).

The size distribution of juvenile horseshoe crabs at Barley Point showed three distinct peaks corresponding to different age classes (Sekiguchi et al. 1988, Shuster and Sekiguchi 2003). The first peak (6 to ~20 mm) represented juveniles in their second year (individuals that hatched in the previous year and overwintered as juveniles). The second peak (~20 to ~35 mm) represented individuals in their third year. Only a few older individuals (year 4 and older) were observed. This is consistent with the general belief that older juveniles likely migrate from tidal flats to deeper waters as they grow (Rudloe 1981, Shuster 1982). As expected, no newly hatched (year 1, <10 mm prosomal width, after Sekiguchi et al. 1988) were observed. Peak spawning in 2009 occurred in mid-May, and larvae would not have emerged from the nests until about mid-July (6 weeks after peak spawning) or later, which was after the samples for juveniles were collected.

The population trends in Pleasant Bay (e.g., low spawning indices, extreme male-biased spawning sex ratios, presence of single females on spawning beaches) have raised concerns for fishery managers (Commonwealth of Massachusetts 2008, Leschen and Correia 2010). Pleasant Bay has been harvested for biomedical purposes for the past 30 years (the bait harvest in Pleasant Bay was terminated as of 2006) (Leschen and Correia 2010). Recent evidence indicates that mortality from biomedical bleeding is higher (30% mortality) than previously thought (15% mortality) (Rudloe 1983, Thompson 1998, Hurton and Berkson 2004, Leschen and Correia 2010). There are concerns that the preferential harvest of females combined with the extended period of biomedical bleeding on the Pleasant Bay population has caused a higher mortality than previously thought or has had a sub-lethal effect on female spawning behavior that may be contributing to the extreme male-biased spawning sex ratios. Furthermore, there is strong

evidence that the Pleasant Bay population is a localized, non-migratory population (James-Pirri et al. 2005, James-Pirri 2010) and that influx of new individuals from offshore may be limited, thus restricting the resilience of the population to harvest pressures. This is especially important to the New England horseshoe crab stock(s), as trawl data suggest a limited or non-existent migration to the continental shelf (Botton and Ropes 1987) and tagging and telemetry data show that horseshoe crabs in New England either remain or return to the embayment where they spawn (James-Pirri et al. 2005, Moore and Perrin 2007, James-Pirri 2010, Schaller et al. 2010, this report). Adaptive fishery management and/or more conservative fishery regulations for localized populations such as the one in Pleasant Bay may be required to ensure the persistence of population and the sustainability of the fishery.

Sources of Expertise

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