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Spawning Densities, Egg Densities, Size Structure, and Movement Patterns of Spawning Horseshoe Crabs, *Limulus polyphemus,* within Four Coastal Embayments on Cape Cod, Massachusetts

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ABSTRACT: Spawning densities, spawning indices, egg densities, size distributions, and movement patterns of horseshoe crabs (*Limulus polyphemus*) were quantified for four coastal embayments (Monomoy National Wildlife Refuge, Pleasant Bay, Nauset Estuary, and Cape Cod Bay) on Cape Cod, Massachusetts from 2000 to 2002. Spawning activity was highest from mid May through mid June, but densities varied throughout the Cape Cod region. Average spawning densities (male and female crabs combined), measured using 25-m^2 quadrats, were lower than 1 crab 25 m^{-2}, although certain locations had consistently higher densities averaging 2 to 3 crabs 25 m^{-2} with individual survey densities recorded as high as 17 crabs 25 m^{-2}. Spawning densities during night surveys were either similar or slightly higher than day surveys, except at a few sites within Pleasant Bay. Spawning indices were considerably lower ranging from 0 to 1.3 females 25 m^{-2} throughout the Cape Cod region. Spawning sex ratios varied from 1:1.6 to 1:3.1 (females:males) throughout the region, except within Pleasant Bay where highly male skewed ratios were observed (e.g., 1:5.8, 3-yr average). Egg densities were low overall (<1 egg cm^{-2}) throughout Cape Cod and egg densities tended to be higher in deeper sediments (5–20 cm deep) compared to shallow sediments (0–5 cm deep) at most locations. Over 7,800 horseshoe crabs were tagged on Cape Cod from 2000 to 2002. Average size and size frequency distributions of tagged crabs varied among regions. Larger individuals were observed at Monomoy National Wildlife Refuge while the smallest individuals were from Cape Cod Bay. We documented an overall recapture rate of 6.7% and our tag-recapture data indicated that 62% of crabs were recaptured at the original tagging location and 70% of recaptures traveled less than 2 km from the original tagging location, providing evidence for localized populations on Cape Cod. We have observed that horseshoe crabs differ among embayments within a regional area, suggesting the potential need for management plans specific to embayments or subregions depending on the characteristics of a population.

Introduction

Recently, there has been a renewed interest in the American horseshoe crab (*Limulus polyphemus*), primarily due to concern over increases in harvest that have coincided with decreases in spawning abundance and density of eggs in beach sediments (Michels 1996; Swan et al. 1996; ASMFC 1998). Conservation and management of this species is controversial since horseshoe crabs are important to a variety of user groups, such as commercial bait fisherman, the biomedical industry, migrating shorebirds, and the mid Atlantic states that receive substantial revenue related to ecotourism (Novitsky 1984; Clark 1996; ASMFC 1998; Walls et al. 2002; USFWS 2003). In 1998, the Atlantic States Marine Fishery Commission (1998) developed stock assessment and management guidelines for the horseshoe crab. This prompted federal agencies on Cape Cod, Massachusetts (National Park Service and United States Fish and Wildlife Service), to assess their management policies, initiate the collection of baseline data, and develop long-term monitoring programs for horseshoe crabs within their jurisdictions. This has also caused a renewed research interest in horseshoe crabs on Cape Cod as studies previous to 1998 dated back to over 40 yr ago (e.g., Shuster 1950; Baptist et al. 1957). In 1999, a collaborative was formed among

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the University of Rhode Island, National Park Service at Cape Cod National Seashore (NS), U.S. Fish and Wildlife Service at Monomoy National Wildlife Refuge (NWR), and Massachusetts Audubon Society's Wellfleet Bay Wildlife Sanctuary with the objective of collecting quantitative data on the spawning populations of horseshoe crabs to assist with the development of management plans for areas managed by these agencies. Here we report the findings of a 3-yr study of spawning horseshoe crab populations on Cape Cod.

Horseshoe crabs spawn on sandy, protected beaches around new and full moon high tides from May through June (Shuster 1982; Shuster and Botton 1985; Barlow et al. 1986; Badgerow and Sydlik 1989). Females excavate nests on beaches and lay their eggs in clusters of 3,000–4,000 eggs, for an estimated annual reproductive output of 88,000 eggs per female (Shuster 1982; Shuster and Botton 1985). In Delaware Bay, horseshoe crab eggs provide a critical food resource for migratory shorebirds (Castro et al. 1989; Castro and Meyers 1993; Clark et al. 1993; Tsipoura and Burger 1999; USFWS 2003). It is not known how many shorebirds on Cape Cod use horseshoe crab eggs as a food resource; there is evidence, from gut content studies, that shorebirds at Monomoy NWR consume horseshoe crab eggs (Koch unpublished data).

Extensive research on spawning horseshoe crabs has been conducted in Delaware Bay and the southeast U.S. (Rudloe 1980; Shuster and Botton 1985; Botton et al. 1988, Wenner and Thompson 2000; Smith et al. 2002b), but there has been no comprehensive regional work done for spawning populations in New England. Prior to 1999, most of the existing information on New England horseshoe crab populations was dated (Shuster 1950, 1957, 1982; Baptist et al. 1957). These studies present important historical information on size distributions (Shuster 1982), spawning sex ratios (Shuster 1950), and movement patterns within the New England region (Baptist et al. 1957), but do not provide data on spawning densities. Widner and Barlow (1999) have studied a population of spawning horseshoe crabs in Bourne, Massachusetts, for more than 15 yr and suggest that spawning densities may be declining in the Cape Cod region. These researchers reported a decline in spawning activity since 1984 by more than 95% (3,171 individuals in 1984 to 148 individuals in 1999) and a decrease in the spawning period from 56 to 11 d (Widner and Barlow 1999). Since 2000, there has been a renewed interest in horseshoe crabs on Cape Cod, primarily due to legal issues regarding the harvest of crabs from the waters of Cape Cod NS and Monomoy NWR and subsequent closure of these areas to harvest.

On Cape Cod, as elsewhere throughout their range, horseshoe crabs are harvested for conch and eel bait and are used by the biomedical industry. Crabs have been harvested from the waters of Monomoy NWR, Cape Cod NS (Pleasant Bay), and Cape Cod Bay, for both of these purposes for over 25 yr (Finley personal communication). The waters of Cape Cod NS and Monomoy NWR were closed to harvesting in 2000. The biomedical industry uses blood from horseshoe crabs to produce *Limulus* amoebocyte lysate for the detection of gram-negative bacterial endotoxins in pharmaceutical products as well as for diagnostic tests for diseases such as spinal meningitis (Novitsky 1984; Berkson and Shuster 1999). Mortality rate associated with biomedical bleeding has been estimated at approximately 15% (Rudloe 1983; Walls and Berkson 2000, 2003).

Our efforts focused on four regional Cape Cod embayments: Monomoy NWR, Pleasant Bay and Nauset Estuary both within Cape Cod NS, and Cape Cod Bay. We surveyed several spawning beaches within the Cape Cod region in 2000, 2001, and 2002 to determine spawning densities and spawning sex ratios. Sediment samples were collected at survey beaches in 2001 and 2002 to determine egg densities and to evaluate if eggs were available to foraging shorebirds. Over 7,800 adults were tagged during the 2000–2002 spawning seasons to track movements within and among embayments. All tagged crabs were measured and sexed to collect information on size distributions. We compared characteristics of horseshoe crabs among the Cape Cod coastal embayments in an effort to evaluate if these populations differ, which could influence how management plans are developed for this species on Cape Cod as well as elsewhere throughout their range.

Materials and Methods

Spawning Surveys

To monitor spawning horseshoe crabs we surveyed spawning beaches within Monomoy NWR, Cape Cod NS, and Cape Cod Bay (Fig. 1) during the late spring and summer of 2000, 2001, and 2002 using quadrat sampling. The U.S. Fish and Wildlife Service was responsible for surveying sites within Monomoy NWR, researchers from the University of Rhode Island surveyed sites within Cape Cod NS, and Massachusetts Audubon Society's Wellfleet Bay Wildlife Sanctuary staff surveyed sites within Cape Cod Bay. In 2000, a total of 11 sites were surveyed for spawning horseshoe crabs: 2 sites at Monomoy NWR, 3 sites within Cape Cod

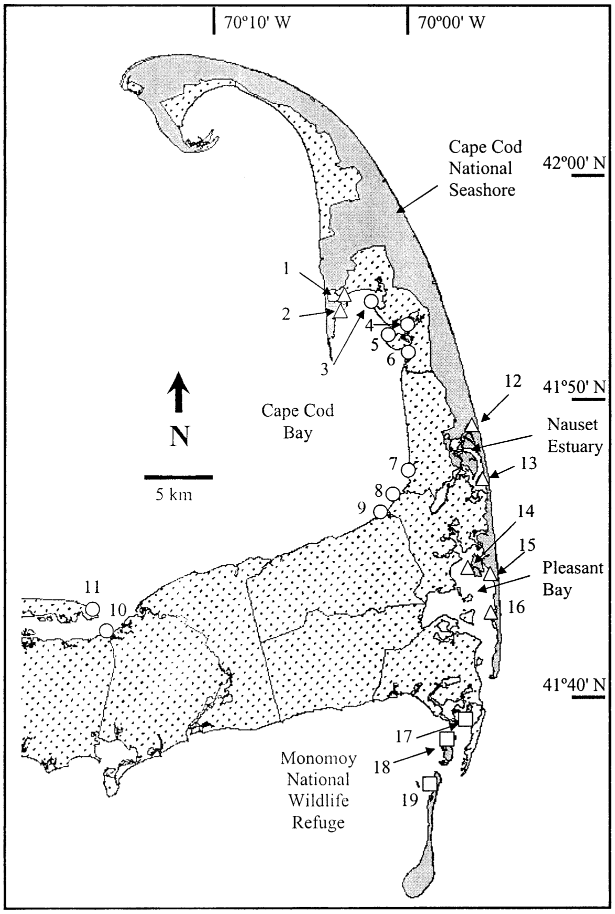

Fig. 1. Locations of spawning surveys for horseshoe crabs within Cape Cod Bay (1–11), Nauset Estuary (12 and 13), Pleasant Bay (14–16), and Monomoy National Wildlife Refuge (17–19). Triangles indicate surveys coordinated by University of Rhode Island; circles indicate surveys coordinated by Massachusetts Audubon Society, Wellfleet Bay Wildlife Sanctuary; squares indicate surveys coordinated by U.S. Fish and Wildlife Service. Survey locations and years surveyed were: 1. Tavern Cove (2001); 2. Saw Point (2001); 3. Chipman's Cove (2000 and 2001); 4. Old Wharf (2001); 5. Lieutenant Island (2000 and 2001); 6. Wellfleet Bay Wildlife Sanctuary (2000 and 2001); 7. First Encounter Beach (2000); 8. Crosby Lane (2000); 9. Cole Road (2001); 10. Bonehill Road (2000); 11. Sandy Neck (2001); 12. North Nauset Beach (2001 and 2002); 13. South Nauset Spit (2001); 14. Hog Island (2000, 2001, and 2002); 15. Marsh 2–3 (2000, 2001, and 2002); 16. Northville (2000, 2001, and 2002); 17. Morris Island (2000, 2001, and 2002); 18. North Monomoy Island (2001 and 2002); and 19. South Monomoy Island (2000, 2001, and 2002).

NS (all located in Pleasant Bay), and 6 sites within Cape Cod Bay. In 2001, some survey sites were dropped due to the absence of spawning crabs, access, or safety issues, and additional sites were added resulting in a total of 16 beaches that were surveyed. At Monomoy NWR an additional survey site was added for a total of 3 sites. Within Cape Cod NS, all 3 sites within Pleasant Bay were resurveyed in 2001, and 2 new sites were added within Nauset Estuary. Within Cape Cod Bay, 3 survey sites were dropped and were replaced by 5 new sites for a total of 8 sites. In 2002, the same sites surveyed in 2001 were surveyed within Monomoy NWR and Pleasant Bay. One site within Nauset Estuary (South Nauset Spit) was not surveyed due in 2002 to staff constraints. No surveys were conducted in Cape Cod Bay in 2002 due to a lack of funding at the Massachusetts Audubon Society. Spawning survey locations were selected based upon prior knowledge of spawning sites and anecdotal information of probable spawning locations. Due to the immediate needs of resource management staff of Cape Cod NS and Monomoy NWR to obtain baseline information for specific areas (i.e., Pleasant Bay and Nauset Estuary, Cape Cod NS, North and South Monomoy Islands, Monomoy NWR), some areas were purposefully chosen as survey locations. Since survey locations were not randomly selected and given that some sites were not monitored in all years, we present no statistical comparisons of spawning indices or densities among sites or regions. Descriptive statistics and graphical comparison of regions are presented as this is the only recent (within 40 yr) and one of the few existing data sets of spawning densities for Cape Cod.

The method we used for spawning surveys was a modification of the Delaware Bay survey method (Smith et al. 2002b). The modification for the Cape Cod region primarily involved increasing the size of the sample quadrat from 1 m^2 (1×1 m) to 25 m^2 (5×5 m), since spawning densities on Cape Cod were much lower than those observed in Delaware Bay, and in Delaware Bay only the top 1 m of beach was available for counting spawning horseshoe crabs due to deep water beyond 1 m of the tide line (Smith personal communication). On Cape Cod, the majority of spawning beaches had a gentle slope and deep water was not as problematic.

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 quadrat was located all subsequent quadrats were systematically placed 10 m apart (some exceptions to this method were necessary at Monomoy NWR and are detailed below). Each quadrat was located adjacent to the water's edge (swash zone), with the quadrat extending into the water. Over the course of the survey the leading edge of the quadrat would move slightly down the beach slope in order to keep in line with the receding tide.

In 2000, surveys at Monomoy NWR used 10 $\times$

10 m quadrats instead of 5 × 5 m quadrats. Surveys at Monomoy NWR using this method were started prior to the coordination among the agencies and it was decided that changing the quadrat size to 5 × 5 m in the midst of the 2000 survey season would be detrimental. At Monomoy NWR in 2000, twenty to twenty-five 10 × 10 m quadrats were randomly placed within a 1,400 m stretch of beach and all crabs within the quadrats were noted. The sex of crabs within each quadrat was not recorded, so a spawning index (estimate of female density) cannot be calculated for this year for the Monomoy NWR data. The total number of pairs, satellite, and solitary crabs encountered along the entire length of the survey beach were recorded and sex ratios can be estimated for 2000 if it is assumed that each pair contained one female and one male, and solitary and satellite crabs were males. Position of the quadrats relative to the waterline was as previously described. In 2001 and 2002, 5 × 5 m quadrats spaced 30 m apart (with the first quadrat randomly located) were used to survey sites at Monomoy NWR. Quadrats were spaced 30 m apart in order to cover the entire beach length within 1 h of peak high tide. All other survey protocols were similar to those used within Cape Cod NS and Cape Cod Bay. An additional survey location at North Monomoy Island was added in 2001. This location was a broad expanse of tidal flat where spawning crabs were found throughout the flats and not just at the waters edge. In order to survey this location, a 100 × 50 m area of the tidal flat was randomly delineated and five randomly located 100-m transects were surveyed within the area. The first quadrat in each transect was randomly located and each subsequent quadrat was spaced 10 m apart.

We conducted spawning surveys during new and full moon high tides, with surveys taking place during a 5-d period around the new or full moons (2 d prior, the day of, and 2 d after). Surveys commenced at peak high tide and were done during both nighttime and daytime tides. Environmental conditions (i.e., weather, wave height, water temperature) were recorded prior to the start of each survey. During night surveys, flashlights were used to illuminate the quadrats. Horseshoe crabs were easily detected during night surveys using flashlights since water clarity, wave height, and water depth rarely caused conditions where the crabs were not visible. Daytime surveys were conducted to augment the nighttime surveys and at some sites were the only surveys done due to the logistical constraints of nighttime surveys, such as insufficient manpower, access, and safety issues. We counted all horseshoe crabs within each quadrat, and recorded the number of pairs (a female with an attached male), satellite males (males in close proximity to a pair), and solitary males. A horseshoe crab was counted as being in a quadrat if half or more of its body was within the quadrat boundary when the quadrat was first laid down. Counting horseshoe crabs within the 25-m^2 quadrat typically took less than 10 s, since usually fewer than 2 crabs were present in any one quadrat. All surveys were completed within 1 h of peak high tide.

We calculated spawning density, defined as the average number of crabs (females and males combined) within a 25-m^2 quadrat, and spawning index, defined as the average number of female crabs within a 25-m^2 quadrat. Spawning densities and spawning indices were calculated by averaging densities from individual surveys for each site (day and night surveys calculated separately) within each moon period (e.g., May new moon, June full moon, June new moon) for each year. A yearly average for each site was calculated by averaging the densities for each moon period within each year. Each site was not monitored during every moon period so some yearly summaries may be an average of four moon periods, while others may be an average of three. It was desirable to achieve some general descriptive statistics on yearly and regional scales, so these summaries are reported. In graphical comparisons among sites and regions, averages were weighted by the number of quadrats sampled since this was reflective of the length of beach that was surveyed at each site. Sex ratios (female:male) were also calculated from survey data.

At Monomoy NWR (Morris Island and South Monomoy Island) beach length ranged from 1,000 to 1,400 m. At North Monomoy Island, we sampled a 100 × 50 m section of the tidal flat area. Due to logistical constraints and safety issues with night sampling, North Monomoy Island was only surveyed during the day. In Cape Cod NS beach length varied from 150 to 700 m. All locations were surveyed during night and day, except one location (Marsh 2–3) where only day surveys were conducted due to safety issues. In Cape Cod Bay beach lengths varied from 200 to 700 m and all locations were surveys during night and day.

Egg Densities from Beach Sediments

To determine densities of horseshoe crab eggs in beach sediments, we collected sediment cores from survey beaches within Monomoy NWR, Cape Cod NS, and Cape Cod Bay in 2001 and 2002. We refer to egg density as the combined total of eggs, embryos, and trilobite larvae. Sediment cores were collected from 9 locations in 2001 and 5 locations in 2002. At each location, a 100-m length of beach, centered mid beach on a 10-m wide strip between the low and high tide line, was randomly selected (except for the site at North Monomoy Island, see below). This portion of the beach was chosen since Shuster (1950, 1982) observed horseshoe crabs on Cape Cod to spawn in a broad band centered at mid beach. A composite core consisting of 5 cores (10 cm diameter, 20 cm deep) was taken from a 1 × 1 m section that was randomly chosen along the length and width of the mid beach section. Samples at the North Monomoy Island (Monomoy NWR) were randomly taken from within the section of tidal flats that was established for the spawning surveys. A composite core was taken to increase the area sampled while reducing the amount of cores taken and later sorted. The 5 cores that comprised the composite sample were taken at each corner and in the center of the 1-m square. Each core was divided into shallow sediment (0–5 cm) and deep sediment (5–20 cm). We divided cores into shallow and deep sediments in order to evaluate if horseshoe crab eggs were present in the top 5 cm of sediment where they would be available to foraging shorebirds. The top 5 cm of sediment from all 5 cores were placed in a bucket, mixed thoroughly, and a subsample representing the same volume (10 cm in diameter, 5 cm deep) was taken from the bucket. The remaining portion of each core (5–20 cm) was placed in second bucket, mixed thoroughly, and a subsample representing the same volume (10 cm diameter, 15 cm deep) was taken. Sediment representing the top 5 cm and bottom 5–20 cm were placed in separate plastic bags and frozen until sorted. Samples were taken in early to mid July of both years and 9–15 composite cores were taken at each location. Sediments were sifted through a 1-mm sieve and all eggs, embryos, and trilobites were enumerated for each sample. Average numbers of eggs (eggs, embryos, and trilobites combined) were calculated for each beach and are presented as the average number per core for shallow and deep sediments.

Beach Slope

To monitor the slope profile of spawning beaches over time, the slope of selected survey beaches was determined using an autolevel (Path Instruments, model 16F352) in 2000. Transects were oriented perpendicular to the water's edge and started at vegetation line (dune border) and extended to where the beach slope leveled out at approximately mean low water. Elevations were determined at 1-m increments along each transect by differential leveling from benchmarks. Transects were spaced at intervals of 15, 30, or 50 m depending on the length of the survey site. Benchmarks and starting locations for each transect were georeferenced by a global positioning unit and compass headings for all transects were noted to enable long-term monitoring of the spawning beach slopes. Beach slopes were measured for two sites within Monomoy NWR (South Monomoy Island and Morris Island), two locations within Pleasant Bay, Cape Cod NS (Hog Island and Northville), and three Cape Cod Bay sites (First Encounter, Chipman's Cove, and Lieutenant Island). Average percent slope of each transect was calculated as the elevation differential divided by the length of transect. The average of all transects for a site was calculated to estimate the slope for each beach.

Tagging Methods

We tagged 7,860 spawning adults throughout Cape Cod from 2000 to 2002 in order to obtain information on the movements of spawning horseshoe crabs. All tagged crabs were sexed and measured in order to collect information on the size structure of spawning horseshoe crabs. Horseshoe crabs were primarily tagged at spawning survey beaches (Fig. 1) but were also tagged at other sites where they were encountered during daytime flood tides. All adults (attached pairs, single females, solitary males, satellite males) were tagged as they were encountered on or approaching beaches. We assume that the sample of tagged individuals was a random and representative sample of the spawning population within each region. Horseshoe crabs were tagged within all 4 embayments on Cape Cod, although crabs were not tagged in all regions in all years. Regions and years where horseshoe crabs were tagged were: Monomoy NWR: 2001 and 2002; Pleasant Bay: 2000, 2001, and 2002 and Nauset Estuary: 2001 and 2002, both within Cape Cod NS; and Cape Cod Bay: 2000, 2001, and 2002 (Fig. 1). We tagged crabs by drilling a small hole (approximately 4 mm) with a battery operated drill through the right posterior point of the prosoma. If possible, the prosoma and drill bit were swabbed with an antibiotic solution prior to drilling each animal. A double T-bar anchor tag (model SHD, FLOY TAG Inc., 4616 Union Bay Place NE, Seattle, Washington 98105, http://www.floytag.com) was inserted through the hole with a tagging gun. Each tag had a unique tag number, research agency identification, and phone number. After tagging, the tag number and location were recorded, the sex of the individual was determined (based on the morphology of the pedipalps), and size was measured as prosomal width (mm) at the widest point across the bottom of the prosoma.

We obtained recapture information of tagged crabs from a variety of sources, but the majority of recaptured crabs were encountered by individuals affiliated with this study during spawning surveys and tagging sessions from May through August of each year. Additional information on recaptured crabs was obtained from a local biomedical company (Associates of Cape Cod), horseshoe crab harvesters, and the general public who reported information throughout the years. Recapture information was limited to the tag number on the crab, the recapture location and date, and whether the crab was alive or dead. Information on recaptured live crabs was used to determine straight-line distance traveled between release and recapture location (calculated by GIS) and days at liberty. From this information we were able to map the straight-line distance movement patterns for horseshoe crabs within each embayment, to determine if crabs moved far from the initial tagging location or returned to the same spawning sites each year.

Statistical Analyses

Chi-square analyses were used to evaluate sex ratios. If significant Chi-square values were found, Freeman-Tukey deviates (Sokal and Rohlf 1995) were calculated to determine what sex occurred at a higher or lower frequency than expected. Nonparametric permutation analyses were used to evaluate differences in egg densities between shallow and deep cores. A macro in Microsoft Excel was programmed to run five hundred permutations to compare the original test statistic (difference between the average of each survey or region) to the permuted distribution of test statistics from which probability values were calculated (Heltshe personal communication). The relationship between average spawning densities and egg density, as well as continuous environmental variables (e.g., beach slope, water temperature) were analyzed by nonparametric (Spearman's rho) correlation or linear regression. Ranked analysis of variance (ANOVA) tests were performed to determine if spawning densities were influenced by wave height. Size data were evaluated using ANOVA followed by least squared means tests, and size frequency distributions were evaluated by pair-wise Kolmogorov-Smirnov tests. Where appropriate, alpha levels were adjusted for multiple pair-wise comparisons using the Bonferroni method (Sokal and Rohlf 1995).

Results

Spawning Surveys

We conducted spawning surveys during a 5-d period around the new and full moons high tides (2 d prior, the day of, and 2 d after). Previous work in Delaware Bay has observed that spawning activity was not equally distributed among the 7-d period around a full or new moon, but rather disproportionately more spawning occurred within 3 d of the new or full moon (Smith et al. 2002b). In Chesapeake Bay, peak spawning does not occur consistently on any one day around the new or full moon (ASMFC 1998). On Cape Cod, due to staff and logistical constraints, all beaches could not be surveyed on exactly the same night. This presents a potential problem for the Cape Cod data in that if surveys for any particular beach and moon period are averaged to obtain summary information (whether by moon period or year), it is possible that the average estimate may be biased due to the different survey dates during any particular moon period (i.e., day of survey may be confounding factor). Since we do not know if there was differential spawning around new or full moons on Cape Cod, we explored our data to determine if there were any locations that were surveyed on the same nights to evaluate if spawning densities differed among days for any particular moon period. Data for 4 beaches surveyed on Cape Cod Bay (Chipman's Cove, Cole Road, Old Wharf, and Wellfleet Bay Wildlife Sanctuary) for night surveys for the June 5, 2001, full moon period fit these criteria (James-Pirri et al. 2002). The night surveys for this full moon period were conducted on the nights of June 3 and June 5 and the early morning hours of June 8, 2001. ANOVA was used to evaluate if the date of the survey during the June 2001 full moon influenced total density or spawning index. The ANOVA results for total density and spawning index were not significant among survey days (density: $F = 0.43$, $p = 0.6603$; spawning index: $F = 0.66$, $p = 0.5404$). These data indicate that there were no detectable statistical differences for total density or spawning index on the different survey dates for the June 2001 full moon period. It should be noted that the failure to reject the null hypothesis of no difference may be a result of a large between site error or a small sample size ($n = 4$ beaches). Since these are the only comprehensive data on spawning densities for Cape Cod at this time, we must conclude that the day of survey did not significantly influence spawning densities at these beaches. Based on the above analyses, we made the following assumptions for all statistics and inferences related to spawning densities. The June 2001 full moon survey data from Chipman's Cove, Cole Road, Old Wharf, and Wellfleet Bay Wildlife Sanctuary were representative of spawning patterns for all moon periods (2000–2002) for all Cape Cod beaches. Since there were no statistical detectable differences in spawning densities (total density and spawning indices) with survey day, averages for beaches during individual moon periods where surveys did not occur on exactly the same day were not biased due to the day of the survey (i.e., day of survey was not a confounding factor).

For simplicity, we summarized spawning indices, spawning densities, and sex ratios for four regions

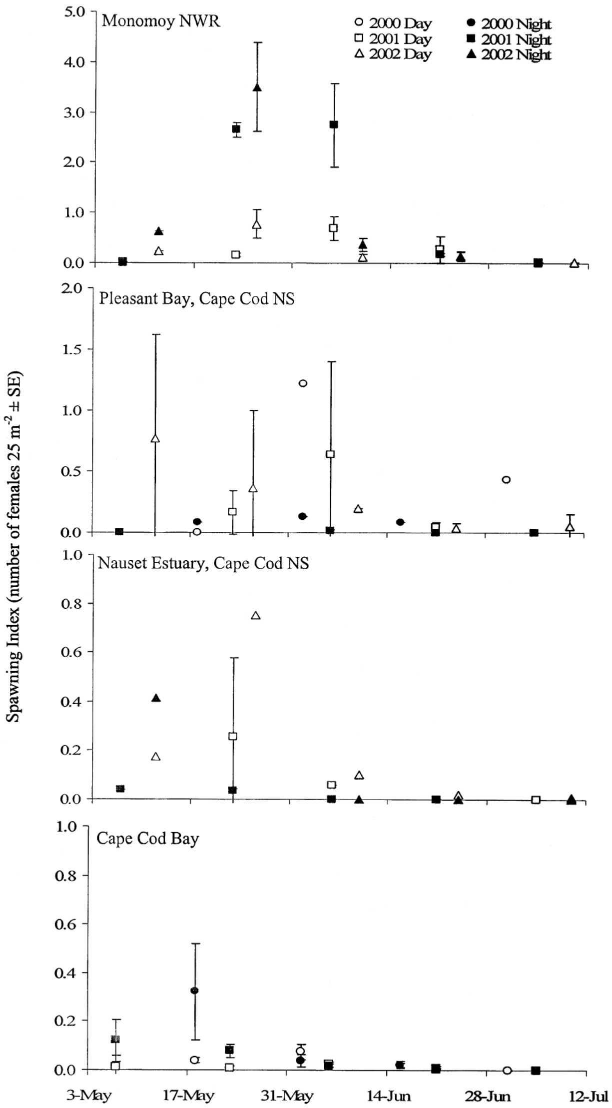

Fig. 2. Average (weighted) day and night spawning indices (number of female crabs 25 m^{-2} ± SE) for each moon phase during the 2000 to 2002 sampling season for embayments on Cape Cod. Spawning indices were weighted by beach length. Indices are plotted by the date of the new and full moon for each month.

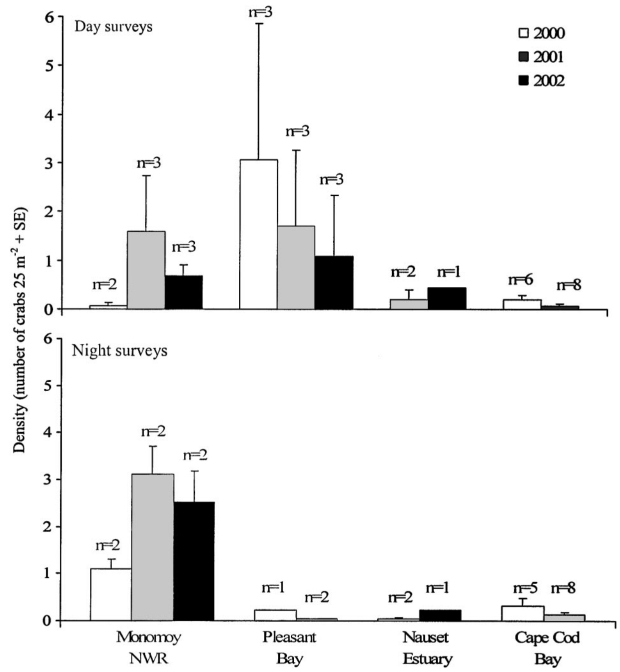

Fig. 3. Average (weighted) spawning densities (number of crabs 25 m^{-2} + SE) of horseshoe crabs for embayments on Cape Cod during day and night surveys. Densities were weighted by beach length (n is number of beaches surveyed). Note: One site within Pleasant Bay (Marsh 2–3) could only be surveyed during day tides.

(Monomoy NWR, Pleasant Bay, Nauset Estuary, and Cape Cod Bay) rather than by individual beach locations. Average (weighted) spawning indices (number of female crabs 25 m^{-2}) for each moon period (e.g., new moon or full moon) within each region and year are shown in Fig. 2. Based on our data, spawning commences on Cape Cod in early May, peaks from mid May through mid June, and was usually completed by mid July. Monomoy NWR had high spawning indices, ranging from 0.1 to 1.0 females 25 m^{-2} for day surveys and 0.8 to 1.3 females 25 m^{-2} for night surveys. Pleasant Bay also had relatively high spawning indices ranging from 0 to 1.2 females 25 m^{-2} for day surveys and 0 to 0.1 females 25 m^{-2} for night surveys. Nauset Estuary had spawning indices intermediate between Pleasant Bay and Cape Cod Bay, ranging from < 0.01 to 0.2 females 25 m^{-2} for day surveys and 0.01 to 0.1 females 25 m^{-2} for night surveys. Cape Cod Bay had relatively low spawning indices, ranging from 0 to 0.1 females 25 m^{-2} for day surveys and 0.01 to 0.25 females 25 m^{-2} for night surveys.

Average (weighted) yearly spawning densities for day and night surveys are shown in Fig. 3. Monomoy NWR and Pleasant Bay had relatively high daytime spawning densities compared to other regions. Pleasant Bay had noticeably higher daytime spawning compared to night spawning surveys; this may be due to the Marsh 2–3 site that could only be surveyed during day tides. We recorded highest daytime spawning densities (e.g., 17 crabs 25 m^{-2}) of all sites surveyed on Cape Cod at Marsh 2–3. This site consistently had high spawning densities (more than 10 crabs 25 m^{-2}) during the peak spawning season. Nauset Estuary had densities similar to Pleasant Bay during night surveys, but not during day surveys when fewer crabs were ob-

TABLE 1. Sex ratios from spawning surveys of horseshoe crabs on Cape Cod. Nauset Estuary was not surveyed in 2000 and Cape Cod Bay was not surveyed in 2002.

Location	Sex Ratios (Female:Male)			
	2000	2001	2002	All Years
Monomoy NWR	1:1.9	1:2.0	1:1.6	1:1.9
Pleasent Bay, Cape Cod NS	1:4.5	1:9.0	1:5.3	1:5.8
Nauset Estuary, Cape Cod NS	—	1:1.6	1:1.6	1:1.6
Cape Cod Bay	1:3.1	1:2.6	—	1:2.9

served. Cape Cod Bay had the lowest densities of all the regions.

Female to male sex ratios from spawning surveys (all surveys combined) from each of the four regions are shown in Table 1. Pleasant Bay consistently had the highest ratios, with a 3-yr average of 1:5.8. Monomoy NWR and Nauset Estuary consistently had the lowest ratios (Monomoy NWR 1:1.9, Nauset Estuary 1:1.6). Sex ratios within each of the regions were fairly consistent among years, with the exception of Pleasant Bay, where a very high male to female ratio of 1:9 was observed in 2001 (Table 1). We found significant differences in spawning sex ratios among the regions in all years. In 2000, Monomoy NWR had significantly lower ratios (more females to males) than either Pleasant Bay or Cape Cod Bay (Chi-square, $p < 0.0167$, Bonferroni adjusted alpha). Freeman-Tukey deviates indicated that there was a lower frequency of females and a higher frequency of males in both Pleasant Bay and Cape Cod compared to Monomoy NWR. There was no difference in the sex ratios between Pleasant Bay and Cape Cod Bay in 2000 (Nauset Estuary was not sampled in 2000). In 2001, Pleasant Bay had significantly higher sex ratios than the three other regions (Chi-square, $p < 0.0083$, Bonferroni adjusted alpha). Freeman-Tukey deviates indicated that the other three regions (Monomoy NWR, Nauset Estuary, and Cape Cod Bay) all had higher frequencies of females and lower frequencies of males compared to Pleasant Bay. There was no difference in the sex ratios among Monomoy NWR, Nauset Estuary, or Cape Cod Bay in 2001. In 2002, we observed a pattern similar to that in 2001. Pleasant Bay had significantly different sex ratios than either Monomoy NWR or Nauset Estuary (Cape Cod Bay was not sampled in 2002; Chi-square, $p < 0.0167$, Bonferroni adjusted alpha). Freeman-Tukey deviates indicated that Pleasant Bay had a lower frequency of females and a higher frequency of males than the other two regions.

Egg Densities

We observed egg densities (eggs, embryos, and trilobites combined) of individual sediment cores ranging from 0 to 17 eggs cm^{-2} (0–1,406 eggs per core) for deep sediments and 0 to 4.2 eggs cm^{-2} (0–350 eggs per core) for shallow sediments; the majority of samples had densities of less than 1 egg cm^{-2} (approximately 83 eggs per core). Average counts of eggs from shallow (top 5 cm) and deep (5–20 cm) sediments are presented in Table 2. Although there was a trend of higher egg densities in deeper sediments at most beaches where eggs were found, only North Nauset Beach and Hog Island in 2001 had statistically higher densities in deep sediments (permutation test, $p < 0.05$). Due to the low number of beaches sampled for egg densities, statistical comparisons among regions were not performed. Cape Cod Bay had very low egg densities with only 1 of the 4 beaches sampled (Tavern Cove Beach) having cores where any eggs were observed. Beaches within Cape Cod NS (North Nauset Beach and Marsh 2–3) had comparably high densities (averaging 2–5 eggs cm^{-2} or 150–450 eggs per core). A significant, but weak, positive relationship was found between spawning index (the average of annual day and night spawning indices for each beach) and total (shallow and deep sediments combined) egg density (Spearman's rho = 0.549, $p = 0.042$).

Beach Slopes and Environmental Variables

Average beach slopes ranged from 6% to 11%. A regression of average beach slope versus annual (2000–2002) day and night spawning densities was not significant ($p = 0.0534$). There was a trend of higher spawning densities associated with beaches that had lower slope profiles. There was no relationship between spawning density or spawning index and water temperature for either day (density: $p = 0.2332$, $R^2 = 0.012$; spawning index: $p = 0.6228$, $R^2 = 0.003$) or night (density: $p = 0.3724$, $R^2 = 0.009$; spawning index: $p = 0.5618$, $R^2 = 0.005$) surveys. There was also no relationship between day or night spawning densities or spawning indices and wave height (ranked ANOVA, $p > 0.05$), although there was a trend of higher spawning densities and spawning indices associated with lower wave heights.

TABLE 2. Average count of horseshoe crabs eggs (eggs, embryos, and trilobites combined) per composite core (± SE) and replicate sample sizes for 9 beaches sampled throughout Cape Cod in 2001 and 2002. Composite cores were 10 cm in diameter (approximately 83 cm^2) and 9–15 replicate composite cores were randomly taken at each sampling location. The top 0–5 cm of the core represents shallow sediments while the bottom 5–20 cm of the core represents deep sediments. Sediment samples were collected in early to mid July in both years. Cape Cod Bay beaches were sampled only in 2001.

Beach	2001		2002	
	Shallow Sediment Count	Deep Sediment Count	Shallow Sediment Count	Deep Sediment Count
Monomoy NWR				
North Monomoy	3.9 (1.9) 10	4.4 (2.6) 10	5.1 (4.2) 15	42.1 (41.7) 15
Pleasant Bay, Cape Cod NS				
Hog Island	0.7 (0.4) 12	78.0 (33.9) 12	0.4 (0.4) 10	0.1 (0.1) 10
Marsh 2–3	0.3 (0.3) 10	0 (0) 10	26.3 (13.0) 10	455.2 (192.1) 10
Northville	0 (0) 10	0 (0) 10	0.2 (0.2) 10	2.2 (2.2) 10
Nauset Estuary, Cape Cod NS				
North Nauset Beach	29.2 (16.9) 10	359.9 (110.3) 10	74.4 (42.1) 10	160.3 (54.3) 10
Cape Cod Bay				
Chipman's Cove	0 (0) 10	0 (0) 9	—	—
Lieutenant Island	0 (0) 10	0 (0) 10	—	—
Old Wharf	0 (0) 8	0 (0) 8	—	—
Tavern Cove	0.1 (0.1) 10	0.1 (0.1) 10	—	—

Size Structure

Size of female horseshoe crabs varied among regions (ANOVA, $p < 0.0001$) but not among years (ANOVA, $p = 0.3000$; interaction term, $p = 0.5332$). Since there were no differences among years, we combined the size data for all years for females in the following analyses, however sizes for individual years are presented in Table 3. Females from Monomoy NWR were significantly larger than those from the other 3 regions (least squared means, $p < 0.0001$ for all comparisons). Females from Nauset Estuary were significantly larger than those from Pleasant Bay and Cape Cod Bay (least squared means, $p = 0.0115$ and $p < 0.0001$, respectively). Females from Pleasant Bay were similar in size to those in Cape Cod Bay (least squared means, $p = 0.1518$).

Size of male horseshoe crabs varied among regions (ANOVA, $p < 0.0001$) and years (ANOVA, $p = 0.0004$). Since the interaction term was significant (ANOVA, $p = 0.0356$) we analyzed male size separately by year to determine if there were differences in size among the regions. Prosomal widths of male horseshoe crabs are presented in Table 3. In 2000, males from Pleasant Bay were significantly larger than those from Cape Cod Bay (ANOVA, $p < 0.0001$). In 2001, there were significant differences in the size of males among all regions (ANOVA, $p < 0.0001$). Males from Monomoy NWR were significantly larger than males

TABLE 3. Prosomal width in mm (± SE) and number of individuals measured for female and male horeshoe crabs tagged on Cape Cod during the 2000 to 2002 spawning seasons. Horseshoe crabs were not tagged (and no size data are available) at Monomoy NWR or Nauset Estuary in 2000.

Region and Sex	Prosomal Width				Size Range (mm)
	2000	2001	2002	All Years	
Monomoy NWR					
Females	—	242 (1.1) 328	242 (1.8) 149	242 (1.0) 477	186–304
Males	—	188 (0.7) 510	187 (0.8) 399	188 (0.5) 909	135–287
Pleasant Bay, Cape Cod NS					
Females	230 (2.1) 136	230 (2.0) 135	222 (4.8) 27	229 (1.4) 298	170–295
Males	180 (0.5) 841	179 (0.5) 663	177 (0.8) 270	179 (0.3) 1775	120–235
Nauset Estuary, Cape Cod NS					
Females	—	234 (1.5) 184	234 (2.6) 72	234 (1.3) 256	185–292
Males	—	177 (1.0) 304	171 (1.4) 129	175 (0.8) 433	134–249
Cape Cod Bay					
Females	227 (1.5) 174	227 (0.8) 539	226 (3.1) 46	227 (0.7) 759	161–286
Males	174 (0.5) 818	175 (0.4) 1847	173 (0.9) 277	174 (0.3) 2942	119–250

TABLE 4. Total number of tagged horseshoe crabs, recapture rates (live and dead recaptures), and percent of recaptures found at or near the original tagging location on Cape Cod. Data are from all sampling years combined for each embayment.

Region	Total Number Tagged	Total Number (Live and Dead) and Overall Recapture Rate (%)	Number (and Percentage) of Live Recaptures	Percent of Live Crabs Recaptured at Tagging Location	Percent of Live Crabs Recaptured within 2 km of Tagging Location	Percent of Recaptures at Liberty more than 9 mo (Live Crabs Only)
Monomoy NWR	1387	46 (3.3%)	38 (2.7%)	68%	76%	23%
Pleesant Bay	2074	110 (5.3%)	103 (5.0%)	33%	43%	16%
Nauset Estuary	697	80 (11.5%)	69 (9.9%)	98%	100%	37%
Cape Cod Bay	3702	288 (7.8%)	236 (6.4%)	49%	60%	23%
All Regions Combined	7860	524 (6.7%)	446 (5.7%)	62%	70%	25%

from the four regions (least squared means, $p < 0.0001$). Pleasant Bay had males that were larger than both Nauset Estuary and Cape Cod Bay (least squared means, $p < 0.05$). Males from Nauset Estuary were significantly larger than those from Cape Cod Bay (least squared means, $p = 0.0402$). In 2002, a similar pattern to 2001 was observed. Males from Monomoy NWR were larger than those from Pleasant Bay, Nauset Estuary, or Cape Cod Bay (ANOVA, $p < 0.0001$; least squared means, $p < 0.0001$). Males from Pleasant Bay were larger than those from Nauset Estuary and Cape Cod Bay (least squared means, $p < 0.05$). Males from Nauset Estuary were similar in size to those from Cape Cod Bay ($p = 0.0953$).

To compare size frequency distributions among embayments, we first analyzed the prosomal width data to determine if we could combine data from all years for each embayment. There were no differences in size frequency distributions for female crabs in any of the yearly comparisons within embayments, so we combined data from all years in the following analyses for female crabs. Female size frequency distributions among pair-wise comparisons for all embayments were significantly different (Kolmogorov-Smirnov test, $p < 0.0083$, Bonferroni adjusted alpha). We observed significant differences in the size frequency distributions for male crabs between years from two embayments (Nauset Estuary, 2001 versus 2002; Pleasant Bay, 2000 versus 2002 [Bonferroni adjusted alpha for Pleasant Bay, $p = 0.0167$]; Kolmogorov-Smirnov test, $p = 0.022$ and 0.001, respectively). Even though there were differences within years in these embayments, we observed the same trends for all pair-wise comparisons for each year. Male size frequency distributions were significantly different among all embayments for each year (Kolmogorov-Smirnov test, $p < 0.05$ for 2000, $p < 0.0083$ for 2001 and 2002, Bonferroni adjusted alpha) with the exception of the comparison between Cape Cod Bay and Nauset Estuary in 2001 (Kolmogorov-Smirnov test, $p = 0.327$).

Recapture Data

A total of 7,860 horseshoe crabs were tagged in 2000, 2001, 2002 throughout Cape Cod and 542 individuals were recaptured (6.7% overall recapture rate), 446 were alive at the time of recapture (5.7% live recapture rate) as of December 2002 (Table 4). Overall recapture rates differed among the four regions. Nauset Estuary had the highest recapture rate (11.5%) while Monomoy NWR had the lowest recapture rate (3.3%; Chi-square, $p < 0.0083$, Bonferroni adjusted alpha). Recapture rates were similar between Pleasant Bay and Cape Cod Bay (5.3% and 7.8%, respectively). Thirty-four individuals (31% of recaptures) were reported by Associates of Cape Cod (the local biomedical facility). More tags from Pleasant Bay were reported by Associates of Cape Cod in 2000 (30 individuals) than in 2001 or 2002 (4 and 0 individuals, respectively). A lower percentage of the recaptures were reported by Associates of Cape Cod for horseshoe crabs found within Cape Cod Bay (4%) and Monomoy NWR (3%). The remaining individuals were reported by the general public or were sighted during spawning surveys or tagging sessions. Four individuals were found with 1–888-LIMULUS button tags (3 alive in 2001, 1 dead in 2002) within Monomoy NWR. These crabs were tagged under the U.S. Fish and Wildlife Service's Cooperative Horseshoe Crab Tagging Program at Associates of Cape Cod (and released in Chatham, Massachusetts) during the summer of 1998.

The majority of the live recaptured horseshoe crabs were found at the same location (62% overall) or within 2 km (70% overall) of the beach where originally tagged (Table 4). Average straight-line distance traveled (as estimated by GIS) by tagged crabs within Pleasant Bay and Monomoy NWR was between 2 and 3 km, and for Cape Cod Bay was 7.2 km. Average distance traveled was not calculated for Nauset Estuary since the majority of recaptures (98%) were found at the original tagging location (the high proportion of individuals recaptured at the same location within Nauset Estuary was most likely due to accessibility of the tagging site and its proximity to a National Park Service nature trail along Nauset Marsh). The majority of recaptures were reported during the same year in which the individual was tagged, but 16% to 37% of live recaptures were reported after 9 or

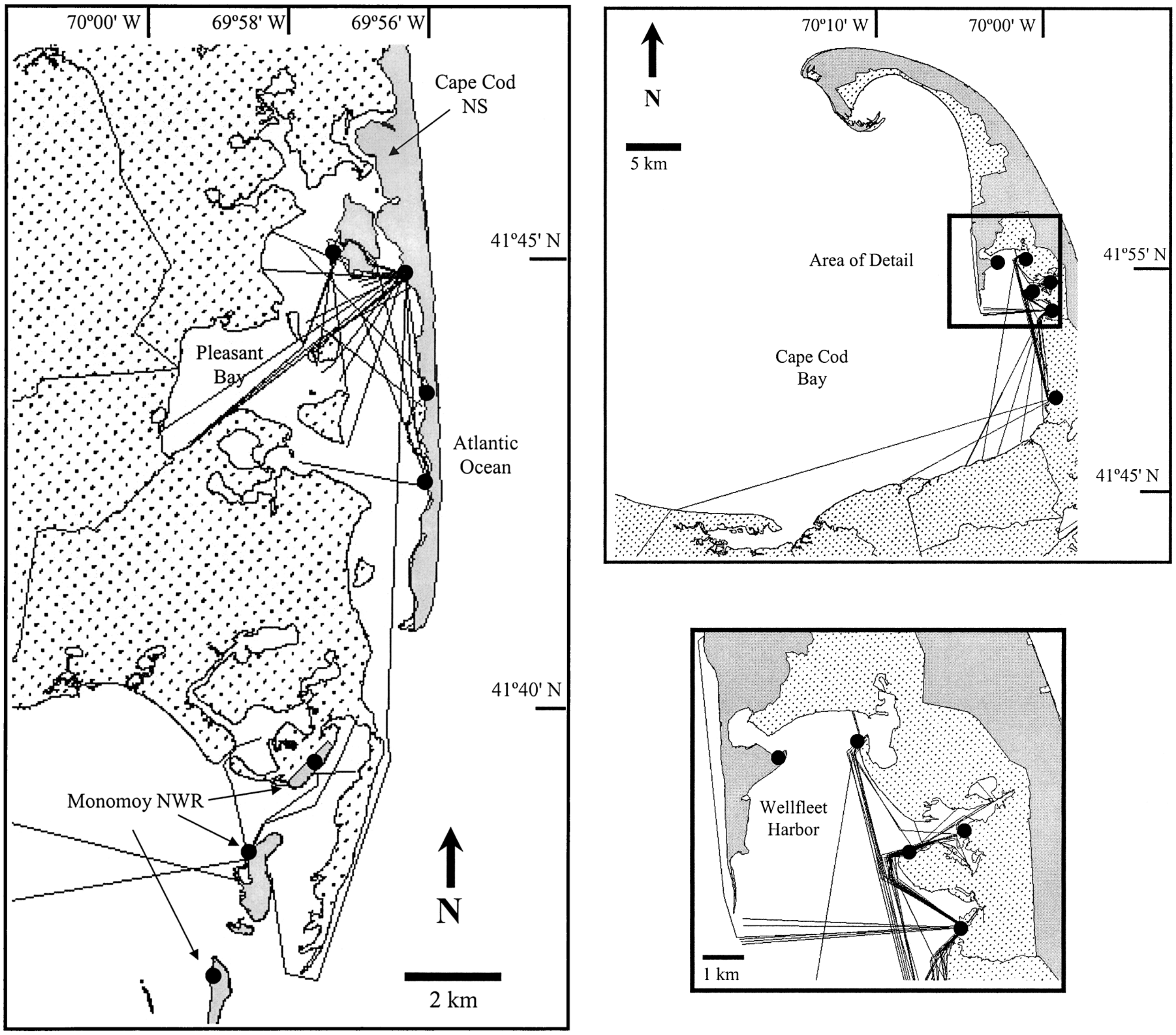

Fig. 4. Straight-line distance traveled (as estimated by GIS) from tagging location to recapture location for horseshoe crabs tagged within Pleasant Bay and Monomoy NWR and Cape Cod Bay. Shaded area indicates Cape Cod NS and Monomoy NWR properties. Circles indicate tagging locations. Lines were drawn only for crabs that were recaptured at a different location than where they were tagged.

more months at liberty (Table 4). Some of these individuals were recaptured at the same spawning beach where they were originally tagged. One individual from Pleasant Bay was recaptured at the same spawning beach where it was originally tagged 2 yr earlier, and 3 other crabs were recaptured at the same spawning beach in Pleasant Bay 1 yr later. Twenty-five of the 26 crabs from Nauset Estuary recaptured after 9 mo were found at same spawning beach. Two individuals recaptured at Monomoy NWR after 9 mo at liberty were found at the same spawning beach; within Cape Cod Bay, 4 individuals were recaptured at the same spawning beach where they had been tagged 1 yr earlier.

Occasionally, horseshoe crabs were recaptured far from their tagging location. At Monomoy NWR, 2 individuals traveled from North Monomoy to Lewis Bay (Yarmouth) and Harwich (29 and 10.5 km, respectively; Fig. 4). In Pleasant Bay, the majority of the recaptured individuals were reported from the middle and northern portions of the Bay. Three individuals tagged within Pleasant Bay were recaptured at North Monomoy Island (approximately 17 km). In Cape Cod Bay, the majority of the recaptured crabs were observed in the northern and eastern (Wellfleet Bay) area of the Bay. One individual was recaptured 33 km from that tagging location. This individual was taken by a biomedical harvester (after it was tagged), and it is possible that it was released in Dennis, Massachusetts (the location where the Associates of Cape Cod releases Cape Cod Bay harvested horseshoe crabs after bleeding) rather than traveling to Dennis under its own power. Although this tag number was not reported by Associates of Cape Cod, not all crabs collected for biomedical purposes are selected for bleeding (i.e., ones that are damaged or have extensive amounts of epibionts on the prosoma are rejected), so this crab could have been released in Dennis by the harvester. The next furthest distance traveled within Cape Cod Bay was 17 km. With the exception of the 3 crabs that traveled from Pleasant Bay to Monomoy NWR, we observed no other movement of tagged crabs among these 4 embayments on Cape Cod.

Discussion

Spawning activity peaked around the May new and full moons, similar to the pattern observed in other populations of horseshoe crabs (Rudloe 1980; Shuster and Botton 1985; Barlow et al. 1986; Wenner and Thompson 2000; Smith et al. 2002b). Rudloe (1980) observed peak spawning activity during the night high tides in Apalachee Bay, Florida; Botton (1982) observed spawning on both the day and night high tides in Delaware Bay. We observed a relatively high amount of spawning during the day high tides on Cape Cod, especially in Pleasant Bay. Barlow et al. (1986) observed that spawning activity on a beach near Woods Hole, Massachusetts, was more strongly correlated with tide height than light or darkness and concluded that horseshoe crabs generally prefer to spawn on the highest tides of the day regardless of when they occur. Carmichael et al. (2003b) estimated the spawning period for horseshoe crabs within Pleasant Bay from the percent of paired crabs throughout the Bay (not from spawning beaches) and extrapolated the start and end of the spawning season based on a polynomial curve. They estimated that the start of spawning was in early April and lasted until early July. While our data are in agreement with the end of spawning in early July, we did not observe horseshoe crabs spawning on beaches until early May. Since Carmichael et al. (2003b) relied upon extrapolated data to determine the start of the spawning season it is likely that they overestimated the beginning of spawning given our empirical observations of spawning starting in early May.

Monomoy NWR, particularly North and South Monomoy Islands, had the highest densities of spawning horseshoe crabs throughout the spawning period. Maximum densities at these locations ranged from 14 to 17 crabs 25 m^{-2} in all years surveyed. Certain sites within Pleasant Bay (Marsh 2–3 and Hog Island) also had high spawning densities, with yearly averages ranging from 6 to 9 crabs 25 m^{-2} (James-Pirri et al. 2002). Marsh 2–3 was an interesting site. This site was a small area (200 × 15 m) characterized by a sandy substrate with irregular patches of salt marsh cordgrass (*Spartina alterniflora*). Horseshoe crabs spawned at the interface of the low marsh zone and the adjacent sand flats where the water depth ranged from 10 to 40 cm, as well as within the patches of *S. alterniflora*. During individual surveys densities were as high as 17 crabs 25 m^{-2}; many more crabs (more than 1,500 individuals during one survey, but averaging approximately 150–350 individuals) were counted in the near vicinity of the quadrats on several survey days. Due to safety issues (i.e., walking across a flooded, ditched marsh in the dark) this site was surveyed only during the day. It is not known how much spawning occurred at this site during night high tides; the area where horseshoe crabs were observed spawning would be submerged under approximately 0.5–1 m of water during night high tides so it is possible that crabs may only spawn at this location during the day. These spawning hot spots within Monomoy NWR and Pleasant Bay may be extremely important as they could be responsible for the majority of the spawning that occurs within these areas. Relative to other areas on Cape Cod, Cape Cod Bay had low spawning densities (<1 crab 25 m^{-2}). Only a few locations in Cape Cod Bay (Chipman's Cove, Lieutenant Island) had surveys where densities over 2 crabs 25 m^{-2} were recorded. We did not observe any relationships among spawning densities or spawning indices with either water temperature or wave height, although there was a trend of higher densities associated with lower wave heights.

We can compare our spawning density data to other studies by converting our spawning densities to number of individuals per square meter and our spawning indices into the Index of Spawning Activity (ISA), which is the number of females per square meter, and calculating yearly averages. Compared to other regions, Cape Cod had very low spawning densities and spawning indices. Spawning densities in Delaware Bay, Florida, and South Carolina average from 1 to 3 crabs m^{-2} (Rudloe 1980; ASMFC 1998; Thompson 1998), whereas yearly spawning densities on Cape Cod were much lower, ranging from 0.03 to 0.04 crabs m^{-2} for day surveys and 0.02 to 0.08 crabs m^{-2} for night surveys. The yearly ISA in Delaware Bay from 1999 to 2001 ranged from 0.73 to 0.90 females (Smith and Bennett 2001) and averaged 0.07 females m^{-2} in 1997 and 1999 on Otter Island, South

Carolina (Wenner and Thompson 2000). On Cape Cod, we recorded yearly ISA densities an order of magnitude lower than those from Delaware Bay and South Carolina, ranging from 0.006 to 0.028 females m^{-2} for day surveys and 0.004 to 0.01 females m^{-2} for night surveys from 2000 to 2002. The overall low densities and ISA observed on Cape Cod highlight the potential importance of spawning hot spots, as specific spawning beaches where higher densities are consistently observed may be responsible for the majority of spawning and recruitment that occurs for a particular population. We estimated from our data that 3 beaches (Marsh 2–3, North and South Monomoy Islands) were responsible for 57% of the total spawning that occurred on the Cape Cod during 2001. Areas of high spawning activity may be of particular importance in the more northern portions of the range for horseshoe crabs where population sizes are smaller.

We observed spawning sex ratios for 3 of the 4 regions (Monomoy NWR, Nauset Estuary, and Cape Cod Bay) of 1 female to every 2–3 males. This was similar to what Barlow et al. (1986) reported for a population in Bourne in (1 female to 2.5 males) in 1986 and were comparable to those observed in other regions. In Delaware Bay, average spawning sex ratios ranged from 1:1.5 to 1:5 (female:male; Shuster and Botton 1985; Swan et al. 1996; Smith et al. 2002b). In Apalachee Bay, Florida, Rudloe (1980) observed ratios of 1:3.6, and in South Carolina, sex ratios have been reported to range from 1:2 to 1:3.5 (Thompson 1998; Wenner and Thompson 2000). Since males congregate along the shoreline to intercept spawning females, sex ratios of spawning horseshoe crabs are usually inherently biased towards males (Brockmann 1990; Shuster 1996). Compared to other areas of Cape Cod, spawning sex ratios for Pleasant Bay, especially in 2001, were highly skewed towards males (1:9) and may be a reflection of other pressures on the Pleasant Bay population. Historical spawning sex ratios for two regions we surveyed (Pleasant Bay and Barnstable Harbor) from the 1950s ranged from 1:1.9 to 1:2.5 for Pleasant Bay and 1:1.5 to 1:2.4 for Barnstable Harbor (Shuster 1950, 1979; Shuster personal communication). Recent female to male spawning sex ratios for Barnstable Harbor (Sandy Neck and Bonehill Road) were 1:6.3 and 1:2.4 in 2000 and 2001, respectively (James-Pirri et al. 2002). The lowest spawning sex ratio we observed in Pleasant Bay, 1:4.5 (2000 data), was much higher than the 1:1.9 and 1:2.5 ratios observed by Shuster in the 1950s in this same embayment (Shuster 1979; Shuster personal communication). The change in spawning sex ratios at these local areas could be evidence that spawning population dynamics have changed in the past 50 yr on Cape Cod. Carmichael et al. (2003b) surveyed horseshoe crabs throughout Pleasant Bay using a grid system and reported a male dominated sex ratio of 1:2.3 in nonspawning adults. Shuster (1996) suggested that a deviation away from a 1:1 ratio in nonspawning adults (e.g., trawl surveys) towards one with fewer females per male could be indicative of overfishing of females. Horseshoe crabs within Pleasant Bay have been harvested by both the biomedical and bait industry for over 25 yr (Finley personal communication). The majority of crabs harvested from Pleasant Bay are used for biomedical purposes (crabs are also taken for scientific research and for bait) with the Pleasant Bay harvest accounting for 22% of all biomedical harvest on the Atlantic Coast in 2001 (Rutecki 2002; Rutecki et al. 2004). The highly skewed spawning ratios observed in this study and the male dominated nonspawning sex ratio reported by Carmichael et al. (2003b) for Pleasant Bay may be a result of this pressure, as both the biomedical and bait fishery preferentially select females at a ratios of 1:0.3 and 1:0.5, respectively (Rutecki 2002; Rutecki et al. 2004). If biomedical bleeding causes additional mortality, estimated to be at least 5–15% (Rudloe 1983; Thompson 1998; Walls and Berkson 2000, 2003; Wenner and Thompson 2000), it is possible that females may have a higher mortality rate causing a skewed sex ratio for this embayment. Kurz and James-Pirri (2002) reported that bleeding may cause disorientated behavior after release that may result in fewer females reaching spawning beaches. It is interesting to note that in Nauset Estuary, a population that has not been exploited by harvesting, the spawning sex ratio was 1:1.6 and this ratio was not significantly different from a 1:1 ratio (Chi-square, $p = 0.150$).

We sampled both shallow (0–5 cm) and deep (5–20 cm) sediments to collect information on egg densities and to determine if horseshoe crab eggs were available to foraging shorebirds within the Cape Cod region. Horseshoe crab eggs provide a critical food resource for migrating shorebirds in Delaware Bay (Castro et al. 1989; Castro and Meyers 1993; Clark et al. 1993; Tsipoura and Burger 1999), and even though horseshoe crabs deposit their eggs in sediments deeper (15–20 cm) than most shorebirds can reach (Clark 1996), wave action and bioturbation (by spawning crabs and other organisms) bring eggs towards the surface where they become available to foraging shorebirds (Botton et al. 1994). We observed eggs in shallow (0–5 cm) sediments, but at low densities (an overall average of 0.1 eggs cm^{-2} for the Cape Cod region). Horseshoe crab eggs have been found in the stomachs of some shorebird species (semipalmated sandpiper *Calidris pusilla,* short-billed dowitcher *Limnodromus griseus,* dunlin *Calidris alpine,* and sanderling *Calidris alba*) at Monomoy NWR, providing evidence that migrant shorebird species consume horseshoe crab eggs during their migration stops at Monomoy NWR (Koch unpublished data). This indicates that in the northern portions of the horseshoe crab's range, although most likely not a primary food resource, they could be a predictable food resource used by shorebirds, but further research needs to be conducted to determine the importance of horseshoe crab eggs to shorebirds in the New England region.

Cape Cod had relatively low egg densities compared to those observed in Delaware Bay. Botton et al. (1994) observed egg densities in Delaware Bay ranging from 30 to 50 eggs cm^{-2}, and Pooler et al. (2003) observed densities of 0.6 eggs cm^{-2} in shallow sediments and 23 eggs cm^{-2} in deep sediments (estimated from 32 beaches, over 2 sampling periods); we observed an average of 0.1 eggs cm^{-2} in shallow sediments and 0.9 eggs cm^{-2} in deep sediments (estimated from 14 beaches, over 2 sampling years). We found a weak but significant relationship of spawning index and egg density. Smith et al. (2002a) also observed a relationship between spawning horseshoe crabs and egg densities in Delaware Bay, but beach morphology and wave energy also were critical variables in explaining egg density in their study.

We do not believe that the time frame (early to mid July) of egg collection is responsible for the observed low densities. Peak Cape Cod wide spawning index occurred during the end of May through mid June, while the majority of the sediment samples were collected in early to mid July. Egg development time varies from 2 to 4 wk, depending on environmental conditions (Botton 1995), and it was likely that some eggs hatched and trilobites emerged from nests prior to sampling. We believe that our samples represent an accurate assessment of egg densities on Cape Cod. If our samples were collected too late in the season, we would expect that the samples collected later in July would have a higher proportion of later stages (i.e., trilobites) than those samples collected earlier. The proportion of stages among the sites with measurable counts of stages did not show any trend with the time of sample collection. Samples collected latest in the year in 2001 (Hog Island in Pleasant Bay, collected on July 25, 2001) had 83% eggs, 1% embryos, and 16% trilobites, while the sample collected earliest for this season (North Nauset Beach collected on July 5, 2001) had 40% eggs, 40% embryos, and 20% trilobites.

The low egg densities observed in this study may be due to the overall low spawning densities and the broad mid beach area over which horseshoe crabs spawn in this region. Even though the core samples used in this study (10 cm diameter) were larger than those used in Delaware Bay (5 cm diameter; Smith et al. 2002a; Pooler et al. 2003) and composite cores (each core was a subsample from a mixture of 5 individual cores from a 1-m^2 area) were taken to increase the area sampled, more replicate cores are most likely required to better estimate egg density in this region; however, we feel our estimates are a good indication of the egg densities for this region. In Delaware Bay, researchers suggested sampling a 100-m segment of beach with 40 replicate, 5 cm diameter cores for deep sediments (0–20 cm) and more than 60 replicate cores for shallow sediments (0–5 cm) (Pooler et al. 2003). The amount of time and labor involved in processing this amount of replicate sediment cores may be a constraint for monitoring egg densities on a regular basis on Cape Cod.

Carmichael et al. (2003b) modeled egg densities for Pleasant Bay arriving at an estimate of 1.1×10^5 eggs m^{-1} of linear shoreline for Pleasant Bay based on the perimeter of Pleasant Bay, proportion of females observed in pairs throughout the bay in 2001 (not on spawning beaches), and an annual fecundity of 88,000 eggs per female. If we assume that their linear estimate of egg density is distributed across a 1-m wide band of beach (equivalent to 1.1×10^5 eggs m^{-2} or 11 eggs cm^{-2}), their estimate is one to two orders of magnitude higher than the actual egg densities we observed in Pleasant Bay at 3 survey beaches (Marsh 2–3, Hog Island, and Northville). Our yearly estimates of egg density for Pleasant Bay (an average of 3 beaches, shallow and deep cores combined) were 0.3 eggs cm^{-2} in 2001 and 1.9 eggs cm^{-2} in 2002, for a 2-yr average of 1.1 eggs cm^{-2}. Even the highest egg densities observed in Pleasant Bay (Marsh 2–3 in 2002, shallow and deep cores combined: 5.7 eggs cm^{-2}) were still lower than their modeled estimate egg density. Carmichael et al. (2003b) assumed that the entire shoreline of Pleasant Bay was suitable for spawning, which is not the case. Approximately 6.8 km or 8% of Pleasant Bay's shoreline is armored by erosion control structures (e.g., bulkheads, revetments, riprap), making these areas unsuitable for spawning habitat (Pleasant Bay Resource Management Alliance 1998). There are most likely other areas along the embayment's shoreline (e.g., peat banks at the edge of the marshes in the northern portion of the bay and southern beaches of Pleasant Bay) where spawning does not occur. The reduction in the amount of shoreline available for spawning would further increase the estimate of egg density presented by these authors, making it even more disparate when compared to our empirical data. The discrepancy between the modeled estimate of Carmichael et al. (2003b) and our empirical egg densities does bring up an interesting question. If the reproductive potential of the Pleasant Bay population is indeed one to two orders of magnitude higher than what is observed in the sediments, this provides further evidence for some sort of spawning disruption (e.g., disorientated behavior after bleeding as reported by Kurz and James-Pirri [2002]) or lack of spawning by mature females within the Pleasant Bay population that may be leading to low egg densities in the sediments and skewed spawning sex ratios.

Shuster (1982) observed that the largest sized horseshoe crabs were found off of South Carolina, with size decreasing both northward and southward of this point. On Cape Cod, we observed larger sized individuals of both sexes at the southern most location (Monomoy NWR) with smaller sized individuals found at the most northern location (Cape Cod Bay), mirroring Shuster's (1982) observation of decreasing size with increasing latitude. Size frequency distributions followed the same trend, with distributions shifted towards larger sizes at Monomoy NWR and smaller sizes in Cape Cod Bay. Differences in sizes between Cape Cod Bay and the other embayments may be explained by genetic variation. Cape Cod Bay is more geographically separated than the other 3 embayments on the Atlantic side of Cape Cod, and we observed no movement of crabs between this embayment and other areas. King et al. (2004) examined microsatellite DNA markers from horseshoe crabs collected from Maine to Florida (including samples from Pleasant Bay) and determined that horseshoe crabs north of Cape Cod (Gulf of Maine) form a discrete genetic population unit from southern New England populations (Atlantic side of Cape Cod and Rhode Island) and mid Atlantic populations. King et al. (2004) suggested that the zone of genetic discontinuity between *L. polyphemus* populations north and south of Cape Cod may be a reflection of restricted gene flow caused by the geographic landform barrier of Cape Cod. We do not know the reason for the size differences observed among the other 3 embayments on the Atlantic side of Cape Cod (Monomoy NWR, Pleasant Bay, and Nauset Estuary), but it is unlikely to be genetic since we observed movement of crabs between 2 of these 3 embayments (Monomoy NWR and Pleasant Bay). It is possible that embayment-specific growth rates may explain the observed size differences; this hypothesis needs further investigation.

The size ranges observed in this study were similar to those reported by Carmichael et al. (2003b) and Shuster (1957, 1979, 1982) for horseshoe crabs measured in Pleasant Bay. The average size for females from Pleasant Bay reported by Carmichael et al. (2003b) is slightly smaller (216 mm; size range 135–288 mm) than the size we observed (229 mm; size range 170–295), but average male size was equivalent. The size discrepancy for females is most likely due to the inclusion of nonspawning subadult females in the Carmichael et al. (2003b) study since they measured crabs throughout the bay whereas we focused on spawning adults.

We observed different recapture rates among the Cape Cod regions that were probably a reflection of the accessibility to the different regions rather than differences in environmental conditions or population characteristics. Sites at Monomoy NWR and Pleasant Bay where tagged horseshoe crabs were likely to be encountered were remote and not easily accessed by the general public. In Nauset Estuary, the small spawning beach where the majority of tagged crabs were observed was adjacent to a National Park Service nature trail heavily used by the general public; the proximity of the natural trail to the beach most likely contributed to the high proportion of recaptures at the same tagging location within Nauset Estuary. The same holds true for Cape Cod Bay, where the majority of spawning locations were in areas easily accessed and frequented by the general public.

The high proportion of horseshoe crabs that were recaptured near (within 2 km) the original location, and (with the exception of 3 individuals that traveled between Pleasant Bay and North Monomoy Island) the observation that all were recaptured within the same region where they were tagged, provides evidence for site fidelity to embayments and possibly to spawning locations and is suggestive of localized populations on Cape Cod. Rudloe (1980) also observed that the crabs did not undertake long migrations, citing the lack of long-range recoveries and high proportion of recoveries near the initial release area as evidence for a localized population. Isotopic signatures of horseshoe crabs from embayments within Pleasant Bay, Pleasant Bay proper, Stage Harbor (Chatham, Massachusetts), and Barnstable Harbor (Cape Cod Bay) were recently found to be significantly distinct, suggesting that crabs remain within localized areas long enough to acquire the isotopic signature of the embayment (Carmichael et al. 2003a; O'Connell et al. 2003). Genetic population subdivision over a relatively small geographic area has been documented for horseshoe crab populations in the mid Atlantic (Pierce et al. 2000). Botton and Loveland (2003) observed that planktonic horseshoe crab larvae have limited dispersal abilities and tend to remain close to shore and suggested that it is unlikely that larvae disperse far from the spawning beach where they were hatched. A concurrent study in 2001 using sonar telemetry observed that horseshoe crabs within Nauset Estuary had very limited movement patterns within that estuary (Kurz and James-Pirri 2002). Previous studies have also suggested that New England populations of horseshoe crabs may be localized and may not undertake extensive migrations. Widener and Barlow (1999) noted that crabs tagged on Cape Cod have returned to the same spawning location in subsequent seasons and that some crabs were observed during the winter months in a nearby harbor. Baptist et al. (1957) studied horseshoe crabs in Plum Island Sound, Massachusetts, and observed that the individuals in that area undertook a limited migration and were most likely a localized population. Botton and Ropes (1987) similarly suggested that migrations of horseshoe crabs among estuaries in New England may be limited, potentially resulting in discrete and isolated populations within local areas that may be sensitive to overexploitation.

This study represents the first comprehensive assessment of spawning horseshoe crabs on Cape Cod, and although this study focused on the Cape Cod region, findings from this work can be applied to horseshoe crabs throughout their range. We have shown that even though egg densities on Cape Cod are low, eggs were present in shallow sediments indicating that they are available to foraging shorebirds. This provides evidence that in the northern portions of the horseshoe crab's range, eggs may be a predictable food resource for shorebirds. Differing trends in size and spawning sex ratios among the four embayments indicate that even over small distances, population characteristics can differ. We observed spawning hot spots where high densities of spawning horseshoe crabs were consistently observed at specific locations and our recapture data provide evidence that horseshoe crabs may have fidelity to specific spawning beaches year after year. Sites with very high spawning densities and sites that are visited by the same individuals year after year may represent beaches that are responsible for the majority of recruitment to the population, and may be a higher priority for management and protection. This highlights the need for management plans specific to embayments or subregions depending on the population's characteristics.

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