

Seasonal movements of American horseshoe crabs *Limulus polyphemus* in the Great Bay Estuary, New Hampshire (USA)

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Abstract The goal of this study was to determine the year round movement patterns of American horseshoe crabs, *Limulus polyphemus*, in the Great Bay Estuary, New Hampshire (USA) by using acoustic telemetry to track the movements of 37 adult *Limulus*, for periods ranging from 2 to 31 months. During the winter (December-March) horseshoe crabs moved very little. In the spring, when water temperatures exceeded 11°C, horseshoe crabs moved at least 1 km further up into the estuary to shallower subtidal areas about a month prior to spawning. The mean distance traveled during spring migrations was 2.6 ± 0.5 ($n=20$) km up the estuary. Mating occurred in May and June and during these months animals spent most of their time in shallow subtidal areas adjacent to mating beaches. In the summer (July-August), animals moved 1.5 ± 0.5 ($n=26$) km down the estuary, towards the ocean, and ranged widely, using extensive portions of the estuary. In the fall (September-November) movement was more limited (0.5 ± 0.5 km; $n = 24$) while animals settled into wintering locations, where they remained until spring. The mean annual linear range for all animals was 4.5 ± 0.3 km ($n=35$) and the maximum distance traveled by an individual horseshoe crab within one year was 9.2 km. There was no evidence that any of the horseshoe crabs tracked during this study left the estuary [*Current Zoology* 56 (5): 587–598, 2010].

Key words *Limulus polyphemus*, Acoustic telemetry, Horseshoe crab, Tracking, Estuary, Home range

American horseshoe crabs *Limulus polyphemus* are commercially important in the United States where they are used for biological specimens, biomedical products, and fish bait—the latter being their largest source of mortality (Manion et al., 2000¹). Horseshoe crabs also have valuable ecological relationships with migratory shorebirds which prey on their eggs and young, and loggerhead turtles which prey on adults. In estuarine ecosystems they have a significant impact on bottom sediments and communities as a result of their foraging behavior and bioturbation (Shuster, 1990; Castro and Myers, 1993; Lee, 2010), and along the east coast of the USA they are closely linked to the successful migration of birds.

The spring migration of red knots from wintering grounds along the coastlines of South America, to nesting territories in arctic Canada, coincides with horseshoe crab spawning in the mid-Atlantic states. In Delaware Bay, the birds have been documented feeding on

Limulus eggs to restore muscle and fat reserves mid-journey (Morrison et al., 1980). As harvesting of horseshoe crabs intensified on mid-Atlantic beaches through the 1990s, and their abundance declined, the reduced availability of eggs to shorebirds resulted in poor shorebird survival and lower nesting success (Castro and Myers, 1993), particularly for red knots (Morrison et al., 1980). In response to this problem a number of states implemented catch regulations beginning in the mid-1990's, culminating with new federal regulations under the Atlantic States Marine Fishery Commission in 2001 (ASMFC, 1998; Kreamer and Michels, 2009).

A thorough understanding of *Limulus* movement patterns is vital for appropriate management of this valuable marine resource. Botton and Ropes (1987) reviewed fisheries trawl data along the continental shelf from southern New England to North Carolina and found that the majority of horseshoe crabs were taken in depths of 30 m or less; only 7% were found at depths up

¹ Manion MM, West RA, Unsworth RE, 2000. Economic Assessment of the Atlantic Coast Horseshoe Crab Fishery. Report by Industrial Economics, Inc., Cambridge, Massachusetts, to: Division of Economics, U.S. Fish & Wildlife Service, Arlington VA.

Received July 03, 2010; accepted Aug. 25, 2010

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to 200 m and fewer than 1% were taken at depths greater than 200 m. Botton and Ropes (1987) also noted that where animals were taken at the greatest depths (290 m), on the edge of the continental shelf off North Carolina, the continental slope is closer to the shore than at any other shelf location surveyed. Hata and Berkson (2003) conducted trawl surveys that extended 22.2 km offshore from New Jersey to Maryland and they also found abundant *Limulus* in their surveys. The earlier work by Botton and Ropes (1987) extended further north than the Hata and Berkson study and, because they found no horseshoe crabs offshore north of Montauk Point (New York), they concluded that offshore migrations were unlikely among northern populations. Taken together with other studies (summarized below), it appears that horseshoe crabs in waters north of New York and throughout New England may behave differently than more southern animals, with respect to seasonal movements and wintering habitats.

Swan (2005) conducted the largest study to date of *Limulus* migrations, tagging more than 30,000 animals over 17-years, at sites from New York to Delaware and Chesapeake Bays. In this study 3.7% (1,119) of the tagged animals were sighted again alive. Evidence for within-season spawning-site fidelity was inferred from the fact that 46% of the live re-sightings took place within a month of tagging near the beach where the animals had been released, while only 8% were found near other shorelines in that same time period (the remaining 46% were recovered over a longer period of time). Plausible long distance movements were documented for 14 individuals that moved distances ranging from 104–265 km from their release sites, over multiple years. Of these, four individuals were documented crossing Delaware Bay, and none of these crossings occurred within the same spawning season. Maximum dispersion distances within Delaware Bay ranged from 43.3 to 98.3 km for animals recovered up to 16 months after tagging. Dispersion distances for animals recovered 5–10 years after tagging ($n=7$) fell within this range. Swan also found that females had a tendency to travel longer distances than males.

Genetic analysis by King et al. (2005), based on *Limulus* populations throughout their range (Maine, USA to the Yucatan, Mexico), identified three regionally distinct stocks. These were: 1) the Gulf of Maine north of Cape Cod Massachusetts; 2) populations from Cape Cod to the Carolinas, including the mid-Atlantic coast and; 3) populations in the Gulf of Mexico. The authors also reported a pattern of male-biased gene flow among

populations, suggesting that in the long run males travel further than females.

In contrast to horseshoe crabs in the mid-Atlantic States, *Limulus* populations in New England appear to be more localized and move shorter distances, and are not known to undertake offshore migrations (Baptist et al., 1957; Botton and Ropes, 1987; James-Pirri et al., 2005; Moore and Perrin, 2007). In Massachusetts, Baptist et al. (1957) conducted a three-year tagging study which established that the vast majority of horseshoe crabs remained inside the estuary year round, with fewer than 0.01% (12 of 1780) relocated outside the estuary where they were tagged (Parker River, Massachusetts). James-Pirri et al. (2005) studied dispersion of tagged *Limulus* ($n=7800$) in four embayments on Cape Cod, Massachusetts, recovering ~7% (of which 6% were alive), and 70% of the live recaptures were within 2 km of the original tagging site. Overall, animals traveled 2–3 km and there was no evidence that horseshoe crabs left their home embayments. A twenty-six day companion study by Kurz and James-Pirri (2002) of animals fitted with ultrasonic transmitters found that most traveled less than 3 km ($n=17$). This same pattern was documented by Moore and Perrin (2007) in Taunton Bay, Maine. All 26 horseshoe crabs they tracked with acoustic telemetry remained within the bay. They did not specifically include the distances traveled by individual *Limulus*, but maps indicate that most animals were detected within 3 km of their release site, and that none traveled further than 4 km. Furthermore, a fixed station receiver near the mouth of the bay did not log any individuals leaving the estuary. These findings concur with data from an ongoing long term tagging study (Schaller, unpublished data) at the same location, which indicates these populations are residential and horseshoe crabs do not leave the bay.

The Great Bay estuary in New Hampshire is also the site of a large horseshoe crab population. The goals of this study were twofold. First, to determine movement patterns expressed by horseshoe crabs during different times of year. Second, to determine if any of the animals tagged and released in the estuary left the estuary, or whether they remained residents within all year long.

1 Materials and Methods

Thirty-seven adult horseshoe crabs, 13 males and 24 females, were fitted with ultrasonic transmitters and tracked over a three year time period (in 2005, three males and five females were tagged; in 2006, three males and sixteen females, and in 2007, seven males

and three females). More females were fitted with transmitters than males for two reasons. First, females tend to be larger than males so the transmitters were likely to have less of an influence on their activity and it was easier to attach them externally or insert them internally. Second, because males often remain attached to females for long periods we were concerned that data obtained from males might actually represent the movements of the females to which they were attached. To avoid this problem in 2007, we glued and taped shut the claspers males use to amplex with females.

Animals were fitted with one of two kinds of ultrasonic transmitters (VEMCO Division AMIRIX Systems, Inc., Halifax, Nova Scotia, Canada; V13L coded tags, 13 mm diameter, 36 mm long, 6 g in water, estimated battery life > 616 days, or V13TP coded tags with temperature and depth sensing capability, 13 mm diameter, 45 mm long, same weight and longevity as V13Ls). Eight V13L transmitters, and all depth and temperature sensing tags ($n=4$), were externally secured to the dorsal carapace of the prosoma using a combination of epoxy, cyanoacrylate glue and duct tape. Most ($n=25$) transmitters were inserted into the frontal area of the prosoma of each animal. This method was preferred so the tag would remain with the animal if it molted, and because we were concerned that externally attached

transmitters would become entangled on underwater debris or plants. This anterior prosoma was chosen because this was the largest body cavity that did not appear to contain a vital organ, like the heart. After cleaning the area with alcohol, a small slit (~2 cm) was made in the lower anterior prosoma and the transmitter, after being dipped in an antiseptic, was inserted. The incision was then covered with a piece of duct tape that was coated with cyanoacrylate glue to facilitate adhesion. Animals were allowed to recover for fifteen to thirty minutes and then released at the point of capture. This procedure was fast and simple enough that some pairs remained amplexed throughout the process, and animals that left the mating beaches often returned within ten minutes.

The Great Bay estuary primarily consists of Great Bay, Little Bay, the Piscataqua River and six other rivers that empty into the Gulf of Maine through the estuary (Fig. 1). All of the tracking data presented in this paper were obtained from horseshoe crabs residing in Great Bay and Little Bay. These areas have very large shallow regions and a deeper, central channel that runs the length of both bays. Throughout the paper “up-estuary” refers to animals moving away from the ocean towards Great Bay or one of the rivers, while “down-estuary” refers to movements towards the ocean.

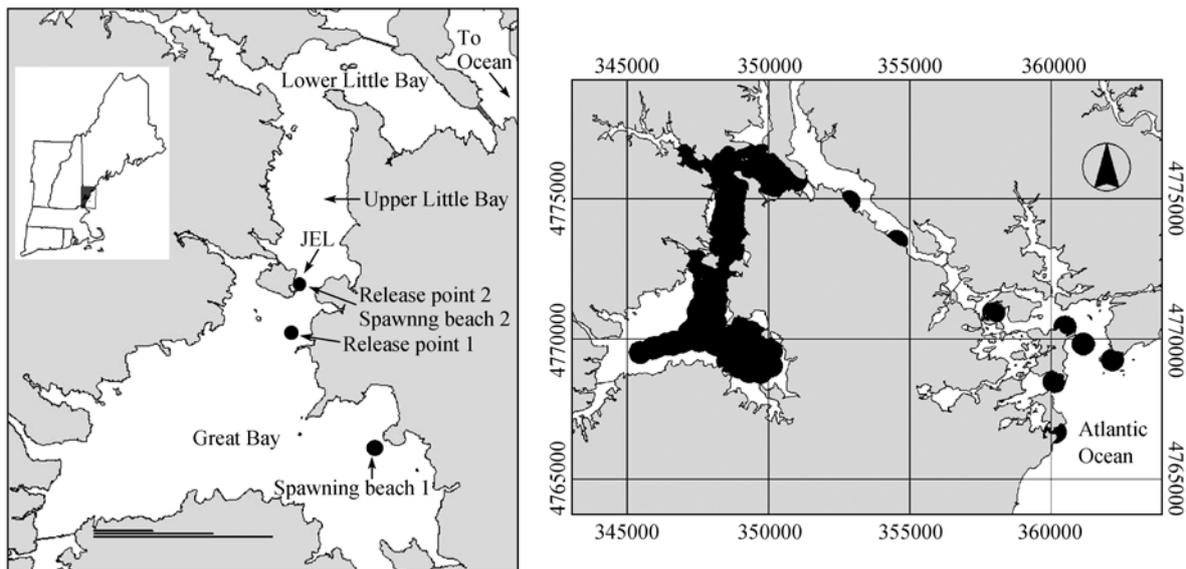


Fig. 1 Map of the Great Bay estuary where the study took place

Left panel: The insert shows the location of the New Hampshire (USA) seacoast. The area expanded is the upper portion of the Great Bay estuary consisting primarily of the largest portion, Great Bay, and both upper and lower Little Bay. The upper right hand corner of this map shows the Piscataqua River, which empties into the Gulf of Maine. Release point 1: animals were captured in traps and released in the fall of 2005. Release point 2: site of Jackson Estuarine Laboratory (JEL) where most animals were fitted with ultrasonic transmitters and released [this was also a spawning beach (2) for these animals]; Spawning beach 1: the location where some of the 2005 animals spawned. The scale bars show distances of 1, 2 and 3 km. Right panel: The blackened portions of the map indicate areas where tracking took place. Dark circles in the river indicate the locations of additional fixed receivers (VR2s). Map coordinates are provided in Universal Transverse Mercator (UTM) Zone 19N and each grid = 5 km².

Animals tagged in the fall of 2005 (three males, five females) were trapped in Little Bay, fitted with transmitters, and released at the same location (Release location 1; Fig. 1, left panel). In 2006 and 2007 all 29 animals (10 males and 19 females) were captured on the shoreline near the Jackson Estuarine Laboratory (JEL) while spawning, and immediately released after tagging at the same location (Fig. 1, left panel; Release location 2, Spawning location 2).

Animals were tracked and relocated using a three different types of hydrophone/receiver systems (manufactured by VEMCO) including: 1) a high resolution, three-buoy, fixed-array positioning system (product name: Vemco Radio Acoustic Positioning system or VRAP) that triangulates signals to identify the location of individual transmitters (Watson et al., 2009; Watson and Chabot, 2010); 2) manual tracking by boat using a handheld hydrophone with a VEMCO receiver (product name: VR100) and; 3) a number of single receiver listening stations (product name: VR2), fixed at various locations within the estuary, that detected the presence or absence of transmitters when they were within range of the receiver. The focus of this study was on the large-scale, seasonal, movements of horseshoe crabs and thus the high resolution data obtained from the fixed array system were used to calculate just one location per day and not their fine-scale movement patterns, which are reported elsewhere in this volume (Watson and Chabot, 2010). The most frequently used data were

obtained either from the buoy array (VRAP) or through manual tracking (with the VR100 and a hydrophone). Fewer data points were used from the fixed station single receivers (VR2s) because of their lower spatial resolution. Table 1 provides a summary of all the detections used for analyses, for all animals and all years. Detection of transmitters by all receivers varied with the tide, currents, wave action, water depth, and whether or not animals had burrowed into the sediment. In general, animals could be detected by the individual fixed station receiver units when they were up to 400 m away, but this diminished to ~15–50 m when animals were in very shallow water or buried (see below). The fixed buoy array used triangulation to improve the resolution of detections to 2 m, and the manual tracking receiver (VR100) had a resolution of ~30 m.

A simple range test was conducted to determine the ability of a VR100 to detect a transmitter in shallow water, either exposed on the bottom, or buried. A transmitter placed on sandy bottom, near a channel where it was inundated 2 m at high tide, was detected at a maximum distance of 144 m. When the same transmitter was buried 10 cm in the sediment in the same location, detection range was reduced to 15 m. While these tests were rudimentary, they demonstrated that tracking in shallow portions of the estuary required more concentrated efforts than tracking in deep water, and that if animals were buried it was necessary to be closer to them to log any detections with the VR100.

Table 1 A summary of the telemetry data that were used for analyses

Acoustic Data Summary	Tag-Year Class			Combined
	2005	2006	2007	
Year in which animals were tagged	2005	2006	2007	Totals
Tagged Individuals	8	19	10	37
Detections Used For Analysis				
Days Detected	102	431	167	700
Months with Detections	73	226	69	368
Number of Seasonal Records	45	132	43	220
Detections Used by Season				
Winter Detections	15	40	6	61
Spring Detections	23	71	20	114
Summer Detections	11	47	19	77
Fall Detections	24	54	22	100
Span of Months Detected	16 to 25	10 to 31	3 to 10	3–31

Only one tag detection was used per day for each animal. The values are expressed in terms of the total for each year class of animals tagged, for all the days, months and seasons that data were obtained from that year class, not in terms of values for just that year. Days, months and season detection values refer to the number of animal detections that occurred. So, for example, “days detected” is a sum of all the days when each animal was detected, totaled for all animals.

Tracking was conducted weekly from May to October, and approximately every other week during the spring and fall. The area covered during tracking, with all methods combined, is depicted in Fig. 1. The only area that was not covered routinely was the western portion of Great Bay, which is very shallow (1–2 m at low tide). Tracking during the winters of 2006–2007 and 2007–2008 was conducted as weather and ice conditions permitted, which only allowed data to be obtained once per month. However, as animals moved little during the coldest months of the year, this frequency of data gathering was sufficient to keep track of animal positions.

Each horseshoe crab was relocated during an average of 10.5 ± 5.4 (Mean \pm Standard Error of the Mean or SEM, used here and throughout) months. Typically data were not obtained continuously for 10.5 months, because data were often missing from some of the winter months when tracking was more limited. On average, data were collected from a given animal over a time span of 20.4 ± 1.0 months (range of 3–31 months; the median span of data was 21 months). Because it was possible for transmitters to continue providing data after animals died or molted, seven data files were truncated to the location at which each animal was last believed to have been alive, based on their movements. Two had been externally tagged (one was found on shore), and five were internally tagged; four were males and three were females. One died during spawning season and all the others failed to move from wintering locations during the subsequent spring and summer.

Location data were consolidated to yield one location per day. The VRAP system usually recorded multiple locations per day for each animal, if it was within range, so the position that logged the highest number of observations per day was used as the only location for that day. Data collected by manual tracking (VR100) were filtered using a Signal to Gain Ratio (SGR), so that the location used for any given day was the one that had the highest SGR value (if the signal was high, with a low gain, then the animal probably closer than if the signal was low with a high gain). Data obtained with fixed station receivers (VR2s) were consolidated to yield the number of observations logged per *Limulus*, per day. If more than one fixed station receiver logged data from the same animal on any one day, the location with the highest number of observations was selected as most representative of the position of that animal on that date. Comparison of data from the array (VRAP), manual tracking (VR100) and individual fixed station receivers

(VR2s) demonstrated that when VR2 units logged ~1000 detections per day, animals were in very close proximity to the unit. If a VR2 logged 100 positions, the animal could be as much as 0.5 km away from the receiver. All of the aforementioned data filtering reduced more than 1 million data points to several thousand. The resulting points were then combined, mapped with ArcView 3.2 GIS, and manually edited to select the daily location with highest resolution. Again, VRAP and VR100 locations were most often used because they were more accurate.

Net distances traveled each season were determined by using the maximum distance moved by each animal in a given season. If an animal moved further up into the estuary from the last known location where it was detected in the prior season, it was considered a positive value and if it moved down-estuary, a negative value. Thus, both positive and negative values were averaged together to yield a net distance moved in each season. The following four time periods were used to designate the seasons, based on previous data on seasonal fluctuations in water temperature and *Limulus* movements (see, for example, James-Pirri, 2010): Winter=December–March, Spring=April–June, Summer=July–August, Fall=September–November.

Because animals tended to move in, or adjacent to, channels which run down the middle of the estuary, movements tend to be of a linear, up-estuary, down-estuary, nature. Therefore, annual home ranges were calculated as linear distances rather than areas. In each year, the two most distant points obtained for each animal were used to calculate its annual linear range. These values were then averaged to calculate the mean annual linear range for all animals in the study. Because some animals were tracked for more than one year, we also calculated the total linear range, over more than one year, for these animals.

2 Results

Typically, horseshoe crabs expressed a seasonal pattern of behavior, moving further up into the estuary from their winter locations to spawn, then roaming down-estuary from spawning locations in the summer and fall. Such a pattern, for an individual female horseshoe crab that was tagged while spawning at JEL in June of 2006, and tracked for over a year, is illustrated in Fig. 2, while data from all animals tracked from 2005–2007 are shown in Figs. 3 and 4. The majority of animals (31) overwintered in locations down-estuary from where they spawned. During the winter months

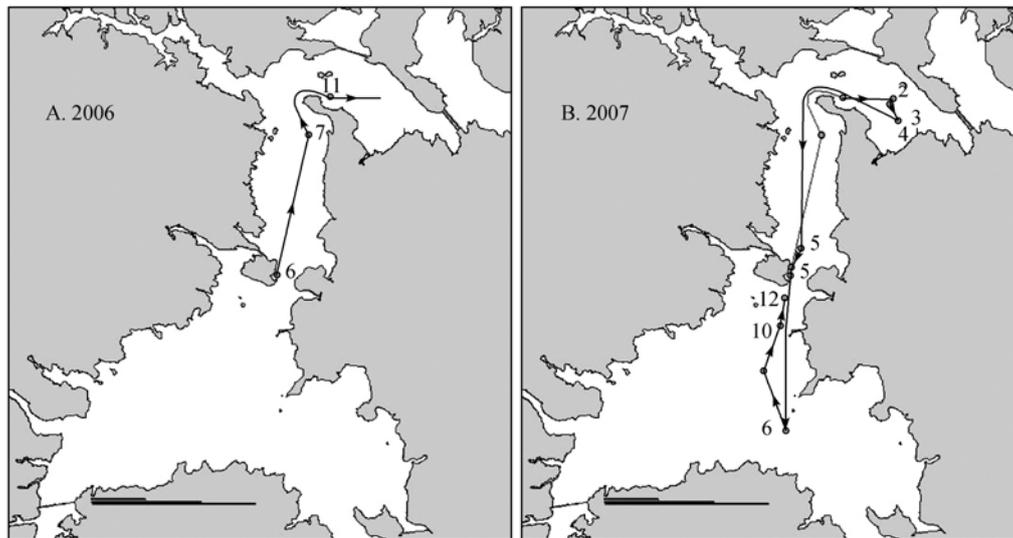


Fig. 2 The movements of one female horseshoe crab in 2006 and 2007

This animal was fitted with an ultrasonic transmitter in June of 2006, at JEL. The numbers beside each point indicate the month in which each positional fix was obtained (1=January, 2 February, etc.).

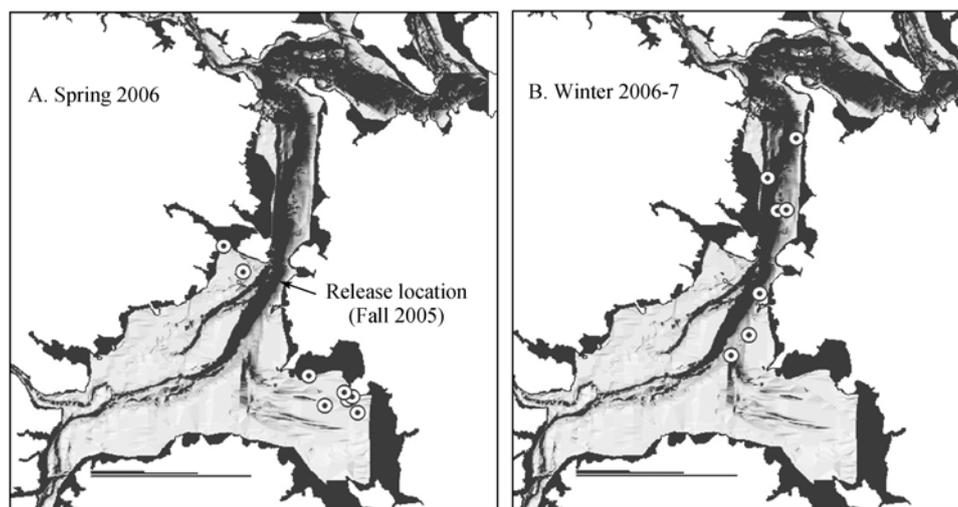


Fig. 3 Locations of horseshoe crabs that were fitted with ultrasonic transmitters in the fall of 2005, and localized the following spring and the next winter

Locations are shown on a bathymetry map of the Great Bay estuary, with shallower areas in lighter shades of gray. Black areas along shorelines are typically < 1–2 m deep and have not been mapped. Scale bars indicate distances of 1, 2 and 3 km.

when the water temperatures were $\sim 8^{\circ}\text{C}$ (December–March), the horseshoe crabs moved very little (Fig. 5). This sedentary state was confirmed in 2007 by manual tracking from January through March, and by tracking using the fixed array VRAP system for continuous monitoring from mid-March through April. During this period of time animals were located in, or adjacent to, the main channel that runs through the center of the estuary. During the winter of 2008, locations for a number of animals ($n=8$) were obtained with high enough reso-

lution to determine their depth, using the depth finder on the vessel. These animals resided at depths ranging from 8–23 m.

Over the course of this study 33% ($n=11$) of animals that we were able to locate during the winter months overwintered in lower Little Bay, 30% ($n=10$) were in upper Little Bay, and 36% ($n=12$) were in Great Bay. In general the overwintering locations of animals were related to their spawning locations the following spring; the further up-estuary they wintered, the further up-estuary

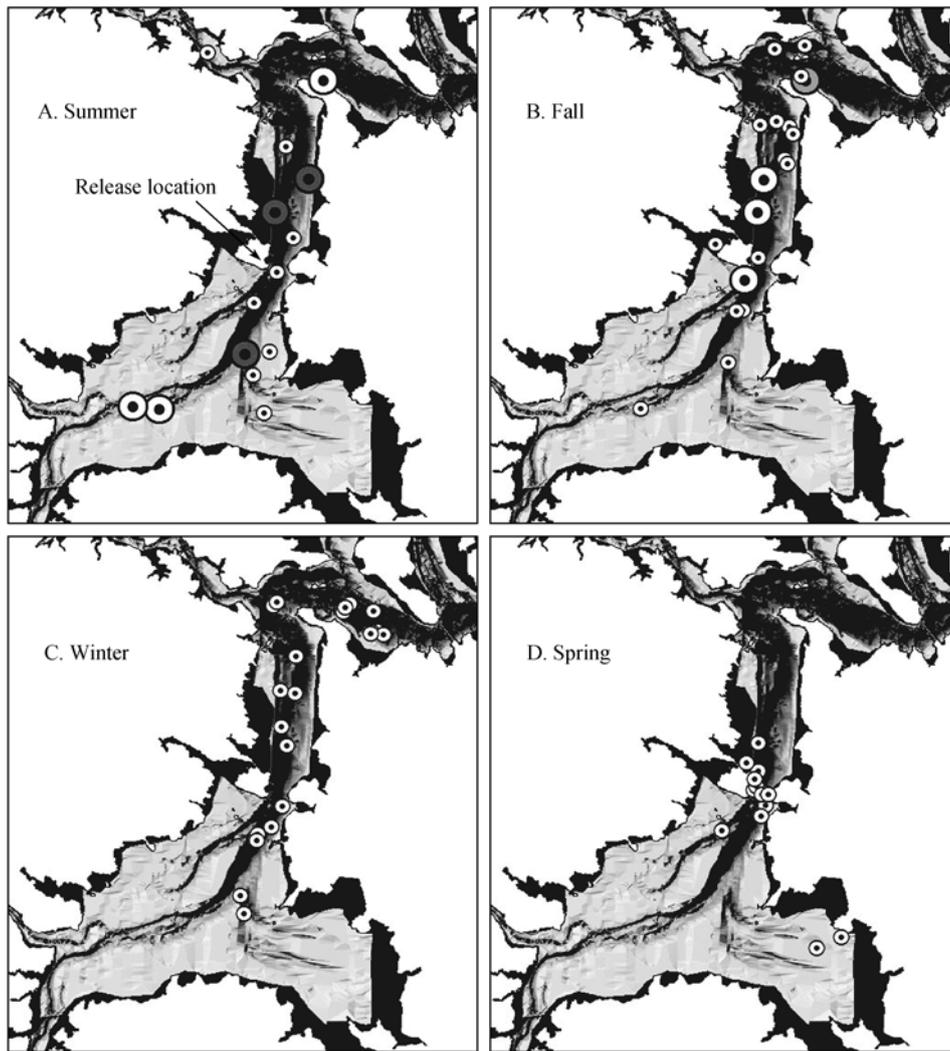


Fig. 4 Seasonal movements of animals released at the JEL (indicated on map A and Fig. 1), in the spring of both 2006 and 2007

Data from both years, for all seasons, are pooled in this figure and there is ~ one point per animal. Large white dots indicate the presence of two animals in that location and large gray dots, three individuals as detected by individual fixed station receivers. Note that most animals are further down the estuary and in deeper water in the fall and winter. Scale bars indicate distances of 1, 2 and 3 km.

they were relocated in the subsequent spring (compare spring and winter locations in Figs. 3, 4). None of the animals that we tracked during this study ever left the estuary or moved farther downstream than the location where lower Little Bay meets the Piscataqua River (upper right hand corner of the left panel in Fig. 1). Because we did not relocate every animal, in every month, it is possible that some moved past our last VR2 listening station, located at the confluence of Little Bay and the Piscataqua River. However, there were at least four additional listening stations, that were part of another tracking study, located in the river between Little Bay and the Gulf of Maine (Fig. 1, right panel), and none of these ever detected a horseshoe crab that we fitted with

a transmitter.

In the spring, when water temps exceeded 11°C, animals moved out of wintering locations and traveled up-estuary an average of 2.6 ± 0.5 km ($n=20$; Fig. 5; range: 0.4–7.4 km) to shallow subtidal mudflats prior to spawning. In some instances ($n=6$) animals that over-wintered in the upper portions of the estuary moved down-estuary short distances (0.1 to 0.9 km) prior to spawning. The transition from winter locations to spawning areas was most obvious in 2007 when all tagged animals ($n=9$) that spent the winter in lower Little Bay moved out of that area within a 10-day period (see Fig. 2 for an example of one of these animals). Five of these animals were relocated in upper Little Bay

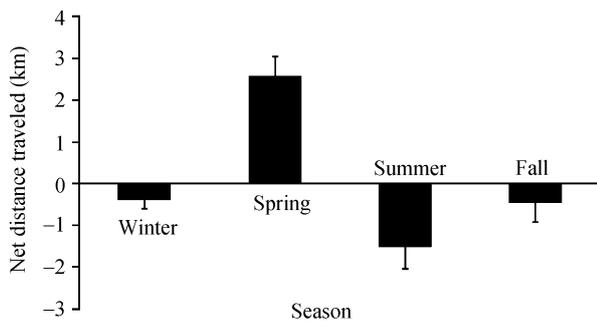


Fig. 5 Net distance moved by horseshoe crabs during four seasons

If a horseshoe crab moved upstream, it was considered a positive value, while downstream movements were given negative values. Winter=Dec–Mar, Spring=Apr–Jun, Summer=Jul–Aug, Fall=Sep–Nov.

during May and June, while the others may have moved into one of the rivers that feed into the estuary, where tracking efforts were limited. The aforementioned spring movements shifted animals into shallower water about a month prior to the onset of spawning.

After spawning, during July and August, animals ranged widely throughout the study area (Figs. 2, 3, 4). While, on average, animals moved down-estuary 1.5 ± 0.5 km ($n=26$), there was a considerable amount of variability in the magnitude and direction of their movements. For example, examination of each movement event (more than one seasonal record per individual) showed that, while most excursions were down-estuary (an average of 2.8 ± 0.3 km; $n=32$; range 0.1 to 7.4 km), in a number of instances animals moved up-estuary (2.1 ± 0.5 km; $n=8$; range = 0.1 to 4.5 km). At this time there were no detections in most shallow upstream mudflats of Great Bay, in contrast to the spring. Shallow flats used in the summer and fall were closer to the deep channels in the estuary than some of the most upstream shallow areas where animals were found only during spawning. During the fall animals covered less of the estuary and net movements were -0.5 ± 0.5 km down-estuary (Fig. 5). By mid-November most animals were at or near locations where they would overwinter (Figs 3, 4). Minor winter movements were logged in December and late March and were less than 0.5 km.

Annual linear ranges were calculated using the distances between each animal's furthest up-estuary and down-estuary locations during the year. The average annual linear range for all animals was 4.5 ± 0.3 km ($n=35$, median = 4.6). There was no statistically significant difference between the annual linear ranges of males and females (Mann-Whitney unpaired *t*-test, $P=0.32$, $n=13$ males and 22 females). The maximum

distance traveled by any individual over the course of this study was 9.2 km and was logged over a nine month period.

VRAP data were examined to determine the amount of time animals spent in a given area. This system has a detection range of ~ 200 meters from the center of the array, because animals must be detected by each of the three buoys to accurately calculate a position. The advantage of the system is that it can automatically track animals with a resolution of $\sim 2\text{--}10$ m (Watson et al., 2009). During March and April 2007 the system was deployed near the over-wintering site in lower Little Bay and the three animals within range moved little, if at all, for all 32 days during which data were collected. The system was then moved to JEL during the spawning season and animals in this location were detected for an average of 10.5 days ($n=9$, range = 5–25 days). Over the summer and fall the array was deployed at several locations in upper Little Bay. In July–September animals were detected for an average of 8.6 days ($n=8$; range = 4–15), and in October–November for 8.0 days ($n=4$; range = 4–13). Therefore, during the time of the year when horseshoe crabs were most active, they tended to occupy an area of about 12 hectares (\sim the detection range of the VRAP array) for a little over a week before moving to a new area.

There was no clear evidence of site fidelity to over-wintering locations in this study. However, this conclusion is only based on data obtained from five individuals over two consecutive winters. Of these five, only one wintered twice in the same section of the bay—at locations that were 1.44 km apart. The average distance between wintering sites in consecutive years was 3.19 ± 1.02 km ($n=5$, all females; also see Fig. 2 for example). There was a stronger expression of spawning site fidelity, with 70% of relocations in consecutive seasons separated by an average of only $0.44 \text{ km} \pm 0.08$ ($n=17$ records, 14 individuals, 1 male, 11 females; we had three consecutive seasons of spring data for three individuals). However, these data only indicate that animals passed by a given spawning location. Our method, unlike the radio telemetry method of Smith et al. (2010), does not allow us to determine if they are actually spawning. The other 30% of records showed animals spawning in different regions of the bay separated by an average of $3.8 \text{ km} \pm 0.17$ ($n=6$, three males, three females). Therefore, while most individuals appeared to spawn from one season to the next in locations that were within ~ 1 km of each other, a significant number of individuals spawned in sites that were nearly 4 km apart

from one year to the next. Moreover, while the area where a horseshoe crab overwintered was highly variable, there did appear to be a relationship between an animal's over-wintering location and where it moved to spawn in the subsequent spring.

3 Discussion

This study, in which acoustic telemetry was used to quantify the movements of > 30 adult horseshoe crabs year-round, demonstrates that horseshoe crabs remain resident within the Great Bay estuary throughout the year. This is consistent with previous horseshoe crab movement data obtained from other New England bays and estuaries (Baptist et al., 1957; Botton and Ropes, 1987; Kurz and James-Pirri, 2002; James-Pirri et al., 2005; Moore and Perrin, 2007; Schaller et al., 2006²; James-Pirri, 2010), and it seems likely that these more localized movements are typical of *Limulus* populations in New England.

In this study, most animals also expressed a consistent pattern of seasonal movements. In the spring, animals moved up the estuary to shallow areas, and then to spawning shorelines. This was followed by movements down the estuary in the summer and fall. As water temperatures dropped in early winter, animals settled into locations that they occupied until the following spring. Interestingly, several crustaceans that inhabit estuaries express similar seasonal movement patterns including lobsters (*Homarus americanus*; Watson et al., 1999) and blue crabs (*Callinectes sapidus*; Hines et al., 1987; Archambault et al., 1990) on the east coast of the USA, and Dungeness crabs on the west coast (*Cancer magister*; Diamond and Hankin, 1985; Smith and Jamieson, 1991; Stone and O'Clair, 2001). Therefore these seasonal movements might be serving some similar purposes for a range of species. For example, because estuaries are typically warmer in the summer, while deeper ocean water is warmer and more stable in the winter, these movements might serve to maximize the number of days animals are at, or above, a given temperature. This, in turn, may favor development and growth of larvae and juveniles in some species, or optimize the energetic costs of adults by allowing them to behaviorally adjust their body temperature to be as close as possible to their preferred temperature (Crossin et al.,

1998).

The clearest movements documented in this study were those that occurred in the spring when almost all animals moved up the estuary at least 2–4 km to shallow subtidal areas adjacent to spawning areas. This dramatic shift from being sedentary in the winter, to very active in the spring, took place when the water temperatures exceeded 10–11°C in April. While a few individuals moved slightly downstream because they over-wintered further up into the estuary, all animals moved to positions that were shallower than where they had been all winter. Horseshoe crabs may make these upstream migrations about a month before spawning for two reasons. First, to assure that most of them are fully synchronized to the tides, because changes in water depth appears to be the main entraining cue for tidal rhythms in this species (Chabot et al., 2008; Chabot et al., 2010; Chabot and Watson, 2010). Secondly, they move close to their spawning shorelines so that when the appropriate combination of water temperatures (for example $\geq 13^\circ\text{C}$ in Maine; Schaller et al., 2002³; $\sim 15^\circ\text{C}$ in Delaware, Smith et al., 2010), lunar phase and weather occur, they are prepared to maximize the limited time span they have for depositing and fertilizing eggs. While it is unclear what triggers the onset of horseshoe crab spawning in the spring of each year, our findings indicated that animals were poised in shallow water adjacent to appropriate shorelines and not just moving inshore on the first high tide following their arrival near a mating beach.

While traditional manual telemetry systems or listening stations do not have the resolution to determine if animals are truly sedentary, the fixed array VRAP system used for aspects of this study does (see Watson and Chabot, 2010). To confirm that animals were not moving in the winter the VRAP system was deployed for six weeks during March and April of 2007 at locations where some animals resided. We were able to confirm that the animals being tracked moved no more than transmitters anchored to the bottom in the same location during this time (for more detail about VRAP and reference transmitters see Watson and Chabot, 2010). However, as soon as the water temperature exceeded 10–11°C, these animals began to be active and rapidly moved up the estuary towards their spawning areas. A

² Schaller SY, Thayer P, LaTulippe S, Solet E, 2006. Maine Horseshoe Crab *Limulus polyphemus* Spawning Surveys, 2005. Report to Maine Department of Marine Resources, West Boothbay Harbor, Maine, by Bar Mills Ecological, P. O. Box 771, Bar Mills, Maine 04004.

³ Schaller SY, Thayer P, Hanson S, 2002. Survey of Maine Horseshoe Crab Spawning Populations, 2001. Report to Maine Department of Marine Resources, West Boothbay Harbor, Maine, by Bar Mills Ecological, P. O. Box 771, Bar Mills, Maine 04004.

similar influence of water temperature on horseshoe crab movements has been documented in outdoor tanks at the JEL (Schaller et al., in preparation) and indoor tanks in concurrent studies (Watson et al., 2009; Chabot and Watson, 2010). In these studies when the water temperature exceeded $\sim 11^{\circ}\text{C}$ horseshoe crabs became active and expressed a variety of biological rhythms, while below 11°C they were typically quite sedentary.

Net seasonal changes in the average position of animals in the estuary were 2.6 km upstream in the spring and 1.5 km downstream in the summer. These values are similar to those obtained both in Cape Cod and Maine embayments. James-Pirri et al. (2005) and Kurz and James-Pirri (2002) found that horseshoe crabs in Pleasant Bay MA moved 2–3 km in the summer and Moore and Perrin (2007) documented animals in Taunton Bay (Maine) moving ~ 3 km from the location where they were tagged, with none going further than 4 km. The maximum linear annual range documented in this study averaged 4.6 km, which is somewhat greater than found in previous studies. This difference might be due to the size and configuration of the Great Bay estuary, as well as the fact that we were able to track animals for more than a year and in a few cases for more than two years. In contrast, the horseshoe crabs in the Great Bay estuary moved markedly less than those horseshoe crabs tagged by Swan (2005) in the Middle Atlantic Bight (range of mean values of 16–46 km, for males and females, in New Jersey and Delaware, for animals at large for months). This large difference in mean distances traveled by most New England horseshoe crabs, in comparison to those further south, may be due to the limitations of their habitat, or the adaptive significance of the migrations, rather than their drive to move different distances. While Smith et al. (2009) concluded that “the greatest proportion of the Delaware Bay horseshoe crabs appears to migrate to the continental shelf”, this is clearly not the case in more northern, geographically constrained, populations. Perhaps, in the south, if horseshoe crabs migrate offshore, they can find water temperatures and other conditions that are suitable enough that they can remain active throughout the year, while in the north, even if they moved offshore, water temperatures would still be limiting, prey would be scarce, and they would have expended a considerable amount of energy for little gain.

When comparing the movements of animals tagged and released in 2005 (Fig. 2) with those from 2006–2007, it appears that subpopulations of horseshoe crabs prefer certain areas of the estuary. For example,

many of the animals that were fitted with transmitters in fall 2005 and released in lower-Great Bay spawned on shorelines in the upper reaches of Great Bay (see spawning site 1, Fig. 1), while only a small percentage (5 of 29) of the animals that were fitted with transmitters at JEL in the spring of 2006 and 2007 spawned at those beaches. Moreover, while a number of the animals tagged at JEL over-wintered in lower Little Bay, none of the 2005 animals over-wintered that far down the estuary. However, despite the apparent separation of these two groups during spawning and overwintering, there was also considerable overlap in their ranges. In this study the limited number of animals for which we obtained winter locations demonstrated variability and shifts in their choice of location wintering location suggesting that animals are not necessarily resident within a certain portion of the estuary from one year to the next, which differs from the conclusions of Moore and Perrin (2007) for animals in Taunton Bay.

While the majority (70%) of horseshoe crabs we tracked expressed some degree of inter-annual spawning site fidelity, returning to spawn in areas that were within ~ 1 km of where they had spawned previously, others did not. Moreover, just because animals returned to the same location where they spawned previously, given the limitations of acoustic telemetry, we are not sure if they actually spawned there or just passed by on the way to other beaches in the estuary. Horseshoe crab mating season lasts about one month, with females reported to return to the same beach on multiple tides, to deposit batches of eggs (Brockmann, 1990; Brousseau et al., 2004; Leschen et al., 2006; Schaller et al., 2006). Data from an ongoing tagging study in (Taunton Bay) Maine found that some animals moved ~ 0.5 km from one side of the bay to the other in a three day period during spawning season (Schaller, unpublished data), so it is possible that, after depositing multiple batches of eggs in one location, they move to another beach to deposit additional eggs at a subsequent time (Smith et al., 2010). For example, Brousseau et al. (2004) found that all females moved away from their mating beach study site within five days after the new moon (they also tracked them for the five days prior to the new moon). Similarly, most animals in this study did not remain around the spawning beach where they were fitted with transmitters and released for more than eight days. Thus, if they do not remain at the same mating beach for more than eight to ten days and mating season lasts for about a month, either they spawn at multiple beaches or they only spawn for five to eight days at a single beach. This is

worth further investigation utilizing a combination of radio and ultrasonic tags.

Acknowledgements We would like to thank the divers and others who assisted with this project including but not limited to Tom Langley, Chris Rillahan, Conor O'Donnel, Darren Scopel, and Jason Goldstein. Thanks also to Gary Blazon for database assistance, and especially to Dave Shay, Lab Manager at Jackson Estuarine Lab for help with boats and gear and keeping it all in dependable working order. Bathymetry maps for Great Bay were obtained from the University of New Hampshire Center for Coastal and Ocean Mapping Joint Hydrographic Center. David R. Smith, Frank Dorsey and two anonymous reviewers made helpful comments to earlier drafts. This project was supported by National Science Foundation grants NSF IOB 0517229 and NSF IOS 0920342 to WHW III and CCC.

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