

Tracking the elusive Great Bay Horseshoe crab *Limulus polyphemus* with Ultrasonic Telemetry:

What do horseshoe crabs do after the spawning season?

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Abstract

For reasons ranging from biomedical applications to important ecological links, the horseshoe crab *Limulus polyphemus* is an exceptionally important species. However, *Limulus* numbers appear to have been declining for more than a decade. At the same time conch and eel fisheries have increased the use of *Limulus* as bait, and there have been notable declines in some shorebirds which use *Limulus* eggs as a food source during long migrations from South America to the Arctic. Because most previous studies of *Limulus* have centered on spawning beaches, our objective was to observe the underwater movements of *L. polyphemus* through ultrasonic acoustic telemetry. Six adult horseshoe crabs were tagged with Lotek CAFT11_2 and CAFT11_3 ultrasonic transmitters, and were tracked using fixed and mobile hydrophones and SRX_400 receivers. A laboratory-based tag retention study was run concurrently. Tagged *Limulus* exhibited a broad range of movement patterns ranging from very little movement to significant up/down bay movements. Two *Limulus* moved rather little after tagging and we expect that they found good feeding areas. Other *Limulus* moved often, especially in late June and early July during an upwelling which advected cooler water into Great Bay. Three *Limulus* disappeared during this event and may have left the estuary or burrowed in response to the cooling. During the tag retention study, both tag loss and tag breakage occurred. This pilot study suggests that *Limulus* may respond to tides, upwelling, and/or feeding.

Materials and Methods

Limulus in Great Bay estuary, New Jersey were selected for this study due to the existence of an ultrasonic telemetry array established by RUMFS and the occurrence of spawning beaches within the estuary. The project had two components running concurrently: a tag retention study and a field tracking study.

Tag attachment: Live and dummy tags, purchased from Lotek, Inc. were attached to the carapace in a location which insured that the tag did not interfere with spawning or daily movements. The tag's vertical orientation allowed the tag to project a loud, clear signal even when an individual *Limulus* was burrowed. To attach the tag, the carapace was dried, roughened with sandpaper, and washed with ethanol. A moldable, marine-grade epoxy was then mixed and shaped to the tag. A PVC sleeve was placed around the tag to protect it from breakage and molded in with epoxy. A bar tag with Rutgers contact information was also embedded in the epoxy.

Tag Retention: Ten adult horseshoe crabs ranging in size from 35 to 60 cm were captured from Great Bay and held in tanks to observe mortality and tag loss rates. Tag mimics were affixed to the carapace. *Limulus* were held in 1.5 x 3 m tanks with flow-through seawater and natural/artificial light through the first half of the experiment and then separated and moved to larger, outdoor 4 foot diameter tanks for the second half.

Tag Deployment: Two different models of Lotek ultrasonic tags were used in this study: four CAFT11_3 tags (11mm diameter, 40 mm length, 8 g in air, 4.3 g in water, battery life 229 days) and three CAFT11_2 tags (11mm diameter, 46 mm length, 8.4 g in air, 4.2 g in water, battery life 92 days). Each tag projects a unique code and transmits at 77 kHz and 150 dB. Tags were divided evenly between females and males to look at gender differences (Table 1). *Limulus* were caught on/around a spawning beach in Sheepshead Creek, tagged, and held in captivity from 24-48 hours in 1.5 x 3m tanks to allow the epoxy to cure. After the tags were secure the *Limulus* were released at the location of capture, and immediately tracked to insure that the tags were functioning and the animals were moving.

Tracking: During June and July, *Limulus* were tracked using two methods. First, the RUMFS ultrasonic array provided real-time monitoring from 12 moored Lotek WHS_1000 wireless units capable of recording the tags within a 500m radius and transmitting the returns to RUMFS via VHF. Some receivers "gated" the openings to the estuary and provided information on animals leaving the system. Second, mobile tracking using a boat-deployed Lotek hydrophone/receiver was used to locate and follow individual *Limulus*. When an individual was located, depth, salinity, location, dissolved oxygen concentration, and tides were recorded. If we failed to locate an individual, or if a signal was lost, we made a 360° scan every 100m along a 1km transect from the last known location. Once a week, the entire bay and nearby rivers were scanned by mobile trackers for tagged individuals.

Data Analysis: Data from the two tracking methods were incorporated to show movements and paths of individual horseshoe crabs. Home range size and the timing of movement are examined.

Crab Name	Code	Tag	Sex	Weight(kg)	Total Length (cm)	Distance Moved (km)	Home Range (km ²)
Mama Cass	115	11-3	F	3.5	55.0	0.43 in one week	0.13
Pumba	111	11-3	M	1.25	44.0	2.32 in eight weeks	1.11
Billy Bob	89	11-3	F	2.25	43.9	4.49 in eight weeks	5.75
Sebastian	101	11-3	M	1.25	42.6	5.03 in six weeks	3.54
Oprah	82	11-2	F	2.5	49.0	0.41 in one week	0.94
Jack Sparrow	73	11-2	M	1.0	38.8	5.35 in six weeks	2.67

Table 1. Tagged horseshoe crabs and their physical characteristics.



Figure 1. A Lotek CAFT11_3 ultrasonic transmitter is affixed to the carapace of a *Limulus* using a marine-grade, moldable epoxy and a pvc-sleeve. The protective sleeve was needed to prevent tag breakage by the horseshoe crab.

Introduction

Limulus polyphemus is an important component of the ecosystem along the east coast of the United States, particularly in Delaware Bay where shorebirds migrating from South America to Arctic breeding grounds stop to feed on *Limulus* eggs deposited on beaches in the spring and early summer. These birds gain weight rapidly and these local beaches are critically linked to the success of these migrants' life history strategy. *Limulus* is also tremendously important to our medical establishment. *Limulus Amebocyte Lysate* (LAL) is derived from the blood of horseshoe crabs, and LAL is used as a diagnostic for bacterially-produced pyrogens on materials such as intravenous fluids to catheters - LAL is arguably one of the more important products derived from a wild organism.

Unfortunately, the only Atlantic population of *Limulus* is in decline. Until recently, there was no unified effort to assess the abundance of this population throughout its range. Increased exploitation, largely as bait for eel and conch fisheries, has created landings in states such as Pennsylvania which had not previously reported any *Limulus* catch. In recent years, the decline in *Limulus* has become a critical complication for the survival of endangered red knots (*Calidris canutus*). The population estimates for these shorebirds, which feed heavily on *Limulus* eggs, declined from 95,000 in 1989 to approximately 15,000 in 2004, and some believe red knots are headed for extinction.

Despite the importance of *Limulus* in so many arenas, rather little is known of its migratory behaviors or habitat requirements. While spawning beach surveys, designed to assess the reproductive potential and egg density, have been undertaken on the shores of the Delaware Bay since 1997, very few studies have focused on *Limulus* away from the beach (Pooler et al. 2003).

Ultrasonic acoustic telemetry makes it possible to follow the movements of individual *Limulus* to observe behavior, distances traveled, swimming activities and site selections (Zamora et al. 2002). Understanding *Limulus* home range size, habitat utilization, and behavior in response to environmental influences would be helpful in developing effective management programs for this important species.

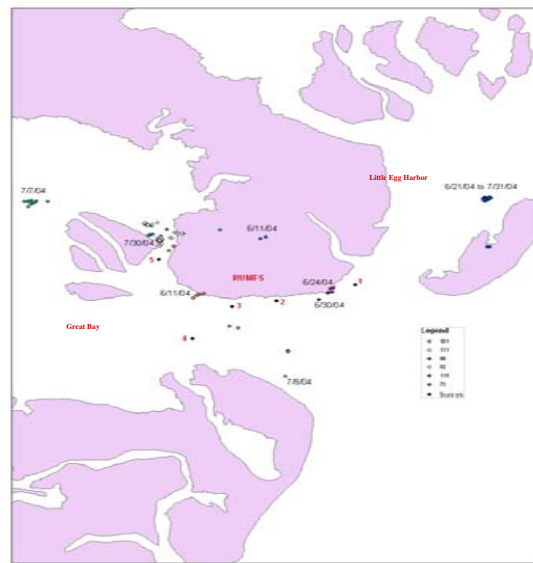


Figure 2. Locations of tagged *Limulus* and moored hydrophones in Great Bay and Little Egg Harbor.

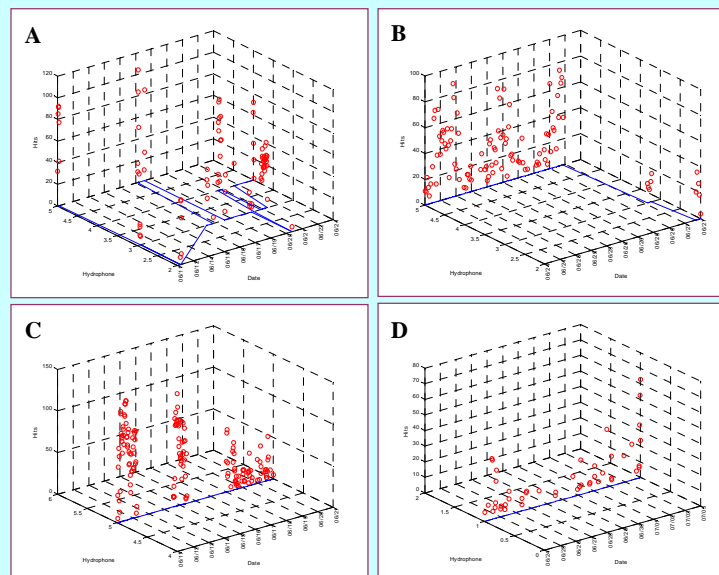


Figure 3. Movements of *Limulus* 101 (A), 82 (B), 111 (C), 115 (D) over time in Great Bay.

Results

During the length of the tag retention study, mortality observed was minimal. One individual died within 24 hours of being caught, most likely due to stress. Tag loss was high, six tags out of ten fell off due to crowding of the animals in the holding tanks. Vertical tags although better positioned for transmitting, seem to have a higher chance of breaking. In future studies, tags that are placed vertically should be better secured - perhaps by attaching screws to the carapace or epoxying in a flat plate to provide greater surface area.

Tagged horseshoe crabs moved far greater and faster than initially expected. Upon release, crabs moved out of the creek, most likely due to disorientation. Fig. 2 represents the location of each horseshoe crab over time and the locations of the moored hydrophones. Every crab, except for 82 and 115, were caught and released in Sheepshead Creek on 6/11/04. 82, at the abandoned fish factory, and 115, at Little Egg Inlet, were caught and released on 6/24/04 and moved out of the estuary on 6/30/04 (see Fig. 3). Fig. 3 represents the time ultrasonic tags were heard at the moored buoys and the red circles represent the number of times the hydrophone heard an ultrasonic tag at that specific location. 111 moved out of the creek after being released and remained under the pier of the fish factory for the duration of the study. 89 similarly moved out of the creek and remained in Little Egg Harbor by can 113. Benthic samples should be analyzed from these spots to look at the reasons for these favorable habitats. *Limulus* 73 spent the first three weeks in the creeks nearby RUMFS. This horseshoe crab, possibly a late spawner, eventually moved out of the creeks on 6/28/04 and moved around the fish factory. It too remained near the pier for a period of time. 101 immediately started on a track out of the bay upon release and was last seen on 7/8/04. However, Fig. 3 A represents the movements of 101 picked up by the stationary hydrophones, and the crab has moved a different path than observed from mobile tracking. Overall, tags 101, 82, and 115 most likely left the bay during the course of this study. No gender differences were observed among movements.

The system array and RUMFS data supplied long-term environmental database through the LEO-15 observatory and long-term field sampling programs provided us with background information. During end of June and early July, upwelling was observed off the coast of New Jersey, bringing cooler, nutrient-enriched waters into the bay. During this time, tags 101, 73, 82, and 115 were unaccounted for, and crabs may have moved further into the estuary to warmer water. Movements of *Limulus* also may have been influenced by tides, food, or spawning. Tagged individuals still remain in the system array and movements will be observed throughout the fall. With time, there might be a sufficient amount of data to understand the underwater movements of *Limulus* or observe more favorable habitats.

Tag loss may have occurred to codes 111 and 89 that stayed in one place. Other tagged crabs, such as 115 and 82, may have left the estuary or burrowed deep enough to block the signal of the ultrasonic tag. For future research, acoustic tracking should begin earlier in the spring to obtain more data, tags should be placed horizontally to avoid tag loss or vertical tags should be better secured.

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