# Maine Horseshoe Crab (Limulus polyphemus) Spawning Surveys, 2004

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Sherry Hanson with volunteers and beachgoers at Thomas Point Beach →



# **Executive Summary**

The Maine Horseshoe Crab Surveys began in 2001 and have been conducted annually since. The purpose of the surveys is to establish quantitative baseline population data, and determine whether horseshoe crab populations are stable or declining. Sites were selected on the basis of a 1977 report by John Born, prepared for the Maine State Planning Office. Spawning counts are conducted at sites from Casco Bay to Frenchman's Bay.

The study has collected the first quantitative data on this species in Maine, and established an index of relative abundance among sites. The sites identified with the most abundant remaining populations in Maine are in Middle Bay, Harpswell/Brunswick; Thomas Point Beach, Brunswick; and in the Damariscotta River, Damariscotta /Nobleboro. Healthy but less abundant populations persist in Taunton Bay, Franklin-Hancock-Sullivan, and on the Bagaduce River, Penobscot/Sedgwick/Brooksville. A number of sites last surveyed in 1976 no longer have spawning populations of horseshoe crabs.

In addition, an intensive tagging study has been conducted on Taunton Bay (Hancock County). Taunton Bay offers a unique opportunity to study horseshoe crabs in a population with no known history of human intervention. The Bay is effectively closed to immigration or emigration (by horseshoe crabs) by its physical configuration which includes 22 miles of shoreline, accessed through a narrow, rocky entrance with a deep channel and strong currents. The horseshoe crabs at this site are sparse enough that it is possible to tag every animal encountered (during spawning), although it is not possible to tag all individuals in the embayment (of 3,282 acres / 1,329 ha). During 4 field seasons, 6176 observations have been logged on 3883 individuals (2595 males, 1288 females). The goal of the tagging study is to continue long enough to determine normal variations in abundance in a natural population (i.e.—without removal by fishing), and the longevity of individuals tagged during the first season. Data on longevity, male:female ratios, and return rates of tagged animals from year to year will provide accurate information that can be used to model horseshoe crab populations. Mathematical models are a valuable tool in managing living resources because they permit testing of the impact of different management scenarios, helping managers to make better decisions with more predictable results.

Returns on tagged individuals have varied between 6.7 and 10.6% of the original year tag year class. The 2001 year class was the largest, and had returns of 8.7, 8.5 and 7.5% (of the original class of 1333 individuals) in the years from 2002 to 2004. Of the individuals tagged in 2001, 26% were females, 74% males. In successive years, females comprised 28% and 33% of the returning 2001 tagees, until 2004 when only 13% of the returns were females—fewer than half of the number returning in the prior two seasons (only 1% of the original 2001 tag year class). The cause has not been attributed but possibilities include differential survival, differential mortality associated with gender, or adult molting since the tags used would remain with the shell in the event of adult molting. Tags are intended to be shed with the shell in the event of molting, and would be expected to leave a minor scar on the edge of the new genal angle after molting. Two untagged individuals were encountered during 2004 that had clean, new shells with a notch in the edge, suggestive of molting out of a tagged shell. Still, unless low levels of molting by adult horseshoe crabs (females in particular) can be confirmed during future field seasons, mortality must be assumed.

Environmental factors have been tracked in an effort to quantify triggers for peak spawning events. The ability to predict these events would facilitate planning and scheduling the 70-80 volunteers each season who conduct counts at 12-15 sites. Lunar phase is most useful in predicting seasonal onset of spawning, but is less useful after spawning is initiated. Temperature appears to function primarily as a limiting factor. Periods of peak activity were facilitated by weather that was sunny, partly cloudy or partly sunny, while days that had intermittent rain, continuous rain or were stormy, coincided with marked declines in spawning activity. On days with complete cloud cover, spawning tended to decline if the overall weather pattern was declining, and tended to increase if the overall weather pattern was improving.

Horseshoe crab populations in Maine persist sparsely and at isolated sites. Unregulated use of this species for bait prior to establishment of limits in 2001 and closure in 2002 may be responsible for the species' decline. With no records of the numbers taken, no determination can be made after the fact. During 2004, 25% of the spawners at the tagging site had been tagged during the prior three field seasons. In a species that requires 9-10 years to reach sexual maturity, removal of adults through fishing would significantly impact the remaining population. In 1976, Born (1977) observed horseshoe crabs being taken in the Damariscotta River for bait "by the truckload". The current population is drastically sparser than that level of abundance and will require a more than a decade of protection (because of the delayed maturation) if it is to recover.

Continued protection and monitoring are recommended to assure the survival of this species in Maine. Middle Bay in Harpswell and Brunswick; Thomas Point Beach in Brunswick, and the Damariscotta River provide critical habitats. Middle Bay in particular is recommended for addition to Maine's Register of Critical Areas. Taunton Bay is worthy of continued protection since the population there has been identified as a genetically isolated subgroup (King et al. 2002); only the inner Hog Bay embayment is included in the Register although horseshoe crabs have been found at other sites in the Bay (Schaller and Thayer 2004, Moore and Perrin unpublished data).

Horseshoe crabs are ecologically valuable to shorebirds and fish which prey on their eggs, and in the diversity they add to ecosystems where they are present. Commercially they have been used for bait, but their highest commercial use is for biomedical purposes in which blood is taken, and the animals are released (with limited mortality). Horseshoe crab blood is currently used to test surgical implants and injectable drugs for gram-negative bacterial contamination. It is reasonable to expect that new uses will be discovered with time, and that there will be a greater emphasis on subtle differences in genetic material. Genetic differences between populations may become extraordinarily valuable, in which case Maine's horseshoe crab populations would be of particular interest.

Tagged adult horseshoe crab from Taunton Bay →



Horseshoe crab at Thomas Point Beach jostled by a wave, will use its tail or telson to right itself →



#### Abstract

The Maine Horseshoe Crab Surveys were begun in 2001 to establish quantitative baseline data and determine whether horseshoe crab (*Limulus polyphemus*) populations are stable or declining. Spawning counts were conducted at sites from Casco Bay (southern Maine) to Frenchman's Bay (downeast), to establish an index of relative abundance. Few (relatively) abundant populations remain in Maine at Middle Bay (Harpswell/Brunswick), Thomas Point (Brunswick), and in the Damariscotta River. Healthy but less abundant populations persist in the Bagaduce River (Brooksville/ Sedgwick) and in Taunton Bay (Hancock/Franklin/Sullivan).

An intensive tagging study has been conducted on Taunton Bay, which offers a natural population with no known history of harvest, and which is effectively closed to immigration and emigration by the physical characteristics of the Bay. During four field seasons, 6176 observations have been logged on 3883 individuals (2595 males, 1288 females; 66.8%, 33.2%). Returns by the 2001 tag year class have varied between 8.7 and 7.5 % in subsequent years. Females comprised 28% (of 116) of the returning 2001 tagged animals in 2002, and 33% (of 113) in 2003, but declined to only 13% (of 100) returning in 2004—just 1% of the original 2001 tag year class of 1333 (individuals). A cause has not yet been attributed, but adult molting is suspected. However, unless adult molting is confirmed, mortality must be assumed instead.

Return rates of individuals tagged in 2001 were analyzed to evaluate spawning site fidelity. While return rates from year to year did not exceed 8.7% of the original tag year class of 1333 individuals, 22% of the individuals were observed again in the years from 2002 to 2004.

Similarly, observations for 2004 were analyzed to determine the ratio of new individuals to those observed that had been tagged in the three prior years. In 2004, there were 1384 observations of 915 individuals (592 males, 323 females) at the tagging site, of which 25% had been tagged in prior field seasons. This suggests that a significant number of the adult horseshoe crabs in the vicinity of Shipyard Point may now be tagged, and each additional season of data will increase the value of the existing data for understanding the population dynamics of horseshoe crabs in Taunton Bay, and other sites in Maine.

# Introduction

This report provides results for the 2004 Maine Horseshoe Crab Surveys, and the associated intensive tagging study at Taunton Bay (Franklin, Hancock County). Begun in 2001, this project has completed a fourth successful season monitoring horseshoe crab populations during periods of peak spawning activity. The purpose of this work is to support management and conservation of this species in the northern extent of its range, to establish quantitative baseline population data for Maine, and to contribute to the understanding of this species' biology and behavior.

The Maine Horseshoe Crab Surveys are divided into two parts. One part consists of counts conducted during spawning, along preset transects on estuarine shorelines. The counts are used to identify extant horseshoe crab spawning populations, and to establish an index of relative abundance for those populations. Counts in 2004 were conducted for 3 periods of 8-9 days each, associated with the late May and June lunar phases (new and full moons).

The other aspect of the project is an intensive tagging study in which all adult horseshoe crabs encountered at one site are marked with individually numbered tags. Tagging is conducted at Taunton Bay, in Franklin (Hancock County), and begins on the same date as the counts. Tagging continues daily on the daytime high tide until June 30<sup>th</sup>. Data from the tagging study distinguish between the number of individuals observed, and the number of observations, since individuals are often observed on more than one day during the spawning season, and in successive seasons.

Tagging data provides more accurate information on population status and trends than can be obtained by simple counts. In exchange, tagging is more time consuming and has associated equipment and tag expenses. Data from the tagging site are used to generate a daily count for the site, which is included in the index of relative abundance. Comparison of the more detailed population information from tagging, to the simple observation data, from the counts, demonstrates the value of both, as well as the limitations of the simple count data.

The Taunton Bay tagging site is remarkable in that it appears to offer a nearly 'closed' population of horseshoe crabs, i.e.—one without immigration or emigration of individuals due to seasonal movement. It also appears to offer a unique opportunity to study a natural population of horseshoe crabs, i.e.—one without any known history of harvest influence. By comparison, horseshoe crab populations from Massachusetts southward through the mid-Atlantic states have had decades of commercial harvest (Schrading et al. 1998, Manion et al. 2000). The regular removal of varying numbers of spawning adults from these populations makes it difficult to separate the impact of excessive harvest from natural fluctuations. With the variables of weather, water quality, food supply, and disease it is a challenge to understand how long it would take a horseshoe crab population to recover from declines induced by these fluctuations. Adding annual harvest to the scenario would compound the difficulty of the challenge. The Taunton Bay tagging site appears to offer both an unharvested population, and a relatively closed site, due to the physical configuration of the Bay, it's rocky mouth, and deep, narrow, entrance subject to heavy currents.

Some wintering horseshoe crabs, particularly in the mid-Atlantic, are known to migrate seasonally from bays and estuaries to sea bottom as far out as the continental shelf (Botton and Ropes, 1987), while others remain in local bays and estuaries, burrowed shallowly in the sediments (Born 1977). Limited information in Maine suggests that horseshoe crabs overwinter in estuaries and embayments (Slade Moore and Steve Perrin, unpublished data). This difference may be due to the harshness of Maine's marine boreal habitats, it's narrow continental shelf, steeper shorelines and rocky bottoms as compared to the wide, sandy continental shelf of the mid- and south-Atlantic shorelines.

### **Biological Background**

Horseshoe crabs range intermittently from Maine to Mexico's Yucatan Peninsula (Shuster 1982), with Taunton Bay (Hancock County), representing the northernmost documented spawning site (Born 1977, Schaller 2002). So far as is currently known, Frenchman's Bay (of which Taunton Bay is an inner embayment) is now the northernmost extent of horseshoe crab spawning (Born 1977, Schaller et al.2002). Born (1977) documented the occasional presence of horseshoe crabs as far northeast as Cobscook Bay, but efforts in 2000 to verify this indicate it was an error. When contacted for verification, Born's source (A. West, now deceased) had no recollection of ever having seen horseshoe crabs in Cobscook Bay (Thomas Trott, personal communication). Telephone interviews of long-time residents of the area (i.e.--specifically long-term employees at Cobscook Bay State Park and Roque Bluffs State Park), failed to substantiate any observations of horseshoe crabs north of Frenchman's Bay (Stu Wagner pers. comm.; Larry Hunter, pers. comm.). Carl Merrill, Director of the Friedman Field Station (Suffolk University) in Edmunds, Maine (on Cobscook Bay) was also consulted, with similar result (pers. comm.).

Horseshoe crabs spawn in the spring, coming to the water's edge at high tide, burrowing in the sand (or gravel or mud), and depositing a cluster of hundreds or thousands of eggs (Rudloe, 1980). During spawning, a male clasps a female in amplexus. Riding piggy-back as she burrows into the sand (or gravel or mud), the male sheds milt that fertilizes the eggs externally. Eggs develop and hatch two weeks or more after spawning (Shuster 1990), the rate of development being slowed by low salinity, low temperatures, or low levels of dissolved oxygen (Jegla and Costlow 1982, Penn and Brockman 1994). Egg development requires 10-15 ppt salinity; optimal levels for development are 20-30 ppt (Jegla & Costlow 1982). Upon

hatching, the tiny trilobite-like larvae are free swimming in the water column, mostly swimming up and then drifting down for approximately six days, at the end of which time they molt to an instar resembling a miniature adult, and settle to the bottom (Rudloe 1979, Shuster 1990). Juveniles molt up to five times the first year, two or three times the second year, a couple of times the third year, and once yearly thereafter. They reach maturity in 15 or 16 molts and 9 to 10 years, respectively, for males and females (Shuster 1990).

Following spawning, adult horseshoe crabs stay in shallow embayments feeding, foraging and resting (Shuster 1982). Juveniles use shoal waters and flats up to 12' deep as nursery areas through the first summer and winter (Brady & Schrading, 1997). Horseshoe crabs feed on infauna (worms, clams, mussels) they encounter by either moving over the bottom or by burrowing through the sediments (Shuster 1950). During the tagging study, one animal was picked up with a partially consumed clamworm, *Nereis* spp., dangling from its mouth (SS). Another horseshoe crab was found eating what appeared to be a cluster of fish eggs (PT).

On the rocky coast of Maine, spawning habitat is the most limited of the required habitats for horseshoe crabs. Horseshoe crabs seek spawning sites that are relatively protected from wind and wave action, in back bays and estuaries (Shuster 1982). Surf conditions on exposed outer beaches are unsuitable to spawning because the animals would be tumbled and injured by the wave action. Horseshoe crabs require a substrate that females can excavate with their feet, or burrow into with their bodies, so that the eggs will be buried by the collapsing wet substrate as they leave (Shuster 1990). Suitable substrates must also be free of anoxic (reducing) sediments (Botton et al. 1988).

Horseshoe crab eggs require a regime of suitable moisture, salinity, temperature, and availability of oxygen for development (Jegla and Costlow 1982). All of these are co-influenced by frequency of inundation, grain-size of the substrate, and depth at which eggs are buried. In turn, these are influenced by proximity to the high tide line, regional variations in tide height, physical characteristics of the embayment, and the lunar phase. Eggs buried too high on a beach may dry out, and those buried too low (i.e.-- in saturated substrate, or saturated fine sediment) may not get enough oxygen for development (Schrading et al. 1999). In fine sediments, buried egg clusters will get more oxygen if the sediments are periodically drained at low tides, than if the substrate remains saturated by water at all times.

The misperception exists among some fans of horseshoe crabs that peak spawning activity is directly tied to the days with the highest monthly tides—this is incorrect, at least in Maine. In northern Maine where tidal amplitude reaches 11-13 feet in height, it would be disadvantageous for horseshoe crabs to deposit eggs where they would not be inundated again for a full month, since the eggs require moisture for development. Days of peak spawning activity have not coincided with the highest of the monthly high tides. In fact, spawning activity in Maine has been minimal on some days with exceptionally high tides. (Schaller et al. 2002, Schaller 2002, Schaller et al. 2004)

Horseshoe crabs spawn on both daytime and nighttime tides. Looking at daily activity patterns, Barlow et al. (1986) found on Cape Cod that horseshoe crabs were more active on the higher of the two *daily* tides, qualified by some preference for the time of day.

"The animals' preference for the afternoon tide grew as the tidal inequality increased, and continued as this tide progressed through the evening and into the early morning hours. Limulus did not, however, always prefer the higher high tides. Even though the tidal inequality diminished and reversed, the majority of animals continued to populate the early morning tides, which were slightly lower than the afternoon tides. They switched to the afternoon high tide... three days after tidal reversal. This behavior was repeated during the next tidal cycle, when they again switched to the afternoon high tide three days after reversal of the tidal inequality at the lunar quadrature..."

The reason for the delayed response is unclear. Surveys have not been conducted overnight in Maine so there is no data to determine whether a similar pattern of behavior occurs here with respect to tidal reversal.

Looking for seasonal patterns of activity, Barlow et al. counted large numbers of animals each day, before and during the new moon at the end of May, and around the full moon in mid-June. A smaller peak of activity occurred near the new moon at the end of June. Data from Maine concur with patterns of large counts in late May and early June, with a smaller peak later in June, locally termed "the straggler's peak".

Horseshoe crabs locate mates visually and have an array of light-sensing organs with which to accomplish this, day or night. Barlow and Powers (2003) provide a good summary in The American Horseshoe Crab (Shuster, Barlow and Brockman, eds. 2003). In addition to the horseshoe crab's well-known pair of dorsal compound eyes, there are two anterior median ocelli near the midline of the carapace, and six primitive eyes. The median ocelli sense ultraviolet light from the moon and stars, and enhance nighttime light sensitivity of the compound eyes. Three other eyes include two rudimentary lateral eyes and an endoparietal eye, which are prominent in juveniles, and may help them see until their compound eyes fully develop. The remaining eyes include two on the ventral side, near the midline anterior to the mouth, and one in the tail or telson. There are also a number of organs distributed along the tail that help synchronize the animals to circadian rhythm. Horseshoe crabs have been found to be as much as 1,000,000 more sensitive to nighttime light levels than daytime levels—displaying "a pronounced circadian rhythm in retinal sensitivity". (Barlow and Powers, 2003)

#### Methodology

The methodology developed in 2001 is still followed (Schaller et al, 2001). For the survey sites, where counts are conducted on the daytime high tide, volunteers are instructed to start 20-30 minutes before high tide. The goal is to complete the work at, or close to, the time of high tide. It is important to finish before the tide begins to ebb noticeably because on Maine's relatively steep shorelines, horseshoe crabs leave the shoreline quickly for deeper water as the tide begins to drop.

In contrast to the survey volunteers who conduct the counts, the tagging crew varies its start time from 30-45 minutes before high tide. This is to accommodate the additional handling time involved in tagging, measuring, and releasing animals. Work is conducted on daytime high tides only, because of the roughness of the shoreline terrain, water turbidity, and issues related to using lights at night.

Data are collected along shoreline transects of 100m or more, and recorded in 10m segments. Animals seen in the area from the water's edge to 1m seaward (perpendicular to the shoreline) are considered to be "In" the transect, and animals observed more than one meter from the water's edge are also recorded, but in a column labeled "Out". In order to limit observational bias in tabulating the results, data for observations only within 1m of shore ("In") along the transect are used to construct the index of relative abundance. The results are standardized to 100m of distance to limit bias in comparing the sites. For some aspects of the tagging study, the individually numbered animal is the statistical unit, permitting tabulation of return rates from year to year. In other analyses, such as the number of observations on a particular day, the number of individuals, frequency of visits, and male-female ratios, all the data are considered.

Tagging is conducted using standard fish marking tags, FD-94, by Floy Tag and Manufacturing of Seattle, Washington. Tags are attached by drilling a small hole (less than 2mm) through a genal angle (preferably the right side). The genal angles are laterally posterior, sharp bony points, on each side of the trailing edge of the head or prosoma. These bony points on the shell contain little tissue and no apparent sensory organs. The animals seem unaware of the tags or the tagging process, and often resume mate searching within

minutes of being released. The tags are individually numbered, stating "Report number XXXX" on one side (XXXX representing the actual tag number), and "HorseshoeCrabs@aol.com" on the other side. The drill bit is dipped in 5% Povidone-Iodine solution between each animal to avoid potential cross contamination. The tagging method was selected in accordance with best recommended practices in wildlife marking techniques to minimize interference with the survival and behavior of the animal (Nietfeld et al. 1994).

Morphological data are collected as the animals are tagged, using a fish board to determine prosomal width. Males are identified by the presence of claspers on their anterior-most set of walking legs; females have an undifferentiated anterior pair of walking feet. Animals missing both anterior legs are examined closely to determine gender, since the loss of the anterior pair of legs is attributed to an injury having occurred while amplexed. Males are often found missing one or more claspers—and the rest of the leg as well. Males are smaller on average than females (Schaller et al. 2001), and females develop patterns of surface erosion (wear marks or scarring) on their shells from the ventral surface of male shells during amplexus, so relative size, the absence of female pattern mating scars, and satellite-male behavior are used to confirm gender.

#### **Results of Surveys**

Spawning Surveys (counts) were conducted in 2004 at 14 sites. The geo-referenced locations are provided in Table 1, and shown on Maps 1, 2 and 3. New to the counts this season was a site in Cumberland at Wildwood, a second site in Yarmouth on Cousin's Island at Sandy Point (in addition to the south of Blaney Point), and three sites in Freeport. There were initially two Freeport sites at Winslow Memorial Park, one on the seaward shore and one on the Staples Cove shoreline, each with different characteristics in substrate and exposure to wind and waves. No spawning activity was observed at either of these sites during the first two counting periods. Both were discontinued in favor of a third site off Cushing Briggs Road in Freeport, known as Sandy Beach. Counts were conducted at Sandy Beach only during the last survey period, because of its late addition. Sandy Beach had abundant evidence of horseshoe crab spawning activity including tracks, nests, and a few animals present at low tide. It was discovered by volunteer Carol Steingart. With the addition of Middle Bay at the Brunswick - Harpswell line, the sites from Cumberland to Middle Bay provide the available data for Casco Bay. Other sites in Casco Bay were scouted, but no evidence of horseshoe crabs was found (Schaller and Thayer, 2003).

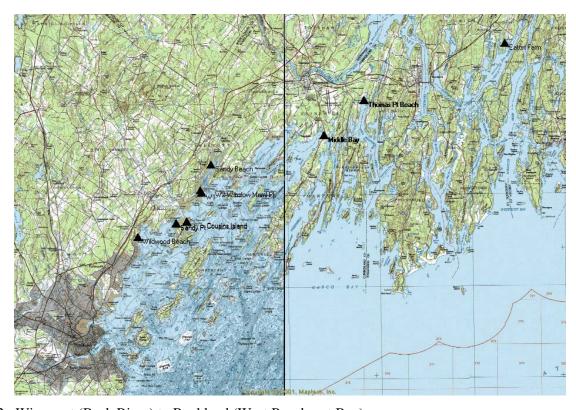
Table 1: 2003 Horseshoe Crab Spawning Survey Sites, Georeferenced by Datum

Town	Site Description	2003 Survey Name	WGS84 N	WGS84 W	NAD83 N	NAD83 W
Cumberland	Wildwood Park Beach	Cousin's Island	43.7590357	-701949804	43 45 32.51	70 11 41.91
Yarmouth	Sandy Pt at Snodgrass Bridge	new in 2004	43.7733140	-70.1443200	43 46 24.83	70 08 39.88
Yarmouth	cove sw of Blaney Point	Cousin's Island	43.7743429	-70.1302621	43 46 27.62	70 07 48.93
Freeport	Winslow Memorial Park, seaward	new in 2004	43.8034806	-70.1104267	43 48 12.52	70 06 37.52
Freeport	Winslow Memorial Park, Staples Cove	new in 2004	43.8034806	-70.1104267	43 48 20.33	70 06 42.74
Freeport	Sandy Beach off Cushing Briggs Road	new in 2004	43.8309308	-70.0974228	43 49 51.34	70 05 50.71
Brunswick	Middle Bay, eastern cove	Middle Bay	43.8593299	-69.9447254	43 51 33.57	69 56 41.00
Brunswick	Thomas Point Beach	Thomas Pt. Beach	43.8939318	-69.8909125	43 53 38.14	69 53 27.27
Wiscasset	Eaton Farm on Back River	Bailey Cove	43.9491529	-69.7011517	43 56 56.94	69 42 04.13
Damariscotta	Day's Cove, shoreline behind hospital	Days Cove	44.0245795	-69.5325076	44 01 28.47	69 31 57.01
Nobleboro	Salt Bay, Damariscotta Mills sewer filter	Damariscotta Mills	44.0614377	-69.5225716	44 03 41.16	69 31 21.24
Sedgwick	behind Bagaduce Lunch	Bagaduce River	44.3985850	-68.7027723	44 23 54.89	68 42 09.97
Franklin	Shipyard Point, start of tagging transect	Taunton Bay (tag site)	44.5817858	-68.230541	44 34 54.41	68 13 49.93
Franklin	Shipyard Point, end of tagging transect	Taunton Bay (tag site)	44.5831980	-68.2319333	44 34 59.50	68 13 54.95
East Franklin	South Bay Rd at Hog Bay	Taunton Bay, So.Bay Rd	44.5711586	-68.2248982	44 34 16.16	68 13 29.62

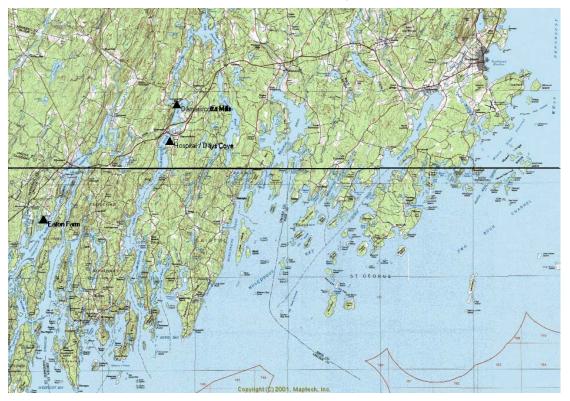
All of the 2003 Survey sites east of Casco Bay were surveyed again in 2004: Thomas Point Beach, Brunswick; Eaton Farm, Wiscasset; Days Cove behind the hospital in Damariscotta; Damariscotta Mills at the sewer filter in Nobleboro; the Bagaduce River in Sedgwick; and Taunton Bay, Franklin, both at Shipyard Point and along South Bay Road. The two sites on the Damariscotta River are relatively close, separated by just under 3.5 miles of water. In spite of their proximity, the counts at these sites do not particularly mimic each other. The site at the Mills is close to a natural falls, terminating the freshwater

portion of the Damariscotta River. As a result, this part of Salt Bay is subject to periodic freshwater inflows and short periods of low salinity following heavy rainfalls. Horseshoe crabs can survive for only a

Map 1: Casco Bay to Wiscasset (Back River)



Map 2: Wiscasset (Back River) to Rockland (West Penobscot Bay)







few hours when salinity levels decline to 4-6ppt (Jegla and Costlow 1982, Sugita 1988, Shuster 1982), and spawning activity halts while the animals survive the low salinity event. Counts at Damariscotta Mills are subject to greater fluctuation than other sites. Interestingly, there is a thick layer of old sawdust in the shoreline at the Mills site that does not seem to deter spawning. No doubt the locale is named for former sawmills on the shoreline.

The Days Cove site, behind the hospital in Damariscotta, was less favored by horseshoe crabs during the first three years of the counts than the site at the Mills. The Days Cove site is unaffected by freshwater inflows, the shoreline is easier for volunteers to walk, and counts appear to be gradually increasing there. Born (1977) noted that horseshoe crabs were removed from this site "by the truckload in 1976 for bait". During the 1980's, the site was said to have been affected by intermittent poor water quality from an overloaded septic system at the hospital (Neal Campbell, pers. comm.). Habitat appears to be improving, coincidental with the addition of a public sewer in the 1990's, and without harvest, gradual recovery of the population can be expected. A decade or more without harvest may need to pass before density reaches a level at which even one "truckload" of horseshoe crabs could be gathered at this site on a single day. Based on comments by Born (1977) it seems possible that horseshoe crabs have already been harvested to the extent of causing long term damage to populations.

Counts were conducted during 2004 for three periods of 8-9 days each. The dates were: Thursday, May  $20^{th}$  – Friday, May  $28^{th}$ ; Friday June  $4^{th}$  – Friday June  $11^{th}$ ; and Friday, June  $18^{th}$  – Saturday June  $26^{th}$ . Dates were chosen because they were subsequent to the May  $19^{th}$  new moon, June  $3^{rd}$  full moon, and June  $17^{th}$  new moon. A six-day period is usually adequate to capture key dates during which spawning activity increases, peaks and declines. Days of peak spawning vary by a few days among sites, so extended data collection periods are used to assure that peak activity is documented at all sites.

Table 2 shows the 2004 data from the counts for all 14 sites. Data were standardized to a 1m x 100m transect. Data for the low tide counts conducted at Middle Bay are presented last; all other sites

Table 2: Counts for all Sites, Standardized to 100m Transect

2004	Wild	wood /10	00m	Cousins	Isl-bridg	je /100m	Cousins	IsI-Blane	y /100m	Staple	es Cove /1	00m	Winslow-seaward /100m		
	Cı	umberlar	nd	`	armouth	า	Υ	armouth			Freeport			Freeport	
	Temp	In	Out	Temp	In	Out	Temp	In	Out	Temp	In	Out	Temp	In	Out
20-May	15.0	0	0	16.0	0	0	15.5	0	1	16.0	0	0	16.0	0	0
21-May	14.0	0	1	16.0	0	0	16.0	0	0	15.0	0	0	13.0	0	0
22-May	12.0	0	0	11.0	0	0	12.0	0	0	12.0	0	0	12.0	0	0
23-May	17.0	0	0	13.0	0	0	13.0	0	0	no temp	0	0	no temp	0	0
24-May	12.0	0	0	12.0	0	0	13.0	0	0	12.0	0	0	12.0	0	0
25-May	11.0	0	0	11.0	0	0	11.5	0	0		no data			no data	
26-May	10.0	0	0	10.5	0	0	11.0	0	0	11.0	0	2	11.0	0	0
27-May	14.0	0	0	17.0	0	0	13.0	0	0	14.0	0	2	12.0	0	0
28-May	10.0	0	0	11.5	0	0	11.5	0	0	11.5	0	0	12.0	0	0
4-Jun	16.0	0	0	no temp	0	0	no temp	0	0	15.0	0	0	13.0	0	0
5-Jun	14.0	2	3	13.0	0	0	14.0	0	6	14.0	0	0	16.5	0	0
6-Jun	14.0	0	0	14.0	0	0	15.0	0	0	19.0	0	0	16.0	0	0
7-Jun	12.0	0	0	12.5	0	0	12.0	0	0	13.0	0	0	14.5	0	0
8-Jun	17.0	1	0	15.0	0	2	17.0	0	0	15.5	0	0	20.0	0	0
9-Jun	18.0	1	3.5	17.0	0	0	24.5	0	0	19.0	0	0	15.5	0	0
10-Jun	16.5	1	1	16.0	0	0	22.0	0	0	20.0	0	0	16.0	0	0
11-Jun	15.0	0	0	14.0	0	0	19.0	0	0						
18-Jun	21.0	2	2	18.0	0	0	18.0	0	0						
19-Jun	16.0	0	0	16.0	0	0	17.0	0	0						
20-Jun	16.0	1.5	0	17.0	0	0	18.0	2	0						
21-Jun	15.0	0	0	17.5	0	0	19.0	0	0						
22-Jun	14.0	0	0	15.0	0	0	16.0	0	0						
23-Jun	no temp	0	2	18.0	0	0	25.0	0	0						
24-Jun	no temp	0	0	17.0	0	0	15.5	0	0						
25-Jun	no temp	0	1.5	19.5	0	0	18.0	0	0						
26-Jun				16.0	0	0	16.5	0	0						
Best 5 Day	ys Total:	4			0			2			0			0	

2004	Sandy	Beach /	100m	Thoma	as Pt Be	/100m	Eato	n Farm /	100m	Day	s Cove /10	00m	Dan	Damar.Mills /100m		
		Freeport		E	Brunswic	:k		Wiscasse	t	D	amariscot	ta		Nobleboro		
	Temp	In	Out	Temp	In	Out	Temp	In	Out	Temp	In	Out	Temp	In	Out	
20-May				20.0	690	38				18.0	25	11	21.0	44	7	
21-May				17.0	514	no data				17.0	23	6	20.0	152	43	
22-May				14.5	7	0				14.0	2	7	15.0	5	27	
23-May				14.5	12	0				14.0	2	9	16.0	0	4	
24-May				13.0	0	3				13.8	0	0	14.0	0	0	
25-May				13.5	0	7				14.0	0	0	13.0	0	0	
26-May				13.0	0	0				12.0	0	0	12.0	0	0	
27-May				20.0	334	no data				16.0	6	9	16.0	0	0	
28-May				12.0	0	4				13.0	0	1	11.5	0	0	
4-Jun				19.5	99	124	13.5	2	4	16.0	17	13		no data		
5-Jun				20.0	453	no data	13.5	4	3	17.5	52	21	20.0	40	82	
6-Jun				20.0	412	no data	13.0	4	6	16.5	106	49	19.0	no data		
7-Jun				18.0	130	no data	14.5	0	2	15.0	16	38	16.0	10	108	
8-Jun				20.0	315	no data		no data		18.5	54	42		no data		
9-Jun				22.0	172	no data				25.0	62	15	21.0	69	42	
10-Jun				20.0	119	no data				19.0	25	7	21.0	71	41	
11-Jun				19.0	23	8				18.5	25	5	no temp	69	35	
18-Jun	no temp	19	1	22.0	80	59				22.0	42	17	24.0	1	4	
19-Jun	no temp	6	2	19.0	12	13				19.0	12	8	21.0	0	0	
20-Jun	no temp	7	3	19.0	14	24				20.0	7	7	23.0	15	16	
21-Jun	17.0	5	10	23.0	15	18				22.0	7	12	24.0	45	7	
22-Jun	15.5	1	2	19.0	2	18				19.0	1	0	20.0	2	19	
23-Jun	19.0	0	7	20.0	3	12				21.5	8	0	24.0	23	6	
24-Jun	18.0	4	2	21.0	6	16				20.0	0	9	21.0	2	6	
25-Jun	16.5	2	0	23.0	1	8							21.0	0	9	
26-Jun	16.0	0	2	20.0	0	9										
Best 5 Day	ys Total:	38			1482			10			290			219		

Table 2 continued: Counts for all Sites, Standardized to 100m Transect

2004	Bagaduce River /100m			SoBa	ayRoad / 1	00m	Shipyard Point / 100m			
		Penobscot		E:	ast Frankl	in		Franklin		
	Temp	In	Out	Temp	In	Out	Temp	In	Out	
20-May	15.0	6	1		no data		16.5	3	0	
21-May	15.0	6	0	no temp	0	0	16.0	7	1	
22-May	12.0	2	0	no temp	0	0	12.5	1	0	
23-May	12.5	1	1	13.0	1	0	12.5	1	0	
24-May	10.0	0	0	12.0	0	0	11.5	0	0	
25-May	9.0	0	0	no temp	0	0	10.5	0	1	
26-May	9.0	0	0		0	0	no temp	0	0	
27-May	12.0	0	4		0	0	11.0	0	0	
28-May	9.5	0	0		no data	0	10.0	0	0	
29-May				10.0	0	0	10.5	1	0	
30-May					0	0	10.5	0	0	
31-May				13.0	1	0	11.5	0	0	
1-Jun					no data		10.0	1	0	
2-Jun					0	0	10.5	0	0	
3-Jun					0	0	11.5	0	0	
4-Jun	13.0	4	4		5	4	14.0	7	5	
5-Jun	13.0	40	19		12	20	15.0	42	1	
6-Jun	13.0	44	46		19	30	15.5	48	5	
7-Jun	14.0	29	19		6	19	14.0	25	14	
8-Jun	13.0	32	13		25	20	16.0	22	10	
9-Jun	16.0	47	25		32	5	17.0	25	17	
10-Jun	17.0	36	21		12	16	17.5	8	13	
11-Jun	16.0	16	20		0	0	14.0	1	1	
12-Jun					no data	_	14.5	0	2	
13-Jun					8	2	15.5	2	1	
14-Jun					5	5	15.5	2	1	
15-Jun					4	6	16.0	7	3	
16-Jun					3	4	19.5	15	4	
17-Jun					2	5	19.5	12	0	
18-Jun	18.0	31	26		3	5	19.5	15 -	1	
19-Jun	18.0	10	1		no data	_	16.5	5	1	
20-Jun	17.0	12	4		8	0	15.5	4	1	
21-Jun	18.0	36	16		3	3	17.5	5	1	
22-Jun	17.0	9	11		0	0	17.0	3	1	
23-Jun	20.0	25	6		no data	•	18.0	3	0	
24-Jun	17.0	9	19		0	0	16.5	1	0	
25-Jun	16.0	6	1		0	0	15.5	1	0	
26-Jun					0	0	14.5	0	0	
27-Jun					0	0	16.0	0	0	
28-Jun					0	0	15.0	0	0	
29-Jun					0	0	14.5	0	0	
30-Jun	Total	404			0	0	15.5	1	1	
Best 5 Days	rotal:	191			94			163		

2004	Middle	Bay <b>LOV</b>	V TIDE
2004		wick / Hai	
		ek, per 10	•
20-May	Ole	21	70111
21-May		34	
22-May		51	
23-May		14	
24-May		1	
25-May		0	
26-May		0	
27-May		0	
-		0	
28-May		- 0	
29-May			
30-May			
31-May		440	
1-Jun		119	
2-Jun		no data	
3-Jun		112	
4-Jun		176	
5-Jun		183	
6-Jun		162	
7-Jun		103	
8-Jun		43	
9-Jun		37	
10-Jun		9	
11-Jun		5	
12-Jun		1	
13-Jun		4	
14-Jun		5	
15-Jun		5	
16-Jun		84	
17-Jun		69	
18-Jun		128	
19-Jun		102	
20-Jun		33	
21-Jun		22	
22-Jun		18	
23-Jun		12	
24-Jun		26	
25-Jun		23	
26-Jun		12	
27-Jun			
28-Jun			
29-Jun			
30-Jun			
5 Day Max	Count:	736	

were surveyed at high tide. Extended datasets are shown for the sites at Taunton Bay and Middle Bay because there were willing and available volunteers for the extended period. With the exception of Middle Bay, the Casco Bay counts were negligible during May, in spite of the fact that shallow water temperatures appeared conducive to spawning activity. It appears that horseshoe crabs winter in parts of Casco Bay that are slow to warm. Horseshoe crabs are cold-blooded, and individuals wintering on the bottom of a bay or estuary are not mobile until temperatures begin to rise at whatever location and localized depth they are in.

Limited water temperature data from the Friends of Casco Bay indicate that Casco Bay water temperatures stay cold into June. Data from two Casco Bay sites showed that water temperatures between May 10<sup>th</sup> – May 20<sup>th</sup>, hovered below 10 °C, at 10 feet or more of depth, (Clapboard Island and Little Flying Point,

nine dates; Friends of Casco Bay, unpublished data, 2001-2004). Spawning in Maine requires a minimum of 12  $^{\circ}$ C, and predominantly occurs at 14  $^{\circ}$ C (Schaller et al. 2002). U.S. Coast & Geodetic Survey charts indicate that except for Middle Bay, the sites in Casco Bay all have waters deeper than 20 feet mlw, within 1 mile of the survey site.

Middle Bay and the surveyed sites east of Casco Bay have extensive shallow water areas adjacent to them that are less than 20' deep at mean low water (mlw) for more than a mile. All of theses sites documented some horseshoe crabs during May of 2004, and they appear to warm a little earlier than most of Casco Bay.

Surprisingly, although the habitat at the Casco Bay sites in Cumberland and Yarmouth appears to offer some of the best horseshoe crab spawning habitat in the State, remarkably low counts were seen in June. This warrants additional investigation. It is possible that oil spills, historic industrial contamination, or current practices have damaged horseshoe crab populations in these areas. The Cumberland site at Wildwood receives storm water runoff from the residential neighborhood, and commercial pesticide application trucks were seen in this neighborhood on multiple days during the survey period. Horseshoe crabs are sensitive to buried anoxic sediments and possibly they are sensitive to other substances that reach shorelines in storm water runoff. It would be interesting to know if some of the pesticides being applied in the neighborhood are chitin inhibitors that block insect growth. Like insects, horseshoe crabs are arthropods with a chitin based shell, and they grow by molting.

Data from the Damariscotta Mills site for 2004 was incomplete, since there were three days during the early June survey period when volunteers did not collect data. Most of the volunteers are of retirement age, and some are less physically sound than other. When faced with a very high tide, some volunteers felt unsafe walking in the water, and did not collect data on those days.

Volunteer safety is of primary concern; the lost opportunity to document this site was unfortunate. It is coincidental that the three days when data were not collected occurred during the period that had the highest activity. Effectively the whole season of data for this site was lost since the peak period (as indicated by several high single-day counts) was incompletely documented. A similar loss of data occurred during the 2003 field season for one day at Day's Cove. In that case, the lost data did not matter because the higher counts clearly occurred in another survey period for which there were five consecutive days of data. Elimination of the site at the Mills and consolidation of the available volunteers is recommended for the future.

A summary of spring counts for 2001-2004 is presented in Table 3A. Spawning populations of horseshoe crabs persist at Middle Bay, Thomas Point Beach, in the Damariscotta and Bagaduce Rivers, and Taunton Bay. Sandy Beach in Freeport is the only survey site inside Casco Bay's Hussey Sound that was seen to host a spawning population. Sites around Wolf's Neck in Freeport also appear to support spawning but are unsuitable to volunteer monitoring. More field work might yield additional sites. Sandy substrate occurs intermittently in Casco Bay, along with rock, dense patches of clay, and mud. The possibility exists that in response to limited habitat, horseshoe crabs are spawning during the high tide on habitat in mid- or lower-intertidal zones.

No clear population trend emerges from the count data, even when presented as a percentage of the year with the highest count in Table 3B. The yearly counts have fluctuated substantially, and even when instances of incomplete data are discounted, additional data are needed. Overall, no local or dramatic short-term decline has been documented in the 4 years of surveys. At the same time, there are very few locations statewide where horseshoe crab spawning can be seen predictably. Perhaps the best that can be said is that the known robust populations remain at Thomas Point Beach, Middle Bay, and the Damariscotta River (in order of relative abundance), while smaller populations persist in Taunton Bay, and the Bagaduce River.

Table 3A: Summary of Counts, 2001-2004

<b>Highest Combined Cou</b>	ints for 5 Consecutive Days	s (per 1x100m)	Sites cou	nted at high t	ide except Middle Bay in 2004		
Town	Site Name		2001	2002	2003	2004	
Cumberland	Wildwood	Casco Bay				4	
Yarmouth	Cousin's IsI - Sandy Pt	Casco Bay				0	
Yarmouth	Cousin's Isl - Blaney Pt.	Casco Bay			3	2	
Freeport	Winslow Mem'l Park	Casco Bay				0	
Freeport	Sandy Beach	Casco Bay				38 ***	
Brunswick / Harpswell	Middle Bay **	Casco Bay	282 **	422 **	93 **	736 (Low)	
Brunswick	Thomas Pt Beach	New Meadows River	171 *	1865	2248	1482	
Wiscasset	Eaton Farm	Back River			23	0 *	
Damariscotta	Days Cove (Hospital)	Damariscotta River	76 *	178	65	290	
Nobleboro	Damariscotta Mills	Damariscotta River	606	403	209	219 *	
Penobscot	Bagaduce River	Bagaduce River	25 *	50	23 *	192	
Franklin	Shipyard Point	Taunton Bay	176	104	67	163	
Franklin	Hog Bay at South Bay Rd	Taunton Bay		32	2 *	94	

<sup>\*</sup> Incomplete data series for the site (see Table 1)

Table 3B: Survey Site Totals as a Percent of the Highest Year's Count

Highest Combined Coun	ts for 5 Consecutive Days	(per 1x100m)	Sites counted at high tide except Middle Bay in 2004					
Town	Site Name		2001	2002	2003	2004		
Brunswick / Harpswell	Middle Bay **	Casco Bay	66.8%	100.0%	22.0%	(no data)		
Brunswick	Thomas Pt Beach	New Meadows River	7.6%	83.0%	100.0%	65.8%		
Damariscotta	Days Cove (Hospital)	Damariscotta River	26.2%	61.4%	22.4%	100.0%		
Nobleboro	Damariscotta Mills	Damariscotta River	100.0%	66.5%	34.5%	36.1%		
Penobscot	Bagaduce River	Bagaduce River	13.0%	26.0%	12.0%	100.0%		
Franklin	Shipyard Point	Taunton Bay	100.0%	59.1%	38.1%	92.6%		

Episodes of peak spawning activity appear to have been captured for all sites by the dates chosen for the 2004 surveys. In 2001, the initial survey dates were chosen based on typical activity patterns in Massachusetts and on Delaware Bay, which begin with the lunar phase (Barlow et al. 1982, Smith: http://ael.er.usgs.gov/group/stats/ Limulus, downloaded April 2001; see also Smith et al. 2002). Spawning in Maine typically initiates a day or two after the lunar phase, and later in the season, with the later arrival of spring (Schaller et al. 2004). Longer survey periods were selected in 2002 after it became apparent that different sites peak in activity on different days (Schaller et al 2002, Schaller 2002, Schaller et al 2004).

Credit is due to the volunteers for successful data collection for the counts. Volunteers who return in successive seasons add stability and consistency to the data collection process. Many new volunteers were needed this season to staff the Casco Bay sites. Their energy, enthusiasm, and support were responsible for the success of the project.

# 2004 Scouting

A number of sites were scouted for spawning activity during late June. More detail is provided in Appendix A. On the lower Damariscotta River, a few horseshoe crabs were seen at Pleasant Cove (3 pairs, 3 males; 600-700m transect), and in Lower Dodge Cove (8 pairs, 1 female, 6 males, 400m transect). Similar numbers were found on the Bagaduce River, near Penobscot Church (5 pairs, 2 females, 4 males; 300m transect) and on Salt Pond in North Sedgwick (2 pairs, 5 males; 100m transect). Horseshoe Cove in Bell's Marsh in Brooksville (Cape Rosier) was scouted and suitable habitat was found but no evidence of horseshoe crabs. (PT)

<sup>\*\*</sup> Middle Bay high tide counts are understated: the water is opaque at high tide due to suspended microfine sediment.

<sup>\*\*\*</sup> Counts conducted only for the third survey period, so the site is incompletely documented.

Born (1977) reported occasional occurrences of horseshoe crabs in the Medomak River, which was scouted in 2004 at eleven sites. Sites were selected based on a horseshoe crab habitat model developed by Banner and Schaller (2001) for the Gulf of Maine Habitat Analysis. The GIS model indicated suitable spawning habitat at a number of sites in the Medomak. Five sites were surveyed along Dutch Neck; 2 shells were found along the east point of Meetinghouse Cove (150m transect) and a pair was found near the Waldoboro town boat ramp. On the east side of the Medomak River, a few pairs were found north of Sampson's Cove (2 pair; 90m transect), and along Pitcher's Cove at Cove Road (1 pair; 100m transect), and one dead animal was found at the boat launch on outer Deaver Road (100m transect). (PT)

Willard Beach in South Portland was scouted because it offers suitable substrate, but no evidence of horseshoe crabs was found. Back Cove in Portland and the mouth of the Stroudwater River were also scouted, with no evidence of horseshoe crabs. Back Cove is reported to have had "many" horseshoe crabs approximately 15 years ago (Nancy Agan, pers. comm.). (SS)

Some sites have been reported that will be worth scouting during 2005. A volunteer reported having been told that horseshoe crabs can be seen at the Town Landing in Yarmouth, at the end of Princes Point Road. Boaters have reported horseshoe crab shells in Broad Cove, Yarmouth. Both of these sites should be scouted in 2005.

Born's (1977) field work noted horseshoe crab spawning at a number of sites where horseshoe crabs have not been found in recent years. These include Stover Cove and Merepoint Neck in Harpswell. Although it is not possible to determine the relative densities that Born saw during his field work, it is possible to say that numbers have declined if former spawning sites now host only a few scattered animals, as on the Medomak River and Salt Bay, in Blue Hill.

Anecdotal reports were received in 2004 of horseshoe crabs having populated sites on Prince Edward Island, Canada as recently as 20 years ago (Linda Barton, pers. comm.). Subsequent inquiries to Canadian tourists visiting Maine from P.E.I. have agreed, but no one has reported seeing horseshoe crabs there in over a decade. Nor prior mention of horseshoe crabs in Canada in recent history is known.

# **Tagging Study Results**

### **Summary of Observations, Individuals, Male: Female Ratios**

2004 was the fourth season of horseshoe crab tagging at Taunton Bay. Tagging began on May 20<sup>th</sup> and continued daily through June 30<sup>th</sup>, for a total of 42 days. Prior seasons varied from 33 to 42 days, but each field season has documented activity associated with three lunar phases. The year 2001 continues to stand out for having had the highest activity—the highest number observations, and the highest number of individuals observed. Table 4 summarizes the number of observations and individuals, categorized by males and females (based on individually numbered tags). The Taunton Bay tagging site is unique in that all horseshoe crabs at the site can be tagged each day, and individually numbered tags enable identification of individuals returning to the site on other days and during other years.

The numbers presented in Table 4 vary slightly from those presented in prior reports, because all four seasons of data were moved into a database for analysis this year. A few duplicate observations of the same tag number on the same day were deleted during data cleanup, and a few problem records were reassigned and used in the analysis. Data on prosomal width, gender, tag number, and review of the original data sheet, were used to reassign the records.

Tag numbers are occasionally difficult to read. They are manufactured using a clear heat-shrink plastic over the individual tag number, which is printed on colored plastic. The clear heat-shrink over wrap binds the tag components together, and protects the number against abrasion. If the clear heat-shrink over wrap is heated slightly too long, the plastic number may be slightly compressed, making it difficult to read. If this

happens, a number nine can be mistaken as an eight, a six as an eight, a five as a three, and so on. Initial record errors are unlikely because tag numbers are assigned sequentially; data recording errors and tag reading errors are more likely to occur on returns. Larger print tags are not currently available. Handheld magnifiers are carried with the tagging equipment.

Table 4: Tagging Study Summary Statistics for Years 2001 - 2004

Year	Dates	No. Days	Males (M)	Females (F)	# Individuals	M Obs	F Obs	# Observations	M per F	M Obs./ F Obs.	Avg.Obs./ Indiv.
2001	5/21-6/23 (-2days)	33	982	351	1333 (100%)	1774	659	2433 (100%)	2.8	2.7	1.8
2002	5/19-6/29	42	465	276	741 (56%)	696	411	1107 (45%)	1.7	1.7	1.5
2003	5/20-6/30	42	556	338	894 (67%)	758	494	1252 (51%)	1.6	1.5	1.4
2004	5/20-6/30	42	592	323	915 (69%)	877	507	1384 (57%)	1.8	1.7	1.5

2001 continues to have been the year when the most observations were logged (2433) and the highest number of new individuals tagged (1333). The <u>number of observations</u> in 2002 was 45% of the 2001 totals, improving to 51% in 2003 and gaining to 57% in 2004. The <u>number of individuals</u> observed in 2002 dropped to 56% of the 2001 totals, recovered somewhat to 67% in 2003, and edged slightly up to 69% in 2004. While the number of females has varied from year to year, the number of males has gradually increased between 2002 and 2004.

The highest spawning counts were obtained in 2001 and the lowest in 2002, but it is unclear whether one was an exceptionally good year or the other was an exceptionally poor year. 2001 was the only season in which water temperatures were warm enough in May to support significant levels of spawning activity. Clearly delineated peak spawning events were documented during both late-May and early June. In subsequent years, water has been slow to warm and spawning did not get underway until June.

It is worth noting that no data collection is conducted on overnight tides. Horseshoe crabs rely on their vision to locate mates (Barlow et al. 1986). Tag numbers would be illegible without bright lights, which would blind the animals for 20-30 minutes, disrupting normal spawning behavior. The variation in the terrain at the tagging site also makes it hazardous for a tagging crew, and volunteers could not be found to assist on overnight counts for a six week period.

Table 5 shows the composition of each tag year class, and the frequency with which tagged horseshoe crabs have been documented in successive years. By the end of the 2004 field season, 3361 individuals had been tagged over four field seasons. Return rates have ranged from 6.7 to 10.2% of a tag-year class returning to the tagging site in successive spawning seasons. In 2002, 8.7% of the 2001 tag year class returned, 8.5% in 2003 and 7.5% in 2004. The decline in 2004 is attributable to fewer returning females.

Table 5: Return Rates Relative to Original Tag Year

Observation	Tag	Number o	of Individuals (	Observed	% by	Original Yea	r Class	% M-F by	Year Class	Returning	M/F
Year	Year	Male	Female	Total	Male	Female	Total	Male	Female	Total	Sex Ratio
Tagged in 2001	2001	982	351	1333	73.7%	26.3%	100%	73.7%	26.3%	100%	2.8
Returned in 2002		83	33	116	6.2%	2.5%	8.7%	71.6%	28.4%	100.0%	2.5
Returned in 2003		76	37	113	5.7%	2.8%	8.5%	67.3%	32.7%	100.0%	2.1
Returned in 2004		87	13	100	6.5%	1.0%	7.5%	87.0%	13.0%	100.0%	6.7
Tagged in 2002	2002	382	243	625	61.1%	38.9%	100%	61.1%	38.9%	100%	1.6
Returned in 2003		41	23	64	6.6%	3.7%	10.2%	64.1%	35.9%	100.0%	1.8
Returned in 2004		28	14	42	4.5%	2.2%	6.7%	66.7%	33.3%	100.0%	2.0
Tagged in 2003	2003	439	277	716	61.3%	38.7%	100%	61.3%	38.7%	100%	1.6
Returned in 2004		38	35	73	5.3%	4.9%	10.2%	52.1%	47.9%	100.0%	1.1
Tagged in 2004	2004	432	255	687	62.9%	37.1%	100%	62.9%	37.1%	100%	1.7
Total number of individua	als tagged:	2235	1126	3361	66.5%	33.5%	100.0%				2.0

Both the 2001 and 2002 tag year classes showed a decline in the number of females who returned in 2004. Females comprised 26% (351) of the 2001 tag year class of 1333 (individuals). Of these, 2.5% (33) returned in 2002, 2.8% (37) returned in 2003, and only 1% (13) returned in 2004. In the 2002 tag year class, females made up 39% (243) of the original class; in 2003, 3% (22) returned, but only 1.9% (14) returned in 2004. Another way of looking at this is by the percentages of males and females returning in any one year, by tag year class. The decline is most dramatic in the 2001 class where returning females dropped from 2.8% to 1% of the original class 1333 (individuals). In 2002, 116 animals tagged in 2001 returned, 28% of which were female; in 2003, 113 returned, 33% of which were female, but in 2004 when 100 returned, only 13% were female.

The cause of the dramatic decline in the percentages of returning females from the 2001 tag year class has not yet been attributed. Possible causes include differential survival, differential mortality based on gender, or adult molting. Older females may be dying off, or being preyed upon, so that by comparison males simply live longer. Another possibility is that some growth occurs in the early years following sexual maturity, and that some of the females molted. However, no molted shells of adult males have been found at Taunton Bay.

Adult molting seems the most likely explanation for the decline in returning 2001 females. If tagged animals molted, their numbered tags would remain in the shed shell. During molting, the molting hormone (ecdysone) separates living tissue from the shell. The point of the genal angle where tags are attached has little living tissue in it and is sometimes a dry cavity. Whatever living tissue is perforated by tagging, would be thin and easily separated during molting, allowing the animal to successfully complete the molt. The result would be a small scar in the edge of the genal angle when the tissue hardened into the new shell after the molt. Two untagged animals have been found at the tagging site with clean new shells that each had a notch in the genal angle, suggestive of adult molting from a tagged shell.

When comparing Tables 4 and 5 it is important to remember that Table 5 shows the number of newly tagged individuals each season. Individuals from prior years that return to the tagging site are included in the data on observations, and are tabulated in the counts of the number of individuals observed, but they already have tags on them – with few exceptions.

Tables 4 and 5 are useful in considering how harvest would impact horseshoe crabs. In 2004 there were 1384 observations of 915 individuals, of which 75% (687) were newly tagged, and 25% (228) had been tagged in previous seasons. Each additional season of tagging data will be useful in providing a tool to model the impact of harvest on this species. Considering that horseshoe crabs need 9-10 years to mature, and that 25% of the spawners seen in 2004 were already tagged (in only 4 field seasons), it is easy to see that removal of adults would significantly impact small populations in a short of time.

# **Tag Retention and Day Tags**

Tags are occasionally lost, but in very small numbers. During 2003 only one tag is known to have been lost. A single horseshoe crab returned with the point of a genal angle broken off, and a portion of the tagging hole was still visible along the otherwise ragged edge of the break. It is likely that the tag became entangled, and as the horseshoe crab struggled, the tagging hole broke, and the tag was lost. The animal escaped whatever entanglement it was in, and the loss of a research tag is desirable in such a circumstance. In 2004, 13 horseshoe crabs returned to the tagging site that had lost tags because the tip of the genal angle had broken away. It is possible that most of the lost tags are from the 2001 tag year class. These tags have been on those shells longest, and may cause wear on the shell that eventually results in breakage, or the longer an animal is tagged, the greater the probability of the tag becoming entangled and then breaking away. The 14 lost tags were evenly distributed between seven males and seven females, so lost tags do not explain the reduced percentages of returning 2001 females.

To date, only 15 tags have been lost from 3361 tagged individuals, which is an acceptably low rate of 4.4 tags per thousand issued (0.45%). Since the original tag year cannot be determined, records for these individuals are not included in analysis of the percentage of tag year classes. One tag is known to have separated from a horseshoe crab during 2001, because it was found floating at the water's edge. It occurred within the first days of tagging and was apparent that the T-bar on the end of the tag had not been 'set' when attached.

Day tags present a minor challenge. During the first week of tagging in 2001, many more horseshoe crabs were encountered than were expected, and the inventory of 500 tags was exhausted during the first peak event. Temporary tags were devised—a different tag for each of five days, and size and sex were recorded. Numbered tags were resumed as soon as a rush shipment could be obtained. Each day tag was replaced with a numbered tag when the animal returned to the tagging site. One of the observation records (from the date the day tag was issued) was assigned to the numbered tag records for the individual (based on sex and prosomal width).

Some of the day-tags were metal wire, while others were plastic or plastic-coated wire. All the day tags held up well during 2002 and 2003 during which no animals were encountered at the tagging site with empty tagging holes. In 2004, after 3 years in salt water, no animals returned with intact plain wire day tags. Only day tags made of plastic or plastic-coated wire returned intact during 2004. A number of animals returned with empty tag holes however, which were attributed to rusted-out plain wire day tags issued on May 27<sup>th</sup>, 2001. Unassigned day tag records for May 27<sup>th</sup>, 2001 were assigned to these horseshoe crabs and a number of unassigned records remain for May 27<sup>th</sup>, 2001.

## **Tagging Sex Ratios and Relevance to Survey Counts**

The ratio of the number of male observations to female observations is similar to the ratio of the number of individual males to females, tracking it relatively closely, from 2.7 in 2001 to 1.7, 1.6 and 1.7 in successive years. This suggests that counts might adequately represent the relative numbers of males to females in a horseshoe crab population. Without this information, one might think that the ratio of males to females in horseshoe crab populations were somewhere around 1:1, and that the prevalence of males at spawning sites was a behavioral effect. Instead, the data demonstrate that males did not necessarily frequent the spawning sites more often than females, but rather that spawning males are more abundant than females. This raises the question of whether the prevalence of males is caused by a sex-linked behavior, by environmental factors, or by an unidentified aspect of resource competition that enhances the survival of males over females. In other states, where horseshoe crabs are harvested commercially for bait, females are taken preferentially over males because they are larger. A female can be cut into more portions of bait, and when quartered, eggs will be shed into the water, acting as an additional attractant. If there were a commercial harvest, it would be easy to think that this explained the greater number of males to females. In the population at the tagging site, where there is no known history of harvest, the larger number of males to females is unexplained.

The tagging data provides valuable insight to interpretation of the counts conducted at the survey sites elsewhere in the State. Tagging data indicate that the Taunton Bay population is larger than would be expected based solely on interpretation of the number of observations in any one week. On rare days (twice) the counts have exceeded 200, but on most days they do not exceed 100, and more often they number fewer than 50 (380m transect). Considering the relatively low number of daily observations, it is surprising that the number of individuals seen at the tagging site each season has ranged from 714 to 1333 each season—many more than might be expected from the daily counts. The number of observations has ranged from 1107 to 2433 each season. Nonetheless, the Taunton Bay population is clearly less abundant (per hectare) than those at Thomas Point, Middle Bay, or in the Damariscotta River (in that order).

#### **Environmental Variables**

The relationship between peak spawning activity and environmental variables continues to be useful in predicting dates of peak spawning activity. The information is valuable in scheduling survey dates for the sites where counts are conducted, and scheduling the 70-80 volunteers each season for those sites.

Charts 1-4 graph the daily counts at the tagging site, and the environmental data for 2004, 2003, 2002 and 2001, respectively. Environmental data includes water temperature, lunar phase and tidal amplitude. Charts 1 and 2 for 2004 and 2003 include daily weather data, ranked from sunny (7) to stormy (1). Supporting data tables are provided in Appendix B. Data for tidal amplitude were taken from the Maine Harbors website (www.maineharbors.com, on various download dates). Data for tide height on that website are given in decimal feet. Bar Harbor data are used for the Taunton Bay site, since this is the closest available location, and equipment for recording tide height inside Taunton Bay is currently beyond the budget of this work.

Charts 5 shows the tagging activity for all four years, with lunar phase and the tidal amplitude for each year. Tagging activity is presented as a percentage of observations on the peak day during the season. It is clear from the charts that the first peak spawning period at Taunton Bay for each of the past four seasons has occurred within three days of a full moon/new moon lunar phase. When spring has been delayed, spawning did not initiate until early June, instead of late May. After the initial spawning event each season, there is a post-peak period of minimal activity, and then spawning activity increases again. However, after the initial spawning period, subsequent periods of increased activity do not follow the lunar phase.

Tide heights are greater downeast than in southern Maine, occasionally exceeding 13 feet in Frenchman's Bay and in Taunton Bay (an inner embayment). Although tidal amplitude does not appear to be a limiting factor to spawning activity, the days of the highest activity did not necessarily coincide with the highest of the high tides. It follows that there would be no evolutionary advantage to spawning at the upper edge of the highest tides, since eggs buried that high along the shoreline would be more likely to desiccate than to develop. Nonetheless, a few individuals spawn where the eggs have no chance of survival, in the current environment—a behavior which may explain how this species has survived so long.

Chart 6 compares the daily tagging activity to water temperature and lunar phase. Low water temperatures appear to suppress spawning activity, as do marked decreases in temperature. During 2004, the Friends of Taunton Bay collected temperature data for a bottom location in Taunton Bay that is shallowly submerged at mean low water. Minimum and maximum daily bottom temperatures are shown for 2004 based on Taunton Bay data. For prior years, bottom temperature data from Boothbay Harbor was used as a surrogate, along with a Taunton Bay temperature reading taken in 3 feet of depth during high tide. The Boothbay Harbor data was from a more deeply submerged site, but it was the only data available, and it documents seasonal sea bottom warming in mid-Maine.

Horseshoe crabs loiter at different depths and different locations, and localized temperatures vary considerably. Until water temperatures have warmed, spawning periods lasting 3 or more days and including hundreds of horseshoe crabs do not regularly occur. Data from Taunton Bay and other survey sites in 2001 and 2002 indicated that 12°C (taken in 3 feet of depth at high tide) was the minimum temperature for peak spawning events, and 14°C was the usual threshold (Schaller et al. 2002, Schaller 2002).

Daily weather was recorded for Taunton Bay during 2004 and 2003 in an effort to quantify the influence of sunlight in facilitating high activity spawning periods. Horseshoe crabs are known to avoid spawning during stormy weather. Stormy weather generally includes wind and wave action, and waves would tumble and jostle horseshoe crabs at the shoreline. Horseshoe crabs do not spawn on high-energy shorelines, i.e. surf beaches or sites with long reaches of open water exposed to wind-driven waves. Instead, they are found in sheltered bays and estuaries. If clear sunny days indicate favorable spawning weather, and stormy days are avoided because they are accompanied by wind and waves, it is reasonable to expect that

Chart 1: 2004 Taunton Bay Tagging Results

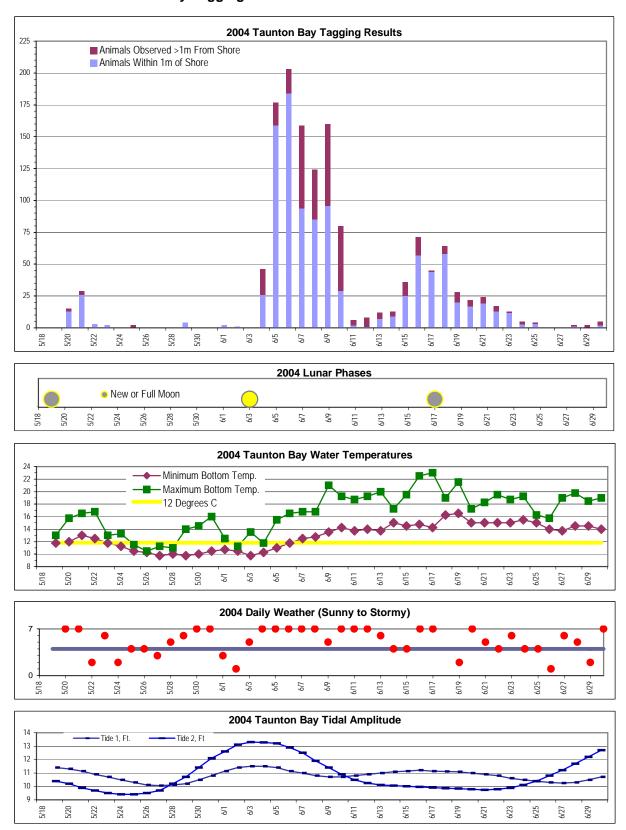


Chart 2: 2003 Taunton Bay Tagging Results

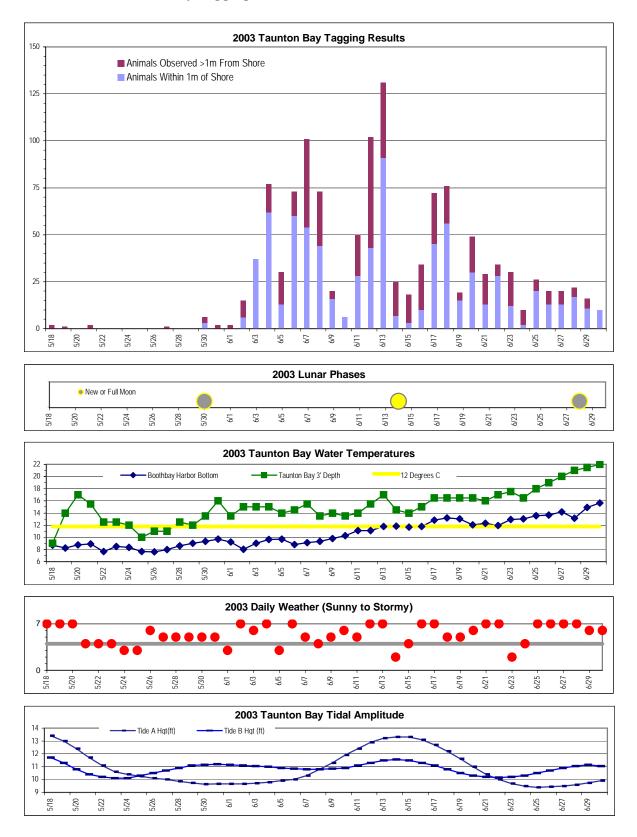


Chart 3: 2002 Tagging Counts and Environmental Data

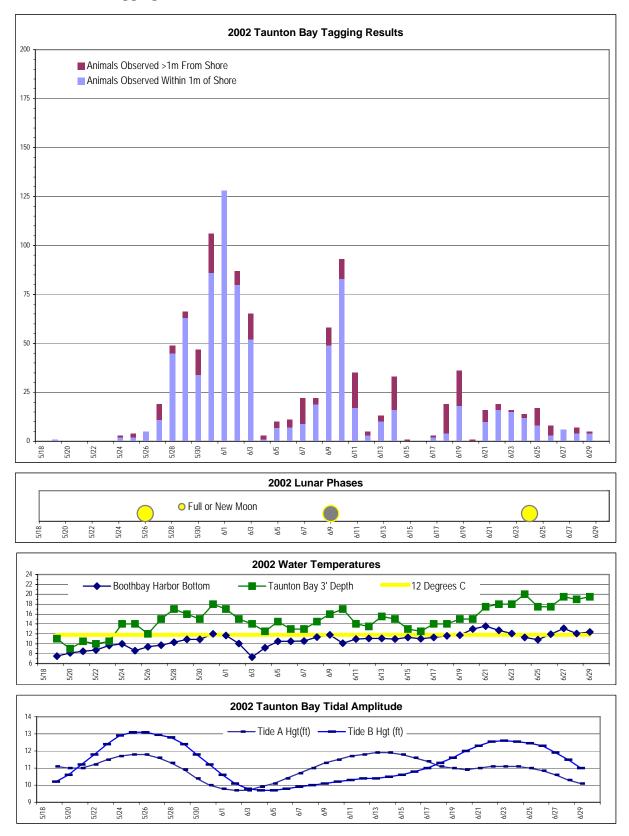


Chart 4: 2001 Tagging Counts and Environmental Data

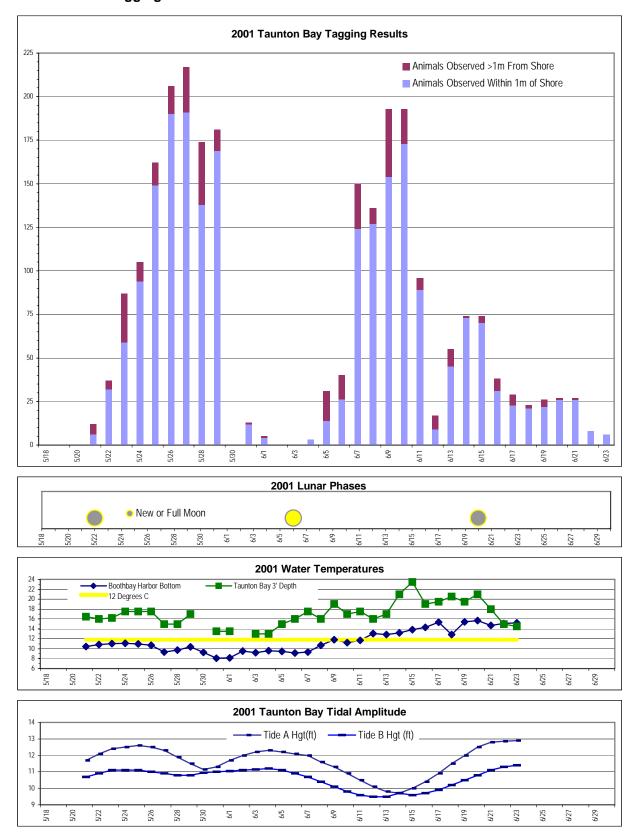
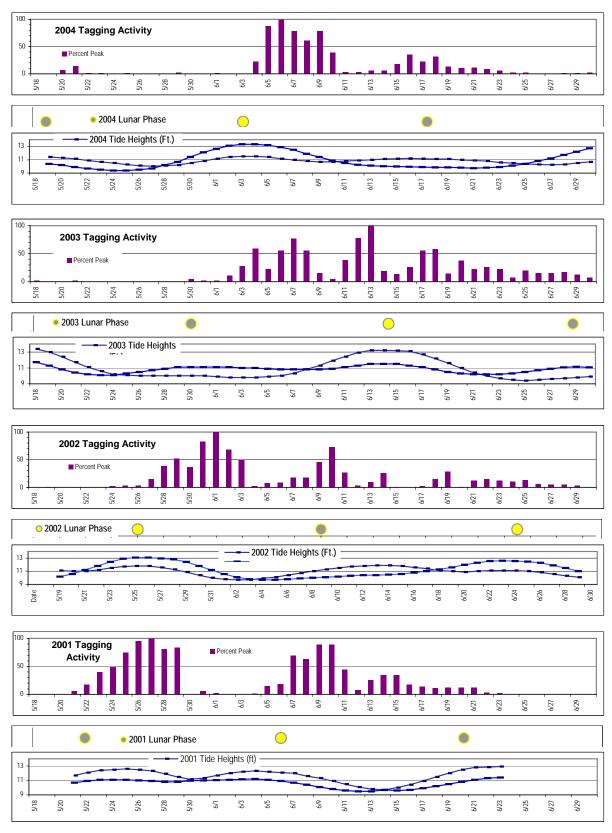
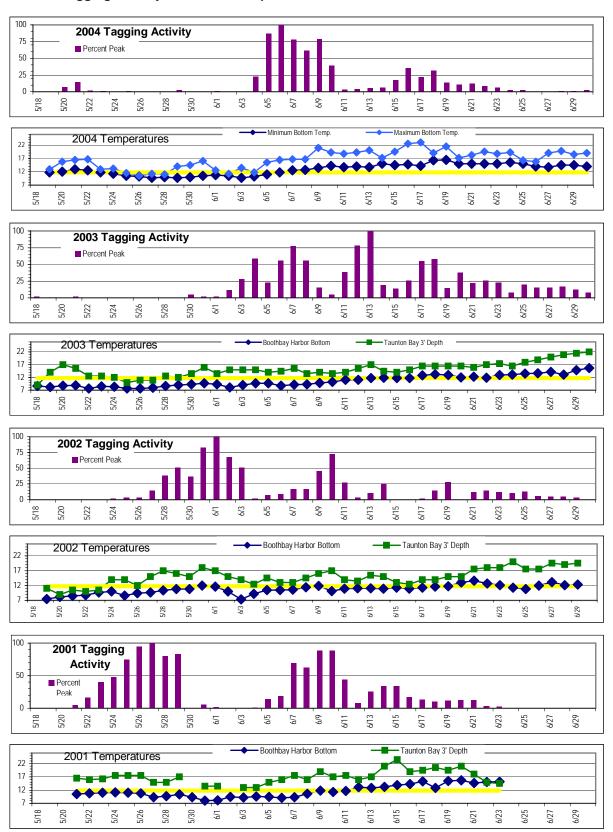


Chart 5: Tagging Activity, Lunar Phase and Tidal Amplitude 2001-2004



**Chart 6: Tagging Activity and Water Temperature** 



horseshoe crab spawning activity would be higher on bright sunny days than on days with heavy cloud cover, or light rain, even if other environmental factors (lunar phase, temperature, and tidal amplitude) were favorable. It is evident that horseshoe crabs can differentiate between cloudy days and night, based on their photosensitivity and circadian clocks, as described by Barlow and Powers (2003). Furthermore, "wind-driven overhead waves act like lenses, creating beams of light that strobe the underwater scene at frequencies of ~2-6Hz" (Passaglia et al., 1997, in Barlow and Powers, 2003), so horseshoe crabs are visually cued to the weather above even when they are deeply submerged.

Weather was recorded as sunny, partly cloudy, partly sunny, overcast, light rain, rain, or stormy, and assigned a value between 7 and 1, based on these gradations. The values were plotted, and are included in charts 1 and 2. Once spawning initiated during the season, and so long as water temperatures did not markedly decline, weather that was sunny, partly cloudy or partly sunny was associated with elevated levels spawning activity (scores of 7, 6 or 5). Cloudy days (a score of 4) did not preclude spawning, but activity levels on cloudy days trended lower than on bright days, even if temperatures remained consistently above 14°C. Rainy days, whether light rain, extended rainfall, or stormy conditions (scores of 3, 2 or 1) were predominantly associated with decreased spawning activity.

One could argue that weather and temperature are not independent-- that sunlight warms the water, thereby increasing water temperatures and fostering higher levels of spawning, and that the effects of one cannot be separated from the effects of the other. Levels of spawning activity increased on cloudy days when they were associated with overall patterns of improvement in the weather. Spawning levels did not increase on rainy days. It is reasonable to think that horseshoe crabs are predominantly responsive to light, because of their many photosensitive organs. But it is also possible that horseshoe crabs are responsive to changes in barometric pressure (through changes in ambient water pressure).

#### **Visits and Spawning Site Fidelity**

Horseshoe crabs do not exhibit strong spawning site fidelity from year to year, but within a season, they will often return to the same spawning site. For all four year classes, visits were tallied by individuals and showed that 1134 females were documented by 2031 observations at the tagging site, for an average of 1.79 visits per female; 2320 males were documented making 4079 spawning visits to the tagging site, for an average of 1.76 visits per male.

Of all four year classes, only one individual was seen during all four tagging seasons, a female, observed five times. Individuals observed over three field seasons included 160 observations of 33 males (4.85 visits per male) and 42 observations of 12 females (3.5 visits per female). For individuals seen during two field seasons, there were 959 observations of 366 males (2.62 visits per male) and 416 observations of 132 females (3.15 visits per female). All other individuals were seen during only one field season, for which there were 2960 observations of 1921 males (1.54 visits per male) and 1568 observations of 989 females (1.59 visits per female).

Looking at the visitation records for the 2001 class only, 10 females (0.8% of 1333 individuals) returned during 3 or more field seasons (one was seen during all four field seasons) and 29 males (2.2% of 1333); 66 females (5%) and 267 males (20.0%) returned during 2 field seasons, while 278 females (20.9%) and 764 males (57.3%) have not been seen since 2001. Overall, 21% of the animals tagged in 2001 have been seen in the subsequent three field seasons. The low rate of return from year to year suggests that horseshoe crabs move randomly and make use of favorable resources as they encounter them.

Tagging records for 2004 were analyzed to determine how many of the individuals seen in 2004 had been tagged during prior seasons. Reiterating the data from tables 4 and 5, 25% of the spawners seen in 2004 had been tagged in prior seasons. This suggests that a significant number of the adult horseshoe crabs in the vicinity of Shipyard Point may now be tagged.

#### **Other Findings**

Males are often found missing one or more claspers and the attached leg(s). This suggests that the clasper muscles rest in the clasped position, and that the animals elect open their claspers to attach or detach themselves. This injury can easily be explained by the fact that some males remain amplexed to females beyond the spawning season. If an amplexed pair is exposed to heavy turbulence, the male could be torn from the female, losing one or both anterior legs in the process. The loss of claspers does not prevent males from spawning, because they can continue to do so as a satellite male.

Partial limb regeneration has been seen on a very few animals, where one or two terminal sections had been lost. Regeneration appears to occur slowly, over multiple seasons, and was not observed to occur where entire legs had been severed.

#### Conclusion

The Maine Horseshoe Crab Surveys have successfully developed an index of relative abundance for known spawning sites in the State. New volunteers continue to join the effort, making it possible to add new sites. The scarcity of horseshoe crab spawning in most of Casco Bay is puzzling; particularly in light of the fact the some of the best available habitat is found there. Toxicology studies are expensive but might shed some light on the scarcity of horseshoe crabs on seemingly optimal habitat. Continued surveys are recommended for 2005. Based on the unusually cold winter, no spawning activity is anticipated during May 2005, and volunteer effort might be best used to survey for one extended period in June.

Continued conservation measures are recommended for horseshoe crabs in Maine. The number of sites where horseshoe crab spawning can predictably be seen are limited in Maine. Middle Bay, the New Meadows River near Thomas Point Beach, the Damariscotta River, Bagaduce River and Taunton Bay all would benefit from habitat protection measures. Middle Bay in particular is recommended for addition to the Register of Critical Areas.

Species density is sparse throughout Maine, and harvest would likely result in additional localized extinctions. Three sites identified by Born (1977) as having breeding populations of horseshoe crabs no longer appear to support them. These include: Merepoint Neck in Brunswick, Stover Cove in Harpswell and Sam's Cove in Breman. Back Cove in the Portland and the mouth of the Stroudwater River offer suitable habitat with no sign of horseshoe crabs (Schaller and Thayer, 2003) although anecdotal accounts have stated that they were there 20 years ago.

The tagging study now has four seasons of data and each additional season of data increases the value of the data for identifying trends in the Taunton Bay horseshoe crab population. It is fortunate that the 2001 season had the highest counts of any season to date, since the 2001 year class will be critical to the development of longevity estimates for horseshoe crabs in Maine. In turn, this information is a valuable component in the development of population models for managing this species.



#### **Citations**

- Banner, A. and S. Schaller, March 2001, Gulf of Maine Watershed Habitat Analysis, U.S. Fish & Wildlife Service, Gulf of Maine Program, Falmouth, Maine. A G.I.S. model identifying important habitat for 64 species, http://r5gomp.fws.gov/gom/habitatstudy/gulf\_of\_maine\_watershed\_habitat\_analysis.htm.
- Barlow, R.B., Jr.; L. C. Ireland & L. Kass. 1982. Vision has a Role in *Limulus* Mating Behavior. Nature 296 (65-66).
- Barlow, Robert B. and Maureen K. Powers. 2003. Seeing at night and finding mates: the role of vision, Chapter 4 in: <u>The American Horseshoe Crab</u>, Carl N. Shuster, Jr., Robert B. Barlow and H. Jane Brockman, eds., Harvard University Press, Cambridge, Massachusetts, pp.83-102.
- Barlow, Robert B. Jr., Maureen K. Powers, Heidi Howard, and Leonard Kass. 1986. Migration of *Limulus* for Mating: Relation to Lunar Phase, Tide Height, and Sunlight. Biological Bulletin 171: 310-329.
- Born, John. 1977. Significant Breeding Sites of the Horseshoe Crab (*Limulus polyphemus*) in Maine and Their Relevance to the Critical Areas Program of the State Planning Office (Planning Report Number 28). Maine State Planning Office, Augusta, Maine. Reissued 1982. 45 pp.
- Botton, M.L., R. E. Loveland, and T. R. Jacobs. 1988. Beach erosion and geochemical factors: influence on spawning success of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay. Marine Biology 99:325-332.
- Botton, M. L. and J. W. Ropes. 1987. Populations of horseshoe crabs, *Limulus polyphemus*, on the northwestern Atlantic and continental shelf, Fisheries Bulletin 85(4):805-812.
- Brady, J. T. and E. Schrading, Feb. 1997, Habitat Suitability Index Models: Horseshoe Crab (Spawning Beaches) Delaware Bay, New Jersey and Delaware, Unpublished, Eric Schrading: USFWS, Pleasantville, NJ Field Office. 10 pps.
- Jegla, T. C. and J. D. Costlow. 1982. Temperature and Salinity Effects on Development and Early Posthatch Stages of Limulus, *In*: Physiology and Biology of Horseshoe Crabs: Studies on Normal and Environmentally Stressed Animals, Alan R. Liss, New York, p. 103-113.
- King, T. L.; M. S. Eackles, and C. R. Callahan. 2002. Genetic Stock Identification in the Horseshoe Crab (*Limulus polyphemus*): A range-wide perspective from microsatellite DNA variation. Poster presented at American Fisheries Society Annual Meeting, August 18-22, 2002; Baltimore, MD
- Manion, Michelle M., Rebecca A. West, and Robert E. Unsworth. 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. 70pp.
- Nietfeld, Marie T., Morley W. Barrett, and Nora Silvy. 1994. Wildlife Marking Techniques, In: T. A. Bookhout, ed., <u>Research and Management Techniques for Wildlife and Habitats</u>, 5<sup>th</sup> edition. The Wildlife Society, Bethesda, Maryland, pp 140-168.
- Passaglia, C., F. Dodge, E. Herzog, S. Jackon, and R. B. Barlow. 1997. Deciphering a neural code for vision. Proceedings of the National Academy of Sciences, 94:12649-15654, *In* Robert B. Barlow and Maureen K. Powers. 2003. Seeing at night and finding mates: the role of vision, Chapter 4 in: <u>The American Horseshoe Crab</u>, Carl N. Shuster, Jr., Robert B. Barlow and H. Jane Brockman, eds., Harvard University Press, Cambridge, Massachusetts, pp.83-102.

- Penn, Dustin and H. Jane Brockman. 1994. Nest-Site Selection in the Horseshoe Crab, Limulus polyphemus. Biological Bulletin 187:373-384. Rudloe, A. 1979. Locomotor and light responses of larval horseshoe crabs, *Limulus polyphemus* (L.). Biological Bulletin 157:494-505.
- Rudloe, A. 1979. Locomotor and light responses of larval horseshoe crabs, *Limulus polyphemus* (L.). Biological Bulletin 157:494-505.
- \_\_\_\_\_ 1980. The breeding behavior and patterns of movement of horseshoe crabs, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. Estuaries 3(3):177-183
- Schaller, Susanne Y. 2002 Maine Horseshoe Crab (*Limulus polyphemus*) Spawning Surveys, 2002. Report to Report to Maine Department of Marine Resources, West Boothbay Harbor, Maine, by Bar Mills Ecological, P. O. Box 771, Buxton (Bar Mills), Maine 04004. 16pp.
- Schaller, Susanne Y., Peter Thayer, and Sherry Hanson. 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, Buxton (Bar Mills), Maine 04004. 34 pp. \_\_\_\_\_ 2004. Maine Horseshoe Crab (Limulus polyphemus) Spawning Surveys, 2003 Report to Maine Department of Marine Resources, West Boothbay Harbor, Maine, by Bar Mills Ecological, P. O. Box 771, Buxton (Bar Mills), Maine 04004. 26 pp.
- Schrading, E, T. O'Connell, S. Michels, and P. Perra. 1998. Interstate Fishery Management Plan for Horseshoe Crab, Atlantic States Marine Fisheries Commission, Fishery Mgt Rpt 32. 59pp.
- Shuster, Carl. N., Jr. 1950 Observations on the natural history of the American horseshoe crab, *Limulus polyphemus* in 3<sup>rd</sup> Report on Investigations of methods of improving the shellfish resources of Massachusetts, Woods Hole Oceanographic Institution: 18-23.
- \_\_\_\_\_ 1982. A pictorial review of the natural history and ecology of the horseshoe crab *Limulus* polyphemus, with reference to other *Limulidae*, in Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals, Alan R. Liss, Inc, NY: 1-52.
- \_\_\_\_\_ 1990 The American horseshoe crab, *Limulus polyphemus*. In R.B. Prior, ed., Clinical applications of the *Limulus* amoebocyte lysate test. CRC Press, Boston: 15-25.
- Smith, David R., Penelope S. Pooler, Benjie L. Swan, Stewart F. Michels, William R. Hall, Peter J. Himchak, and Michael J. Millard. 2002. Spatial and Temporal Distribution of Horseshoe Crab (Limulus polyphemus) Spawning in Delaware Bay; Implications for Monitoring. Estuaries 25:1, p. 115-125.

### **Personal Communications**

- Agan, Nancy. April 3, 2004, Observed "many horseshoe crabs in Back Cove" .... "about 15 years ago".
- Campbell, Neal. March 25, 2005. Code Enforcement Officer, Town of Damariscotta, Maine.
- Hunter, Larry. August 25, 2000. Park Manager, Roque Bluffs State Park, Roque Bluffs, Maine.
- Merrill, Carl. August 24, 2001. Director, Robert S. Friedman Field Station of Suffolk University at Cobscook Bay, Suffolk Drive, R.R.1, Box 102, Edmunds, Maine 04628
- Trott, Thomas. November 15, 2000. Research Associate, Robert S. Friedman Field Station of Suffolk University at Cobscook Bay, Suffolk Drive, R.R.1, Box 102, Edmunds, Maine 04628
- Wagner, Stu. August 21, 2000. Park Manager, Cobscook Bay State Park, Lubec, Maine.

### Appendix A: Horseshoe Crab Scouting Notes 2004, P. Thayer

- 6/20/04 Pleasant Cove, Damariscotta R. (northwest shoreline of cove). Walked 6-700m transect southward from point of land at house at end of access driveway. Mostly marshgrass/bank at edge of mud. Water temp. 15 C. at north end; 15 C. at south end of transect. At small pocket beach at north end of transect (next to the house), found 1 female, 3 males, 1 mating pair; also were depressions in mud, probably from spawning activity. Found 1 mating pair midway in transect. At south end of transect saw 1 male.
- 6/20/04 Lower Dodge Cove, Damariscotta R. Water temp 14.5 C. Walked northerly app. 400m to marshgrass/point. 16 C. at both south and north ends. 6 males (2 newly dead); 1 female (newly dead); 7 mating pair. Most crabs seen on southern end. One male was very large and feeding on possibly a large fish egg sac. 2 pairs were mating right in the cobbled brick area. Not a great volunteer survey site.
- 6/21/04 Penobscot Church, Bagaduce River. (northerly shore of cove). App. 300m, marshgrass/bank at edge of mud. 5 mating pairs; 4 males; 2 females. 16 C.
- 6/21/04 Horseshoe Cove (Bell's Marsh), Brooksville (Cape Rosier) (upper east side). Good access (Bell Marsh Rd, first house on right, number 175 or 75), walked shore southerly 300m, to point at 'narrows'. Suitable habitat (sand/mud sediment). No crabs or shells. 15 C.
- 6/21/04 Salt Pond, N. Sedgwick (southwest end survey station). 100 m transect, marshgrass/bank at edge of mud. 2 mating pairs; 5 males. 20 C.
- 6/22/04 Broad Cove, Medomak River (west side of river, from ramp at end of Storer Rd). 15.5 C at ramp. Walked ~ 500m to north of ramp, into Western Branch marsh grass/bank area, 15.5 C. No crabs or shells. Walked ~ 500m to south of ramp gravel, sand, small rock. 1 male; 1 recently dead male; 1 newly dead female (eaten). Continued~500m more into flat-then-marshy north shore of Broad Cove. 1 mating pr. 16 C.
- 6/23/04 Dutch Neck, Medomak River Inner Meetinghouse Cove Too wet, marshy.
- 6/23/04 Dutch Neck, Medomak River east point of Meetinghouse Cove (driveway/house at tip). Mostly marsh; some ledge. Walked ~ 150m. 2 shells. 20 C.
- 6/23/04 Dutch Neck, Medomak River southeast boatramp. Shoreline all ledge.
- 6/23/04 Dutch Neck, Medomak River Osborn Finch Preserve Too far of a walk in for volunteers.
- 6/23/04 Waldoboro Town Boatramp (west side Medomak R.) Poor visibility brown water. Walked ~100m, mostly marshgrass. 19.5 C. One mating pair at ramp according to kids present.
- 6/24/04 East Side Medomak River (jellyfish abundant; mostly either marshgrass or rocky shore). First stop just above Sampson's Cove, trio of large estates (same family). Good shore gravel/sand/stones. Caretaker (Chester Merrifield) used to see many HSCs ~ 20 years ago, not much now. 18 C. Walked ~90m northerly from bottom of stairs. 2 mating pair, having quite large females.
- 6/24/04 East Side Medomak River Pitcher's Cove (Cove Rd.) Mostly ledge; some good sediment. Walked ~ 100m. 1 mating pair.
- 6/24/04 East Side Medomak River Back River/Cove Shoreline all ledge/rocky.
- 6/24/04 East Side Medomak River south of Havener Ledge (take Deaver Rd. continuing from Cove Rd., towards The Narrows). Small cove; some sand (~ 20m) but mostly rocky/ledge. No crabs/shells seen. 15 C. Boy present said he sees a few shells sometimes, but no live crabs.
- 6/24/04 East Side Medomak River boat launch, outer Deaver Rd. (Clammer parking area). South side of road walked ~ 400m; excellent shoreline/substrate. No crabs/shells seen. Waves limited visibility somewhat. 15.5 C. North side of road quiet marshy area. Walked ~ 100m; found 1 dead male.

Appendix B: Table 1, 2004 Data in Charts 1, 5, & 6 (380m transect)

2004	Н	ISC Coun	its	Tide Heights	(Feet)	T.B. Bottom T	emp. C		
Date	In	Out	All	Tide 1, Ft.	Tide 2, Ft	Minimum	Maximum	Moon	Weather
5/19/04				11.40	10.40	11.75	13.00	New	
5/20/04	13	2	15	11.30	10.20	12.00	15.75		7 (full sun)
5/21/04	26	3	29	11.15	9.90	13.00	16.50		7
5/22/04	3		3	10.90	9.70	12.50	16.75		2
5/23/04	2		2	10.70	9.50	11.75	13.00		6
5/24/04			0	10.50	9.40	11.25	13.25		2
5/25/04		2	2	10.30	9.40	10.50	11.50		4
5/26/04			0	10.10	9.50	10.25	10.50		4
5/27/04			0	10.05	9.70	9.75	11.25		3
5/28/04			0	10.10	10.20	10.00	11.00		5
5/29/04	4		4	10.20	10.70	9.75	14.00		6
5/30/04	0		0	10.50	11.40	10.00	14.50		7
5/31/04			0	10.80	12.10	10.50	16.00		7
6/1/04	2		2	11.15	12.60	10.75	12.50		3
6/2/04	1		1	11.40	13.10	10.50	11.25		1
6/3/04			0	11.50	13.30	9.75	13.50	full	5
6/4/04	26	20	46	11.50	13.29	10.25	11.75		7
6/5/04	159	18	177	11.40	13.20	11.00	15.50		7
6/6/04	184	19	203	11.15	12.90	11.75	16.50		7
6/7/04	94	65	159	11.00	12.50	12.50	16.75		7
6/8/04	85	39	124	10.80	11.90	12.75	16.75		7
6/9/04	96	64	160	10.70	11.40	13.50	21.00		5
6/10/04	29	51	80	10.70	10.90	14.25	19.25		7
6/11/04	2	4	6	10.80	10.50	13.75	18.75		7
6/12/04	1	7	8	10.90	10.25	14.00	19.25		7
6/13/04	7	5	12	11.00	10.10	13.75	20.00		6
6/14/04	9	4	13	11.10	10.05	15.00	17.25		4
6/15/04	25	11	36	11.15	10.00	14.50	19.50		4
6/16/04	57	14	71	11.20	9.95	14.75	22.50		7
6/17/04	44	1	45	11.15	9.93	14.25	23.00	new	7
6/18/04	58	6	64	11.13	9.88	16.25	19.00		
6/19/04	20	8	28	11.10	9.85	16.50	21.50		2
6/20/04	17	5	22	11.00	9.80	15.00	17.25		7
6/21/04	19	5	24	10.90	9.75	15.00	18.25		5
6/22/04	13	4	17	10.80	9.80	15.00	19.50		4
6/23/04	12	1	13	10.60	9.90	15.00	18.75		6
6/24/04	3	2	5	10.50	10.10	15.50	19.25		4
6/25/04	3	1	4	10.40	10.40	15.00	16.25		4
6/26/04				10.30	10.80	14.00	15.75		1
6/27/04				10.25	11.20	13.75	19.00		6
6/28/04	1	1	2	10.30	11.70	14.50	19.75		5
6/29/04	0	2	2	10.50	12.20	14.50	18.50		2
6/30/04	2	3	5	10.70	12.70	14.00	19.00		7

Appendix B: Table 2, 2003 Data in Charts 2, 5, & 6 (380m transect)

2003	F	ISC Coun	ts	Tide Heights	(Feet)	BBH Temp	TB Temp		
Date	In	Out	All	Tide 1, Ft.	Tide 2, Ft	Sea Bottom	High Tide, 3'	Moon	Weather
5/18/03	0	2	2	13.4	11.7	8.7	9.0		7
5/19/03	0	1	1	13.0	11.3	8.3	14.0		7
5/20/03	0	0	0	12.4	10.8	8.8	17.0		7
5/21/03		2	2	11.7	10.4	8.9	15.5		4
5/22/03			0	11.1	10.2	7.7	12.5		4
5/23/03			0	10.6	10.1	8.5	12.5		4
5/24/03			0	10.4	10.1	8.4	12.0		3
5/25/03			0	10.3	10.3	7.7	10.0		3
5/26/03			0	10.1	10.5	7.6	11.0		6
5/27/03		1	1	10.0	10.7	8.0	11.0		5
5/28/03			0	9.9	10.9	8.6	12.5		5
5/29/03			0	9.8	11.1	9.0	12.0		5
5/30/03	3	3	6	9.7	11.2	9.4	13.5	New	5
5/31/03		2	2	9.7	11.2	9.7	16.0		5
6/1/03		2	2	9.7	11.2	9.2	13.5		3
6/2/03	6	9	15	9.7	11.1	8.0	15.0		7
6/3/03	37		37	9.7	11.1	9.0	15.0		6
6/4/03	62	15	77	9.8	11.0	9.7	15.0		7
6/5/03	13	17	30	9.9	10.9	9.7	14.0		3
6/6/03	60	13	73	10.0	10.9	8.8	14.5		7
6/7/03	54	47	101	10.3	10.8	9.2	15.5		5
6/8/03	44	29	73	10.8	10.8	9.3	13.5		4
6/9/03	16	4	20	11.3	10.9	9.8	14.0		5
6/10/03	6		6	11.9	10.9	10.3	13.5		6
6/11/03	28	22	50	12.4	11.1	11.1	14.0		5
6/12/03	43	59	102	12.9	11.3	11.1	15.5		7
6/13/03	91	40	131	13.2	11.5	11.8	17.0		7
6/14/03	7	18	25	13.3	11.6	11.8	14.5	Full	2
6/15/03	3	15	18	13.3	11.5	11.7	14.0		4
6/16/03	10	24	34	13.1	11.3	11.8	15.0		7
6/17/03	45	27	72	12.7	11.1	12.9	16.5		7
6/18/03	56	20	76	12.2	10.8	13.2	16.5		5
6/19/03	15	4	19	11.6	10.5	13.0	16.5		5
6/20/03	30	19	49	11.0	10.3	12.1	16.5		6
6/21/03	13	16	29	10.4	10.2	12.3	16.0		7
6/22/03	28	6	34	10.0	10.2	11.9	17.0		7
6/23/03	12	18	30	9.7	10.2	12.9	17.5		2
6/24/03	2	8	10	9.5	10.3	13.1	16.5		4
6/25/03	20	6	26	9.4	10.5	13.6	18.0		7
6/26/03	13	7	20	9.5	10.7	13.7	19.0		7
6/27/03	13	7	20	9.5	10.9	14.2	20.0		7
6/28/03	17	5	22	9.6	11.1	13.1	21.0	New	7
6/29/03	11	5	16	9.8	11.2	14.9	21.5		6
6/30/03	10	-	10	9.9	11.1	15.6	22.0		6

Appendix B: Table 3, 2002 Data in Charts 3, 5, & 6 (380m transect)

2002	Н	SC Counts	 3	Tide Heights	(Feet)	BBH Temp	TB Temp	
Date	In	Out	All	Tide 1, Ft.	Tide 2, Ft	Sea Bottom	High Tide, 3'	Moon
5/19/02	1			11.1	10.2	7.49	11	
5/20/02				11.0	10.6	8.15	9	
5/21/02				11.0	11.2	8.47	10.5	
5/22/02				11.2	11.8	8.74	10	
5/23/02				11.5	12.4	9.65	10.5	
5/24/02	2	1		11.7	12.9	10	14	
5/25/02	2	2		11.8	13.1	8.61	14	
5/26/02	5			11.8	13.1	9.42	12	Full
5/27/02	11	8		11.6	12.95	9.69	15	
5/28/02	45	4		11.3	12.8	10.33	17	
5/29/02	63	3		10.9	12.4	10.87	16	
5/30/02	34	13		10.40	11.8	10.86	15	
5/31/02	86	20		10.00	11.2	11.98	18	
6/1/02	128			9.80	10.6	11.68	17	
6/2/02	80	7		9.70	10.1	10.07	15	
6/3/02	52	13		9.70	9.8	7.3	14	
6/4/02	1	2		9.90	9.7	9.22	12.5	
6/5/02	7	3		10.10	9.7	10.47	14.5	
6/6/02	7	4		10.40	9.8	10.47	13	
6/7/02	9	13		10.70	9.9	10.55	13	
6/8/02	19	3		11.00	10.0	11.38	14.5	
6/9/02	49	9		11.30	10.1	11.83	16	New
6/10/02	83	10		11.50	10.2	10.12	17	
6/11/02	17	18		11.70	10.3	10.98	14	
6/12/02	3	2		11.80	10.4	11.11	13.5	
6/13/02	10	3		11.90	10.4	11.1	15.5	
6/14/02	16	17		11.90	10.5	10.97	15	
6/15/02		1		11.80	10.6	11.31	13	
6/16/02				11.60	10.8	10.99	12.5	
6/17/02	2	1		11.40	11.0	11.27	14	
6/18/02	4	15		11.10	11.3	11.6	14	
6/19/02	18	18		11.00	11.6	11.73	15	
6/20/02		1		10.90	12.0	12.97	15	
6/21/02	10	6		11.00	12.3	13.56	17.5	
6/22/02	16	3		11.10	12.6	12.7	18	
6/23/02	15	1		11.10	12.6	12.08	18	
6/24/02	12	2		11.10	12.6	11.29	20	Full
6/25/02	8	9		11.00	12.5	10.82	17.5	
6/26/02	3	5		10.85	12.3	11.95	17.5	
6/27/02	6			10.60	11.9	13.12	19.5	
6/28/02	4	3		10.30	11.5	12.08	19	
6/29/02	4	1		10.10	11.0	12.38	19.5	
						13.17		

Appendix B: Table 4, 2001 Data in Charts 4, 5 & 6 (380m transect)

2001	HSC Counts			Tide Heights	Tide Heights (Feet)		TB Temp	
Date	In	Out	All	Tide 1, Ft.	Tide 2, Ft	Sea Bottom	High Tide, 3'	Moon
5/18/01								
5/19/01								
5/20/01								
5/21/01	6	6		11.70	10.7	10.47	16.5	
5/22/01	32	5		12.10	10.9	10.82	16	New
5/23/01	59	28		12.40	11.1	11.03	16.2	
5/24/01	94	11		12.50	11.1	11.13	17.5	
5/25/01	149	13		12.60	11.1	11	17.5	
5/26/01	190	16		12.50	11.0	10.71	17.5	
5/27/01	191	26		12.30	10.9	9.36	15	
5/28/01	138	36		11.90	10.8	9.72	15	
5/29/01	169	12		11.50	10.8	10.4	17	
5/30/01				11.15	11.0	9.3		
5/31/01	12	1		11.30	11.0	8.09	13.5	
6/1/01	4	1		11.70	11.1	8.14	13.5	
6/2/01				12.00	11.1	9.51		
6/3/01				12.20	11.2	9.22	13	
6/4/01	3			12.30	11.2	9.59	13	
6/5/01	14	17		12.20	11.1	9.44	15	
6/6/01	26	14		12.10	10.9	9.14	16	Full
6/7/01	124	26		12.00	10.7	9.32	17.5	
6/8/01	127	9		11.60	10.4	10.7	16	
6/9/01	154	39		11.30	10.1	11.8	19	
6/10/01	173	20		10.90	9.8	11.23	17	
6/11/01	89	7		10.50	9.6	11.67	17.5	
6/12/01	9	8		10.10	9.5	13.09	16	
6/13/01	45	10		9.80	9.5	12.9	17	
6/14/01	73	1		9.70	9.7	13.19	21	
6/15/01	70	4		10.00	9.6	13.87	23.5	
6/16/01	31	7		10.40	9.7	14.31	19	
6/17/01	23	6		10.90	9.9	15.35	19.5	
6/18/01	21	2		11.50	10.2	12.9	20.5	
6/19/01	22	4		12.00	10.5	15.4	19.5	
6/20/01	26	1		12.50	10.8	15.7	21	New
6/21/01	26	1		12.80	11.1	14.7	18	
6/22/01	8			12.85	11.3	15.1	15	
6/23/01	6			12.90	11.4	15.2	14.5	