

**Maine Horseshoe Crab (*Limulus polyphemus*)  
Spawning Surveys, 2003**

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## Abstract

Maine spawning surveys of horseshoe crabs (*Limulus polyphemus*) continued for a third season in 2003. Survey sites were located in Yarmouth, Brunswick (2 sites), Woolwich, Nobleboro, Damariscotta, Sedgwick, Franklin and Sullivan. The purpose of this project is to establish quantitative baseline data on horseshoe crab populations in Maine as a basis for management of this species. The 2003 season recorded cooler than normal water temperatures through the end of May, which resulted in negligible activity during the initial historical May spawning period. Warmer water temperatures in June supported normal levels of spawning activity, as documented by the June counts. The tagging study on Taunton Bay in Franklin continued for a third year, with more observations (1255) and more individuals observed (900) than in 2002 (1119 and 741, respectively), but fewer than in 2001 (2273 and 1292 respectively). The overall data indicate that horseshoe crab populations are declining at all but two of the sites, both of which are in Brunswick.

## Introduction

This report provides the results for the 2003 Maine Horseshoe Crab Surveys, and the associated tagging study at Taunton Bay (Franklin, Hancock County). Begun in 2001, this work is in its third year conducting spawning counts of horseshoe crabs during periods of expected peak activity. The purpose of this project is to establish quantitative baseline data on horseshoe crab populations in Maine, to support management of this species, and to increase the base of biological knowledge of this species in the northern extent of its range.

This project depends on some 50-70 volunteers to collect data at sites ranging from Casco Bay in Cumberland County to Taunton Bay in Hancock County. Effective use of volunteer time commitments to obtain high quality data of these events depends upon predicting when these naturally-driven events will occur. The annual survey dates are based on predicted dates of peak spawning activity. Field work preparation included volunteer training sessions, setting up 8-10 sites with transects, determining shoreline access, and determining the daily high tide schedules for each site. Data collection times vary each day with high tide, which crests at different times for most sites. Accordingly, nearly every site has a different schedule on any given day, making the project more challenging for volunteers to fit into their daily routines.

Periods of peak horseshoe crab spawning activity in Maine are associated with the new moon and full moon lunar phases of late May and June. Further south, where ocean temperatures are warmed by the Gulf Stream, the season begins earlier. Within Maine, periods of peak activity vary between sites, and not necessarily on a south to north gradient. Sites within a watershed can vary in dates of peak activity, for reasons that are unclear. Lunar phase seems to be the primary factor triggering the spawning season, but other factors appear to be involved and water temperature can be limiting.

Horseshoe crabs are euryhaline, meaning that they tolerate a wide range of salinity. While Maine's estuaries typically range between 25-33ppt, salinity occasionally drops to 15ppt. At levels below 8-10ppt, horseshoe crabs approach minimal tolerance ranges for survival. Tolerances for salinity levels of 4-6ppt are usually described in terms of the number of hours horseshoe crabs can survive. (Jegla and Costlow 1982, Sugita 1988, Shuster 1982)

Only one site, Damariscotta Mills, has periodically documented salinity levels below 10ppt, and occasionally as low as 5ppt (May 20<sup>th</sup> and 23<sup>rd</sup>, 2003). Survey sites typically record salinities of 25-33ppt (Schaller et al. 2002, Schaller 2002). The fluctuations in salinity can be attributed to freshwater inflows from the Damariscotta River which empties into Salt Bay relatively nearby.

Tidal amplitude-- the height of the tide-- does not appear to have any bearing on peak activity, as shown by the 2001 and 2002 data for Maine, insofar as it relates to the highest monthly tide, also known as the spring

tide. Spring tides were so named in reference to lively or springing water, versus the season of the year per se (Clancy 1968).

Horseshoe crab eggs require a regime of suitable moisture, temperature and salinity for development (Jegla and Costlow 1982, Sugita 1988). They can hatch in as few as 2-3 weeks of spawning (Shuster 1990), or as long as several months (Botton et al. 1992). In northern Maine where tidal amplitude reaches 11-13 feet in height, it would be disadvantageous for horseshoe crabs to deposit eggs in sediments that would not be over washed again for a month, when the eggs require moisture for development. Nonetheless, the misperception persists among some fans of horseshoe crabs that there is some correlation between the highest monthly tides and spawning activity.

Horseshoe crabs do spawn more actively on the higher of the two *daily* tides, qualified by some preference for the time of day (Barlow et al. 1986). Working on Cape Cod, 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. The inequality of daily high tides was greatest during the full moon, minimal near the quarter moon, and increased again towards the new moon. A preference by horseshoe crabs was described for the higher of the two *daily* high tides, and a conflicting preference for tides in the (pre-daylight) early morning hours.

“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...”

In addition to the counts that volunteers conduct at survey sites, an intensive tagging study is conducted at Taunton Bay. Tagging is conducted daily from the late May lunar phase until the end of June, and an individually numbered tag is attached to each animal captured. By tracking every individual over several weeks, and following them over several years, the tagging study provides a more complete dataset with which to interpret the surveys.

## **Methodology**

The methodology developed in 2001 is still followed (Schaller et al, 2001). For the survey sites, counts are conducted on the daytime high tide, starting approximately 20-30 minutes before high tide. The goal is to complete the work at or close to the time of high tide, because on Maine’s relatively steep shorelines, horseshoe crabs retreat to deeper water within an hour as the tide begins to ebb. The tagging crew varies its start time from 45-30 minutes before high tide 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 (Barlow et al. 1986).

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”. To limit observation bias in tabulating the results, only data within the “In” transect are used to construct the index of relative abundance. For other aspects of this study, such as the number of observations, 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 through a genal angle. The genal angles are the laterally posterior sharp bony points on each side of the trailing edge of the prosoma, or head. These bony points on the shell contain little tissue or sensory organs and the animals 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.

Morphological data are collected as the animals are tagged, by 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 assumed to be males, since the loss of the anterior pair of legs could be attributed to an injury having occurred during amplexus. In the absence of the anterior pair of legs, additional observations are used to confirm gender. Specifically, 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.

Ancillary issues relating to project management and the methodology are discussed in Appendix C. This includes routinely asked questions about tagging. It also includes an explanation of temporary tags that were used for part of the 2001 field season when the supply of numbered tags was temporarily exhausted.

## Results of Surveys

Spawning surveys (counts) were conducted at eight sites in 2003, adding a new site in Casco Bay. The list of 2003 survey sites is provided in Table 1, with georeferencing. Locations are shown in Maps 1 and 2. Although there are two sites in the Damariscotta River, they do not appear to be redundant as spawning activity varies between these sites. One is in Damariscotta Mills, at the sewer filter, and the other is on shoreline behind the hospital and adjacent to Day’s Cove. Surveys at the Mills occasionally document sudden drops in spawning activity associated with temporary drops in salinity and temperature. The site at Day’s Cove appears to be free of these fluctuations. The latter has recorded higher levels of activity later in the season, associated with the late June lunar phase, when activity has dropped off at the Mills site. There is no way of knowing whether this represents a seasonal shift in spawning behavior or a seasonal shift in habitat use, with incidental spawning behavior.

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

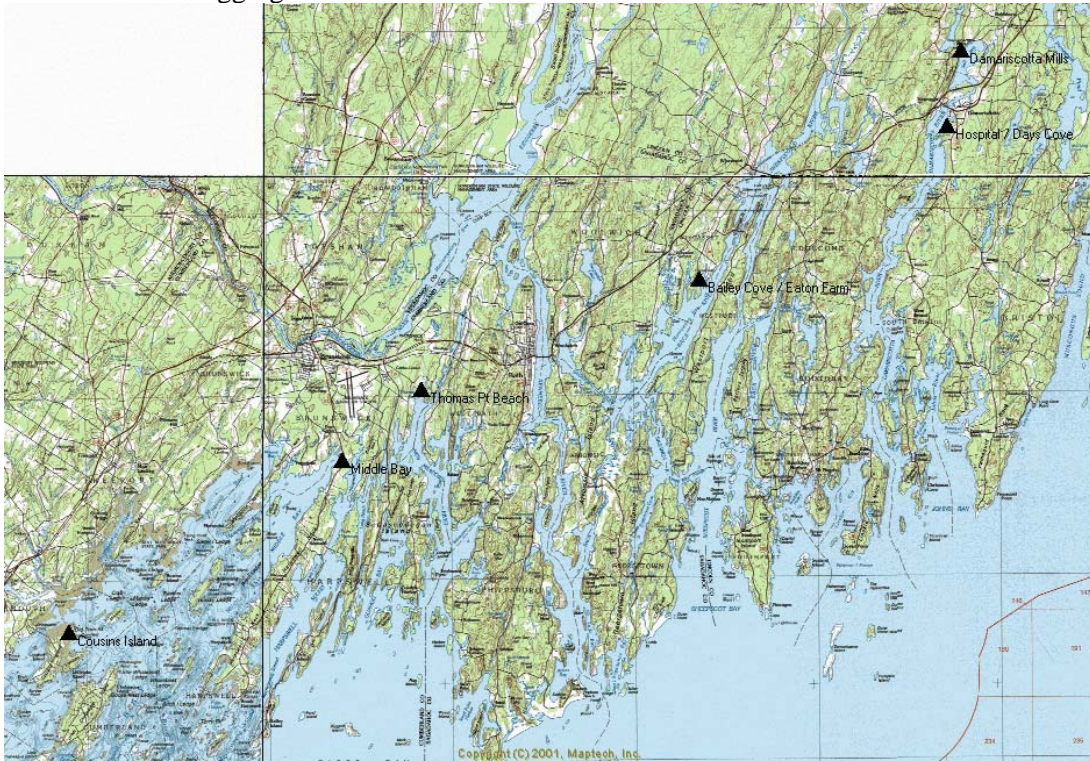
2003 Survey Name	Town	Site Description	WGS84 N	WGS84 W	NAD83 N	NAD83 W
Cousin’s Island	Yarmouth	cove sw of Blaney Point	43.7743429	-70.1302621	43 46 27.62	70 07 48.93
Middle Bay	Brunswick	Middle Bay, eastern cove	43.8593299	-69.9447254	43 51 33.57	69 56 41.00
Thomas Pt. Beach	Brunswick	Thomas Point Beach	43.8939318	-69.8909125	43 53 38.14	69 53 27.27
Bailey Cove	Wiscasset	Eaton Farm on Back River	43.9491529	-69.7011517	43 56 56.94	69 42 04.13
Days Cove	Damariscotta	shoreline behind hospital	44.0245795	-69.5325076	44 01 28.47	69 31 57.01
Damariscotta Mills	Nobleboro	Gt Salt Bay at D. Mills, sewer filter	44.0614377	-69.5225716	44 03 41.16	69 31 21.24
Bagaduce River	Sedgwick	behind Bagaduce Lunch	44.398585	-68.7027723	44 23 54.89	68 42 09.97
Taunton Bay (tag site)	Franklin	Shipyards Point, start of tagging transect	44.5817858	-68.230541	44 34 54.41	68 13 49.93
Taunton Bay (tag site)	Franklin	Shipyards Point, end of tagging transect	44.583198	-68.2319333	44 34 59.50	68 13 54.95
Taunton Bay, So.Bay Rd	East Franklin	South Bay Rd at Hog Bay	44.5711586	-68.2248982	44 34 16.16	68 13 29.62

A similar pattern of differential activity at neighboring sites was seen at Taunton Bay, in 2003. High levels of activity were recorded at the Shipyards Point tagging site following the early June lunar phase, declining in late June. On the opposite shore at South Bay Road (a half-mile away), low counts were recorded in early June, and higher counts following the late June lunar phase.

Map 1: 2003 Northern Tagging Sites



Map 2: 2003 Southern Tagging Sites



The Shipyard Point tagging site is located inside Taunton Bay, at the mouth of Hog Bay. The South Bay Road site is located inside Hog Bay, on shoreline adjacent to flats that drain at low tide. Low tide exposes 70% or more of the Hog Bay bottom. The South Bay transect looks north to Shipyard Point. Shorelines at Shipyard Point are steeply sloped, consistent with historic use of the site. The turning of the tide at Shipyard Point is readily apparent within the first two hours, and there is a marked retreat of horseshoe crabs from the shallows. On the shoreline along South Bay Road, a one-foot vertical drop in the tide is barely noticeable. The flat remains fully inundated for several hours after high tide before emerging. Even after being exposed, the fine sediments of the flat drain slowly and some horseshoe crabs wait out the tide by burrowing shallowly into the flat.

A site at Cousin’s Island in Yarmouth was added to begin documenting horseshoe crab populations south of Brunswick. A high count of only two animals was observed at this site during a week of surveys. However, over 100 juvenile shells were collected at this site during late summer molt surveys (Schaller et al., 2003). Juvenile horseshoe crabs were also observed foraging at this site during low tide in early June. The absence of adults is puzzling with so many juveniles at the site, and it is worth monitoring to see what happens over the next several seasons.

Survey data for all three years are shown in Appendix A. The 2001 project indicated that peak activity was associated with lunar phases, and that more than four consecutive days of data collection were needed to document an increase in activity levels, a peak, and a decline. The 2002 data were collected over two seven-day periods, and documented that spawning activity peaked on different days at different sites. All but one site documented a bell-curve pattern of increasing activity, peak activity, and two of three days of declining activity during the data collection period (Schaller 2002).

The highest single day count (per 1m by 100m transect), and the average for the highest five consecutive days are shown in Table 2. Table 2 indicates a decline for all the sites, except Thomas Point Beach. The 2001 high count of only 103 (at Thomas Point Beach) should be disregarded at that site since the data for that year did not document a peak and a decline. It therefore may not have documented the maximum counts for that year.

**Table 2: 2003 Horseshoe Crab Spawning Surveys, High Counts**

Site Name	Highest Single Day Count (per 1x100m)			Mean Count, Highest 5 Consecutive Days		
	2001	2002	2003	2001	2002	2003
Cousin's Island			2			1
Middle Bay***	57**	103	39	*	84	19
Thomas Pt. Beach	103**	703	722	34**	373	448
Bailey Cove - Eaton Farm		15	9		8	5
Hospital, by Day's Cove	50**	102	19	*	36	13
Damariscotta Mills	192	143	98	121	81	42
Bagaduce River	16	16	12	*	10	5
Taunton Bay - Shipyard Pt.	46	34	24	35	21	13
Hog Bay - South Bay Rd			28			6

\* Fewer than 5 consecutive days of data  
 \*\* 2001 counts did not confirm observation of peak date, as indicated by a lower count the following day.  
 \*\*\* Biased by poor visibility; water is opaque at high tide due to suspended micro-fine sediment.

The Middle Bay population is problematic because silt renders the water opaque at high tide. Low tide counts were conducted there in 2002 and 2003. The highest counts there were 1389 and 918 respectively, with 5 day means of 486 and 472. This population also appears to be holding it's own based on low tide counts. Populations at Bailey Cove, Day's Cove, Damariscotta Mills, the Bagaduce River and Taunton Bay all appear to be declining, based on the survey data.

Charts 1 through 8 graph the survey data as a percent of the peak day (highest counted) activity. Data for Cousins Island (Yarmouth) are omitted because of the limited data. Charts 1, 2, 5 and 7 appear to show some similar patterns in the timing of spawning activity, but no particular consensus emerges when the data are combined in Chart 9 (for eight sites during the early June survey dates). Note that Chart 9 graphs a limited time period, and that the maximum counts recorded for the Bagaduce River and Taunton Bay sites occurred in the later lunar phase, shown on Chart 10. Chart 10, combines the survey data for the four sites that had extended datasets—i.e. where some counts were conducted following the full moon on June 14<sup>th</sup>.

It appears from three years of data that the late May and early June spawning events are episodic; they usually follow the lunar phase and are marked by a distinct period of inactivity between them. Within the five to seven day event, extremes in environmental parameters can be limiting. As the early June spawning event winds down, activity levels may or may not zero out at a site, and they often continue at decreasing levels though the end of June, with or without a bell-curve of activity following the late June lunar phase.

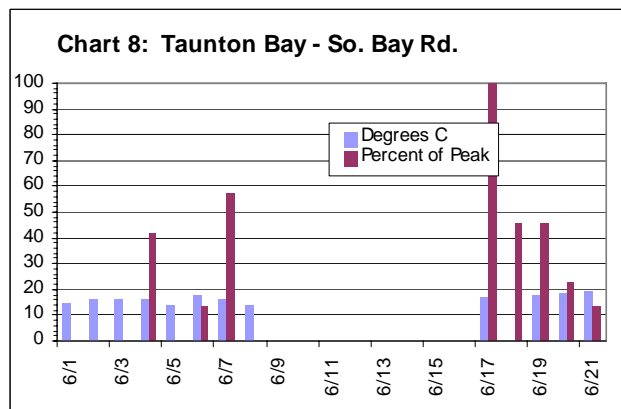
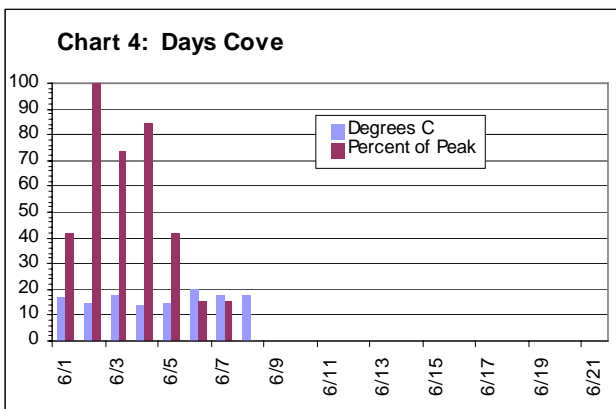
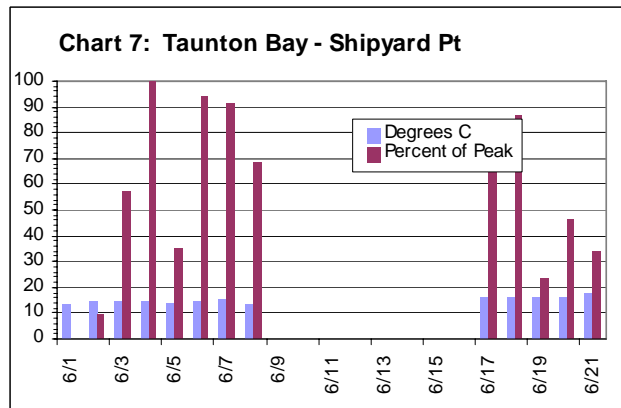
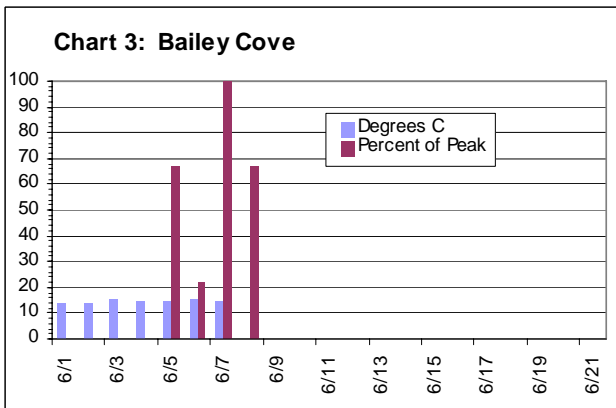
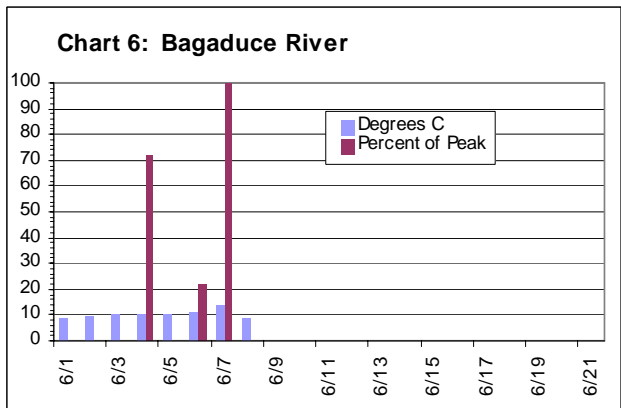
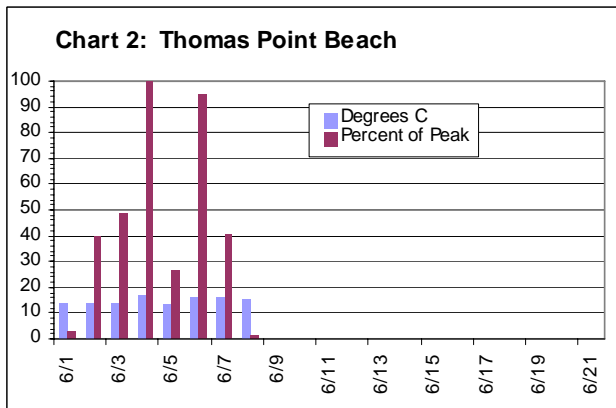
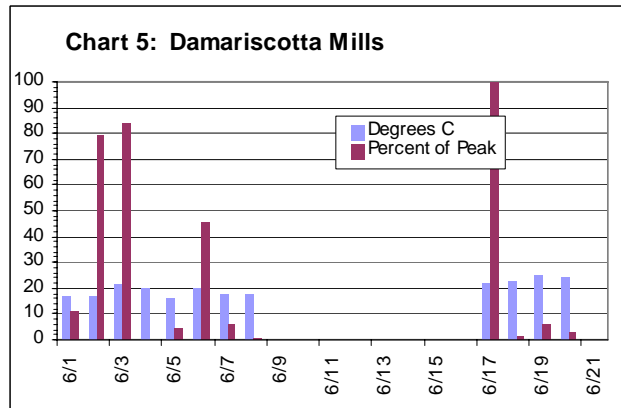
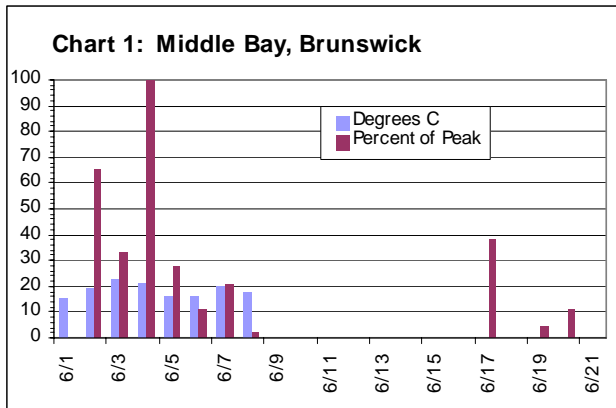
Peak spawning activity continues to be difficult to predict. In 2001, survey dates were chosen based on peak activity dates on Cape Cod and in Delaware Bay, which initiate with the lunar phase (Barlow et al.1986, and <http://ael.er.usgs.gov/group/stats/Limulus>, downloaded April 2001; see also Smith et al. 2002. The onset of spawning activity in Maine is seasonally delayed by the later advancement of spring. It also typically begins a day or two after the lunar phase, instead of with the lunar phase.

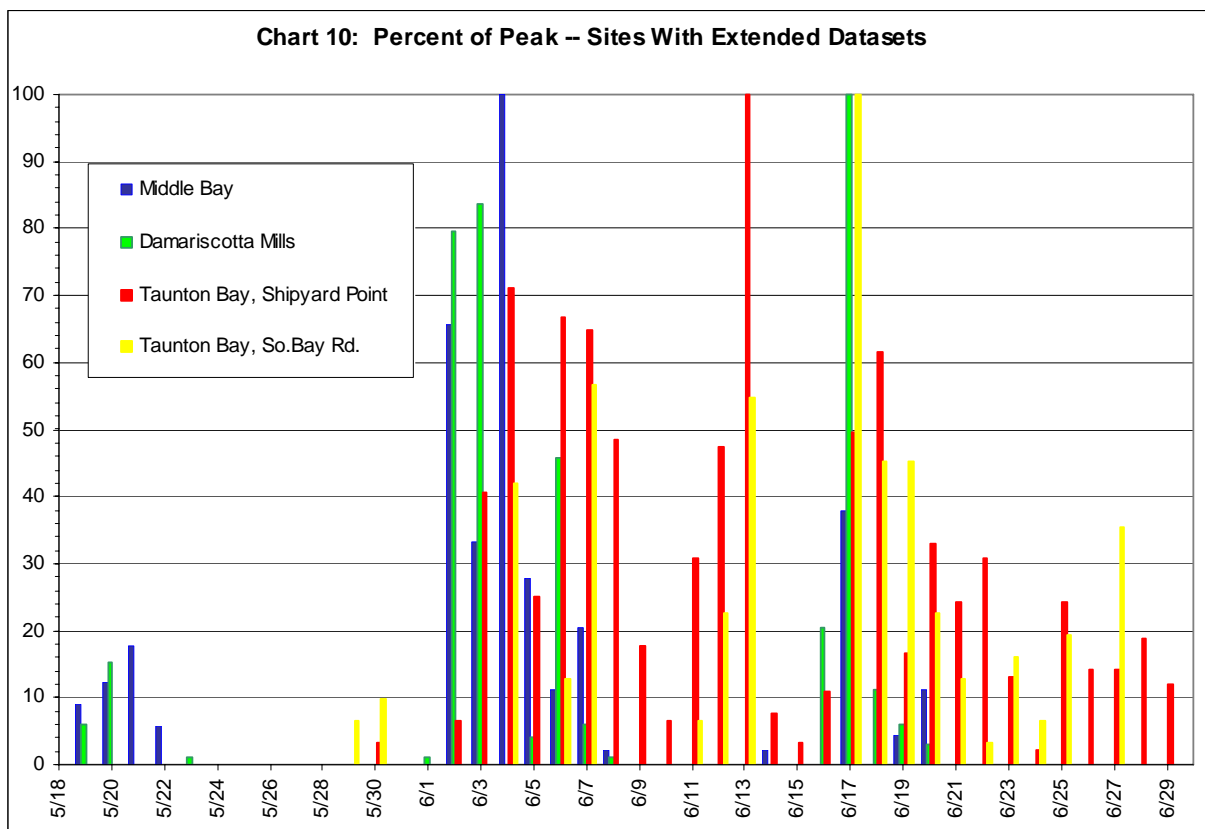
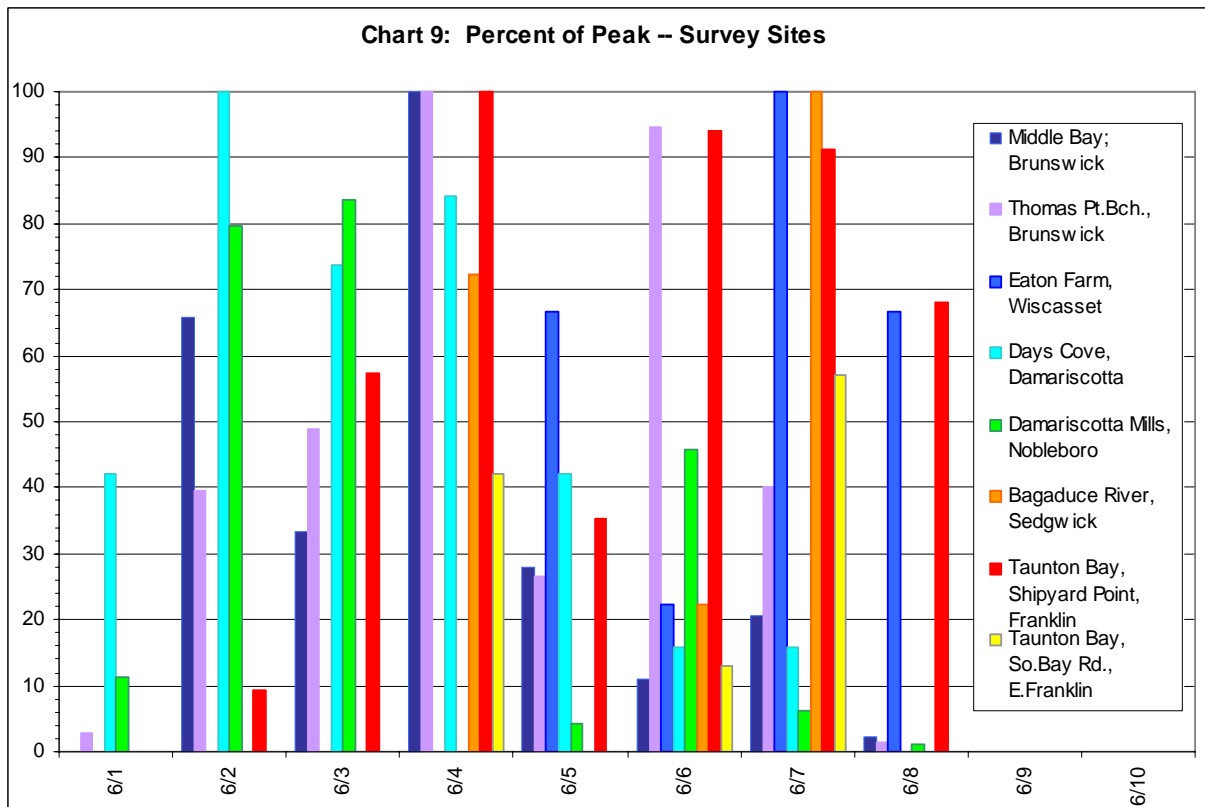
In 2003, the survey periods were extended to nine days; all sites appeared to have documented peaks and declines in activity. A severely cold winter resulted in colder spring water temperatures, and effectively cancelled the late May spawning. Although a few horseshoe crabs were observed in May (at Middle Bay, Damariscotta Mills, and Thomas Point Beach), they were a fraction of the counts that would be seen during the early June survey dates. None were observed during the May survey dates on Cousin's Island (Yarmouth), at Bailey Cove (Wiscasset), the Bagaduce River (Sedgwick), or in Taunton Bay (Franklin).

The surveys in early June provided consistent data for all the sites, and for the first time captured the activity cycle for all the sites. Credit for this success is due to the volunteers who staff these sites on a daily basis for the duration of the surveys. The return of a number of volunteers from one season to the next has added stability to the surveys, and consistency in the data collection.









## Results of Tagging

The Taunton Bay data for Shipyard Point is the largest dataset, both in terms of counts and because of the tagging data. Tagging was conducted for 42 days in 2003, from May 20<sup>th</sup> to June 30<sup>th</sup>. In 2002, tagging was conducted for 42 days, and in 2001 for 32 days. Although the number of days has varied from year to year, data associated with two complete lunar phases has been captured each year. Summary statistics for the tagging study are presented in Table 3. The highest numbers were documented in the initial year of the study. During 2002, observations dropped nearly 50% for no apparent reason, and in spite of an increase in the number of days of field work. The numbers recovered somewhat in 2003, and might have been closer to those seen in 2001 if there had been a late May spawning period. The entire east coast was affected, and spawning in Delaware Bay was also delayed two weeks (Smith, pers. comm., 2004).

**Table 3: Tagging Study Summary Statistics for Years 2001 - 2003**

Year	Dates	No. Days	Individuals	Females	Males	Unidentified	Observatons	Obs./ Indivs.
2001	5/21-6/23 (-2days)	32	1292	337	955	3	2273	1.76
2002	5/19-6/29	42	741	269	472	0	1119	1.51
2003	5/20-6/30	42	900	342	557	1	1255	1.39

Table 4 details the observations by tag year and gender. Of the 1255 observations in 2003, 978 (78%) were of new individuals, 103 (8%) were animals tagged in 2002, and 173(14%) were from 2001. Within the 2003 observations, the ratios of females/males, by tag year, were 2.26 observations of year 2001 females to males, 1.42 observations of 2002 females to males, and 1.44 observations for the 2003 year class. It would be incorrect to conclude from Table 3 that as the year classes age, the senior females are observed less frequently at the same spawning grounds than males in their same age class, because the number of individuals tagged in each of the three years has varied so widely. The declining sex ratios are of interest, but inconclusive when based on observations alone.

**Table 4: Observations in 2003, Distribution by Tag Year & Gender**

2003 Season Yr Class	Number of Observations				Observation Percentages				Year Classes, Sex Ratios by Tag Year				
	Female	Male	Unk	Total	Female	Male	Unk	Total	Female	Male	Unk	Total	M / F
2001	53	120		173	4.2%	9.6%		13.8%	30.6%	69.4%		100.0%	2.26
2002	42	61		103	3.3%	4.9%		8.2%	40.8%	59.2%		100.0%	1.45
2003	401	577	1	979	32.0%	46.0%	0.1%	78.0%	41.0%	58.9%	0.1%	100.0%	1.44
Total:	496	758	1	1255	39.5%	60.4%	0.1%	100.0%					1.53

Table 5 reports the number of individuals tagged each field season, and the composition of the age classes by gender. The ratio of males per female is shown to have declined steadily, from 2.83 in 2001, to 1.64 in 2002, to 1.58 in 2003. If horseshoe crabs were being fished for bait in Taunton Bay, and females were being taken preferentially as they are elsewhere, these data would suggest that fishing was impacting the population. In the absence of any known fishing of horseshoe crabs in the estuary, the declining sex ratios bear more investigation. It is possible that there is some mechanism related to density, resource richness, or environmental contamination that influences gender determination in this species during development. If so, this steady decline in sex ratios may warn of ongoing disturbance in the ecosystem, or of one that occurred years ago while these age classes were developing.

**Table 5: Seasonal Tabulation of Number of Individuals, Composition by Year Class and Gender**

Year Classes		Number of Individuals Observed				Composition by Year Class & Gender				M / F
		Female	Male	Unk	Total	Female	Male	Unk	Total	Sex Ratio
<b>2001 Season</b>										
New Tagees, 2001	<i>Total</i>	337	953	3	1293	26.1%	73.7%	0.2%	100.0%	2.83
<b>2002 Season</b>										
	TagYear	Female	Male	Unk	Total	Female	Male	Unk	Total	
New Tagees, 2002	2002	236	386	0	622	31.8%	52.1%	0.0%	83.9%	1.64
Returning: 2001	2001	33	86	0	119	4.5%	11.6%	0.0%	16.1%	
	<i>Total</i>	269	472	0	741	36.3%	63.7%	0.0%	100.0%	
<b>2003 Season *</b>										
	TagYear	Female	Male	Unk	Total	Female	Male	Unk	Total	
New Tagees, 2003	2003	278	438	1	717	30.9%	48.7%	0.1%	79.8%	1.58
Returning: 2002	2002	26	41	0	67	2.9%	4.6%	0.0%	7.5%	
Returning: 2001	2001	38	77	0	115	4.2%	8.6%	0.0%	12.8%	
	<i>Total</i>	342	556	1	899	38.0%	61.8%	0.0%	100.0%	

\* In 2003, one escaped untagged, so one record is removed from the tagging statistics.

Another measure of survival is the return rates of individuals as a percentage of their class year. Table 6 shows that 9.2% of 2001 tagees returned in 2002, and 8.7% in 2003; 10.8% of the 2002 tagees returned in 2003. As with the Observations in Table 4, the analysis of the Number of Individuals in Table 5 shows a decline in the ratio of males per female in the tagging year classes. The ratio of the returning males per female is also declining. This may be a reflection on gender-biased survival rates, or it may be that males range more widely and fewer return to the same spawning site.

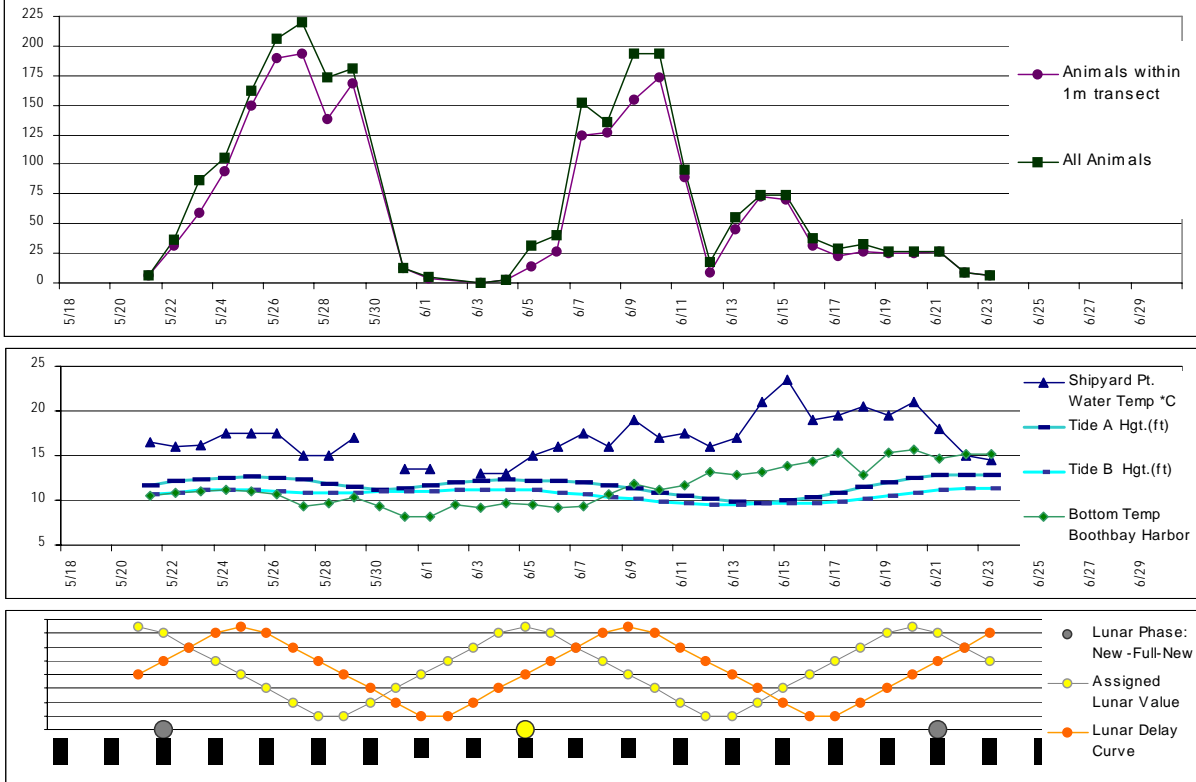
**Table 6: Return Rates Relative to Original Class Size**

Returning Classes	Year Class	Number of Individuals				Percentages of Original Year Class				M / F
		Female	Male	Unk	Total	Female	Male	Unk	Total	Sex Ratio
Tagged in 2001	2001	337	953	3	1293	26.1%	73.7%	0.2%	100.0%	2.83
Returned in 2002	2001	33	86	0	119	2.6%	6.7%	0.0%	9.2%	2.6
Returned in 2003	2001	37	76	0	113	2.9%	5.9%	0.0%	8.7%	2.1
Tagged in 2002	2002	236	386	0	622	37.9%	62.1%	0.0%	100.0%	1.64
Returned in 2003	2002	26	41	0	67	4.2%	6.6%	0.0%	10.8%	1.6
Tagged in 2003	2003	278	438	1	717	38.8%	61.1%	0.1%	100.0%	1.58
Tagged in Taunton Bay		851	1777	4	2632	32.3%	67.5%	0.2%	100.0%	2.09

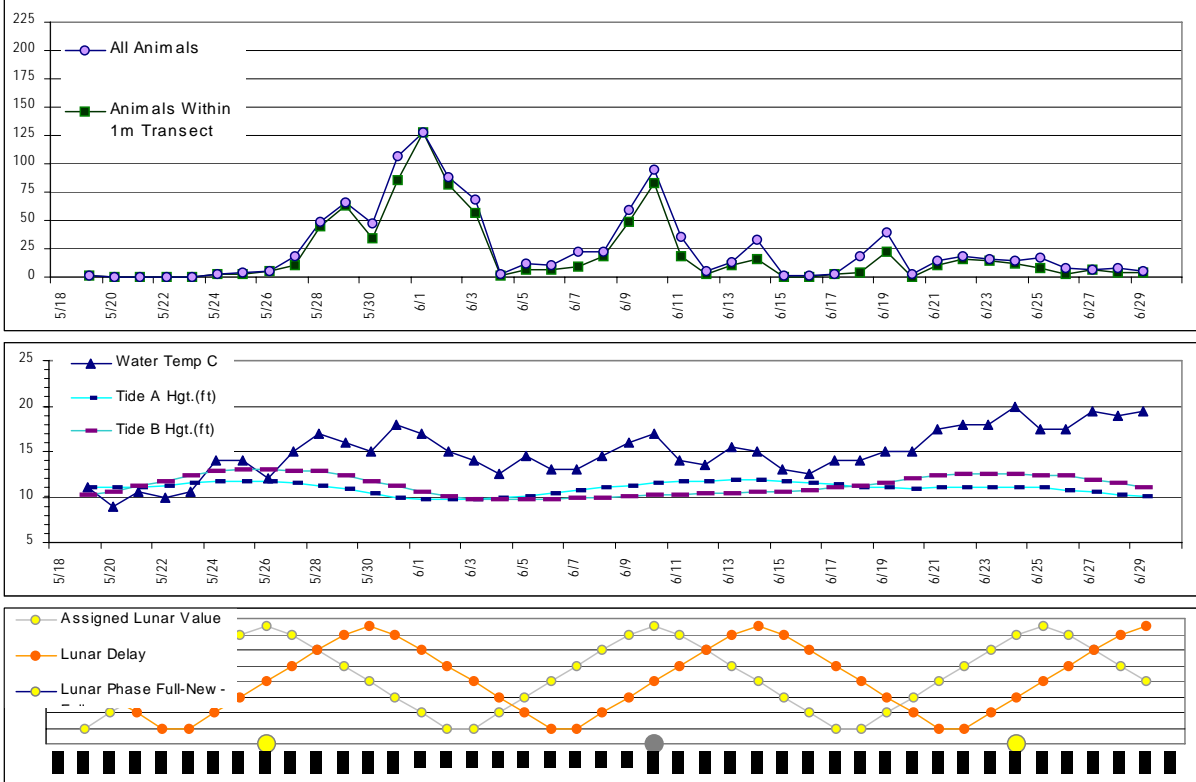
\* In 2003, one escaped untagged, so one record is removed from the tagging statistics.

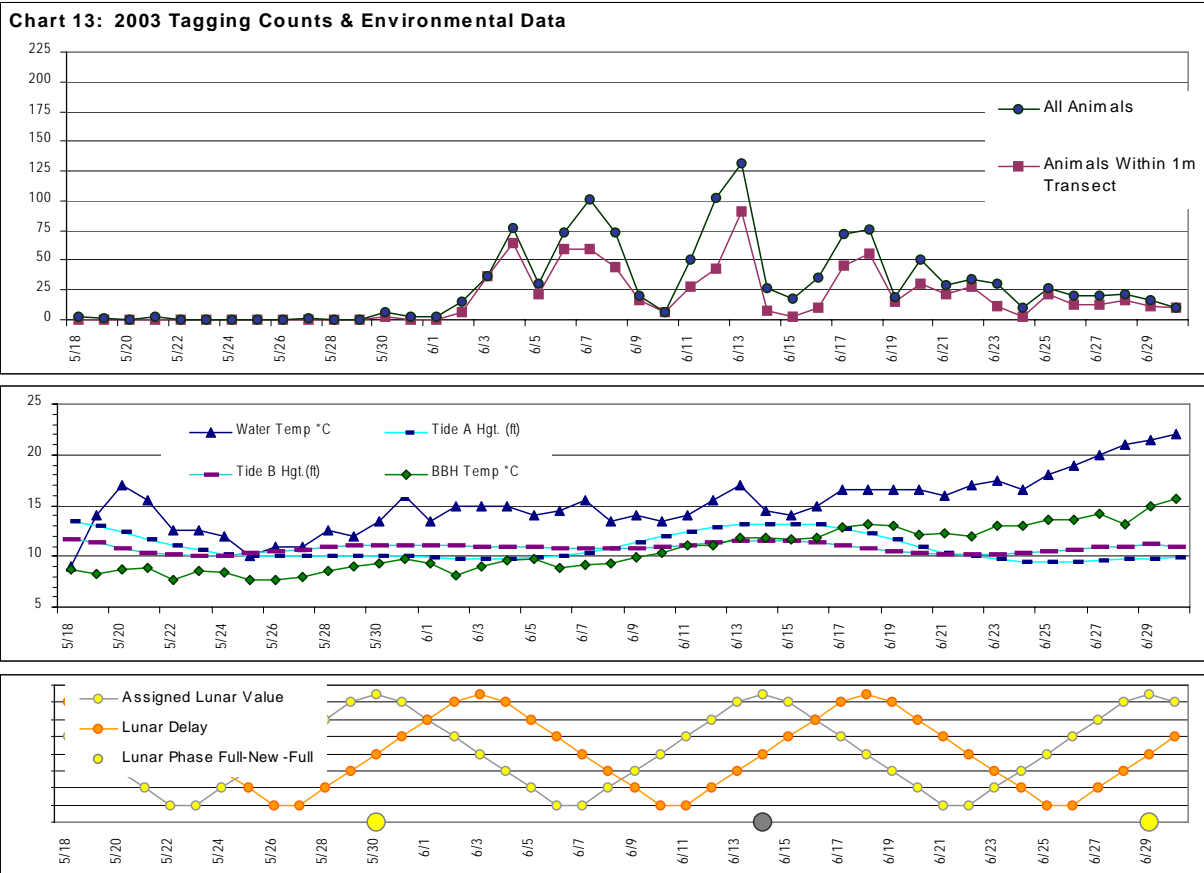
The relationship between peak spawning activity and environmental variables is of ongoing interest because of its usefulness in determining the annual survey dates. The daily counts and the environmental data for 2003 are shown in Chart 13, preceded by plots for 2001 and 2002 in Charts 11 and 12, for comparison. The corresponding data tables are provided in Appendix B. The effective cancellation of the May spawning period due to the cold is obvious. In the absence of this typical start to the season, the usual pattern was thrown off. When spawning did occur, it was subsequent to the lunar phase, it extended through the two-week period to the next lunar phase without an interim period of no-activity, and the peaks of activity more closely followed temperature than any other parameter considered.

**Chart 11: 2001 Tagging Counts & Environmental Data**



**Chart 12: 2002 Tagging Counts and Environmental Data**



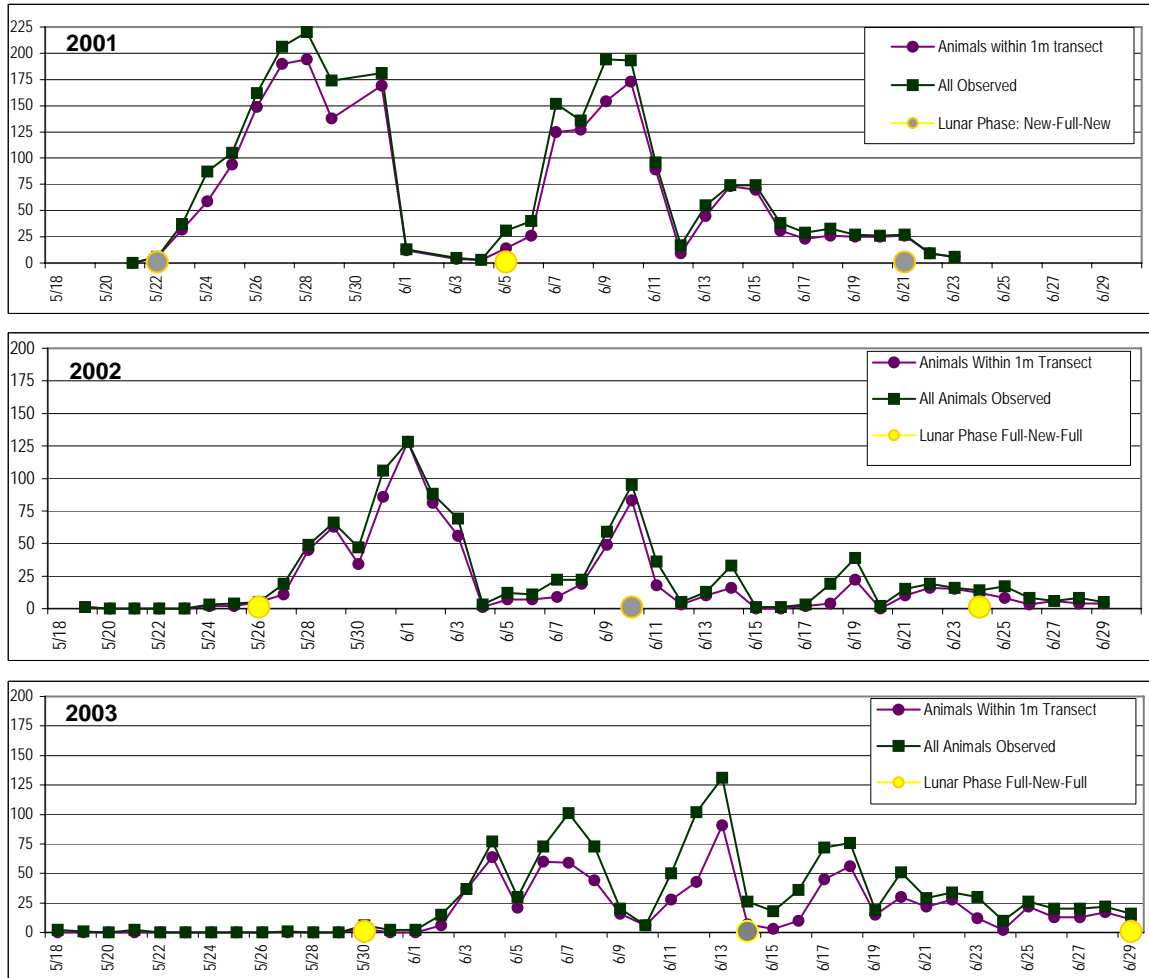


It is important to note that the lunar phases shift from year to year. The 2001 data documented spawning activity associated with two lunar phases. The 2002 data documented activity for most of three phases, and the 2003 data again documented two periods. To see the relationship graphically, the Shipyard Point counts are presented in Chart 14 for all three years with the lunar phases. The lunar phase is arguably the key triggering factor in the initiation of the spring spawning period. The interaction of the shift from year to year may have some influence on other mechanisms associated with spawning activity. Temperature has a role as well, but is not singly influential.

The tagging crew has noticed that days with high counts tend to be bright, sunny days, as well as falling within the other parameters. Horseshoe crabs have been documented as not spawning during periods of foul weather. Stormy weather creates wind and wave conditions in which animals would be jostled and tumbled. Horseshoe crabs have a high degree of sensory capability in vision and light sensing organs, with ten different eyes and light sensors (Barlow et al. 1986). Their ability to detect sunlight, even underwater, may be much greater than we realize. Accordingly, sunlight may contribute to high levels of spawning activity, in combination with other parameters of lunar phase and temperature. Data on light levels and a multivariate analysis would be beneficial, but is beyond the current scope of the project.

Data on bottom temperature could clarify the relationship between temperature and the other parameters. In 2001 it appeared that spawning followed lunar phase, with a delay. In 2002 and 2003 that pattern appeared to hold for the onset of spawning, but high counts more closely following water temperature thereafter. It may be that horseshoe crabs have survived the millennia by being behaviorally flexible in response to shifting environmental conditions.

**Chart 14: Three Years of Spawning Counts and Shifting Lunar Phase**

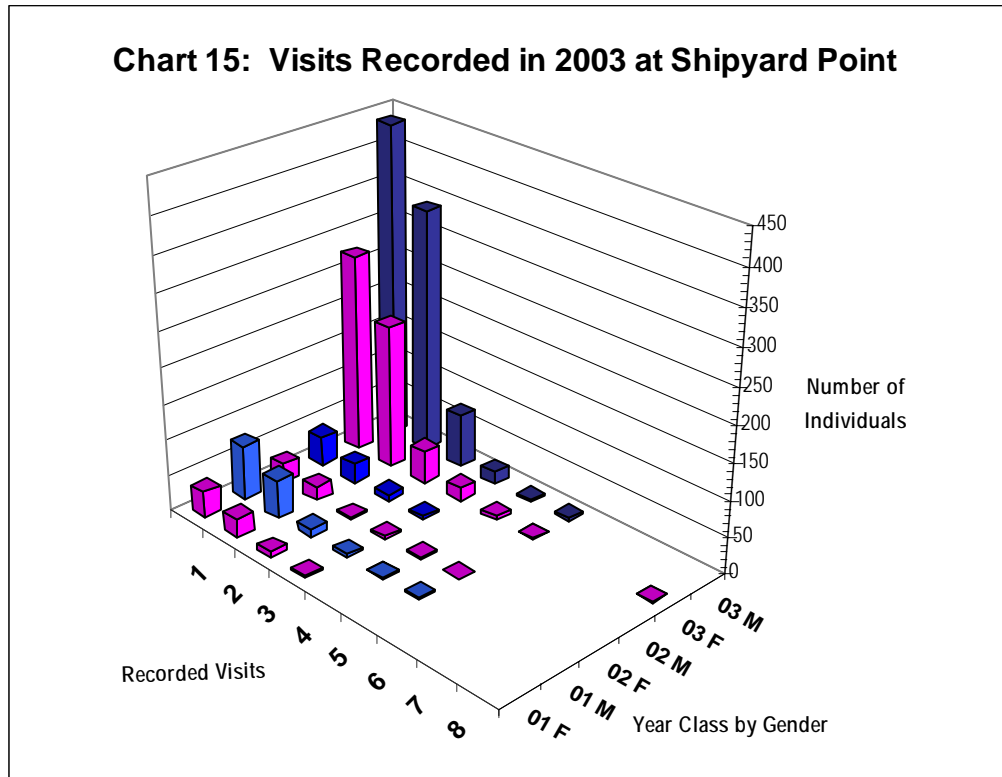


**Visits**

Analysis of the 2003 spawning site visits documented that 246 females made 488 spawning site visits, averaging 1.98 visits each, and 421 males made 745 site visits, averaging 1.77 visits each. By the end of the 2003 season, 2933 individuals had been tagged at Shipyard Point (2033 were tagged in prior years). From the tagging year classes of 2001 and 2002, 282 were documented as returning to Shipyard Point in a subsequent season. Only 15 individuals (1.2% of 1292) of the 2001 year class have returned in both 2002 and 2003; all the others were documented in only one season. Ten of those fifteen were observed only once in each year. This very low return rate indicates that horseshoe crabs do not exhibit spawning site fidelity. It suggests that they choose suitable habitat as they encounter it, or move on to other habitat. The frequency of visits is provided in Chart 15, and is similar to what has been seen in prior years.

Data from a 100m transect on the south shore of Hog Bay documented returns on 46 tagged horseshoe crabs from across the Bay at Shipyard Point. Of these, 24 were tagged in 2001, 15 tagged in 2002, and 7 tagged in 2003. Only 2 of the 2001 tageses and 2 of the 2002 tageses seen on the Hog Bay Road transect in 2003 were also seen at Shipyard Point. The majority of animals seem to have ranged less widely. The fact that 7 (0.7%) of the 2003 tageses were seen on the other side of the Bay in the same season shows that a few tended to be travelers nonetheless.

**Chart 15: Visits Recorded in 2003 at Shipyard Point**



**Conclusion**

Horseshoe crabs in Maine appear to exist in isolated populations. Overall, numbers appear to be gradually eroding, and appear to be doing so in the absence of any significant harvesting by fishermen either for bait or live trade. Only the two largest populations, located in Middle Bay and Thomas Point Beach (Brunswick) appear to have remained stable, based on three years of data. The tagging data from Taunton Bay show that when consistent data collection is applied, many more individual animals are documented than any average single day of data collection would suggest are present. Nonetheless, data for the past three years indicates that this population is declining as well.

Data collected through this project continues to benefit management of horseshoe crabs in Maine. The high variation in counts from season to season justifies continued data collection. So far, the data supports anecdotal information that horseshoe crabs are declining in Maine. The tagging data has been helpful in describing the relationship between the number of observations and the number of individuals observed at the site. Continuation of the tagging study will be useful in documenting longevity of this species in Maine. Many additional questions are raised, including whether the declining sex ratios seen in Taunton Bay are occurring elsewhere, and whether these are related to population density, forage habitat richness, environmental contamination or some other factor.



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Appendix A: Survey Data for 2003, 2002, 2001

2001 Dates	Middle Bay Cove*			Middle Bay Farm**			Stover Point			Mere Point			Thomas Pt Beach		
	Brunswick			Harpwell			Harpwell			Brunswick			Brunswick		
	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total
5/21/01							0	0	0				5	53	58
5/22/01							0	0	0	2	0	2	43	159	202
5/23/01							0	0	0	2	3	4	31	180	211
5/24/01										<u>10</u>	5	15	72	144	216
6/3/01													0	2	2
6/4/01	0	0	0	blocked visibility						0	0	0	3	0	3
6/5/01	19	2	21	<u>14</u>	2	16	0	0	0	1	2	3	2	12	14
6/6/01	56	30	86	blocked visibility						2	2	4	5	14	19
6/7/01	57	33	90	7	3	10				5	0	5	35	66	101
6/8/01	approx 100 total						0	0	0				26	68	94
6/9/01	approx 50 total												<u>103</u>	76	179
6/19/01															
6/20/01															
6/21/01	2	0	2							0	0	0	1	13	14
6/22/01													0	8	8
6/23/01										0	0	0	0	12	12
6/24/01													4	5	9
6/25/01	2	0	2	(data adj to 100 m									0	3	3
6/26/01	0	0	0	from 200 m transect)									3	0	3
Born, 1977:	Breeding Site			Breeding Site			Breeding Site			Breeding Site			Breeding Site (+)		

2001 Dates	Hospital (Day's) Cove			Mills Sewer Filter			Graham Creek			Gt Salt Bay: 3A			Gt Salt Bay: 3B		
	Damariscotta River			Damariscotta River			Damariscotta River			Damariscotta River			Damariscotta River		
	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total
5/21/01	3	3	6	16	19	35	1	0	1				8	7	15
5/22/01	16	12	28	4	8	12	10	0	10				4	2	6
5/23/01	27	3	30	39	66	105	14	0	14				3	0	3
5/24/01															
6/3/01															
6/4/01	8	4	12	16	24	40	24	12	36						
6/5/01	18	10	28	60	43	103	35	0	35						
6/6/01	<u>50</u>	8	58	167	63	230	<u>75</u>	5	80						
6/7/01				192	83	275									
6/8/01				171	37	208									
6/9/01															
6/19/01										0	0	0	1	7	8
6/20/01	17	9	26	42	34	76	56	20	76	0	20	20	<u>21</u>	10	31
6/21/01	16	16	32	49	29	78	40	2	42	<u>8</u>	0	8	6	8	14
6/22/01	11	6	17	0	4	4	43	5	48	5	12	17	3	0	3
Born, 1977:	Breeding Site (+)									Breeding Site (+)			Breeding Site (+)		

2001 Lunar Phases: New: 5/22, Full: 6/5, New: 6/21.

Appendix A (continued)

2001 Dates	Bryant's Beach Damariscotta River			Pleasant Cove Boothbay			Salt Pond Blue Hill			Bagaduce River Sedgwick			Shipyard Point Franklin		
	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total
	5/21/01	combined		34	0	0	0	0	0	0	3	0	3	1.6	0.0
5/22/01	combined		77	0	0	0	<u>13</u>	6	19	7	3	10	8.6	1.4	10.0
5/23/01	combined		87	0	0	0	1	25	26	11	1	12	15.9	8.1	24.1
5/24/01										2	8	10	25.4	3.0	28.4
6/3/01													0.0	0.0	0.0
6/4/01	combined		36	0	0	0	0	0	0	0	0	0	0.8	0.0	0.8
6/5/01	combined		75	0	0	0				2	8	10	3.7	4.5	8.2
6/6/01	combined		78	2	0	2	0	5	5	<u>16</u>	13	29	6.8	3.7	10.5
6/7/01							0	4	4	7	10	17	32.6	6.8	39.5
6/8/01													33.4	2.4	35.8
6/9/01													40.5	10.5	51.1
6/10/01													<u>45.5</u>	5.3	50.8
6/11/01													23.4	1.8	25.3
6/12/01													2.4	2.1	4.5
6/19/01															
6/20/01	23	16	39	<u>2</u>	0	2									
6/21/01	<u>58</u>	27	85	see errata: no data 6/21						0	5	5			
6/22/01	45	20	65							0	2	2			
6/23/01										0	0	0			
6/24/01													(selected dates only)		
6/25/01							0	0	0	2	0	2	(data adj to 100 m from 380 m transect)		
6/26/01															

Born,1977:

Breeding Site

Breeding Site (+)

Breeding Site

2001 Lunar Phases: New: 5/22, Full: 6/5, New: 6/21.

Appendix A continued: Survey Data for 2003, 2002, 2001

2002 1x100m	Middle Bay		Thomas Pt. Be.		Bailey Cove		Days Cove		Damar. Mills		Bagaduce R.		Taunton Bay*	
	Temp	Count	Temp	Count	Temp	Count	Temp	Count	Temp	Count	Temp	Count	Temp	Count
5/15/02									12.0	0				
5/16/02									14.0	0				
5/17/02									14.0	2				
5/18/02										0				
5/19/02									13.0	4				0
5/20/02									7.0	0				0
5/21/02									12.0	0				0
5/22/02									14.0	0				0
5/23/02		0							16.0	0				0
5/24/02		32							18.0	14			14.0	1
5/25/02		38							16.0	37			14.0	1
5/26/02		46	12.0	2	12.0	0	13.0	0	15.0	2	12.5	0	12.0	1
5/27/02	99		16.0	335	13.4	6	13		19.0	2	15.0	4	15.0	3
5/28/02	80		17.0	465	14.0	5	23		20.0	23	15.0	11	17.0	12
5/29/02	80		18.0	703	15.3	6	18.0	23	20.5	70	16.5	16	16.0	17
5/30/02	103		18.0	327	14.9	2	17.5	17	19.0	82	15.0	9	15.0	9
5/31/02	60		20.0	35	16.2	15	102		20.5	98	16.1	10	18.0	23
6/1/02	10				17.3	13			20.0	99			17.0	34
6/2/02	16								20.0	54			15.0	21
6/3/02	0								17.0	10			14.0	15
6/4/02									20.5	12				0
6/5/02									17.0	18				2
6/6/02									17.0	23				2
6/7/02									16.5	26				2
6/8/02									21.0	42				5
6/9/02					16.0	0			20.0	43				13
6/10/02			17.0	8	16.0	2	18.0	35	21.0	143	15.0	16		22
6/11/02			15.0	6		0	16.0	11	17.0	14	14.0	10		5
6/12/02	34		13.0	3			15.0	3	15.0	3	12.0	1		1
6/13/02	0		16.0	12			15.5	26	19.0	19	14.0	8		3
6/14/02	42		16.0	5			15.5	5	17.0	1	14.0	5		4
6/15/02	2						14.0	7	14.0	3				0
6/16/02														0
6/17/02	13													1
6/18/02	10													1
6/19/02														6
6/20/02														0
6/21/02														3
6/22/02														4
6/23/02														4
6/24/02														3
6/25/02														2
6/26/02														1
6/27/02														2
6/28/02														1
6/29/02														1

\* Taunton Bay data calculated for 1x100m transect from 1x380m transect. 2002 Lunar Phases: Full: 5/26, New: 6/10, Full: 6/24.

Appendix A continued: Survey Data for 2003, 2002, 2001

2003 1x100m	Cousins Isl.		Middle Bay*		Thomas Pt.Bch.		Eaton Farm		Days Cove		Damar.Mills		Bagaduce River*		TB: Shipyd Pt*		TB: So BayRd*	
	Temp	In	Temp	In	Temp	In	Temp	In	Temp	In	Temp	In	Temp	In	Temp	In	Temp	In
5/18/03	15.0	0	20.0	0	12.0	6	12.1	0	16.0	0	21.0	0	12.0	0	9.0	0		
5/19/03	12.0	0	19.0	3	16.5	27	11.9	0	18.0	0	18.0	6	13.0	0	14.0	0		
5/20/03	13.0	0	22.0	5	17.0	108	12.9	0	18.0	0	20.0	15	9.0	0	17.0	0		
5/21/03	13.0	0	18.0	7	14.0	12	12.5	0	17.5	0	19.0	6	8.0	0	15.5	0		
5/22/03	13.0	0		2	14.0	130	12.9	0	17.0	0	17.0	0	8.0	0	12.5	0		
5/23/03	11.0	0	13.5	0	11.0	1	12.8	0	13.0	1	16.0	1	7.0	0	12.5	0		
5/24/03	10.0	0	11.5	0	11.0	0	12.9	0	12.0	0	13.0	0			11.0	0		
5/25/03	10.5	0							12.0	0	12.0	0			10.5	0		
5/26/03	11.0	0							13.0	0	14.0	0			11.5	0	12.0	0
5/27/03															11.0	0	12.0	0
5/28/03															12.0	0	12.5	0
5/29/03															12.0	0	13.0	2
5/30/03															14.0	1	15.0	3
5/31/03															14.5	0	15.5	0
6/1/03	13.0	0	15.5	0	14.0	20	14	0	17.0	8	17.0	11	9.0	0	13.5	0	14.5	0
6/2/03	15.5	1	19.0	26	14.0	286	13.7	0	15.0	19	17.0	78	9.5	0	14.5	2	16.0	0
6/3/03	15.0	0	23.0	13	14.0	352	15.5	0	18.0	14	21.0	82	10.0	0	14.5	10	16.5	0
6/4/03	12.5	0	21.5	39	17.0	722	14.4	6	14.0	16	20.0	0	10.0	8	15.0	17	16.0	12
6/5/03	13.5	0	16.0	11	13.5	191	14.6	2	15.0	8	16.0	4	10.0	0	14.0	6	14.0	0
6/6/03	23.5	2	16.0	4	16.0	683	15.8	9	20.0	3	20.0	45	11.0	3	14.5	16	18.0	4
6/7/03	15.0	0	19.5	8	16.0	290	14.4	6	18.0	3	18.0	6	14.0	12	15.5	16	16.0	16
6/8/03	14.0	0	18.0	1	15.5	10			18.0	0	18.0	1	9.0	0	13.5	12	14.0	0
6/9/03	13.0	0			15.0	22			18.0	1			13.0	0	13.0	4		
6/10/03															13.5	2	14.0	0
6/11/03															14.0	7	16.0	2
6/12/03															15.5	11	18.5	6
6/13/03															17.0	24	18.0	15
6/14/03				1											14.5	2		
6/15/03															14.0	1		
6/16/03											21.0	20			15.0	3		
6/17/03				15							22.0	98			16.5	12	17.0	28
6/18/03											23.0	11			16.5	15		13
6/19/03				2							25.0	6			16.5	4	17.5	13
6/20/03				4							24.5	3			16.5	8	18.5	6
6/21/03															18.0	6	19.0	4
6/22/03															17.0	7	18.0	1
6/23/03															17.5	3	17.0	5
6/24/03															16.5	1	18.0	2
6/25/03															18.0	6	20.0	5
6/26/03															19.0	3	22.0	0
6/27/03															20.0	3	23.0	10
6/28/03															21.0	4		
6/29/03															21.5	3		
6/30/03															22.0	3		

\* Denotes sites with transects longer than 100m; data recalculated to be comparable. Moons: Full 5/30, New: 6/14, Full: 6/29.

Appendix B: Data Tables for Taunton Bay Tagging Transect, 380m.

2001 Data	Transect 380x1m	Total Observed	Shipyard Pt H2O °C	Salinity ppt	Tide A Hgt. (Ft)	Tide B High Tide	Temp. BBH	Lunar Phase
5/21/01	6	6	16.5	26.9	11.7	10.7	10.47	
5/22/01	32	37	16.0	29.7	12.1	10.9	10.82	New
5/23/01	59	87	16.2	30.0	12.4	11.1	11.03	
5/24/01	94	105	17.5	31.0	11.1		11.13	
5/25/01	149	162	17.5	30.8	12.6	11.1	11.00	
5/26/01	190	206	17.5	31.8	12.5	11.0	10.71	
5/27/01	191	217	15.0	32.3	12.3	10.9	9.36	
5/28/01	138	174	15.0	31.2	11.9	10.8	9.72	
5/29/01	169	181	17.0		11.5	10.8	10.40	
5/30/01		no data			11.2	11.0	9.30	
5/31/01	12	13	13.5	29.5	11.3	11.0	8.09	
6/1/01	4	5	13.5	28.2	11.7	11.0	8.14	
6/2/01		no data			12.0	11.1	9.51	
6/3/01	0	0	13.0	29.4	12.2	11.2	9.22	
6/4/01	3	3	13.0	30.9	12.3	11.2	9.59	
6/5/01	14	31	15.0	28.7	12.2	11.1	9.44	Full
6/6/01	26	40	16.0	29.0	10.9		9.14	
6/7/01	124	150	17.5	29.4	12.0	10.7	9.32	
6/8/01	127	136	16.0	30.6	11.6	10.4	10.70	
6/9/01	154	194	19.0	29.0	11.3	10.1	11.80	
6/10/01	173	193	17.0	30.6	10.9	9.8	11.23	
6/11/01	89	96	17.5	30.1	10.5	9.6	11.67	
6/12/01	9	17	16.0	28.6	10.1	9.5	13.09	
6/13/01	45	55	17.0	34.4	9.8	9.5	12.90	
6/14/01	73	74	21.0	30.4	9.7	9.7	13.19	
6/15/01	70	74	23.5	29.2	10.0	9.6	13.87	
6/16/01	31	38	19.0	30.7	10.4	9.7	14.31	
6/17/01	23	29	19.5	30.3	10.9	9.9	15.35	
6/18/01	26	33	20.5	28.9	11.5	10.2	12.90	
6/19/01	25	27	19.5	28.5	12.0	10.5	15.40	
6/20/01	25	26	21.0	28.6	12.5	10.8	15.70	
6/21/01	26	27	18.0		12.8	11.1	14.70	New
6/22/01	9	9	15.0	28.5	11.3		15.10	
6/23/01	6	6	14.5	28.4	12.9	11.4	15.20	
Total Obs:	2122	2451						
Survey Dates (Counts): May 21st to June 23rd (less 2 days). Tagging: May 22nd to June 17th (less 2 days).								
Total Observed includes observations within the 380x1m transect, and beyond the transect.								
Tide height and lunar phase downloaded 3.7.01 from: <a href="http://www.maineharbors.com/maybar01.htm">www.maineharbors.com/maybar01.htm</a> and <a href="http://www.maineharbors.com/junbar01.htm">/junbar01</a> , based on Bar Harbor.								
Temp. BBH = Bottom temperatures at Boothbay Harbor, degrees Celsius.								
Lunar Value is a number assigned to graphically display changes in the lunar phase.								

Appendix B continued: Data Tables for Taunton Bay Tagging Transect, 380m.

2002 Data	380m x 0-1m	All Observed	Shipyard Pt. H2O * C.	Salinity (ppt)	Tide Hgt1(ft)	Tide Hgt 2 (ft)	Lunar Phase
5/19/02	1	1	11.0		11.1	10.2	
5/20/02	0	0	9.0		11.0	10.6	
5/21/02	0	0	10.5		11.0	11.2	
5/22/02	0	0	10.0		11.2	11.8	
5/23/02	0	0	10.5		11.5	12.4	
5/24/02	2	3	14.0	28.0	11.7	12.9	
5/25/02	2	4	14.0	29.5	11.8	13.1	
5/26/02	5	5	12.0	26.5	11.8	13.1	Full
5/27/02	11	19	15.0	28.5		11.6	
5/28/02	45	49	17.0	30.3	12.8	11.3	
5/29/02	63	66	16.0	30.0	12.4	10.9	
5/30/02	34	47	15.0	32.0	11.8	10.4	
5/31/02	86	106	18.0	30.0	11.2	10.0	
6/1/02	128	128	17.0	31.0	10.6	9.8	
6/2/02	81	88	15.0	30.0	10.1	9.7	
6/3/02	56	69	14.0	30.0	9.8	9.7	
6/4/02	1	3	12.5	30.0	9.7	9.9	
6/5/02	7	12	14.5	31.0	9.7	10.1	
6/6/02	7	11	13.0	32.0	9.8	10.4	
6/7/02	9	22	13.0	31.0	9.9	10.7	
6/8/02	19	22	14.5	31.0	10.0	11.0	
6/9/02	49	59	16.0	31.0	10.1	11.3	
6/10/02	83	95	17.0	32.0	10.2	11.5	New
6/11/02	18	36	14.0	32.0	10.3	11.7	
6/12/02	3	5	13.5	32.0	10.4	10.4	
6/13/02	10	13	15.5	32.0	11.9	10.4	
6/14/02	16	33	15.0	32.0	11.9	10.5	
6/15/02	0	1	13.0	29.0	11.8	10.6	
6/16/02	0	1	12.5	25.0	11.6	10.8	
6/17/02	2	3	14.0	26.0	11.4	11.0	
6/18/02	4	19	14.0	26.0	11.1	11.3	
6/19/02	22	39	15.0	26.0	11.0	11.6	
6/20/02	0	2	15.0	26.0	10.9	12.0	
6/21/02	10	15	17.5	25.0	11.0	12.3	
6/22/02	16	19	18.0	27.0	11.1	12.6	
6/23/02	15	16	18.0	27.0	11.1	12.6	
6/24/02	12	14	20.0	25.0	11.1	12.5	Full
6/25/02	8	17	17.5	27.0	11.0	11.0	
6/26/02	3	8	17.5	28.0	12.3	10.8	
6/27/02	6	6	19.5	27.0	11.9	10.6	
6/28/02	4	7	19.0	27.0	11.5	10.3	
6/29/02	4	5	19.5	28.0	11.0	10.1	
Counts	842	1068	On June 2-3rd, both tides occurred during daylight, and				
6/2 am	41	48	surveys were conducted on both tides; only the pm data is				
6/3 am	3	3	included in the analysis of the counts. Data from both tides				
Observed	886	1119	is included in the tagging analysis.				

Appendix B continued: Data Tables for Taunton Bay Tagging Transect, 380m.

2003 Data	Start Time	am/pm	Count: In 0-1m	All Observed	Water Temp C.	Tide Height Tide A (ft)	Tide Height Tide B (ft)	Boothbay Hbr bottom temp	Lunar Phase
5/18/03	12:45	pm	0	2	9.0	13.4	11.7	8.7	
5/19/03	14:50	pm	0	1	14.0	13.0	11.3	8.3	
5/20/03	15:40	pm	0	0	17.0	12.4	10.8	8.8	
5/21/03	16:35	pm	0	2	15.5	11.7	10.4	8.9	
5/22/03	17:45	pm	0	0	12.5	11.1	10.2	7.7	
5/23/03	18:40	pm	0	0	12.5	10.6	10.1	8.5	
5/24/03	19:40	pm	0	0	12.0	10.2	10.1	8.4	
5/25/03	8:00	am	0	0	10.0	10.1	10.3	7.7	
5/26/03	9:00	am	0	0	11.0	10.0	10.5	7.6	
5/27/03	9:45	am	0	1	11.0	10.0	10.7	8.0	
5/28/03	10:30	am	0	0	12.5	10.0	10.9	8.6	
5/29/03	11:15	am	0	0	12.0	10.1	11.1	9.0	
5/30/03	11:50	am	3	6	13.5	10.0	11.1	9.4	New
5/31/03	12:30	pm	0	2	16.0	10.0	10.0	9.7	
6/1/03	13:10	pm	0	2	13.5	11.1	9.9	9.2	
6/2/03	13:40	pm	6	15	15.0	11.1	9.8	8.0	
6/3/03	14:20	pm	37	37	15.0	11.0	9.8	9.0	
6/4/03	15:15	pm	64	77	15.0	11.1	9.8	9.7	
6/5/03	15:50	pm	21	30	14.0	10.9	9.8	9.7	
6/6/03	16:50	pm	60	73	14.5	10.8	10.0	8.8	
6/7/03	17:30	pm	59	101	15.5	10.8	10.3	9.2	
6/8/03	18:45	pm	44	73	13.5	10.8	10.8	9.3	
6/9/03	19:30	pm	16	20	14.0	10.8	11.3	9.8	
6/10/03	7:55	am	6	6	13.5	10.9	11.9	10.3	
6/11/03	8:50	am	28	50	14.0	11.1	12.4	11.1	
6/12/03	9:55	am	43	102	15.5	11.3	12.9	11.1	
6/13/03	10:55	am	91	131	17.0	11.5	13.2	11.8	
6/14/03	11:50	am	7	25	14.5	11.5	13.2	11.8	Full
6/15/03	12:50	pm	3	18	14.0	11.5	11.5	11.7	
6/16/03	13:35	pm	10	35	15.0	13.1	11.3	11.8	
6/17/03	14:27	pm	45	72	16.5	12.7	11.1	12.9	
6/18/03	15:26	pm	56	76	16.5	12.2	10.8	13.2	
6/19/03	16:25	pm	15	19	16.5	11.6	10.5	13.0	
6/20/03	17:09	pm	30	50	16.5	11.0	10.3	12.1	
6/21/03	18:13	pm	22	29	16.0	10.4	10.2	12.3	
6/22/03	19:10	pm	28	34	17.0	10.0	10.2	11.9	
6/23/03	19:50	pm	12	30	17.5	9.7	10.2	12.9	
6/24/03	8:14	am	2	10	16.5	9.5	10.3	13.1	
6/25/03	9:10	am	23	26	18.0	9.4	10.5	13.6	
6/26/03	10:07	am	13	20	19.0	9.5	10.7	13.7	
6/27/03	10:42	am	13	20	20.0	9.6	10.9	14.2	
6/28/03	11:30	am	17	22	21.0	9.7	11.0	13.1	
6/29/03	12:10	am	11	16	21.5	9.8	11.2	14.9	New
6/30/03	12:49	am	10	10	22.0	9.9	9.9	15.6	
Dawn counts removed, to present only 1 count per day:					6/9/03	7:05	am	1	2
5/23/03	18:00	am	0	0	6/21/03	5:26	am	2	4
5/24/03	7:30	am	0	0	6/22/03	6:26	am	4	4
6/8/03	5:50	am	3	4	6/23/03	7:20	am	0	0

Counts were conducted on May 18-19th, without tagging. They are removed from tagging data and included in graphed counts.



## **Appendix C: Ancillary Issues in Project Management**

The use of marking techniques to study fish and wildlife routinely raises standard questions regarding animal welfare. The optimal marking technique should meet as many of the following criteria as possible: it should be minimally stressful and minimally painful to the study animals, should not produce adverse effects on the animals' behavior or survival, should be durable and should stay with the animal, be easily recognizable, easily obtained and applied, and relatively inexpensive (Nietfield et al. 1994). For horseshoe crabs, because they grow by molting, the decision was made for this study to select a marker (tag), which could be shed with the old shell. The question of whether or not adult horseshoe crabs molt has not been answered yet, but the consensus is that adult molting occurs infrequently if at all (Shuster 1990).

The tags used in this project would meet all of the optimal criteria, if there were a way to assure that they would stay with the animal through molting. The tags are thin, light in weight, and flexible. They are attached through the shell, near the edge of the prosoma. The location near the edge of the shell avoids interference with muscle or organ tissue. While no molted shells with attached tags have yet been found, one animal was found with a notch in the edge of the genal angle, suggesting that it had been previously tagged and had molted out of the tagged shell. This was a 20.5cm female, and it is reasonable to conclude that she had molted to have reached that size, as compared to the more usual 16-19cm range of prosomal width. Female horseshoe crabs larger than 20cm prosomal widths are uncommon in Maine. (Schaller et al., 2002).

Horseshoe crabs are often found with traumatic damage to their shells. Broken telsons are common; missing legs and holes in the shells are also seen on a regular basis. Occasionally individuals are found which have survived injuries that clearly might have been fatal. One example, was an individual with a large section torn from its prosoma, including the whole genal angle, forming a missing triangle of two inches per side.

Another example, is the shell of a female that had been predated by gulls. Only the dorsal portion of the prosoma remained, but a few green eggs remained dried on the inside of the shell indicating this had been a fertile female when it died. Most remarkable was a 2.5cm hole through the shell, midway up from the edge of the prosoma to the orbital ridge. Smooth edges around this hole and a keyhole-shaped opening to the edge of the indicated that the injury had healed cleanly. It appears that either the original injury was larger, and the shell healed around it, or that the animal molted sometime after being injured.

Looking at the kinds of injuries that horseshoe crabs experience as bottom dwellers, it seems reasonable to suggest that level of disturbance caused by tags in this study is analogous to a person of getting an ear pierced (once). The portion of the tag which anchors it to the shell is relatively small, and moves freely, flexing easily. The hole through which it attaches is barely large enough to insert the tagging device.

Other types of tags have been used during this project when the supply of numbered tags was temporarily exhausted. During the 2001 season, many more horseshoe crabs showed up at the tagging site than were anticipated. The supply of numbered tags was exhausted within the first week, and a rush order was placed for additional tags. For several days, tags were made of a variety of materials until more numbered tags arrived. The substitute tags were attached in the same location, and are referred to as day tags because a different color tag was used for each of 5 days. Initially, animals were measured, identified by gender, and a wire twist tie was attached. On successive days, plastic cable ties were used—short (1”) white cable ties, short black (1”) cable ties, long white (2”) and long black (2”) cable ties. As animals with day tags returned on successive days, they were given numbered tags, and the numbered record was associated with an original record based on gender and prosomal width. Tag retention in all cases was good. Only one tag is known to have been lost, and it occurred during the first year with a numbered tag that appeared not to have been checked before releasing the animal.

During 2002, more horseshoe crabs with day tags returned and were given numbered tags. Only one animal appeared at the tagging site during 2002 with an empty tagging hole. During 2003, twelve horseshoe crabs appeared at the tagging site with empty tag holes. These were attributed to animals that had been tagged with twist ties—the kind that come with some brands of plastic food storage bags. Some of the twist-ties used were plastic-covered wire, and a number of these were recovered intact and still attached in 2003. The ones that were not plastic-covered presumably rusted out. Twenty-eight records remain unassigned from animals that received twist-ties as temporary tags. If more than twenty-eight animals return in the future with empty holes, it would be attributed to tag loss versus twist-ties that had deteriorated to rust. A total of 127 day tag records remain unassigned from 2001.

Other exceptions that came up during the first season of tagging is that there were two peak days on which many more animals showed up on the transect than previously and handling was slowed by the varying degrees of facility among volunteers. When picked up, horseshoe crabs typically fold into a defensive position to minimize the amount of exposed underbelly. They often flail with their tail (telson), which may be a response to being upside down, and an effort to flip right-side-up. Some volunteers adapt more easily than others to handling and processing the animals.

Tagging and measuring were slowed on some peak days in 2001 by having over 200 animals instead of the more typical 50-100, in combination with handling anxiety by the volunteers, and a succession new helpers. The result was that progress along the transect sometimes slowed depending on the counts and the composition of the day's field crew. As progress slowed, it became increasingly important to complete the transect before the tide ebbed. In rare instances, to expedite processing groups of 3 animals were prepared for tagging (drilled) and then the drill bit was sanitized before the next 3 animals, for an estimated maximum of 30 animals over the entire 2001 season. Handling times have improved in successive seasons, and an experienced core tagging team has been emerged.

Beginning in 2003, tagged animals were measured each time they were captured. Although this is redundant, it simplified the handling routine, and streamlined the process on busy days. This resulted in prosomal width being measured and recorded more than once for animals with repeat visits. Sometimes the results were identical time after time, and sometimes the measurements were slightly different. Variation occurs because the animals are often trying to escape while being measured, and they need to be pressed gently against the fishboard so that the prosomal edges are in contact with the underlying ruler. Not pressing firmly enough can result in a measurement that is slightly too small. Conversely, pressing too firmly will flex the shell slightly and generate a larger measurement. Consistency improves with repetition, and development of a small group of experienced and dedicated volunteers to staff the tagging crew has been key to the continued success of this project.

