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NOTE

Distribution of juvenile American horseshoe crabs *Limulus polyphemus* in the Great Bay Estuary, New Hampshire, USA

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ABSTRACT: In order to effectively manage a fishery, it is important to know the essential habitats used by all life history stages of the species being managed. However, this is often a challenge for animals such as the American horseshoe crab *Limulus polyphemus*, whose juvenile stages are difficult to find and quantify. Visual surveys and suction sampling have been used in previous studies, but in the Great Bay Estuary, New Hampshire, they are less effective, and so little is known about the habitats occupied by juvenile horseshoe crabs in this area. To fill this knowledge gap, we used SCUBA-dive surveys to locate juvenile horseshoe crabs. We found them on subtidal and some intertidal mudflats in the upper regions of the estuary, both adjacent to, and at some distance from, known spawning beaches. However we did not find them in either intertidal or subtidal areas adjacent to spawning beaches just 1–2 km closer to the coast. These data should be useful for future conservation efforts designed to protect essential horseshoe crab habitats in the Great Bay and other similar estuaries.

KEY WORDS: *Limulus polyphemus* · American horseshoe crab · Juveniles · Essential habitats · Estuary

1. INTRODUCTION

The quality and quantity of the habitats occupied by marine organisms have direct effects on the size and resiliency of their populations. Nursery habitats are generally characterized by high food availability and a low risk of predation, and are often adjacent to adult habitats to facilitate the transition from juveniles to adults (Beck et al. 2001). However, while mapping the distribution of most adult fish or shellfish species is fairly straightforward, it is often a challenge for juvenile life stages because they tend to be small and difficult to find or catch (Beck et al. 2001). American horseshoe crabs *Limulus polyphemus* are a good example of a commercially important species that is easy to quantify as an adult, but difficult as a juvenile. During the spawning season, horseshoe crabs appear at specific beaches at high tide to mate, and so it is relatively easy to observe and quantify both their abundance and egg densities (Carmichael et al. 2003, James-Pirri et al. 2005, Botton et al. 2018). In fact, since 1990, annual horseshoe crab spawning surveys have been conducted to track the abundance of breeding adults in order to inform management (Shuster 2009).

In contrast to adults, much less is known about juvenile horseshoe crabs because they are small, cryptic, and often buried in intertidal and shallow subtidal sediments (Rudloe 1981, Botton et al. 2010). Methods used to study and count juveniles, such as handpicking, shoveling, and sieving at low tide, are only possible in areas with firm sand and clear water (Rudloe 1981, Botton et al. 2003, Carmichael et al.

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2003). Traditional trawls and suction-dredge trawls have been successful in deeper water (Burton et al. 2009), but are only possible in some areas and can be somewhat destructive and underestimate densities. As a result of these limitations, the distribution of *Limulus* nursery habitats is not well defined in many areas, which prompted the Atlantic States Marine Fisheries Commission (ASMFC) to state that there is a need to 'identify juvenile horseshoe crab habitats throughout its range and document extent of use' (ASMFC 2015).

Previous field studies documenting juvenile American horseshoe crab habitats have been focused primarily in Florida, Delaware–New Jersey, and Massachusetts, USA (Rudloe 1981, Gaines et al. 2002, Botton et al. 2003, Carmichael et al. 2003, 2004, Burton et al. 2009). The consensus from these studies is that juvenile horseshoe crabs are found in the sandy intertidal zone adjacent to spawning beaches. However, a few studies, using different methods, have also found them in the subtidal zone in Delaware Bay (Burton et al. 2009).

The Great Bay Estuary (GBE), New Hampshire, is located near the most northern part of the American horseshoe crab's geographic range (National Marine Fisheries Service 2010). As with many estuarine systems, the extensive inter- and sub-tidal mudflats of the GBE are potentially suitable habitats for developing horseshoe crabs because they are rich in sedimentary organic matter, benthic algae, and meiofauna prey that can support their shifting diets as they develop (Gaines et al. 2002, Carmichael et al. 2004, Lee 2010). Nevertheless, juveniles have not been documented in the GBE, most likely because of the difficulty conducting surveys walking on mudflats with very soft sediments and turbid waters. In fact, in visual shoreline surveys from 2006–2010, no juvenile horseshoe crabs were sighted (NHFG 2010).

In this study, we used a SCUBA-diving method which allowed us to swim over the intertidal mudflats at high tide without disturbing the sediment and also survey in deeper subtidal areas that are otherwise not accessible. This approach was very effective and enabled us, for the first time, to document the habitats occupied by juvenile horseshoe crabs in the GBE.

2. MATERIALS AND METHODS

2.1. Survey sites

In total, 7 locations within the GBE, New Hampshire, were chosen as survey sites. These sites were distributed throughout the 2 main areas of the GBE: Little Bay and Great Bay (see Fig. 1, Table 1). Three sites were located in Little Bay and 4 in Great Bay. Some sites were adjacent to known horseshoe crab spawning beaches (Cheng et al. 2016), while others were in deeper water at least 0.5 km from spawning beaches. Additionally, some areas that were surveyed were exposed at low tide (intertidal) and others were always submerged (subtidal).

2.2. SCUBA-dive surveys

SCUBA-dive surveys were conducted from July to October 2012 and 2013, during daytime slack-high tides when juvenile horseshoe crabs have been shown to be most active (Dubofsky et al. 2013), and when the intertidal mudflats are submerged. Depths of the study sites ranged from 2–5 m at high tide and visibility was typically limited to ~1–2 m (see Fig. 2, Video 1 in the Supplement at www.int-res.com/articles/suppl/m662p199_supp/). During surveys, 2 divers swam parallel to each other while holding a

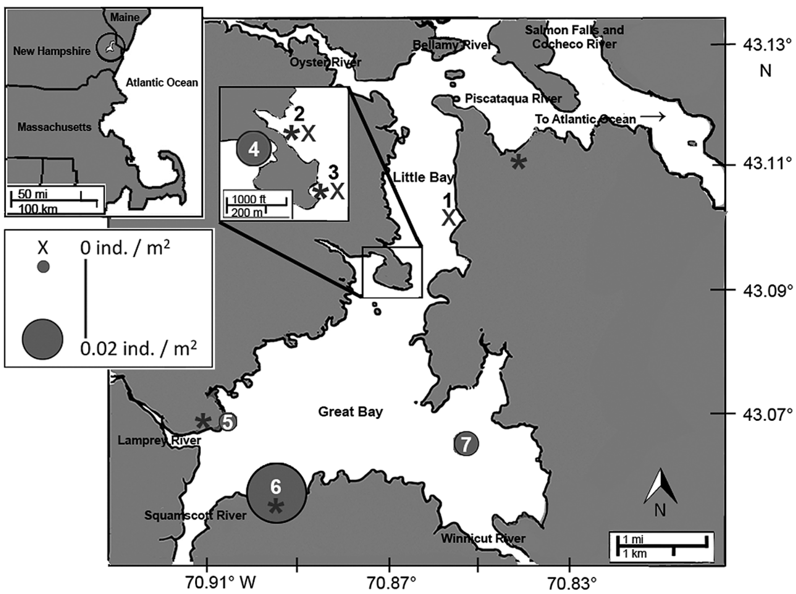

Fig. 1. Study location in the Great Bay Estuary, NH, USA. Numbers indicate where SCUBA-dive surveys were done (see Table 1). Graduated circles show juvenile horseshoe crab abundances (ind. m^{-2}); 'X' indicates no horseshoe crabs were found at that site; asterisks indicate locations where horseshoe crab spawning surveys were conducted by Cheng et al. (2016)

Table 1. Juvenile horseshoe crab survey site locations and characteristics. Surveys <0.1 km from a known spawning beach, based on Cheng et al. (2016), are indicated with Y (yes). The table also designates whether dives were conducted subtidally, in the intertidal, or both, as well as the number of surveys conducted at each survey site, and whether juvenile horseshoe crabs were found at the site

Site no.	Site name	Location	Near known spawning sites?	Survey area (m^2)	Subtidal or both?	No. of surveys conducted	Juveniles found?
1	White Wall	Little Bay	N	1180	Subtidal	3	N
2	Boat Launch	Little Bay	Y	1570	Both	4	N
3	JEL Cove	Little Bay	Y	540	Both	3	N
4	Adams Point Cove	Great Bay	N	740	Subtidal	9	Y
5	Moody Point	Great Bay	Y	1030	Subtidal	3	Y
6	Sandy Point	Great Bay	Y	440	Both	4	Y
7	Portsmouth Country Club	Great Bay	N	530	Subtidal	3	Y

1 m long rope that was kept taught between them. Due to limited visibility, each diver examined a 1 m area, yielding a 2 m wide transect for the pair of divers. Each diver was equipped with a catch bag to collect horseshoe crabs for subsequent examination.

A person positioned at the surface recorded the GPS coordinates of where the dive started and estimated and recorded the path taken based on divers' surface bubbles and landmarks. This path was then mapped with Google Earth and the length of the transect was determined using Google Earth tools. Transect areas (the transect length multiplied by 2 m) for each site ranged from ~550–1500 m^2 (see Table 1).

2.3. Data analyses

Live horseshoe crabs that were ≤125 mm in prosomal width (PW), light in color, and showed no signs of wear, bio-fouling, or mating scars were considered as juveniles (see Fig. 2, Video 1 in the Supplement). Juvenile horseshoe crabs collected during each survey were counted, and the mean density at each survey site was calculated in terms of individuals per square meter. The PW of all juveniles were measured, and the median sizes from each survey site were calculated and compared using the Kruskal-Wallis nonparametric ANOVA, followed by a Dunn's pairwise multiple comparison test. Statistical analyses were conducted using InStat v.3.1a (GraphPad Software) and Microsoft Excel Analysis ToolPak (Microsoft).

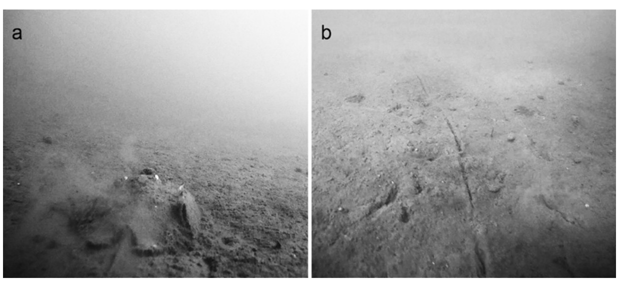

Fig. 2. Photographs taken during SCUBA-dive surveys of (a) a juvenile *Limulus polyphemus* and (b) the trail left by its tail on the sediment. For video, please view Video S1 in the Supplement at www.int-res.com/articles/suppl/m662p199_supp/

3. RESULTS

A total of 116 juvenile horseshoe crabs were found during both survey years (mean ± SE PW: 72.6 ± 2.0 mm; range: 26–125 mm; Fig. A1 in the Appendix). Juvenile horseshoe crab molts (n = 101) were also collected, measured, and are reported in Fig. A1, but were not further analyzed for the present study. All live juveniles were found in Great Bay and, while most were collected on the subtidal mudflats, they were also found adjacent to, and at least 0.5–2.5 km from a known spawning beach (Fig. 1, Table 1).

The highest densities of juveniles were found in Adams Point Cove and near Sandy Point (Fig. 1, Table 1). PWs were significantly different between sites (Kruskal-Wallis nonparametric ANOVA, df = 3, $p < 0.0001$; Table 2), with the smallest juveniles found at Sandy Point compared with Adams Point Cove and Portsmouth Country Club (Dunn's pairwise multiple comparison test, $p < 0.01$).

4. DISCUSSION

In this study, we demonstrated that SCUBA-diving is an effective technique for surveying both subtidal areas and intertidal mudflats at high tide when juvenile horseshoe crabs are generally more active and visible (Dubofsky et al. 2013). Whereas juveniles were not found in the GBE in previous surveys using other methods (NHFG 2010), with SCUBA-dive sur-

Table 2. Prosomal widths (PW), with standard deviation (SD) and standard error of means (SEM), of juvenile horseshoe crabs (n = 116) collected from Great Bay sites

Site name	PW (mean, mm)	SD	SEM	PW (mm) (min.)	(max.)
Adams Point Cove	77.9	21.7	2.5	26.0	120.0
Moody Point	83.0	11.3	6.5	76.0	96.0
Sandy Point	55.7	11.5	2.1	32.5	84.0
Portsmouth Country Club	84.5	23.0	9.4	55.0	125.0

veys we successfully collected 116 juvenile horseshoe crabs ranging in size from 26–125 mm in PW (Table 2, Fig. A1) in both subtidal and intertidal areas in the upper portion of the GBE. However, no juveniles were captured in Little Bay, even though we conducted 7 SCUBA-dive surveys in areas that were adjacent to known spawning beaches (Table 1).

Previous studies have documented the presence of small juvenile horseshoe crabs in both the intertidal zone and subtidal waters adjacent to known spawning beaches (Rudloe 1981, Carmichael et al. 2003, Botton et al. 2003). In the same location where Botton et al. (2003) found small juveniles in shallow water, Burton et al. (2009), using a suction-sampling approach, found the greatest abundance of juvenile horseshoe crabs in nearshore subtidal habitats adjacent to spawning beaches. Our data from Great Bay are very similar, even though we used a different method. However, in Great Bay, juvenile horseshoe crabs were also present in large numbers in subtidal areas at least 0.5 km from known spawning beaches. This might be true in other locations as well, but to our knowledge, extensive surveys have not been carried out in subtidal areas distant from spawning sites in other locations, so we are not certain whether this is a common pattern or not.

Surprisingly, we failed to find any juveniles on the mudflats adjacent to spawning locations in Little Bay during the 7 surveys we conducted there. Our current working hypothesis is that, due to strong currents in Little Bay (0.75–1.5 m s^{-1}; Short 1992), larvae that hatch there and are in the water column are transported up into Great Bay where the water is calmer (0.5 m s^{-1}) and there are vast subtidal mudflats that are optimal for subsequent development and growth. Drifters, designed to mimic the passive drift of pelagic larvae, have been released in Little Bay to investigate American lobster *Homarus americanus* larval transport (Goldstein 2012), and they all ended up in Great Bay, regardless of whether they were released on a flood or ebb tide. Since *Limulus* larvae spend about 7–10 d in the plankton (Botton et al. 2010), there is plenty of time for them to be carried into Great Bay where they can settle. This might also be how they enter the tidal creeks, where we have recently found juveniles (authors' unpubl. data). Juveniles have also been collected in similar tidal creeks by Meury & Gibson (1990) and Dubofsky et al. (2013) on Cape Cod, Massachusetts, for their respective studies.

While we found that the mean PW of juvenile horseshoe crabs captured at Sandy Point was significantly smaller than at other sites, this difference may be due to how we conducted these surveys. Due to its accessibility, we initiated dives at this site from shore, covering both intertidal and subtidal areas. These data are consistent with the hypothesis put forth by Rudloe (1981) that younger individuals develop in shallow nearshore habitats, and as they develop, they gradually move further from shore to deeper subtidal habitats. Clearly, more extensive surveys of Great Bay, including the aforementioned tidal creeks and more nearshore intertidal areas, are necessary to fully understand the complete life cycle of horseshoe crabs in this northern estuary.

This study represents the first successful effort to locate juvenile horseshoe crabs in a large northern estuary using SCUBA-dive surveys. Our results suggest a similar approach might be useful in other similar estuaries and embayments, and such efforts might enhance our understanding of the distribution of juveniles, as well as their critical nursery habitats. Furthermore, we are now in a better position to evaluate activities that might disturb these habitats, such as dredging and coastal development (Beck et al. 2003). Finally, conducting annual surveys to monitor the abundance of juveniles might also make it possible to adjust management practices in a timely manner, rather than waiting 7–11 yr for new recruits to reach sexual maturity and appear on spawning beaches (Shuster 1950).

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Appendix.

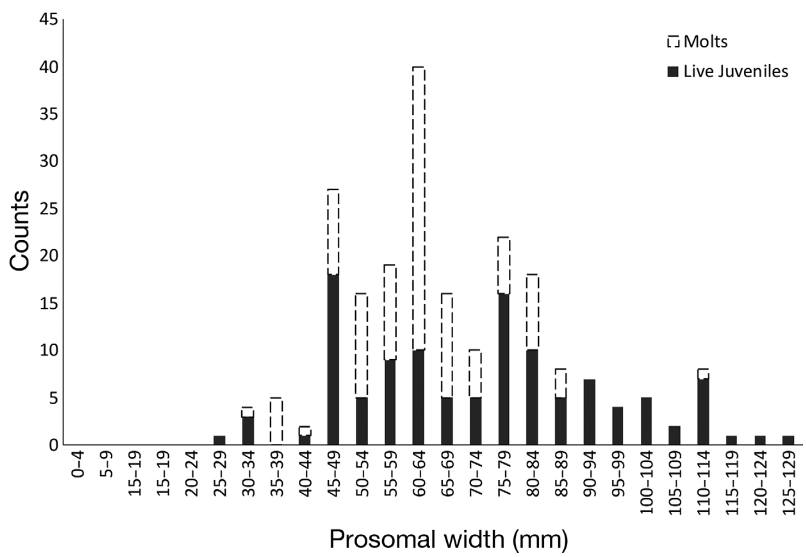

Fig. A1. Size–frequency distribution of prosomal widths for live juvenile horseshoe crabs (n = 116) and molts (n = 101) from 2012 and 2013 combined

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