Identifying the Major Threats to American Horseshoe Crab Populations, with Emphasis on Delaware Bay

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1 Introduction

The objective of this review is to examine the major threats to American horseshoe crabs, *Limulus polyphemus*, particularly in the Delaware Bay region of the United States. We begin with a brief overview of the past and present commercial exploitation of horseshoe crabs for fertilizer and bait, current management practices, and horseshoe crab population status and trends. We then discuss what we believe to be the existential threat to horseshoe crabs, which is the erosion and degradation of essential spawning habitat. The consensus of the scientific community is that overfishing and habitat loss pose the most acute threats to horseshoe crab populations, both in North America (Smith et al. 2016a, b) and Asia (Akbar John et al. 2018). This does not, however, preclude the possibility that specific local factors could stress particular populations and exacerbate the threats posed by overfishing and habitat loss.

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The public perception of these effects is driven, at least in part, by assertions made in the media, through blogs, websites, or articles published in newspapers and magazines. As one example, both biomedical bleeding and oyster aquaculture are strongly implicated in the following:

> The welk [*sic*] and eel bait industry gobbles up the lion share at a quota of more than 600,000 each year, although the actual kill has gone down over the last few years. The medical industry bleeds over 600,000, probably kills about a third, and almost certainly diminishes reproduction of the other 400,000 because they bleed only females. The aquaculture industry expanded their reach and area over the last two years taking more than a half mile of prime crab spawning habitat. (Conservewildlifenj 2017)

The same source also writes that "the oyster aquaculture industry ruins horseshoe crab breeding habitat through industrial level ATV (all-terrain vehicle) use on the intertidal flats used by crabs to feed and impeding crabs from easily reaching the shoreline to breed." In an otherwise well-sourced article, the alarming headline "Medical Labs May Be Killing Horseshoe Crabs" implicates that the biomedical bleeding of horseshoe crabs contributes to their decline (Carson 2016).

Here, we evaluate the evidence that rack-and-bag oyster culture and biomedical bleeding may be having significant negative impacts on horseshoe crab populations in comparison to habitat loss and overfishing by the bait industry.

1.1 Background

Horseshoe crabs are an ancient group of marine chelicerates with a deep fossil ancestry extending to at least the Late Ordovician, ca. 445 million years before present (Rudkin and Young 2009). Representatives of the genus *Limulus* have been dated to the Late Jurassic (Błażejowski 2015), but today the genus has only one extant species, the American horseshoe crab, *L. polyphemus*. These occur along the east coast of North American, ranging from the Gulf of Maine to the Yucatán Peninsula. Three other species of horseshoe crabs (*Tachypleus tridentatus*, *T. gigas*, and *Carcinoscorpius rotundicauda*) are found in Asia, ranging from southern Japan to the Bay of Bengal (Akbar John et al. 2018).

In a comprehensive review of population genetic, morphometric, and other data, Smith et al. (2016a) recognized six Atlantic coast subpopulations of American horseshoe crabs: Gulf of Maine, mid-Atlantic, southeast Atlantic, Florida Atlantic, Northeast Gulf of Mexico, and Yucatán Peninsula. Delaware Bay, in the approximate center of the mid-Atlantic region, has long been recognized as having the largest concentrations of spawning horseshoe crabs throughout its range (Shuster Jr and Botton 1985). The large size of the Delaware Bay population is believed to be a consequence of the bay's long sandy shoreline, favorable hydrographic features (e.g., moderately large tidal range, ideal temperature, and salinity), abundant areas of intertidal sand flats that serve as juvenile nursery habitats, and the richness of food resources in the bay and nearby continental shelf (Shuster Jr 2015).

1.2 Commercial Exploitation and Management

The high quantity of horseshoe crabs in Delaware Bay has fostered several periods of commercial fisheries and has contributed to their central ecological importance to the ecosystem. In the mid-nineteenth and early twentieth centuries, millions of horseshoe crabs were collected each year for use as fertilizer and livestock feed (Shuster Jr. 2003; Kreamer and Michels 2009). Following several decades of relatively modest fishing pressure between the 1940s and 1970s (Botton and Ropes 1987a; Kreamer and Michels 2009), the harvesting of horseshoe crabs as bait for American eel (*Anguilla rostrata*) and whelk (*Busycon carica* and *Busycotypus canaliculatus*) fisheries expanded rapidly in the 1980s and early 1990s, reaching a peak of over 6.1 million pounds (2.8 million kg, or approximately three million individuals) in 1997 (ASMFC 1998). Because egg-bearing females were preferentially targeted during the spawning season by the bait fishermen, concerns were raised about the sustainability of this fishery. The potential threat to horseshoe crabs themselves was especially heightened by the growing awareness of the importance of their eggs as food for migratory shorebirds in Delaware Bay (Myers 1986; Castro and Myers 1993; Clark et al. 1993; Botton et al. 1994; Botton and Harrington 2003; Mizrahi and Peters 2009), and the potentially catastrophic impacts that a reduced supply of eggs would have on the ability of shorebirds to gain sufficient mass during their Delaware Bay stopover. Intensive foraging on horseshoe crab eggs is a strategy used by many birds to successfully complete their migration to their Arctic breeding grounds (Baker et al. 2004; Niles et al. 2009).

The coast-wide fishery management plan (FMP) that was initiated by the Atlantic States Marine Fisheries Commission (ASMFC) in 1998 specifically addressed the size of the horseshoe crab population sufficient to meet the energetic needs of migratory shorebirds in Delaware Bay (ASMFC 1998). Among other measures, the FMP established maximum allowable horseshoe crab harvests for each member state and, through an ongoing adaptive management process, ASMFC technical committees regularly review the status and trends for both horseshoe crabs and migratory shorebirds, thus modifying the allowable harvest in accordance with these data (McGowan et al. 2011, 2015; Millard et al. 2015). A no-take area in federal waters adjacent to the mouth of Delaware Bay, known as the Carl N. Shuster Jr. Horseshoe Crab Reserve, was also established to protect horseshoe crabs that seasonally migrate between Delaware Bay and the continental shelf (Botton and Ropes 1987b). The 2019 regulations for the four states bordering the Delaware Bay region (New Jersey, Delaware, Maryland, and Virginia) stipulate that only male horseshoe crabs can be collected. States may enact even more conservative measures: for example, since 2006, New Jersey has had a moratorium on the collection of all horseshoe crabs from State waters, extending to a distance of 3 nautical miles (5.6 km) from the shoreline. Since the implementation of the FMP, harvests of horseshoe crabs have averaged about 700,000 per year, or about 1/3 of the annual harvest prior to 2000 (Fig. 1). The horseshoe crab population in Delaware Bay now appears to be stable and increasing in the most recent surveys (Smith et al. 2016a,

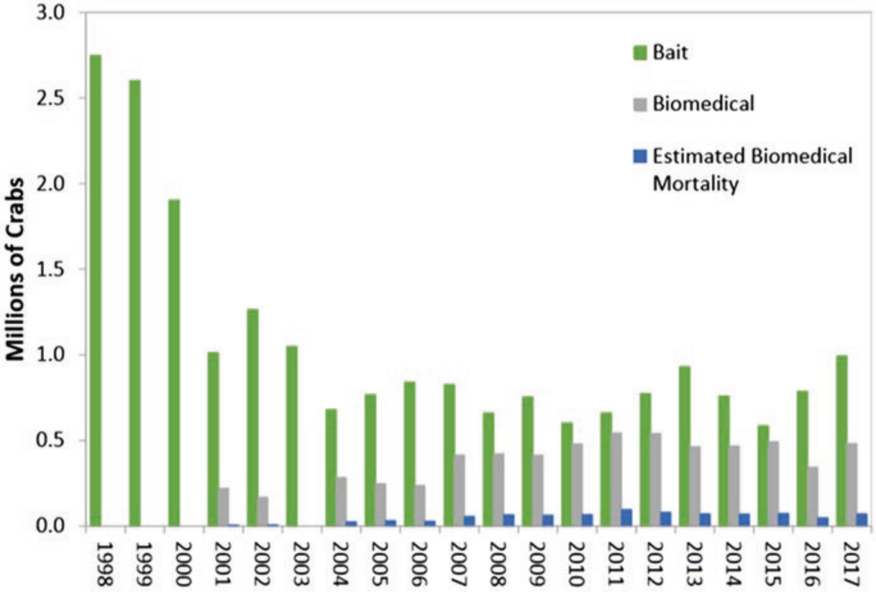

Fig. 1 Landings of horseshoe crabs, 1988 to present. (Source: ASMFC). Biomedical mortality is estimated at 15% of the number of bled animals

b; ASMFC 2019), but there are concerns about whether the population (especially the number of females) has rebounded sufficiently to sustain migratory shorebirds (e.g., Niles et al. 2009; Smith et al. 2009; Karpanty et al. 2011). The most recent (2016) IUCN Red List classification of *Limulus polyphemus* as Vulnerable is based primarily on threats faced by the species in the extreme northern (Gulf of Maine) and southern (Yucatán) portions of its range, rather than on the mid-Atlantic and southeast Atlantic where populations appear to be more stable (Smith et al. 2016b). The *rufa* Red Knot, one of six subspecies of Red Knots globally, is acknowledged to be the shorebird that is most reliant on Delaware Bay horseshoe crab eggs and was listed as a Threatened species in 2014 (USFWS 2014). The link to declines in *rufa* Red Knots with declines in horseshoe crab eggs is confounded by increases in timing mismatches between relatively fixed Red Knot migrations and variable timing in horseshoe crab spawning driven by local weather patterns (Tucker et al. 2019).

2 Loss and Degradation of Spawning Habitat

Sandy estuarine beaches are the optimal spawning habitats for *Limulus polyphemus*; factors such as beach width, sediment grain size, hardness (compaction), depth of oxygen penetration, and wave energy are among the factors that contribute to the selection of particular locations for egg-laying (Botton et al. 1988, 2018; Penn and

Brockmann 1994; Smith et al. 2011). In Delaware Bay, sea level rise and beach erosion have exposed areas of intertidal peat (Botton et al. 1988), which are the remnants of several-thousand-year-old salt marshes (Knebel et al. 1988). Peat banks, or beaches with only a thin veneer of sand overlaying peat, tend to have far fewer eggs than more optimal beaches (Botton et al. 1988). The avoidance of peat is most likely a consequence of the reduced developmental success of horseshoe crab eggs in low O_2/high H_2S environments (Vasquez et al. 2015; Funch et al. 2016). The encroaching bay has also compelled the use of various shoreline armoring practices along Delaware Bay, including the use of revetments and bulkheads, that have diminished the suitability of the habitat for horseshoe crabs (Loveland and Botton 2015). Shoreline armoring is also a major factor in the declining populations of horseshoe crabs in Asia (Akbar John et al. 2018).

As we have recently discussed the importance of sea level rise and beach erosion to horseshoe crabs in Delaware Bay (Loveland and Botton 2015), we will concentrate our discussion here on the central portion of the Cape May Peninsula (New Jersey), in the vicinity of the Rutgers Cape Shore Laboratory (Fig. 2). There are several reasons why we focus on this area. First, this beach has been the primary study site for horseshoe crabs in New Jersey, beginning with studies by Carl N. Shuster Jr. in the late 1940s (Shuster Jr 1955), followed by a series of ecological and behavioral studies by Botton and Loveland from the 1970s through the 2000s (reviewed by Botton 2009). We therefore have substantial ground truth documentation of the changes in the habitat that have occurred over these several decades of study, which we supplement with Google Earth imagery. Second, this area has historically been, and is once again, an area in Delaware Bay where intertidal culturing

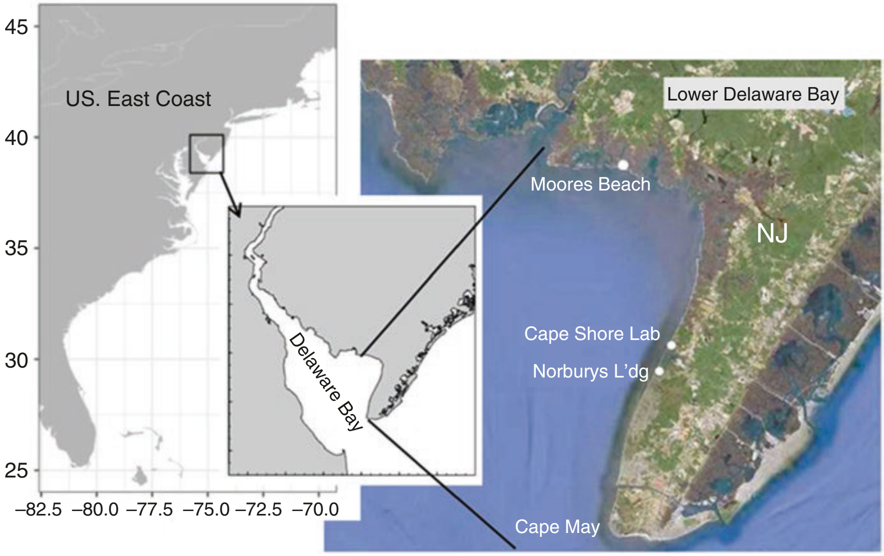

Fig. 2 Map of lower Delaware Bay, NJ, showing locations of the beaches noted in the text

of American oysters (*Crassostrea virginica*) has taken place. The potential impacts of oyster culture on horseshoe crabs (and shorebirds) are discussed in Sect. 3. Finally, collection of horseshoe crabs for fertilizer (Shuster Jr. 2003) once took place in this region; in fact, some older residents still use the location name “King Crab Landing” (a/k/a Highs Beach) when referencing the small town just north of the Cape Shore Laboratory. In the 1980s and 1990s, horseshoe crabs were hand-collected for biomedical bleeding from this location, but the majority of the Delaware Bay area crabs are now collected offshore during the summer and fall (J. Cooper, pers. comm.). The biomedical use of horseshoe crabs, and its possible impact on the population, is discussed in Sect. 4.

2.1 Habitat Loss Along the Cape May Peninsula

Early photographs from the 1930s confirm that there was a largely uninterrupted and continuous area of sandy beach in the vicinity of the Cape Shore Laboratory (Fig. 3). Experimental culture of oysters has taken place in this area of Delaware Bay since the 1930s; this has included rack culture (Fig. 4a) and the placement of large quantities of surf clam shell on the intertidal sand flats for oyster spat collection (Fig. 4b). This region was also notable for its large horseshoe crab population that supported the local harvesting of the animals for the fertilizer industry (Shuster Jr. 2003). When Shuster Jr and Botton (1985) conducted their high tide surveys of spawning activity in the late 1970s, this region had the highest density of horseshoe crabs in all of Delaware Bay. Peak spawning counts in the range of 20,000–35,000

Fig. 3 Photograph of the Cape Shore Laboratory beach ca. 1930s. The original Oyster Research Laboratory is at the left; in the foreground are oyster racks and a railway leading from the intertidal flats to the beach. (Photo credit: T. C. Nelson, courtesy of W. J. Canzonier)

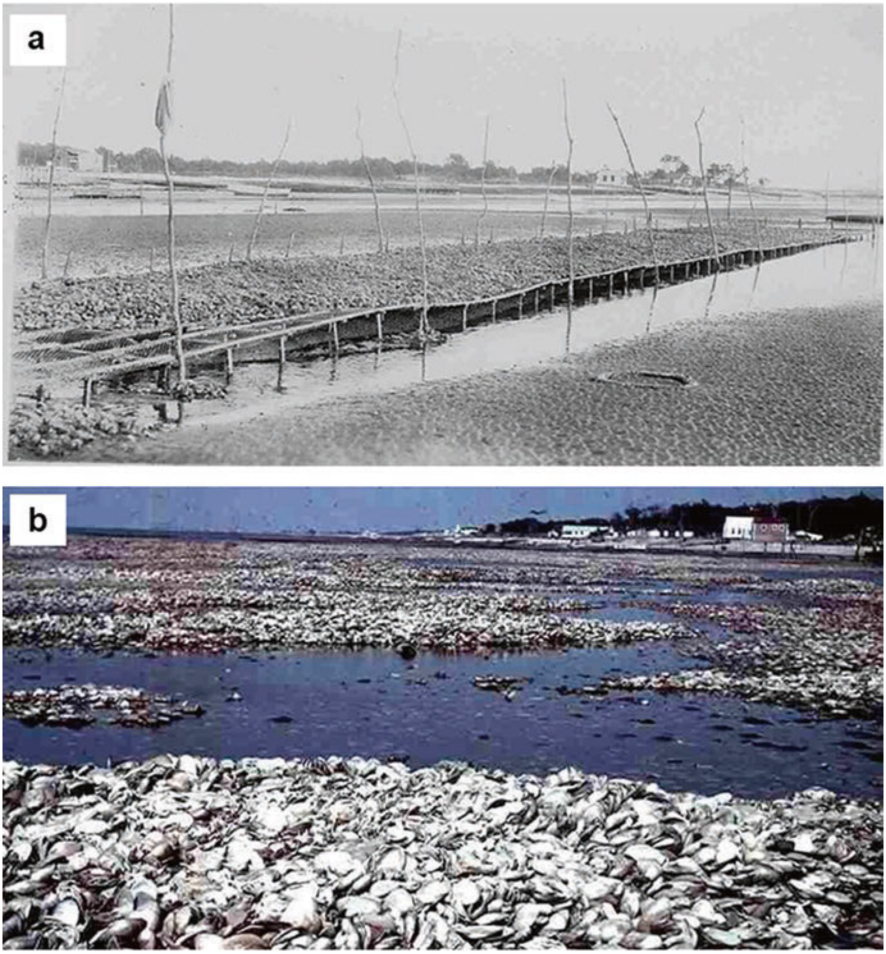

Fig. 4 Photographs documenting the presence of shellfish culture in the vicinity of the Cape Shore Laboratory. (**a**) Oyster conditioning racks on the intertidal flats ca. 1930s. (Photo credit: T. C. Nelson, courtesy of W. J. Canzonier). (**b**) Aggregations of surf clam (*Spisula solidissima*) shell on the intertidal flats, 1964. Shell was collected from local clam processing facilities and placed on the flats to serve as substrate for oyster spat, which were later moved to subtidal leased areas elsewhere in Delaware Bay. (Photo credit: H. Hidu, courtesy of W. J. Canzonier)

animals per km were recorded in the 1970s and 1980s (Shuster Jr and Botton 1985), and even neap tide abundance in the late 1980s often exceeded 7000 animals per km (Botton et al. 1988). Well into the 1990s, it was common to see an almost solid carpet of horseshoe crabs along the beach at high tide, with numerous satellite males jostling for position around the mated pairs. Small patches of peat began to be noticeable in the 1980s, leading to the initial observations about the avoidance of these sediments by spawning adults. Nonetheless, the vast majority of this habitat was optimal spawning beach (Shuster Jr and Botton 1985; Botton et al. 1988) because of its favorable sediment texture, width, and lack of shoreline armoring.

Google Earth images of the Cape Shore region from 1991 and 2016 (Fig. 5) show important changes in the beach and shoreline. In 1991, there was a wide band of sand along a relatively straight and uniform dune line protecting the marshes and forests behind it (Fig. 5a). In fact, the marsh was a functioning freshwater marsh with little to no saltwater intrusion (D. Bushek, personal observation). A tidal inlet at Green Creek (Fig. 5b) was constructed in 1994 to replace a former pipeline that

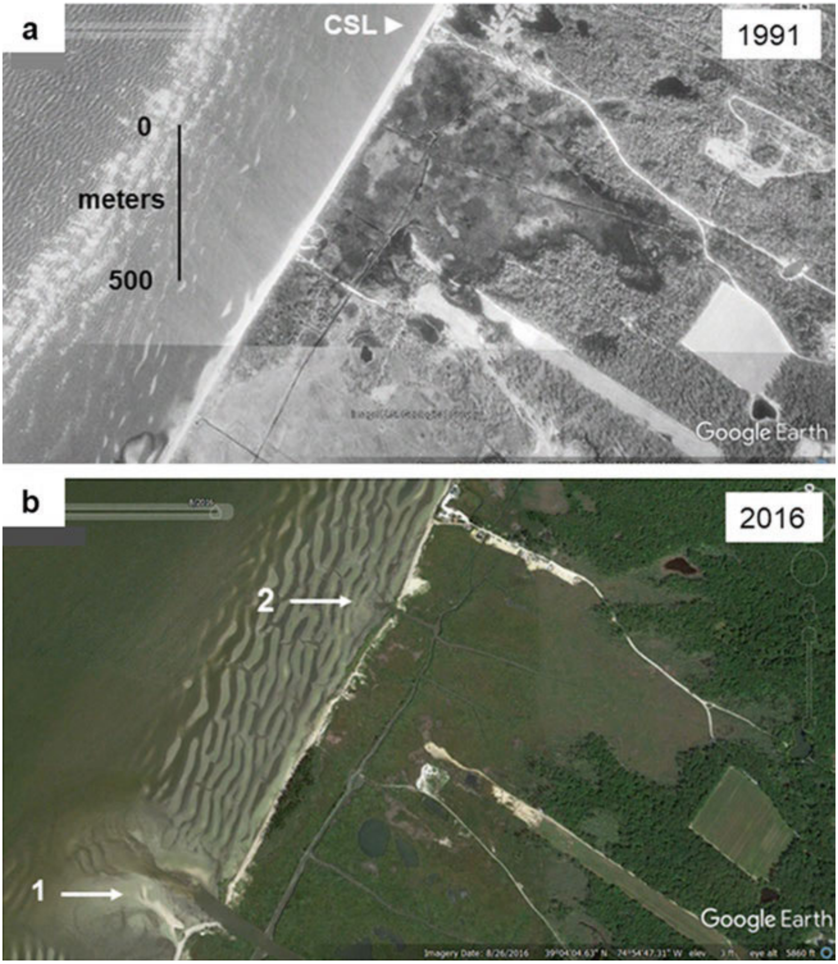

Fig. 5 Google Earth images showing changes to the shoreline at the Cape Shore Laboratory (CSL) region between (**a**) 1991 and (**b**) 2016. The locations of the 1994 and 2010 dredged tidal creeks are shown by arrows 1 and 2, respectively. Note the overall loss of sandy beach and the increasing areas of shoreline peat, marsh, and overwashed sand. (Images are taken at an altitude of 5860 ft (1.78 km))

drained the freshwater marsh above the high tide line, thus converting it to salt marsh (Weggel 2011). The Schellenger's Creek inlet, closer to Norbury's Landing (south of the area shown on the map), was dredged in 1995 by the Cape May County Mosquito Commission and a culvert was placed closer to the Cape Shore lab to allow saltwater to convert that portion of former freshwater marsh, all as a mechanism for mosquito control. The culvert near the Cape Shore Laboratory failed and by 2010 another tidal inlet had formed (Fig. 5b). As these tidal inlets expanded, shoreline erosion accelerated as evidenced by increasing areas of overwash through the time series of images (Fig. 6). Shoreline that was formerly sandy beach has transitioned into peat banks and salt marsh, with many areas of overwashed sand (Fig. 6a–c). The beach immediately fronting the Cape Shore Laboratory has been stabilized by a gabion wall in order to protect the facilities (Fig. 6d). In brief, in less than three decades, the nearly 3 km beach from the Cape Shore Laboratory to Norbury's Landing has transformed from one of the most productive horseshoe crab habitats in the world to a locale that is marginal or unsuitable for horseshoe crab spawning.

Although the recession of the shoreline near the Cape Shore Laboratory may have been accelerated by the entrainment of sediments at the mouths of the tidal

Fig. 6 Recent (2017) photographs of the Cape Shore Laboratory region. (**a**) A large peat outcrop to the south of the Cape Shore Laboratory (approximately the same location shown in Fig. 5). (**b**) Area of salt marsh that has developed near tidal creek 2 labeled in Fig. 5b). (**c**) One of the large sand overwash areas in this region. (**d**) A portion of the ~100 m gabion wall at the Cape Shore Laboratory beach. (Photo credit: M. Botton)

creeks (Weggel 2011), erosion is not at all atypical. To the contrary, many other Delaware Bay beaches have experienced similar alterations (Loveland and Botton 2015). Some bay shore communities (e.g., Fortescue, East Point, and Pierce's Point) have installed stone revetments, or wooden and sheet steel bulkheads to protect property, which have diminished the suitability of these beaches for horseshoe crabs (Botton et al. 1988; Jackson and Nordstrom 2009). Other communities have lost the battle against the rising sea level and have been abandoned (e.g., Moores Beach, Thompsons Beach, Bay Point). It is also the case that the effects of sea level rise along the bay were obvious long before Hurricane Sandy in late October 2012 although some narratives suggest otherwise (e.g., Palmquist 2018). We note that a closer inspection of the Google Earth images reveals a shoreline retreat of 20–50 m between 1991 and 2016 and that much of this shoreline retreat was evident in Google Earth by 2011. Under virtually all scenarios of CO_2 and other greenhouse gas emissions, sea level will continue to rise some 0.7–1.2 m before the year 2300 (Mengel et al. 2018). This will have the potential to inundate much of the New Jersey coast (Cooper et al. 2008) and have devastating consequences for shallow-water and shoreline ecosystems.

The ongoing erosion and degradation of essential spawning habitat thus poses a significant threat to American (Smith et al. 2016a, b) and Asian (Akbar John et al. 2018) horseshoe crabs. It is against this backdrop that we now examine two contentious issues: the effects of oyster culture and biomedical bleeding.

3 Horseshoe Crabs and Oyster Farms

3.1 Background

The Delaware Bay has approximately 261 km of shoreline (Lathrop et al. 2013) with one third (33.0%) of that suitable for horseshoe crab spawning. Only a small portion of the suitable crab habitat (~5%) is also home to intertidal oyster farming (Munroe et al. 2017). Oyster farms currently occupy approximately 10 acres along the lower Delaware Bay of New Jersey on which they produce over 1.8 million market-sized oysters annually (Calvo 2016). During the first half of the twentieth century, large wooden intertidal racks were used to cultivate oysters over wide expanses of this region (Fig. 4). That practice stopped following the onset of MSX (multinucleated sphere unknown) disease in 1957, which killed as many as 95% of the oysters cultivated on commercial leases (Ford and Haskin 1982). In 1962, Dr. Harold Haskin began breeding oysters that survived MSX, creating the first disease-resistant lines of oysters that set the stage for the rejuvenation of oyster aquaculture in Delaware Bay and elsewhere along the mid-Atlantic coast (Haskin and Ford 1979; Ford and Haskin 1987). Shellfish aquaculture along the Cape Shore region of the Delaware Bay in New Jersey, like other forms of molluscan aquaculture, is viewed as a low-impact, sustainable food production system (Shumway et al. 2003;

Hilborn et al. 2018; van der Schatte et al. 2018). As farm production rebuilds in New Jersey and expands regionally, nationally, and globally, the industry faces challenges in assuring ecological sustainability and social license (Billing 2018). In particular, the nature of the interaction among farms and wildlife such as birds and mammals that may use habitat near to or occupied by farms is in many cases poorly understood (Price et al. 2017; Barrett et al. 2018).

Despite the historical use of the lower Delaware Bay for intertidal oyster cultivation during a period when horseshoe crabs were heavily harvested for fertilizer (Shuster Jr. 2003; Kreamer and Michels 2009), little data has been collected specifically addressing the ability of horseshoe crabs to traverse intertidal oyster farms. Modern farms use rack and bag methods, a farming activity that grows oysters in specialized cultivation bags elevated off the bottom on top of metal racks (Fig. 7). If crab migration activity is impeded or harmed by farm gear as they move past farms to reach spawning habitat, spawning may be inhibited and population-level consequences may result. Precautionary measures were recently implemented in response to a dearth of science pertaining specifically to the issue (Walsh 2016). To address

Fig. 7 Horseshoe crabs swim among and below oyster racks as tide floods in the Delaware Bay, spawning habitat can be seen in the background of the photo. (Photo credit: D. Munroe)

this information gap, and to better understand the interactions among crabs and intertidal oyster farms in the Delaware Bay, a series of experiments were conducted in 2016 (Munroe et al. 2017) and then expanded upon in 2018 at the Rutgers University Cape Shore Laboratory and on adjacent active commercial oyster farms, co-located along the Delaware Bay in New Jersey during active horseshoe crab spawning activity from May through mid-June in each year.

The primary goal of the experiments was to assess the ability of crabs to move around and among oyster farms during low- and high-tide conditions as they migrate to and from the beach where they spawn. Experiments included surveys of the distribution of crabs on the tidal flats within and outside farms during low tide, and controlled behavioral experiments to assess the ability of crabs to move among and past farm gear both under water and in dry (low tide) conditions.

Surveys

Low-tide crab surveys were done using paired transects on two farms in 2016 and four farms in 2018. These low-tide surveys were conducted because Delaware Bay water conditions are sufficiently turbid that crabs cannot be observed visually when water is present (high tide). The survey design assumed that crabs moving through adjacent control and farm transects were equally distributed, and those that remain during low tide are not sufficiently mobile to redistribute substantially after the tide recedes. Thus, the low-tide distribution represented the distribution (not abundance) of crabs as the tide receded, and that any differences observed between paired transects would thus be attributable to the presence of farms. At each farm site, paired, 1-meter-wide transects were oriented perpendicular to the shore, mapped, and marked with poles. All mapping and marking of transects was performed prior to the arrival of spawning horseshoe crabs. Each pair included one transect that intersected a farm and a parallel control transect passing through adjacent unfarmed intertidal habitat, ~25 m away. The locations of habitat features (e.g., sloughs and sandbars) and farm gear (e.g., racks) along each transect were noted. Here, and in subsequent surveys and experiments, controls were selected to have an equivalent bottom type, habitat features, and distance from high tide as the paired farm. The inshore edge of each farm was offset from the high-tide line by at least 91.5 m (300 feet), an area in which no farm gear is permitted due to precautionary restrictions. Complete descriptions, including schematics of transect layout, are provided in Munroe et al. (2017, 2020).

During daytime low tides, starting in early May and continuing through the end of crab spawning activity, transects on all four farms were walked and all crabs encountered along the transect were documented, and their location (inshore of, or within farm) was noted. Walks were repeated at least weekly through the duration of the spawning season. The data collected during these surveys were used to test for differences in the number of crabs observed inshore and within farm gear among paired farm and control transects.

Two of the four paired sets of transects were also surveyed during high tides over the course of 1 week between late May and early June in 2018, a period of high horseshoe crab activity. These high-tide surveys were performed using dual-frequency identification sonar (DIDSON) mounted beneath a small boat. This sonar technology is a nondestructive, nonintrusive tool that creates echograms capable of visualizing submerged habitat and organisms moving within that habitat (Moursund et al. 2003). During each survey, the boat motored slowly along each transect, following the same path that was walked during low-tide using poles located at each end of the transects as guiding markers. In addition, counts of crabs over time (15 minutes) were repeatedly made using the sonar from the boat at fixed stations within the farm and at a nearby control location with no farm gear. Only two farms were able to be surveyed by sonar in this manner because of logistical limitations due to battery and boat speed constraints.

In total, six replicate high-tide events were surveyed and the sonar videos used to count the number of single and paired horseshoe crabs along each transect. Because these sonar videos were taken from above as the boat motored over the tidal flats, oyster bags on the racks sometimes obscured the ability of the counter to see portions of the bay floor beneath the bags; therefore, videos along farm segments were corrected for area obscured. The crab counts collected during these high-tide surveys were then used to test for differences in the number of crabs along paired farm and control transects.

Behavior Experiments

As a compliment to the surveys, agility and behavior experiments were conducted during low tide on the beach, under water in tanks in controlled conditions, and under water in natural conditions at farm and control sites.

The low-tide beach experiment was performed on hard sand habitat during a daytime low tide in late April 2016. In this experiment, described in detail in Munroe et al. (2017), the height of oyster racks were varied between 7.5 and 30.5 cm above the sand. Horseshoe crabs ranging in prosoma width from 17.5 to 23.0 cm were placed right side up approximately 1 m from the oyster rack, then observed as each walked beneath the rack. The rack height and success or failure of each crab to pass beneath or around the rack was recorded each time the experiment was repeated. The goal of this experiment was to determine at what clearance height crabs of varying sizes could pass beneath an oyster rack during low tide.

The second experiment performed in a tank filled with water aimed to test whether mature horseshoe crabs (including amplexed mating pairs) can pass beneath, around, or over oyster racks of varying heights when under water. These agility experiments were conducted in a large fiberglass tank in 2018. Twenty crabs, collected at random, were used for each trial. Before being placed into the tank, the prosomal width of each crab was measured and the sex determined. Each crab was marked with an identification number, which allowed observers to identify behaviors of individual crabs during the course of the trial. Marked animals were placed into the tank and allowed to acclimate for 15 minutes before each trial was initiated. During this acclimation period, many of the males attached to females, creating amplexed pairs and these pairings were also noted.

A total of 11 oyster gear treatments were tested in the tank. Ten farm gear treatments of varying heights and configurations were used, plus a control in which the footprint of an oyster rack was drawn on the tank bottom but no physical structure was placed in the tank. The suite of treatment types included three rack heights (7 cm, 12 cm, and 20 cm) with and without oyster bags attached, an oyster bag on the bottom (no rack), a floating oyster bag tethered to the bottom with 6.35 mm (¼ inch) braided sinking line, oyster bags leaning on the side of a rack, and a rack on its side (no bag). These various gear configurations encompassed the gear types typically used in the intertidal farms in Delaware Bay, and others currently disallowed due to the restrictions and precautionary measures to protect red knots (Walsh 2016). All of the gear used were identical to those used by farmers with the exception that all were shorter than those used on farms. All gear also had bungee cords with metal hooks attached mimicking what farmers use to hold bags onto the racks. All oyster bags used in treatments were plastic mesh with 1 cm openings, measured 8 cm × 48 cm × 90 cm (3″ × 19″ × 36″), and contained adult oyster shells to mimic live oysters in the bags (see Munroe et al. (2020) for schematic details of gear configurations).

During each agility experiment, a given treatment was placed in the tank with the 20 acclimated crabs. The crabs were observed continuously for 15 minutes as they moved about the tank and interacted with the oyster gear. A record for each individual crab was kept noting each time that an individual crab passed to the side, beneath, or over a treatment structure. Each treatment was replicated between 9 and 14 times, for a total of 139 trials. Horseshoe crabs used in the agility trials ranged in size from 16 to 28 cm prosomal width for males (mean = 20.1 cm), and from 21 to 30.5 cm for females (mean = 25.8 cm), and had sex ratios consistent with those in the spawning populations. Details of the agility trials are documented in Munroe et al. (2020).

The behavior experiments that observed crab behavior under water in natural conditions were performed using DIDSON sonar to record crabs moving among real oyster farm gear and control sites between May 13 and 23, 2019. Two sonars were used concurrently to collect paired video at two locations equidistant from the high-tide line and at the inshore edge of a commercial farm and at a comparable control location. The sonars were fixed in place, were set to image the bottom, and were tethered by cable to land where each fed live video to computers and recording devices. In total, seven high-tide events were observed and recorded, with recording starting as the tide submerged both sonars, and ending when the tide dropped below the units.

The tracks of single crabs and pairs in amplexus in these videos were analyzed to evaluate the path followed by the crabs (distance over ground), the speed of movement, and the direction. These metrics were used to compare behavior of horseshoe crabs at farm and control sites on the flats. Further details of how these videos were collected and analyzed can be found in Munroe et al. (2020).

Survey Results

The number of horseshoe crabs observed during low tide along transects at each farm varied throughout the spawning season, among years, and among farm sites. Numbers of horseshoe crabs along the transects ranged from a low of zero at the beginning and end of the observation period, to a high of 135 per transect during the observation made on May 21, 2018; a period falling between the new and full moon in late May. In total, over all transects and across the entire 2016 observation period, 853 crabs were observed on the two farms (Munroe et al. 2017), whereas 1176 crabs were observed in 2018 on the four farms studied (Munroe et al. 2020). No difference was found among the number of crabs counted inshore of farm gear compared to controls, nor within the farm footprint compared to controls. The fact that no difference is observed among crab counts at paired farm and control survey sites at low tide, regardless of whether the observations are inshore of or within the farm footprint, indicates that crabs do not differentially use intertidal habitats in locations where farm gear is present.

High-tide surveys using DIDSON sonar occurred during the week of May 28, 2018, encompassing the full moon in late May. At the same time, high abundances of crabs were observed spawning along the entire length of beaches within the study area. In 2018, two of the paired farm transects were surveyed during high tide using DIDSON sonar. Sonar video of horseshoe crabs during high tide showed large aggregations of crabs in sloughs (muddy depressions) and sparsely distributed crabs moving independent of other crabs in seemingly random directions outside of sloughs. Crabs did not move *en masse* as a unit; rather they crossed the sand flats in all directions moving as singles or pairs with few other horseshoe crabs nearby. On many occasions, horseshoe crabs were observed to move under and out from farm gear unimpeded. On other occasions, a crab was observed to bump into the leg of a rack or another horseshoe crab, after which they would alter direction slightly and continue moving.

Similar to the results found in the low-tide transect survey, differences in numbers of crabs were observed over time (among high tides) and among farms, but no difference was observed between paired farm and control areas (Munroe et al. 2020). Within the intertidal region inshore of the farm gear, no significant difference was detected among control and farm counts of single horseshoe crabs nor amplexed pairs. Similarly, within the area of the farm gear (outer intertidal), no significant difference was detected among control and farm counts of single or amplexed horseshoe crabs.

Counts of horseshoe crabs made at fixed stations over time, standardized to crabs/minute, ranged from 0 to nearly 2 crabs per minute moving through an area of bottom approximately 10 m^2. No significant difference was detected among paired control and farm counts for single crabs, nor amplexus pairs. When counts were corrected for view obstruction by farm gear, no significant difference among single horseshoe crab counts at control versus farm was detected; however, significantly more amplexed pairs were observed at the farm station. In agreement with the low-tide surveys, no difference was observed among horseshoe crab counts at paired farm and control sites during high tide, indicating that crabs use these intertidal habitats consistently, regardless of the presence of farm gear.

Behavioral Experiments: Results

The low-tide experiment on hard sand demonstrated that horseshoe crabs of all sizes tested could pass beneath racks that had a clearance above the sand of 10 cm or more (Fig. 7) (Munroe et al. 2017). Six of the 48 horseshoe crabs in the experiment did not pass beneath or around the rack, and all of those occurred when they encountered racks with only very small clearance (7.5 cm). In all six of those cases, the crabs bumped up against the rack edge, stopped, then buried slightly, and stayed in place. Four other horseshoe crabs, when bumping against the lowest rack height, changed course and continued around the rack, a behavior commonly observed in the survey experiment described above when horseshoe crabs bumped into one another underwater.

In the tank experiments, across all 11 gear configurations, male, female, and amplexed pairs were all observed moving around and under/over/through the oyster farm gear without difficulty. Interestingly, this included single female horseshoe crabs and crabs engaged in amplexus successfully passing both under and over the racks with the least clearance. In general, amplexed pairs and females tended to move along the walls of the tank when no gear was present, likely due to edge effects of the tank. When the tallest racks (greatest clearance) were used horseshoe crabs tended to pass under them, whereas when the shortest racks were used crabs tended to pass over them. This behavior is likely due to the fact that prosoma heights of spawning male and female horseshoe crabs (7.5 and 10.0 cm, respectively, Kraeuter and Fegley 1994) are greater than the shortest rack height tested, and less than the tallest rack height tested. Among all of the 128 trials performed in this experiment, no horseshoe crab was ever observed to be stuck or impeded from moving past or through the oyster farm gear (Munroe et al. 2020). This experiment demonstrates that rack and bag or floating farm gear are not obstacles that impede horseshoe crab movement whether elevated above the sediment or not.

Finally, the behavior experiments conducted in 2019 using fixed DIDSON sonars provided a novel and informative new look at horseshoe crab behavior under unmanipulated conditions (Fig. 8). In those experiments, horseshoe crabs were observed as they moved across the tidal flats at a farm site and nearby nonfarm (control) site. These concurrent observations of horseshoe crabs at a farm and control location allowed comparison of the numbers of crabs moving around, and evaluation of their behavior including speed, path straightness, and direction. No difference was observed in the number of crabs moving around at the farm compared to the control location (Munroe et al. 2020). Likewise, horseshoe crab speed (~12 cm/second) and direction (generally following tidal currents) did not change as they moved through the farm. Path straightness was slightly altered (~3% less straight) as they moved through the farms, likely due to occasionally needing to navigate around the legs of racks (Munroe et al. 2020).

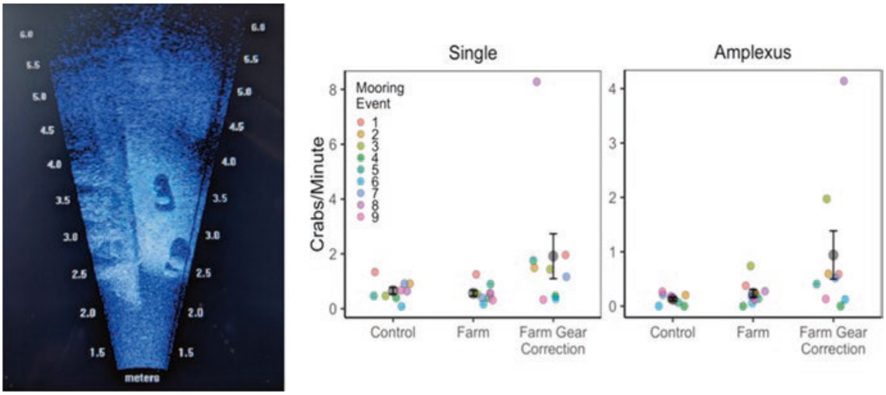

Fig. 8 Screenshot of sonar video showing crabs in amplexus beside bags of oysters on a rack (left). Single and amplexus crab counts from all paired mooring events (right). Gray points represent the mean ± standard deviation. Colored points show average per observation event

3.2 Assessing the Impacts of Oyster Culture

Across all of the surveys and experiments performed, our results indicate that horseshoe crabs can successfully traverse farms and reach spawning beaches, and that horseshoe crabs do not avoid farm gear when accessing spawning beaches. The studies discussed here show no difference in the numbers of horseshoe crabs reaching inshore spawning habitat due to farm gear, suggesting that reproductive behavior and capacity is unimpacted by oyster farms. When sonar counts of horseshoe crabs at farm sites were corrected for obstruction of the view of the bottom, there in fact appears to be more crabs moving within farms at high tide compared to control sites without gear. Attractiveness of farm structures in marine habitats, such as fish net pens and shellfish gear, to mobile fish is well documented (Callier et al. 2017). It is possible that horseshoe crabs also find oyster farm gear attractive due to increased foraging opportunities, shelter, or other cues; future research may address this possibility.

4 Biomedical Use of Horseshoe Crabs

4.1 Discovery of a Reagent for Detection of Bacterial Endotoxin

For centuries, physicians experimented unsuccessfully with injection therapy because patients developed devastating infections and high fevers called “injection fever.” Florence Seibert (1925) proved that the fevers were caused by Gram-negative bacteria (GNB) and sought to make injectable fluids safe by eliminating GNB and their pyrogenic (fever inducing) extracts that contained endotoxin. She avoided the fever reactions caused by endotoxin (pyrogen) by making pyrogen-free water by distillation, using a rabbit fever test to verify safety, and producing sterile solutions of saline and dextrose in glass bottles by steam sterilization. Under Seibert's influence, Baxter began production of sterile IV fluids (LVPs, large volume parenterals) in 1933 at Glenview IL. LVPs were essential for managing the wounded in WWII. It is inconceivable that sterile, pyrogen-free IV solutions were first produced less than a century ago.

Horseshoe crabs and mankind have a unique bond. Physician scientists from The Johns Hopkins University, while working at the Woods Hole Marine Biological Laboratory (MBL), discovered that blood cells of the horseshoe crab had a unique way of recognizing and destroying certain bacteria. While studying the innate immunity of horseshoe crabs at MBL, Frederick Bang (1956) observed that injection of GNB or their extracts caused them to die, not by infection, but by coagulation of their hemolymph. In collaboration with hematologist Jack Levin in 1963, they observed that endotoxin caused this unexpected phenomenon by inducing the amebocytes to release an enzyme coagulation cascade that produced clotting (Levin and Bang 1964). This finding led to the creation of *Limulus* amebocyte lysate (LAL). Levin envisioned a simple test for endotoxin in septic patients, but this application never materialized.

Cooper et al. (1971) collaborated to show that LAL reagent was the optimum tool for screening injectable drugs, vaccines, and implantable devices for the presence of life-threatening endotoxin. Tens of thousands of rabbits were then used annually to test for endotoxin pyrogen as a potential contaminant in all injectable fluids. Levin's novel reagent was compared with the required rabbit pyrogen test (RPT) for endotoxin. LAL was consistently a more simple, sensitive, and specific test than the costly, variable RPT. The potential for LAL to replace the RPT was intensely studied by the parenteral drug industry and the Food and Drug Administration (FDA). The public was generally unaware of horseshoe crabs until they learned about the value of their blood to healthcare. In contrast, thousands of people today volunteer their time for horseshoe crab surveys along our coast and in Asia, and become part of citizen science events that heighten their awareness of horseshoe crabs and their important ecological relationships (Kreamer and Kreamer 2015; Nishimura and Iwaoka 2015; Liao et al. 2019; Zaukia et al. 2019).

4.2 FDA Elects to License and Regulate LAL as a Biological Product

The FDA became a stakeholder in LAL when Cooper collaborated with the Agency's Biologics Division to establish an LAL test capability (Cooper et al. 1972). Seligmann envisioned LAL reagent as the endotoxin test of the future and began a program in 1973 that developed regulations for the production of LAL reagents. A firm wishing to market LAL had to submit for review and approval two detailed documents, an application for a suitable facility and a submission detailing the LAL production process. FDA began licensing in the LAL industry in 1977. The regulations required a catch-and-release policy for horseshoe crabs. The FDA conducts biannual inspections of LAL firms to review compliance with drug regulations, such as Good Manufacturing Practices (GMPs), and the firms' written, FDA-approved procedures (FDA 2018). LAL was first used for drug testing in 1974 when it was required as a safety test for influenza vaccine.

Baxter Travenol, world's largest producer of injectables and medical devices, made a corporate decision to go from rabbit to LAL testing in the 1970s. A global study totaling 356,548 LAL tests and 66,594 rabbit tests proved LAL's specificity and sensitivity; this report led the FDA to approve LAL as an alternative to rabbits (Pearson and Weary 1980).

4.3 The LAL Industry

The first commercial LAL production was established by Cooper at Chincoteague VA in 1971. Five horseshoe crab bleeding facilities are located on the eastern US coast from Massachusetts to South Carolina that produce LAL for FDA-approved reagent. The approval by the FDA in 1987 for the use of LAL reagent as an official test for bacterial endotoxin (pyrogen) led to increased production of LAL during the 1990s to meet the growing needs of the pharmaceutical firms. Approximately 450,000 horseshoe crabs are now collected annually by US biomedical LAL firms (Fig. 1). Male and female donors are bled in about equal proportion, For example, males comprise approximately 60% of the crabs processed at two market-leading LAL firms. The worldwide market for amebocyte lysates is approximately $500 million, including TAL (*Tachypleus* amebocyte lysate). LAL and TAL firms have the crucial responsibility of providing >70 million test units annually for assuring the safety of injectable products. Horseshoe crabs bled for LAL production in the United States are returned to sea in a timely manner. Biomedical business provides livelihood for many watermen as an alternative to a bait fishery.

Tachypleus tridentatus used in China for TAL are most often diverted to commercial markets rather than return to sea because there is no regulatory policy for conservation (Gauvry 2015). The sharp decline of *T. tridentatus* in Asian waters led the IUCN to add them to their list of Endangered Species (Laurie et al. 2019). Eight Chinese firms produce about 15% of the amebocyte lysate global market. There will be pressure to turn to the use of LAL as the horseshoe crab population is exhausted in the South China Sea (Gauvry 2015).

The biomedical community has minimized its impact on horseshoe crab populations through 45 years of consistent conservation practices. From the outset, biomedical firms used a return-to-sea policy to minimize impact on horseshoe crabs. The FDA made this policy a condition for licensure for LAL production. In 1990, Jim Finn and Benjie Swan of Finn-Tech, a New Jersey LAL producer, introduced the Delaware Bay Spawning Survey that provides critical data on horseshoe crab population and migration (Swan 2005). The survey continues under the coordination of Swan. Initially used as an educational tool, the survey has become a management tool.

4.4 *Fishery Management Plan (FMP) and Biomedical Uses of the Horseshoe Crab*

The advent of the horseshoe crab bait industry raised the concern of LAL firms for a diminished horseshoe crab stock. At the urging of South Carolina's LAL firm, Endosafe, Inc., the State enacted legislation in 1991 for possession of horseshoe crabs, which must be collected by hand harvest, and limited their collection for biomedical and research applications. These regulations became a model for oversight of horseshoe crab use by the ASMFC. New Jersey also banned collection of horseshoe crabs for the bait industry in 2007.

The ASMFC created a FMP for limiting the horseshoe crab bait harvest (ASMFC 1998). The biomedical industry was exempted from ASMFC harvest limits because of low mortality and the critical need to assure safety of injectable medications. In anticipation of a growing LAL market, the FMP included a mortality threshold of 57,500 (not a limit); the average total estimated mortality for the past 5 years is 67,500. In response, the ASMFC sponsored a meeting of state marine resource leaders and scientists from biomedical firms to write Biomedical Best Management Practices (BMPs) for LAL firms (ASMFC 2011). All aspects of crab collection, handling, bleeding, and return-to-sea were addressed in the practices. Although LAL firms operate in diverse conditions and locations, basic operating procedures and conservation steps were identified and agreed upon. Biomedical firms use all possible conservation measures to assure the continued availability of healthy crab populations. Only healthy crabs are bled to avoid bacterial contamination of valuable LAL reagent.

4.5 *Estimated Mortality from LAL Processes*

LAL-related mortality is widely debated and estimates range from 0% to 30% (Smith et al. 2016a, b) (Table 1). The bleeding step does not result in immediate death because specimens are prescreened for health and lack of injury. However, the stress of collection and transport processes may cause mortality in horseshoe crabs that are unhealthy; death that occurs up to point of release is reported as LAL mortality and usually constitutes 2–3% of total catch.

Marine resource managers became interested in postrelease mortality to aid stock assessment of horseshoe crabs and assure the public that LAL processing was

Table 1 Summary of estimated postbleeding mortality studies relative to LAL Biomedical Firms and Best Management Practices (BMPs)

Author and date	Location	Number	Mortality	Relevance of methodology to biomedical practices
Rudloe 1983	Florida Gulf Coast	10,062	11%	1. Release and recapture from bay. Recovery of 1415 crab with 85 dead
		80	2.5%	2. Bled and unbled crab held in small pen for 30 days
Thompson 1998	Charleston, SC	40	15%	Bled and unbled held in open sea-water enclosure for 7 days
Yadon and Endosafe 1999	Charleston	252	8.3%	Bled and unbled crab held in sea pond for 2 weeks
Walls and Berkson 2003	Hampton, VA	400	8%	Bled and unbled crab held in replicated flow-through tanks for 2 weeks
Hurton and Berkson 2006	Blacksburg, VA	200	0	1. Bled and unbled crab held in tanks for 2 weeks; "low stress conditions"
		195	8.3%	2. Bled and unbled held in tanks under "high stress conditions"
Leschen and Correia 2010	Woods hole, MA	281	29.8%	Crab excessively stressed and held in tanks. Methods not representative of biomedical BMP due to excessive stress. Unexplained among-tank variation
Anderson et al. 2013	Durham, NH	56	17.9	Crab excessively stressed and held in various small tanks. Methods not representative of biomedical BMP due to elevated temperature and air exposure
Hamilton et al. 2019	Mariculture center, Bluffton, SC	100	11%	Bled and unbled crab held in seawater ponds at low densities for up to 8 weeks. Observed negative impact of heavy epibiont coverage

not a threat to their populations. Table 1 summarizes the results of 10 estimated biomedical mortality studies and relates their methods to best management practices. Two of the ten mortality studies addressed in Table 1 reported estimated mortality rates that were conspicuously high as outliers.

Challenges to conducting simulated postrelease studies include (1) containment in marine environments that allow for prompt renourishment of donors after being bled; (2) design of simulated processing steps that are representative of LAL industry practices (BMPs); and (3) storage in a nontoxic environment that has sufficient oxygen, salinity, and other requirements.

When these and other conditions are not met, anomalously high mortality rates are observed. For example, a small study of 56 crabs reported an 18% loss (Anderson et al. 2013). The excessive stress and containment in multiple small tanks rendered the experimental conditions as being nonrepresentative of biomedical LAL practices and unaligned with BMPs. Specimens were subjected to long periods out of water and high temperatures, when kept in a barrel in mid-day sun for 4 hours. The study reported observations termed “sublethal” effects of bleeding. This simply meant that horseshoe crabs were less active for a day or two after bleeding (Smith et al. 2016a). The study stated that animals no longer spawned after bleeding, but no data to supported this conclusion. In contrast, Hamilton et al. (2019) and Swan (photo by personal communication 2018) observed spawning activity within a week of bleeding for tagged specimens (Fig. 9). The 2019 Stock Assessment presented tagging data indicating that horseshoe crabs bled, tagged, and released did not experience a reduction in long-term survival due to bleeding when compared to animals that were just caught, tagged, and released.

A study by Leschen and Correia (2010) reported the effects of two LAL treatment methods on horseshoe crabs held in saltwater tanks at the MBL. The results indicated toxicity in at least four of the holding tanks. The methods section specified that three groups of horseshoe crabs were held in six flow-through seawater tanks that contained 5 cm of sediment. Tanks differed by volume but shared a common source and flow of seawater. A similar number of animals from each treatment group and a control was assigned to each tank. Although the mortality of horseshoe crabs was similar for the two treatment groups, there was a significant difference in mortality with respect to the tanks. Mortality did not align with treatment group. Mortality by tank varied from 8.7% to 48%. The mortality rate for tanks 1 and 4 averaged 12.7%, whereas the rate for tanks 3 and 5 averaged 45%; one control died in each of the tanks. This unexplained difference in mortality indicated that there was an apparent risk factor in at least two of the tanks, such as a chemical or microbial contaminant, overcrowded conditions, or failure to maintain a required provision condition, such as oxygen, that impacted negatively on female horseshoe crabs that were stressed by bleeding. Potentially, these conditions produced anomalously high, estimated postrelease mortality.

The 2019 Horseshoe Crab Benchmark Stock Assessment and Peer Review Report (ASMFC 2019) found no discernible evidence of adverse effects upon either horseshoe crabs or migratory birds from LAL production. The most salient finding

Fig. 9 A horseshoe crab bled and tagged June 28, 2018, and released to the south of Moores Beach, Delaware Bay, NJ. It was found spawning July 1, 2018, on Moores Beach. (Photo credit: J. Cooper)

was that the ASMFC estimated mortality is less than 1% of total estimated horseshoe crab mortality, rendering the 15% estimate a moot point. The Assessment and Peer Review Report's findings were that the natural mortality (24%), loss of habitat, bait fishing, and discards from various fisheries are the major threats to horseshoe crab sustainability. The major limitation of simulated holding studies is that the bled, stressed horseshoe crabs are not restored to their habitat for foraging and recovery, such as tidal flats. A recent study by Owings et al. (2019) found that the behavioral impacts of bleeding were short-lived, with bled crabs exhibiting similar biological rhythms and seasonal migratory behaviors to unbled crabs within 1–2 weeks after bleeding.

4.6 The Future of LAL Products

Technical advances reduce LAL needs. Charles River Labs attained FDA approval for a miniaturized LAL-cartridge-based system that reduces LAL content by 95%**.** Recombinant LAL products (rFC) are being evaluated for robustness, specificity, and sensitivity (e.g., Li et al. 2015; Tsuchiya 2020). The FDA has zero tolerance for endotoxin contamination and will not approve these products until they are validated as equivalent and specific as LAL for endotoxin detection, as done in the Baxter study described above. The US Pharmacopeia committed to introducing an Informational Chapter that provides a guideline for comparing recombinant LAL products with the horseshoe crab-derived LAL using reference standards and naturally occurring endotoxin in pharmaceutical water samples (Akers et al. 2020).

5 Summary and Conclusions

Rack-and-bag oyster culture on Delaware Bay tidal flats does not have deleterious effects on adult horseshoe crabs. Field studies (Sect. 3) indicate that the animals can successfully move in or around oyster farms and reach spawning beaches, and that crabs do not become entrained in farm gear when accessing spawning beaches. Moreover, the assertion that oyster aquaculture in the vicinity of the Cape Shore Laboratory is having harmful impacts to *Limulus* in an area of "prime horseshoe crab habitat" (Conservewildlifenj 2017) is undermined by indisputable evidence (Sect. 18.1) that this area has transitioned from the optimal sandy beach studied from the 1970s through the early 2000s to unsuitable habitat (peat banks and salt marsh) today. Some authors (e.g., Burger et al. 2015; Burger 2018) suggest that migratory shorebirds might show avoidance of oyster racks, but it should be noted that these field studies were conducted in a different portion of Delaware Bay (Reeds Beach), and deployed experimental racks that differed significantly in several ways from current aquaculture practices at the Cape Shore region. Historically, many more shorebirds have been found at Reeds Beach than at the Cape Shore

(Botton et al. 1994), and the absence of broad intertidal sand flats at Reeds Beach would, in any event, make this an unlikely area for the potential future expansion of oyster aquaculture. Interestingly, an oyster reef project at south Reeds Beach is being promoted as beneficial to both horseshoe crabs and shorebirds (e.g., Mirin 2015; Post 2015).

With regard to the effects of biomedical bleeding, simulated postbleeding mortality studies that are generally compatible with the biomedical BMPs indicate that the estimated biomedical mortality is less than 15%. The mortality caused by the biomedical industry is small in comparison to the bait fishery (Fig. 1). The most recent Horseshoe Crab Benchmark Stock Assessment and Peer Review Report (ASMFC 2019) found no credible evidence that biomedical use threatens the sustainability of the horseshoe crab or availability of their eggs for migratory birds. If approximately half of the 440,000 estimated horseshoe crabs collected for LAL production comes from the Delaware Bay stock, and half of these are female, then the number of Delaware Bay females bled would be about 110,000. Assuming 15% LAL-related mortality, then the number of females lost would be no more than 16,500 (under worst circumstances). That is about 0.2% of the 7.6 million female crabs estimated by the 2019 Assessment findings (ASMFC 2019). When one compares 0.2% with the 24% total mortality, LAL-related loss is small. Although further quantitative studies are required, discards (bycatch) of horseshoe crabs from various fisheries are likely to have a far more significant negative impact on horseshoe crab populations than biomedical bleeding (ASMFC 2019). The deleterious effects of the biomedical industry for horseshoe crab sustainability are minimized because of consistent and unique conservation efforts, such as the catch-and-release policy, support for banning the bait fishery, adherence to LAL good management practices, and coordination of the Delaware Bay horseshoe crab spawning survey.

In conclusion, we believe that the ongoing loss of high-quality spawning habitat is a greater threat to horseshoe crabs in Delaware Bay than oyster culture and biomedical mortality. We believe that future discussion needs to focus on the preservation and, if feasible, replenishment of the remaining optimal habitats. Given the inevitable landward retreat of beaches in an era of ongoing sea level rise, planners must envision where future sandy beach will likely be located – these are not necessarily the same places where beaches are found today. This will entail further studies of the sediment budget along Delaware Bay. We also believe that conversations and hard decisions should be made concerning the relative merits of shoreline armoring, beach nourishment, and buy-outs and abandonment of heavily impacted areas (Loveland and Botton 2015).

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References

B. Akbar John, B.R. Nelson, H.I. Sheikh, S.G. Cheung, Y. Wardiatno, B.P. Dash, K. Tsuchiya, Y. Iwasaki, S. Pati, A review on fisheries and conservation of Asian horseshoe crabs. Biodivers. Conserv. **27**, 3573–3598 (2018). https://doi.org/10.1007/s105331-018-1633-8

J. Akers, D. Guilfoyle, D. Hussong, K. McCullough, R. Mello, D. Singer, E. Tidswell, R. Tirumalai, Functinal challenges for alternative bacterial endotoxins tests. American Pharm Review **2020**, 76–81 (2020)

R.L. Anderson, W.H. Watson, C.C. Chabot, Sublethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, *Limulus polyphemus*. Biol. Bull. **225**, 137–151 (2013)

Atlantic States Marine Fisheries Commission (ASMFC), Interstate fishery management plan for horseshoe crab. Atlantic States Marine Fisheries Commission, Fishery Management Report No. 32. Washington, DC (1998)

Atlantic States Marine Fisheries Commission (ASMFC), Horseshoe crab biomedical ad-hoc working group report (2011), www.asmfc.org/uploads/file/biomedAdHocWGReport_Oct2011.pdf. Accessed May 2018

Atlantic States Marine Fisheries Commission (ASMFC), 2019 horseshoe crab benchmark stock assessment and peer review report (2019), www.asmfc.org/uploads/file/5cd5d6f1HSCAssessment_PeerReviewReport_May2019.pdf. Accessed July 2019

A.J. Baker, P.M. González, T. Piersma, L.J. Niles, I. de Lima do Nascimento, P.W. Atkinson, N.A. Clark, C.D.T. Minton, M.K. Peck, G. Aarts, Rapid population decline in red knots: Fitness consequences of decreasing refuelling rates and late arrival in Delaware Bay. Proc. Biol. Sci. **271**, 875–882 (2004)

F.B. Bang, A bacterial disease of *Limulus polyphemus*. Bull. Johns Hopkins Hosp. **98**, 325–351 (1956)

L.T. Barrett, S.E. Swearer, T. Dempster, Impacts of marine and freshwater aquaculture on wildlife: A global meta-analysis. Rev. Aquac., 1–23 (2018). https://doi.org/10.1111/raq.12277

S.L. Billing, Using public comments to gauge social licence to operate for finfish aquaculture: Lessons from Scotland. Ocean Coast. Manag. **165**, 401–415 (2018)

B. Błażejowski, The oldest species of the genus *Limulus* from the Late Jurassic of Poland, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 3–14

M.L. Botton, The ecological importance of horseshoe crabs in estuarine and coastal communities: A review and speculative summary, in *Biology and Conservation of Horseshoe Crabs*, ed. by J. T. Tanacredi, M. L. Botton, D. R. Smith, (Springer, New York, 2009), pp. 45–64

M.L. Botton, C.P. Colón, J. Rowden, S. Elbin, D. Kriensky, K. McKown, M. Sclafani, R. Madden, Effects of a beach nourishment project in Jamaica Bay, New York on horseshoe crab (*Limulus polyphemus*) spawning activity and egg deposition. Estuar. Coasts **41**, 974–987 (2018). https://doi.org/10.1007/s12237-017-0337-8

M.L. Botton, B.A. Harrington, Synchronies in migration: Shorebirds, horseshoe crabs, and Delaware Bay, in *The American Horseshoe Crab*, ed. by C. N. Shuster, R. B. Barlow, H. J. Brockmann, (Harvard Press, Cambridge, 2003), pp. 6–32

M.L. Botton, R.E. Loveland, T.R. Jacobsen, Beach erosion and geochemical factors: Influence on spawning success of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay. Mar. Biol. **99**, 325–332 (1988)

M.L. Botton, R.E. Loveland, T.R. Jacobsen, Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and the abundance of horseshoe crab (*Limulus polyphemus*) eggs. Auk **111**, 605–611 (1994)

M.L. Botton, J.W. Ropes, The horseshoe crab, *Limulus polyphemus*, fishery and resource in the United States. Mar. Fish. Rev. **49**, 57–61 (1987a)

M. L. Botton, J. W. Ropes, Populations of horseshoe crabs, *Limulus polyphemus*, on the Northwestern Atlantic continental shelf. Fish Bull., **85**, 805–812 (1987b)

J. Burger, L.J. Niles, A.D. Dey, T. Dillingham, A.S. Gates, J. Smith, An experiment to examine how Red Knots *Calidris canutus rufa* and other shorebirds respond to oyster culture at Reeds Beach, Delaware Bay, New Jersey. Wader Stud **122**, 89–98 (2015)

J. Burger, Use of intertidal habitat by four species of shorebirds in an experimental array of oyster racks, reefs and controls on Delaware Bay, New Jersey: Avoidance of oyster racks. Sci. Total Environ. **624**, 1234–1243 (2018)

M.D. Callier, C.J. Byron, D.A. Bengtson, P.J. Cranford, S.F. Cross, U. Focken, H.M. Jansen, P. Kamermans, A. Kiessling, T. Landry, F. O'beirn, Attraction and repulsion of mobile wild organisms to finfish and shellfish aquaculture: A review. Rev Aquacult **10**(4), 924–949 (2017)

L.M. Calvo, New Jersey shellfish aquaculture situation and outlook report. 2015 Shellfish aquaculture survey results. Haskin Shellfish Research Laboratory, Port Norris, pp. 1–12 (2016), https://hsrl.rutgers.edu/outreach/aquaculture/AquacultureReports/NJAquaculture_Survey_Yr2016.pdf. Accessed 1 Dec 2018

C. Carson, Medical labs may be killing horseshoe crabs (2016), https://www.scientificamerican.com/article/medical-labs-may-be-killing-horseshoe-crabs/. Accessed 12 Jan 2019

G. Castro, J.P. Myers, Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. Auk **110**, 927–930 (1993)

K.E. Clark, L.J. Niles, J. Burger, Abundance and distribution of migrant shorebirds in Delaware Bay. Condor **95**, 694–705 (1993)

Conservewildlifenj, 20 years on Delaware Bay: Shorebird need more horseshoe crabs (2017), http://www.conservewildlifenj.org/blog/wp-content/uploads/2017/06/Del-Bay-blog-5-img-6.htm. Accessed 21 January 2019

J.F. Cooper, J. Levin, H.N. Wagner Jr., Quantitative comparison of in vitro (*Limulus)* and in vivo (rabbit) methods for detection of endotoxin. J. Lab. Clin. Med. **78**, 138–148 (1971)

J.F. Cooper, H.D. Hochstein, E.B. Seligmann Jr., The limulus test for endotoxin (pyrogen) in radiopharmaceuticals and biologicals. Bull Parenter Drug Assoc **34**, 153–162 (1972)

M.J.P. Cooper, M.D. Beevers, M. Oppenheimer, The potential impacts of sea level rise on the coastal region of New Jersey, USA. Clim. Chang. **90**, 475–492 (2008)

Food and Drug Administration (FDA), CFR Title 21 Part 210 Current good manufacturing practices (2018), https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=210.1

S.E. Ford, H.H. Haskin, Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). J. Parasitol. **73**, 368–376 (1987)

S.E. Ford, H.H. Haskin, History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. J. Invertebr. Pathol. **40**(1), 118–141 (1982)

P. Funch, T. Wang, C. Pertoldi, A.B. Middelbo, Low oxygen levels slow embryonic development of *Limulus polyphemus*. Biol. Bull. **231**, 113–119 (2016). https://doi.org/10.1086/690091

G. Gauvry, Current horseshoe crab harvesting practices cannot support global demand for TAL/LAL: The pharmaceutical and medical device industries' role in the sustainability of horseshoe crabs, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 475–500

K.L. Hamilton, L.E. Burnett, K.G. Burnett, R.E.G. Kalisperis, A.E. Fowler, Physiological impacts of time in holding ponds, biomedical bleeding, and recovery on the Atlantic horseshoe crab, *Limulus polyphemus*. Comp. Biochem. Physiol. A **239** (2019). https://doi.org/10.1016/j.cbpa.2019.110554. Accessed June 2020

H. Haskin, S.E. Ford, Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. Mar. Fish. Rev. **41**, 54–63 (1979)

R. Hilborn, J. Banobi, S.J. Hall, T. Pucylowski, T.E. Walsworth, The environmental cost of animal source foods. Front. Ecol. Environ. **16**(6), 329–335 (2018)

L. Hurton, J. Berkson, Potential causes of mortality for horseshoe crabs (*Limulus polyphemus*) during the biomedical bleeding process. Fish. Bull. **104**, 293–298 (2006)

N.L. Jackson, K.F. Nordstrom, Strategies to conserve and enhance sandy barrier habitat for horseshoe crabs (*Limulus polyphemus*) on developed shorelines in Delaware Bay, USA, in *Biology and Conservation of Horseshoe Crabs*, ed. by J. T. Tanacredi, M. L. Botton, D. R. Smith, (Springer, New York, 2009), pp. 399–416

S.M. Karpanty, J. Cohen, J.D. Fraser, J. Berkson, Sufficiency of horseshoe crab eggs for red knots during spring migration stopover in Delaware Bay USA. J. Wildl. Manag. **75**, 984–994 (2011)

H.J. Knebel, C.H. Fletcher III, J.C. Kraft, Late Wisconsinian-Holocene paleogeography of Delaware Bay: A large coastal plain estuary. Mar. Geol. **83**, 115–133 (1988)

J.N. Kraeuter, S.R. Fegley, Vertical disturbance of sediments by horseshoe crabs (*Limulus polyphemus*) during their spawning season. Estuaries **17**, 288–294 (1994)

G. Kreamer, S. Michels, History of horseshoe crab harvest on Delaware Bay, in *Biology and Conservation of Horseshoe Crabs*, ed. by J. T. Tanacredi, M. L. Botton, D. R. Smith, (Springer, Boston, 2009), pp. 299–313

G. Kreamer, S. Kreamer, Green eggs and sand, team *Limulus*, and more: Educating for horseshoe crab conservation in the United States, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 557–574

R.G.J. Lathrop, L. Niles, D. Merchant, T. Farrell, C. Licitra, Mapping and assessing critical horseshoe crab spawning habitats of Delaware Bay: Update to 2007/10 and post-Sandy. Walton Center for Remote Sensing and Spatial Analysis, Rutgers University, New Brunswick (2013), Accessed online https://crssa.rutgers.edu/projects/hcrab/reports/CRSSAreport_20130916_Horseshoe_Crab_Habitat_in_DelBay_Update.pdf 17 June 2020

K. Laurie, C.-P. Chen, S.G. Cheung, V. Do, H. Hsieh, A. John, F. Mohamad, S. Seino, S. Nishida, P. Shin, M. Yang, *Tachypleus tridentatus*. The IUCN Red List of Threatened Species 2019: e.T21309A149768986 (2019), https://doi.org/10.2305/IUCN.UK.2019-1.RLTS.T21309A149768986.en. Accessed 8 Aug 2019

A.S. Leschen, S.J. Correia, Mortality in female horseshoe crabs (*Limulus polyphemus*) from biomedical bleeding and handling: Implications for fisheries management. Mar. Freshw. Behav. Physiol. **43**, 135–147 (2010)

J. Levin, F.B. Bang, The role of endotoxin in the extracellular coagulation of *Limulus* blood. Bull. Johns Hopkins Hosp. **115**(3), 265–274 (1964)

P. Li, B. Ho, J.L. Ding, Biotechnology efforts to conserve horseshoe crabs through the development of recombinant factor C-based endotoxin test, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 501–512

Y. Liao, H.L. Hsieh, S. Xu, Q. Zhong, J. Lei, M. Liang, H. Fang, L. Xu, W. Lin, X. Xiao, C.P. Chen, S.G. Cheung, B.K.Y. Kwan, Wisdom of crowds reveals decline of Asian horseshoe crabs in Beibu Gulf, China. Oryx **53**, 222–229 (2019)

R.E. Loveland, M.L. Botton, Sea level rise in Delaware Bay: Adaptations of spawning horseshoe crabs (*Limulus polyphemus*) to the glacial past, and the rapidly changing shoreline of the bay, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Managemen*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 41–63

C.P. McGowan, D.R. Smith, J.A. Sweka, J. Martin, J.D. Nichols, R. Wong, J.F. Lyons, L.J. Niles, K. Kalasz, J. Brust, Multispecies modeling for adaptive management of horseshoe crabs and red knots in the Delaware Bay. Nat. Resour. Model. **24**, 117–156 (2011)

C.P. McGowan, D.R. Smith, J.D. Nichols, J.E. Lyons, J. Sweka, K. Kalasz, L.J. Niles, R. Wong, J. Brust, M. Davis, B. Spear, Implementation of a framework for multi-species, multi-objective adaptive management in Delaware Bay. Biol. Conserv. **191**, 759–769 (2015)

M. Mengel, A. Nauels, J. Rogelj, C.-F. Schleussner, Committed sea-level rise under the Paris Agreement and the legacy of delayed mitigation action. Nat. Commun. **9**, 601 (2018). https://www.nature.com/articles/s41467-018-02985-8

M.J. Millard, J.A. Sweka, C.P. McGowan, D.R. Smith, Assessment and management of North American horseshoe crab populations, with emphasis on a multispecies framework for Delaware Bay, U.S.A. populations, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 407–431

B. Mirin, Red Knot rescue (2015), https://www.audubon.org/news/red-knot-rescue. Accessed 12 Jan 2019

D.S. Mizrahi, K.A. Peters, Relationships between sandpipers and horseshoe crabs in Delaware Bay: A synthesis, in *Biology and Conservation of Horseshoe Crabs*, ed. by J. T. Tanacredi, M. L. Botton, D. R. Smith, (Springer, New York, 2009), pp. 65–87

R.A. Moursund, T.J. Carlson, R.D. Peters, A fisheries application of a dual-frequency identification sonar acoustic camera. ICES J. Mar. Sci. **60**(3), 678–683 (2003)

D. Munroe, D. Bushek, P. Woodruff, L. Calvo, Intertidal rack-and-bag oyster farms have limited interaction with horseshoe crab activity in New Jersey, USA. Aquac. Environ. Interact. **9**, 205–211 (2017). https://doi.org/10.3354/aei00227

D.M. Munroe, T.M. Grothues, N.E. Cleary, J. Daw, S. Estrada, Oyster aquaculture does not impede spawning beach access for Atlantic horseshoe crabs *Limulus polyphemus*. Aquac. Environ. Interact. **12**, 81–90 (2020). https://doi.org/10.3354/aei00351

J.P. Myers, Sex and gluttony on Delaware Bay. Nat. Hist. **95**(5), 68–77 (1986)

L.J. Niles, J. Bart, H.P. Sitters, A.D. Dey, K.E. Clark, P.W. Atkinson, A.J. Baker, K.A. Bennett, K.S. Kalasz, N.A. Clark, J. Clark, S. Gillings, A.S. Gates, P.M. González, D.E. Hernandez, C.D.T. Minton, R.I.G. Morrison, R.R. Porter, R.K. Ross, C.R. Veitch, Effects of horseshoe crab harvest in Delaware Bay on Red Knots: Are harvest restrictions working? Bioscience **59**, 153–164 (2009)

C. Nishimura, C. Iwaoka, Building local community awareness of horseshoe crabs in Kujukushima, Nagasaki, Japan, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 575–585

M. Owings, C. Chabot, W. Watson III, Effects of the biomedical bleeding process on the behavior of the American horseshoe crab, *Limulus polyphemus*, in its natural habitat. Biol. Bull. **236**, 207–223 (2019). https://doi.org/10.1086/702917

D. Palmquist, Nature returns: Restoration brings back birds (2018), https://medium.com/usfishandwildlifeservicenortheast/nature-returns-restoration-brings-back-birds-ed715091c9b2/. Accessed 12 Jan 2019

F.C. Pearson, M. Weary, The significance of *Limulus* amebocyte lysate specificity on the pyrogen evaluation of parenteral drugs. Bull. Parenter. Drug Assoc. **34**(2), 103–108 (1980)

D. Penn, H.J. Brockmann, Nest-site selection in the horseshoe crab *Limulus polyphemus*. Biol. Bull. **187**, 373–384 (1994)

J. Post, Future of horseshoe crabs looks better (2015). Cape May County Herald, 12 Feb 2015, https://www.capemaycountyherald.com/news/environment/article_5f595461-a7e0-5537-9c15-c928f7c6ec9c.html. Accessed 12 Jan 2019

C.S. Price, E. Keane, D. Morin, C. Vaccaro, D. Bean, J.A. Morris Jr, Protected species and marine aquaculture interactions (2017). NOAA Technical Memorandum NOS NCCOS 211. Beaufort, NC. p 85, https://doi.org/10.7289/V5/TM-NOS-NCCOS-211

A. Rudloe. The effect of heavy bleeding on mortality of the horseshoe crab, Limulus polyphemus, in the natural environment. J. Invert. Pathol. **42**, 167–176 (1983)

D.M. Rudkin, G.A. Young, Horseshoe crabs – An ancient ancestry revealed, in *Biology and Conservation of Horseshoe Crabs*, ed. by J. T. Tanacredi, M. L. Botton, D. R. Smith, (Springer, New York, 2009), pp. 25–44

F.B. Seibert, The cause of many febrile reactions following intravenous injections. Am. J. Phys. **71**, 621–651 (1925)

C.N. Shuster Jr, On morphometric and serological relationships within the Limulidae, with particular reference to *Limulus polphemus* L. PhD thesis, New York University, New York. Diss Abstr 18, pp. 371–372 (1955)

C.N. Shuster Jr., King crab fertilizer: A once-thriving Delaware Bay industry, in *The American Horseshoe Crab*, ed. by C. N. Shuster Jr., R. B. Barlow, H. J. Brockmann, (Harvard University Press, Cambridge, MA, 2003), pp. 341–357

C.N. Shuster Jr., The Delaware Bay area, U.S.A.: A unique habitat of the American of horseshoe crab, *Limulus polyphemus*, in *Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management*, ed. by R. H. Carmichael, M. L. Botton, P. K. S. Shin, S. G. Cheung, (Springer International, Switzerland, 2015), pp. 15–39

C.N. Shuster Jr., M.L. Botton, A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.), in Delaware Bay. Estuaries **3**, 363–372 (1985)

S.E. Shumway, C. Davis, R. Downey, R. Karney, J. Kraeuter, J. Parsons, R. Rheault, G. Wikfors, Shellfish aquaculture–in praise of sustainable economies and environments. World Aquacult. **34**(4), 8–10 (2003)

D.R. Smith, E.M. Hallerman, M.J. Millard, J.A. Sweka, R.G. Weber, An incomplete analysis: Response to Niles et al. 'Are harvest restrictions working?'. Bioscience **59**, 541 (2009)

D.R. Smith, N.L. Jackson, K.F. Nordstrom, R.G. Weber, Beach characteristics mitigate effects of onshore wind on horseshoe crab spawning: Implications for matching with shorebird migration in Delaware Bay. Anim. Conserv. **14**, 575–584 (2011)

D.R. Smith, H.J. Brockmann, M.A. Beekey, T.L. King, M.J. Millard, J. Zaldivar-Rae, Conservation status of the American horseshoe crab, (*Limulus polyphemus*): A regional assessment. Rev. Fish Biol. Fish. **27**, 135–175 (2016a)

D.R. Smith, H.J. Brockmann, M.A. Beekey, T.L. King, M.J. Millard, J. Zaldivar-Rae (2016b) *Limulus polyphemus*. The IUCN Red List of Threatened Species 2016: e.T11987A80159830, https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T11987A80159830.en. Downloaded on 04 January 2019

B.L. Swan, Migrations of adult horseshoe crabs, *Limulus polyphemus*, in the middle Atlantic bight: A 17-year tagging study. Estuaries **28**, 28–40 (2005)

M. Thompson, Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in the South Atlantic Bight. M.S. thesis, 136 p. Univ. Charleston, Charleston (1998)

M. Tsuchiya, Innovative mechanism of *limulus* amebocyte lysatae activation to achieve specificity and sensitivity to endotoxin; comparison with recombinant factor c reagents. Int. J. Develop. Res. **14**, 36751–36756 (2020). https://doi.org/10.37118/ijdr.19019.05.2020

A.M. Tucker, C.P. McGowan, M.J. Catalano, A. DeRose-Wilson, R.A. Robinson, J. Zimmerman, Foraging ecology mediates response to ecological mismatch during migratory stopover. Ecosphere **10**(10), e02898 (2019). https://doi.org/10.1002/ecs2.2898

US Fish and Wildlife Service (USFWS), Endangered and threatened wildlife and plants; threatened species status for the rufa Red Knot. Fed. Regist. **79**(238), 73706–73748 (2014)

O.A. van der Schatte, L. Jones, L.L. Vay, M. Christie, J. Wilson, S.K. Malham, A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac., 1–23 (2018). https://doi.org/10.1111/raq.12301

M.C. Vasquez, A. Murillo, H.J. Brockmann, D. Julian, Multiple stressor interactions influence embryo development rate in the American horseshoe crab, *Limulus polyphemus*. J. Exp. Biol. **218**, 2355–2364 (2015)

E.A. Walls, J. Berkson, Effects of blood extraction on horseshoe crabs (*Limulus polyphemus*). Fish. Bull. **101**, 457–459 (2003)

W.L. Walsh, Biological opinion on the effects of existing and expanded structural aquaculture of native bivalves in Delaware Bay, Middle and Lower Townships, Cape May County, New Jersey on the federally listed red knot (*Calidris canutus rufa*). US Fish and Wildlife Service, New Jersey Field Office, Galloway, p. 175 (2016), Accessed online (https://www.fws.gov/northeast/njfieldoffice/pdf/AquaculturePBO_20160401.pdf) 17 June 2020

J.R. Weggel, The impact of opening an inlet in the Delaware Bay at Norbury's Landing, NJ. J Coast. Res. **59**(S1), 273–279 (2011)

D. Yadon, An evaluation of Limulus post-bleeding mortality experiment data: statistical analyses performed by South Carolina Department of Natural Resources (SCDNR) in cooperation with Endosafe, Inc. SCDNR report, 4 pp. (1999)

N.A.M. Zaukia, B. Satyanarayanaa, N. Fairuz-Fozia, B.R. Nelson, M.B. Martin, B. Akbar-John, A.J.K. Chowdhury, Citizen science frontiers horseshoe crab population regain at their spawning beach in East Peninsular Malaysia. J. Environ. Manag. **232**, 1012–1020 (2019). https://doi.org/10.1016/j.jenvman.2018.12.002