Chapter 28
Biomedical Implications for Managing the *Limulus polyphemus* Harvest Along the Northeast Coast of the United States

Thomas J. Novitsky

Abstract North American horseshoe crabs (*Limulus polyphemus*) have been continuously harvested in Massachusetts for the production of *Limulus* amebocyte lysate (LAL); since the mid 1970s as bait for American eel (*Anguilla rostrata*); and since the mid 1990s as bait for whelk (*Busycon* ssp.). Harvest regulations were promulgated by the Atlantic States Marine Fisheries Commission (ASMFC) in 1998 over concern for the observed decline of the Red Knot (*Calidris canutus*), a correlation between its feeding behavior (horseshoe crab eggs during the spring migration), and a decline in spawning horseshoe crabs likely due to harvesting for bait. Generally, horseshoe crab harvest for the production of LAL (biomedical use) is exempted from harvest regulations, since the animals bled for LAL production are returned alive to the ocean, and mortality is considered low. However, evidence is accumulating that mortality of bled horseshoe crabs is higher than originally thought (29 % vs 15 %); that females may have an impaired ability to spawn following bleeding and release; and that bled crabs become disoriented and debilitated for various lengths of time following capture, handling, bleeding, and release. This paper reviews the latest evidence for mortality and impairment of horseshoe crabs bled for biomedical use, especially in Massachusetts where horseshoe crab populations in small bays and inlets are particularly vulnerable, and since State regulations allow for using bled crabs as bait rather than returning to the site of capture. A novel management plan is proposed that can satisfy all affected parties as well as insure a continued supply of horseshoe crabs for the vitally important production of LAL. This plan may also serve as a model for other areas of the Atlantic coast where biomedical harvest occurs.

Keywords Limulus amebocyte lysate (LAL) • Biomedical • Atlantic States Marine Fisheries Commission (ASMFC) • Massachusetts • Best Management Practices (BMP) • Mortality • Sublethal effects • Artificial bait • Alternative assays • Food and Drug Administration (FDA) • Open reporting • Dual use

T.J. Novitsky (✉)
Horseshoe Crab Conservation Association, P.O. Box 2334, Orleans, 02653-2334 MA, USA
e-mail: tjnovitsky@gmail.com

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28.1 Introduction

The American horseshoe crab, *Limulus polyphemus*, has long been of interest to researchers due to the properties of its blood (hemolymph) and blood cells (amebocytes) (Howell 1885; Loeb 1902). In addition to the interest generated in studying a "living fossil" since the horseshoe crab was recognizable in the fossil record back some 445 mya, and nearly identical in gross morphology to fossils from 250 mya (Rudkin et al. 2008), use of *Limulus* as a research tool was facilitated by its abundance, size, ease of collection, suitability to be maintained in aquaria, and, perhaps most importantly, by the design of its circulatory system and large blood volume. These attributes most likely contributed to the decision by Dr. Frederik Bang in 1953 to use the horseshoe crab as a surrogate for studying the clotting mechanisms of human blood (Bang 1953). Subsequent discoveries by Bang and colleague Dr. Jack Levin (Levin and Bang 1968) led to an assay for endotoxin (pyrogen) employing an amebocyte extract, *Limulus* amebocyte lysate (LAL).

In October 1978, a symposium on the "Biomedical Applications of the Horseshoe Crab (Limulidae)" was held at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts (Cohen et al. 1979). This symposium's location, timing, and subject matter reflected an incredible growth in interest in the horseshoe crab, especially LAL. At the time of the symposium, it had been only just over 1 year since the United States Food and Drug Administration (FDA) licensed the first manufacturer, Associates of Cape Cod, Inc, also located in Woods Hole, MA to produce LAL for use to detect pyrogen (endotoxin) in injectable pharmaceuticals (Novitsky 1991, 2009; Levin et al. 2003). Although the symposium covered all aspects of horseshoe crab biology, the majority of presentations related to the biomedical use of the recent commercial availability of LAL. It is notable, even at this early stage of the LAL industry, that concern over the survival of the species was raised. Anne Rudloe (1979) who would later be contracted by the FDA to conduct a study examining the mortality associated with horseshoe crabs bled to produce LAL stated in her presentation: "The emerging importance of *Limulus* to biomedical research, as the source of *Limulus* lysate, requires more complete knowledge of the biology of the species, so that it can be wisely managed as a natural resource." She went on to say: "There has developed in recent years a substantial harvest of horseshoe crabs for this purpose. Such harvesting *has most often occurred on breeding beaches*." In another paper, Sydney R. Galler (1979), Assistant Secretary for Environmental Affairs, US Department of Commerce, called on the conference participants as well as the scientific community to develop a practical plan for the conservation and protection of the horseshoe crab in parallel with their efforts to disseminate information about biomedical applications. Thus from the very beginning of the LAL industry, population depletion was a concern. To be fair, Jack Levin released the horseshoe crabs he used for his research immediately following bleeding and delighted in watching them swim away from the dock near the MBL (personal communication). Likewise, Associates of Cape Cod, Inc. instituted a policy of immediate release followed by the rest of the industry. This practice also became part of the

FDA's LAL licensing requirements (Levin et al. 2003) until the FDA relinquished authority for the general handling of horseshoe crabs prior to bleeding and the disposition of horseshoe crabs following bleeding to the ASMFC in 2002 (Horseshoe crab technical committee, Atlantic States Marine Fisheries Commission Draft, March 5–6, 2002). Biomedical use, i.e. the production of LAL, continues to be regulated by the FDA but only with respect to the quality of the reagent, not to the harvest or final disposition of the horseshoe crabs used as a raw material.

It was not until 1998 with the institution of a Horseshoe Crab Working Group organized under the auspices of the Atlantic States Marine Fisheries Commission (ASMFC) that coast-wide regulations for the harvest of horseshoe crabs was instituted (ASMFC 1998). Prior to 1998, certain states (Rhode Island, Massachusetts, New York, and Florida) had no catch limit and no reporting, but required a license. Virginia also had no catch limit but required a license and prohibited capture by trawl and dredge. Other Atlantic states (New Hampshire, Delaware, Maryland, and South Carolina) had various regulations covering limits as well as other aspects of capture (ASMFC 1998). Also, up until 1998 regulation in federal waters varied along the coast. For example, harvest in certain National Wildlife Refuges and National Seashores was allowed either by precedent, i.e. horseshoe crab harvest had occurred historically, or as was the case in the Monomoy National Wildlife Refuge (NWR), allowed by permit (Compatibility Determination Eastern Massachusetts National Wildlife Refuge Complex 2002; James-Pirri 2002). Prior to 1998, commercial horseshoe crab harvesting had been ongoing for many years on Monomoy NWR. Officially, however, commercial harvest of horseshoe crabs was never allowed on refuge land or waters due to the fact that a compatibility determination was never completed by the United States Fish and Wildlife Service, which had authority over the refuge. However, after discussions with the refuge staff, Jay Harrington was issued a 'Special Use Permit' in 1991 to legally harvest horseshoe crabs in "closed areas" of the refuge, specifically for the biomedical company, Associates of Cape Cod, Inc. Permits were renewed annually, and a scheduled compatibility review conducted in 1994 reconfirmed that the harvest of horseshoe crabs for biomedical use was compatible. However in 1999, due to public concern over reports of excessive horseshoe crab harvesting in the refuge, it was determined that the Monomoy NWR did not have the authority to regulate intertidal areas within the refuge boundary. As a result, in 2000, the United States Fish and Wildlife Service refused to renew a harvest permit for Jay Harrington (Compatibility Determination Eastern Massachusetts National Wildlife Refuge Complex 2002). This action was accompanied by a near simultaneous denial for harvest by the National Park Service in the nearby waters of the Cape Cod National Seashore. Facing the loss of a significant portion of their raw material supply, Associates of Cape Cod, Inc. and Jay Harrington, whose very livelihood mainly depended on harvesting horseshoe crabs, brought legal action against the United States Department of Interior (DOI) and were granted an injunction to continue harvesting in these areas for the 2000 and 2001 seasons (Associates of Cape Cod, Inc. 2000). The DOI prevailed, however, and in May of 2001 the government's win resulted in the prohibition of horseshoe crab harvest for any reason in the National Seashore and in the Monomoy National

Wildlife Refuge (actually a ban was instituted for the refuge until a new compatibility study could be completed) (Compatibility Determination Eastern Massachusetts National Wildlife Refuge Complex 2002). The ruling eventually resulted in a ban for the harvesting of horseshoe crabs in all federal waters (James-Pirri 2012).

Coast-wide harvest regulations begun in 1998 were promulgated not from a concern for the future of the increasingly important LAL (biomedical) industry, but from an environmental concern over a potential lack of food (horseshoe crab eggs) for migratory shorebirds, in particular the Red Knot (*Calidris canutus*), that stopped in the Delaware Bay during migration to their Arctic nesting grounds. This migration synchronized perfectly with the annual horseshoe crab spawning event, and the birds needed this critical stopover and the excellent nutrition from horseshoe crab eggs for a successful migration (Mizrahi and Peters 2009).

To further protect the LAL industry that used far fewer crabs than the bait industry, and since mortality from bleeding was considered insignificant (most bled crabs were returned to their environment alive), the biomedical industry was exempt from restrictions on harvesting horseshoe crabs with the exception of a requirement to report the number of horseshoe crabs bled (ASMFC 1998; Novitsky 2009). The extremely small number of horseshoe crabs harvested specifically for research is considered inconsequential and taking for research purposes is completely exempt.

Currently harvest restrictions in the US seem to have stabilized horseshoe crab populations, although in the Northeast where there are many small populations restricted to bays and inlets, the ASMFC and State-specific limits may not be sufficient (Smith et al. 2009). The 'stock status determination' of the ASMFC concluded "…the evidence from New York and New England suggest that the current harvest within these regions is not sustainable." In Wellfleet Harbor, for example, over-fishing for bait has resulted in a horseshoe crab decline when compared to other nearby areas (James-Pirri 2002; James-Pirri et al. 2005). The horseshoe crab harvest is thought to have degraded the Wellfleet Harbor's bottom, which is thought to adversely affect the shellfish harvest (Town of Wellfleet, The Wellfleet Shellfish Advisory Board Letter to Paul J. Diodati, Director of Massachusetts DMF, 2012). In addition, there is no longer a biomedical harvest in the bay since the females are too small (personal communication, Town of Wellfleet, The Wellfleet Shellfish Advisory Board 2012 Letter to Paul J. Diodati, Director of Massachusetts DMF). Other environmental factors on Cape Cod, Massachusetts, in particular poorly located shoreline stabilization and beach nourishment projects, may also reduce horseshoe crab populations by their impact on critical spawning habitat (Fabie 2009).

Other examples abound. In a relatively compact area near Mashnee Dike on the upper reaches of Buzzards Bay Massachusetts, a small but stable breeding population that had been studied for years by scientists from the Marine Biological Laboratory in Woods Hole was virtually wiped out using a hand harvest during spawning (Widener and Barlow 1999). A similar situation was found at Stage Harbor (Widener and Barlow 1999). Two other surveyed bays in Massachusetts, Wellfleet Harbor and Cape Cod Bay have also shown serious declines related to spawning indices (Faherty 2012). This type of extermination of local populations most likely repeats itself in many other bays and inlets in the Northeast even though state-wide harvest quotas initially set by the ASMFC are never reached, i.e. demand for bait seems to be satisfied (ASMFC 2013). Basically the horseshoe crab is an easy fishing target during its most vulnerable spawning period. Crabs come into shallow water on sandy beaches that are well known to fishermen and where they are easily captured by hand/rake from small boats. This type of harvest should raise an immediate concern for the continued survival of this species (Fabie 2009). Harvest numbers from the other two Northeastern states, Maine and New Hampshire, while having small populations of horseshoe crabs and regulated bait harvests, are considered *de minimis* by the ASMFC Interstate Fisheries Management Fisheries Program Charter. *De minimis* is defined as "a situation in which, under existing condition of the stock and scope of the fishery, conservation, and enforcement actions taken by an individual state would be expected to contribute insignificantly to a coastwide conservation program required by a Fishery Management Plan or amendment." Maine and New Hampshire reported to the ASMFC bait harvest totals from 1998 to 2003 and 1998 to 2002 respectively until they were granted *de minimis* status. Application for this status can be made if average horseshoe crab bait landings for two consecutive years constitute less than one percent of the coastwide total. Once granted, ASMFC member states are no longer required to report their harvests as long as the conditions for maintaining their status continue (ASMFC 2001).

Although it was recognized that there was mortality associated with biomedical use, these limits were regarded as insignificant and initially based on the 10–15 % reported by Rudloe (1983). Several recent papers have reported mortalities between 10 and 30 %. Most of these studies attempted to control conditions for bleeding and handling crabs and some have attempted to mimic conditions currently employed by the LAL industry (Walls and Berkson 2003; Hurton and Berkson 2006; Leschen and Correia 2010; Anderson et al. 2013). Due to the variations in methodology, it is difficult to compare these studies. It would be best to copy the tagging and release modality used originally by Rudloe (1983), i.e. tagging experimental (bled) and controls (unbled) horseshoe crabs then releasing them back into the ocean environment and subsequently calculating mortality based on the numbers of recovered tagged animals. However, with Rudloe's (1983) method, it is extremely difficult to recover sufficient animals to reach statistical significance without initially using a very large number of animals. Rudloe (1983) for example, barely achieved statistical significance with her 2-year study. Unfortunately nearly all of the mortality studies following Rudloe's study (1983) have used ponds or aquaria for observing bled horseshoe crabs and controls. These studies, of course, can produce their own experimental bias, and the ones showing highest mortalities are often criticized by the LAL industry by employing the unproven argument that bled horseshoe crabs released to their "natural" environment would fare much better than those released to "artificial" environments. For now the ASMFC "accepts" mortality for bled horseshoe crabs released to the environment as 15–30 % (ASMFC 2013).

In addition to mortality, the vitality of crabs released following bleeding for biomedical use may now be cause for concern. The strongest circumstantial evidence for this may be the extremely skewed ratios of female to male crabs in Pleasant Bay, an area in Massachusetts that has been traditionally harvested for biomedical use (Carmichael et al. 2003) and since 2006 has been set aside solely for biomedical harvest, i.e. no bait harvest allowed (James-Pirri et al. 2005; Leschen et al. 2005; Massachusetts Division of Marine Fisheries 2006; James-Pirri 2012). Recently, direct experimental evidence has been published (see Sect. 28.4, below) that supports an adverse effect on bled crabs that have been released (Kurz and James-Pirri 2002; Leschen and Correia 2010; James-Pirri et al. 2012; Anderson et al. 2013).

Today, there are four companies operating in the United States that produce LAL from *Limulus polyphemus* harvested from various locations along the Atlantic Coast. It should be noted that there exists a similar industry in Southeast Asia where other species of horseshoe crabs, namely *Tachypleus tridentatus*, *Tachypleus gigas*, and *Carcinoscorpius rotundicauda* are, or can potentially be used to make an LAL equivalent, *Tachypleus* amebocyte lysate (TAL) and *Carcinoscorpius* amebocyte lysate (CAL).

This paper reviews the current status of the horseshoe crab harvest along the Atlantic coast of the United States compared to that in the Northeast, in particular Massachusetts, with respect to all stakeholders. In the Northeast especially, the concept of "sustainable yield" may need to be replaced with "population recovery" if a slow but certain decline of the horseshoe crab is to be reversed.

28.2 Effect of Bleeding on *Limulus* Mortality

Several studies followed Rudloe's (1983) initial mortality assessment, many of which attempted to more closely mimic conditions used by LAL manufacturers (Kurz and James-Pirri 2002; Walls and Berkson 2003; Leschen and Correia 2010). These additional studies, while not strictly comparable due to the different methods used, found mortalities ranging from 5 to 30 %. Despite one recent (Leschen and Correia 2010), very carefully controlled study conducted at a functioning biomedical facility and using only female horseshoe crabs (females are preferred by the biomedical industry due to their larger size and hence larger hemolymph content) that found a 29 % mortality, the ASMFC has retained their initial estimate of 15 % mortality for bled crabs not returned to their native environment (ASMFC 2013). In 1998 the ASMFC Fisheries Management Board established a mortality threshold for bled and returned horseshoe crabs of 57,500. This figure is based on the 15 % mortality estimate (ASMFC 1998) and has been exceeded in 2007 and every year since (ASMFC 2013). With an increase in crabs bled for biomedical comes an increase in mortality. Although mortality following bleeding is only an estimate, additionally biomedical harvesters are required to report actual mortality from capture to return (ASMFC 2013). In 2012 estimated mortality (15 %) was 79,786, well above the threshold of 57,500 (ASMFC 2013). In 2011, 545,164 horseshoe crabs were harvested for biomedical use compared with 650,539 for bait (ASMFC 2013). This represents 45.6 % of the total harvest. If horseshoe crabs harvested for biomedical use and subsequently used for bait are included, the total number for biomedical becomes 52.6 %. Actual mortality, i.e., not estimates from numbers of bled and released horseshoe crabs, have gone from 4,391 in 2004 to 9,665 in 2010, a 220 % increase.

At their summer meeting on 4 Aug 2011, the ASMFC Horseshoe Crab Management Board approved formation of an ad-hoc working group, made up of technical committee members and biomedical representatives, to develop best management practices (BMP) to minimize coast-wide mortality from the practice of collecting horseshoe crab blood for biomedical use (ASMFC 2011b). In October the newly formed Horseshoe Crab Biomedical Ad-hoc Working Group issued a report with recommended best management practices (BMP) (ASMFC 2011a). Many of the recommendations, e.g. keeping transit times to a minimum, keeping crabs cool and moist during transit and storage between collection and return, and returning crabs to the ocean as soon as practical following bleedings, were already standard practice for most of the biomedical industry. BMPs were never formalized, and the BMPs listed by the ad-hoc group were not made mandatory nor was there any reporting requirement. While it would have been too soon to attribute a positive effect due to the implementation of BMP, harvest-related mortality actually fell to 6,917 in 2011 (ASMFC 2012). Mortality fell again in 2012 to 6,819 when biomedical users should have been employing the BMP. However, the lowest mortality number was recorded in 2008 (2,973) well before the BMP recommendations were made. It is therefore too early to tell whether BMP as currently recommended will significantly affect mortality.

Despite the variability of mortality rates from more recent studies, and a biomedical industry that may now be the major user of the horseshoe crab, the ASMFC Horseshoe Crab Stock Assessment Subcommittee continues to endorse the 15 % mortality rate despite the 85 % increase in the biomedical harvest since 2004 with a corresponding increase in mortality of 75 % (ASMFC 2013).

While the Massachusetts bait harvest as a percentage of the coast-wide harvest has remained fairly consistent–ranging between 9 and 21 % with no clear trend up or down (Table 28.1), there is no clear idea of the number of horseshoe crabs harvested for ‘biomedical use only’ in the state. Unlike the fishing industry, the biomedical industry argues it would hurt competition if its use numbers were released. Due to this industry secrecy and its acceptance by the ASMFC, critical review outside the jurisdiction of the ASMFC has been impossible. However, from the data publically available, a comparison made between horseshoe crabs counted against the bait quota and horseshoe crabs harvested only for biomedical use, a range of 12.7–23.3 % from 2004 to 2012 was observed, with no clear trend apparent (Table 28.2). ASMFC reports also indicate that biomedical use (all manufacturers) has increased nearly twofold, from 343,126 crabs in 2004 to 611,827 crabs in 2012 (ASMFC 2013). If the total bait harvest is compared to all horseshoe crabs bled, the numbers have also been steadily increasing–from a low of 42 % in 2005 to over 83 % in 2012 (Table 28.3). Massachusetts however, is currently the only state that *consistently* allows crabs bled for biomedical use to be subsequently used as bait (dual-use) and be counted against the bait quota as allowed by the ASMFC since 2004 (Marin Hawk, Fishery Management Plan Coordinator ASMFC, personal

Table 28.1 Massachusetts (MA) bait harvest compared with coast-wide bait harvest 1998–2012 (ASMFC 2013)

Year	MA bait	Coast-wide bait	% MA bait
1998	400,000	2,743,585	14.6
1999	545,715	2,600,914	21.0
2000	272,930	1,656,967	16.5
2001	134,143	1,013,697	13.2
2002	138,613	1,265,926	10.9
2003	125,364	1,052,493	11.9
2004	69,436	681,323	10.2
2005	73,740	769,323	9.6
2006	171,906	840,944	20.4
2007	150,829	827,554	18.2
2008	103,963	660,983	15.7
2009	98,332	817,265	12.0
2010	54,782	605,511	9.0
2011	67,087	662,622	10.1
2012	106,821	729,100	14.7

Table 28.2 Coast-wide comparison of number of bait horseshoe crabs bled vs. number of biomedical-only horseshoe crabs bled (ASMFC 2013)

Year	Bait	Biomedical	Total	% Bait bled
2004	50,366	275,194	325,560	18.3
2005	39,429	270,496	309,925	12.7
2006	58,625	296,958	355,583	16.5
2007	71,379	398,844	470,223	15.2
2008	87,864	402,080	489,994	21.9
2009	110,350	362,291	472,641	23.3
2010	66,047	438,417	504,464	13.1
2011	83,312	492,734	576,046	14.5
2012	81,030	485,965	566,995	14.3

communication). The Massachusetts Department of Marine Fisheries (DMF) believes this dual-use exemption results in a significant reduction in the total number of horseshoe crabs harvested in the state (Massachusetts 2008 compliance report to the Atlantic States Marine Fisheries Commission). Thus the 81,030 crabs most recently reported by the ASMFC as "number of bait crabs bled" coast-wide in 2012, may therefore be due in large part to the Massachusetts harvest (ASMFC 2013). The number of bait crab landings reported for Massachusetts for the same year was 106,821 (number includes bait crabs bled for biomedical). Thus, if the assumption that the Massachusetts biomedical industry is using most of the total number of horseshoe crabs counted against the bait quota as reported for the entire biomedical industry, then up to 80 % of the Massachusetts bait harvest may be attributed to the biomedical use. If one adds in the numbers harvested for biomedical use only, the

Table 28.3 Coast-wide bait harvest vs. coast-wide biomedical harvest, 2004–2012 (ASMFC 2013)

Year	Total bait[a]	Biomedical[b]	% Biomedical
2004	681,323	343,126	50.4
2005	769,429	323,149	42.0
2006	840,944	367,914	43.8
2007	827,554	500,251	60.4
2008	660,983	511,478	77.4
2009	817,265	512,552	62.7
2010	605,511	548,751	90.6
2011	662,622	628,476	94.8
2012	729,100	611,827	83.9

[a]Includes crabs harvested for biomedical use but counted against bait quota
[b]Includes all biomedical crabs, i.e. biomedical use only plus those counted against bait quota

Massachusetts industry may easily be the largest user of crabs in the state. It is unfortunate that only estimates of the numbers of horseshoe crabs used by region can be made due to the secrecy surrounding the biomedical industry and their operations. If the percent of biomedical crabs bled actually is mainly attributable to the Massachusetts harvest, the Massachusetts DMF may be correct in assuming their "dual-use" policy helps reduce the total number of crabs harvested in their state. However, without better disclosure, there can be no independent verification. In any case, if the coast-wide number of horseshoe crabs harvested for bait is compared to those harvested solely for biomedical use and the biomedical crabs counted against the bait quota, the biomedical industry as a whole is now using a significant percentage of the total horseshoe crab harvest in the United States–as much as 94.8 % in 2011 (Table 28.3).

28.3 Harvest Effects on Spawning *Limulus*, Especially Females

Female horseshoe crabs have been preferentially used for bait and biomedical bleeding, albeit for different reasons. Fishing lore attributes females more attractive as bait than males, most likely due to the presence of eggs and/or a female specific pheromone thought to attract predators as well as males prior to and during spawning. For the biomedical industry, sexually mature females are preferred as the larger females yield more blood than the smaller males for the same amount of work. It is thought that females have an additional molt compared to males before their final, i.e. terminal ecdysis, and hence grow larger (Shuster and Sekiguchi 2003). Data collected from the Massachusetts bait harvest yearly since 2000 confirm this size difference (Table 28.4). Calculated average prosomal width (mm) of harvested females vs. males was 251.9 ± 8.2 vs. 197.6 ± 2.7 respectively.

Table 28.4 Relative size (prosoma width) difference between sexes from Massachusetts bait harvest 2000–2012 (ASMFC 2013)

Year	Female/male
2000	1.32
2001	1.32
2002	1.32
2003	1.29
2004	1.26
2005	1.33
2006	1.28
2007	1.29
2008	1.26
2009	1.26
2010	1.22
2011	1.22
2012	1.20

Table 28.5 Female to male ratios from Massachusetts (MA) bait harvest 2003–2011 ((ASMFC 2012); 2013 data from Vincent Malkoski, Senior Marine Fisheries Biologist, Massachusetts Division of Marine Fisheries, personal communication)

Year	% Female	% Unclassified[a]
2003	51.4	0
2004	55.9	0.06
2005	50.4	0
2006	49.5	0.05
2007	48.8	0.06
2008	52.8	0.02
2009	53.2	0.08
2010	62.0	10.7
2011[b]	NR[c]	100
2012	52.8	29.8
2013	58.9	33.4

[a]Unclassified count not used to calculate % Female
[b]Preliminary data
[c]Not Reported by MA to ASMFC

Massachusetts and Rhode Island bait harvest ratios (% Females) are shown in Tables 28.5 and 28.6 respectively. The greater percentage of females harvested, albeit small, supports the claim that females are preferred as bait over males. Also, beginning in 2010, bait harvest percentage in Massachusetts of "unclassified" spiked well above 0.1 % (the 100 % figure reported for 2011 is an anomaly due to the fact that no numbers according to sex were reported) and was much higher compared to percentages reported by the biomedical industry over the same time period (Table 28.7). This most likely reflects a more careful reporting by the biomedical industry but may be related to the dual use of horseshoe crabs harvested for biomedical then used as bait rather than being released following bleeding. Horseshoe crabs used for this purpose are typically marketed by bait dealers and not by fishermen directly, so counts may be missed due to lack of a clear reporting authority. However, while sex ratio data is unavailable for industry-wide biomedical, there is

Table 28.6 Female to male ratios from Rhode Island bait harvest 2009–2011 (ASMFC 2012)

Year	% Female	% Unclassified[a]
2009	58.2	9.8
2010	56.2	11.5
2011[b]	51.4	0

[a]Unclassified count not used to calculate % Female
[b]Preliminary data

Table 28.7 Female to male ratios from Massachusetts biomedical harvest 2008–2013 (Massachusetts Compliance Report to ASMFC 2008–2012; 2013 data from Vincent Malkoski, Senior Marine Fisheries Biologist, Massachusetts Division of Marine Fisheries, personal communication)

Year	% Female	% Unclassified
2008	64	1.5
2009	66	<1
2010	62	10
2011	NR	NR
2012	72	1
2013	66	0

NR not reported

still a greater female horseshoe crabs preference for biomedical use when compared to those harvested for bait in Massachusetts (Table 28.7).

Spawning surveys in Massachusetts also indicate a disproportional effect on female horseshoe crab abundance. Massachusetts DMF reported that 48 % of all 2008 surveys in the state recorded no female crabs. In addition, only 12 % of these surveys recorded more than 10 females (Massachusetts Compliance Report to ASMFC 2012). Surveys of Wellfleet and Cape Cod Bay indicated an even smaller fraction of female crabs on average compared with those for other embayments in the state (Faherty 2012).

A feature unique to the horseshoe crab harvest in Massachusetts is a provision for biomedical harvest only since 2006 in Pleasant Bay (Massachusetts Division of Marine Fisheries 2006). Horseshoe crabs for bait and biomedical have been harvested in Pleasant Bay for over 30 years (Leschen and Correia 2010). Sex ratios for this area differ dramatically from other areas in Massachusetts as well as other areas along the Atlantic coast in that they are skewed toward males (James-Pirri 2012). Data from the 1950s indicate a female:male ratio of 1:2.5, which was similar to that observed in Cape Cod Bay (1:2.4). More recent data find ratios of 1:5.8 (2000–2001) and 1:9.5 (2008) compared with 1:2.9 and 1:1.7 for Pleasant Bay and Cape Cod Bay respectively for the same time periods (Massachusetts Division of Marine Fisheries 2008; James-Pirri 2012). These differences suggest a higher mortality and/or morbidity for female horseshoe crabs bled for biomedical use and released. Even before Pleasant Bay was closed to harvesting for bait, mortality associated with the biomedical harvest (assuming 10–15 % of crabs bled and released) was higher simply due to the fact that the biomedical harvest was up to 25 times greater (Rutecki et al. 2004). These observations, i.e. a selective effect on females, and overall mortality, should be given priority for further investigation. As an example, biomedical horseshoe crab harvest in the Monomoy NWR was allowed by permit from 1991 until 2001 when populations were reevaluated and the practice of biomedical harvest only, even when the bled horseshoe crabs were supposedly returned to the area where they were captured, was deemed to be detrimental (Compatibility Determination Eastern Massachusetts National Wildlife Refuge Complex 2002).

Extensively harvested areas in Massachusetts also indicate an impact on spawning horseshoe crabs. Spawning indices, summarized by the Massachusetts Audubon Society from their annual surveys indicate a decline in spawning females in Wellfleet Bay and Cape Cod Bay relative to other areas, e.g. Pleasant Bay where regular surveys are conducted (Faherty 2012; James-Pirri 2012).

One especially dramatic example showed not only a decline in spawning, but the elimination of nearly an entire local population in the Mashnee Dike area of Buzzards Bay, MA. This particular population had been studied for years by vision researcher Robert Barlow at the MBL but was essentially decimated in a relatively short period of time with a 95 % reduction of the population between 1984 and 1999 (Widener and Barlow 1999).

28.4 Non-lethal Effects of Bleeding on *Limulus*

Traditionally the biomedical industry has bled crabs only once per year. Early studies indicated that it took at least a week for the crab to regain blood volume and several weeks to regain baseline amebocyte counts (Anderson et al. 2013). Since it is impractical to maintain crabs in holding ponds until they regain blood volumes and cell count, a practice of one bleed per year became the norm. Although bled crabs were seldom tagged, a fresh scar or needle puncture mark on the arthrodial membrane was quite apparent so that even if a bled crab was recaptured in the same year, a trained technician could avoid a second bleeding if a scar was in evidence. However, there is no provision in the proposed BMPs for preventing crabs being bled twice or more during a single season, and the effect on crab mortality is unknown. Likewise, due to the design of the horseshoe crab's circulatory system (open, i.e. no separate veins with capillaries connected to the arteries to circulate hemolymph back to the cardiac sinus), once the cardiac sinus (large tubular heart) and 11 major arteries (Shuster 2003) are empty, the blood flow slows to a drip or stops completely. It has been estimated that no more than 30 % of the entire blood of an individual crab is ever removed using a gravity flow as opposed to vacuum aspiration (Novitsky 1991). This type of bleeding, i.e. using gravity flow, appears to have become an industry standard (Levin et al. 2003), but due to the secrecy associated with the biomedical industry and a lack of a provision in the BMPs, it remains unclear whether this method is used universally. While equations have been developed to calculate the amount of blood that can be removed from an individual crab

(Hurton et al. 2005), use of this for relating blood volume removed to mortality or other physiological effects is unreliable as the amount of blood removed from an individual crab is extremely variable for reasons unknown (personal observation).

In one study conducted on female crabs using actual bleeding protocols employed by the biomedical manufacturer in Massachusetts (with some additional modifications to comply with the proposed BMP, i.e. employing a refrigerated truck for crab transport, loading fewer crabs per transport container to reduce crushing, and keeping the crabs moist by covering the transport containers with wet burlap), a significant female mortality following bleeding from 22.5 to 29.8 % was found compared to the 3 % for unbled controls (Leschen and Correia 2010).

In another study that examined selected hemolymph components of biomedically bled vs. wild caught female horseshoe crabs (using the same crabs as the aforementioned study), it was found that a number of constituents differed between bled and unbled crabs, in particular, protein concentration (James-Pirri et al. 2012). These authors concluded that the lower protein concentrations found in bled horseshoe crabs and prolonged biomedical bleeding may impact crab physiology. In a study examining specific effects of bleeding on behavior and physiology, results suggested that biomedical bleeding may decrease female horseshoe crab fitness (Anderson et al. 2013). These authors found reduced activity in horseshoe crabs following bleeding measured by several parameters. They also found unexpected mortality in one group. Physiological parameters were also affected, e.g. hemocyanin content. The authors do point out that thermal stress in combination with bleeding may have also affected activity level and physiology. Indeed, Coates et al. (2012) found horseshoe crabs (unbled) were adversely affected by extended captivity, and the effects were exacerbated with increasing temperature. While the maximum temperature tested in the Coates et al. (2012) study was only 23 °C (a maximum of 38 °C was reached with one group in the Anderson et al. (2013) study), captivity in the Coates et al. (2012) study covered 56 days, a much longer time than Anderson et al. (2013). Also, the thermal death point measured in horseshoe crabs collected from Woods Hole, Massachusetts was found to be 41 °C (Mayer 1914). It is unlikely therefore that temperature accounted for the mortality observed by Anderson et al. (2013).

28.5 Summary and Recommendations

Despite state-by-state quotas imposed by the ASMFC and additional harvest restrictions imposed by individual states, horseshoe crabs, especially females, continue to decline in the Northeast, in particular Massachusetts (Massachusetts Division of Marine Fisheries 2009, 2010, 2011, 2012). In New England, horseshoe crab habitat is comprised of a series of coastal embayments with limited movement of populations between them. Because of this, horseshoe crab populations would benefit from more local management, e.g., embayment by embayment, rather than overall harvest quota/restrictions for the entire State (Smith et al. 2009). Likewise, harvesting females during spawning, where populations spawn in relatively small and easily accessible harbors, bays and inlets, may explain the decline in females as well as the overall number of horseshoe crabs. Where numbers are not necessarily declining, e.g., Pleasant Bay, due to the return of horseshoe crabs following bleeding and a lack of a bait harvest, the ratio of spawning females to males is highly skewed when compared to other areas, even where both bait and biomedical harvest is permitted. This latter phenomenon may in fact be related to yearly, preferential bleeding of females. Female horseshoe crabs captured during the act of spawning may be at additional risk. This may be particularly true in Pleasant Bay. The Massachusetts DMF should set a priority to review the current data in Pleasant Bay and if females are at particular risk due to biomedical use, institute additional restrictions in this unique area, an area that has perhaps been studied with respect to horseshoe crabs more than any other. Grady and Valiela (2007) using a matrix modeling method on data collected from horseshoe crab populations on Cape Cod, including Pleasant Bay, concluded that low harvest pressure and restricting harvest to sexually mature individuals would be sufficient to sustain populations. However, the biomedical industry already accepts only larger, sexually mature animals, and low harvest pressure may be impossible given the fact that ASMFC quotas have not been reached in years, and small isolated populations, without additional protection, will continue to be threatened. Thus, the single most effective step to ensure sustainability of the horseshoe crab harvest in the Northeast, particularly Massachusetts, is to completely restrict all commercial harvest during the spawning season. This restriction would include no harvest for a time prior to spawning when the horseshoe crab is coming out of its winter inactivity and beginning to feed and move toward spawning beaches, as well as during the actual spawning period. Current restrictions that cover only small periods around the monthly tides do not protect the horseshoe crabs that remain near the breeding beaches waiting for the next tidal cycle. To accomplish this and to facilitate enforcement, a no harvest period from January 1 through July 31 is suggested. Crabs captured after July 31 by trawling in deeper water would provide sufficient numbers of animals to satisfy both the bait and biomedical industries.

While the fate of horseshoe crabs used for bait is clear, there is much uncertainty surrounding both the mortality and subsequent fitness of horseshoe crabs bled for biomedical use. Thus, an immediate updating of BMPs to remove ambiguity and subsequent inclusion in an enforceable regulation is imperative. In Leschen and Correia's (2010) study for example, the authors' needed to secure a refrigerated truck for horseshoe crab transport and insist on packing fewer horseshoe crabs per container than were currently being used in the biomedical facility.

The practice of "dual-use," i.e., allowing biomedical horseshoe crabs to be used for bait following bleeding, should be completely eliminated. While the concept of dual-use would suggest a decrease in the total number of horseshoe crabs harvested, this is not evident from harvest data. In fact, in Massachusetts, the biomedical harvest may be close to equal that of the bait harvest. Dual-use may also encourage and sustain large-scale commercial horseshoe crab operations, since a more sophisticated operation is required to supply both industries. Of course dual-use horseshoe crabs command a higher price as they are "sold twice."

"Open" reporting needs to be applied to the biomedical industry. If small commercial fishermen and bait dealers are required to report their catches, sex ratios, and other information, there can be no valid reason for biomedical manufacturers to be exempt from full disclosure. Since the entire bait industry is several orders of magnitude smaller by revenue than the biomedical industry, claiming confidentiality due to company size, as is the case in Massachusetts, would be more appropriately applied to fishermen rather than the biomedical industry.

The ASMFC should officially encourage development and use of an artificial bait, especially one that does not contain horseshoe crabs specifically sacrificed for this purpose. An artificial bait currently exists (University of Delaware 2013). There is also bait that can be made from the cell-free hemolymph that results from the production of LAL. This "waste" product, when added to a binder has be shown to be an effective replacement bait (Novitsky et al. 2002).

Finally, in the age of genetic engineering, there is no reason why a synthetic replacement for LAL cannot be designed. In fact, one LAL manufacturer currently sells a synthetic substitute along with a traditional LAL (Lonza 2014). This synthetic substitute was invented by scientists using one of the horseshoe crab genes responsible for the main enzyme component of LAL to engineer a reagent produced in yeast (Ding et al. 1977). According to the FDA however, this synthetic reagent is not by definition, LAL, i.e. a lysate (L) of *Limulus* (L) amebocytes (A) and thus cannot be licensed. The major users of LAL, the pharmaceutical industry (various lots of intravenous solutions, biologics, and medical devices are required to be tested with FDA-licensed LAL prior to release for distribution and use) do not have a choice of using a synthetic substitute until the FDA changes regulations. It is interesting to note that LAL may be one of only a few diagnostic reagents (if not the only one) that is regulated on its composition (LAL) rather than what it detects (endotoxin). As endotoxin has been standardized as to its toxicity (pyrogenic dose in humans), and an official reference standard is commercially available and accepted by several pharmacopeias and the FDA, any reagent that can accurately and routinely detect the pyrogenic dose of endotoxin, i.e. by testing with the reference standard, should be a ready substitute for LAL. The PyroGene™ synthetic reagent already does this (Lonza 2014), as do some other endotoxin tests currently under development or that have been described in the literature, such as the in vitro pyrogen test (Daneshian et al. 2006). Thus all those concerned with horseshoe crab conservation, especially state agencies responsible for the regulation of horseshoe crab harvest and the ASMFC, should actively encourage the FDA to allow LAL substitutes as long as the substitutes can be properly validated (i.e., shown to detect a pyrogenic level of endotoxin in an actual pharmaceutical drug and device).

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