Species Listing Proposal Form:
Listing Endangered, Threatened, and Special Concern Species in Massachusetts

Scientific Name: *Limulus polyphemus* Current Listed Status: None

Common Name: American Horseshoe Crab

Proposed Action:

Add the species, with the status of: Special Concern Species

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Proponents:

Sharl Heller, President, Southeastern Massachusetts Pine Barrens Alliance
Address: The Center at the Center Hill Preserve, 158 Center Hill Road, Plymouth, MA 02360
Telephone: Cell: 617-449-8816; Center Office: 774-773-9982 Email: slheller@comcast.net

Signature Sharl Heller Date Submitted: February 24, 2023

Barbara Brennessel, Ph.D., Professor Emerita of Biology, Wheaton College

- *Diamonds in the Marsh: A Natural History of the Diamondback Terrapin*
- *Good Tidings: The History and Ecology of Shellfish Aquaculture in the Northeast*
- *The Alewives' Tale: The Life History and Ecology of River Herring in the Northeast*
- *The Adventures of Allie the Alewife*
- *Tidal Water: A history of Wellfleet's Herring River*

Deborah Cramer, Author

- *Great Waters: An Atlantic Passage* (W.W. Norton)
- *Smithsonian Ocean: Our Water Our World* (Harper Collins/Smithsonian Books)
- *The Narrow Edge: A Tiny Bird, an Ancient Crab, and an Epic Journey* (Yale University Press)
  - National Academy of Sciences Best Book
  - Society of Environmental Journalists Rachel Carson Book Award
  - *Volando a Orillas del Mar: El viaje épico de un ave playera que une continentes* (Vázquez Mazzini, Buenos Aires)
  - 绝境 (Commercial Press, Beijing)
  - Forthcoming (Yeren Press, Taiwan)

Tim Simmons, Conservation Ecologist

Restoration ecologist with the Massachusetts Division of Fisheries and Wildlife Natural Heritage and Endangered Species Program (NHESP), retired

JUSTIFICATION

There are several persuasive arguments for listing the American horseshoe crab as a Species of Special Concern in Massachusetts, including population data from government agencies, conservation organizations, and scientific research, including research demonstrating the effects of declining horseshoe crabs to coastal ecosystems, and links between the population of crabs and the disappearance of a variety of shorebirds from their traditional migrating routes.

The most up-to-date data is derived from trawl surveys conducted by the Massachusetts Division of Marine Fisheries (DMF), spawning surveys conducted by various organizations under DMF's aegis, data analysis by the Atlantic States Marine Fisheries Commission (ASMFC) which conducts stock assessments coastwide, published scientific research, and population assessments from the International Union for the Conservation of Nature (IUCN).

Another, perhaps unique argument for the listing of *Limulus polyphemus*, is the absence of critical data. For the past several decades—associated with the development of our coastlines and the increased importance of this species' primordial blood to biomedical research—horseshoe crabs have suffered from a dramatic case of benign neglect. The disappearance of this species from our beaches, where once it was so ubiquitous that municipalities paid bounties for its removal, should have been met with alarm, and with armies of researchers. Instead, it was met with relentless stories of the species' use in biomedical research, and with its relegation by fisheries' regulators to the status of by-catch and insistence that the species is doing well, despite the lack of evidence necessary to prove that.

For those reasons and others, which we discuss further in the **Trends** and **Threats** sections of this proposal, while the data do not demonstrate an immediate risk of extinction, the mismanagement of this species makes its own persuasive argument. There is also abundant evidence that the quantity of horseshoe crabs taken from Massachusetts waters each year has dramatically interrupted their breeding activity, threatening both the sustainability of the horseshoe crabs themselves, and their essential role in our coastal ecosystems. And while the effect of decreasing horseshoe crabs in the coastal food web has not been fully characterized in Massachusetts, multiple studies in other waters draw a clear line between the decline of horseshoe crabs and the decline of a variety of other coastal species (Mattei et al.2020, 2022).

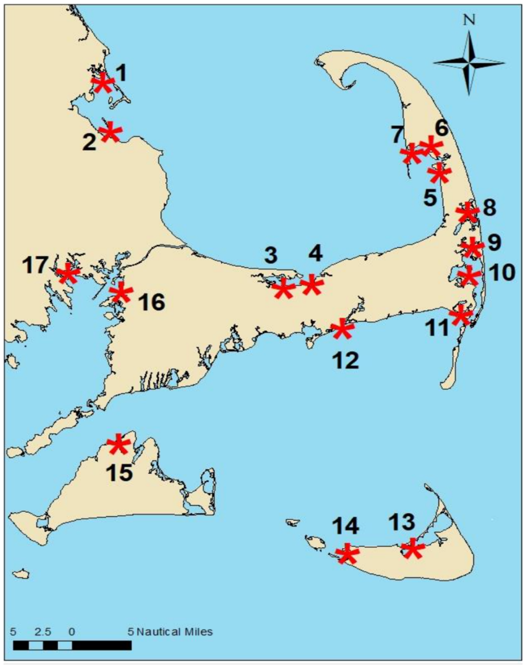
(1) Taxonomic Status.

Taxonomy of *Limulus* has been stable for over one hundred years (Kin 2014 and Bicknell 2021).

(2) Recentness of records.

Spawning beach Surveys

The locations of spawning beach surveys are shown in the map supplied by the DMF.



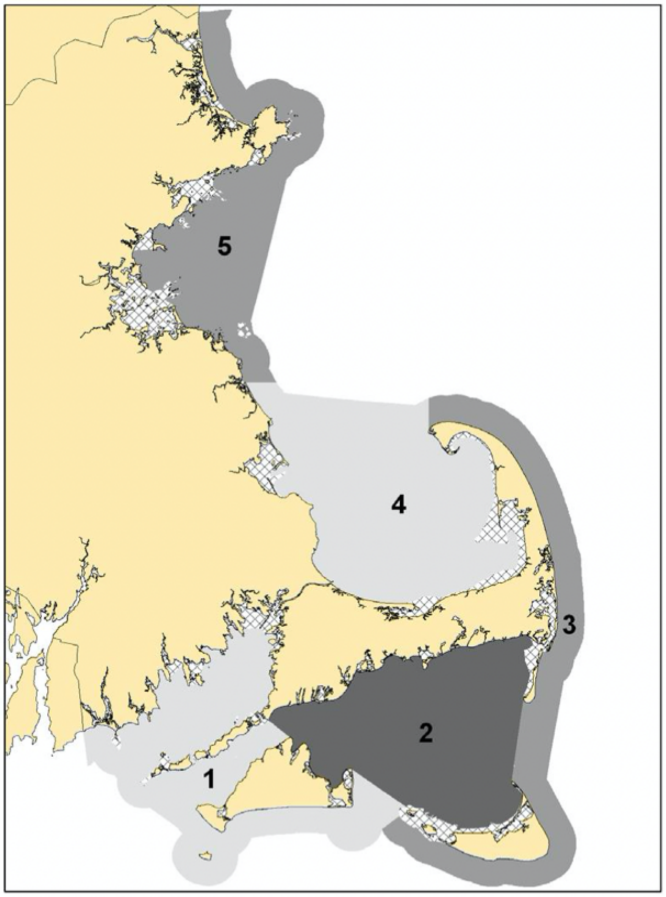
Key to Map: 1. Duxbury; 2. Long Beach; 3. Milway; 4. Long Pasture; 5. Sanctuary Beach; 6. Indian Neck; 7. Great Island; 8. Priscilla's Landing; 9. Marsh 2-3; 10. Erica's Beach; 11. Morris Island; 12. Bass River; 13. Monomoy Beach; 14. Warren's Landing; 15. Tashmoo; 16. Tahanto; 17. Swifts Beach.

The methodology for these volunteer-run spawning surveys is described in Appendix 3. Data from the surveys is compiled and analyzed by DMF and submitted in their annual compliance reports to the Atlantic States Fishery Commission (ASMFC) (Appendix 4 for 2021 is the latest report). Several of the spawning surveys were only recently initiated (Bass River, Long Beach, and Chatham (not on map), but others have a much longer trajectory.

Overall, the spawning survey indices are very low, with many sites reporting averages of just fractions of a female horseshoe crab in each surveyed quadrant (Appendix 5). The sites that have higher spawning indices are areas that have either been closed to take (Priscilla's Landing in Nauset Estuary) or in the biomedical-only area of Pleasant Bay (Marsh 2-3). It is notable that Great Island, in the Cape Cod National Seashore, is also closed to take but spawning indices remain very low. Most of Great Island, in Wellfleet Harbor, is open to take. Although there is some year-to-year variability, with some sites showing modest increases, there are no significant long-term changes in these spawning indices over the duration of the spawning surveys, even those where lunar closures were put in place over a decade ago.

Trawl Surveys

The DMF has conducted bottom trawl surveys since 1978. These surveys are not specifically designed for horseshoe crabs but have captured horseshoe crab data offshore. Trawl surveys are conducted in spring and fall of most years. The methodology for the trawl surveys is described in the ASMFC report 2021 (Appendix 4) and the locations are depicted in the map below, which is also found in the ASMFC Report 2021. Data for the Gulf of Maine include Cape Cod Bay, while the rest of the state is considered as Southern New England.



Map of regions for DMF's bottom trawl survey. For the attached report, regions 1–3 are considered Southern New England (SNE) and regions 4–5 are Gulf of Maine (GOM).

The results of the trawl surveys are depicted in the ASMFC report (Appendix 4) and are summarized as follows:

"Horseshoe crab survey results from the 2021 DMF spring and fall trawl surveys were mixed (Figure 2 though Figure 5). South of Cape Cod, mean number and weight of spring caught males and females in SNE (Figure 2 and Figure 3) remain near their respective time series highs, but at or below time series medians in the fall. North of Cape Cod, 2021 mean number and weight data points were at or below their time series medians during the spring and fall surveys. Size distribution data are given in Figure 6 through Figure 13. Crabs south of Cape Cod are usually larger (and more numerous) than crabs north of Cape Cod."

The population data for Massachusetts horseshoe crabs described above may be interpreted in several ways. As a response to the perceived substantial decreases in the number of horseshoe crabs observed by the public and by shellfish aquaculturists, in 2012 the Town of Wellfleet Shellfish Advisory Board and Select Board jointly petitioned the DMF for a moratorium on the take of horseshoe crabs in Wellfleet Harbor. In response, Paul Diodoti, then Director of DMF, denied the petition, writing: "I have substantial concerns regarding the consistency with which population data is collected as well as the variability of the spawning survey indices." In essence, Diodati acknowledged that their data was flawed. Furthermore, the public has no data from the biomedical industry.

Due to the scarcity of horseshoe crabs, it is difficult to achieve statistical significance in spawning survey trends, while the trawl surveys collect horseshoe crabs as bycatch and are not optimized for a horseshoe crab population study.

Horseshoe crabs as essential parts of coastal ecosystems

Horseshoe crabs are, or were, what scientists call a “dominant” species in coastal ecosystems, with many animals dependent upon them. In “superabundance,” they support a rich web of life. Long before they are in danger of extinction, their loss reverberates through coastal food webs. When their numbers are diminished, other threatened or endangered species are deprived of essential food. The horseshoe crab, scientists are now beginning to realize, is “in need of conservation, not to prevent extirpation, but to increase population density to restore its dominant status.” (Mattei et al., 2022).

Loggerhead Sea Turtles (*Caretta caretta*)

Loggerhead sea turtles are threatened in Massachusetts. https://www.mass.gov/doc/loggerhead-sea-turtle/download. Horseshoe crabs were once the primary prey of loggerheads, more than 40% of their diet (Seney and Musick 2007). Loggerheads, once rare north of Cape Hatteras, have been moving north, into Chesapeake Bay, Long Island Sound, Narragansett Bay, and into waters off Cape Cod. When horseshoe crabs were abundant, the turtles came into the mouths of estuaries to feed upon them. (Keinath, p152, in Shuster, Barlow and Brockman, 2003). When the horseshoe crab population plummeted, loggerheads were forced to eat bycatch from fin fisheries. (Seney and Musick, 2007).

American Eel (*Anguilla rostrata*)

The American eel is listed as endangered on the IUCN Red List, “depleted with the stock being at or near historically low levels.” https://www.iucnredlist.org/species/191108/121739077#population. The Atlantic States Marine Fisheries Commission finds that the American eel continues “to remain depleted." The causes are many and include many food web alterations.” http://www.asmfc.org/species/american-eel. Horseshoe crab eggs are, or were, food for eels between May and August. When both were abundant, eels could be seen eating horseshoe crab eggs as they were laid (Botton and Schuster, 2003).

Roseate Tern (*Sterna dougallii*)

The roseate tern is endangered in Massachusetts. https://www.mass.gov/doc/roseate-tern/download. It feeds on a variety of fish, including Atlantic silversides, which in turn feed on horseshoe crab eggs.

Fish

Horseshoe crab eggs and larvae are food for forage fish, killifish, and Atlantic silversides, that in turn feed larger perch, weakfish, northern kingfish, puffers, summer and winter flounder, and striped bass. When they are available, larger fish will eat entire eggs masses of horseshoe crabs (Botton and Shuster, 2003). These fish occur in the waters of Massachusetts (Eastern MA National Wildlife Refuge Complex, 2002). Killifish, puffers, and flounder have all been documented feeding on eggs or larvae. Shark eat horseshoe crabs whole. In the 1960s, one writer for *Audubon Magazine* witnessed on a Massachusetts beach, fish nearly strand themselves at the tideline hurtling themselves at freshly laid clusters of horseshoe crab eggs, and half a dozen other fish nuzzling beneath a female horseshoe crab jus[i]t as she dropped her eggs (Botton and Schuster, 2003).

Other Fauna

Sand shrimp, fiddler crab, and blue crab, all occurring in the waters of Massachusetts, eat horseshoe crabs, horseshoe crab eggs, and/or horseshoe crab larvae. These faunae are important food for shorebirds whose populations are declining.

Shorebirds

At least 10 species of shorebirds are known to rely on horseshoe crab eggs when horseshoe crabs are abundant during their migrations along the Atlantic coast. Species especially dependent on horseshoe crab eggs are red knot, sanderling, semipalmated sandpiper, and ruddy turnstone. Also using horseshoe crab eggs are short-billed dowitchers, and dunlin. Their populations are all dropping (Andrews et al., 2012) for many reasons, including the loss of horseshoe crab eggs, their most nutrient-rich food. A number of these birds migrate through Massachusetts, either in the spring, when they eat horseshoe crab eggs, or in the fall, when they eat eggs and larvae.

Semipalmated Sandpipers and **ruddy turnstones** have a history of eating horseshoe crab eggs in Massachusetts, with the semipalmated sandpipers also heavily dependent on sand shrimp that eat horseshoe crab eggs. Both species have declined so rapidly they scientists find the birds now meet IUCN Red List standards for Vulnerable, semipalmated sandpipers migrating along the northeastern U.S. dropping by 80 percent since 1980 and ruddy turnstones dropping by 85 percent (Smith et al., 2023). A Species of Concern listing can make a difference for these birds. After a 20-year ban on the horseshoe crab take from the Monomoy National Wildlife Refuge, the population began rebuilding there, and in June 2022, a large flock of ruddy turnstones were seen in the refuge eating horseshoe crab eggs. (Manomet, personal communication). However, this hard-won improvement is now again jeopardized by the expansion of a second biomedical company taking horseshoe crabs for bleeding in waters just outside the refuge.

Short-billed dowitchers, whimbrel, and Hudsonian godwit all pause to refuel in Massachusetts on their long migrations, and all now qualify as Endangered by IUCN standards, the short-billed dowitcher population along the northeast U.S. dropping by 90%, whimbrel 86%, Hudsonian godwit by 95% (Smith et all, 2003). Whimbrel eat sand shrimp, fiddler crabs, and other crabs that eat horseshoe crab eggs. When horseshoe crabs were plentiful, short-billed dowitchers were eating their eggs in Massachusetts in July, most likely eggs laid in June (Eastern MA National Wildlife Refuge Complex, 2002; Mallory and Schneider, 1979).

Red knots were listed as threatened under the U.S. Endangered Species Act in 2014, primarily because as so many horseshoe crabs were taken from Delaware Bay, the knots lost the energy-rich eggs they needed to double their weight for the last leg of their journey to the Arctic. These birds are threatened in Massachusetts. In the Five Year Review of the Status of the Red Knot, the U.S. Fish and Wildlife Service found it still “depleted" (Rufa Red Knot Five Year Review, 2021). Unlike Delaware Bay and South Carolina, important foraging areas for red knots migrating north, Massachusetts is in the unique position, if its horseshoe crab population was restored, of providing the birds with rich food both on the north and south bound migrations, when eggs and larvae would still be available. Knots once migrated through Massachusetts in both the spring and the fall (Harrington et al., 2010), with some 25,000 birds passing through Wellfleet Bay. Few horseshoe crabs spawn in Wellfleet Bay now. Massachusetts regulations governing the take of horseshoe crabs have been too weak to enable the crabs to return. As many as 7,000 red knots came through western Cape Cod Bay (Duxbury, Scituate, and Plymouth) in the fall in 1969, but then the numbers dropped, possibly associated with a horseshoe crab bounty program that removed thousands of horseshoe crabs from Massachusetts beaches every year. It is likely that if horseshoe crabs were restored to abundance in Massachusetts, the knots would return. That knots were in Massachusetts in the 1980s, eating horseshoe crab eggs, is evidenced by two videos taken in the Monomoy National Wildlife Refuge by artist Robert Verity Clem, which show red knot, ruddy turnstones, Hudsonian godwits, and other shorebirds eating horseshoe crab eggs.

A few horseshoe crabs spawning on a beach is not enough to provide food for shorebirds. While the turnstones are capable of digging down to the eggs, if there are enough, the knots and other shorebirds depend on masses of eggs being overturned by waves or by the horseshoe crabs themselves as, crowded on the beach, they crawl over each other and expose some of the eggs. In Delaware Bay, horseshoe crab regulations have not been tight enough to bring back the horseshoe crabs and the density of their eggs has not increased (Smith et al., 2022).

Research in Long Island Sound indicates that horseshoe crab densities are now so low that very few shorebirds are seen eating the eggs (Beekey et al., 2013; Mattei et al., 2022). Horseshoe crabs are no longer fulfilling their ecological function and scientists working in the Sound are calling for a moratorium on the horseshoe crab fishery there (Mattei al., 2022).

In addition to the value of horseshoe crabs in and of themselves, and their essential role in coastal ecosystems, horseshoe crabs also play a critical role in modern medical science. They are the source of LAL (Limulus Amebocyte Lysate), used by the pharmaceutical industry in testing for bacterial endotoxins in injected drugs such as vaccines, intravenous chemotherapies and antibiotics, insulin injections, and implanted medical devices such as heart stents, and hip and knee replacements. Millions of people benefit from the exquisite endotoxin sensitivity of the horseshoe crab today. However, medical science's complete dependence on LAL is coming to an end. The development of a synthetic version already in use by four major pharmaceutical industries inside the U.S., which is now included in the European pharmacopeia for use in Europe, may soon be approved for general use in the U.S (Cramer, 2023).

Worldwide, there are two sources of horseshoe crabs for lysate, the China Sea, and the east coast of the United States. In Asia, where they are killed for food and for their blood, the IUCN classifies the primary horseshoe crab used for blood, *Tachypleus tridentatus,* as endangered, facing extinction from "relentless and unremitting" threats from humans. China's scientists and drug regulators consider the resource "exhausted," and "on the brink of extinction." Its horseshoe crabs "largely depleted," China is, according to the IUCN, targeting Vietnam, where exporting the crab is illegal, where its numbers have already declined by 50% and its range contracted by 50%. This depletion leaves American horseshoe crabs, themselves already diminished, to fill the rising gap. At the same time the supply of horseshoe crabs is decreasing, the demand for endotoxin tests is rising, fueled by explosive growth in the pharmaceutical industry.

The horseshoe crab take in Massachusetts has a long history. The baseline had already shifted by the time Massachusetts began counting horseshoe crabs.

Bounty program

Sifting through old histories begins to provide an idea of the former abundance of horseshoe crabs. Long before the Atlantic States Marine Fisheries Commission realized that dramatic losses of horseshoe crabs along the eastern seaboard necessitated the implementation of quotas in the horseshoe crab fishery, and long before the Massachusetts whelk fishery (which uses horseshoe crab as bait) was underway, Massachusetts was destroying horseshoe crabs. In the 1960s and 1970s, the Commonwealth subsidized cities and towns offering a bounty on horseshoe crabs, paying fishermen three or four cents a telson in this predator control program designed to protect soft shell clams for fishermen, a misguided idea, since clams and horseshoe crabs lived on the same areas of seafloor for thousands of years.

The Massachusetts Division of Marine Fisheries estimates that during these years, participating fishermen killed more than half a million horseshoe crabs every year. In 1960, in Chatham alone, fishermen killed 50,000 horseshoe crabs (DMF News Second Quarter 2006). The Chatham shellfish constable reports that “tens of thousands of horseshoe crabs” were removed from its waters during this program. In the 1960s, Duxbury took 14,000, 16,000, and 20,000 from the town’s small beaches (Massachusetts Division of Marine Fisheries 2006 bounty data). A writer for *Audubon* went to the Duxbury dump and found “hundreds of crabs, many on their backs and waving their legs feebly in the sun.” The number of crabs recorded in the bounty program is probably an underestimate. The same writer observed children encouraged to crush, stone, smash, or attempt to spear horseshoe crabs. They did this for fun, not for the bounty (Rood 1967).

Town ordinances

The killing of horseshoe crabs continued after the bounty program ended. Several coastal Massachusetts towns—including Brewster, Chatham, Chilmark, Eastham, and Gayhead—all had ordinances requiring people who found live horseshoe crabs to strand them above the high tide line (Massachusetts Division of Marine Fisheries Service bounty data). It’s entirely possible that the combination of the bounty program and these ordinances caused a depletion serious enough to cause the red knots to abandon Cape Cod Bay.

Spawning beaches

The Massachusetts Division of Marine Fisheries (DMF) identified beaches with high densities of spawning horseshoe crabs in 2007, for example, high levels of spawning on Back River, Duxbury and Bradford Street beaches, along with Ship Yard Lane Beach, in Duxbury. The DMF doesn’t appear to have updated and published this list. Without returning to the Massachusetts spawning and nursery beaches listed in 2007, and surveying them, it’s impossible to determine whether there’s been any significant increase in spawning. Further, many beaches were already depleted by the time this survey was made. The shellfish reports for Duxbury in the 1960s and 1970s mention heavy spawning on the Back River, but also at Cedar Pond, Bluefish River, and Goose Flats (MA DMF News, 2006).

Trends in female/male horseshoe crab ratios a sign of continuing horseshoe crab decline

In one area of Massachusetts, Pleasant Bay, there are data going back before the bounty program and before the dramatic increase in the take of horseshoe crabs for bait in the whelk fishery, beginning in the 1970s, and before the increase in the take of horseshoe crabs for bleeding to make the endotoxin assay Limulus amebocyte lysate. This older data provides a context for the status of spawning horseshoe crabs today. We have come to assume, for example, that the larger number of males seen on spawning beaches is normal, when the historical data suggests otherwise.

Before the ratio of spawning male to female horseshoe crabs became high, even the ASMFC agreed that "shifts in the normal 1:1 sex ratio toward less than one female per male becomes an important criterion, pointing specifically to overfishing of females (ASMFC, 1998). In other heavily fished regions, the proportion of males to females on spawning beaches has increased (in Delaware Bay from two females to three females in 1999 to two females to five males in 2017), but the increases in Massachusetts are alarmingly skewed by comparison (ASMFC, 2019). In the 1950s, female to male sex ratios of spawning horseshoe crabs in Pleasant Bay ranged from 1:19 to 1:2.5. In a study carried out 2000 - 2002, the ratio had risen to 1:4.5. Both the biomedical and bait horseshoe crab fisheries preferentially select females when females are available. In Nauset Estuary, where, in 2000 - 2002, there was no horseshoe crab take, the spawning female to male sex ratio was 1:1.6. In a follow up study, 2006-2008, the spawning sex ratios in Nauset Estuary, which had just opened to a horseshoe crab fishery, were essentially the same, 1: 1.3, while those in Pleasant Bay were becoming highly skewed towards males. On Hog Island, the ratio was 1 female to 3.9- 5.3 males, and on two other sites, it had increased to 1 female to 8.5-14.0 males with one female to 12 satellite males were occasionally observed, and clusters of up to one female to 30 males were recorded (Mass Audubon data as reported in MA DMF Compliance Report 2009).

In 2000, biomedical fishermen were caught poaching in the Cape Cod National Seashore. The national seashore prohibited them from returning, a federal court backed that decision, and a few years later, Pleasant Bay was open only to the biomedical take. However, these prohibitions were not extensive enough to fully protect Pleasant Bay's horseshoe crabs. The boundaries of the seashore extend only a short distance into the bay, enabling fishermen to take the horseshoe crabs as they enter or leave the seashore's protected waters. The study suggested that tighter regulations might be required to ensure the persistence of horseshoe crabs in Pleasant Bay, yet in 2022, the spawning sex ratio continues to be alarmingly skewed.

To determine whether bleeding was causing a greater mortality than had been currently understood, the MA DMF studied mortality of unbled females vs. those handled and bled by Associates of Cape Cod, the local biomedical company. The results documented a mortality rate of 30%, substantially higher than the 5-15% estimate currently used for management of this fishery (Leschan and Correia, 2010). Yet, the DMF did not take further action to reduce the take of horseshoe crabs. Other studies both confirm the 30 percent mortality rate and suggest that horseshoe crabs, dazed from bleeding, wander aimlessly on the seafloor, unresponsive to the tidal rhythms calling them to spawn (James-Pirri, et al., 2005).

(3) Native Species Status.

"Horseshoe crabs have been crawling ashore in Massachusetts for about 350 million years, and they look the same now as they did when living side-by-side with dinosaurs.

In fact, horseshoe crabs are commonly referred to as "living fossils" because they are one of the most ancient creatures still living today.

The species that currently calls Massachusetts home is *Limulus polyphemus*. Unfortunately, the Commonwealth's population of these incredible marine animals is in decline and facing increasing threats to their survival." Mass Audubon— https://www.massaudubon.org/our-conservation-work/wildlife-research-conservation/horseshoe-crab-monitoring.

(4) Habitat in Massachusetts.

Past and Present Population Status and Distribution in Massachusetts

In Massachusetts horseshoe crab populations have historically been distributed along Nantucket Sound, Buzzards Bay, Cape Cod Bay and the Gulf of Maine: the largest assemblages in the south, lesser amounts on the North Shore. Unfortunately, there is no statewide baseline population data for the period prior to the increased exploitation of horseshoe crabs in the 1990s. There is also a lack of reliable, current information about population sizes, spawning stock biomass, recruitment, and actual mortality after bleeding.

A survey of spawning beaches in Massachusetts was completed in 2004 (Appendix 1). It is not clear if there are discrete subpopulations. Some studies of localized embayments have been conducted to establish baseline values. There is evidence that Massachusetts' horseshoe crabs display a degree of site fidelity so they may exist as subpopulations within specific embayments, but they also appear capable of movement to adjacent embayments (James-Pirri et al., 2005).

Baseline data does exist for the population within Pleasant Bay (Carmichael et al, 2003). A study (James-Pirri et al 2005) in four Massachusetts estuaries (Monomoy Wildlife Refuge, Nauset Estuary, Pleasant Bay, and Cape Cod Bay) indicated that there is site fidelity within embayments. James-Pirri's study also documented male dominant sex ratios (1 female to 5.8 males) in Pleasant Bay, where only biomedical take is permitted.

The larger female crabs are preferred for bleeding and this observation may have an impact on the sex ratios in Pleasant Bay. More recent documentation shows larger ratios than 5.8 males to 1 female: as much as 12:1 and up to 20:1 in some years (Appendix 2, Mark Faherty: Mass Audubon, personal communication). Sex ratios in Massachusetts are the highest of all states surveyed, with most operational sex ratios for the species ranging from nearly 1:1 in Maine to 3.5:1 in Delaware Bay (Mattei et al. 2010). All management decisions about horseshoe crabs are based on two types of population data: spawning beach surveys conducted by volunteers using DMF protocols, and trawl surveys, conducted by DMF.

(5) Federal Endangered Species Act status.

Not listed under the National Endangered Species Act.

(6) Rarity and geographic distribution.

Range-wide Distribution and Debate

Globally there are several horseshoe crab species, only one of which is found in the U.S.

Limulus polyphemus is found along the Atlantic Coast from Maine to Florida, and along the Gulf Coast from Florida to Texas.

The largest concentration found along the Atlantic Coast is in Delaware Bay, but declining numbers throughout their range (due in part to their exploitation for bait and biomedical uses) has led to public debates, legislative battles, and legal challenges related to its management or the lack thereof.

NatureServe State rankings below indicate the varying, general responses of those states:

<table>
<tr><td>Maine</td><td>SNR</td><td rowspan="10">N5 = Nationally Secure

SNR = State Not Ranked

S2 = Imperiled: At high risk of extirpation in the jurisdiction due to restricted range, few populations or occurrences, steep declines, severe threats, or other factors.

S3 = Vulnerable: At moderate risk of extirpation in the jurisdiction due to a fairly restricted range, relatively few populations or occurrences, recent and widespread declines, threats, or other factors.</td></tr>
<tr><td>Massachusetts</td><td>No data available</td></tr>
<tr><td>Rhode Island</td><td>SNR</td></tr>
<tr><td>Connecticut</td><td>S2S3</td></tr>
<tr><td>New York</td><td>S3</td></tr>
<tr><td>New Jersey</td><td>SNR</td></tr>
<tr><td>Delaware</td><td>SNR</td></tr>
<tr><td>Maryland</td><td>SNR</td></tr>
<tr><td>South Carolina</td><td>SNR</td></tr>
<tr><td>Florida</td><td>S3</td></tr>
</table>

Several State Wildlife Action Plans have classified horseshoe crabs as a Species of Greatest Conservation Need (SGCN).

ME	SGCN Priority 1	https://www.maine.gov/ifw/wildlife/reports/pdfs/SGCN_Reports/SGCN/Horseshoe%20Crab__Limulus%20polyphemus.pdf
NH	SGCN	https://www.wildlife.state.nh.us/wildlife/profiles/wap/marine-horseshoecrab.pdf
RI	SGCN	https://dem.ri.gov/sites/g/files/xkgbur861/files/programs/bnatres/fishwild/swap/sgcnsci.pdf
CT	SGCN	
DE	SGCN Tier 1	Species are in the highest need of conservation action. These include the rarest species in the state, species that are highly globally imperiled, and species with regionally important Delaware populations that are also under high threat from climate change
MD	SGCN	
SC	SGCN	https://www.dnr.sc.gov/swap/index.html

(7) Trends.

The International Union for the Conservation of Nature (IUCN) includes American Horseshoe Crab on their Red List, noting that, "as a result of overharvesting for use as food, bait and biomedical testing, and because of. habitat loss, the American horseshoe crab is listed as Vulnerable to extinction." https://www.iucnredlist.org/species/pdf/80159830/attachment.

When they added *Limulus polyphemus* to their Red List in 2020, the IUCN noted that the "breadth of declines was highest in the New England area and diminished generally from the northern to southeastern areas with indications of negative slopes for Florida Atlantic and Northeast Gulf regions (Table 3 and Figure 4)."

South Carolina's full ban of their horseshoe crab bait fishery is directly relevant to consideration of the crab's listing as a species of special concern—and the necessary protections that would follow—in Massachusetts. The legislature of South Carolina banned the horseshoe crab bait fishery in 1989 but took no other actions to protect the species. That gave Charles River Laboratories, now a multi-billion, multinational company and the largest manufacturer of LAL in the United States, a monopoly on horseshoe crabs in that state. Beginning in the 1990s the horseshoe crab bleeding fishery began to steadily expand and today the horseshoe crab population there can no longer support that industry without severely impacting the crab's viability.

Three trawl surveys, each made in the spring and fall, track horseshoe crab populations in South Carolina. The ASMFC stock assessment threw out some of those surveys because too few horseshoe crabs were caught in the towns to make the data remotely meaningful. The surveys that remained all demonstrated downward trends.

The pressure to provide crabs for the biomedical industry was also seen on the state's spawning beaches (Hunt, 2022). Charles River Labs was discovered illegally removing horseshoe crabs from the Cape Romain National Wildlife Refuge without a permit. In a span of ten years the beaches on Marsh Island and White Banks within the refuge were virtually emptied of spawning horseshoe crabs. Recently only males have been seen on the beaches in the spring. As a result, a critical stopover for red knots was lost and those birds disappeared from that refuge.

The Turtle Island Wildlife Management Area near the Georgia border was also a critical feeding area for large flocks of red knots every spring. In only a few years, fishermen taking horseshoe crabs to bleed, emptied Turtle Island of horseshoe crabs, and bird populations plummeted as well. The Defenders of Wildlife and the Southern Environmental Law Center successfully sued the U.S. Fish and Wildlife Service to stop the horseshoe crab take in the Refuge and is now suing the state of South Carolina and Charles River Laboratories, asserting that the thousands of horseshoe crabs kept for weeks at a time in holding ponds where they spawn, losing billions of eggs into the water, constitutes a take under the Endangered Species Act, depriving federally listed birds of food they desperately need (Smith et al., 2019).

Further, while lysate manufacturers claim the mortality of bled horseshoe crabs is low (a fisherman asserted at a recent horseshoe crab stakeholders meeting run by the Massachusetts Division of Marine Fisheries that only 1 or 2 percent died in the bleeding process), a study in South Carolina jointly run by the state and the bleeding company found that 20% of bled female horseshoe crabs died in the process.

These experiences have implications for Massachusetts, where the pressure on horseshoe crabs is arguably greater than in South Carolina. South Carolina has no bait industry, while Massachusetts has one of the largest bait fisheries on the eastern seaboard, and now not one, but two biomedical companies are bleeding horseshoe crabs. Many of these horseshoe crabs – the exact number isn't known – are kept in holding pens in the water. These pens are above the seafloor, so the horseshoe crabs are not eating, and as in South Carolina, while the crabs are in the pens, they are not spawning on the beaches where they can sustain or rebuild the population.

Experience in states other than South Carolina is instructive as well.

Delaware Bay (Delaware and New Jersey) has less biomedical pressure than Massachusetts – one large company, and two small ones – and its bait fishery is much more restrictive than Massachusetts. New Jersey has prohibited a bait fishery since 2008. Delaware's bait fishery is prohibited during the horseshoe crab spawning season, and when the bait fishery is open taking female horseshoe crabs is prohibited. This population was so heavily exploited by 2000 that the U.S. Fish and Wildlife Service listed the Red Knot as threatened under the Endangered Species Act. Despite that listing and the other regulations put in place, the population of crabs only stabilized at its depleted level, and after 20 years has not recovered (ASMFC stocks neutral in Delaware Bay).

Regulations governing the horseshoe crab take in Delaware Bay - a ban on the take of female horseshoe crabs for bait, and a full bait moratorium in New Jersey, millions of dollars' worth of beach renourishment and rebuilding in Virginia have not been sufficient to rebuild the population of horseshoe crabs. The horseshoe crab takes for bait and biomedical is still too high there, as evidenced by the Virginia Tech Horseshoe Crab Trawl Survey, the only dedicated horseshoe crab survey along the entire east coast, and one which measures trends in juvenile, newly mature, and mature horseshoe crabs. This survey, which has run since 2002, shows no statistically significant increase in the population of adult female horseshoe crabs, the population needed to restore this species (Hallerman and Jiao, 2022).

In 2021, the U.S. Fish and Wildlife Service still considered the red knot "depleted," Horseshoe crab egg densities on New Jersey beaches, which need to be at 50,000 per square meter to provide sufficient nourishment for impacted shorebirds, is mired at 5,000 eggs per square meter (Smith et al, 2022).

Connecticut has mismanaged its horseshoe crabs (Beekey and Mattei, 2015) and they have been designated as a species of concern. The Connecticut General Assembly is considering a bill to ban both the bait and biomedical take. Those actions would increase the pressure on MA horseshoe crabs. Seikagaku (Associates of Cape Cod) is already finding it difficult to meet its needs in Massachusetts and is taking horseshoe crabs from Rhode Island and New York.

(8) Threats and vulnerability.

In Massachusetts, horseshoe crabs face many challenges. Historically, they were collected for use as fertilizer and as additives to livestock feed. Aquaculturists have long held the belief that horseshoe crabs are responsible for declining oyster and clam resources, therefore it was their custom to collect them and dispose of them in the local dump or to snap off their telsons so that they couldn't right themselves if overturned in shallow water.

The most serious immediate impact on the species comes from exploitation as the result of commercial taking, which includes egg-bearing females which take 10 years to mature. Compared to the other Atlantic states described above, Massachusetts has the least restrictions on take. There are currently two commercial fisheries in Massachusetts: one for bait take and one for biomedical take of horseshoe crabs in order to extract their blood. It is also more common today to participate in the so-called a "rent-a-crab" approach to taking, wherein horseshoe crabs are bled first, then sold as bait. There are a few site exceptions: all take is prohibited in the Monomoy National Wildlife Refuge and in the Cape Cod National Seashore. In Pleasant Bay, only biomedical take is permitted.

Bait Take

In Massachusetts, horseshoe crabs spawn in the spring, as early as mid-April to the end of June or early July. They are thought to move from offshore locations to low energy, well aerated sandy shores such as beaches and intertidal areas along the coast. They are harvested in both locations; offshore by trawlers and by hand when they arrive on shore to spawn.

Beginning in the 1990s, there was unprecedented demand for both whelks and eels from Asian buyers. Horseshoe crabs are the preferred bait for both whelk and eel fisheries. Localized studies shed some light on the effect of the increased horseshoe crab take pressure that resulted from that new demand. Widener and Barlow (1999) assessed the horseshoe crab population at Mashpee Dike in Bourne from 1984-1999. They documented a precipitous decline of 95% of the spawning population and 80% of the general population. The decline was attributed to a massive increase in hand takes of horseshoe crabs at Mashpee Dike.

In a study of age structure and threats to the population, Grady and Valiela (2006) proposed that horseshoe crab populations were most vulnerable when individuals were taken prior to sexual maturity, and that the population growth rate is most sensitive to the survival of older juveniles: likely the very horseshoe crabs that are collected during offshore bait take.

Biomedical Take

Massachusetts has an area designated for biomedical bleeding only. The bait take was banned in Pleasant Bay in 2006. We are not aware of studies that have tracked the effects of bleeding on mortality of horseshoe crabs in Pleasant Bay, the effects of the biomedical fishery on spawning, or the effects of bleeding on population trends in Pleasant Bay. There are studies, however, that have tracked the ratio of male to female spawning horseshoe crabs in Pleasant Bay (described above and in Appendix 2). The trend is concerning.

Until recently, there was only one biomedical operation in Massachusetts: Associates of Cape Cod in Falmouth. Recently however, the largest biomedical bleeding company in the U.S., Charles River Laboratories, has set up operations in Harwich. Due to confidentiality issues with the industry (the so-called 'Rule of Three), data for biomedical takes in Massachusetts was unavailable to the public. Just recently however, the DMF released the total number of horseshoe crabs sold to biomedical companies in 2022, allowed now because the data came from three or more harvesters. In a recent memo (McKiernan, 2023) to the Massachusetts Marine Fisheries Advisory, *Proposal to Adjust Commercial Horseshoe Crab Limits for 2023,* the DMF reported that in 2022, the biomedical horseshoe crab landings in Massachusetts approached 175,000 crabs. The DMF proposes to cap the 2023 biomedical take at 200,000. This is higher than the biomedical take in South Carolina where there is no bait take. The DMF currently allows 165,000 crabs to be taken as bait and will continue to allow bait takes in 2023 of 140,000 crabs.

The regulations for the biomedical fishery require that horseshoe crabs are returned to their site of take after bleeding. However, the mortality of horseshoe crabs after up to 40% of their blood is removed and their subsequent handling and transport, is estimated to be 15-30%. The Massachusetts Division of Marine Fisheries undertook a mortality study – unbled crabs vs. bled crabs from the Associates of Cape Cod and found a 30% mortality rate (MA ASMFC Compliance Report, 2009). Some studies in Massachusetts have also addressed the health and reproductive capabilities of horseshoe crabs after bleeding. For example, Kurz and James-Pirri (2002) observed disorientation after bleeding and posited that this behavior may affect reproductive capability. Anderson et al. (2013) demonstrated effects on movement and activity, as well as decreases in hemocyanin levels in bled crabs. They concluded that sub-lethal effects resulting from extraction of LAL may decrease fitness and reproduction. Using acoustic tags, Owings et al. (2019) found that bled female horseshoe crabs approached mating beaches less frequently than controls with the largest impacts seen 1-2 weeks after bleeding.

Other Threats

Losses of spawning habitat such as coastal wetlands and beaches due to development, construction of revetments and climate change related issues such as sea level rise, are also important factors affecting the vitality of the horseshoe crab population and present unique challenges to coastal zone managers at the municipal, state, and national level.

In 2012, as a response to Massachusetts communities concerned with the observed decrease in horseshoe crabs at known spawning sites, the DMF instituted lunar closures for horseshoe crab takes. The underlying rationale was that horseshoe crabs spawn predominantly during new and full moons, an observation made in Delaware Bay. There is no evidence that this is the case in Massachusetts, and in a study conducted in a neighboring state, New Hampshire (Cheng, 2014), spawning appeared not to be dependent on moon phase, but more so on environmental conditions, such as wind speed, wave action and water temperature.

The Massachusetts lunar closures instituted in 2010 were thought to curtail the numbers of horseshoe crabs collected as bait. Horseshoe crabs take 9-11 years to reach sexual maturity, so the lunar closures were expected to have an impact by 2019-2020. Recent spawning surveys in many Massachusetts locations do not show significant positive effects resulting from this management decision.

As noted earlier, it is difficult to make management decisions about takes without a population baseline or a clear indication of the sustainability of the takes. The collective data depict a population that has **not** recovered after changes in management practices (lunar closures, catch limits, etc.) put in place by the DMF.

After 20 years of regulation, horseshoe crab data in Massachusetts do not indicate that the population is recovering from decades of heavy exploitation. There are no data, or the existing data are not robust. The most recent horseshoe crab stock assessment carried out by the Atlantic States Marine Fisheries Commission repeatedly emphasized the need to track horseshoe crabs not only by sex, but by level of maturity, distinguishing between juvenile, newly mature, and mature female horseshoe crabs. The Massachusetts trawl surveys do not and cannot do this.

The ASMFC has determined that too few horseshoe crabs are caught to make these distinctions meaningful. However, without them, the only way of assessing trends in spawning females is spawning surveys on the beaches. 1) Those surveys did not begin until the population was depleted, so there is no baseline, and 2) the confidence intervals are so close in most of the surveys, that it is impossible to detect any real trends in the population overall, and 3) there are so few crabs caught in these surveys that according to the ASMFC stock assessment analysis, it's impossible to detect trends in the overall population of female horseshoe crabs, let alone trends in mature females. In summary, there is no indication that the population is rebounding.

Horseshoe crabs are dominant contributors to the health of coastal ecosystems and currently essential for human health until the recombinant LAL substitute comes into wider use. We are concerned that the take of horseshoe crabs not only cannot rebuild the diminished population but may also lead to further declines. Because female horseshoe crabs take at least ten years to mature, their absence on spawning beaches can be a sign that the population is declining. On beaches in South Carolina, horseshoe crabs spawned until there were no more mature females, and then within a couple of years, the beaches were empty. We are concerned that this could happen in Massachusetts and is happening already in Cape Cod Bay.

To prevent a further decline, to restore the population to robustness, and the coastal ecosystem that relies on an abundance of horseshoe crabs, particularly, the shorebirds whose numbers are also currently in serious decline, we request the designation of the American horseshoe crab as a **Species of Special Concern** in Massachusetts.

CONSERVATION GOALS

Removal from the list will be predicated by populations returning to historical densities of spawning horseshoe crabs throughout its range in Massachusetts and the recovery of red knot and other shorebird populations dependent on horseshoe crab.

Management measures required to meet horseshoe crab conservation goals:

- End horseshoe crab bait take
- Reduce horseshoe crab bycatch limit
- Cap biomedical take
- Require biomedical take and mortality data to be transparent and publicly available
- Prohibit biomedical take during spawning season (April through July)
- Prohibit holding pens
- Expand existing protected areas and designate additional, new ones
- Restore horseshoe crab spawning and nursery habitats
- Fund and implement a dedicated horseshoe crab trawl survey
- Implement a monitoring program for the biomedical take that ensures the use of best management practices in the biomedical take
- Expand the horseshoe crab monitoring program
- Conduct research to determine the minimum and optimal densities of horseshoe crab eggs on the beach that can sustain and rebuild those rapidly declining shorebird populations that rely on horseshoe crab eggs, and whose population declines meet the IUCN qualifications for threatened or endangered status
- Raise public awareness of the importance of horseshoe crabs in coastal ecosystems at spawning beaches and nurseries coastwide

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APPENDIX

1) Horseshoe Crab Spawning Beaches and Nurseries Excerpted from the Massachusetts 2009 Compliance Report to the Atlantic States Marine Fisheries Commission – Horseshoe Crab Submitted by: Robert Glenn, Massachusetts Division of Marine Fisheries. (A survey of spawning beaches in Massachusetts was completed in 2004 (Appendix 1)).

2) Annual Horseshoe Crabs Sex Ratios

3) *Horseshoe Crab Spawning Survey Instructions*

4) Massachusetts 2021 Compliance Report to the Atlantic States Marine Fisheries Commission – Horseshoe Crab

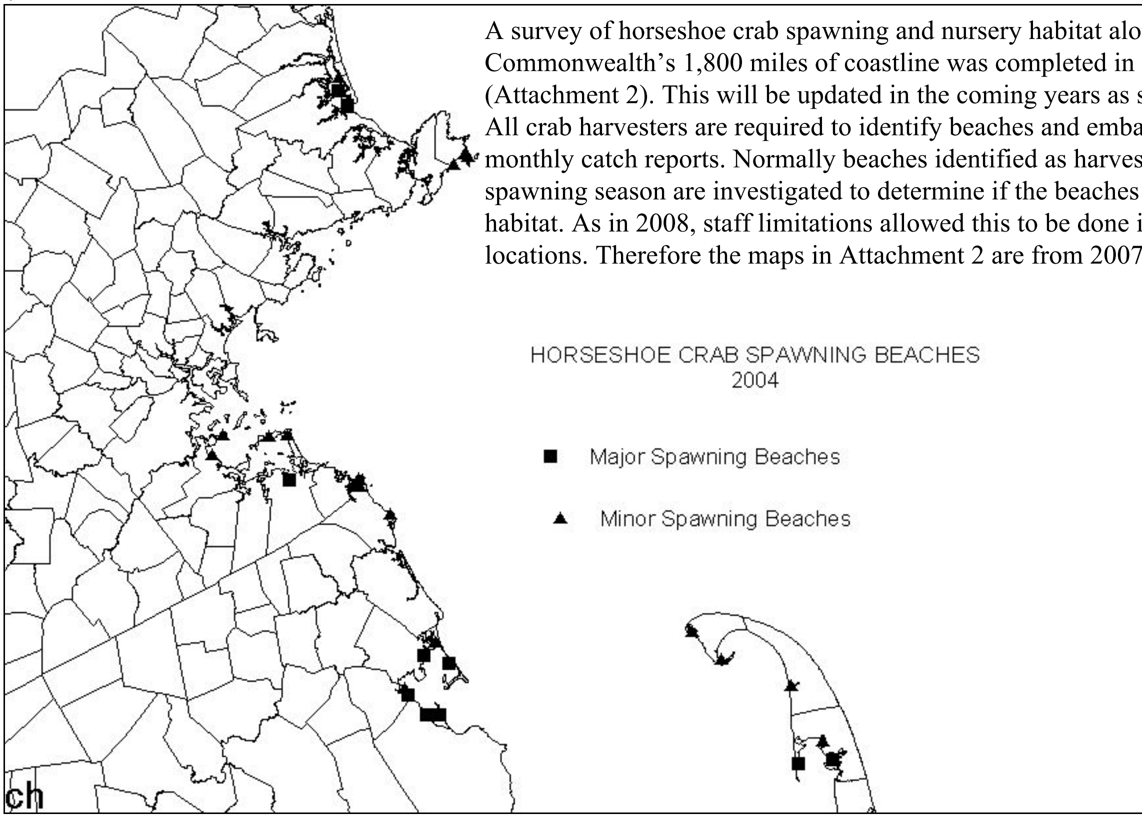
5) Spawning Data 2

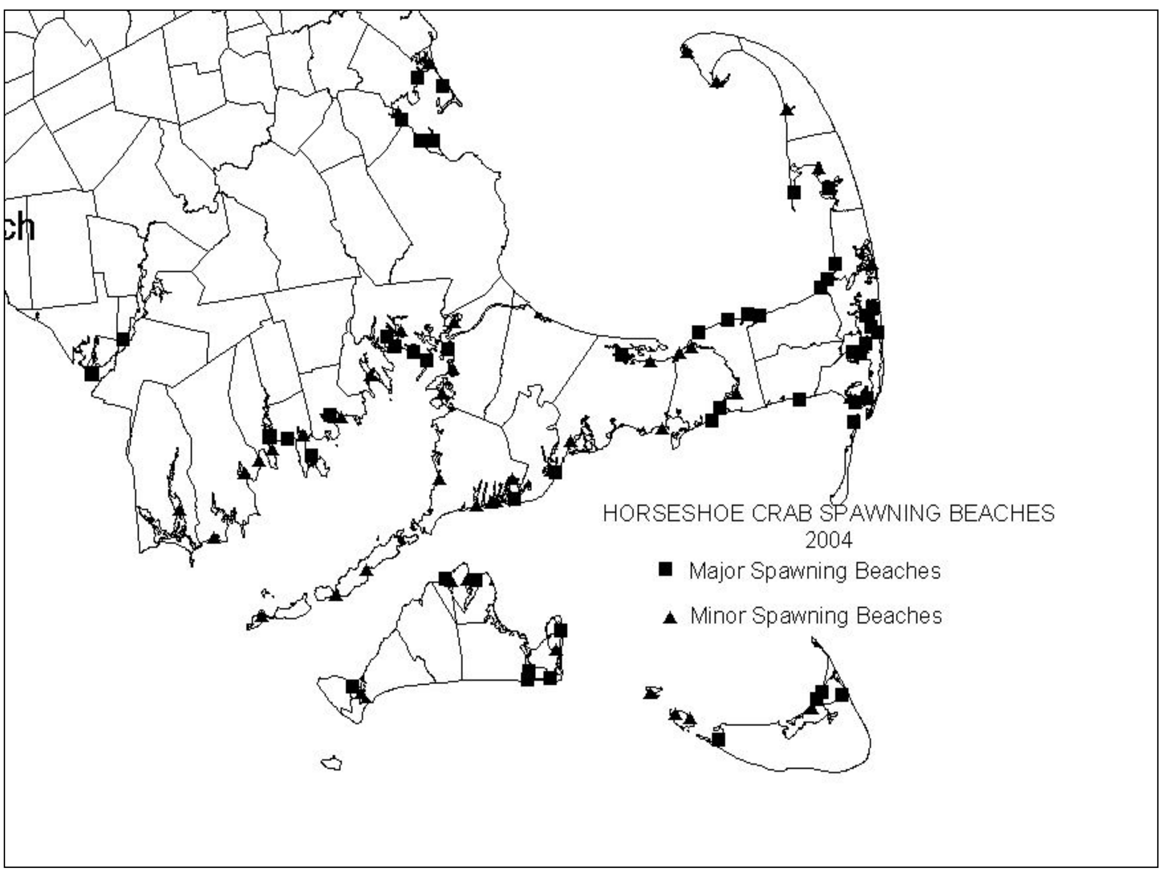
6) IUCN Red List - American Horseshoe Crab *(Limulus polyphemus)*

Appendix 1

Horseshoe Crab Spawning Beaches and Nurseries
Excerpted from the Massachusetts 2009 Compliance Report to the Atlantic States Marine Fisheries Commission – Horseshoe Crab
Submitted by: Robert Glenn, Massachusetts Division of Marine Fisheries

A survey of horseshoe crab spawning and nursery habitat along the Commonwealth's 1,800 miles of coastline was completed in 2004 (Attachment 2). This will be updated in the coming years as staffing allows. All crab harvesters are required to identify beaches and embayments on the monthly catch reports. Normally beaches identified as harvest sites during the spawning season are investigated to determine if the beaches are spawning habitat. As in 2008, staff limitations allowed this to be done in only a few locations. Therefore the maps in Attachment 2 are from 2007.





HORSESHOE CRAB SPAWNING BEACHES
2007

MOUNT HOPE BAY			
Town	**Embayment**	**Beach**	**Density**
Somerset	Mount Hope Bay	Brayton Pt Beach	High
	Taunton River	Pierce Town Beach	High
Fall River	Taunton River	Ark Bait Cove	High
Swansea	Coles River	Bluffs Beach	High
	Coles River	Ocean Grove	High
	Coles River	Cedar Cove	Moderate
BUZZARDS BAY			
Bourne	Buttermilk Bay	Hideaway Village	Moderate
	Phinney's Harbor	Monument Beach	High
	Phinney's Harbor	Mashnee Dike	High
	Phinney's Harbor	Toby Island	Moderate
	Pocasset Harbor	North Cove	Moderate
	Pocasset Harbor	Tahanto Beach	Moderate
Dartmouth	Allen's Pond	South Beaches	Moderate
	Clarks Cove	Anthony Beach	Moderate
	Apponagansett Bay	Apponagansett Park Beach	Moderate
	Little River	Beach at mouth	Reported
	Slocum River	Demarest Lloyd State Beach	Reported
	Clarks Cove	Jones Beach	Moderate
	Apponagansett Bay	Little Bridge Beach	Reported
Fairhaven	Nasketucket Bay	Deacon's Cove	High
	Nasketucket Bay	Edgewater Ramp Beach	Moderate
	Acushnet River	Fairhaven Common's Beach	High
	NB Outer Harbor	Fort Phoenix Beach	Reported
	Nasketucket Bay	Knomere Beach	Reported
	NB Outer Harbor	Priest Cove, Red Rock Beach	High
	Nasketucket Bay	Raymond Street Beach	Reported
	NB Outer Harbor	Silver Shell Beach	Reported
	Acushnet River	Tin Can Island	High
	Nasketucket Bay	Association Beach	Moderate
Falmouth	Great Sippewisset	Black Beach	Reported
	Buzzards Bay	Old Silver Beach	Reported
Gosnold	Cuttyhunk Pond	Church Beach	Reported
	Pasque Pond	Beach	Reported
	Vineyard Sound*	Tarpaulin Cove	Moderate
Mattapoisett	Aucoot Cove	Hollywood Beach	Moderate
	Mattapoisett Harbor	Neds Point	Reported
	Mattapoisett Harbor	Shining Tides Beach	Moderate
	Buzzards Bay	Point Connett Beach	Reported

Marion	Sippican Harbor	Meadow Island	Moderate
	Sippican Harbor	Ram Island	Reported
	Sippican Harbor	Planting Island	Moderate
	Aucoot Cove	Converse Pt Beach	Reported
New Bedford	Acushnet River	Palmer Cove	High
	NB Outer Harbor	East Beach	Moderate
	NB Outer Harbor	Ebb Tide Beach	Moderate
	NB Outer Harbor	Davy's Locker Beach	Reported
Wareham	Buttermilk Bay	Jefferson Shores	Moderate
	Wareham River	Long Beach	High
	Little Harbor	Little Harbor Beach	High
	Wareham River	Pine Hurst Beach	Reported
	Wareham River	Swifts Beach	High
	Buzzards Bay	Stony Point Dike	Moderate
Westport	Westport Harbor	Cherry & Webb Beach	Moderate
	East Branch West Branch	Upper Islands Sanford Flat Area	Reported Reported
	Westport Harbor	Boat Ramp Beach	Reported
SOUTH CAPE			
Barnstable	Centerville Harbor	Craigville Beach(5th Ave)	Reported
	Cotuit Bay	Ropes Beach	Reported
	East Bay	Dawes Beach	Moderate
	Hall Creek	Backside Beach	Reported
	Hyannis Harbor	Kalmus Beach	Reported
	Cotuit Bay	Sampson Island	High
	Cotuit Bay	Pirate Cove	High
Chatham	Nantucket Sound	Cockle Cove Beach	High
	Nantucket Sound	Monomoy Island	High
	Stage Harbor	Harding Beach	High
	Stage Harbor	Morris Island	High
	Oyster Pond	Beaches at mouth	Reported
	Oyster River	Sear's Point Beach	High
Dennis	Bass River	Opposite High Bank	Moderate
	Bass River	Old Field Point	Reported
	Bass River	W. Dennis Beach	High
	Bass River	Georgetown Flats	Reported
Falmouth	Bourne's Pond	Old Mouth	Reported
	Great Pond	Entrance Beaches	Moderate
	Green Pond	Entrance Beaches	Moderate
	Eel River	Washburn Island	Reported
	Waquoit Bay	WBNERR Beach	Reported
Mashpee	Waquoit Bay	Sage Lot Pond	High
	Nantucket Sound	South Cape Beach	Reported
	Popponesset Bay	Popponesset Beach	Reported
	Popponesset Bay	Daniel Island Beach	Reported

	Popponesset Bay	Pirates Cove Landing	Moderate
Yarmouth	Bass River	Wind Mill Beach	Moderate
	Nantucket Sound	Sea Gull Beach	Moderate
	Nantucket Sound	Lighthouse Beach	Moderate
	Parker River/ Lewis Pond	Landing Beach	Reported
MARTHA'S VINEYARD			
Aquinnah	Menemsha Pond	Red Beach	High
Chilmark	Menemsha Pond	Landing	Moderate
Edgartown	Cape Poge Bay	Simon Point	Moderate
	Katama Bay	SE Corner	High
	Katama Bay	South Side	High
	Salt Pond	Fuller Street Beach	Reported
Oak Bluffs	Lagoon Pond	Worcester St.	Reported
	Vineyard Haven	Eastville Point	Moderate
Tisbury	Lake Tashmoo	Flats at mouth	High
	Lagoon Pond	Cedar Neck	Reported
NANTUCKET			
Nantucket	Madaket Harbor	Hither Creek	Moderate
	Muskeget Island	Coves	Reported
	Nantucket Harbor	Backside Outer Beach	Reported
	Nantucket Harbor	Pocomo Point Beach	High
	Tuckernut Island	Coves	Reported
OUTER CAPE			
Chatham	Bassing Harbor	Fox Hill	Moderate
	Crows Pond	Nickerson Neck	Moderate
	Chatham Harbor	North Beach	Reported
	Chatham Harbor	Outermost Marine Cove	High
	Pleasant Bay	Muddy Creek Landing	Reported
	Chatham Harbor	South Beach	Reported
	Pleasant Bay	Strong Island, East Side & Creeks	High
	Pleasant Bay	Ryders Cove	Reported
Eastham	Nauset Harbor	Stony Island	Reported
	Nauset Harbor	Outer Beach	Reported
Harwich	Round Cove	Landing	Reported
Orleans	The River	Barley Neck	High
	Little Pleasant Bay	Hog Island	High
	Little Pleasant Bay	Jack Knife Cove	High
	Kesczyogansett Pond	Town Landing	Moderate
	Town Cove	YC Landing	Moderate
	Little Pleasant Bay	National Seashore	High
	Little Pleasant Bay	Pochet Island	High
	Little Pleasant Bay	Sampson Island	High
	Little Pleasant Bay	Old Field Point	Reported
	Pleasant Bay	Strong Island	High

Cape Cod Bay			
Barnstable	Barnstable Harbor	Scudder Lane	High
	Barnstable Harbor	Sand Island	High
	Barnstable Harbor	The Cove	Reported
	Barnstable Harbor	Calves Pasture Point	High
	Barnstable Harbor	Bone Hill	Reported
	Barnstable Harbor	Eastern end	High
	Barnstable Harbor	Salten Point	High
Brewster	Cape Cod Bay	Brewster Flats	High
	Cape Cod Bay	Ellis Landing	High
	Cape Cod Bay	Namskaket Creek	High
	Cape Cod Bay	Paine's Creek	Moderate
Dennis	Cape Cod Bay	Chapin Beach	High
	Cape Cod Bay	Corporation Beach	High
	Cape Cod Bay	Cold Storage Beach	Moderate
	Cape Cod Bay	Quivett Creek	Moderate
	Cape Cod Bay	Chase Garden Creek	Reported
Duxbury	Duxbury Bay	Back River	High
	Duxbury Bay	Duxbury Beach	High
	Duxbury Bay	Ship Yard Lane	High
	Duxbury Bay	Bradford Street	High
Eastham	Cape Cod Bay	First Encounter	High
	Cape Cod Bay	Sunken Meadow	High
	Cape Cod Bay	Boat Meadow Sand Spit	Reported
Kingston	Kingston Bay	Gray's Beach	Reported
	Kingston Bay	Rocky Nook Association Beach	Reported
Orleans	Cape Cod Bay	Rock Harbor Beach	Moderate
	Cape Cod Bay	Skaket Beach	Reported
Plymouth	Plymouth Harbor	Plymouth Beach	High
	Duxbury Bay	Saquish Cove	Reported
	Plymouth Harbor	Steven's Field	High
Provincetown	Hatches Harbor	Entrance Beach	Reported
	Inner Harbor	Wood's End	Moderate
Truro	Pamet Harbor	Harbor Bar	Reported
	Pamet Harbor	Landing Beach	Reported
	Cape Cod Bay	Corn Hill Beach	Reported
Wellfleet	Wellfleet Harbor	Chipman Cove	High
	Wellfleet Harbor	Great Island	Moderate
	Wellfleet Harbor	Mayo Beach	Reported
	Wellfleet Harbor	WBWS	High
	Wellfleet Harbor	Indian Neck	Moderate
Yarmouth	Cape Cod Bay	Bass Creek	Moderate
	Chase Garden Creek	Gray's Beach	Moderate
MASSACHUSETTS BAY			
Cohasset	Cohasset Harbor	Bassing Harbor Beach	Moderate

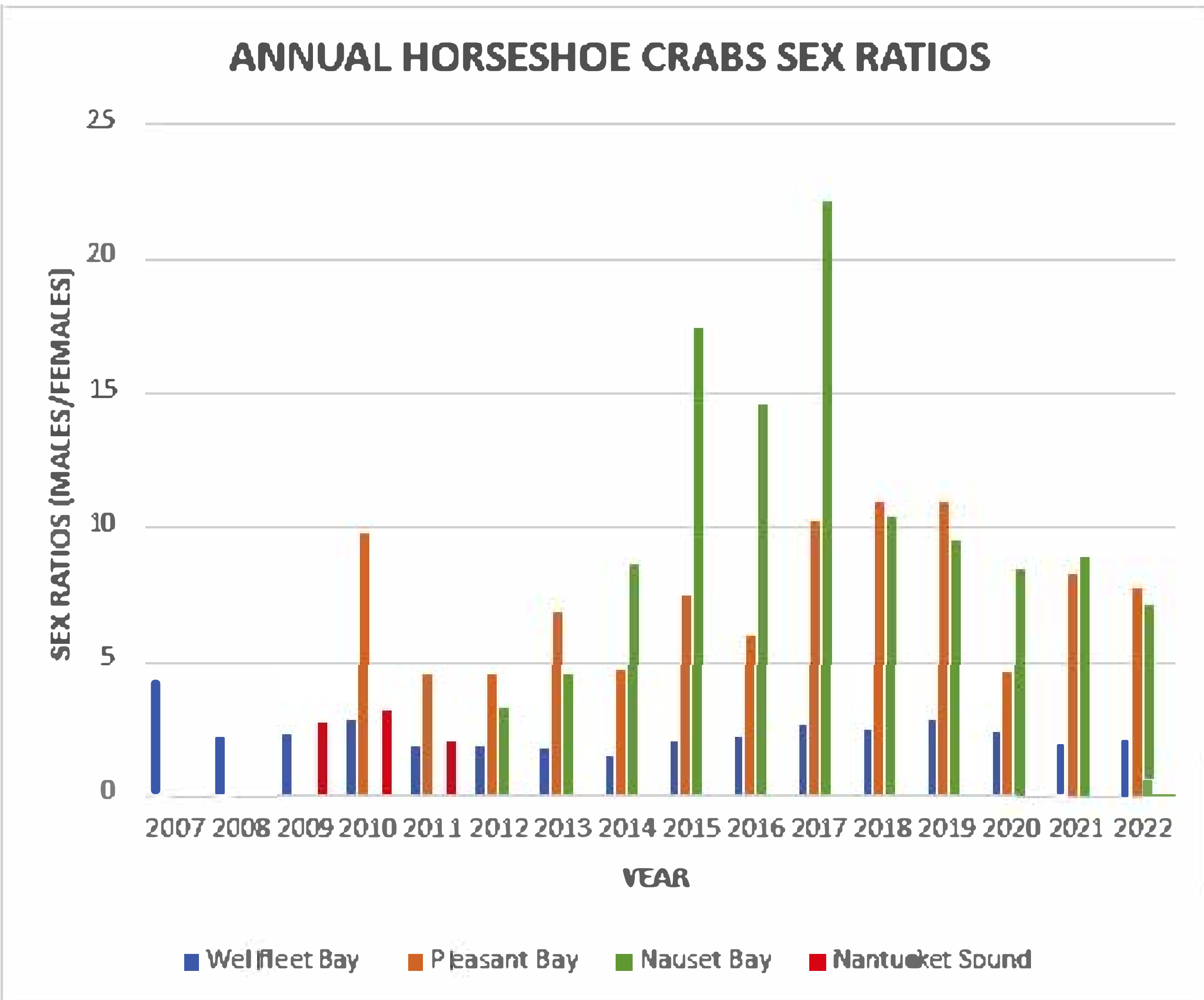
	Cohasset Harbor	Briggs Cove	Reported
Hingham	Hingham Harbor	Hingham Beach	Reported
Hull	Hull Bay	Pt Allerton Beach	Reported
	Hull Bay	Windmill Pt Beach	Reported
Scituate	Scituate Harbor	Jericho Landing Beach	Reported
NORTH SHORE			
Ipswich	Ipswich Bay	Cranes Beach	Reported
Newbury	Plum Island Sd.	Parker River Refuge	High
Quincy	Quincy Bay	Wollaston Beach	Reported
Rockport	Sandy Bay	Old Garden Beach	Moderate
		Back Beach	Reported
		Front Beach	Reported

HORSESHOE CRAB NURSERY AREAS 2007

MOUNT HOPE BAY		
TOWN	**EMBAYMENT**	**DENSITY**
Somerset	Mount Hope Bay	High
	Taunton River	High
Swansea	Mount Hope Bay	High
	Coles River	High
BUZZARDS BAY		
Bourne	Buttermilk Bay	High
	Phinney's Harbor	High
Fairhaven	Nasketucket Bay	High
	Outer Harbor	Moderate
Mattapoisett	Mattapoisett Harbor	Reported
New Bedford	Outer Harbor	Moderate
Wareham	Buttermilk Bay	Reported
	Outer Wareham River	Moderate
Westport	Westport Rivers	Reported
SOUTH CAPE		
Barnstable	Lewis Bay	Reported
	Cotuit Bay	Moderate
Chatham	Cockle Cove Beach	High
	Stage Harbor	High
	Monomoy Island	High
Dennis	Bass River	High
Falmouth	Waquoit Bay	Reported
Mashpee	Waquoit Bay (Sage Lot Pond)	High
	Popponesset Bay	Moderate
Yarmouth	Bass River	High

MARTHA'S VINEYARD		
Aquinnah	Menemsha Pond	Moderate
Chilmark	Menemsha Pond	Moderate
Edgartown	Cape Poge Bay	Moderate
	Katama Bay	High
Oak Bluffs	Lagoon Pond	Reported
	Vineyard Haven Harbor	Reported
Tisbury	Lake Tashmoo	Moderate
NANTUCKET		
Nantucket	Madaket Harbor	Reported
	Muskeget Island	Reported
	Nantucket Harbor	High
	Tuckernuck Island	Reported
OUTER CAPE		
Chatham	Bassing Harbor	Reported
	Chatham Harbor	High
	Crowes Pond	Reported
	Pleasant Bay	High
Eastham	Nauset Marshes	Moderate
Harwich	Pleasant Bay	High
Orleans	Little Pleasant Bay	High
	Pleasant Bay	High
CAPE COD BAY		
Barnstable	Barnstable Harbor	High
Brewster	Brewster Flats	High
Dennis	Dennis Flats	High
Duxbury	Duxbury Bay	High
	Back River Marsh	High
Eastham	Eastham Flats	High
Kingston	Kingston Bay	Moderate
Orleans	Orleans Flats	High
Plymouth	Plymouth Harbor	High
Provincetown	Hatches Harbor	Reported
Truro	Pamet Harbor	Reported
Wellfleet	Wellfleet Harbor	High
Yarmouth	Yarmouth Flats	High
	Chase Garden Creek	Reported
MASSACHUSETTS BAY		
Cohasset	Cohasset Harbor	Reported
Hull	Hull Bay	Reported
Scituate	Scituate Harbor	Reported
NORTH SHORE		
Ipswich	Plum Island Sound	High
Newbury	Plum Island Sound	High

Appendix 2


ANNUAL HORSESHOE CRABS SEX RATIOS
25
20
15
10
5
0
SEX RATIOS (MALES/FEMALES)
2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022
YEAR
Wellfleet Bay
Pleasant Bay
Nauset Bay
Nantucket Sound

adapted from : http://www.lsc.usgs.gov/aeb/2065/protocol.asp

Appendix 3 Horseshoe Crab Spawning Survey Instructions

CONTENTS:

1. Instruction sheet and pacing estimation calculation
2. Random Numbers Table
3. Table of dates and times of surveys (to be included a later date)
4. Copy of Spawning Survey Data Sheet

Supplies for each site:
5 m^2 quadrat
thermometer
pens/pencils
datasheets and clipboard
flashlights
hip or knee boots

PREPARATION FOR THE SURVEY:

Determine your pace
Follow the instructions before your first assigned time to survey and preferably on a beach since pace length is affected by the surface you are walking on. This should take 15-20 minutes to complete.

*Note: Each person has to determine their pace **ONCE** for the whole season.*
*Note: All measurements are **metric***

1. Lay out the 20 **meter** measuring tape straight on the beach.
2. Count the number of paces it takes you to walk the length of the string using your normal stride. Remember that a pace is two steps. Enter this number in the space next to TRIAL 1 on the Pacing Trial Form.
3. Repeat this process twice more. Enter the second number next to TRIAL 2 and the third number next to TRIAL 3.
4. These three numbers are A, B, and C on the Pacing Trial Form. Add A, B, and C, and divide this number by three to find D, your average number of paces per 20 meters.
5. Divide D by 20 to find your average number of paces per meter (E).

NAME:____
NUMBER OF PACES IT TAKES TO WALK 20 METERS OR APPROXIMATELY 66 FEET:
TRIAL 1 = _____________ (A)
TRIAL 2 = _____________ (B)
TRIAL 3 = _____________ (C)
AVERAGE PACES PER 20 METERS:
TOTAL (A+B+C) = _____________ / 3 = ______________ (D) PACES PER 20 METERS
NUMBER OF PACES PER METER:
(D) ______________ / 20 = ________________ (E) PACES PER METER

Note: The average number of paces per meter is probably different for each person, because it depends on your stride length. It is important that you do this yourself and know these numbers before you arrive at the beach.

adapted from : http://www.lsc.usgs.gov/aeb/2065/protocol.asp

Clothing and accessories

- Wear appropriate clothing for weather and wet conditions at the water's edge. Consider using sunscreen during the day. *If thunderstorms are present do not go onto the beach.*
- For night surveys bring a headlamp or a flashlight. Headlamps are most useful because they free up both hands.
- Bring a clipboard or hard surface to write on. Also, bring a few pencils and pens.
- Shoes are a necessity. We recommend boots, water shoes or old sneakers. *Do not go barefoot*.
- An accurate wristwatch is needed for recording arrival time, as well as start and stop times of the survey.

SURVEY PROTOCOL:

Surveys should be conducted within 2 days of the FULL or NEW moon. This gives a 5-day (2 days prior, the day of, and 2 days after the moon) window to complete the surveys for each moon period. It is desirable to survey each location 2 to 3 times during each moon, preferably both day AND night (4 to 6 surveys) surveys at each location). Priority for surveys should be given to those dates closer to or on the full or new moon. The minimum number of surveys for a location and moon is 1 DAY and 1 NIGHT survey. If it is logistically impossible to conduct night surveys (for example, access to Marsh 2-3 or Hog Island) that the minimum is 2 DAY surveys per moon.

It is preferred that all surveys take place on the SAME day or night.

Setup

- Arrive at the beach at least 30 minutes before high tide. Record the time you arrive in the space marked ARRIVAL AT SITE on the Beach Site Sheet.

- Fill out the Survey Data sheet as completely as possible. The thermometers are used for both air and water temperature. Even if the weather prevents you from doing the survey, please fill out the survey sheet with all possible information and explain why the survey could not be completed.

- To survey the horseshoe crabs, you will start at one end of a section of beach, walk to the other end, and along the way place quadrats to count horseshoe crabs.

- Flip a coin to decide which end of the beach section you will start: if heads, start at the south or west end of the beach, depending on the orientation of the beach; if tails, start at the north or east end of the beach. Fill that information out on the SURVEY START POINT line of the Beach Site Sheet.

- Go to the end of the survey beach to begin depending on the results of the coin toss. This site will be either marked by a pole or described to the team members before the surveys. Specific instructions will be provided for each particular beach.

- As you walk to the starting location, find a stick (or use a flag stick) that you can use to determine high tide. When you get to the starting location, push the stick into the sand at the tide line. The tide line is the highest point on the beach that the water reaches. Move the stick up the beach as the water reaches higher on the beach. Begin the survey when the tide begins to recede and the water no longer reaches the stick. Record your starting time on the Beach Site Sheet where it says START OF SURVEY.

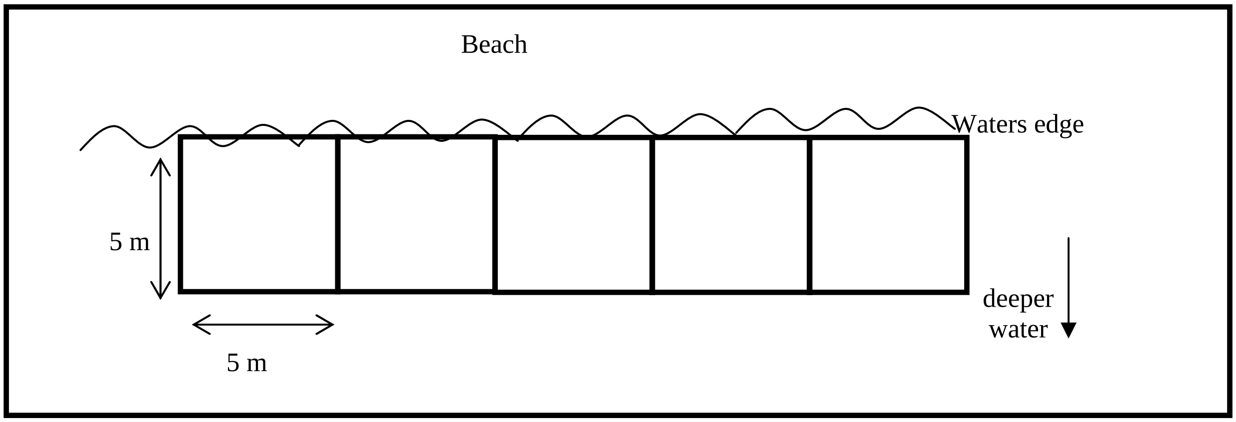
adapted from : http://www.lsc.usgs.gov/aeb/2065/protocol.asp

Beaches Less than 100m in Length

(This can also be done for beaches up to 200m in length)

Survey Protocol #1: Strip Transects with 5m blocks specific to Cape Cod (modified from Delaware Bay protocol)

- You will be surveying in groups with usually 3 people. A survey protocol diagram (below) illustrates the placement of quadrats. You will be recording the number of horseshoe crabs within EACH 5m quadrat or block of beach. It is important to record each block so the distribution of the crabs on the beach can be known.



1) **Flip a coin** to decide which end of the designated beach length to start sampling.

2) **Choose 1 random number between 0 and 10 from the random number table**. This number is the random start point (in meters) where the **1st quadrat begins** within the first 10 meters of beach. Pace to the location of the 1st quadrat (calculating the paces you take by multiplying your paces per meter by the random number chosen). All remain quadrats immediately adjacent to each other. *We are only choosing 1 random starting location.*

3) Begin sampling just as the water begins to recede from peak high tide – use a stick to determine this time.

4) The quadrat size is 5 x 5 meters in area. The **first quadrat begins at the toe of your last step**. You place one of the end stakes in the sand at the water line (high tide line for the first quadrat). One person then walks out into the water perpendicular to the shore line 5 meters and places the middle stake in the sand. He or she then walks parallel with the shoreline to place the third stake in the sand (*making sure to form a 90 degree angle as best as possible*). This forms half of the square where the horseshoe crabs will be sampled. Follow the last stake back to the shoreline to imagine where the square ends. The third person or recorder will stand where a fourth stake would be in order to visualize a complete square. Mark the spot in the sand where the fourth stake would go with your foot.

5) One person is recording the information on the survey sheet, one is in the quadrat counting crabs, the third person (if available) is assisting with male/female identification, the placement of stakes, and is standing at the position of where the fourth stake would be at the water's edge.

6) First count the number of males then the number of females in the quadrat.

7) For every quadrat count the number of **single** horseshoe crabs (single individuals), the number of **doubles or pairs** (ONE female with ONE male clasped on), and the number of **satellites** (males surrounding females which are not attached to her but trying to dislodge the clasped male).

8) Record all data on the Tally Sheet specific for your beach. Be sure to record the number of crabs in EACH quadrat or 5m block. If there are no crabs in the quadrat record a zero (0).

9) Once you are done with the count at a given quadrat, pull up the stakes and mark out the next adjacent quadrat. **The edge of the quadrat closest to land will line up with the receding water's edge as you work down to the other end of the site**. Repeat the quadrat setup and record the counts. Continue until you have sampled the entire beach section.

10) Fill out the Beach Site Sheet as appropriate.

Counting Horseshoe Crabs

- Once the quadrat is in place try not to move it again until you are done counting.

- You will count all the horseshoe crabs 'in the quadrat'. A horseshoe crab is considered 'in the quadrat' if **more than half of its body is inside the quadrat.**

- When there are numerous animals, you may have to lift some up to assure you've counted all of those underneath. Heavy work gloves are useful for this. Try to minimize disturbance to the spawning horseshoe crabs. Spawning females will be partially buried in the sand while laying eggs. ***DO NOT LIFT UP A PARTIALLY BURIED HORSESHOE CRAB***.

- Count the animals of each sex separately. If a horseshoe crab is not buried, the two most common ways to determine its sex are its size and position. Males are, for the most part, smaller and 'clasped' or crowding on top of females. There also tends to be more males than females.

- Report your count of pairs, satellites, and singles and tally by total number of males & females to the recorder who will record the information for each quadrat (see data sheet). If the recorder is working with another observer, keep the tally in your head until the recorder can record quadrat counts for you. Don't pick up the quadrat and move to the next quadrat location until you know the recorder has recorded all the information for your present quadrat.

- If there are horseshoe crabs outside the quadrats keep a tally of the total number that are observed outside, place this number on the TOTAL # CRABS OUTSIDE space. This means all crabs that you can see, regardless how deep the water. Try to record the number of crabs by pairs, satellites, and singles. This information is used to estimate population size on the spawning beach.

- If you see a horseshoe crab with a tag, record the tag number and color of the tag. Tags are attached to the right rear point (although some animals were tagged on the left point). Record the tag numbers in the TAG #'S OF TAGGED CRABS space. DO NOT remove the TAG.

- Report zero (0) when there are no horseshoe crabs within the quadrat. Do not move the quadrat from the preselected quadrat location to include nearby animals. Empty quadrats are just as important as those with horseshoe crabs because they will help reflect changes in the population.

Once you are done surveying

- Record the time in the space marked END OF SURVEY on the Beach Site Sheet.

Return all of the original data sheets to your survey coordinator:

Massachusetts Audubon: ________________________________

Massachusetts Department of Marine Fisheries _________________________________

US Fish & Wildlife Service: _____________________________________

University of Rhode Island: _________________________________

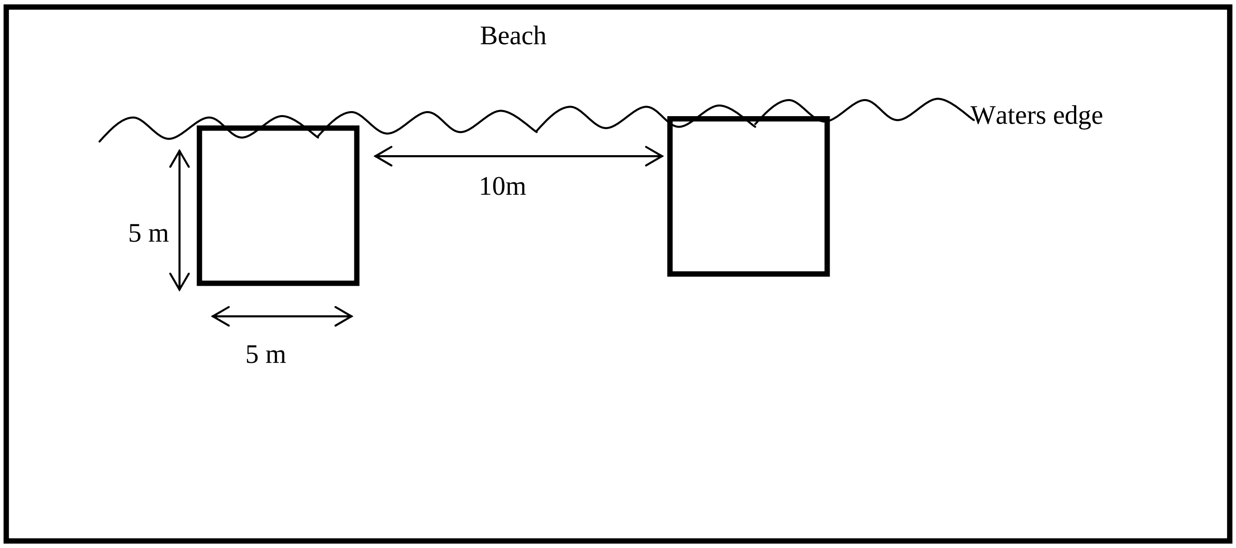
Other ____

adapted from : http://www.lsc.usgs.gov/aeb/2065/protocol.asp

Beaches Greater than 100m to 200m in Length

Survey Protocol #2: Placing the Quadrats with 10m intervals – specific to Cape Cod (modified from Delaware Bay protocol)

- You will be surveying in groups with usually 3 people. A survey protocol diagram (below) illustrates the placement of quadrats.



1) **Flip a coin** to decide which end of the designated beach length to start sampling.

2) **Choose 1 random number between 0 and 10 from the random number table**. This number is the random start point (in meters) where the 1st quadrat begins within the first 10 meters of beach. Pace to the location of the 1st quadrat (calculating the paces you take by multiplying your paces per meter by the random number chosen). All remain quadrats will be placed 10 meters apart. *We are only choosing 1 random starting location.*

3) Begin sampling just as the water begins to recede from peak high tide – use a stick to determine this time.

4) The quadrat size is 5 x 5 meters in area. The **first quadrat begins at the toe of your last step**. You place one of the end stakes in the sand at the water line (high tide line for the first quadrat). One person then walks out into the water perpendicular to the shore line 5 meters and places the middle stake in the sand. He or she then walks parallel with the shoreline to place the third stake in the sand (*making sure to form a 90 degree angle as best as possible*). This forms half of the square where the horseshoe crabs will be sampled. Follow the last stake back to the shoreline to imagine where the square ends. The third person or recorder will stand where a fourth stake would be in order to visualize a complete square. Mark the spot in the sand where the fourth stake would go with your foot.

5) One person is recording, one is in the quadrat counting, the third person is assisting with male/female identification, the placement of stakes, and is standing at the position of where the fourth stake would be at the water's edge.

6) First count the number of males then the number of females in the quadrat.

7) For every quadrat count the number of **single** horseshoe crabs (single individuals), the number of **doubles** (a female with a male clasped on), and the number of **satellites** (males surrounding females which are not attached to her but trying to dislodge the clasped male).

8) Record all data on the Tally Sheet specific for your beach.

9) Once you are done with the count at a given quadrat, pull up the stakes and stretch out the three stakes to form a 10 m length line to measure the distance to where the next quadrat starts. Measure the 10 m beginning at the spot marked in the sand where the fourth stake would have been. **The edge of the quadrat closest to land will line up with the receding water's edge as you work down to the other end of the site**. Repeat the quadrat setup and record the counts. Continue with 10 meters between quadrats and do as many as are specified for your particular site.

10) Fill out the Beach Site Sheet as appropriate.

Appendix 4

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Massachusetts
Division of Marine Fisheries

MarineFisheries
Commonwealth of Massachusetts

Massachusetts 2021 Compliance Report to the Atlantic States Marine Fisheries Commission – Horseshoe Crab

Submitted by:
Derek Perry, Marine Fisheries Biologist
Massachusetts Division of Marine Fisheries
South Shore Field Station
836 South Rodney French Boulevard
New Bedford, MA 02744

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I. Introduction

Massachusetts Division of Marine Fisheries (DMF) staff and numerous volunteer groups conducted spawning beach surveys at 15 beaches during the full and new moons of May and June. April spawning beach surveys were discontinued in 2021 because observations of April spawning activity have been low since the survey expanded in 2013 to include the second half of April. Prosomal widths were taken from 1,815 bait crabs and 904 biomedical crabs as part of our market sampling program. The bait fishery harvested 95% of the 2021 Massachusetts self-imposed quota. The number of crabs bled for biomedical purposes remains confidential due to the limited number of biomedical facilities in the state (one). A second biomedical firm is expected to open in 2022; Charles River Laboratories plans to open a bleeding facility in Harwich, Massachusetts.

II. Request for *de minimis* status – not applicable

III. Previous calendar year's fishery

a. <u>Bait Harvest</u>

In 2021, 44 of 210 horseshoe crab bait permits issued by DMF were actively fished, representing an increase of five active permits and a decrease of five issued bait permits from 2020. Nine fishermen with Coastal Access Permits also participated in the fishery in 2021. See Table 1 for the associated harvests. Based on dealer data, 47% of the quota issued by ASMFC to Massachusetts (330,377 crabs), and 95% of the more restrictive state quota voluntarily self-imposed by Massachusetts (165,000 crabs) was harvested. Dealers reported purchasing 11,080 more crabs than harvesters reported selling (Table 2). This is attributed to harvester trips where catch was not reported but was reported by the dealer. Bait crabs were harvested primarily by mobile gear (trawl or dredge; 62% of harvest) or by hand (including rakes, dipnets, and hand tongs; 36%), with 2% harvested by other means (gill net, weirs, pots, etc.) (Table 3). Bait crabs harvested in May and June accounted for 48% of all bait crabs landed in 2021 (Table 4).

Table 1. Number of permits issued, number of permits actively fished, and number of crabs fishermen reported harvesting by permit type (data source: Massachusetts Trip Level Reports and NMFS Vessel Trip Reports). Confidential data has been replaced with an asterisk.

Permit Type	# of Permits Issued	# of Permits Fished	# of Crabs Harvested
Biomedical	14	4	*
Commercial	210	44	119,239
Coastal Access	N/A	9	25,694

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Table 2. Number of bait crabs reported by bait harvesters (data sources: Massachusetts Trip Level Reports, NMFS Vessel Trip Reports).

	Female	Male	Unclassified	Total
Bait Harvest	29,458	7,433	108,042	144,933

Table 3. Number of bait crabs captured by method, as reported by harvesters (data source: Massachusetts Trip Level Reports and NMFS Vessel Trip Level Reports).

Harvest Method	# of Crabs	# of Total
Hand	52,546	36%
Mobile	89,603	62%
Other	2,784	2%

Table 4. Number of bait crabs harvested by month, as reported by harvesters (data sources: Massachusetts Trip Level Reports and NMFS Vessel Trip Reports). Confidential data has been replaced with an asterisk.

	# of Crabs
JAN	*
FEB	*
MAR	*
APR	1,990
MAY	42,338
JUN	26,948
JUL	18,552
AUG	27,994
SEP	13,684
OCT	4,840
NOV	2,886
DEC	2,508

b. <u>Scientific and Research Harvest</u>

As a condition of permit renewal, researchers that wish to harvest horseshoe crabs in Massachusetts are required to report the number of horseshoe crabs taken for scientific purposes. In 2021, two research organizations applied for scientific permits to collect horseshoe crabs. Under these permits, 15 crabs were collected. Another organization released 46 age-1 crabs collected as eggs in 2020.

c. Biomedical Fishery

In 2021, DMF issued 14 biomedical harvest permits, four of which were actively fished. This represents a decrease of one issued permit and no change to the number of active permits from 2020.

Associates of Cape Cod (ACC) was the only biomedical company producing *Limulus* Amebocyte Lysate (LAL) in Massachusetts in 2021. ACC filed monthly catch reports listing the dealers from whom they purchased crabs, location of harvest, the number and sex of crabs purchased, and the ultimate disposition of the crabs (released or returned to bait market). ACC also reported the number of crabs they rejected or received dead. Per the terms of the Letter of Authorization issued to ACC, they must adhere to the following conditions: keep crabs moist during transport and storage, transport crabs in a temperature-controlled truck with the thermostat set between 50 and 60° F, keep crabs in the laboratory at ≤70° F, and hold crabs in barrels no more than approximately 2/3 full.

Confidential data has been removed from this section.

d. Shorebird monitoring- Not applicable

e. Benthic Sampling

Except for 2020, which was missed due to the Covid-19 pandemic, DMF's Resource Assessment Project has conducted seasonal spring (May) and fall (September) bottom trawl surveys in state waters since 1978. Approximately 100 tows are made during each season in five bio-geographic areas (Figure 1), using a stratified random sampling design, with 22 total strata. The net's design (¾-sized two seam 39' x 51' otter trawl with 3 ½" cookies on a chain sweep, ¼" knotless codend liner) is appropriate for sampling horseshoe crabs; however, the vessel size precludes towing inside most shallow embayments less than approximately 25 feet. For this report, areas 1–3 are considered Southern New England (SNE), and areas 4–5 are the Gulf of Maine (GOM). All data reported are from the survey's two shallowest depth strata (0–30' and 30–60', combined) because nearly all horseshoe crabs caught in this survey since 1978 have come from these two strata.

Horseshoe crab survey results from the 2021 DMF spring and fall trawl surveys were mixed (Figure 2 though Figure 5). South of Cape Cod, mean number and weight of spring caught males and females in SNE (Figure 2 and Figure 3) remain near their respective time series highs, but at or below time series medians in the fall. North of Cape Cod, 2021 mean number and weight data points were at or below their time series medians during the spring and fall surveys. Size distribution data are given in Figure 6 through Figure 13. Crabs south of Cape Cod are usually larger (and more numerous) than crabs north of Cape Cod.

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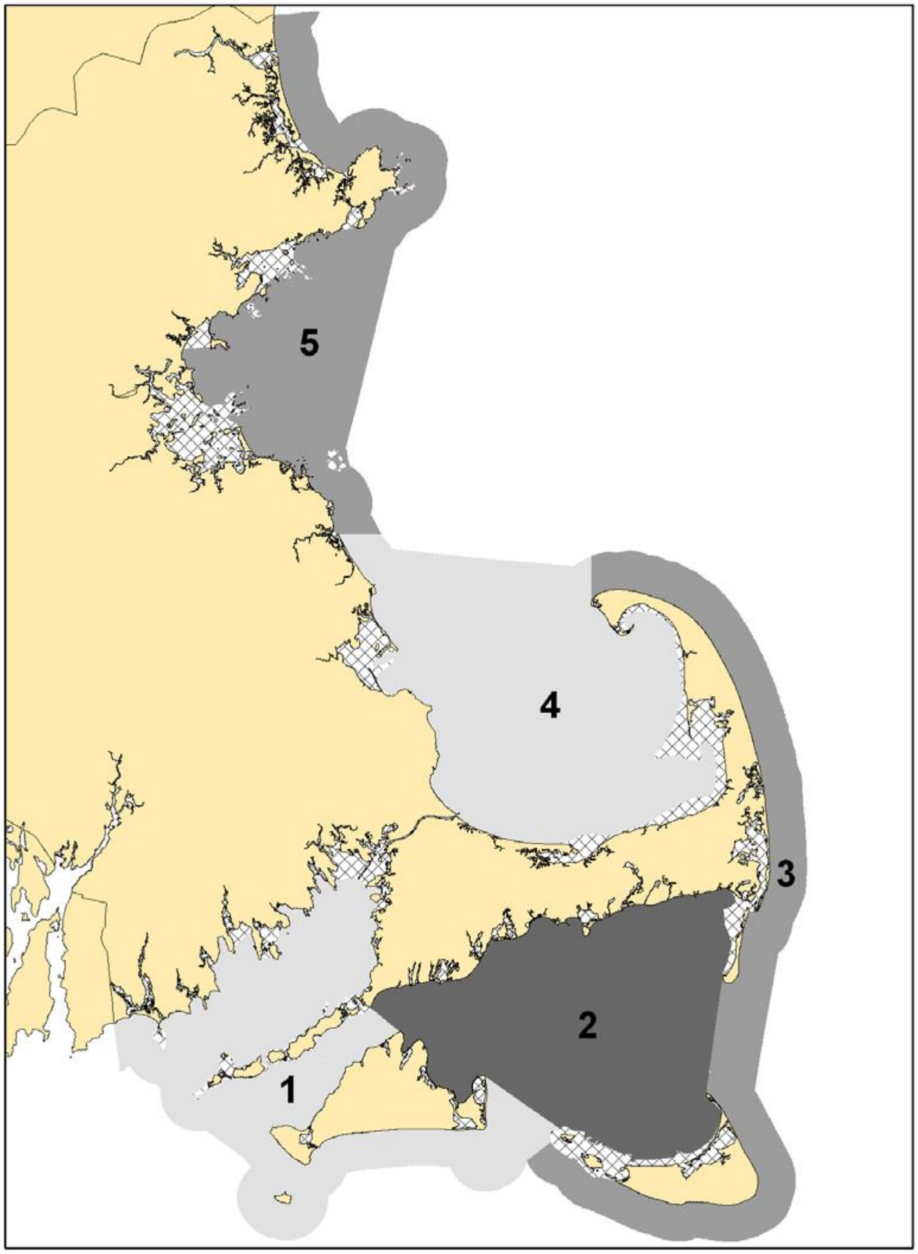

Figure 1. Map of regions for DMF's bottom trawl survey. For this report, regions 1–3 are considered Southern New England (SNE) and regions 4–5 are Gulf of Maine (GOM).

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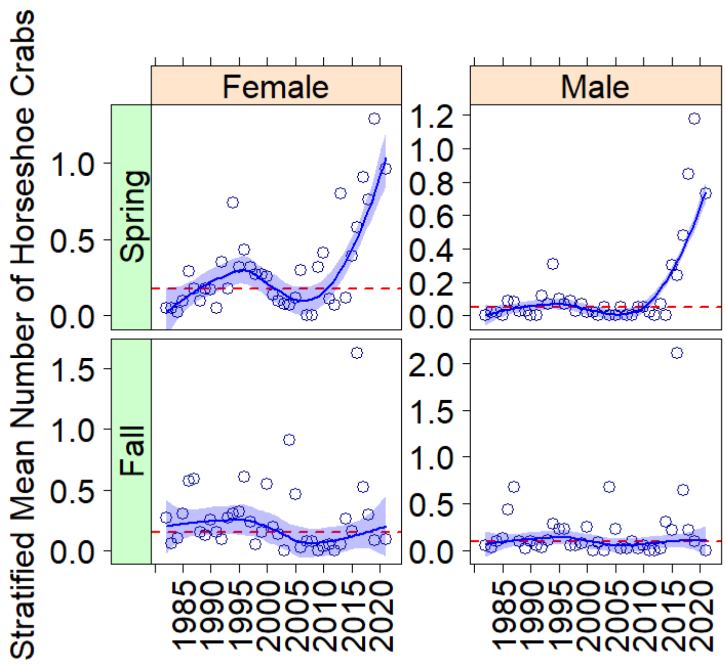


Figure 2. Bootstrapped mean number of horseshoe crabs per tow from the two shallowest depth strata (0–30’ and 30–60’ combined) of the DMF bottom trawl survey in SNE, by survey season and crab sex. The survey was not conducted in 2020 due to the Covid-19 pandemic. Red, dashed line is the time series median, blue line is a loess fit using family=symmetric, and span=0.66. These settings provide a resistant fit to outliers at the end of the time-series. Blue shaded area is an approximate 95% confidence interval for the fit.

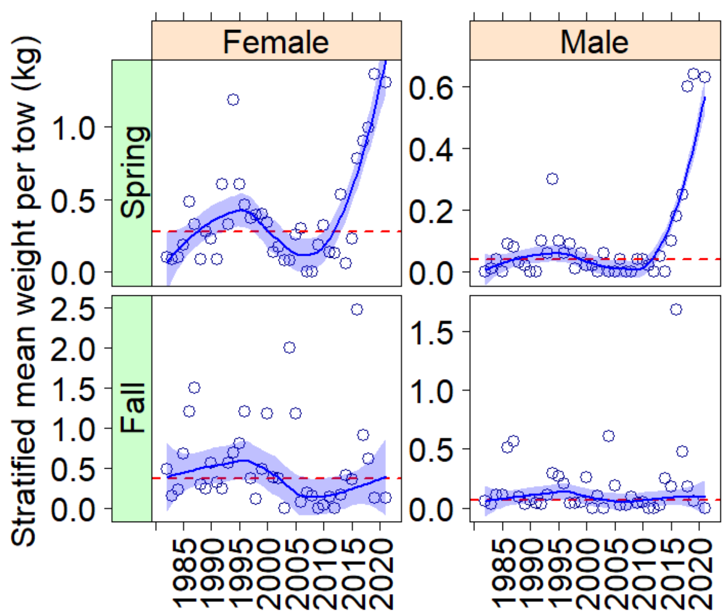


Figure 3. Bootstrapped horseshoe crab mean weight (kg) per tow from the two shallowest depth strata (0–30’ and 30–60’ combined) of the DMF bottom trawl survey in SNE, by survey season and crab sex. The survey was not conducted in 2020 due to the Covid-19 pandemic. Red, dashed line is the time series median, blue line is a loess fit using family=symmetric, and span=0.66. These settings provide a resistant fit to outliers at the end of the time-series. Blue shaded area is an approximate 95% confidence interval for the fit.

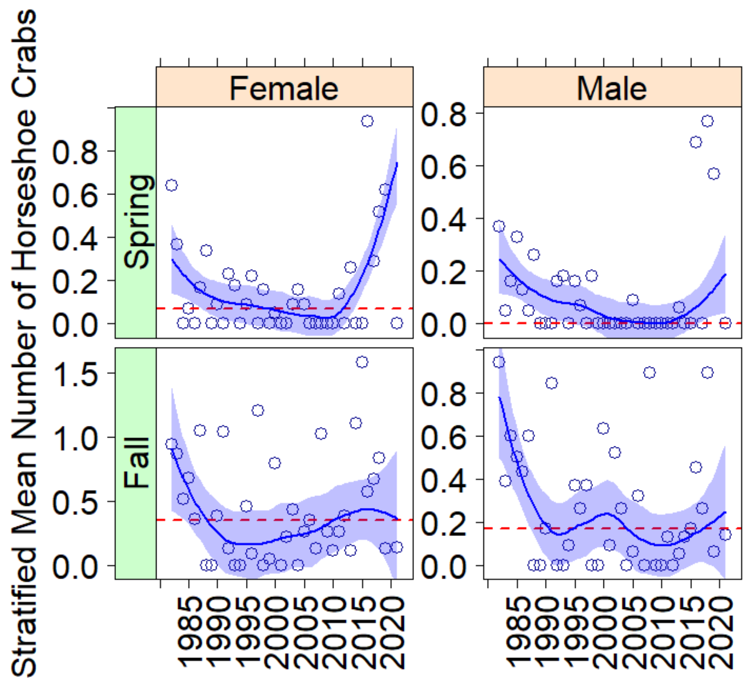


Figure 4. Bootstrapped mean number of horseshoe crabs per tow from the two shallowest depth strata (0–30' and 30–60' combined) of the DMF bottom trawl survey in GOM, by survey season and crab sex. The survey was not conducted in 2020 due to the Covid-19 pandemic. Red, dashed line is the time series median, blue line is a loess fit using family=symmetric and span=0.66. These settings provide a resistant fit to outliers at the end of the time-series. Blue shaded area is an approximate 95% confidence interval for the fit.

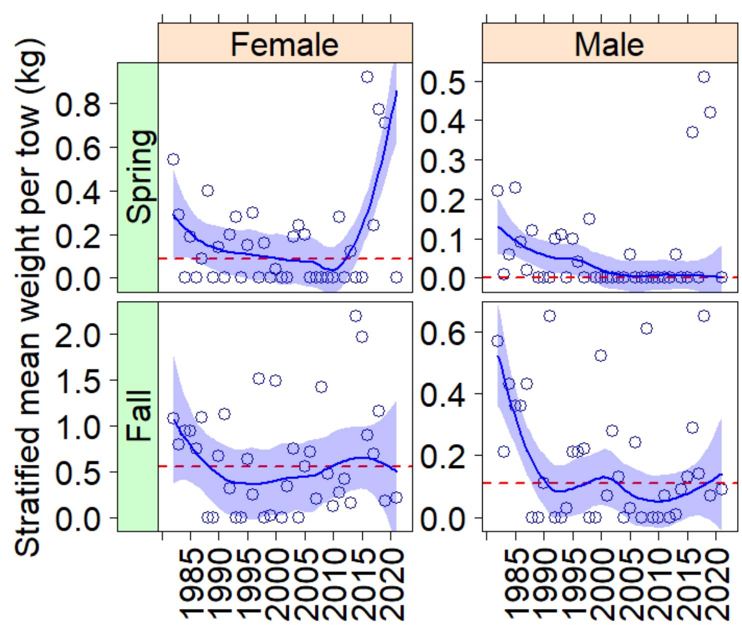


Figure 5. Bootstrapped horseshoe crab mean weight (kg) per tow from the two shallowest depth strata (0–30 and 30–60' combined) of the DMF bottom trawl survey in GOM, by survey season and crab sex. The survey was not conducted in 2020 due to the Covid-19 pandemic. Red, dashed line is the time series median, blue line is a loess fit using family=symmetric and span=0.66. These settings provide a resistant fit to outliers at the end of the time-series. Blue shaded area is an approximate 95% confidence interval for the fit.

Confidential Data Has Been Removed from This Report

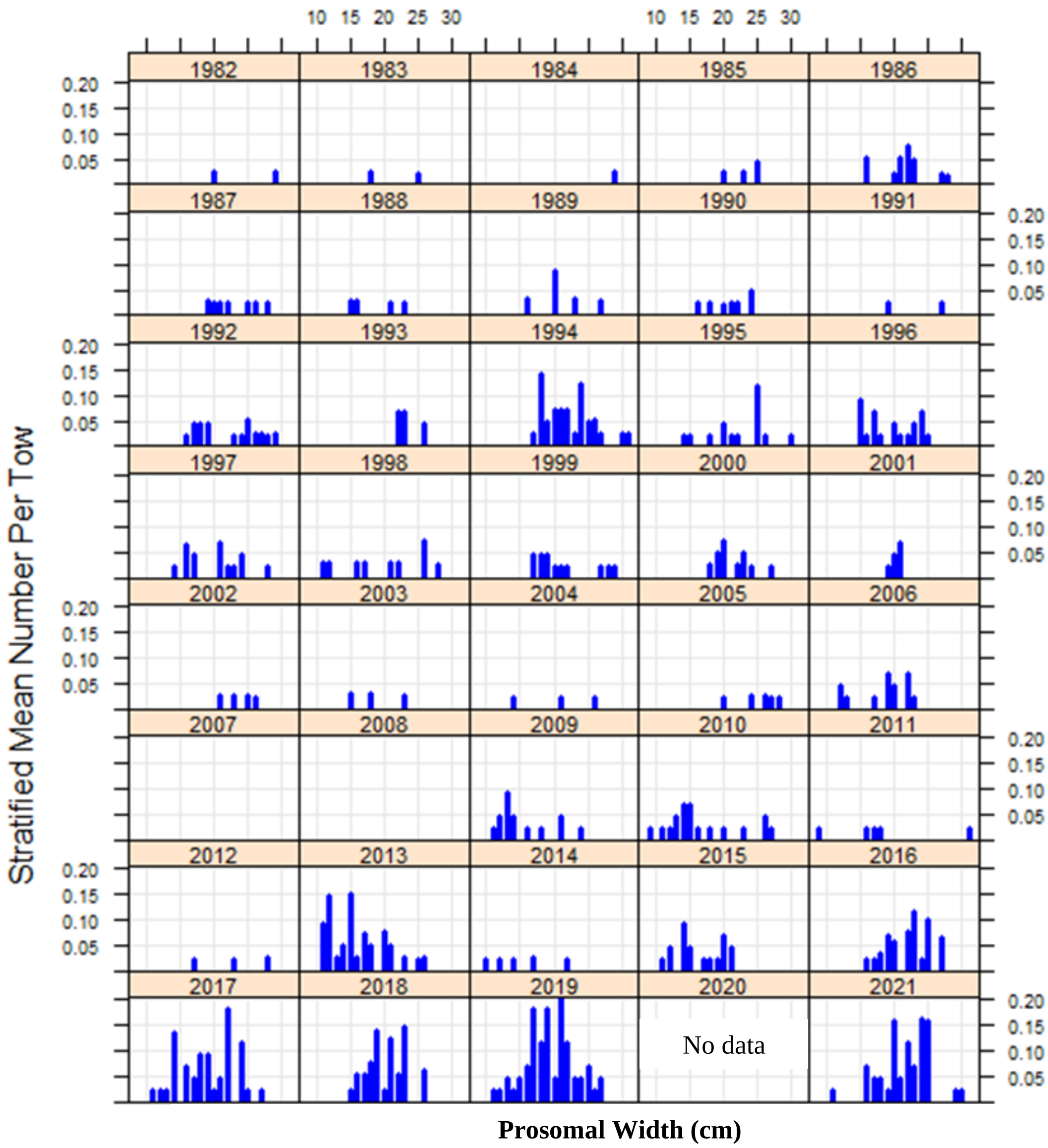


Figure 6. SNE female horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF spring bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

Confidential Data Has Been Removed from This Report

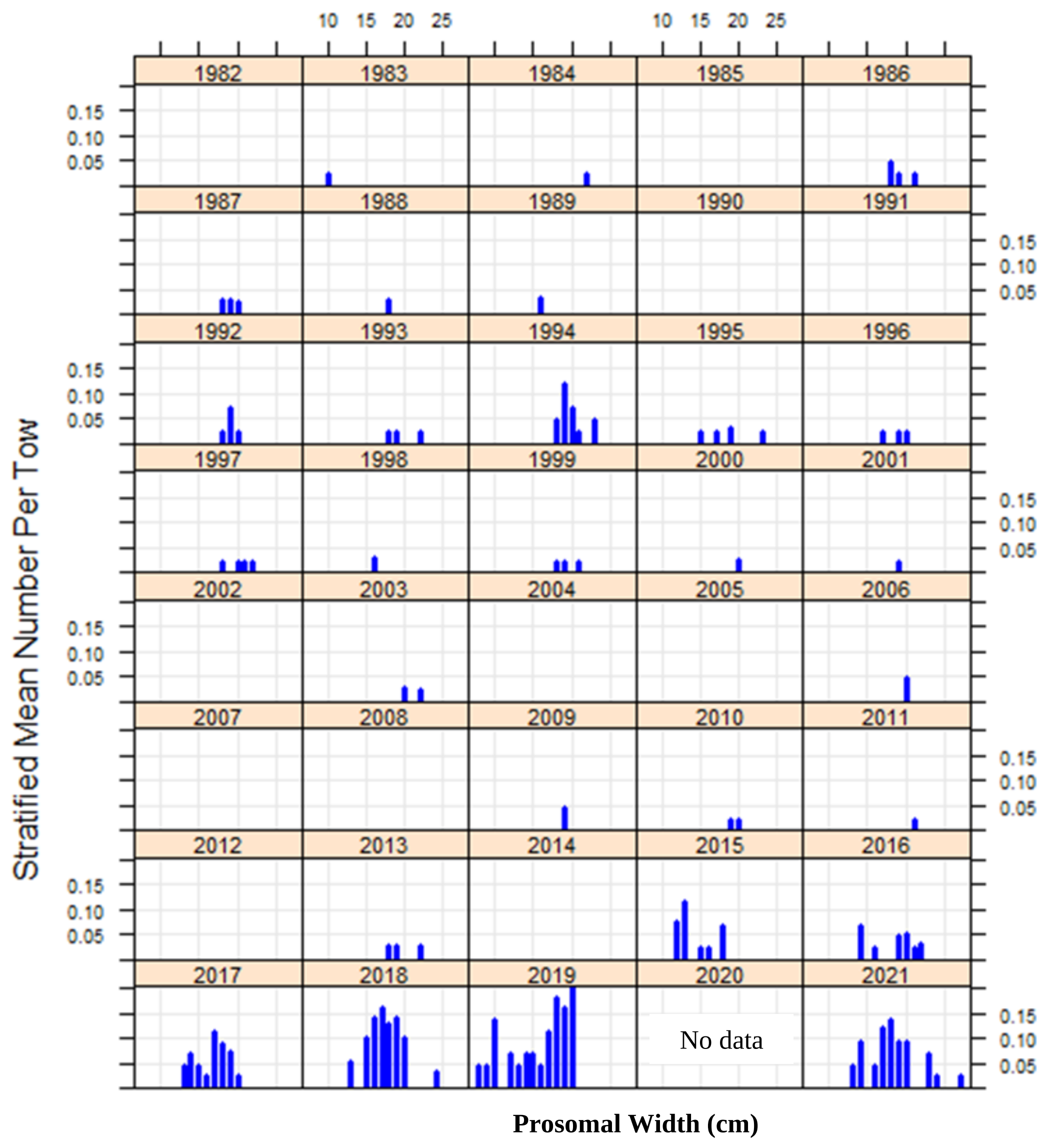


Figure 7. SNE male horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF spring bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

This Report Contains Confidential Data

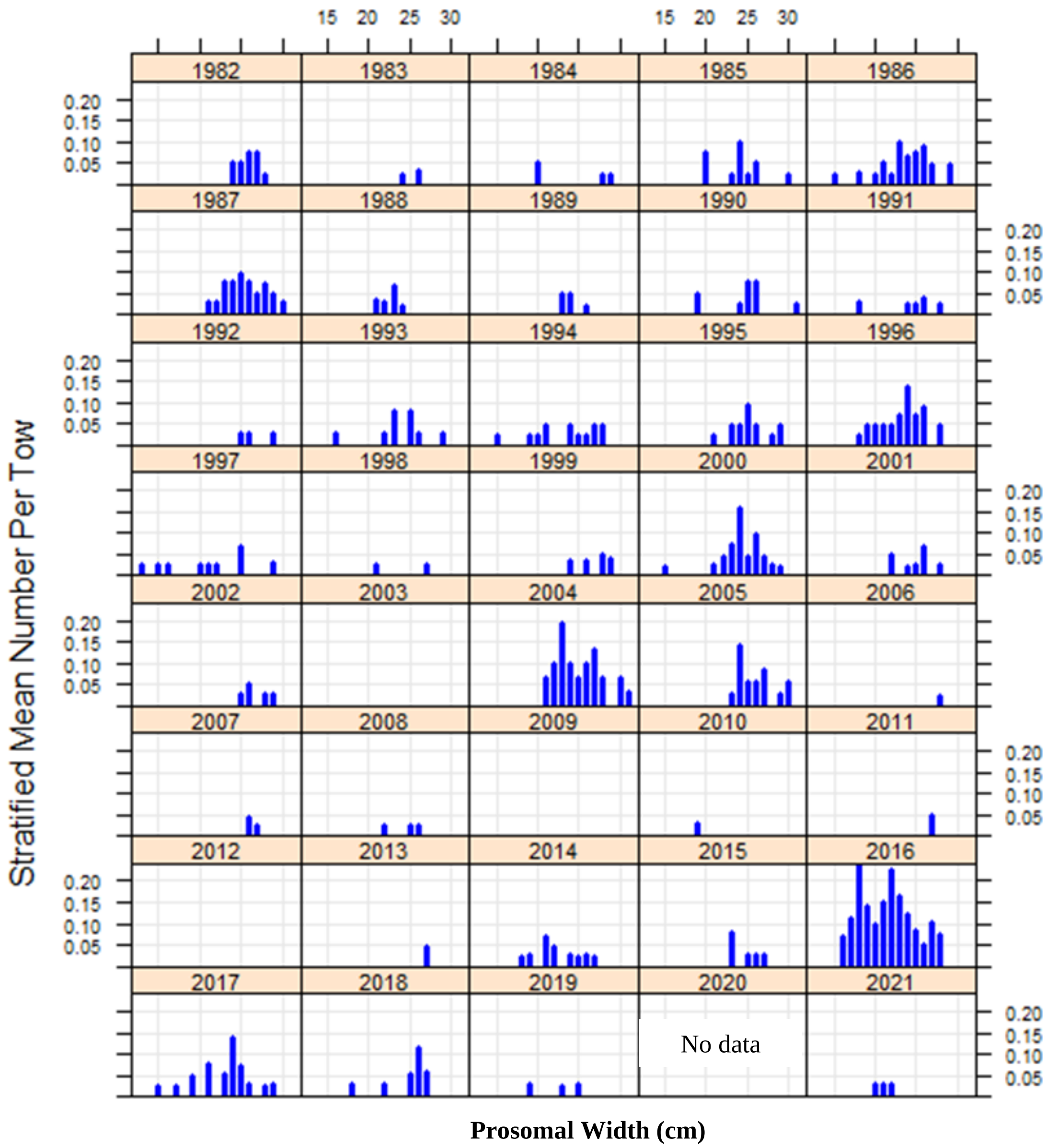


Figure 8. SNE female horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF fall bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

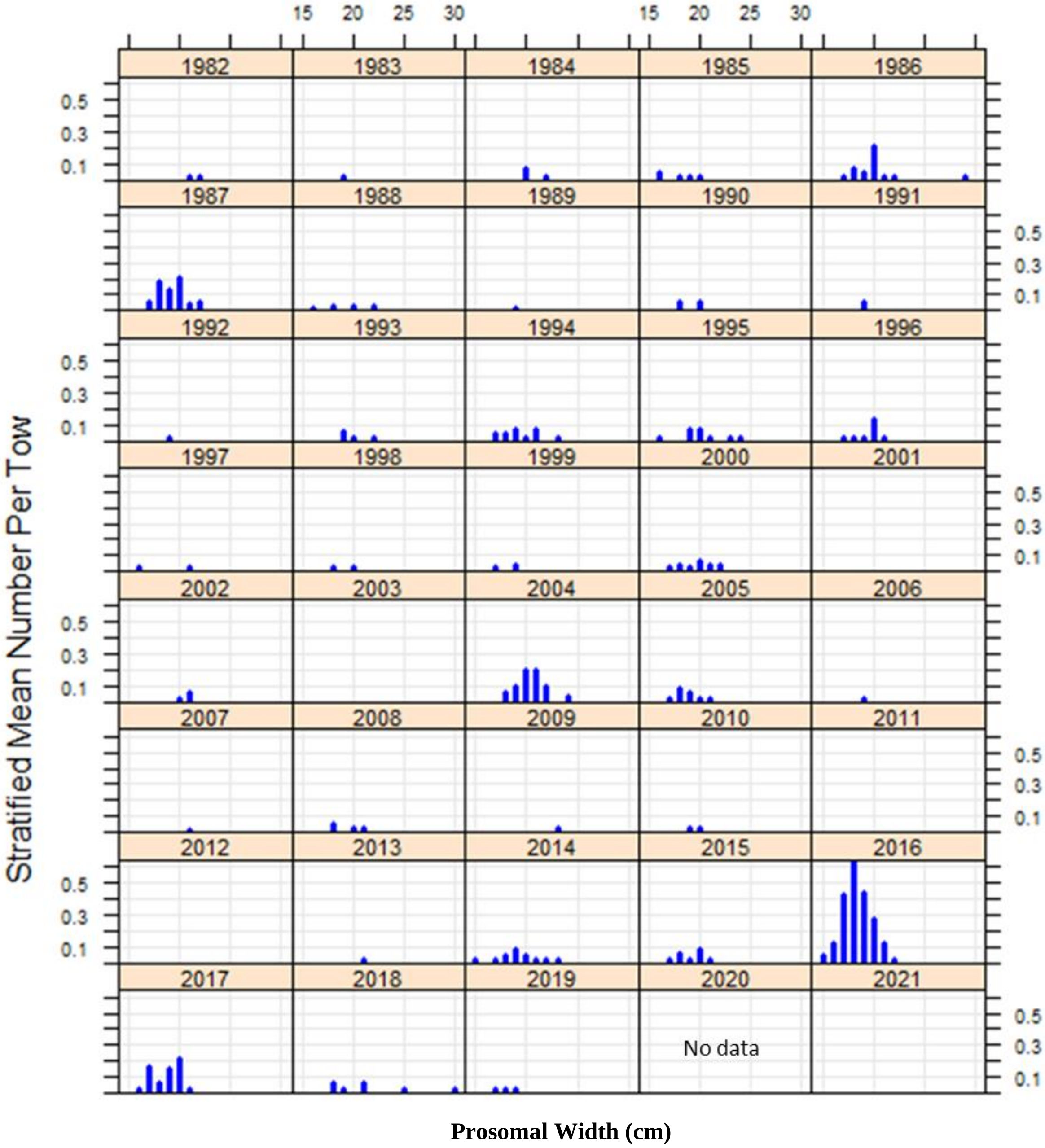


Figure 9. SNE male horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF fall bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

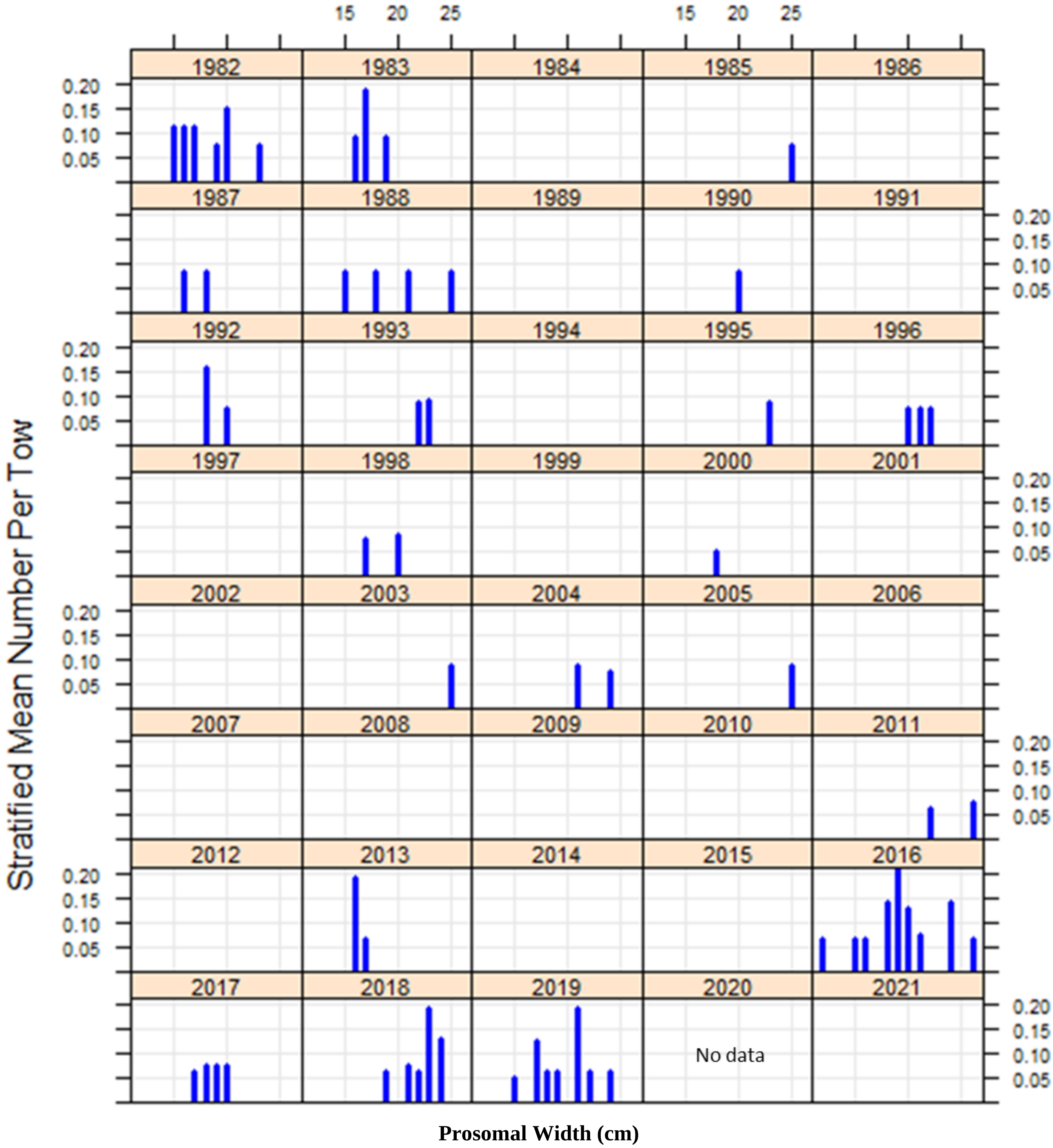


Figure 10. GOM female horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF spring bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

This Report Contains Confidential Data

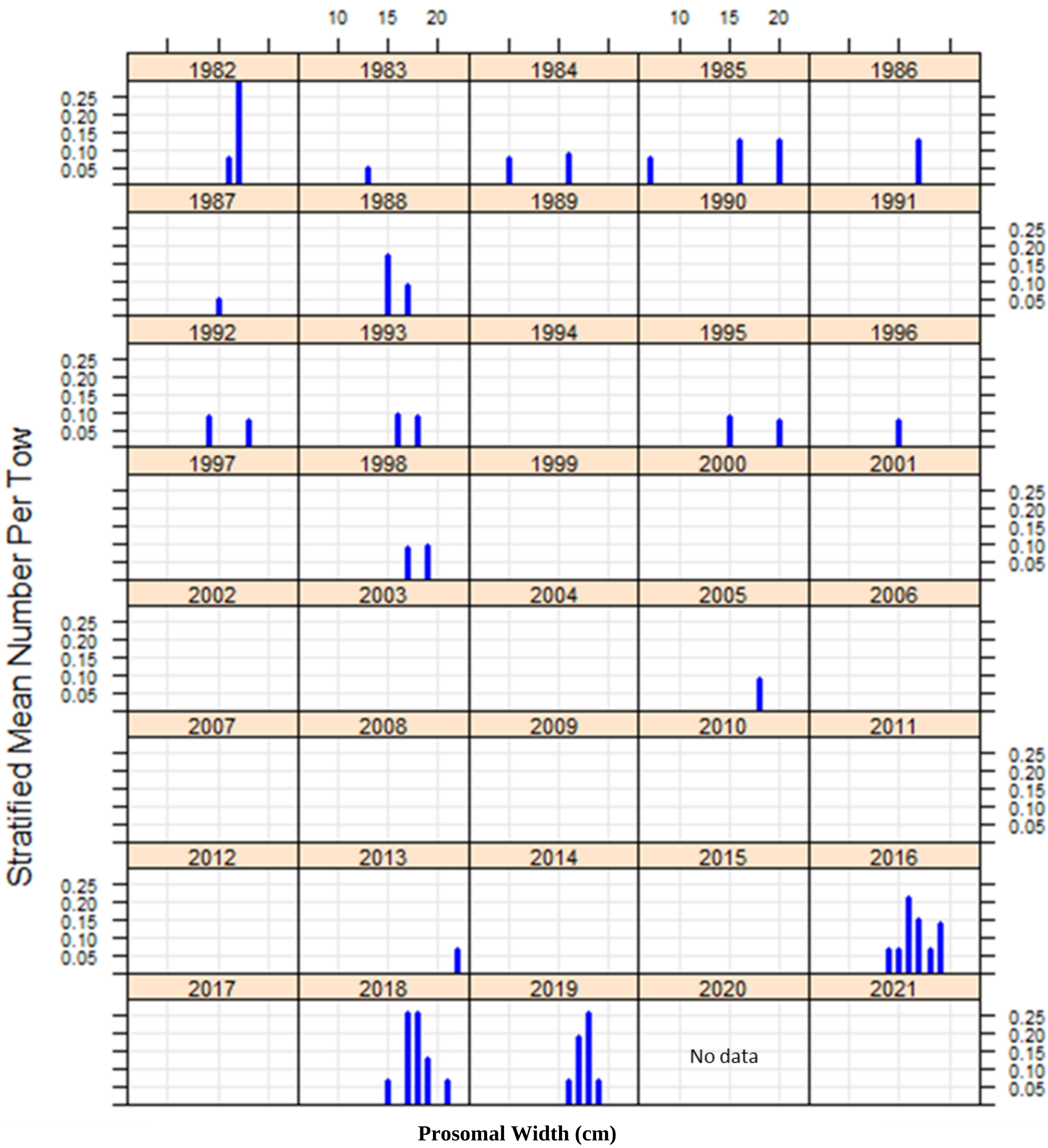


Figure 11. GOM male horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF spring bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

This Report Contains Confidential Data

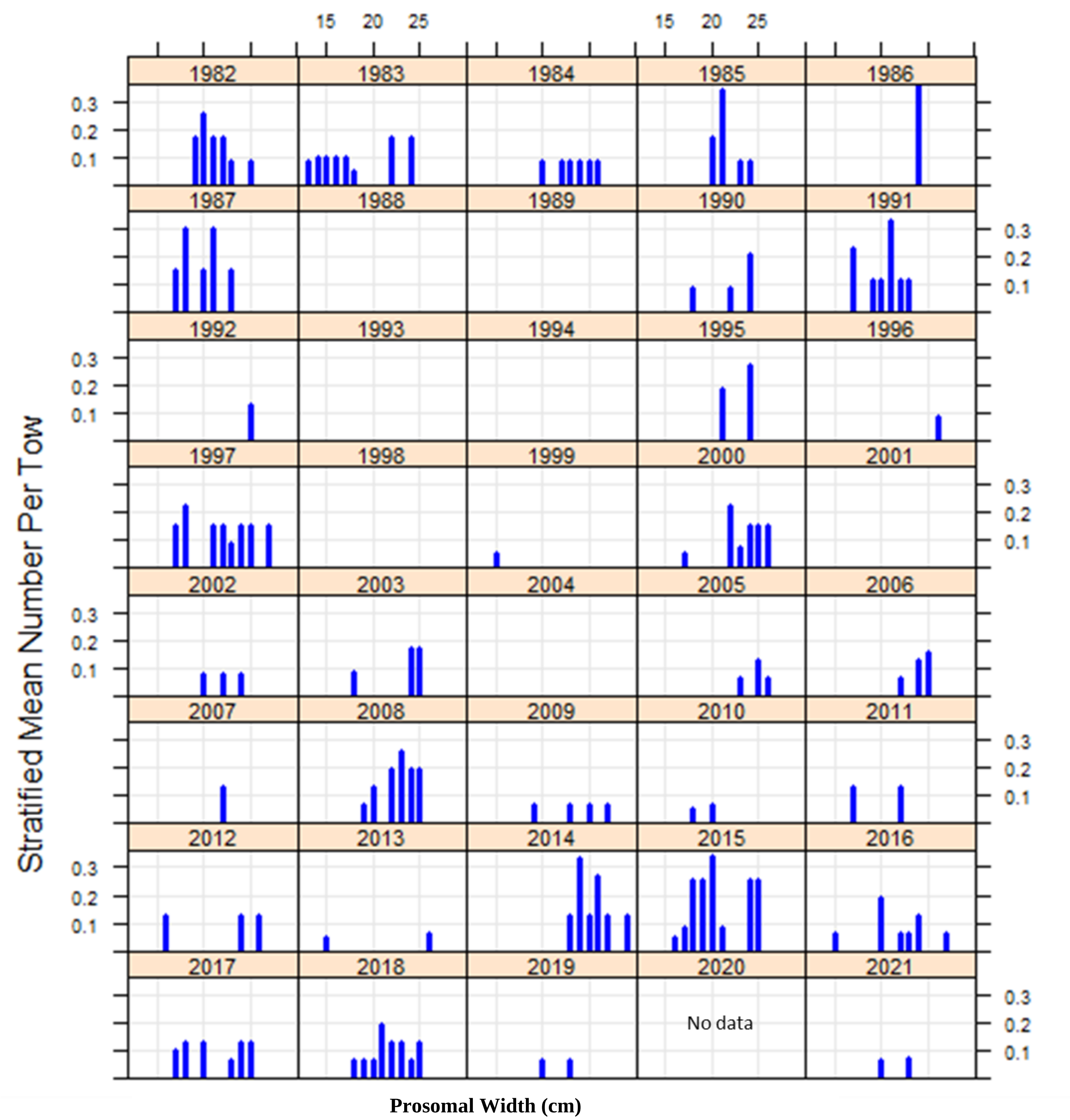


Figure 12. GOM female horseshoe crab size distribution from the two shallowest strata (0–30' and 30–60' combined) of the DMF fall bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

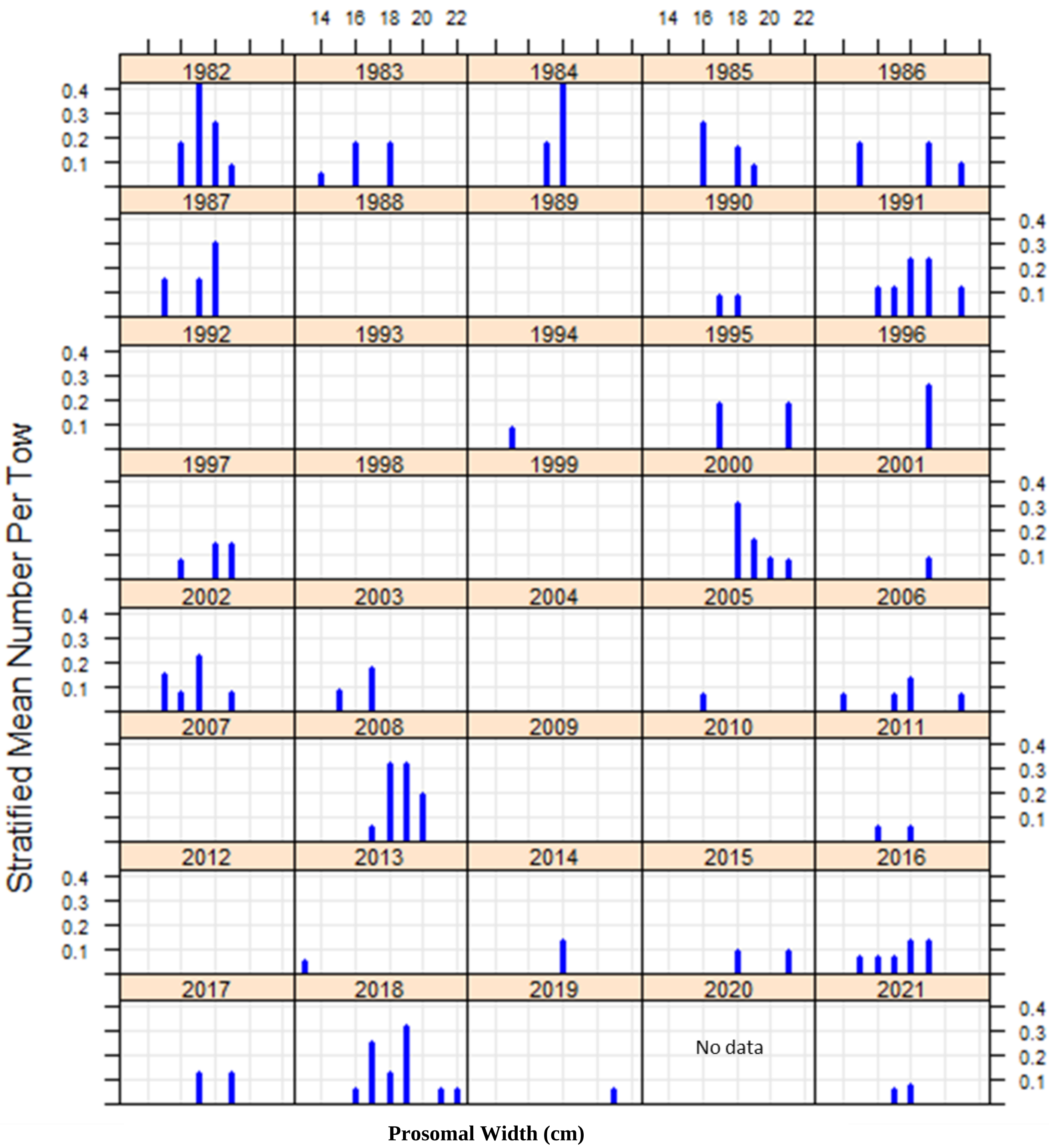


Figure 13. GOM male horseshoe crab size distribution from the two shallowest strata (0–30’ and 30–60’ combined) of the DMF fall bottom trawl survey. The survey was not conducted in 2020 due to the Covid-19 pandemic.

IV. Planned management programs for the current calendar year

a. Summary of changes from previous years

There were no regulation changes from previous years directly related to horseshoe crabs.

b. Summary of monitoring programs that will occur

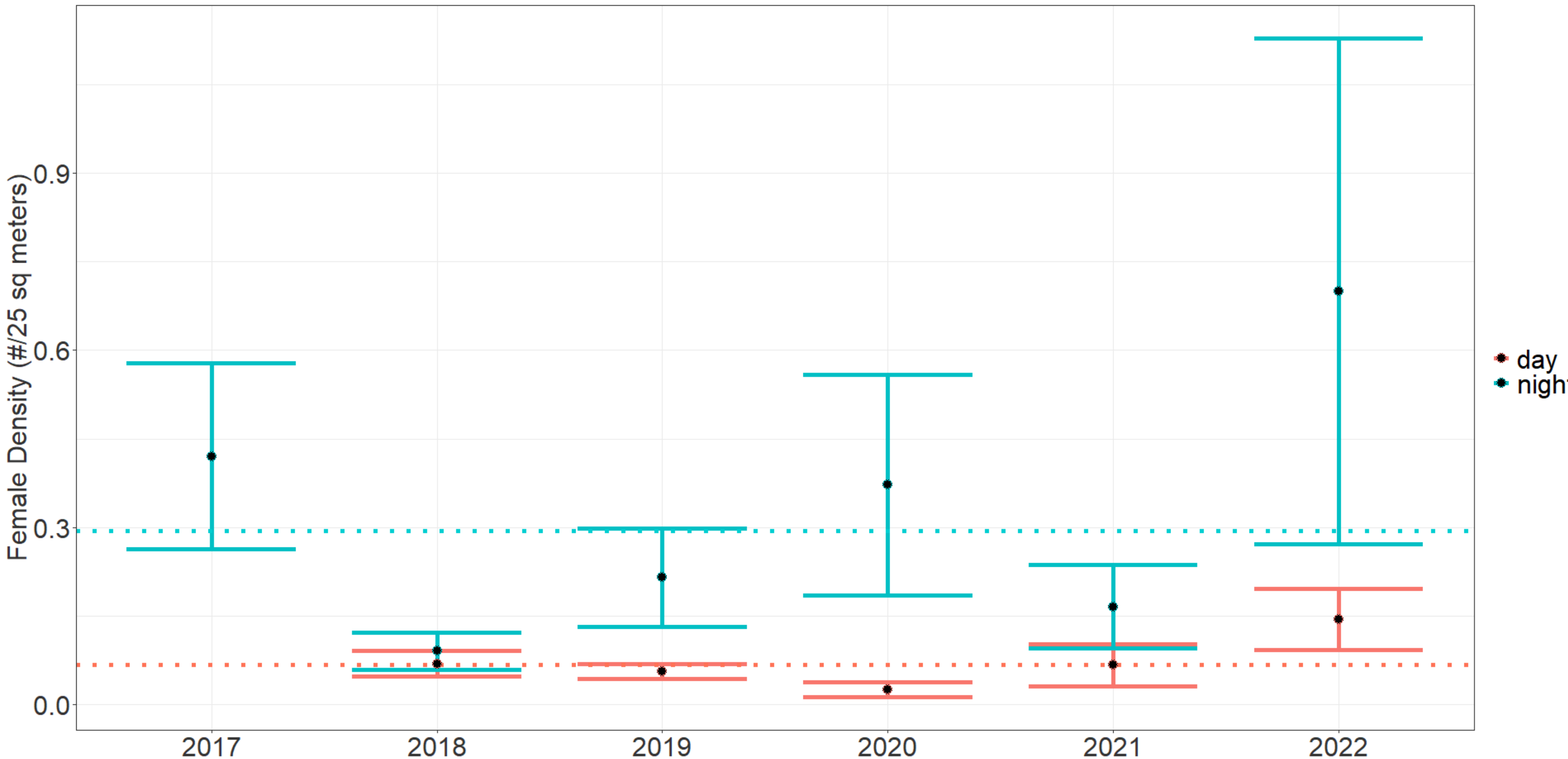
- DMF will continue collecting catch reports from all crab harvesters, dealers, and scientific permit holders.
- DMF will continue to collect monthly reports from the biomedical industry.
- DMF will also continue to characterize the commercial fishery through market sampling.
- DMF spring and fall trawl surveys will continue to monitor and record weight, number and prosomal width by sex of individuals collected.
- DMF will continue to coordinate and support spawning beach surveys conducted in cooperation with various volunteer organizations.

V. Law Enforcement reporting requirements

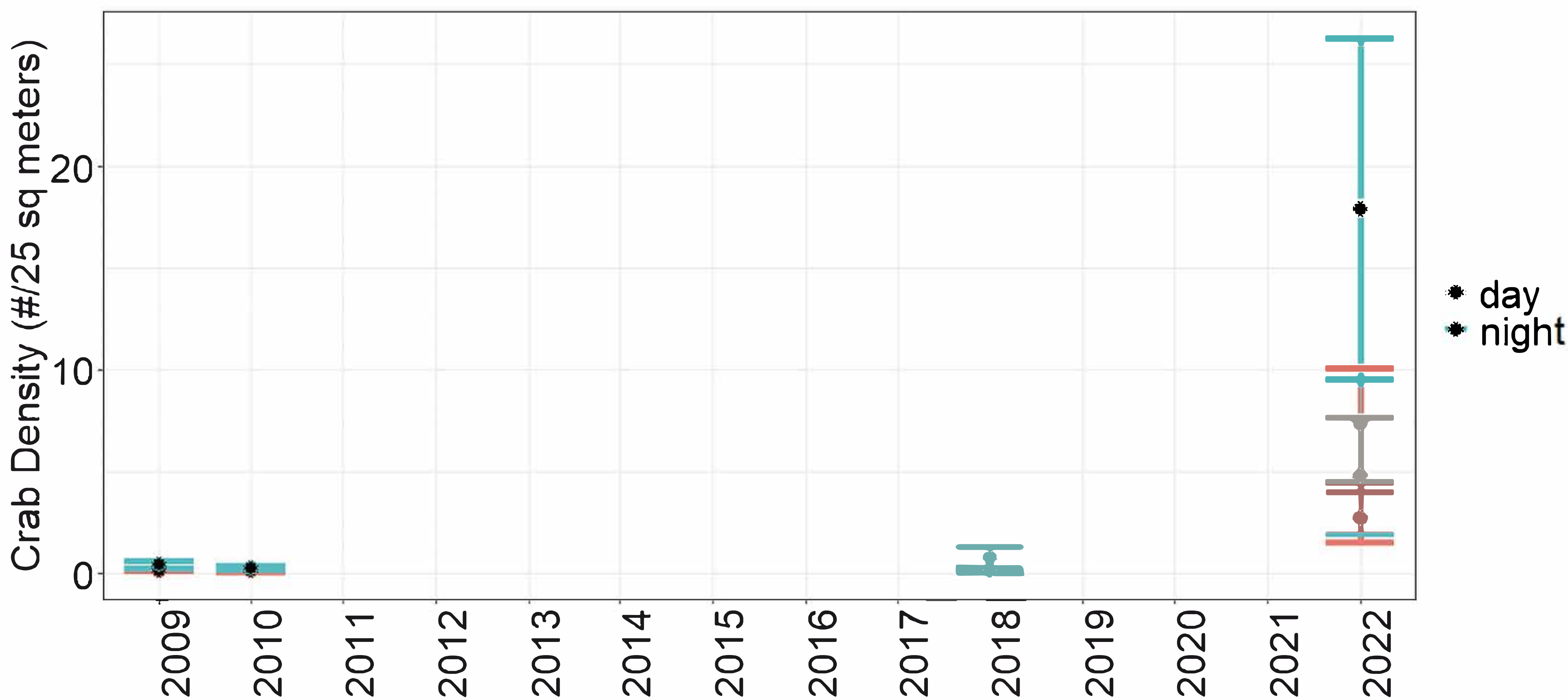
The Massachusetts Environmental Police did not report any horseshoe crab related violations in 2021.

Appendix 5

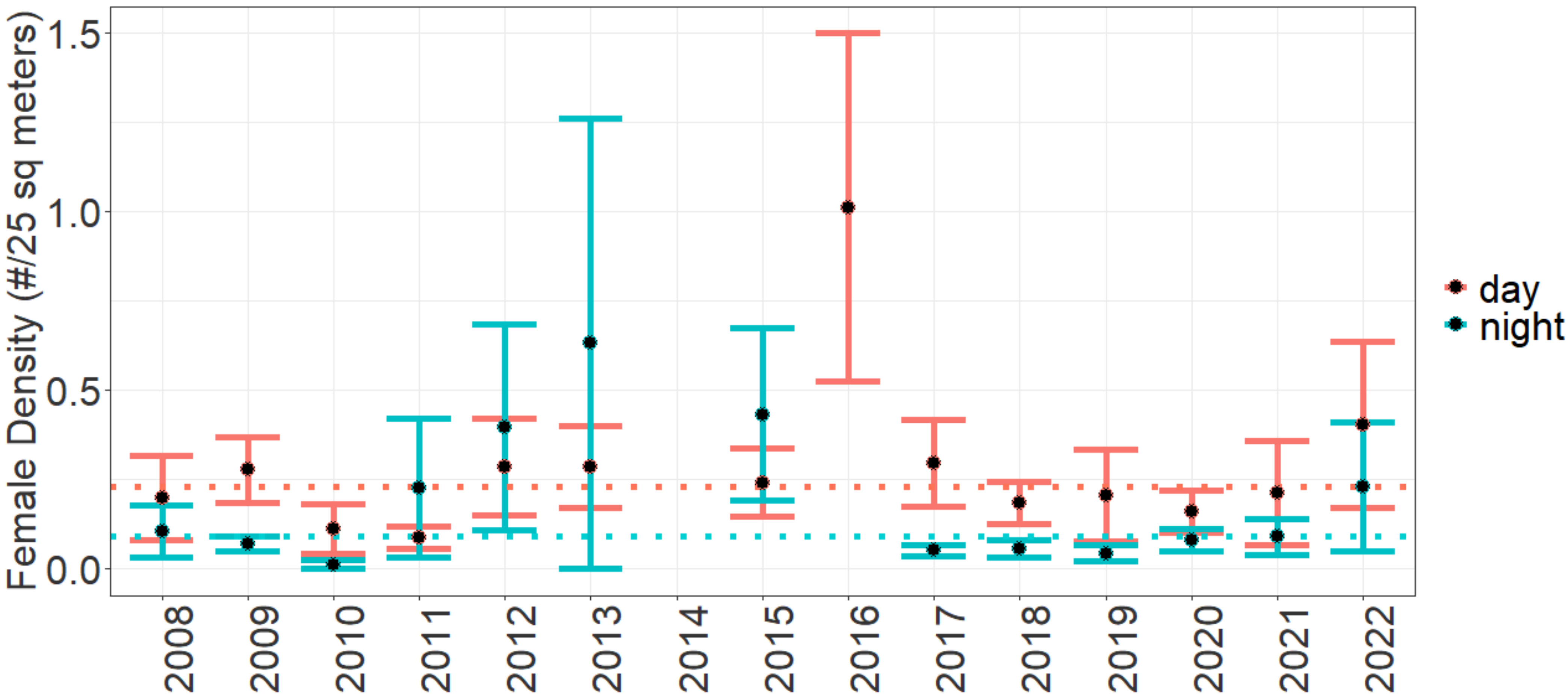
Bass River



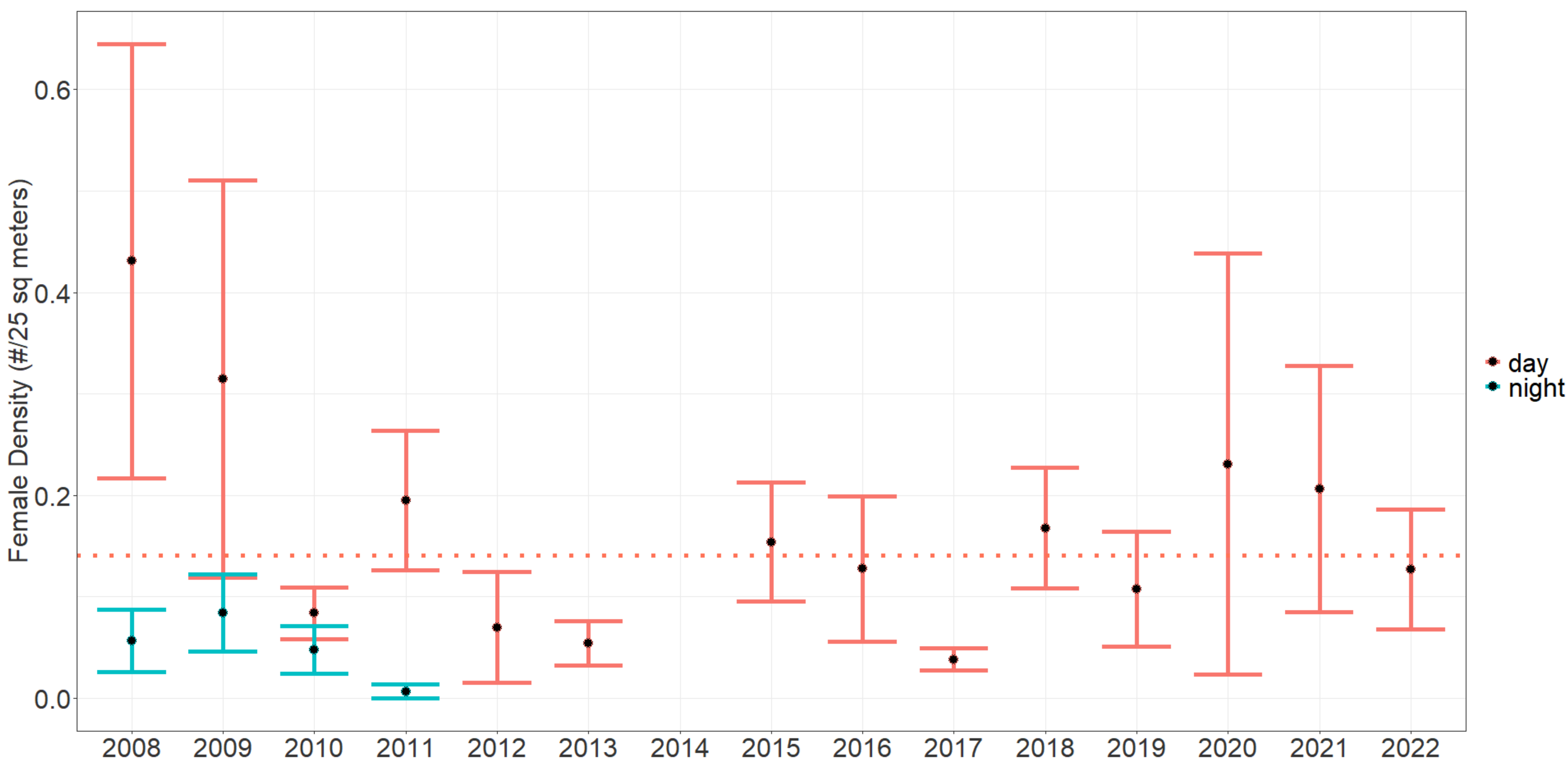
Chatam USFWS



Duxbury Beach



Erica's



Female Density (#/25 sq meters)
0.0
0.2
0.4
0.6
2008
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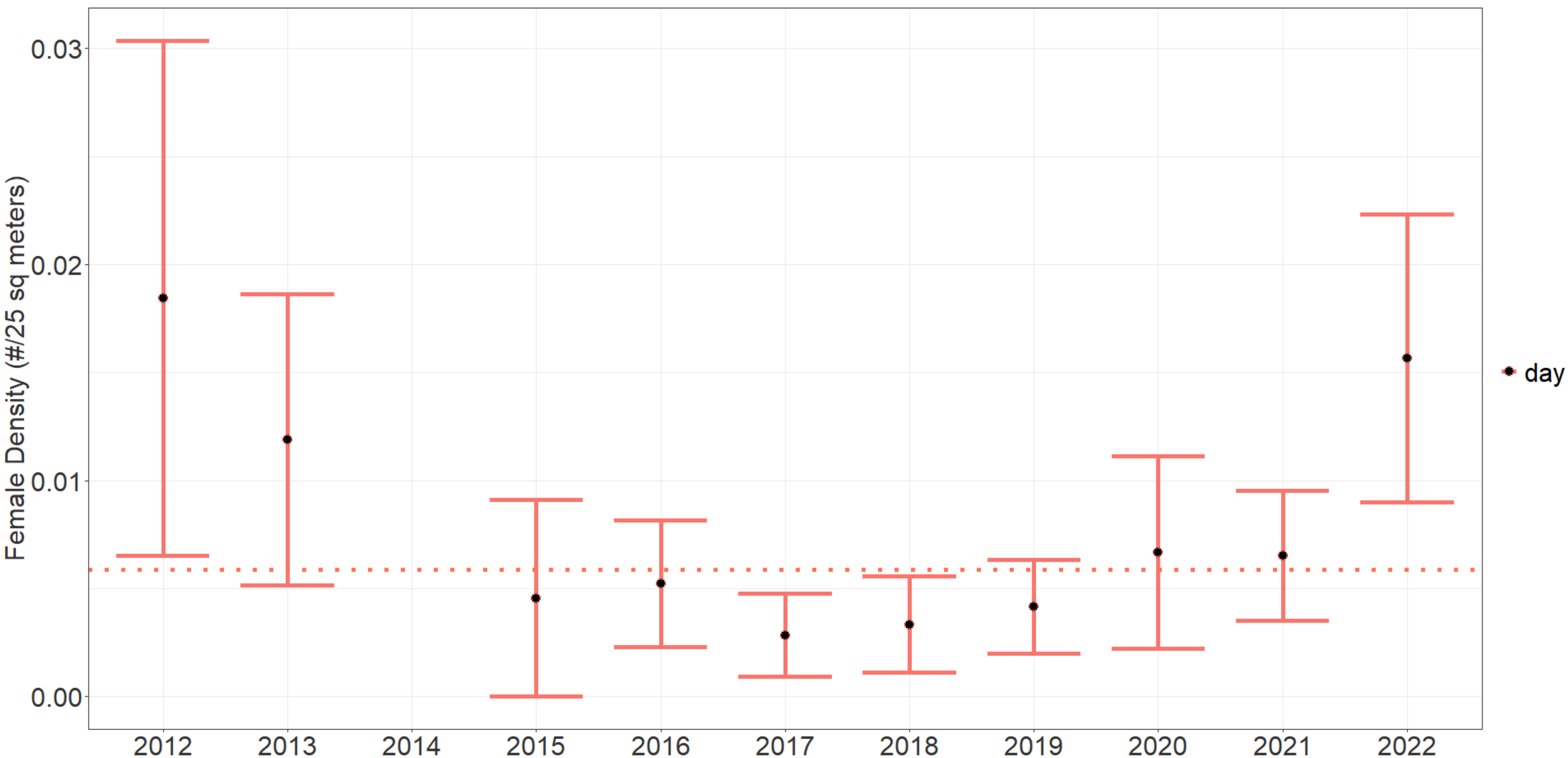
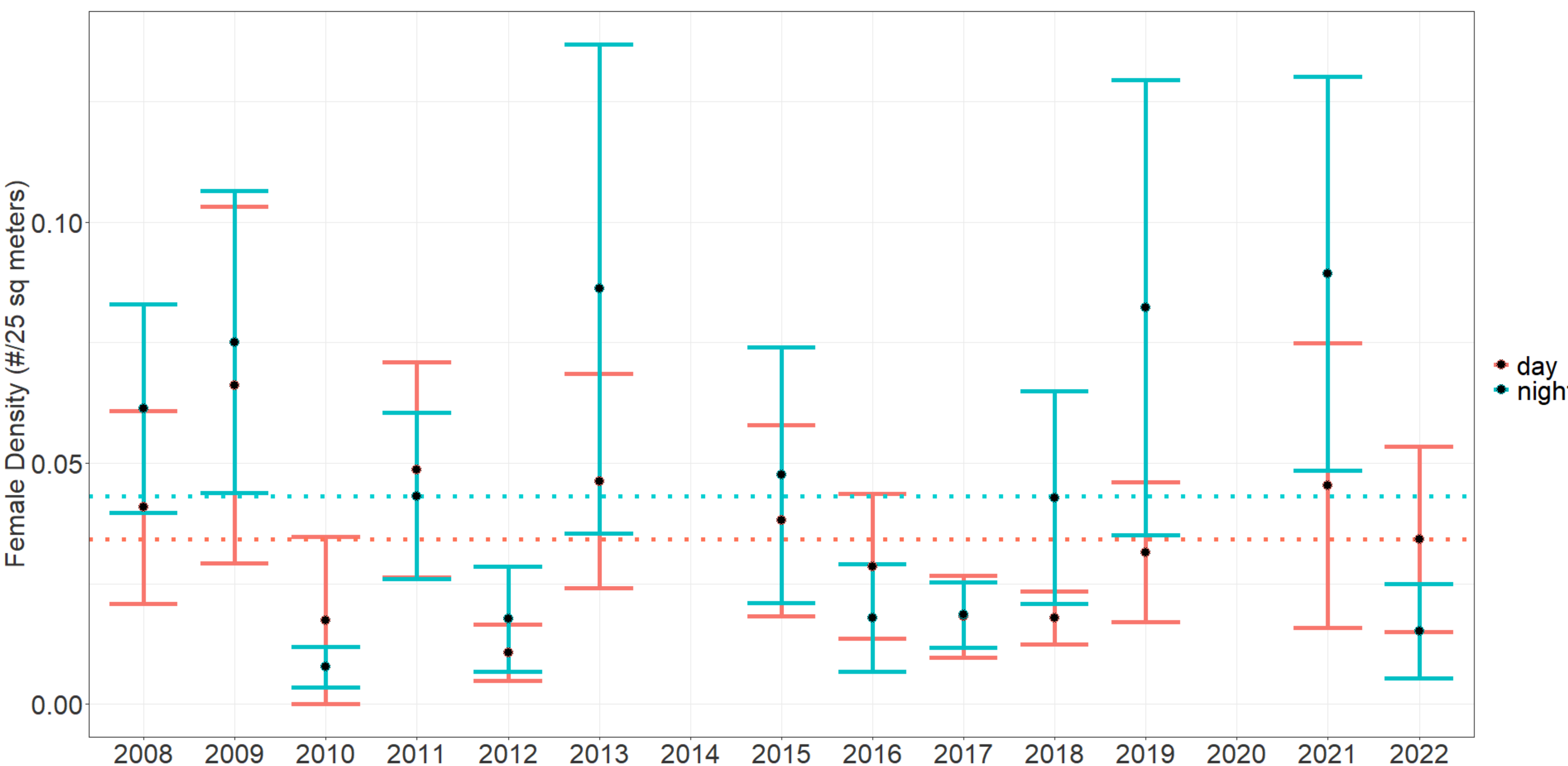
Great Island



Indian Neck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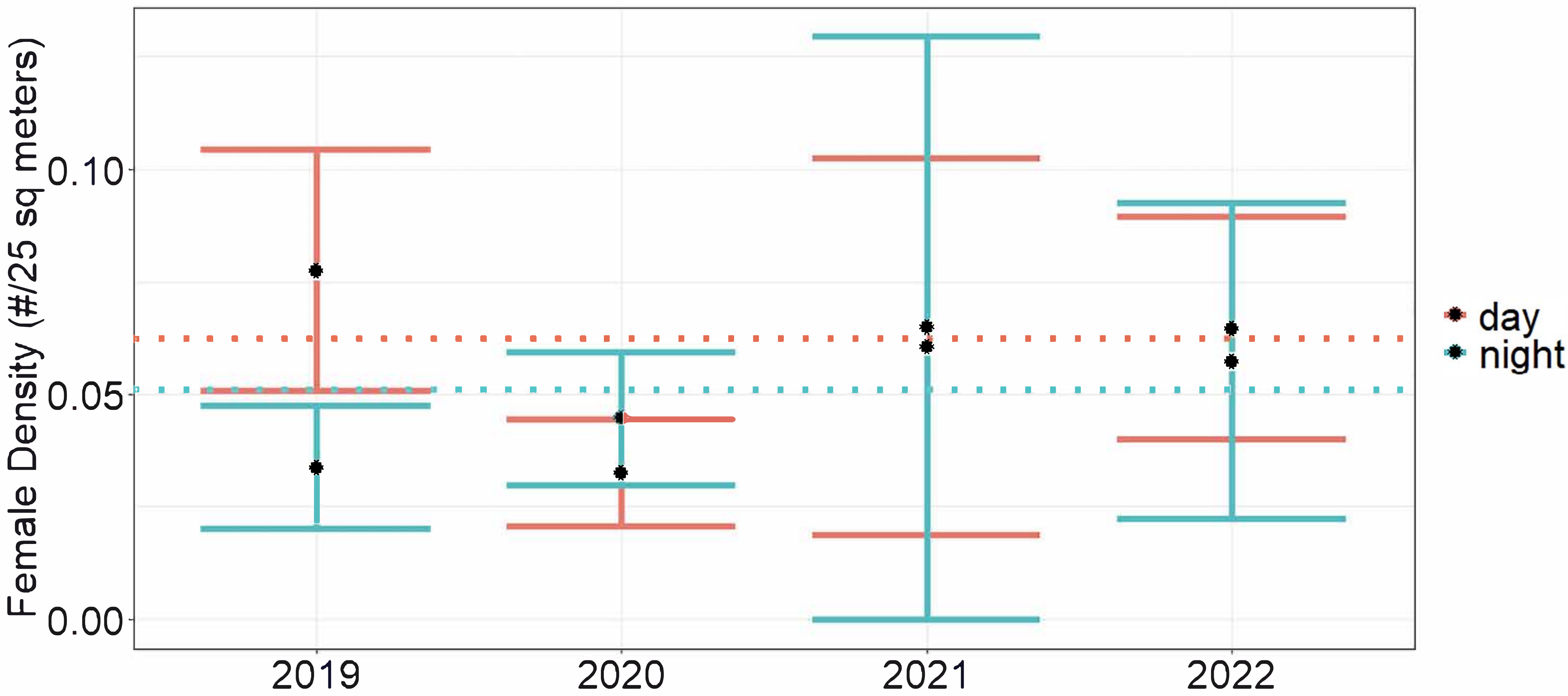
Long Beach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2019
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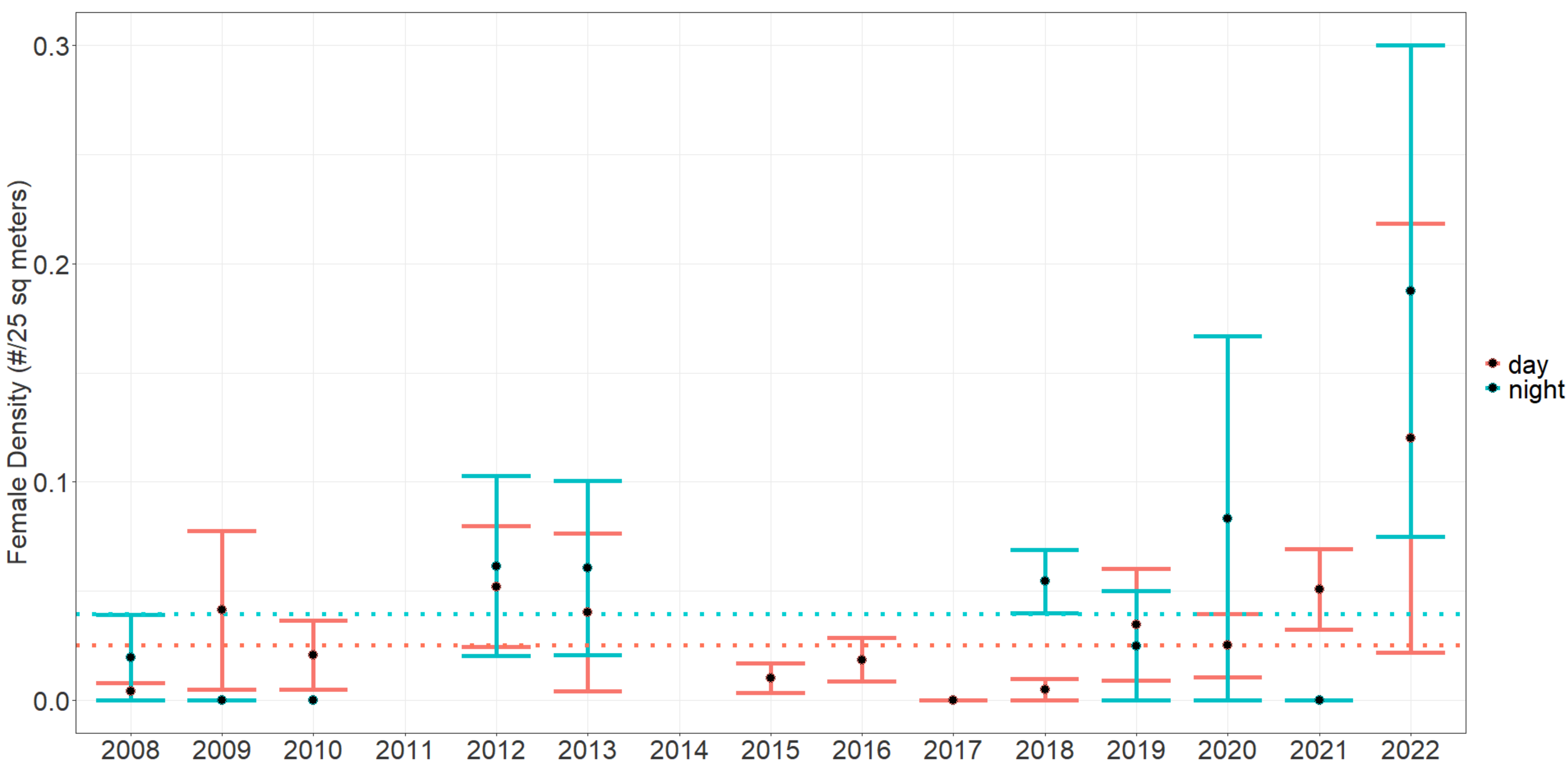
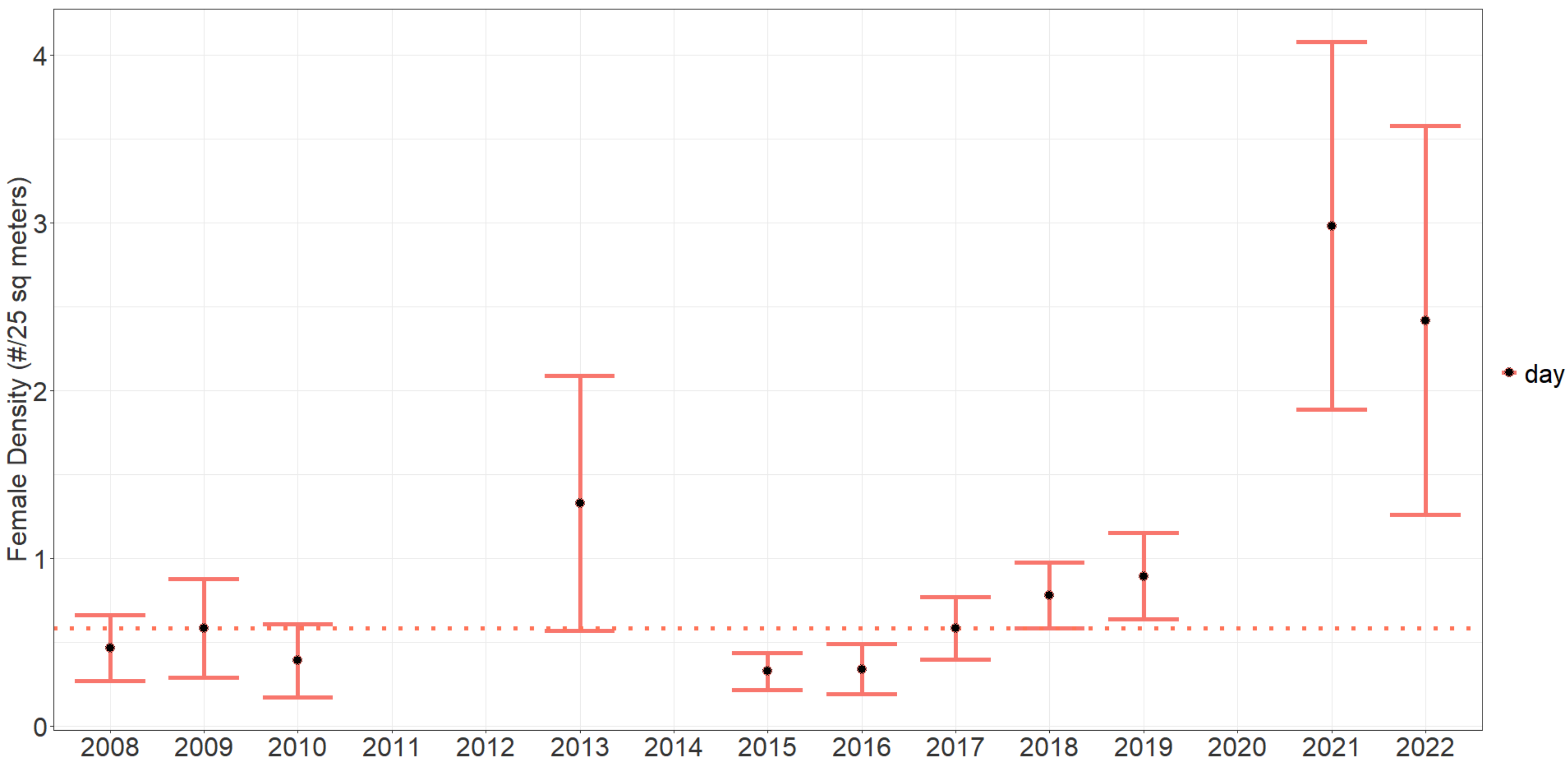
Long Pasture



Marsh 2-3



Female Density (#/25 sq meters)
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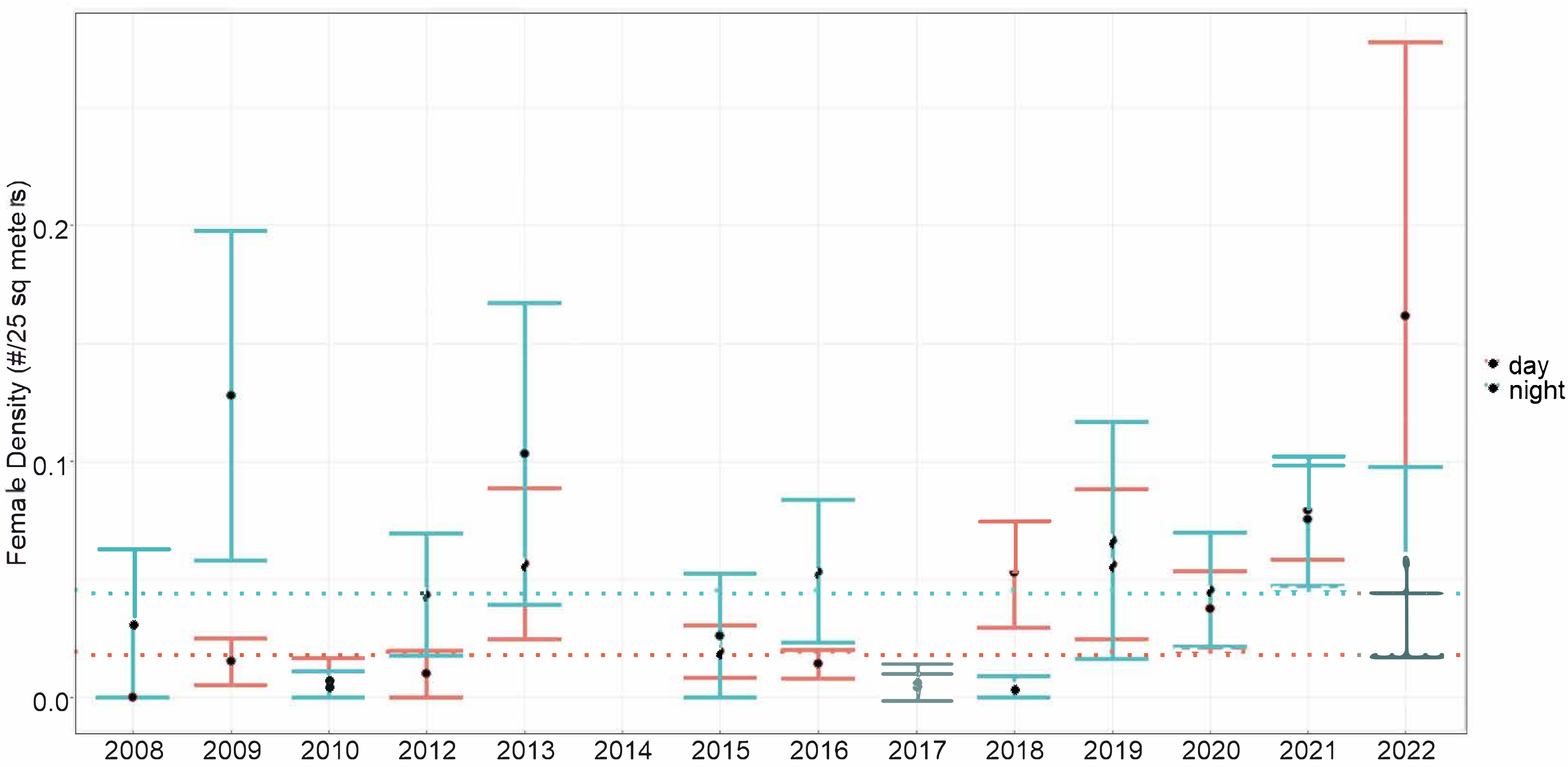
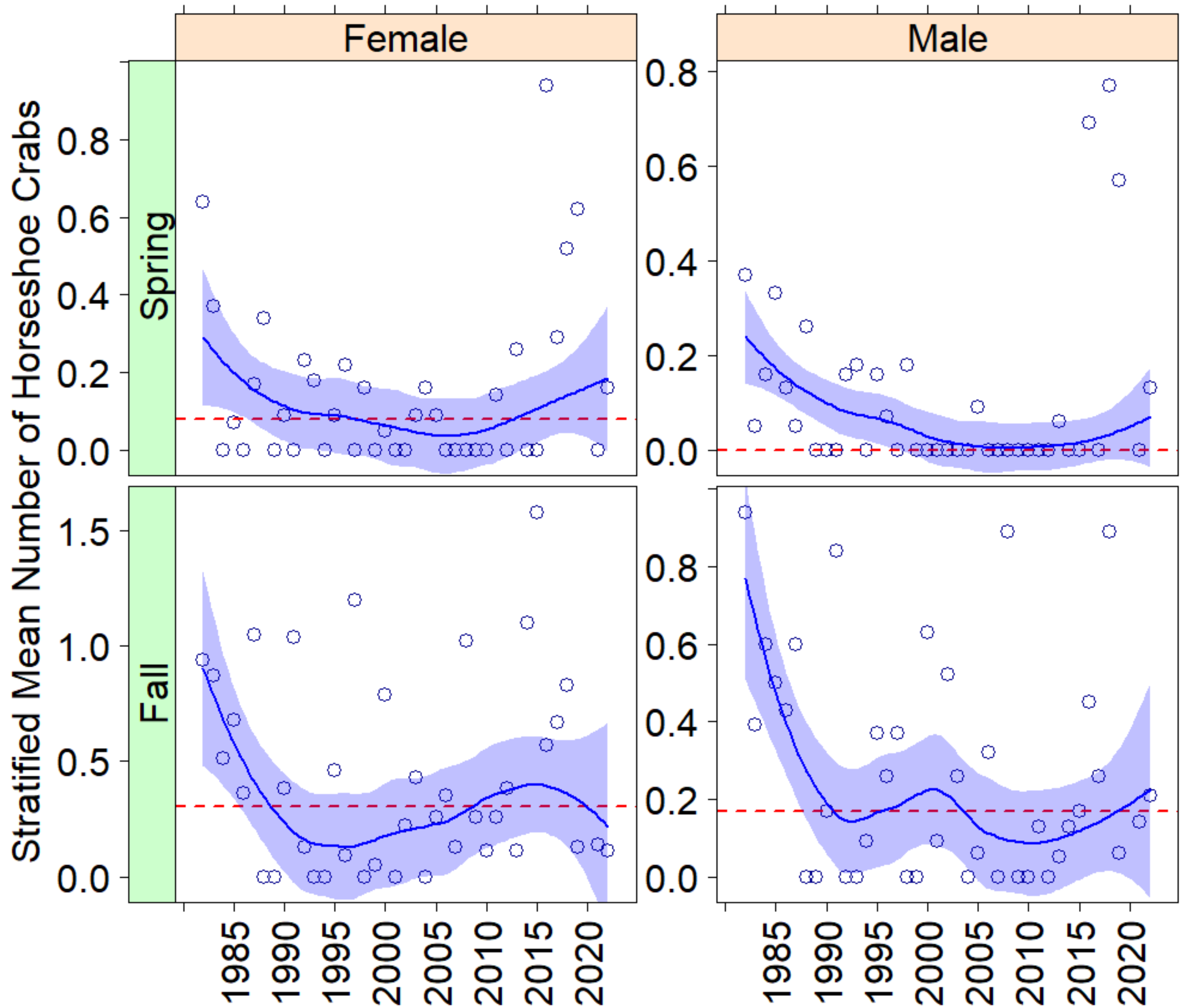
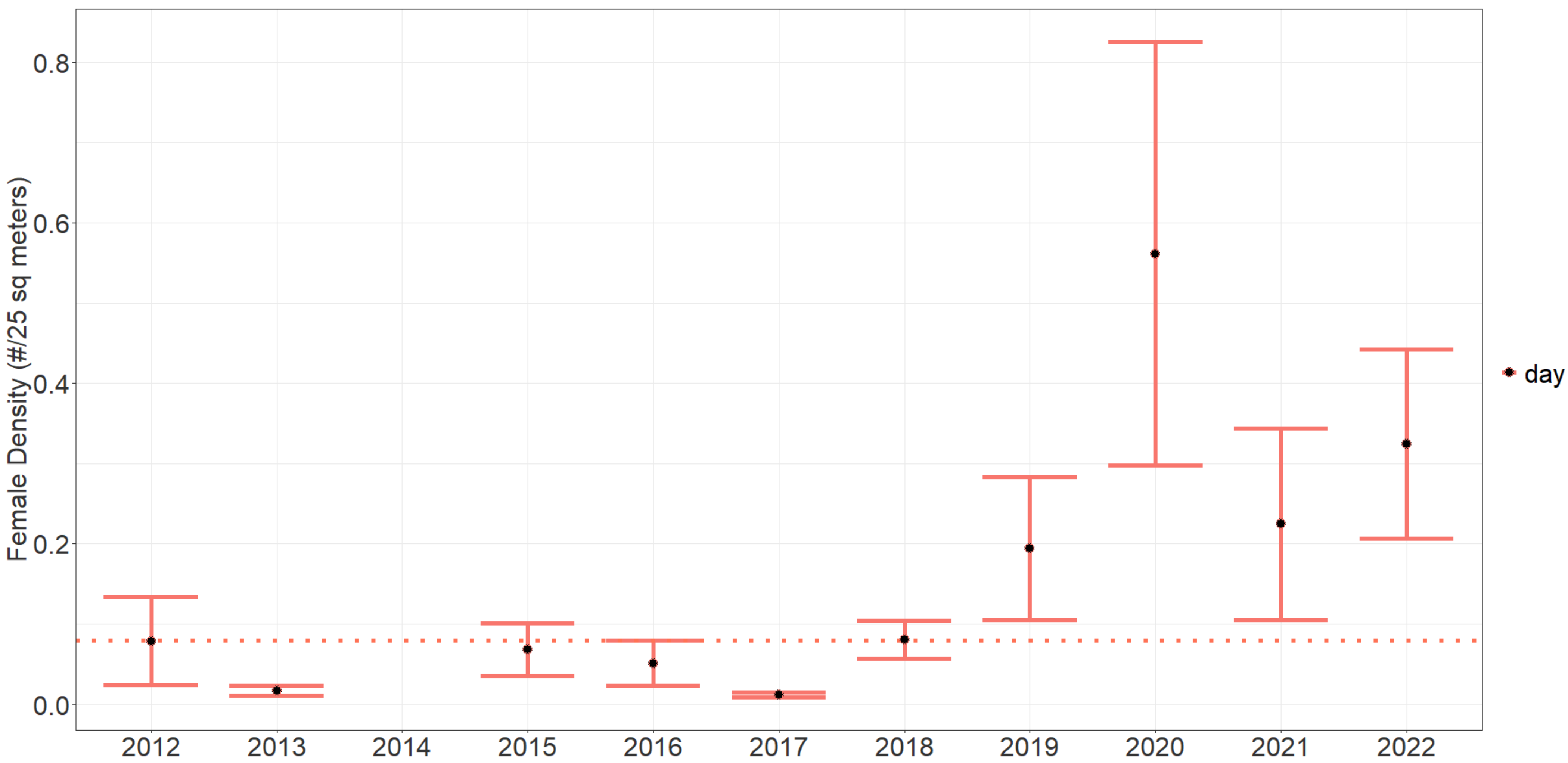
Millway Beach



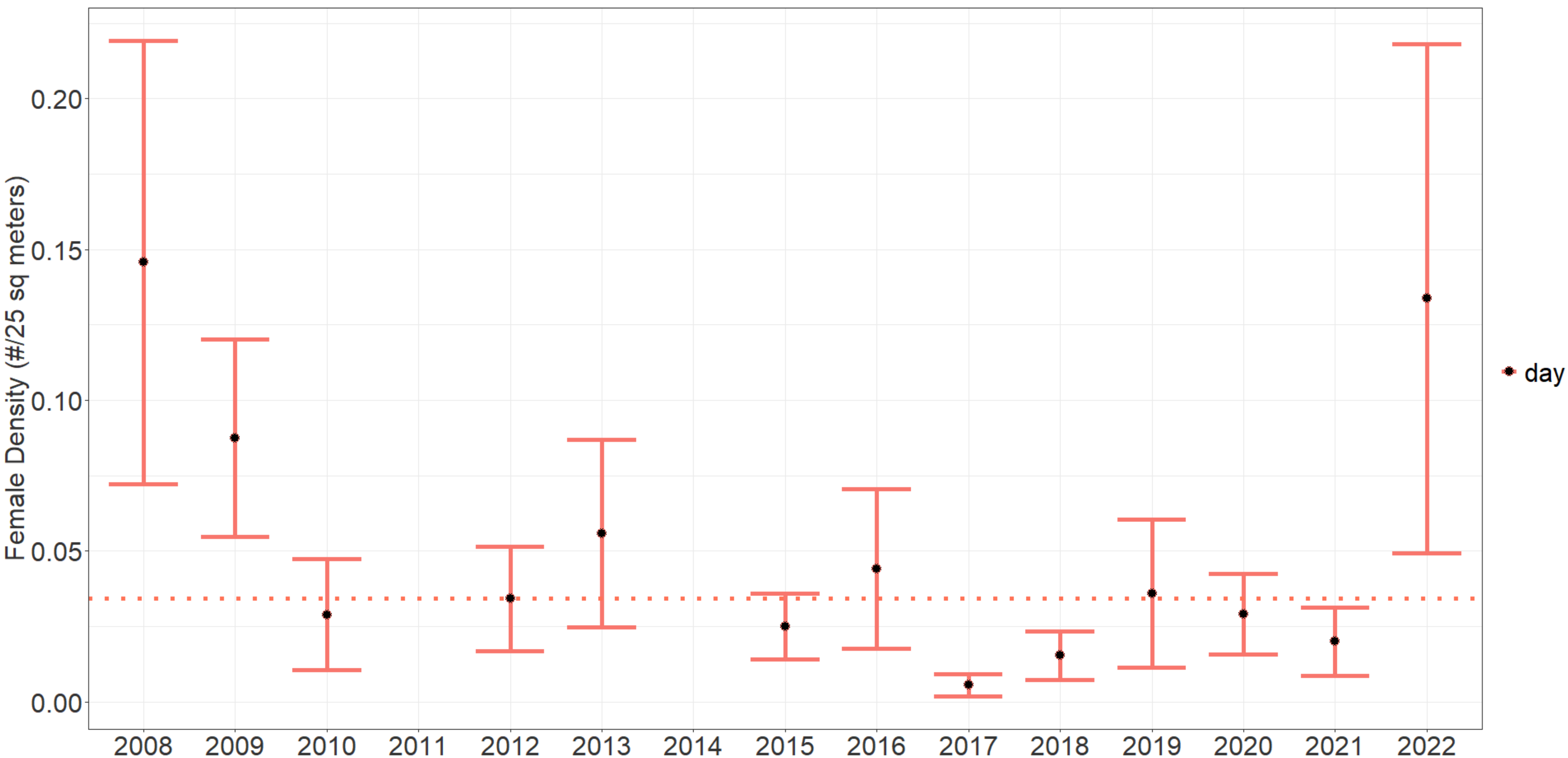
North of Cape Cod MA DMF Trawl Survey dices (0-60')



Priscilla Landing



Sanctuary



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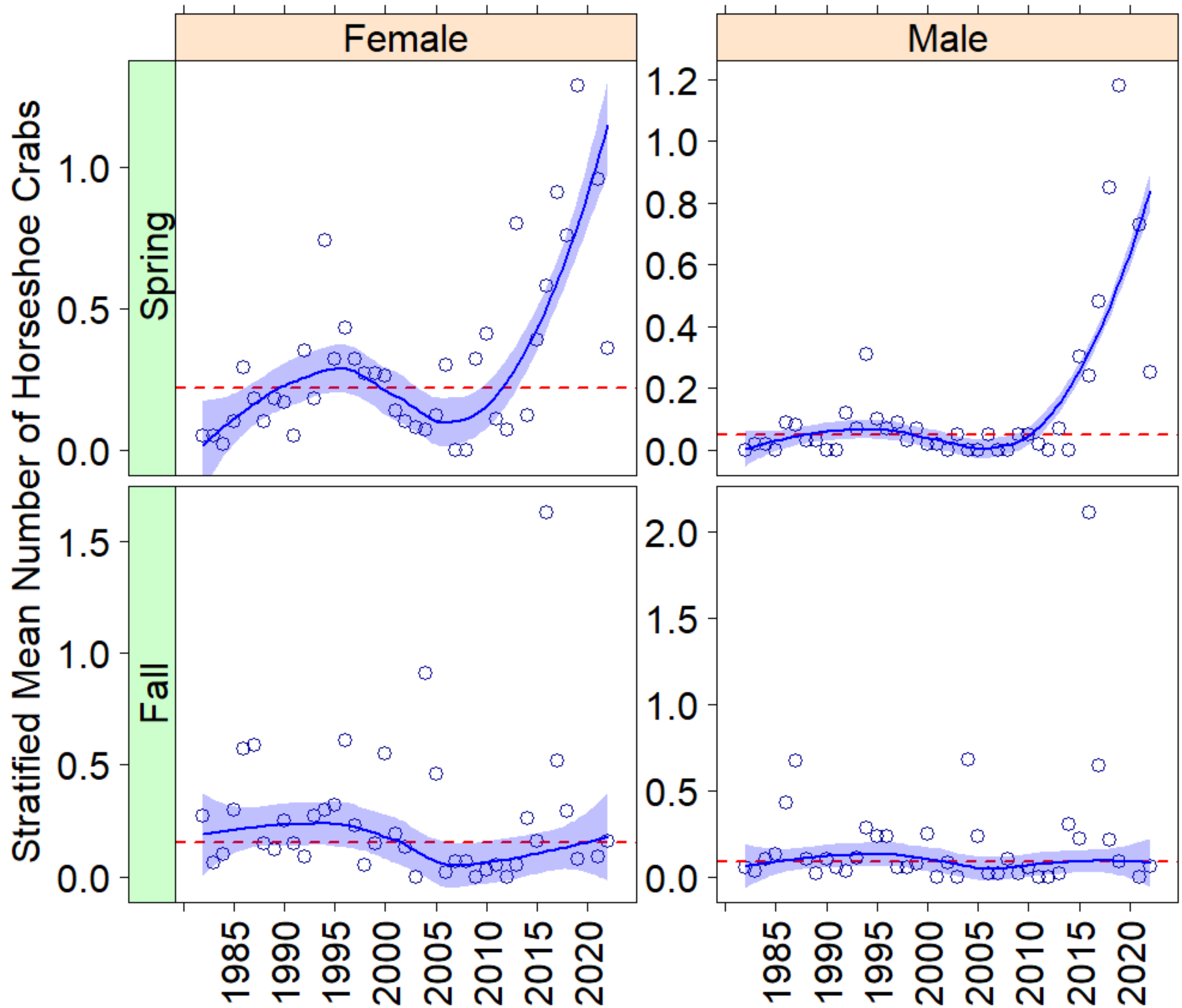
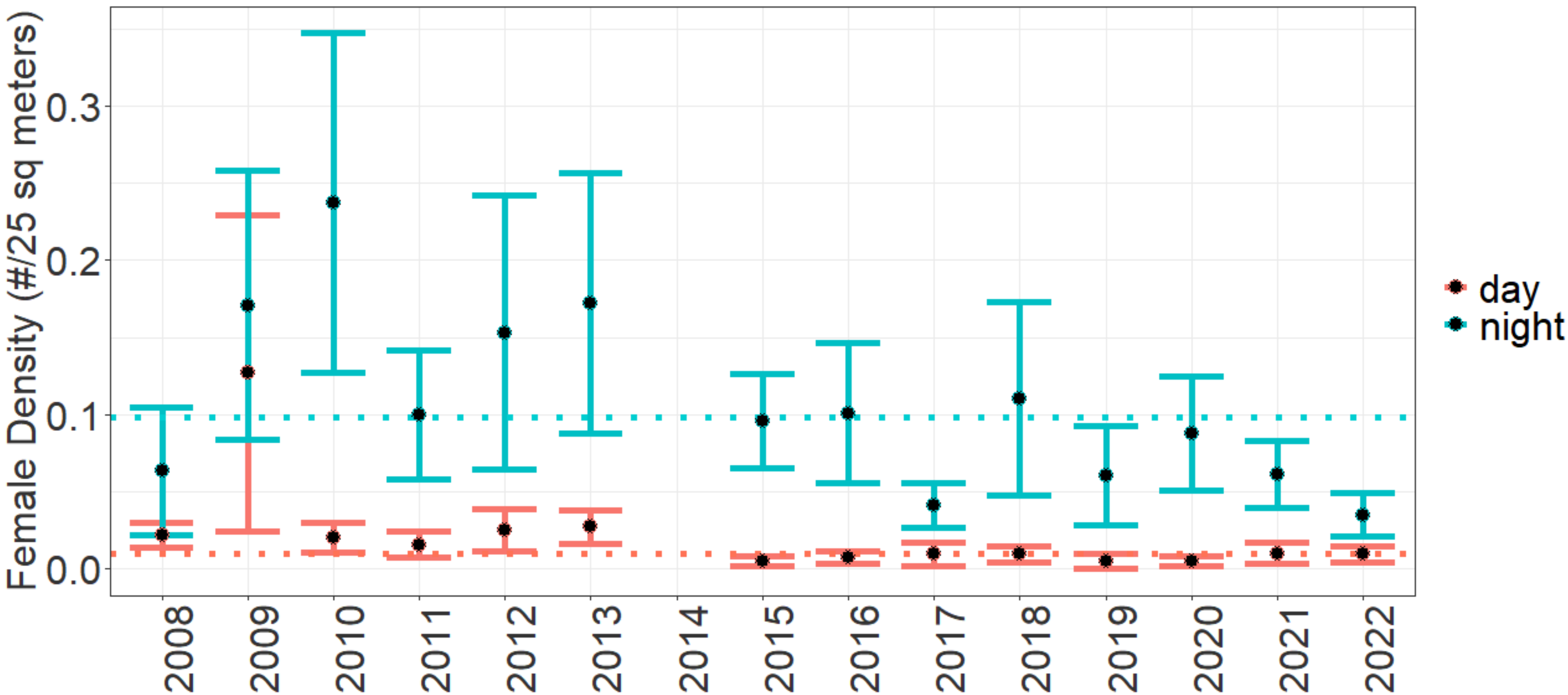
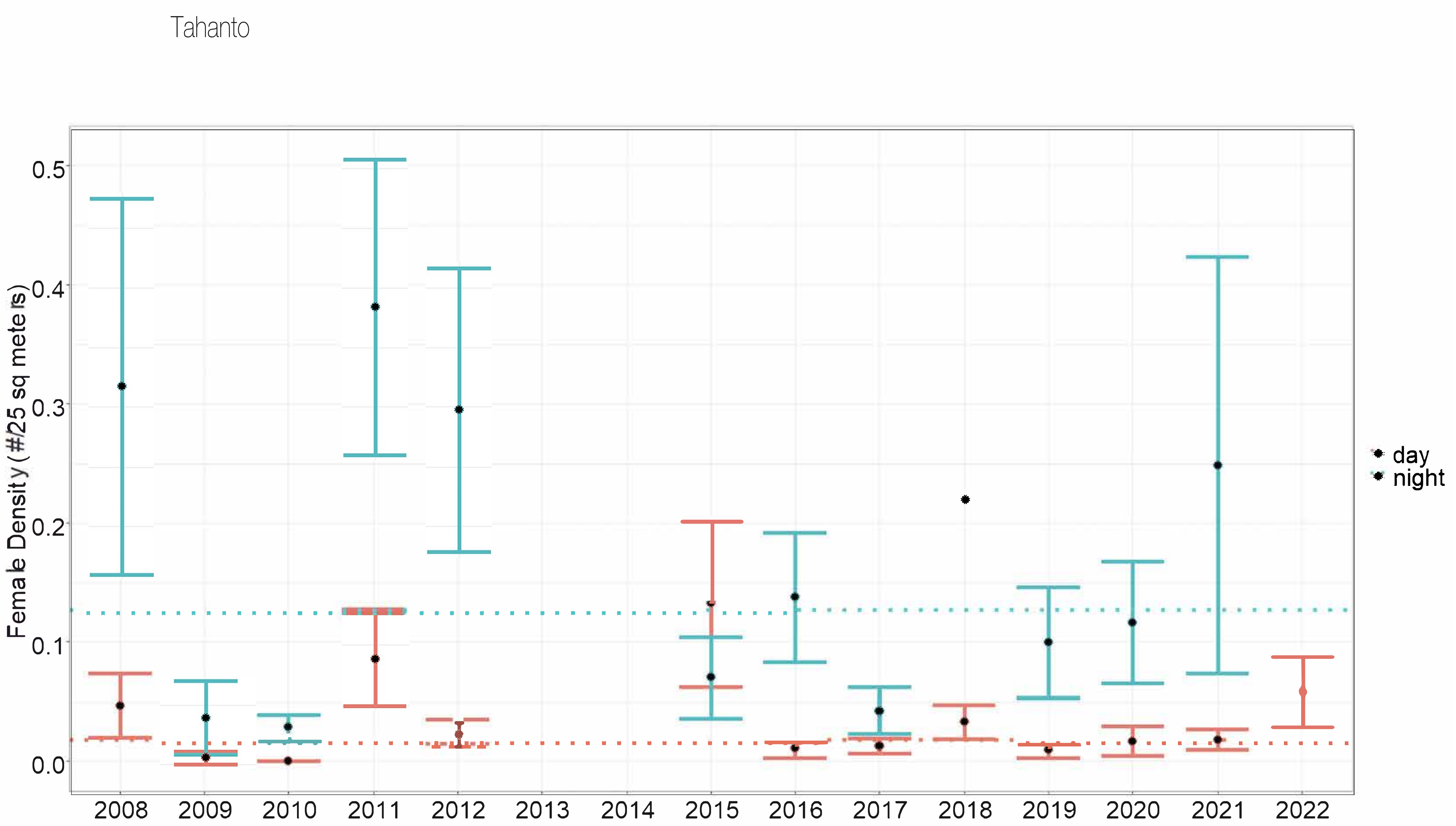
South of Cape Cod MA DMF Trawl Survey dices (0-60')



Swifts Beach

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Female Density (#/25 sq meters)
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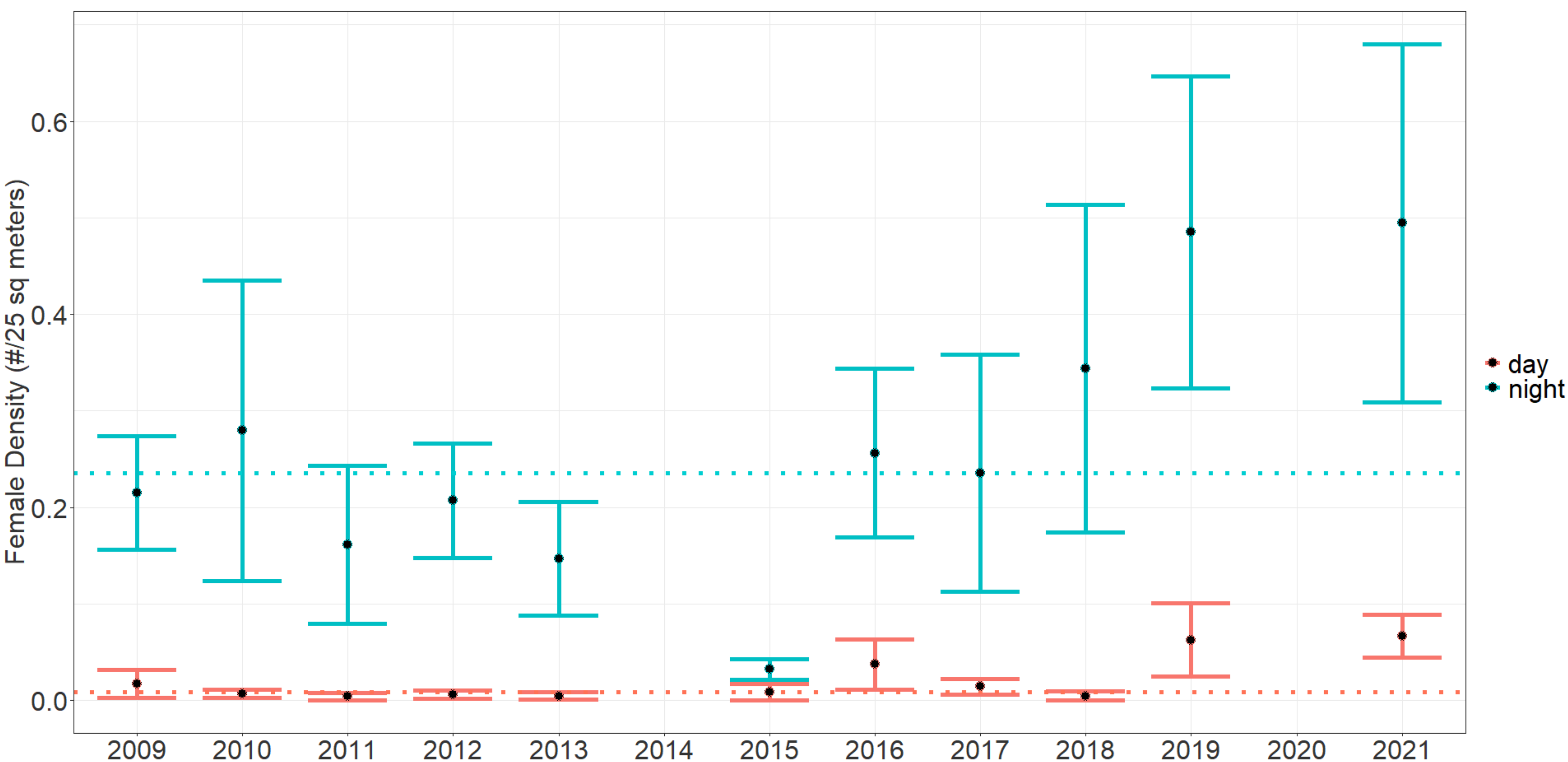
Tashmoo



Female Density (#/25 sq meters)
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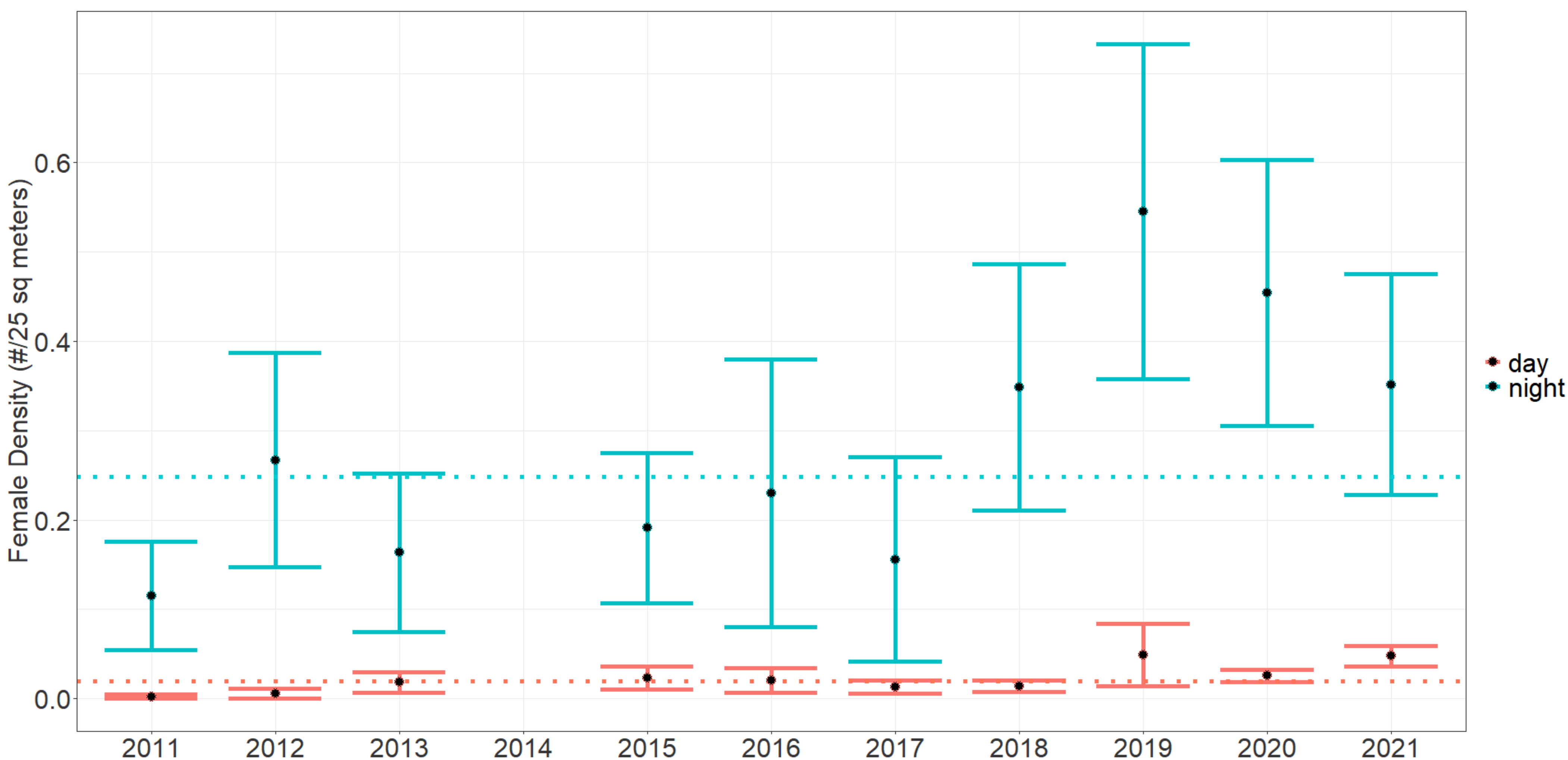
Warren's



Female Density (#/25 sq meters)
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American Horseshoe Crab (*Limulus polyphemus*)

Meta-analysis used to determine quantitative trends in Gulf of Maine (New Hampshire), Mid-Atlantic, Southeast, Florida-Atlantic, and Northeast Gulf of México Regions

Data were available from 40 fishery-independent data sets covering Mid-Atlantic and Florida regions (New Hampshire to Florida; regions as defined above and in Figure 1) over a range of years. The fishery-independent data sets were selected by the Atlantic States Marine Fisheries Commission (ASMFC) for stock assessment (ASMFC; Sweka *et al*. 2013). ASMFC selects datasets that are overseen or conducted by state or federal agencies or academic institutions using standardized methodology and survey design. State agencies rely on these datasets to comply with ASMFC monitoring requirements. The basic data were individual counts of Horseshoe Crabs within sampling units; the demographic (age-class, sex) and temporal and spatial resolution of each dataset is described in Sweka *et al*. (2013: Appendix B) and summarized in Table 2.

We analyzed trends from each dataset and then used meta-analysis techniques to summarize inference at the regional or sub-regional level because the data came from many independent monitoring programs. We grouped the datasets from the Mid-Atlantic region into sub-regions because of geographic differences in harvest pressure and environmental conditions. The sub-regions were New England states (NH, RI, MA), New York area (CT, NY), and Delaware Bay area (NJ, DE, MD, VA). In addition, datasets represented the Southeastern (NC, SC, GA), Florida Atlantic (FL), and Gulf of México (FL) regions. There were no state-specific datasets from NC; however, data from an offshore monitoring program (SEAMAP) included waters off the NC coast. The time series varied among the datasets. The New England area included the longest time series, with one data set from 1959 and several that started in the 1970s. Data sets from the New York and Delaware Bay areas started in the late 1980s. Data sets from the Southeast included several that started in the mid-1990s.

The objective of the meta-analysis of regional trends was to determine change in horseshoe crab populations during the periods defined by the available data. The trend analyses involved fitting a linear regression to the data, which had been standardized by subtracting the mean and dividing by the standard deviation. Standardization was required for the trend analysis results based on individual datasets to be combined using meta-analysis techniques.

We used the following three meta-analysis techniques described by Manly (2001:123-125):

- Fisher's method addressed the hypothesis that at least one of the indices showed a significant decline. The test statistic was calculated by $S_1 = -2\sum 1n(p_i)$ where p_i was the one-tailed p-value that tested for a significantly negative regression slope for the i^{th} index.
- Stouffer's method addressed the hypothesis that there was a consensus for a decline supported by the set of indices. Here the individual one-tailed p-values were converted to z-scores, which under the null hypothesis were distributed as a Normal random variable with mean of zero and a variance of $1/\sqrt{n}$, where n was the number of datasets. The test statistic was $S_2 = \bar{z}/(1/\sqrt{n})$. A version of the Stouffer's method incorporated weighting into the calculation of the test statistic. We used a measure of precision (the inverse of the root mean square error, i.e., the RMSE) as the weight (w_i). The weighted test statistic was $S_3 = (\sum w_i z_i)/\sqrt{\sum w_i^2}$.
- A weighted standardized slope along with confidence intervals addressed the hypothesis that the datasets showed a significant decline on average. We used a measure of precision as the weight (inverse of the RMSE) so that the datasets with the higher precision received greater weight. The calculation of the weighted slope was $\bar{b}_w = \sum w_i b_i / \sum w_i$, where b_i was the slope for the i^{th} dataset. The standard error was $(\bar{b}_w) = \sqrt{\sum w_i (b_i - \bar{b}_w)^2 / (\sum w_i (n-1)}$. The t-distribution was used to calculate confidence intervals.

Results indicated that there have been significant declines in at least one dataset in all areas except the Southeast and Florida as evidenced by test S_1 (Table 3). The breadth of decline was evidenced by S_2, S_3, and weighted slope, which all indicate that breadth of declines was highest in the New England area and diminished generally from the northern to southeastern areas with indications of negative slopes for Florida Atlantic and Northeast Gulf regions (Table 3 and Figure 4). The uncertainty in the Florida Atlantic estimates was high, in part, because of the low number of and variation in trends among available datasets (Figure 4). Although the sub-regional level inference for Florida Atlantic suggested no significant decline in the Horseshoe Crab population, the datasets from Jacksonville indicated an embayment-specific decline.

For those regions or sub-regions with negative weighted slope (i.e., Gulf of Maine (NH), New England area, New York area, Northeast Gulf region), population reduction over 40 years, which approximates three generations based on age-structured population models (Sweka *et al*. 2007), can be projected assuming the current linear trends continue and the index represents population abundance. The formula used for this projection was

$$\text{Percent projected population change} = ((1+\lambda^{40})-1)*100,$$

Where λ denoted weighted slope and 40 years coincided with three generations. Continuation of these negative trends would result in projected population reductions of 100% in Gulf of Maine (NH), 92% in New England, 11% in New York, 55% in Florida Atlantic, and 32% in Northeast Gulf of México. Although not accounting for carrying capacity limits to population growth, projections indicate population increases in the Delaware Bay of 116% and in the Southeast region of 218% over 40 years.

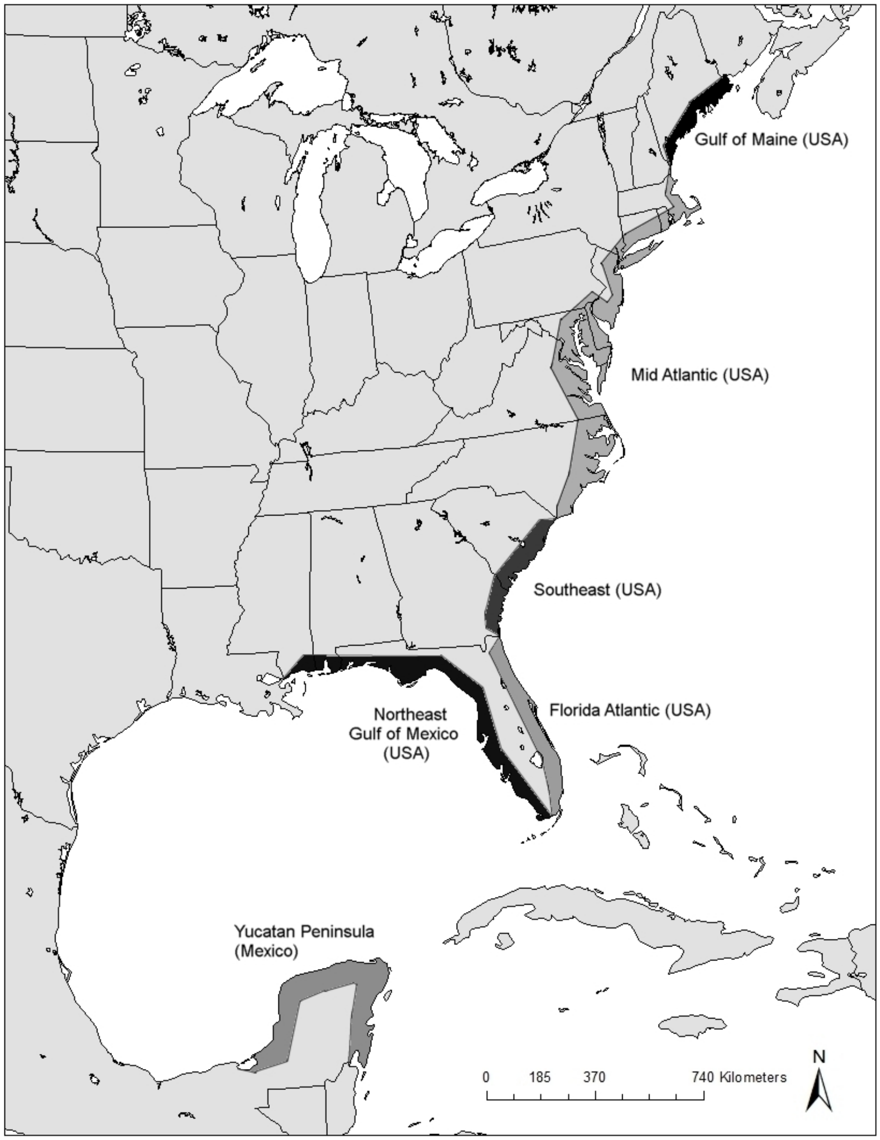

Figure 1. Range map for the American Horseshoe Crab (*Limulus polyphemus*), including genetically-defined regions used in the IUCN Red List assessment. Shading is included to contrast each region and indicate geographic extent.

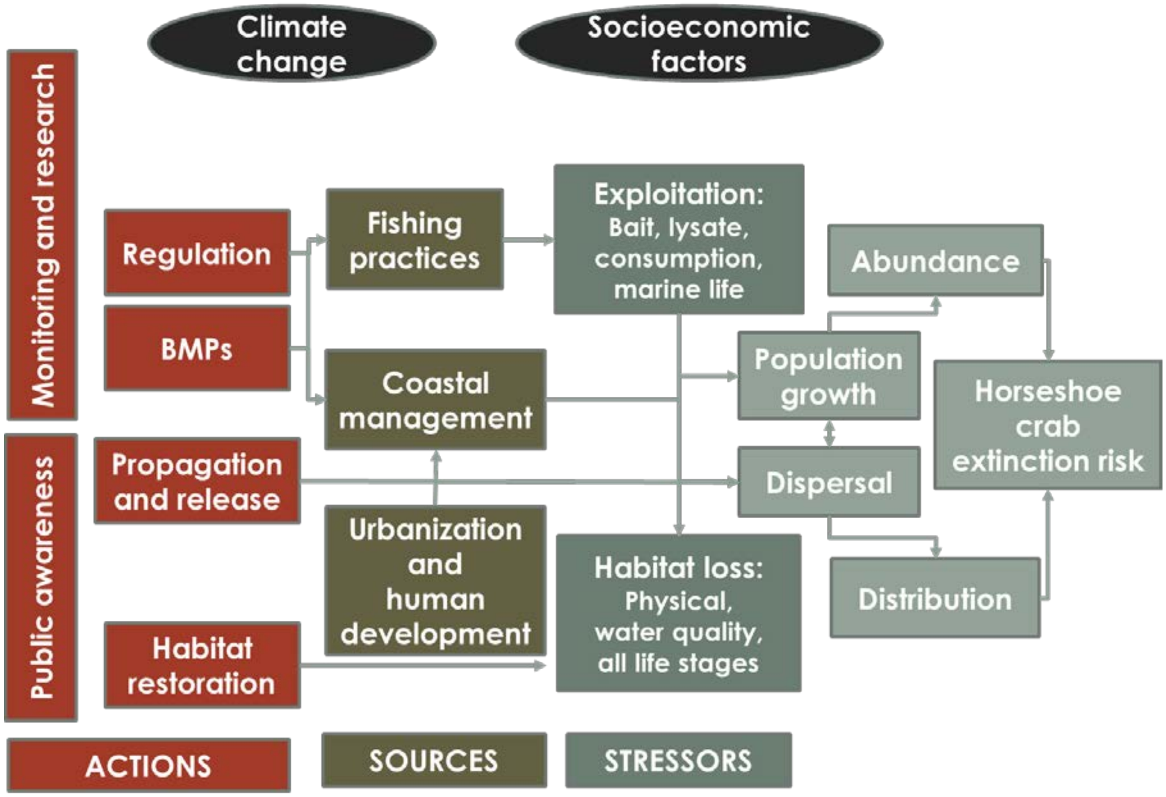

Figure 2. Conceptual model (influence diagram) for the American Horseshoe Crab assessment showing influence of stressors, sources, and actions on population extinction risk. Population risk determines regional risk, which rolls up to determine species level extinction risk.

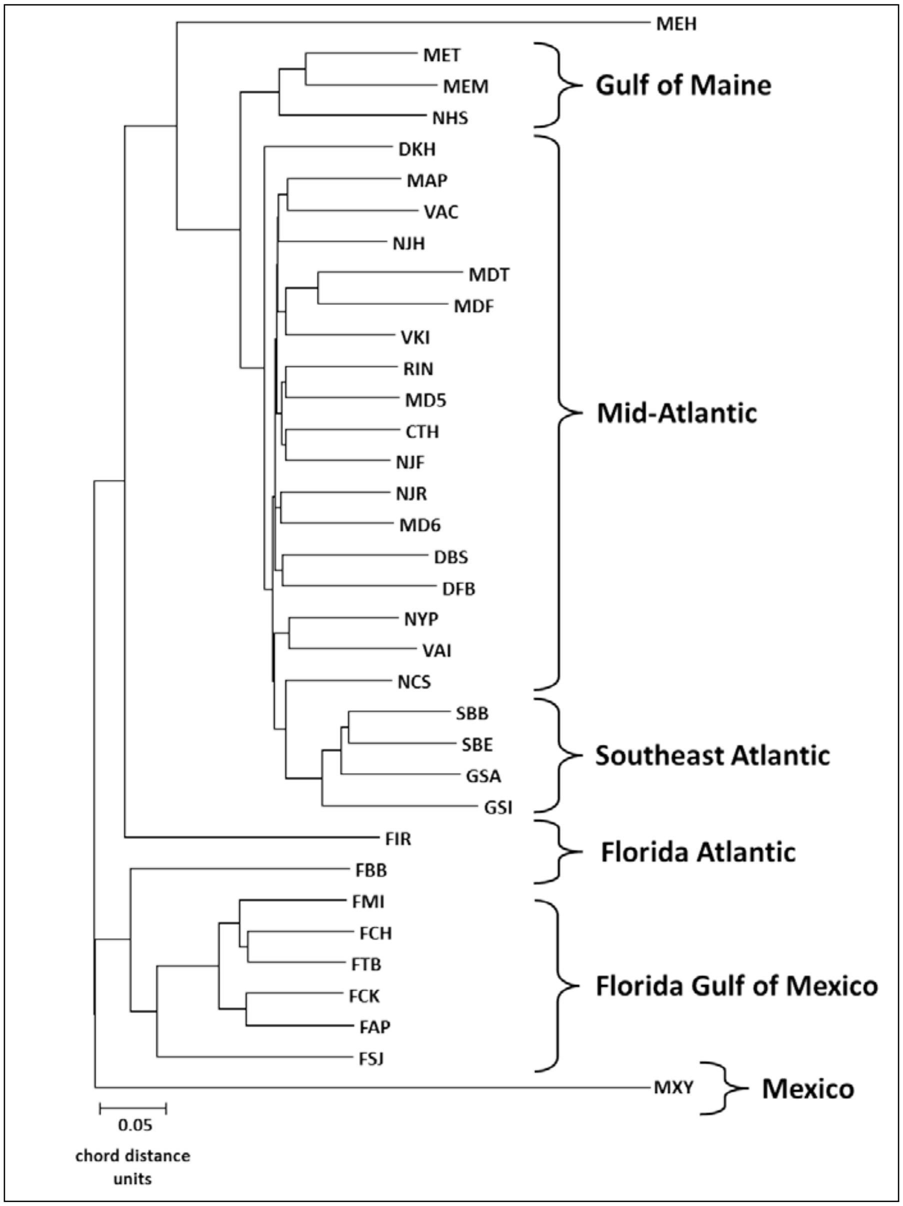

Figure 3. Neighbour-joining phenogram depicting genetic distance (chord, Cavalli-Sforza and Edwards 1967) among 35 *Limulus polyphemus* collections sampled from the Atlantic and Gulf coasts of the United States and Ria Lagartos and San Felipe, Yucatán, Republic of México. Brackets group collections into suggested management units. Abbreviations for spawning site collections are found in Table 1.

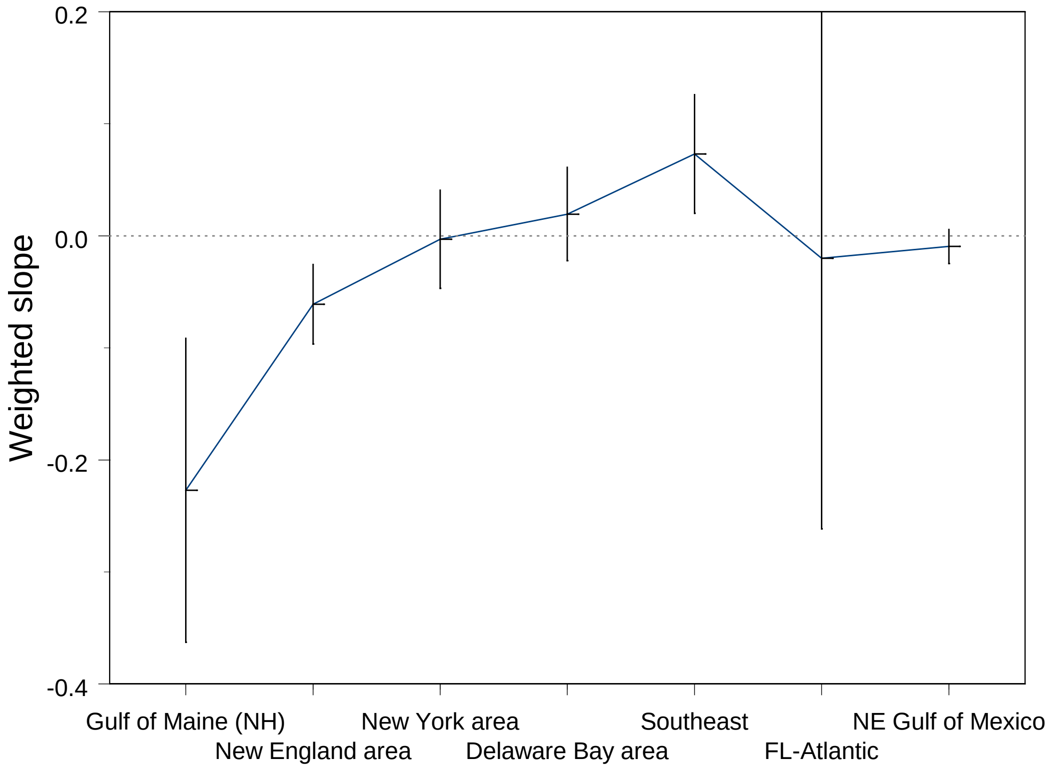

Figure 4. Weighted standardized slope with 90% confidence bars from meta-analyses of multiple datasets from New Hampshire (NH) in the Gulf of Maine region to the Northeast (NE) Gulf of México region with time series spanning different years. Regions and areas with regions are described in the text and in Figure 1. The datasets were grouped and oriented generally north to south on the x-axis. The datasets from Gulf of Maine New Hampshire are from the Great Bay. The New England, New York, and Delaware Bay constitute areas within the Mid-Atlantic region. The Southeast, Florida Atlantic, and Northeast Gulf are separate regions.

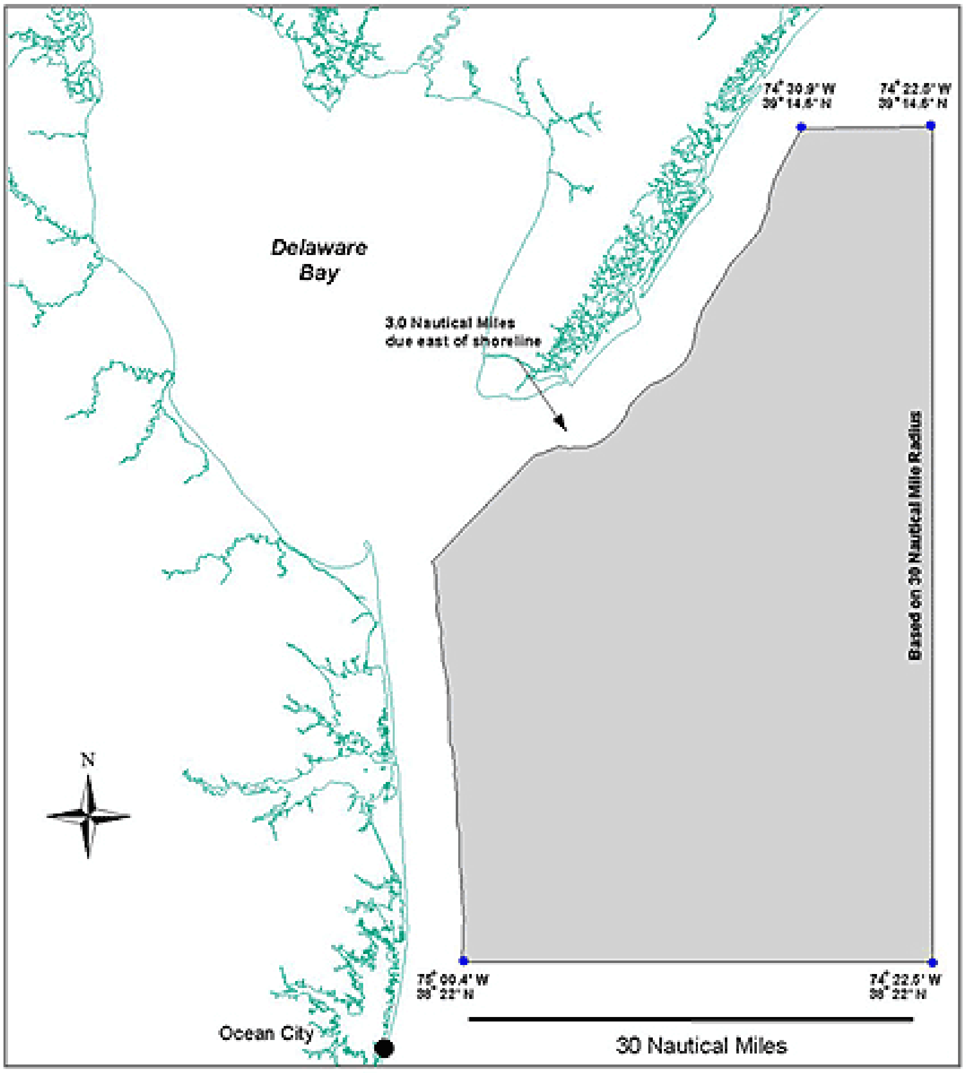

Figure 5. The Carl N. Shuster Jr. Horseshoe Crab Reserve (gray area) off the mouth of Delaware Bay, which is a marine protected area where harvest of Horseshoe Crabs is prohibited.

Table 1. Abbreviation, general location, and sample size for 35 spawning and 5 near- or off-shore dredge or trawl collections of horseshoe crabs *Limulus polyphemus* genotyped at 13 microsatellite DNA loci to assess population structuring.

Abbreviation	Spawning collection site	Sample size
MEH	Hog Bay, Franklin, Maine	47
MET	Thomas Point Beach, Maine	45
MEM	Middle Bay, Brunswick, Maine	48
NHS	Chadman's Landing, Squamscott River, New Hampshire	48
MAP	Pleasant Bay, Massachusetts	48
RIN	Green Island, Narragansett Bay, Rhode Island	48
CTH	Housatonic River, Milford Point. Connecticut	48
NYP	Great Peconic Bay, Long Island, New York	48
NJF	Fortescue Beach, New Jersey	48
NJR	Reeds Beach, New Jersey	48
NJH	Highs Beach, New Jersey	49
DHK	Kitt's Hummock Beach, Delaware	36
DBS	Big Stone Beach, Delaware	31
DFB	Fowler Beach, Delaware	47
MDT	Turkey Point, Chesapeake Bay, Maryland	30
MDF	Flag Pond State Park, Chesapeake Bay, Maryland	29
MD5	Ocean City, Maryland – 2005	48
MD6	Ocean City, Maryland – 2006	48
VAC	Chincoteague, Virginia	48
VKI	Kiptopeke St. Park, Chesapeake Bay, Virginia	48
VAI	Tom's Cove, Assateague Island, Virginia	48
NCS	Shackleford Banks, North Carolina	55
SBB	Bull's Bay, South Carolina	53
SBE	Beaufort, South Carolina	48
GSA	Savannah, Georgia	48
GSI	Sapelo Island, Georgia	32
FIR	Indian River, Florida (Atlantic coast)	47
FBB	Biscayne Bay	20
FMI	Tiger Tail Beach, Marco Island, Florida (Gulf coast)	81
FCH	Charlotte Harbor, Florida	51
FTB	Tampa Bay, Florida	201
FCK	Seahorse Key, Cedar Keys NWR, Florida	132

Abbreviation	Spawning collection site	Sample size
FAP	Alligator Point, Apalachicola Bay, Florida	92
FSJ	St. Joseph Bay, Florida	23
MXY	Ria Lagartos and San Felipe, Yucatán, Mexico	20
	Subtotal	*1,841*
	Near- or Off-shore Dredge or Trawling Collection	
NYL	Offshore Long Island, New York (trawl)	46
NJC	Offshore Cape May Inlet, New Jersey (trawl)	48
MOC	Ocean City, Maryland (trawl)	48
VCH	Chincoteague Island (commercial dredge)	46
FWS	US Fish and Wildlife Service Cruise 2007 (trawl)	48
	Subtotal	*236*
	TOTAL	**2,077**

Table 2. Summary of fishery-independent data used in the quantitative trend analysis, data are separated by sub-region within genetically distinct regions including the Gulf of Maine, Mid-Atlantic, Southeast Atlantic, Florida Atlantic, and Northeastern Gulf of Mexico. Additional details can be found in Sweka *et al*. (2013: Appendix B).

Region	**Dataset**	**Years of data**	**Survey method (dredge, trawl, beach count, etc.)**	**Notes**
Gulf of Maine, New Hampshire	New Hampshire spawning survey	2001 - 2012	Spawning count	Counts along three 100 m beaches in Great Bay, NH during new and full moons May through September
Mid-Atlantic: New England area	Massachusetts (MA) University of RI (URI) Marine Research Inc (MRI) Power plant (PR) RI DFW (DFW) Stout (ST)	• (MA) 1978-2012 • (URI) 1959-2012 • (MRI) 1988-2012 • (DFW) 1998-2012 • (PR) 1992-2012 • (ST) 1975-2012	• (MA, URI, MRI, DFW) trawl • (PR, ST) count	• (MA) stratified random; 94 stations per year; spring and fall • (URI) fixed station; 2 sampled weekly for 12 months • (MRI) fixed station; 60-70 tows per 6 month period; April-October • (DFW) stratified random component and fixed station component; 84 stratified random (split spring and fall), 150 fixed stations about 13 per month; year round • (PR) fixed site; 3 water intakes at power station; 3 counts per week; year round • (ST) fixed site; 2 ponds; 1 count per year during spawning season
Mid-Atlantic: New York area	New York: Peconic Bay (PB), Jamaica Bay (JB), Little Neck Bay (LNB), Manhassett (MH) Connecticut Long Island Sound (LIS)	• (PB, JB, LNB, MH) 1987-2012 • (LIS)1992-2012	• (PB, LIS) trawl • (JB, LNB, MH) seine	• (PB) constrained random; 16 stations; May-October • (JB, LNB, MH) fixed site; 5 to 10 seine sites per beach per sampling trip; May-October • (LIS) stratified random; 40 per month; spring (April-June) and fall (September-October)
Mid-Atlantic: Delaware Bay area	New Jersey trawl (NJ) Delaware trawl (DE) Delaware Bay spawning survey (DB) Ocean trawl (OC)	• (NJ) 1998-2012 • (DE) 1990-2012 • (DB) 1999-2012 • (OC) 2002-2011	• (NJ) trawl • (DE) trawl • (DB) spawning count • (OC) trawl	• Adult males, adult females, and juveniles analyzed separately • (NJ) Fixed stations; 11 per month; April-October • (DE) Fixed stations; 16 foot trawl: 40 per month; August-October; 30 foot trawl: 9 per month; April-July • (DB) 24 accessible beaches throughout DB; 12 nights per year; 100 quadrats per night; May-June • (OC) stratified random stations NJ to VA from shore to 12 NM; 40-50 stations; September-October

Region	Dataset	Years of data	Survey method (dredge, trawl, beach count, etc.)	Notes
Southeast	South Carolina (SC), Georgia (GA), Southeast Area Monitoring and Assessment Program (SEAMAP)	• (SC) 1995-2012 • (GA) 1999-2012 • (SEAMAP) 1998-2008	Trawl	• (SC) Fixed stations; 200 per year; March-June, October and December • (GA) Fixed stations; 36 per month; monthly • (SEAMAP) Stratified random, fixed stations; 78 per season; spring (April-May), summer (July-August), fall (October-November)
Florida Atlantic	Jacksonville (JX), Indian River (IR), Tequesta (TQ)	• (IR & TQ) 1997-2013 • (JX) 2001-2013	Seine	Multiple samples per month
Northeast Gulf	Apalachicola (AP), Cedar Key (CK), Charlotte Harbor (CH)	• (AP) 1998-2013 • (CK) 1997-2013 • (CH) 1996-2013	Seine	Multiple samples per month

Table 3. Meta-analysis of trends based on 40 datasets ranging from New Hampshire (NH) in the Gulf of Maine to Northeast (NE) Gulf of México. The datasets were grouped by region, and for the Mid-Atlantic region, the datasets were further subdivided into sub-regions to reflect differences in harvest pressure and environmental condition. The number of years covered by each dataset varied. Definitions of the test statistics are in text.

Region	Sub-region	S_1: Tests whether at least one of datasets shows decline			S2: Tests whether there is a consensus for decline among datasets				S_3: Like S2, but weights inverse to RMSE		Weighted slope: Estimates an overall standardized slope weighted inverse to RMSE. Shown with 90% confidence intervals				
		S_1	df	Pr(X2>S_1 | df)	z-bar	S_2	Pr(Z<S_2)	wt z-bar	S_3	Pr(Z<S_3)	Weighted slope	Variance	SE	LCL	UCL
Gulf of Maine	**Great Bay, NH**	19.5505	4	0.0006	-2.4073	-3.4044	0.0003	-2.4277	-3.4333	0.0003	-0.2271	0.0005	0.0215	-0.3627	-0.0914
Mid-Atlantic	**New England**	121.5815	14	0.0000	-2.9998	-7.9367	0.0000	-3.6450	-9.6438	0.0000	-0.0610	0.0003	0.0183	-0.0966	-0.0255
	New York	23.3928	12	0.0246	-0.2029	-0.4970	0.3096	-0.3513	-0.8605	0.1948	-0.0030	0.0005	0.0218	-0.0469	0.0408
	Delaware Bay	72.2429	32	0.0001	0.1231	0.4924	0.6888	-0.1231	-0.4923	0.3113	0.0194	0.0006	0.0237	-0.0222	0.0611
Southeast		17.2080	18	0.5088	0.4672	1.4015	0.9195	1.0831	3.2494	0.9994	0.0294	0.0006	0.0253	-0.0178	0.0765
FL-Atlantic		10.5370	6	0.1038	-0.3499	-0.6061	0.2722	-0.4099	-0.7100	0.2389	-0.0200	0.0068	0.0827	-0.2614	0.2215
NE Gulf of Mexico		5.1771	6	0.5213	-0.1877	-0.3252	0.3725	-0.1883	-0.3261	0.3722	-0.0094	0.0000	0.0052	-0.0246	0.0058

Table 4. State-specific bait harvest quotas based on Addendum IV of the Atlantic States Marine Fisheries Commission's (ASMFC) Fishery Management Plan for Horseshoe Crabs. Addendum IV was enacted in 2006. Average reported landings (animals) are shown for 2008 to 2012.

State	Landings in 1998	ASMFC harvest quota enacted 2006	Average landings (2008-2012)
Maine	13,500	13,500	0
New Hampshire	350	350	8
Massachusetts	440,503	330,377[a]	86,197
Rhode Island	26,053	26,053[a]	15,744
Connecticut	64,919	48,689	26,618
New York	488,362	366,272[a]	142,380
New Jersey	604,049	100,000[a,b]	0
Pennsylvania	0	0	0
Delaware	482,401	100,000[b]	92,488
Maryland	613,225	170,653[b]	166,083
Virginia	203,326	152,495[b]	141,544
North Carolina	24,036	24,036	23,826
South Carolina	0	0	0
Georgia	29,312	29,312	0
Florida	9.455	9,455	209
Coastwide	2,999,491	1,371,192	695,096

a = States have set a more conservative quota

b = New adaptive management quota set annually

Table 5. Sex-specific bait harvest quota (animals) for Delaware Bay area states based on adaptive resource management framework adopted in 2012 by the Atlantic States Marine Fisheries Commission.

	Delaware Bay Origin Quota		Total Quota	
State	**Male**	**Female**	**Male**	**Female**
Delaware	162,136	0	162,136	0
New Jersey	162,136	0	162,136	0
Maryland	141,112	0	255,980	0
Virginia	34,615	0	81,331	0