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 $\left(\bar{b}_w\right) = \sqrt{\sum w_i (b_i - \bar{b}_w)^2 / (\sum w_i (n-1)}$. 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.

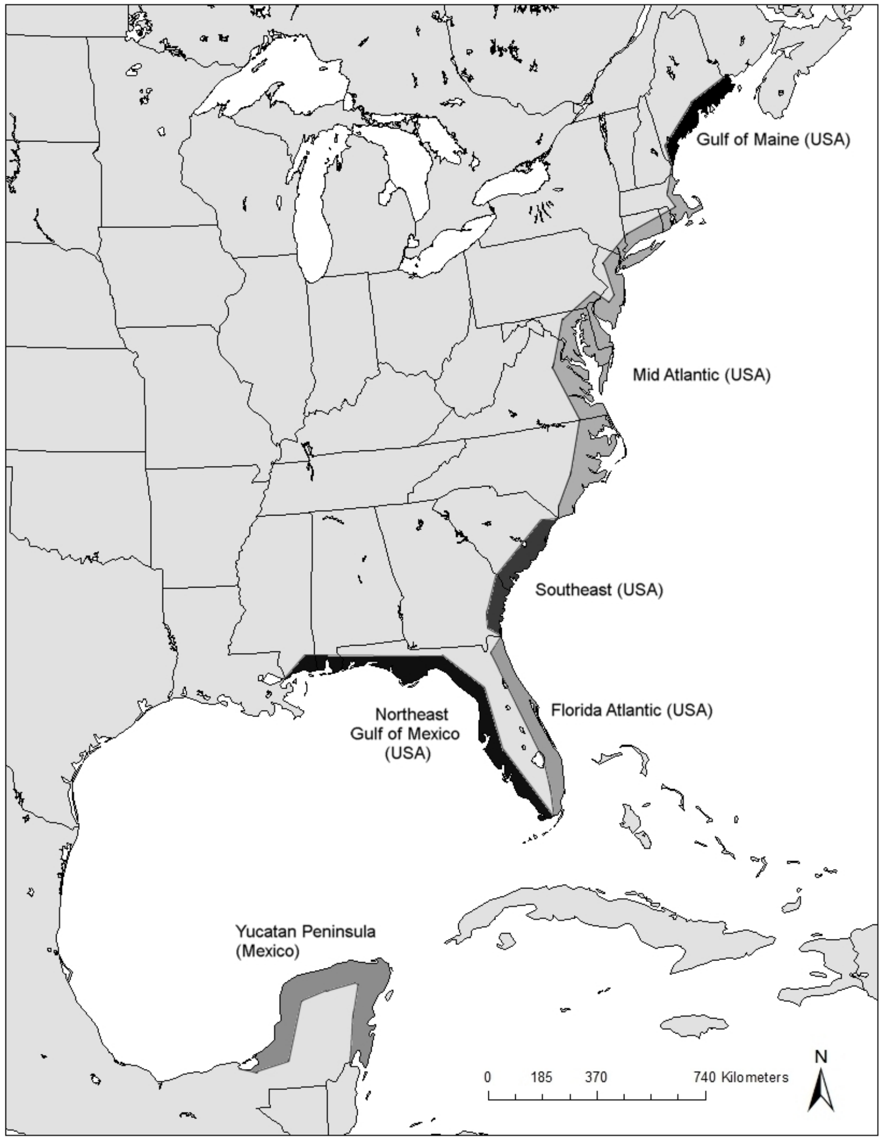

Figure 1. Range map for the American Horseshoe Crab (*Limulus polyphemus*), including genetically-defined regions used in the IUCN Red List assessment. Shading is included to contrast each region and indicate geographic extent.

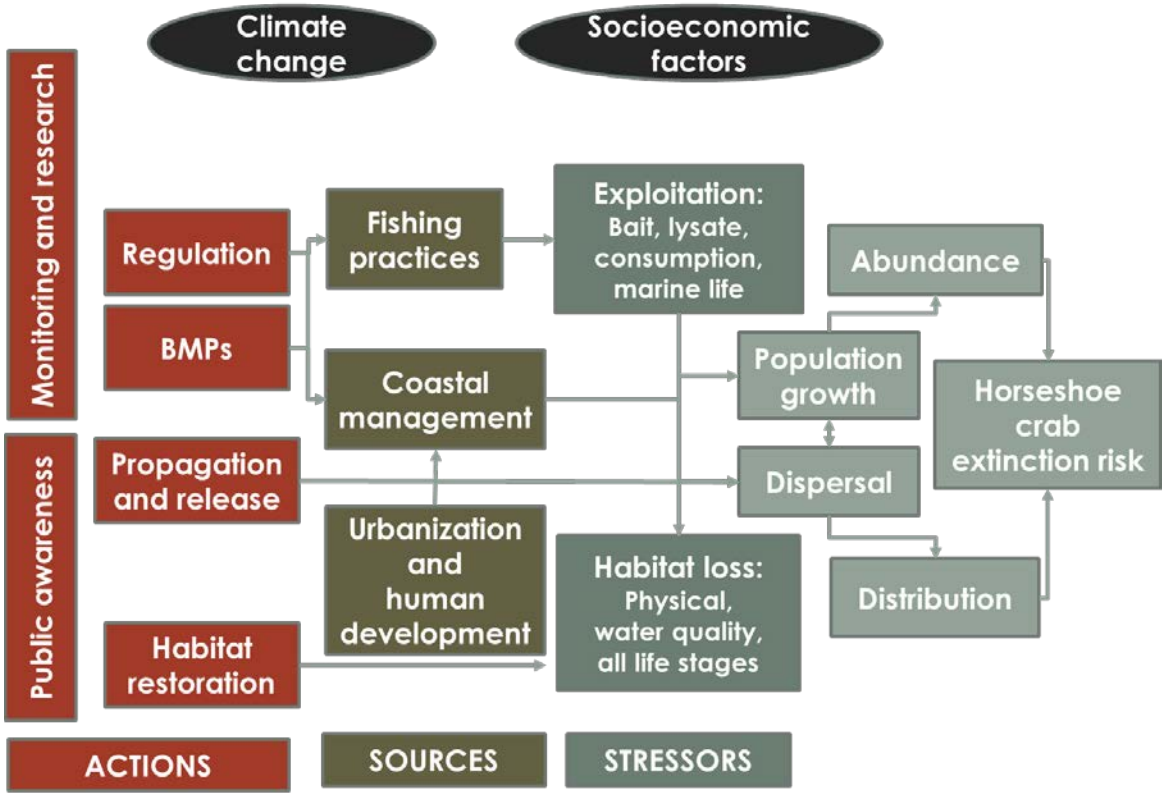

Figure 2. Conceptual model (influence diagram) for the American Horseshoe Crab assessment showing influence of stressors, sources, and actions on population extinction risk. Population risk determines regional risk, which rolls up to determine species level extinction risk.

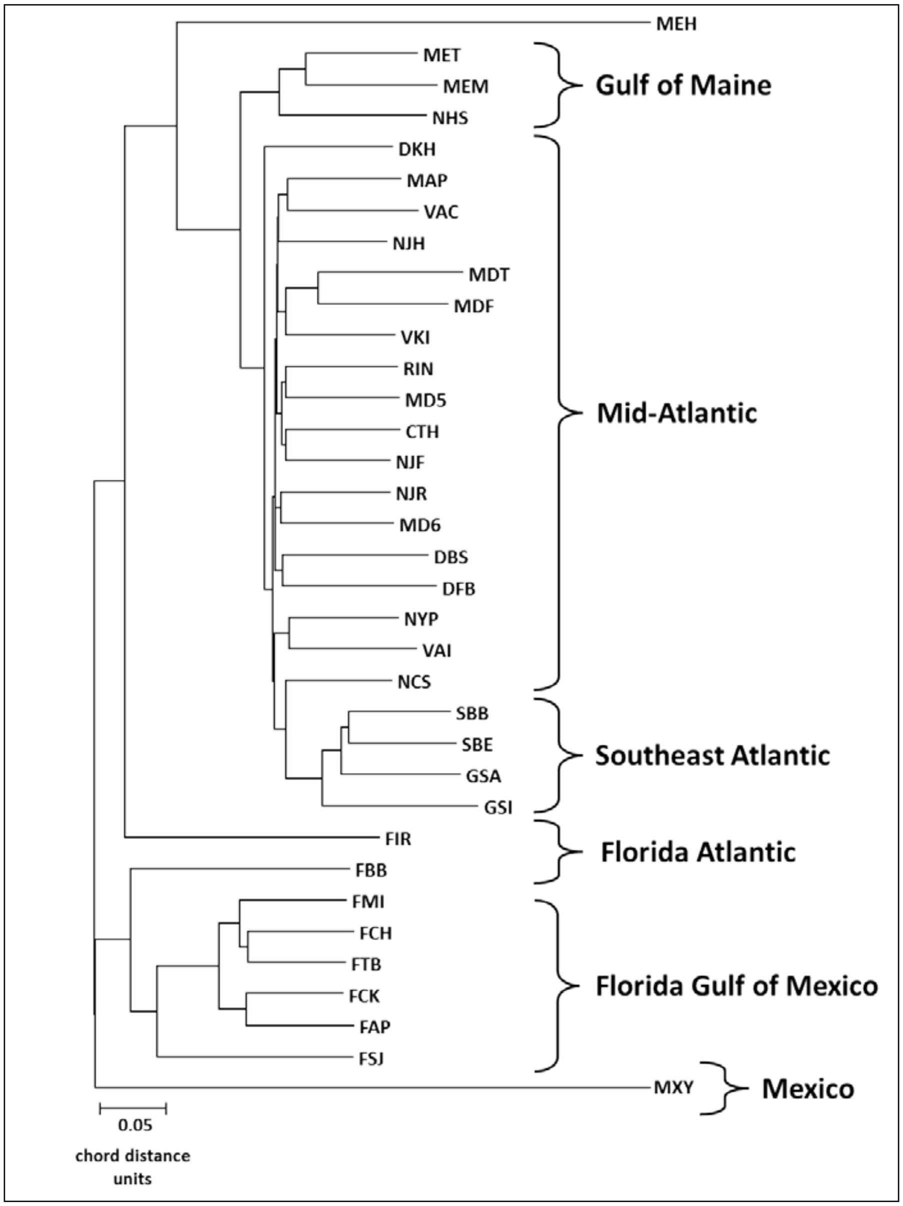

Figure 3. Neighbour-joining phenogram depicting genetic distance (chord, Cavalli-Sforza and Edwards 1967) among 35 *Limulus polyphemus* collections sampled from the Atlantic and Gulf coasts of the United States and Ria Lagartos and San Felipe, Yucatán, Republic of México. Brackets group collections into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

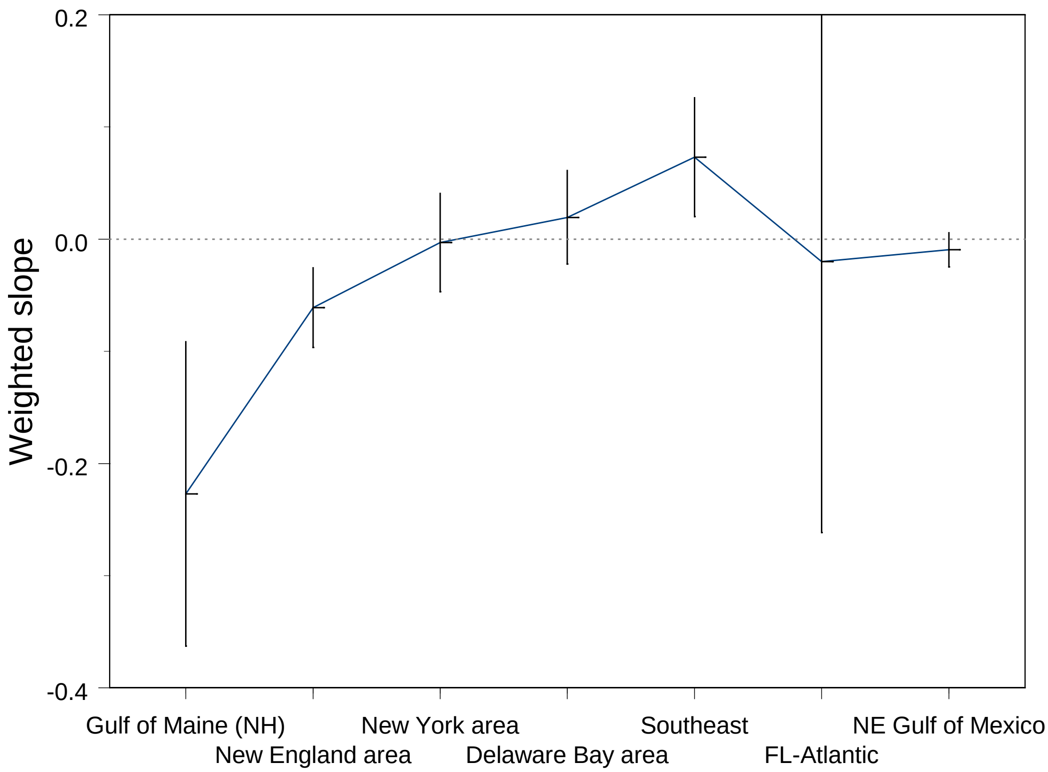

Figure 4. Weighted standardized slope with 90% confidence bars from meta-analyses of multiple datasets from New Hampshire (NH) in the Gulf of Maine region to the Northeast (NE) Gulf of México region with time series spanning different years. Regions and areas with regions are described in the text and 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.

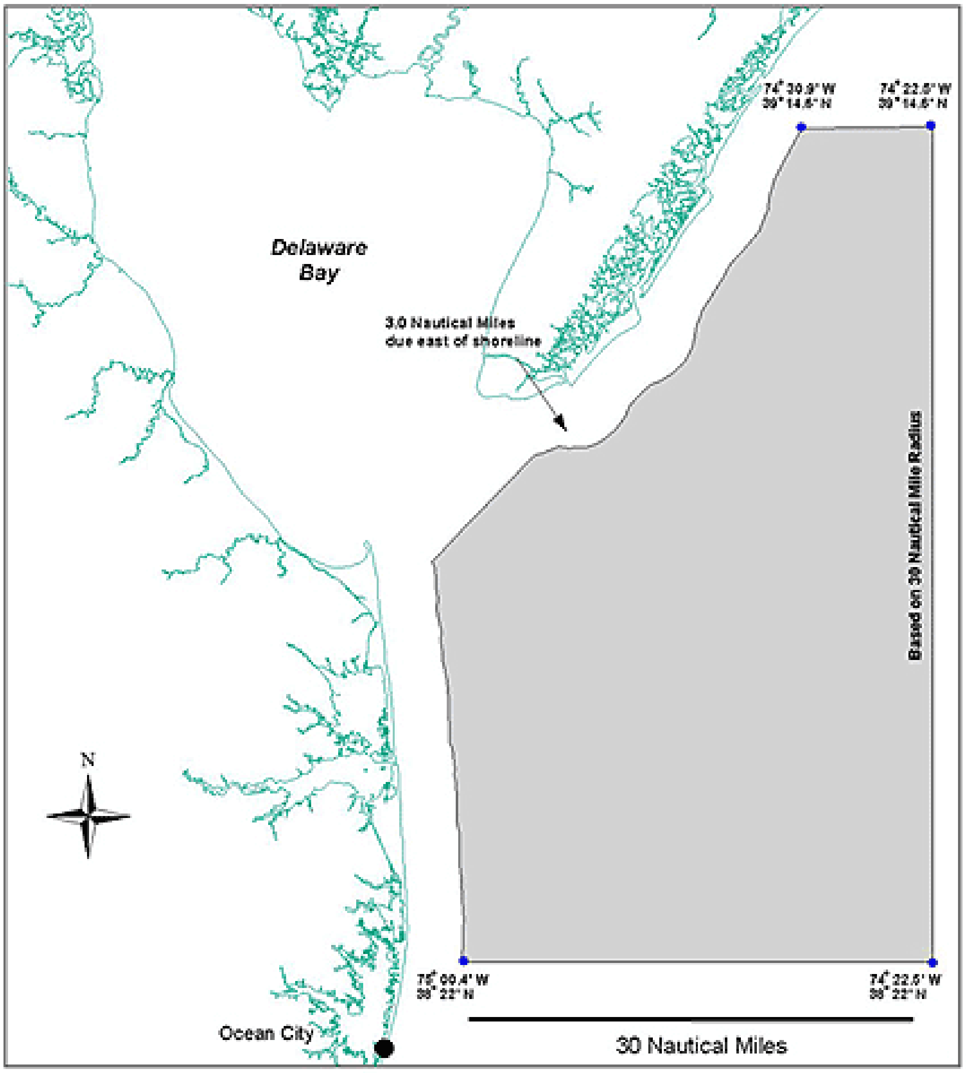

Figure 5. The Carl N. Shuster Jr. Horseshoe Crab Reserve (gray area) off the mouth of Delaware Bay, which is a marine protected area where harvest of Horseshoe Crabs is prohibited.

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	$Pr(X2>S_1 \mid df)$	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