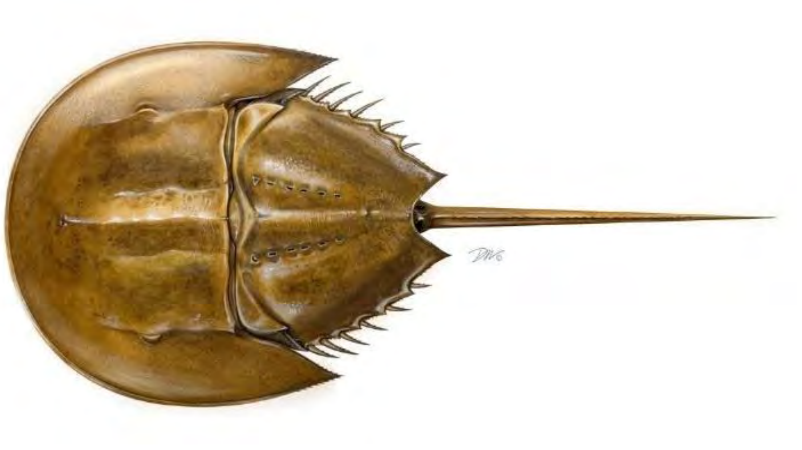
Atlantic States Marine Fisheries Commission

2019 Horseshoe Crab Benchmark Stock Assessment and Peer Review Report



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Sustainable and Cooperative Management of Atlantic Coastal Fisheries

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ASMFC Horseshoe Crab Stock Assessment Subcommittee
John Sweka (Chair), U.S. Fish and Wildlife Service
Natalie Ameral, Rhode Island Division of Fish and Wildlife
Kristen Anstead, Atlantic States Marine Fisheries Commission
Linda Barry, New Jersey Division of Fish and Wildlife
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Derek Perry, Massachusetts Department of Fish and Game
Linda Stehlik, National Marine Fisheries Service
Chris Wright, National Marine Fisheries Service
Catherine Ziegler, New York State Department of Environmental Conservation
Jordan Zimmerman, Delaware Division of Fish and Wildlife

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Atlantic States Marine Fisheries Commission

2019 Horseshoe Crab Benchmark Stock Assessment Peer Review Report

Conducted on
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Arlington, Virginia

Prepared by the
ASMFC Horseshoe Crab Stock Assessment Review Panel

Dr. Lawrence Jacobson (Chair), National Marine Fisheries Service (retired), Northeast Fisheries Science Center, Woods Hole, Massachusetts
Dr. Ruth Carmichael, University of South Alabama, Dauphin Island, Alabama
Dr. Matthew Cieri, Maine Department of Marine Resources, West Boothbay Harbor, Maine

ACRONYMS

ARIMA	Auto-Regressive Integrated Moving Average (stock assessment model)
ARM	Adaptive Resource Management
B_{MSY}	Biomass at maximum sustainable yield
CMSA	Catch Multiple Survey Analysis (stock assessment model)
CV	Coefficient of Variation
F	Annual fishing mortality rate
F_{MSY}	Fishing mortality rate at maximum sustainable yield
MSY	Maximum Sustainable Yield
N_{MSY}	Abundance at maximum sustainable yield
NEFOP	Northeast Fisheries Observer Program
TOR	Term of Reference

EXECUTIVE SUMMARY

The assessment splits the range of horseshoe crabs into four regions; Northeast (Maine, Massachusetts, Rhode Island), New York (Connecticut, New York), Delaware Bay (New Jersey, Delaware, Maryland, Virginia), and Southeast (North Carolina, South Carolina, Georgia, Florida).

The Panel recommends using horseshoe crab trend estimates for females and males combined from Autoregressive Integrated Moving Average (ARIMA) models fit to survey data for stock status determination, relative to abundance in 1998. Examination of results from multiple surveys within individual regions is necessary. Stock status was based on the proportion of surveys above or below their 1998 reference point when ASMFC management began. Stock status was considered poor if 33% of the surveys were below their reference point (red), good if 66% were above their reference point (green), and neutral (yellow) otherwise.

Stock status differs among regions based on the recommended 1998 reference point and ARIMA-based relative abundance estimates (see Figure 1 in Advisory Report). Based on this recommended approach, horseshoe crab relative abundance in the Northeast and Delaware Bay regions are in a neutral condition, New York is in a poor condition, and the Southeast is in a good condition. On a coastwide basis, horseshoe crab relative abundance is likely in a neutral condition.

ARIMA and Catch Multiple Survey Analysis (CMSA) model estimates were both available for female horseshoe crabs in the Delaware Bay region. The Panel recommends CMSA results when abundance and fishing mortality estimates are required, such as in the Adaptive Resource Management (ARM) model used by managers (Note the ARM model was described during review discussions but not reviewed by the Panel). CMSA results were not used for status determination because comparable reference points were not available. However, given the increasing survey trends, low landings, and CMSA results of low fishing mortality and relatively high abundance, overfishing and an overfished status are unlikely for female horseshoe crabs in the Delaware Bay region.

The magnitude of horseshoe crab discards in the targeted horseshoe crab fishery and other fisheries is potentially the most important uncertainty and highest priority research recommendation identified in the assessment to improve abundance estimates. Preliminary results show discard mortality may be comparable to or greater than combined mortality from other sources.

The stock assessment could not determine overfished stock status in terms of B_{MSY}, N_{MSY} or proxy reference points because biomass, abundance estimates and MSY reference points were not available. Trend-based relative abundance reference points were used instead, following common practice in many fisheries.

The stock assessment could not determine if overfishing is occurring in terms of F_{MSY} for horseshoe crabs as mortality estimates and suitable reference points were not available. It was not possible to determine if overfishing was occurring based on trends because discards are uncertain.

It is important to continue survey data collection for horseshoe crabs (particularly the Virginia Tech survey), promote consistent survey sampling among locations, and expand survey data collection to include size, sex, and information on female reproductive condition (primiparous vs. multiparous).

COMMENTS BY TERMS OF REFERENCE

1. **Evaluate the thoroughness of data collection and the presentation and treatment of fishery-dependent and fishery-independent data in the assessment, including the following but not limited to:**
    a. **Presentation of data source variance (e.g., standard errors).**
    b. **Justification for inclusion or elimination of available data sources,**
    c. **Consideration of data strengths and weaknesses (e.g., temporal and spatial scale, gear selectivities, ageing accuracy, sample size),**
    d. **Calculation and/or standardization of abundance indices.**

The Review Panel examined a number of different fishery-dependent and fishery-independent data sources. Data used for ARIMA models and the Delaware Bay region were sufficient to support the analysis presented. The Panel noted the ARIMA approach was better than the Conn (2010) method in application to horseshoe crabs because of spatial variation in population dynamics within regions. Review efforts were therefore focused on the ARIMA approach.

Fishery-independent data included survey information and stock abundance from multiple different surveys (see Table 33 of Assessment Report), which are the primary data for ARIMA models. The assessment team presented the data clearly and handled the data appropriately. However, many surveys did not identify primiparous (first time female spawners) or multiparous individuals (repeat female spawners) nor record sex. This deficiency will hamper future assessment efforts, and a recommendation to add sex and maturity sampling was made by the Review Panel (see TOR 8).

Fishery-dependent data included bait landings, biomedical collection, and discards. Biological sampling for the bait fishery and biomedical collection seemed adequate given the limited use of commercial data in the assessment, although the assessment team did highlight several improvements from past stock assessments. Discards are a substantial uncertainty (see below). The development and use of bait bags may have reduced bait harvest numbers by 50-75% in the early years of management (when benchmark was set). The assessment team should provide a description of this change in the overview of the history of the stock (e.g., Fisher and Fisher 2006; Gerhart 2007).

The assessment team presented data and analysis on the proposed 15% biomedical mortality rate, which appears to be a robust estimate determined by a simple and fairhanded approach based on the best available data. The Review Panel agreed with the assessment team's approach, but noted some covariates such as season of harvest, size/condition of crabs, and location that are worth investigating. However, additional data and analyses are not likely to significantly alter assessment results due to the modest magnitude of biomedical mortality. As such, while an uncertainty, the biomedical mortality rate should receive less focus in future assessments.

By far the largest source of data uncertainty was regional and coastwide discards and the associated mortality of discarded horseshoe crabs. Losses due to discards may be similar or greater than losses from bait harvest and biomedical collection combined. The Review Panel highlights the importance of discard mortality for assessment and management of horseshoe crabs.

With respect to discards, the Review Panel recommends:

1) Expanding the analysis of discards to the entire stock unit, beyond the Delaware Bay region.
2) Further examination of discard mortality rates by gear, area, and season. This effort should include a literature review as well as field studies as time allows.
3) Stratification of observer data by season, area, and fleet is critical in discard estimation and it will be important to develop and test approaches for horseshoe crabs. It is important to exclude fleets incapable of harvesting horseshoe crabs (i.e., offshore fisheries, midwater or raised foot rope trawls). These tasks will require thorough examination of data from the Northeast Fisheries Observer Program (NEFOP) data and state at-sea observer programs.
4) Future assessment teams should include an analyst who has direct access to the NEFOP database and the experience necessary to conduct discard analyses. In addition, it would be useful to provide data training and access to ASMFC analytical staff.

2. **Evaluate the methods and models used to estimate population parameters (e.g., F, biomass, abundance) and biological reference points, including but not limited to:**
    a. **Evaluate the choice and justification of the preferred model(s). Was the most appropriate model (or model averaging approach) chosen given available data and life history of the species?**
    b. **If multiple models were considered, evaluate the analysts' explanation of any differences in results.**
    c. **Evaluate model parameterization and specification (e.g., choice of CVs, effective sample sizes, likelihood weighting schemes, calculation/specification of M, stock- recruitment relationship, choice of time-varying parameters, plus group treatment).**

Models and modeling decisions for stock status determination were appropriate and acceptable. ARIMA models were the primary modeling technique used in this and the last two assessments to estimate relative stock abundance and define limit reference points based on trends for horseshoe crabs (Helper et al. 2002). The models were fit to selected survey data for males and females combined in each stock region and are intended to smooth the data, reduce noise, and estimate underlying trends in stock size. ARIMA models with three lags are well-suited for horseshoe crabs because: the statistical approach is objective, the model complexity is reasonable given the length and noise in the survey data, the method accommodates years with missing data, and results can be used to estimate stock status and reference points that are comparable. One attribute of the method is that it estimates multiple trends (one for each survey) instead of a single trend for each region. Conn (2010) models produce a single trend for each region and were evaluated for horseshoe crabs but rejected in favor of ARIMA models. The assumption with Conn models of identical trends in each survey was sometimes violated, likely due to heterogeneous population dynamics within regions.

Estimates from a Catch Multiple Survey Analysis (CMSA) model are the best available estimates of abundance and fishing mortality for female horseshoe crabs in the Delaware Bay region. The CMSA estimates may be biased low, however, due to the assumption of 100% capture efficiency in the Virginia Tech trawl survey. Other uncertainties include missing years of Virginia Tech survey information (2012-2015), lack of a stock recruitment relationship, short time series of data, and discards. For these reasons, and as indicated in the assessment report, the Panel notes uncertainty in model results. The Panel further recommends caution in using this model to interpret stock status or develop management reference points at this time. However, the CMSA results are based on multiple survey time series, with data for some surveys available for all years. The model takes advantage of the ability to define new recruits in terms of primiparous individuals and the high probability that catchability is equivalent between primiparous and multiparous horseshoe crabs. Of note, the Virginia Tech survey is specifically designed for horseshoe crab collection and has a higher capture efficiency than other surveys (see research recommendations). The Panel agrees the CMSA model estimates are suitable for input to models such as the Adaptive Resource Management (ARM) model.

The Panel reviewed a theoretical simulation model used to estimate MSY-based reference points from a published density dependent relationship, including improved estimates of natural mortality. A similar method for making short-term stock abundance forecasts was also reviewed. Earlier versions of the reference point model were used in ARM management. The Panel agreed the new estimates of natural mortality and other changes were improvements that could be considered in the ARM framework. However, the reference points from the simulation approach should not be directly compared to abundance and fishing mortality estimates from the CMSA for status determination because calculations between the two models may not be comparable (see below). For the same reasons, the forecast model should not be used to make short-term stock size projections based on CMSA results. It is wiser to use the CMSA itself for short-term projections to ensure comparability and because variances for the predictions can be directly calculated. The theoretical population model and reference points may provide useful information in other circumstances.

There was considerable discussion about comparing stock estimates from one model to reference points calculated in another. The Reviewer's advice to avoid this practice is based on the possibility of errors in status determination that can be reduced or avoided using a single model to calculate stock size and reference points. As an example, if we ignore random estimation errors and say the stock size estimate from the first model is $B' = gB$ where B is the true biomass and g is a multiplicative bias due to model misspecification and data errors. The stock size and B_{MSY} reference point estimates from the second model are $B''=hB$ and $B_{MSY}''=hB_{MSY}$ where B_{MSY} is the reference point and h is the bias. The status determination ratio B'/B_{MSY}'' based on two models is in error by the factor g/h which might amount to substantial over- or underestimation. In contrast, using stock size and reference point from just the second model, for example, gives $B''/B_{MSY}'' = hB/hB_{MSY} = B/B_{MSY}$ which is likely more accurate because the bias h in the numerator and denominator cancels out.

3. **Evaluate the diagnostic analyses performed, including but not limited to:**
    a. **Sensitivity analyses to determine model stability and potential consequences of major model assumptions**
    b. **Retrospective analysis**

Residuals from ARIMA models used for status determination were normally distributed and had acceptable temporal patterns. Retrospective patterns generally are not a problem in ARIMA models. Historical analyses demonstrated that the ARIMA models were stable from one assessment to the next.

There was no evidence of retrospective patterns in CMSA results and the model fit to survey data was acceptable. Extensive sensitivity analysis demonstrated that the CMSA model was robust to assumptions about catchability, selectivity, natural mortality, and survey variance. The stability was due to assumptions that primiparous and multiparous females had the same catchability in the Virginia Tech survey and that the survey, which was designed for horseshoe crabs, captures nearly 100% of the horseshoe crabs in its path between the trawl sweeps. Sensitivity analysis showed the two assumptions were compatible because results were similar when one of the assumptions was eliminated.

4. **Evaluate the methods used to characterize uncertainty in estimated parameters. Ensure that the implications of uncertainty in technical conclusions are clearly stated.**

The uncertainty in ARIMA model fits was displayed graphically in terms of confidence intervals. Uncertainty in status determination based on ARIMA model results considered the uncertainty in both the stock status measure and the reference point. The criterion used to identify stocks below their reference point was relatively stringent (50% probability of being less than the reference point with 80% confidence), but appropriate and consistent with Helser and Hayes (1995).

Variance and CVs for CMSA results were estimated using the delta method in AD-Model Builder for presentation in the final report. The assessment authors were asked to depict CMSA results using asymmetric confidence intervals and to provide CVs for estimates in tables. The variances for recruitment estimates in years with missing Virginia Tech survey data were large, as expected, but variances for total stock size were reasonable.

5. **If a minority report has been filed, review minority opinion and any associated analyses. If possible, make a recommendation on current or future use of the alternative assessment approach presented in minority report.**

No minority reports were submitted.

6. **Recommend best estimates of stock biomass, abundance, and exploitation from the assessment for use in management, if possible, or specify alternative estimation methods.**

The Panel recommends using horseshoe crab trend estimates for females and males combined from ARIMA models fit to survey data for stock status determination, relative to abundance in 1998. Examination of results from multiple surveys within individual regions is necessary due to the lack of comprehensive, consistent survey methods through time. Stock status was based on the proportion of surveys above or below their 1998 reference point when ASMFC management began. Stock status was is considered poor if 33% of the surveys are below their reference point (red), good if 66% are above their reference point (green), and neutral (yellow) otherwise.

ARIMA and Catch Multiple Survey Analysis (CMSA) model estimates were both available for female horseshoe crabs in the Delaware Bay region. The Panel recommends CMSA results when abundance and fishing mortality estimates are required, such as in the Adaptive Resource Management (ARM) model used by managers. CMSA results were not used for status determination because comparable reference points were not available. However, given the increasing survey trends, low landings, and CMSA results (low fishing mortality and relatively high abundance), overfishing and an overfished status are unlikely for female horseshoe crabs in Delaware Bay.

Exploitation estimates were available for females in the Delaware Bay region only. Simple catch/survey, catch/ARIMA and catch/swept area abundance exploitation rates were not calculated because of difficulties in estimating catch including discards.

7. **Evaluate the choice of reference points and the methods used to estimate them. Recommend stock status determination from the assessment, or, if appropriate, specify alternative methods and measures.**

For the coastwide and regional assessments using ARIMA models, the Review Panel endorses the use of reference points for each stock region based on relative abundance in 1998, when

ASMFC management commenced. A second alternative of using quartiles was examined but was not favored given the short timeframe of the indices.

Further, the Panel recommends using horseshoe crab trend estimates for females and males combined from ARIMA models fit to survey data for stock status determination relative to abundance in 1998. Examination of results from multiple surveys within individual regions is necessary. Stock status is based on the proportion of surveys above or below their 1998 reference point when ASMFC management began. Stock status is poor if 33% of the surveys are below their reference point (red), good if 66% are above their reference point (green), and neutral (yellow) otherwise. The color code system is useful in tables that summarize stock status results.

To help managers determine if changes in harvest practices or other population pressures have affected horseshoe crabs in recent years, the Review Panel requested a table comparing regional status results in the current and previous stock assessment.

For the Delaware Bay region, the Panel reviewed a reference point approach based on a theoretical population model, which was used to estimate N_{MSY} and F_{MSY}. The modeling indicated F_{MSY} for Delaware Bay is below 0.1 and population growth occurs slowly, over decades. While informative, the reference points from the theoretical approach should not be directly compared to abundance and fishing mortality estimates from the CMSA for status determination because calculations in the two models may not be comparable. Alternative, history-based reference points could be explored, but given the short time series, the Review Panel and assessment team expressed concern about the historical approach. Ultimately, the Review Panel did not make any recommendations on Delaware Bay region-specific reference points.

8. **Review the research, data collection, and assessment methodology recommendations provided by the TC and make any additional recommendations warranted. Clearly prioritize the activities needed to inform and maintain the current assessment and provide recommendations to improve the reliability of future assessments.**

RESEARCH RECOMMENDATIONS

The Review Panel commends the assessment team for development of a thorough set of research recommendations under the categories of future research, data collection, and assessment methodology. In contrast to the recommendation of the SAS, however, the Review Panel recommends that a benchmark stock assessment be considered in five years. The potential for improved discards estimation and associated model updates to significantly affect horseshoe crab stock assessment was the primary reason for this recommendation. Also the Review Panel supports the assessment team's plan to remain proactive about maintaining surveys and research programs particularly focused on three main areas: 1) refining estimates of bycatch and discard mortality through literature review and experimentation, 2) better defining the constraints of existing trawl surveys, and improving the efficiency and consistency of surveys among locations and through time, particularly to include data on both primiparous and multiparous females whenever possible, and 3) improving the assessment methodology to support future model applications.

The Panel also noted there is a meaningful need for data on the juvenile and subadult components of this stock that are not well captured in either trawl or spawning surveys. While trawl surveys are likely to continue to serve as the primary basis of tracking abundance through time, it is important to continue to support research to better define these poorly understood stock components such as natural mortality and recruitment.

The Review Panel cautions the assessment team to avoid broad-brushing when discussing survey results. Remember that surveys are necessarily an index of change based on specific locations and segments of each population.

Climate change is already likely affecting horseshoe crab populations, habitat, and food resources in undefined ways. While not as much of a priority for study as discard estimates, the Review Panel appreciated the assessment team's inclusion of research recommendations on this topic and thinks the concept must be a consideration in all ongoing and future research. Of particular importance are the effects of temperature and sea level rise on the extent of available spawning and foraging habitat.

To improve data analyses and subsequent assessment, the Review Panel noted some constraints that could be improved for future assessments:

1) In some cases, additional data needed to address questions were available, but not readily accessible to the ASMFC assessment team. The Review Panel recommends ensuring that existing resources such as fisheries observer (discard) and NEFOP data be made directly available to the assessment team.
2) The inability to publicly show regional biomedical collection and mortality data and derivative stock assessment results presents a material constraint to fully explaining the stock assessment results. The assessment team could consider alternative approaches to share mortality data such as by reporting biomedical and bycatch estimated mortality together. Efforts should be made to improve data access and use however possible.
3) Given the evidence of links (as yet poorly defined) between the Atlantic coast and Gulf coast horseshoe crab populations, which will likely increase if the effects of climate change prompt large-scale alteration of habitat or animal movement, and the likelihood of future harvest pressure in the Gulf, the Review Panel encourages the assessment team to enhance communication with Gulf States Marine Fisheries Commission and encourage data collection in anticipation of future need.

The Review Panel prioritized the following research recommendations from the assessment report:

Data Collection

- Better characterize discards, landings, and discard mortality by gear. This effort could be accomplished through a combination of literature research for other commercial species such as blue crabs and other invertebrates and experimentation.
- Continue biosampling for sex and weight, particularly by primiparous and multiparous, and expand where possible, using standardized protocols across regions and surveys.
- Continue to fund and operate the full Virginia Tech Trawl Survey annually.
- Conduct a gear efficiency study of the Virginia Tech Trawl Survey given the importance of using swept-area estimates of abundance in modeling the Delaware population.
- Determine the sampling constraints of all surveys used in horseshoe crab stock assessment, particularly better defining the area and type of habitat represented by each survey and the portion of the population sampled (by size, sex, maturity status to the extent possible). This could be done at the cost of staff time only.
- Define the features among existing trawl surveys and compare them to the demographics of the sampled populations to determine which survey approaches (timing, gear type or size, etc.) are effective to encourage consistent and most effective sampling methodology among locations. This could be done at the cost of staff time only.
- Expand coastwide tagging studies to better define movement (extent of range), population mixing among regions (including greater tag and recapture effort in the Gulf of Mexico), mortality and maximum age. Mortality estimates from tagging are particularly important when other estimates are not available, and they should be emphasized in future assessments. These data will support use of the MARK and JSC models outside of Delaware Bay and inform applicability of management zones.

Assessment Data and Methodology

The configuration of the Northeast region, which includes the Rhode Island and Massachusetts surveys, should be reconsidered in the next assessment. Declining trends in the Rhode Island survey are like trends in the New York region to the south and markedly different from the increasing trend in the more northern Massachusetts survey. In addition, the small Rhode Island survey has a disproportionate effect on status determination for the much larger Northeast region.

Some potential improvements to the CMSA model should also be considered. Survey data are weighted in aggregate based on standardized variances from preliminary Conn models and then individually based on estimated annual CVs. Sensitivity analyses showed that model results were robust to configuration of weights. However, it is not clear whether uncertainties were double counted or that the product of the two types of inverse variance weights (one standardized the other not) is appropriate. The assessment team should consider whether these conventions and assumptions affect the delta method variances for abundance and fishing mortality estimated in the model.

Survey data for primiparous horseshoe crabs in the Virginia Tech trawl survey are important in CMSA for estimating recruit abundance. The Virginia Tech survey is the only survey that distinguishes between primiparous and multiparous horseshoe crabs. The survey was not conducted during 2012-2015. Therefore, the variance of model recruitment estimates is very large for these years. Alternate approaches to estimating recruitment and more realistically appraising its variance should be considered. For example, a spawners-recruit formulation or a random walk model that assumes similar recruitment in adjacent years might be appropriate. It might be advantageous to individually weight recruitment deviations to control problematic estimates. Fortunately, as demonstrated by sensitivity runs, the uncertainty of recruitment estimates in years with missing survey data had very little effect on total stock abundance estimates because the recruitment estimates in adjacent years tend to be negatively correlated such that an underestimate in year *t* results in an overestimate in year *t+1* that cancels the potential error in total abundance. The changes suggested could increase the realism of the estimated recruit time series but would probably have little effect on the overall abundance estimates.

If use of the CMSA model continues or is expanded, then it should be modified to include short-term projection capabilities so that projections and historical model estimates are guaranteed to be comparable. It is easy to calculate the variance of projected estimates, including uncertainty in recruitment, terminal stock size, catchability, etc. Also, it would be good to compute any new reference points directly in the CMSA to ensure comparability of reference points and stock status measures.

CMSA models for male horseshoe crabs in Delaware Bay and other areas should be developed. The best approach may be to use a two-sex version of the model so that combined male and female abundance can be compared to catch and surveys with no sex data.

The CMSA for horseshoe crabs took advantage of aspects of female horseshoe crab biology (terminal molt at maturity) and the Virginia Tech survey carried out in Delaware Bay which distinguishes between primiparous (newly mature = recruits in CMSA) and multiparous crabs (post-recruits in CMSA). Unfortunately, primiparous and multiparous crabs are not distinguished in other surveys and the methods used for Delaware Bay are not applicable elsewhere. Other approaches to tracking abundance of new recruits (e.g. cohort slicing) could be tested so that the model can be applied to other areas and sexes.

If the CMSA model is too difficult to apply in other areas, then a two sex and length-based (or possibly age based counting age from recruitment to the fishery) approach should be considered. Alternately, and considering data and staff limitations, it may be best to continue using the robust and simple ARIMA model approach.

9. Recommend timing of the next benchmark assessment and updates, if necessary, relative to the life history and current management of the species.

The Review Panel recommends that a benchmark stock assessment be considered in five years given the potential for improved discard estimates and associated model updates to significantly improve the horseshoe crab stock assessment.

Special Comments

To facilitate communication, the Review Panel recommends using consistent and accurate terminology such as N_{MSY} rather than B_{MSY} when referring to counts as opposed to biomass data. Similarly, the Panel suggests, to the extent possible, displaying comparable data on the same axis range (or scale) to facilitate data interpretation.

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ADVISORY REPORT

A. Status of the Stock: Current and Projected

Based on the recommended modeling approach (see below), horseshoe crab in the Northeast and Delaware Bay regions are in a neutral condition, New York is in a poor condition, and the Southeast is in a good condition (Table 1). On a coastwide basis, horseshoe crab relative abundance is likely in a neutral condition.

Fishing pressure was estimated for female horseshoe crabs in the Delaware Bay region but not for males or horseshoe crabs in other regions because discard mortality in the horseshoe crab and other fisheries is unknown and may be substantial.

B. Stock Identification and Distribution

The Atlantic States Marine Fisheries Commission (ASMFC) manages the horseshoe crab stock from Maine to eastern Florida (Figure 1). Genetics, isotope analyses, and tagging data suggest the horseshoe crab population is comprised of multiple units, some distributed across multiple states and others embayment-specific that are linked to varying degrees. Due to varying levels of data at these levels, the assessment splits the range of horseshoe crabs into four regions; Northeast (Maine, Massachusetts, Rhode Island), New York (Connecticut, New York), Delaware Bay (New Jersey, Delaware, Maryland, Virginia), and Southeast (North Carolina, South Carolina, Georgia, Florida). This was a pragmatic decision that balances data availability and biological realism.

C. Landings

Since the mid- to late-1900s, horseshoe crabs have been harvested commercially primarily for use as bait and for use in the biomedical industry (Figure 2). Bait harvest is used primarily in the conch and American eel pot fisheries. The biomedical industry uses crabs to manufacture Limulus Amebocyte Lysate (LAL) which is used to test pharmaceuticals for the presence of gram-negative bacteria.

Early harvest records should be viewed with caution due to potential under-reporting. Between the mid-1800s and mid-1900s harvest ranged from approximately 1 to 5 million crabs annually, then dropped to between 250,000 and 500,000 crabs annually in the 1950s. About 420,000 crabs were harvested annually during the early 1960s.

Commercial landings declined after 1998 when ASMFC management began and then fluctuated around an average of 753,000 crabs from 2004-2017. The 2017 harvest level was the largest harvest since 2003 but still over 500,000 crabs less than the coastwide quota of 1.587 million crabs.

Biomedical losses are modest (<13% of bait landings assuming 15% bleeding mortality) but are not shown due to confidentiality concerns.

D. Data and Assessment

Relative abundance trends were estimated by fitting ARIMA models to survey data for horseshoe crabs taken during multiple research surveys in each of the four regions. Relative abundance in 2017 was compared to relative abundance during 1998 when ASMFC management began where the estimates for 1998 and 2017 were both from ARIMA models.

Additional information about abundance and exploitation are available for female horseshoe crabs in the Delaware Bay area from a CMSA model. The results were not used for status determination but are recommended for use where biomass and fishing mortality estimates are required for management.

E. Biological Reference Points

The recommended biological reference point for horseshoe crabs is the relative abundance of male and female horseshoe crabs during 1998 from ARIMA models. Stock status is based on the proportion of surveys in a region or coast wide that are above or below their 1998 reference point. Stock status is poor if 33% of the surveys are below their reference point (colored red in tables), good if 66% are above their reference point (green), and neutral (yellow) otherwise (Table 1).

F. Fishing Mortality

CMSA results indicate low fishing mortality for female horseshoe crabs in Delaware Bay in recent years (Figure 3). It was not possible to develop trend based or other measures of fishing pressure on males in Delaware Bay or for other areas due to uncertainty about discards.

G. Recruitment

CMSA model estimates for female horseshoe crabs indicate roughly average recruitment during 2017-2018 but the estimates are uncertain due to missing Virginia Tech survey data for 2013-2016 (Figure 4). No other direct information about recruitment is available.

H. Spawning Stock Abundance

Based on CMSA estimates, female spawning biomass in Delaware Bay is relatively high (Figure 5). No other direct estimates of spawning stock abundance are available.

I. Bycatch

The assessment provided the first estimates of discard mortality in the horseshoe crab and other fisheries. Preliminary results are uncertain but suggest that discard mortality may be comparable to or greater than mortality from other sources (bait landings plus biomedical collection). The magnitude of horseshoe crab discards in the horseshoe crab and other fisheries is the most important uncertainty and research recommendation identified in the assessment.

J. Other Comments

It is important to continue survey data collection for horseshoe crabs (particularly the Virginia Tech survey), determine how current survey methods differ (and implications for assessment across sites), define which methods are most effective to promote consistent survey sampling among locations, and to expand survey data collection to include size, sex, and female reproductive condition (primiparous vs. multiparous) information.

K. Tables

Table 1. Stock status determination for the coastwide and regional stocks based on the 1998 index-based reference points from ARIMA models.

Region	2009 Benchmark	2013 Update	2019 Benchmark	2019 Stock Status
Northeast	2 out of 3	5 out of 6	1 out of 2	Neutral
New York	1 out of 5	3 out of 5	4 out of 4	Poor
Delaware Bay	5 out of 11	4 out of 11	2 out of 5	Neutral
Southeast	0 out of 5	0 out of 2	0 out of 2	Good
Coastwide	7 out of 24	12 out of 24	7 out of 13	Neutral

L. Figures

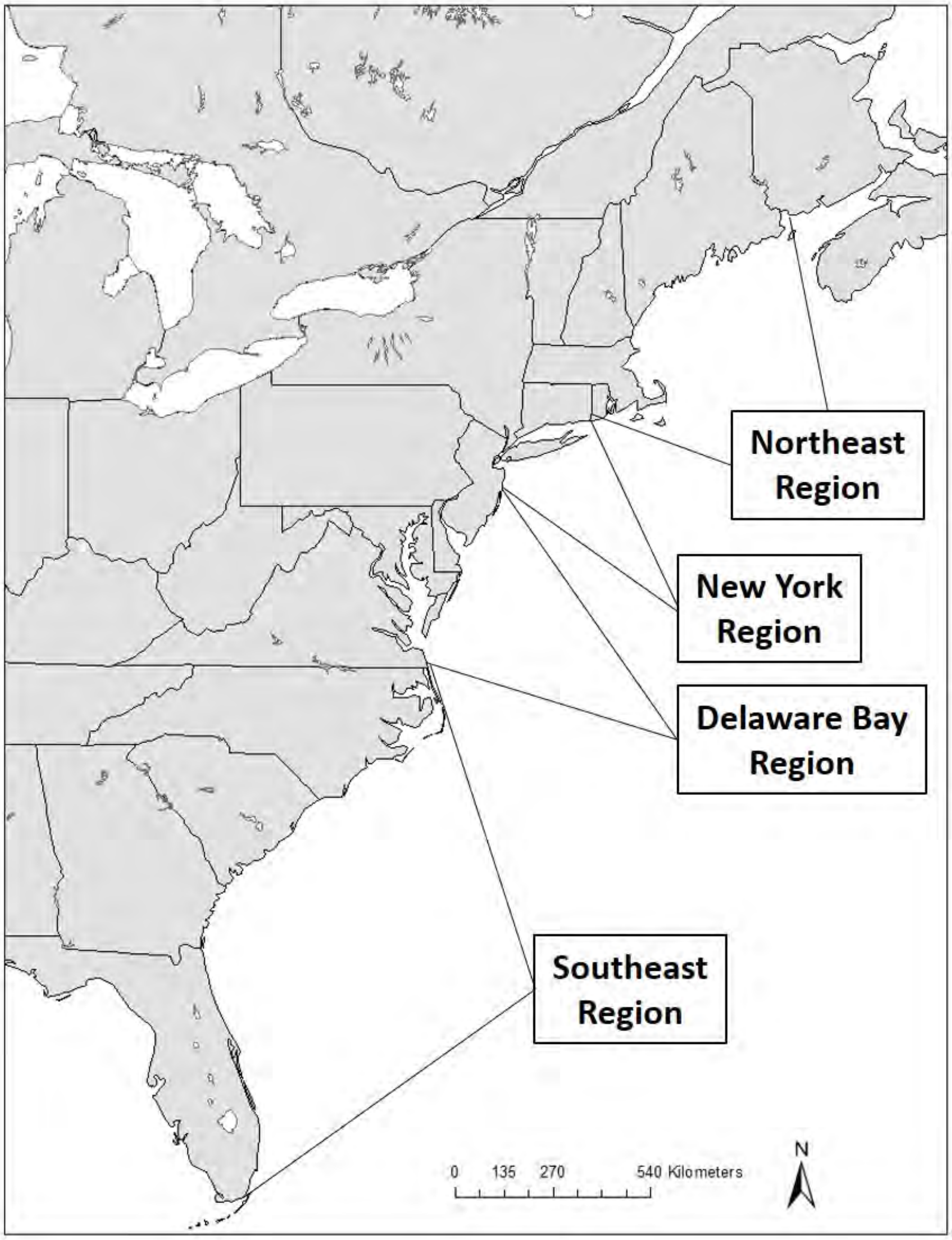


Figure 1. Map of the Atlantic coast showing the regions for horseshoe crab assessment.

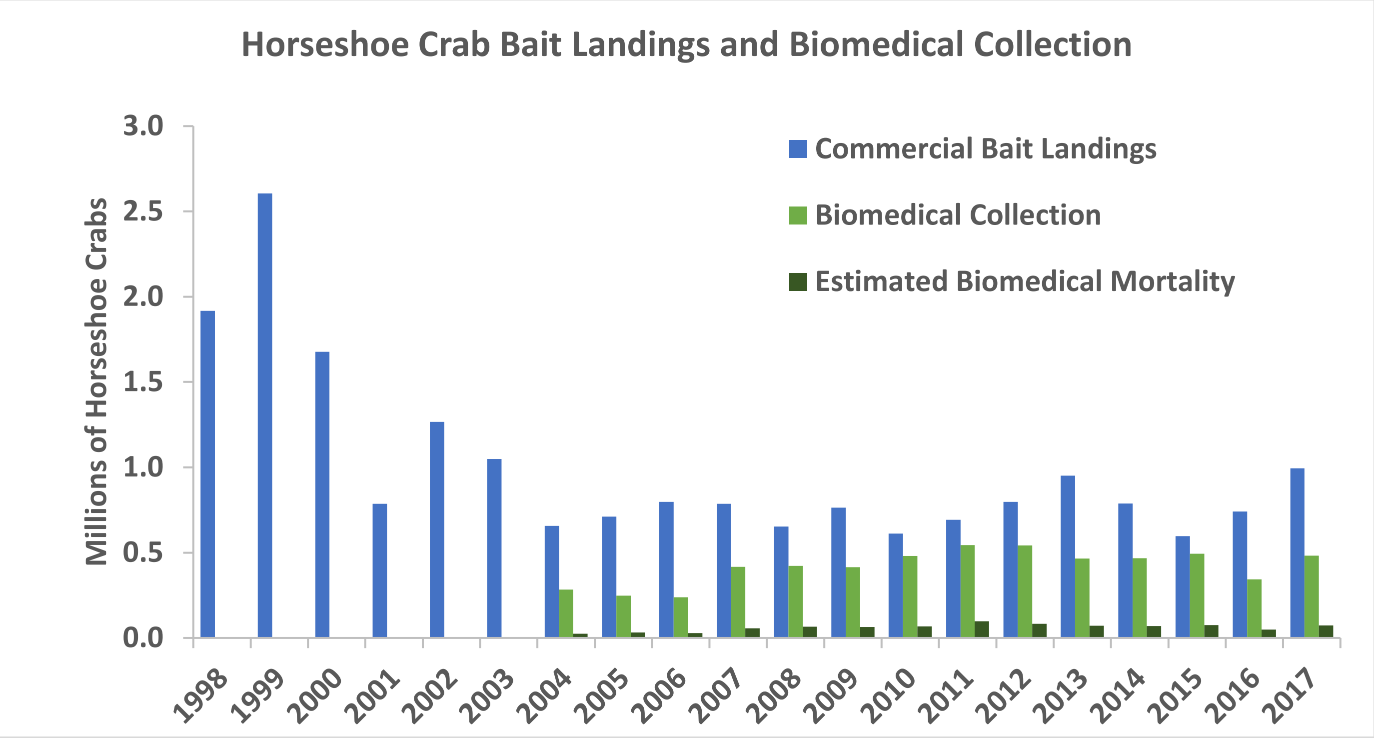


Figure 2. Coastwide horseshoe crab bait landings, biomedical collection, and estimated mortality attributed to the biomedical industry. Biomedical data has been reported to ASMFC since 2004 and a 15% rate is applied to the number of horseshoe crabs bled and released alive to estimate mortality from the industry.

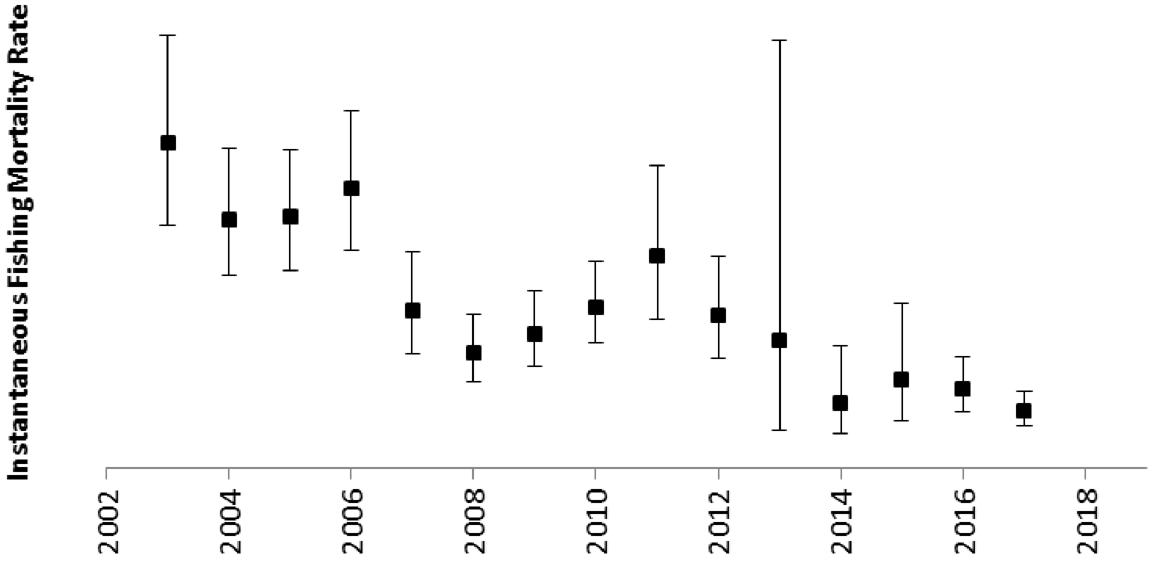


Figure 3. CMSA model estimated instantaneous fishing mortality rate F with lower and upper 95% confidence limits. Y-axis values have been removed due to CONFIDENTIAL data.

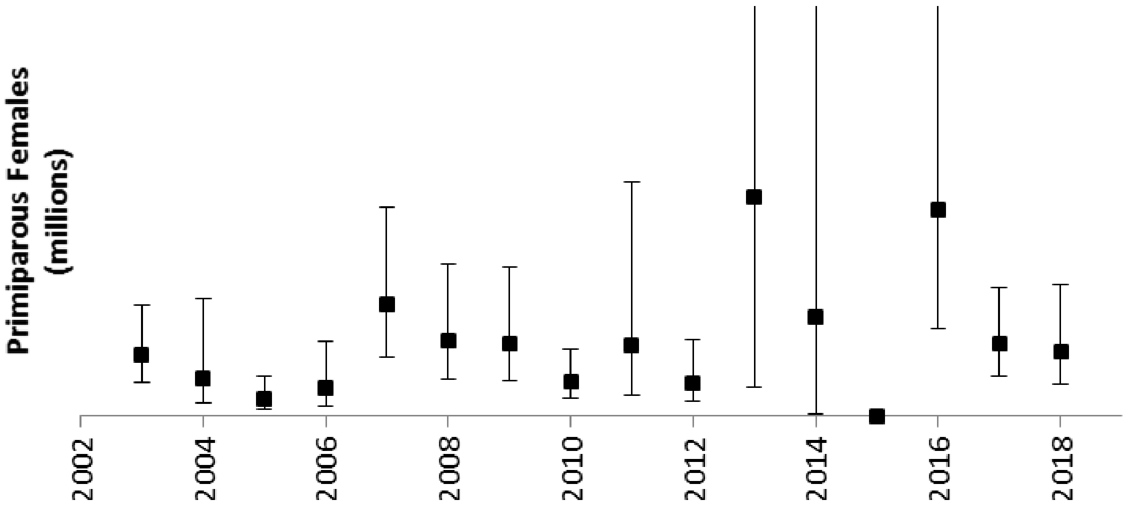


Figure 4. CMSA model estimated primiparous female abundance with lower and upper 95% confidence limits. Upper confidence limits for 2013, 2014, and 2016 extend beyond y-axis with values of CONFIDENTIAL. Y-axis values have been removed due to CONFIDENTIAL data.

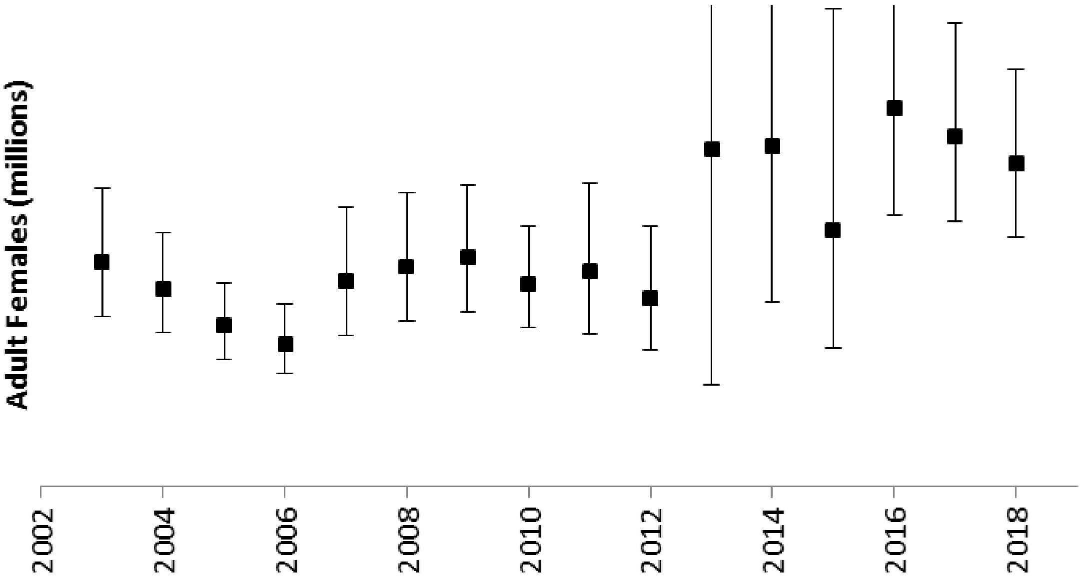
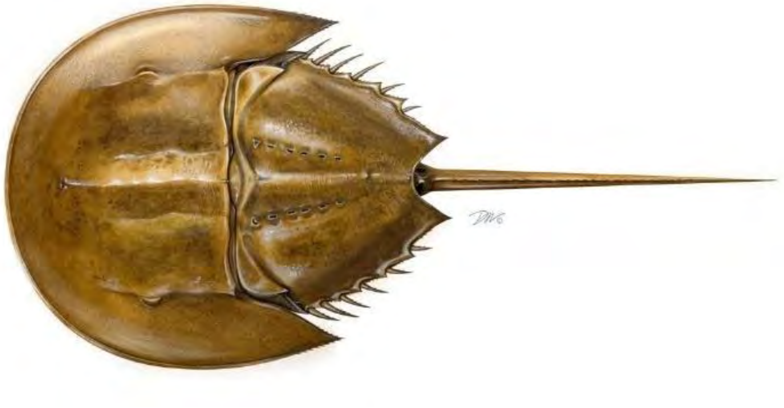


Figure 5. CMSA model estimated adult (primiparous + multiparous) female abundance with lower and upper 95% confidence limits. Upper confidence limits for 2013, 2014, and 2016 extend beyond the y-axis with values of CONFIDENTIAL. Y-axis values have been removed due to CONFIDENTIAL data.

Atlantic States Marine Fisheries Commission

2019 Horseshoe Crab Benchmark Stock Assessment Non-Confidential Report



Prepared by the
ASMFC Horseshoe Crab Stock Assessment Subcommittee

John Sweka (Chair), U.S. Fish and Wildlife Service
Natalie Ameral, Rhode Island Division of Fish and Wildlife
Kristen Anstead, Atlantic States Marine Fisheries Commission
Linda Barry, New Jersey Division of Fish and Wildlife
Jeffrey Dobbs, North Carolina Division of Marine Fisheries
Michael Kendrick, South Carolina Department of Natural Resources
Kim McKown, New York State Department of Environmental Conservation
Michael Schmidtke, Atlantic States Marine Fisheries Commission
Dave Smith, U.S. Geological Survey
Rich Wong, Delaware Division of Fish and Wildlife

STATEMENT REGARDING CONFIDENTIAL DATA

Note: The stock assessment and peer review was conducted with the inclusion of biomedical data, which is confidential. Much of the report that details confidential data has been redacted for this public report and noted as **CONFIDENTIAL**. Results have been summarized when data was removed. Confidential data are data such as commercial landings, including biomedical harvest, which can be identified down to an individual or single entity. Federal and state laws prohibit the disclosure of confidential data, and the Atlantic States Marine Fisheries Commission abides by those laws. In determining what data are confidential, most agencies use the “rule of 3” for commercial catch and effort data. The “rule of 3” requires three separate contributors to fisheries data in order for the data to be considered non-confidential. This protects the identity of any single contributor. In some cases, annual summaries by state and species may still be confidential because only one or two dealers process the catch. Alternatively, if there is only one known harvester of a species in a state, the harvester’s identity is implicit and the data for that species from that state is confidential.

EXECUTIVE SUMMARY

The purpose of this assessment was to evaluate the current status of horseshoe crab (*Limulus polyphemus*) along the U.S. Atlantic coast. Data from a variety of fisheries-dependent and –independent sources were reviewed and used to develop bait landings, commercial discard estimates, indices of abundance, and biomedical collection and mortality estimates as well as perform trend analyses, survival estimates, and a catch survey model.

Stock Identification and Management Unit

The Atlantic States Marine Fisheries Commission (ASMFC) manages the horseshoe crab stock from Maine to eastern Florida. Genetics, isotope analyses, and tagging data suggest that the horseshoe crab population is comprised of multiple units, some distributed across multiple states and others embayment-specific. Due to varying quantity and quality of data at these levels, for the purpose of this assessment, horseshoe crabs are evaluated on a coastwide and regional level consisting of the Northeast, New York, Delaware Bay, and the Southeast.

Commercial Fisheries

Horseshoe crabs are primarily harvested commercially as bait for the commercial American eel and whelk/conch fisheries along the Atlantic coast. Since 1998, states have been required to report annual landings to ASMFC through the compliance reporting process and bait landings were validated from Maine to Florida for 1998-2017 for this assessment. The majority of horseshoe crab harvest comes from the Delaware Bay region, followed by the New York, the Northeast, and the Southeast regions. Trawls, hand harvests, and dredges make up the bulk of commercial horseshoe crab bait landings. In recent years, the Delaware Bay region has been limited to male-only harvest through an adaptive management process that constrains the value of horseshoe crab harvest based on the needs of shorebirds. Horseshoe crab landings for 1998-2017 peaked in 1999 at 2.6 million horseshoe crabs and have decreased since the late 1990s. Landings have remained under 1 million horseshoe crabs since 2003 and from 2004-2017 average landings were 752,886 horseshoe crabs.

Horseshoe crabs are also collected by the biomedical industry to support the production of Limulus amebocyte lysate (LAL), a clotting agent that aids in the detection of endotoxins in patients, drugs, and intravenous devices. Blood from the horseshoe crab is obtained by collecting and extracting a portion of their blood. Most crabs collected and bled by the biomedical industry are, as required by the FMP, released alive to the water from where they were collected; however, a portion of these crabs die from the procedure. Crabs harvested for bait are sometimes bled prior to being processed and sold by the bait industry; these crabs are counted against the bait quota. Biomedical use has increased since 2004, when reporting began, but has been fairly stable in recent years. Previous assessments and management documents have applied a mortality rate of 15% to the number of horseshoe crabs bled and released alive to estimate the number of crabs that die each year during the process and this assessment maintains the 15% mortality rate based on an updated meta-analysis of available literature on this topic.

Horseshoe crabs are also encountered in several other commercial fisheries. Discard mortality occurs in various dredge fisheries and may vary seasonally with temperature, impacting both mature and immature horseshoe crabs; however, the actual rate of discard mortality is unknown. Commercial discards were estimated for the Delaware Bay region as part of this assessment with data from the NMFS' Northeast Fisheries Science Center's Northeast Fisheries Observer Program. Estimates indicate a significant amount of horseshoe crabs are captured and discarded in other fisheries, although a large amount of uncertainty is associated with the estimates.

Indices of Relative Abundance

There are spawning beach surveys available to monitor horseshoe crab spawning activity and one trawl survey designed to directly measure horseshoe crab abundance in the Delaware Bay region. These surveys were used to develop indices of relative abundance for the species. Additionally, several other fishery-independent surveys along the Atlantic coast that encounter horseshoe crabs were used to develop abundance indices. Many of these data sets had a high proportion of zero catches per tow in the survey and therefore all indices were developed using the delta distribution for the mean and variance for each year of a survey to specifically take into account the number of zero catches.

Assessment Methods

Tagging data from the USFWS horseshoe crab database were explored by region to estimate survival. The highest survival rates were in Delaware Bay and coastal Delaware-Virginia regions. The lowest were in coastal New York-New Jersey and the Southeast.

The horseshoe crab population was primarily evaluated using autoregressive integrated moving average models (ARIMA) on the coastwide-level and a catch multiple survey analysis (CMSA) for the Delaware Bay region. The CMSA modelling approach could only be developed in the Delaware Bay region due to the availability of the Virginia Tech Trawl Survey that collects stage-based data.

The results of ARIMA indicated that, in general, the Northeast surveys had conflicting trends, New York surveys showed decreasing trends, Delaware Bay surveys indicated increasing or neutral trends, and the Southeast showed increasing or neutral trends.

The CMSA indicated that adult abundance in the Delaware Bay was stable from 2003-2012 and then began increasing considerably in the last few years. This finding is consistent with stock rebuilding due to a period of significantly reduced commercial landings and tight management controls on the fishery beginning in the early 2000s in this region. Recruitment is less stable throughout the time series due to the missing years of data from the survey.

Prior to this assessment, biomedical data were not included in the modeling efforts as a source of harvest. For this assessment, the CMSA was run with and without the biomedical and discard estimates to evaluate the contribution of these other sources of mortality. Population estimates were largely unaffected by the estimated biomedical or discard numbers. Omitting biomedical harvest resulted in a decrease of fishing mortality (*F*) by a small number that did not affect stock status. Commercial discards had a larger effect on *F* and omitting the discard estimates decreased *F* by more than omitting the biomedical data. Commercial discards are likely a larger source of removals than biomedical mortality although much uncertainty is associated with the estimates. Sensitivity runs around varying levels of biomedical mortality rates and the discard estimates indicate that harvest in the region, including biomedical, bait, and discard estimates, appear to be sustainable at current levels and management strategies.

Stock Status

To date, no overfishing or overfished definitions have been adopted by the Management Board. For this assessment, biological reference points were developed for the Delaware Bay horseshoe crab population using a theoretical model and comparing to CMSA estimates. The comparison approach was not endorsed by the Peer Review Panel for use in management. Stock status was determined on coastwide and regional stocks based on the results from the ARIMA and in comparison to similar analysis in past assessments. The current stock status indicates that the Northeast region, which has two surveys with conflicting results, is in a neutral state whereas the horseshoe population in the New York region is poor and has been declining in status from previous assessments. Based on ARIMA results, the Delaware Bay region is in a neutral state and the Southeast region is in a good state.

Region	2009 Benchmark	2013 Update	2019 Benchmark[1]	2019 Stock Status
Northeast	2 out of 3	5 out of 6	1 out of 2	Neutral
New York	1 out of 5	3 out of 5	4 out of 4	Poor
Delaware Bay	5 out of 11	4 out of 11	2 out of 5	Neutral
Southeast	0 out of 5	0 out of 2	0 out of 2	Good
Coastwide	7 out of 24	12 out of 24	7 out of 13	Neutral

[1] The number of surveys below the index based 1998 reference point in the terminal year from ARIMA modeling

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TERMS OF REFERENCE REPORT SUMMARY

Terms of Reference for the Horseshoe Crab Assessment

1. Define population structure based on available data. If alternative population structures are used in the models (e.g., coast-wide, regional, sub-regional or estuary-specific), justify use of each population structure.

2. Characterize precision and accuracy of fishery-dependent and fishery-independent data, including biomedical data, that are used in the assessment, including the following but not limited to:
    a. Provide descriptions of each data source (e.g., geographic location, sampling methodology, potential explanation for outlying or anomalous data)
    b. Describe calculation and potential standardization of abundance indices.
    c. Discuss trends and associated estimates of uncertainty (e.g., standard errors)
    d. Justify inclusion or elimination of available data sources.
    e. Discuss the effects of data strengths and weaknesses (e.g., temporal and spatial scale, gear selectivities, aging accuracy, sample size) on model inputs and outputs.

3. Develop models used to estimate population parameters (e.g., F, biomass, abundance) and biological reference points, and analyze model performance.
    a. Describe stability of model (e.g., ability to find a stable solution, invert Hessian)
    b. Justify choice of CVs, effective sample sizes, or likelihood weighting schemes.
    c. Perform sensitivity analyses for starting parameter values, priors, etc. and conduct other model diagnostics as necessary.
    d. Clearly and thoroughly explain model strengths and limitations.
    e. Briefly describe history of model usage, its theory and framework, and document associated peer-reviewed literature. If using a new model, test using simulated data.
    f. If multiple models were considered, justify the choice of preferred model and the explanation of any differences in results among models.
    g. State assumptions made for all models and explain the likely effects of assumption violations on synthesis of input data and model outputs.
    h. Incorporate biomedical data into the models used. Reassess associated mortality of bled crabs coast-wide, or regionally if possible.

4. Characterize uncertainty of model estimates and biological or empirical reference points.

5. Perform retrospective analyses, assess magnitude and direction of retrospective patterns detected, and discuss implications of any observed retrospective pattern for uncertainty in population parameters (e.g., F, SSB), reference points, and/or management measures.

6. Recommend stock status as related to reference points (if available). For example:
   a. Is the stock below the biomass threshold?
   b. Is F above the threshold?

7. Other potential scientific issues:
   a. Compare trends in population parameters and reference points with current and proposed modeling approaches, including the results of the ARM model for the Delaware Bay. If outcomes differ, discuss potential causes of observed discrepancies.
   b. Evaluate the sub-lethal effects of biomedical bleeding on horseshoe crabs.
   c. Compare reference points derived in this assessment with what is known about the general life history of the exploited stock. Explain any inconsistencies.

8. If a minority report has been filed, explain majority reasoning against adopting approach suggested in that report. The minority report should explain reasoning against adopting approach suggested by the majority.

9. Develop detailed short and long-term prioritized lists of recommendations for future research, data collection, and assessment methodology. Highlight improvements to be made by next benchmark review.

10. Recommend timing of next benchmark assessment and intermediate updates, if necessary relative to biology and current management of the species.

Terms of Reference for the Horseshoe Crab Peer Review

1. Evaluate the thoroughness of data collection and the presentation and treatment of fishery-dependent and fishery-independent data in the assessment, including the following but not limited to:
   a. Presentation of data source variance (e.g., standard errors).
   b. Justification for inclusion or elimination of available data sources,
   c. Consideration of data strengths and weaknesses (e.g., temporal and spatial scale, gear selectivities, aging accuracy, sample size),
   d. Calculation and/or standardization of abundance indices.

2. Evaluate the methods and models used to estimate population parameters (e.g., F, biomass, abundance) and biological reference points, including but not limited to:
    a. Evaluate the choice and justification of the preferred model(s). Was the most appropriate model (or model averaging approach) chosen given available data and life history of the species?
    b. If multiple models were considered, evaluate the analysts' explanation of any differences in results.
    c. Evaluate model parameterization and specification (e.g., choice of CVs, effective sample sizes, likelihood weighting schemes, calculation/specification of M, stock-recruitment relationship, choice of time-varying parameters, plus group treatment).

3. Evaluate the diagnostic analyses performed, including but not limited to:
    a. Sensitivity analyses to determine model stability and potential consequences of major model assumptions
    b. Retrospective analysis

4. Evaluate the methods used to characterize uncertainty in estimated parameters. Ensure that the implications of uncertainty in technical conclusions are clearly stated.

5. If a minority report has been filed, review minority opinion and any associated analyses. If possible, make recommendation on current or future use of alternative assessment approach presented in minority report.

6. Recommend best estimates of stock biomass, abundance, and exploitation from the assessment for use in management, if possible, or specify alternative estimation methods.
7. Evaluate the choice of reference points and the methods used to estimate them. Recommend stock status determination from the assessment, or, if appropriate, specify alternative methods/measures.

8. Review the research, data collection, and assessment methodology recommendations provided by the TC and make any additional recommendations warranted. Clearly prioritize the activities needed to inform and maintain the current assessment, and provide recommendations to improve the reliability of future assessments.

9. Recommend timing of the next benchmark assessment and updates, if necessary, relative to the life history and current management of the species.

10. Prepare a peer review panel terms of reference and advisory report summarizing the panel's evaluation of the stock assessment and addressing each peer review term of reference. Develop a list of tasks to be completed following the workshop. Complete and submit the report within 4 weeks of workshop conclusion.

1 INTRODUCTION

1.1 Brief Overview and History of the Fisheries

Historically, horseshoe crabs (*Limulus polyphemus*) were harvested commercially for fertilizer and livestock feed. Between the mid-1800s and mid-1900s harvest ranged from approximately 1 to 5 million crabs annually (Shuster 1960; Shuster 1982; Shuster and Botton 1985; Finn et al. 1991). Harvest numbers dropped to between 250,000 and 500,000 crabs annually in the 1950s (Shuster 1950) and 42,000 crabs were reported annually by the early 1960s (Finn et al. 1991). Early harvest records should be viewed with caution due to potential under-reporting. The period between 1950 and 1960 is considered the lowest period of horseshoe crab abundance. The substantial commercial-scale harvesting of horseshoe crabs ceased in the 1960s (Shuster 1996).

Since the mid to late 1900s, horseshoe crabs have been commercially harvested primarily for use as bait and to support a biomedical industry. Horseshoe crabs are commercially harvested primarily for use as bait in the conch (*Busycon* spp.) and American eel (*Anguilla rostrata*) pot fisheries, although they are also harvested to a lesser extent for use as bait in the catfish (*Ictalurus* spp.) and killifish (*Fundulus* spp.) fisheries. The biomedical industry uses crabs, most notably, for the manufacture of Limulus Amebocyte Lysate (LAL), a product used to test pharmaceuticals for the presence of gram-negative bacteria. Since 1998, horseshoe crabs have been managed under the Interstate Fishery Management Plan (FMP) for Horseshoe Crab (1998) and its subsequent addenda (Addenda I-VII) by the Atlantic States Marine Fisheries Commission (ASMFC).

Commercial harvest information prior to 1998 is available through the National Marine Fisheries Service and the previous ASMFC stock assessments (ASMFC 2009a, 2013). Commercial landings from 1998-2017 were validated through the Atlantic Coastal Cooperative Statistics Program (ACCSP) by the states during this assessment process, and non-validated landings were not used in any models or analyses. Shortly after establishment of the Interstate Fishery Management Plan (FMP) for Horseshoe Crab in 1998, commercial landings declined until approximately 2004, after which they fluctuated without a long-term directional trend around an average of 753,000 crabs from 2004-2017 (Table 1, Figure 1). A notable increase in coastwide harvest occurred in 2017, with the largest harvest since 2003. However, this harvest was still over 500,000 crabs less than the coastwide quota established by the FMP (1.587 million crabs).

Horseshoe crabs from the Delaware Bay region (New Jersey-Virginia) have been of particular concern due to their relationship with red knots (*Calidris canutus*), a shorebird species currently listed as Threatened by the US Fish and Wildlife Service (USFWS). In 2012, the Adaptive

Resource Management (ARM) model was approved for use, beginning with the 2013 fishing season. The ARM model determines bait harvest levels for the Delaware Bay using population estimates of horseshoe crabs and red knots in that region. Prior to the ARM model's use, New Jersey enacted a commercial harvest moratorium (2006) and Delaware instituted regulations allowing commercial harvest of male crabs only (2008) through state laws. Since use of the ARM model began, the model has recommended and the Horseshoe Crab Management Board (Board) has annually specified harvest package 3 (500,000 male-only crabs) for the Delaware Bay. This regional quota has been allocated among states or areas where crabs of Delaware Bay origin are harvested (New Jersey, Delaware, Maryland, and Virginia east of the COLREGS line). Although they receive a share of the Delaware Bay quota, the commercial moratorium in New Jersey remains in effect.

1.2 Management Unit Definition

The fishery management unit includes the horseshoe crab stock(s) of the Atlantic coast of the United States (Maine to eastern Florida). The coastwide stock is currently managed on state by state, multi-state (e.g., Delaware Bay region), and embayment levels. See section 2.1 Stock Definition for more information.

1.3 Regulatory History

1.3.1 Interstate Management

Prior to 1998, horseshoe crab harvest was unregulated in most states. The Horseshoe Crab Management Board approved the Horseshoe Crab FMP in October 1998. The goal of the FMP is "management of horseshoe crab populations for continued use by: current and future generations of the fishing and non-fishing public (including the biomedical industry, scientific and educational research) migratory shorebirds; and other dependent fish and wildlife (including federally listed sea turtles)" (ASMFC 1998a). The FMP outlined a comprehensive monitoring program and maintained controls on the harvest of horseshoe crabs put in place by New Jersey, Delaware, and Maryland prior to the approval of the FMP. These measures were necessary to protect horseshoe crabs within and adjacent to the Delaware Bay, which is the epicenter of spawning activity along the Atlantic coast. However, subsequent increased landings in other states largely negated these conservation efforts.

In April 2000, the Management Board approved Addendum I to the Horseshoe Crab FMP (ASMFC 2000a). This Addendum established a coastwide, state-by-state annual quota system to further reduce horseshoe crab landings. Through Addendum I the Board recommended to the federal government the creation of the Carl N. Schuster Jr. Horseshoe Crab Reserve, an area of nearly 1,500 square miles in federal waters off the mouth of Delaware Bay that is closed to horseshoe crab harvest. In May 2001, the Board approved Addendum II, which established criteria for voluntary quota transfers between states (ASMFC 2001). In March 2004, the Board approved Addendum III to the FMP (ASMFC 2004a). This addendum sought to further the conservation of horseshoe crab and migratory shorebird populations in and around the Delaware Bay. It reduced harvest quotas, implemented seasonal bait harvest closures in New Jersey, Delaware, and Maryland, and revised monitoring components for all jurisdictions.

Addendum IV was approved in May 2006 (ASMFC 2006a). It further limited bait harvest in New Jersey and Delaware to 100,000 crabs (male only) and required a delayed harvest in Maryland and Virginia. Addendum V, adopted in September 2008, extended the provisions of Addendum IV through October 31, 2009 (ASMFC 2008a). Through a vote, the Board extended the provisions of Addendum IV through October 31, 2010. Addendum VI further extended Addendum IV provisions through April 30, 2013. It also prohibited directed harvest and landing of all horseshoe crabs in New Jersey and Delaware from January 1 through June 7, and female horseshoe crabs in New Jersey and Delaware from June 8 through December 31 (ASMFC 2010). Addendum VI also mandated that no more than 40% of Virginia's annual quota may be harvested east of the COLREGS line in ocean waters. It also requires that horseshoe crabs harvested east of the COLREGS line and landed in Virginia must be comprised of a minimum male to female ratio of 2:1.

Addendum VII was approved in February 2012 (ASMFC 2012). This addendum implemented the Adaptive Resource Management (ARM) Framework for use during the 2013 fishing season and beyond. The Framework considers the abundance levels of horseshoe crabs and shorebirds in determining the optimal harvest level for the Delaware Bay states of New Jersey, Delaware, Maryland, and Virginia (east of the COLREGS). The Board annually reviews recommended harvest levels from the ARM Subcommittee, who run the ARM model, and specifies harvest levels for the following year in New Jersey, Delaware, Maryland, and Virginia. Since initial implementation in 2013, the ARM model has recommended harvest package 3, and the Board has acted in accordance with this recommendation, specifying annual Delaware Bay harvests of 500,000 male-only horseshoe crabs in every year. State quotas throughout the Atlantic coast, with regards to the interstate FMP, have been specified through 2019 (Table 2) and have generally remained the same since 2013. In accordance with the FMP, any overages of quotas set by the FMP have been accounted for through Board-approved quota transfers between states or by a crab-for-crab quota reduction for the state with the overage in the following year.

1.3.2 State Management

Summaries of state-specific horseshoe crab management regulations are provided below. These summaries are not intended to be comprehensive. For complete sets of regulations, please reference states' marine fisheries agencies.

1.3.2.1 Massachusetts

Massachusetts is issued an annual bait harvest quota of 330,377 crabs, but voluntarily imposes a more restrictive quota of 165,000 crabs. The biomedical fishery is not subjected to an annual quota. There are two permits under which horseshoe crabs can be harvested, a limited entry fishery regulated permit endorsed for horseshoe crab bait harvest, or a biomedical harvest permit. A permit is not required to harvest or possess six or fewer crabs per day. Licensed pot fishermen may possess more than six crabs without a regulated horseshoe crab permit as long as the source of the crabs is a documented permitted wholesale or bait dealer.

After they are bled, crabs collected under the biomedical harvest permit are required to be released back in to the waters from which they were collected. Mobile gear fishermen harvesting with a permit endorsed to harvest horseshoe crabs for bait are subjected to a possession and landing limit of 300 crabs per calendar day or fishing trip (whichever is longer). Non-mobile gear bait harvesters are prohibited from landing or possessing more than 400 crabs per day. Biomedical harvest permit holders are prohibited from landing or possessing more than 1,000 crabs per day. Regardless of permit type, there is a 7-inch minimum legal size. The import of Asian horseshoe crabs is prohibited.

Bait harvesters can only sell to bait dealers, and biomedical harvesters can only sell to biomedical dealers. However, bait dealers can loan bait crabs to biomedical dealers in what is known as the “rent-a-crab” program, where crabs intended for the bait market can be sold to biomedical dealers, bled, and then returned to the bait dealer. Rent-a-crabs are counted against the bait quota.

Permit restrictions are issued annually through a letter of authorization (LOA) to those permitted to receive crabs for biomedical purposes. This LOA states that crabs collected by the biomedical fishery must be returned in good condition to the embayment in which they were collected. All bled horseshoe crabs must be marked after bleeding with a distinct marking (changing each year) to avoid re-bleeding within a season. Crabs with the current year’s marking cannot be re-bled during the same year. Crabs also must be transported in temperature-controlled trucks set to between 50-60 F˚, and temperature in lab and holding areas cannot exceed 70 F˚. Containers holding crabs cannot be more than 2/3 full to reduce the chance of crushing crabs at the bottom of a container. Crabs also must be kept moist. Horseshoe crabs cannot be harvested during five-day lunar closures, starting two days prior and ending two days after the new and full moons from mid-April through the end of June. In addition, those using mobile gear cannot harvest on Fridays or Saturdays during the summer flounder season (beginning June 10th and lasting until the summer flounder quota is reached). Pleasant Bay, located in Eastern Cape Cod has been closed to bait harvest since 2007.

1.3.2.2 Rhode Island

Commercial harvest of horseshoe crabs in Rhode Island is currently managed using seasons, quota, and mandated reporting. In addition to possessing either a Rhode Island Multipurpose license or a Principal Effort/Commercial fishing license with a non-lobster crustacean endorsement, commercial harvesters must also obtain a horseshoe crab permit approving their participation in either bait, biomedical, or both fisheries. As of the 2017 season, commercial bait harvest has been closed during the month of May and restricted to 60 crabs per day when open. The commercial biomedical harvest is closed from two days before to two days after new and full moons (a five-day closure) during the month of May and does not entail a daily possession limit. Reporting of commercially harvested crabs is required via phone call to the Department of Marine Fisheries every Monday for the previous calendar week’s landings and monthly via paper report delivered no later than 15 days after the close of the month being reported. Minimum size limit remains at seven inches in prosomal width.

1.3.2.3 Connecticut

All horseshoe crab harvest from Connecticut waters requires a commercial license, and directed hand harvest of horseshoe crabs also requires an additional Horseshoe Crab Endorsement. All applicable license types are restricted to those with previous history, although license transfer is allowed under specific conditions. When taken under a commercial horseshoe crab trawl license, the possession limit is 25 crabs per vessel per trip or per day, whichever is the longer period of time. No transfer at sea is allowed. When taken under a commercial horseshoe crab hand-harvest license, the possession limit is 500 crabs per license holder per 24-hour period that begins at 12:00 pm. No person taking horseshoe crab under a hand-harvest license shall use any tool, except that gloves may be worn by the license holder. Any person that does not hold a commercial hand-harvest license and an endorsement letter is prohibited from entering the water to assist a licensee. Such unlicensed or unendorsed persons are not prohibited from carrying crabs that have been placed on the beach by the license holder to a storage container or vehicle or taking crabs from a license holder for storage while remaining in a boat. Since December 2000, hand-harvest of horseshoe crabs is not allowed from three closed areas; (1) Menunketesuck Island in Westbrook; and (2) the area known as Sandy Point in West Haven; and (3) the area known as Milford Point in Milford.

Connecticut’s quota is 48,689 crabs, as set by Addendum IV in 2001. From 2001-2006 the open harvest season included only June, and since 2007 it extends from May 22 through July 7, exclusive of weekends. Since 2000, all commercial license holders have been required to report horseshoe crab landings (numbers of crabs) monthly by gear type and fishing area. All harvest is recorded as commercial landings regardless of whether it is sold for any purpose or kept for personal use.

1.3.2.4 New York

To commercially harvest horseshoe crabs for bait a person must have a commercial crab permit and a commercial horseshoe crab permit. Five or less horseshoe crabs may be harvested for personal use without a commercial bait permit. To harvest horseshoe crabs for biomedical purposes a person must have a biomedical harvester permit and must sell to a company that has a biomedical user permit. A person must have a valid commercial crab license to be eligible for a biomedical harvester permit. A person must be approved by the FDA to produce LAL to be eligible for a biomedical user permit. Biomedical user permit holders must ensure all horseshoe crab used in the production of LAL are either returned to the location of harvest as soon as possible after the bleeding process or sold as bait and reported as bait harvest. A person may only apply for and hold one horseshoe crab permit type in a calendar year.

The total annual commercial fisheries bait harvest of horseshoe crabs may not exceed the amount annually allocated to New York State by ASMFC pursuant to the FMP (currently 366,272 crabs). For more than a decade New York has voluntarily limited the commercial harvest quota to 150,000 crabs. The Department of Environmental Conservation (DEC) is authorized to set seasonal quota caps and daily trip limits.

Commercial bait harvest permit holders must file monthly harvest reports, except during May, June, and July, when harvest reports must be submitted weekly. Biomedical harvest permit holders must file monthly harvest reports. In addition, they must notify the DEC 24 hours in advance with details on the planned harvest. Biomedical user permit holders must file monthly reports. In addition, they must notify the DEC 24 hours in advance of releasing horseshoe crabs back into the water.

Horseshoe crabs may only be taken for commercial and biomedical purposes by: hand harvest, pound net, trap net, gill net, otter trawl, seine or dredge. Dredges used to harvest horseshoe crabs shall not be greater than six feet in width. Except during the months of September and October, dredges may not be used to harvest horseshoe crabs in the Atlantic Ocean. The possession or landing of horseshoe crabs from any vessel having a dredge onboard is also prohibited while the dredge fishery is closed.

The DEC may establish closed areas for commercial hand-harvest of horseshoe crabs if it determines that the area receives significant use by spawning horseshoe crabs or shorebird species for which horseshoe crab eggs are an important food source. The DEC may also close harvest in areas managed by a local, state, or federal agency or governing body as public recreation areas, at the request of that agency or governing body.

1.3.2.5 New Jersey

A moratorium is in place on the harvest of horseshoe crabs and horseshoe crab eggs for an indeterminate period of time. The law prohibits the possession of horseshoe crabs and horseshoe crab eggs except for those individuals in possession of a scientific collecting permit, allowing them to possess horseshoe crabs or horseshoe crab eggs for research or educational purposes only. Those fishermen utilizing horseshoe crabs as bait must provide adequate documentation that the horseshoe crabs in their possession were not harvested in New Jersey. For those commercial fishermen in possession of horseshoe crabs, documentation shall include a receipt or bill that provides the name, address, and phone number of the person or company that provided the horseshoe crabs, the permit or license number of the person or company named, and the state and, if possible, the location where the horseshoe crabs were harvested.

1.3.2.6 Delaware

Delaware’s annual horseshoe crab harvest is determined in accordance with the annual sex-specific allocations identified in Addendum VII to the FMP. Harvest is required to be reported by phone to the Delaware Department of Natural Resources Division of Fish and Wildlife (DNREC DDFW) on a daily basis. Upon reaching 95% of the annual allocation, DNREC establishes a date and time to close the fishery, based on recent fishery performance and landings. Any overages incurred are subtracted from the following year’s horseshoe crab quota allocation.

Two methods of harvest are permitted and employed in Delaware’s horseshoe crab fishery. Hand harvest licenses were capped in 1998, although transfer of licenses between qualified individuals is lawful. Individuals that have a current commercial eel license are also allowed to harvest horseshoe crabs for personal bait use. Harvest by eel licensees may not be sold or commingled with any other commercial harvest of horseshoe crabs. Annual hand harvest may not begin until June 8 and ends upon reaching the quota allocation. No more than 300 cubic feet of horseshoe crabs may be collected in a 24-hour period. If the quota has not been reached by June 30, five horseshoe crab dredge permits are issued via lottery, if more than five applications are received. Only current holders of oyster harvesting licenses are eligible for horseshoe crab dredge permits. Dredge harvest is limited to 1,500 horseshoe crabs per day. No harvest, by any method, is allowed to occur between sunset and sunrise.

Delaware has prohibited the use of more than one-half of a female horseshoe crab or one male horseshoe crab as bait in any type of pot on any one day. Bait saving devices are mandatory in all whelk pots employed in the state. Possession of Asian horseshoe crabs or parts thereof are prohibited without written authorization from the Director of the Division of Fish and Wildlife.

1.3.2.7 Maryland

The annual quota of male horseshoe crabs for the commercial fishery is 255,980 male crabs. There is no female harvest permitted. Harvest is subject to daily catch limits, determined by whether the harvester has a valid landing permit. Non-permitted harvesters may not land more than 25 horseshoe crabs per day. Permitted harvesters may not land more than 150 horseshoe crabs per day from May 1-July 9. From July 10-November 30, permitted harvesters are subject to daily limits as designated on their respective permits.

The bait fishery is subject to seasonal restrictions. From May 1-July 9, horseshoe crabs from outside one mile of the Atlantic coast or from Maryland’s coastal bays and tidal tributaries may be caught and landed, but crabs may not be caught within one mile of the Atlantic Coast or the Chesapeake Bay and its tidal tributaries. From July 10-November 30, horseshoe crabs from the state tidal waters may be caught and landed. From December 1-April 30, horseshoe crabs may not be caught or landed in Maryland.

Horseshoe crabs used for scientific purposes (including biomedical use) must be collected by individuals with scientific collection permits. These permits are only granted with proof that collected crabs are being supplied to a facility approved by the US Food and Drug Administration (FDA). Only male crabs may be collected from January 1-June 6. Crabs must be transported in a refrigerated truck and returned within 48 hours. A chain of custody form must follow the crabs from collection to release, and an annual report detailing use of horseshoe crabs is due to the state by January 31 of the following year.

1.3.2.8 Potomac River Fisheries Commission (PRFC)

Potomac River commercial watermen are required to keep an accurate and complete daily account of their catches and releases and submit these reports to the PRFC on a weekly basis.

1.3.2.9 Virginia

Virginia allocates its quota annually among five different harvest gear types including trawl, dredge, pound nets, by-hand, and by other gear. Each one of these gear types is limited entry and requires a gear-specific harvesting permit to participate in the fishery. The harvest of horseshoe crabs in Virginia requires a Commercial Fishing Registration License as well as a gear-specific horseshoe crab harvesting permit. The daily landing limits for each gear-specific license are 2,500 crabs by Trawl Permit, 2,500 crabs by Class A Dredge Permit, 1,000 crabs by Class B Dredge Permit, 500 crabs by Hand Harvest Permit, 500 crabs by Pound Net Permit, and 250 crabs by General Category Permit.

Daily harvest of horseshoe crabs in Virginia must be reported to the agency on a monthly basis through the Virginia Mandatory Reporting Program. Individuals also must call in daily harvests of horseshoe crabs to the agency each day. Each dealer must obtain a Horseshoe Crab Buying Permit in order to buy horseshoe crabs in Virginia. These permitted buyers must supply daily reports of all horseshoe crabs bought on a monthly basis.

The landing of horseshoe crabs in Virginia by trawl is prohibited from January 1 through June 7 of each year and is limited to male only harvest. Virginia prohibits the harvest of horseshoe crabs within 1,000 feet in any direction of the mean low waterline from May 1 through June 7 of each year. Individuals must obtain a Scientific Collection Permit from the Virginia Marine Resources Commission in order to harvest horseshoe crabs for biomedical purposes.

1.3.2.10 North Carolina

Commercial harvest regulations are set by proclamation of the Division of Marine Fisheries Director as stated in North Carolina Marine Fisheries Commission Rule 15A NCAC 03L .0207. The current harvest season is January 1 to April 30 each year with a 50 crab per day limit. An additional opening can occur later in the year if sufficient quota remains uncaught.

Biomedical use crabs are subject to the same harvest regulations as the commercial harvest. Additionally, a biomedical use permit is required as outlined in North Carolina Marine Fisheries Commission Rule 15A NCAC 03O .0503 (a) pursuant to the ASMFC Horseshoe Crab FMP.

1.3.2.11 South Carolina

Taking or possessing horseshoe crabs is unlawful except under permit granted by the South Carolina Department of Natural Resources (SCDNR). Horseshoe crabs may be possessed for educational purposes or for use in LAL production, with appropriate permits. There is no commercial harvest or sale of horseshoe crabs in South Carolina.

Educational permits allow harvest and possession of no more than 25 horseshoe crabs or parts of horseshoe crabs taken in South Carolina state waters.

Horseshoe crabs from which blood is collected for production of LAL may be held in facilities approved by the SCDNR and must be handled so as to minimize injury to the crab. Horseshoe crabs collected must be returned unharmed to state waters of comparable salinity and water quality as soon as possible after bleeding unless subsequent retention is permitted. Horseshoe crabs must be collected by hand outside of restricted areas. Facilities permitted to use horseshoe crabs for LAL production are required to submit monthly reports of collection activity and any mortality that occurs while crabs are possessed.

1.3.2.12 Georgia

All Georgia salt waters are closed to the taking of horseshoe crabs for bait except during those times when the salt waters or portions thereof are opened to the taking of shrimp, whelk, or blue crab by trawling. All horseshoe crab harvest by gear other than a trawl requires a commercial license with a horseshoe crab endorsement. Harvest by trawl requires a commercial trawl license.

It is unlawful for any person taking horseshoe crabs to take or possess more than 25 horseshoe crabs at any one time or for there to be on board the boat used for the taking more than 75 horseshoe crabs at any one time, whichever is less. The taking or catching of horseshoe crabs incidentally during legal fishing operations of other marine species is not a violation of this Rule if the horseshoe crabs so taken in excess of the limits are immediately returned to the water from which they were taken without being intentionally or negligently harmed by the taker or the equipment being used. Horseshoe crabs landed in other states may be imported with appropriate documentation.

Collections of crabs for biomedical use must be conducted by harvesters licensed by the Georgia Department of Natural Resources (GADNR). Individuals that possess crabs for biomedical use must also have a license from GADNR. There are no restrictions on the number of horseshoe crabs that may be taken for biomedical use. Crabs collected for biomedical use are to be returned unharmed to state waters of comparable salinity and water quality as soon as feasible after blood extraction.

1.3.2.13 Florida

Harvest, possession, and sale of horseshoe crabs within Florida state waters requires a current Saltwater Product License (SPL), and no recreational harvest is allowed. Horseshoe crabs must be harvested by hand or gig; all other gear and methods are prohibited. Those possessing a current SPL, can harvest 25 crabs per day. An SPL holder with a Marine Life endorsement can harvest 100 crabs per day, and SPL holders with a permit to harvest eels commercially in freshwater may harvest 100 crabs per day. Harvesting crabs for biomedical purposes require a Horseshoe Crab Biomedical Collecting Permit. This permit has no bag or possession limits if the crabs are maintained and released alive in the area where collected. Biomedical permits are valid for one year and require an activity report detailing the number of crabs collected, areas of collection, and percent mortality up to the point of release, to be submitted by May 1 each year.

1.4 Assessment History

1.4.1 Previous stock assessments

The initial stock assessment for horseshoe crab was completed and peer reviewed in 1998 (ASMFC 1999; ASMFC 1998b). A new assessment framework was proposed in 2000 (ASMFC 2000b), and an internally peer-reviewed assessment was produced in 2004. The most recent externally peer-reviewed benchmark stock assessment was completed in 2009 (ASMFC 2009a) and updated in 2013 (ASMFC 2013).

The ARM model currently used to provide management advice for horseshoe crab in the Delaware Bay region (ASMFC 2009b). Since the first year of implementation of the ARM, the model is renewed annually to set harvest specifications in the region.

1.4.2 Summary of Previous Assessment Models

1.4.2.1 Model Description

The 2013 stock assessment update consisted of trend analyses using autoregressive integrated moving averages (ARIMA). In previous assessments (ASFMC 2004b, 2009a), linear trend analyses were also conducted and a meta-analysis (Manly 2001) was used to evaluate consensus among trends. The peer-review panel for the 2009 assessment concluded that the ARIMA modeling was a good advancement in trend analysis and superseded other trend analyses (ASMFC 2009a, 2009c).

The 2009 benchmark stock assessment also included the application of a surplus production model (Prager 1994) and a catch-survey model (Collie and Sissenwine 1983) for the Delaware Bay region. Those models were not included in the 2013 stock assessment update because of improvements that needed to be made as per peer review comments which could be addressed only as part of a benchmark stock assessment. Previous application of these models to the Delaware Bay region did not include mortality due the biomedical industry – an oversight.

Multispecies models have been developed to support adaptive management of horseshoe crab harvest and recovery of the migratory shorebird populations that rely on horseshoe crab eggs in Delaware Bay (primarily Red Knot). The predictive horseshoe crab models are stage-based models based on Sweka et al. (2007). The ARM Framework is described in separate reports developed by the ARM workgroup and reported through the Delaware Bay Ecosystem Technical Committee. The ARM Framework, established through Addendum VII (2012), incorporates both shorebird and horseshoe crab abundance levels to set optimized harvest levels for horseshoe crabs of Delaware Bay origin and is fully described in ASMFC 2009b. This model is updated annually to set harvest specifications and operates outside of the ASMFC benchmark and update stock assessment processes.

1.4.3 Results of the Previous Assessment

No overfishing or overfished definitions have been adopted by the Management Board. Models that could be used in determining overfishing and overfished status were not run as part of the stock assessment update in 2013, the last time the stock was assessed. The 2013 stock assessment update found that horseshoe crab abundance trends varied regionally/sub-regionally based on the ARIMA results. Positive trends were observed in the Southeast and for some indices in Delaware Bay regions. In the Southeast region there was evidence that abundance has remained stable or continued to increase since the 2009 stock assessment. In Delaware Bay, there was evidence for demographic-specific increases in abundance through the time series of data, but trends have been largely stable since the 2009 stock assessment. An exception was the continued sharp increase in abundance indices from the New Jersey Surf Clam Dredge Survey. Declining abundance was evident in the New York and the Northeast regions. These declines were evident in the previous 2004 and 2009 stock assessments, and trends have not reversed. The status of horseshoe crabs in the Northeast region appeared worse in 2013 than what it was during the 2009 stock assessment, with more indices likely less than their Q_{25} and 1998 reference points.

1.4.4 Previous Peer Review Comments

The 2009 peer review panel commended the SAS on advances they made during the benchmark stock assessment including the development of the ARM model and the use of ARIMA. They encouraged the continued development of the catch survey analysis (CSA) and made several recommendations during the 2009 benchmark stock assessment for the application of trend analyses, ARIMA, the surplus production model, and the CSA for future assessments (ASMFC 2009c).

2 LIFE HISTORY

Horseshoe crabs are characterized by high fecundity, high egg and larval mortality, and low adult mortality (Botton and Loveland 1989; Loveland et al. 1996). They breed in late spring on low-energy coastal beaches along the Atlantic and Gulf of Mexico coasts, laying eggs in nests buried in the sand. Larvae hatch from the eggs within 2-4 weeks, although some larvae may overwinter within nests and hatch out the following spring (Botton et al. 1992). Planktonic larvae typically settle within one to two weeks of hatching and begin molting. Juvenile crabs remain in the intertidal flats, usually near breeding beaches. Older individuals move out of intertidal areas to deeper waters (Botton and Ropes 1987). Crabs are thought to mature around 10 years of age and may live up to 20 or more years.

2.1 Stock Definitions

This stock assessment is for the Atlantic coast horseshoe crab populations that range from Gulf of Maine to Florida. The species range extends into the Gulf of Mexico from Florida west into Louisiana and south to the Yucatán Peninsula. The species is considered to be absent from Texas to Tabasco, México.

Ecological processes, genetic patterns, and tagging analyses suggest a regional or sub-regional population structure. Botton and Loveland (2003) examined abundance and dispersal of horseshoe crab larvae in Delaware Bay. They found a strong tendency for larvae to stay close to spawning beaches. This finding suggests that larval dispersal is not the mechanism for mixing populations (Botton and Loveland 2003). Studies revealing high genetic diversity among populations allow assessments of sex-specific gene flow patterns, which indicate that males disperse at higher rates than females (Pierce et al. 2000, King et al. 2005). This sex-biased dispersal of sexually mature individuals implies that if a population becomes extirpated, gene flow alone may not be sufficient to repopulate an area due to limited larval dispersal potential (Botton and Loveland 2003) and female migration (Swan 2005) among embayments (King et al. 2005).

King et al. (2005), with the intent to account for the genetic structure at a scale relevant to conservation and management, suggested that the distribution of the American horseshoe crab is comprised of multiple population units divided among large geographic regions. Based on the major zones of discontinuity in the genotypic patterns of nDNA, Smith et al. (2017) structured a rangewide risk assessment into the following regions and then integrated the regional assessments to the species level. The transnational genetically-informed regions were:

- Gulf of Maine (USA), including embayments from Great Bay estuary in New Hampshire and north into Maine
- Mid-Atlantic (USA), including all embayments south of New Hampshire to and including North Carolina
- Southeast (USA), including embayments in South Carolina and Georgia, but note that the Georgia population extends into northern Florida
- Florida Atlantic (USA), including embayments along the Atlantic coast of Florida south of the Georgia population
- Northeast Gulf of Mexico (USA), including embayments along the Gulf coast of Florida, Alabama, barrier islands of Mississippi, and easternmost barrier island of Louisiana.
- Yucatán Peninsula (México), including embayments on the western, northern, and eastern portions of the peninsula (the Mexican states of Campeche, Yucatán, and Quintana Roo) and Mexican portion of the Caribbean Sea.

Also, tagging data indicate that a majority of adult crabs remain within local regions and some overwinter in local embayments (ASMFC 2004; James-Pirri et al. 2005; Swan 2005; Smith et al. 2006; Moore and Perrin 2007). Tag release and recapture data from the United States Fish and Wildlife Service horseshoe crab tagging database was used to examine patterns in release and recapture location. Tag recaptures after more than three months at large were examined for the following regions: Northeast, coastal New York-New Jersey, coastal Delaware-Virginia, Delaware Bay, Chesapeake Bay, and Southeast (Table 3 and Table 4).

More than 93% of recaptures were within the region of release except for those released in the coastal Delaware-Virginia. Among those released in coastal Delaware-Virginia, 66% were recaptured in coastal Delaware-Virginia and 31% were recaptured in Delaware Bay. These results are consistent with a regional horseshoe crab population structure. Rutecki et al. (2004) argued for management to consider harvest rates and population abundances possibly down to the embayment level.

Evidence of regional differences are further supported by stable isotope analyses, which indicate adult crabs are loyal to local feeding grounds (Carmichael et al. 2004; O'Connell et al. 2003). Trends in horseshoe crab abundance and population dynamics differ among regions (ASMFC 2004; Smith et al. 2017). Smaller sized populations such as those in Cape Cod waters may be localized based on spawning densities, size structure, and movement patterns (Carmichael et al. 2003; James-Pirri et al. 2005).

Finally, different embayments and regions are subject to different types and levels of harvest for different purposes. Since different types of harvest (bait, biomedical, or scientific) select for different size and sex segments of the population, different populations may experience different harvest pressures due to their location-specific population dynamics (Rutecki et al. 2004). Widener and Barlow (1999) studied a population of horseshoe crabs that appeared to be a local one. They concluded, "Harvesting large numbers of animals from such a local population would have significant impact on its size” (Widener and Barlow 1999). In Delaware Bay waters, commercial harvest is conducted by hand and dredge (Kraemer and Michels 2009), while in areas such as Cape Cod most harvest is conducted by hand from local beaches (Rutecki et al. 2004). In Delaware Bay, the majority of harvested crabs are collected for bait. In contrast, among Cape Cod populations, the primary purpose for which crabs are harvested (bait, biomedical, or scientific) varies by embayment (Rutecki et al. 2004) with bait harvest predominating except in Pleasant Bay where only biomedical harvest is permitted (A. Leschen, personal communication). Since mortality associated with each harvest type varies, the extent of harvest pressure and depletion by overharvest also necessarily varies among embayments (Widener and Barlow 1999; Rutecki et al. 2004). Hence, there is strong support for local management based on regional or sub-regional population structure and harvest pressures.

For purposes of this assessment, the coastwide stock of horseshoe crabs was divided into four geographic regions based on genetic analysis, data availability, and state boundaries. These four regions include: 1) Northeast – Maine south to Rhode Island; 2) New York – Connecticut south to northern New Jersey; 3) Delaware Bay – northern New Jersey south to Virginia; and 4) Southeast – North Carolina south to the Florida Keys (Figure 2).

2.1.1 Genetics

A range of molecular genetic techniques applied across multiple studies has been used in attempts to assess population structure (stock identification) in horseshoe crabs. These studies now include the first range-wide surveys of nuclear DNA variation in any horseshoe crabs (King et al. 2015). King et al. (2003, 2005, 2015) found that the correlation of genetic and geographic distance among horseshoe crab populations sampled along the Atlantic coast suggests isolation by distance as the driving force behind population structure. The more recent findings (King et al. 2005, 2015) suggest the presence of similar levels of genetic diversity and variation among the collections, punctuated with a series of genetic discontinuities of varying “depth” across the species’ range that could indicate demographic independence or regional adaptation, and reflect vicariant geographic events. Populations sampled within these regional groupings exhibit shallow but statistically significant differentiation. Moreover, populations at the ends of the range are more differentiated from nearby populations than are populations in the middle of the range from their neighbors. A separate study showed possible subdivision between collections from the upper Chesapeake Bay and near the entrance of Delaware Bay (Pierce et al. 2000). However, this finding is in contrast to what King et al. found. Pierce et al. (2000) also suggest that the samples from the upper Chesapeake Bay show a resident population. In addition, based on electrophoretic evidence, gene flow does occur between widely separated populations, although considerable genetic variation exists within and between populations of horseshoe crabs (Selander et al. 1970). Saunders et al. (1986) found no evidence for genetic divergence between New England and middle Atlantic populations based on mitochondrial DNA analysis.

2.1.2 Morphometric Information

Shuster (1979) suggested that each major estuary along the coast had a discrete horseshoe crab population, which could be distinguished from one another by adult size, carapace color and eye pigmentation. Differences between the morphologic characteristics of discrete populations were seen among geographically distinct populations (Riska 1981). Larger animals and populations are reported in the middle of the species' distribution (Maryland to New York), while smaller animals and populations are found in the southern and northern extent of its range (Shuster 1982). However, based on morphometric data collected in South Carolina the greatest mean adult size occurs in the South Atlantic Bight and decreases in size north and south (Shuster 1950; Thompson 1998). Thompson (1998) hypothesized that larger individuals occur in the South Atlantic Bight due to optimal temperature and salinity for horseshoe crab development in this region.

Due to their morphological similarity to mid-Mesozoic taxa, horseshoe crabs are considered to be evolutionarily static (Kin and Błażejowski 2014) and have been referred to as phylogenetic relics (Selander et al. 1970). However, close inspection has revealed the presence of considerable morphological and genetic variability (Shuster 1979; Riska 1981; Selander et al. 1970; King et al. 2005; Faurby et al. 2010). Recent genetic studies (King et al. 2015), reveal a pattern of genetic variation that is consistent with patterns of morphological variation identified previously (Shuster 1979; Riska 1981).

2.1.3 Tagging Information

Tagging data from the USFWS horseshoe crab database were analyzed by region to estimate survival and evaluate the dataset for movement analysis. The regions identified in the database are Northeast, coastal New York-New Jersey, Delaware Bay, coastal Delaware-Virginia, Chesapeake Bay, North Carolina, Southeast, and Gulf (Table 3). The Northeast, Delaware Bay, Southeast, and Gulf showed high rates (>93%) of within-region recaptures (Table 4).

Survival analysis was conducted using program MARK (White and Burnham 1999) which showed regional variation in annual survival rate (Table 5). The Jolly-Cormack-Seber (JCS) model was fit to all data. Releases were sufficient to support survival analysis for the Northeast, coastal New York-New Jersey, Delaware Bay, coastal Delaware-Virginia, and the Southeast. The numbers of years of release varied by region. Models were fit for each region separately and then combined for the years 2009-2017, which are the years that all regions had in common. The survival analysis showed that models with regional and time-specific survival and probability of capture fit best based on AIC (Table 5). The highest survival rates were in Delaware Bay and coastal Delaware-Virginia regions. The lowest were in coastal New York-New Jersey and the Southeast.

Movement rates that were estimated by fitting multi-state models in program MARK (Lebreton et al. 2009) showed significant exchange between coastal areas and Delaware Bay (Table 6). Multi-state models have been used to estimate within-region movement for Long Island populations (J. Bopp, SUNY, personal communication). Problems with convergence were encountered and further analysis is needed. However, results for the Delaware Bay region under constant rate model are shown in Table 6.

2.2 Migration Patterns

The current understanding of horseshoe crab migratory patterns is that juveniles move from shallow estuarine waters to deeper estuarine or ocean waters as they grow and mature, reaching sexual maturity either in their natal estuary or ocean waters (Baptist et al. 1957; Shuster 1979; Shuster and Botton 1985; Botton and Ropes 1987; Botton and Loveland 2003; Smith et al. 2009). After maturation, adults migrate annually from the deeper estuary or ocean waters to spawn on estuarine beaches. It is currently unclear why some horseshoe crabs remain within natal estuary waters to mature while others migrate to ocean water to mature. The vast majority of horseshoe crabs from Delaware Bay, for example, migrate to the continental shelf to grow and mature (Botton and Ropes 1987; Smith et al. 2006; Hata and Hallerman 2008), but this population may exhibit some sex-specific migratory patterns. While all juveniles tend to remain within the Bay. Smith et al. (2009) showed that at about eight years of age, females were more likely than their male counterparts to migrate to the continental shelf to mature and males tended to reach sexual maturity without leaving the bay.

While the continental shelf is an important area for maturing horseshoe crabs from the Delaware Bay population, horseshoe crabs from other regions appear to remain within local embayments while maturing (Botton and Ropes 1987; James-Pirri et al. 2005; Swan 2005; Smith et al. 2006; Moore and Perrin 2007; Beekey and Mattei 2009; Schaller et al. 2010; Beekey and Mattei 2015). The importance of local embayments to horseshoe crabs was shown by Landi et al. (2015), who found that spawning locations within Long Island Sound tended to be close to offshore locations where adults had been caught in trawl surveys. Stable isotope analyses also show that adult crabs are loyal to their local feeding grounds (O'Connell et al. 2003; Carmichael et al. 2004). In addition, acoustic telemetry has demonstrated that many animals remain year-round within one bay or estuary (Rudloe 1980; Ehlinger et al. 2003; Beekey and Mattei 2009; Schaller et al. 2010; Watson et al. 2016). The annual migration of mature horseshoe crabs from deeper waters to estuarine spawning beaches appears to be triggered, at least in part, by the onset of warm water temperatures (Smith and Michels 2006; Watson et al. 2009).

Microsatellite genotyping has shown the presence of distinct regional populations for horseshoe crabs, as well as evidence for some gene flow among these regional populations (King et al. 2005; Smith et al. 2017). A low level of gene flow among regional populations is also supported by an analysis of USFWS tagging database showing that horseshoe crabs may migrate significant distances as mature crabs. Crabs tagged in the Gulf of Mexico, for instance, were later recorded from the Southeast and Delaware-Virginia regions while horseshoe crabs tagged in the Southeast region have been documented along the Atlantic coast up to the Northeast region. In addition, horseshoe crabs tagged in the Northeast region have been documented in the Southeast, and horseshoe crabs tagged in New York and New Jersey have also been documented to move towards the Southeast region. Additional genotyping analysis within the southeastern population showed no evidence of genetic structuring across the study area and indicated significant gene flow was occurring across multiple estuaries in South Carolina (Cushman et al., *in review*). While the vast majority of horseshoe crabs appear to stay within or near their natal estuaries, genetic and tagging data highlight the importance of movement within and among regional populations of horseshoe crabs. Because the boundaries separating regional populations of horseshoe crabs may not align with state-level management zones, it is important to understand how horseshoe crab movement might affect horseshoe crab populations in different management zones. As such, further research is needed to better understand the movement patterns of horseshoe crabs both within and among areas of distinct management jurisdiction.

Adult horseshoe crabs are known to be important predators of a variety of benthic macrofauna (Carmichael et al. 2004, 2009; Botton 2009). Primary prey for adult horseshoe crabs are blue mussels (*Mytilus edulis*) and surf clams (*Spisula solidissima*; Botton and Haskin 1984, Botton and Ropes 1989). Horseshoe crabs serve as prey for endangered sea turtles (Keinath 2003; Witherington and Witherington 2015), and their eggs are consumed by migrating shorebirds (Haramis et al. 2007). Their burrowing activities are a form of bioturbation that affects the habitat available for other species (Gilbert and Clark 1981; Kraeuter and Fegley 1994), and predatory activities affect the intertidal and subtidal meio- and macrofaunal communities (Wenner and Thompson 2000; Ehlinger and Tankersley 2009).

2.3 Age

No reliable method is available to directly age horseshoe crabs. Botton and Ropes (1988) and Grady et al. (2001) used epifaunal *Crepidula fornicata* (shell length / shell weight) on the crab's prosoma to indirectly determine age. Shuster (2000) developed criteria for assigning approximate age based on carapace color and the extent of carapace wear. Hata and Berkson (2003) used shell wear, color and structural changes of the pedipalps (males) to stage horseshoe crabs by maturity in conjunction with the Virginia Polytechnic Institute and State University's horseshoe crab trawl survey. Smith et al. (2009) used shell wear, color, size, structural changes of pedipalps and egg presence to characterize maturity and approximate age. Several researchers have proposed the use of ommatidia (units that compose the compound eye) to age juvenile horseshoe crabs, but funding sources are necessary to more formally investigate this possibility. Research using lipofuscin for aging has not been shown to be reliable (Smith et al. 2009). Estimating age by length/width measurements, at least over a wide geographical range, is complicated by the apparent latitudinal differences in size (Shuster 1954; Botton et al. 1992).

Indirect aging methods have provided estimates of longevity. Botton and Ropes (1988) estimated that Delaware Bay horseshoe crabs live at least 17 to 19 years using *C. fornicata*. Swan (2005) found a similar range for Delaware Bay horseshoe crabs based on tagging data. Grady et al. (2001) estimated that Pleasant Bay, New Hampshire, crabs live at least 17 years using *C. fornicata*. Ropes (1961) estimate longevity at 14 to 19 years using tagging data from Pleasant Bay. Shuster and Sekiguchi (2003) reported that horseshoe crabs may live for 20 years in the northern part of their range. Recent tagging data have shown adult crabs at large for up to 17 years before recapture (D. Smith, personal communication), indicating an individual at least 27 years of age.

2.4 Growth

Horseshoe crabs undergo stepwise growth, with females typically attaining larger sizes than males. Smith et al. (2009), reviewing several studies, reported the average prosomal width growth increment for all instars was 1.28 (range: 1.15 – 1.52). Growth is relatively rapid during the first several years progressing through stages I-V in the first year, stages VI – VII the second year, stages VII – IX the third year, with a single molt per year until reaching maturity (Shuster 1982). Shuster (1950) citing "different" sources and a series of exuviae from a captive specimen, approximated that it took 9 to 12 years for horseshoe crabs to reach sexual maturity. Sekiguchi et al. (1982) concluded that male horseshoe crabs molt 16 times and mature in their ninth year; females molt 17 times and mature in their tenth year. Smith et al. (2009) found that males in Delaware Bay tended to mature at age 10 and 11, while females tended to mature at ages 10, 11, and 12.

Carmichael et al. (2003) concluded that male and female horseshoe crabs may continue to molt upon maturation and that males and females had differential growth rates with females also molting more times than males. Female exuviae from crabs of a mature size with amplexus scars have been encountered (G. Breese, G. Gauvry, and C. Shuster, personal communication; Carmichael et al. 2015), further supporting the conclusions of Carmichael et al (2003). The steeply decreasing tag return rates among older adult crabs, and shiny shells with possible tag scars found in a tagging study conducted by Schaller and Dorsey (2011) provide more evidence for this conclusion. However, Smith et al. (2009) examined the hypotheses of differential maturity, differential growth and indeterminate molting and also concluded that females did not grow at a faster rate than males, but rather underwent an additional molt. Although they could not confirm or rule out post-amplexus molting, they did find that it is likely uncommon (<1% of population) and had no discernable population-level effect within the Delaware Bay population.

To test how prosomal width-to-weight relationships vary by sex and region, width and weight data were separated by sex, and split into four regions; Northeast (Maine, Massachusetts, Rhode Island), New York (Connecticut, New York), Delaware Bay (New Jersey, Delaware, Maryland, Virginia), and Southeast (North Carolina, South Carolina, Georgia, Florida).

Graham et al. (2009) established a log-transformed prosomal width-to-weight relationship using the form

$$log_e(Wt) = log_e(PW) * \alpha + log_e (b)$$

where Wt = weight of a horseshoe crab (kg); PW = prosomal width (mm); α = slope; and b = y-intercept.

Linear regressions were used to determine the regional and sex-specific slopes and y-intercepts for the width-to-weight relationships. Two-way ANCOVAs were used to test whether sex specific and regional differences existed in the prosomal width-to weight relationship. The ANCOVAs revealed a significant difference by sex (*P*<0.001). Male prosomal width-to-weight relationships showed no significant difference when specific regions were compared to a coastwide aggregate relationship, although the Northeast region was significantly different from the Southeast ($P=0.021$), Delaware Bay ($P<0.001$), and New York ($P=0.004$) regions when compared region-to-region. Females showed no regional differences.

Regional and sex specific width-to-weight relationships were calculated to be;

Coastwide, female: $\log_e(Wt) = \log_e(PW) * 2.8659 - 15.1802$

Northeast, male: $\log_e(Wt) = \log_e(PW) * 2.8357 - 15.1309$

Southeast, Delaware Bay, New York, male: $\log_e(Wt) = \log_e(PW) * 2.4381 - 12.9439$

2.5 Reproduction

Warming spring temperatures often provide a cue for adult horseshoe crabs to move from deep bays and shelf waters that serve as overwintering habitat to the intertidal zone of beaches where spawning occurs (Shuster 1982; Moore and Perrin 2007; Watson et al. 2009; Schaller et al. 2010; Cheng et al. 2015). In the Gulf of Mexico, spawning extends from February until October, with peaks in March or April (Rudloe 1980; Brockmann et al. 2015). In south Florida, spawning can occur throughout the year (Ehlinger and Tankersley 2007) whereas spawning activity in Georgia and South Carolina occurs from March to July (Thompson 1998). In the Delaware Bay area the crabs spawn from April through at least July, with peak spawning occurring in May and June (Shuster and Botton 1985, Michels et al. 2008; Smith and Michels 2006) and in Long Island Sound, spawning generally begins in May (Beekey and Mattei 2009). In Cape Cod, Massachusetts, spawning begins in May and continues into July (Barlow et al. 1986; Widener and Barlow 1999; James-Pirri et al. 2005), although Carmichael et al. (2003) reported the spawning season in Pleasant Bay, Massachusetts may span from late March through mid-July, based on observations of pairs of horseshoe crabs in amplexus. Variability in the timing of horseshoe crab spawning migrations is associated with water temperature (Smith et al. 2017). Because the current warming trend of estuarine and ocean temperatures is expected to continue, it will be important to understand how increases in water temperatures will affect the timing of horseshoe crab migrations and spawning activity. As such, further research is necessary to understand how temperature sensitivity might vary regionally and how climate warming will affect the timing and magnitude of annual horseshoe crab migrations.

Horseshoe crabs prefer to spawn during high tides, using changes in water depth as a cue (Chabot et al. 2008; Chabot and Watson 2010; Chabot et al. 2011). Some researchers have also reported that peak spawning is associated with the highest tides of the month on the new and full moons (Rudloe 1980, Shuster and Botton 1985, Barlow et al. 1986, Smith et al. 2002a). Lunar period, however, may not always be a valid predictor of horseshoe crab spawning. For example, Leschen et al. (2006) and James-Pirri et al. (2005) found similar levels of spawning activity during all daytime high tides regardless of lunar phase in the vicinity of Cape Cod. Similarly, in Great Bay Estuary, New Hampshire, temperature was shown to be an important determinant of spawning activity with little relationship with lunar phase or time of day (Watson and Chabot 2010; Cheng et al. 2016). The higher of the two daily tides can also be related to spawning activity (Barlow et al. 1986; Rudloe 1980; Chabot and Watson 2010; Brockmann and Johnson 2011). In Delaware, however, the highest levels of spawning activity occur during the evening high tides (Shuster and Botton 1985; Smith et al. 2010). In microtidal areas, wind-blown surge can have a greater effect on water level than tides. Under these conditions, wind-blown surge can strongly influence the numbers of spawning horseshoe crabs (Brockmann and Johnson 2011).

Males are known to locate females using both visual and chemoreceptive cues (Brockmann 2003a; Saunders et al. 2010) and female crabs often arrive at the spawning beach with a male attached to the opisthosoma (Cohen and Brockmann 1983; Loveland and Botton 1992; Brockmann 2003a; Shuster 1982; Cheng 2014). Often several satellite males accompany the attached pair on the beach (Cohen and Brockmann 1983; Brockmann and Penn 1992). Males in amplexus are not shown to differ in size from satellite males, but males in amplexus are generally in better condition, more active, have a higher sperm concentration, remain attached longer and are more recently molted into the adult phase than males not in amplexus (Cohen and Brockmann 1983; Brockmann and Penn 1992; Loveland and Botton 1992; Brockmann 2002; Duffy et al. 2006; Sasson et al. 2012). The males externally fertilize the eggs as they are being deposited. Although a single attached male can fertilize all of a female's eggs, satellite males, when present, may fertilize a majority of eggs (Brockmann et al. 1994, 2000).

Female horseshoe crabs prefer to lay their eggs in well-drained sandy beaches that are protected from surf, although they are also known to spawn in cobble, mud, and peat. It is currently unclear how important these non-sandy habitats are to the reproductive potential of horseshoe crabs across their range. On a single tide, females can excavate a pit and deposit from two to five clusters of about 1000 – 4000 eggs at depths from 5 to 20 cm (Rudloe 1979; Brockmann 1990; Leschen et al. 2006; Brockmann 2003b). However, estimates of eggs per cluster vary: Shuster and Botton (1985) reported 3,650 to 4,000 eggs per cluster and Weber and Carter (2009) reported an average of 5,786 ± 2,834 eggs per cluster. Egg cluster size was 1,644 – 1,739 eggs/cluster in Florida (Johnson and Brockmann 2010), 2,365–5,836 eggs/cluster in Delaware Bay (Shuster and Botton 1985; Weber and Carter 2009), 3,741 eggs/cluster in Long Island Sound (Beekey et al. 2013), and 640–1,280 in Cape Cod, Massachusetts (Leschen et al. 2006). There does not appear to be a relationship between cluster size and female size (Brockmann 1996; Leschen et al. 2006), but larger females carry more eggs and lay more clusters per spawning season than smaller females. Leschen et al. (2006) found a correlation between female size and the number of eggs laid by horseshoe crabs in Pleasant Bay, Massachusetts. Overall, much of the variability in horseshoe crab fecundity appears to be related to female size and latitude (Botton et al. 2010; Smith et al. 2017). Because female size can vary with latitude, more research is needed to understand how latitude, and thus temperature, interacts with female size to affect fecundity in horseshoe crabs.

Female horseshoe crabs typically complete their spawning activity during one tidal cycle (5 days of high tide around new or full moon; Brockmann and Penn 1992; Brousseau et al. 2004; Smith et al. 2010; Beekey and Mattei 2015). In Florida, females return to beaches to nest on average 3.4 times and most spawn during only one tidal cycle (Brockmann 1990). Female horseshoe crabs in Delaware Bay were shown to spawn over two to five consecutive nights, remaining within 50 to 715m of their established spawning beach before moving away from the beaches several days after the new moon (Brousseau et al. 2004; Smith et al. 2010). In Long Island Sound, females were found returning to the same beach up to six days after their initial appearance (Beekey and Mattei 2015). Significant beach fidelity over successive years, however, has not been demonstrated.

Egg development is dependent on temperature, salinity, moisture, and oxygen content (Vasquez et al. 2015b). Larval horseshoe crabs, termed trilobites, generally hatch from the eggs within 2–4 weeks, with a small proportion of larvae overwintering within nests and hatching the following spring (Botton et al. 1992; Shuster 1950). Hatching of eggs is triggered by environmental cues related to high water conditions including hydration, physical disturbance, and hypoosmotic shock, which facilitate survival of newly-hatched larvae (Ehlinger and Tankersley 2003; Botton et al. 2010). Trilobite larvae do not appear to be strong swimmers, relying on vertical movements to take advantage of selective tidal stream transport. Larvae that become planktonic settle to benthic habitats within approximately one week of hatching (Shuster 1982). Larval and juvenile crabs appear to show little dispersal because they remain in the intertidal flats near breeding beaches (Botton and Loveland 2003; Cheng et al. 2015). After approximately two weeks as larvae, they molt to the juvenile (second instar) stage where the telson is formed. As they grow, the older juveniles move out of intertidal areas (Botton and Ropes 1987).

2.6 Natural Mortality

Two field studies have published direct estimates of survival rates of horseshoe crabs. Botton et al. (2003) reported only 3 of 100,000 trilobite larvae were found as fourth instars on adjacent tidal flats by the end of their first summer in New Jersey. Carmichael et al. (2003) calculated annual survival rates for juvenile and adult horseshoe crab stages based on size-based cohort progressions in Pleasant Bay, MA. Very low mortality was reported on juvenile horseshoe crabs after instar 7 (age 1) through the sub-adult stage (age 8), with increasing mortality on adult stages (Table 7) (Carmichael et al. 2003). No significant difference in mortality rates were seen between adult males and females. A natural mortality rate schedule based on these survival estimates along with an assumed 20-year lifespan has been employed in subsequent horseshoe crab operational models (Sweka et al. 2007), stock assessments (ASMFC 2009a), and adaptive resource models (McGowan et al. 2011) (Table 7).

Horseshoe crab egg predation/consumption by shorebirds is well documented (Botton 1984; Botton et al. 1994; Haramis et al. 2007; Botton 2009; Beekay et al. 2013). Despite significant shorebird predation on eggs, such activity probably has little impact on the horseshoe crab population since consumption is mostly relegated to surface eggs, which would not survive regardless of predation (Botton et al. 1994; Botton 2009). Egg burial depths (>5 cm) in Delaware Bay generally outreach the bill penetration of shorebirds (Loveland et al. 1996; Weber and Carter 2009), while successive horseshoe crab spawning and wave action produce high levels of naturally exhumed eggs unrelated to predation (Jackson et al. 2005; Smith 2007; Botton 2009).

Eggs and trilobite larvae are also preyed upon by numerous surf zone fishes and crustaceans including eels, catfish, juvenile striped bass, white perch, killifish, weakfish, Atlantic silversides, bluefish, sand shrimp, blue crabs, spider crabs, and hermit crabs (*summarized in:* Botton 2009). In Delaware Bay, eggs or trilobites were found in stomachs of 95% of killifish (*Fundulus heteroclitus*) and 96% of Atlantic silverside (*Menidia menidia*) (Botton and Loveland unpublished).

Evidence of post-larval horseshoe crabs has been found in stomachs of bluefish (*Pomatomus saltatrix*) (Friedland et al. 1988) and bonnethead sharks (*Sphyrna tiburo*) (Cortes et al. 1996). Horseshoe crabs can be a major (>40%) component in the diet of loggerhead turtles (*Caretta caretta*) (Seney and Musick 2007). Botton and Loveland (1993) also observed direct predation on adult horseshoe crabs by Herring Gulls and Great Black-backed Gulls in Delaware Bay. Abundant numbers of durophagous, benthic, and large opportunistic predators are found in Delaware Bay with horseshoe crabs, such as black drum, cownose rays, bullnose rays, spiny dogfish, smooth dogfish, sandbar sharks, sand tiger sharks (McElroy 2009), various skate species, striped bass, Atlantic sturgeon, blue crabs, summer resident sea turtles, bullnose rays (Szczepanski and Bengtson 2014). Some predation by these species is likely, but to what extent has not been studied in Delaware Bay. American eel and whelks are also potentially significant predators on the Delaware Bay population, given the importance of horseshoe crabs as the preferred bait in these commercial fisheries.

A major source of adult natural mortality is related to spawning, as excessive energy expenditure, stranding, desiccation, and predation are elevated during mating and egg-burying behaviors. Botton and Loveland (1989) estimated nearly 200,000 mortalities related to stranding on New Jersey beaches in 1986. They believed this could be responsible for up to 10% of the adult population in Delaware Bay, although this is likely an overestimation based on a very conservative population estimate. The population estimate of 2.3 to 4.5 million individuals was based on scaled-up NMFS trawl survey catches that admittedly lacked sufficient sampling in inshore strata containing highest densities of horseshoe crabs (Botton and Ropes 1987). Botton and Loveland (1989) suggested this stranding percentage likely varies among estuaries due to population density, weather and tidal conditions, and beach geomorphology. The condition of the individual, which is probably age-related, is also a factor in stranding-related mortality (Penn and Brockmann 1995). Natural and man-made impingements are also factors that affect stranding-related mortality. The reTURN The Favor program implemented by The Wetlands Institute has rescued over 197,000 horseshoe crabs in the 5 years since its establishment. Of these rescued horseshoe crabs, it was found that approximately 3.7-7.2% of crabs were entrapped in natural impingements and 14-20% of crabs were entrapped by man-made impingements over the years (Ferguson et al. 2017).

Recent mark-recapture analyses (summarized in Section 2.1.3) produced annual survival rates of adult horseshoe crabs ranging from 59% to 79% across various embayments (D.R. Smith, unpublished). In Delaware Bay, the instantaneous natural mortality rate (M) was M=0.274 (from the estimate of survival 76%), which is considerably lower than the adult M=0.47 employed in modeling to-date (Table 7). A lower M (e.g. <0.47) is supported by the empirical ratio of multiparous to primiparous females (ratio=3.8) observed in the Virginia Tech Trawl Survey. Given its biology, newly mature primiparous females will spawn in the upcoming year and exhibit multiparous indicators thereafter, generally occurring between ages 9 and 10. Given a longevity of 20 or 27 years, *M* would need to be 0.215 or 0.231 to produce a 3.8 multiparous (ages 10+) to primiparous (age 9) ratio.

Protracted at-large durations were also noted in the mark-recapture analyses (up to 17 years), which sheds new light on potential longevity. A mark-recapture duration of 17 years suggests a longevity of roughly 27 years given a minimum age-at-tagging of nine to 11 (based on onset of maturity (Shuster 1950)). Maximum age has heretofore been assumed to be 20 years (Ropes 1961; Botton and Ropes 1988; Swan 2005).

Greater longevity changes the understanding of natural mortality. Indirect estimates of age-invariant, constant, *M* based on a maximum age of 27 years would range between *M*=0.11 and *M*=0.17 (depending on selected mortality model), as opposed to a range of 0.15 to 0.22 given a maximum age of 20 years (Hoenig 1983; Hewitt and Hoenig 2005). Other indirect estimates of constant *M* can be generated from models that incorporate von Bertalanffy (LVB) growth parameters and environmental information (Pauly 1980; Jensen 1996) (Table 8 and Table 9), although these estimates appear too high to allow for the population to reach maximum ages of 20 plus years.

Von Bertalanffy (LVB) parameters were fit to Carmichael et al.'s (2003) sub-adult growth trajectory, with the assumption of asymptotic size occurring at instars 18 and 20 for males and females. These instars correspond to ages 9 and 11 for males and females, consistent with longstanding expectations about maturity and terminal molting (Shuster 1950; Botton and Ropes 1988; Schuster and Sekiguchi 2003). Stockpiling of males and females also occurred at these instar stages in Pleasanton Bay (Carmichael et al. 2003), further supporting the timing of growth cessation. Asymptotic sizes of adult stages were based on average carapace widths of adult horseshoe crabs (males=203 mm and females=245 mm) observed in the Delaware Division of Fish and Wildlife 30-foot trawl survey from 1966-2018 (M. Greco unpublished data).

Age-variable mortality models allow for *M* to vary inversely with size (Peterson and Wroblewski 1984; McGurk 1986; Lorenzen 1996; 2000; Gislason et al. 2010). Age-based mortality schedules, utilizing von Bertalanffy parameters and width:weight relationships (Graham et al. 2009), were calculated using Lorenzen (1996) and Gislason et al. (2010) models (Table 10).

These mortality schedules did not accommodate higher adult mortality rates caused by excessive spawning mortality. Replacing the size-based mortality rates for adults (ages ≥10) with mortality estimates (*Z*=0.238 to 0.528) from recent mark and recapture analyses of adult tagged crabs (D.R. Smith, unpublished) is an option that would better describe mortality in adult age classes.

However, both models, the Lorenzen (1996) model especially, generate mortality rates that appear too high to suit the life history and extended longevity of horseshoe crabs. The extremely elevated early stage mortality rates do not allow for enough survival for the population to reach maturity (age 10) or its maximum age (20-27 years). Extremely high Age 0 mortality (M=8, 11) from the Gislason et al. model does correspond well with Botton et al.'s (2003) field estimate of M=10.4. Other Age 0 estimates of M=4.6 (equivalent to 99% mortality) in Pleasant Bay, MA (Carmichael et al. 2003) and M=3.6 in Delaware Bay (R. Wong, unpublished) mesh well with the Lorenzen (1996) model. Future work is needed to better understand size and age-based natural mortality rates for horseshoe crabs.

2.7 Sex Ratio

Two types of sex ratios are useful for understanding horseshoe crab ecology and informing management decisions. The population sex ratio is the ratio of males to females among individuals in the population. The operational sex ratio is the ratio of males to females among adults that are actively spawning. While juveniles show a balanced population sex ratio (Shuster and Sekiguchi 2003; Smith et al. 2009), the population sex ratio among adults has been observed to be somewhat skewed toward males in Delaware Bay (2.2:1 M:F; Smith et al. 2006) and Pleasant Bay, MA (2.3:1 M:F; Carmichael et al. 2003). This difference has been attributed to higher fishing or natural mortality among adult females compared to males, but also might be due to males maturing earlier than females and living as long as females (Smith et al. 2009). The operational sex ratio of horseshoe crabs on the spawning beaches is highly skewed toward males because of behavior and population demographics (Brockmann and Smith 2009). One male attaches to a female in amplexus prior to spawning. During fertilization, however, the amplexed pair is often surrounded by unattached (i.e. satellite) males (Brockmann and Penn 1992). Hence, the operational sex ratio on spawning beaches is expected to be male biased compared to the population sex ratio among adults.

A population sex ratio over 1 is likely to be required among adults to ensure that reproduction is not limited by sex ratio. Brockmann (1990) found that female horseshoe crabs will tend not to nest unless they are in amplexus with a male, and that satellite males are not needed to fertilize eggs. Some males (approximately 30%) are not capable of amplexus because of their condition (Brockmann and Smith 2009). Thus, there needs to be an excess of males in the population to ensure a sufficient number of males capable of amplexus to pair with the females ready to spawn. In the Delaware Bay population, the operational sex ratio averaged 3.8 M:F (SD = 0.51) over 1999 to 2008 (Michels et al. 2008). In contrast, the population sex ratio averaged 2.0 M:F (SD = 0.19) over 2002 to 2008 (Hata and Hallerman 2008). Thus, on average, the operational sex ratio is 1.88 times (SD = 0.19) the population sex ratio for the Delaware Bay population (Hata and Hallerman 2008; Michels et al. 2008).

Sex ratios in estuarine habitats sampled in the Delaware Bay Adult Trawl Survey for the 1990-2017 time period were significantly different for the spring and fall seasons averaging 1.27 and 2.2 M:F in the spring and fall, respectively (Paired t-test; $P<0.001$; Table 11). The seasonal difference in sex ratios for the Delaware Bay Adult Trawl Survey indicates that the relative abundance of females in estuarine habitats is greater during the spring, compared to the fall. This finding is broadly consistent with previous research showing that female horseshoe crabs are more likely than their male counterparts to migrate out of estuarine waters (Smith et al. 2009). While the Delaware Bay Adult Trawl Survey shows seasonal differences in sex ratio, the

New Jersey Ocean Trawl Survey that samples coastal habitats showed no significant difference between seasons with sex ratios of 1.13 and 1.03 in the spring and fall, respectively (Paired t-test; P=0.20; Table 11). The presence of seasonal shifts in sex ratios for the Delaware Bay, but not for the New Jersey Ocean Trawl Survey, could reflect differences in habitat where sex-specific migration patterns may be more likely to occur within estuarine habitats such as Delaware Bay. Annual average sex ratio in offshore habitats sampled in the New Jersey Surf Clam Survey was 0.51 M:F, much lower than sex ratios for the Delaware Bay and New Jersey Ocean Trawl Surveys. It is unclear why the New Jersey Surf Clam survey has lower sex ratio compared to other surveys. Together, these data provide further evidence for sex-specific migration patterns in horseshoe crabs that warrant further study in order to better understand behavior and migration patterns of male and female horseshoe crabs.

Temporal trends in sex ratios for surveys used in this assessment were conducted using Mann-Kendall analysis for the New Jersey Surf Clam Survey as well as both the spring and fall surveys of the Delaware Bay Adult Trawl and the New Jersey Ocean Trawl. Only one of the fishery-independent surveys analyzed showed a significant temporal trend in sex ratio with the available data (Table 11). A significant increase in the sex ratio for the spring season (March-August) of the Delaware Bay Adult Trawl Survey from 1990 – 2017 was documented (Table 11; Figure 3). These data show a sex ratio for the spring of 1990 of 0.76 M:F (CL: 0.30-1.23) increasing to 2.0 M:F (CL: 1.31-2.68) in the spring of 2017 (Table 12). While Mann-Kendall analysis found significant increases in sex ratio in these data (τ=0.39, P=0.004, sen-slope=0.033), breakpoint analysis was also conducted to assess shifts in the stability of the linear relationship. Breakpoint analysis fits linear models to sections of the data and detects locations of breaks in the relationship by minimizing residual sums of squares and determines the optimal number of breaks by minimizing information criterion (Bai and Perron 2003; Zeileis et al. 2003). This breakpoint analysis indicated the presence of a single breakpoint at the year 2006 for the spring season of the Delaware Bay Adult Trawl survey. This breakpoint year is consistent with the regulatory change that reduced the total harvest of horseshoe crabs in Delaware Bay and implemented male-only harvest for portions of Delaware Bay. Mean sex ratio for this survey from 1990-2006 was 0.94 M:F, whereas mean sex ratio from 2007-2017 was 1.79 M:F.

Significant increases in the M:F sex ratio were also observed in some of the fishery-dependent data, specifically, the Virginia off-shore waters and Virginia landings data (Table 11, Table 12). These changes in the sex ratio are not necessarily representative of the population, but rather, reflect changes in the regulations concerning collection and harvest of horseshoe crabs in these regions.

3 HABITAT DESCRIPTION

3.1 Brief Overview of Habitat Requirements

Essential habitat is defined as those waters and substrate necessary for fish spawning, breeding, feeding, or growth to maturity. Habitat requirements change throughout the horseshoe crab life cycle, extending from intertidal beach fronts and tidal flats in coastal embayments for eggs and larvae, to the edge of the continental shelf for adults. Limulus has been described as an ecological generalist (Sekiguchi and Shuster 2009) able to tolerate a wide range of environmental parameters throughout its distribution. Various environmental tolerances have been documented for horseshoe crabs in several areas; however, Sekiguchi and Shuster (2009) suggest that individual sub-populations may have a narrower tolerance than the species.

3.1.1 Spawning, egg, larval habitat

Spawning adults prefer sandy beach areas within bays and coves that are protected from wave energy (Shuster and Botton 1985; Smith et al. 2002b; Jackson et al. 2002; Landi et al. 2015). Nests are primarily located between the low tide terrace (tidal flat) and the extreme high tide water line (Penn and Brockmann 1994; Weber and Carter 2009). Weber and Carter (2009) found that 85% of nests were deposited between the tidal flat and the nocturnal high tide wrack line on the western shore beaches of Delaware Bay. Penn and Brockmann (1994) found similar results in Delaware Bay, but noted that nest deposition occurred in a narrower band within the beach front on Seashore Key, Florida. The differences in nest site selection between Florida and Delaware can be explained by differences in beach morphology, particularly sediment grain size, and its effect on interstitial conditions (Penn and Brockmann 1994). In Massachusetts, New Jersey, and Delaware, beaches are typically coarse-grained and well drained, as opposed to Florida beaches which are typically fine-grained and poorly drained. Spawning is sometimes observed on offshore sandbars and oyster bars (Wenner and Thompson 2000). In Long Island Sound, nests can be found on beaches ranging from coarse-grained and well drained to cobble-dominated substrates to fine grained and poorly drained muddy substrates (Beekey and Mattei 2009).

Beach habitat also must include a sufficient depth of porous, well-oxygenated sediments to provide a suitable environment for egg survival and development (Botton et al. 1988). Nest depth on the western shore of Delaware Bay generally ranged between 3.5 and 25.5 cm (mean 15.5, SD 3.5), although nest depth may be affected by wave energy, bioturbation, or other factors after deposition (Weber and Carter 2009). These results are similar to those found by previous investigators on Delaware Bay beaches (e.g., Hummon et al 1976; Penn and Brockmann 1994; Botton et al 1994). Sediment grain size, in particular, can influence spawning site selection as environmental conditions in the sand affect development (moisture, temperature, and oxygen gradients) (Penn and Brockmann 1994; Jackson et al. 2005). Previous studies suggest that females avoid laying eggs in eroded beaches that are high in hydrogen sulfide and where sediment pore water is low in oxygen, factors that are known to affect development (Botton et al. 1988; Penn and Brockmann 1994, Vasquez et al. 2015).

Rate of egg development is dependent on interstitial environmental parameters including temperature, moisture, oxygen, and salinity (French 1979; Jegla and Costlow 1982; Laughlin 1983; Penn and Brockmann 1994) and disturbance (bioturbation) from external forces (Jackson et al 2005). Placement of nests in the intertidal zone subjects horseshoe crab eggs to a wide range of environmental parameters, making it necessary for eggs and larvae to have wide tolerance ranges; however optimum egg development occurs within a much narrower range of conditions. Studies have shown that optimal development occurs at salinities between 20 and

30 ppt (Jegla and Costlow 1982; Laughlin 1983), although populations from microtidal lagoon systems that often experiences high salinities (>50 ppt) had an optimal range of 30 to 40 ppt, with hatching occurring at salinities as high as 60 ppt (Ehlinger and Tankersly 2004). Egg development occurs most readily at temperatures ranging from 25 to 30°C (Jegla and Costlow 1982; Laughlin 1983; Penn and Brockmann 1994; Ehlinger and Tankersly 2004), with temperatures of 20 and 40°C showing little to no development (Laughlin 1983; Ehlinger and Tankersly 2004). Penn and Brockmann (1994) found optimal development of horseshoe crab eggs from Delaware and Florida to occur at oxygen concentrations between 3 and 4 ppm and moisture content between 5 and 10%.

In addition to the influences of interstitial microhabitat on nest site selection, Thompson (1998) found that preferentially selected spawning sites were located adjacent to large intertidal sand flat areas, which provide protection from wave energy and an abundance of food for juveniles. Most nesting beaches have nearby nursery habitats for juveniles (Botton and Loveland 2003). Geographic differences in nest site selection can be explained by differences in wave energy, beach morphology, and geochemistry (Botton et al. 1988; Penn and Brockmann 1994; Smith et al. 2002a; Beekey and Mattei 2009; Landi et al. 2015).

Horseshoe crab spawning areas are limited by the availability of suitable sandy beach habitat. For example, based on geomorphology, Botton et al. (1992) estimated that only 10% of the New Jersey shore adjacent to Delaware Bay provided optimal horseshoe crab spawning habitat. However, spawning may occur along peat banks if there is sand in the upper intertidal regions and along the mouths of salt marsh creeks (Botton 2009). Shuster (1996) stated that spawning may occur along muddy tidal stream banks, but not on peat banks because adults are sensitive to hydrogen sulfide and anaerobic conditions. Subtidal spawning has been reported, but the extent to which this occurs is unknown. A Habitat Suitability Index model was developed for horseshoe crab spawning habitat within the Delaware Bay (Brady and Schrading 1996).

After hatching, some larvae delay emergence and overwinter within beach sediments, emerging the following spring (Botton et al. 1992). Larvae typically settle in shallow water areas to molt (Shuster 1982).

3.1.2 Juvenile and adult habitats

Nearshore, shallow water, intertidal flats are considered essential habitats for development of juvenile horseshoe crabs (Botton 2009). Juveniles usually spend their first two years on intertidal sand flats (Rudloe 1981; Sekiguchi and Shuster 2009). Thompson (1998) also found significant use of sand flats by juvenile horseshoe crabs in South Carolina. Prime spawning habitat is widely distributed throughout Maryland's Chesapeake and coastal bays, including tributaries. Horseshoe crabs are restricted to salinities that exceed 7 parts per thousand. In the Chesapeake Bay, spawning habitat generally extends to the mouth of the Chester River, but can occur farther north during years of above normal salinity levels. Prime spawning beaches within the Delaware Bay consist of sand beaches between Maurice River and the Cape May Canal in New Jersey and between Bowers Beach and Lewes in Delaware.

Older juveniles and adults are exclusively subtidal, except during spawning. Second and third year instars remain in the vicinity of the spawning beach but move just offshore into shallow subtidal water (Sekiguchi and Shuster 2009), with each succeeding stage moving toward deeper water. In the Delaware Bay, females begin to leave the Bay and move to continental shelf waters around age 7 to 8 to mature in the ocean (Hata and Hallerman 2009a, 2009b, 2009c; Smith et al. 2009). Smith et al. (2009) provide evidence that males remain in the Bay until maturity (age 9), but Hata and Halllerman (2009a, 2009b, 2009c) found evidence of significant numbers of immature males on the shelf one to two years prior to reaching maturity (Hata and Hallerman 2009a, 2009b, 2009c).

The diet of juveniles is varied, including particulate organic matter from algal and animal sources (Gaines et al. 2002; Carmichael et al. 2004). As horseshoe crabs mature, the diet composition shifts to larger prey, and horseshoe crabs are known to be important predators of benthic meiofauna (Carmichael et al. 2004; Carmichael et al. 2009; Botton 2009).

Delaware Division of Fish and Wildlife's 16-foot bottom trawl survey data indicated that more than 99 percent of juvenile horseshoe crabs (<16 cm prosomal width) were taken at salinities >5 parts per thousand.

As ecological generalists living in a shallow water environment over a wide geographic range, *Limulus* is subject to, and therefore adapted to, a wide range of environmental conditions. Specific requirements for adult habitat are not known, but it has been suggested that individual sub-populations may have a narrower tolerance than the species as a whole (Sekiguchi and Shuster 2009). Adult horseshoe crabs range from 21 N to 44 N and 68 W to 90 W (Sekiguchi and Shuster 2009), and have been found as far as 35 miles offshore at depths greater than 200 meters; however, Botton and Ropes (1987) found that 74 percent of the horseshoe crabs caught in bottom trawl surveys conducted by the National Marine Fisheries Service (NMFS), Northeast Fisheries Science Center were taken in water shallower than 20 meters. They are observed in a wide range of salinity regimes, from low salinity (< 10 pp) areas such as the upper Chesapeake Bay, to the hypersaline (>50 ppt) environments of the Indian River Lagoon in Florida. During the spawning season, adults typically inhabit bay areas adjacent to spawning beaches. In Delaware Bay, horseshoe crabs are active in the Bay area at temperatures above 15°C (Sekiguchi and Shuster 2009; Smith et al. 2010), while crabs in Great Bay, NH increase activity at temperatures above 10.5°C (Watson et al 2009). In the fall, adults may remain in bay areas or migrate into the Atlantic Ocean to overwinter on the continental shelf.

Sekiguchi and Shuster (2009) have identified four possible large-scale factors that limit horseshoe crab distribution and habitat, including geomorphology, thermal tolerance, tidal regimes, and currents. Indo-Pacific species of horseshoe crab span the equator, but *Limulus* does not, perhaps due to limited availability of embayments with suitable spawning habitat, or the lack of a broad continental shelf to provide a migratory route. The northern extent of all horseshoe crab species may be limited by duration and severity of winter temperatures. The lack of horseshoe crab populations in the western Gulf of Mexico, which has suitable beach spawning habitat, is thought to be a result of the local tidal regime. Nearly all horseshoe crab populations occur in areas with semi-diurnal tides of moderate amplitude, but tides of this type are not observed in the western Gulf of Mexico.

Habitat degradation is likely an important component of the population dynamics of horseshoe crabs. Groins and bulkheads adversely impact horseshoe crab spawning habitat. Bulkheads may block access to intertidal spawning beaches, while groins and seawalls intensify local shoreline erosion and prevent natural beach migration. An estimated 10 percent of the New Jersey shoreline adjacent to the Delaware Bay has been severely disturbed by shoreline protection structures (Botton et al 1988). Rip-rap and revetments also adversely impact horseshoe crabs by minimizing potential spawning sites and by entrapping and stranding them. A contributing factor in the decline of horseshoe crabs in the Delaware Bay between 1871 and 1981 may be the increased number of jetties and residential development (Shuster and Botton 1985). The Wetland Insitute's reTURN The Favor program records data and information on the locations of impingements that are found while working at New Jersey beaches. Of the 22 beaches that are covered, almost all are affected by structures of variable severity that inhibit the ability for horseshoe crabs to spawn or survive. This data is used to identify beaches that are in need of small-scale restoration projects (Ferguson et al. 2017).

Shoreline erosion combined with shoreline development results in the loss of suitable and potentially suitable spawning beaches. Beach migration is a coastwide phenomenon, where beaches move landward associated with erosional events. However, hard structures (e.g., bulkheads, seawalls, revetments) associated with beach development interfere with the natural beach migration causing habitat loss. Beaches along the New Jersey shore of the Delaware Bay have generally eroded at varying rates ranging from 1 to 12 feet per year for the last 100 years (U.S. Army Corps of Engineers 1997). Erosion rates from 1 to 26 feet per year, averaging approximately 3 to 5 feet per year and the existence of hard structures limiting beach migration have resulted in a decline in Delaware beaches (U.S. Army Corps of Engineers 1991). McCormick and McCormick (1998) report the annual rate of erosion in the Chesapeake Bay averages 1 foot per year. Shoreline areas with high concentrations of silt or peat are less favorable to horseshoe crabs because the anaerobic conditions reduce egg survivability. Horseshoe crabs may detect hydrogen sulfide (which is produced in the anaerobic conditions of peat substrates) or low oxygen conditions, and actively avoid such areas (Botton et al 1988). Erosion affects spawning by influencing beach characteristics that are most important in site selection, such as beach topography, sediment texture, and geochemistry (Botton et al 1988).

Adult horseshoe crabs are known to be important predators of a variety of benthic macrofauna (Carmichael et al. 2004, 2009; Botton 2009). Botton and Haskin (1984) and Botton and Ropes (1989) found that the primary prey for adult horseshoe crabs are blue mussels (*Mytilus edulis*) and surf clams (*Spisula solidissima*). Recent declines in surf clam in the mid-Atlantic are being attributed to climate-change induced increases in water temperatures during late-summer and fall (E. Powell, personal communication). The effects of a declining prey base, in general, and of surf clam populations on horseshoe crab population carrying capacity is unknown.

In summary, horseshoe crabs are an important part of the ecology of the coastal systems in which they are found (Botton 2009). They are prey for endangered sea turtles (Keinath 2003, Witherington and Witherington 2015), and their eggs are consumed by migrating shorebirds (Haramis et al. 2007). Their burrowing activities affect the habitat available for other species through bioturbation (Gilbert and Clark 1981; Kraeuter and Fegley 1994), and predatory activities affect the intertidal and subtidal meio- and macrofauna (Wenner and Thompson 2000; Ehlinger and Tankersley 2009).

4 FISHERY DEPENDENT DATA SOURCES

Commercial fisheries for horseshoe crab consist primarily of directed trawls, hand harvest, and dredge fisheries for use as bait and are the major source of fishery-dependent data for the stock. Landings for horseshoe crabs have been reported since 1970 and fishery-dependent data of the catches have been collected since 1998. Horseshoe crabs are also commercially collected for use in the biomedical industry.

4.1 Commercial Bait Fishery

The commercial bait fishery consists primarily of trawl, hand harvest, and dredge fisheries. State and federal governments collected the fishery-dependent data included in this summary. Since 1998, ASMFC has compiled landings by state in the annual FMP review report. The horseshoe crab fishery supplies bait for the American eel, conch (whelk) and, to a lesser degree, catfish (Ictaluridae) fisheries. The American eel pot fishery prefers female horseshoe crabs to males, while the conch pot fishery uses both male and female horseshoe crabs. The conch fishery uses horseshoe crabs more frequently than the American eel fishery, with eel baits using blue crabs or fish more often than horseshoe crabs (ASMFC 2017).

Most fishing effort for horseshoe crabs is concentrated within the mid-Atlantic coastal waters and adjacent federal waters. However, Massachusetts and New York have also supported a significant fishery. The hand, trawl, and dredge fisheries accounted for 86% of the of the 2017 commercial horseshoe crab bait landings coastwide (by weight) by reported gear type (ASMFC 2018). This pattern is consistent with the distribution of landings by gear since the 1970s. During the past 25 years, the proportion of horseshoe crabs caught by the hand fishery has increased and now accounts for the largest of any reported harvest, while the proportion caught by the trawl fishery has decreased during the same timeframe (ASMFC 2018).

Previous to 1998, commercial landings data for horseshoe crab were collected by the National Marine Fisheries Service (NMFS) by state, year, and gear type. Data were obtained from dealers, logbooks, and state agencies that require fishermen to report landings; however, NMFS records are often incomplete. In addition, the conversion factor used to convert numbers landed to pounds landed has been quite variable among the states and NMFS. Since 1998, states have been required to report annual landings to ASMFC through the compliance reporting process. Landings used in this assessment for 1998 through 2017 were validated by state agencies through ACCSP. Reported landings data show that commercial harvest of horseshoe crabs was high in the late 1990s, declined, and have been relatively stable from 2004 through 2017 (Table 1, Figure 1). Older landings, collected by NMFS, were not incorporated into any models in this assessment due to questionable accuracy of the data.

4.1.1 Data Collection and Treatment

4.1.1.1 Survey Methods

Commercial horseshoe crab landings data collection is a joint state and federal responsibility. The cooperative state-federal fishery data collection systems obtain landings data from state mandated fishery or mollusk trip-tickets, landings weigh-out reports provided by seafood dealers, federal logbooks of fishery catch and effort, shipboard and portside interviews, and biological sampling of catches. State fishery agencies are usually the primary collectors of landings data, but in some states NMFS and state personnel cooperatively collect the data. Statistics for each state represent a census of the horseshoe crabs landed, rather than an expanded estimate of landings based on sampling data. Although the NMFS reports landings in pounds, adoption of the Interstate Fishery Management Plan for Horseshoe Crab (FMP) in 1998 required states to collect and report all horseshoe crab harvest by numbers, pounds, sex, and harvest method (ASMFC 1998a). All states with an operating fishery require mandatory reporting. Horseshoe crab landings reported after 1997 were expressed as numbers of crabs and were obtained directly from the states.

Commercial sampling intensity varies from state to state. Most jurisdictions have implemented mandatory monthly or weekly reporting. Reporting compliance has substantially improved since adoption of the FMP, with all required states (those with landings >5% of the coastwide total) now providing landings by sex each year in compliance reports. In years initially following the adoption of the FMP, some sex information was missing.

4.1.1.2 Biological Sampling Methods

Under the 1998 FMP, states are required to characterize a portion of the commercial catch based on prosomal width and sex. Though many states implemented this compliance component, sampling intensity has been inconsistent among states and between years.

Prosomal width measurements and some sex data from commercial biosampling programs are available from Massachusetts, New York, Delaware, Maryland, Virginia, and North Carolina. These data were included in the growth (Section 2.4) and sex ratio (Section 2.7) analyses for this assessment.

4.1.2 Commercial Bait Landings

The adoption of the FMP in 1998 improved harvest monitoring through mandatory reporting. The adoption of Addendum I to the FMP established reference period landings for the bait fishery that allowed for the implementation of quotas and served as a benchmark to evaluate subsequent bait landings. Addenda III (2004), IV (2006a), and V (2008a) further reduced harvest quotas, implemented seasonal bait harvest closures, and mandated male-only fisheries in some or all of the states in which harvest impacted the Delaware Bay population of horseshoe crabs (New Jersey, Delaware, Maryland, and Virginia).

Commercial bait landings for each state were validated through ACCSP. Inconsistencies between landings in the ACCSP data warehouse and annual compliance reports resulted in a second validation with most of the Atlantic states. For the Delaware Bay Region, ACCSP also validated 2017 landings to support the regional models. Outside the Delaware Bay, landings for 2017 were pulled from compliance reports. Landings previous to 1998 could not be validated by ACCSP and are not included in this assessment or any of the analyses. The coastwide bait landings of horseshoe crabs in Table 1 represent the best data available. Horseshoe crab landings for 1998-2017 peaked in 1999 at 2.6 million horseshoe crabs and have decreased since the late 1990s (Figure 1). Landings have remained under 1 million horseshoe crabs since 2003 and from 2004-2017 landings have averaged 752,886. Sex data were not available for all states, but based on the data available the sex ratio has shifted to predominantly male horseshoe crabs being caught in the bait fishery due to the implementation of the ARM Framework and resulting male-only harvest in the Delaware Bay. At a regional level, on average, commercial bait harvest of horseshoe crabs is predominantly from the Delaware Bay, followed by the New York region, then the Northeast (Table 13, Figure 4). The Southeast historically and presently harvests the smallest number of horseshoe crabs as part of the bait harvest.

Bait landings for the Delaware Bay states was developed to support the catch survey model for that region. Horseshoe crab landings from New Jersey and Delaware are considered to be 100% Delaware Bay origin (i.e., has spawned at least once in Delaware Bay) whereas 51% of Maryland’s harvest and 35% of Virginia’s are believed to be Delaware Bay origin based on genetic data and analysis (ASMFC 2012). These percentages were applied to the Delaware Bay states’ bait harvest. Horseshoe crabs that were not sexed were portioned into males and females based on sex ratios in order to determine how many female horseshoe crabs were harvested in the commercial bait fishery in order to support modeling efforts (Table 14). Similar to the coastwide bait landings, bait landings of Delaware Bay origin were the highest in the late 1990s and have decreased since (Figure 5). The implementation of the ARM Framework through Addendum VII (ASMFC 2012), female harvest in the region has been restricted and this can be seen in the sex ratio of the catch.

4.1.3 Commercial Bait Catch Rates (CPUE)

Commercial catch rates are available from Delaware via the state’s compliance report for 2017 (Figure 6). Delaware commercial catch rates were calculated by state employees by dividing the number of horseshoe crabs landed in the dredge and hand fishery by the respective number of trips for each fishery. The commercial CPUE in Delaware’s dredge fishery peaked in 1996 and were the lowest in 2003, although there are several years since then when there was no dredge fishery. For the hand harvest CPUE, the highest value was in the terminal year of 2017 and the lowest was in 2013.

Interpretation of the Delaware catch rates are complicated by the imposition of regulations after 1997. For example, after 1997 trip limits were established on the dredge fishery of 1,500 crabs per day and the hand fishery was restricted to 300 ft^3 per day. In addition, the dredge fishery, which was capped at five permits issued annually to fishermen that had traditionally harvested using this gear became subject to a lottery that included non-traditional participants.

These non-traditional fishermen tended to be less efficient while they learned various gear nuisances and locations of horseshoe crab concentrations. Further harvest restrictions were imposed from 2004 and on.

No other state provided sufficient information for this assessment or through their 2017 compliance reports to calculate commercial CPUE. The SAS therefore relied entirely on fishery-independent data to characterize regional and coastwide trends for this assessment.

4.2 Commercial Biomedical Fishery

Research on horseshoe crabs for use in the biomedical industry began in the early 1900s (Shuster 1950). Scientists have used horseshoe crabs in eye research, surgical suture wound dressing development, and detection of bacterial endotoxins in pharmaceuticals (Hall 1992). The current major biomedical use of horseshoe crabs is in the production of LAL. LAL is a clotting agent in horseshoe crab blood that makes it possible to detect endotoxins in patients, drugs, and all intravenous devices. The LAL test was commercialized in the 1970s (J. Cooper, personal communication), and is currently the worldwide standard for screening medical equipment for bacterial contamination.

Blood from horseshoe crabs is obtained by collecting horseshoe crabs, extracting a portion of their blood, and typically releasing them alive. Crabs collected for LAL production are typically collected by hand or trawl. Crabs are inspected to cull out damaged or moribund animals, and transported to the bleeding facility. Following bleeding, most crabs are returned near the location of capture; however, some states allow facilities to bleed crabs caught by the bait industry prior to these crabs going to the market for sale (ASMFC 2004). Bled crabs that are caught and sold by the bait industry are counted against that state's bait harvest quota.

There are six companies along the Atlantic coast that extracted horseshoe crab blood during the time period examined by this assessment, 1999-2017: Associates of Cape Cod (MA), Limuli Labs (NJ), Lonza (MD, formerly Cambrex Bioscience), Wako Chemicals (MD, previously VA), Heptest Labs (VA), and Charles River Endosafe (SC). Addendum III requires states where horseshoe crabs are collected for biomedical bleeding to collect and report total collection numbers, crabs rejected, crabs bled (by sex) and to characterize mortality.

Estimates of biomedical harvest prior to 2004 are uncertain due to lack of standardized reporting; however, estimates from several sources are consistent, lending some credence to the estimates. The FDA estimated medical usage increased from 130,000 crabs in 1989 to 260,000 in 1997 (D. Hochstein, personal communication). This was consistent with other estimates ranging between 200,000 and 250,000 crabs per year on the Atlantic coast (B. Swan, personal communication; Manion et al. 2000). A survey of biomedical companies conducted by the Horseshoe Crab Technical Committee (TC) in 2001 indicated that about 280,000 crabs were bled in 1998 and 2000.

Since 2004, ASMFC has required states to monitor the biomedical use of horseshoe crabs to determine the source of crabs, track total harvest, characterize pre- and post-bleeding mortality, and determine fate (bait or release) of crabs used for biomedical purposes. As reported in annual compliance reports, the total number of crabs delivered to biomedical facilities has increased from 335,501 crabs in 2004 to 575,760 crabs in 2017 which includes crabs harvested as bait (Table 15).

Since 2011, biomedical companies along the Atlantic coast operate under a set of Best Management Practices (BMPs). These BMPs were a product of a collaboration between the LAL companies and ASMFC (Appendix A).

4.2.1 Biomedical Mortality Rate

For previous assessments and the annual compliance reports, mortality in the biomedical fishery (bait crabs excluded) was calculated in two steps. First, pre-bleeding mortality was determined from harvest and use reports provided by the biomedical harvesters. Second, a 15% mortality rate was applied to all bled crabs to determine the post-bleeding mortality. The two values were summed to provide a coastwide estimate of mortality from the harvest, transport, handling, and bleeding of horseshoe crabs used for biomedical purposes.

The 1998 FMP (ASMFC 1998a) established a biomedical mortality threshold of 57,500 crabs which, if exceeded, triggers the Management Board to consider action. The threshold has been exceeded every year since 2007 with the exception of 2016, although no management action has occurred. At the Management Board's request, the TC reviewed available literature and other information on mortality associated with the biomedical fishery (ASMFC 2008b). Despite limitations in study methodology and regional differences in results, the TC endorsed the use of a constant 15% mortality rate at that time.

The SAS developed a Biomedical Workgroup (WG) to review all available literature per region where biomedical facilities operate in order to reassess the 15% mortality rate for bled and released crabs. Each member assessed the studies in terms of how similar they were to the way the biomedical facilities in the region handle crabs and their adherence to the BMPs. The WG presented the results to the SAS, and the SAS also reviewed two additional submissions from Dr. James Cooper and Benjie Swan summarizing the literature, previous mortality rates, and a history of biomedical practices. The reports from the WG members as well as the additional submissions can be found in Appendix A.

The SAS discussed how to determine a biomedical mortality rate at length but with the paucity of long-term studies or studies that collaborate with biomedical facilities to mimic their procedure, it remained a challenging task. Despite having multiple studies and opinions from the SAS and some biomedical representatives on which studies should be considered, the SAS decided to expand Swan's approach from her submission of averaging among all biomedical studies without assigning any value to the studies (i.e., which are more in line with biomedical facilities and which are not) but apply a more rigorous statistical analysis than just a calculated mean.

In order to determine what mortality should be applied to crabs that were bled by the facility and released alive, the SAS compiled all the mortality rates and sample sizes (Table 16). Some studies had multiple rates from multiple treatments and each were treated independently. The rates and samples sizes were analyzed used R Markdown where an overall mortality rate distribution was found by simulating each reported rate as a separate random variable with its own binomial distribution. Then the expected values of the quantiles across the separate studies were calculated to determine a biomedical mortality of 15% with a 95% confidence interval of 4-30%. Therefore, the mortality rate of 15% remains unchanged for this assessment.

4.2.2 Sub-lethal Effects

There are few studies regarding the sub-lethal effects of bleeding horseshoe crabs. Anderson et al. (2013) evaluated the behavioral and physiological impact of biomedical harvest on 28 female horseshoe crabs. The results showed similar mortality rates as previous bleeding studies (18%) but also showed that bleeding decreased the horseshoe crabs activity levels, changed the expression of circatidal rhythms, and altered the amounts of hemocyanin in their blood which may have immune function implications. The study concluded that bleeding horseshoe crabs may decrease female fitness, but it did not follow Best Practices currently used by biomedical facilities and may not reflect sub-lethal effects of the industry.

A University of New Hampshire master's thesis by Owings (2017) focused on determining the effects of bleeding on the behavior of horseshoe crabs, impacts on activity and hemocyanin levels, and reduction of the effects by using a food supplement. Comparing 14 bled and 14 control horseshoe crabs, the study found that bled crabs mated less post-release. Additionally, the author noted that awareness of the overall health and hemocyanin levels of individual horseshoe crabs and avoidance of bleeding already-stressed or sick horseshoe crabs decreases mortalities. It should be noted that the Best Practices agrees and stipulates that horseshoe crabs should be sorted during collection so that unhealthy crabs are returned to the water on site (Appendix 12.1). The thesis concludes by suggesting the industry consider using a dietary supplement before or after bleeding to improve the effects of altering the horseshoe crabs physiological status and survivorship. Similar to Anderson et al. (2013), this thesis did not follow Best Practices currently used by the biomedical facilities and may not reflect sub-lethal effects of the industry. Further research should be done to consider sub-lethal effects of biomedical bleeding that adhere to Best Practices.

4.2.3 Biomedical Effect on Survival

The SAS wanted to examine potential differences in recapture rates and survival rates of bled and unbled horseshoe crabs. Current biomedical companies that participate in the US Fish and Wildlife Service's cooperative horseshoe crab tagging program include Lonza and Wako Chemicals. For the tagging study, both companies catch crabs off the coast of Maryland and Virginia mainly via trawl. While most of the other tagging partners tag crabs as they are spawning on beaches, there are additional trawl-caught crabs that are tagged (Table 17). The tagging programs that have captured horseshoe crabs with a trawl include: Maryland Dept. of Natural Resources (MDDNR), North Carolina Cooperative Research Cruise (NCCRUISE), New York Department of Environmental Conservation (NYDEC), Sacred Heart University (SHU), and Virginia Tech (VATECH).

The SAS explored two approaches for preliminary analyses of bleeding effects based on tagging data. For the first approach, the SAS summarized trawl captured and tagged crabs, in order to reduce bias of capture and/or resight probabilities that may occur between hand-captured and trawl-captured horseshoe crabs (Table 17 and Table 18). When horseshoe crabs are recaptured, their disposition is either alive, dead, or unknown. Unknown disposition occurs when a tag is found and it is not attached to a horseshoe crab carapace. Comparisons were made for the percent of reports for alive, dead, and unknown dispositions for bled and unbled, male and female crabs based on the number of years at large (YAL) for the individual crab. Only years where there were greater than 10 total recaptures were included (Table 19 and Table 20).

For the second approach, Cormack-Jolly-Seber (CJS) capture recapture models were fit for the subset of data tagged and released in the coastal region of Delaware, Maryland, and Virginia from 1999 to 2017. All observations regardless of capture and disposition were included in the capture history matrix. This allowed for sufficient data to fit the complex models while controlling for geography because nearly all tagged and bled crabs were released in the coastal Delaware, Maryland, and Virginia geographic area. There were 77,436 tagged animals with known sex and bleeding status: 8,449 unbled females, 20,435 bled females, 14,998 unbled males, and 33,554 bled males. Models, which were fit using RMark, included covariates for sex, bleeding status, and time for apparent survival (Phi) and capture probability (p).

4.2.3.1 Results

4.2.3.1.1 Trawl Captured and Tagged Crabs

There was a higher proportion of unbled horseshoe crabs reported as alive over time for both males and females (Figure 7). The greatest difference in recapture rates appears to be within the first year of release (0 YAL), as the rates generally become more similar with time. This trend also occurred with horseshoe crabs reported as dead (Figure 8). Again, it appears the effect of bleeding may be greatest within the first year of release, as the number of dead reports sharply declines between zero and one year at large, after which there is a steady increase over time for both bled and unbled horseshoe crabs.

There may also be a difference between sexes, as males appear to be captured alive at a higher rate than females (Figure 7), regardless if those males or females were bled. Males are also reported as dead at a lower rate than females (Figure 8).

Bled crabs (both male and female) are reported as unknown at a higher rate than unbled crabs (Figure 9). As time at large increases, the rate of bled female crabs reported as unknown increased from 27% (0 YAL) to 65 % (8 YAL). Bled males reported as unknown also increased, albeit at a lower rate than bled females. There was not much change in the number of unbled crabs reported as unknown for either males or females (Figure 9). It is likely that unknown reports are a combination of both tag loss and mortality.

4.2.3.1.2 Cormack-Jolly-Seber Model

The best fitting models included group-level effects on apparent survival due to bleeding and sex (Table 21). Survival also varied with time; however, year-specific survival was not estimable for many of the years. Thus, years were binned into periods defined by 2, 3, or 4 consecutive years and estimated average survival over the multiple year periods. The model with the binned 3-year periods fit best (Table 21). The estimated apparent survival was higher in most time intervals for crabs that had been bled, particularly for females (Table 22). On average, females had a lower survival rate than males, but the difference was higher for unbled crabs (70% for females and 73% for males) then for bled crabs (75% for females and 76% for males).

4.2.3.2 Discussion and Recommended Next Steps

Preliminary analysis presents some evidence for a short-term reduction in survival due to bleeding based on first year returns. In contrast, annual survival considering multiple years does not indicate a reduction in survival due to bleeding. Rather the multiyear estimates indicate higher survival for bled crabs compared to unbled crabs tagged and released in the coastal Delaware, Maryland, and Virginia geographic area. The pattern of higher survival for bled crabs could be due to confounding factors related to local harvest pressure on unbled crabs tagged on coastal beaches in the fishery or due to the culling of biomedical catches for selection of high condition individuals. Biomedical culling could result in biomedically tagged individuals representing a healthier subset of the overall population and thus having higher survival, all else equal.

These are preliminary analyses, and the SAS recommends continued evaluation of the tagging data by fitting capture-recapture models that include a short-term (1 year) bleeding effect, account for spatial distribution of harvest pressure, account for capture methodology, and account for disposition of recaptured tagged individuals. Potential methodological approaches include use of time-varying individual covariates to indicate which crabs are 1 year from bleeding and use of hierarchical models to estimate interannual variation in survival within time periods defined by major regulatory changes.

4.2.4 Biomedical Data Estimation

For this assessment, the SAS was tasked with evaluating the biomedical collection and mortality by region and use the mortality associated with biomedical bleeding in the modelling approach. In order to use the data regionally (by sex in some cases), consider the full range of biomedical mortality, and extend the time series, some estimation from the data set had to be performed prior to inclusion in analysis. When assessing the biomedical harvest by region, as opposed to coastwide, the data becomes confidential (*see Statement, page iv*) and therefore some information has been removed from this public document.

4.2.4.1 Methods

Data for the biomedical use of horseshoe crabs is reported to the Commission annually in state compliance reports. Under Addendum III, states are required to report biomedical collections by month and sex, along with the number or percent of observed mortality up to the point of release, collection method, disposition of bled crabs, and condition of holding environment of bled crabs prior to release (ASMFC 2004b). Clarity of reported information has improved throughout the years, and the information is now requested using a standardized template for data entry. To include the most extensive and accurate information possible, states were requested to resubmit biomedical data, including years prior to reporting as required in Addendum III, as available. This also gave states the opportunity to confirm or update information that may have been preliminary at the time of submission for past compliance reports. Discrepancies with previous reports were confirmed by the states in coordination with biomedical facilities.

Data on biomedical use of horseshoe crabs were available for 1999-2017, but the amount, quality, and completeness varied. Within this timeframe several facilities had years of missing information on collections, observed mortality, number of crabs bled, and sex ratio of crabs caught and released solely for biomedical use (biomedical-only), i.e. those that did not enter the bait market after being bled. Mortality of crabs that entered the bait market after being bled is included in bait landings, not in biomedical mortality. To extend the time series of all facilities and account for biomedical mortality in as many years as possible, missing years were estimated based on available data. Biomedical company representatives and state permitting records were consulted to confirm whether and which facilities were operating during years without data.

To account for potential annual trends in the biomedical market as a whole, annual collections of biomedical-only crabs from states with incomplete time series from 1999-2017 were regressed against those with complete time series. Regressions were only conducted for relevant years, when data were reported and had the same facility or facilities operating as years requiring estimation. One state requiring estimation only had two years of data relevant to the missing years, thus a regression could not be conducted and values for missing years were estimated as the mean of the two years of relevant, available data. Relationships between facilities or averages of available years were only used to estimate collections of horseshoe crabs used solely for biomedical use. They were not used to estimate numbers observed dead, bled, or sex ratios when such information was missing. These estimates were made based on state-specific data as described below.

Annual state percentages of collected biomedical-only crabs that were observed dead during the biomedical bleeding process (capture to release) were calculated for all years when such data were available. Years when these data were not available were estimated as state averages of relevant reported annual percentages observed dead multiplied by reported or estimated collection numbers.

Annual state percentages of collected biomedical-only crabs that were bled were calculated for all years when such data were available. Years when these data were not available were estimated as state averages of relevant reported annual percentages bled multiplied by reported or estimated collection numbers.

Annual state sex ratios of biomedical-only crabs collected were calculated for all years when such data were available. Sex-specific collections for unreported years or crabs reported as unknown sex were estimated as relevant state average annual sex ratios multiplied by unsexed, reported or estimated collection numbers. Sex ratios estimated for collections are assumed to also be reflective of later stages and data for the biomedical process (e.g. crabs bled, observed mortality, post-bleeding mortality).

4.2.4.2 Results

Collection data were available for 101 (89%) of the 114 state-year combinations considered (Table 23). Sex, bleeding, and observed dead proportions were more available later in the time series. Sex was not reported for every stage of the biomedical process. Thus, all collection, bled, observed dead, and total mortality numbers are assumed to have the same sex ratio within each state-year combination.

Two significant relationships were observed for collection numbers of states requiring imputation with those of states with full time series of data from 1999-2017 (Figure 9). These relationships were used to estimate collections in missing years when collections were known to have been conducted.

Annual biomedical collections trend up early in the time series to a peak in 2011 (Figure 11). From approximately 2010 through 2017 collections have been fairly stable, outside of a significant, single-year decrease in 2016. This decrease was due to known, temporary changes in production. More typical collection numbers resumed in 2017.

The average annual proportion of collected crabs observed dead during the biomedical process and proportion bled had little variation by state and facility (Table 24). All facilities observed mortalities less than 10% while crabs were in their possession, with all currently operating facilities observing mortalities less than 4%. Most states/facilities bled over 90% of crabs collected.

The bleeding mortality estimate from the meta-analysis of bleeding studies (15%) was applied to numbers of bled crabs to estimate bleeding mortality. This was added to the number of crabs observed dead during the biomedical process to estimate the total mortality attributable to biomedical use (Figure 12). As Delaware Bay was the only region in which sex-specific mortality information was used to model the population, these mortality estimates are specified in Figure 12. These mortality estimates include apportioning of Virginia and Maryland crabs, with 35% and 51% of crabs from each state, respectively, being of Delaware Bay origin. These percentages are based on genetic population structure findings (ASMFC 2012).

4.2.5 Biomedical Biological Data

Sex ratios varied considerably among facilities (Table 25). These sex ratios are likely not representative of population sex ratios in collection areas, as gear selectivity and culling of crabs less likely to be selected for bleeding would alter these ratios. Some facilities have size-based criteria that exclude smaller individuals, making females, the larger of the sexes, more likely to be bled. Additionally, some facilities collect crabs by hand, which allows greater ability to select for large females than other gears, such as a dredge or trawl. Time series of sex ratios indicate greater use of male crabs more recently than in the past.

4.2.6 Biomedical Data to Support Modelling Efforts

The bleeding mortality estimate and 95% confidence limits from the meta-analysis of bleeding studies (15%; [4%, 30%]) were applied to numbers of bled crabs to estimate bleeding mortality. Bleeding mortality was added to the number of crabs observed dead during the biomedical process to estimate the total mortality attributable to biomedical use (Figure 12, Table 26). Biomedical mortality accounted for less than 20% of coastwide mortality resulting from directed (bait and biomedical) use of horseshoe crabs in all years (Table 27). The percent of mortality attributed to the biomedical industry did vary by region, but is **CONFIDENTIAL** (Table 28). Annual sex-specific mortalities were estimated (Figure 12, Table 29) and used as inputs for the catch survey analysis, modeling the Delaware Bay population. These mortality estimates include apportioning of Virginia and Maryland crabs, with 35% and 51% of crabs from each state, respectively, being of Delaware Bay origin.

4.3 Commercial Discards

4.3.1 Northeast Fisheries Observer Program

4.3.1.1 Program Description

Discard information from observed commercial fishing trips was obtained from NMFS' Northeast Fisheries Science Center's (NEFSC) Northeast Fisheries Observer Program (NEFOP). The NEFOP program collects data on harvested and discarded catch, gear, effort, and species' lengths and weights using trained fishery observers from Maine to North Carolina. The total catch and a subsample of the total catch from each observation (e.g., towed trawl net) are weighed. The observer program is mandatory for federally-permitted vessels which are selected at random for observation during fishing trips. The program began in 1989 but data on horseshoe crab was available beginning in 2004. Horseshoe crab landings and observed discards were used to develop discard estimates from gillnets, trawls, and dredges in the Delaware Bay states for use in the catch survey analysis (CSA). Estimates for the other regions were attempted but lacked sufficient sampling to produce reliable estimates. See the NEFOP website for additional details about the program (http://nefsc.noaa.gov/fsb/program.html).

4.3.1.2 Methods

The NEFOP data set included all landings from observed trips, including those where no horseshoe crab were encountered, as well as horseshoe crabs discarded and horseshoe crabs kept, all in pounds (Figure 13 and Figure 14). NEFOP observer data were used to develop annual ratios of observed discarded horseshoe crab to observed landings of all species by gill nets, bottom trawls, and dredges from the Delaware Bay states (Delaware, New Jersey, Maryland, and Virginia) for 2004-2017. Ratios were then applied to reported gill net, bottom trawl, and dredge landings of all species from those states for 2004-2017 as queried from the Atlantic Coastal Cooperative Statistics Program (ACCSP; Figure 15) warehouse to estimate total discards of horseshoe crab. Some landings were not available at the gear level ("NOT CODED"). These landings were partitioned into trawl, gillnet, and dredge landings by calculating the annual proportion of landings by these gear categories and then these proportions were applied to the "NOT CODED" landings. Gears that were categorized as "trawl" or "gill net" but are unlikely to capture horseshoe crabs, such as midwater trawls or anchored and drift floating gill nets, were removed from the analysis.

The annual ratios by major gear type were calculated as the ratio of the mean discards of horseshoe crab per observation (i.e., tow or net set), in pounds, to the mean landings of aggregated species per observation, also in pounds (Equation 1).

$$Equation\ 1: R = \frac{\overline{D}}{\overline{L}} = \frac{\sum_1^n D_i}{\sum_1^n L_i}$$

This ratio estimator includes all observations with observed landings of any species, including those where no horseshoe crab were discarded. The variance of the ratio estimator was calculated with Equation 2 (Pollock et al. 1994).

$$Equation\ 2: Var(R) = \frac{1}{n(n-1)\overline{L}^2}\left(\sum_1^n D_i^2 + R^2\sum_1^n L_i^2 - 2R\sum_1^n D_i L_i\right)$$

It was assumed that discarding rates during observed trips were representative of overall discarding rates in these fisheries. Small sample sizes of positive observations precluded developing ratios at finer resolution (e.g., by state or season).

For trawls, annual mean weights were calculated as the total number counted from subsamples divided by the total subsample weight and were applied to the discard estimates in weight to derive discard estimates in numbers. In years with no observer data, averages of all the years combined were used. For gill nets and dredges, there was not sufficient biological sampling to calculate the mean weight of horseshoe crabs caught as bycatch in the gear. The SAS used the state-generated conversion factors of 1 pound for male horseshoe crabs and 2.67 pounds for female horseshoe crabs. Based on commercial biological sampling sex ratio of 48% female horseshoe crabs, the conversion factor of 1.8 pounds per horseshoe crab caught as bycatch in the dredge and gill net gears were used to convert from pounds to numbers.

Ratios estimates, variances, and discard estimates by gear are in Table 30-Table 32. A discard mortality rate of 50% was assumed for both gillnet and trawl discards of horseshoe crab due to the effects of being stuck in a gill or trawl net for extended periods of time or tows. The TC discussed that the trawl discard mortality is likely lower than 50% based on field observations, maybe even as low as 5% (S. Doctor, personal communication). The TC chose to maintain the rate at 50% to be precautionary since the discard estimates are likely biased low since they do not account for biomedical trips that are known to sort and discard catch at sea and observed trips target some species other than horseshoe crabs and may handle crabs differently. A discard mortality rate of 5% was assumed for dredge discards of horseshoe crab (D. Smith, unpublished data). These mortality rates were developed from SAS and TC discussions and members' field experience due to a lack of information about discard mortality rates from various gears for horseshoe crabs. For use in the female-only catch survey analysis, the 48% female sex ratio was applied to the dead discard numbers.

4.3.1.3 Results

Based on the data from NEFOP for the Delaware Bay region, observed landings of all species were variable (Figure 14) and most observed trips were in New Jersey and Virginia and few trips were observed in Maryland and Delaware. Of all the observed trips in the Delaware Bay region, 45% landed scallops, 16% landed short-fin squid, 7% landed Atlantic mackerel, 6% landed summer flounder, 6% landed Atlantic long-fin squid, and the remaining species comprised <5% the observed landings by weight. Horseshoe crab landings comprised <1% of the total observed landings in the data set. From 2004-2017, 51% of observed fishing trips used dredges, 46% used trawls, and 3% used gill nets.

Pounds of kept and discarded horseshoe crabs from observed fishing trips in the NEFOP data set was variable but generally increased in the Delaware Bay region from 2004-2017 (Figure 13). The increase in discards could in part be due to the male-only harvest which began in the 2014 fishing season through the present and the closure of New Jersey's horseshoe crab fishery in 2007 or be an artifact of sampling, particularly since Maryland and Delaware have fewer trips observed that encounter horseshoe crabs compared to New Jersey and Virginia. From 2004-2017, 96% of horseshoe crabs encountered on observed trips in New Jersey were discarded, 81% in Virginia, and 24% in Maryland. Delaware did not have enough encounters with horseshoe crabs to make any generalizations, but of the 136 pounds caught in observed trips in the state, 100% were discarded.

Total landings from gill nets, trawls, dredges, and not coded gears of all species by state in the Delaware Bay region from ACCSP (Figure 15) indicated stability throughout the time series with a slight decrease over time. Average landings from the Delaware Bay region for all fisheries was 558 million pounds from 2004-2017. The majority of all-species landings in the region were from New Jersey (which has had a moratorium on horseshoe crab bait harvest since 2006) followed by Virginia, Maryland, and then Delaware.

The ratio estimators varied by gear and year (Table 30-Table 32). Estimated discards of horseshoe crab also varied by gear and year (Figure 16-Figure 18) where dredges discarded the most horseshoe crabs and trawls discarded the least. Conversely, trawls were the most subsampled trips for weights used to convert discards in pounds to discards in numbers. Discards from dredges increased remarkably in 2014-2017 due to several observed trips with high discarded horseshoe crabs in those years. Estimated discards from gill nets and trawls followed similar patterns with peaks in 2011 and 2013 and decreased discards from 2014-2017. Estimated discards for all three gears combined showed an increase of discards throughout the time series (Figure 19), although those estimates were highly influenced by the dredge discard estimates.

Mortality rates of 50% for trawls and gill nets and 5% for dredges were applied to the estimated numbers of horseshoe crabs discarded to get estimated number of dead horseshoe crabs attributed to discards in the Delaware Bay region. The number of dead horseshoe crabs from discards was 101,100 on average and ranged from a low of 21,937 in 2005 to a high of 216,518 in 2013 (Figure 20). In order to be used in the CSA model, discard estimates need to be proportioned by sex. Few horseshoe crabs were sexed throughout the time series (n=209) and those that were sexed were collected primarily from the trawl fisheries. The SAS instead referred to sex data from commercial sampling programs in the Delaware Bay states to derive a sex ratio of 48% female. Applying that percentage to all years of discards data resulted in an average of 48,527 female horseshoe crabs dead from discards in all gears, ranging from 10,530 in 2005 to 103,928 in 2013 (Figure 21).

4.4 Recreational

There is no recreational fishery for horseshoe crabs. Some states allow a minimal number of crabs to be retained for personal use. Landings of this type are not quantified.

5 INDEPENDENT DATA SOURCES

5.1 Stock Assessment Subcommittee Criteria

The SAS established the following set of criteria for evaluating data sets and developing indices of relative abundance for horseshoe crab:

1. Time series: Ideally, the time series should be 20 years long to account for the lifespan of horseshoe crab. Recognizing that would eliminate many surveys, the SAS recommended at least 10 years of data be available in a survey.
2. Survey design: Surveys with statistical designs are preferred, such as surveys with random stratified sampling.
3. Gear: Surveys should operate with gear that is capable of catching horseshoe crabs and to which horseshoe crabs are available.
4. Temporal and spatial coverage: Only surveys that operate during a time and place where horseshoe crab are available for capture should be considered. Examining the precision or proportion of zero catches of horseshoe crabs in a survey can be tools for evaluating this.
5. Methodology: Survey methodology should be consistent throughout the time series or changes should be able to be accounted for in the standardization process.

The SAS evaluated several data sets for developing indices of abundance for horseshoe crab. After some preliminary analysis, nine were rejected for various reasons as indicated in Table 33 and abundance indices were developed from the remaining surveys. When possible, indices of abundance were developed by season and sex. There were also efforts to develop surveys by stage to support modelling approaches; however, stage-based indices were not able to be developed due to insufficient data. The SAS explored using nominal and GLM standardized indices, but encountered issues with these methods due to the high proportion of zero catches in many of the tows in the surveys. Therefore, all indices were developed using the delta distribution for the mean and variance for each year of a survey to specifically take into account the number of zero catches (Pennington 1983). Maps of the surveys were included when they were supplied from the data provider. A summary of the gear used and size range of horseshoe crabs caught for the surveys included in this assessment can be found in Table 34 and Figure 22, as requested by the Peer Review Panel.

5.2 Surveys

5.2.1 New Hampshire Spawning Beach Survey

5.2.1.1 Survey Design and Methods

The New Hampshire spawning survey operated for 11 years from 2002 to 2012. During the months of May through September five beach locations were surveyed in 150, 300, or 450 meter transects.

5.2.1.2 Biological and Environmental Sampling

All horseshoe crabs visible in the transects were counted and sexed. Prosomal widths were taken when possible. Eggs were recorded using a presence/absence description. Temperature, weather, cloud cover, wave action, moon stage, and salinity were recorded for environmental factors.

5.2.1.3 Evaluation of Survey Data

A spring (May and June) index was developed from this survey. Male and female indices were calculated separately for this survey because 100% of the individuals recorded were sexed. July, August, and September were dropped due to high occurrences of zero sightings. The subset data resulted in 57% zero sightings over the entire time series.

5.2.1.4 Abundance Index Trends

Abundance peaked for both male and female horseshoe crabs in 2004. Between 2005 and 2012 abundance remained relatively low (Figure 23 and Figure 24).

5.2.2 Massachusetts Resource Assessment Trawl

5.2.2.1 Survey Design and Methods

The Massachusetts Resource Assessment Trawl is an otter trawl survey which began operating annually during the months of May and September in 1978. The study area is stratified based on five bio-geographic regions and six depth zones (Figure 25). Sampling intensity is approximately 1 station per 19 square nautical miles. A minimum of two stations are assigned to each stratum. A standard tow of 20-minute duration at 2.5 knots is attempted at each station during daylight hours.

5.2.2.2 Biological and Environmental Sampling

The total weight and length-frequency of each species are recorded directly into Fisheries Scientific Computer System (FSCS) data tables. Horseshoe crab sex identification and prosomal width measurements began in 1982. Temperature and depth are recorded for each tow.

5.2.2.3 Evaluation of Survey Data

Two fall (September-October) indices were developed from this survey to reflect the differences in the horseshoe crab populations north and south of Cape Cod. The data was split into north and south based on strata so that the north was zones 4-5 and the south was zones 1-3 (Figure 25) and subset to include only tows occurring at depths between 6-14 meters where horseshoe crabs were predominantly encountered. Tows that occurred outside of these parameters had very low frequencies of horseshoe crab catch. Throughout the time series and strata, fall tows were 10% positive for horseshoe crab presence. Nearly all crabs were sexed after 1982, however the sample sizes were still small and the SAS could not justify using such small numbers to calculate a sex based index from this survey.

5.2.2.4 Abundance Index Trends

Horseshoe crab abundance varied by region. In the North Cape index (Figure 26), abundance peaked in 1980 and declined since then although the last four years exhibited mid-range abundance. There were multiple years in the survey that did not encounter any horseshoe crabs. The South Cape index shows a different pattern from the North Cape index where it begins relatively low and then experiences high abundance in 2016-2017 (Figure 27).

5.2.3 Rhode Island Costal Trawl Survey (monthly segment)

5.2.3.1 Survey Design and Methods

The Rhode Island Coastal Trawl Survey began operating in 1990. The monthly segment of the survey samples 13 fixed stations, 12 inside Narragansett Bay and 1 in Rhode Island Sound (Figure 28). At each station, an otter trawl is towed for twenty minutes.

5.2.3.2 Biological and Environmental Sampling

All catch is identified by species then measured and weighed. Horseshoe crab sex and prosomal width recordings began in 1998. Temperature, depth, salinity, and weather conditions are all recorded for environmental factors.

5.2.3.3 Evaluation of Survey Data

A spring (April - July) and fall (August – October) index was developed from this survey. The data was subset to include only tows with recorded bottom temperatures greater than 10.1°C thus eliminating a large proportion of zero catch tows. Ultimately, the SAS decided to use only the fall index due to a higher rate of percent positive tows. Throughout the time series, fall tows were 22% positive while spring tows were 13% positive. Sexes were kept combined as there are not enough crabs caught in this survey to support sex specific indices.

5.2.3.4 Abundance Index Trends

Horseshoe crab abundance has remained relatively steady according to this survey. Throughout the time series, abundance remains relatively low between 0.5 and 1.5 crabs per tow (Figure 29).

5.2.4 Connecticut DEEP Long Island Sound Trawl Survey

5.2.4.1 Survey Design and Methods

This survey began operation in 1984 and continues to sample Connecticut and New York waters during the spring (April – June) and fall (September – October) seasons. The sampling area is divided into 1x2 nautical mile sites with each site assigned to one of 12 strata defined by depth interval (0-9.0 m, 9.1-18.2 m, 18.3- 27.3 m, or 27.4+ m) and bottom type (mud, sand, or transitional) (Figure 30). Forty samples are collected each month resulting in 200 sites annually. It should be noted that this survey did not operate in the fall of 2010.

5.2.4.2 Biological and Environmental Sampling

All catch is identified by species and weighed in aggregate by species. Horseshoe crab counts began in fall 1997 while lengths and sex records began in fall 1998. Depth, salinity, temperature, and sediment type are recorded for environmental factors.

5.2.4.3 Evaluation of Survey Data

A spring (April – June) and fall (September – October) index were developed from this survey. The data was subset to include only tows with depths less than 43.5m and bottom temperatures greater than 4.3°C thus eliminating a large proportion of zero catch tows. Ultimately, the SAS decided to use only the fall index due to a higher rate of percent positive tows. Throughout the time series, fall tows were 39% positive while spring tows were 29% positive. Sexes were kept combined as there were not enough sexed crabs to support separate indices.

5.2.4.4 Abundance Index Trends

Abundance of horseshoe crabs in this survey is relatively high compared to surveys operating with similar gears. Horseshoe crabs caught per tow remained fairly steady between two and four individuals until 2010 when numbers dropped to consistently catching between one and two horseshoe crabs per tow (Figure 31).

5.2.5 New York DEC Peconic Small Mesh Trawl Survey

5.2.5.1 Survey Design and Methods

This survey began operating in 1987 and continues to sample 16 randomly selected stations in the Peconic Bay on a weekly basis from May through October. The survey area was divided into 77 sampling blocks with each block measuring 1' latitude and 1' longitude (Figure 32). The 4.8 meter semi-balloon shrimp trawl net is towed for 10 minutes at approximately 2.5 knots using a 10.7 meter lobster style workboat.

5.2.5.2 Biological and Environmental Sampling

All catch is identified by species and counted. Horseshoe crab sex and prosomal width have been recorded since 1997. Temperature, salinity, depth, dissolved oxygen, and secchi disc readings are recorded for environmental factors.

5.2.5.3 Evaluation of Survey Data

Spring (May-July) and fall (August-October) indices were developed from this survey. Tows with missing salinity, salinity greater than 32.12 (unit), missing temperature, and temperatures less than 11°C were eliminated in attempts to use only data points with complete environmental information and a higher likelihood of catching horseshoe crabs. Both seasons of the survey were fairly similar in regard to total horseshoe crabs caught and percent zeros. Throughout the entire time series, the spring survey caught 7,270 crabs with 57% of tows catching zero horseshoe crabs. The fall survey caught 7,200 crabs with a 60% zero catch rate. The SAS decided to use the fall portion of the survey to remain consistent with regional time series usage of fall inclusion only. Sexes were kept combined as there were not enough sexed crabs to support separate indices. Throughout the entire time series only 34% of crabs were sexed.

5.2.5.4 Abundance Index Trends

Horseshoe crab abundance peaked in 1991 after which numbers have been steadily decreasing. Since 2004, horseshoe crab catch per tow has been consistently below one (Figure 33).

5.2.6 New York DEC Western Long Island Beach Seine Survey

5.2.6.1 Survey Design and Methods

The New York Seine Survey began operation in 1984 in Jamaica Bay (Figure 34), Manhasset Bay (Figure 35), and Little Neck Bay (Figure 36). Pre-2000 sampling was conducted 2 times per month during May and June, once a month July through October and then 2 times per month from May through October for 2000 – 2002. Currently, 5-10 seine sites are sampled in each bay on each sampling trip. From 1984 – 1998 a 500 ft x 12 ft seine with stretch mesh in the wings and stretch mesh in the bag was used for one sampling round generally in the spring. Currently a 200 ft x 10 ft beach seine with ¼ inch square mesh in the wings, and 3/16 inch square mesh in the bunt is being used. The seine is set by boat in a “U” shape along the beach and pulled in by hand.

5.2.6.2 Biological and Environmental Sampling

All finfish species are identified and counted. Starting in 1987, invertebrates were consistently counted. Since 1998, horseshoe crabs have been counted, measured, and sex has been identified. Environmental information (air and water temperature, salinity, dissolved oxygen, tide stage, wind speed and direction, and wave height) has been recorded at each station. Bottom type, vegetation type, and percent cover have been recorded qualitatively since 1988.

5.2.6.3 Evaluation of Survey Data

Two indices of abundance were developed from this survey based on geographic location: a Jamaica Bay index and a Manhasset and Little Bays index. The latter two Bays were combined due to proximity to each other. Horseshoe crabs were most reliably caught in all three regions in May and June, although the survey runs from April through November. Without subsetting, the survey had on average 30% positive tows but when restricted to the spring months the proportion positive tows increased to 45%.

5.2.6.4 Abundance Index Trends

The Jamaica Bay index of horseshoe crab abundance shows variable abundance through the 1990s with a lot of fluctuation between high peaks and low values in the 2000s (Figure 37). From 2006 through the terminal year, the index exhibits lower abundances of horseshoe crabs in this region. For the Manhasset and Little Neck Bays index, abundance was variable with some high values from 1987-2003 (Figure 38). After 2003, the index decreased dramatically and has remained low through 2017.

5.2.7 Northeast Area Monitoring and Assessment Program Trawl Survey

5.2.7.1 Survey Design and Methods

The Northeast Area Monitoring and Assessment Program (NEAMAP) Trawl Survey began sampling the coastal ocean from Martha's Vineyard, MA to Cape Hatteras, NC since the fall of 2007 (Figure 39). The survey area is stratified by both latitudinal/longitudinal region and depth. A four-seam, three-bridle, 400x12 cm bottom trawl is towed for 20 minutes at each sampling site with a target speed-over-ground of 3.0 kts. The net is outfitted with a 2.54cm knotless nylon liner to retain the early life stages of the various fishes and invertebrates sampled by the trawl. The survey conducts two cruises a year, one in the spring (April-May) and one in the fall (September-November). A total of 150 sites are sampled per cruise, except 160 sites were sampled in the spring and fall of 2009 as part of an investigation into the adequacy of the program's stratification approach.

5.2.7.2 Biological and Environmental Sampling

For each tow, the catch is sorted by species. Horseshoe crab are measured for prosomal width and sex when possible. A number of variables (profiles of water temperature, salinity, dissolved oxygen, and photosynthetically active radiation), atmospheric data, and station identification information are recorded at each sampling site.

5.2.7.3 Evaluation of Survey Data

A spring (April- May) and a fall (October) index were developed from this survey. Horseshoe crabs were caught in the fall with 56% positive tows and in the spring when there were 72% positive tows. The SAS decided to use the fall portion of the survey to be consistent with other surveys in the region. The fall portion of the NEAMAP survey was further split to develop two indices from this data set: a New York index (strata 3-5) and a Delaware Bay index (strata 8-11). Nearly half of horseshoe crabs caught in the survey were sexed, but due to the subset data there were not enough to justify developing sex-specific indices. Based on the prosomal widths provided, this survey catches primarily adults in the fall.

5.2.7.4 Abundance Index Trends

The survey of relative abundance of horseshoe crab in the New York portion of the NEAMAP survey began with high values in 2007-2008 and the lowest value in 2010 (Figure 40). The index was variable throughout the 2010s. For the Delaware Bay index developed from the fall portion of the NEAMAP survey, horseshoe crab abundance was variable with the highest abundance in 2009 and 2015 and the lowest abundance in 2013 (Figure 41).

5.2.8 New Jersey Ocean Trawl Survey

5.2.8.1 Survey Design and Methods

New Jersey's Ocean Trawl Survey has been operating since August of 1988 and collects samples during five survey cruises per year (30 samples in January, 39 samples each in April, June, August and October) in the nearshore ocean waters of New Jersey. It uses a three-in-one design, two-seam trawl net with forward netting of 12 cm stretch mesh, rear netting of 8 cm, and a 6.4 mm bar mesh liner in the cod end. The survey incorporates a random stratified design with sampling sites selected within 15 strata (Figure 42) with longitudinal boundaries consisting of 5, 10 and 15 fathom isobaths. The latitudinal boundaries are identical with the NMFS groundfish survey except the extreme southern and northern ends of the sampling area. These strata are further divided into blocks which are 2.0 minutes longitude by 2.5 minutes latitude for the midshore and offshore strata, and 1.0 minutes longitude by 1.0 minutes latitude for the inshore strata. The standard duration of each sample is a 20-minute tow.

5.2.8.2 Biological and Environmental Sampling

Catches are sorted to species level whenever possible, enumerated, weighed (gross weight per species) and measured for length/width (cm) data. Certain species are sexed and horseshoe crabs have consistently been sexed since 1999. Environmental data include depth (m), surface and bottom water temperature (degrees Celsius), salinity (0/00) and dissolved oxygen (mg/L) along with air temperature, wind direction and speed, weather conditions, wave height, and swell direction and height.

5.2.8.3 Evaluation of Survey Data

This survey catches mainly adult horseshoe crabs, and the SAS concluded that the paucity of juvenile crabs negated development of juvenile indices from this program. A spring/summer (April and August) and a fall (October) index were developed from this survey for female adult (>= 19 cm pw), male adult (possessing male pedipalps), and all horseshoe crabs combined. The indices for all horseshoe crabs combined used the years 1989-August 2018, while the sexed indices only used the years in which the crabs were consistently sexed (1999-August 2018). Overall, horseshoe crabs were caught slightly more often in the fall (58.5% positive tows) than in the spring (51.4% positive tows). This pattern continued for female adults (46.6% positive tows in the fall, 43.7% positive tows in spring) and male adults (40.0% fall positive tows, 38.1% spring positive tows).

5.2.8.4 Abundance Index Trends

For all horseshoe crabs combined in the spring, abundance was higher in the years 1990 through 2005 peaking at 1997 and 1999, before falling between 2006 through 2010. After 2011, the abundance has been on an upward trend with a survey-high peak in 2013 (Figure 43). However, the fall index shows high abundance from 1989-1992, then fluctuations at lower levels thereafter (Figure 44). The spring index for adult female horseshoe crabs shows a trend similar to the spring index for all crabs: higher abundance in the early years followed by declines through 2010 before trending higher through 2017 with a peak during 2013 (Figure 45). This pattern is also seen in the spring indices for adult males (Figure 46). The fall indices for the sexed crabs generally followed the same patterns as for all horseshoe crabs combined but with subtle differences. While the fall indices for adult females (Figure 47) showed a peak in 2004 and adult males (Figure 48) showed peaks a year later in 2005. All of the fall indices showed fluctuating abundances with steep decreases for 2017 after a rise of varying scales in 2016. All the indices for spring and fall showed noticeable declines in 2010.

All the indices showed generally increasing trends after 2012, though the fall indices all showed steep declines for 2017. This result may have been an artifact of the timing of this survey cruise missing the fall migrations of this species.

5.2.9 New Jersey Surf Clam Dredge Survey

5.2.9.1 Survey Design and Methods

New Jersey's surf clam dredge survey has been operated by New Jersey's Bureau of Shellfisheries since 1988, with horseshoe crab catches recorded since 1998. The sample area includes the state waters component of New Jersey's ocean waters from Cape May north to the Shrewsbury Rocks off Monmouth County, NJ. The standard sample duration is a 5-minute tow. The gear type is a commercial hydraulic dredge equipped with a 72" knife and 2" X 2" steel mesh liner on the dredge floor. Through 2012, this survey collected on average 328 samples per year with the sampling conducted between June and August. In 2013, due to funding and staff shortages, the number of samples fell by more than half to 122 samples, with the subsequent years' averaging about 165 samples each. Due to this change in methodology, only the data from 1998 through 2012 were used for this assessment.

5.2.9.2 Biological and Environmental Sampling

This survey is focused on surf clam abundance and size data collection, but also records catch, sex and prosomal width (mm) information on all horseshoe crabs caught. A Peterson grab sample is taken at the end of each sample tow for bottom sediment analysis.

5.2.9.3 Evaluation of Survey Data

As this survey catches mainly adult horseshoe crabs, no juvenile indices were developed from this program. With the timing of the survey occurring mainly in the summer months of June, July and August, only one index (considered to be spring in this assessment) was developed for each of the following categories: all combined, female adult (> 180 mm pw), and male adult (possessing male pedipalps) horseshoe crabs. Positive tows for all horseshoe crabs combined made up 31.5% of all samples. The sexed indices all followed a pattern of higher positive tows for females than males: adults (21.7% female, 11.1% males).

5.2.9.4 Abundance Index Trends

For all horseshoe crabs combined, the abundance index trended upward from 2002 through 2012 after generally decreasing from 1998 to 2001 (Figure 49). The index rose above all the previous years' values in 2006 and remained above that level through 2012. The index for the adult females (Figure 50) shows fluctuations trending lower from 1998 through 2003 then rising from 2004 through 2007. There was a drop to an intermediate level of abundance in 2008 followed by another fluctuating but general trend upward through 2012. The abundance index for the adult male (Figure 51) showed generally declining fluctuations from 1998 through 2005. The index then increased through 2007, decreased to an intermediate level through 2009 before entering a general increase from 2010 through 2012.

5.2.10 Delaware Fish and Wildlife Adult Trawl Survey

5.2.10.1 Survey Design and Methods

Delaware has conducted the Adult Trawl Survey in three discrete time spans: 1966 – 1971, 1979 – 1984, and continuously since 1990. This assessment used the data from the latest time period (1990 – 2017) and was updated through 2018 for the spring portion of the survey. The survey samples 9 fixed stations monthly from March through December for an annual total of 72 samples. This survey uses a 30 foot, 2-seam otter trawl with a 3 inch stretch mesh in the wings and body and a 2 inch stretch mesh in the cod end. The sampling area includes the Delaware waters of the Delaware Bay at depths ranging from 7 – 35 m (Figure 52). The standard duration for each sample is 20 minutes at a speed of 3 knots.

5.2.10.2 Biological and Environmental Sampling

Catch is sorted to species level, enumerated, and weighed (aggregate per species) and measured for length/width to the nearest 0.5 cm. Horseshoe crabs are sexed, enumerated and measured (prosomal width). Environmental data include tide stage, water temperature (degrees Celsius), salinity (ppt), cloud cover and depth (m).

5.2.10.3 Evaluation of Survey Data

As this survey catches mainly adult horseshoe crabs. Spring (March through August) and fall (September through December) indices were developed from this survey for the following categories of horseshoe crabs: all adults combined, adult female, and adult male. Overall, the proportion positive tows varied little between the seasons with the spring showing slightly higher values than the fall (43.6% spring, 39.5% fall). A similar pattern was seen for males (36.8% spring, 36.6% fall). The pattern was reversed for females (32.6% spring, 33.0% fall). Another pattern emerged of higher respective proportion positive tows values for the males than for the females.

5.2.10.4 Abundance Index Trends

For all adult horseshoe crabs combined in the spring (Figure 53), abundance was highest in 1990 and 1991, and then a downward trend began from 1992 through 1995. It rebounded with an increase in 1996 before continuing the general trend downward through 2005. There was a moderate increase in 2006 and 2007 before dropping to low abundance levels from 2008 through 2013. Since 2014 there has been a generally upward trend with a steep increase in 2018. A similar pattern was seen for the spring indices of adult females (Figure 54) and males (Figure 55).

The fall index for all adult horseshoe crabs combined (Figure 56) showed a higher level abundance in 1990 and 1991, then dropped in 1992 and began fluctuating between low and intermediate levels through 2005. Abundance climbed steeply to a high level in 2006 before dropping again to previous low levels from 2007 through 2012. The index began a general increase from 2013 through 2015 before jumping to higher levels culminating in the time series high in 2017. A similar pattern was seen for the fall index for adult females (Figure 57) and males (Figure 58).

5.2.11 Delaware Bay Horseshoe Crab Spawning Survey

5.2.11.1 Survey Design and Methods

The ASMFC's FMP for Horseshoe crab (ASMFC 1998) required that the states of Delaware, Maryland, and New Jersey implement pilot horseshoe crab spawning surveys based on "standardized and statistically robust methodologies." In January 1999, the ASMFC convened a workshop that established a framework for such surveys in the Mid-Atlantic region. The framework built upon existing horseshoe crab spawning survey efforts by Finn et al. (1991) and Maio (1998). The survey began in 1999 and has continued through the present. Approximately 25 beaches are sampled in the Delaware Estuary during nighttime high tides in May-June. The goals are to provide an index of spawning activity and distribution in the region, increase the understanding of environmental factors on spawning activity and distribution, and promote public awareness of the role crabs play in shorebird dynamics. The survey has been shown to provide levels of spatial and temporal coverage essential for understanding trends in spawning activity (Smith and Michels 2006).

5.2.11.2 Biological and Environmental Sampling

The survey collects environmental data including water temperature, tidal height, wave height and biological data such as sex and spawning activity.

5.2.11.3 Evaluation of Survey Data

The SAS was primarily interested in this survey for the sex ratio data it provides in order to inform control rules in the Delaware Bay region. The SAS determined that this survey provides the most reliable data available for spawning beach sex ratios. For other data provided by this survey, the full annual reports are available at https://www.delawarebayhscsurvey.org/surveyreports/.

5.2.11.4 Sex Ratio Trends

Annual sex ratios from the spawning beach survey are available in Table 35. Current horseshoe crab harvest management strategies in the Delaware Bay area limit the harvest to predominantly male crabs. Concern was expressed that these strategies may cause spawning sex ratios (M:F) to drop and yet the sex ratio has increased in recent years. Annual sex ratios have ranged from 3.1:1 in 2001-2002 to 5.2:1 in 2017 over the course of the survey. M:F ratio in 2017 (5.2:1) was above the time series average (4:1).

5.2.12 Virginia Tech Horseshoe Crab Trawl Survey

5.2.12.1 Survey Design and Methods

The trawl survey conducted by Virginia Polytechnic Institute and State University (Virginia Tech) is the only survey available that is designed specifically to characterize the horseshoe crab population in coastal and lower Delaware Bay (Figure 59). The survey has operated from 2002-2011 and then again from 2016-2017 due to a lack of funding during the missing years. The survey area is stratified by distance from the shore and bottom topography. Tows are 15-minutes long and the survey only operates in the fall (mid-September-late October).

5.2.12.2 Biological and Environmental Sampling

All horseshoe crabs are counted and a subset are measured for prosomal width and identified by sex and maturity. Immature, newly mature, and mature crabs are differentiated in the data set.

5.2.12.3 Evaluation of Survey Data

This is the only survey specifically designed to catch and characterize the horseshoe crab population in its sampling region. The SAS decided to accept the indices as provided by Virginia Tech since they also used the delta distribution to model the mean and error of the annual catch.

5.2.12.4 Abundance Index Trends

The indices of abundance developed by sex and stage for horseshoe crabs in the Virginia Tech trawl survey can be found in Figure 60. Abundance varied by stage and sex, although there is a slight increase in abundance across the stages throughout the time series.

5.2.13 Maryland Coastal Bays

5.2.13.1 Survey Design and Methods

The 16’ otter trawl survey has been operating since 1989 and collects samples monthly in April through October in the coastal bays from the Delaware to the Virginia line at 20 fixed sites (Figure 61).

5.2.13.2 Biological and Environmental Sampling

All catch is identified by species and counted. Horseshoe crabs are sexed when possible and a prosomal width is measured. Tide stage, weather conditions, wind speed, depth, temperature, dissolved oxygen, and salinity are recorded for each sampling event.

5.2.13.3 Evaluation of Survey Data

A spring (April- May) and a fall (August-October) index were developed from this survey. Horseshoe crabs were more reliably caught in the spring with 14% positive tows than in the fall when there were 6% positive tows so the SAS decided to use only the spring portion of this survey in the assessment. Nearly all horseshoe crabs caught in the survey were sexed after the fall of 1993, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population in the region. Based on the prosomal widths provided, this survey catches primarily adults in the spring. The SAS abbreviated the survey to 1990-2017 due to high catches of horseshoe crabs that were not consistent with the following years and biased trend analyses. Maryland Department of Natural Resources supported the exclusion of the 1989 data point as well (S. Doctor, MD DNR, personal communication).

5.2.13.4 Abundance Index Trends

Abundance was high for 1994-1995, 2003, and 2010 and otherwise was relatively low (Figure 62).

5.2.14 North Carolina Estuarine Gill Net Survey

5.2.14.1 Survey Design and Methods

This floating gill net survey has been in operation since 2000 and samples in the Pamlico Sound and several river sites. Each region is overlaid with a one-minute by one-minute grid system (equivalent to one square nautical mile) and delineated into shallow (<6 feet) and deep (>6 feet) strata using bathymetric data from NOAA navigational charts and field observations (Figure 63). Gear is typically deployed within one hour of sunset and fished the next morning to keep soak times within 12 hours. Sampling initially occurred during all 12 months but was abbreviated in 2002 to no longer sample between December 15-February 14 due to low catches and unsafe working conditions.

5.2.14.2 Biological and Environmental Sampling

All horseshoe crabs caught in this survey are counted, measured for prosomal width, weighed, and sexed. Latitude, longitude, water temperature and salinity, and depth are recorded.

5.2.14.3 Evaluation of Survey Data

A spring (April- June) and a fall (August-October) index were developed from this survey. Horseshoe crabs were more reliably caught in the spring with 14% positive tows than in the fall when there were 5% positive tows, so the SAS decided to use only the spring portion of this survey in the assessment. Due to low catches of horseshoe crabs, depths over 3 m were excluded and only the Pamlico Sound region was used in this assessment. Nearly all horseshoe crabs caught in the survey were sexed, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population in the region. Based on the prosomal widths provided, this survey catches primarily adults in the spring. The survey encountered no horseshoe crab in the spring of 2000, the first year of the survey, so it was dropped from the analysis.

5.2.14.4 Abundance Index Trends

Horseshoe crab abundance was low from 2001-2007 and began to increase to the highest abundance in the time series in 2014 (Figure 64). The index began to decrease again after 2014 but still remains higher than the early part of the survey.

5.2.15 South Carolina Crustacean Research and Monitoring Survey

5.2.15.1 Survey Design and Methods

The Crustacean Research and Monitoring Survey (CRMS) has been operating in the Charleston Harbor and St. Helena, Port Royal, and Calibogue Sounds and since 1995. It samples monthly in the Harbor and in April, May, August, and December in the Sounds. The survey consists of 15 minutes trawls at each station. There was a vessel change in 2002 but the data was calibrated to accommodate that change.

5.2.15.2 Biological and Environmental Sampling

All catch for this survey is sorted and horseshoe crabs are counted, weighed, sexed, and measured for prosomal widths. The survey collects and reports latitude, longitude, water temperature, salinity, depth, and air temperature although not all variables are recorded consistently throughout the time series.

5.2.15.3 Evaluation of Survey Data

A spring (March-April) and a fall (August-December) index were developed from this survey. Horseshoe crabs were more reliably caught in the spring with 34% positive tows than in the fall when there were 22% positive tows, so the SAS decided to use the spring portion of this survey in the assessment. Data was subset to regions that encountered horseshoe crab. Nearly all horseshoe crabs caught in the survey were sexed, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population in the region. Based on the prosomal widths provided, this survey catches primarily adults in the spring.

5.2.15.4 Abundance Index Trends

The index of relative abundance of horseshoe crab developed from the CRMS indicated high abundance throughout the 2000s with lower abundance from 2010-2017 (Figure 65).

5.2.16 South Carolina Trammel Net Survey

5.2.16.1 Survey Design and Methods

The Trammel Net Survey has been operating monthly since 1995 and covers nine lower-estuarine strata along the coast of South Carolina (Figure 66). Each month, 10- 12 stations per stratum are chosen for sampling, although this number was not always achieved due to weather, tide, or time restrictions. Monthly sites were selected at random (without replacement) from a pool of 22-30 possible sites per stratum. Occasionally it was necessary to add new sites to the pool as others were lost due to changing coastal features (e.g., erosion, new docks).

5.2.16.2 Biological and Environmental Sampling

All catch for this survey is sorted and horseshoe crabs are counted, weighed, sexed, and measured for prosomal widths. The survey collects and reports depth, air temperature, water temperature, salinity, DO (1998 onwards), set duration, and tide.

5.2.16.3 Evaluation of Survey Data

A spring (March-May) and a fall (July-September) index were developed from this survey. Horseshoe crabs were more reliably caught in the spring with 13% positive tows than in the fall when there were 6% positive tows, so the SAS decided to use the spring portion of this survey in the assessment. Due to low catches of horseshoe crabs, depths over 2.2 m were excluded and data was subset to waterbodies that encountered horseshoe crab. Nearly all horseshoe crabs caught in the survey were sexed, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population in the region. Based on the prosomal widths provided, this survey catches primarily adults in the spring.

5.2.16.4 Abundance Index Trends

The index of abundance began relatively low in 1995 and began to increase in the late-2000s (Figure 67). The index reached its highest value in 2012 and decreased to another low in the terminal year of 2017.

5.2.17 Southeast Area Monitoring and Assessment Program

5.2.17.1 Survey Design and Methods

The Southeast Area Monitoring and Assessment Program (SEAMAP) South Atlantic Coastal Trawl Survey has been sampling from Cape Hatteras, North Carolina, to Cape Canaveral, Florida since 2001 (Figure 68). Trawls operate in the spring (early April-mid-May), summer (mid-July-early August), fall (October-mid-November). Stations are randomly selected from a pool of stations within each of 24 strata. The number of stations sampled in each stratum is determined by optimal allocation. A total of 102-112 stations are sampled each season (306-336 stations/year).

5.2.17.2 Biological and Environmental Sampling

Contents of each net are sorted separately to species and counted. Only total biomass is recorded for all other miscellaneous invertebrates (excluding cannonball jellies) and algae, which are treated as two separate taxonomic groups. Measurements of finfish are recorded as total length or fork length, measured to the nearest centimeter. Additional data are collected on individual specimens of priority species including horseshoe crabs (prosomal width in mm, individual weight, and sex). Latitude, longitude, water and air temperature, salinity, tow duration, and depth are recorded on each tow.

5.2.17.3 Evaluation of Survey Data

A spring (April-July) and a fall (October-November) index were developed from this survey. Horseshoe crabs were caught in the spring with 19% positive tows and in the fall when there were 25% positive tows. The SAS decided to use the fall-portion of the SEAMAP data. Depth was subset to 5-11 m due to low catches of horseshoe crab outside of those depths. The SAS split the data set further to develop two indices from SEAMAP: a South Carolina index and a Georgia-Florida index. A high proportion of horseshoe crabs caught in the survey were sexed, especially in the later years, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population when the survey was split by state. Based on the prosomal widths provided, this survey catches primarily adults in the fall.

5.2.17.4 Abundance Index Trends

The index of horseshoe crab abundance for South Carolina developed from the SEAMAP survey indicated low abundance at the beginning of the time series, an increase from 2009-2012, and a decreased abundance from 2013 through the terminal year (Figure 69). The index developed from the fall portion of the Georgia-Florida data indicates a low abundance of horseshoe crab with increased abundance in 2011, 2012, and 2016 with otherwise low abundance including in the terminal year (Figure 70).

5.2.18 Georgia Ecological Monitoring Trawl Survey

5.2.18.1 Survey Design and Methods

The Ecological Monitoring Trawl Survey (Georgia Trawl) conducted by GA DNR has operated along the Georgia coastline since 1999 (Figure 71). The survey operates monthly in creek, sound, and beach stations. There are 36 fixed stations that are sampled monthly.

5.2.18.2 Biological and Environmental Sampling

Catch is sorted by species and total number and weight are recorded. Selected finfish, shrimp and crabs are measured. Horseshoe crab counts, weights, sex are recorded. Tow duration, latitude, longitude, tide stage, water and air temperature, and salinity are recorded.

5.2.18.3 Evaluation of Survey Data

A spring (March-May) and a fall (September-November) index were developed from this survey. Horseshoe crabs were caught in the spring with 42% positive tows and in the fall with 38% positive tows. The SAS decided to use the spring-portion of the Georgia Trawl data. Depth was subset to 5-14 m due to low catches of horseshoe crab outside of those depths. All of horseshoe crabs caught in the survey were sexed, but the SAS concluded that too few horseshoe crabs were collected in total to justify using the sex ratio from the catch as representative of the population. Based on the prosomal widths provided, this survey catches primarily adults in the fall.

5.2.18.4 Abundance Index Trends

For the spring index developed from the Georgia Trawl data, abundance of horseshoe crabs varied but appeared to be increasing in recent years (Figure 72).

5.3 Index Correlations

Association of abundance indices for horseshoe crab was evaluated with Spearman's rank correlation coefficient, or Spearman's rho (ρ). This is a nonparametric test to evaluate association of two ranked variables over time (i.e., indices of abundance). Associations were evaluated between indices by region.

5.3.1 Northeast Region

There were three indices developed for the Northeast region: Massachusetts's Trawl North Cape, Massachusetts's Trawl South Cape, and the Rhode Island Monthly Trawl. The North and South Cape indices were positively correlated with each other but negatively correlated with the Rhode Island Trawl (Figure 73) although all of the correlations were insignificant ($P>0.05$).

5.3.2 New York Region

Five indices of horseshoe crab abundance were developed for the New York Region for this assessment: Connecticut Long Island Sound Trawl Survey, New York's Peconic Bays, Seine Jamaica Bay, and Little Neck and Manhasset Bays, and NEAMAP New York portion. All surveys were positively correlated with each other (Figure 74) although all were insignificant ($P>0.05$) except for the positive correlation between the Connecticut Long Island Sound Trawl Survey and New York's Peconic Bays (ρ=0.59, P=0.020) and New York's NEAMAP portion and the New York Seine Jamaica Bay (ρ=0.52, P=0.026).

5.3.3 Delaware Bay Region

Eight indices of horseshoe crab abundance were developed for the Delaware Bay region. For the correlation analysis, only the following combined sexes and adult surveys were tested: Delaware's Adult Trawl spring and fall indices, New Jersey's Ocean Trawl spring and fall indices, New Jersey Surf Clam, Maryland Coastal Bays, NEAMAP portion that operates in the Delaware Bay, and the Virginia Tech Trawl Survey. All correlations are insignificant (P>0.05) except for the correlations between the Delaware Adult Trawl spring and fall indices (ρ=0.69, p<0.001), Delaware Adult Trawl spring and New Jersey Ocean Trawl fall (ρ=0.28, P<0.001), New Jersey Ocean Trawl spring and Surf Clam surveys (ρ=-0.77, P=0.011), and NEAMAP and the Virginia Tech Trawl (ρ=0.36, P=0.020) (Figure 75).

5.3.4 Southeast Region

Six surveys were developed into abundance indices for horseshoe crab for the Southeast region: South Carolina's Trammel, CRMS, and SEAMAP (South Carolina portion), North Carolina's Gill Net Survey, Georgia Trawl Survey, and SEAMAP (Georgia-Florida portion). There were both positive and negative correlations among the surveys in this region (Figure 76) but all were insignificant (P>0.05) except for the correlations between the North Carolina Gill Net and South Carolina Trammel indices (ρ=0.53, P=0.036), the North Carolina Gill Net and South Carolina CRMS indices (ρ=-0.62, P=0.010), and SEAMAP's South Carolina and Georgia-Florida indices (ρ=0.81, P<0.001).

6 METHODS

6.1 Power Analysis

6.1.1 Background of Analysis and Model Description

Power analysis was used to calculate the probability of detecting trends in the abundance indices developed from fishery-independent data using the methods of Gerrodette (1987). Using this approach, changes in abundance can take place due to constant increments (linear model) or at a constant rate (exponential model). Linear trends were modeled as Ai=A1[1+r(i-1)] where Ai represents the abundance as a function of an index of time (i) and r is a constant increment of changes as a fraction of the starting abundance index (A1). Exponential trends were modeled as Ai=A1(1+r)i-1. For a linear change, r=R/(n-1) where R is the overall fraction change in abundance. For an exponential change, r=(R+1)1/(n-1) – 1. For each survey, the median CV can be calculated as the median proportional standard error or (SE(Ai)/Ai). The SAS established a reference point of a power of 0.80 for surveys to detect an increasing trend.

6.1.2 Model Configuration

All fishery-independent surveys that were developed into abundance indices were tested in the power analysis including any season or sex specific variants. Variability in abundance as a function of both linear and exponential change was tested using a one-tailed test. Power was calculated for a change (R) of ±50% over a 20-year time period for both a linear and exponential trend.

6.1.3 Model Results

Median CVs, or proportional standard error, ranged from 0.132-0.817 for the surveys analyzed and power values ranged from 0.18 to 1.0 (Table 36). Surveys with low CVs had higher power and those with high CVs had lower power as was expected. Exponential trends indicated slightly higher power than linear trends. For both linear and exponential trends, the ability to detect decreasing trends was higher than that of increasing trends. The surveys with greater than a 0.80 power of being able to detect a 50% increase in abundance were Connecticut LISTS, New York Peconic Bay and Seine Survey for Little Neck and Manhasset Bays, Delaware Adult Trawl (spring portions), NEAMAP, portions of the New Jersey Ocean Trawl, New Jersey Surf Clam, Virginia Tech Trawl, North Carolina Gill Net, South Carolina CRMS, and Georgia Trawl. The remaining surveys all fell below the desired power of 0.80 and therefore the ability to detect trends in the past 20 years is limited for many of the surveys used in this assessment.

6.2 Conn Method

6.2.1 Background of Analysis and Model Description

When several population abundance indices provide conflicting signals, hierarchical analysis can be used to estimate a single population trend. The abundance indices for horseshoe crab were combined into regional composite indices using hierarchical modeling as described in Conn (2009). This method assumes each index samples a relative abundance but that the abundance is subject to sampling and process errors. It can be used on surveys with different time series, but it does assume that indices are measuring the same relative abundance.

6.2.2 Model Configuration

Abundance indices for horseshoe crabs from each region were standardized to their means. Indices were combined using the methods of Conn in R and WinBUGS. The Massachusetts Trawl North Cape, Massachusetts’s Trawl South Cape, and Rhode Island Monthly Trawl were combined to form a northeast region composite index for 1978-2017. For the New York Region, the Connecticut LISTS, New York Peconic, NEAMAP (New York strata only), New York Seine Jamaica Bay, and New York Seine Little Neck and Manhasset Bay indices were combined for a New York region composite index for 1987-2016. For the Delaware Bay Region, several Conn indices were developed in order to support the models for that area. An adult composite was developed from the spring and fall components of the New Jersey OT and Delaware Adult Trawl, the NJ Surf Clam, NEAMAP (Delaware Bay strata only), VT Tech Trawl, and Maryland Coastal Bays surveys. Additionally, female-only and male-only indices were developed using the sex-specific indices developed from the New Jersey OT and Surf Clam, VT Tech Trawl, and Delaware Adult Trawl surveys. A southeast region Conn for 1995-2017 was developed from the North Carolina Gill Net, South Carolina Trammel, CRMS, and SEAMAP (South Carolina strata only), Georgia Trawl, and SEAMAP (Georgia-Florida strata only).

The estimates of process error variance for each of the indices were also examined. High sigma (σp) values, or the standard deviation of the process errors, suggest that the index may be a poor index for tracking abundance or may be measuring a different subpopulation whereas lower values indicate indices that may be better tracking the population or are consistent with the other indices included.

6.2.3 Model Results

The hierarchical index developed for the Northeast region predicted variable but stable abundance from 1978-2017, with moderate peaks in the terminal year estimates (Figure 77). The standard deviation of the process errors for the surveys used in the Northeast region Conn were higher for the Massachusetts Trawl, both the North and South Cape indices, than those of the Rhode Island Monthly Trawl (Table 37), indicating that the surveys may be tracking different populations or it may be reflecting differences in sampling programs (see Conn 2009 for a more thorough discussion).

The hierarchical index developed for the New York region predicted stable abundance throughout the time series with a slight increase in recent years (Figure 78). The standard deviation of the process errors for the surveys used in the New York region Conn had similar sigma values with the New York portion of NEAMAP being slightly higher (Table 37). This may indicate that the offshore NEAMAP trawl may be slightly out of line with the other more inshore surveys, but the sigma is still within an acceptable range.

The hierarchical indices developed for the Delaware Bay region for males and females combined, males-only, and females-only followed similar trends (Figure 79 - Figure 81). The indices predicted high abundance in the 1990s decreasing to a stable but low abundance in the early 2000s. The index is variable from 2005 through the terminal year but appears to have a slight increase from 2014-2016. The standard deviation of the process errors for the surveys used in the Delaware Bay region Conn ranged from 0.171 to 0.948 with the Virginia Tech Trawl survey having the lowest sigma values and the Delaware Adult Trawl having the highest (Table 37). The Virginia Tech Trawl survey is the only non-spawning beach survey that is specifically designed to monitor horseshoe crab and its low sigma value indicates that it is the most informative survey available.

The hierarchical index developed for the Southeast region predicted low abundance from the mid-1990s through the late 2000s when abundance starts increasing until a slight downtick in the terminal years (Figure 82). The standard deviation of the process errors for the surveys used in the Southeast region Conn had similar sigma values except for both SEAMAP indices, which had very high sigma values (Table 37). These indices may not be a good measure of horseshoe crab abundance in the region, or they may be measuring something else such as an offshore population (see Conn 2009 for a more thorough discussion).

6.3 Autoregressive Integrative Moving Average (ARIMA)

6.3.1 Background of Analysis and Model Description

Fishery independent surveys for horseshoe crabs can be quite variable, making inferences about population trends uncertain. Observed time series of abundance indices represent true changes in abundance, within survey sampling error, and varying catchability over time. One approach to minimize measurement error in the survey estimates is by using autoregressive integrated moving average models (ARIMA, Box and Jenkins 1976). The ARIMA approach derives fitted estimates of abundance over the entire time series whose variance is less than the variance of the observed series (Pennington 1986). This approach is commonly used to gain insight in stock assessments where enough data for size or age-structured assessments (e.g. yield per recruit, catch at age) are not yet available.

Helser and Hayes (1995) extended Pennington's (1986) application of ARIMA models to fisheries survey data to infer population status relative to an index-based reference point. This methodology yields a probability of the fitted index value of a particular year being less than the reference point [P(indext<reference)]. Helser et al. (2002) suggested using a two-tiered approach when evaluating reference points whereby not only is the probability of being below (or above) the reference point is estimated, the statistical level of confidence is also specified. The confidence level can be thought of as a one-tailed a-probability from typical statistical hypothesis testing. For example, if the P(indext<reference) = 0.90 at an 80% confidence level, there is strong evidence that the index of the year in question is less than the reference point. This methodology characterizes both the uncertainty in the index of abundance and in the chosen reference point. Helser and Hayes (1995) suggested the lower quartile (25th percentile) of the fitted abundance index as the reference point in an analysis of Atlantic wolfish (Anarhichas lupus) data. The use of the lower quartile as a reference point is arbitrary but does provide a reasonable reference point for comparison for data with relatively high and low abundance over a range of years.

The purpose of this analysis was to fit ARIMA models to time series of horseshoe crab abundance indices to infer the status of the population(s).

6.3.2 Model Configuration

Relative abundance indices included in this analysis are shown in Table 38. The ARIMA model fitting procedure of Pennington (1986) and bootstrapped estimates of the probability of being less than an index-based reference point (Helser and Hayes 1995) and corresponding levels of confidence (Helser et al. 2002) were coded in R (R code developed by Gary Nelson, Massachusetts Division of Marine Fisheries). ARIMA models were fit to ln transformed index values in the majority of surveys but were fit to ln+0.01 transformed index values for surveys that had an index value of 0 in one or more years. An 80% confidence level was chosen for evaluating P(indext<reference). Two index-based reference points were considered: 1) the lower quartile of the fitted abundance index (Q25) as proposed by Helser and Hayes (1995); and 2) the fitted abundance index from 1998 – the time of development of the ASMFC Interstate Management Plan for horseshoe crabs. The use of two reference points allowed

evaluation of the status of the horseshoe crabs with respect to historic levels, and just prior to the implementation of harvest restrictions to determine if such restrictions have resulted in an increase in abundance.

6.3.3 Model Results

The ARIMA models provided adequate fits to nearly all of the horseshoe crab indices. In two cases (Table 38), residuals from the ARIMA model fits were not normally distributed and subsequent bootstrapped probabilities of being below reference point values should be considered with caution. The survey whose residuals were not normally distributed were MA DMF Trawl survey north of Cape Cod and the GA Spring Trawl survey.

Trends in fitted abundance indices from ARIMA models showed much variation among surveys (Figure 83-Figure 89) both between and within regions. In the Northeast Region (Figure 83), indices generally displayed a decreasing trend with the exception of the MA DMF Trawl which showed an increasing trend after 2013 south of Cape Cod. All indices in the New York Region showed a decreasing trend (Figure 84) with the Peconic Trawl survey showing the greatest relative decrease. Trends in the Mid-Atlantic region (Figure 85–Figure 88) were either increasing in recent years (e.g. DE 30 ft. Trawl survey, NJ Surf Clam Dredge, NJ Spring Ocean Trawl) or stable (e.g. MD Coastal Bays Trawl). The Virginia Tech Trawl Survey (Figure 88) showed relatively large fluctuations prior to its interruption after 2011. Once it resumed in 2016, index values increased over those observed in 2011 and 2016 and 2017 values were similar. Indices in the Southeast Region were generally increasing prior to 2010 across all surveys (Figure 89), but since then have fluctuated or showed a slight decreasing trend in recent years.

Bootstrapped probabilities that the terminal year of indices were below reference points also varied greatly among surveys (Table 39). To generalize the probabilities of terminal year being below reference points, the SAS considered a probability of ≥0.50 as being “likely” to be below reference points (Table 40). Only those surveys whose residuals from fitted ARIMA indices were normally distributed, were overall combined-sex surveys (i.e. not double counting surveys who separated sexes), and whose terminal year was either 2016 or 2017 were considered. In the Northeast Region, 1 out of 2 surveys were likely less than the 1998 reference point and 1 out of 2 surveys were likely less than the Q25 reference point. In the New York Region, 4 out of 4 surveys were likely less than the 1998 reference point and 4 out of 5 surveys were likely less than the Q25 reference point. In the Mid-Atlantic Region, 2 out of 5 surveys were likely less than the 1998 reference point and no survey was likely less than the Q25 reference point. Finally, in the Southeast Region, no survey was below either the 1998 or the Q25 reference point. Coastwide, 7 out of 13 surveys were likely less than the 1998 reference point and 5 out of 19 surveys were likely less than the Q25 reference point.

6.4 Horseshoe Crab Operating Model

6.4.1 Background of Analysis and Model Description

The horseshoe crab is a long-lived species with females reaching sexual maturity at approximately ten years of age (Sweka et al. 2007). A major difficulty in stock assessments of horseshoe crabs is that individuals in the catch and in fishery-independent surveys cannot be aged, thus negating the application of age-structured assessment models. Application of surplus production models to horseshoe crabs has been questioned due to their long age to maturity. Following the 2009 ASMFC horseshoe crab benchmark stock assessment, the peer-review panel recommended the development of an operating model of horseshoe crab population dynamics to generate known data sets of catch and fishery-independent surveys which could then be used as input data to a surplus production model to test if such a simple model could accurately estimate fishing mortality, biological reference points, and be used to determine stock status (i.e., overfishing, overfished). Also, attempts were made in this assessment to apply an index method (Rago and Legault *unpublished manuscript,* https://www.nefsc.noaa.gov/nft/AIM.html) and a catch survey model (Collie and Sissenwine 1983) in some areas and an operating model would also be useful in evaluating the merits of these models as well.

6.4.2 Model Configuration

An operating model for horseshoe crab population dynamics was developed largely from the model described by Sweka et al. (2007). This was an age-structured model and only modeled female crabs. Life history parameters are provided in Table 41. The maximum age of crabs in the model was set to 20 years. Natural mortality (*M*) varied with age and crabs began maturing at age 10 and were fully mature by age 12. For individuals in maturing age classes (ages 10 and 11), natural mortality was lower for immature individuals compared to mature individuals. Partial recruitment to the fishery followed the same schedule as maturity. Fecundity of mature crabs was 80,300 eggs.

The number (*N*) of age class *i* at time *t* was:

$$N_{i,t} = N_{i-1,t-1}e^{(M_i+R_i\cdot F)}$$

where *R* is the age-specific partial recruitment to the fishery and *F* is the fishing mortality. Because natural mortality differed between mature and immature individuals within an age class, the model separated age 10, 11, and 12 into immature, primiparous (first time spawners), and multiparous (spawing at least once before) individuals.

$$N_{10imm,t} = N_{9,t-1}e^{(M_9+R_9\cdot F)}(1-m_{10})$$

$$N_{10primi,t} = N_{9,t-1}e^{(M_9+R_9\cdot F)}(m_{10})$$

$$N_{11imm,t} = N_{10,t-1}e^{(M_{10imm}+R_{10imm}\cdot F)}(1-m_{11})$$

$$N_{11primi,t} = N_{10imm,t-1}e^{(M_{10imm}+R_{10imm}\cdot F)}(m_{11})$$

$$N_{11multi,t} = N_{10primi,t-1}e^{(M_{10primi}+R_{10primi}\cdot F)}$$

$$N_{12primi,t} = N_{11imm,t-1}e^{(M_{11imm}+R_{11imm}\cdot F)}(m_{12})$$

$$N_{12multi,t} = N_{11primi,t-1}e^{(M_{11primi}+R_{11primi}\cdot F)} + N_{11multi,t-1}e^{(M_{11multi}+R_{11multi}\cdot F)}$$

Age-specific catch was calculated using Baranov's catch equation:

$$C_{i,t} = \left(\frac{R_i \cdot F}{M_i + R_i \cdot F}\right) \cdot (1 - e^{M_i+R_i\cdot F}) \cdot N_{i,t}$$

The number of female eggs produced in a year was equal to the number of sexually mature individuals multiplied by fecundity and divided by 2.

$$E_t = \left(\sum N_{i,mature,t} \cdot f\right)/2$$

Density-dependence was incorporated into the model through density-dependent egg mortality as described in Sweka et al. (2007) and Smith (2007) and depended on the number of mature crabs. As the number of spawning crabs increases, nest disturbance increases, thus bringing more eggs to the surface which do not survive and more female crabs spawn in less optimal habitat with lower egg survival. Survival of eggs to hatching as age 0 crabs was described by the function:

$$S_{egg,t} = 1 - \left(0.0957 \cdot \ln\left(\sum N_{i,mature,t} \cdot m_i\right) - 0.995\right)$$

The number of age 0 female crabs at the beginning of the year was:

$$N_{0,t} = E_t \cdot S_{egg,t}$$

The model was coded in a MS Excel spreadsheet and the carrying capacity (K) of the simulated population was determined by allowing the population to grow under no fishing mortality until the number of mature females reached an asymptote. To determine the maximum sustainable yield of this simulated population, the population started at K and was projected 400 years into the future. The fishing mortality associated with maximum sustainable yield (Fmsy) was solved for by maximizing the total catch in year 400 and the associated biomass at maximum sustainable yield (Bmsy) was equal to the total number of mature female crabs in year 400 when catch was maximized.

Given the life history parameters of this simulated population, the carrying capacity was determined to be 14,569,967 mature female crabs. Maximum sustainable yield was determined to be 647,609 female crabs which corresponded to an Fmsy of 0.1613 and a Bmsy of 5,433,439 crabs (Figure 90).

6.4.3 Simulated Data

Following development of the operating model, four data sets were simulated to examine how accurately a surplus production model (ASPIC; Prager 1994) and a catch survey model could estimate population parameters. In each of these scenarios, the model started with the population at equilibrium at the carrying capacity. Fishing mortality, F, was then allowed to vary annually according to a uniform distribution with bounds described in Table 42. The data time series used in the surplus production and catch survey models started 10 years after harvesting of the population began and ran for a total of 50 years. The harvest scenarios simulated were:

1) Constant F and a "one-way trip" of a declining population

2) Decreasing F through time

3) Very low F after initial harvest followed by a period of increased F

4) Decreasing F followed by very low F. Age-specific natural mortality was allowed to slightly vary according to a normal distribution with a CV = 0.01.

Fishery-independent surveys were generated for the surplus production and catch survey models by assuming values of the catchability coefficient (q) equaled 0.00012. Simulations for testing the surplus production applied q to total number of mature females while simulations for the catch survey model applied q separately for primiparous and multiparous individuals in order to generate an index for newly recruited individuals and previously recruited individuals, respectively. A fifty-year time series of each scenario's catch and fishery-independent indices were then used as input data to an index method, surplus production model, and catch survey model to evaluate model performance.

6.5 Application of an Index Method for Horseshoe Crab

The SAS attempted to apply An Index Method (AIM) model to horseshoe crabs in each region along the coast. This method was developed by Rago and Legault (unpublished manuscript) and is available in the NOAA Fisheries Toolbox (https://www.nefsc.noaa.gov/nft/AIM.html). This is a data poor stock assessment model that only requires a time series of catch data and a corresponding index of abundance and is typical of the situation in all regions of the coast other than the Delaware Bay for horseshoe crabs. AIM fits a linear relationship between the replacement ratio derived from a smoothed index of abundance and relative F (catch/abundance index) and characterizes the population response to varying levels of fishing mortality. If the relationship between the replacement ratio and relative F is valid, AIM can be used to estimate the level of relative F at which point the population is likely to be stable and catch recommendations can be made.

Although the minimal data requirements of AIM were attractive to use in the assessment of horseshoe crabs, the SAS abandoned its application for multiple reasons. In the New York region, AIM was not a suitable stock assessment model because of the general continuous decline in abundance indices despite changes in catch (i.e., a "one-way trip" situation). There are no general guidelines on the number of years to smooth the abundance index when calculating the replacement ratio, and the SAS attempted different numbers of years in smoothing. The significance of the linear relationship between the replacement ratio and relative F varied greatly depending on both the number of years used in smoothing and the fishery-independent surveys used in the model even when those surveys all assessed the same population of horseshoe crabs. It made intuitive sense that the number of smoothing years should reflect the life history of the horseshoe crabs with a long time to maturity and a 10-year smoothing was tested with simulated data from the operating model. Results of this testing were very inconsistent between simulated data series and the SAS determined that AIM did not adequately capture the dynamics of a long-lived species such as horseshoe crab and further application of this model was dropped from this assessment.

6.6 Testing of Surplus Production Model with the Operating Model

6.6.1 Background of Analysis and Model Description

The surplus production model was developed for horseshoe crabs in the Delaware Bay region because of its relatively simple modeling approach. Surplus production models combine the effects of recruitment, growth, and mortality into a single function and assume no size or age structure in the population. It requires a time-series of fishery removals and one or more time-series of catch-per-unit effort from a survey. The model assumes that the population is closed, the environment is constant, abundance indices are proportional to the true population abundance, total catch is known without error, the stock responds instantaneously to changes, and that the intrinsic rate of increase (r) and carrying capacity (K) remain constant.

The 2009 benchmark stock assessment included the application of a surplus production model for the Delaware Bay region (ASMFC 2009a). The model was not included in the 2013 stock assessment update because the benchmark did not include mortality due to the biomedical industry which was considered an oversight. Additionally, in 2009, the Peer Review Panel expressed concern about the long time period (~9 years) horseshoe crabs spend before they recruit to the fishery and questioned if this is a suitable model for the species. They suggested that the SAS further evaluate the violation of the assumption that “the stock reacts instantaneously to changes in conditions” given only mature crabs are included in the model and it takes the species 9-11 years to mature. Additionally, the Panel stated that the potential for this model to provide good estimates of stock status relative to reference points (e.g., FMSY) in regions outside the Delaware Bay would be challenging due to lack of contrast in the time series that were available for those regions.

The Panel made several suggestions for testing the surplus production model for horseshoe crab before using it to assess the stock. They recommended that an operating model for evaluating the performance of the surplus production model should be explored such as the simple age-structured operating model similar to Sweka et al. (2007). They suggested the development of an operating model of horseshoe crab population dynamics to generate known data sets of catch and fishery-independent surveys which could then be used as input data to a surplus production model to test if such a simple model could accurately estimate fishing mortality, biological reference points, and be used to determine stock status (i.e., overfishing, overfished).

6.6.2 Model Configuration

The SAS tested the application of the surplus production model with an age-structured operating model adapted from Sweka et al. (2007), described in Section 6.3, before developing it for horseshoe crab by region for this assessment.

All four simulated data sets were analyzed with the surplus production model in ASPIC (Prager 1994). The non-equilibrium Graham-Schaefer, or logistic, form was used to test this model for horseshoe crab. For inputs into the model, the simulated catch and abundance index were used. The starting values for the model were calculated as follows:

1) B_1/K =0.05

2) MSY=1/2*Maximum Catch

3) K=10*Maximum Catch

4) q=Average Index Value/(2*Maximum Catch)

Both MSY and K had minimum and maximum constraints of 1/8 and 8 times their values. The surplus production model estimates MSY and the associated MSY-based references points of B_{MSY}, the stock biomass associated with MSY, and F_{MSY}, the fishing mortality that maximizes the yield from the population. These absolute values are usually imprecise (Prager 1994) since they require good estimates of catchability (q). Relative biomass (B/B_{MSY}) and relative fishing mortality (F/F_{MSY}) can be used to determine overfishing and overfished status. All of the calculations for horseshoe crab were done in numbers, not weight, although “biomass” will still be referenced in the model outputs.

6.6.3 Model Results

6.6.3.1 Simulation 1

The first simulation represented a constant F and a “one-way trip” of a declining population. The pattern of the true F and the ASPIC-estimated F followed similar patterns but were on different scales with the true F being higher than the surplus production estimated F (Figure 91). True population numbers and ASPIC-estimated numbers had a similar result where the patterns were alike, but the scales were different with the estimated population numbers being higher than the true numbers. The application of a surplus production model often results in imprecise absolute values of fishing mortality and biomass, but relative fishing mortality and biomass usually can be used to determine overfishing and overfished status. Both the relative fishing mortality and biomass followed similar patterns throughout the time series when comparing the true values to the ASPIC-estimated values. Both relative F’s indicated overfishing (F/F_{MSY} >1) but the true values indicated that the stock was not overfished (B/B_{MSY}>1) whereas

ASPIC determined the stock was overfished for most years ($B/B_{MSY}<1$). The difference in overfished status between ASPIC and true values from the operating model is a concern for the application of the surplus production model for horseshoe crab.

6.6.3.2 Simulation 2

The second simulation represented a decreasing *F* through time. The pattern of the true *F* and the ASPIC-estimated F followed similar patterns but were on different scales with the true *F* being higher than the surplus production estimated *F* (Figure 92). True population numbers and ASPIC-estimated numbers had a similar result where the patterns were alike, but the scales were different with the ASPIC-estimated population numbers being higher. The application of a surplus production model often results in imprecise absolute values of fishing mortality and biomass, but relative fishing mortality and biomass usually can be used to determine overfishing and overfished status. Both the relative fishing mortality and biomass followed similar patterns throughout the time series when comparing the true values to the ASPIC-estimated values, but true relative *F* indicated some overfishing in the early years ($F/F_{MSY} >1$) whereas ASPIC indicated no overfishing. True and ASPIC-estimated relative biomass were similar in pattern and values with both indicating that the stock was not overfished ($B/B_{MSY} >1$).The difference in overfishing status is a concern for the application of the surplus production model for horseshoe crab.

6.6.3.3 Simulation 3

The third simulation represented an institution of a moratorium followed by a low *F*. The pattern of the true *F* and the ASPIC-estimated *F* followed similar patterns but were on different scales with the true *F* being higher than the surplus production estimated *F* (Figure 93). True population numbers and ASPIC-estimated numbers had a similar result where the patterns were alike, but the scales were different with the ASPIC-estimated population numbers being higher. The application of a surplus production model often results in imprecise absolute values of fishing mortality and biomass, but relative fishing mortality and biomass usually can be used to determine overfishing and overfished status. Both the relative fishing mortality and biomass followed similar patterns throughout the time series when comparing the true values to the ASPIC-estimated values, but true relative *F* indicated some overfishing in the early years ($F/F_{MSY} >1$) whereas ASPIC indicated no overfishing. True and ASPIC-estimated relative biomass were similar in pattern and values with both indicating that the stock was not overfished ($B/B_{MSY}>1$).The difference in overfishing status is a concern for the application of the surplus production model for horseshoe crab.

6.6.3.4 Simulation 4

The fourth simulation represented a high *F* flowed by a moratorium. The pattern of the true *F* and the ASPIC-estimated *F* followed similar patterns but were on different scales with the true *F* being higher than the surplus production estimated *F* (Figure 94). True population numbers and ASPIC-estimated numbers had a similar result where the patterns were alike, but the scales were different with the ASPIC-estimated population numbers being higher. The application of a surplus production model often results in imprecise absolute values of fishing mortality and biomass, but relative fishing mortality and biomass usually can be used to determine overfishing and overfished status. Both the relative fishing mortality and biomass followed similar patterns throughout the time series when comparing the true values to the ASPIC-estimated values, but true relative *F* indicated some overfishing in the early years ($F/F_{MSY} > 1$) whereas ASPIC indicated no overfishing. True and ASPIC-estimated relative biomass were similar in pattern and values with both indicating that the stock was not overfished ($B/B_{MSY} > 1$). The difference in overfishing status is a concern for the application of the surplus production model for horseshoe crab.

6.6.3.5 Summary of Model Results

The application of the surplus production model for assessing the status of horseshoe crabs was tested using simulated data from an operating model as suggested by the 2009 Peer Review Panel. The simulated data results indicated that the surplus production model is poor at estimating absolute values of horseshoe crab population numbers and fishing mortality. In all four scenarios, ASPIC overestimated population numbers and underestimated *F*. For relative fishing mortality and biomass, ASPIC suggested a different overfishing or overfished status from the true simulated values for all four scenarios. For simulation 1 where *F* was variable but stable and population numbers were decreasing, ASPIC results suggested the stock was overfished when the true values from the operating model did not. Conversely, for simulations 2-4 where *F* decreased throughout the time series in different ways, ASPIC underestimated relative fishing mortality and failed to show overfishing in the first decade of the simulation. Ultimately, when comparing the true values and the estimated values, the surplus production model did not successfully estimate relative quantities compared to the true quantities. The simulation work confirms the suspicions of the 2009 Peer Review Panel and indicates that the application of the surplus production model for horseshoe crab is not appropriate. The results are likely due to the violation of the assumption that “the stock reacts instantaneously to changes in conditions” given only mature crabs are included in the model and it takes the species 9-11 years to mature. Therefore, the surplus production model was not further developed for horseshoe crab in this assessment.

6.7 Catch Survey Analysis

6.7.1 Background of Analysis

Initial attempts at modeling Delaware Bay horseshoe crab stock dynamics using a catch-survey analysis (CSA) began in 2008 (ASMFC 2009a) adhering largely to the methods described in Collie and Sissenwine (1983). The horseshoe crab’s unique life history was well-suited to the two-stage modeling approach, as newly mature horseshoe crabs (termed ‘primiparous’) exhibit readily-identifiable secondary sexual characteristics, cease molting, and recruit into the spawning population in the ensuing year (Schuster and Sekiguchi 2003). Horseshoe crabs that have spawned at least once (termed ‘multiparous’) bear identifiable, permanent, mating abrasions (Hata and Hallerman 2009b, 2009c). Relative abundances of primiparous and multiparous crabs are measured in the Virginia Tech horseshoe crab trawl survey (VT survey) in the fall directly outside of the population's major spawning grounds (Hata and Hallerman 2018). Primiparous and multiparous females were used as indices of pre-recruits and full-recruits in the CSA model. The original model contained a limited survey time series (8 years) and lacked some sources of harvest information (most notably biomedical mortalities). Realistic outputs were producible, although model instability was an issue (due to the shortened time series and survey variability) that could be overcome by allowing a freely-estimable primiparous catchability parameter (R. Wong, unpublished). Given the favorable horseshoe crab life history and early modeling work, the 2009 Stock Assessment Peer Review Panel encouraged the continued development of the CSA in future assessments (ASMFC 2009a).

6.7.2 Model Description

A catch multiple survey analysis (CMSA) was developed for this stock assessment tailored to available horseshoe crab survey and harvest information in order to produce estimates of Delaware Bay adult female abundance and fishing mortality rates (poor fit to survey indices prevented the development of male-only and combined split-sex models.). The CMSA contains a similar, simplified model structure to the Chesapeake Bay Blue Crab sex-specific catch multiple survey analysis by Miller et al. (2011). The model tracks the dynamics between two horseshoe crab stages: a) primiparous (newly mature yet spawning-naive) females; and b) multiparous (spawning-experienced) females. The broad assertion is that all primiparous females will participate in the proceeding spring spawning event, thus fully entering the multiparous stage within a single year (12-month period). It is also widely accepted that horseshoe crabs undergo a terminal molt at maturity (Shuster and Sekiguchi 2003). Therefore, multiparous abundance in a given year is a direct function of the primiparous and multiparous abundance in the previous year minus harvest and natural mortality. These adjacent reproductive stages are readily-identifiable in the field (Hata and Hallerman 2009b, 2009c), making horseshoe crabs well-suited to the catch-survey model dynamics.

The catch multiple survey model is based on the first order difference equation:

$$N_{y+1} = \left(\left(N_y + R_y\right)e^{-Mt} - C_y\right)e^{-M(1-t)} \quad (1)$$

which relates the fully-recruited abundance at the beginning of the year (N_{y+1}), to the fully-recruited abundance at the beginning of the previous year (N_y), plus pre-recruit abundance in the previous year(R_y), minus catch (C_y), all decremented by natural mortality, M, with t representing the fraction of the year corresponding to the harvest midpoint.

Minimum data requirements for the model include: i) annual indices of relative abundance for each size stage; ii) relative selectivities of size stages to the survey gear; iii) annual harvest; and iv) an estimate of instantaneous natural mortality rate.

Survey indices of abundance are assumed proportional to absolute stock sizes and are described by

$$r_{i,y} = s_i q_i R_y e_i^{\delta y} \qquad (2)$$

and

$$n_{i,y} = q_i N_y e_i^{\eta y} \qquad (3)$$

where $r_{i,}$ and $n_{i,}$ are the observed indices of pre-recruit and fully-recruited horseshoe crabs from survey *i*, q_i is the survey catchability coefficient, and $e^{\eta y}$ and $e^{\delta y}$ are lognormally distributed random variables, which represent survey measurement errors. The term *s* relates the pre-recruit catchability to the full-recruit catchability expressed as the ratio of q_r/q_n (Conser 1994).

$$s = q_r/q_n \qquad (4)$$

Annual exploitation rates μ were calculated as

$$\mu_y = {C_y}\Big/{(R+N)_y} \qquad (5)$$

Instantaneous fishing mortality rates F were calculated from relationships between μ, instantaneous total mortality rate Z, and annual mortality rate A.

$$Z_{y+1} = \ln\left(\frac{(R_y+N_y)}{N_{y+1}}\right) \qquad (6)$$

$$A_y = 1 - e^{-Z_y} \qquad (7)$$

$$F_y = \mu_y \frac{Z_y}{A_y} \qquad (8)$$

Parameters are estimated by minimizing the objective function, which is the sum of the likelihood components for each data source. Each likelihood component consists of

$$L_i = k_i + \tfrac{1}{2}\textstyle\sum_{y\in i}\left(\left(\ln O_{i,y} - \ln P_{i,y}\right)^2/cv_{i,y}\right) \qquad (9)$$

where *O* and *P* are observed and predicted values of the indices of abundance for each survey *i*. Constants *k* were ignored to simplify the equations. Empirical survey *cv* (coefficient of variations) were used for each year of the index *i,y*. Likelihood weightings λ were employed to best use available horseshoe crab data sources.

6.7.3 Model Configuration

The unit stock being modeled in the CMSA was the Delaware Bay horseshoe crab population. The region, for purposes of defining the boundaries of this unit stock, included states from New Jersey to Virginia. All horseshoe crabs found in Delaware Bay and ocean waters of New Jersey and Delaware are considered part of the Delaware Bay stock. A significant proportion of horseshoe crabs found in ocean areas of Maryland and Virginia also belong to this unit stock.

After a review of genetics and tagging work, the Delaware Bay Ecosystem Technical Committee of the ASMFC concluded that 51% and 35% of horseshoe crabs found in the ocean areas of Maryland and Virginia are likely of Delaware Bay origin, as necessary to determine quota allocations across the region (ASMFC 2012). This assessment operated under this allocation arrangement for purposes of defining the unit stock and its harvest removals from across States within this region.

A one-year model time step based on the January to December calendar year was used. All model parameters were estimated in the log scale.

The CMSA model was implemented in ADMB version 12.0. Log-scale standard deviations of parameters and derived values were generated in ADMB as described in Fournier et al. (2012).

Three fishery-independent surveys provided information about Delaware Bay adult female abundance: the VT survey (see 5.2.12), Delaware Fish and Wildlife Adult Trawl Survey (see 5.2.10), and New Jersey Ocean Trawl Survey (see 5.2.8) (Figure 95 and Figure 96). Stage-specific, swept-area abundance estimates of primiparous and multiparous females from the VT survey (Hata and Hallerman 2018) were used as pre-recruit (*r*) and full-recruit (*n*) indices (Table 43). VT swept-area estimates were based on mean crab densities (assuming a lognormal delta-distribution) expanded to the Delaware Bay survey area, 5,127 km^2. The ratio *s* was set to unity, given no evidence to support differences in catchability between stages of similar size and, ostensibly, distribution. Since VT collections occur in October, these indices were lagged forward to represent *n* and *r* at the start of the ensuing calendar year (January). The VT survey did not operate from 2012 to 2015 due to funding limitations. Aggregate stage (*r*+*n*) indices were constructed from the DE and NJ trawl surveys, since mature animals were not specifically categorized as primiparous or multiparous in the field. Aggregate stage indices were based on spring trawl collections and were assumed to reflect abundance at the start of the model time-step. Empirical annual survey CVs were incorporated into the modeling framework.

Three sources of harvest were included in the CMSA model: i) commercial bait landings (see 4.1.2); ii) commercial discard mortalities (see 4.3.1.3); and iii) biomedical mortalities (see 4.2.6). All harvest data were partitioned to only adult female horseshoe crabs of Delaware Bay origin (Figure 97). Data collection and harvest quantification methods are described in detail in section 4. Discard data were unavailable for 2003, so it was assumed that discard mortalities equaled the 3-year average value estimated in 2004-2006.

Instantaneous natural mortality rate (*M*) on adult females was assumed to be *M*=0.274 based from empirical estimates of survival rates (mean =0.76) of tagged adult Delaware Bay horseshoe crabs from 2009-2017 (D.R. Smith, unpublished; see 2.1.3 and 2.6) and also on aligning mortality rate with long-held assumptions about maturity and longevity (see 2.6). *M* was assumed constant across years and equal for primiparous and multiparous females since both stages will experience spawning-related mortality, the primary source of adult natural mortality. A comprehensive review of natural mortality is provided in 2.6.

6.7.4 Testing of CMSA with the Operating Model

The SAS tested the application of the CMSA with an age-structured operating model adapted from Sweka et al. (2007), described in Section 6.3, before developing it for horseshoe crab for this assessment. Four simulated data sets were analyzed using CMSA in ADMB version 12.0 and the results are described below. To match the development of the operating model, the CSA used *M*=0.47 for simulation testing. Simulated primiparous and multiparous indices were provided along with catch values as inputs to the model. Comparisons were made between true population size and *F* and the estimated values calculated by the CMSA.

After reviewing the testing of the CMSA with the operating model, the SAS was satisfied with its performance and found it to be appropriate for further development and use in this assessment.

6.7.4.1 Simulation 1

The first simulation represented a constant *F* that ranged from 0.18-0.22 and a “one-way trip” of a declining population. The pattern of true and CMSA-estimated *F*, population estimates, and index estimates were nearly identical throughout the time series (Figure 98). To get total horseshoe crab numbers, the estimated primiparous and multiparous numbers were added together for the CMSA-estimated values and compared to the true values from the operating model.

6.7.4.2 Simulation 2

The second simulation represented a decreasing *F* through time. The pattern of true and CMSA-estimated *F* was nearly identical, with the CMSA slightly overestimating *F* in the beginning of the time series but otherwise predicting *F* to be similar to the true values (Figure 99). To get total horseshoe crab numbers, the estimated primiparous and multiparous numbers were added together for the CMSA-estimated values and compared to the true values from the operating model. The CMSA slightly underestimated the population but was very close to the true numbers. The index fits were very close to the true values.

6.7.4.3 Simulation 3

The third simulation represented an institution of a moratorium followed by a low *F*. The pattern of true and CMSA-estimated *F* was nearly identical, with the CMSA slightly overestimating *F* in the beginning of the time series but otherwise predicting *F* to be similar to the true values (Figure 100). To get total horseshoe crab numbers, the estimated primiparous and multiparous numbers were added together for the CMSA-estimated values and compared to the true values from the operating model. The CMSA slightly underestimated the population but was very close to the true numbers. The model fits to the indices were very close to the true values as well.

6.7.4.4 Simulation 4

The fourth simulation represented a high *F* followed by a moratorium. The pattern of true and CMSA-estimated *F* was nearly identical, with the CMSA slightly overestimating *F* in the beginning of the time series but otherwise predicting *F* to be similar to the true values (Figure 101). To get total horseshoe crab numbers, the estimated primiparous and multiparous numbers were added together for the CMSA-estimated values and compared to the true values from the operating model. The CMSA slightly underestimated the population but was very close to the true numbers. The model fits to the indices were very close to the true values as well.

6.7.5 Base Model Run

A base model was selected from extensive model building and testing of inputs, starting values, bounds, and choice of CVs and likelihood weights λ (Table 43).

The use of swept-area abundance estimates as inputs for r and n in lieu of mean catch-per-tow or densities was highly influential in the evolution of the base model. Given the artifact of unusually low magnitudes of annual landings, the use of swept area, scaled-up primiparous and multiparous estimates was needed in order to properly scale model-estimated population size. Catch is the critical input in model equation eq. (1) for scaling the population size. The CMSA time series occurs during a period of severe landings restrictions relative to historic levels and commercial moratoria (2007-present) on female harvest, which has resulted in marginal commercial landings (and elevated commercial discard rates). Given the use of swept-area estimates, a catchability coefficient was not estimated for the VT survey.

Survey indices and annual CVs from 2003--2018 were used in the base model (except 2013-2016 for the VT survey) (Table 43, Figure 95, Figure 96). The VT survey was not conducted in 2013-2016.

Model catch consisted of all commercial bait landings, commercial discard mortalities, and biomedical mortalities of Delaware Bay adult female horseshoe crabs from the unit region from 2003-2017 (Table 43, Figure 97). A 15% mortality rate was used for bled females reported by the biomedical industry based on a comprehensive literature review and analysis (Section 4.2.1).

Likelihood weights λ_i were based on results of a hierarchical analysis of adult female indices from the VT, DE, and NJ trawl surveys (Conn 2009). The Conn (2009) hierarchical analysis produces a composite index from multiple indices, whereby process error variances (σ^p) generated for each index can be used as an inverse measure of how well the index contributes to the composite (Conn 2009). The inverse Conn variances $(\sigma^p)^{-1}$ for VT, DE, and NJ survey indices (viz. 4.3, 1.12, and 1.8) were proportioned to sum to 1 (viz. 0.59, 0.16, 0.25) and used as λ_i for each likelihood component in the base model (Table 43). Twenty parameters were estimated: median primiparous abundance (1); primiparous abundance for each year (16); catchability coefficients (2) for the Delaware and New Jersey surveys; and multiparous abundance for the start of time series (1), summarized in Table 44.

6.7.6 Model Results

The base model produced excellent convergence criteria and was highly stable and robust to a wide range of starting parameter input values and bounds. Model predictions fit indices well, with excellent agreement with the primiparous index and well-behaved fits through observed multiparous indices (Figure 102-Figure 105).

Estimated primiparous abundance is fairly stable through the time series (Table 45, Figure 106). Rising multiparous abundance is evident and reflects some of the large increases seen in the multiparous trawl indices in later years (Table 45, Figure 107, Figure 108). Fishing mortality rates are very low (average F=**CONFIDENTIAL**[1]), seemingly properly reflecting the current period of highly protective fishery restrictions and moratoria (Figure 109).

6.7.7 Retrospective Analysis

Minor retrospective error or bias was detected from a data peel to 2009 (Figure 110-Figure 112). Mohn's (1999) ρ statistic for total, multiparous, and primiparous abundance was **CONFIDENTIAL** (Table 46). This is consistent with very little retrospective error seen in CSA estimates using simulated population data (Mesnil 2003).

6.7.8 Sensitivity Runs

Several sensitivity runs of the CMSA were conducted to evaluate effects of assumptions on natural mortality, harvest, λ, CVs, q, and starting values (Table 47, Figure 113).

A likelihood profile of M sensitivity runs showed best fit to data between $0.15 \leq M \leq 0.25$, much lower than the previously assumed M=0.47 for adults and supporting the base model M=0.274 (Figure 114). This lower level of M is in better agreement with the understanding of the horseshoe crab's extended longevity (>20y) and late maturity.

Varying catch inputs had little effect on model outputs given the low overall magnitude of removals. Model outputs of terminal F ranged from 0.007 when excluding biomedical data to **CONFIDENTIAL** when testing different assumed mortality rates of bled biomedical harvest ranging from 4%, 15%, and 30% (Table 47).

Commercial discard mortalities were a newly added source of harvest in this assessment. Beginning in 2007, discard mortalities have consistently been the biggest source of removals on the stock following the implementation of a commercial moratorium on female harvest in Delaware Bay. When discard mortalities were removed from the base model, terminal year

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[1] Benchmark base run values are CONFIDENTIAL because they are based on harvest that includes numbers of horseshoe crabs attributed the biomedical industry. Values without biomedical data are F_{2017}=0.007 and B_{2018}=8,718,040. The benchmark values of F_{2017} and B_{2018} with the biomedical data, although minimally different, represent the best data but are CONFIDENTIAL.

fishing mortality was *F*= **CONFIDENTIAL**, a **CONFIDENTIAL** % reduction from the base model *F* (Table 47).

An equal weight λ_i =1 model produced considerably higher terminal stock size estimates since greater emphasis on the VT survey was no longer specified, allowing the model to more closely fit the sharply rising DE and NJ trawl indices. A base model using the unproportionalized Conn weights (4.3, 1.16, 1.8; VT, DE, NJ) predictably had little impact on outputs (Table 47).

Using fixed survey-wide CVs rather than annual CVs for each year of the index was tested. Survey-wide CVs [0.35, 0.258, 0.353, 0.258; VT_r, VT_n, DE, NJ] based on empirical average annual CVs produced slightly higher terminal N estimates (Table 47). Implementing survey-wide CVs reflecting the group's subjective confidence in each survey [0.25, 0.5, 0.5; VT, DE, NJ] resulted in similar outputs to the base model run (Table 47).

Allowing the base model to freely estimate the VT survey catchability coefficient resulted in inflated (roughly 3X) stock size estimates (Table 47). This is an interesting result as the model is seeking a larger stock size in relation to catch, beyond the credible range of expected values. Excessive observation error in surveys, over-specified harvest, or over-specified *M* in the base model could contribute to this situation.

Model runs that excluded parameter estimations in 2013-2016 due to the missing VT survey years were explored. Terminal year outputs were nearly identical to base model outputs.

The base model was highly robust to large variations in starting values of R, N, and q. Model convergence and parameter estimations were unchanged from changes in starting values ranging by more than an order of magnitude (Table 47).

6.7.9 Discussion

Rising adult abundance is evident in model outputs. Stock rebuilding is not surprising given an extended period of significantly reduced commercial landings and tight management controls on the fishery beginning in the early 2000s. Delaware Bay female commercial bait landings in the late 1990s easily exceeded 500,000 per year (see 4.1.2), while bait landings during the model period have averaged 78,000 crabs. Estimated multiparous abundance is stable from 2003 to 2012 and then rises considerably by 2017 (Figure 103). A delayed rebuilding response in multiparous abundance is consistent with slow maturity, long life span, and density-dependent recruitment.

Estimated primiparous abundance occurs in a fairly narrow range around **CONFIDENTIAL** crabs in years with available primiparous and multiparous indices (2003-2012; Figure 106). Although aggregate survey indices are available in 2013-2016, estimates of primiparous and multiparous abundance during this time block (2013-2016) are highly uncertain given the lack of survey indices to allocate abundance between stages. This generally stable recruitment is consistent with a life history dependent on relatively finite amounts of beach habitat for yearly egg burial and incubation. As Sweka et al. (2007) demonstrate, there is an upper cap on the amount of egg production in the population due in part to the maximum capacity of spawning habitat and density-dependent egg mortality. Fairly stable primiparous recruitment with incrementally expanding multiparous abundance would be expected from a species in the mid to later-stages of rebuilding, due to capped recruitment potential, slow growth, low mortality, and long lifespan.

6.7.10 Caveats

The CMSA model is understandably highly levered on the VT survey, as this survey is the only source of information about primiparous and multiparous stages. The magnitude of the VT swept area estimates is assumed to be representative of the Delaware Bay population size, R, N. This assumption was critical in informing the model about population scale. Although q_vt is input to 1, the model can freely estimate R, N above or below r_vt and n_vt in order to best fit all available data. As seen in sensitivity runs, R and N become more inflated as less weight is given to the VT survey (i.e. equal λs) or when the model is allowed to freely estimate q_vt. In reality, the VT swept area estimates are likely minimum estimates of abundance given: 1) the VT trawl gear efficiency is less than 100%; and 2) the VT survey spatial area may be a low estimate of Delaware Bay unit stock spatial area (excludes inside waters of Delaware Bay).

Natural mortality *M* is a critical input in the CMSA model. Although *M* is generally specified well according to sensitivity runs, and is supported by empirical survival estimates, there is some evidence *M* could still be over-specified given the mean ratio of 3.48 multiparous to primiparous females observed in the VT survey along with long-held assumptions about maturity and longevity. For example, assuming maturity starts at 10 years and lifespan ends at 20 years, the *M* needed to achieve this ratio is M=0.23 (closer to the preferred *M* in the likelihood profile). Another possible caveat is the assumption of a constant *M* for both stages, since *M* may increase with age to some extent given higher spawning mortality associated with declining condition as horseshoe crabs age (Penn and Brockmann 1995).

Model catch is assumed known with no error. The biggest source of uncertainty in harvest inputs was associated with discard mortalities. Annual discard mortalities were the products of observer discard rates and reported fishery trips, further proportioned by sex using fishery-independent sex-ratios. It was assumed that 100% of discards were adult stage horseshoe crabs, although this almost certainly is an overestimation. It was also required to make broad assumptions about discard mortality rates, basing mortality rates (i.e. 50% trawl, 5% dredge) on the SAS's collective experience in managing Mid-Atlantic fisheries combined with an understanding of horseshoe crab biology. Since data were unavailable, it was assumed 2003 discard mortalities were equal to the average of the next three years of estimates (2004-2006). High variability in discard rates, use of external sex ratios, and judgment-based mortality rates are clear caveats to consider and warrant study to refine future estimates. Whereas estimates of discard mortalities may be biased high from assuming 100% adult status, commercial bait harvest may be underestimated from undocumented illegal horseshoe crab harvest caused by the short commercial quota seasons and the high value of adult females as bait in eel and whelk fisheries.

The missing time block (2013-2016) of VT survey information in the model is not ideal, but it isn't as problematic as it could be since the model only tracks two stages rather than multiple cohorts through a time-age matrix. The most obvious problem it presents is that the 2017 estimate of multiparous abundance is based only on the three observed survey indices without the aid of information about R and N from the previous year, 2016. Ultimately, these missing years deprive a fuller understanding of the observed rising population trajectory, since a large increase occurs between 2012 and 2017. This multiparous increase is observed in both aggregate survey indices and male horseshoe crab indices in Delaware Bay, and is further supported by excellent spawning beach numbers in the 2018 Delaware Spawning Beach Survey based on anecdotal observation (J. Zimmerman, personal communication).

7 STOCK STATUS

7.1 Current Overfishing, Overfished/Depleted Definitions

To date, no overfishing or overfished definitions have been adopted by the Management Board.

7.2 Development of Reference Points for Horseshoe Crab

For this assessment, biological reference points were developed for the Delaware Bay horseshoe crab population. Reference points for other populations were not developed because of insufficient information on life history and a lack of suitable stock assessment models to gauge status relative to reference points. Two general methods to develop reference points for female horseshoe crabs were used: 1) reference points derived from a population projection model for Delaware Bay female horseshoe crabs and 2) egg-per-recruit (EPR) and yield-per-recruit (YPR) models. Male horseshoe crab reference points were based on the sex ratio of male:female horseshoe crabs.

7.2.1 Methods

The projection model was based on the age-structured horseshoe crab model of Sweka et al. (2007) and used as an operating model to determine the efficacy of the stock assessment models used in this assessment. Age-0 natural mortality was equal to 10.4143 which came from an estimate of age 0 survival in Delaware Bay from Botton et al. (2003). Estimates of natural mortality at the juvenile (M_{juv}) and mature (M_{mat}) ages in the Sweka et al. (2007) model were based on a study by Carmichael et al. (2003) from Pleasant Bay, MA and may not accurately reflect those in Delaware Bay. In the present projection model, to develop reference points, M_{mat} was reduced from 0.470 in Sweka et al. (2007) to 0.274 to match the value used in the CMSA in this assessment. Justification for the use of this value comes from analysis of tagging data (Section 2.1.3).

There is no empirical estimate of the carrying capacity (K) for female horseshoe crabs in Delaware Bay and previous estimates of the carrying capacity (~14 million) were based on projecting the Sweka et al. (2007) model forward until an equilibrium was reached under no fishing mortality. This level was a function of both the age-specific natural mortality schedule and an assumed density-dependent egg mortality function (Smith 2007). Because M_{mat} was reduced in this current model and there are no estimates of M_{juv} specific to the Delaware Bay, the SAS was very uncertain as to what the actual female carrying capacity of the Delaware Bay is, which makes development of biological reference points difficult.

In order to derive biological reference points from the current projection model, three different levels of female horseshoe crab K were considered and values of M_{juv} required to stabilize the population at those levels under a situation of no fishing mortality were solved for. The lowest level of K was 10 million horseshoe crabs, which is a level slightly greater than the current estimate of female abundance from the CMSA model results. An intermediate level of 14 million was chosen to represent the current management of Delaware Bay horseshoe crabs under the Adaptive Resource Management (ARM) framework whereby 80% of K gives value to the harvest of female horseshoe crabs in the optimization routine. Finally, an upper level of 18 million was chosen to acknowledge that current management's estimate of K may be an underestimate. For each level of K, M_{juv} was determined by setting the population level at the K and solving for a value of M_{juv} that resulted in a finite population growth rate (λ) of 1.0 from a population projection matrix (leslie matrix).

The population projection model was coded in a MS Excel spreadsheet and began with a stable age distribution at a given level of K. To determine the maximum sustainable yield of this simulated population, the population was projected 400 years into the future. The fishing mortality associated with maximum sustainable yield (F_{msy}) was solved for by maximizing the total catch in year 400, and the associated number at maximum sustainable yield (N_{msy}) was equal to the total number of mature female crabs in year 400 when catch was maximized. F_{20} and F_{40} reference points were estimated by solving for the F that resulted in 20% or 40% of K in year 400, and the number associated with F_{20} and F_{40} was also estimated. This process was completed for each of three possible levels of K explored and resulted in three suites of biological reference points.

Life history parameters used in the projection model (Table 48) were used to generate parameters in per-recruit models (Table 49). The difference between these two tables of life history parameters was that those of the projection model separated ages 10 and 11 into immature and mature individuals while those of the per-recruit model combined them into a single age 10- and 11-year classes. Maturity in the projection model represented the probability of an individual becoming mature at age i if it was immature at age i-1 whereas maturity in the per-recruit models represented the proportion of the age class that was mature at age i.

Per-recruit modeling was performed according to the methods of Gabriel et al. (1989) in the R package fishmethods. It was assumed that 30% of natural mortality occurred before spawning and 0% of fishing mortality occurred before spawning. The EPR model estimated the F rate that preserved 20% (F_{20}) and 40% (F_{40}) of the maximum EPR of an unfished population. In the YPR model, it was assumed that individuals did not recruit to the fishery until they were sexually mature and once sexually mature, a terminal molt occurred after which the weight of individuals remained the same throughout the remainder of their life. Thus, age-specific weights were simply set to 1.0 for all ages and the YPR values could be interpreted as the number of individuals per recruit.

7.2.2 Results and Discussion

The reference points from the projection model varied with the assumed level of *K* (Table 50). F_{MSY} ranged from 0.0695 to 0.0796 and values of F_{40}, which is often used as a proxy for F_{MSY} were of similar magnitude (range = 0.0632 to 0.0724).[2]

As an additional check on the coding of the projection model, life history parameters were input into the population projection matrix (leslie matrix). The effects of the various *F* reference points on population growth rates (λ or the dominant eigenvalue from the projection matrix) were tested using the R package demogR. The population number was set at the estimated *K*, N_{msy}, N_{40}, and N_{20} to appropriately include density dependent egg mortality in the projection matrix and corresponding *F* values of 0, F_{msy}, F_{40}, and F_{20} were used. In all cases $\lambda = 1.0$ indicating a stable population at those levels of *F* and confirming the coding of the operating model was capturing the population dynamics as expected.

The EPR and YPR models were determined unsuitable in determining reference points for a species such as horseshoe crab. The EPR model estimated F_{20} ranging from 2.2508 to 2.2676 and F_{40} ranging from 0.6444 to 0.6465, depending on the juvenile natural mortality used. All of these values appeared to be excessively high given the natural mortality of the species (Table 51). When these values of *F* were input into the projection model, the population crashed to less than 1% of the carrying capacity. Also, the plot of YPR vs. *F* showed no declining trend in YPR as *F* increased (Figure 115). The life history of horseshoe crabs, with greater mortality on mature individuals compared to immature individuals, density dependent egg mortality not accounted for in a traditional EPR model, and a terminal molt and lack of increasing weight with age are responsible for these questionable per-recruit results and reference points based on traditional per-recruit models should be avoided.

Management of horseshoe crabs can call for sex specific harvest rates because sexes are easily distinguishable, and ideally, separate sex-specific reference points would be developed and used. Unfortunately, the catch survey model could not estimate the abundance and fishing mortality for male horseshoe crabs. In lieu of having male reference points which could be compared to the CMSA results, the SAS recommends using the sex ratio of male:female crabs from the Delaware Bay spawning survey as a reference point for male horseshoe crabs. This sex ratio reference point would be 2:1. If the sex ratio is >2:1 on the spawning beaches, it can safely be assumed that adequate egg fertilization is occurring, and the abundance of male horseshoe crabs is not limiting the growth of the horseshoe crab population. This assumption is consistent with current management of horseshoe crabs in the Delaware Bay area under the ARM model.

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[2] The Peer Review Panel did not endorse the use of the reference points developed for this stock assessment.

7.3 Stock Status Determination

Although reference points were developed for the Delaware Bay population as described above, the Peer Review Panel recommended that these not be used for comparison to CMSA model output and recommended status determinations be based on ARIMA analyses within each region and coastwide. The reference point from the ARIMA fits was the 1998 index-based reference point because this reference point represents the point in time when horseshoe crabs became actively managed by the ASMFC and status relative to this reference point gives an indication of the effects of management on populations. ARIMA results from surveys used to determine stock status included those surveys with combined-sex indices, residuals of ARIMA model fits were normally distributed, time series extending back to at least 1998, and terminal years were 2016 or 2017.

Stock status was based on the percentage of surveys within a region (or coastwide) having a >50% probability of their terminal year fitted value being less the 1998 index-based reference point. “Poor” status was >66% of surveys meeting this criterion, “Good” status was <33% of surveys, and “Neutral” status was 34 – 65% of surveys (Table 53). The stock status of the Northeast region was neutral; New York region was poor; Delaware Bay region was neutral; and Southeast region was good. The overall coastwide status was neutral.

Applying these stock status criteria to summary ARIMA results from the 2009 benchmark assessment and 2013 update assessment gives a general idea of how status has changed through time. The status of the Delaware Bay region and Southeast region has remained consistently neutral and good, respectively, through time. The status of the Northeast region has changed from neutral, to poor, and back to neutral. The status of the New York region has trended downward from good, to neutral, and now poor. These trends in time should be viewed with caution because the number of surveys in each region has changed in the current assessment and the index values have changed due to our use of the delta distribution for estimates of the mean and variance of each survey index. Previous assessments used index values as given to the SAS by state TC members with no standardization. Previous assessments also included all subsets of a survey (e.g., male and female indices from the same survey) which resulted in “double counting” of individual surveys.

A more detailed description of the surveys used to determine stock status is provided in Table 54. Recent trends (5 year and 10 year) were characterized for each survey by linear regression fitted ARIMA values. An alpha level of 0.10 used to determine if a significant trend occurred over these recent time periods. The Northeast region contained only two surveys meeting the criteria for use in stock determination (MA DMF trawl south of Cape Cod and the RI monthly trawl survey from the fall) and these surveys had conflicting trends. The MA DMF trawl survey south of Cape Cod showed an increasing trend in recent years while the Rhode Island monthly trawl survey continued to show a declining trend. There was consistency among New York region surveys with three out of four showing declining trends in the past five years and all showing declining trends in the past 10 years. Surveys from the Delaware Bay and Southeast regions showed either no trend or increasing trends.

Despite the aforementioned caveats when interpreting changes in regional status through time in Table 53, it is clear that the status of the New York region has declined through time. The surveys in the New York region are largely the same since the 2009 benchmark assessment and have consistently been combined-sex surveys. The difference in this assessment was that the Little Neck and Manhasset Bay surveys were combined into a single survey whereas they were considered separate surveys in previous assessments. The status of the New York survey has gone from good, to neutral, to poor. There is no mortality associated with biomedical collections in the New York region and bait harvest has been reduced from historic levels with a current NY state mandated quota of 150,000 per year. Two hypotheses for the continued decline in abundance are: 1) bait harvest remains at a level that is not sustainable in the New York region; or 2) the habitat has changed and cannot support the number of horseshoe crabs it once did.

7.3.1 Uncertainty

ARIMA results give some indication of stock status (whether the populations are increasing or decreasing) and the probability of the current state of the populations being less than an index-based reference point. However, specific reasons for continued decline, as seen in the New York region, remain elusive and it cannot be determined if these declines are a result of excessive exploitation or changes in habitat suitability.

There also remains much uncertainty about embayment and region-specific populations that could not be modelled as part of this assessment because of a lack of data. Maine, New Hampshire, and Florida were grouped into regions that may not reflect the abundance of horseshoe crabs in those areas. Additionally, the regional groupings used in this assessment reflect the SAS and TC’s best efforts to reflect biology and management units but the states are encouraged to consider the embayment-specific populations of horseshoe crabs that are in their state’s waters. There is evidence that there are embayment-specific populations in Maine, New Hampshire, and Florida, as well as in other states (see section 2.1), and yet there are no sufficient surveys to track abundance for these populations. These issues can persist even when there is sufficient data available for tracking abundance. For example, populations of horseshoe crab north and south of Cape Cod in Massachusetts exhibit different patterns, as does the abundance index in Rhode Island, and yet these indices were combined in this stock assessment to represent the Northeast region. The Gulf of Maine could be considered its own region in future assessments if there are any additional suitable indices from that area and the Massachusetts North Cape index may be better categorized to that region. Similar considerations could be made for Florida if there was data to support it. All of the Atlantic states are encouraged to monitor and manage the horseshoe crab populations at appropriate levels and collect additional data as needed.

7.4 Comparison of Assessment Management Advice to ARM Model

Management advice that may stem from this stock assessment versus the Adaptive Resource Management (ARM) model represents two different, and somewhat competing, management objectives (Table 55). This stock assessment can form the basis for single species management in the Delaware Bay, while the ARM model represents multi-species management with the harvest of horseshoe crabs constrained by the needs of shorebirds such as the red knot. Currently, management of horseshoe crabs in the Delaware Bay falls under Addendum VII of the fisheries management plan, which calls for the use of the ARM model when making annual harvest recommendations.

Underlying the ARM model are population models for both red knots and horseshoe crabs. The optimization routine in the ARM model determines the best choice among five potential harvest packages (numbers of male and females that can be harvested) given the current abundance of each species in order to maximize the long-term value of horseshoe crab harvest. The ARM model values female harvest only when the abundance of Red Knots reaches 81,900 birds (a value related to the historic abundance of red knots in the Delaware Bay) or when the abundance of female horseshoe crabs reaches 80% of their carrying capacity (11.2 million assuming a carrying capacity of 14 million). On an annual basis, the ARM model is used to select the optimum harvest package to implement for the next year given the current year's estimate of horseshoe crab abundance from the swept area estimate from the VA Tech trawl survey and a mark-resight estimate of red knot abundance.

At the present time, neither the 81,900 red knot threshold nor the 11.2 million female horseshoe crab thresholds are met. This assessment estimates there are **CONFIDENTIAL** female horseshoe crabs and the ARM workgroup estimated there were 45,221 red knots in Delaware Bay in 2018. While the Peer Review Panel did not endorse the use of the reference points developed for this stock assessment, they did suggest that the ARM Workgroup consider using the population estimates from the CMSA as the best available population estimates of horseshoe crabs in the Delaware Bay region.

8 RESEARCH RECOMMENDATIONS

The SAS identified several research recommendations that would benefit horseshoe crab and future stock assessments. Research recommendations have been categorized as future research, data collection, and assessment methodology and listed in order of priority. The SAS recommends that an update be considered in five years and a benchmark stock assessment considered in ten years given the life history of horseshoe crab and the need for more data. The SAS and TC recommend that during the years between this assessment and the next, members remain proactive about maintaining surveys and research programs and continuing to initiate or participate in activities that accomplish some of the research recommendations listed below.

Future Research

- Determine relationship between age, stage, and size for horseshoe crabs.
- Compare densities of horseshoe crabs nearshore, offshore, and in bays, compare different stages (i.e., primiparous and multiparous), and look at movements among embayments within regions (i.e., around Cape Cod, Long Island).

- Characterize the proportion of states' landings that comprise crabs of Delaware Bay origin. This can be done through a directed tag/release study, genetics/microchemistry study, or both.
- Collect more life history information, particularly for juveniles, on growth, molt timing, and distribution.
- Evaluate the effect of warming temperatures on distribution and timing of spawning for horseshoe crabs.
- Address the issue of gear saturation for spawning beach surveys and/or explore analyses that would be less sensitive to gear saturation. Explore the methodology and data collection of spawning beach surveys and the ability of these surveys to track spawning abundance.
- Determine if there is illegal take-and-use at sea, transfer at sea, and poaching from spawning areas for horseshoe crabs and estimate the amount if possible.

Data Collection

- Continue to fund and operate the full Virginia Tech Trawl Survey annually.
- Conduct a gear efficiency study of the Virginia Tech Trawl Survey given the importance of using swept-area estimates of abundance in modeling the Delaware population.
- Better characterize the discards, landings, and discard mortality by gear.
- Increase the priority of maintaining and managing horseshoe crab data in and among states, both fishery-dependent and –independent, and improve communication between data providers.
- Continue current biosampling for sex and weight and expand where possible.
- Develop a standardized biosampling protocol to cover different seasons and obtain weights, ages, stages, and widths of horseshoe crabs using a random sampling design.
- Expand or implement fishery-independent surveys (e.g., spawning, benthic trawl, tagging) to target horseshoe crabs throughout their full range including estuaries. Highest priority should be given to implementing directed surveys in the Northeast and New York regions.
- Collect sex and stage data in fishery-independent surveys. Surveys should consider using similar methods as the Virginia Tech Trawl Survey and collect biological data by sex and stage, particularly by primiparous and multiparous.
- Continue to evaluate biomedically bled crabs' mortality rates. Consider a tagging study of biomedically bled horseshoe crabs to obtain relative survival and collaborations between researchers and biomedical facilities that would result in peer-reviewed mortality estimates.
- Maintain consistent data collection and survey designs for spawning beach surveys each year and encourage spawning beach surveys to conduct the data collection for the survey and tagging resights separately.

Assessment Methodology

- The ARM working group should consider using the population estimates from the CMSA model as an input to the ARM model as well as estimated mortality from discards and the biomedical industry.
- Further develop the catch-survey analysis and apply assessment modeling beyond the Delaware Bay region, which would require more stage-based data collection.
- Develop a stage-based or length-based model specific for horseshoe crabs that addresses their life history characteristics.
- Estimate the survival of early life stages (e.g., age-zero, juveniles) and growth rates.
- Explore the possibility of using a delay-difference model for future assessments. Because of the life history of horseshoe crab, this would require 20-30 years of data before it could be developed.
- Continue to evaluate tagging data by fitting capture-recapture models that include a short-term (1 year) bleeding effect, account for spatial distribution of harvest pressure, account for capture methodology, and account for disposition of recaptured tagged individuals. Potential methodological approaches include use of time-varying individual covariates to indicate which crabs are 1 year from bleeding and use of hierarchical models to estimate interannual variation in survival within time periods defined by major regulatory changes.

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10 TABLES

Table 1. Coastwide horseshoe crab (HSC) commercial bait landings in numbers, 1998-2016, as validated by ACCSP. The 2017 landings are from state compliance reports.

Year	Female HSC (#s)	Male HSC (#s)	Unclassified Sex (#s)	Total HSC (#s)
1998	382,199	413,698	1,120,553	1,916,450
1999	388,280	466,540	1,750,460	2,605,280
2000	189,653	392,123	1,095,137	1,676,913
2001	155,561	280,626	349,220	785,407
2002	299,296	558,704	408,794	1,266,795
2003	233,583	415,456	399,061	1,048,100
2004	146,399	201,252	308,790	656,441
2005	142,303	258,774	309,457	710,534
2006	201,063	212,478	383,870	797,411
2007	141,705	191,574	452,325	785,604
2008	89,817	229,265	333,781	652,863
2009	115,590	355,323	293,741	764,654
2010	97,546	269,886	245,067	612,499
2011	79,827	315,679	297,364	692,870
2012	135,266	287,991	373,610	796,867
2013	83,161	477,844	390,357	951,362
2014	38,314	423,265	325,819	787,397
2015	33,398	247,593	315,655	596,646
2016	42,636	353,061	345,065	740,762
2017	160,726	675,241	158,524	994,491

Table 2. State bait harvest quotas for 2019 as determined by the interstate FMP (ASMFC) and state-specific regulations (state).

Jurisdiction	**ASMFC Quota 2019**	**State Quota 2019**
MA	330,377	165,000
RI	26,053	8,398
CT	48,689	48,689
NY	366,272	150,000
NJ*	162,136	0
DE*	162,136	162,136
MD*	255,980	255,980
VA**	172,828	172,828
NC	24,036	24,036
SC	0	0
GA	29,312	29,312
FL	9,455	9,455
TOTAL	1,587,274	1,025,834

***Male-only harvest**

****Virginia harvest east of the COLREGS line is limited to 81,331 male-only crabs under the ARM harvest package #3. Value shown is the total state quota.**

Table 3. Numbers of tags released and recaptured by region.

		Recaptured by region								
Release region	# Released	Ches Bay	Coast DE-VA	Coast NY-NJ	Del Bay	Gulf	NC	North east	South east	Unk
Ches Bay	840	105	6	1	0	0	0	0	0	0
Coast DE-VA	96,095	18	5,983	123	2856	0	9	85	5	5
Coast NY-NJ	27,765	0	18	2872	44	1	1	142	1	0
Del Bay	78,841	5	506	291	14,006	1	4	27	3	17
Gulf	1,853	0	2	0	0	142	0	0	2	0
NC	280	1	1	0	1	0	4	1	0	0
Northeast	98,274	2	17	965	31	0	0	19,158	3	7
Southeast	13,305	0	5	6	9	3	0	6	1,713	0
Unknown	17	0	0	8	0	0	0	8	0	1

Table 4. Recapture (%) relative to total recaptures for each region of release.

		Recapture Region								
Release region	**Released**	**Ches Bay**	**Coast DE-VA**	**Coast NY-NJ**	**Del Bay**	**Gulf**	**NC**	**Northeast**	**Southeast**	**Unk**
Ches Bay	840	93.75	5.36	0.89	0.00	0.00	0.00	0.00	0.00	0.00
Coast DE-VA	96,095	0.20	65.86	1.35	31.44	0.00	0.10	0.94	0.06	0.06
Coast NY-NJ	27,765	0.00	0.58	93.28	1.43	0.03	0.03	4.61	0.03	0.00
Del Bay	78,841	0.03	3.41	1.96	94.25	0.01	0.03	0.18	0.02	0.11
Gulf	1,853	0.00	1.37	0.00	0.00	97.26	0.00	0.00	1.37	0.00
NC	280	12.50	12.50	0.00	12.50	0.00	50.00	12.50	0.00	0.00
Northeast	98,274	0.01	0.08	4.78	0.15	0.00	0.00	94.92	0.01	0.03
Southeast	13,305	0.00	0.29	0.34	0.52	0.17	0.00	0.34	98.34	0.00
Unknown	17	0.00	0.00	47.06	0.00	0.00	0.00	47.06	0.00	5.88

Table 5. **Regional apparent annual survival rates, averaged among years 2009-2017.**

Region	Phi-hat	SE	LCL	UCL
Coastal DE-VA	0.71	0.0118	0.6874	0.7335
Coastal NY-NJ	0.62	0.0162	0.5884	0.6516
Delaware Bay	0.76	0.0137	0.7275	0.7813
Northeast	0.67	0.0058	0.6587	0.6813
Southeast	0.63	0.0350	0.5545	0.6907

Table 6. **Annual survival and movement rates for Delaware Bay and coastal embayments in Delaware and Virginia for the years 2003 to 2017 estimated from multi-state model using program MARK.**

Parameter	Location	Estimate	Standard error	Lower confidence limit	Upper confidence limit
Annual survival rate	Coastal DE-VA	0.61	0.0148	0.5820	0.6400
	Delaware Bay	0.79	0.0103	0.7677	0.8080
	Other areas	0.59	0.0349	0.5182	0.6541
Annual movement rate	Coastal embayments to Delaware Bay	0.28	0.0478	0.1944	0.3804
	Coastal embayments to other areas	0.03	0.0014	0.0286	0.0339
	Delaware Bay to coastal embayments	0.23	0.0344	0.1741	0.3085
	Delaware Bay to other areas	0.02	0.0008	0.0233	0.0263
	Other areas to coastal embayments	0.70	0.1048	0.4643	0.8581
	Other areas to Delaware Bay	0.27	0.1038	0.1169	0.5097

Table 7. Instantaneous natural mortality rate (M) schedule.

Age	*S*	*M*	Reference
Age 0 to Age 1	0.00003	10.4143	Botton et al. 2003
Ages 1 to 8	0.9738	0.0265	Carmichael et al. 2003 (Table 13)
Age 9 to Age 10	0.7994	0.2239	Mean of 1-8 and 11-17 - assumption
Age 10 to Age 11	0.7994	0.2239	Mean of 1-8 and 11-17 - assumption
Ages 11 to 17	0.6250	0.4700	Carmichael et al. 2003 (Table 10 -mean of instars 20-23)
Ages 18 to 19	0.08	2.5257	Carmichael et al. 2003 (Table 10 –Instar 24)
Age 20	0		All dead - assumption

Table 8. Inputs for estimating natural morality for horseshoe crabs. NOAA average water temperatures for Lewes DE (https://www.nodc.noaa.gov/dsdt/cwtg/all_meanT.html).

Inputs	Combined-sex	Females	Males
Maximum Observed Age	27	27	27
Average Water Temp C*	12.99	12.99	12.99
K	0.15	0.14	0.17
L_inf cm	23.08	26.39	21.12
T0	0.10	0.12	0.09

Table 9. Models and estimates of age-invariant instantaneous natural mortality rates for horseshoe crabs.

Model	Formula	M (combined-sex)	M (females)	M (males)
Hoenig (1983)	Z = exp(1.44-0.982*ln(tmax)), 134 stocks	0.166	0.166	0.166
	Z = exp(1.46-1.01*ln(tmax)), 84 fish stocks	0.154	0.154	0.154
Longevity-Based ROTs	Z = ln(1.5%)/tmax or 4.22/tmax	0.156	0.156	0.156
	Z = ln(5%)/tmax or 3/tmax	0.111	0.111	0.111
Pauly (1980)	ln(M) = -0.0066-0.279*ln(Linf)+0.6543*ln(K)+0.4634*ln(T)	0.399	0.359	0.440
	ln(M) = -0.0152-0.279*ln(Linf)+0.6543*ln(K)+0.4634*ln(T)	0.396	0.356	0.436
Jensen (1996)	M = gK; g = 1.598	0.246	0.221	0.275

Table 10. Hypothetical instantaneous natural mortality rate schedules for horseshoe crab based on von Bertalanffy growth.

	Gislason, et al. 2010	Gislason, et al. 2010	Lorenzen 1996	Lorenzen 1996
Age	**Male M**	**Female M**	**Male M**	**Female M**
0	8.95	11.13	3.73	4.05
1	1.42	1.55	1.44	1.42
2	0.68	0.72	0.98	0.94
3	0.44	0.45	0.79	0.74
4	0.33	0.33	0.68	0.62
5	0.27	0.26	0.61	0.55
6	0.23	0.22	0.56	0.50
7	0.20	0.19	0.53	0.46
8	0.18	0.17	0.50	0.44
9	0.17	0.16	0.48	0.42
10	0.16	0.14	0.47	0.40
11	0.15	0.14	0.45	0.39
12	0.15	0.13	0.45	0.37
13	0.14	0.12	0.44	0.37
14	0.14	0.12	0.43	0.36
15	0.14	0.11	0.43	0.35
16	0.13	0.11	0.42	0.35
17	0.13	0.11	0.42	0.34
18	0.13	0.11	0.42	0.34
19	0.13	0.10	0.41	0.34
20	0.13	0.10	0.41	0.33
21	0.13	0.10	0.41	0.33
22	0.12	0.10	0.41	0.33
23	0.12	0.10	0.41	0.33
24	0.12	0.10	0.41	0.33
25	0.12	0.10	0.41	0.32
26	0.12	0.10	0.40	0.32
27	0.12	0.10	0.40	0.32

Table 11. Data and results for the Mann-Kendall test of temporal trends in sex ratios, defined as the ratio of males to females. Significant p-values are presented in bold. Trends test not applicable for biomedical data due to the low number of years with sex-specific harvest data. Survey type refers to fisheries independent (FI) and dependent (FD) data. Confidential biomedical data have been removed from this public document.

Source	Type	State	Location	Sex Ratio	tau	p value	Years included in analysis
Trawl	FI	NJ	DE_Bay Fall	2.16	0.18	0.19	1990-2017
Trawl	FI	NJ	**DE_Bay Spr**	**1.27**	**0.38**	**0.00**	1990-2017
Trawl	FI	NJ	NJ_Ocean_Fall	1.13	0.18	0.28	1999-2017
Trawl	FI	NJ	NJ_Ocean_Spr	1.03	0.13	0.46	1999-2017
Trawl	FI	NJ	NJ_SurfClam	0.51	-0.05	0.84	1998-2012
Spawning	FI	NH	Beaches	1.55	0.31	0.21	2002-2012
Landings	FD	MD	MD	1.49	-	-	1998-2016
Landings	FD	VA	**VA**	**1.30**	**0.45**	**0.02**	2001-2016
Landings	FD	NJ	NJ	2.52	-0.17	0.60	1998-2006
Landings	FD	DE	DE	1.87	-	-	1998-2016
Commercial	FD	VA	VA_SIW	0.64	0.28	0.14	2000:2003, 2006:2017
Commercial	FD	VA	**VA_SOW**	**1.28**	**0.69**	**0.00**	2000, 2002, 2005, 2006, 2008, 2010:2017
Biomed	FD	**CONFIDENTIAL**					
Biomed	FD						
Biomed	FD						
Biomed	FD						
Biomed	FD						
Biomed	FD						

Table 12. Sex ratio and proportion female information, along with associated confidence limits, for each survey of available fisheries-independent and –dependent data sources.

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Trawl	NJ_SurfClam	1998	57.9%	45.7%	70.1%	0.73	0.36	1.09
Trawl	NJ_SurfClam	1999	63.4%	51.7%	75.1%	0.58	0.29	0.87
Trawl	NJ_SurfClam	2000	60.0%	52.6%	67.4%	0.67	0.46	0.87
Trawl	NJ_SurfClam	2001	65.0%	54.5%	75.5%	0.54	0.29	0.79
Trawl	NJ_SurfClam	2002	68.3%	58.4%	78.3%	0.46	0.25	0.68
Trawl	NJ_SurfClam	2003	73.2%	64.7%	81.6%	0.37	0.21	0.52
Trawl	NJ_SurfClam	2004	80.1%	74.7%	85.4%	0.25	0.17	0.33
Trawl	NJ_SurfClam	2005	86.5%	80.6%	92.5%	0.16	0.08	0.24
Trawl	NJ_SurfClam	2006	74.2%	68.5%	80.0%	0.35	0.24	0.45
Trawl	NJ_SurfClam	2007	64.2%	53.7%	74.6%	0.56	0.30	0.81
Trawl	NJ_SurfClam	2008	62.4%	55.0%	69.8%	0.60	0.41	0.79
Trawl	NJ_SurfClam	2009	72.2%	61.3%	83.1%	0.39	0.18	0.59
Trawl	NJ_SurfClam	2010	60.8%	54.4%	67.2%	0.64	0.47	0.82
Trawl	NJ_SurfClam	2011	69.8%	61.2%	78.4%	0.43	0.26	0.61
Trawl	NJ_SurfClam	2012	53.6%	36.9%	70.3%	0.87	0.28	1.45
Trawl	NJ_Ocean_Spr	1996	59.9%	51.6%	68.2%	0.67	0.44	0.90
Trawl	NJ_Ocean_Spr	1999	44.2%	36.3%	52.1%	1.26	0.86	1.67
Trawl	NJ_Ocean_Spr	2000	48.8%	43.4%	54.3%	1.05	0.82	1.28
Trawl	NJ_Ocean_Spr	2001	45.5%	38.2%	52.7%	1.20	0.85	1.55
Trawl	NJ_Ocean_Spr	2002	62.4%	50.5%	74.2%	0.60	0.30	0.91
Trawl	NJ_Ocean_Spr	2003	48.0%	40.8%	55.1%	1.08	0.77	1.40
Trawl	NJ_Ocean_Spr	2004	50.8%	45.2%	56.5%	0.97	0.75	1.19
Trawl	NJ_Ocean_Spr	2005	47.5%	41.1%	54.0%	1.10	0.82	1.39
Trawl	NJ_Ocean_Spr	2006	54.0%	38.0%	70.0%	0.85	0.30	1.40
Trawl	NJ_Ocean_Spr	2007	52.9%	40.5%	65.4%	0.89	0.45	1.33
Trawl	NJ_Ocean_Spr	2008	50.1%	45.4%	54.9%	1.00	0.81	1.18
Trawl	NJ_Ocean_Spr	2009	44.4%	37.4%	51.4%	1.25	0.90	1.61
Trawl	NJ_Ocean_Spr	2010	41.5%	37.7%	45.2%	1.41	1.19	1.63
Trawl	NJ_Ocean_Spr	2011	55.9%	46.7%	65.1%	0.79	0.49	1.08
Trawl	NJ_Ocean_Spr	2012	46.4%	40.5%	52.2%	1.16	0.89	1.43
Trawl	NJ_Ocean_Spr	2013	53.7%	44.0%	63.4%	0.86	0.53	1.20
Trawl	NJ_Ocean_Spr	2014	51.6%	40.4%	62.8%	0.94	0.52	1.36
Trawl	NJ_Ocean_Spr	2015	46.2%	32.4%	60.0%	1.16	0.52	1.81
Trawl	NJ_Ocean_Spr	2016	48.6%	42.8%	54.3%	1.06	0.82	1.30
Trawl	NJ_Ocean_Spr	2017	45.0%	29.1%	60.9%	1.22	0.44	2.00

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Trawl	NJ_Ocean_Fall	1999	51.9%	46.1%	57.7%	0.93	0.71	1.14
Trawl	NJ_Ocean_Fall	2000	50.6%	41.2%	60.0%	0.98	0.61	1.34
Trawl	NJ_Ocean_Fall	2001	51.6%	43.4%	59.9%	0.94	0.63	1.25
Trawl	NJ_Ocean_Fall	2002	49.9%	42.1%	57.7%	1.00	0.69	1.32
Trawl	NJ_Ocean_Fall	2003	45.6%	37.6%	53.6%	1.19	0.81	1.58
Trawl	NJ_Ocean_Fall	2004	51.1%	46.5%	55.8%	0.96	0.78	1.13
Trawl	NJ_Ocean_Fall	2005	38.0%	31.8%	44.2%	1.63	1.20	2.06
Trawl	NJ_Ocean_Fall	2006	43.9%	36.6%	51.1%	1.28	0.90	1.66
Trawl	NJ_Ocean_Fall	2007	43.9%	38.8%	49.1%	1.28	1.01	1.54
Trawl	NJ_Ocean_Fall	2008	58.8%	49.2%	68.4%	0.70	0.42	0.98
Trawl	NJ_Ocean_Fall	2009	49.7%	35.6%	63.8%	1.01	0.44	1.58
Trawl	NJ_Ocean_Fall	2010	46.2%	31.1%	61.3%	1.16	0.46	1.87
Trawl	NJ_Ocean_Fall	2011	42.8%	30.7%	54.9%	1.34	0.68	2.00
Trawl	NJ_Ocean_Fall	2012	45.1%	30.6%	59.7%	1.22	0.50	1.93
Trawl	NJ_Ocean_Fall	2013	65.0%	42.2%	87.9%	0.54	0.00	1.08
Trawl	NJ_Ocean_Fall	2014	43.3%	34.2%	52.4%	1.31	0.83	1.80
Trawl	NJ_Ocean_Fall	2015	47.2%	36.5%	57.9%	1.12	0.64	1.60
Trawl	NJ_Ocean_Fall	2016	39.7%	27.6%	51.8%	1.52	0.75	2.29
Trawl	NJ_Ocean_Fall	2017	47.1%	32.6%	61.7%	1.12	0.47	1.77
Trawl	DE_Spr	1990	56.7%	41.7%	71.7%	0.76	0.30	1.23
Trawl	DE_Spr	1991	48.8%	41.3%	56.3%	1.05	0.74	1.36
Trawl	DE_Spr	1992	55.2%	46.8%	63.6%	0.81	0.54	1.09
Trawl	DE_Spr	1993	44.0%	33.9%	54.1%	1.27	0.75	1.79
Trawl	DE_Spr	1994	38.4%	27.4%	49.4%	1.60	0.86	2.35
Trawl	DE_Spr	1995	49.6%	41.5%	57.8%	1.01	0.68	1.34
Trawl	DE_Spr	1996	65.2%	55.4%	74.9%	0.53	0.30	0.77
Trawl	DE_Spr	1997	44.4%	34.4%	54.4%	1.25	0.75	1.76
Trawl	DE_Spr	1998	52.5%	41.7%	63.4%	0.90	0.51	1.30
Trawl	DE_Spr	1999	42.9%	34.5%	51.4%	1.33	0.87	1.79
Trawl	DE_Spr	2000	46.7%	39.3%	54.1%	1.14	0.80	1.48
Trawl	DE_Spr	2001	48.6%	39.6%	57.6%	1.06	0.68	1.44
Trawl	DE_Spr	2002	65.0%	29.5%	100.5%	0.54	0.00	1.38
Trawl	DE_Spr	2003	52.5%	36.6%	68.5%	0.90	0.32	1.48
Trawl	DE_Spr	2004	75.0%	0.0%	100.0%	0.33	0.00	1.77
Trawl	DE_Spr	2005	71.4%	26.3%	100.0%	0.40	0.00	1.28
Trawl	DE_Spr	2006	48.8%	38.4%	59.2%	1.05	0.61	1.49
Trawl	DE_Spr	2007	37.0%	26.8%	47.1%	1.70	0.96	2.45
Trawl	DE_Spr	2008	41.7%	20.4%	62.9%	1.40	0.18	2.62
Trawl	DE_Spr	2009	38.8%	26.4%	51.2%	1.58	0.75	2.40

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Trawl	DE_Spr	2011	25.5%	13.6%	37.4%	2.93	1.09	4.76
Trawl	DE_Spr	2012	45.5%	31.0%	60.0%	1.20	0.50	1.90
Trawl	DE_Spr	2013	37.5%	7.7%	67.3%	1.67	0.00	3.78
Trawl	DE_Spr	2014	39.2%	30.2%	48.2%	1.55	0.97	2.14
Trawl	DE_Spr	2015	36.1%	26.0%	46.3%	1.77	0.99	2.55
Trawl	DE_Spr	2016	42.7%	34.4%	50.9%	1.34	0.89	1.80
Trawl	DE_Spr	2017	33.4%	25.8%	41.0%	2.00	1.31	2.68
Trawl	DE_Fall	1990	39.5%	30.9%	48.0%	1.53	0.99	2.08
Trawl	DE_Fall	1991	41.5%	31.2%	51.8%	1.41	0.81	2.01
Trawl	DE_Fall	1992	26.1%	16.6%	35.5%	2.83	1.45	4.22
Trawl	DE_Fall	1993	30.5%	24.3%	36.8%	2.28	1.61	2.95
Trawl	DE_Fall	1994	26.5%	4.6%	48.3%	2.78	0.00	5.90
Trawl	DE_Fall	1995	46.1%	36.2%	56.0%	1.17	0.71	1.64
Trawl	DE_Fall	1996	29.1%	23.2%	35.0%	2.43	1.74	3.13
Trawl	DE_Fall	1997	46.3%	37.4%	55.2%	1.16	0.75	1.57
Trawl	DE_Fall	1998	33.3%	20.3%	46.4%	2.00	0.83	3.17
Trawl	DE_Fall	1999	35.1%	23.1%	47.2%	1.85	0.87	2.82
Trawl	DE_Fall	2000	50.9%	40.3%	61.6%	0.96	0.55	1.37
Trawl	DE_Fall	2001	44.4%	0.0%	96.1%	1.25	0.00	3.87
Trawl	DE_Fall	2002	35.3%	0.0%	72.7%	1.83	0.00	4.83
Trawl	DE_Fall	2003	23.3%	9.9%	36.6%	3.30	0.82	5.78
Trawl	DE_Fall	2004	33.3%	0.0%	100.0%	2.00	0.00	27.41
Trawl	DE_Fall	2005	42.9%	0.0%	100.0%	1.33	0.00	4.50
Trawl	DE_Fall	2006	27.0%	18.7%	35.2%	2.71	1.57	3.85
Trawl	DE_Fall	2007	27.3%	11.9%	42.6%	2.67	0.60	4.73
Trawl	DE_Fall	2008	37.5%	0.0%	76.4%	1.67	0.00	4.43
Trawl	DE_Fall	2009	26.5%	7.5%	45.4%	2.78	0.07	5.48
Trawl	DE_Fall	2010	31.8%	0.4%	63.2%	2.14	0.00	5.25
Trawl	DE_Fall	2011	18.8%	0.0%	41.2%	4.33	0.00	10.71
Trawl	DE_Fall	2012	22.7%	0.0%	47.5%	3.40	0.00	8.20
Trawl	DE_Fall	2013	41.6%	27.6%	55.6%	1.41	0.60	2.22
Trawl	DE_Fall	2014	31.1%	18.7%	43.5%	2.21	0.93	3.50
Trawl	DE_Fall	2015	43.4%	33.1%	53.7%	1.31	0.76	1.85
Trawl	DE_Fall	2016	27.0%	22.2%	31.8%	2.71	2.04	3.37
Trawl	DE_Fall	2017	25.1%	17.7%	32.5%	2.99	1.81	4.16

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Survey	NH_Spawn	2002	40.4%	32.4%	48.4%	1.47	0.98	1.96
Survey	NH_Spawn	2003	48.8%	46.4%	51.2%	1.05	0.95	1.15
Survey	NH_Spawn	2004	48.8%	47.1%	50.4%	1.05	0.98	1.12
Survey	NH_Spawn	2005	46.5%	43.1%	49.9%	1.15	0.99	1.31
Survey	NH_Spawn	2006	46.2%	42.8%	49.5%	1.17	1.01	1.32
Survey	NH_Spawn	2007	49.7%	39.1%	60.3%	1.01	0.58	1.44
Survey	NH_Spawn	2008	32.1%	27.4%	36.8%	2.11	1.66	2.57
Survey	NH_Spawn	2009	28.0%	19.9%	36.0%	2.58	1.55	3.61
Survey	NH_Spawn	2010	23.7%	13.5%	33.9%	3.21	1.40	5.03
Survey	NH_Spawn	2011	47.8%	45.5%	50.2%	1.09	0.99	1.19
Survey	NH_Spawn	2012	45.7%	42.7%	48.7%	1.19	1.04	1.33
Landings	MD	1998	69.2%	-	-	0.45	-	-
Landings	MD	1999	82.6%	-	-	0.21	-	-
Landings	MD	2000	53.2%	-	-	0.88	-	-
Landings	MD	2001	50.3%	-	-	0.99	-	-
Landings	MD	2002	36.5%	-	-	1.74	-	-
Landings	MD	2003	43.3%	-	-	1.31	-	-
Landings	MD	2004	40.1%	-	-	1.49	-	-
Landings	MD	2005	36.0%	-	-	1.78	-	-
Landings	MD	2006	65.7%	-	-	0.52	-	-
Landings	MD	2007	59.0%	-	-	0.69	-	-
Landings	MD	2008	40.5%	-	-	1.47	-	-
Landings	MD	2009	30.8%	-	-	2.25	-	-
Landings	MD	2010	26.2%	-	-	2.82	-	-
Landings	MD	2011	21.3%	-	-	3.69	-	-
Landings	MD	2012	32.4%	-	-	2.09	-	-
Landings	MD	2013	0.0%	-	-	Inf	-	-
Landings	MD	2014	0.0%	-	-	Inf	-	-
Landings	MD	2015	0.0%	-	-	Inf	-	-
Landings	MD	2016	0.0%	-	-	Inf	-	-
Landings	VA	2001	30.2%	-	-	2.31	-	-
Landings	VA	2002	58.0%	-	-	0.72	-	-
Landings	VA	2003	87.1%	-	-	0.15	-	-
Landings	VA	2004	84.8%	-	-	0.18	-	-
Landings	VA	2005	67.7%	-	-	0.48	-	-

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Landings	VA	2007	50.5%	-	-	0.98	-	-
Landings	VA	2008	44.1%	-	-	1.27	-	-
Landings	VA	2009	34.6%	-	-	1.89	-	-
Landings	VA	2010	38.5%	-	-	1.60	-	-
Landings	VA	2011	36.6%	-	-	1.73	-	-
Landings	VA	2012	53.0%	-	-	0.89	-	-
Landings	VA	2013	53.1%	-	-	0.88	-	-
Landings	VA	2014	26.4%	-	-	2.79	-	-
Landings	VA	2015	32.7%	-	-	2.06	-	-
Landings	VA	2016	33.1%	-	-	2.02	-	-
Landings	NJ	1998	28.1%	-	-	2.56	-	-
Landings	NJ	1999	33.1%	-	-	2.02	-	-
Landings	NJ	2000	23.9%	-	-	3.19	-	-
Landings	NJ	2001	26.1%	-	-	2.83	-	-
Landings	NJ	2002	28.2%	-	-	2.54	-	-
Landings	NJ	2003	25.8%	-	-	2.87	-	-
Landings	NJ	2004	27.6%	-	-	2.63	-	-
Landings	NJ	2005	27.9%	-	-	2.59	-	-
Landings	NJ	2006	41.1%	-	-	1.43	-	-
Landings	DE	1998	53.9%	-	-	0.85	-	-
Landings	DE	1999	44.4%	-	-	1.25	-	-
Landings	DE	2000	45.6%	-	-	1.19	-	-
Landings	DE	2001	41.4%	-	-	1.41	-	-
Landings	DE	2002	39.4%	-	-	1.54	-	-
Landings	DE	2003	34.4%	-	-	1.91	-	-
Landings	DE	2004	35.0%	-	-	1.86	-	-
Landings	DE	2005	30.7%	-	-	2.25	-	-
Landings	DE	2006	17.9%	-	-	4.58	-	-
Landings	DE	2007	0.0%	-	-	Inf	-	-
Landings	DE	2008	0.0%	-	-	Inf	-	-
Landings	DE	2009	0.0%	-	-	Inf	-	-
Landings	DE	2010	0.0%	-	-	Inf	-	-
Landings	DE	2011	0.0%	-	-	Inf	-	-
Landings	DE	2012	0.0%	-	-	Inf	-	-
Landings	DE	2013	0.0%	-	-	Inf	-	-
Landings	DE	2014	0.0%	-	-	Inf	-	-
Landings	DE	2015	0.0%	-	-	Inf	-	-
Landings	DE	2016	0.0%	-	-	Inf	-	-
Commercial	SIW	2000	50.6%	-	-	0.98	-	-

Type	Source	Year	Proportion Female	LCL	UCL	Sex Ratio	LCL	UCL
Commercial	SIW	2002	58.2%	-	-	0.72	-	-
Commercial	SIW	2003	63.3%	-	-	0.58	-	-
Commercial	SIW	2004	-	-	-	-	-	-
Commercial	SIW	2005	-	-	-	-	-	-
Commercial	SIW	2006	83.2%	-	-	0.20	-	-
Commercial	SIW	2007	74.2%	-	-	0.35	-	-
Commercial	SIW	2008	75.4%	-	-	0.33	-	-
Commercial	SIW	2009	65.6%	-	-	0.53	-	-
Commercial	SIW	2010	67.1%	-	-	0.49	-	-
Commercial	SIW	2011	61.8%	-	-	0.62	-	-
Commercial	SIW	2012	66.1%	-	-	0.51	-	-
Commercial	SIW	2013	51.6%	-	-	0.94	-	-
Commercial	SIW	2014	48.8%	-	-	1.05	-	-
Commercial	SIW	2015	48.6%	-	-	1.06	-	-
Commercial	SIW	2016	61.8%	-	-	0.62	-	-
Commercial	SIW	2017	55.6%	-	-	0.80	-	-
Commercial	SOW	2000	79.5%	-	-	0.26	-	-
Commercial	SOW	2002	89.3%	-	-	0.12	-	-
Commercial	SOW	2005	56.5%	-	-	0.77	-	-
Commercial	SOW	2006	76.7%	-	-	0.30	-	-
Commercial	SOW	2007	-	-	-	-	-	-
Commercial	SOW	2008	59.3%	-	-	0.69	-	-
Commercial	SOW	2010	66.7%	-	-	0.50	-	-
Commercial	SOW	2011	44.9%	-	-	1.23	-	-
Commercial	SOW	2012	25.4%	-	-	2.93	-	-
Commercial	SOW	2013	52.0%	-	-	0.92	-	-
Commercial	SOW	2014	41.6%	-	-	1.41	-	-
Commercial	SOW	2015	46.5%	-	-	1.15	-	-
Commercial	SOW	2016	23.4%	-	-	3.27	-	-
Commercial	SOW	2017	24.6%	-	-	3.07	-	-

Biomedical **CONFIDENTIAL** Data Removed

Table 13. Commercial bait landings in numbers of horseshoe crabs by region, 1998-2016. The four regions are the Northeast (Maine, Massachusetts, Rhode Island), New York (Connecticut, New York), Delaware Bay (New Jersey, Delaware, Maryland, Virginia), and Southeast (North Carolina, South Carolina, Georgia, Florida).

Year	Region				Coastwide
	Northeast	New York	Delaware Bay	Southeast	
1998	413,700	387,045	1,088,393	27,312	1,916,450
1999	573,618	439,076	1,530,614	61,972	2,605,280
2000	288,310	644,363	718,805	25,435	1,676,913
2001	137,733	140,582	497,962	9,130	785,407
2002	142,770	209,351	900,241	14,432	1,266,795
2003	131,286	149,450	741,369	25,995	1,048,100
2004	75,466	166,002	402,696	12,277	656,441
2005	55,843	170,855	476,123	7,713	710,534
2006	149,851	199,270	437,490	10,800	797,411
2007	109,166	323,320	343,632	9,486	785,604
2008	111,392	181,284	333,946	26,241	652,863
2009	109,996	150,118	471,515	33,025	764,654
2010	75,243	155,404	370,921	10,931	612,499
2011	101,884	167,573	396,286	27,127	692,870
2012	145,218	203,679	423,296	24,674	796,867
2013	166,775	191,242	561,031	32,314	951,362
2014	144,212	155,004	461,579	26,603	787,397
2015	125,596	164,956	280,991	25,103	596,646
2016	131,101	188,767	395,697	25,197	740,762

Table 14. Horseshoe crab commercial bait harvest in numbers for the Delaware Bay states by sex, 1998-2017, validated by ACCSP. The number of female horseshoe crabs of Delaware Bay origin was developed to support the catch survey analysis for that region. See section 4.1.3 for how these numbers were developed.

Year	Female HSC (#s)	Male HSC (#s)	Unclassified Sex (#s)	Total HSC (#s)	DB Origin HSC (#s)	Female DB Origin HSC (#s)
1998	382,199	413,698	292,496	1,088,393	867,959	435,810
1999	388,280	466,540	675,794	1,530,614	1,041,126	530,743
2000	189,653	392,123	137,029	718,805	560,745	189,434
2001	155,561	280,626	61,775	497,962	375,546	120,932
2002	299,296	558,704	42,241	900,241	736,242	257,378
2003	233,583	415,456	92,330	741,369	592,206	220,354
2004	146,399	201,252	55,045	402,696	261,560	108,843
2005	142,303	258,774	75,046	476,123	335,971	116,577
2006	201,063	212,478	23,949	437,490	253,187	104,048
2007	141,705	191,574	10,353	343,632	200,858	67,674
2008	89,817	229,265	14,864	333,946	209,414	44,329
2009	115,590	355,323	602	471,515	268,547	48,663
2010	97,546	269,886	3,489	370,921	196,307	41,385
2011	79,827	315,679	780	396,286	235,358	33,728
2012	135,266	287,991	39	423,296	241,717	56,112
2013	83,161	477,844	26	561,031	341,199	29,111
2014	38,314	423,265		461,579	294,504	13,410
2015	33,398	247,593		280,991	201,066	11,689
2016	42,636	353,061		395,697	235,009	14,923
2017	48,447	524,359		572,806	369,161	16,956

Table 15. Numbers of horseshoe crabs collected, bled, and estimated mortality for the biomedical industry as reported in annual FMP Reviews.

	2004	*2005*	*2006*	*2007*	*2008*	*2009*	*2010*	*2011*	*2012*	*2013*	*2014*	*2015*	*2016**	*2017*
A. Number of crabs brought to biomedical facilities (bait and biomedical crabs)	335,501	282,723	282,787	478,911	491,543	521,330	551,052	600,772	622,098	525,667	534,702	563,631	426,286	575,760
B. Number of bait crabs bled	40,572	36,103	46,600	63,424	69,062	106,365	71,989	78,005	81,433	61,297	67,143	69,731	77,946	95,231
C. Number of biomedical-only crabs collected (not counted against state bait quotas)	284,215	248,475	237,822	416,824	422,958	414,959	480,914	545,164	541,956	464,657	467,897	494,123	344,495	483,245
D. Reported observed mortality of biomedical-only crabs from collection to release	10,145	3,030	2,450	4,663	6,476	6,318	6,829	24,139	7,370	5,447	5,658	5,362	1,004	6,057
E. Number of biomedical-only crabs bled	101,020	190,362	177,599	352,645	397,809	386,118	412,781	486,850	497,956	440,402	432,340	464,506	318,523	444,115
F. Estimated post-bleeding mortality of bled biomedical-only crabs (15% est. mortality)	15,153	28,554	26,640	52,897	59,671	57,918	61,917	73,028	74,693	66,060	64,851	69,676	47,778	66,617
G. Total estimated mortality on biomedical crabs not counted against state bait quotas (15% est. mortality)	25,298	31,584	29,090	57,560	66,147	64,236	68,746	97,166	82,063	71,507	70,509	75,038	48,782	72,674

***Some biomedical collections were reduced in 2016 due to temporary changes in production.**

Table 16. Summary of studies that estimate a mortality rate of crabs bled for biomedical purposes and the same size of crabs bled to obtain the rate. See Appendix A for complete citations for each published paper.

Author(s)	Year	Mortality Rate	Sample Size
Rudloe	1983	0.10	4822
		0.03	40
Thompson	1998	0.15	20
		0.00	594
SCDNR	1999	0.07	132
Wenner and Thompson	2000	0.08	75
Kurz and James-Pirrri	2002	0.20	10
Walls and Berkson	2003	0.00	10
		0.30	10
		0.00	30
		0.00	30
		0.20	30
		0.00	30
		0.07	30
		0.17	30
Hurton and Berkson	2005	0.00	40
		0.00	40
		0.00	40
		0.00	40
		0.03	39
		0.05	39
		0.10	39
		0.15	39
Leschen and Correia	2010	0.15	15
		0.23	19
		0.40	13
		0.07	14
		0.31	14
		0.20	14
		0.20	17
		0.29	21
		0.49	14
		0.10	9
		0.40	15
		0.27	18
DeLancey and Floyd	2012	0.20	50
Anderson et al.	2013	0.00	7
		0.14	7
		0.14	7
		0.43	7
Linesh	2017	0.11	48
Owings	2017	0.00	8
		0.06	17
		0.14	8
		0.13	8
		0.44	9
		0.75	8

Table 17. All trawl captured horseshoe crabs that have been tagged and released since 1999. Shaded gray columns indicate biomedical companies that tag bled crabs (Lonza and Wako).

Release Year	Lonza	MDDNR	NCCRUISE	NYDEC	SHU	VATECH	Wako
1999	2,500	975	0	0	0	0	0
2000	2,500	0	0	0	0	0	0
2001	2,500	0	0	0	0	0	0
2002	2,499	0	0	0	0	0	0
2003	0	0	6	0	0	450	0
2004	2,500	0	3	0	0	330	0
2005	5,496	0	0	0	0	219	0
2006	5,000	0	9	0	0	196	0
2007	5,596	0	16	961	0	202	0
2008	5,496	0	8	257	0	233	75
2009	4,076	0	0	14	2	1,169	102
2010	4,950	0	0	26	3	0	68
2011	5,000	0	0	303	0	408	34
2012	4,150	0	0	65	11	0	153
2013	4,350	0	3	0	125	0	332
2014	2,400	0	0	0	123	0	437
2015	1,275	0	1	43	89	0	636
2016	2,449	0	0	0	51	0	275
2017	2,814	0	0	32	41	37	219
Totals	**65,551**	**975**	**46**	**1,701**	**445**	**3,244**	**2,331**

Table 18. List of recaptured trawl-tagged crabs since 1999. Shaded gray columns indicate biomedical companies that tag bled crabs (Lonza and Wako).

Recover Year	Lonza	MDDNR	NCCRUISE	NYDEC	SHU	VATECH	Wako
1999	16	1	0	0	0	0	0
2000	59	24	0	0	0	0	0
2001	65	18	0	0	0	0	0
2002	124	11	0	0	0	0	0
2003	117	5	0	0	0	2	0
2004	114	3	0	0	0	8	0
2005	140	3	0	0	0	9	0
2006	392	1	0	0	0	13	0
2007	261	0	0	20	0	22	0
2008	371	1	1	11	0	14	0
2009	505	3	0	18	0	11	5
2010	432	1	0	9	1	50	0
2011	470	0	1	9	0	40	4
2012	283	0	1	4	5	24	9
2013	371	1	0	3	0	46	10
2014	282	0	0	2	2	20	15
2015	237	0	0	2	4	13	22
2016	212	0	0	2	1	6	31
2017	250	0	0	0	3	13	18
Totals	**4,701**	**72**	**3**	**80**	**16**	**291**	**114**

Table 19. Total recaptures by years at large (YAL) for all trawl captured, bled male and female horseshoe crabs since 1999.

	Females			Males		
YAL	Alive	Dead	Unknown	Alive	Dead	Unknown
0	348	377	263	657	298	211
1	176	48	131	391	59	122
2	52	31	71	243	58	91
3	51	24	50	150	41	62
4	38	18	39	107	39	43
5	18	12	27	83	23	29
6	8	8	18	76	21	32
7	1	5	19	50	15	10
8	3	3	11	32	15	11
9	1	0	9	13	7	9
10	1	1	8	7	5	6
11	3	3	7	7	6	5

Table 20. Total recaptures by years at large (YAL) for trawl captured, unbled male and female horseshoe crabs since 1999.

	Females			Males		
YAL	Alive	Dead	Unknown	Alive	Dead	Unknown
0	37	23	6	67	20	6
1	14	8	4	52	7	4
2	10	7	4	25	6	2
3	10	4	1	31	15	2
4	8	9	0	17	4	3
5	4	1	1	10	4	2

Table 21. Model statistics for the top 6 out of 70 models fit to the capture recapture data for horseshoe crabs tagged in the coastal Delaware and Virginia geographic area between 1999 and 2017. Model names include group and time effects for apparent survival (Phi) and capture probability (p); npar=number of parameters; AICc=corrected Akaike Information Criteria; Delta AICc=0 indicates the best fitting model.

Model	npar	AICc	Delta AICc
Phi(~sex * bled * timebin3) p(~sex * bled * time)	144	52255.95	0
Phi(~sex * time) p(~sex * bled * time)	162	52264.21	8.263303
Phi(~bled * timebin3) p(~sex * bled * time)	120	52286.22	30.26859
Phi(~sex * bled * timebin4) p(~sex * bled * time)	138	52288.65	32.70104
Phi(~sex * bled * timebin3) p(~bled * time)	72	52289.79	33.83709
Phi(~sex * time) p(~bled * time)	90	52310.64	54.68489

Table 22. Apparent survival (Phi_hat) estimated from the best fitting model (Table 21). Estimates are annual survival within 3-year periods with standard error (SE) and 95% confidence intervals (LCL, UCL).

		Not bled				Bled			
Sex	Years	Phi_hat	SE	LCL	UCL	Phi_hat	SE	LCL	UCL
F	1999-2001	0.5576	0.1386	0.2953	0.7914	0.7747	0.0667	0.6191	0.8791
F	2002-2004	0.6263	0.1078	0.4046	0.8051	0.8212	0.0527	0.6945	0.9027
F	2005-2007	1.000*	0.0001	0.0000	1.0000	0.5068	0.0227	0.4623	0.5512
F	2008-2010	0.6483	0.0488	0.5480	0.7371	0.7472	0.0313	0.6811	0.8036
F	2011-2013	0.7036	0.0770	0.5352	0.8303	0.8434	0.0547	0.7050	0.9238
F	2014-2017	0.7022	0.3896	0.0577	0.9891	0.8126	0.1769	0.3079	0.9769
M	1999-2001	0.7068	0.0729	0.5474	0.8276	0.9161	0.0408	0.7940	0.9687
M	2002-2004	0.7243	0.0870	0.5278	0.8606	0.7215	0.0280	0.6636	0.7729
M	2005-2007	0.9010	0.0752	0.6357	0.9793	0.7472	0.0210	0.7039	0.7860
M	2008-2010	0.6365	0.0268	0.5825	0.6873	0.6731	0.0208	0.6311	0.7125
M	2011-2013	0.6804	0.0438	0.5892	0.7596	0.8624	0.0358	0.7762	0.9189
M	2014-2017	0.7813	0.1789	0.3145	0.9653	0.6660	0.0790	0.4986	0.7999

* Survival for unbled females during 2005-2007 was not estimable.

Table 23. Annual biomedical data availability by state. State-year combinations filled green indicate that number of crabs collected, number or percent bled, number or percent observed dead, and sex ratio (for at least a subsample) were all reported. State-year combinations filled yellow indicate that number of crabs collected was reported and at least one of the following, indicated within the cell, was not reported: number or percent bled (NB), number or percent observed dead (ND), or sex ratio (NS). State-year combinations filled red indicate that number of crabs collected was not reported.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 24. Proportions of horseshoe crabs collected for biomedical use that were observed dead and bled by state.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 25. Reported sex ratios of horseshoe crabs used for biomedical purposes by state and year, shown as percent female. No sex ratios were reported prior to 2004.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 26. Regional (NE: Northeast, DB: Delaware Bay, SE: Southeast; CONFIDENTIAL data removed) and coastwide estimates of biomedical mortality (numbers of crabs) using bleeding mortalities of 4%, 15%, and 30%. Delaware Bay estimates include all crabs caught from New Jersey through Virginia, not only those of Delaware Bay origin.

Year	Biomedical Mortality with 4% Bleeding Mortality				Biomedical Mortality with 15% Bleeding Mortality				Biomedical Mortality with 30% Bleeding Mortality			
	NE	DB	SE	Coastwide	NE	DB	SE	Coastwide	NE	DB	SE	Coastwide
1999				7,511				22,528				43,007
2000				10,236				31,563				60,644
2001				12,500				36,316				68,791
2002				20,783				46,150				80,742
2003				19,579				43,479				76,069
2004				32,431				66,450				112,838
2005				22,557				54,772				98,702
2006				23,351				56,189				100,965
2007				26,922				74,936				140,409
2008				22,388				66,148				125,818
2009				21,762				64,236				122,153
2010				23,340				68,747				130,664
2011				43,613				97,166				170,195
2012				27,288				82,064				156,757
2013				23,063				71,507				137,568
2014				24,020				71,577				136,429
2015				26,511				77,608				147,283
2016				13,745				48,782				96,561
2017				23,822				72,674				139,291

Table 27. Directed (bait and biomedical) use mortality by numbers of crabs using biomedical bleeding mortalities of 4%, 15%, and 30%. Biomedical mortalities are also shown as annual percentages of total directed use mortality.

Year	Bait Harvest	Biomedical Use					
		Total Mortality with 4% Bled Mortality	% Directed Use Mortality	Total Mortality with 15% Bled Mortality	% Directed Use Mortality	Total Mortality with 30% Bled Mortality	% Directed Use Mortality
1999	2,605,280	7,511	0.29%	22,528	0.86%	43,007	1.62%
2000	1,676,913	10,236	0.61%	31,563	1.85%	60,644	3.49%
2001	785,407	12,500	1.57%	36,316	4.42%	68,791	8.05%
2002	1,266,795	20,783	1.61%	46,150	3.51%	80,742	5.99%
2003	1,048,100	19,579	1.83%	43,479	3.98%	76,069	6.77%
2004	656,441	32,431	4.71%	66,450	9.19%	112,838	14.67%
2005	710,534	22,557	3.08%	54,772	7.16%	98,702	12.20%
2006	797,411	23,351	2.85%	56,189	6.58%	100,965	11.24%
2007	785,604	26,922	3.31%	74,936	8.71%	140,409	15.16%
2008	652,863	22,388	3.32%	66,148	9.20%	125,818	16.16%
2009	764,654	21,762	2.77%	64,236	7.75%	122,153	13.77%
2010	612,499	23,340	3.67%	68,747	10.09%	130,664	17.58%
2011	692,870	43,613	5.92%	97,166	12.30%	170,195	19.72%
2012	796,867	27,288	3.31%	82,064	9.34%	156,757	16.44%
2013	951,362	23,063	2.37%	71,507	6.99%	137,568	12.63%
2014	787,397	24,020	2.96%	71,577	8.33%	136,429	14.77%
2015	596,646	26,511	4.25%	77,608	11.51%	147,283	19.80%
2016	740,762	13,745	1.82%	48,782	6.18%	96,561	11.53%
2017	994,491	23,822	2.34%	72,674	6.81%	139,291	12.29%

Table 28. Commercial bait harvest and biomedical harvest by region in numbers of horseshoe crabs, 1999-2016. The numbers for biomedical harvest represent the total number of horseshoe crabs bled and released with the 15% mortality applied. % Biomed represents the percent amount of directed harvest (bait + biomedical) attributed to biomedical regionally and coastwide.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 29. Estimated biomedical mortality (numbers of crabs) for crabs of Delaware Bay origin, with bleeding mortalities of 4%, 15%, and 30%, used as inputs in the Catch Multiple Survey Analysis model. This includes all biomedical mortality from New Jersey, 51% of biomedical mortality from Maryland, and 35% of biomedical mortality from Virginia.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 30. Estimated horseshoe crab dredge discards in weight (lbs) and numbers. Data collected in 2010 was used to convert weight to discards in numbers for all years. To convert pounds (lbs) to numbers, a conversion of 1.8 pounds/crab was used.

Year	Ratio	Ratio CV	Discards (lbs)	Discards LCI	Discards UCI	n Fish Counted	Total Subsample Weight (lbs)	n Subsamples	Mean Weight (lbs)	Discards (numbers)
2004	0.00081	0.22006	583,410	326,642	840,178	NA	NA	NA	NA	317,935
2005	0.00065	0.19863	342,233	206,277	478,189	NA	NA	NA	NA	186,503
2006	0.00232	0.47539	1,223,591	60,219	2,386,964	NA	NA	NA	NA	666,807
2007	0.00031	0.34298	172,505	54,173	290,836	NA	NA	NA	NA	94,008
2008	0.00079	0.28886	432,739	182,734	682,743	NA	NA	NA	NA	235,825
2009	0.00118	0.23483	603,889	320,266	887,512	NA	NA	NA	NA	329,095
2010	0.00164	0.59808	811,481	0	1,782,147	21	75	1	3.57	442,224
2011	0.00079	0.31310	389,230	145,492	632,969	NA	NA	NA	NA	212,115
2012	0.00049	0.55345	217,559	0	458,378	NA	NA	NA	NA	118,561
2013	0.00017	0.31907	62,813	22,729	102,896	NA	NA	NA	NA	34,230
2014	0.00594	0.87940	2,237,922	0	6,173,968	NA	NA	NA	NA	1,219,576
2015	0.00380	0.34944	1,406,693	423,577	2,389,809	NA	NA	NA	NA	766,590
2016	0.01193	0.37253	4,523,910	1,153,293	7,894,527	NA	NA	NA	NA	2,465,346
2017	0.00568	0.55577	2,003,434	0	4,230,343	NA	NA	NA	NA	1,091,790

Table 31. Estimated horseshoe crab gill net discards in weight (lbs) and numbers. Data collected in 2005 was used to convert weight to discards in numbers for all years. To convert pounds (lbs) to numbers, a conversion of 1.8 pounds/crab was used.

Year	Ratio	Ratio CV	Discards (lbs)	Discards LCI	Discards UCI	n Fish Counted	Total Subsample Weight (lbs)	n Subsamples	Mean Weight (lbs)	Discards (numbers)
2004	0.01899	0.42285	239,909	37,018	442,801	NA	NA	NA	NA	130,741
2005	0.00373	0.29202	35,358	14,707	56,008	1	4	1	4.00	19,268
2006	0.00225	0.38654	13,853	3,144	24,562	NA	NA	NA	NA	7,549
2007	0.01465	0.38903	175,329	38,913	311,745	NA	NA	NA	NA	95,547
2008	0.00926	0.39576	90,751	18,920	162,581	NA	NA	NA	NA	49,455
2009	0.01389	0.49618	147,298	1,126	293,471	NA	NA	NA	NA	80,272
2010	0.03066	0.21314	246,878	141,641	352,115	NA	NA	NA	NA	134,538
2011	0.04753	0.29030	392,901	164,784	621,017	NA	NA	NA	NA	214,115
2012	0.01197	0.30259	76,634	30,257	123,010	NA	NA	NA	NA	41,762
2013	0.05793	0.38904	416,868	92,513	741,222	NA	NA	NA	NA	227,176
2014	0.00990	0.44947	128,300	12,967	243,634	NA	NA	NA	NA	69,918
2015	0.00933	0.24701	86,424	43,728	129,120	NA	NA	NA	NA	47,098
2016	0.00301	0.16393	16,613	11,167	22,060	NA	NA	NA	NA	9,054
2017	0.00324	0.23918	34,092	17,784	50,399	NA	NA	NA	NA	18,579

Table 32. Estimated horseshoe crab trawl discards in weight (lbs) and numbers. Year-specific data was used to convert weight to numbers for 2012-2016. For the remaining years, data was pooled among all years of available data for the conversions.

Year	Ratio	Ratio CV	Discards (lbs)	Discards LCI	Discards UCI	n Fish Counted	Total Subsample Weight (lbs)	n Subsamples	Mean Weight (lbs)	Discards (numbers)
2004	0.00173	0.35004	1,495	448	2,541	NA	NA	NA	NA	1,700
2005	0.00659	0.65466	5,235	0	12,089	NA	NA	NA	NA	5,954
2006	0.00214	0.48793	2,729	66	5,392	NA	NA	NA	NA	3,104
2007	0.02139	0.43254	15,591	2,104	29,079	NA	NA	NA	NA	17,734
2008	0.02147	0.36827	47,298	12,461	82,135	NA	NA	NA	NA	53,798
2009	0.02243	0.32233	62,144	22,082	102,207	735	237	4	0.32	77,605
2010	0.02183	0.46159	46,695	3,587	89,802	NA	NA	NA	NA	53,112
2011	0.03961	0.32002	170,758	61,465	280,050	NA	NA	NA	NA	194,225
2012	0.02051	0.31988	67,766	24,412	111,120	1751	1906	14	1.09	62,255
2013	0.04386	0.29299	112,787	46,695	178,879	2791	1555	13	0.56	202,436
2014	0.01057	0.31140	20,617	7,777	33,458	488	456	6	0.93	22,064
2015	0.04630	0.27952	89,541	39,484	139,598	3386	3,244	33	0.96	93,467
2016	0.03534	0.23252	51,907	27,768	76,045	1739	1,823	27	1.05	49,520
2017	0.010384	0.22696	32,090	17,524	46,656	1,711	1,192	22	0.70	30,614

Table 33. Surveys considered for developing abundance indices for horseshoe crab. Table indicates which surveys were accepted for index development and which were rejected.

Data Source	Survey	Accepted	Rejected	Reason(s) Rejected			
				Time series too short or broken	Rare occurance of HSC	Inconsistent methods, gear changes	Better survey available with similar coverage
ME DMR	ME-NH Trawl		X		X		
NH F&G	Habitat Monitoring Survey		X	X			
NH F&G	Spawning Survey	X					
MA DMF	Resource Assessment Trawl	X					
MA DMF	Spawning Beach Survey		X	X		X	
RI DEM	Coastal Trawl Survey (seasonal segment)		X				X
RI DEM	Coastal Trawl Survey (monthly segment)	X					
Sacred Heart Univ	Project limulus		X			X	
CT DEEP	Long Island Trawl Survey	X					
NYS DEC	Peconic Bay Small Mesh Trawl Survey	X					
NYS DEC	Western Long Island Beach Seine Survey	X					
NYS DEC	Horseshoe Crab Spawning and Tagging Survey		X			X	
NJ DFW	Ocean Trawl	X					
NJ DFW	Delaware Bay Trawl Survey		X				X
NJ DFW	Surf Clam Survey	X					
DE DFW	Adult Trawl Survey (30')	X					
DE DFW	Juvenile Trawl Survey (16')		X				X
MD DNR	Coastal Bays	X					
Virginia Tech	Virginia Tech Mid-Atl HSC Benthic Trawl	X					
NC DMF	North Carolina fisheries independent gillnet survey	X					
SC DNR	Crustacean Research and Monitoring large trawl survey	X					
SC DNR	SEAMAP- South Atlantic Coastal Trawl Survey	X					
SC DNR	Trammel Net Survey	X					
GA DNR	Ecological Monitoring Trawl Survey	X					
FL FWC	Fisheries- Independent Monitoring Program (FIM)		X	X	X		
NMFS	NEFSC Trawl		X		X		X
NEAMAP	NEAMAP	X					

Table 34. List of fishery-independent surveys that were developed in relative abundance indices for this stock assessment, the gear used in the survey, the minimum and maximum prosomal width, and median prosomal width of horseshoe crabs caught.

Survey	Gear	Range of widths (cm)	Median width (cm)
MA DMF	3/4 size North Atlantic type two seam otter trawl; codend has a 6.4 mm knotless liner	4-53	16
RI Trawl	Otter trawl with a ¼ mesh inch line; survey net is 210 x 4.5”, 2 seam (40’ / 55’), mesh size 4.5”	4-31	23
CT LISTS	Otter trawl with 102 mm mesh in wings and belly, 76 mm mesh in tailpiece, 51 mm mesh codend	5-34	22
NY Peconic	Trawl - 4.8 meter semi-balloon shrimp trawl net	4-53	23
NY WLIS	Seine - ¼ inch square mesh in the wings, 3/16 inch square mesh in the bunt	2-53	17
NEAMAP	Trawl - four-seam, three-bridle, 400x12 cm bottom trawl	4-53	17
NJ OT	Two-seam trawl with forward netting of 12 cm stretch mesh, rear netting of 8 cm, lined with 6.4 mm bar mesh liner	3-53	20
NJ Surfclam	Commercial hydraulic clam dredge	2-53	15
DE Adult	30 ft 2-seam otter trawl, 3" (7.6cm) stretch mesh in wings and body, 2" (5.1cm) stretch mesh in cod end	4-53	19
Virginia Tech	Two-seam trawl with net body of 15.2 cm stretched mesh, bag 14.3 cm stretched mesh	2-53	16
MD Coastal	Otter trawl, usually 5.5 or 6 inch mesh	6-38	19
NC Esturine	Floating gill nets with 30-yard segments of 3, 3 ½, 4, 4 ½, 5, 5 ½, 6, and 6 ½ inch stretched mesh	1-50	20
SC CRMS	20-foot trawl net, with 1” stretch mesh	2-53	23
SC Trammel	183 x 2.1 m trammel net	2-48	23
SEAMAP	Paired 75-ft (22.9-m) mongoose-type Falcon trawl nets with 1.875-in (47.6-mm) stretch mesh	2-53	23
GA Trawl	40' flat beam trawl	4-53	22

Table 35. Indices of bay-wide male and female horseshoe crab spawning activity (ISA), number of beaches surveyed, standard deviation (SD), coefficient of variations (CV), 90% confidence intervals (CI) and sex ratio for the Delaware Bay from 1999 to 2017 (Source: DE DFW).

Year	Beaches Surveyed	Male				Female				Annual Sex Ratio (M:F)
		ISA	90% CI	SD	CV (%)	ISA	90% CI	SD	CV (%)	
1999	17	2.5	1.86, 3.37	0.45	18	0.77	0.62, 0.97	0.1	13	3.2
2000	22	2.96	2.31, 3.80	0.45	15	0.91	0.74, 1.13	0.12	13	3.2
2001	22	2.37	1.91, 2.95	0.31	13	0.75	0.63, 0.90	0.08	10	3.1
2002	23	2.86	2.45, 3.34	0.27	9	0.91	0.79, 1.04	0.07	8	3.1
2003	23	2.89	2.50, 3.33	0.25	9	0.8	0.71, 0.91	0.06	8	3.6
2004	24	2.93	2.55, 3.36	0.24	8	0.77	0.68, 0.87	0.06	7	3.8
2005	23	3.23	2.79, 3.74	0.29	9	0.82	0.72, 0.93	0.07	9	3.9
2006	24	3.99	3.49, 4.56	0.33	8	0.99	0.89, 1.10	0.07	7	4
2007	24	4.22	3.63, 4.90	0.38	9	0.89	0.78, 1.01	0.07	8	4.7
2008	25	2.3	1.83, 2,90	0.32	14	0.68	0.59, 0.78	0.06	9	3.4
2009	26	4.67	4.11, 5.29	0.36	8	1	0.89, 1.11	0.06	6	4.7
2010	25	3.39	2.93, 3.94	0.31	9	0.8	0.70, 0.92	0.07	8	4.2
2011	25	3.31	2.83, 3.87	0.31	10	0.64	0.57, 0.72	0.05	7	5.2
2012	25	2.44	1.97, 3.01	0.31	13	0.56	0.47, 0.67	0.06	10	4.4
2013	25	3.2	2.98, 3.44	0.14	4	0.85	0.80, 0.91	0.03	4	3.8
2014	25	2.28	2.09, 2.48	0.12	5	0.54	0.50, 0.59	0.03	5	4.2
2015	23	2.75	2.59, 2.92	0.1	4	0.66	0.62, 0.70	0.02	4	4.2
2016	25	4.1	3.86, 4.36	0.2	4	0.9	0.85, 0.95	0.03	3	4.6
2017	25	3.68	3.37, 4.02	0.2	5	0.71	0.65, 0.78	0.04	6	5.2

Table 36. **Results of the power analysis by survey for linear and exponential trends in horseshoe crab abundance indices over a twenty-year period. Power were calculated as the probability of detecting a 50% change following the methods of Gerrodette (1987). Sex includes all mature horseshoe crab or multiparous (M) or primiparous (P) if indicated.**

State	Survey	Season	Sex	Time Period	Median CV	Linear Trend		Exponential Trend	
						+50%	-50%	+50%	-50%
NH	Beach Spawner	Spring	Female	2002-2012	0.488	0.33	0.46	0.35	0.51
NH	Beach Spawner	Spring	Male	2002-2012	0.488	0.33	0.46	0.35	0.51
MA	Trawl North Cape	Fall	All	1978-2017	0.817	0.18	0.24	0.20	0.30
MA	Trawl South Cape	Fall	All	1978-2017	0.574	0.27	0.37	0.29	0.42
RI	Monthly Trawl	Fall	All	1998-2016	0.365	0.48	0.66	0.50	0.70
CT	CT LISTS	Fall	All	1997-2016	0.254	0.74	0.91	0.75	0.92
NY	Peconic Bay	Fall	All	1987-2016	0.132	1.00	1.00	1.00	1.00
NY	Seine - Jamaica	Spring	All	1987-2017	0.418	0.40	0.56	0.42	0.60
NY	Seine - LN & Man	Spring	All	1987-2017	0.302	0.61	0.80	0.63	0.82
DE	Adult Trawl	Fall	All	1990-2017	0.341	0.53	0.71	0.54	0.74
DE	Adult Trawl	Spring	All	1990-2017	0.272	0.69	0.87	0.70	0.88
DE	Adult Trawl	Fall	Female	1990-2017	0.337	0.54	0.72	0.55	0.75
DE	Adult Trawl	Spring	Female	1990-2017	0.275	0.68	0.86	0.70	0.88
DE	Adult Trawl	Fall	Male	1990-2017	0.380	0.46	0.63	0.47	0.67
DE	Adult Trawl	Spring	Male	1990-2017	0.281	0.67	0.85	0.68	0.87
NY	NEAMAP	Fall	All	2007-2017	0.303	0.61	0.80	0.62	0.82
DB	NEAMAP	Fall	All	2008-2016	0.213	0.86	0.97	0.87	0.97
NJ	Ocean Trawl	Fall	All	1989-2017	0.329	0.55	0.74	0.57	0.77
NJ	Ocean Trawl	Spring	All	1989-2017	0.284	0.66	0.84	0.67	0.86
NJ	Ocean Trawl	Fall	Female	1999-2017	0.298	0.62	0.81	0.64	0.83
NJ	Ocean Trawl	Spring	Female	1999-2017	0.250	0.75	0.91	0.76	0.92
NJ	Ocean Trawl	Fall	Male	1999-2017	0.373	0.47	0.65	0.49	0.68
NJ	Ocean Trawl	Spring	Male	1999-2017	0.298	0.62	0.81	0.64	0.83
NJ	Surf Clam	Summer	All	1998-2012	0.135	1.00	1.00	1.00	1.00
NJ	Surf Clam	Summer	Female	1998-2012	0.141	0.99	1.00	0.99	1.00
NJ	Surf Clam	Summer	Male	1998-2012	0.199	0.90	0.98	0.90	0.99
MD	Coastal Bays	Spring	All	1990-2017	0.500	0.32	0.45	0.34	0.50
NJ-VA	Virginia Tech Trawl	Fall	Female - M	2002-2017	0.262	0.72	0.89	0.73	0.90
NJ-VA	Virginia Tech Trawl	Fall	Female - P	2002-2017	0.300	0.62	0.81	0.63	0.83
NJ-VA	Virginia Tech Trawl	Fall	Male - M	2002-2017	0.281	0.67	0.85	0.68	0.87
NJ-VA	Virginia Tech Trawl	Fall	Male - P	2002-2017	0.336	0.54	0.73	0.55	0.75
NC	Gillnet	Spring	All	2001-2016	0.152	0.99	1.00	0.99	1.00
SC	SEAMAP	Fall	All	2001-2017	0.435	0.54	0.38	0.58	0.40
GA-FL	SEAMAP	Fall	All	2001-2017	0.390	0.61	0.44	0.65	0.46
SC	CRMS	Spring	All	1995-2017	0.291	0.64	0.83	0.65	0.85
SC	Trammel	Spring	All	1995-2017	0.344	0.52	0.71	0.54	0.74
GA	Trawl	Spring	All	1999-2017	0.176	0.95	1.00	0.95	1.00

Table 37. List of surveys used in the regional Conn indices and their associated sigma values, or the standard deviation of the process error. All surveys are for combined sexes and adult horseshoe crabs unless specified in the parentheticals.

Survey	σ^p
MA Trawl North Cape (Fall)	4.097
MA Trawl South Cape (Fall)	2.651
RI Monthly (Fall)	0.308
CT LISTS (Fall)	0.224
NY Peconic (Fall)	0.641
NY Seine Jamaica Bay (Spring)	0.466
NY Seine Little N & Manh (Spring)	0.298
NY NEAMAP (Fall)	0.705
DB NEAMAP (Fall)	0.680
NJ Ocean Trawl (Spring)	0.602
NJ Ocean Trawl (Fall)	0.467
NJ Ocean Trawl (Spring, F only)	0.535
NJ Ocean Trawl (Spring, M only)	0.626
NJ Ocean Trawl (Fall, F only)	0.541
NJ Ocean Trawl (Fall, M only)	0.709
NJ Surf Clam	0.579
NJ Surf Clam (F only)	0.429
NJ Surf Clam (M only)	0.362

Survey	σ^p
DE Adult Trawl (Fall)	0.918
DE Adult Trawl (Spring, F only)	0.820
DE Adult Trawl (Spring, M only)	0.806
DE Adult Trawl (Fall, F only)	0.714
DE Adult Trawl (Fall, M only)	0.817
VT Tech Trawl	0.171
VT Tech Trawl (F only)	0.233
VT Tech Trawl (M only)	0.155
MD Coastal Bays (Spring)	0.561
NC Gillnet (Pamlico Sound, Spring)	0.423
SC Trammel (Spring)	0.280
SC CRMS (Spring)	0.819
SEAMAP (SC only, Fall)	4.281
GA Trawl (Spring)	0.651
SEAMAP (GA & FL, Fall)	3.551

Table 38. Results of autoregressive integrated moving average (ARIMA) model fits for horseshoe crab surveys. W is the Shapiro-Wilk test statistic for normality of residuals (p value in parentheses); n is the number of years in the time series; r1, r2, and r3 are the first three autocorrelations; θ is the moving average parameter; SE is the standard error of θ; and σ^2c is the variance of the index.

Survey	Years	n	W	p	r_1	r_2	r_3	θ	SE	σ^2_c
Northeast Region										
MA DMF Trawl – North of Cape Cod	1978-2017	40	0.82	0.01	-0.43	-0.11	0.06	1.00	0.13	3.82
MA DMF Trawl – South of Cape Cod	1978-2017	40	0.96	0.17	-.39	-0.01	-0.10	0.63	0.21	1.79
NH Spawner - Female	2002-2012	11	0.96	0.74	-0.31	-0.22	0.06	0.59	0.27	0.71
NH Spawner - Male	2002-2012	11	0.90	0.20	-0.36	-0.19	0.05	0.62	0.23	0.31
RI Monthly Trawl - Fall	1998-2016	19	0.96	0.58	-0.29	-0.29	0.24	0.64	0.24	0.40
New York Region										
CT Long Island Sound Trawl - Fall	1997-2016	20	0.93	0.15	-0.24	-0.20	-0.12	0.49	0.23	0.17
NEAMAP - Fall	2007-2017	11	0.92	0.34	-0.31	-0.13	-0.08	0.78	0.70	0.71
NY Jamaica Bay Seine	1988-2016	29	0.99	0.96	-0.52	-0.10	0.38	0.75	0.15	0.57
NY Little Neck and Manhasset Bay Seine	1988-2016	29	0.96	0.36	-0.40	-0.17	0.07	0.64	0.15	0.29
NY Peconic Trawl	1987-2016	30	0.97	0.53	-0.52	0.32	-0.16	0.21	0.19	0.20
Mid-Atlantic Region										
DE 30 ft Trawl - Fall	1990-2017	28	0.97	0.49	-0.24	-0.11	0.17	0.62	0.16	1.22
DE 30 ft Trawl - Fall Female	1990-2017	28	0.95	0.20	-0.32	-0.04	0.07	0.63	0.15	1.11
DE 30 ft Trawl - Fall Male	1990-2017	28	0.96	0.35	-0.28	-0.11	0.17	0.64	0.15	1.32
DE 30 ft Trawl - Spring	1990-2017	28	0.96	0.34	0.09	-0.07	0.19	0.57	0.18	1.15
DE 30 ft Trawl - Spring Female	1990-2017	28	0.96	0.40	-0.10	-0.22	0.17	0.61	0.17	1.02
DE 30 ft Trawl - Spring Male	1990-2017	28	0.94	0.09	-0.29	-0.13	0.20	0.63	0.17	1.32
MD Coastal Bays Trawl - Spring	1990-2017	28	0.96	0.31	-0.44	-0.10	0.14	1.00	0.11	0.40
NEAMAP - Fall	2007-2017	11	0.92	0.30	-0.45	-0.12	0.12	1.00	0.29	0.48
NJ Ocean Trawl - Fall	1989-2017	29	0.97	0.49	-0.55	0.28	-0.28	0.75	0.15	0.45
NJ Ocean Trawl - Fall Female	1999-2017	19	0.94	0.32	-0.19	-0.19	-0.13	1.00	0.15	0.21
NJ Ocean Trawl - Fall Male	1999-2017	19	0.97	0.87	-0.34	0.12	-0.30	1.00	0.16	0.27

Table 38 ***Continued***

Survey	Years	n	W	p	r_1	r_2	r_3	θ	SE	σ^2_c
Mid-Atlantic Region										
NJ Ocean Trawl - Spring	1989-2017	29	0.97	0.50	-0.42	-0.04	0.00	0.48	0.18	0.32
NJ Ocean Trawl - Spring Female	1999-2017	19	0.93	0.16	-0.37	0.01	-0.05	0.45	0.22	0.34
NJ Ocean Trawl - Spring Male	1999-2017	19	0.93	0.15	-0.22	-0.09	-0.10	0.27	0.30	0.29
NJ Surf Clam Dredge	1998-2012	15	0.97	0.90	-0.36	-0.09	0.17	0.41	0.19	0.15
NJ Surf Clam Dredge - Female	1998-2012	15	0.96	0.74	-0.47	0.20	-0.23	0.68	0.23	0.15
NJ Surf Clam Dredge - Male	1998-2012	15	0.97	0.92	-0.38	0.06	0.00	0.54	0.28	0.28
VA Tech Trawl	2002-2017	12	0.92	0.32	-0.49	-0.05	0.19	0.64	0.29	0.21
VA Tech Trawl - Immature Female	2002-2017	12	0.97	0.91	-0.52	-0.05	0.21	1.00	0.30	0.39
VA Tech Trawl - Immature Male	2002-2017	12	0.96	0.78	-0.51	-0.10	0.24	1.00	0.32	0.47
VA Tech Trawl - Mature Female	2002-2017	12	0.92	0.26	0.04	-0.33	-0.46	0.00	0.47	0.17
VA Tech Trawl - Mature Male	2002-2017	12	0.89	0.13	-0.14	-0.06	-0.65	0.45	0.59	0.25
VA Tech Trawl - Newly Mature Female	2002-2017	12	0.92	0.28	-0.14	0.10	-0.71	0.03	0.37	0.47
VA Tech Trawl - Newly Mature Male	2002-2017	12	0.93	0.41	-0.27	-0.19	-0.04	0.60	0.31	1.10
Southeast Region										
GA Trawl - Spring	1999-2017	19	0.87	0.02	-0.50	0.16	-0.22	0.77	0.15	0.44
NC Gillnet - Spring	2001-2016	16	0.90	0.08	0.10	-0.27	-0.30	0.10	0.30	0.12
SC CRMS	1995-2017	23	0.96	0.53	-0.25	-0.20	0.09	0.32	0.27	0.43
SC Trammel Net	1995-2017	23	0.96	0.54	-0.33	-0.33	0.18	0.73	0.14	0.31
SEAMAP - SC Fall	2001-2017	17	0.93	0.19	-0.14	-0.09	-0.32	0.52	0.17	3.48
SEAMAP GA-FL - Fall	2001-2017	17	0.97	0.75	-0.13	-0.35	-0.23	0.42	0.24	2.55

Table 39. Reference points from the ARIMA model for each survey and the probability that the terminal year's fitted index (i_f) is below the reference point. The 1998 reference is i_{1998} and the lower quartile reference is Q_{25}. Reference points are based on ln transformed index values. Surveys that began after 1998 do not have a 1998 reference value.

Survey	i_f	i_{1998}	$P(i_f<i_{1998})$	Q_{25}	$P(i_f<Q_{25})$
Northeast Region					
MA DMF Trawl – North of Cape Cod	-0.70	-0.66	0.41	-0.59	0.21
MA DMF Trawl – South of Cape Cod	-0.11	-1.13	0.08	-1.60	0.04
NH Spawner - Female	0.73			0.69	0.34
NH Spawner - Male	1.14			1.18	0.44
RI Monthly Trawl - Fall	-1.16	-0.88	0.62	-0.92	0.56
New York Region					
CT Long Island Sound Trawl - Fall	0.06	0.86	1.00	0.32	0.83
NEAMAP - Fall	1.19			0.98	0.23
NY Jamaica Bay Seine	-0.69	0.10	0.96	-0.34	0.64
NY Little Neck and Manhasset Bay Seine	0.33	1.47	1	0.48	0.60
NY Peconic Trawl	-1.65	0.38	1.00	-0.81	0.97
Mid-Atlantic Region					
DE 30 ft Trawl - Fall	1.90	0.59	0.02	0.19	0.00
DE 30 ft Trawl - Fall Female	0.70	-0.45	0.03	-0.82	0.00
DE 30 ft Trawl - Fall Male	1.40	0.02	0.02	-0.26	0.01
DE 30 ft Trawl - Spring	1.28	1.07	0.33	0.10	0.04
DE 30 ft Trawl - Spring Female	0.33	0.25	0.50	-0.66	0.06
DE 30 ft Trawl - Spring Male	0.61	0.21	0.21	-0.52	0.04
MD Coastal Bays Trawl - Spring	-1.14	-1.00	0.36	-1.30	0.01
NEAMAP - Fall	2.82			2.69	0.05
NJ Ocean Trawl - Fall	1.48	1.89	0.82	1.42	0.32
NJ Ocean Trawl - Fall Female	0.72			0.67	0.11
NJ Ocean Trawl - Fall Male	0.79			0.71	0.07
NJ Ocean Trawl - Spring	2.42	2.36	0.51	1.62	0.00
NJ Ocean Trawl - Spring Female	1.53			0.66	0.00
NJ Ocean Trawl - Spring Male	1.76			0.57	0.00
NJ Surf Clam Dredge	0.85	0.11	0.00	-0.06	0.00
NJ Surf Clam Dredge - Female	-0.52	-0.60	0.12	-0.75	0.04
NJ Surf Clam Dredge - Male	-1.13	-1.02	0.54	-1.70	0.01
VA Tech Trawl	4.65			4.46	0.04
VA Tech Trawl - Immature Female	3.02			2.87	0.02
VA Tech Trawl - Immature Male	2.66			2.44	0.01
VA Tech Trawl - Mature Female	2.83			2.08	0.00
VA Tech Trawl - Mature Male	3.80			3.16	0.00
VA Tech Trawl - Newly Mature Female	1.26			0.46	0.04

Table 39 ***Continued***

Survey	i_f	i_{1998}	$P(i_f<i_{1998})$	Q_{25}	$P(i_f<Q_{25})$
Mid-Atlantic Region					
VA Tech Trawl - Newly Mature Male	1.24			0.76	0.03
Southeast Region					
GA Trawl - Spring	0.89			0.54	0.03
NC Gillnett - Spring	-0.47			-1.30	0.00
SC CRMS	0.22	-1.00	0.00	-0.25	0.13
SC Trammel Net	-0.67	-1.39	0.00	-1.12	0.00
SEAMAP - SC Fall	0.60			-0.36	0.02
SEAMAP GA-FL - Fall	-0.11			-1.08	0.02

Table 40. Number of surveys with terminal year having a greater than 0.50 probability of being less than the reference point (i.e. likely less than the reference point). Time series were only included in this summary if the terminal year was 2016 or 2017, residuals from ARIMA model fits were normally distributed, and combined-sex surveys. Those surveys that did not begin until after 1998 were not included in the $P(if<i_{1998})>0.50$ summary.

Region	$P(i_f<i_{1998})>0.50$	$P(i_f<Q_{25})>0.50$
Northeast	1 out of 2	1 out of 2
New York	4 out of 4	4 out of 5
Mid-Atlantic	2 out of 5	0 out of 7
Southeast	0 out of 2	0 out of 5
Coastwide	7 out of 13	5 out of 19

Table 41. Horseshoe crab life history parameters used in the operating model.

Age	Natural mortality (*M*)	Probability of Maturing[3] (m)	Fishery Recruitment (*R*)	Fecundity (*f*)
0	10.4143	0.00	0.00	0
1 – 9	0.0265	0.00	0.00	0
10	0.0265[1]; 0.4700[2]	0.20	0.00[1];1.0[2]	80,300
11	0.0265[1]; 0.4700[2]	0.6577	0.00[1];1.0[2]	80,300
12	0.4627	1.00	0.99	80,300
13 – 17	0.4700	1.00	1.00	80,300
18 – 20	2.5257	1.00	1.00	80,300

[1] immature individuals; [2] mature individuals

[3] The probability of maturing represents the probability of becoming a mature individual at age *i* if that individual was immature at age *i*-1.

Table 42. Scenarios of *F* simulated by the operating model to generate data sets used in a surplus production model and a catch survey model. Fishing mortality varied annually according to a uniform distribution with bounds described below.

	Scenario 1		Scenario 2		Scenario 3		Scenario 4	
Years	**Min *F***	**Max *F***	**Min *F***	**Max *F***	**Min *F***	**Max *F***	**Min *F***	**Max *F***
2001 - 2020	0.18	0.22	0.18	0.22	0.18	0.22	0.18	0.22
2021 - 2040	0.18	0.22	0.08	0.12	0.01	0.02	0.06	0.10
2040 - 2060	0.18	0.22	0.02	0.06	0.02	0.06	0.01	0.02

Table 43. Catch multiple survey analysis base model inputs. *Values shown in millions. CONFIDENTIAL biomedical data has been removed.

	Lambdas			Starting Values					
M	VT	DE	NJ	R	N	q_de	q_nj	s	Biomed.
0.274	0.59	0.16	0.25	2.0E+06	3.6E+06	2.3E-07	5.0E-07	1	15%

	Harvest			Survey Indices				Coefficient of Variation			
Year	Commercial	Discard	Biomedical	VT*, r	VT*, n	DE	NJ	VT, r	VT, n	DE	NJ
2003	220,354	35,941		1.537	4.959	1.203	2.246	0.24	0.26	0.492	0.188
2004	108,843	39,416		0.794	3.379	0.056	2.502	0.45	0.22	0.566	0.229
2005	116,577	10,530		0.358	2.735	0.093	2.77	0.32	0.2	0.43	0.241
2006	104,048	18,560		0.479	3.138	1.411	1.856	0.34	0.22	0.305	0.258
2007	67,674	29,444		2.051	6.611	1.284	1.474	0.33	0.31	0.274	0.249
2008	44,329	30,441		2.373	7.746	0.185	2.37	0.33	0.25	0.379	0.32
2009	48,663	45,789		2.571	6.311	0.34	1.368	0.36	0.4	0.356	0.289
2010	41,385	55,649		0.885	2.975	0.206	0.579	0.26	0.33	0.492	0.302
2011	33,728	103,092		1.338	5.178	0.25	2.215	0.74	0.26	0.385	0.256
2012	56,112	27,810		0.845	5.29	0.275	1.804	0.34	0.2	0.296	0.249
2013	29,111	103,928		-	-	0.111	7.996	-	-	0.448	0.347
2014	13,410	51,346		-	-	1.218	3.358	-	-	0.266	0.239
2015	11,689	52,134		-	-	0.439	3.145	-	-	0.289	0.249
2016	14,923	73,226		-	-	1.079	3.989	-	-	0.215	0.244
2017	16,956	38,009		1.608	6.024	1.6	5.613	0.22	0.17	0.216	0.25
2018	-	-	-	1.48	7.185	3.127	3.104	0.27	0.27	0.237	0.226

Table 44. The number of parameters estimated in the catch multiple survey analysis: median primiparous abundance (1); primiparous abundance for each year (16); catchability coefficients (2) for the Delaware and New Jersey surveys; and multiparous abundance for the start of the time series (1).

Parameter	No. Estimates	Description
R_{median}	1	Median primiparous abundance (log-scale)
Φ	16	Deviations from median primiparous abundance (log-scale)
N_0	1	Initial multiparous abundance (log-scale)
q_de	1	Catchability coefficient for the Delaware trawl survey (log-scale)
q_nj	1	Catchability coefficient for the New Jersey survey (log-scale)

Table 45. Selected catch multiple survey analysis based model outputs: q=catchability coefficients; R=primiparous abundance; N=multiparous abundance; u=exploitation rate; Z= instantaneous total mortality rate; A=annual mortality rate; and F=instantaneous fishing mortality rate.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 46. Mohn's p statistic for total, multiparous, and primiparous abundance.

[Table Removed Due to **CONFIDENTIAL** Data]

Table 47. Sensitivity runs for the catch multiple survey analysis model. All runs that included CONFIDENTIAL biomedical data have been removed.

		M	λ			Biomed	Starting Values				Terminal Output Values			
	Name		VT	DE	NJ		*R*	*N*	*q_de*	*q_nj*	*NegLL*	*R*	*N*	*F*
	Base													
	alt_base													
M [0.10-0.80]	**M_0.10**													
	M_0.15													
	M_0.19													
	M_0.195													
	M_0.198													
	M_0.199													
	M_0.20													
	M_0.201													
	M_0.202													
	M_0.203													
	M_0.204													
	M_0.205													
	M_0.206													
	M_0.21													
	M_0.25													
	M_0.30													

	M_0.35													
	M_0.40													
	M_0.45													
	M_0.47													
	M_0.50													
	M_0.80													
Harvest	**Biomed_0%**	0.274	0.59	0.16	0.25	0%	14.5	15.1	-15.3	-14.5	25.16	1,587,760	7,145,540	0.007
	Biomed_4%													
	Biomed_30%													
	Discard=0													
λ	**λ=Conn unadj.**													
	λ=1													
cv	**cv_average**													
	cv_fixed													
	cv_off													
q_VT	**q_vt**													
	q_vt_s													
s [0.5-1.5]	**s_0.5**													
	s_0.6													
	s_0.7													
	s_0.8													

	s_0.9					
	s_1.0					
	s_1.1					
	s_1.2					
	s_1.3					
	s_1.4					
	s_1.5					
	s_free					
Starting Values	**R_14.0**					
	R_14.3					
	R_14.7					
	N_14.4					
	N_15.8					
	N_17.0					
	q_DE_-14.6					
	q_DE_-16.0					
	q_DE_-17.5					
	q_NJ_-13.8					
	q_NJ_-15.5					
	q_NJ_-17.0					

Table 48. Horseshoe crab life history parameters used in the projection model to estimate biological reference points.

Age	Natural mortality (*M*)[1]	Probability of Maturing (m)[2]	Fishery Recruitment (*R*)	Fecundity (*f*)
0	10.4143	0.00	0.00	0
1 – 9	0.0817, 0.0744, 0.0685	0.00	0.00	0
10_{immature}	0.0817, 0.0744, 0.0685	0.00	0.00	80,300
10_{mature}	0.274	0.20	1.00	80,300
11_{immature}	0.0817, 0.0744, 0.0685	0.00	0.00	80,300
11_{mature}	0.274	0.66	1.00	80,300
12	0.274	1.00	1.00	80,300
13 – 20+	0.274	1.00	1.00	80,300

[1] Three levels of natural mortality corresponding to K = 10, 14, 18 million female horseshoe crabs, respectively.

[2] The probability of maturing represents the probability of becoming a mature individual at age *i* if that individual was immature at age *i*-1.

Table 49. Horseshoe crab life history parameters used in the egg- and yield-per-recruit modeling.

Age	Natural Mortality (*M*)[1]	Proportion Mature (m)	Fishery Recruitment (R)	Fecundity (f)	Weight
1	0.0817, 0.0744, 0.0685	0	0	0	1
2	0.0817, 0.0744, 0.0685	0	0	0	1
3	0.0817, 0.0744, 0.0685	0	0	0	1
4	0.0817, 0.0744, 0.0685	0	0	0	1
5	0.0817, 0.0744, 0.0685	0	0	0	1
6	0.0817, 0.0744, 0.0685	0	0	0	1
7	0.0817, 0.0744, 0.0685	0	0	0	1
8	0.0817, 0.0744, 0.0685	0	0	0	1
9	0.0817, 0.0744, 0.0685	0	0	0	1
10	0.1173,0.1112, 0.1064	0.2000	0.2000	80,300	1
11	0.2156, 0.2131, 0.2111	0.7163,0.7159, 0.7156	0.7050	80,300	1
12	0.274	1	1	80,300	1
13	0.274	1	1	80,300	1
14	0.274	1	1	80,300	1
15	0.274	1	1	80,300	1
16	0.274	1	1	80,300	1
17	0.274	1	1	80,300	1
18	0.274	1	1	80,300	1
19	0.274	1	1	80,300	1
20	0.274	1	1	80,300	1

[1] Three levels of natural mortality corresponding to K = 10, 14, 18 million female horseshoe crabs, respectively.

Table 50. Reference points for female horseshoe crab harvest in the Delaware Bay generated from a population projection model. Reference points were generated for a range of possible carrying capacities (*K*) and associated juvenile mortalities (M_{juv}) needed to stabilize an unfished population at those carrying capacities.

K	M_{juv}	N_{msy}	F_{msy}	MSY	u_{msy}	N_{40}	F_{40}	N_{20}	F_{20}
10,000,000	0.0817	3,664,522	0.0695	215,498	0.0588	4,000,000	0.0632	2,000,000	0.1140
14,000,000	0.0744	5,132,293	0.0749	324,508	0.0632	5,600,000	0.0682	2,800,000	0.1232
18,000,000	0.0685	6,600,291	0.0796	442,334	0.0670	7,200,000	0.0724	3,600,000	0.1310

Table 51. Reference points generated from horseshoe crab egg-per-recruit models for the Delaware Bay population under varying levels of juvenile natural mortality (M_{juv}).

M_{juv}	F_{20}	F_{40}
0.0817	2.2675	0.6465
0.0744	2.2582	0.6453
0.0685	2.2508	0.6443

Table 52. Sex specific fishing mortality (*F*) and biomass reference points for the Delaware Bay region generated from a population projection model (Table 50) along with terminal year values from the base run of the catch survey model. [3]

	Delaware Bay Horseshoe Crabs	
	Reference Point	**Benchmark Values**
Females	F_{MSY} = 0.0695 - 0.0796	F_{2017} = **CONFIDENTIAL***
	N_{MSY} = 3,664,522 - 6,600,291	N_{2018} = **CONFIDENTIAL***
Males	Sex Ratio (M:F) = 2:1	2017 Sex Ratio (M:F) = 5.2:1

***Benchmark values are CONFIDENTIAL because they are based on harvest that includes numbers of horseshoe crabs attributed the biomedical industry. Values without biomedical data are F_{2017}=0.007 and B_{2018}=8,718,040. The benchmark values of F_{2017} and B_{2018} with the biomedical data are slightly higher and lower, respectively, and although minimally different, represent the best data but are CONFIDENTIAL. The stock status of not overfished and overfishing not occurring is unchanged with or without the biomedical data.**

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[3] The Peer Review Panel did not endorse the use of the reference points developed for this stock assessment.

Table 53. **Stock status determination for the coastwide and regional stocks based on the 1998 index-based reference points from ARIMA models. Status was based on the percentage of surveys within a region (or coastwide) having a >50% probability of their terminal year fitted value being less than the 1998 index-based reference point. "Poor" status was >66% of surveys meeting this criterion, "Good" status was <33% of surveys, and "Neutral" status was 34 – 65% of surveys. The same criteria were applied to ARIMA results from the 2009 benchmark assessment and 2013 update assessment for comparison purposes. NOTE: The suite of surveys used in each assessment as well as the index values differed between assessments (see Section 7.3 for explanation).**

Region	2009 Benchmark	2013 Update	2019 Benchmark	2019 Stock Status
Northeast	2 out of 3	5 out of 6	1 out of 2	Neutral
New York	1 out of 5	3 out of 5	4 out of 4	Poor
Delaware Bay	5 out of 11	4 out of 11	2 out of 5	Neutral
Southeast	0 out of 5	0 out of 2	0 out of 2	Good
Coastwide	7 out of 24	12 out of 24	7 out of 13	Neutral

Table 54. **Details of surveys used in determining regional stock status. Arrows indicate increasing (↗), decreasing (↘), or stable (↔) trends over the most recent 5 and 10 year periods. $P(i_f<i_{1998})$ represents the probability of the terminal year's fitted index value (i_f) being less than the 1998 index-based reference point from ARIMA modeling. The average of this probabilities within a region is also given.**

Region	SurveyName	5 year trend	10 year trend	$P(i_f<i_{1998})$	Avg. Prob
New England	MA DMF Trawl - South of Cape Cod	↗	↗	0.08	
	RI Monthly Trawl - Fall	↘	↘	0.62	0.35
New York	CT Long Island Sound Trawl - Fall	↘	↘	1.00	
	NY Jamaica Bay Seine	↘	↘	0.96	
	NY Little Neck and Manhasset Bay Seine	↘	↘	1.00	
	NY Peconic Trawl	↔	↘	1.00	0.99
Delaware Bay	DE 30 ft Trawl - Fall	↗	↗	0.02	
	DE 30 ft Trawl - Spring	↗	↗	0.33	
	MD Coastal Bays Trawl - Spring	↗	↔	0.36	
	NJ Ocean Trawl - Fall	↔	↔	0.82	
	NJ Ocean Trawl - Spring	↗	↗	0.51	0.41
Southeast	SC CRMS	↗	↔	0.00	
	SC Trammel Net	↔	↗	0.00	0.00

Table 55. Comparison of the current stock assessment and the adaptive resource management (ARM) model for horseshoe crabs in the Delaware Bay region.

	Coastwide Stock Assessment	**Adaptive Resource Management (ARM)**
Management objective	Maximum sustainable yield	Maximum yield while maintaining ecological function (shorebird constraints)
Model types	Single species models	Multi-species models
Management triggers	Reference points based on HSC biology and life history (F_{msy}, B_{msy}, etc.)	Threshold values based on Red Knot abundance (81,900) **OR** female HSC abundance (80% of K, 11.2 million)
Status conclusions	Not overfished; overfishing not occurring	Thresholds for each species not met – female harvest not valued
Management recommendations	Female harvest could increase	Continued male only harvest (as of 2018)

11 FIGURES

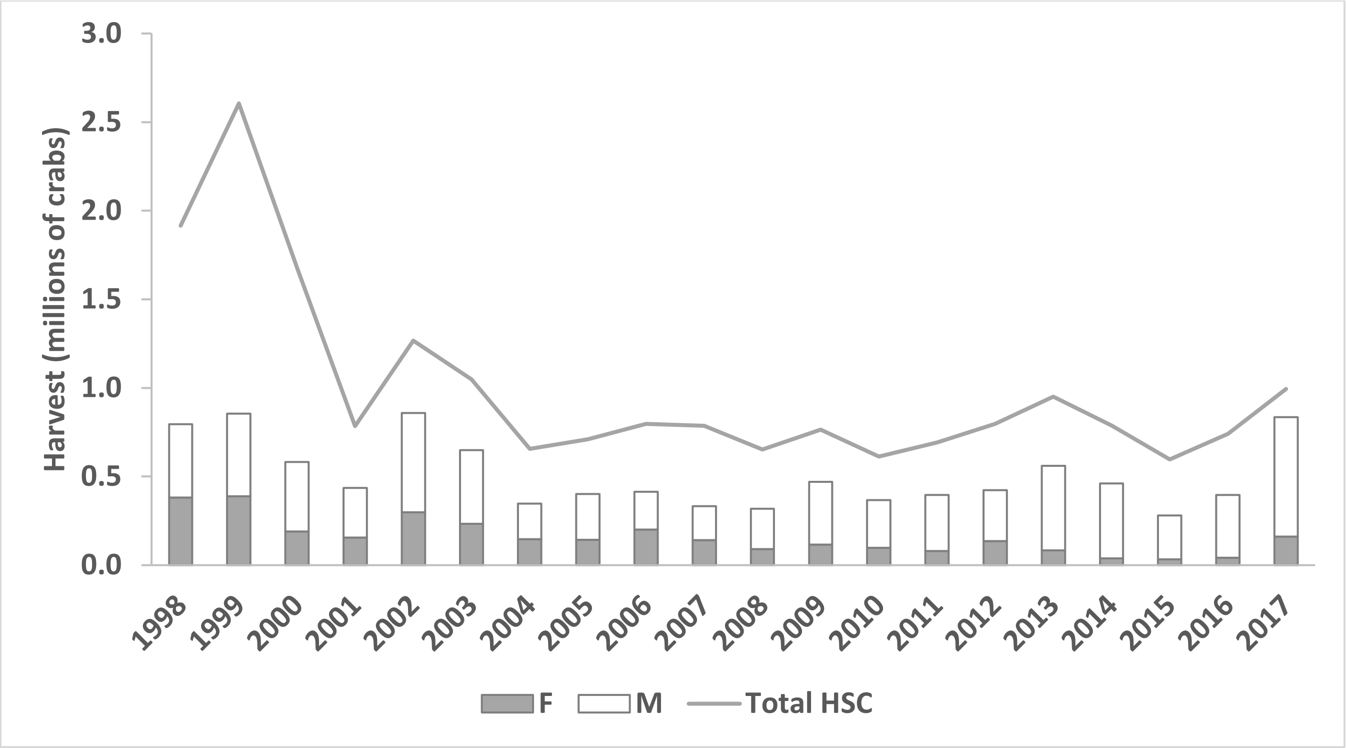


Figure 1. Coastwide horseshoe crab bait landings, 1998-2017, in numbers and by sex. Not every state along the Atlantic coast provides comprehensive sex data and therefore some are unclassified. Landings from 1998-2016 were validated by ACCSP; 2017 landings came from the 2018 FMP Review and state compliance reports.

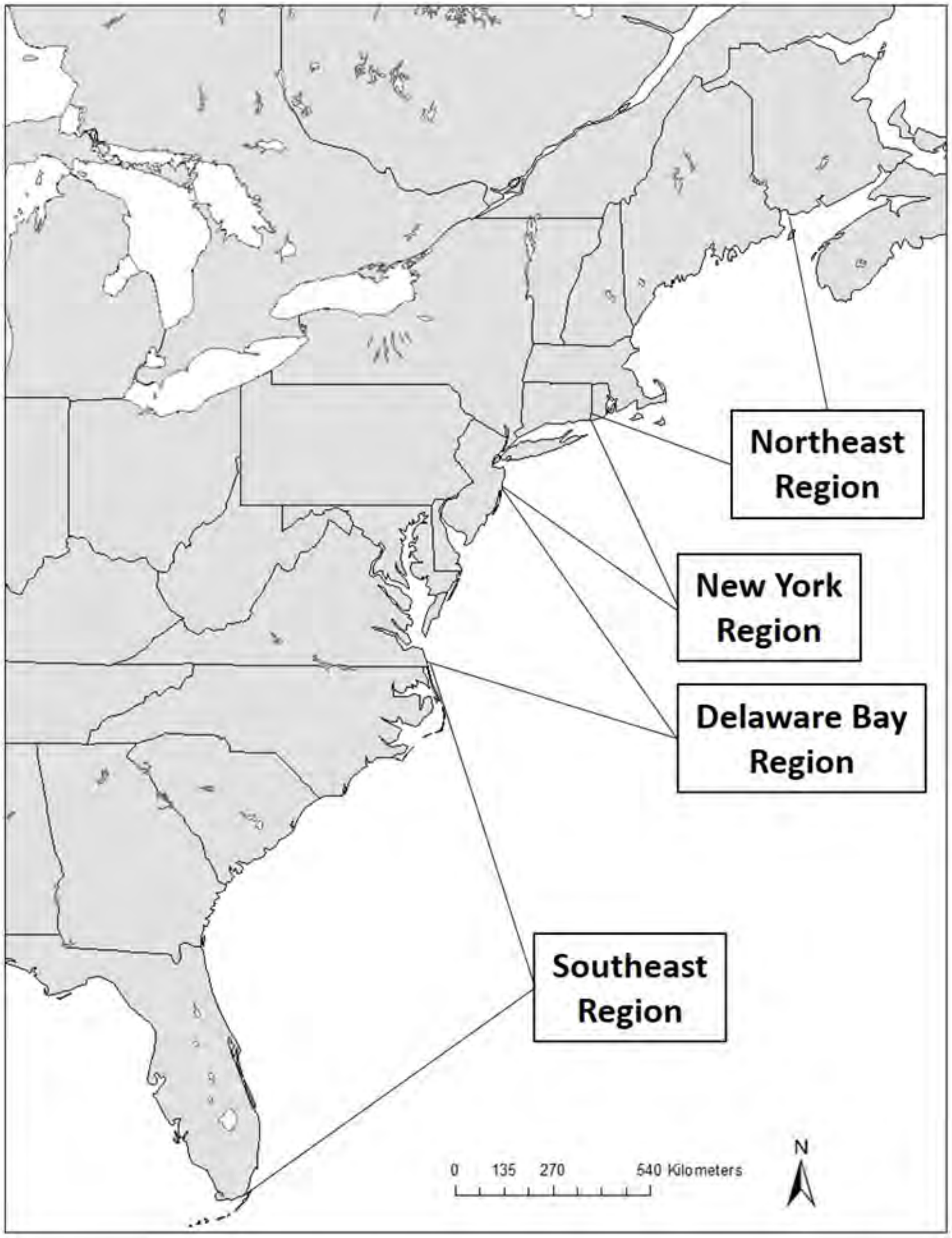


Figure 2. Map of the Atlantic coast showing the regions for horseshoe crab assessment.

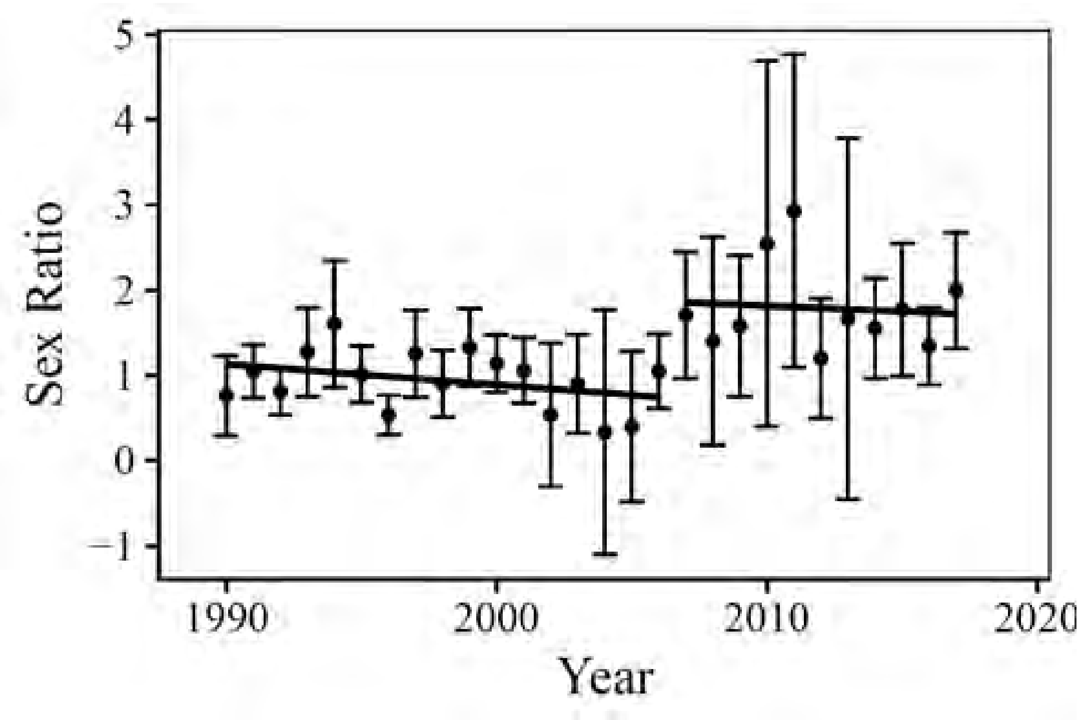


Figure 3. **Annual sex ratio (M:F) and associated confidence intervals for horseshoe crabs collected in the Delaware Bay 30' adult trawl survey from 1990 to 2017. Despite significant increases in sex ratio for these data, breakpoint analysis detected a significant shift in the relationship between these variables in 2006.**

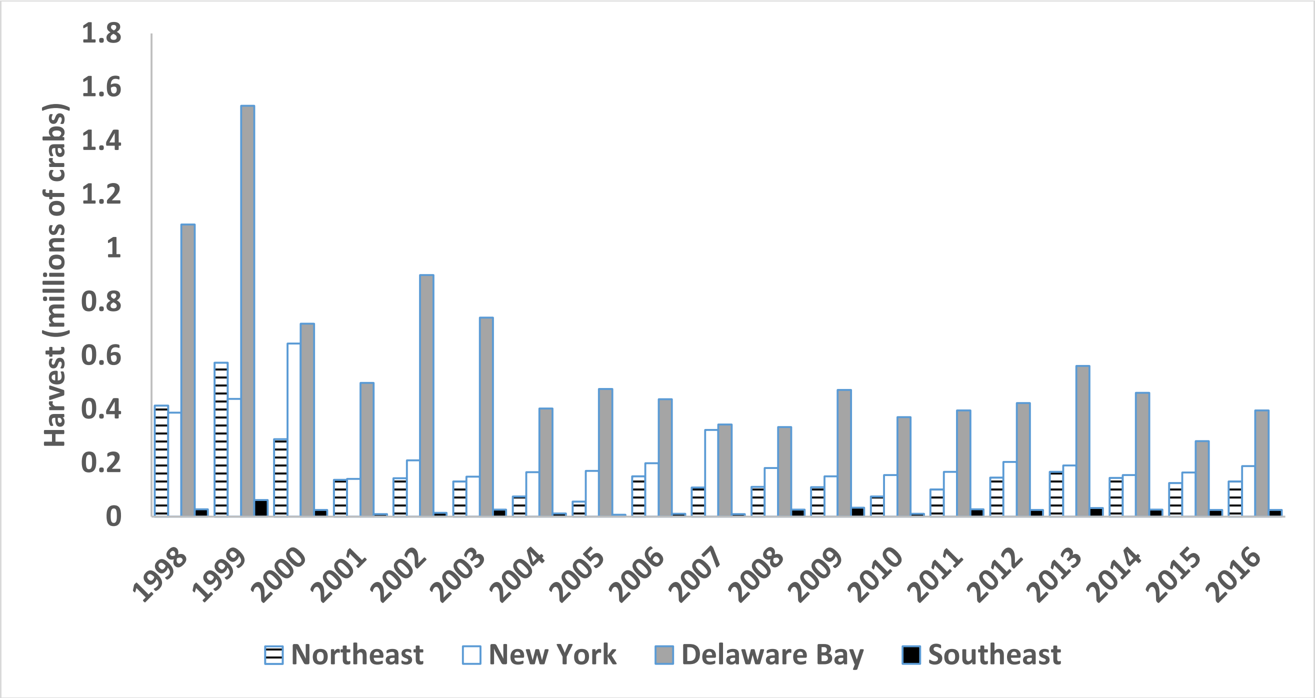


Figure 4. Horseshoe crab bait harvest by region, 1998-2016. The four regions are the Northeast (Maine, Massachusetts, Rhode Island), New York (Connecticut, New York), Delaware Bay (New Jersey, Delaware, Maryland, Virginia), and Southeast (North Carolina, South Carolina, Georgia, Florida).

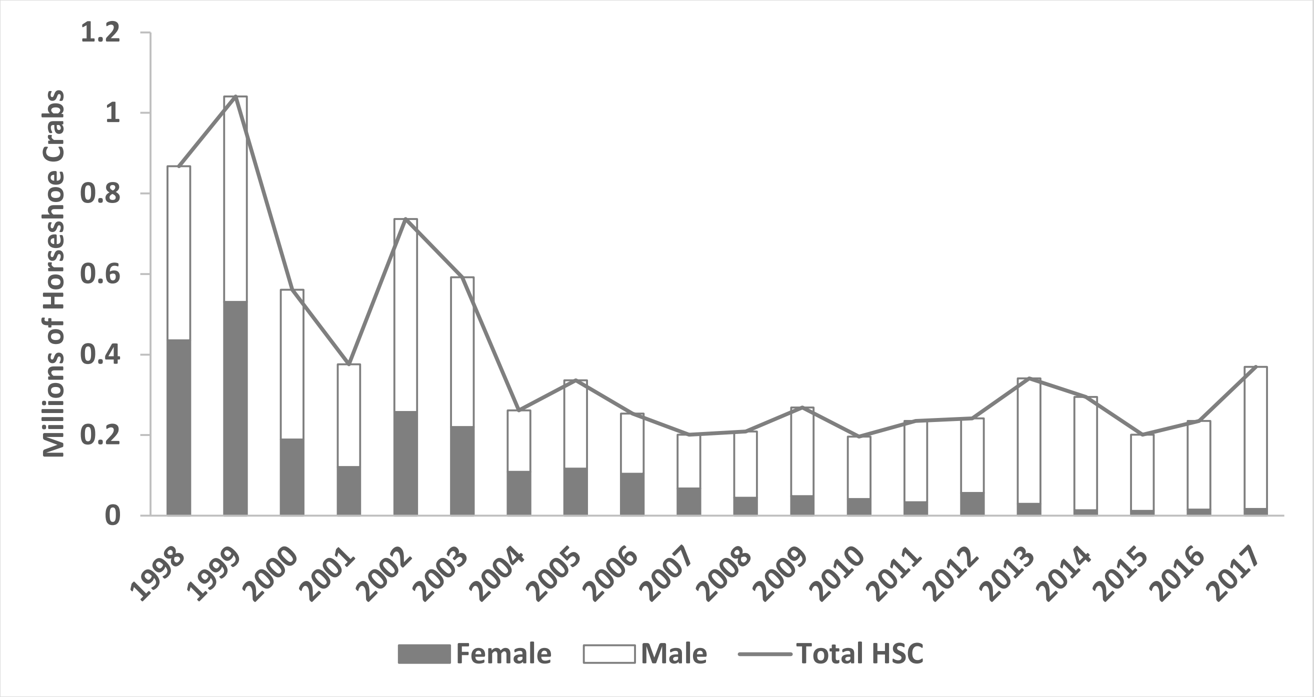


Figure 5. **Horseshoe crab bait landings of Delaware Bay origin, 1998-2017, by sex to support the catch multiple survey model. All landings were validated through ACCSP.**

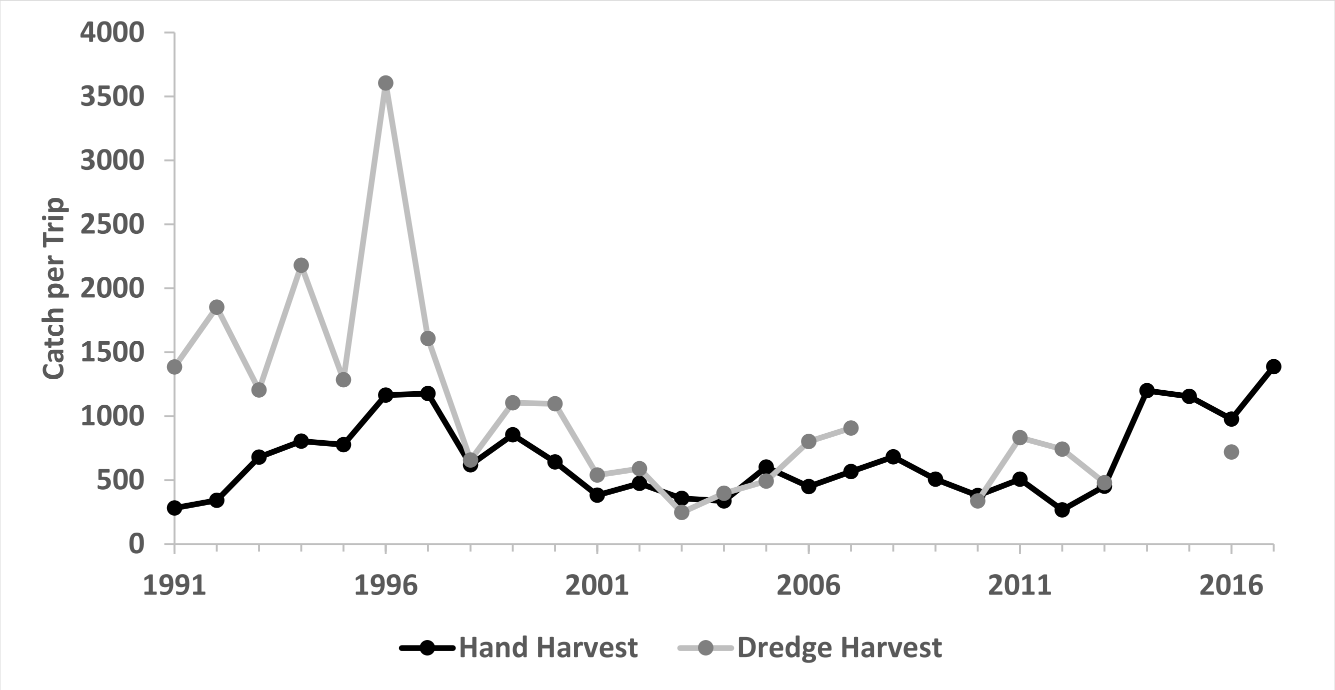


Figure 6. **Delaware's commercial horseshoe crab catch rates (mean number of crabs per trip). Missing values for dredge harvest in 2008, 2009, 2014, 2015, and 2017 are due to no dredge fishery in those years. Source: Delaware's Department of Fish and Wildlife's 2017 Compliance Report.**

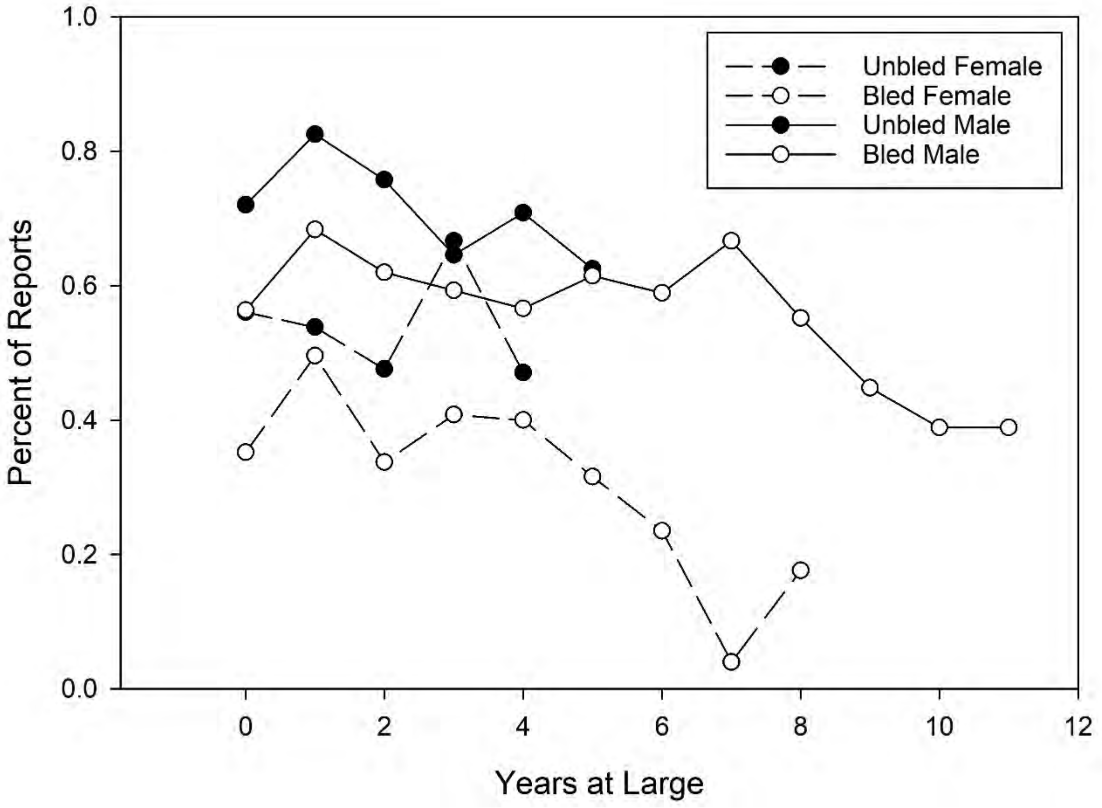


Figure 7. Comparison of bled (open circles) and unbled (filled circles) male (solid line) and female (dashed line) horseshoe crabs recaptured as alive over time.

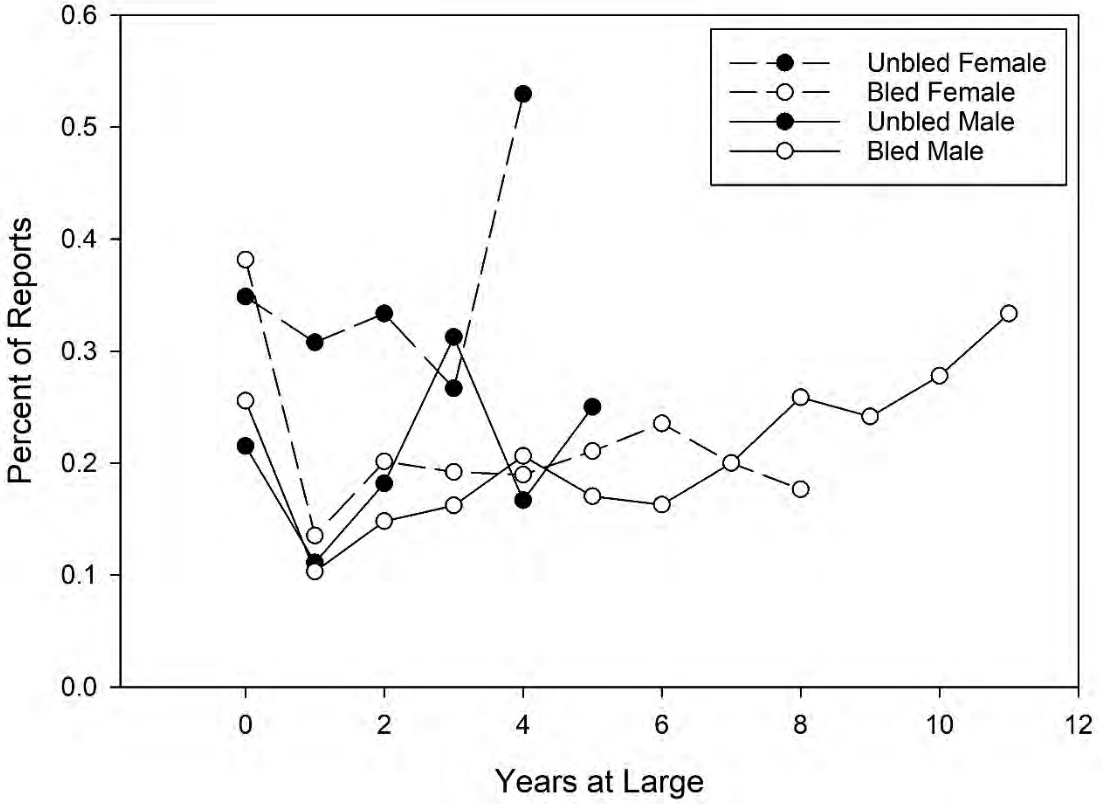


Figure 8. Comparison of bled (open circles) and unbled (filled circles) male (solid line) and female (dashed line) horseshoe crabs recaptured as dead over time.

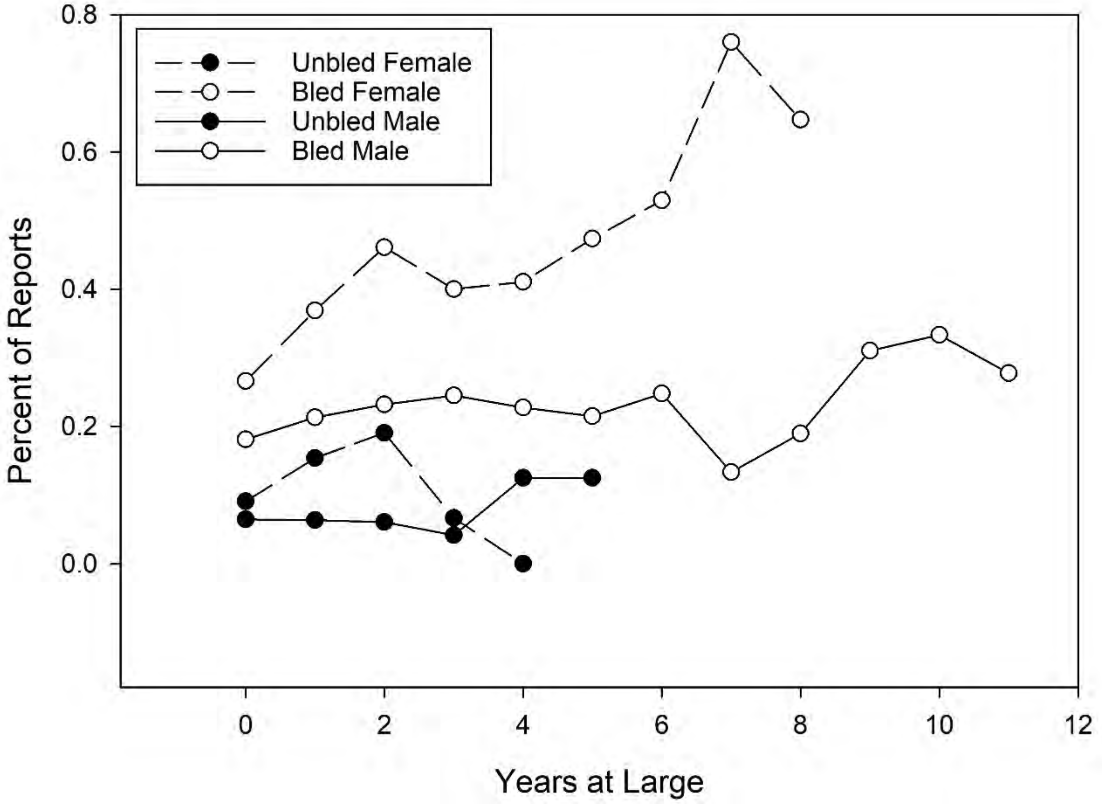


Figure 9. Comparison of bled (open circles) and unbled (filled circles) male (solid line) and female (dashed line) horseshoe crab tags reported as unknown disposition (e.g., tag was found unattached from crab).

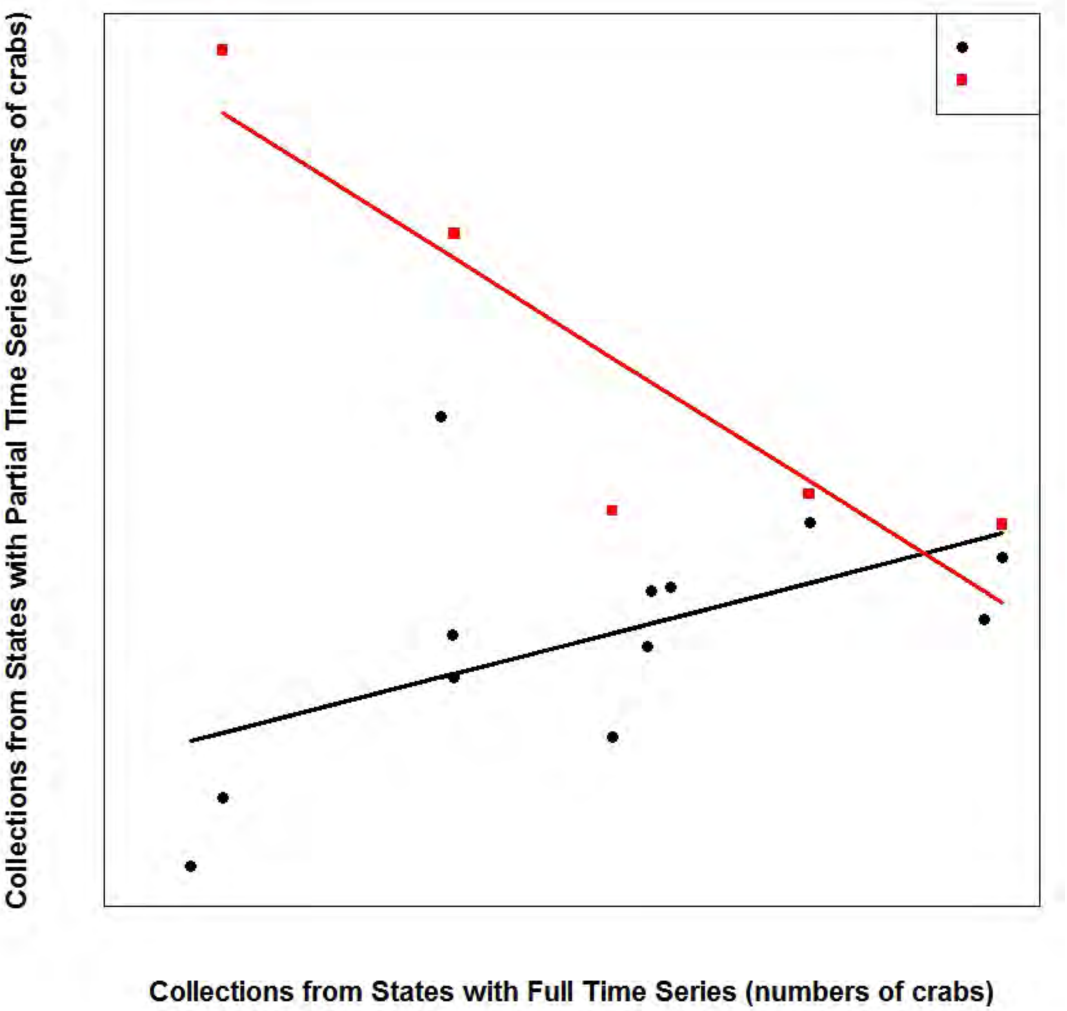


Figure 10. Linear regressions of biomedical collections of horseshoe crabs from states with partial and full time series from 1999-2017. Axis values and state names have been removed due to CONFIDENTIAL data.

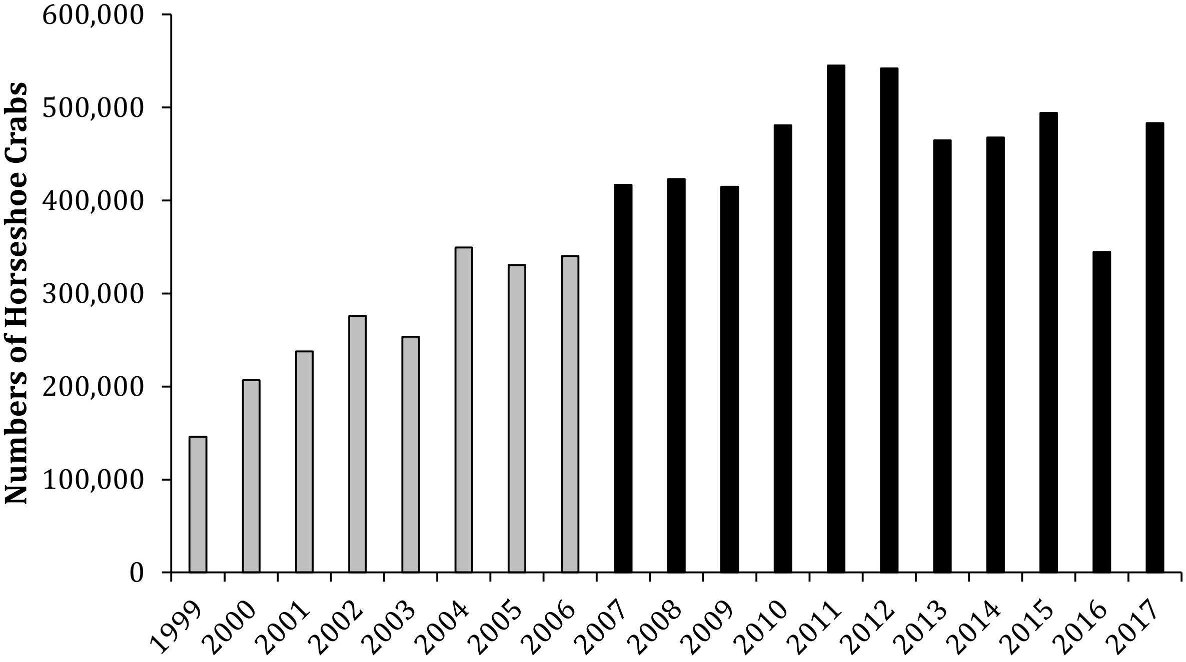


Figure 11. Annual numbers of horseshoe crabs collected solely for biomedical use coastwide. These numbers do not include crabs that entered the bait market after bleeding. Black bars indicate years in which all states reported collection numbers, and grey bars indicate years that include imputed values for at least one state due to missing data when collections were known to have occurred.

[Figure Removed Due to **CONFIDENTIAL** Data]

Figure 12. Estimated mortality attributable to biomedical use of horseshoe crabs along the US Atlantic coast. Sex-specific mortality of crabs of Delaware (DE) Bay origin is highlighted. Delaware Bay origin crabs include 100% of New Jersey, 51% of Maryland, and 35% of Virginia mortality, based on genetic information.

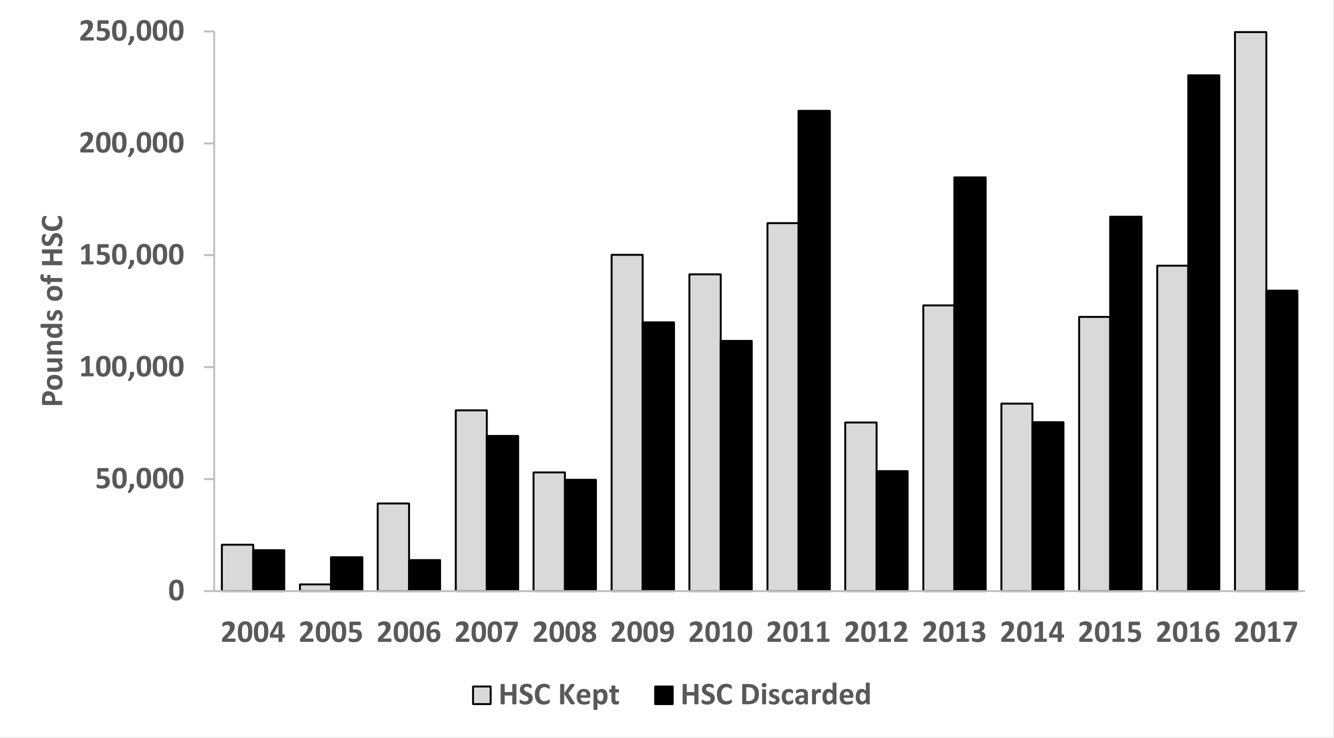


Figure 13. Total pounds of horseshoe crabs (HSC) discarded and horseshoe crabs kept in observed fishing trips in the NEFOP data set for the Delaware Bay states, 2004-2017.

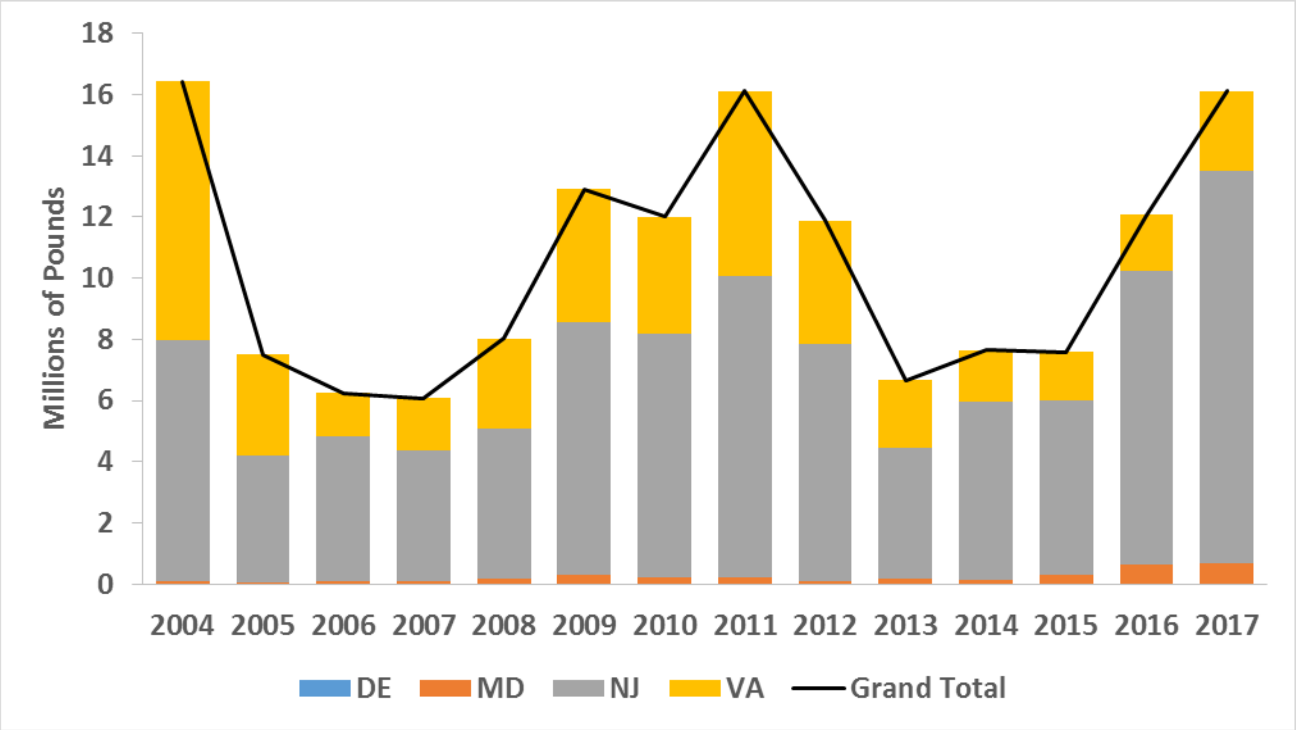


Figure 14. Total pounds of observed landings, all species, from the NEFOP data set for the Delaware Bay states, 2004-2017.

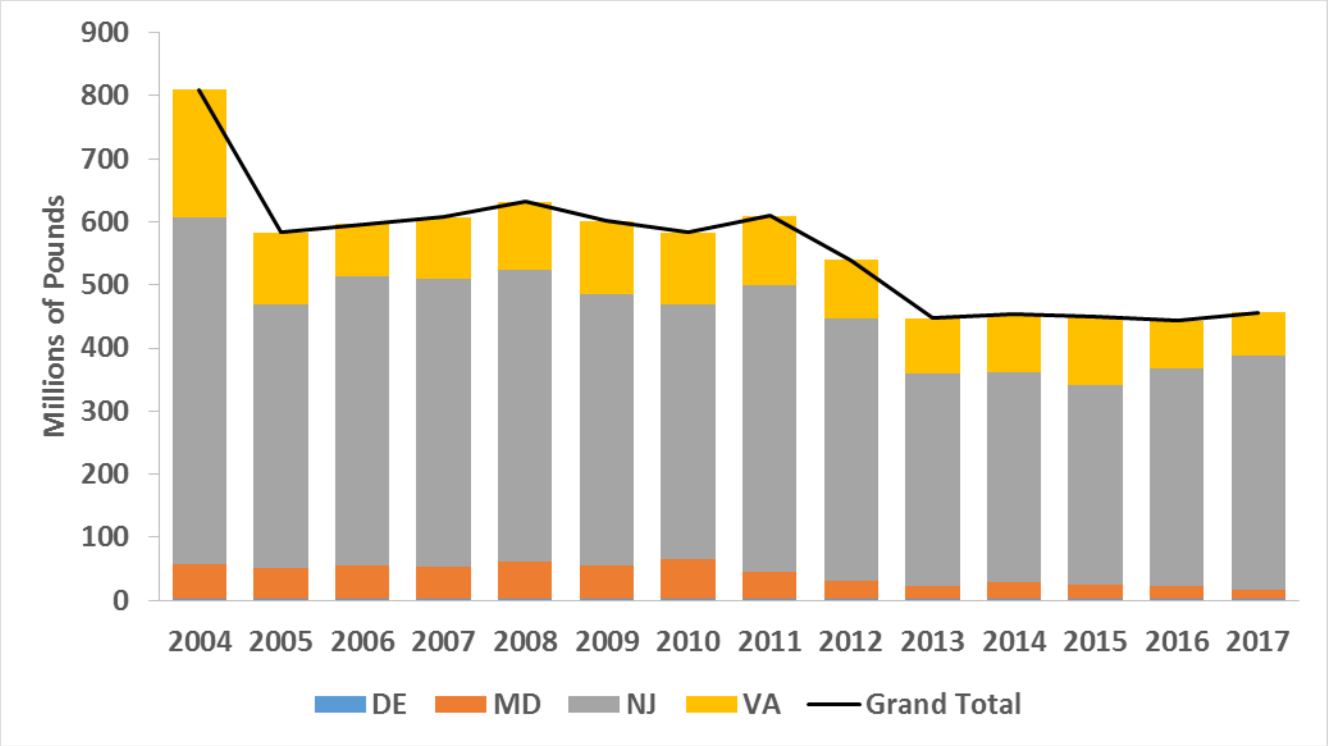


Figure 15. All species landings by state for gillnets, trawls, dredge, and "not coded" for the Delaware Bay region for 2004-2017 (source: ACCSP).

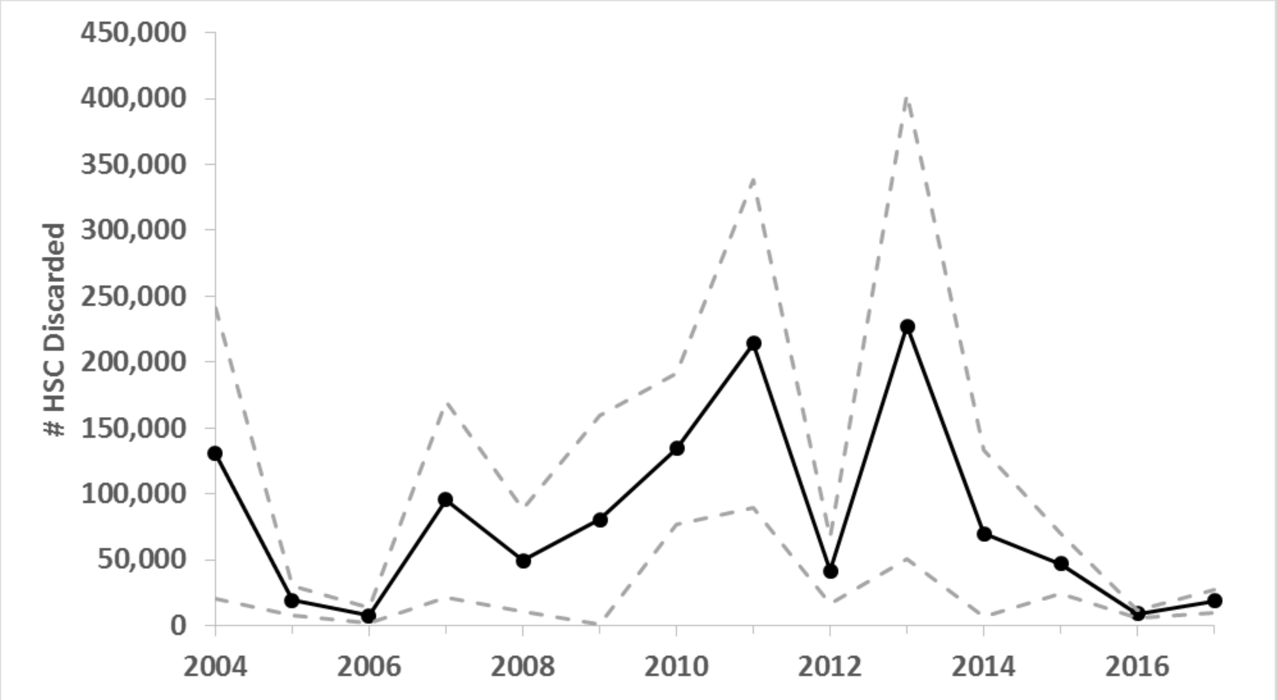


Figure 16. Estimated number of horseshoe crabs discarded from gill nets in the Delaware Bay region, 2004-2017, with 95% confidence intervals.

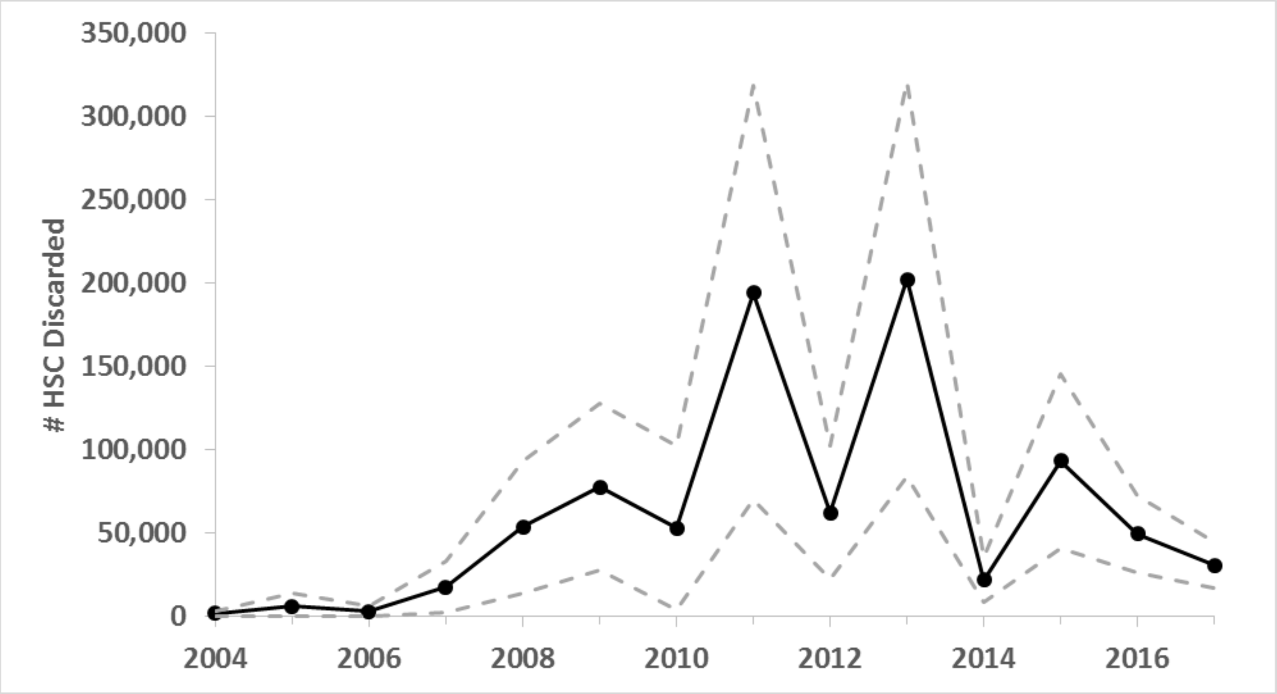


Figure 17. Estimated number of horseshoe crabs discarded from trawls in the Delaware Bay region, 2004-2017, with 95% confidence intervals.

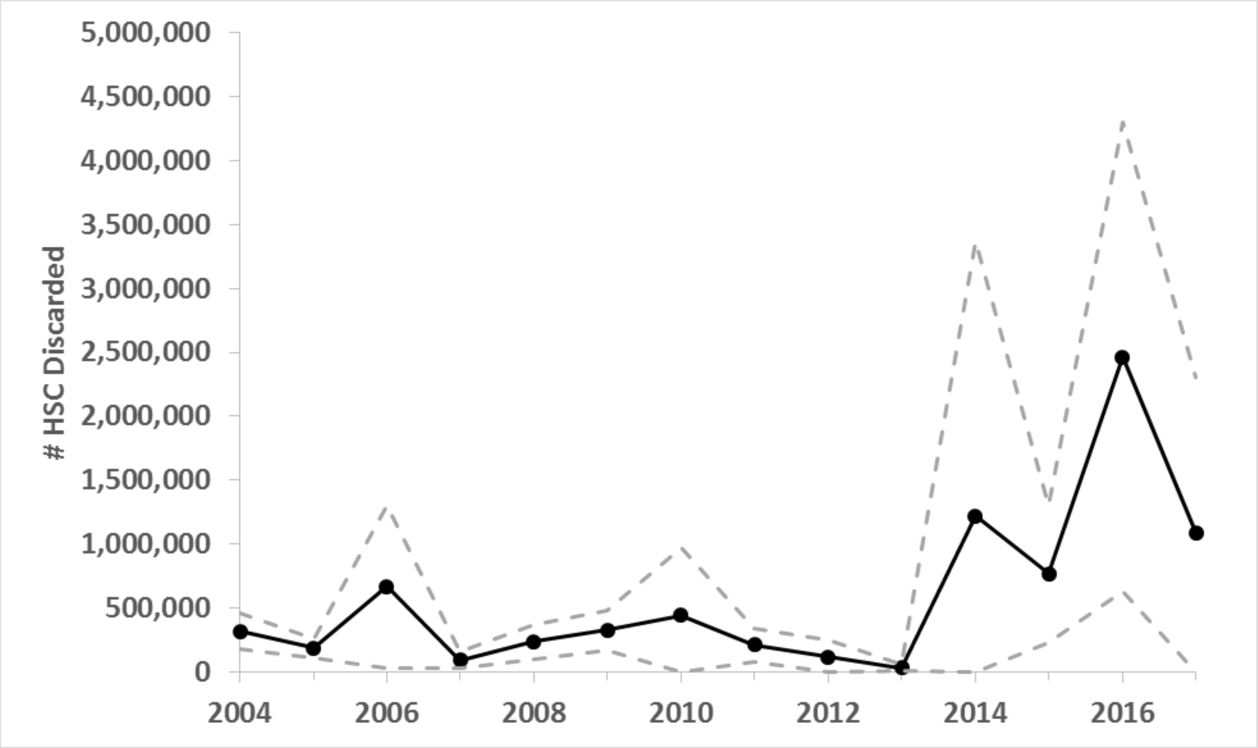


Figure 18. Estimated number of horseshoe crabs discarded from dredges in the Delaware Bay region, 2004-2017, with 95% confidence intervals.

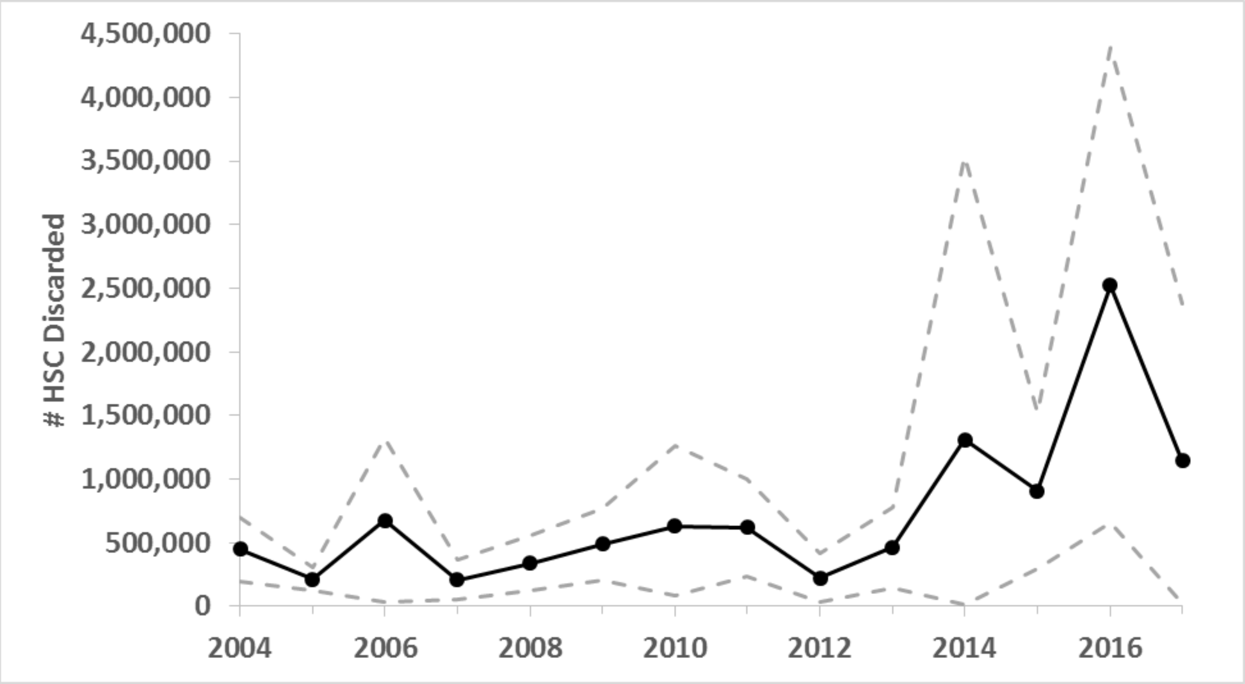


Figure 19. Estimated number of horseshoe crabs discarded from gill nets, trawls, and dredges in the Delaware Bay region, 2004-2017, with 95% confidence intervals.

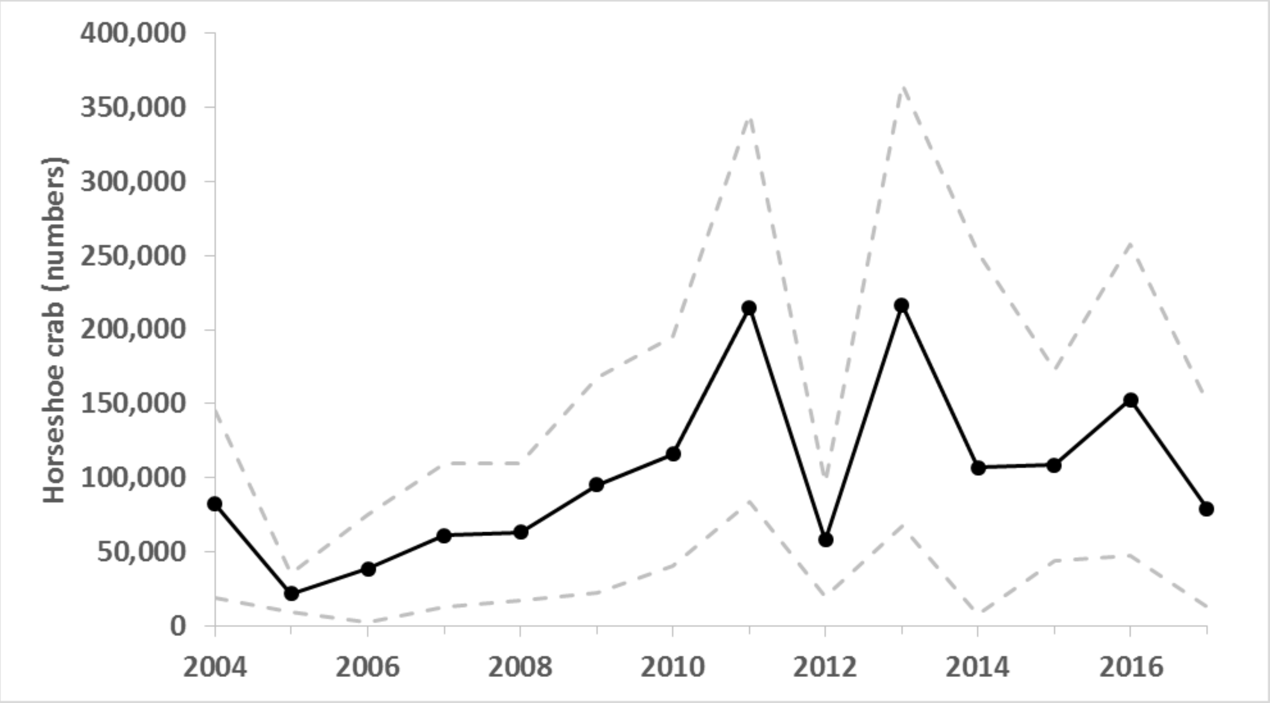


Figure 20. Number of dead discarded horseshoe crabs in the Delaware Bay region, 2004-2017, from gillnets, trawls, and dredges with 95% confidence intervals.

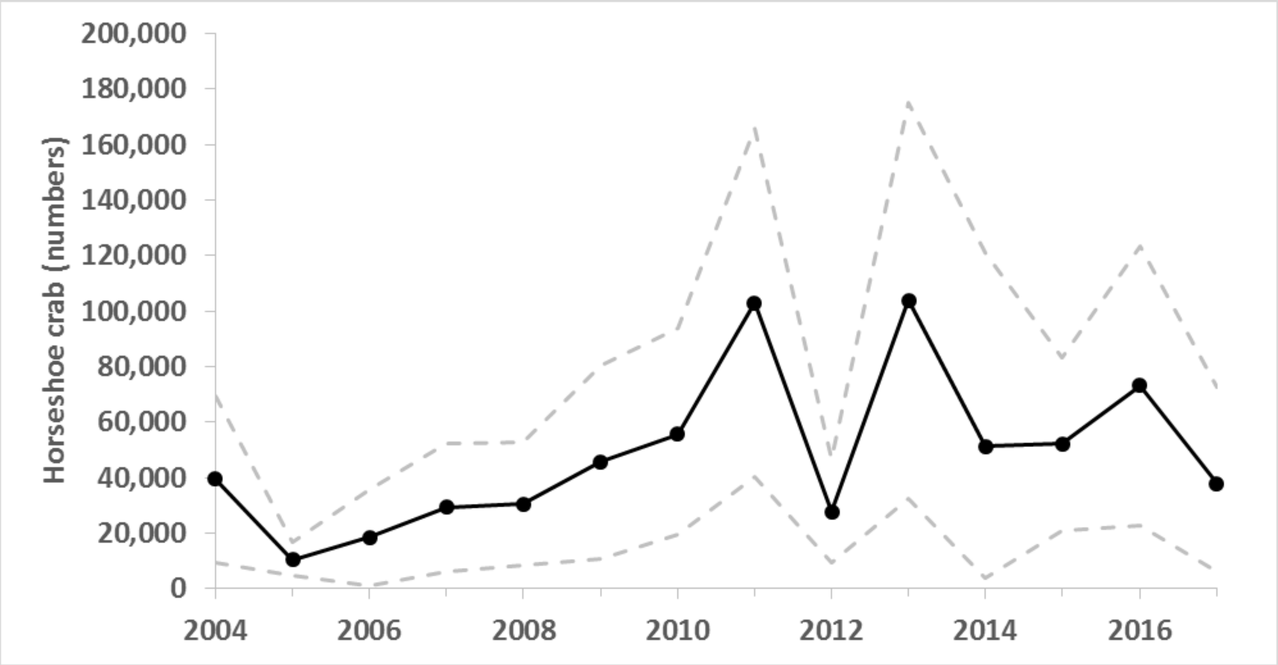


Figure 21. Number of dead discarded female horseshoe crabs in the Delaware Bay region, 2004-2017, from gillnets, trawls, and dredges with 95% confidence intervals.

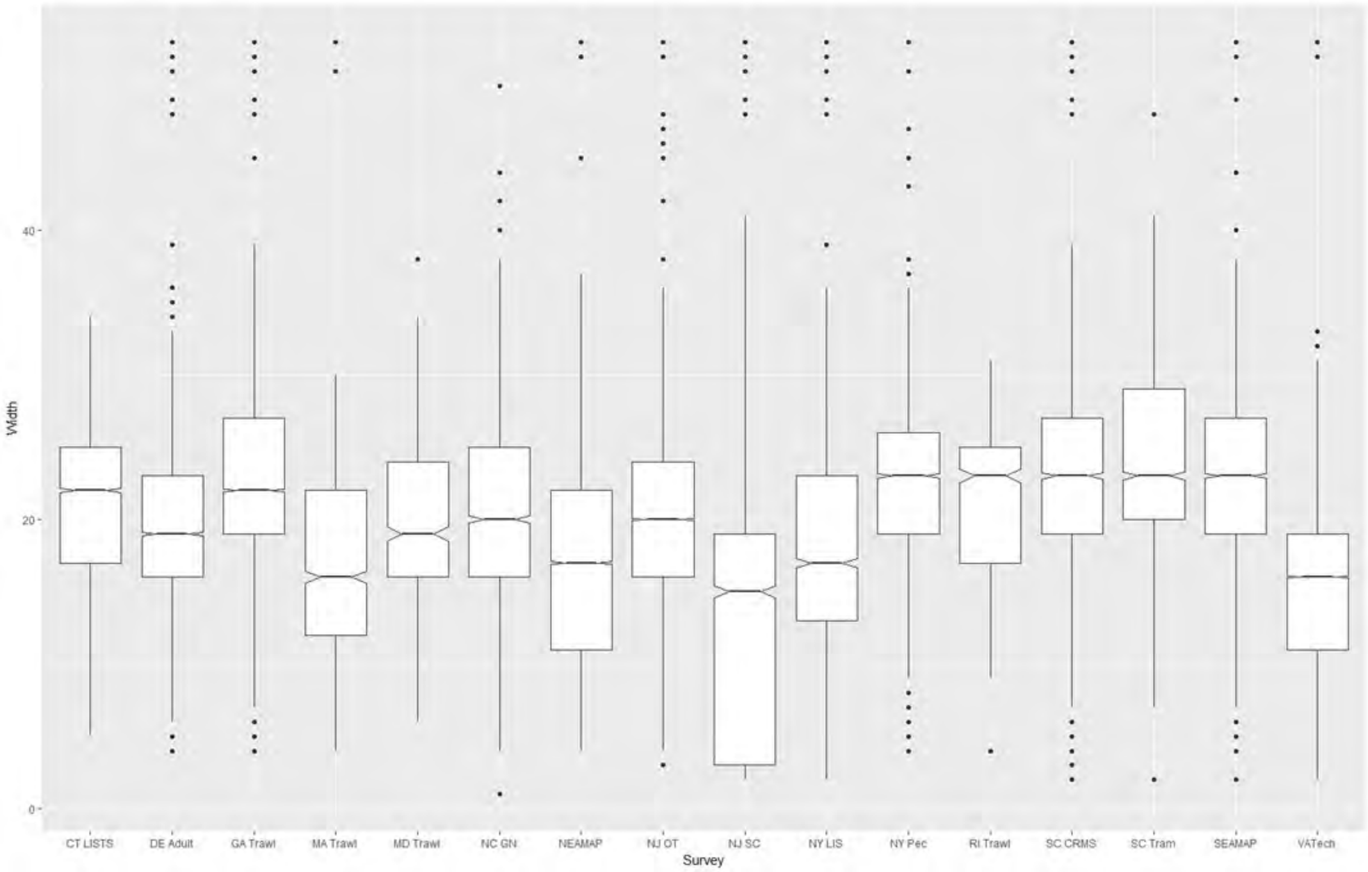


Figure 22. Boxplot of horseshoe crab prosomal widths (cm) caught in each fishery independent survey used in this assessment.

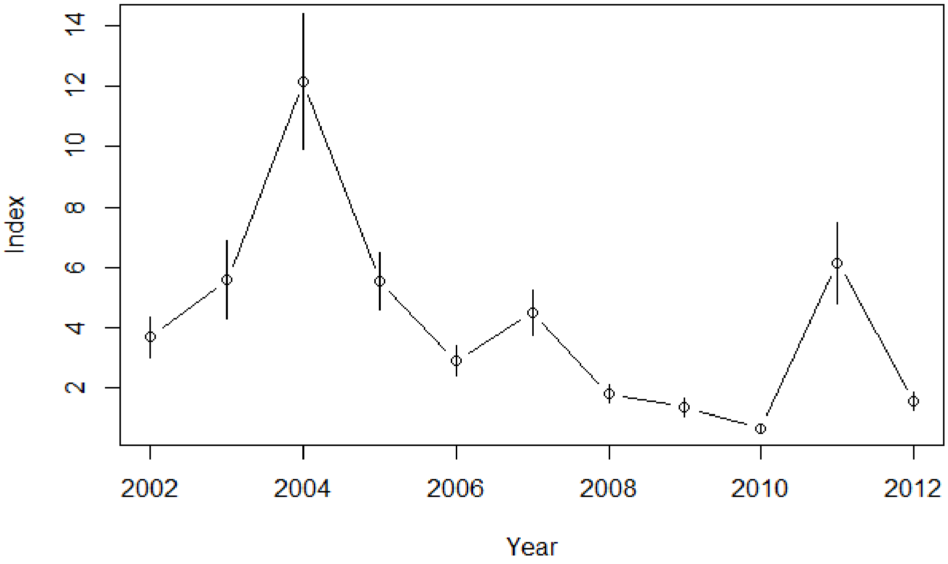


Figure 23. Index of relative abundance of female horseshoe crabs (delta mean crabs per sampling event) developed from the spring portion of New Hampshire's Spawning Beach Survey with 95% confidence intervals.

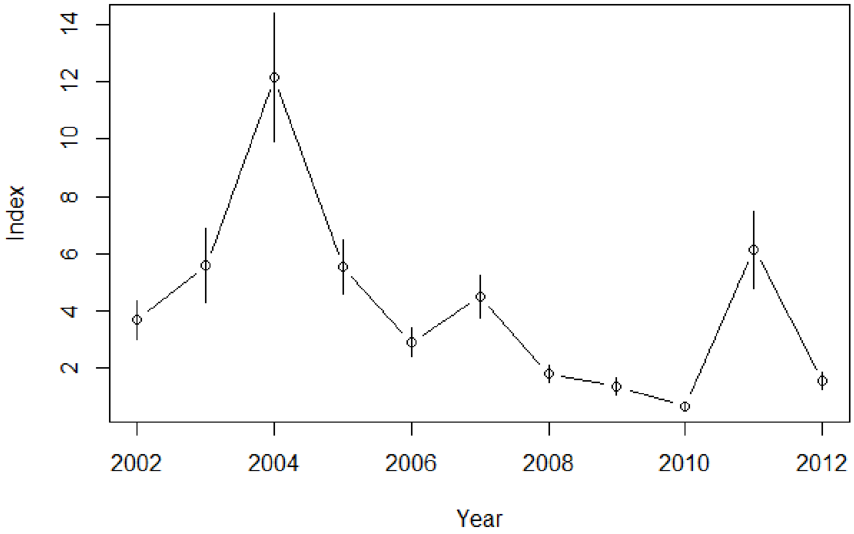


Figure 24. Index of relative abundance of male horseshoe crabs (delta mean crabs per sampling event) developed from the spring portion of New Hampshire's Spawning Beach Survey with 95% confidence intervals.

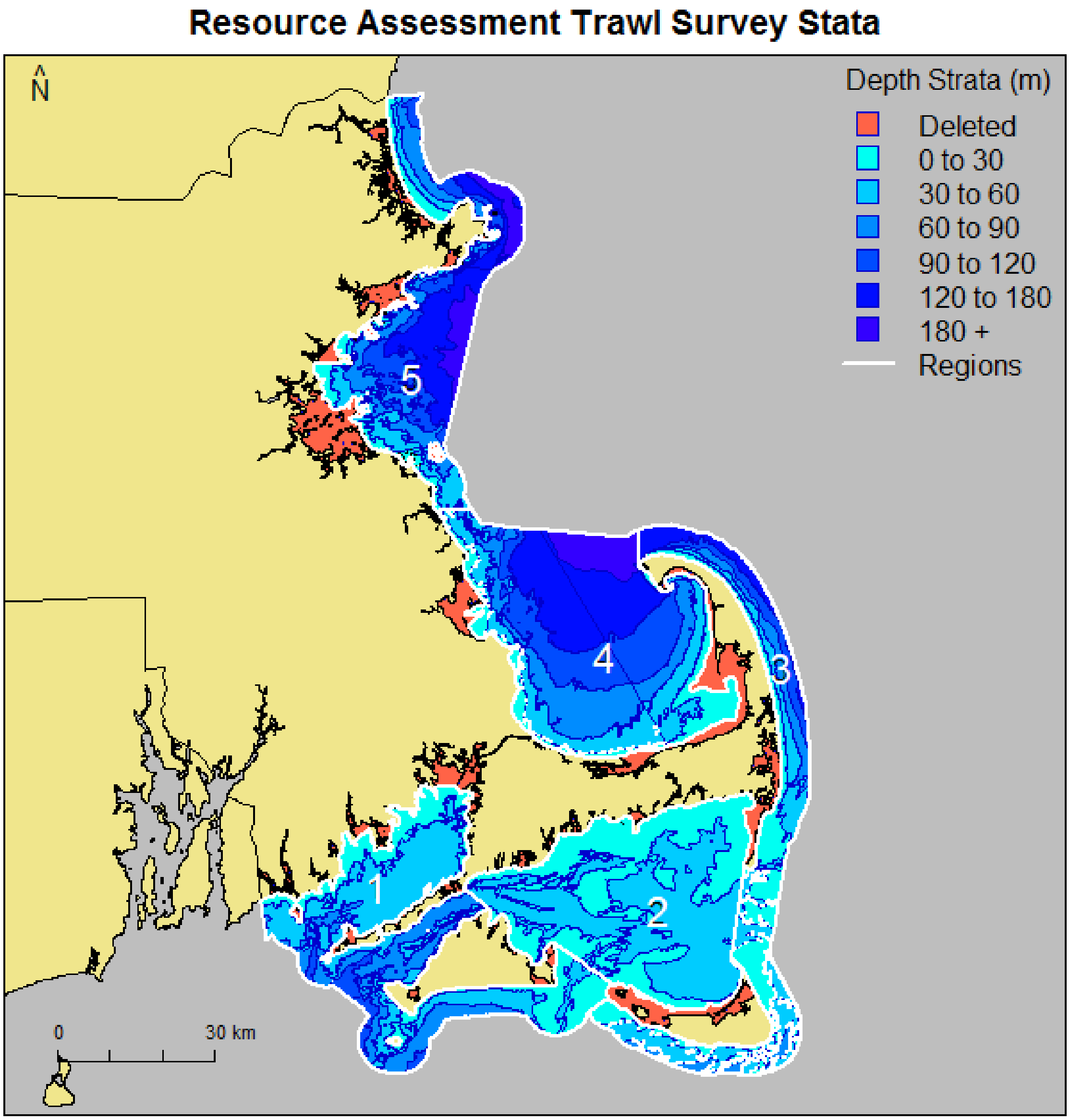


Figure 25. Map of Massachusetts Assessment Trawl Survey Strata.

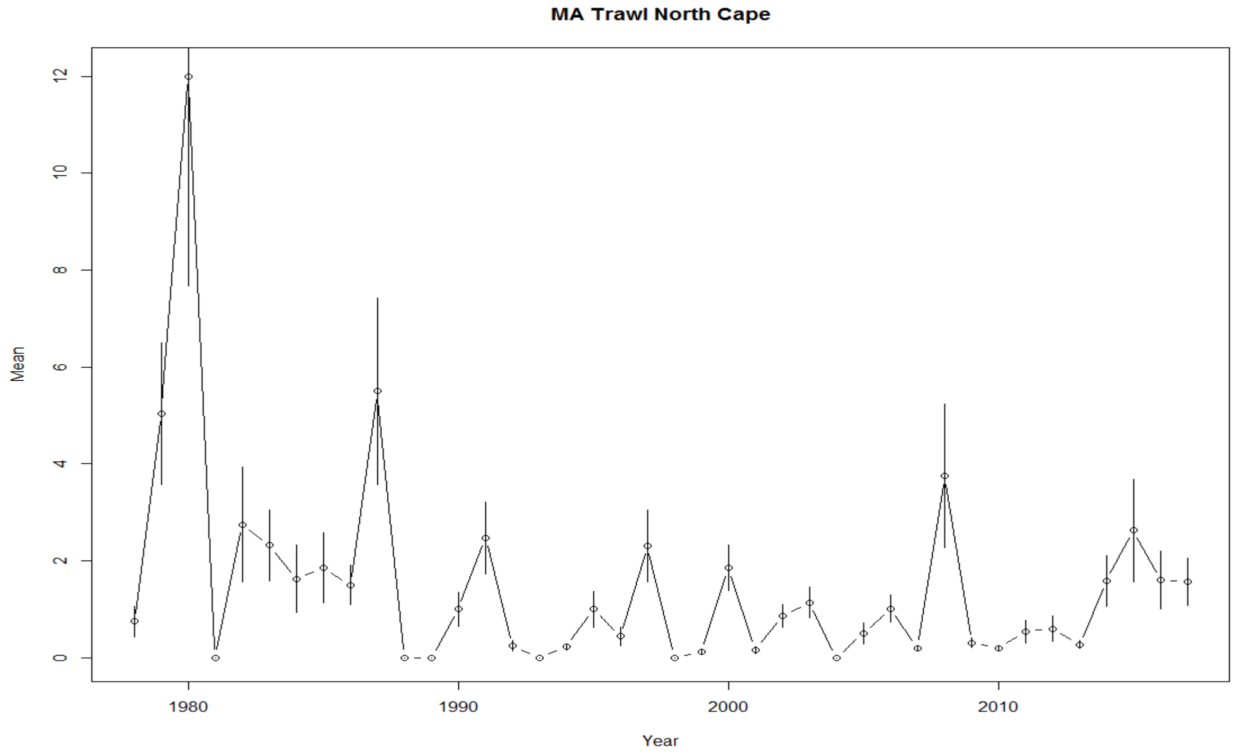


Figure 26. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of Massachusetts' Resource Assessment Trawl Survey in strata north of Cape Cod with 95% confidence intervals.

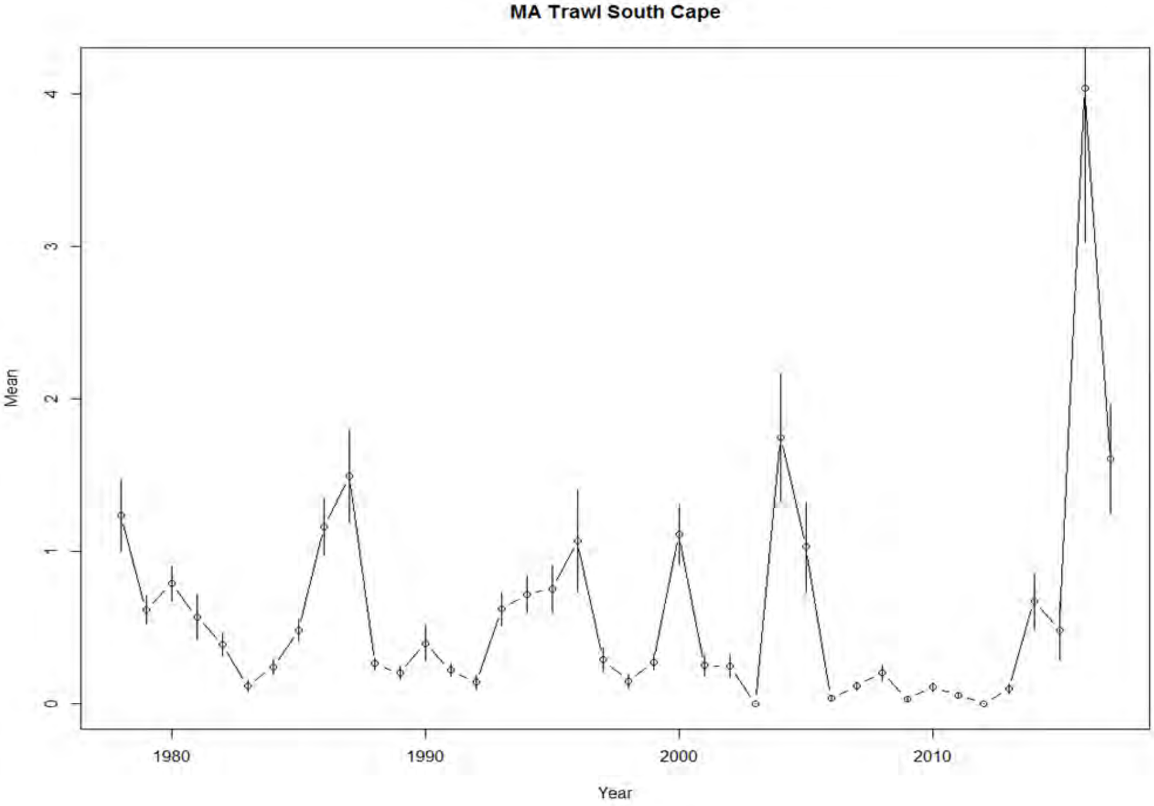


Figure 27. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of Massachusetts' Resource Assessment Trawl Survey in strata south of Cape Cod with 95% confidence intervals.

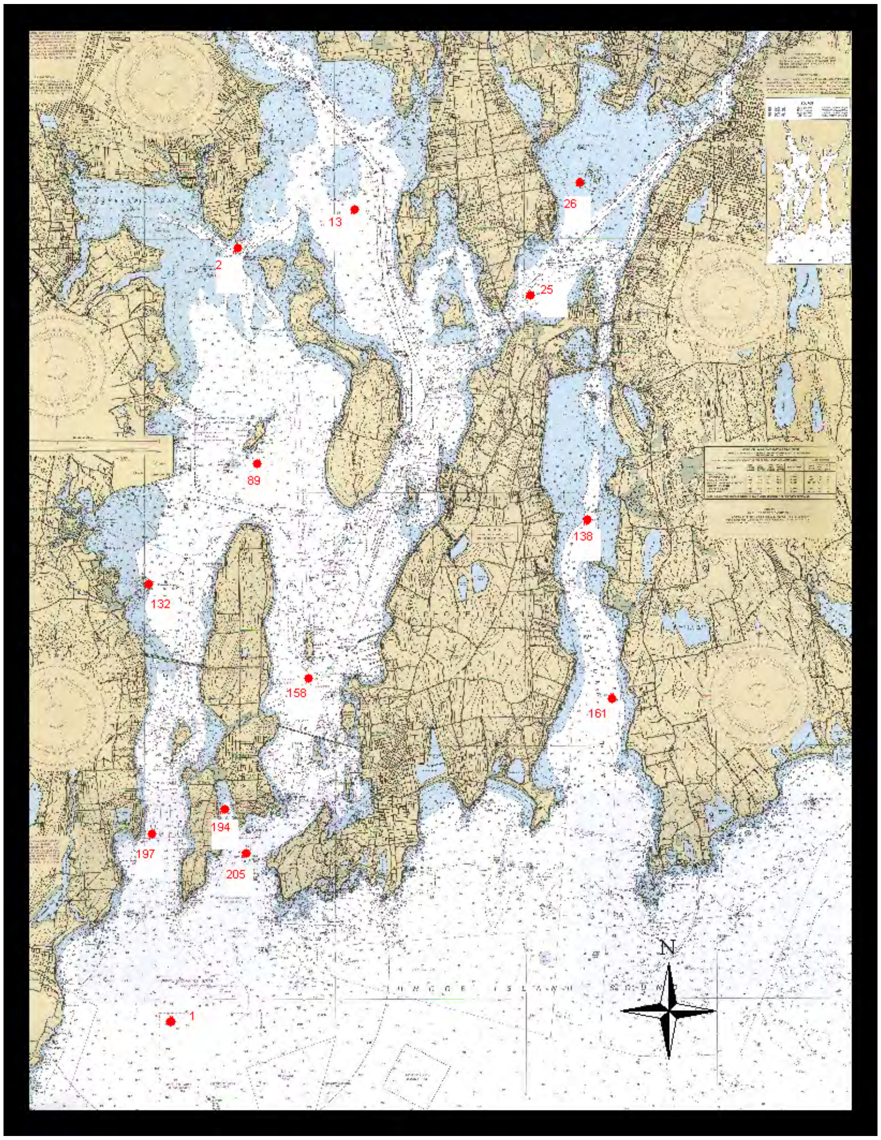


Figure 28. Map of Rhode Island Coastal Trawl Survey Monthly Segment fixed tow stations.

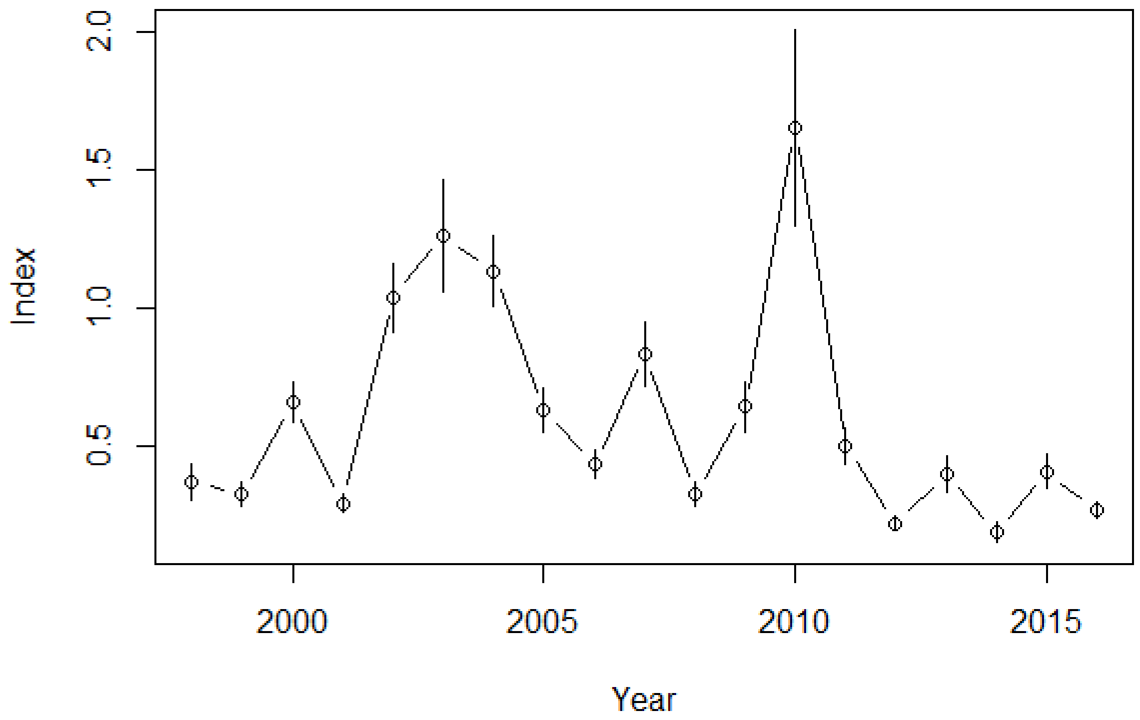


Figure 29. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of Rhode Island's Coastal Trawl Survey Monthly Segment with 95% confidence intervals.

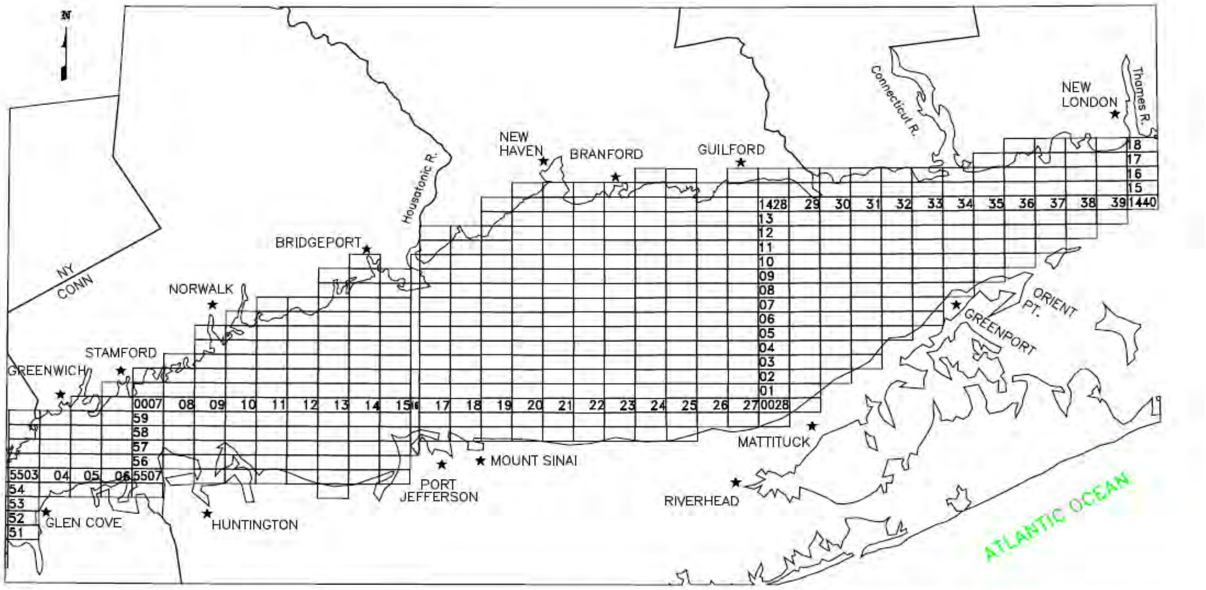


Figure 30. Map pf Connecticut DEEP Long Island Sound Trawl Survey site grid.

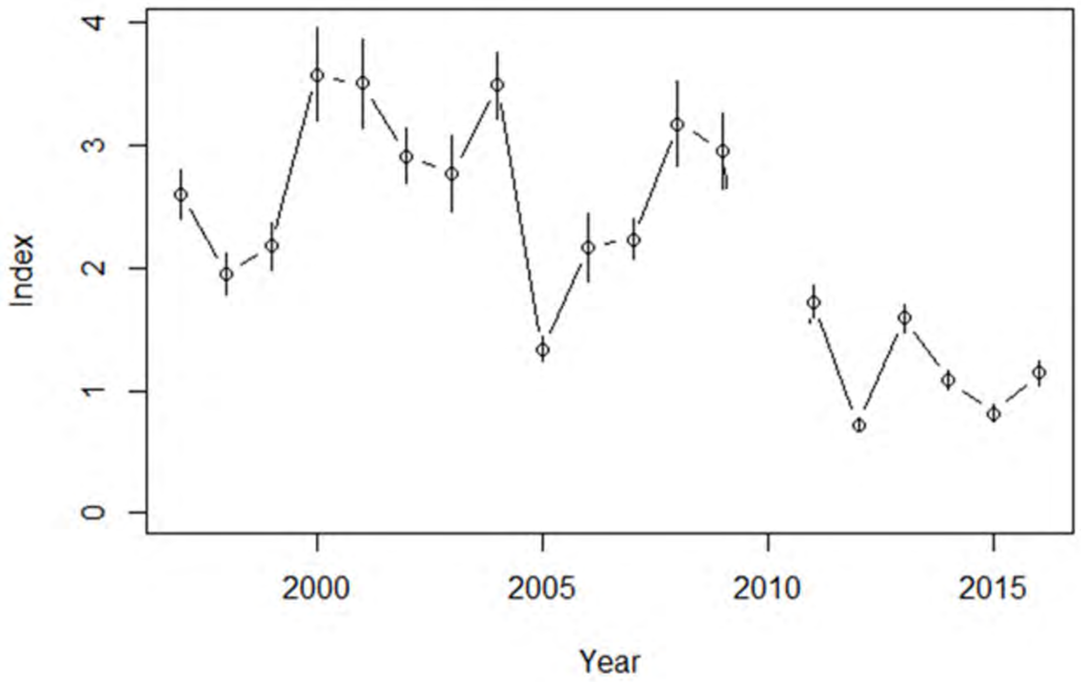


Figure 31. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of Connecticut DEEP Long Island Sound Trawl Survey with 95% confidence intervals.

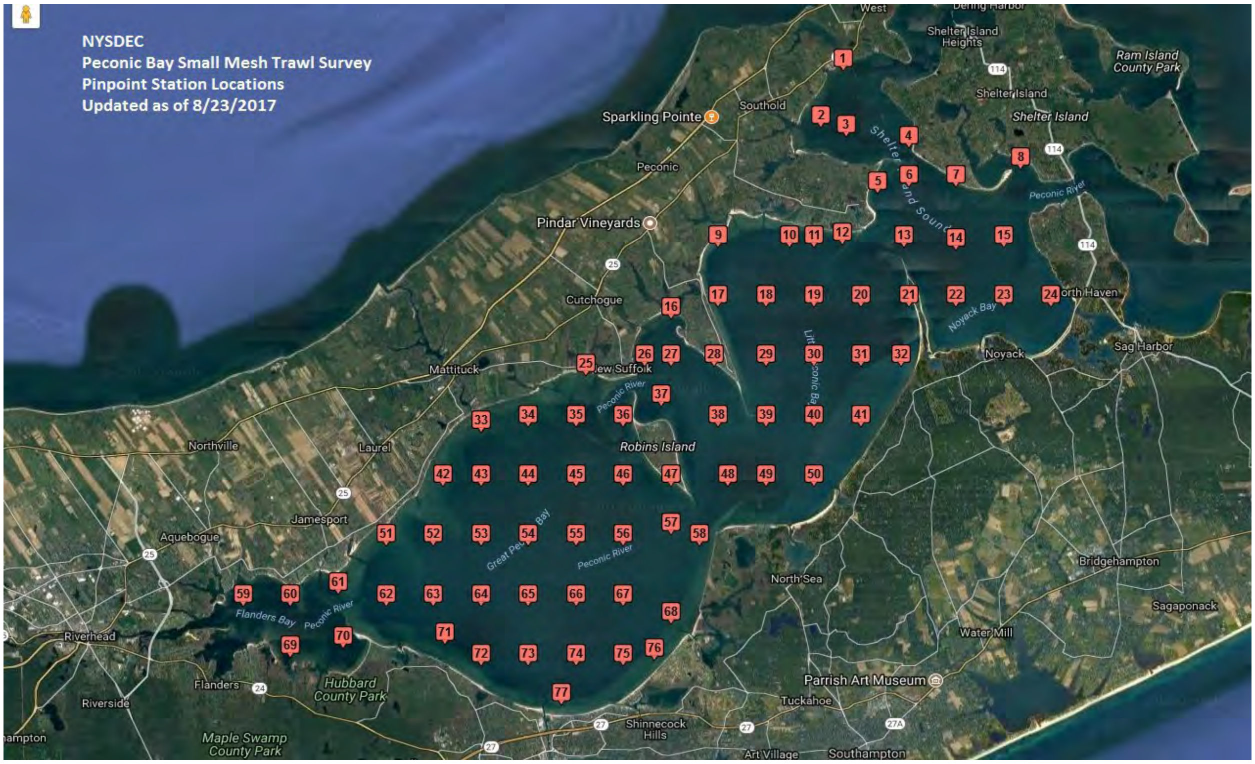


Figure 32. Map of New York Peconic Bay Small Mesh Trawl Survey Sampling Grid.

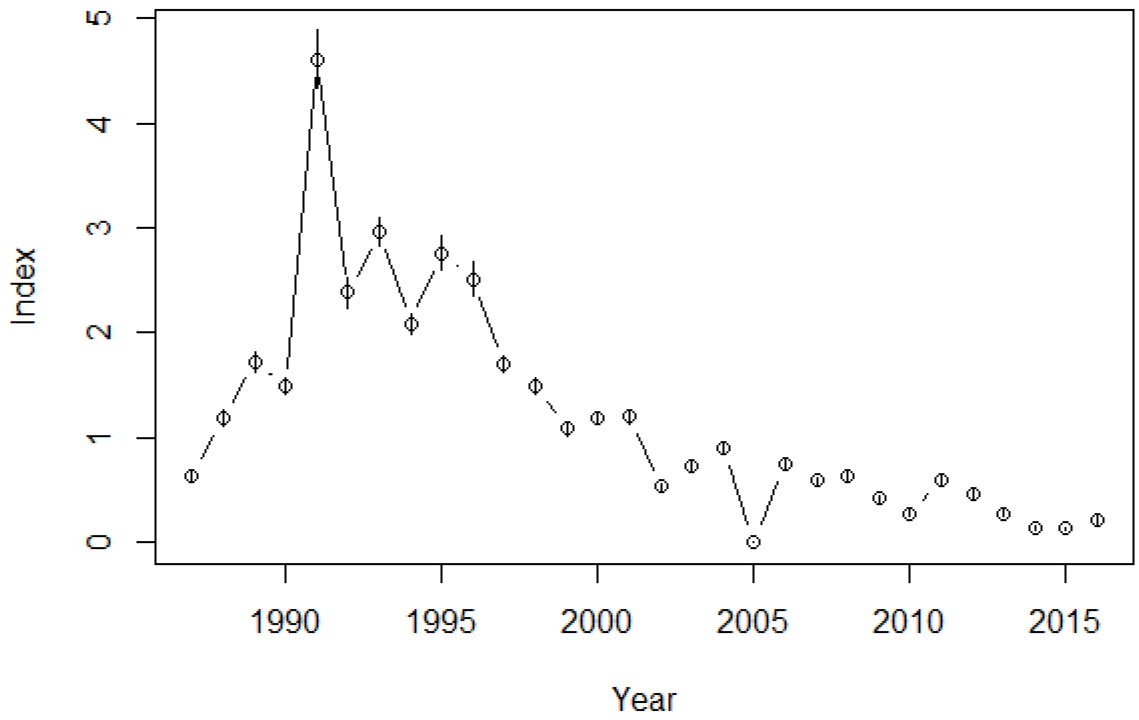


Figure 33. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of the New York DEC Peconic Bay Small Mesh Trawl Survey with 95% confidence intervals.

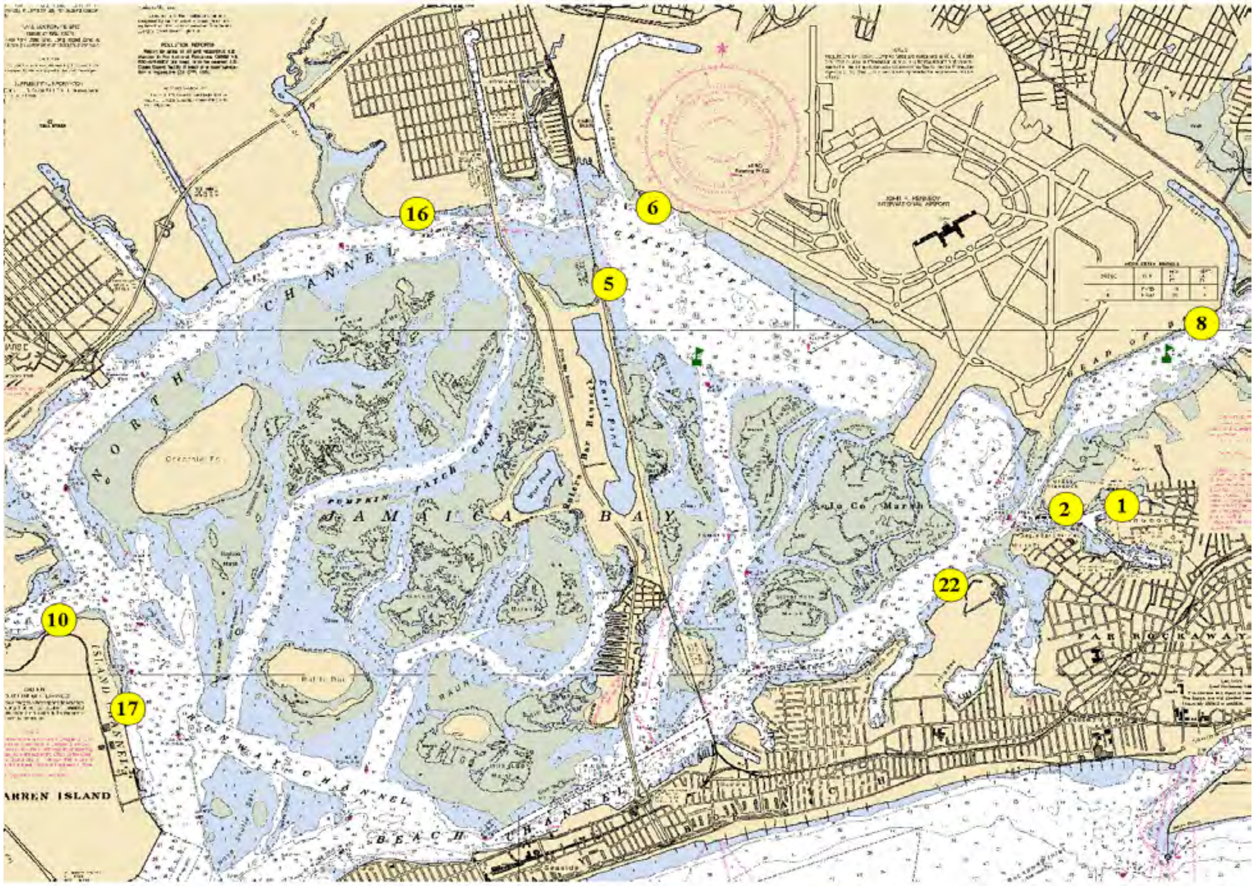


Figure 34. Map of New York DEC Western Long Island Beach Seine Survey Jamaica Bay Stations.

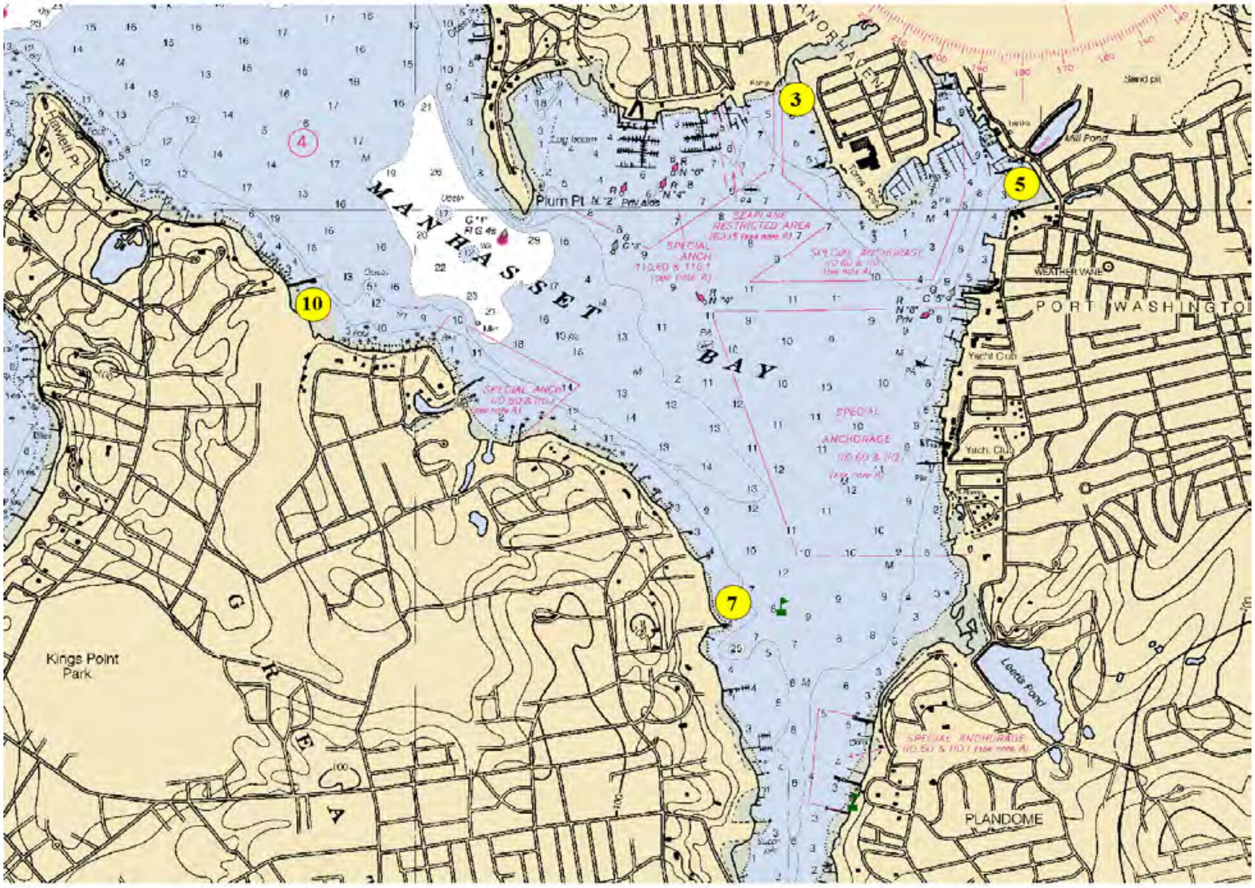


Figure 35. Map of New York DEC Western Long Island Beach Seine Survey Manhasset Bay Stations.

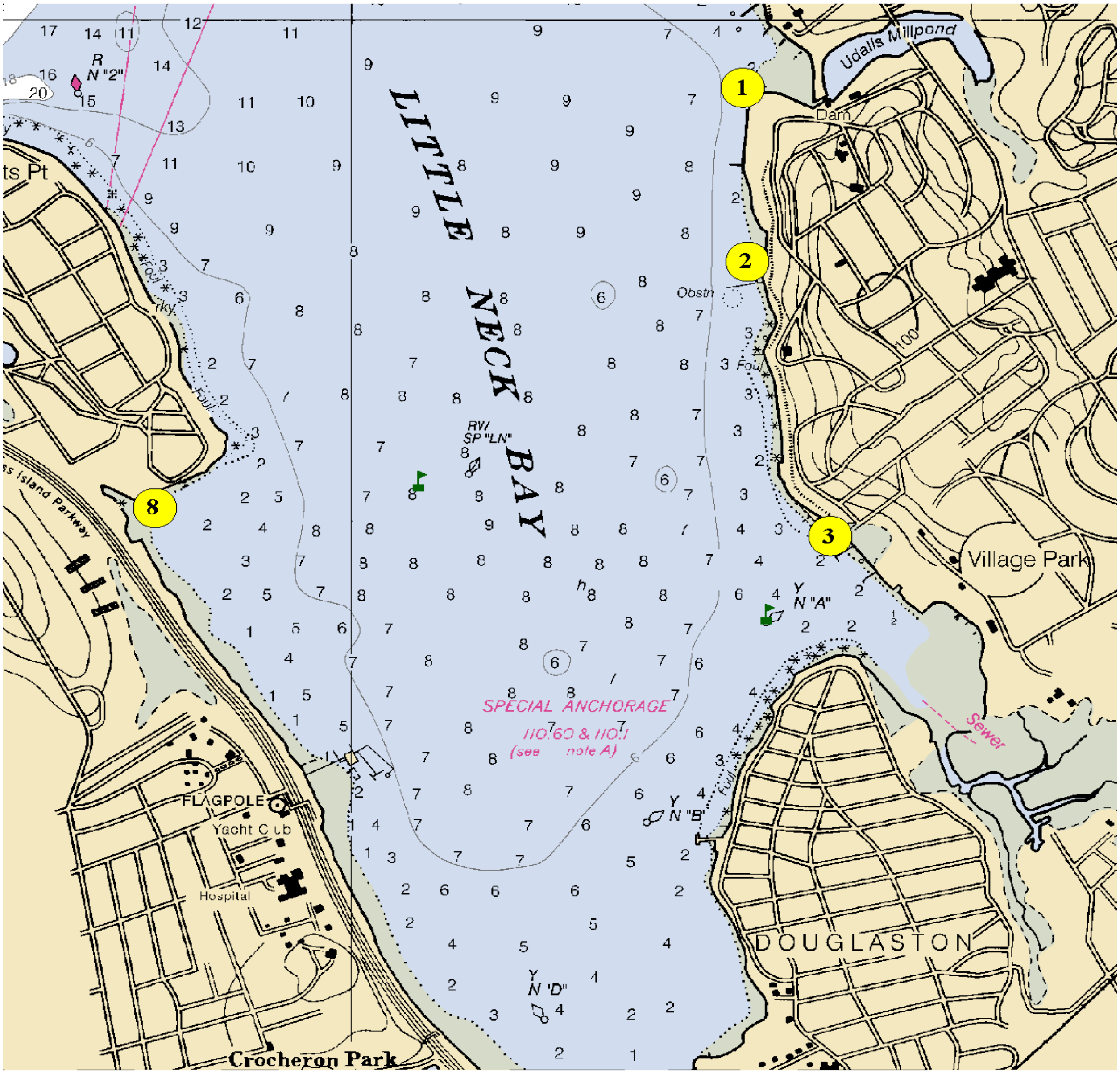


Figure 36. Map of New York DEC Western Long Island Beach Seine Survey Little Neck Bay Stations.

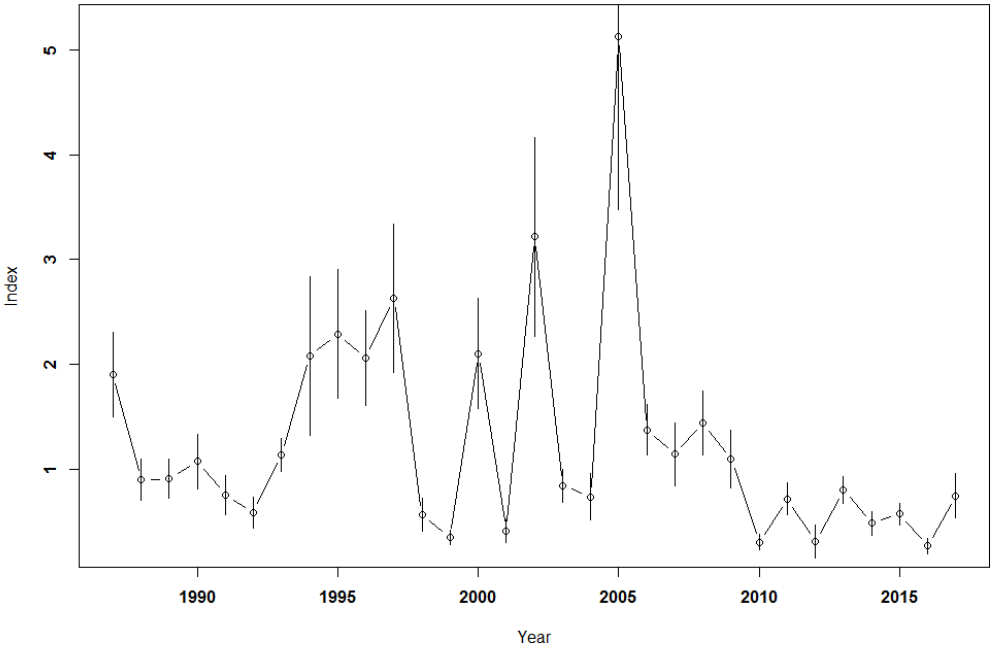


Figure 37. Index of relative abundance of horseshoe crab (delta mean catch per tow) in Jamaica Bay developed from the spring portion of the New York Seine Survey with 95% confidence intervals.

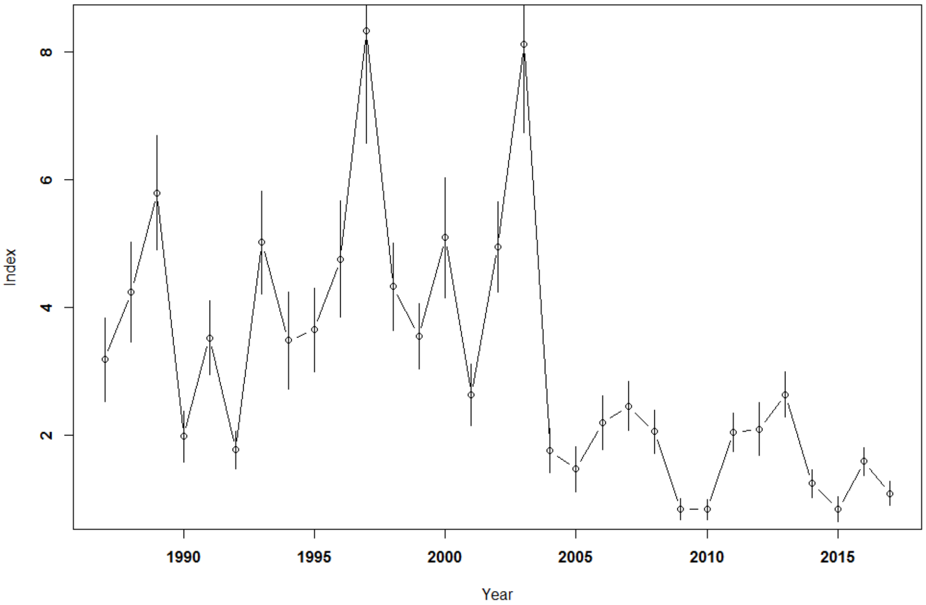


Figure 38. Index of relative abundance of horseshoe crab (delta mean catch per tow) in Manhassett and Little Neck Bays developed from the spring portion of the New York Seine Survey with 95% confidence intervals.

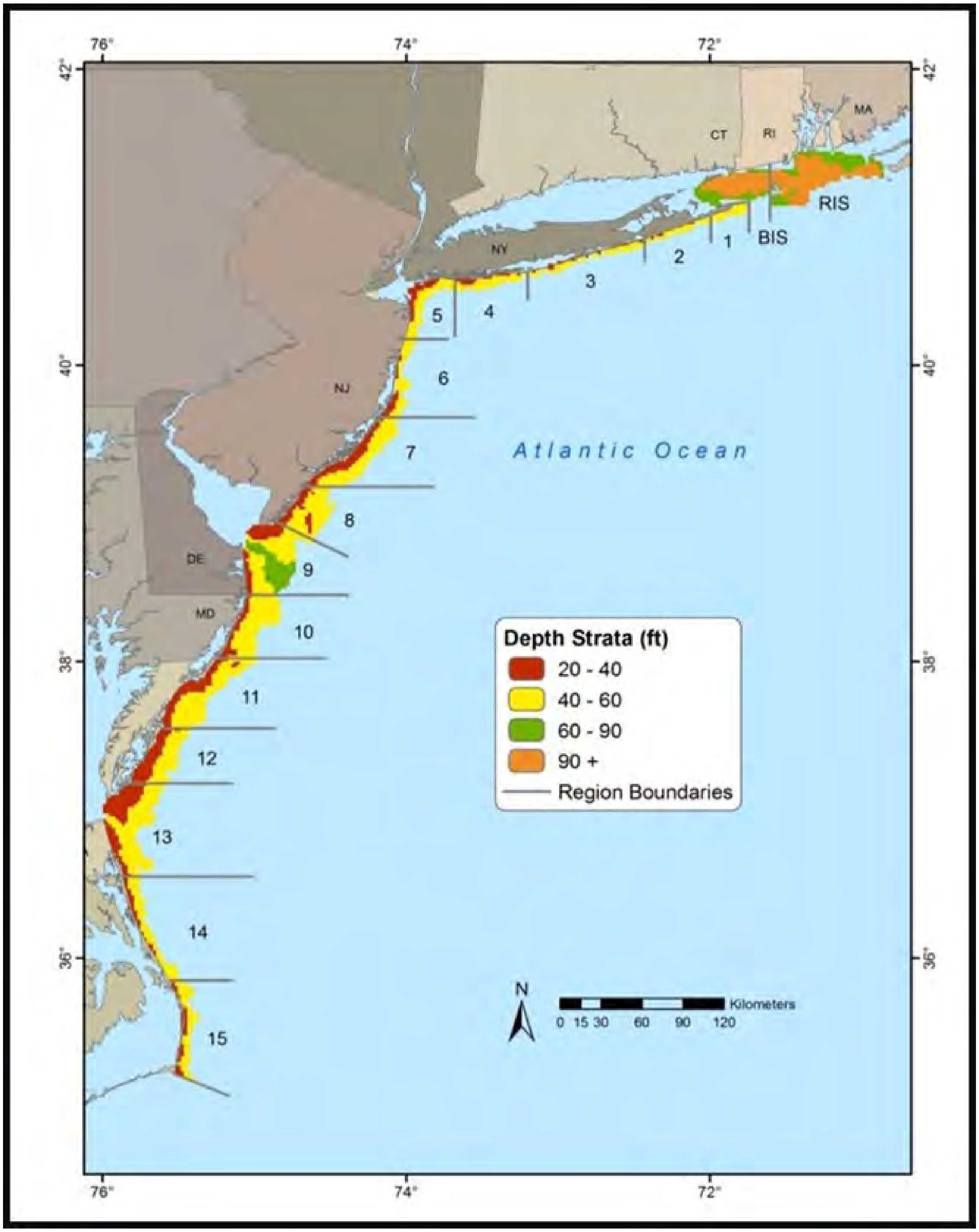


Figure 39. Map of the sampling strata used in the NEAMAP survey (map provided by NEAMAP and available on the website http://www.neamap.net/index.html.

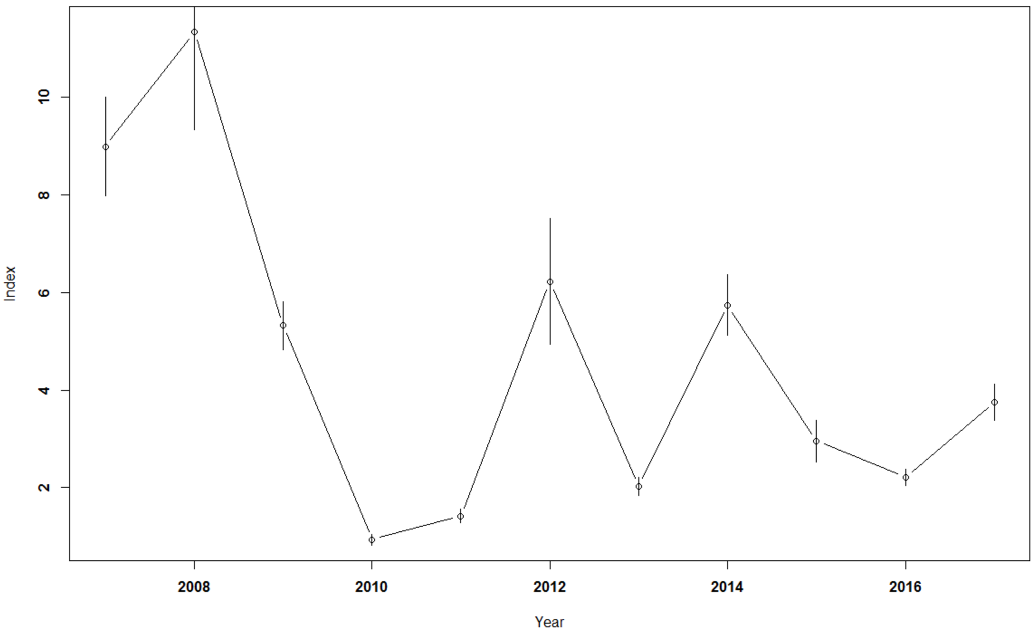


Figure 40. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of NEAMAP for the New York region with 95% confidence intervals.

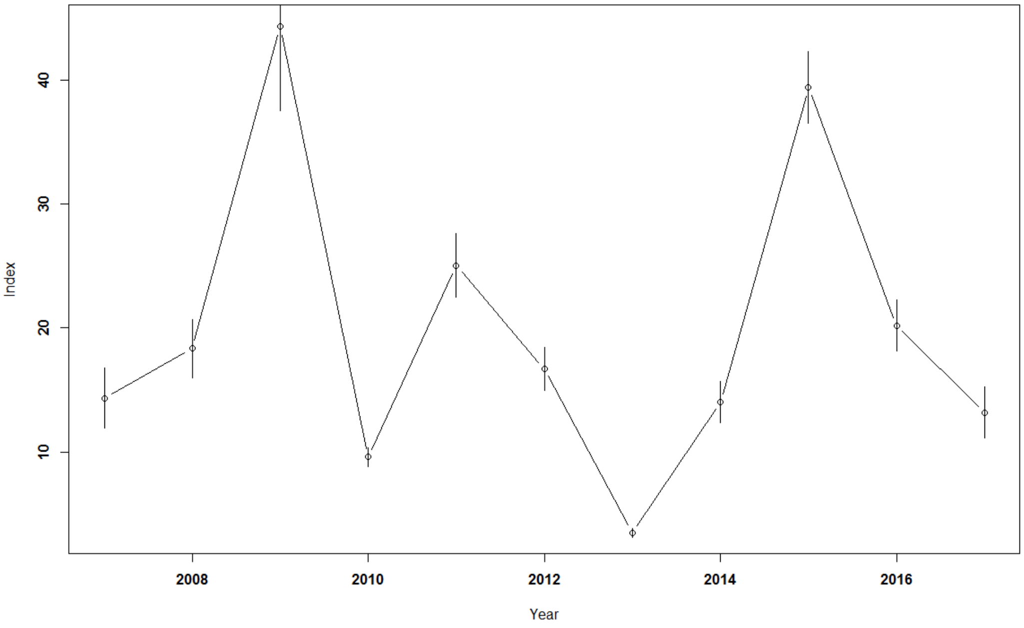


Figure 41. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the fall portion of NEAMAP for the Delaware Bay region with 95% confidence intervals.

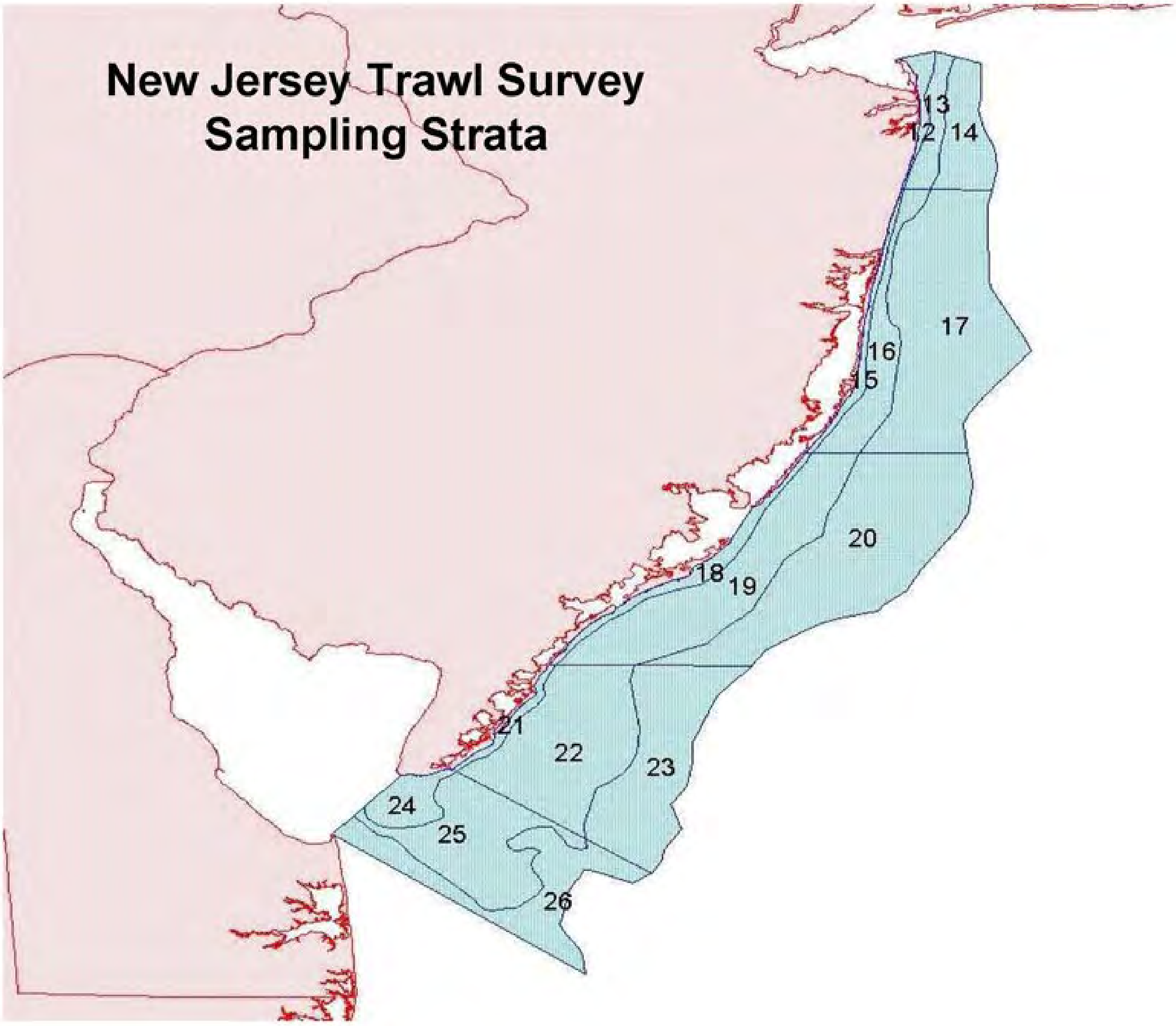


Figure 42. New Jersey Ocean Trawl Survey sampling area with survey strata defined.

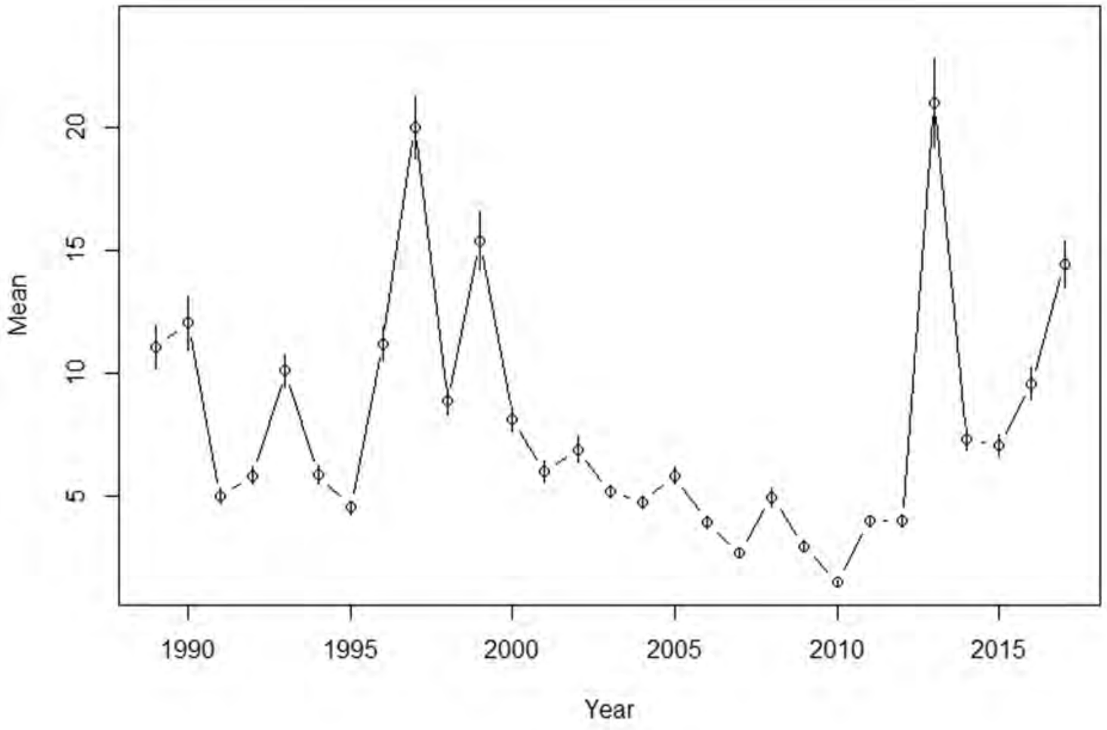


Figure 43. Abundance index for all horseshoe crabs in the spring (April and August) samples from New Jersey's Ocean Trawl Survey.

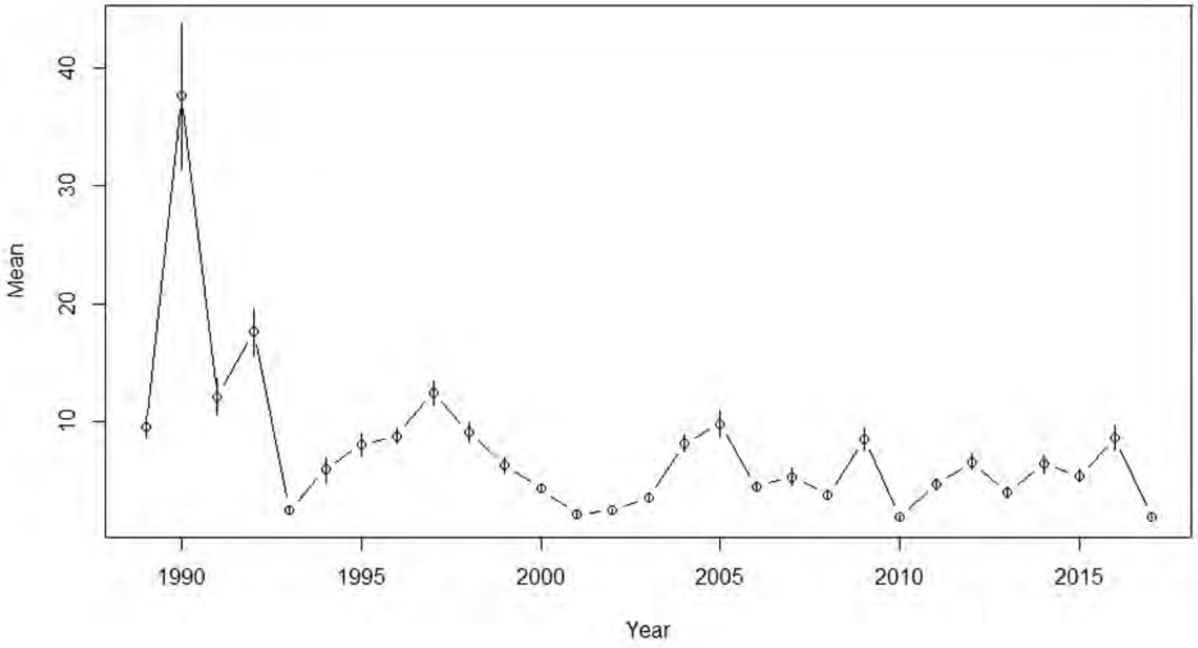


Figure 44. Abundance index for all horseshoe crabs in the fall (October) samples from New Jersey's Ocean Trawl Survey.

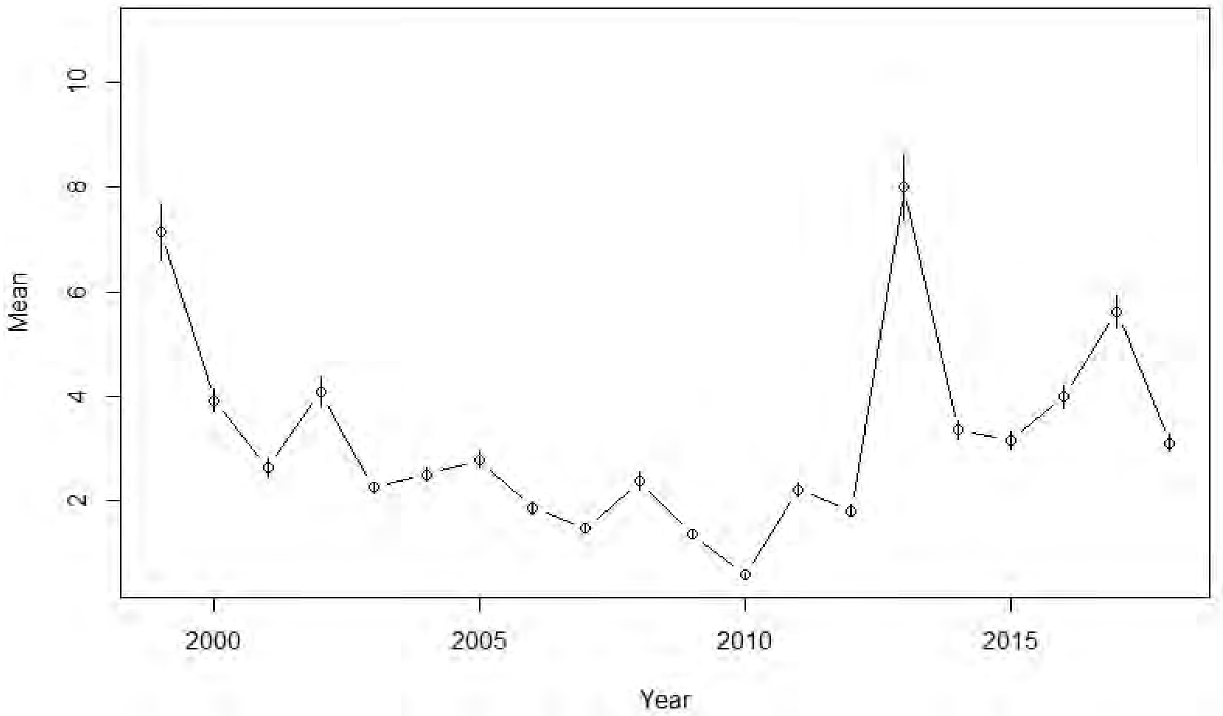


Figure 45. Abundance index for adult female horseshoe crabs (>= 19 cm pw) in the spring (April and August) from New Jersey's Ocean Trawl Survey.

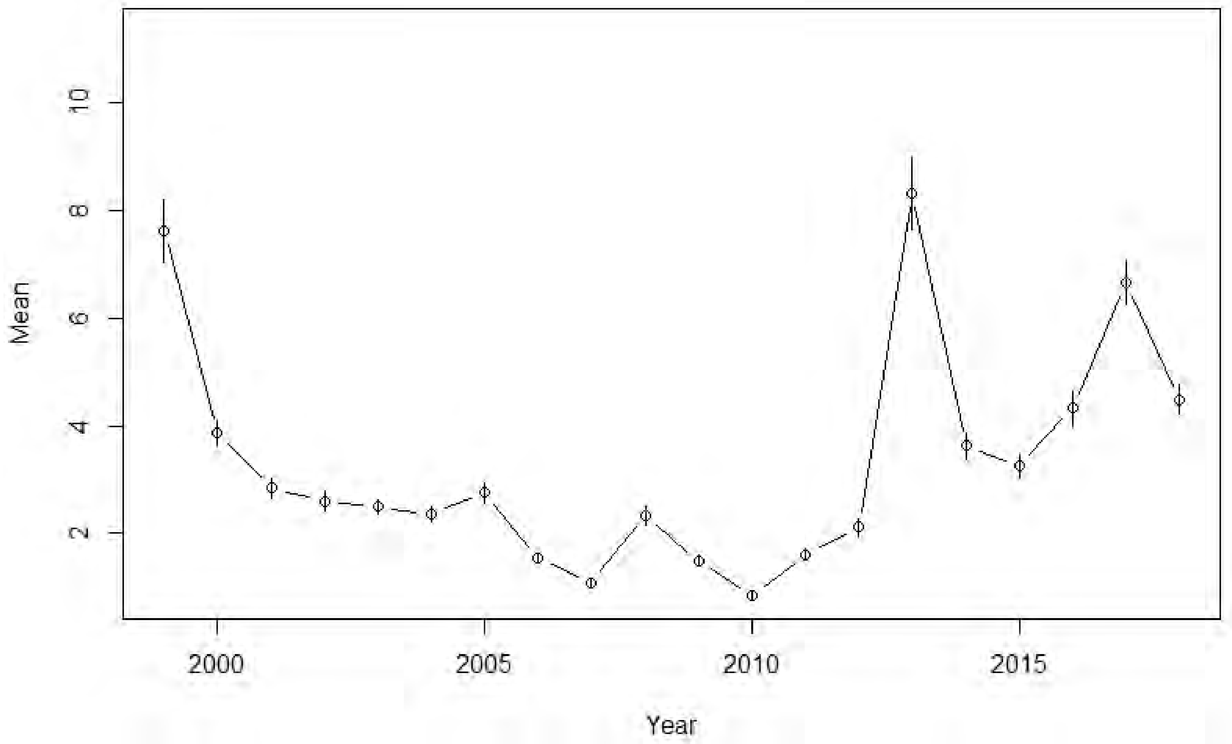


Figure 46. Abundance index for adult male horseshoe crabs in the spring (April and August) from New Jersey's Ocean Trawl Survey.

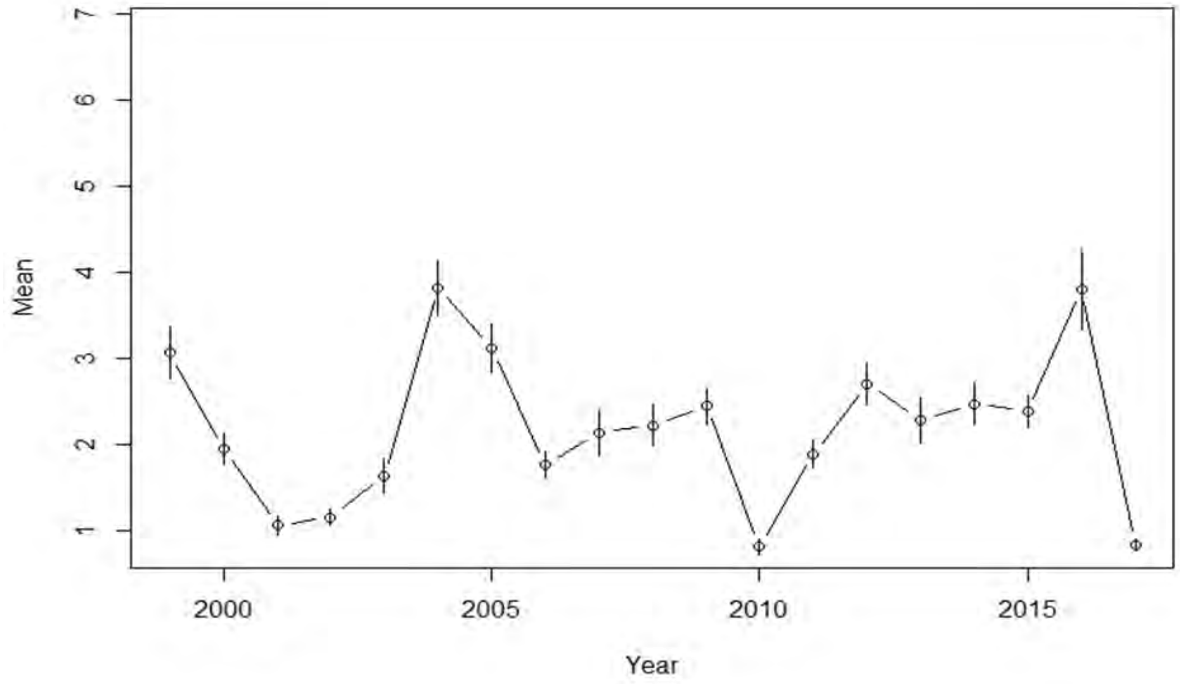


Figure 47. Abundance index for adult female horseshoe crabs (>= 19 cm pw) in the fall (October) from New Jersey's Ocean Trawl Survey.

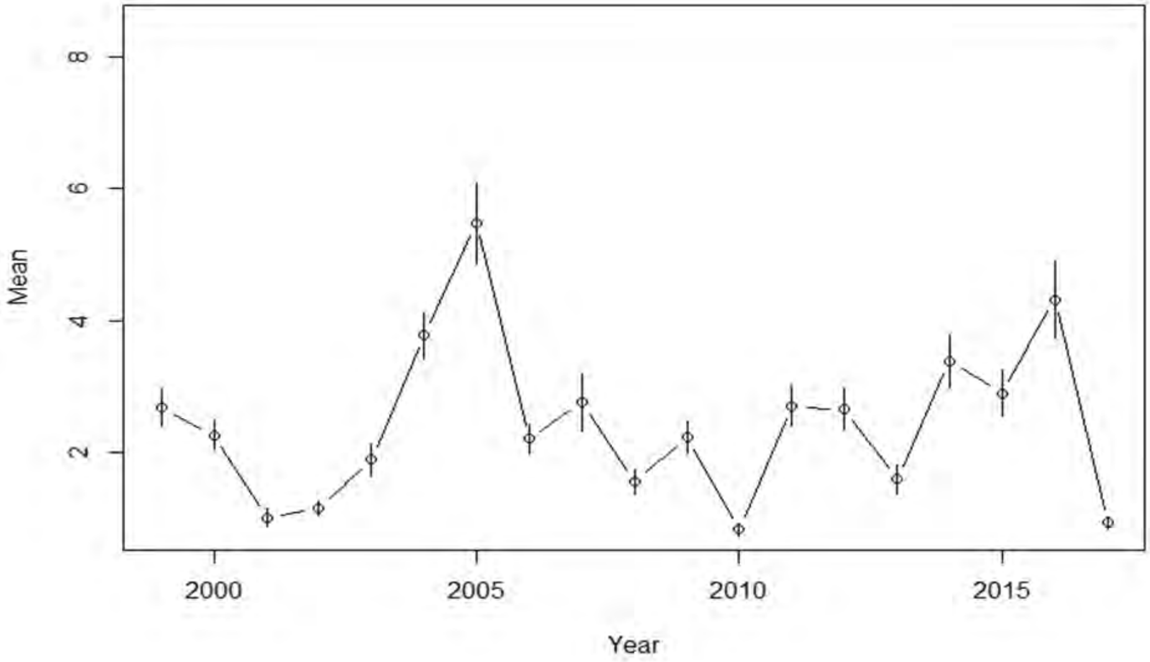


Figure 48. Abundance index for adult male horseshoe crabs in the fall (October) from New Jersey's Ocean Trawl Survey.

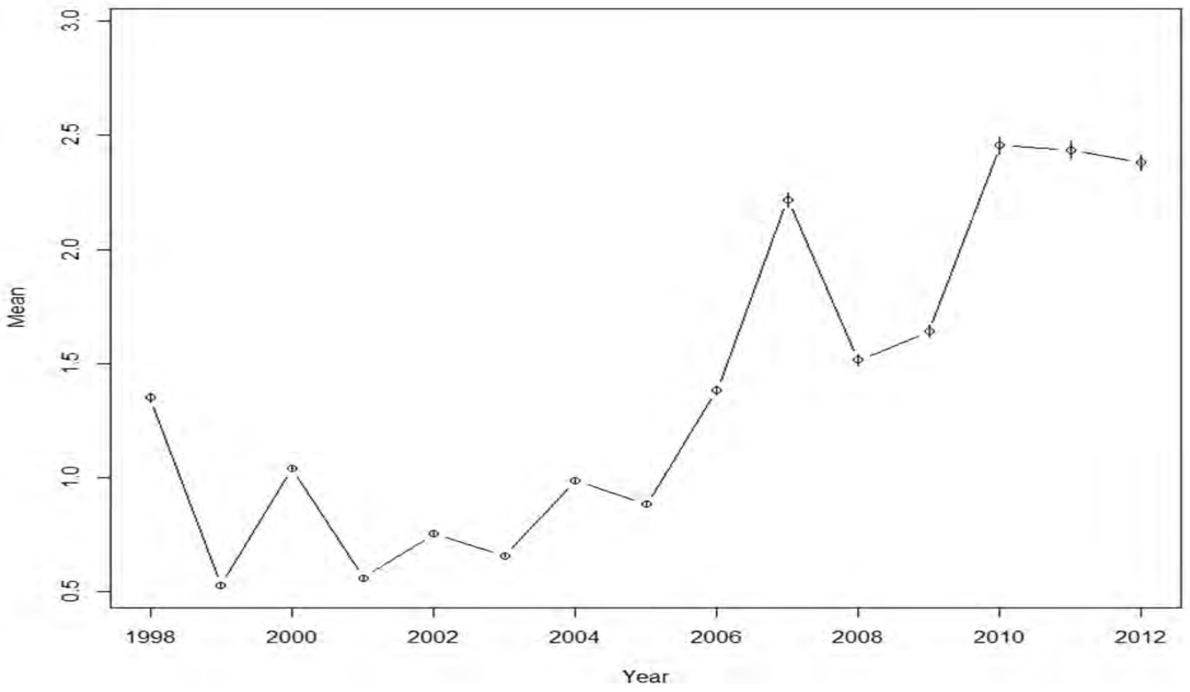


Figure 49. Abundance index for all horseshoe crabs combined in New Jersey's Surf Clam Dredge Survey (June, July, August).

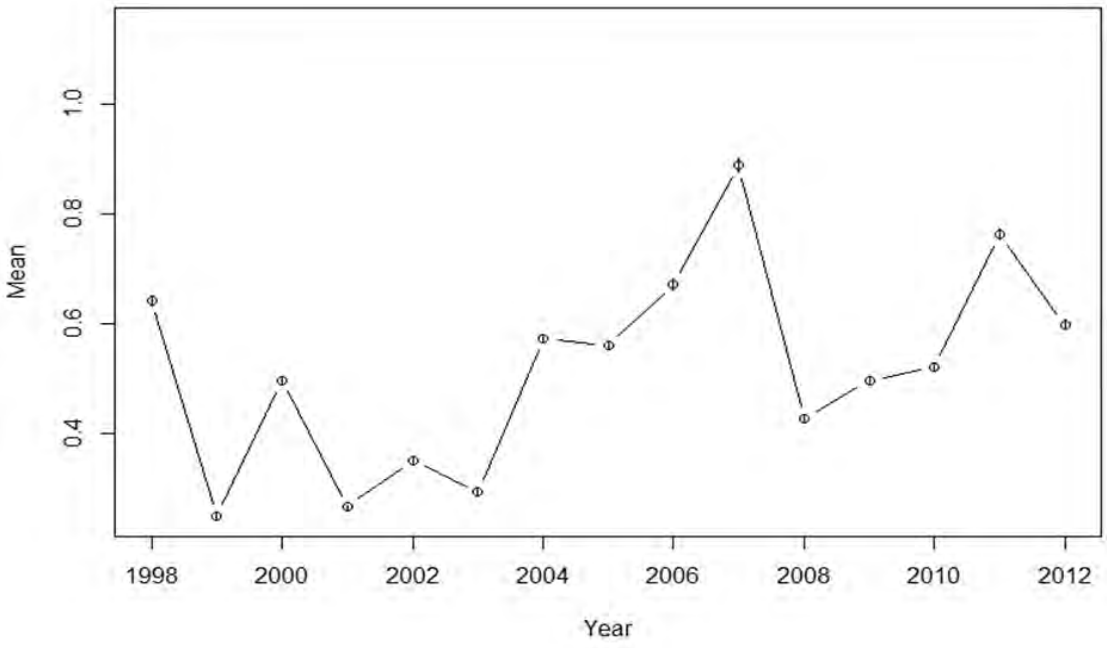


Figure 50. Abundance index for adult female horseshoe crabs (> 180 mm pw) in New Jersey's Surf Clam Dredge Survey (June, July, August).

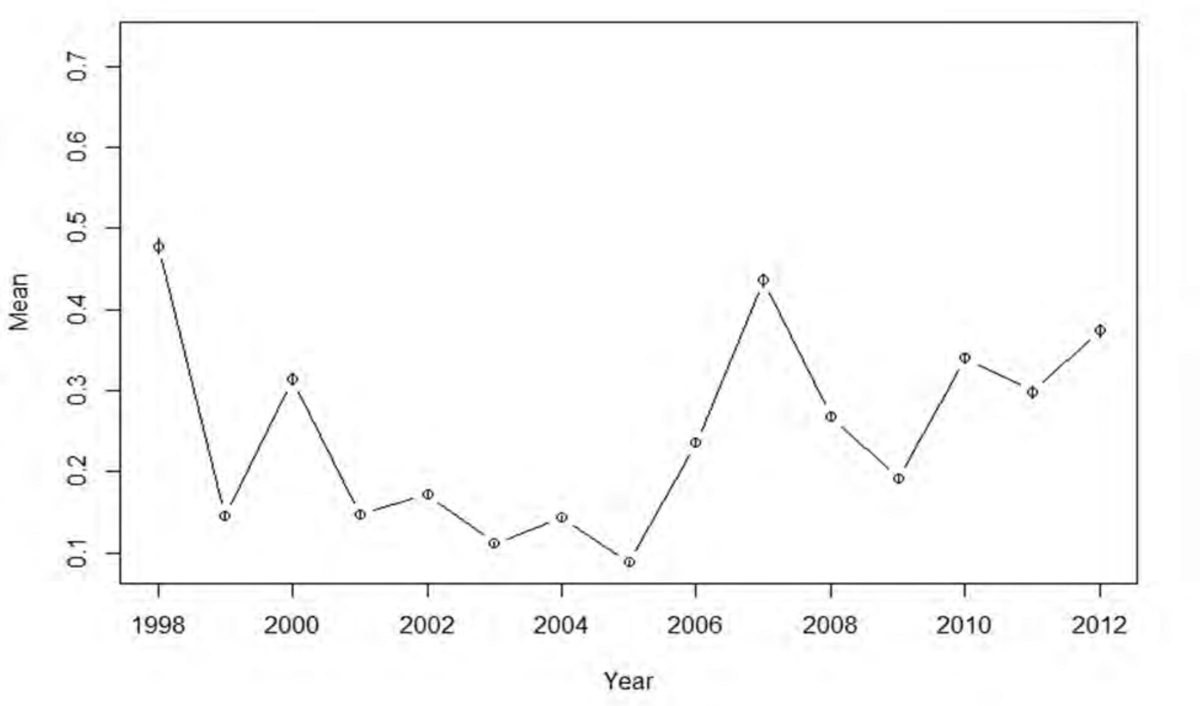


Figure 51. Abundance index for adult male horseshoe crabs (possessing male pedipalps) in New Jersey's Surf Clam Dredge Survey (June, July, August).

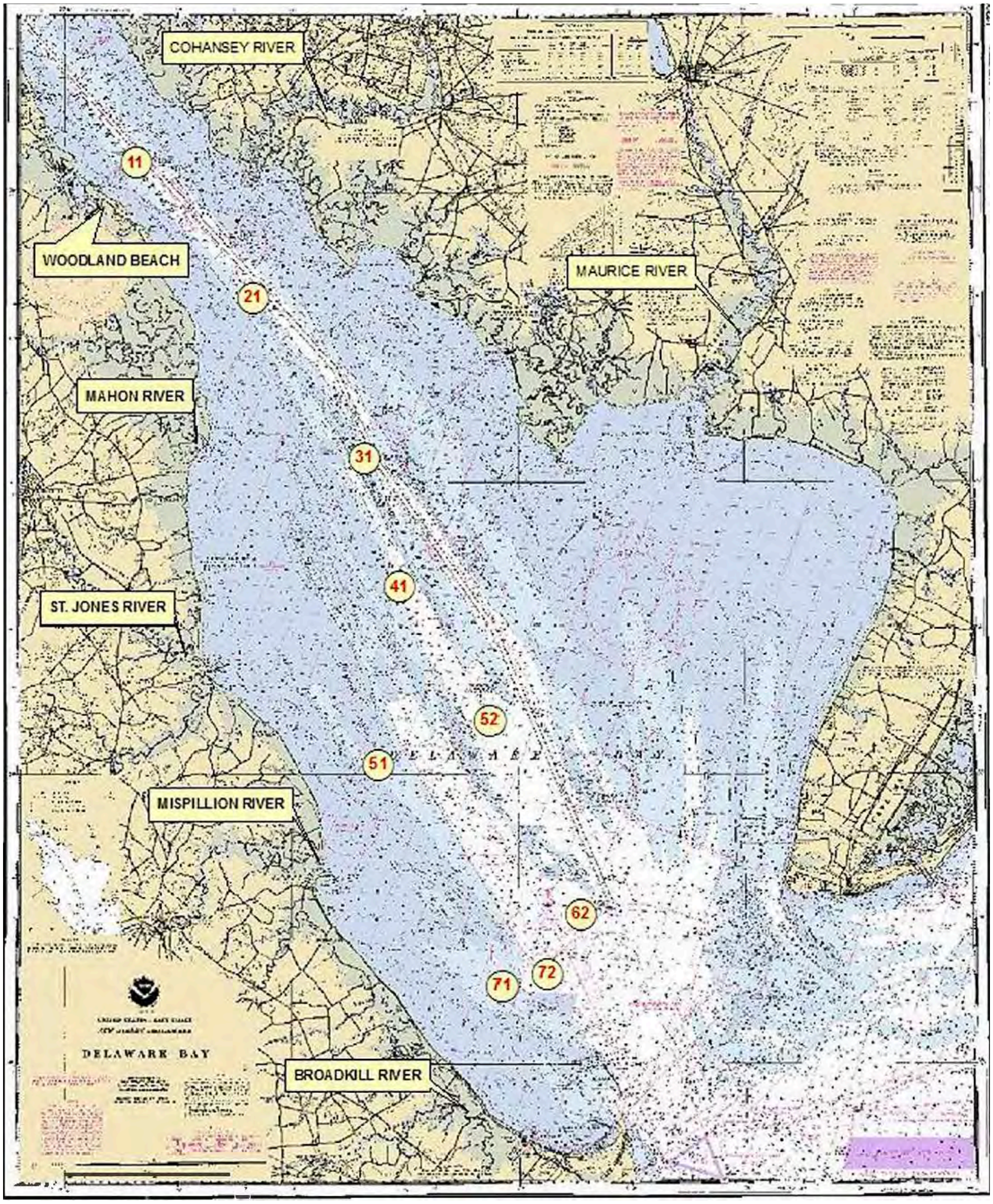


Figure 52. Delaware Fish & Wildlife Adult Trawl Survey sampling area and stations.

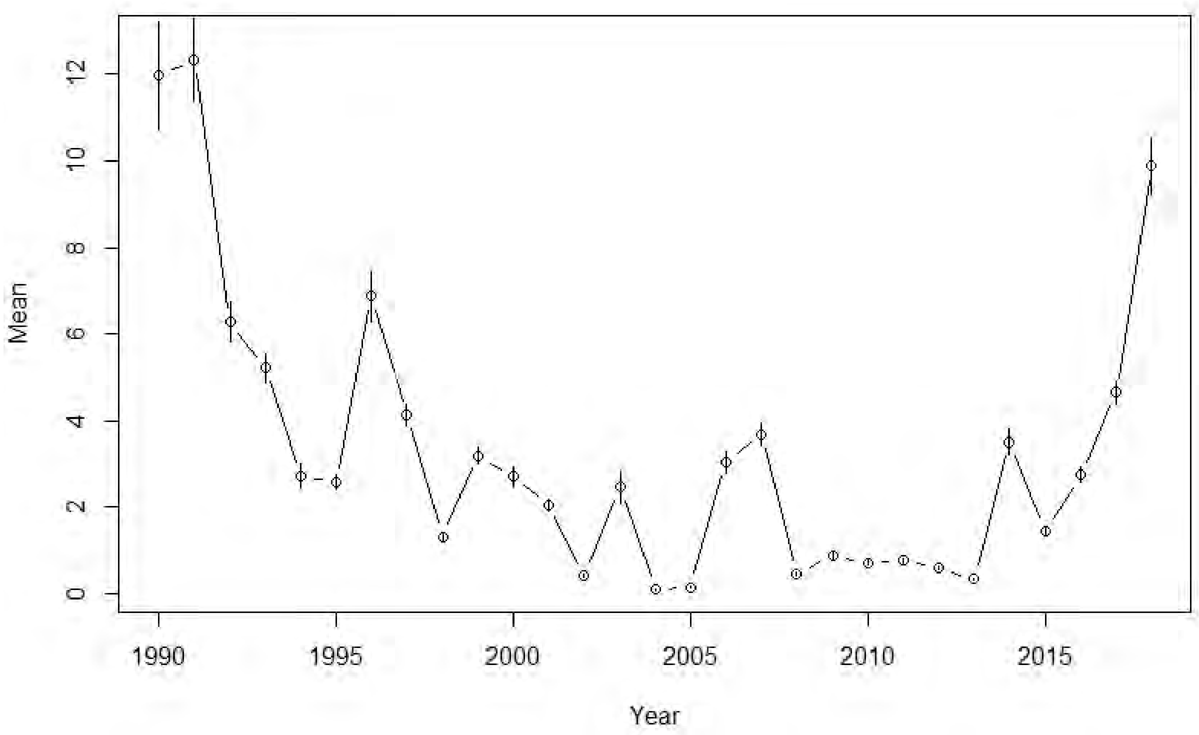


Figure 53. Delaware Fish and Wildlife Adult Trawl Survey abundance index for all adult horseshoe crabs combined in spring (March through August).

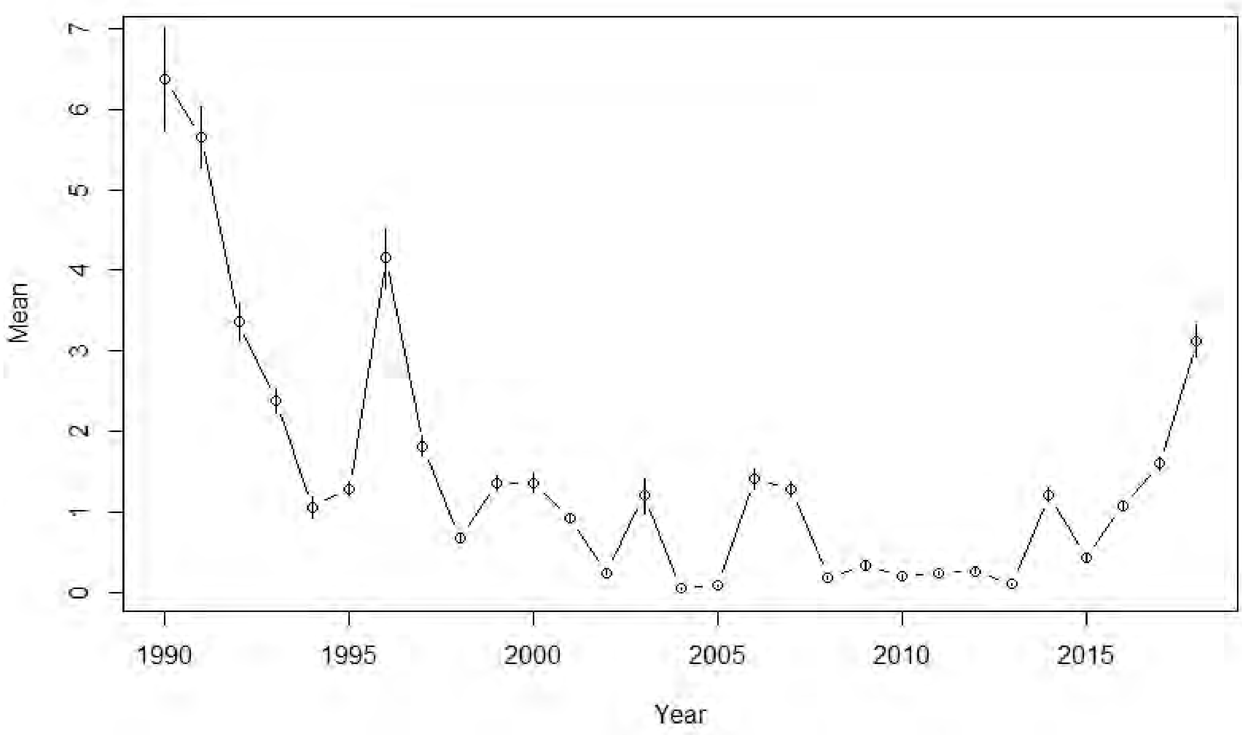


Figure 54. Delaware Fish and Wildlife Adult Trawl Survey abundance index for all adult female horseshoe crabs in spring (March through August).

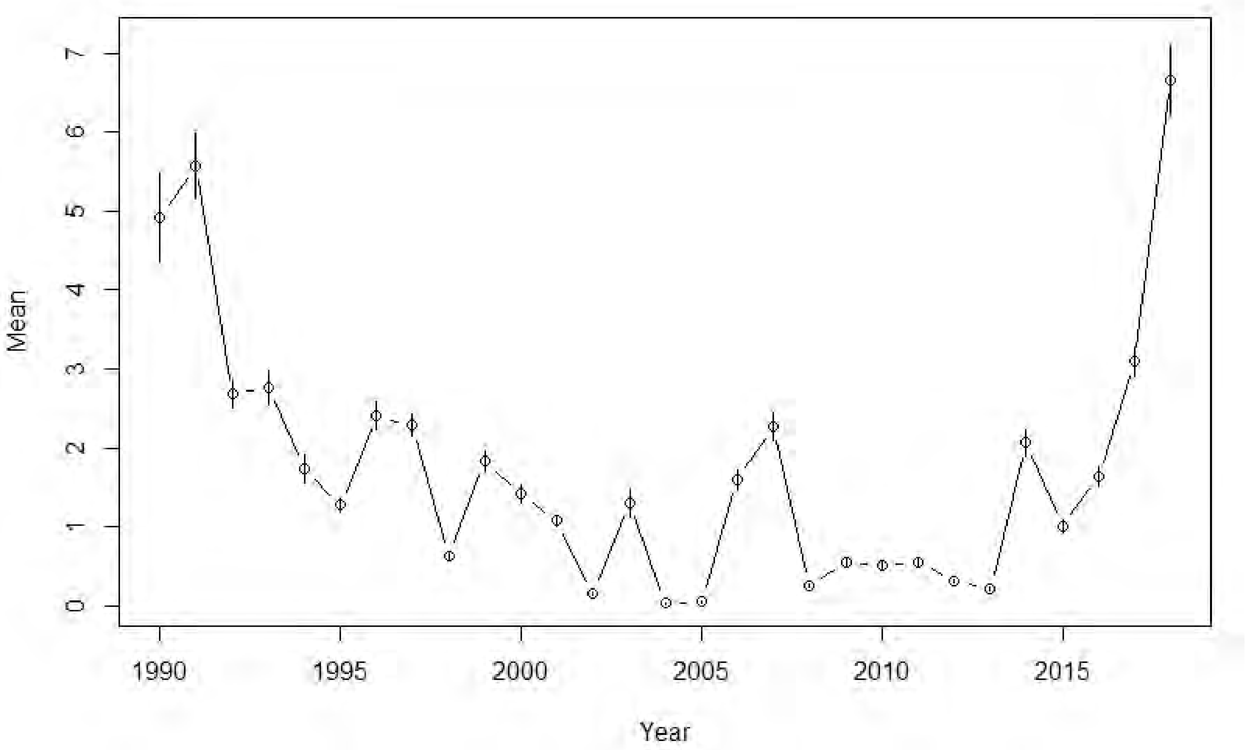


Figure 55. Delaware Fish and Wildlife Adult Trawl Survey abundance index for all adult male horseshoe crabs in spring (March through August).

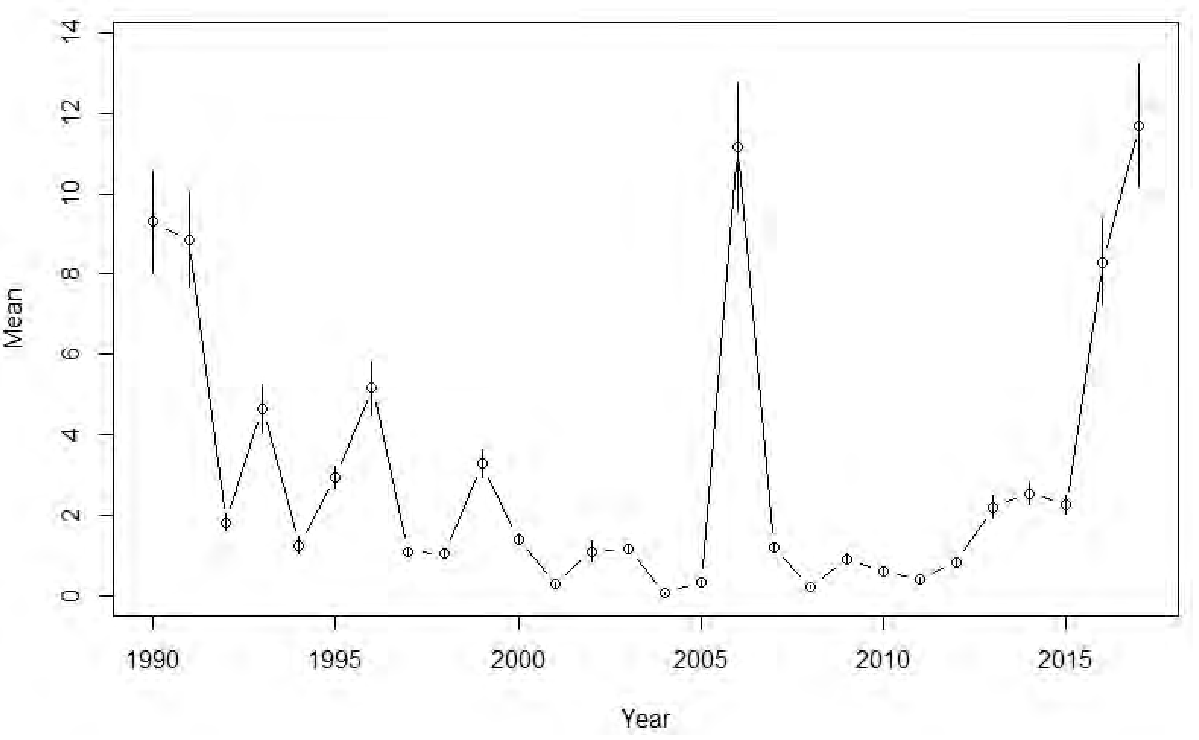


Figure 56. Delaware Fish and Wildlife Adult Trawl Survey abundance index for all adult horseshoe crabs combined in fall (September through December).

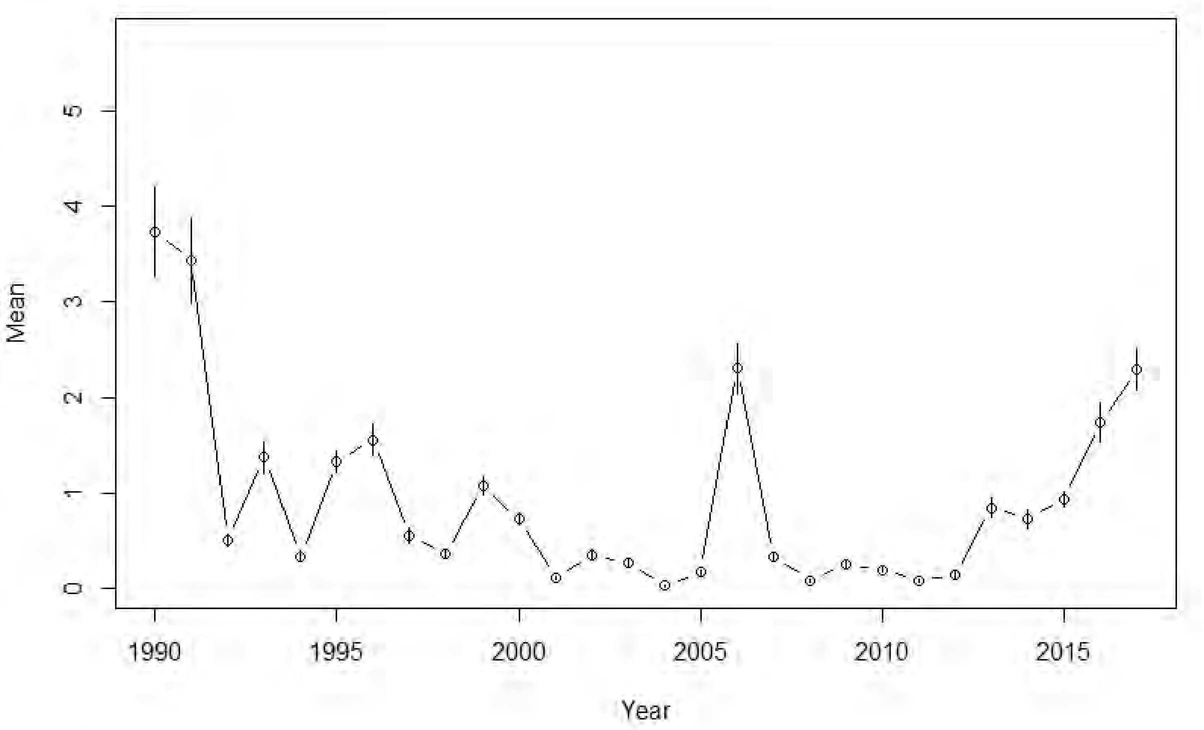


Figure 57. Delaware Fish and Wildlife Adult Trawl Survey abundance index for adult female horseshoe crabs in fall (September through December).

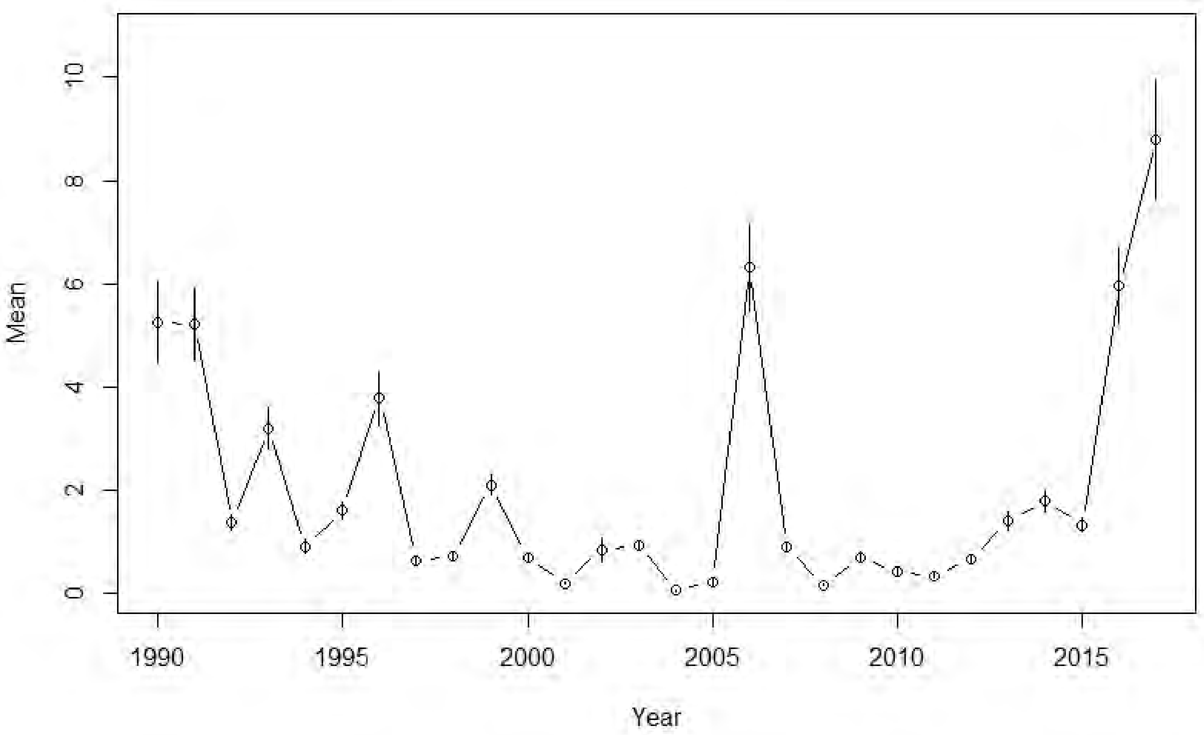


Figure 58. Delaware Fish and Wildlife Adult Trawl Survey abundance index for adult male horseshoe crabs in fall (September through December).

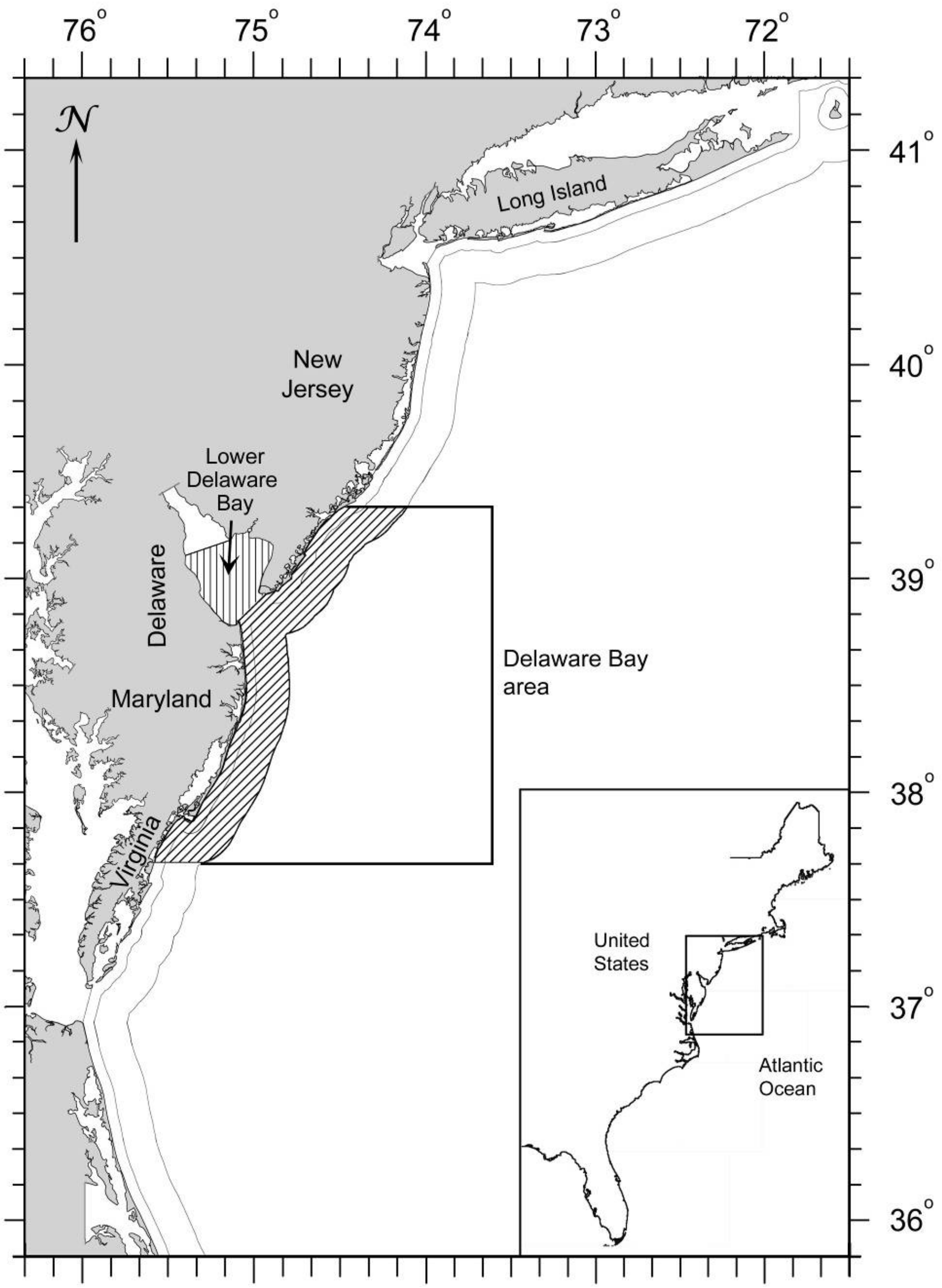


Figure 59. Virginia Tech trawl survey sampling area. The coastal Delaware Bay area (DBA) and Lower Delaware Bay (LDB) survey areas are indicated. Mean catches among years were compared using stations within the shaded portions of the survey area in the annual report (map provided by Virginia Tech).

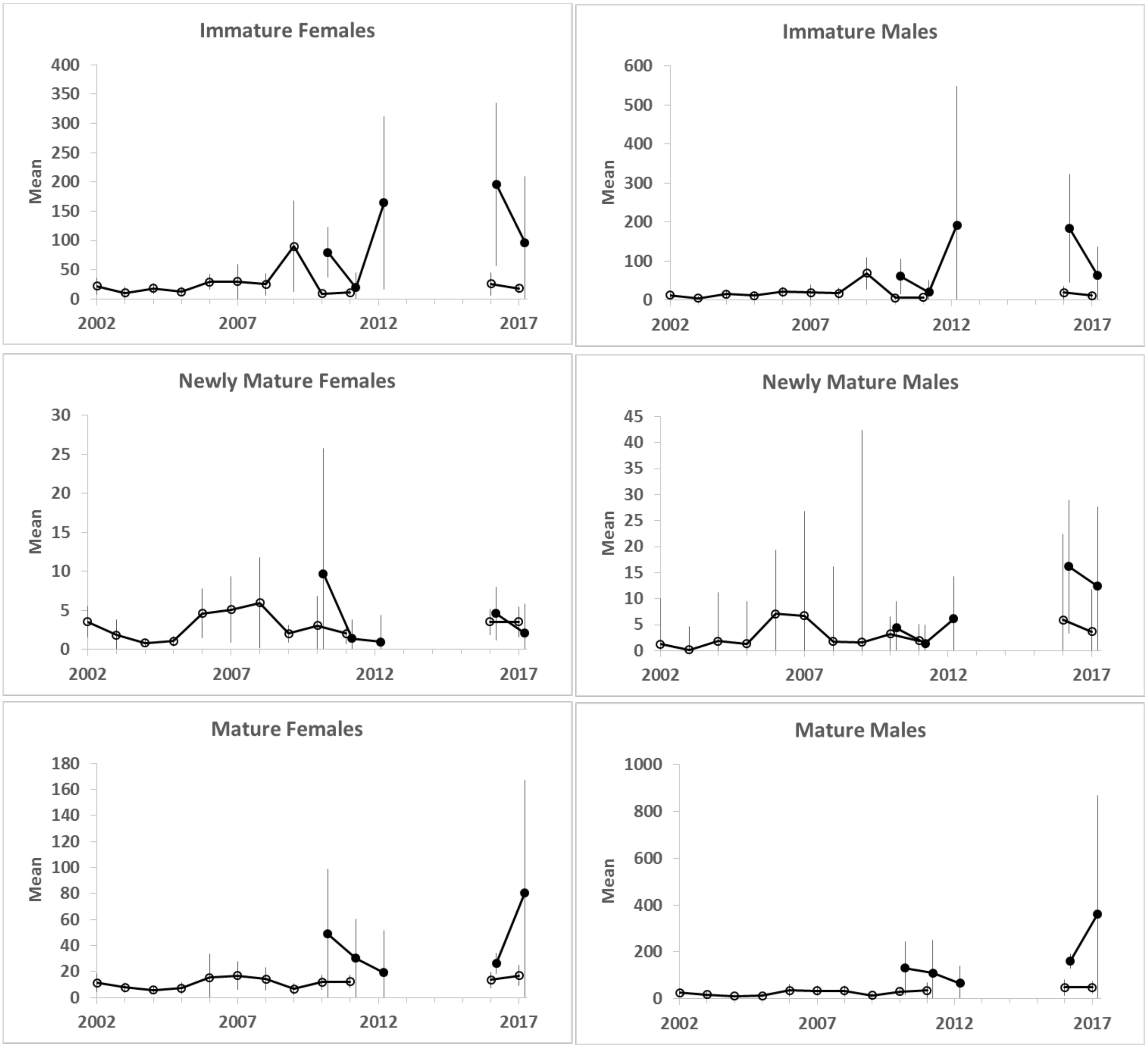


Figure 60. Delta distribution model mean catches per tow of horseshoe crabs in the lower Delaware Bay survey by demographic group with coastal Delaware Bay area survey means for comparion. Vertical lines indicate 95% confidence limits. Solid symbols indicate the lower Delaware Bay survey. Open symbols indicate the coastal Delaware Bay area survey. Note differences in y-axis scales.

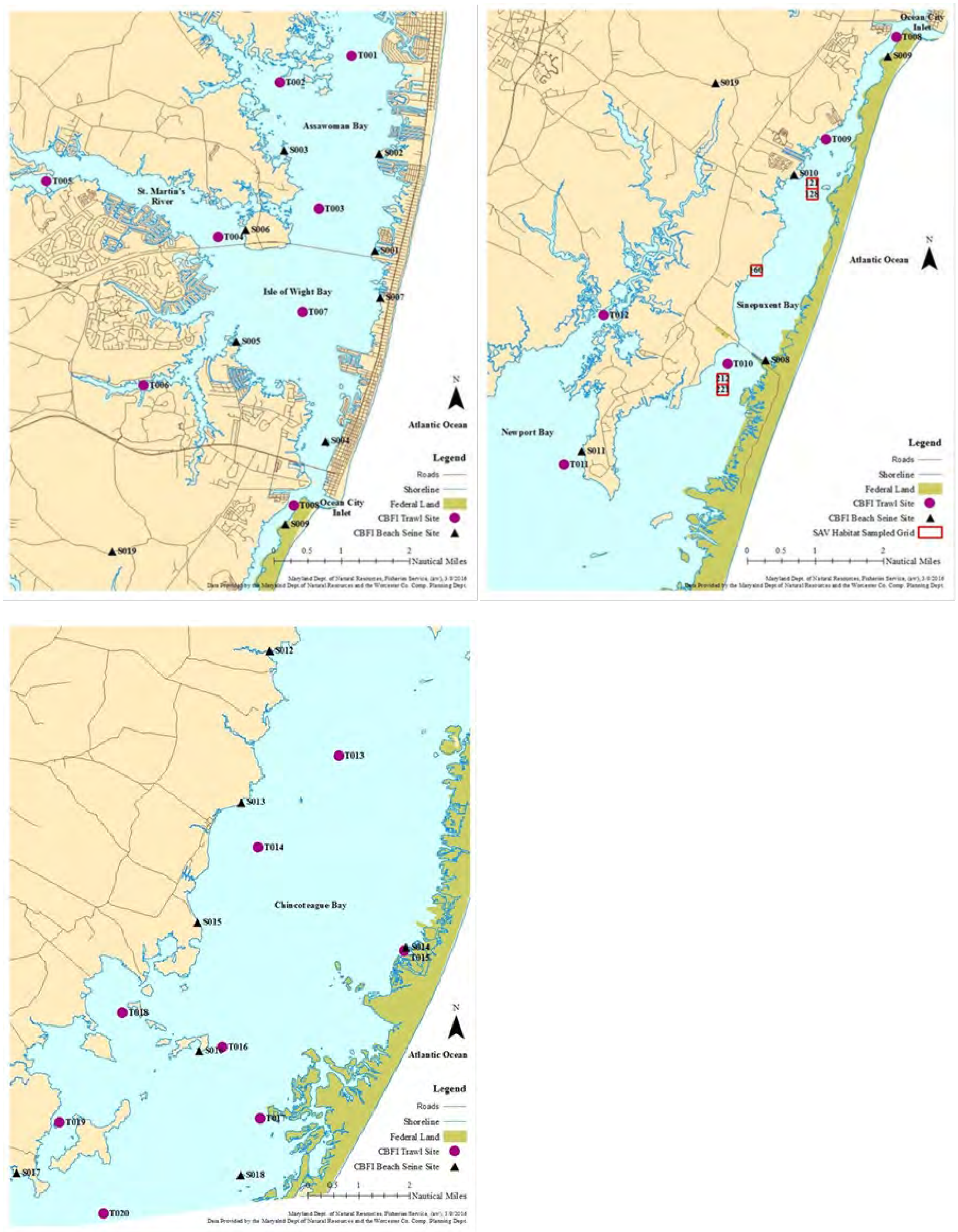


Figure 61. Map of the Maryland Coastal Bays Survey sampling sites. Trawl sites are labled with the prefix of "T" (map from MD DNR).

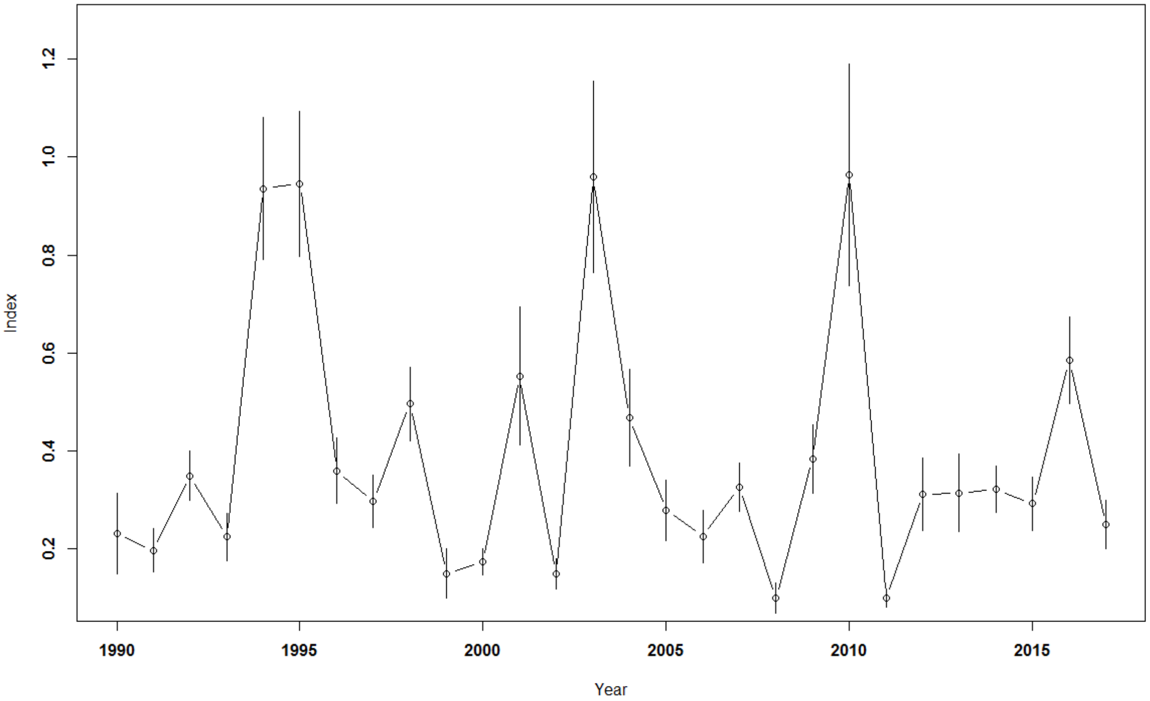


Figure 62. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the spring portion of Maryland's Coastal Bays Survey with 95% confidence intervals.

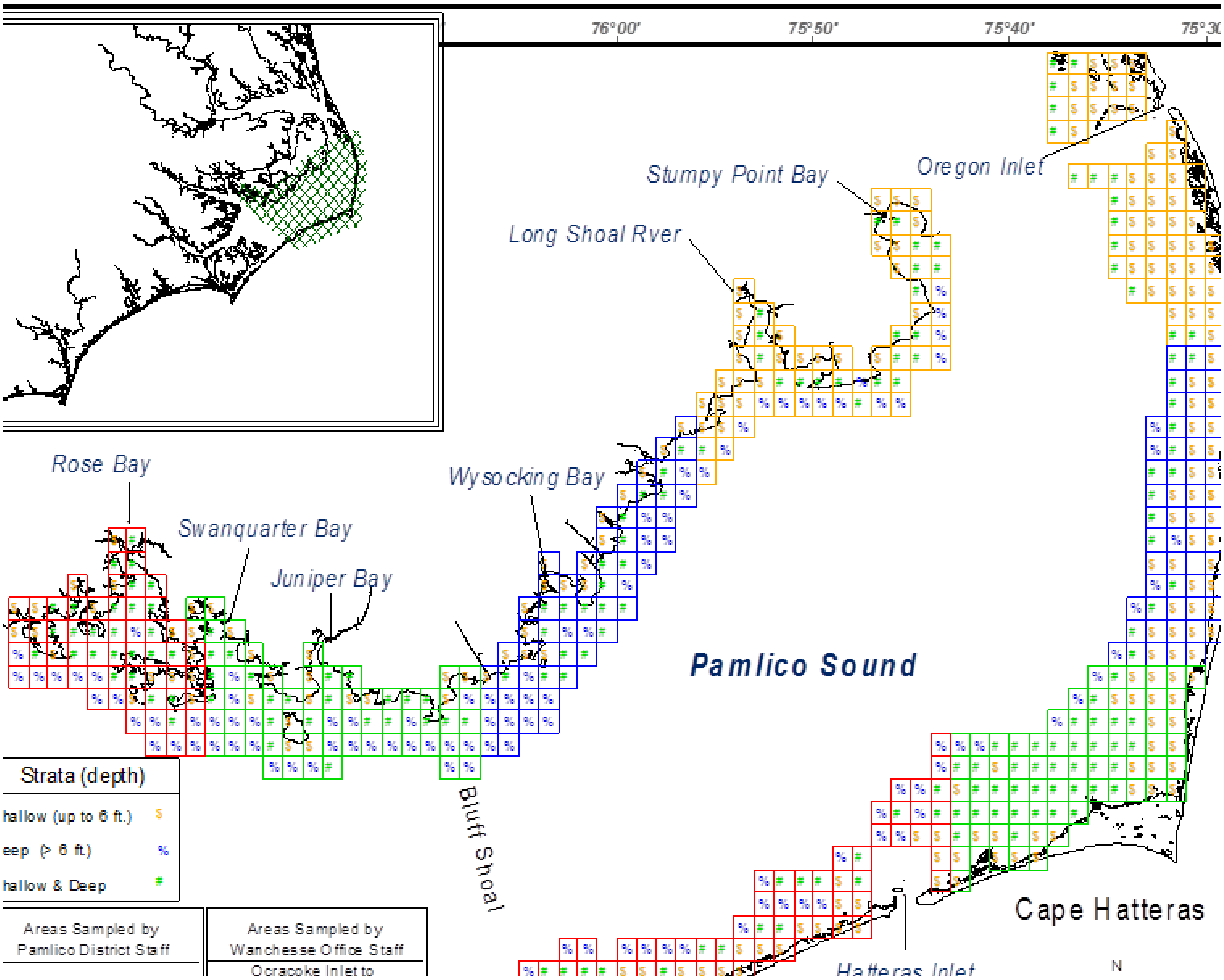


Figure 63. Map of the sampling sites for North Carolina's Estuarine Gillnet fishery independent survey. This survey also operates in several rivers, but only the Pamlico Sound sites were used for developing an index of horseshoe crab abundance for this region (map provided by NC DNR).

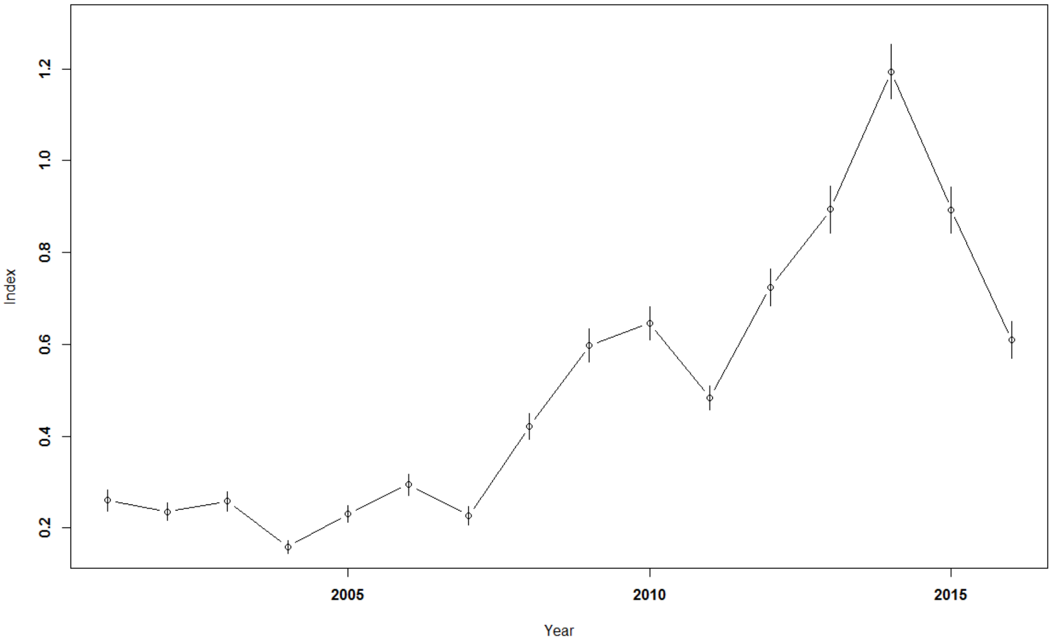


Figure 64. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the spring portion of North Carolina's Estuarine Gill Net Survey with 95% confidence intervals.

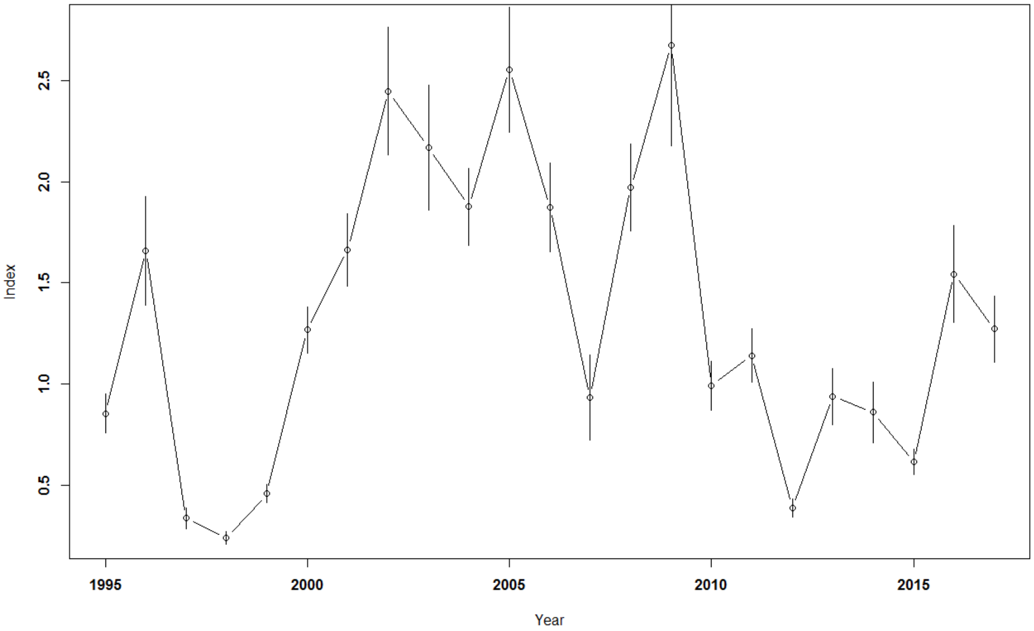


Figure 65. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the spring portion of South Carolina's CRMS with 95% confidence intervals.

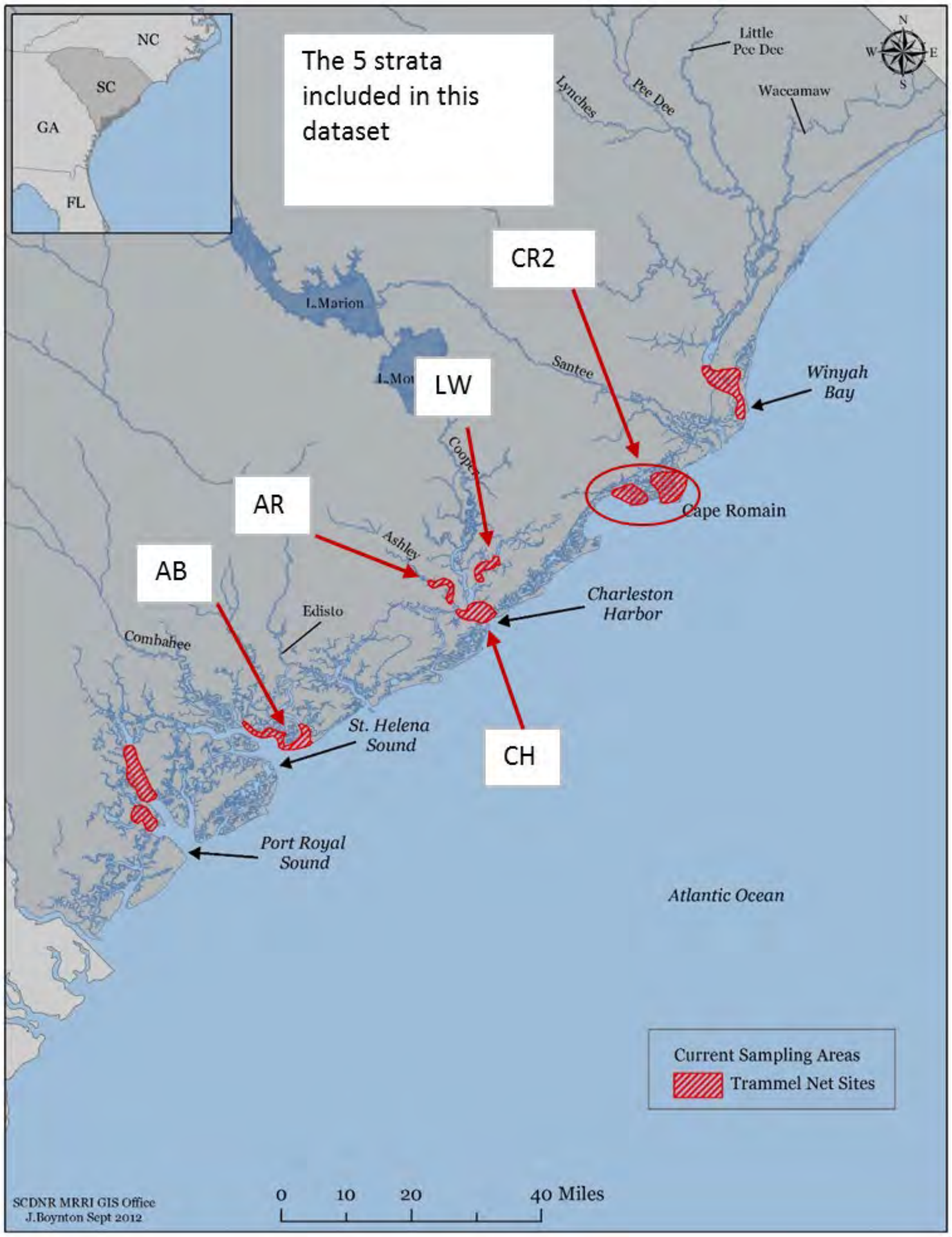


Figure 66. Areas samples by the trammel net, electrofishing, and long-line surveys of the SC DNR Inshore Fisheries Section. Trammel net strata used for analyses in this report: AB - ACE Basin; AR - Ashley River; CH - Charleston Harbor; LW - Lower Wando River; CR2 - Cape Romain (map provided by SC DNR).

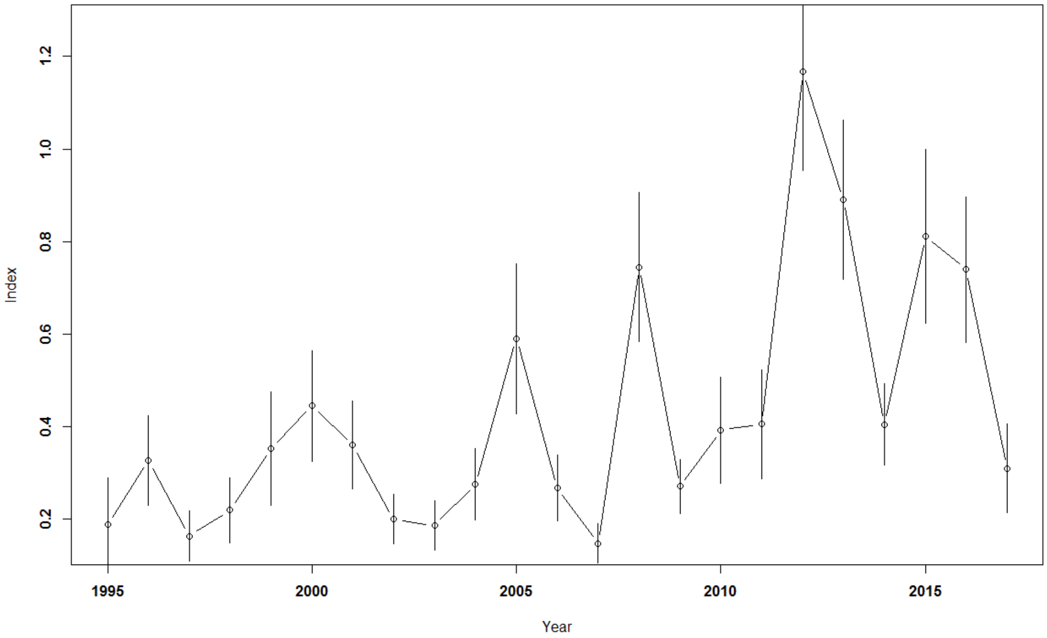


Figure 67. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the spring portion of South Carolina's Trammel Net Survey with 95% confidence intervals.

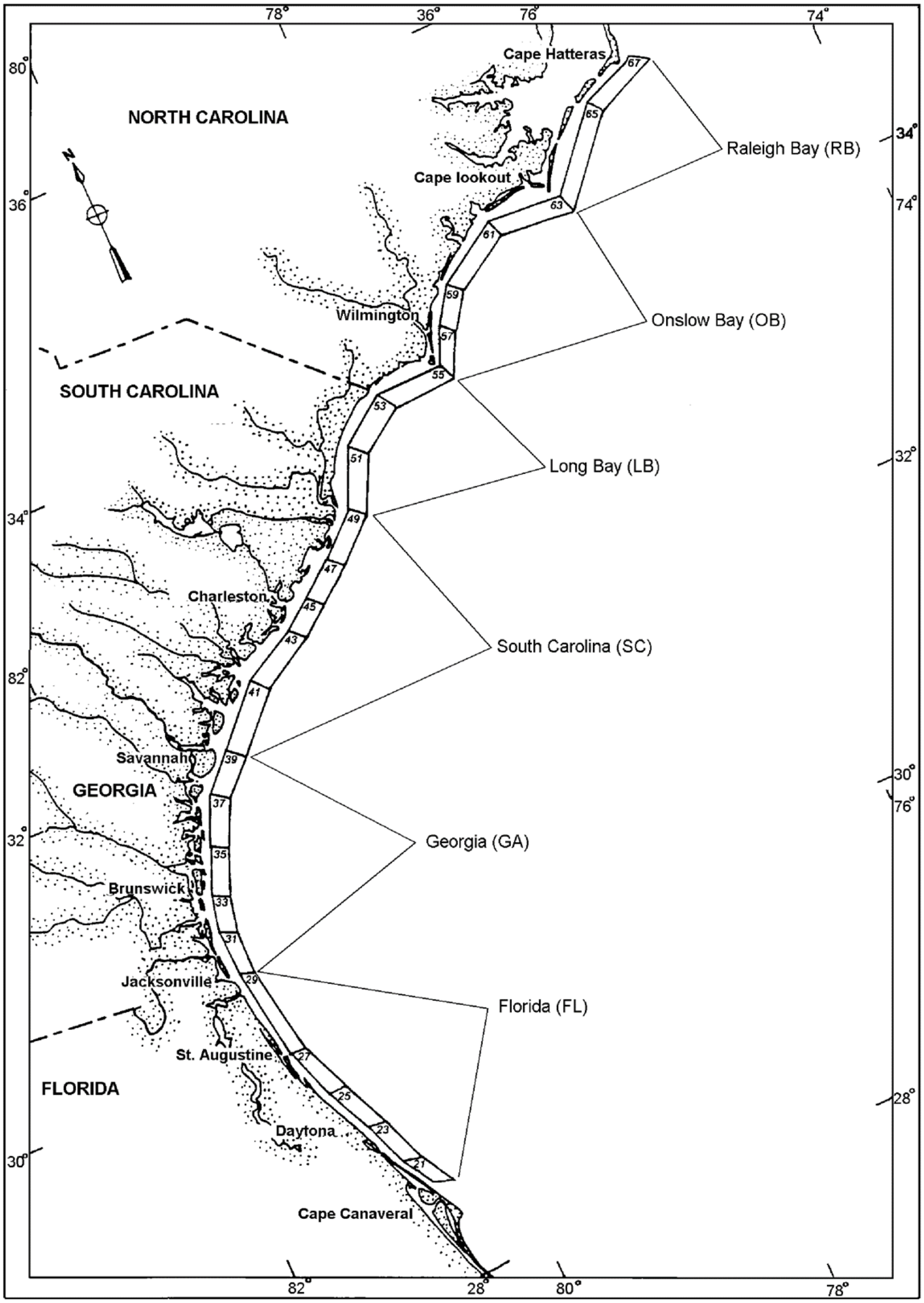


Figure 68. States and stations sampled as part of the SEAMAP trawl survey (map provided by SC DNR and SEAMAP).

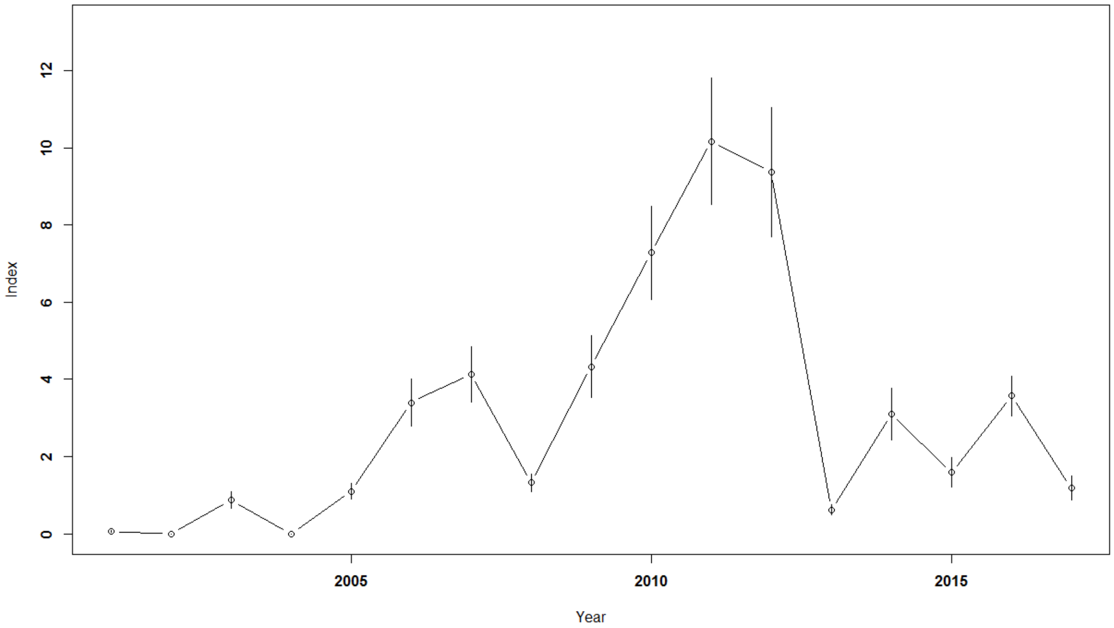


Figure 69. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the South Carolina and fall portion of the SEAMAP survey with 95% confidence intervals.

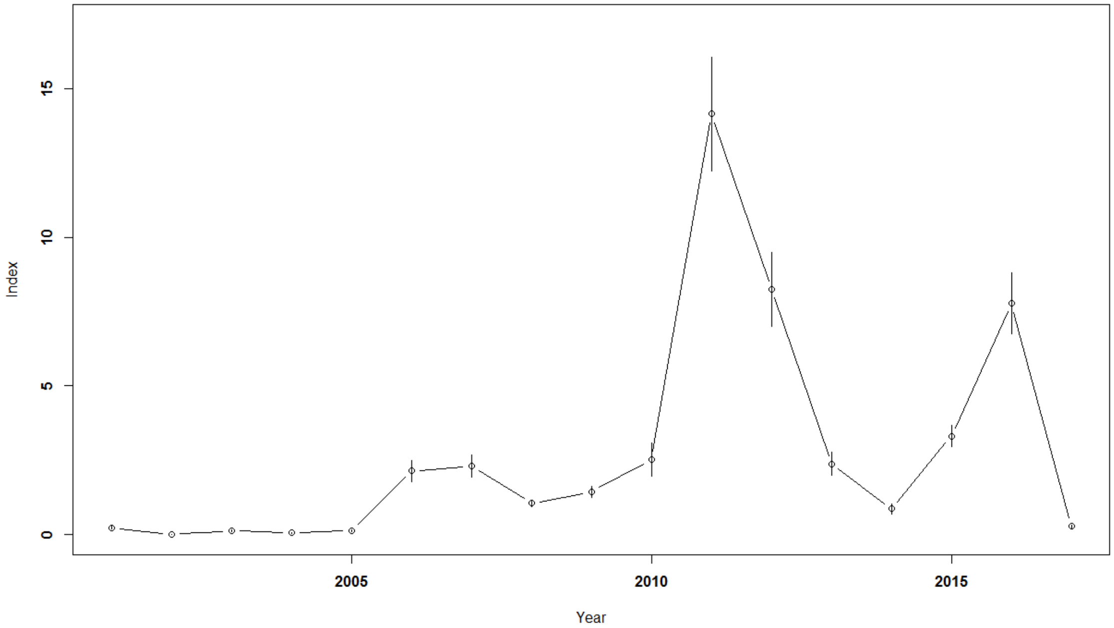


Figure 70. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the Georgia-Florida and fall portion of the SEAMAP survey with 95% confidence intervals.

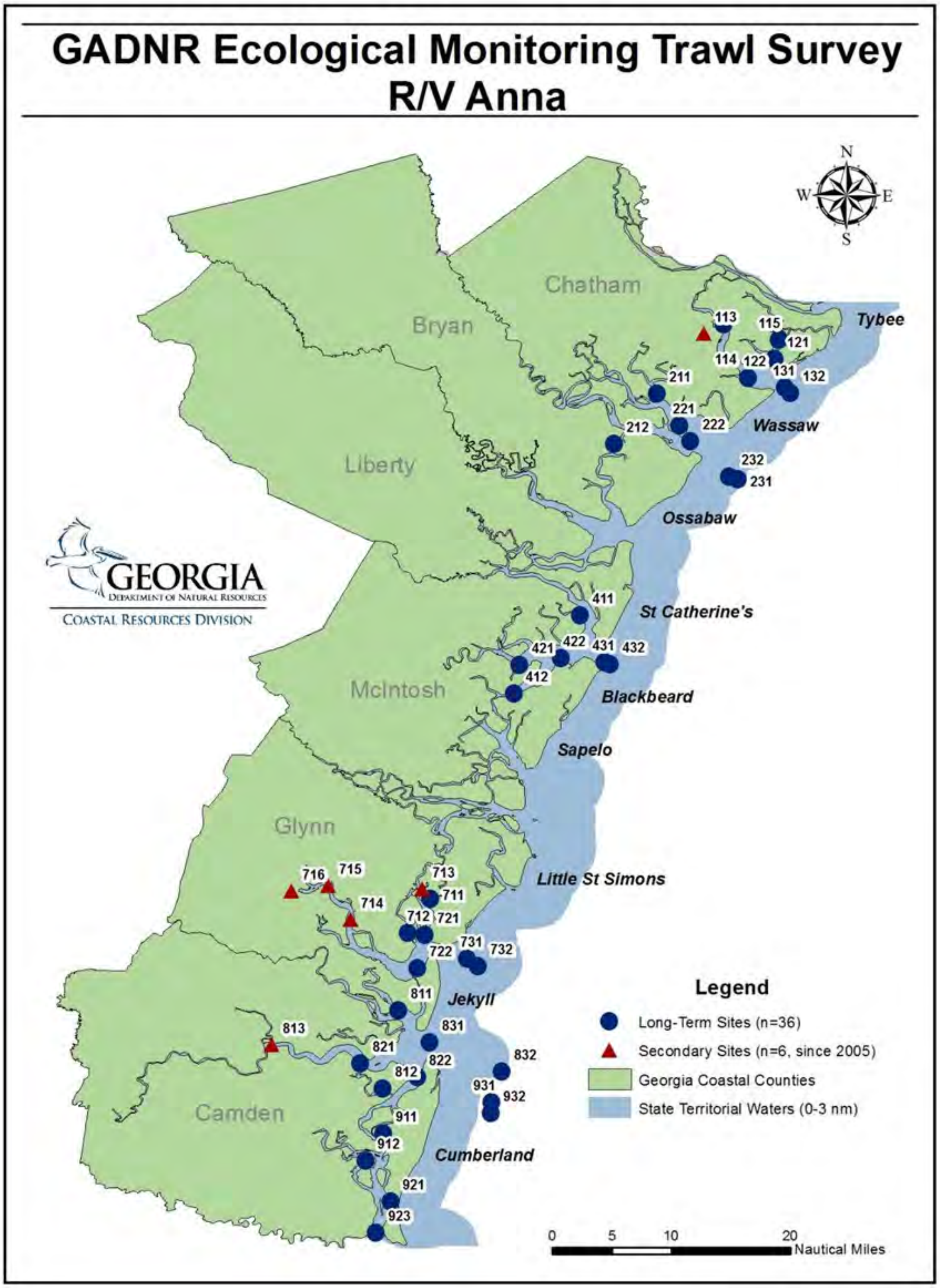


Figure 71. Map of the survey sites for Georgia's Ecological Monitoring Trawl Survey (map provided by GA DNR).

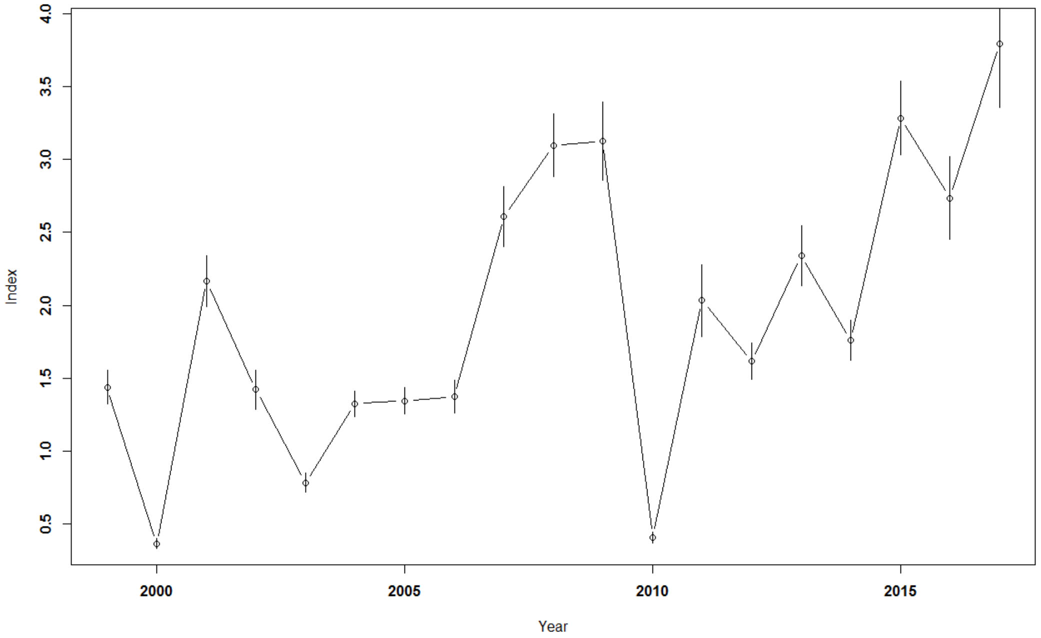


Figure 72. Index of relative abundance of horseshoe crab (delta mean catch per tow) developed from the Georgia Trawl survey with 95% confidence intervals.

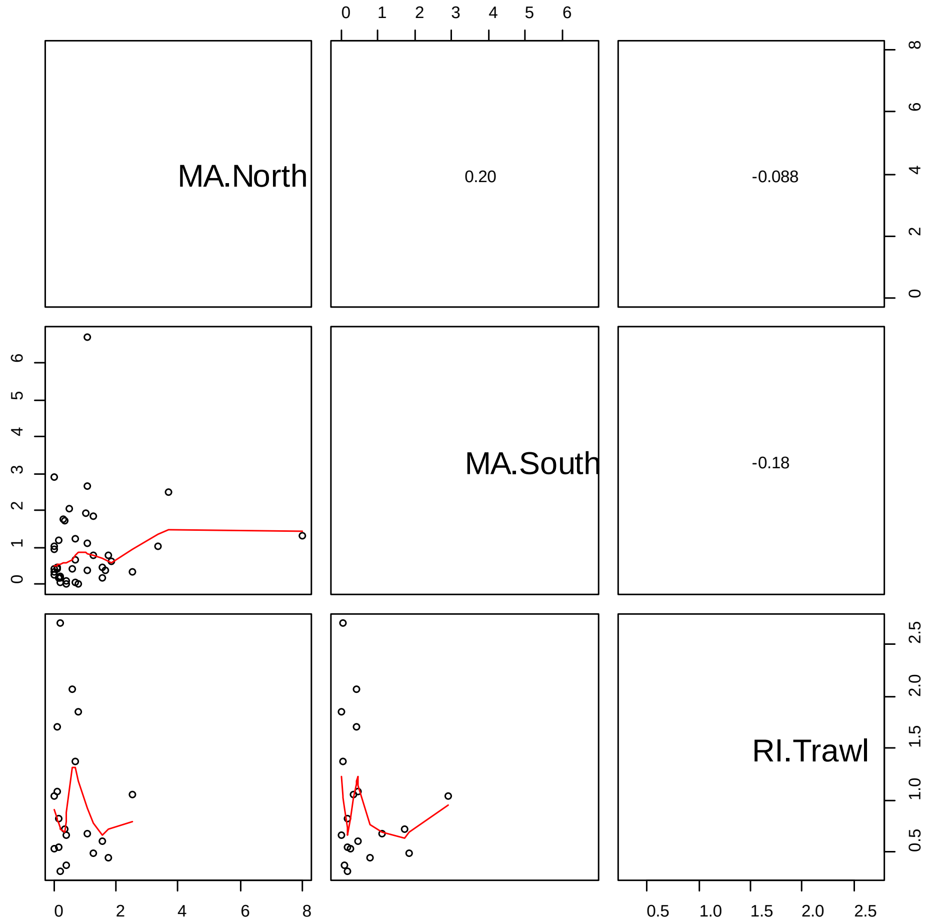


Figure 73. Correlation coefficients and scatter plots for the horseshoe crab abundance indices in the Northeast Region for 1978-2017. All correlations are insignificant (*P*>0.05).

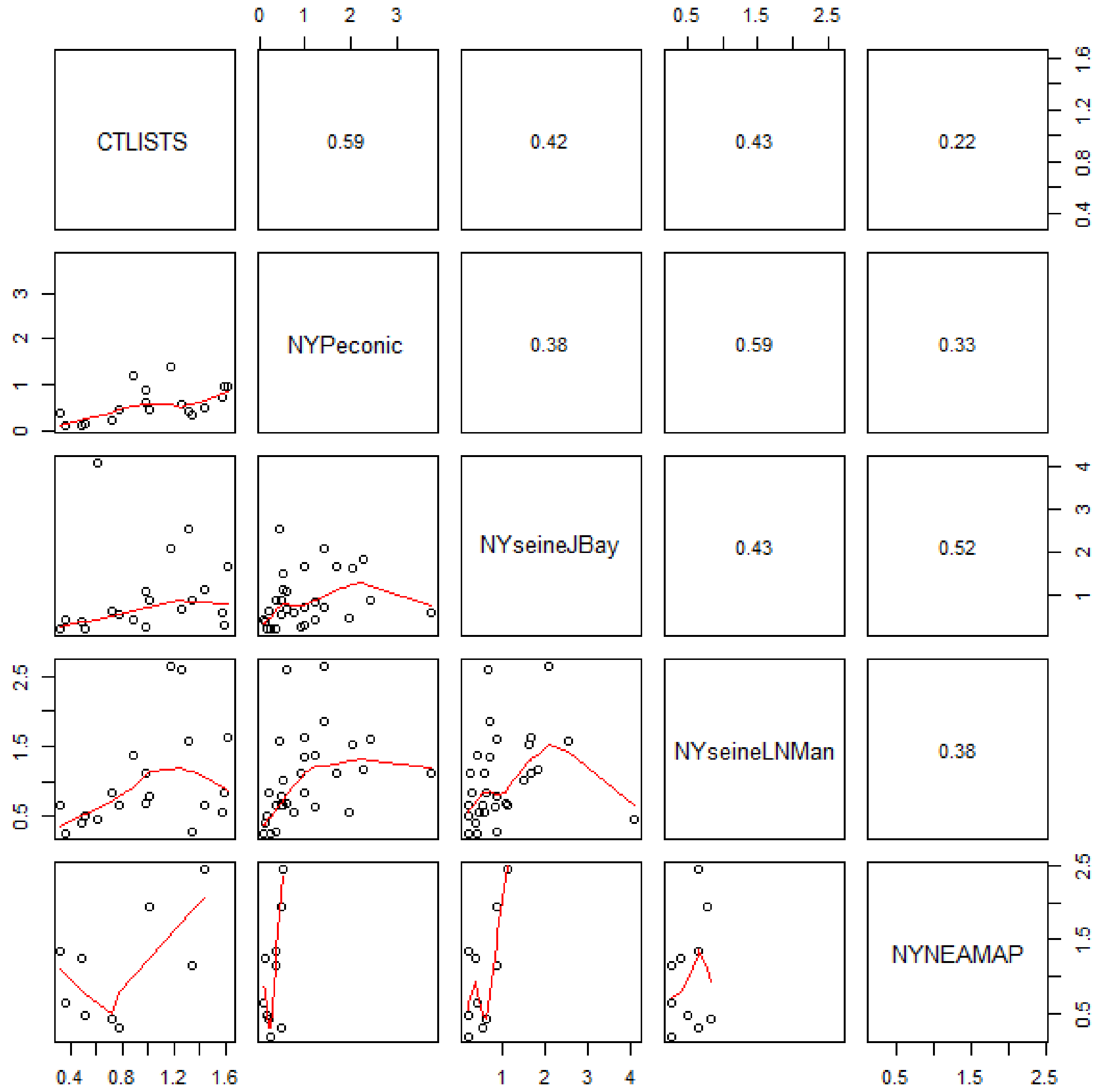


Figure 74. Correlation coefficients and scatter plots for the horseshoe crab abundance indices in the New York Region for 1987-2016. All correlations are insignificant (*P*>0.05) except for the positive correlation between the Connecticut Long Island Sound Trawl Survey and New York's Peconic Bays (*P*=0.020) and New York's NEAMAP portion and the New York Seine Jamaica Bay (*P*=0.026).

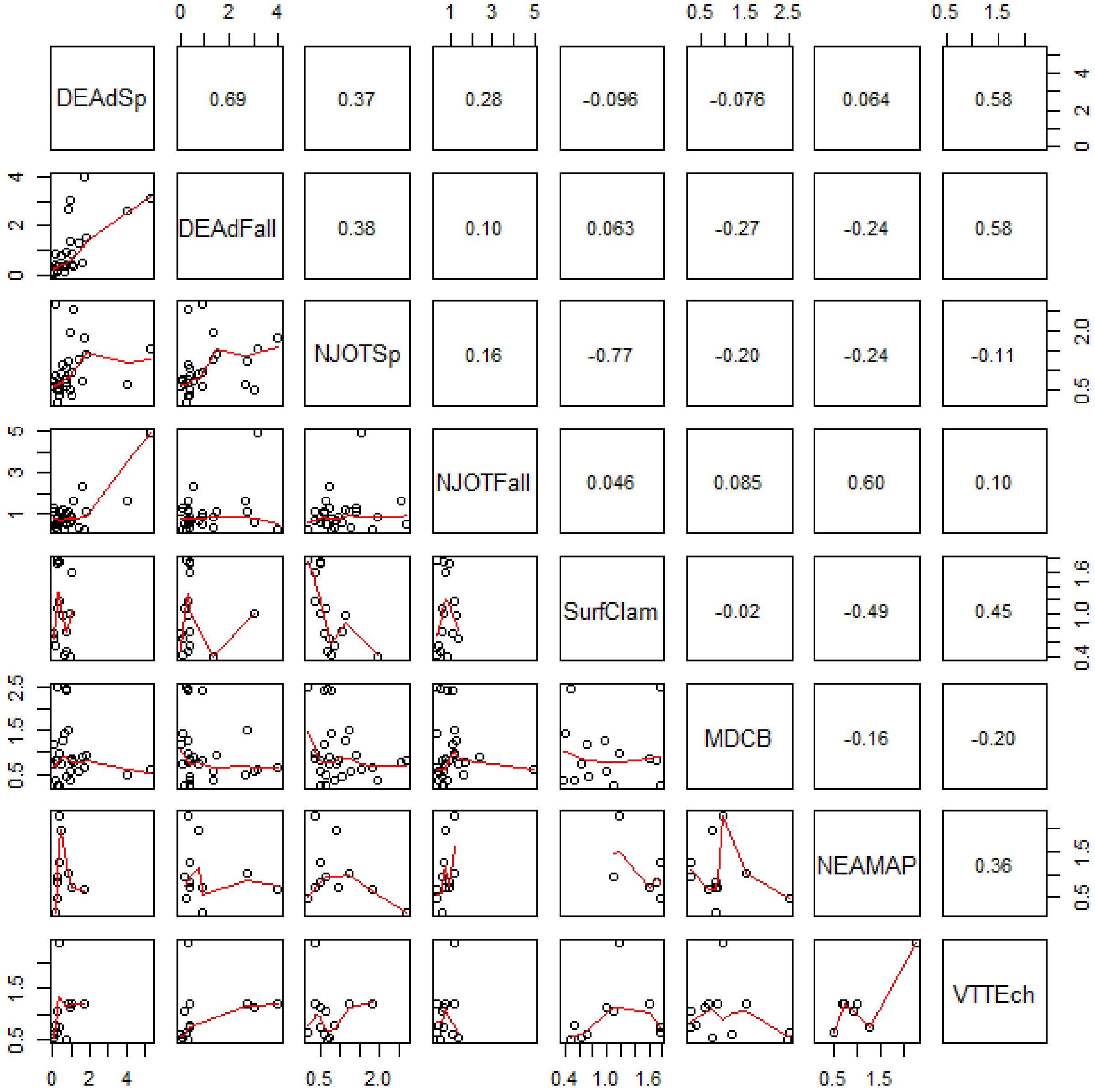


Figure 75. Correlation coefficients and scatter plots for the horseshoe crab abundance indices in the Delaware Bay Region for 1989-2017. All correlations are insignificant (*P*>0.05) except for the correlations between the Delaware Adult Trawl spring and fall indices (*P*<0.001), Delaware Adult Trawl spring and New Jersey Ocean Trawl fall (*P*<0.001), New Jersey Ocean Trawl spring and Surf Clam surveys (*P*=0.011), and NEAMAP and the Virginia Tech Trawl (*P*=0.020).

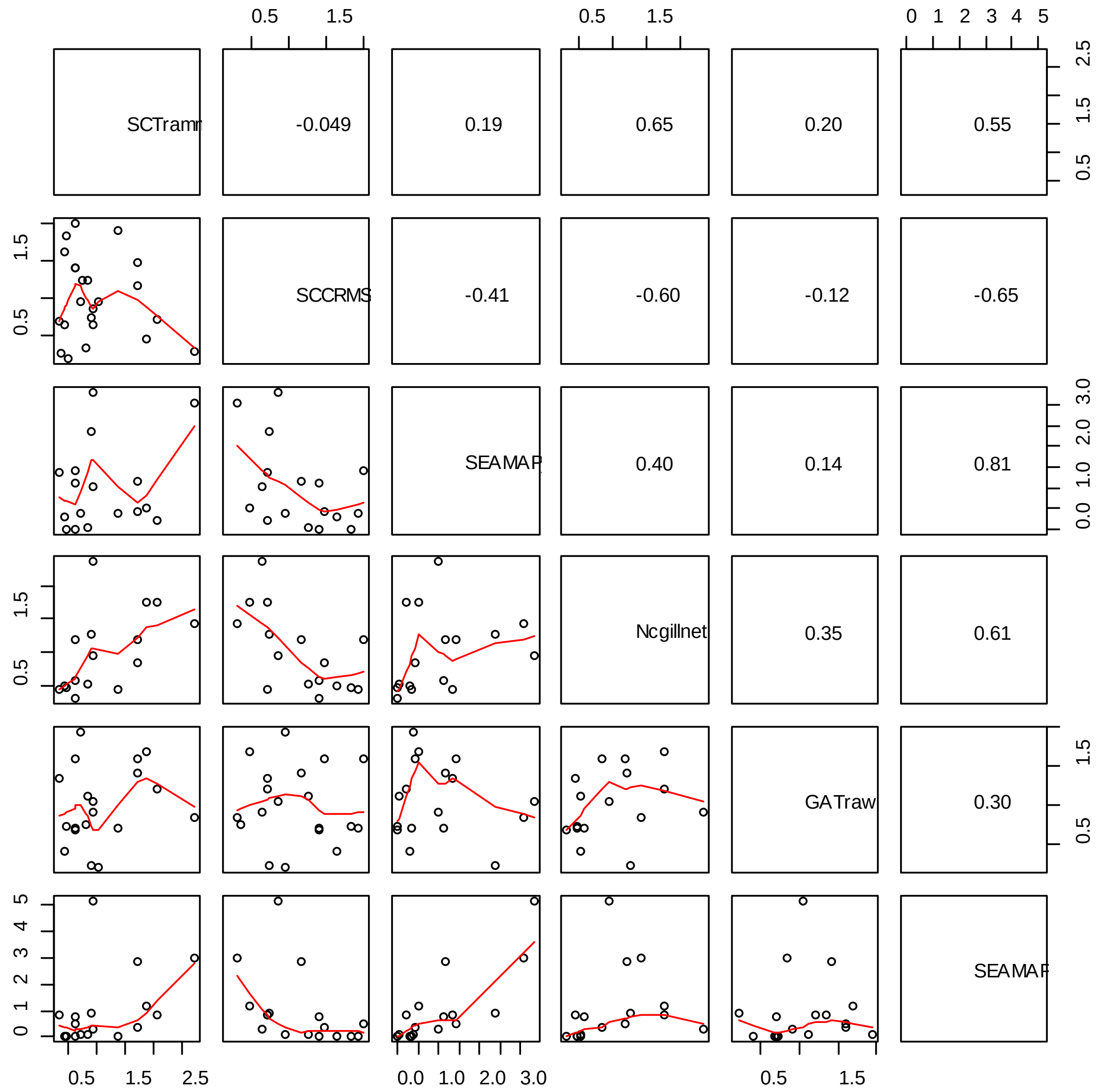


Figure 76. Correlation coefficients and scatter plots for the horseshoe crab abundance indices in the Southeast Region for 1995-2017. All correlations are insignificant (*P*>0.05) except for the correlations between the North Carolina Gill Net and South Carolina Trammel indices (*P*=0.036), the North Carolina Gill Net and South Carolina CRMS indices (*P*=0.010), and SEAMAP's South Carolina and Georgia-Florida indices (*P*<0.001).

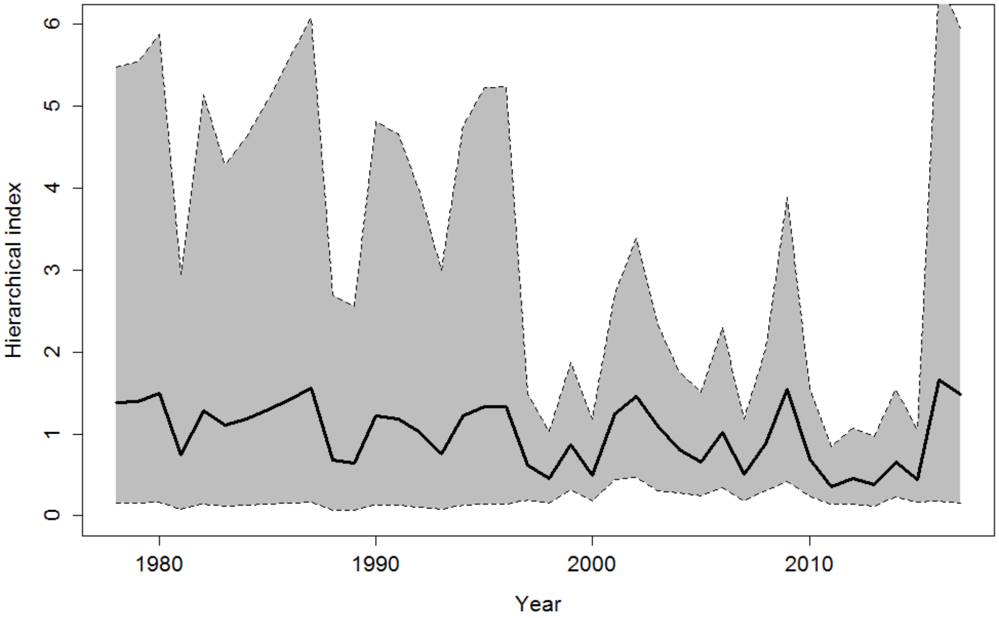


Figure 77. Time series of horseshoe crab relative abundance in the Northeast region as estimated from hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

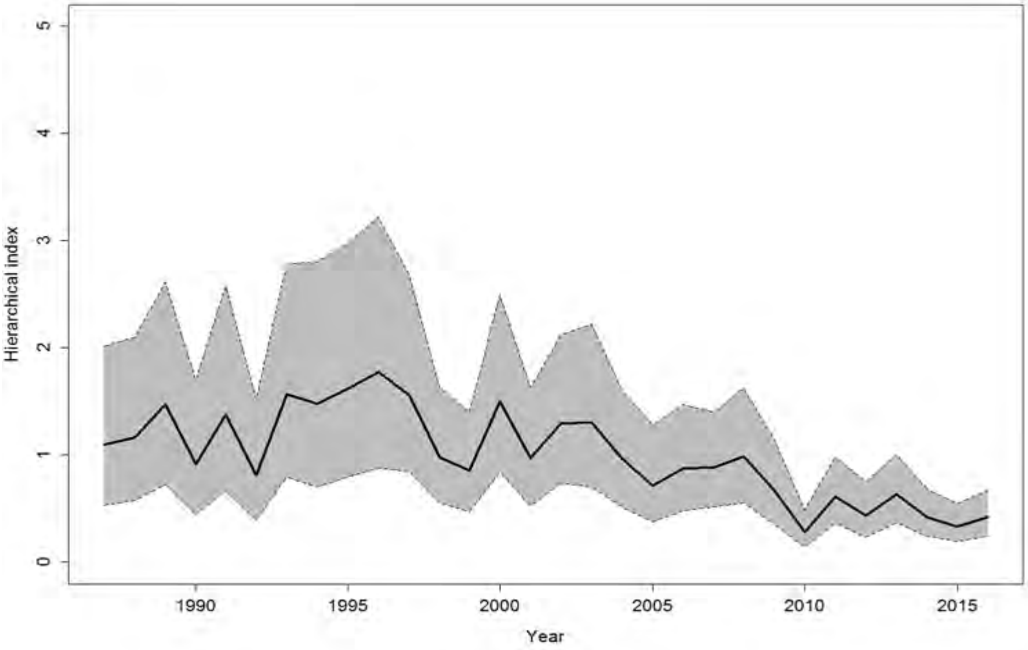


Figure 78. Time series of horseshoe crab relative abundance in the New York region as estimated from hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

Figure 79. Time series of horseshoe crab relative abundance in the Delaware Bay region as estimated from the hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

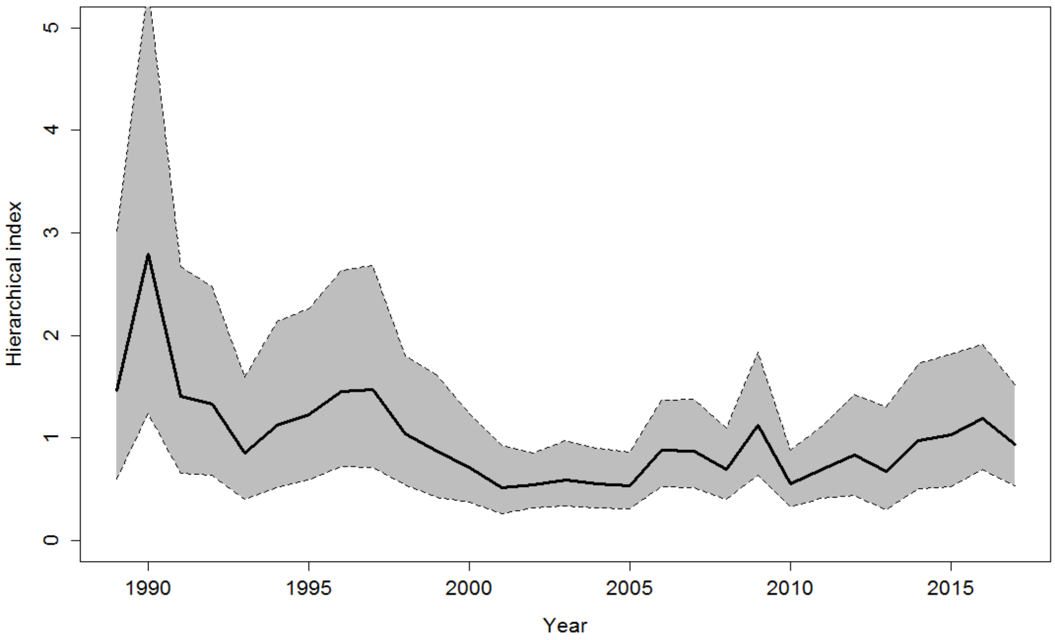


Figure 80. Time series of female horseshoe crab relative abundance in the Delaware Bay region as estimated from the hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

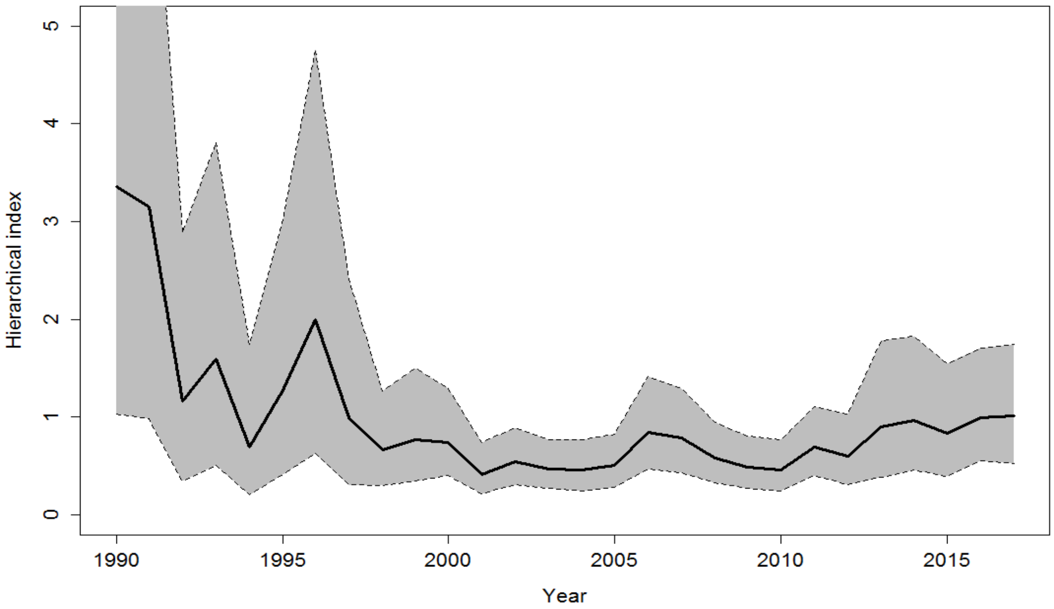


Figure 81. Time series of male horseshoe crab relative abundance in the Delaware Bay region as estimated from the hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

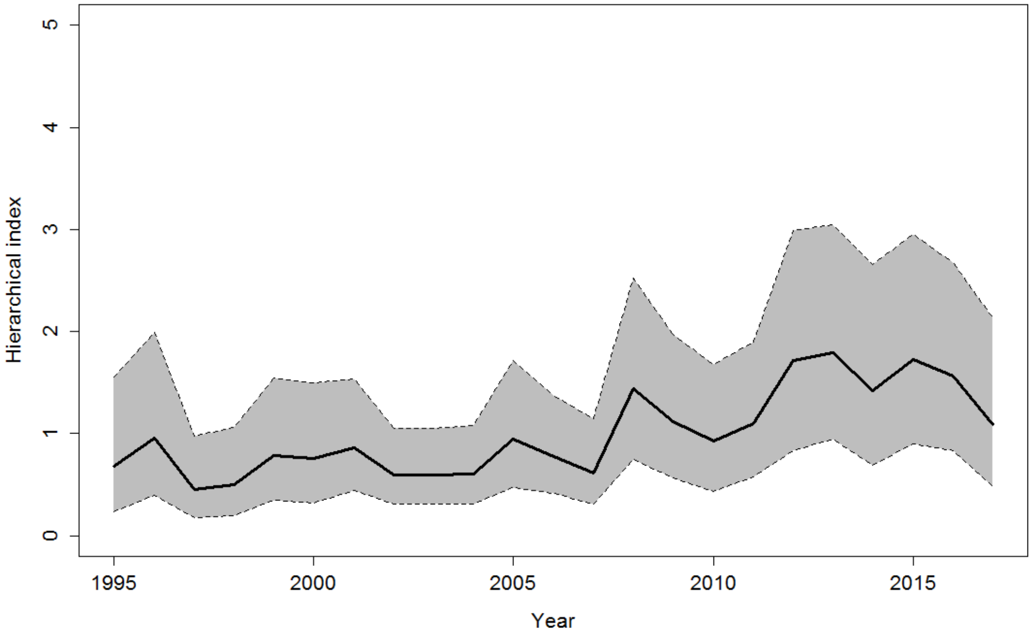


Figure 82. Time series of horseshoe crab relative abundance in the Southeast region as estimated from hierarchical analysis. The black line gives the posterior mean and the grey, shaded area represents a 95% credible interval about the time series.

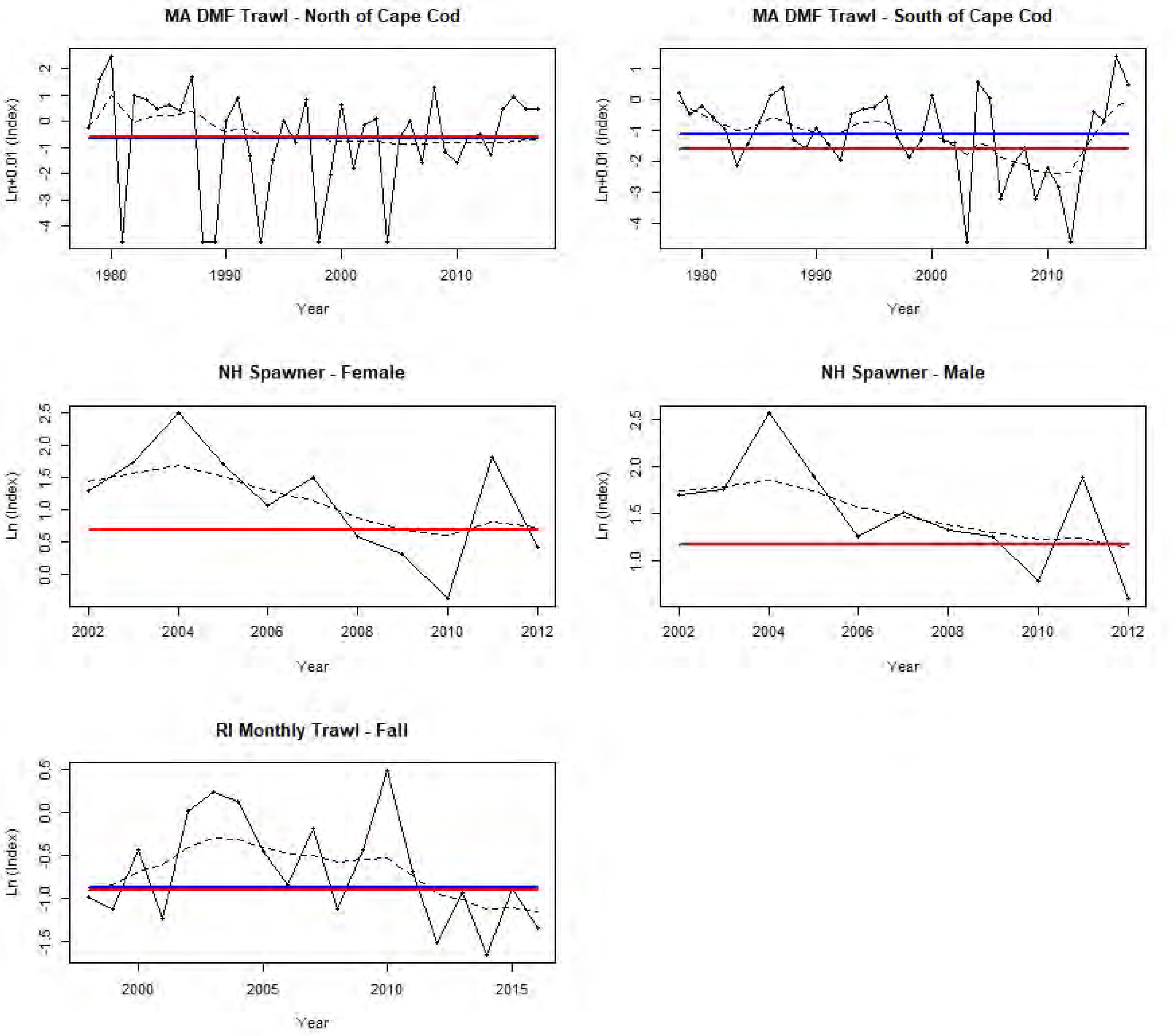
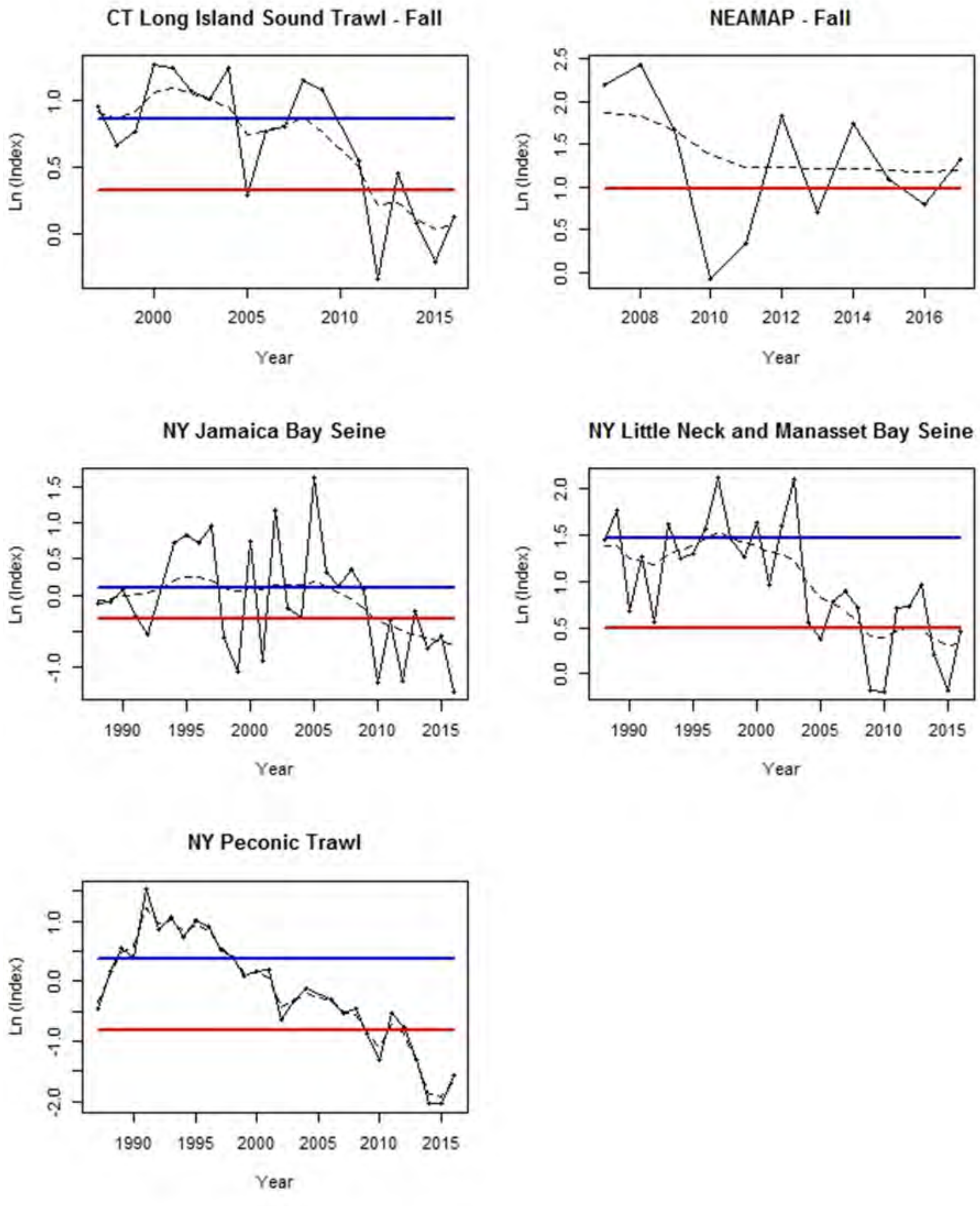


Figure 83. Northeast Region horseshoe crab survey ARIMA model fits. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point. Note: The residuals from the ARIMA model fit to the MA DMF Trawl – North of Cape Cod were not normally distributed.



CT Long Island Sound Trawl - Fall

NEAMAP - Fall

NY Jamaica Bay Seine

NY Little Neck and Manasset Bay Seine

NY Peconic Trawl

Figure 84. New York Region horseshoe crab survey ARIMA model fits. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point.

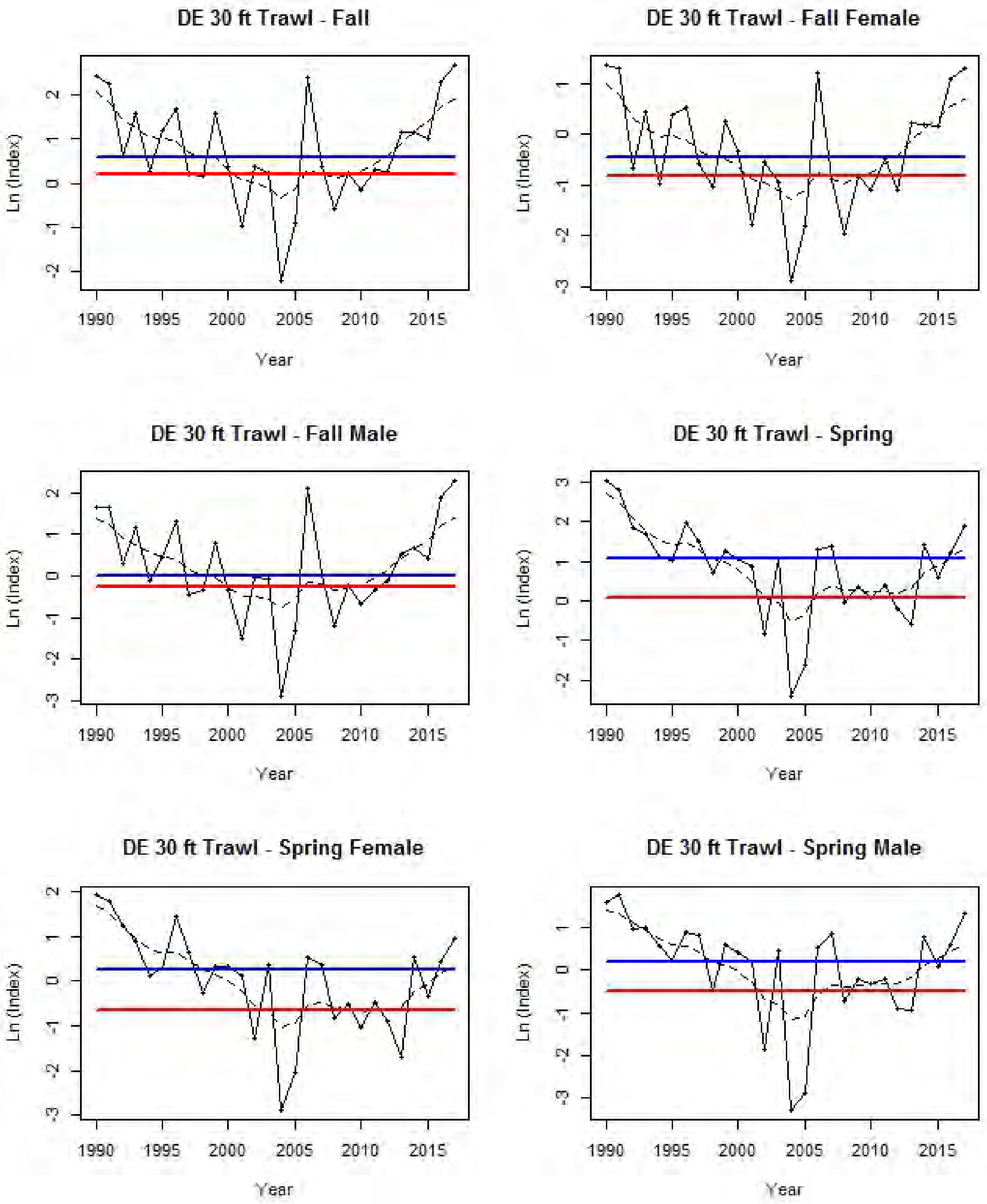


Figure 85. ARIMA model fits to horseshoe crab indices from the DE 30 ft. Trawl survey in the Mid-Atlantic Region. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point.

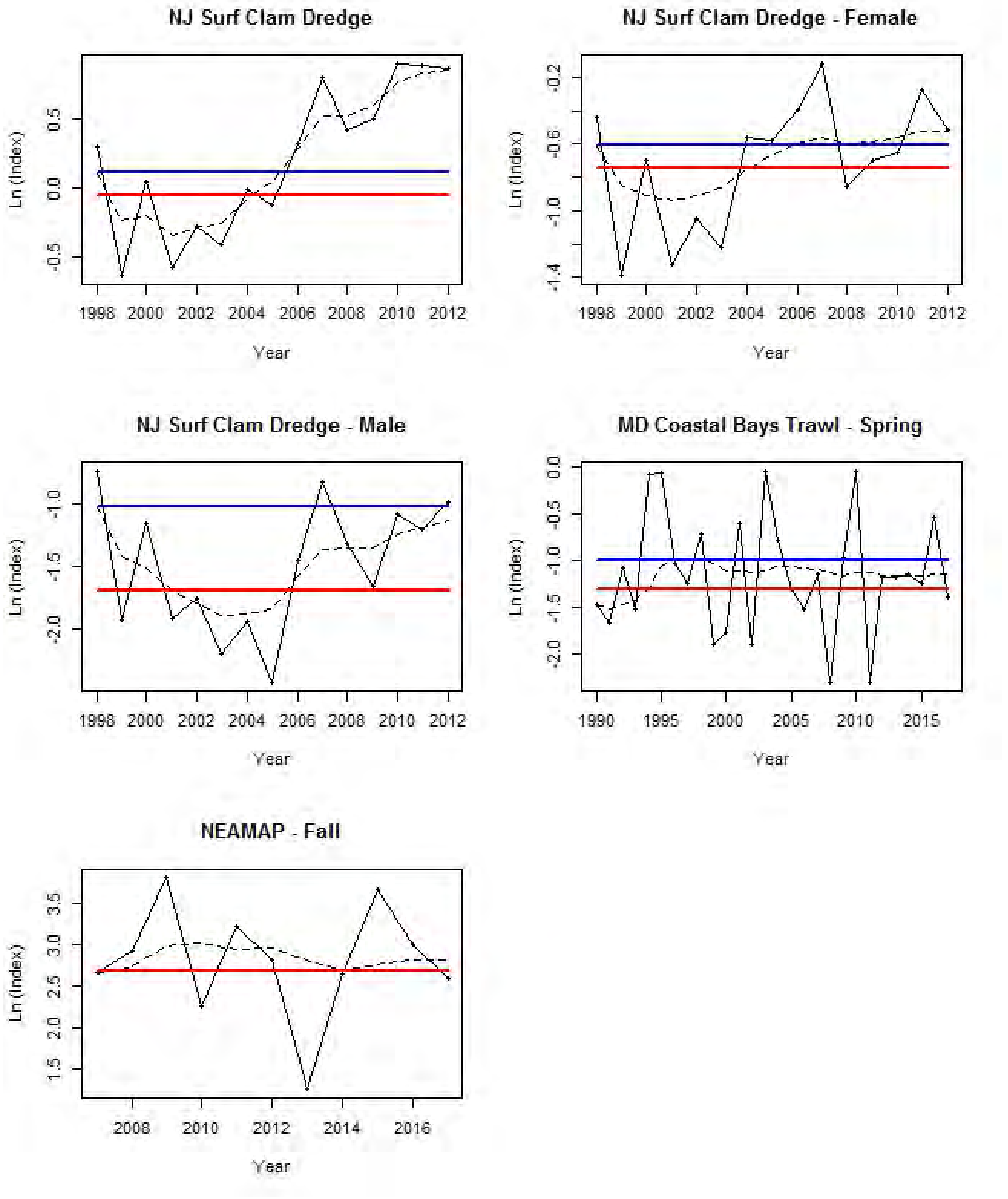


Figure 86. ARIMA model fits to horseshoe crab indices from various surveys in the Mid-Atlantic Region. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point.

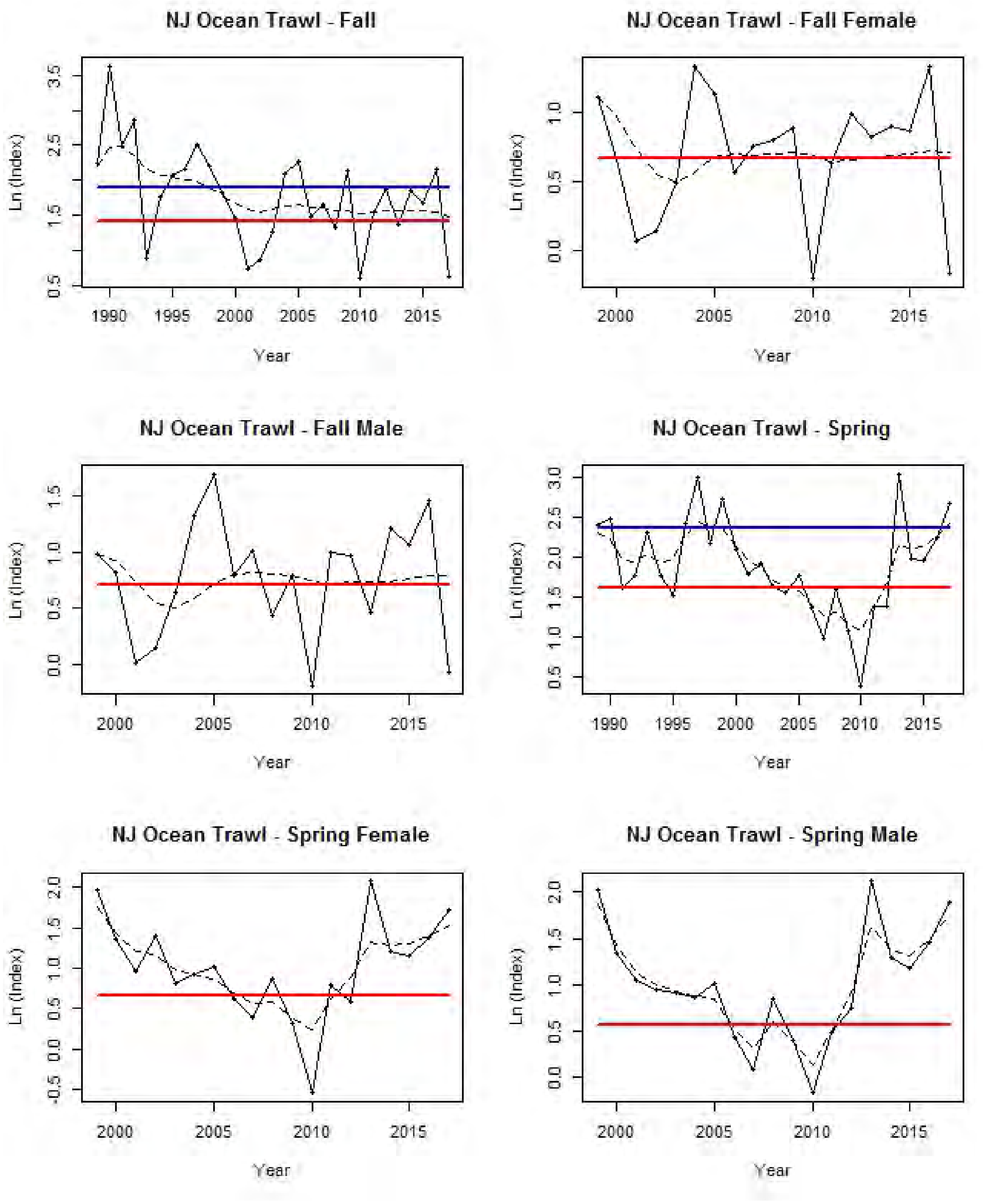


Figure 87. ARIMA model fits to horseshoe crab indices from the NJ Ocean Trawl survey in the Mid-Atlantic Region. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point.

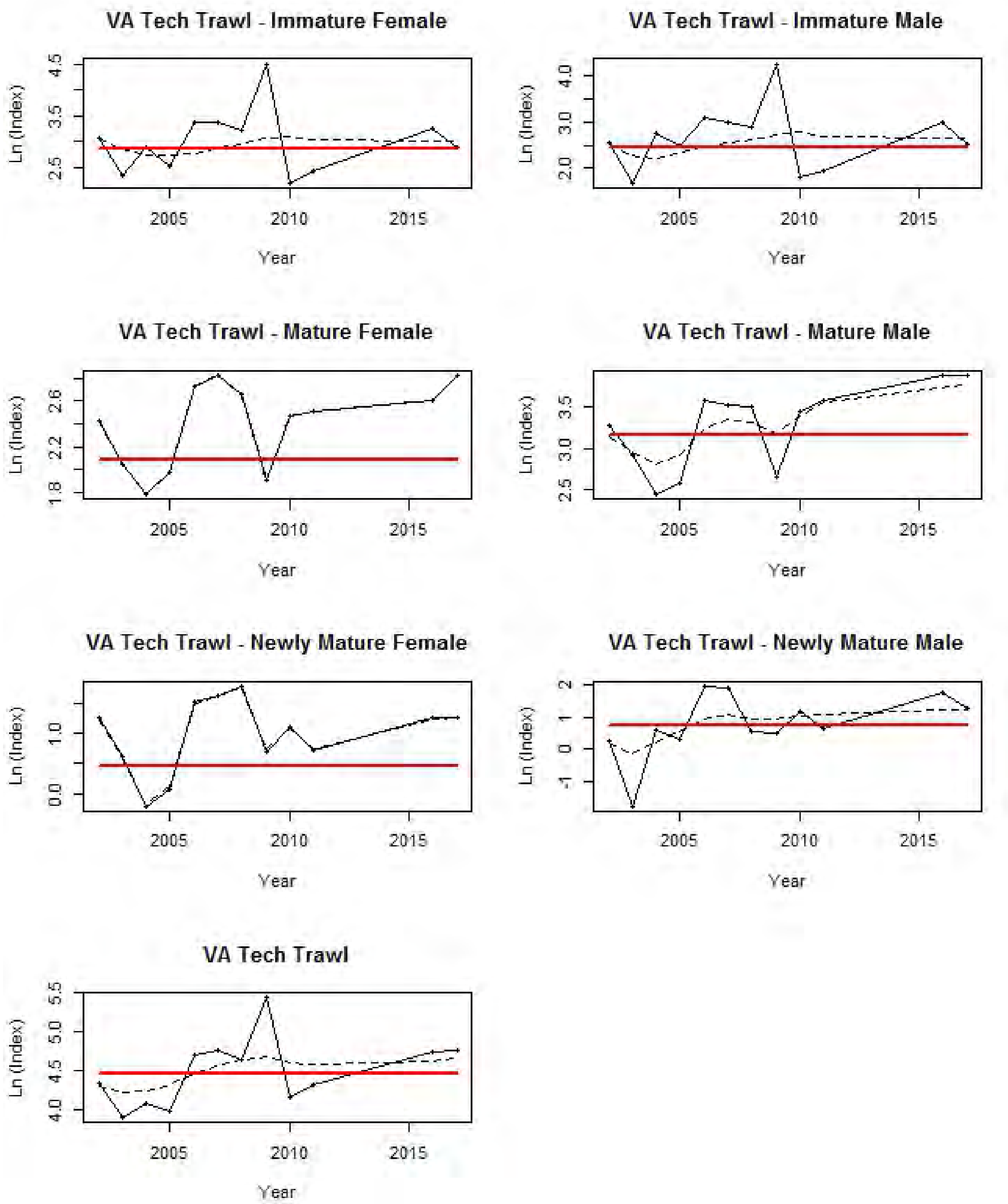


Figure 88. ARIMA model fits to horseshoe crab indices from the VA Tech Trawl survey in the Mid-Atlantic Region. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point.

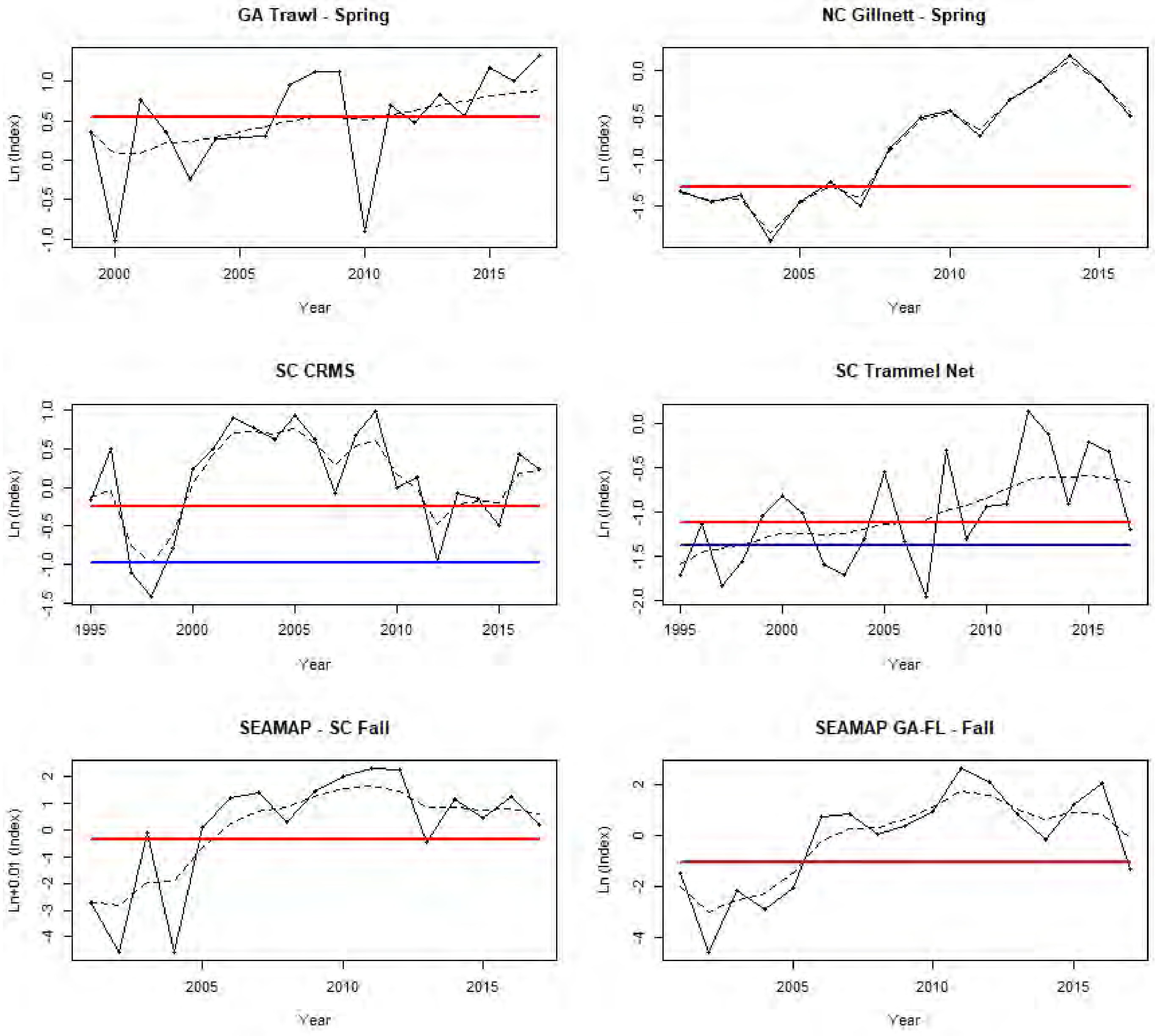


Figure 89. Southeast Region horseshoe crab survey ARIMA model fits. The solid line represents the observed ln transformed indices and the dashed line represents the fitted indices. The red horizontal line represents the Q_{25} reference point and the blue horizontal line represents the 1998 reference point. Note: The residuals from the ARIMA fit to the GA Trawl were not normally distributed.

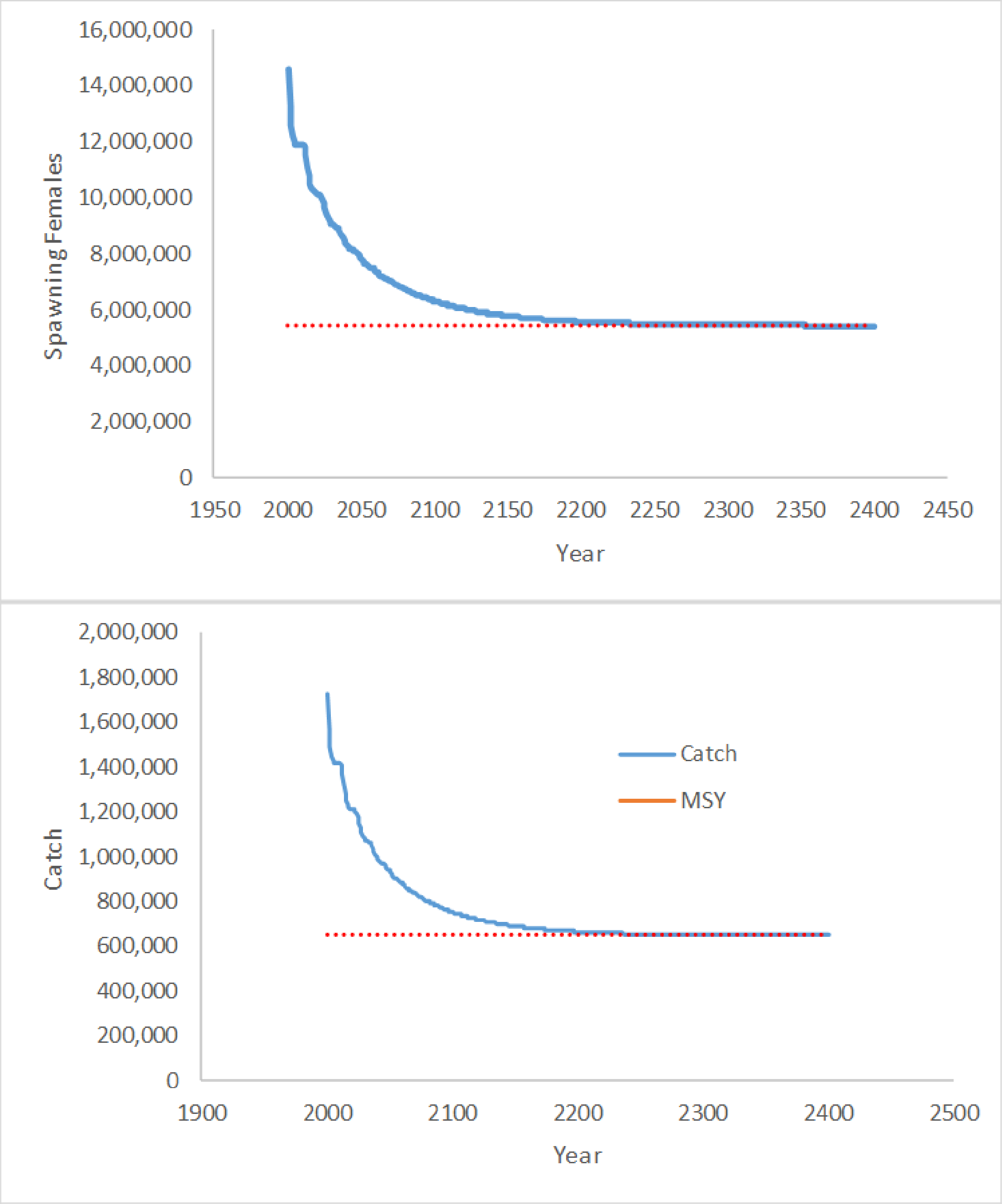


Figure 90. Projections of the horseshoe crab operating model under F_{MSY} (0.1613) showing where the population asymptotes at B_{MSY} (5,433,439) and where catch asymptotes at *MSY* (647,609 crabs).

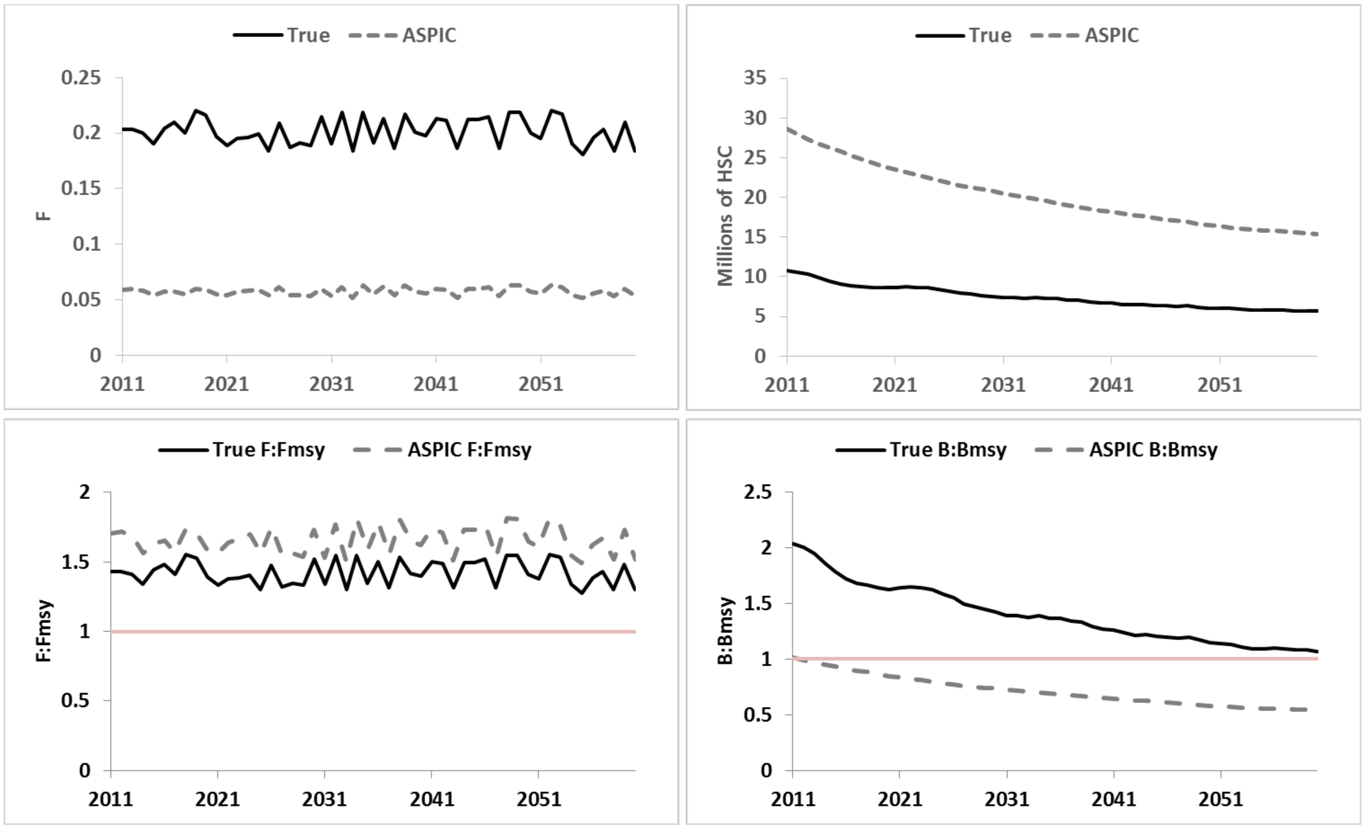


Figure 91. Comparison between simulated "true" data from the operating model and surplus production (ASPIC) results for simulation 1.

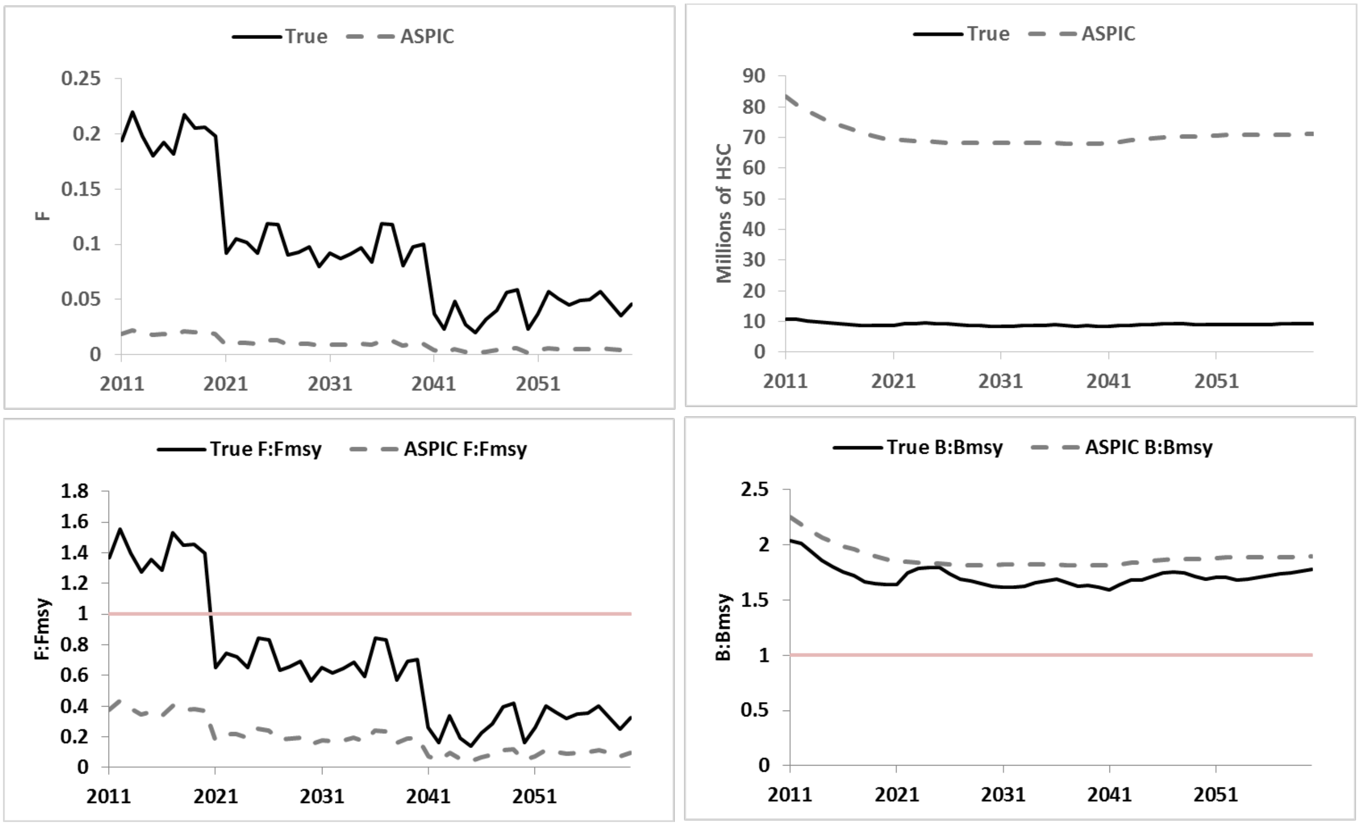


Figure 92. Comparison between simulated “true” data from the operating model and surplus production (ASPIC) results for simulation 2.

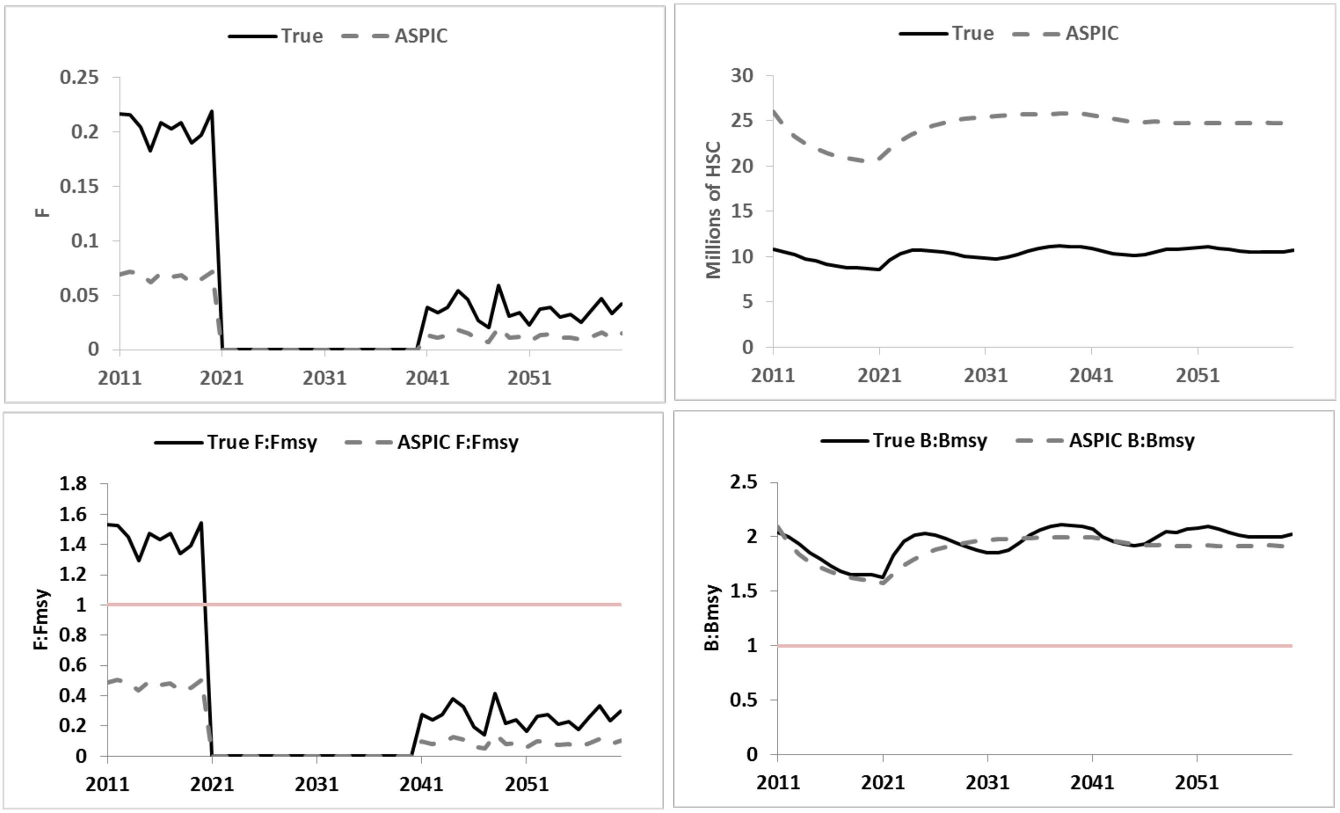


Figure 93. Comparison between simulated "true" data from the operating model and surplus production (ASPIC) results for simulation 3.

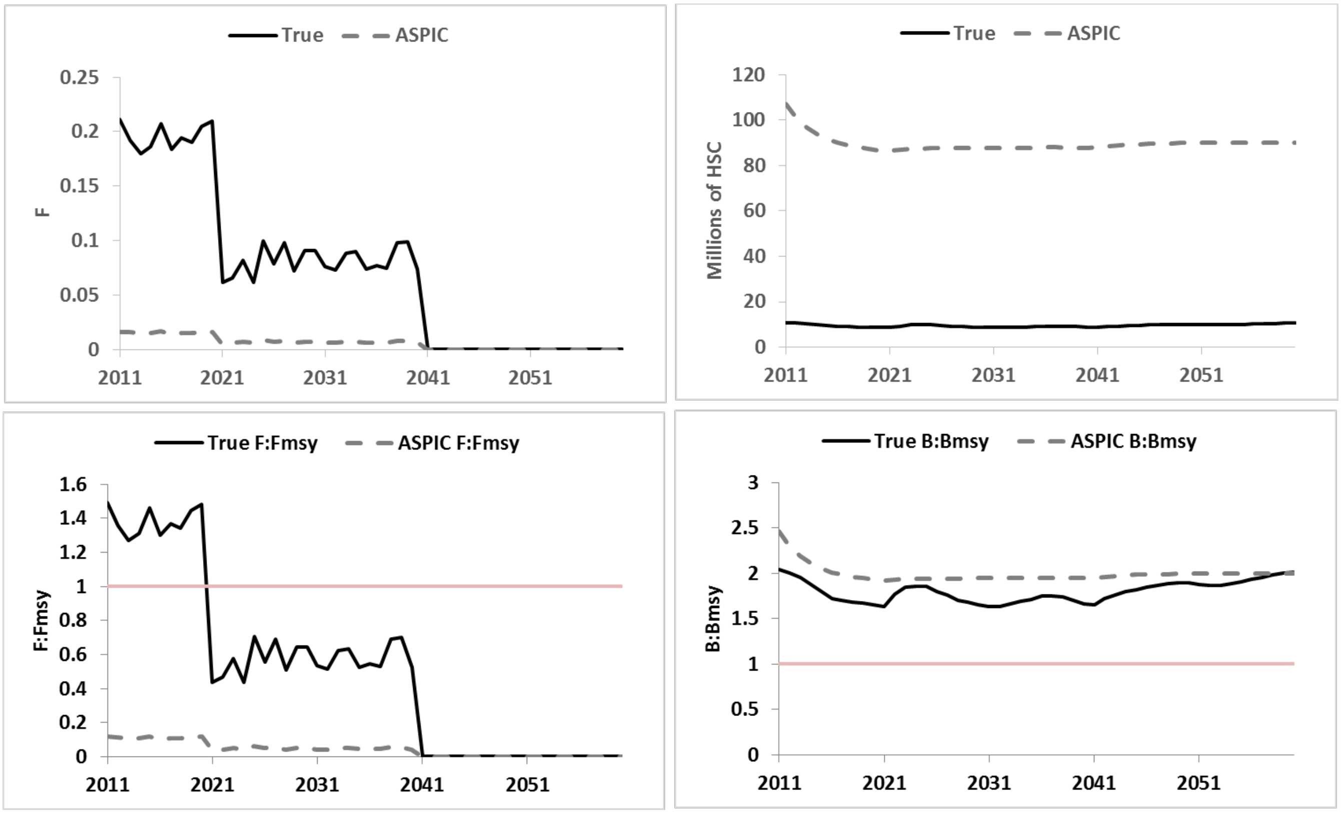


Figure 94. Comparison between simulated "true" data from the operating model and surplus production (ASPIC) results for simulation 4.

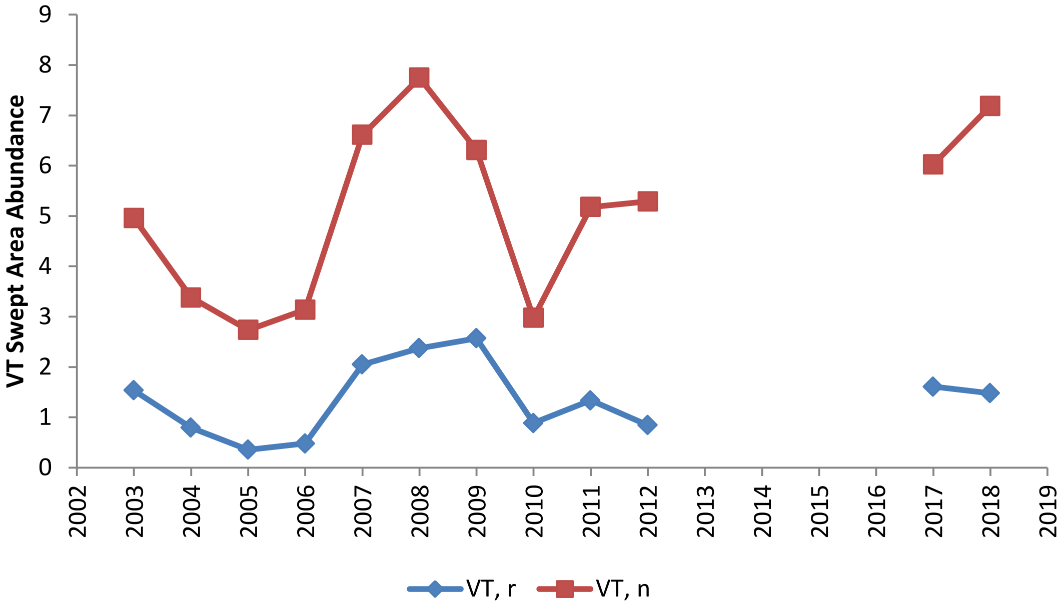


Figure 95. Primiparous and multiparous indices (in millions) from the Virginia Tech Trawl Survey.

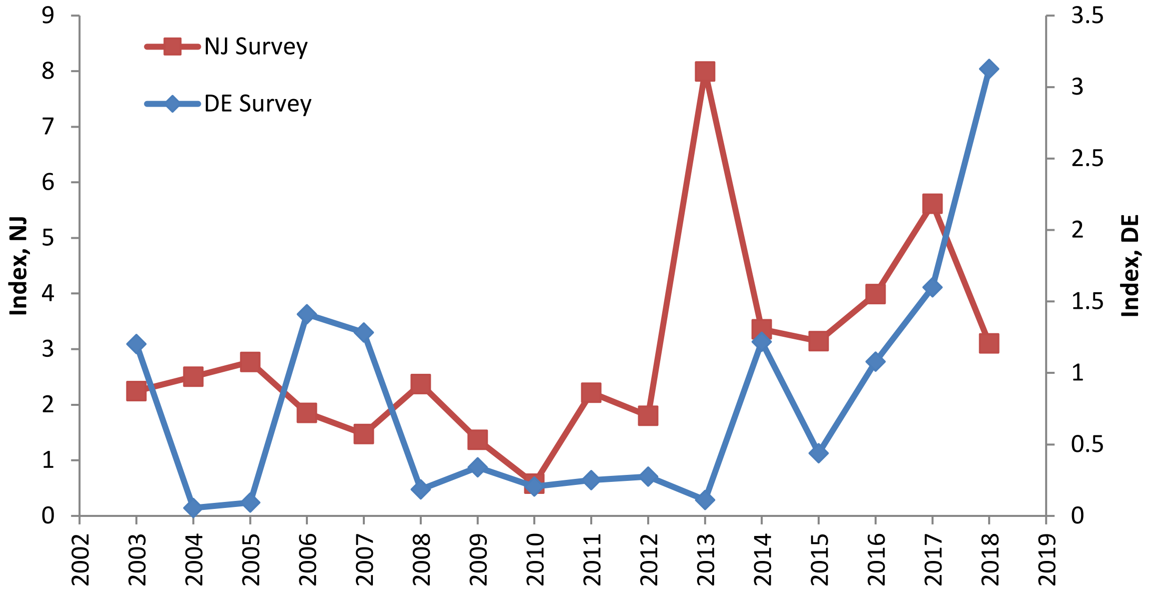


Figure 96. Aggregate stage indices from the Delaware and New Jersey trawl surveys.

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Figure 97. Catch inputs for the base CMSA model.

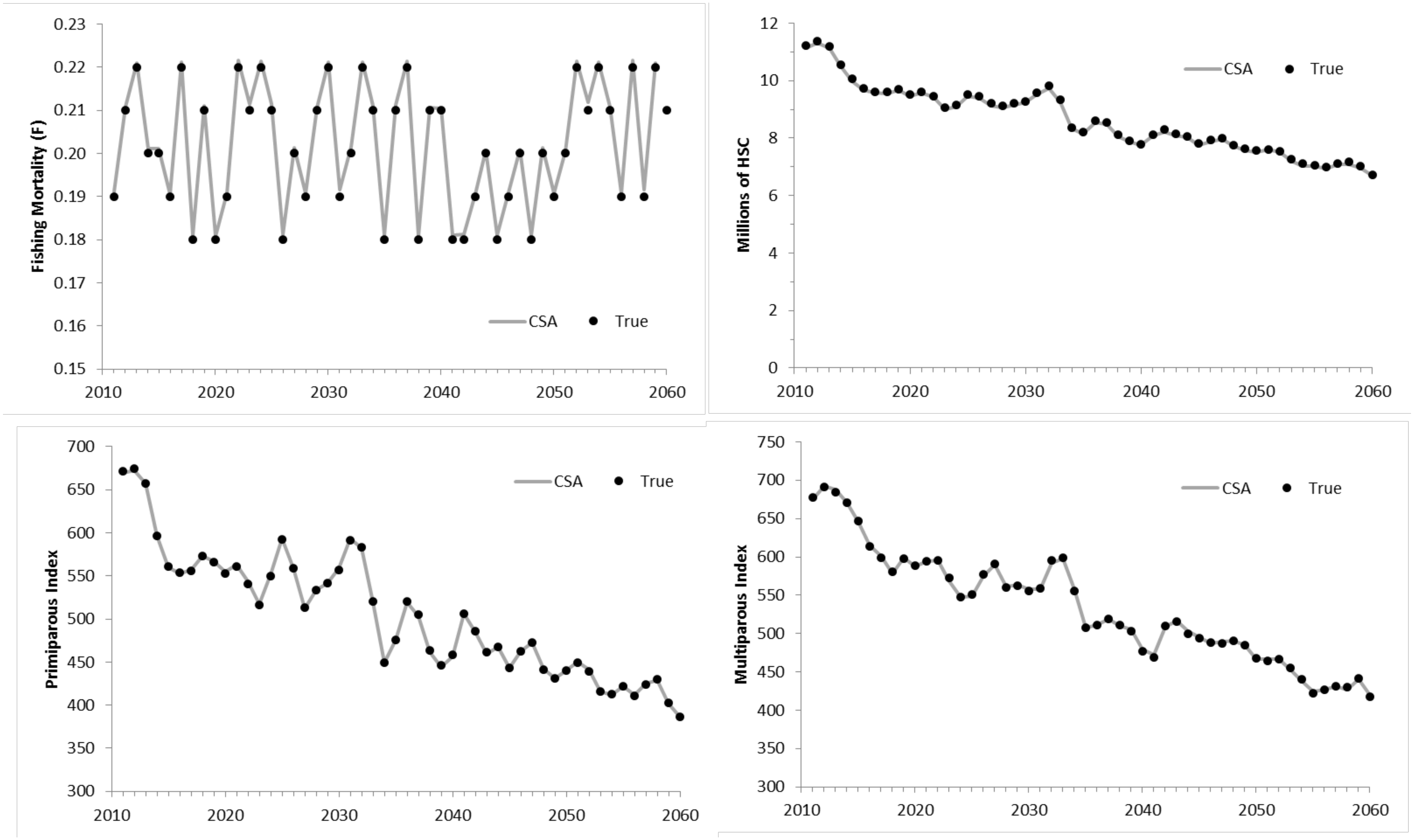

Figure 98. Comparison between simulated "true" data from the operating model and catch survey analysis (CSA) results for simulation 1.

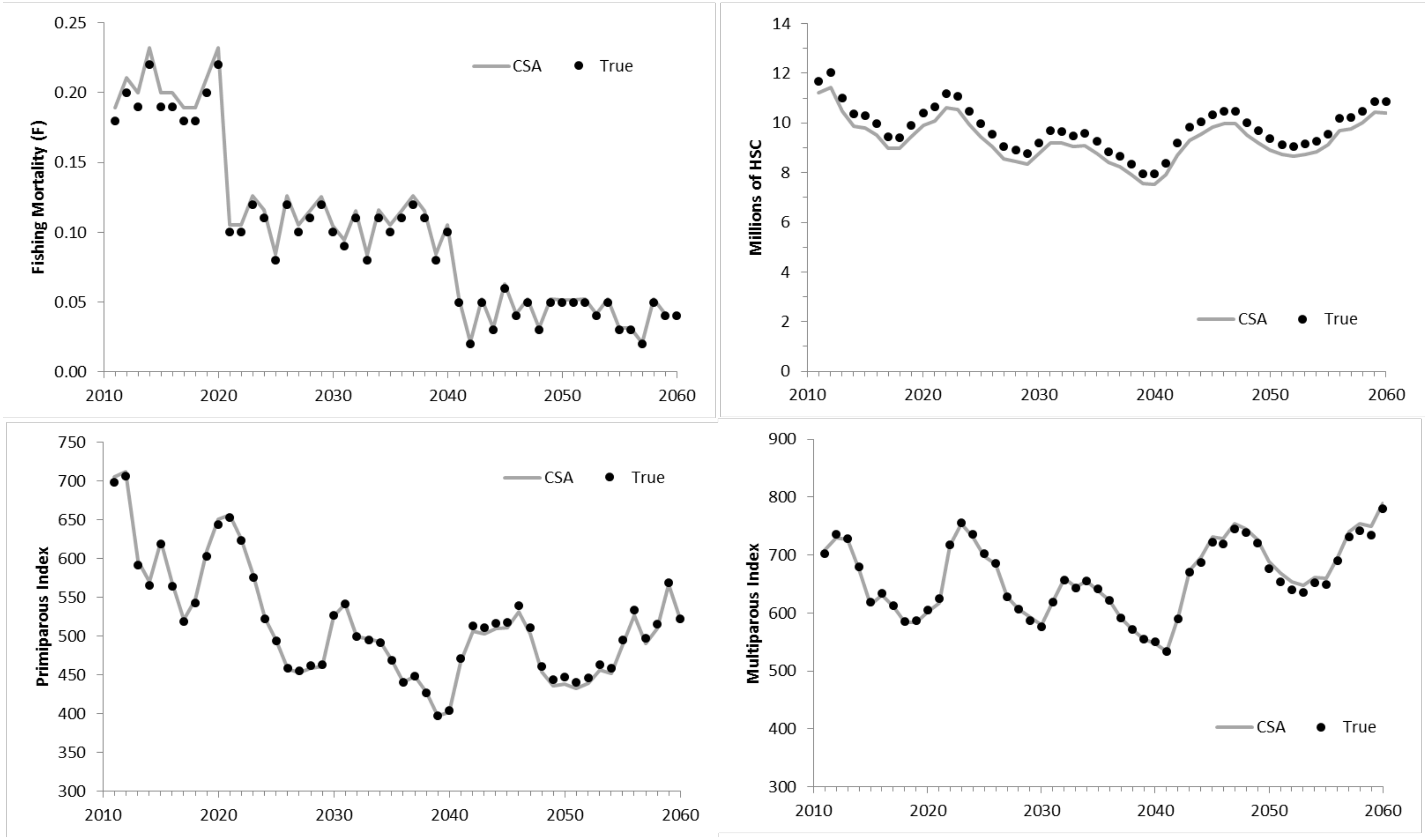

Figure 99. Comparison between simulated "true" data from the operating model and catch survey analysis (CSA) results for simulation 2.

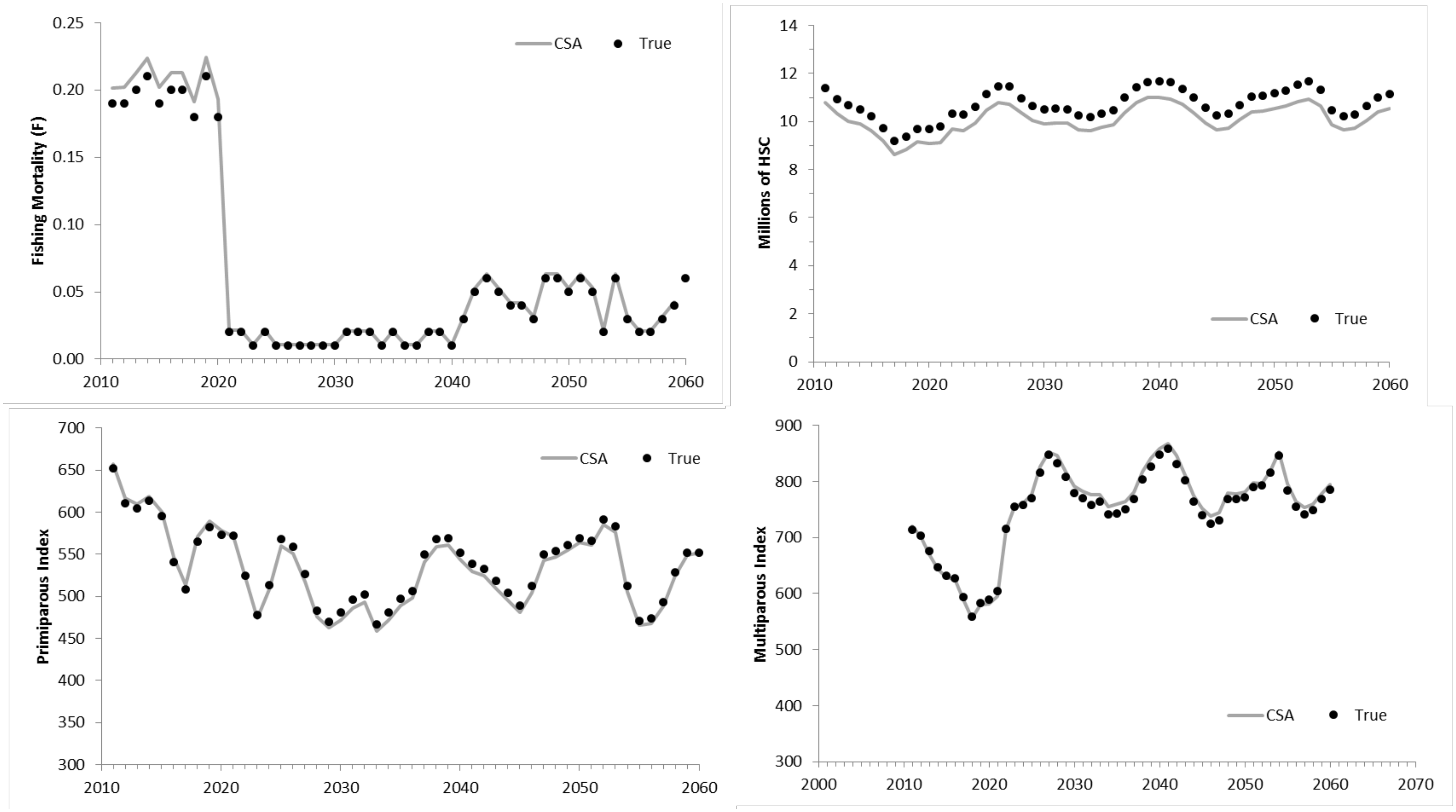

Figure 100. **Comparison between simulated "true" data from the operating model and catch survey analysis (CSA) results for simulation 3.**

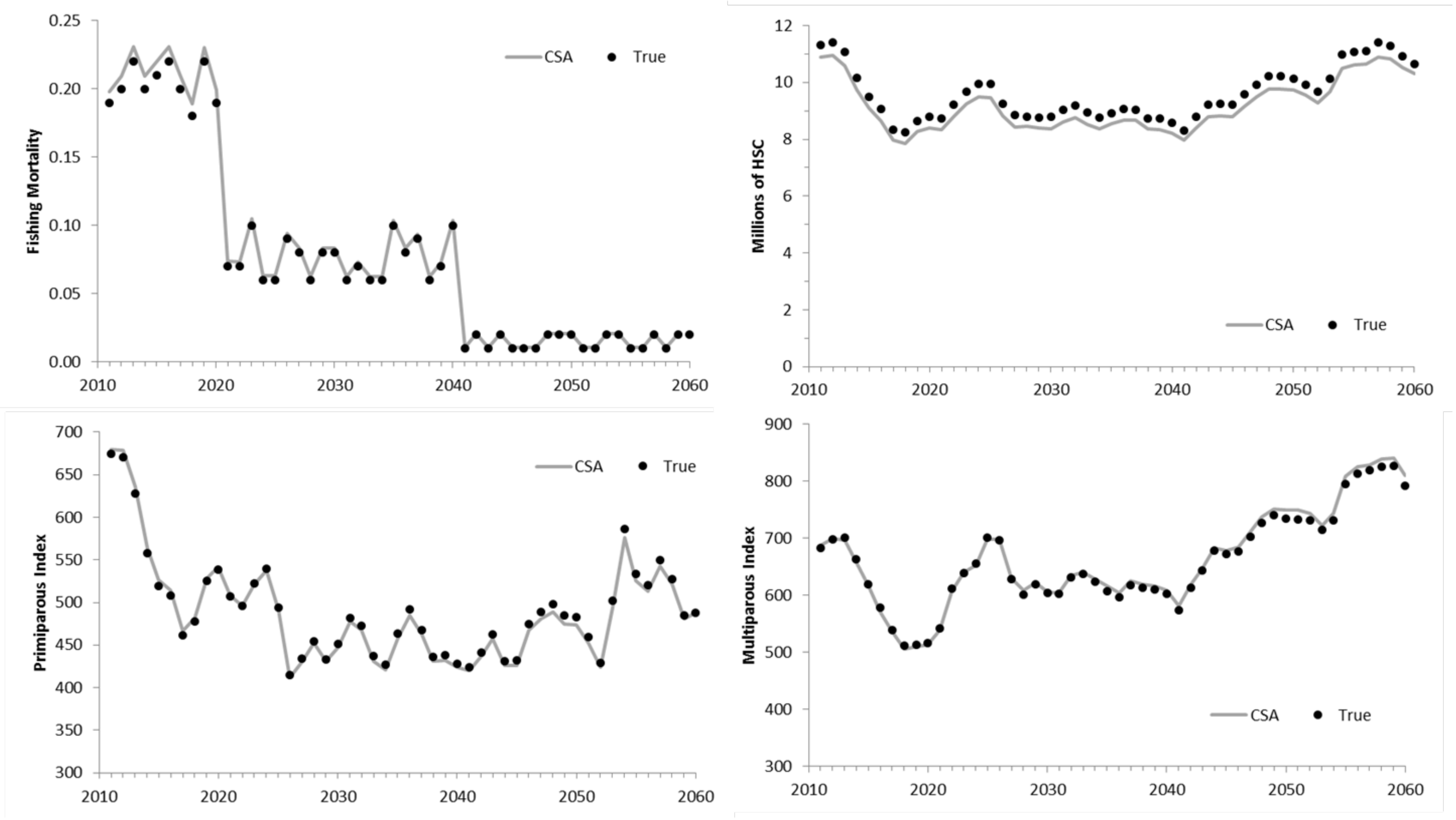

Figure 101. **Comparison between simulated "true" data from the operating model and catch survey analysis (CSA) results for simulation 4.**

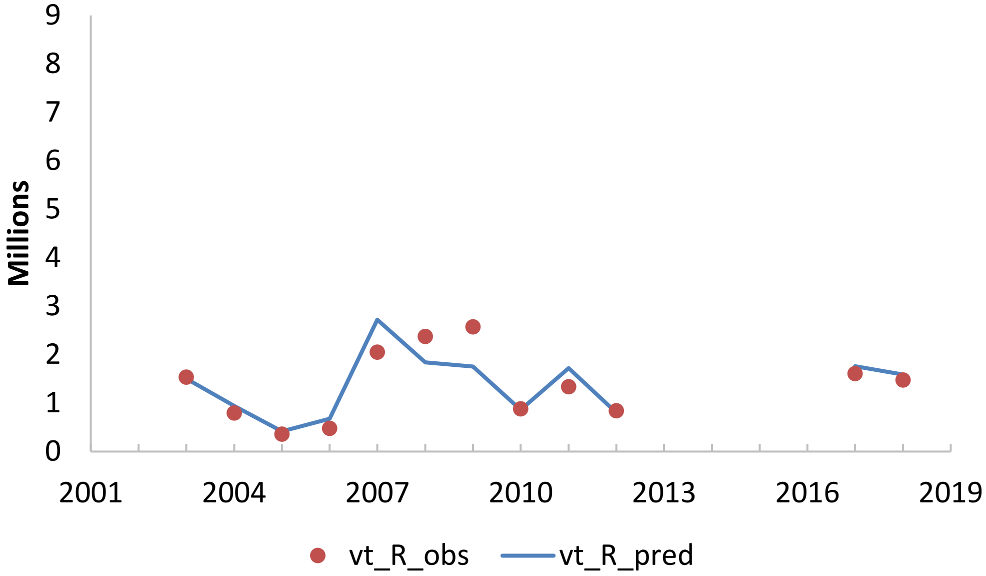

Figure 102. **CMSA model fit to the primiparous female index from the Virginia Tech Trawl Survey.**

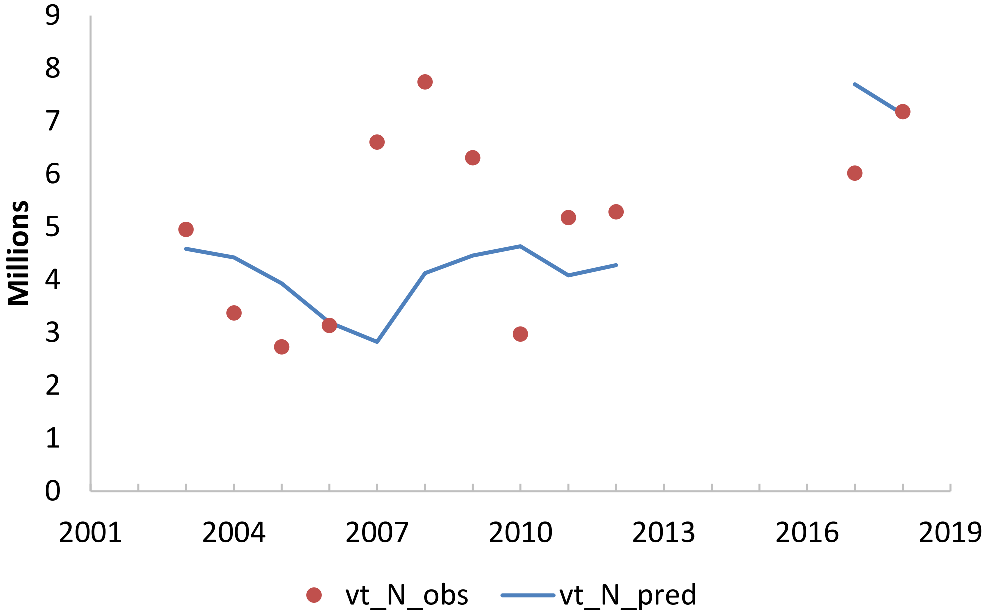

Figure 103. **CMSA model fit to multiparous female index from the Virginia Tech Trawl Survey.**

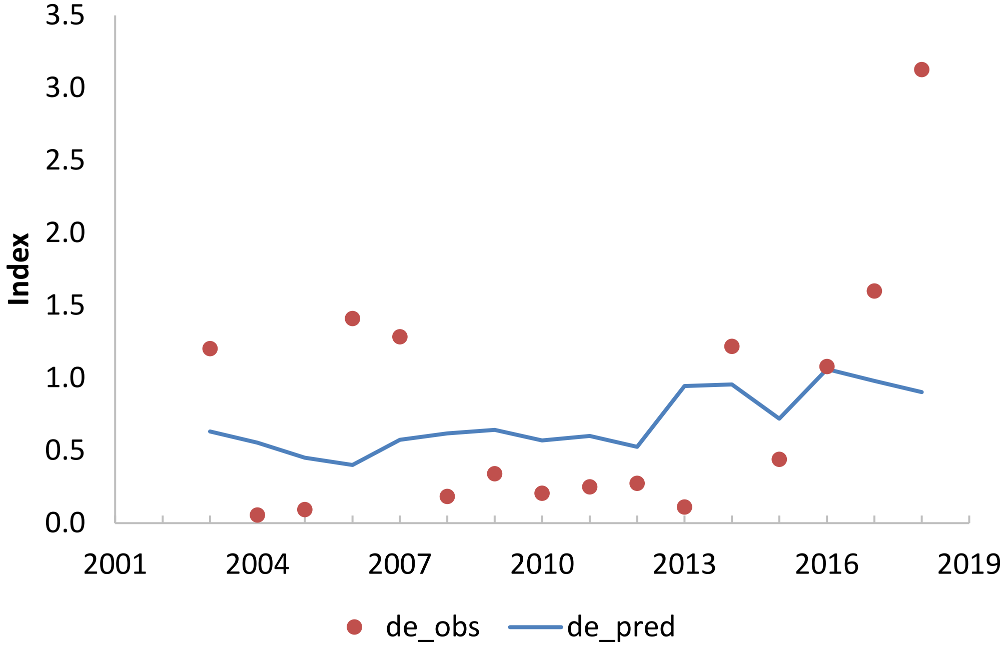

Figure 104. **CMSA model fit to Delaware Bay trawl survey aggregate adult female index.**

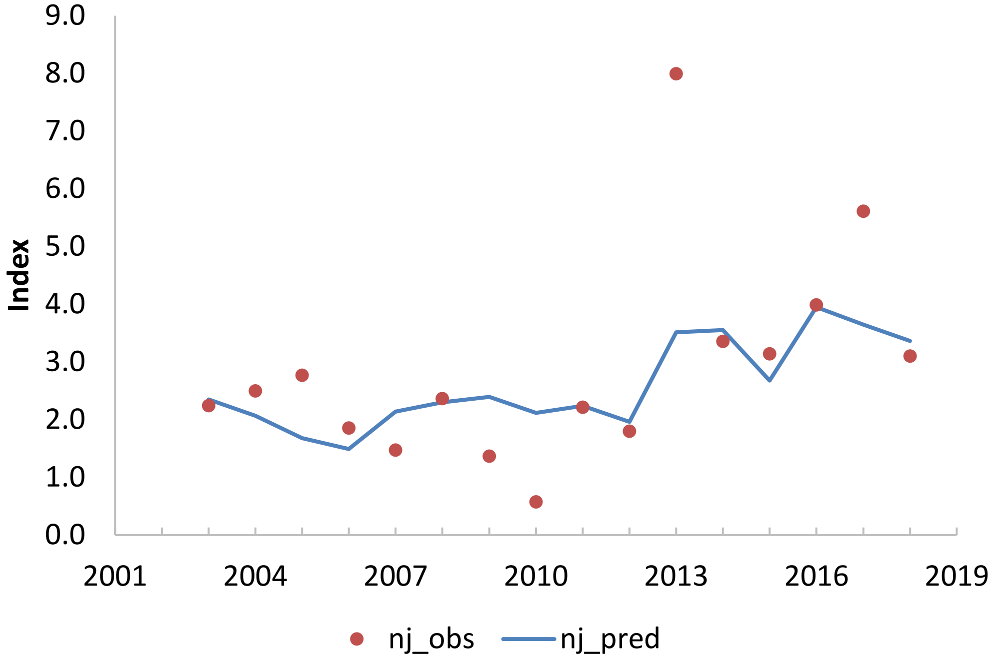

Figure 105. **CMSA model fit to New Jersey Ocean trawl survey aggregate adult female index.**

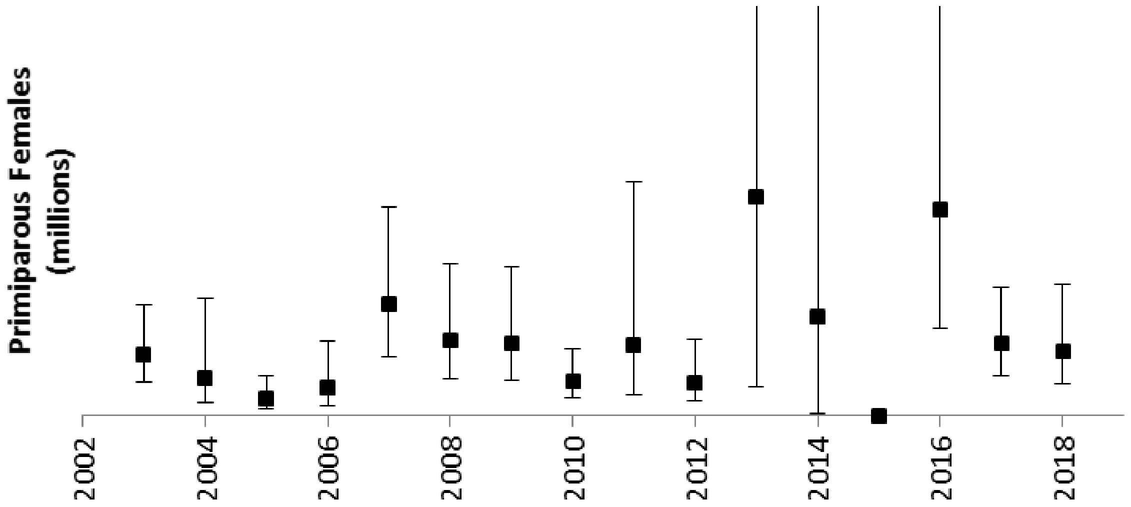

Figure 106. CMSA model estimated primiparous female abundance with lower and upper 95% confidence limits. Upper confidence limits for 2013, 2014, and 2016 extend beyond y-axis with values of CONFIDENTIAL. Y-axis values have been removed due to CONFIDENTIAL data.

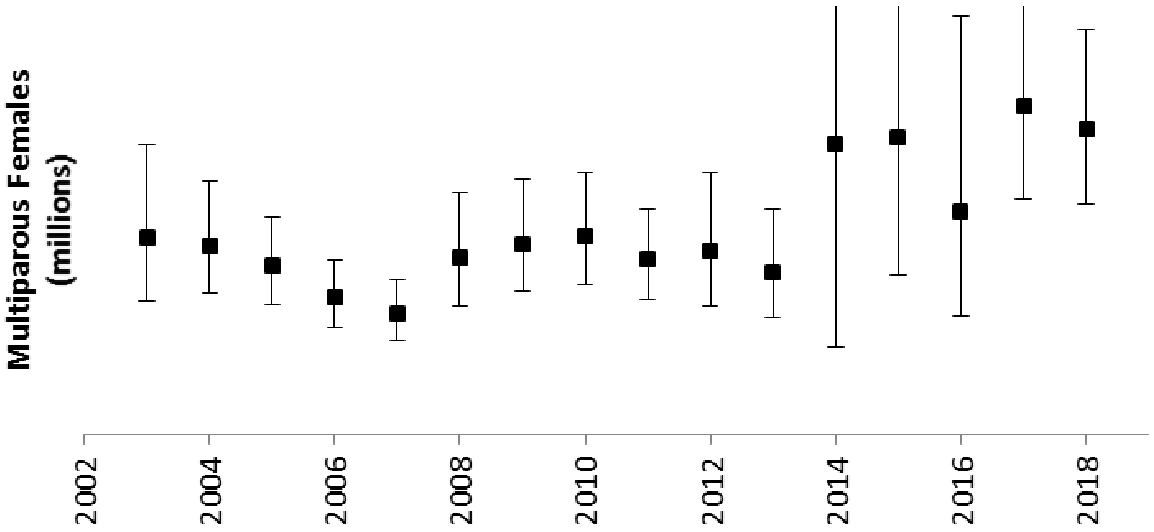

Figure 107. CMSA model estimated multiparous female abundance with lower and upper 95% confidence limits. Upper confidence limits for 2014, 2015, and 2017 extend beyond y-axis with values of CONFIDENTIAL. Y-axis values have been removed due to CONFIDENTIAL data.

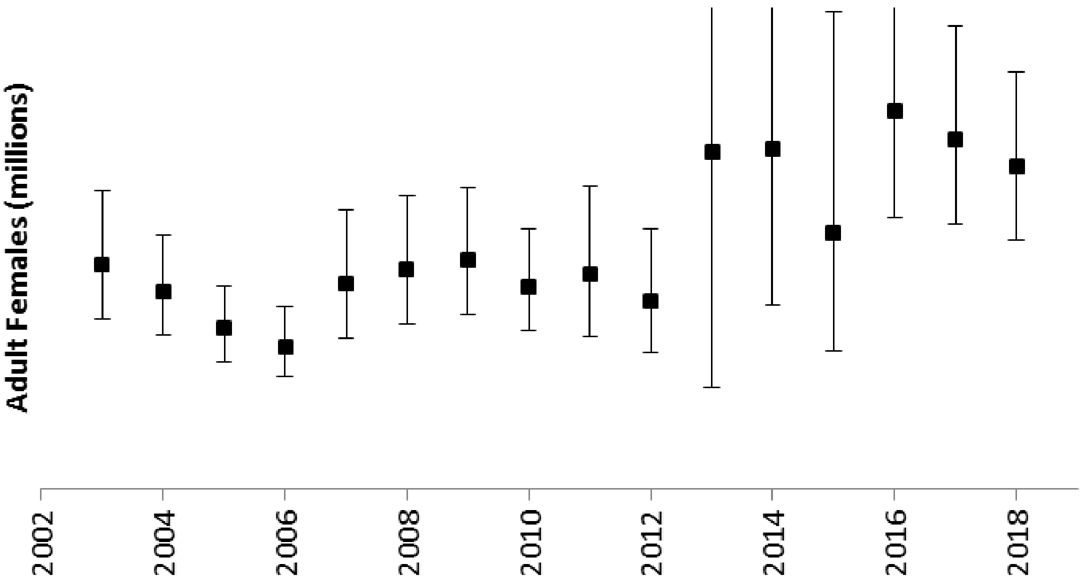

Figure 108. CMSA model estimated adult (primiparous + multiparous) female abundance with lower and upper 95% confidence limits. Upper confidence limits for 2013, 2014, and 2016 extend beyond the y-axis with values of CONFIDENTIAL. Y-axis values have been removed due to CONFIDENTIAL data.

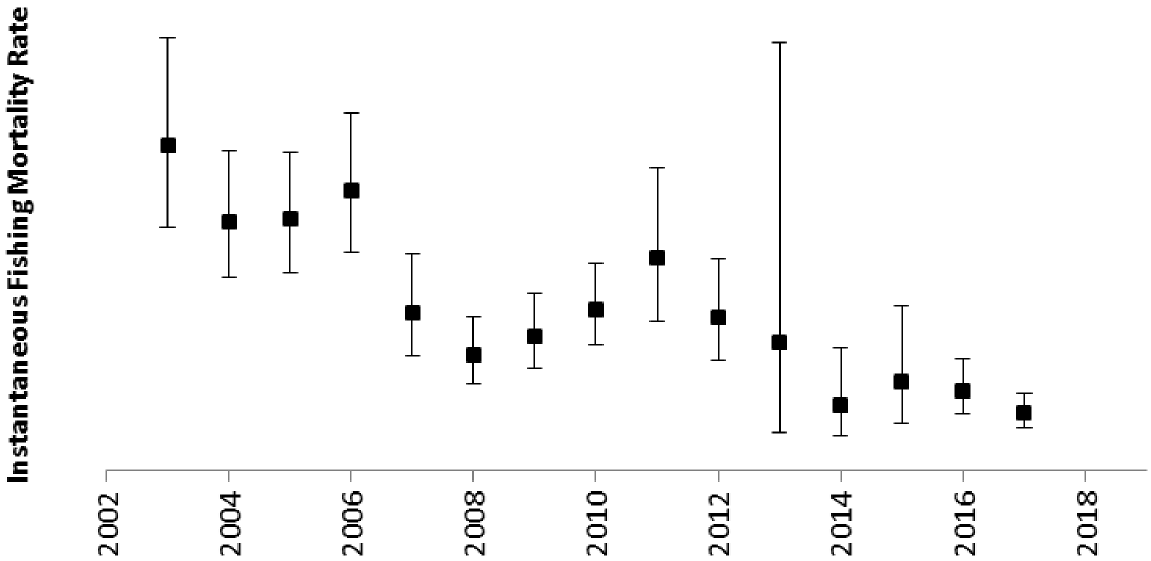

Figure 109. CMSA model estimated instantaneous fishing mortality rate F with lower and upper 95% confidence limits. Y-axis values have been removed due to CONFIDENTIAL data.

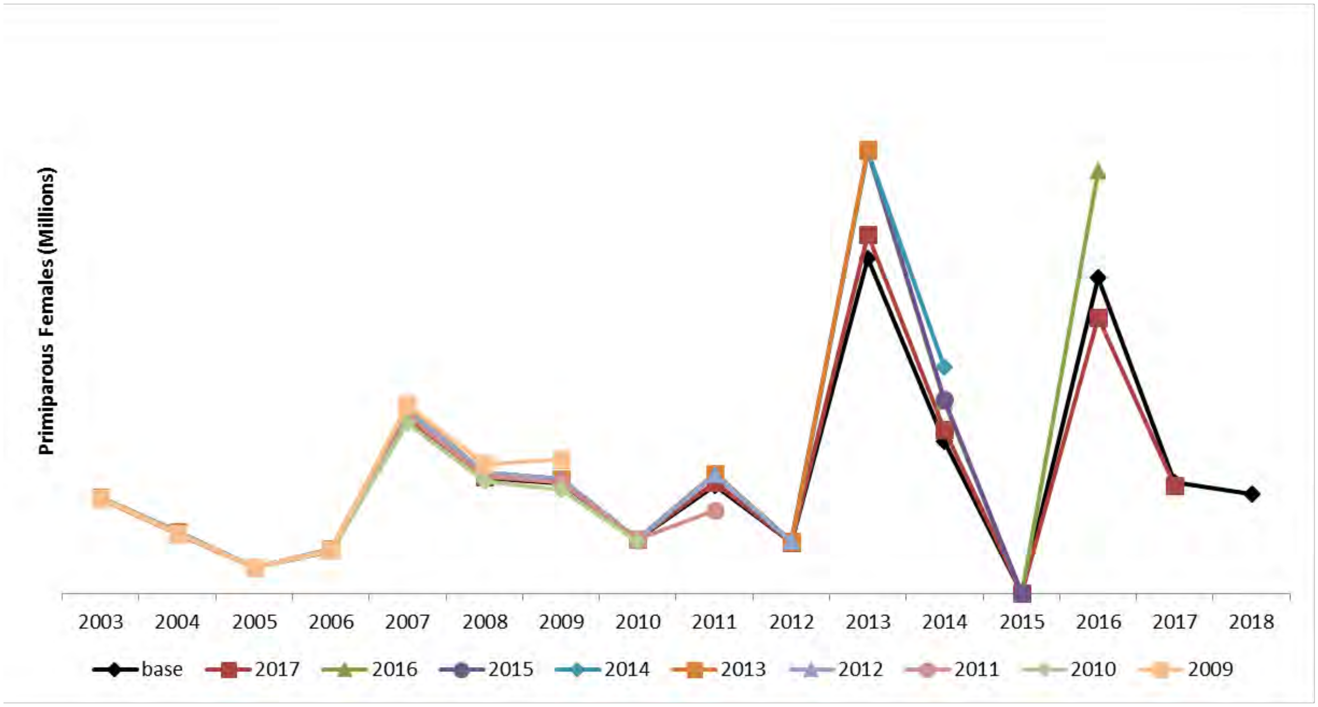

Figure 110. Retrospective peel of estimated primiparous abundance to 2009. Y-axis values have been removed due to CONFIDENTIAL data.

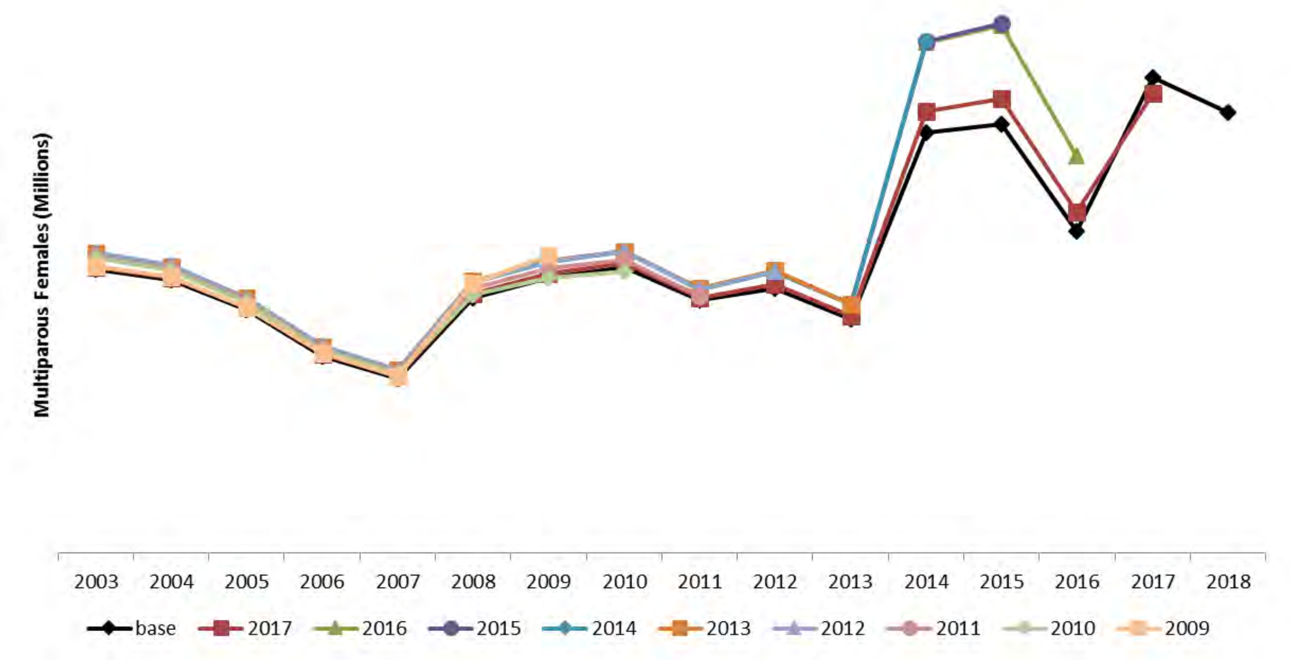

Figure 111. Retrospective peel of estimated multiparous abundance to 2009. Y-axis values have been removed due to CONFIDENTIAL data.

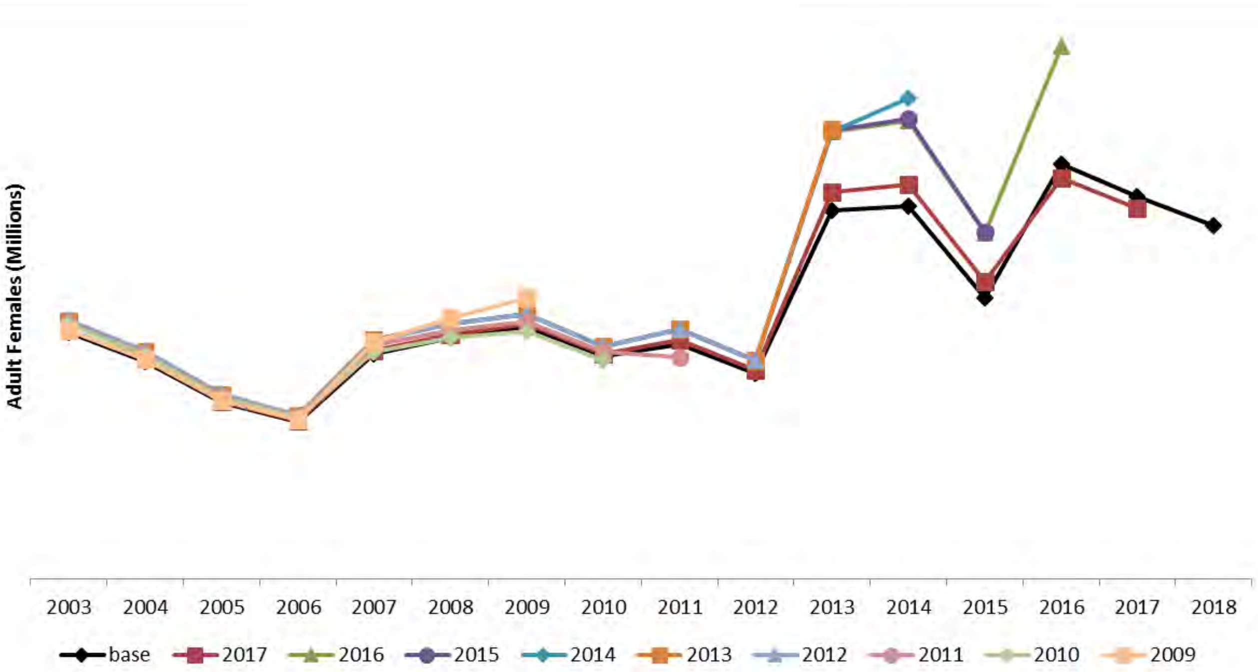

Figure 112. Retrospective peel of estimated total adult female abundance to 2009. Y-axis values have been removed due to CONFIDENTIAL data.

[Figure Removed Due to **CONFIDENTIAL** Data]

Figure 113. Terminal estimates of stock size and instantaneous fishing mortality rate from sensitivity runs.

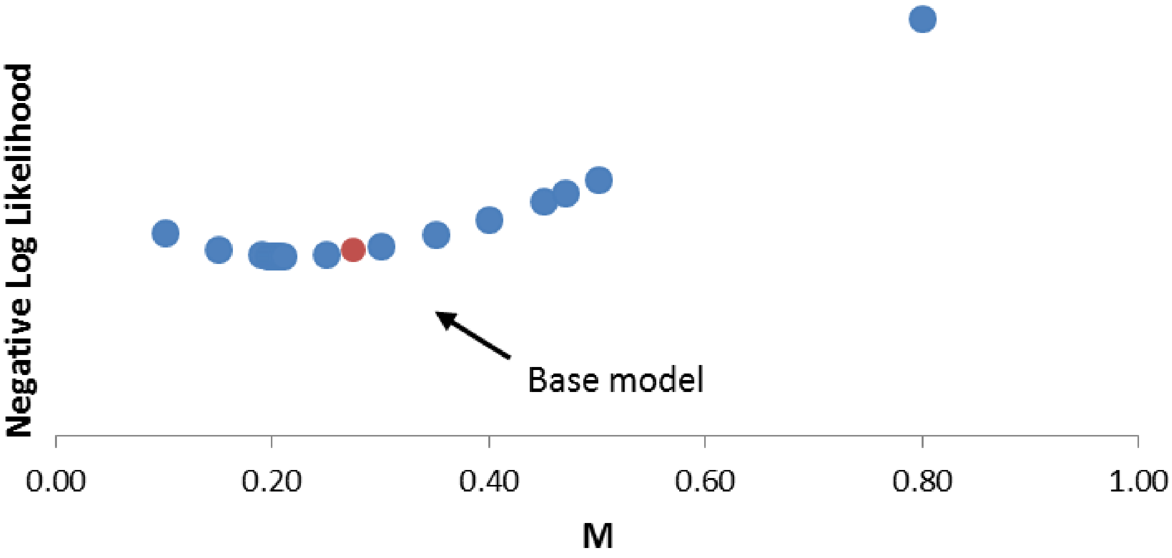

Figure 114. **Likelihood profile of base CMSA model runs with varying M inputs. Y-axis values have been removed due to CONFIDENTIAL data.**

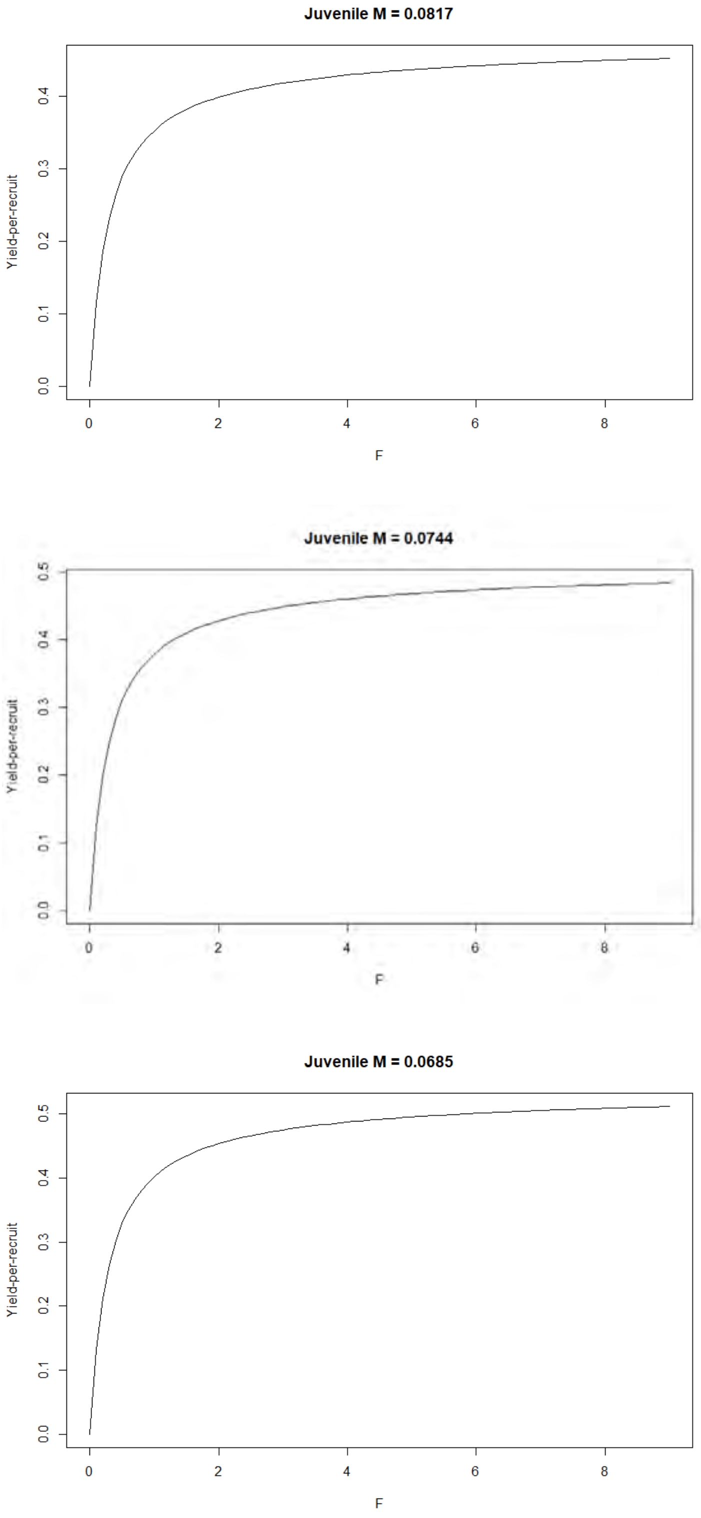

Figure 115. **Yield-per-recruit model results for horseshoe crab for each level of juvenile mortality. The estimated YPR did not decline with high levels of F in any case.**

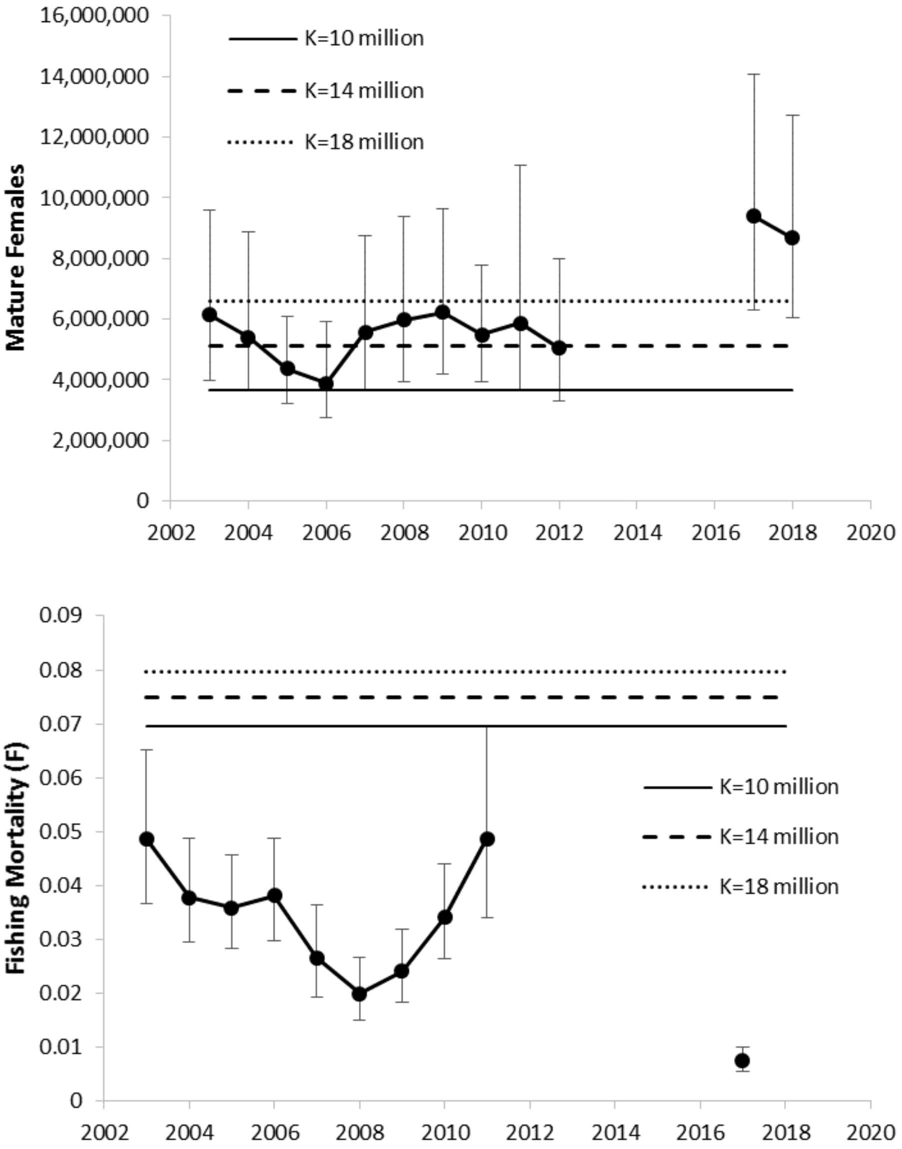

Figure 116. Stock status of female horseshoe crabs in the Delaware Bay with biomedical data is CONFIDENTIAL. Graphs have been replaced with non-confidential data that do not include biomedical data and therefore does not represent the best data for determining stock status. Comparing terminal year estimates of the number of mature females and fishing mortality showed that females are not overfished and overfishing is not occurring. The horizontal lines on the graphs indicate the reference points (B_{MSY} and F_{MSY}) generated from the theoretical population projection model under various assumptions of carrying capacity (K).

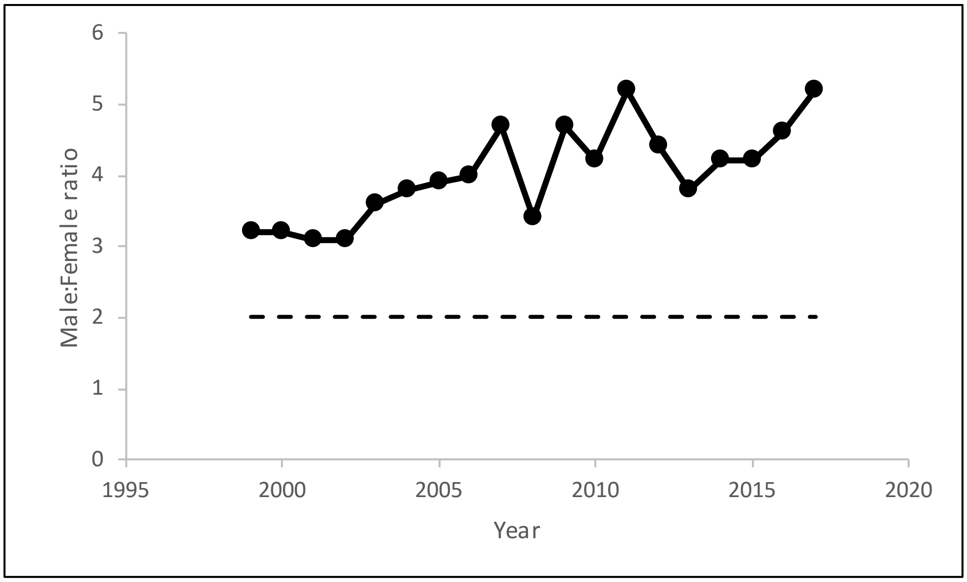

Figure 117. Sex ratio of Delaware Bay horseshoe crabs from the Delaware Bay spawning survey. The terminal year sex ratio was greater than the 2.0 reference point indicating that the male population is not overfished and there was no declining trend in the sex ratio indicating that overfishing was not occurring.

12 APPENDIX A: Biomedical Workgroup Reports

12.1 Biomedical Best Management Practices

The scope of discussion for the best management practices (BMPs) was limited to the collection, bleeding, and release of crabs collected solely for biomedical purposes. However, the WG recognized that these same practices must also be used when collecting crabs that will ultimately go to the bait industry to ensure a quality product for the biomedical and bait industries. However, the focus of this discussion was on biomedical-only crabs.

Collection

- **For targeted horseshoe crab trawl tows, reasonable tow times, recommended at 20-30 minutes bottom time (winches locked)**
- **Proper care and handling of horseshoe crabs while sorting and placing into bins**
- **Avoid exposure to direct sun, extreme temperatures as well as rapid temperature changes**
- **Night harvesting is recommended during periods of excessive heat**
- **During collection, sort out juveniles and do not bleed**
- **Sort out and return to the water individuals that do not appear to be healthy (damaged, slow movement, dull shell/old)**
- **When possible, release juveniles or unhealthy individuals immediately and do not transport to the facility**
- **Educate collectors in proper handling techniques**
- **Specify expectations of collectors in written contracts**
- **Periodically audit horseshoe crab collectors on implementation of BMPs for collecting**

Transport to Facility

- **Maintain temperature between approximately ambient water temperature at time of collection and 10ºF below ambient-water temperature**
- **Maintain good ventilation while stacked in bins**
- **Limit number of horseshoe crabs to a suitable number, dependent on container size and shape, and avoiding over-stacking to minimize damage to other horseshoe crabs**
- **Minimize travel time**
- **Keep bins and horseshoe crabs covered to protect against direct sunlight**
- **Secure containers in transport vehicle**

Holding at Facility/Preparation for bleeding/Bleeding

- **Limit holding time, under normal circumstances, at the facility to less than 24 hours**
- **No prolonged exposure to fresh water**
- **Follow written procedures for proper care and handling when sorting horseshoe crabs and moving them between bins and within the facility**

- **Inspect crabs for health and damage, selecting only undamaged and healthy crabs for bleeding**
- **Maintain clean, sanitary conditions during bleeding**
- **Maintain same level of care for rejected crabs that are not bled while they are being held until released back to sea**
- **Avoid bleeding crabs more than once per year**
- **If crabs are marked to avoid re-bleeding, ensure that the mark is residual and not harmful to the crab**
- **Bleed until rate slows down so that excessive bleeding is prevented**
- **Continue 30-year policy of not attempting to suction additional blood from the horseshoe crabs**
- **Perform internal audits to maintain quality control over written procedures**

Post-Bleeding Holding

- **Recognizing that the horseshoe crabs are now stressed from the bleeding process, maintain the same level of care as that used when transporting horseshoe crabs into the facility for bleeding**
- **Return to the water as soon as possible. If not being returned to the area of capture, ensure that conditions (salinity, water temperature, etc.) are similar to those found at the harvest site**
- **Minimize holding time post-bleeding**
- **While in holding, keep horseshoe crabs in the dark to minimize movement and injury**
- **Keep horseshoe crabs well-ventilated, moist, and allocate only a suitable number of crabs to holding containers**
- **Do not keep crabs out of the water for longer than 36 hours in total**

Return to Sea

- **Use same care in handling and transporting crabs being returned to the water**
- **Include return written instructions and requirements within contract with collectors, if applicable**
- **Periodically audit horseshoe crab collectors on implementation of BMPs for returning**

Overarching practices for all steps

- **Generate written procedures for all handlers of horseshoe crabs, covering all steps in the process from collection to release**
- **Keep horseshoe crabs cool, moist and covered, avoiding direct sunlight**
- **Establish a dialogue among collectors, the biomedical company, and the state regulatory agency to address concerns and challenges**

- Have a written contract between collectors and the biomedical company, outlining practices and expectations
- Perform audits of the various steps and contractors/employees throughout the process
- Ensure proper monitoring and recording of mortality at each step in the chain of custody

Other opportunities-Dual use of bait horseshoe crabs

The WG agreed that dual use of bait horseshoe crabs should be encouraged where possible but not required due to differing state regulations and the challenges of transport, volume, and timing. Depending upon capture and facility location, travel time may exceed what is practicable to maintain the health of the horseshoe crabs during transport to a biomedical facility. Additionally, the bait industry tends to collect a large volume of crabs within a short period, such that a biomedical facility would not be able to keep up with that volume in that time frame. Company representatives felt that licensing issues would not be a major challenge to using more bait crabs in the biomedical process first; rather, it would be the logistics of coordinating harvesters and their volume of catch in order to increase the use of bait crabs.

Review of Bleeding Mortality reports

There was some discussion that given recent findings and the wide variation in testing conditions and mortality results in bleeding studies, a formal peer review of the published studies might be considered. Publication of such a report could reduce some of the conflicting views currently expressed by various interests. Such a report could also frame future research avenues.

Summary

This report establishes BMPs for the various steps throughout the biomedical process, from harvest to release. Many of these practices are already in use by the biomedical companies, in order to sustain the horseshoe crab population and ensure a steady and reliable supply of product to the pharmaceutical market. The WG recommends that biomedical facilities follow these practices and monitor their suppliers. The WG also recommends holding future meetings to discuss opportunities to further decrease mortality. Given the recent and expected future increased demand for LAL, such periodic meetings are essential for continued successful management of the horseshoe crab resource along the Atlantic coast.

12.2 Northeast Region Biomedical Literature Summary

There is only one biomedical facility in the northeast, Associates of Cape Cod (ACC) which is located in Falmouth, Massachusetts. The Massachusetts Division of Marine Fisheries gives ACC a letter of authorization (LOA) each year allowing them to receive horseshoe crabs for biomedical use. These letters follow the Best Management Practices (BMPs) outlined by the ASMFC Biomedical Working Group (http://www.asmfc.org/uploads/file/biomedAdHocWGReport_Oct2011.pdf), and also includes state-level permit requirements. Included in the LOA are specifications as to what temperature the crabs should be held (50-60˚ F during transport, ≤70˚ F in laboratory), a marking requirement to prevent re-bleeding the same crab within the same year, a requirement to keep crabs moist, a limit to how many crabs can be stacked on top of each other while held in barrels, a requirement to release biomedical crabs to the embayment they were collected from, and other requirements. Crabs are typically out of the water for less than 26 hours (personal communication B. Hoffmeister, ACC, March 2018). MA DMF regularly visits ACC to collect data and ensure the terms of the LOA are being followed. Three papers have been published on the impacts of bleeding horseshoe crabs for biomedical purposes in the northeast region; Kurz and James-Pirri (2002), Leschen and Correia (2010), and Anderson et al. (2013). Both male and female crabs are bled by the biomedical industry, but all three papers from the northeast have focused solely on female crabs. The methods and results from these papers have varied (Table 1).

Kurz and James-Pirri (2002) attached an acoustic tag to ten bled and ten non-bled female crabs and released the crabs within half an hour of taking them out of the water. Two bled crabs and one non-bled crab were never detected again. It is unknown if these crabs left the survey area or died out of the water where they could not be detected. Making the assumption that the crabs had left the survey area, the reported mortality rate for bled crabs was 20% and 0% for non-bled crabs (Table 1). The two confirmed mortalities were found dead 28 and 68 days after being bled. There was no significant difference in the amount of movement between bled and un-bled crabs or in the spatial distribution of bled and un-bled crabs. Bled crabs appeared to exhibit more random directional movements compared to un-bled crabs, thus the authors suggested the bled crabs may have been disoriented after bleeding. Crabs in this study were subjected to conditions better than current BMPs. Time out of the water did not exceed half an hour and the crabs spent minimal time being transported to and from the collection site.

Table 1. Summary of three biomedical horseshoe crab bleeding mortality papers from the northeast region. All three studies focused only on female crabs. Control = non-bled crabs.

		Sample Size		Mortality Rate		
Study	Treatment	Control	Bled	Control	Bled	BMPs Followed
Kurz and James-Pirri 2002	1:Control	10		0%		Yes
	2: Bled		10		20%	Yes
Leschen and Corriea 2010	1: Control, 4hr exp.	98		3.1%		Yes
	2: Bled, 6 hr exp		89		22.5%	Yes
	3: Bled, 25 hr exp.		94		29.8%	Yes
Anderson et al. 2013	1: Outdoor ind. enclosures	7	7	Unreported	0%	No
	2: Indoor running wheel	7	7	Unreported	14%	No
	3: Indoor ind. enclosures	7	7	Unreported	14%	No
	4: Outdoor communal	7	7	Unreported	42%	No

Leschen and Correia (2010), in cooperation with ACC, also looked at post-bleeding mortality of female crabs. Three hundred and ten crabs were collected by ACC's supplier, transported to ACC by ACC's staff in an ACC truck, and bled by ACC staff. Crabs with injuries prior to bleeding were removed from the study and current BMP methods were followed. Crabs were then sent to a research laboratory and held in tanks. Mortality rates from this study were 3% for un-bled crabs (control, treatment one), 22.5% for crabs bled and placed in tanks the same day (treatment two), and 29.8% for crabs bled and held overnight before being placed in tanks the next morning (treatment three) (Table 1). Crabs from all three treatments were mixed together amongst six tanks. Within the bled treatments, 84.3% of the mortalities occurred by day six. An analysis of deviance revealed that treatment and tank were significant factors in explaining mortality. While bled crabs had a significantly higher mortality rate than un-bled crabs, the significant tank effect shows that something else also contributed to crab mortality rates.

Anderson et al. (2013) followed the "high stress" methods of Hurton and Berkson (2006) that the authors state "approximated the standard biomedical bleeding procedure". These methods drastically deviate from current BMPs. Fifty-six crabs were collected and split equally among four experiments which were sorted by crab size due to laboratory space restraints. The largest

14 crabs were placed into individual wire enclosures within an outdoor tank under natural light conditions (experiment 1). The smallest 14 crabs were placed on individual running wheels within partitioned indoor tanks (experiment 2). The remaining 28 crabs were split evenly among two experiments, one placed 14 crabs in a communal, indoor tank within individual enclosures (experiment 3), and the final 14 crabs were placed in a communal indoor tank and had 1-2 ml of blood drawn weekly to monitor hemocyanin levels (experiment 4). Half of the crabs in each experiment were bled, the rest were left as a control. Crabs that were bled were exposed to direct sunlight, temperatures reaching 37 ˚C, and held out of the water for 52 hours. Overall post-bleeding mortality rate was 18%. Mortality was highest in the experiment 4, where crabs were held communally at a very high density (27 crabs/m^2) and had hemolymph samples drawn multiple times post-bleeding (42% mortality) compared to experiments that partitioned crabs individually and handled them only once (0-14% mortality). The authors found that bleeding caused the crabs to be more sluggish and impacted their movement patterns when compared to crabs that were not bled and exposed to the “high stress” conditions.

Despite three peer-reviewed papers on the subject, given the wide range of mortality estimates produced there are still many questions as to how the bleeding process affects horseshoe crabs in the northeast region. The work reviewed above was conducted under a wide range of conditions and sample sizes, with varying study goals, making it difficult to compare results. Mortality rates from bled crabs ranged from 0% to 42% while reported mortality rates for un-bled crabs were less than 5%. There is evidence for negative effects of holding conditions (see also Coates et al. 2012), which likely compounded mortality estimates in some cases. There is obviously more work required to accurately estimate bleeding impacts to crabs (both lethal and sublethal). Given the wide range of mortality estimates published in the peer-reviewed literature for this region, any assignment of biomedical mortality rates for the assessment process must incorporate a sensitivity analysis. This would allow the assessment to produce model estimates of stock size over the range of potential biomedical impacts suggested by the best available science.

Literature Cited

Anderson, R. L., W. H. Watson III, and C. C. Chabot. 2013. Sublethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, *Limulus Polyphemus*. *Biological Bulletin* 225: 137-151.

Coates, C. J., E. L. Bradford, C. A. Krome, and J. Nairn. 2012. Effect of temperature on biochemical and cellular properties of captive *Limulus Polyphemus*. *Aquaculture* 334-337:30-38.

Hurton, L., and J. Berskon. 2006. Potential causes of mortality for horseshoe crabs (*Limulus polyphemus*) during the biomedical bleeding process. *Fisheries Bulletin* 104:293-298.

Kurz, W., and M.J. James-Pirri. 2002. The impact of biomedical bleeding on horseshoe crab, *Limulus Polyphemus*, movement patterns on Cape Cod, Massachusetts. *Marine and Freshwater Behaviour and Physiology* 35(4): 261-268.

Leschen, A. S., and S. J. Correia. 2010. Mortality in female horseshoe crabs (*Limulus polyphemus*) from biomedical bleeding and handling: implications for fisheries management. *Marine and Freshwater Behaviour and Physiology* 43(2):135-147.

12.3 Delaware Bay Region Biomedical Literature Summary

The Delaware Bay region is unfortunately lacking in quantity as far as independent biomedical research projects are concerned. However, the quality of the few papers available is fairly high. The two projects available for review are Hurton's 2003 thesis for VA Polytechnical Institute and Walls' and Berkson's 2003 Fisheries Bulletin entry. It should be noted that Hurton's thesis results were used to publish 3 subsequent papers that were also reviewed.

The most important part of these papers to consider when deciding how to judge the accuracy of mortality rates is the methods section. Hurton's methods were well designed and documented which helps lend credibility to the results. Two groups were analyzed for Hurton's experiment. The first group (n= 200, 100M 100F) was designated as the "low stress." This cohort was treated following BMPs. The second group (n= 195, 110M 85F) was designated as "high stress." This cohort was treated with external pressures beyond bleeding, including temperature fluctuation, salinity fluctuation, and other variables that horseshoe crabs may experience during transport and holding. Both groups were subjected to the same bleeding treatments: a control group of 0% bled, a group of 10% total hemolymph extraction, a group of 20% total hemolymph extraction, a group of 30% total hemolymph extraction, and a group of 40% total hemolymph extraction. In the low stress group, total mortality was 0%. In the high stress group, average mortality was 7.2% for combined males (6.4%) and females (8.24%). The highest recorded value of all five high stress treatments occurred with the 40% bled female group at 29.4%. It should also be noted that this is the only mortality value in the whole data set that is over 15% value currently used as the standard biomedical mortality rate.

The Walls Berkson study had comparable results to Hurton's thesis. Overall mortality for bled crabs was 8% (16 crabs) while unbled crabs had a total mortality of .5% (1 crab). The issue with this study is the lack of description in the methods section. The paper only states that the bled cohort "underwent BioWhittaker's normal bleeding process." Due to how drastically the treatment of crabs can vary I think this would have been an important place to include exact treatments, especially because the mortality levels were so low. The whole 3-year study included 8 separate cohorts resulting in a total of 200 unbled crabs and 200 bled crabs being observed. It should also be noted that all crabs in this study were MALE.

Paper	**Reported Mortality Rate**	**Sample Size**	**Adherence to BMPs**
Hurton, Berkson, Smith 2005	0% in low stress group 7.2% in high stress group (bled) 2.6% in high stress (unbled) 29.4% F crabs bled at 40% volume	Low stress group N = 200, bled crabs= 160 High Stress group N=195 , (110M 85 F) bled crabs = 156	BMPs followed in low stress group, purposely not followed in high stress group
Walls, Berkson 2003	unbled HSC average .5% (0-3.3%) bled HSC average 8% (0-30%)	total unbled N = 200 total bled N = 200	crabs "underwent BioWhittaker's normal bleeding process"

Literature Cited

Hurton, L. and J. Berkson 2005. Potential causes of mortality for horseshoe crabs (*Limulus polyphemus*) during the biomedical bleeding process. Fishery Bulletin 104:293-298.

Hurton, L., J. Berkson, and S. Smith 2005. Estimation of total hemolymph volume in the horseshoe crab *Limulus polyphemus*. Marine and Freshwater Behaviour and Physiology 38(2):139-147.

Walls, E.A. and J. Berkson 2003. Effects of blood extraction on the horseshoe crab, *Limulus polyphemus*. Fish. Bull. 101:457-459.

12.4 Southeast Region Biomedical Literature Summary

A total of 5 studies have been conducted through the South Carolina Department of Natural Resources over a >20-year time-frame to assess the mortality associated with biomedical processing of American horseshoe crabs, *Limulus polyphemus*. Most of these studies were conducted in collaboration with the biomedical bleeding facility located in Charleston, SC (Endosafe, Inc.). For these studies, horseshoe crabs were harvested and handled in accordance with industry standards by Endosafe before SCDNR representatives randomly selected individuals for control groups, which were not bled, and treatment groups, which were bled. Following this process, horseshoe crabs were then followed for 7-14 days to assess mortality. One study, Linesch (2017), did not use crabs provided by Endosafe, but rather collected crabs themselves and independently simulated the biomedical bleeding process including holding of crabs in ponds prior to extraction of hemolymph, and then following crabs for 12 days. Estimates of total mortality from these studies range from 6.6% to 20.4%, with a mean mortality estimate of 12.3%. Additional results from the Linesch (2017) study, as well a study conducted in the northeast region (Owings 2017), show that biomedical processing can reduce the physiological fitness of horseshoe crabs that survive biomedical processing. While the mortality of biomedically-bled horseshoe crabs after 14 days has not been assessed in the southeast, the reduced physiological function associated with biomedical processing suggests that there is an increased risk to mortality that extends beyond this 14-day window of previously-conducted experiments. As such, there does not appear to be sufficient evidence to change the current 15% mortality rate for biomedically-processed horseshoe crabs in the Southeast region.

Table 1. Summary of biomedically-related mortality assessments conducted in South Carolina

Citation	# of Crabs	Mortality	Study Description
SCDNR (1999)	267	6.60%	Selected crabs from biomedical facility (133 un-bled, 134 bled). Tracked for 14 days
Thompson (1999)	40	15.00%	Selected crabs from biomedical facility (20 un-bled, 20 bled). Tracked for 7 days
Wenner & Thompson (2000)	150	8.30%	Selected crabs from biomedical facility (75 un-bled, 75 bled). Tracked for 14 days
DeLancey & Floyd (2012)	100	20.40%	Selected crabs from biomedical facility (50 un-bled, 50 bled). Tracked for 14 days
Linesh (2017)	96	11.00%	Hand-harvested crabs from beach (48 un-bled, 48 bled). Tracked for 12 days

Literature Cited

DeLancey L & Floyd B (2012). Tagging horseshoe crabs*, Limulus polyphemus*, in conjunction with commercial harvesters and the biomedical industry in South Carolina. Final report South Carolina Sea Grant Consortium. Project #: R/CF-14.

Linesch K (2017) Evaluating best management practices in the South Carolina horseshoe crab fishery: A field approach to determine physiological impacts of the biomedical bleeding process. Thesis, College of Charleston.

Owings M (2017) Effect of the biomedical bleeding process on the behavior and physiology of the American horseshoe crab, *Limulus polyphemus*. Thesis, University of New Hampshire.

SCDNR (1999). An evaluation of *Limulus* post-bleeding mortality experiment data.

Thompson (1999) Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in South Carolina. Thesis, Medical University of South Carolina.

Wenner E. & Thompson M. (2000). Evaluation of harvesting impacts and population trends for *Limulus polyphemus* in South Carolina. Final report to NOAA, MARFIN Grant #NA87FFO431.

12.5 Biomedical Literature Review by Dr. James Cooper

The LAL Biomedical Industry Impacts Positively on Horseshoe Crab Sustainability

Presented to the Stock Assessment Subcommittee of ASMFC by James F Cooper, PharmD*

About the Author

James Cooper in 1970 pioneered development of the LAL test as a sensitive detector of endotoxin (pyrogen) for injectable drug products. His publications span the history of LAL technology and horseshoe crab (HSC) conservation. He founded Endosafe Inc. in 1987. He is a consultant on endotoxin issues and is a retired Professor of Pharmaceutical Sciences at the Medical University of South Carolina, Charleston. In 1997 he began ASMFC service as a member of the team, headed by Tom O'Connell that drafted the Fisheries Management Plan for HSC. He has served on the HSC Advisory Panel since that time, including position of Chair for the past 12 years.

Introduction

This discussion briefly describes the life cycle of the Horseshoe crab (HSC) and critiques studies that attempt to understand the impact of biomedical bleeding processes upon donor crabs. Our understanding is incomplete, but we know a great deal about where and how HSC live. HSC are significant to the ecology of shallow-water marine life as prey, predators and hosts to a diverse array of epibionts on their shell, appendages and gills (Shuster and Sekiguchi 2003). These hitch-hikers affix to or infest HSC exterior surfaces and are significant factors in the aging and ultimate fate of their hosts. HSC require about nine-to-ten years and 16-18 molts to reach sexual maturity. In their early stages they are highly vulnerable to prey by birds, fish and other crustaceans. Juveniles and adults are prey for loggerhead turtles, sharks and other large sea creatures. Adults that are stranded on the beaches of Delaware Bay are susceptible to attack by laughing gulls. Humans negatively impact by loss of habitat (e.g., commercial and housing development) and exploitation of the resource. During spawning their eggs provide nutrition for a vast array of migratory shorebirds.

Large juveniles and adult HSC are opportunistic foragers in tidal flats and the ocean floor. In tidal flats, they prefer feeding on soft-shell clams and marine worms. A high concentration of large HSC predators, such as in Delaware Bay and the ACE Basin of South Carolina, has great impact on benthic invertebrates. Botton et al. (2003) observed that bivalves were the major diet of HSC in Delaware Bay tidal flats. Their examination of crabs dredged off the New Jersey coast found that their guts were stuffed with an average of 400 blue mussels.

Impact of the LAL Biomedical Industry on Horseshoe Crab Sustainability

The LAL biomedical industry impacts positively on HSC conservation because the importance of LAL makes them extremely valuable to mankind. Cooper and Levin (1971) developed a screening test for bacterial endotoxin in injectable drugs from LAL (*Limulus* amebocyte lysate) reagent. Subsequently, a robust LAL production industry (biomedical) flourished in the 1970s to meet the needs of the pharmaceutical and medical device industry. Five firms currently produce LAL. The LAL producers applied a return-to-sea policy from the outset. In 2011 they met with ASMFC to formalize Best Management Practices (BMPs). Mortality doesn't occur during the bleeding process of donor crabs, which is consistent with human blood donation.

Investigators designed experiments to determine if there was significant post-bleeding mortality after release of donor crabs to the environment. Reported mortality rates varied greatly. (Table 1). Rudloe (1983) observed a 11% loss in a release-and-recapture study in a Gulf Coast bay. Thompson (1998) found 15% mortality in a small study where 40 bled and un-bled HSC were kept for a week in a shallow sea-water tank. Dave Yadon (1999) observed an 8.3% loss where 252 bled and un-bled HSC were retained in a shallow sea-water tank. Walls and Berkson (2003) reported a loss of 8% where 400 HSC were held in replicated flow-through tanks for 2 weeks. Hurton and Berkson (2006) reported no loss under low-stress conditions, but a 8.3% loss under high-stress conditions. The results of these reports prompted the ASMFC to assign 15% as the estimated post-release mortality from biomedical processing.

A robust report by Linesch (2017) is consistent with the above studies. Linesch held 100 bled and 100 un-bled donors in low-density seawater ponds at Waddell Mariculture Center in Bluffton SC for up to 8 weeks. Mortality was 11%. This study generally emulated practices of a South Carolina LAL-production facility. An exception was that Linesch only studied females. In contrast, the LAL facility currently observes a 2.6 male/female ratio so that only 30% of donors are females. She observed that a high carapace epibiont load impacted negatively on physiological health metrics.

Two studies reported mortality significantly greater than previous reports. A small study of 56 crabs reported an 18% loss (Anderson, *et al*., 2013). The excessive stress and containment in multiple small tanks rendered the experimental conditions as not representative of biomedical LAL practices and unaligned with BMPs. For example, specimen were subjected to long periods out of water and high temperatures, methods that are offensive and not justifiable. The report speculated that bleeding suppressed spawning activity, but other reports contradict this claim (Linesch 2017; Spawn 2018; Figure 1).

More puzzling was the study by Leschen and Correia (2010) that reported the effects of two LAL treatment methods on HSC held in salt-water tanks at the MBL (Marine Biological Laboratory) in Woods Hole, MA. Female horseshoe crab were separated into three treatment groups of 99, 89 and 93; treatments were intended to emulate the processing of HSC at a nearby LAL firm.

Group 1 was the control group that was held out of water for 4 hours. Groups 2 and 3 were exposed to conditions mimicking an open boat deck, one-hour drive in a non air conditioned truck, being stored for 24 hours stacked in 30-gallon totes at room temperature, one-hour truck ride and a 15-minute boat ride. The HSC from Group 2 were held for six hours after the biomedical process and the HSC from Group 3 held overnight for 25 hours. Mortality of the un-bled control HSC was low (3%) and differed significantly from that of either bled group (22.5% and 29.8%, respectively).

The methods section specified that three groups of HSC were held in six flow-through seawater tanks that contained 5 cm of sediment. Tanks differed by volume and shared a common source and flow of seawater. A similar number of HSC from each treatment group and control were assigned to each tank. Although the mortality of HSC was similar for the two treatment groups, there was a significant difference in mortality with respect to the six tanks. Mortality did not align with treatment group. Since the author's data in Table 3 obscured the variation by tank, their results were reconfigured to reveal the unexplained variation in mortality by tank (Fig. 2), which the authors admit. The tank rates varied from 8.7% to 48%. There were apparently three populations in the study. The mortality rate for tanks 1 and 4, which contained 55 HSC, was 12.7%. The mortality rate for tanks 2, and 6, which contained 72 HSC, was 26%. The rate for tanks 3 and 5 was 45%; one control crab died in each of these tanks. Mortality was determined by multiplying the predicted mortality rate times the number of crabs per tank per treatment (Table 3 of Leschen and Correia 2010). This unexplained difference in mortality indicated that there was an apparent risk factor in at least two of the tanks, such as a chemical or microbial contaminant, or failure to maintain a condition, such as oxygen, that impacted negatively on female HSC that were stressed by bleeding. The reported 45% mortality rate for tanks 3 and 5 is significantly distinct from the other 4 tanks in the study as well as previously reported estimated mortality studies. In summary, this study encountered unforeseen experimental circumstances that apparently produced falsely-high, post-release mortality estimates. This flawed study should be excluded from reports that are used to define LAL-related mortality estimates.

For an estimated mortality to be applicable to biomedical procedures, it must be consistent with the Best Management Practices (BMPs) accepted by the ASMFC and LAL firms in 2011. These procedures generally describe the collection, inspection, handling, bleeding, training and return policies of biomedical firms. The value of excessive-stress studies that do not follow the BMPs are only indicative of the resilience of donor crabs in the presence of taxing conditions. The great limitation of most HSC mortality studies is that the donor crab is not returned to a preferred foraging site (see above), such as a tidal mud flat, but is retained in an artificial container in a high density. Also, control of ambient salinity, temperature, acidity and oxygen is challenging. HSC are 2-to-5 kg, large animals that generate considerable waste that must be managed. The ideal mortality experiment would require study of recently molted crabs that were returned to a natural environment, a costly and challenging experiment to manage.

Technical advances reduce LAL needs. Charles River Labs attained FDA approval for a LAL-cartridge based system that reduces the need for LAL by 95%. Recombinant LAL products (rFC) are being evaluated for robustness, specificity and sensitivity. The FDA has zero tolerance for endotoxin contamination and will not approve these products until they are validated as equivalent and specific as LAL for endotoxin detection. Several years of product development and costly validation will be required before drug regulators and pharmaceutical industries will rely on a recombinant product.

Finally, the biomedical community has had a positive impact on HSC populations through 45 years of consistent conservation practices. The actual mortality from biomedical procedures is likely in single digits because the reported mortality studies present worst-case estimates. Biomedical efforts have produced either limits or bans for the HSC bait fishery in South Carolina and the Delaware Bay area. Public education is important; by placing a value on HSC for LAL, the public reveres HSC and watermen no longer destroy HSC collected as by-catch.

Conclusions

- Simulated post-bleeding mortality studies that are generally compatible with the biomedical BMPs indicate that the estimated biomedical mortality is less than 15%.
- Post-bleeding mortality studies that are generally incompatible with the biomedical BMPs are irrelevant to a mortality estimate and should not be used for that purpose.
- There is no credible evidence that biomedical use threatens the sustainability of the horseshoe crab or availability of eggs for migratory birds.
- The net effect of the biomedical industry for HSC sustainability is positive because of consistent and unique conservation efforts.

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12.6 Biomedical Literature Review by Benjie Swan

Biomedical Use and Mortality Rates of Horseshoe Crabs, *Limulus polyphemus*

Benjie Swan

Limuli Laboratories

Cape May, New Jersey

Introduction

A horseshoe crab fishery has existed since the late 1800's with millions caught and reported, predominately in the Delaware Bay. The early harvests of horseshoe crabs became fertilizer for farm fields and feed for livestock until the 1900's. The large annual harvests of horseshoe crabs eventually dwindled and attention on the horseshoe crabs focused on its study with respect to human physiology and health which earned Dr. Hartline a Nobel Prize for his work. In the 1980's and 1990's, the horseshoe crab fishery grew as more horseshoe crabs were being harvested for eel and conch bait. At the same time, the link was discovered between horseshoe crabs and migratory shorebirds. The monumental importance of the horseshoe crab became apparent and the need for its management was recognized.

In response to the bait harvest of the horseshoe crabs, State regulations were introduced and eventually, a coast wide management plan was adopted in 1998 by the Atlantic States Fisheries Commission (ASMFC). Unlike other fishery plans, the Interstate Fishery Management Plan (FMP)for the Horseshoe Crab considered both the sustainability of the horseshoe crab population and the dietary demands of the migratory shorebirds. (The shorebirds feed on horseshoe crab eggs that are brought to the surface by large numbers of spawning horseshoe crabs.) Focusing primarily on the Delaware Bay region, seven addendums to the FMP followed, reducing the bait harvest of almost 3 million in 1998. Under the FMP, a current quota of 1,587,274 horseshoe crabs is allowed, however the 2016 actual harvest of 787,223 was much lower due to some states being more restrictive.

Another unique component of the horseshoe crab fishery is that horseshoe crabs are collected and used to manufacture, Limulus Amebocyte Lysate (LAL), a product critically connected to human health. This rather obscure product has tested human injectable drugs and medical devices from potentially life threatening bacteria for almost 50 years. LAL is produced from live horseshoe crabs, a marine species and horseshoe crabs, similar to human blood donors, are bled and then released alive in order to make the product.

Horseshoe crabs collected for manufacturing LAL are categorized as "biomedical" and governed separately from the bait fishery due to its critical use and the low mortality associated with the process. From the ASMFC Fishing Year Reports spanning the years 2004 to 2016, the average number of horseshoe crabs collected for biomedical use is 462,670, with 5,086 reported dead. It is presumed that some of the horseshoe crabs may die after bleeding and that average is 58,721 from the same time frame (Table 1).

Table 1. Biomedical horseshoe crab mortality numbers.

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Reported Observed Mortality of Horseshoe Crabs for Biomedical Use	4,391	4,256	4,639	3,599	2,973	6,523	6,447	8,485	7,396	5,485	5,658	5,250	1,015
Estimated Post Bleeding Mortality of Bled Biomedical-Only Crab (15% est.mortality)	41,279	40,574	44,543	59,833	60,312	53,252	65,319	75,117	74,882	65,535	64,846	70,118	47,765
Number of Crabs Brought to Biomedical Facility (bait and biomedical crabs)	343,126	323,149	367,914	500,251	511,478	512,853	552,083	623,680	624,440	554,419	536,798	564,526	426,195*

As more attention is focused and more information is gathered on the horseshoe crab population and harvest numbers, more precise accounting is being called for, specifically for the Stock Assessment. Although the number of horseshoe crabs that die in order to manufacture LAL is a miniscule fraction of the coast wide population of horseshoe crabs, pressure has been placed to continually revisit the mortality rates. The number of horseshoe crabs that die due to biomedical processing is being analyzed as there is a large discrepancy between what the biomedical companies report as dead and the number of horseshoe crabs that are estimated to die because of the "bleeding" process. This paper examines the mortality studies associated with biomedical processing and assesses their applicability (Table 2).

History of LAL

The use of horseshoe crabs to manufacture LAL began with the discovery by Dr. Frederick Bang, a professor at Johns Hopkins University. He observed that the horseshoe crab's blood would clot when injected with live or dead gram negative bacteria (Bang 1956) and, working with Jack Levin, discovered the clotting phenomenon was localized in the amebocytes or white blood cells of the horseshoe crab (Levin and Bang 1964). James Cooper, a graduate student at Johns Hopkins and an employee of the United States Food and Drug Administration (US FDA) applied the horseshoe crab derived product to test radiopharmaceuticals for bacterial contamination. The in vitro (Limulus) test was more sensitive, easier to use and less costly than the in vivo (rabbit) test used at the time (Cooper et al. 1971 and 1972). In 1973, the US FDA declared that LAL was a biological product and was subject to licensing requirements as provided in Section 351 of the Public Health Service and in 1977, issued licensing for LAL production. Once the FDA Draft Guidelines for the LAL test was published in 1987, its use became more widespread and gradually replaced the Rabbit Pyrogen Test.

Table 2. Mortality studies summary.

Study	Year	Type of Study	Collection Time of Year	Total Time Held for Bleeding Process	Time Held After Study	Sample Size	Sample Size Female	Sample Size Male	Number Unbled	Dead Females	Dead Males	Mortality Rate of Unbled HSC	Number Bled	Dead Females	Dead Males	Mortality Rate of Bled HSC
Rudloe	**1983**	Mortality/Tagging	April 28 to May 30th	Time out of water 30 minutes	0	10,259	*	*	5,437	38 Not Sexed	*	*	4,822	47 Not Sexed	*	10% First Year/11% Second Year
Rudloe	**1983**	Behavior/Tank	*	*	30 days	80	40	40	40	2 Not sexed	*	5%	40	1 Not Sexed	*	2.5%
Thompson	**1998**	Mortality/Tank	May	BMP	7 days	40	20	20	20	0	0	0%	20	0	3	15%
Thompson	**1998**	Mortality/ Tagging	May/June	BMP	0	1,328	851	477	734	15	6	2.86%	594	0	0	0.00%
Endosafe	**1999**	Mortality/Pond	20-May	BMP	14 days	267	133	134	120 recaptured	1	1	1.67%	132 recaptured	4	7	8.33%
Kurz and Pirrri	**2002**	Behavior/ Transmitters	July 2 to 8	Time out of Water 30 minutes	0	20 Total	20	0	10	0	0	0%	10	2	0	20%
Walls and Berkson	**2003**	Mortality/Tank	Jul-Aug	BMP	2 weeks	400 Total	*	400	200	*	1	0.5% Average	200	*	16	8.00% Average
		Trial 1	8-Jul	*	Jul 08-22,1999	*	*	20	10	*	0	0.0%	10	*	0	0.0%
		Trial 2	22-Jul	*	Jul 22-Aug 05, 1999	*	*	20	10	*	0	0.0%	10	*	3	30.0%
		Trial 3	19-Jun	*	Jun 19-Jul 03, 2000	*	*	60	30	*	0	0.0%	30	*	0	0.0%
		Trial 4	7-Jul-2000	*	Jul 07-21, 2000	*	*	60	30	*	0	0.0%	30	*	0	0.0%
		Trial 5	1-Aug-2000	*	Aug 01-15, 2000	*	*	60	30	*	1	3.3%	30	*	6	20.0%
		Trial 6	6-Jun-2001	*	Jun 06-20, 2001	*	*	60	30	*	0	0.0%	30	*	0	0.0%
		Trial 7	20-Jun-2001	*	Jun 20-Jul 04, 2001	*	*	60	30	*	0	0.0%	30	*	2	6.7%
		Trial 8	15-Aug-2001	*	Aug 15-29, 2001	*	*	60	30	*	0	0.0%	30	*	5	16.7%

Table 2. *Continued*.

Study	Year	Type of Study	Collection Time of Year	Total Time Held for Bleeding	Time Held After for Study	Sample Size	Sample Size Female	Sample Size Male	Number Unbled	Dead Females	Dead Males	Mortality Rate of Unbled HSC	Number Bled	Dead Females	Dead Males	Mortality Rate of Bled HSC
Hurton and Berkson	**2005**	Mortality/ Tank	9-Jul-2003	15-20 hours	2 weeks	Low Stressed	100	100	40	0	0	0.0%	160	0	0	0% Average
		Trial 1	*	*	*	10% Blood Extraction	*	*	*	*	*	*	40	0	0	0.0%
		Trial 2	*	*	*	20% Blood Extraction	*	*	*	*	*	*	40	0	0	0.0%
		Trial 3	*	*	*	30% Blood Extraction	*	*	*	*	*	*	40	0	0	0.0%
		Trial 4	*	*	*	40% Blood Extraction	*	*	*	*	*	*	40	0	0	0.0%
		Mortality/ Tank	28-Aug-2003	47 hours	2 weeks	High Stressed	85	110	39	0	1	2.6%	156	7	6	8.33% Average
		Trial 1	*	*	*	10% Blood Extraction	*	*	*	*	*	*	39	1	0	2.6%
		Trial 2	*	*	*	20% Blood Extraction	*	*	*	*	*	*	39	0	2	5.1%
		Trial 3	*	*	*	30% Blood Extraction	*	*	*	*	*	*	39	1	3	10.3%
		Trial 4	*	*	*	40% Blood Extraction	*	*	*	*	*	*	39	5	1	15.4%
Leschen, Correia	**2010**	Mortality/ Tank	2-Jun	4 hours out of water	17 days	Group 1- Control (Unbled)	99	*	99	Not Reported	n/a	3% Average	*	*	*	*
		Trial 1	*	*	*	Tank 1	*	*	16	Not Reported	n/a	1.7%	*	*	*	*
		Trial 2	*	*	*	Tank 2	*	*	21	Not Reported	n/a	2.8%	*	*	*	*
		Trial 3	*	*	*	Tank 3	*	*	16	Not Reported	n/a	6.1%	*	*	*	*
		Trial 4	*	*	*	Tank 4	*	*	13	Not Reported	n/a	0.8%	*	*	*	*
		Trial 5	*	*	*	Tank 5	*	*	16	Not Reported	n/a	4.3%	*	*	*	*
		Trial 6	*	*	*	Tank 6	*	*	16	Not Reported	n/a	2.4%	*	*	*	*

Table 2. *Continued*.

Study	Year	Type of Study	Collection Time of Year	Total Time Held for Bleeding	Time Held After for Study	Sample Size	Sample Size Female	Sample Size Male	Number Unbled	Dead Females	Dead Males	Mortality Rate of Unbled HSC	Number Bled	Dead Females	Dead Males	Mortality Rate of Bled HSC
Leschen, Correia	**2010**	Mortality/ Tank	2-Jun	6 hours	17 days	Group 2	89	*	*	*	*	*	89	Not Reported	*	22.5% Average
Continued		Trial 1	*	*	*	Tank 1	*	*	*	*	*	*	15	Not Reported	*	15.2%
		Trial 2	*	*	*	Tank 2	*	*	*	*	*	*	19	Not Reported	*	22.5%
		Trial 3	*	*	*	Tank 3	*	*	*	*	*	*	13	Not Reported	*	40.0%
		Trial 4	*	*	*	Tank 4	*	*	*	*	*	*	14	Not Reported	*	7.2%
		Trial 5	*	*	*	Tank 5	*	*	*	*	*	*	14	Not Reported	*	31.4%
		Trial 6	*	*	*	Tank 6	*	*	*	*	*	*	14	Not Reported	*	20.3%
		Mortality/ Tank	2-Jun	25 hours	17 days	Group 3	93	*	*	*	*	*	93	Not Reported	*	29.8% Average
		Trial 1	*	*	*	Tank 1	*	*	*	*	*	*	17	Not Reported	*	20.3%
		Trial 2	*	*	*	Tank 2	*	*	*	*	*	*	21	Not Reported	*	29.3%
		Trial 3	*	*	*	Tank 3	*	*	*	*	*	*	14	Not Reported	*	48.7%
		Trial 4	*	*	*	Tank 4	*	*	*	*	*	*	9	Not Reported	*	9.9%
		Trial 5	*	*	*	Tank 5	*	*	*	*	*	*	15	Not Reported	*	39.5%
		Trial 6	*	*	*	Tank 6	*	*	*	*	*	*	18	Not Reported	*	26.5%
Anderson, Watson III, Chabot	**2013**	Mortality/ Tank	May 15-23	52 Hours	6 Weeks	56 Total	56	*	28	0	*	0% Average	28	5	*	17.9% Average
		Trial 1	June 06-08 Date of Bleeding	*	*	OU Tank	14	*	7	0	*	0%	7	0	*	0.0%
		Trial 2	June 01-03 Date of Bleeding	*	*	LRW Tank	14	*	7	0	*	0%	7	1	*	14.3%
		Trial 3	June 01-03 Date of Bleeding	*	*	LU Tank	14	*	7	0	*	0%	7	1	*	14.3%
		Trial 4	June 01-03 Date of Bleeding	*	*	LCT Tank	14	*	7	0	*	0%	7	3	*	42.9%

Manufacture of LAL

In August 1978, the Federal Register publication of proposed rules stated “There will be an adequate and available supply of source material, and to guarantee that the manufacture of LAL will not have an adverse impact on existing crab populations, the horseshoe crabs shall be returned alive to their natural environment after a single collection of blood” (Federal Register 1978). Another rule under general requirements regarding handling of the horseshoe crabs read “The horseshoe crabs (Limulus polyphemus) from which blood is collected for production of the lysate, shall be handled in a manner so as to minimize injury to each crab.” (Federal Register 1980).

Prior to obtaining a license to sell LAL, companies enter a Biologic License agreement that adheres to the US FDA rules and requirements. Within that agreement, companies adhere to practices for the collection, handling, transport, bleeding and release of the horseshoe crabs that maximizes their well being and survival. In 2011, LAL companies in conjunction with ASMFC formalized these practices into Best Management Practices (BMPs). The 42 practices are documented in Table 3.

The manufacturing of LAL is entirely dependent on obtaining live and healthy horseshoe crabs. Depending on the location of the company along the eastern seaboard, the "bleeding season" varies in time of the year and duration. Horseshoe crabs are collected by hand when the horseshoe crabs are along the beaches, or by trawling when the crabs migrate to deep water. The horseshoe crabs are only accessible certain times of the year governed by weather and fishery regulations and cannot be stored or frozen for later use.

The health of the horseshoe crab from the collection to its release is of the utmost importance in order to obtain a quality product and a high survival rate of the bled horseshoe crabs. To avoid the hotter temperatures and sun of the day, the vast majority, if not all of the horseshoe crabs, are collected at night and transported in the early morning hours to the Laboratory for the "bleeding" process. The collected horseshoe crabs are carefully inspected for activity levels and injuries. The inspection is important for two reasons, injured or lethargic animals may introduce contamination into the sterile process and/or create a poor quality product and secondly, injured or slow moving horseshoe crabs may not survive the bleeding process. The rejected horseshoe crabs are not bled and returned to the water, to increase their odds of survival.

Only healthy crabs are bled; the blood flow is fast and steady initially and then slows to a drip. The blood is not extracted, but flows freely from the crab's open circulatory system. The actual "bleeding" of the crab takes minutes while the next steps of isolating and breaking open the white blood cells are labor intensive and require a full day. The bleeding process does not result in the death of the horseshoe crabs, and they are returned to the water adhering to the "Return to Sea" policy established from the onset of LAL manufacture.

Table 3. Best Management Practices (BMPs) for Biomedical Horseshoe Crabs.

Collection

☐ For targeted horseshoe crab trawl tows, reasonable tow times, recommended at 20-30 minutes bottom time (winches locked)

☐ Proper care and handling of horseshoe crabs while sorting and placing into bins

☐ Avoid exposure to direct sun, extreme temperatures as well as rapid temperature changes

☐ Night harvesting is recommended during periods of excessive heat

☐ During collection, sort out juveniles and do not bleed

☐ Sort out and return to the water individuals that do not appear to be healthy (damaged, slow movement, dull shell/old)

☐ When possible, release juveniles or unhealthy individuals immediately and do not transport to the facility

☐ Educate collectors in proper handling techniques

☐ Specify expectations of collectors in written contracts

☐ Periodically audit horseshoe crab collectors on implementation of BMPs for collecting

Transport to Facility

☐ Maintain temperature between approximately ambient water temperature at time of collection and 10ºF below ambient-water temperature

☐ Maintain good ventilation while stacked in bins

☐ Limit number of horseshoe crabs to a suitable number, dependent on container size and shape, and avoiding over-stacking to minimize damage to other horseshoe crabs

☐ Minimize travel time

☐ Keep bins and horseshoe crabs covered to protect against direct sunlight

☐ Secure containers in transport vehicle

Holding at Facility (Preparation for Bleeding/Bleeding)

☐ Limit holding time, under normal circumstances, at the facility to less than 24 hours

☐ No prolonged exposure to fresh water

☐ Follow written procedures for proper care and handling when sorting horseshoe crabs and moving them between bins and within the facility

☐ Inspect crabs for health and damage, selecting only undamaged and healthy crabs for bleeding

☐ Maintain clean, sanitary conditions during bleeding

☐ Maintain same level of care for rejected crabs that are not bled while being held until released back to sea

Holding at Facility (Preparation for Bleeding/Bleeding)(continued)

☐ Avoid bleeding crabs more than once per year

☐ If crabs are marked to avoid re-bleeding, ensure that the mark is residual and not harmful to the crab

☐ Bleed until rate slows down so that excessive bleeding is prevented

☐ Continue 30-year policy of not attempting to suction additional blood from the horseshoe crabs

☐ Perform internal audits to maintain quality control over written procedures

Table 3. *Continued*.

Post-Bleeding Holding

☐ Recognizing that the horseshoe crabs are now stressed from the bleeding process, maintain the same level of care as that used when transporting horseshoe crabs into the facility for bleeding

☐ Return to the water as soon as possible. If not being returned to the area of capture, ensure that conditions (salinity, water temperature, etc.) are similar to those found at the harvest site

☐ Minimize holding time post-bleeding

☐ While in holding, keep horseshoe crabs in the dark to minimize movement and injury

☐ Keep horseshoe crabs well-ventilated, moist, and allocate only a suitable number of crabs to holding containers

☐ Do not keep crabs out of the water for longer than 36 hours in total

Return to Sea

☐ Use same care in handling and transporting crabs being returned to the water

☐ Include return written instructions and requirements within contract with collectors, if applicable

☐ Periodically audit horseshoe crab collectors on implementation of BMPs for returning

Overarching practices for all steps

☐ Generate written procedures for all handlers of horseshoe crabs, covering all steps in the process from collection to release

☐ Keep horseshoe crabs cool, moist and covered, avoiding direct sunlight

☐ Establish a dialogue among collectors, the biomedical company, and the state regulatory agency to address concerns and challenges

☐ Have a written contract between collectors and the biomedical company, outlining practices and expectations

☐ Perform audits of the various steps and contractors/employees throughout the process

☐ Ensure proper monitoring and recording of mortality at each step in the chain of custody

Biomedical Numbers

The use of horseshoe crabs for the manufacture of LAL was fully established after the FDA issued Draft Guidelines in 1987. James J. Finn, a lysate manufacturer, located along the Delaware Bay shore, connected the biomedical use of the crab to fishery management and began reporting the number of horseshoe crabs used for LAL production. Finn's 1991 report on the first Delaware Bay spawning survey of horseshoe crabs estimated biomedical use to be about 130,000 in 1989, the onset of LAL manufacturing. About 280,000 crabs were bled in 1998 and 2000 based on a survey conducted by the ASMFC Horseshoe Crab Technical Committee in 2001.

These early estimates of biomedical use relied on yearly reports submitted by the biomedical companies to their respective State. In 2004, the ASMFC adopted Addendum III which required standardized reporting from the states with biomedical collection in order to obtain the number of biomedical horseshoe crabs collected coast wide. The number of horseshoe crabs collected averaged 462,670 during the years 2004 to 2016. However, even with standard reporting, it is difficult to compare yearly numbers as biomedical collection has changed in response to the FMP and its Addendums. For example, there was an increase in the biomedical numbers in 2006 possibly due to Addendum VI which encouraged the use of more males.

In addition to the submitted fishery information, companies are required to track their mortality numbers from collection to release at six steps.

Step 1. The horseshoe crabs are collected by hand or trawl.

Step 2. They are transported to the Laboratory.

Step 3. They are handled prior to bleeding.

Step 4. They are bled.

Step 5. They are handled and transported prior to release.

Step 6. They are released.

Mortality in the biomedical fishery is computed in two steps. First, mortality is determined from actual numbers of horseshoe crabs reported dead by the biomedical companies. The horseshoe crabs for biomedical use are donors, caught alive and released alive. Inherently, in any fisheries there is a mortality rate associated with the catch and release. This number is easily accountable and is part of the scrutiny of the horseshoe crabs used for bleeding. Both slow moving and dead horseshoe crabs are rejected as unresponsive and their numbers are about one percent of the total number of collected horseshoe crabs. The number of horseshoe crabs rejected for unresponsiveness are listed as mortal horseshoe crabs in the ASMFC tables. There is not an additional mortality associated with slow moving horseshoe crabs.

Besides demise, the crabs are carefully inspected for injuries, even the slightest injuries are noted and the horseshoe crabs are not bled. The horseshoe crabs rejected due to minor injuries will survive. Leschen and Correia (2010) worked with 310 collected horseshoe crabs for biomedical use and rejected 12 of the horseshoe crabs for bleeding. The biomedical company inspected the remaining 298 horseshoe crabs and after their scrutiny, rejected an additional 17 crabs for reasons unseen by the untrained eye. Although the horseshoe crabs were deemed not fit for bleeding, their injuries were so minor that Leschen and Corriea (2010) considered the rejected horseshoe crabs as adequate to use for the control group, an indication of their survival.

The second part for computation is a 15% mortality rate applied to all bled crabs assuming there would be some degree of post-bleeding mortality. Initially, the mortality rate of 10% reported by Rudloe in 1983 was used but after additional mortality studies were published, the post bled mortality rate was raised to 15%. However, the post-bleeding mortality that may occur is much harder, almost impossible to decipher.

Mortality Studies

Rudloe (1983)

The effect of bleeding on the horseshoe crab population was first studied by Ann Rudloe, 35 years ago, funded by the US FDA in response to the use of horseshoe crabs for LAL manufacturing. Her work is the most well known and cited for the mortality rate calculated for the horseshoe crabs.

The bulk portion of Rudloe's study focused on the release and recaptures of 10,062 bled and unbled animals within St. Joseph Bay along Florida's Gulf Coast. Both groups of animals were treated in the same manner and out of the water for about 30 minutes. Half of the animals were bled until the flow slowed to an intermittent drip similar to a "trained bleeder". The number of recaptures was 1,415 with 85 dead. Rudloe attributed a 10% greater mortality with bled crabs than unbled the first year and 11% the second year.

Another part of her study was a pen experiment that held the horseshoe crabs after bleeding to determine their survival. During the course of her study, Rudloe realized the difficulty in maintaining horseshoe crabs and designed a small scale tank study. Eighty adult horseshoe crabs were collected and half were bled. Both sets of crabs were placed in a pen and held for 30 days. Two unbled animals died (5%) and one bled female died (2.5%). The dead animals were noted as having "poor eye and shell condition" indicating they may have been older and in questionable health.

Rudloe also investigated the activity of the bled horseshoe crabs. Sixty horseshoe crabs, half bled and half unbled, were placed in a tank and their activity levels were gauged by chart deflections. Sixteen comparisons were performed between the control and the bled groups, in six cases there was no significant difference between the groups, in six cases the bled horseshoe crabs were more active and in four cases the control group was more active. Her tagging study also observed that the movements of unbled and bled horseshoe crabs were almost identical.

Thompson (1998)

The biomedical company in South Carolina, Endosafe, in conjunction with a graduate student from the College of Charleston, conducted a mortality rate study. Prompted by the acceptance of the FMP in 1998, both a tagging study and a preliminary tank study were performed to determine a mortality rate of horseshoe crabs bled by Endosafe.

Thompson tagged 734 unbled (non LAL) and 594 bled (LAL) horseshoe crabs in 1997. The 594 tagged LAL animals were selected from the horseshoe crabs transported, held and bled at the laboratory. The unbled animals were released along the spawning beaches where the study was being conducted and the bled animals were released at their usual release point in a more remote location. The mortality rate of the non LAL animals was 2.86%, 15 dead females and 6 dead males. The mortality rate for the bled animals was 0% with seven live recaptures reported. It should be noted the recapture rate for the LAL animals (1.18%) was much lower than the non-LAL crabs (12.94%) most likely due to their release in a remote area.

Thompson's preliminary tank study in 1996 used 40 horseshoe crabs specifically collected for biomedical use. The forty horseshoe crabs were collected, transported, and handled the same way except twenty crabs, ten males and ten females, were bled by Endosafe. After the bleeding process, both groups of horseshoe crabs were placed in a single tank that was drained daily to clean the water, and they were fed for seven days. Three of the 20 bled females died, resulting in a 15% mortality rate.

Endosafe, Inc. (1999)

Following Thompson's thesis work, Endosafe conducted another mortality study with the approval of South Carolina Department of Natural Resources (SCDNR) and the AMFC Technical Committee in May of 1999. Horseshoe crabs were randomly selected from horseshoe crabs collected for biomedical use and subjected to the same environmental conditions except half were bled and the other half unbled. The animals are marked with paint and a scratch mark and 133 unbled animals and 134 bled animals were released into a pond for a two week holding period. After the holding period, 120 horseshoe crabs from the control group and 132 bled crabs were accounted for. One male and one female died from the control group resulting in an overall mortality rate of 1.67%, and seven males and four females died from the bled group, 8.33% mortality. (It was noted that the bled animals were left out in the sun longer than the control group waiting for the marking paint to dry.)

Kurz and Pirri (2002)

Kurz and Pirri (2002) studied the movements of bled and unbled horseshoe crabs via transmitters upon release into Nauset Estuary, a small embayment in Massachusetts. Twenty female horseshoe crabs that were greater than 200 mm in size and free of epibionts were hand collected during the spawning season from mid May to early July. Ten of the crabs were bled until the blood flow slowed, losing 90 mL of blood. The crabs were not out of the water for longer than 30 minutes and released from July 2nd to July 8th.

Kurz and Pirri reported a more random movement pattern with the bled group than the unbled group but found no difference in their average rate of movement. There was also no significant difference in their spatial distribution with 17 of the 20 crabs, nine control crabs and eight bled crabs, located in the same spawning area.

During their study, Kurz and Pirri found two of the ten bled crabs were deceased after 28 days and 68 days. They reported a mortality rate of 20% and added that it may have been an artifact of low sample size designed for their behavioral study.

Walls and Berkson (2003)

Walls and Berkson (2003) researchers from Virginia Technological University worked directly with a LAL company, BioWhittaker/currently Lonza, to study the mortality rate of their bled horseshoe crabs. The crabs were trawl collected during July and August 1999, 2000 and 2001 in waters off Chincoteague, Virginia or Ocean City, Maryland. A small number of newly matured male horseshoe crabs were selected for study in order to limit any variation between the control and bled groups. The two groups were handled the same way and under the same conditions with the exception that half were bled according to the biomedical company's usual procedure. After the bleeding procedure, the horseshoe crabs were packed in coolers, transported to Hampton, Virginia where equal numbers of control and bled horseshoe crabs were placed in four replicated flow through holding tanks for a two week holding period. This process was repeated eight times over the course of the study.

The combined mortality rate for the eight two week period is 0.5% for the unbled animals (1 unbled crab died of 200) and 8% for the bled crabs (16 bled crabs died of 200). The mortality rates from the 8 periods were varied, ranging from 5 to 30% mortality for the bled individuals. The results from four of the periods resulted in 0% mortality while the other four periods resulted in 3 out of 10 crabs (30%) , 6 of 30 (20%), 2 of 30 (6.67%) and 5 of 30 crabs (16.67%) die.

Hurton and Berkson (2005)

Hurton and Berkson (2005) along with the biomedical company, Cambrex/ currently Lonza, expanded on their 2003 work and studied if mortality was directly related to the amount of blood taken from the horseshoe crabs and/or the stress level of the horseshoe crabs. The crabs were trawl collected from Ocean City, Maryland and transported to Virginia Tech, Blacksburg, Virginia.

During Experiment 1, a group of 100 males and 100 females were left out of the water for 15-20 hours at 21 degrees C and were considered "lower stressed" animals. They were bled with varying amounts of blood taken based on a predicted blood volume calculated using the crab's intraocular distance, The crabs were separated into five groups; a control group and four groups with 10%, 20%, 30%, 40% blood taken. The bled crabs were returned to the water tanks and monitored for 2 weeks. There were no deaths within any of the groups.

During Experiment 2, a "higher stressed" group of 110 males and 85 females were exposed to varying levels of blood loss. The stress included 47 hours out of the water and temperatures reaching 36 degrees C. Fourteen horseshoe crabs died; 1 unbled male died, 1 bled female died at 10%, 2 bled males died at 20%, 4 bled crabs at 30% and 6 bled crabs died at 40%. The overall resultant mortality was 8.3% compared to 2.6% for unbled animals. The study indicated with high stress more deaths may occur as blood loss is increased. Hurton and Berkson noted that the bleeding volume for a biomedical company would be in the range of 10% blood loss and the mortality rate of 10%.

Leschen and Correia (2010)

Leschen and Correia (2010) researched the mortality rates of bled animals in Massachusetts in response to stressful conditions and if their survival rate increased if they are returned to the water quicker. Leschen and Correia's study separated the horseshoe crabs into three treatment groups, Group 1 of 99 crabs, Group 2 of 89 horseshoe crabs and Group 3 of 93 crabs. Group 1 was the control group that was held out of water for 4 hours and Groups 2 and 3 were exposed to conditions mimicking an open boat deck, one hour drive in non air conditioned truck, bleeding until the blood clots (30% blood extraction), being stored for 24 hours stacked in 30 gallon Rubbermaid totes at room temperature, one hour truck drive and a 15 minute boat ride. The crabs from Group 2 were held for six hours after the biomedical process and the crabs from Group 3 held overnight, 25 hours. The three groups were distributed in six different tanks and monitored.

The mortality rates for the control group was 3.01% (reported) and for Group 2, 22.5% (reported) and Group 3 29.8% (reported). There was no significant differences in the number of crabs per tank, ranging from 6.1 to 7.2, dissolved oxygen levels, ranging from 8.6 to 9.1 or the water temperatures, ranging from 15.4 to 15.7. However, the mortality in Tank 3 was greater than the other Tanks for the three groups, followed by the mortality rates in Tank 5. Tank 4 had the lowest mean Dissolved Oxygen (DO) concentration and the lowest mortality while Tank 3 had the highest DO and highest mortality. It is appears although not significantly different, there was a tank effect.

Anderson, Watson III, Chabot (2013)

Anderson, Watson III and Chabot (2013) studied the impact bleeding has on the horseshoe crabs' locomotion and hemocyanin levels. They collected 56 female horseshoe crabs at spawning beaches at Adams Point, Great Bay, Durham, New Hampshire from 15th to 23rd of May 2012. Due to laboratory restraints, the horseshoe crabs were sorted according to size and distributed into four tanks. The tanks were identified as the Outdoor tank - OU with the largest size animals, the Laboratory Running Wheel tank - LRW with the smallest horseshoe crabs and the Laboratory Unrestrained tank - LU and the Laboratory Communal tank - LCT with the medium size animals.

For the bleeding process, the four groups of horseshoe crabs were exposed to a total of 52 hours of varying temperatures and conditions, meant to mimic biomedical practices. During the 52 hours, the LRW, LU and LCT groups were exposed to sunlight for four hours in a barrel and then kept in the barrel after it was covered for an additional four hours and maximum temperatures reaching 37 degrees C. The Outdoor group was not exposed to the direct sunlight and only subjected to maximum temperatures reaching 28 degrees C. The behavior study resulted in five bled horseshoe crabs dying, three on the second day from the LCT tank and two on the third day from the LRW and LU tanks. No bled crabs (0%) died in OU tank.

Anderson et al. found that the bled horseshoe crabs had decreased activity and expressions of tidal rhythms after two weeks of bleeding. The bled crabs also exhibited decreased linear and angular velocities in the first week after bleeding but resumed normal linear velocities after 3 weeks. The greatest effect of the bleeding process was the long term declines in the hemocyanin concentrations.

Assessing the Mortality Studies

The papers provide differing estimates for the mortality rates associated with unbled and bled horseshoe crabs. Some of the papers reported the average mortality rate from a number of experiments. There were 25 separate mortality rates for the control or unbled groups from the reviewed studies and their individual experiments. Assigning no relevance to the 25 estimates, the mortality rate for the unbled horseshoe crabs averaged 1.34% with 14 rates having zero deaths and the remaining rates ranging from 0.8% to 6.10%. If the average ten mortality rates are used, the average mortality rate was 1.56%, with four zero rates and the remaining rates ranging from 0.5% to 5%.

For bled animals, there were 39 individual rates and 13 estimates that were stated or averaged. The average of the 39 individual experimental rates was 14.25% with 10 rates that were zero and the rest ranging from 2.5% to 48.7%. If the 13 average estimates and stated were averaged, the resultant mortality rate is 11.80% with rates ranging from 2.5% to 29.80%. Nine of the 13 rates were below 15% mortality. The highest rates were from the studies conducted in Massachusetts meant to mimic the biomedical practices. The highest average rates reported were 17.9 % from Anderson et al. (2013), 20% from Kurz and Pirri (2002), and 22.5% and 29.8% from Leschen and Corriea (2010). The estimates illustrate the variability in the mortality rate of control and bled animals and its dependence on many factors.

The most well known and cited mortality rate of 10% is from Rudloe's tagging study. The greatest benefit of the study was the fact that the animals were released into their natural environment after bleeding. The practice most closely resembles the biomedical's "Return to Sea" policy. A policy established 40 years ago that greatly contributes to the survival rate of the bled individuals. Thompson's work also involved a tagging study and found a 0% mortality rate for the bled animals, however recaptures were minimal since the bled animals were released in a remote area. Seven live bled recoveries were found and no dead recoveries were found for the bled horseshoe crabs.

Although Kurz and Pirri's tracking study was similar to a tagging study, its design was set up to study behavior and had a very small sample size. They focused on the movements of twenty horseshoe crabs and found two bled horseshoe crabs dead and noted a mortality rate of 20% adding "However, the slightly higher mortality rate observed in this study may have been an artifact of low sample size."

Another variable to consider with the tagging studies is the natural mortality rate associated with spawning. If the tagging studies are conducted during the spawning season, the natural mortality rate is estimated to be 10% due to stranding (Botton and Loveland 1989). Stranding occurs when the horseshoe crab is overturned by the water and is exposed upside down to predation and the environment. Kurz and Pirri's study conducted during the spawning season found two dead crabs 28 days and 68 days after bleeding. Their death could have been attributed to the 10% spawning risk.

Most of the mortality studies are non tagging studies that held the horseshoe crab specimens in tanks for weeks prior to and after the bleeding process. The control group was kept the same amount of time for comparison, but the multiple stressors on the crabs would make the resultant mortality rate higher. Rudloe established the difficulty in keeping large numbers of horseshoe crabs in a confined area. More recently, Mattei (2011), while studying tag induced mortality penned 105 horseshoe crabs for 44 days and found a mortality rate of 4% for the untagged horseshoe crabs. Her work confirms the difficulty in maintaining horseshoe crabs.

The researchers attempted to combat the challenge of maintaining the horseshoe crabs by using small sample sizes, multiple study periods and/or many holding areas (tanks). Leschen and Correia used multiple tanks, distributing both the control crabs and the bled crabs between six tanks. Based on the mortalities, there was a difference between the Tanks with Tank 4 and

Tank 5 having the greatest mortalities even for the control group. Although, they state there were no significant differences between the tanks, the resultant data strongly suggest the tanks did influence the mortality rate. Anderson et al.'s study also used multiple tanks to keep the horseshoe crabs. The tanks were quite different in size and volume affecting the environmental conditions the horseshoe crabs were exposed to. The difference in tank volumes is listed in the Chart below.

Tank	Number of Tanks	Size	Volume
Outdoor (OU)	7	183 cm x 92 cm x 50 cm	5.89 cubic meters
Laboratory Running Wheel (LRW)	4	80 cm x 65 cm x 32 cm	0.67 cubic meters
Laboratory Unrestrained (LU)	2	1.7 m x 0.9 m x 0.75 m	2.30 cubic meters
Laboratory Communal (LCT)	1	80 cm x 65 cm x 32 cm	0.17 cubic meters

The Outdoor Tank with the least mortalities had the greatest volume, whereas the Laboratory Communal Tank had the least volume and the greatest mortality. The two other Tanks had volumes between the Outdoor tank and the Laboratory Communal Tank and resulted in a mortality rate in the midrange of the other tanks.

In addition to using multiple tanks to maintain the horseshoe crabs in captivity, small sample sizes are necessary. Although, horseshoe crabs are the most studied invertebrate because of their hardiness, only a few can be maintained in tanks. However, small sample size diminishes the value of the studies. Anderson, Watson III and Chabot recorded a lesser blood volume taken from the horseshoe crabs in the laboratory tanks compared to the animals in the outdoor tank indicative of the health of the horseshoe crabs. This concurs that healthy animals must be used for bleeding, if not they do not bleed or survive well.

Ignoring flaws in the study designs, it is imperative to note that many of the studies did not adequately mimic biomedical practices. When comparing the BMPs to the studies' practices, many of the studies did not adhere to the same practices that the LAL manufacturers do. Holding time and temperatures as well as exposure to the elements deviate from the BMPs practices with most of the studies mimicking the worst case scenario for these factors. The two documented practices that are most essential for the survival of the bled horseshoe crabs would be to avoid direct exposure to the sun and to return the crabs to the water as soon as possible.

Leschen and Corriea's study demonstrated the importance of the "Return to Sea" policy and found a 7.5% greater survival rate when the bled horseshoe crabs were returned to the water 14 hours sooner. The rate for animals held 8 hours after bleeding (22.3%) was considerably less than the rate for animals held for 22 hours after bleeding (29.8%).

The studies were conducted during the warmer months of the horseshoe crab's spawning and/or bleeding season and are not reflective of the entire "bleeding" season. The "bleeding" season for some companies may not be conducted during the hottest months or only a portion of the season is conducted during those months. For example, in the Mid-Atlantic region the "bleeding" season starts after the horseshoe crabs' spawning season and may last until late October with July and August being the hottest months. All the studies were conducted during the hottest times of the season.

Some of the studies focused on the amount of blood taken and its effect on the survival of the horseshoe crab and also the time needed for the blood concentration to reach prebleeding levels. Rudloe (1983) rebled 26 horseshoe crabs that were recaptured 13 to 36 days after bleeding. The blood levels were only slightly below the initial blood volume, 49 mL compared to 63 mL for the males and 125 mL compared to 137 mL for the females. Twelve of the recaptured horseshoe crabs bled more the second time. Kurz and Pirri (2002) reported an average of 89.9 mL of blood were taken from ten females with an average prosoma width of 232 mm. Hurton, Berkson and Smith (2005) developed an equation relating the size of the horseshoe crab and the amount of blood taken but indicated that blood volume is affected by season, salinity, health and other environmental conditions.

Although blood volume is variable, Hurton and Berkson (2005) studied the effect the amount of blood extraction has on the survival of the horseshoe crabs. Mortality rates were zero if the horseshoe crab was unstressed even at the highest amount of blood loss, however, mortality rates increased as the amount of blood loss increased if in combination with extreme environmental conditions. They found the highest mortality rate of 15.4% for "stressed" animals when the greatest amount of blood is taken (40%). Hurton et al. noted that the blood loss during bleeding was generally less than the calculated 30% volume.

Leschen and Correia (2010) reported that the five mortalities resulting from their study seemed unrelated to the amount of blood loss. The mean percentage of blood taken from the deceased crabs was within the range of the overall amount of 19.8% for all the studied animals. They also reported that the change in activity levels were not related to amount of blood loss.

Anderson, Watson III and Chabot (2013) focused on blood volumes of the groups before and after bleeding. They reported the amount of blood loss ranged from 14% to 21% and did not differ between the live and dead horseshoe crabs. After six weeks, the laboratory animals did not regain their blood volumes, most likely due to poor holding conditions while the Outdoor group exposed to better holding conditions regained 60% of their original volume.

The studies indicate that blood volume is variable and dependent on many factors, however similar to mortality, under good conditions, blood levels return to normal. A biomedical practice is to bleed horseshoe crabs once in a season, enabling the horseshoe crab to regain their blood volume if necessary over a long period of time. In addition, since the horseshoe crabs with lower blood volumes were not deceased after the six weeks, their survival is most likely.

Summary

Overriding all the studies, is the fact that mortality of bled horseshoe crabs is low and survival is high. The tagging studies show minimal mortality and the laboratory studies in conjunction with the biomedical companies present rates similar to the tagging study mortalities. The studies meant to mimic biomedical practices or expose the horseshoe crabs to additional stressors reported much higher mortality rates, however the results are confounded by captivity and/or variable environmental conditions. The studies demonstrated the difficulty in maintaining horseshoe crabs and the effect environmental conditions have on the mortality rate. The mortality rates are variable across the studies, however the survival rate of the horseshoe crabs can be at their maximum if certain conditions are made.

The studies conducted by Leschen and Correia and Anderson, Watson III and Chabot subject the horseshoe crabs to the worst environmental conditions. Leschen and Correia (2010) exposed the bled horseshoe crabs to poor tank conditions evidenced by the high mortality rates for the control animals. Anderson, Watson III and Chabot intentionally placed the horseshoe crabs in barrels exposed to four hours of direct sunlight and then immediately covered the barrels, essentially "cooking" the horseshoe crabs for another four hours. Even under these extremely harsh conditions, the survival rates were between 60% and 70%.

The biggest obstacle to overcome in conducting mortality studies is how to monitor the horseshoe crabs after bleeding. Tagging studies adhere to the most important biomedical practice of releasing the bled horseshoe crabs into their natural environment. However, recapture of the tagged animals may be too minimal to estimate a mortality rate. Rudloe's study conducted in a small embayment had good recapture rates for both control and bled animals. Thompson's tag study had a minimal recovery rate of 1.2% for the bled animals, seven found alive and no crabs found dead.

The alternative to tagging studies is placing the horseshoe crabs in closed tanks and monitoring their survival. Horseshoe crabs need a large space and good water flow to maintain their health and survival. To achieve these needs, researchers used small sample sizes and, either used more tanks or conducted the study on different days. The use of more tanks introduced a new variable and influenced the mortality rates. Running the experiments during separate periods, introduces differences in the environmental conditions. The differences may be reflected in the variability of the results, ranging from 0% to 30% for one study.

Mortality rates for bled horseshoe crabs should be analyzed with caution understanding the complex nature of horseshoe crabs. The studies do demonstrate that adherence to the BMPs will ensure the horseshoe crab's survival after bleeding. Avoiding direct sunlight, extreme temperatures and excessive time out of the water are extremely important for the survival of the horseshoe crabs.

In conclusion, mortality rates are variable and ever changing dependent on many factors. There will never be one set mortality rate and if so, would the number be meaningful and add to our management of the horseshoe crab population. The number of horseshoe crabs estimated to die due to the bleeding process (average 58,721) is about 7% of the horseshoe crabs that die as bait for eel and conch (787,223 reported 2016 harvest) and a miniscule fraction, 0.2%, of the estimated number of horseshoe crabs in Delaware Bay alone (24,000,000).

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