

**Estimation of Survival of the Atlantic Horseshoe Crab, *Limulus polyphemus*, by Analysis of  
Mark-Recapture Data with Tag Loss**

by

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## Abstract

The Atlantic Horseshoe crab, *Limulus polyphemus*, is a valuable resource along the Mid-Atlantic coast which has, in recent years, experienced new management paradigms due to increased concern about this species role in the environment. While current management actions are underway, many acknowledge the need for improved and updated parameter estimates to reduce the uncertainty within current management models. Specifically, updated and improved estimates of demographic parameters such as adult survival in the regional population of interest, Delaware Bay, could greatly enhance these models and improve management decisions. There is however, some concern that difficulties in tag resighting because of physical or behavioral differences between sexes or a complete loss of tags could be occurring. As defined in the assumptions of a Jolly-Seber model, any apparent loss of tags can bias results, by underestimating survival. Using data from a double tag mark-resight study conducted in Delaware Bay and Program MARK, we conducted three analyses to assess this system.

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## Introduction

The Atlantic horseshoe crab, *Limulus polyphemus*, is a species that has been around for at least 350 million years. Interestingly, it has remained relatively unchanged throughout history deeming it the nickname of a 'living fossil.' It is currently a species of Near Threatened listing on the IUCN Red List and is of value economically and ecologically to the Mid-Atlantic States of the US. Horseshoe crabs are a source of revenue to commercial fishermen and the biomedical industry, who uses the blood of horseshoe crabs to make a reagent called Limulus amoebocyte lysate. Because of their role in our society and the environment it of concern that measures be taken to protect and conserve the horseshoe crab as well as the interest of these stakeholders. As of 1998, the Atlantic State Marine Fisheries Commission put into place rules and regulations on the harvest of horseshoe crabs. Currently a multispecies predictive model developed by McGowan et al. (2011) is being utilized for the management of horseshoe crabs that takes into account not only horseshoe crab harvest, but also their relationship with the shorebird species the red knot. Of course, to ensure that this model is as accurate as possible, life history parameters will continuously need to be updated. Currently, the estimate of adult survival in this model was taken from a study conducted in Pleasant Bay, Massachusetts in 2002. It would be advantageous to estimate an updated and site specific estimate to Delaware Bay to reduce uncertainty of this parameter within the current predictive model.

To estimate adult survival we choose to use two different methods, a Cormack-Jolly-Seber (CJS) model and a multistate model. One of the main assumptions of a Cormack-Jolly-Seber model is that tags are not being lost because it will bias the estimates and result in the

underestimation of survival. To ensure that our estimate of adult survival was as accurate as possible, we first ran an analysis to determine what the rate of tag loss was. Using a multistate model in Chapter 1, we ran an analysis to gain a better understanding of not only if tag loss was occurring but also if tag loss occurred at a constant rate or changed with time. This was important to define initially because it determined how the bias of tag loss should be incorporated into the estimation of survival. Using a CJS model in Chapter 2, we analyzed a 12 year data set to estimate the rate of adult survival. To include a bias of tag loss, a constant rate of tag loss was applied to the estimate of survival a posterior. In Chapter 3, we developed a multistate model that determined adult survival and tag loss simultaneously to even further reduce uncertainty surrounding these parameters. Site specific and updated parameter estimates will help to increase the confidence in the accuracy of the current management models underlying decision making for horseshoe crab harvest.



## Chapter 1: A multistate approach for the estimation of tag loss of the Atlantic horseshoe crab, *Limulus polyphemus*

### Introduction

The Atlantic horseshoe crab, *Limulus polyphemus*, is a valuable resource along the Mid-Atlantic coast which has, in recent years, experienced new management paradigms due to increased concern about this species role in the environment. While current management actions are underway, many acknowledge the need for improved and updated parameter estimates to reduce the uncertainty within current management models (McGowan et al. 2011). Specifically, updated and improved estimates of demographic parameters such as adult crab survival in the regional population of interest, Delaware Bay, could greatly enhance these models and improve management decisions. There is however, some concern that difficulties in tag resighting because of physical or behavioral differences between sexes or a complete loss of tags could be occurring. As defined in the assumptions of a Jolly-Seber model, any apparent loss of tags can bias results by underestimating survival. Given this uncertainty, as a first step towards estimating an unbiased estimate of adult survival we estimated the rate of tag loss.

The most established method for assessing populations is to collect mark-recapture data and with that, estimate life history parameters using the Cormack-Jolly-Seber approach (Williams et al. 2002). This method, like all models, is restricted by specific assumptions to guarantee that parameters are calculated without bias (Pollock et al. 1990). One of the main assumptions of a Cormack-Jolly-Seber model is that there is no loss of marks or tags. A violation of this assumption would result in the underestimation of survival, as individuals who become unintentionally removed from the study will be inaccurately treated as if they emigrated or died (Pollock et al. 1990; Alisauskas and Lindberg 2002). Further analysis is required for any species that exhibits even a minimal potential for tag loss (Williams et al. 2002).

Double marking individuals in a mark-recapture study is a highly effective method for investigating tag loss (Seber 1982). In the field, the use of a second more visible tag can be utilized as an effort to increase the probability for an individual to be resighted, thereby increasing capture history data and reducing the need for expensive or time consuming physical recaptures (Nichols and Hines 1993; McGowan et al. 2011). While this use of different types of individual markers can significantly improve the detection probability in the field, it can also

provide a sufficient amount of information to conduct analyses about tag loss. If the auxiliary tag is treated as an indicator of a main tag, as a main tag would for an individual animal, the same methods as those used to estimate survival of an individual animal can be utilized to estimate the ‘life span’ of a main tag (Nichols and Hines 1993). Also as in a model of survival, information collected on an auxiliary tag can reveal individual characteristics of the main tag. This knowledge of the life span and characteristics of a tag itself can improve the monetary and physical efficiency of a study.

There are many statistical methods established for dealing with tag loss that all require prior knowledge about the performance of the tags over time. For models where tag loss has been determined to be constant over time, survival can be calculated *a posteriori* (Arnason and Mills 1981). In these situations the estimated Jolly-Seber survival rate,  $\phi$ , is simply divided by the probability that an individual retains their tag,  $\theta$ , to yield a ‘corrected’ value of survival,  $\hat{\phi}^C$  (Nichols and Hines 1993; Seber 1982) (1).

$$\hat{\phi}^C = \phi / \theta \quad (1)$$

For some species there may be evidence to support that tag loss varies with time since marking due to physical wear, difference in types of tags, or to misapplication (Nichols et al. 1992; Nichols and Hines 1993; Conn et al. 2004). In these situations, alternative more complex methods must be utilized. Similar to the method for constant tag loss, Nichols et al. (1992) proposes another two step approach that the uses cohorts to define the differences in tagged individuals so that the rate of tag loss can then be applied to each cohort to determine survival estimates over time.

While this technique is more straightforward, they also recommend the use of recapture and resight data modeled together in a single estimation model that estimates both survival and tag loss jointly in the same likelihood equation. A single estimation model would be greatly favored method of estimating survival over other multistep processes as it has the advantage of producing survival estimates directly. The ability to directly estimate parameters not only increases the precision of your results but also allows for the ability to easily test and compare alternative models. A growing interest has been found in the use of a multistate model as a more comprehensive alternative (e.g., Alisaukas and Lindberg 2002; Joe and Pollock 2002; Conn et al.

2004; Kendall et al. 2006). Under multistate approaches, the added complexity of transition parameters provides a natural framework for modeling tag loss (Conn et al. 2004). Here, we developed a multistate model to illuminate just tag loss of the Atlantic horseshoe crab, *Limulus polyphemus*, in Delaware Bay. This analysis will allow us to gain insight about the behavior of tag loss and the model itself can be adjusted to incorporate the estimation of survival in future analyses.

The Atlantic horseshoe crab has become the center of interest to many different user groups. Horseshoe crab blood is used in biomedical testing and research, they are used as bait by the eel and whelk fishing industries, and due to a hypothesized ecological relationship with migrating shorebirds, have become of interest to shorebird biologists and conservationists. With the invested interest of these groups in mind, many acknowledge the need for a better understanding of the horseshoe crab population dynamics specifically in Delaware Bay. The newly adopted management paradigm uses population model for horseshoe crabs to evaluate the effects of harvest management action on horseshoe crab and red knot population viability (ASMFC 2009; McGowan et al. 2011). These projection and optimization models rely of survival and productivity estimates for both species that may be imperfect because they are based on data from different geographic regions or may be subject to estimator bias and assumption violations (ASMFC 2009; McGowan et al. 2011) There is a strong need for more complete and detailed studies with a Delaware Bay specific focus to resolve some of this uncertainty (ASMFC 2009; McGowan et al. 2011). Additionally, crab abundance monitoring in the future may rely on mark-recapture approaches to assess population state and management effectiveness. Smith et al. 2006 acknowledges the possibility of tag loss over time. The lack of conclusive information on if or at what rate tag loss enables further refinement of mark-recapture survival and abundance estimates to account for tag loss bias. The development of this multistate model allows models of constant loss and age dependence to be compared and the most appropriate course of action for modeling survival may be taken in the future. An accurate picture of tag loss for the Atlantic Horseshoe crab will contribute to the improvement of demographic parameter estimates specifically in Delaware Bay and will be beneficial to the future management of this cohort as well as the species.

## Methods

## *Study design*

The data set for this study is composed of individuals captured and marked by researchers of the U.S. Fish & Wildlife Service, Maryland Fisheries (MFRO) and the U.S. Geological Survey in Delaware (USGS). The similarities in training and methodologies of both agencies permitted the combination of the data sets as there is no reason to suspect any differences in the application of tags. The USGS tagged all females with the exception of one male and MFRO tagged both males and females. The identification of sex was performed visually, as females are usually larger in size than males (Carmichael et al. 2003; Swan 2005). Males can also be distinguished by the presence of claspers, a modified version of the first pair of walking legs which aid in attachment to females during spawning (Swan 2005). No other individual covariates were measured consistently enough to be included in this analysis.

All individuals were initially captured and tagged in May or June of 2003. A 4mm diameter hole was drilled into the lower left region of the carapace along the edge of the shell in the field using a rechargeable drill (Smith et al. 2010; Swan 2005). Through this hole, a “Christmas Tree” pin was inserted and the individual was given a standard button tag that is circular metal tag that displays a unique number and methods for reporting a resighting (Brousseau et al. 2004; Smith et al. 2006; Swan 2005). A second flat auxiliary tag was also glued onto the dorsal surface of the carapace. It is expected that mortality is not affected by the presence of a tag (Smith et al. 2006; Mattei et al. 2011).

Resight efforts were conducted by the releasing agencies although anyone could potentially report a tag. The resight data comes from a variety of sources including commercial fisherman, biomedical industries, other research efforts, and the general public. Resight efforts by researchers from the two agencies who released these individuals varied over the years. Researchers from MFRO specifically looked for tags throughout the spawning season in 2003 but did not conduct efforts in the subsequent years. So individuals could accurately be designated to a specific state for the multistate model, resight data was limited to individuals with known presence of both disc and auxiliary tag from 2004 until 2010. Also, individuals who were resighted dead were removed from the analyzed data set for concern that the unknown amount of time they remained on the shore could have accelerated tag loss.

## *Model Development*

All models were run using the program MARK (White and Burnham 1999). A set of models were chosen *a priori* including the covariates of sex, stratum, and time to represent different competing biological hypothesis. The multistate model was defined by four states, hereafter called strata, depending on the number of tags that an individual possessed at a given time (Figure 1). All individuals started in stratum A which is defined as a state of having both tags present. Stratum B was a state of possessing only the main tag, stratum C was having only the auxiliary tag and stratum D was having no tags present. As many have suggested limitations and problems with significance testing (Anderson et al. 2000; Johnson 1999), a more robust alternative can be found in the use of model selection and estimations with confidence intervals. Akaike's Information Criteria (AICc) is a commonly accepted and useful tool in model selection and was used in our analysis of the results (Burnham and Anderson 2002). The model with the lowest AICc value was favored and the difference in AICc values of competing models was used to further rank the models from there on out (Anderson et al. 2000). All models chosen in our candidate set represented a plausible biological scenario of our system.

The flexibility and detail of a multistate model makes it suitable for a wide array of biological systems and questions (Kendall et al. 2006). The use of transition parameters can expose even more detail about a system and allow for creative modeling of ecological systems. For the purpose of this study, we focused our interest on estimating the probability of transiting between tagged strata instead of the probability of being in the strata themselves. In this context, the probability of transition represents the probability of the loss of a specific type of tag, or tag "mortality". Transition parameters can be described as the probability that an individual alive in one stratum at time  $i$  will transition into another stratum at time  $i+1$ . One of the assumptions of a multistate model is that no individuals leave the study so that at any given time if an individual is detected it must be in one of the designated strata. Transition probabilities of a stratum therefore are conditional on survival and must always sum to one. This assumption forces the transition parameters of each stratum to be written as one function of one another (Williams et al. 2002).

In order to get our model to realistically represent this system, many analytical constraints had to be made within the models. After the initial tagging process it was impossible to, at any point, gain a tag. This allowed us to simplify the model by constraining these

transitions that represented a gain of a tag to be equal to zero. Intuitively, it is impossible to ever detect an individual in stratum D and so the probability of detection of this stratum was also constrained to equal zero. Stratum D is classified as an absorbing state as once an individual moves into this stratum, it can never leave. There is no indication to suggest that either tag should be affected by the presence of the other. This knowledge allowed us to make the further simplification of setting corresponding transitions equal to one another. Moving from stratum A to stratum C occurs when an individual has lost a main tag and moving from stratum B (main tag) to stratum D (no tag) is also a scenario in which an individual has lost a main tag. Since there is no reason to believe that transitioning from two tags to auxiliary tag should be any different from transitioning from main tag to no tag, we can constrain  $\psi_{BD}$  to be equal to  $\psi_{AC}$ , further simplifying this model. This same line of thinking allows for the simplification of  $\psi_{AB}$  and  $\psi_{CD}$ . We were then left with only three transition parameters that need to be estimated, the loss of the main tag, the loss of the auxiliary tag, and the loss of both tags at once (Figure 1). As transition parameters must sum to one, the remaining transitions of staying in a stratum are not explicitly calculated.

### *Simulations*

We carried out Monte Carlo simulations with the simulation tool in Program MARK (White and Burnham 1999) to test the effect of increased detection would have on the ability of this data set to model the covariate of time. We were interested in gaining a better understanding about the effect that increased detection rates would have on the confidence intervals of the transition parameter that corresponds to main tag loss. Four scenarios were developed with the intent to inform decisions about future tagging and resighting monitoring effort in this system. All simulations were set up to include the covariates of sex, strata and time, as this model proved to be the top model from our candidate set from a preliminary analysis ( $S(\cdot) p(\text{sex}+\text{strata}+\text{time}) \text{Psi}(\text{main}+\text{aux}+\text{both}+\text{sex}+\text{time})$ ). Due to the inability of this data set to produce estimates for the majority of the transition parameters this model was removed from the final analysis yet provides an interesting topic for this simulation. Each scenario was given initial values taken directly from the beta estimates of this top model for survival, S, and transition, Psi. Detection rates were taken from the original model for scenario 1 to represent the real data. The remaining three scenarios were given a constant rate of detection held at 5%, 10% and 15%. 1,000 simulations were carried

out to produce estimates of the real parameter values and standard error of the real parameter estimates.

## Results

### *Analysis Results*

There were 3,098 individuals tagged and released with both the main tag and the auxiliary tag. 1,749 individuals were tagged by the USGS and 1,349 by the MFRO. 2,027 of the tagged individuals were females and 1,071 were males. Over seven years individuals were resighted 182 times. Of these, 44 were females, 138 were males, and only 33 were resighted with just an auxiliary tag (stratum C).

Twelve models were evaluated to compare hypotheses about sex, stratum, and time (Table 1). Preliminary analysis explicitly modeled time dependence and found that, with our data, the model did not have sufficient data to estimate over half of the parameters. However, models with the inclusion of time produced lower AICc values suggesting that there is an effect of time for and so time was simplified as a linear trend for this analysis. All subsequent competing models differed from the top model by an AICc value of 10.88 or greater and carried no model weight thus all inference and parameter estimates were based on the top model only.

The overall the mean annual probability of losing a main tag was 8.07% (0.0225 SE, 0.0460-0.1367 95% CI). The annual probability of losing the auxiliary tag was higher, as expected, with an average of 45.06% (0.0588 SE, 0.3374-0.5627 95% CI). The mean probability of losing both tags was 0.0% (2.4555E-05 SE, 1.2275E-138-1.000E+00 95% CI). This model included a significant effect of sex on Psi ( $\beta_{\text{Females}}=0.6183$ , 0.1663 SE, 0.2923-0.9442 95% CI). For the main tag, females had a higher mean probability of losing their tag at 10.28% (0.0284 SE, 0.0588-0.1725 95% CI). Males were found to have a mean probability of tag loss at 5.86% (0.0166 SE, 0.0332-0.1009 95% CI). The estimates produced by year suggest that the probability of tag loss follows an increasing linear trend.

The mean annual probability of detection was 13.68% for individuals in stratum A (1.679 SE, 6.306E-05-0.6478 95% CI). The mean probability of detection was roughly the same for both strata B and C at 10.05% and 13.29% respectively (2.4015 SE, 1.8721E-05-0.5722 95% CI

and 1.7827 SE, 4.6168E-05-0.6429 95% CI). However, for all strata the probability of detection for the first year was considerably higher than the others. For males, the mean value of the first year was 94.96% while all subsequent years were below 11%. For females, the mean value of the first year was 62.98% and was below 1% for the remaining years. The model supported an effect of sex on detection ( $\beta_{\text{Females}} = -2.5275$ , 0.1363 SE, -2.2794 - -2.2603 95% CI) and on average males had a higher probability of detection for all strata. The model also supports an effect of observer type (e.g., public, researcher, etc.) on the detection rate. Detection probability by the public was statistically significantly different from the intercept of being detected by a research group, while the other group was not (respectively,  $\beta_{\text{Public}} = 70.46$ , 4.2943 SE, 62.0463-78.8801 95% CI;  $\beta_{\text{Other}} = 49.52$ , 9179.5226 SE, -17,942.338-18,041.391 95% CI).

### *Simulation Results*

The results of the four simulation scenarios demonstrate that increasing detection generally tends to decrease the uncertainty with the transition parameters of main tag loss at each time step. These results show that for both males (Table 2) and females (Table 3) even with an increase of detection rates to 5% for all observable strata, it would greatly reduce uncertainty within these models and allow for more accurate estimation of parameters that include the covariate of time. For each scenario, the transition parameter corresponding to main tag loss was averaged at each time step for all 1000 simulations to produce estimates and a standard error at each time step. Averages of main tag loss for scenarios with increased detection were found to be much lower than that of the simulation using the original data taken from the top model. This is likely an artifact of the initial estimates used in the simulation and so this trend should not be discerning for the overall simulation. The trend of interest for these simulations are the values of the standard error. For males the average value of standard error across all years for the model of the original data (TIME) was found to be 0.2877 (0.0228 SE, 0.2430–0.3323 95% CI). The average value for males in the scenario of increased detection to 5% was found to be higher at 0.3354 (0.0246 SE, 0.2874-0.3837 95% CI). While the average value is higher for the TIME scenario, the results of the data show that all of the values of standard error were lower for the 5% increase in detection with the exception of time step 7. The average standard error for the scenario of 5% detection becomes 0.07866 (0.0074 SE, 0.0641-0.0933 95% CI) when time step 7 is removed. For females the average value of standard error for the original model (TIME) was



found to be 0.6444 (0.0861 SE, 0.4756-0.8130 95% CI). Females with an increase in detection to 5% were found to have an average value of standard error much lower than that of the original data at 0.1731 (0.0152 SE, 0.1433-0.2026 95% CI).

## Discussion

Initial simulations and analyses determined that current detection rates were not sufficient enough to allow for the estimation of time specific transition rates within these models. The results of the initial analyses also showed strong support for the inclusion of time in both detection and transition rates. However due to low detection rates and sparse data, these transition parameters were inestimable in many years for a time dependent model. The ability to compare hypotheses of tag loss as a function of time or at a constant rate was a main objective for this analysis and so the inclusion of a trend was used in place of time for all models within the candidate set to ensure that all real parameters were estimable. For future analysis we would highly recommend including a model with time not simplified as a linear trend. The results of the simulations suggest that an increase in detection rates would be necessary to allow for the estimation of transition parameters using a model with time dependence and increasing the detection to even 5% across all strata would reduce the standard error for most years. Increasing the detection probability might require addition resighting effort in monitoring programs but the ability to more accurately compare models with time dependence to a linear trend would be immensely valuable to future studies. While future studies would greatly benefit from an increased in resight data, this model can be a useful tool for the understanding and management of this species.

Our data analysis supports an effect of sex, strata and a trend of time for detection. However, the ability to detect significant effect sizes has been compromised due to such low detection rates throughout this study. Biologically, there is reason to believe that sex and stratum would influence detection over time. Throughout their lifetime, Horseshoe crabs will molt 16-17 times, reaching sexual maturity in about 9-10 years for males and 11-12 years for females (Swan 2005; Smith et al. 2009). Once mature, the males are generally smaller than females in part because they mature at younger ages (Smith et al. 2011). These differences in development and size between males and females leads to concern that sex, particularly size because of sex, may be an influencing factor on detection.

Alternatively, differing behavior between the genders may also be a factor that affects detection. Fertilization is external by free swimming sperm and multiple males can succeed in fertilization of a female's eggs (Shuster 1982). Consequently, females spend less time on the beach than males on average. When females are found onshore, they tend to be surrounded by multiple satellite males resulting in biased operational sex ratio (Smith et al. 2002). Evidence also suggests the population includes more breeding males than females because males reach sexual maturity earlier (Smith et al. 2009). The observed bias in sex ratio due to males being more numerous and spending more time on the beach could highly influence detection probability. Our results suggest that sex does appear to be a contributing factor for detection but do not convey a difference significant enough to change current field methods. A more in depth analysis to provide more conclusive results would be beneficial for the efficiency of future field studies. If behavior or physical differences in sex do favor the detection of one gender over the other then it may be more efficient to tag just one sex, depending on the objectives of the monitoring program. It could also help improve resight rates which would greatly improve the ability to run statistical analysis on this species.

Results support a linear trend in describing transition rates. More importantly, the results show support that the rates of tag loss are not constant and are affected by time. This is significant for determining how to incorporate tag loss in the estimation of survival. While these results support that the rate of tag loss change over time, future studies would benefit from more consistent and greater resight effort to reduce the uncertainty within this model. The parameter estimates indicate that tag loss is greater in the first few years and then begins to taper. The agencies tagging horseshoe crabs for this study concentrated resighting efforts only during the spawning season that individuals were initially tagged. A greater resight effort would be able to conclude if tags are truly more likely to fall off within the first few years of tagging or if this trend is a result of reduced resight efforts. In addition, long term tag loss could be explained as wear on the tag while short term tag loss is likely to be a reflection of misapplication. The addition of more data would allow for the ability to study this system in more depth and possibly begin to tease apart these different hypotheses about how tags are lost.

This analysis found support for an effect of sex on tag loss ( $\beta_{\text{Females}}=0.62$  (0.17 SE, 0.29 - 0.94 95% CI). Differences in behavior during the spawning season may contribute to an

increased rate of tag loss for females. Males clasp onto the back of females as they make their way to the beach which could contribute to wear on the tag. Also, females often tend to be surrounded with numerous satellite males throughout the spawning season, which could lead to excessive wear on the tag. Knowledge if sex is a contributing factor to tag loss would improve the economic efficiency of field studies if measures could be taken to reduce tag loss or depending on the objective of the study, researchers could reduce tagging only one sex.

### *Management Recommendations*

The flexible framework of a multistate model proved a successful technique to describe this system of tag loss. This analysis brings forth many questions about this system but the support for a trend of time has great implications for future studies of adult survival for this species. With these results the current survival estimate can be recalculated to eliminate tag loss bias. An updated estimation of adult survival to include this bias of tag loss will greatly improve the predictive power of the current management models thereby improving decision making. Further, tag loss bias should be incorporated into mark recapture based abundance estimation. We recommend for future studies the consideration of not only a bias to include the loss of main tags but also an effect of time on tag loss. Based on the simulations of this data set, increased resighting monitoring effort and a greater initial tagging effort would be valuable to improve upon these results and to achieve a deeper understanding of this population. While we recommend first working on improving detection rates future work may also benefit from the use of more covariates to explain some of the variation, such as weather data to explain the effect of time. Finally, the age of an individual affects when and how often Horseshoe crabs come up to the shoreline to spawn it is also likely that age could be a factor in detection probability.

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Figure 1: Movement of the system of tag loss in four stratum.

Stratum A: An individual who possesses both a main tag and an auxiliary tag.

Stratum B: An individual who a main tag and no auxiliary tag.

Stratum C: An individual who an auxiliary tag and no main tag.

Stratum D: An individual who no tags.

$\Psi$ : The probability of an individual transitioning from one stratum to another stratum

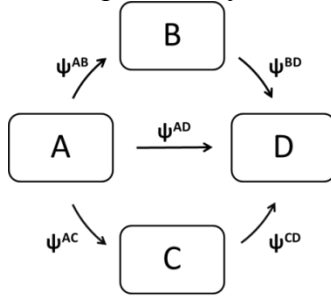


Table 1: Model selection criteria for apparent survival (S), detection (p), and transition (Psi) for the tag loss rates of the Atlantic Horseshoe crab in Delaware Bay.

Model	AICc <sup>a</sup>	$\Delta$ AICc <sup>b</sup>	AICc Weights <sup>c</sup>	Model Likelihood <sup>d</sup>	Num. Par <sup>e</sup>	Deviance
S(.) p(strata+sex+T+COV <sup>f</sup> ) Psi(aux <sup>g</sup> +main <sup>h</sup> +both <sup>i</sup> +sex+T)	7877.93	0.00	1.00	1.00	9.00	7859.88
S(.) p(strata+sex+T+COV) Psi(aux+main+both+T)	7888.81	10.89	0	0	8.00	7872.77
S(.) p(strata+sex+T+COV) Psi(aux+main+both+sex+T)	7903.30	25.37	0	0	7.00	7889.27
S(.) p(strata+T+COV) Psi(aux+main+both+sex+T)	8144.23	266.31	0	0	8.00	8128.20
S(.) p(strata+T+COV) Psi(aux+main+both+T)	8483.78	605.85	0	0	7.00	8469.75
S(.) p(strata+T+COV) Psi(aux+main+both+T)	8483.78	605.85	0	0	7.00	8469.75
S(.) p(strata+T+COV) Psi(aux+main+both)	8488.67	610.74	0	0	6.00	8476.64
S(.) p(T+COV) Psi(aux+main+both+T)	8528.27	650.34	0	0	5.00	8518.25
S(.) p(strata+COV) Psi(aux+main+both)	8905.52	1027.59	0	0	4.00	8897.51
S(.) p(strata+sex+T) Psi(aux+main+both+sex+T)	8975.77	1097.84	0	0	9.00	8957.72
S(.) p(COV) Psi(aux+main+both)	9724.99	1847.07	0	0	3.00	9718.99
S(.) p(.) Psi(aux+main+both)	9973.47	2095.54	0	0	5.00	9963.45

<sup>a</sup> Akaike's Information Criterion

<sup>b</sup> Difference between the AICc value of the selected model and the minimum AICc value

<sup>c</sup> Strength of evidence for selected model / strength of evidence for the competing previous model

<sup>d</sup> The likelihood of the model  $i$  given the data is  $= \exp(-1/2\Delta_i)$

<sup>e</sup> Number of parameters estimated in selected model

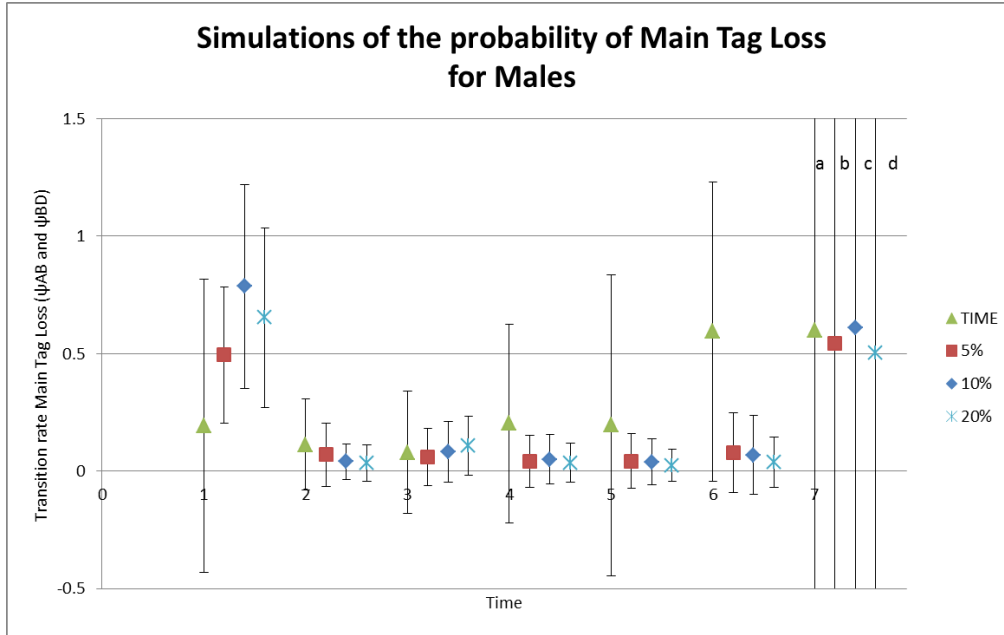
<sup>f</sup> Covariate of reporter type on detection

<sup>g</sup> Transition of auxiliary tag loss ( $\psi^{AB}$  and  $\psi^{CD}$ )

<sup>h</sup> Transition of main tag loss ( $\psi^{AC}$  and  $\psi^{BD}$ )

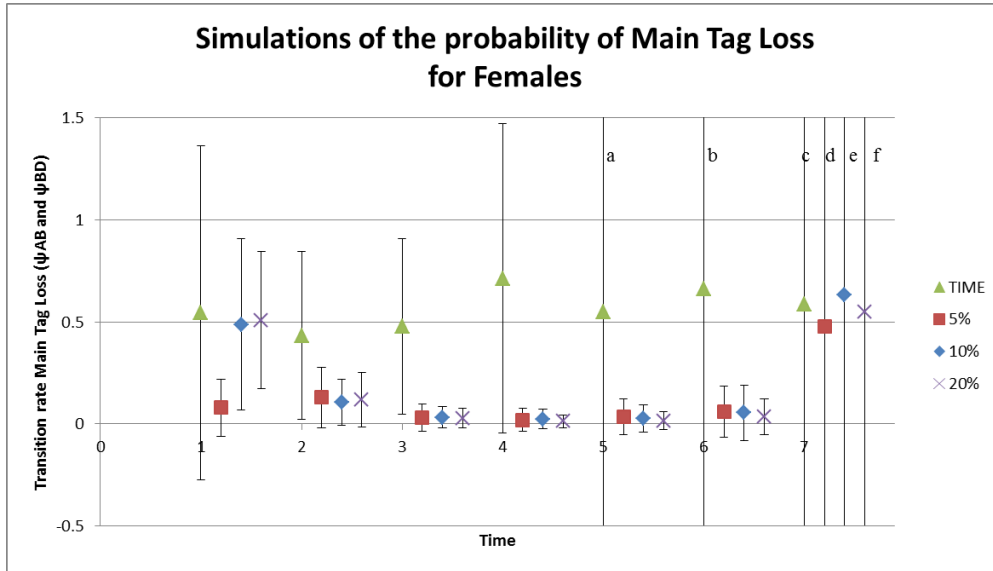
<sup>i</sup> Transition of loss of both tags ( $\psi^{AD}$ )

Figure 2: Simulation Results for the transition parameter of main tag loss for Males from the model,  $S(\cdot) p(\text{sex}+\text{strata}+\text{time}) \Psi(\text{main}+\text{aux}+\text{both}+\text{sex}+\text{time})$  with manipulated detection rates: Initial values of detection were taken directly from the top model of the results (TIME) or held constant at a rate of detection of 5%, 10% or 20%. Each data point represents the average value of main tag loss at each time step over 1000 simulations. Error bars represent two times the average value of standard error at each time step over 1000 simulations.



- <sup>a</sup> The average standard error values for TIME at Time 7 was 0.623
- <sup>b</sup> The average standard error values for 5% detection at Time 7 was 1.876
- <sup>c</sup> The average standard error values for 10% detection at Time 7 was 2.556
- <sup>d</sup> The average standard error values for 20% detection at Time 7 was 1.454

Figure 3: Simulation Results for the transition parameter of main tag loss for Females from the model,  $S(\cdot) p(\text{sex}+\text{strata}+\text{time}) \Psi(\text{main}+\text{aux}+\text{both}+\text{sex}+\text{time})$  with manipulated detection rates: Initial values of detection were taken directly from the top model of the results (TIME) or held constant at a rate of detection of 5%, 10% or 20%. Each data point represents the average value of main tag loss at each time step over 1000 simulations. Error bars represent two times the average value of standard error at each time step over 1000 simulations.



<sup>a</sup> The average standard error values for TIME at Time 5 was 1.097

<sup>b</sup> The average standard error values for TIME at Time 6 was 0.879

<sup>c</sup> The average standard error values for TIME at Time 7 was 1.326

<sup>d</sup> The average standard error values for 5% detection at Time 7 was 0.900

<sup>e</sup> The average standard error values for 10% detection at Time 7 was 2.473

<sup>f</sup> The average standard error values for 20% detection at Time 7 was 1.648



## Chapter 2: Survival of the Atlantic horseshoe crab, *Limulus polyphemus*, in Delaware Bay

### Introduction

The Atlantic horseshoe crab, *Limulus polyphemus*, is a species of biological importance and has had a significant socioeconomic impact on the communities of the mid-Atlantic Coast. As of 2002 the horseshoe crab and related industries, including ecotourism, commercial fishing, and biomedical value, were bringing in between 93 to 123 million dollars to the regional economies of the entire Atlantic Coast (Walls et al. 2002). However, concerns for shorebird population declines, particularly in Delaware Bay, have led to debates about suitable management and harvest regulations (McGowan et al. 2011). Spring, northward migration of a variety of shorebirds along the Atlantic coast of North America coincides with the annual spawning of the Atlantic horseshoe crab (Mizrahi and Peters 2009; Smith et al. 2009). The red knot (*Calidris canutus rufa*) is currently a species of concern among the migrating shorebird species that use Delaware Bay as a stopping over site and is also a candidate subspecies for listing under the U.S. Endangered Species Act due to recently estimated significant population declines (Baker et al. 2004; Morrison et al. 2004; Niles et al. 2009). The main food source for the red knot during stopover in Delaware Bay is the protein and fat rich eggs of the Atlantic horseshoe crab (Karpanty et al. 2006). A marked increase in horseshoe crab harvest efforts in the 1990's and therefore reduction in egg availability was a hypothesized explanation for the observed declines in Delaware (Baker et al. 2004, Niles et al. 2009). The monetary value of the horseshoe crab along with their vital role in the environment highlights the importance and caution that must be taken in deciding management plans.

Growing concerns for migrating shorebirds and the Atlantic horseshoe crab led to the development of management regulations in the 1990's (Walls et al. 2002). In 1998 the Atlantic States Marine Fisheries Commission (ASMFC) developed a coastwise management plan to regulate crab harvest and integrate the diverse interests of the variety of stakeholders (ASMFC 1998). Recent amendments to this 1998 Horseshoe Crab Fishery Management Plan have taken into account the need for multi-species objectives to incorporate red knot recovery. Following the Adaptive Resource Management (ARM) framework, McGowan et al. (2011) constructed a predictive multi-species model to take into account the hypothesized relationship between the red knot and the Atlantic horseshoe crab. Specifically, the model links red knot mass gain, annual survival, and fecundity to horseshoe crab abundance and spawning and the model can be used to

evaluate horseshoe crab harvest actions with respect to red knot and horseshoe crab population objectives.

The new management paradigms of the Mid-Atlantic population of *Limulus polyphemus* have increased the need for further analysis of the current demographic data to achieve a broader, more in depth understanding of the economic and ecological significance of this prehistoric creature. The horseshoe crab model presented by McGowan et al. (2011), as it currently stands, has significant uncertainty due to spatial and temporal variation of life history parameters used. The best available estimates of sub adult and adult survival parameters were estimated from a population in Pleasant Bay, Massachusetts (Carmichael et al. 2003; Sweka et al. 2007). The Atlantic horseshoe crab can be found intermittently along the eastern coast of North America (Shuster 1982). There is genetic and morphological evidence to suggest that there are distinct subpopulations within the Atlantic population (Botton and Loveland 2003; King et al. 2005). This presumption of distinct divisions among the population necessitates the estimation of distinct population parameters if a region is to be modeled accurately. There is a strong need for more complete and detailed studies with a Delaware Bay specific focus to resolve some of this uncertainty. Recommendations to improve the current model call for new estimates of the demographic parameters, such as adult annual survival (ASMFC 2009; McGowan et al. 2011). The current estimate of adult annual survival, from the Pleasant Bay population, is 66.0% (C.V = 0.15%) (Sweka et al. 2007; McGowan et al. 2011).

Our objective for this analysis was to estimate adult survival using data specifically collected in and around Delaware Bay. We also tested for an effect of sex and of biomedical agencies on survival and detection. Previous studies (see Rudloe 1983; Walls and Berkson 2003; Hurton and Berkson 2006; Leshcen and Correia 2010) suggest that bleeding by biomedical industries for the production of *Limulus* Amebocyte Lysate (LAL) does appear to increase mortality. LAL is a reagent prepared from the blood of the horseshoe crab that has the ability to detect nanogram quantities of endotoxin, which is a pyrogen that can be found within the cell wall of gram-negative bacteria (Levin et al. 2003). This reagent replaced rabbit pyrogen testing in the U.S. and is a more efficient and humane method for testing the sterility of products and the safety of drugs (Levin et al. 2003). Knowledge of how sex and agency affect survival and detection will help to improve the effectiveness and efficiency of future field studies as well as contribute to a more accurate model structure of this system. Updated and site specific

demographic parameter estimates will help to reduce uncertainty within current management models making the predictive models more effective in accomplishing conservation and management objectives.

## Methods

### *Study Design*

This data set is a compilation of independent studies conducted by 37 different agencies. These agencies were composed of biomedical companies, independent research institutions, government agencies and academic institutions. We restricted our analysis to data collected between 1999 and 2010. We removed individuals of unknown sex from this data set. The identification of sex is performed visually, as females are usually larger in size than males (Carmichael et al. 2003; Swan 2005). Males can also be distinguished by the presence of claspers, a modified version of the first pair of walking legs which aid in attachment to females during spawning (Swan 2005). No other individual covariates were measured consistently enough across each independent study to be included in this analysis.

Tagging methods were consistent across all agencies. To tag individuals, first a 4mm diameter hole is drilled into the lower left region of the carapace along the edge of the shell in the field using a rechargeable drill (Figure 1) (Swan 2005; Smith et al. 2010). Through this hole, a “Christmas Tree” pin is inserted and the individual is given a standard button tag, a circular metal tag that displays a unique number and methods for reporting a resighting (Brousseau et al. 2004; Swan 2005; Smith et al. 2006). Mortality is not affected by the presence of a tag (Smith et al. 2006; Mattei et al. 2011). The duration and resight effort of each agency varied due to the individual objectives of each study. Resight data was collected by either structured survey efforts of the individual agency, through help from outside sources, or a combination of both. Many agencies offer a way for the general public to report a tag online or by phone and some states require fishermen to report tags. Some agencies offer a reward for reporting a tag to encourage help from the general public (Swan 2005). Only resight data that explicitly stated that an individual was captured or resighted alive was utilized for this analysis.

### *Statistical Modeling*

Using Program MARK (White and Burnham 1999), we used a Cormack-Jolly-Seber (CJS) model to analyze the data and estimate survival and detection probability. *A priori*, we choose a set of models that included the covariates of sex, agency and time on survival and detection

parameters to represent different competing biological hypothesis. Sex was used to define the grouping of this analysis and agency was used as an individual covariate. Agencies were separated into two groups, biomedical and non-biomedical agencies. Models were run with the effect of agency on only the first year, the first two years, the first three years or all years to test the test if there is some initial or long term effect of bleeding on survival (indicated by Biomed 1, Biomed 2, Biomed 3 or Biomed respectively in Table 5).

Akaike's Information Criteria (AICc) is a commonly accepted and useful tool in model selection and was used in our analysis of the results (Burnham and Anderson 2006). The model with the lowest AICc value was favored as the best representation of the data set. The difference in AICc of competing models to the top model was used to further rank the models from there on out (Anderson et al. 2000). The methods of Information-Theoretic approaches, such as AICc, are conditional on the knowledge that there is an adequate fit of the general model to the data (Cooch and White 2012) and so a goodness of fit test was run. Goodness of fit tests can elucidate the underlying patterns of the data set to give evidence if any lack of model fit can be attributed to noise or more importantly if it is due to violations of the CJS model assumptions (Williams et al. 2002). Any violations of the CJS assumptions must be accounted for in models to produce accurate estimates of survival. Using Program RELEASE, a program within Program MARK, a median  $\hat{c}$  test was run for this analysis to test the goodness of fit.

Cormack-Jolly-Seber models assume no loss of marks (Pollock et al. 1990). If tag loss is occurring and not being account for, the result of the estimate becomes the product of the actual survival rate and the rate of tag loss. Conflating these parameters generally tends to underestimate survival (Pollock et al. 1990; Alisauskas and Lindberg 2002). Following the methods of Nichols et al. (1992) and Nichols and Hines (1993), Butler et al., (Chapter 1 of this thesis) conducted an initial study to produce a single estimate of the probability of tag loss which we then applied to the results of this analysis post hoc. We estimated the mean probability of tag loss to be 8.07% per year (Butler et al., Chapter 1 of this thesis). Specifically, females had a higher average probability of losing their tag at 10.28% and males were found to have an average probability of tag loss at 5.86%

## Results

Overall there were 164,415 individuals tagged and released over a span of twelve years. Of these, 59,018 individuals were tagged by government affiliated agencies, 48,801 individuals

were tagged by biomedical industries, and the remaining 56,596 individuals were tagged by research or academic institutions. 63,080 were females and 101,335 were males. During this study there were 5,648 resightings of individuals. There were 4,321 resighting of males and 1,227 resightings of females. 2,442 of the resightings were initially released from a government agencies, 1,708 from a biomedical company and 1,398 were from other research or academic institutions.

20 models that represented plausible and competing hypotheses about the description of this system made up our candidate set (Table 1). The top model within this set was the model that included the covariates of sex, time, and agency for both parameters of survival,  $\Phi$ , and detection,  $p$ , as well as an interaction term between sex and time. The covariate of agency described a long term effect of bleeding (Biomed) as opposed to a more temporary effect (Biomed 1, Biomed 2, or Biomed 3). The results of the goodness of fit tests conducted conveyed that there was an adequate fit of our model to this data set. The  $\hat{c}$  of the most parameterized model possible, also our top model, was within a reasonable range ( $\hat{c} \leq 1$ ) (Burnham and Anderson 2002; Williams et al. 2002). Given this result, no adjustments were necessary for this analysis. All subsequent models after the top model held no model weight or likelihood and so all model inference was based on the top model.

Overall, the mean annual survival rate was 65.81% (S.E. = 0.0671, 95% C.I. = 0.3866 - 0.7636) and adding the correction due to tag loss, is adjusted to 71.07%. This model does show that there is a statistically significant effect of sex ( $\beta_{\text{Male}} = 2.5667$ , S.E. = 0.9489, 95% C.I. = 0.7069 - 4.4265). On average, males were found to have a higher probability of survival compared to females. Males had a mean annual rate of survival of 73.03% (S.E. = 0.0412, 95% C.I. = 0.4510 - 0.7886). With the correction for mean tag loss rate of males this value is adjusted to 77.31%. By year, males had survival rates ranging from as low as 44.98% in 2009 (S.E. = 0.0285, 95% C.I. = 0.3948 - 0.5060) to as high as 99.86% in 2002 (S.E. = 0.0071, 95% C.I. = 0.0348 - 1.00). Females had a mean annual survival rate of 58.60% (S.E. = 0.0931 SE, 95% C.I. = 0.3322 - 0.7386). This value adjusted for the mean rate of female tag loss is 64.62%. Females were found to have a wider range of estimates for survival than males, ranging from 8.04% in 2010 (S.E. = 0.0737, 95% C.I. = 0.0122 - 0.3815) to 97.87% in 2005 (S.E. = 0.0643, 95% C.I. = 0.0992 - 0.9999).

Estimates of mean annual detection were 4.01% (S.E. = 0.0164, 95% C.I. = 0.0218 - 0.0715). Females had a mean annual probability of detection at 4.71% (S.E. = 0.02996, 95% C.I. = 0.0156 - 0.1037). Males had a mean annual probability of detection of 3.21% (S.E. = 0.0029, 95% C.I. = 0.0268 - 0.0386). The rate of detection for males ranged from as low as 1.84% in 2006 (S.E. = 0.0012, 95% C.I. = 0.0163 - 0.0208) to 5.56% in 2001 (S.E. = 0.0059, 95% C.I. = 0.0450 - 0.0685). For females, the rate of detection ranged from 0.3% in 2007 (S.E. = 0.0012, 95% C.I. = 0.0034 - 0.0082) to 32.44% in 2010 (S.E. = 0.2972, 95% C.I. = 0.03256 - 0.8727). There was a statistically significant effect of sex on detection ( $\beta_{\text{Male}}=0.3308$ , 0.1590 SE, 95% C.I. = 0.0191 – 0.6425) though this may be an artifact of an outlier in the estimate of female detection in the year 2010. Removing this outlying value of female detection in 2010, the highest value of female detection never exceeds 5%. This leaves a mean rate of annual detection of 1.93% (S.E. = 0.0032, 95% C.I. = 0.0139 – 0.0268), making the probability of detecting a female lower than that of males.

## Discussion

Sex, time and agency had substantial effects on detection rates. On average, females were found to have a higher probability of detection compared to males although this was due to an outlier for females in 2010. Removing this outlier reduces the mean probability of detection of females that is lower than that of males. Although the results show a statistically significant effect of sex on detection, it is clear that further analysis would be necessary to determine if or which gender has a higher probability of detection. The differences in detection between genders may be due to differences in behavior or morphology. While spawning surveys find more males than females because more males congregate on the beach during spawning (Shuster 1982), females remain longer in deep water during the spawning season making them more easily captured by trawl surveys. Females may also be more likely to be detected from trawl surveys because of their larger size (Carmichael et al. 2003). Horseshoe crabs continue to molt and grow until they reach sexual maturity. For males this occurs at 9-10 years of age and 11-12 years for females (Swan 2005; Smith et al. 2009). Since males reach maturity earlier, the females have more time to continue to molt and grow making them larger in size on average compared to the males (Smith et al. 2011). However, the number of individuals observed on the beach during the breeding season favoring males due to fact that males tend to be more numerous and spend more time on the beach than females. This behavior would favor a hypothesis that the probability of

detecting a male should be greater than that of a female. Multiple males can succeed in fertilization the eggs of a female. Consequently, males spend more time on the beach than females (Shuster 1982). Not only do females spend less time on the shore but when they are onshore they tend to be surrounded or covered by multiple satellite males. Evidence also suggests the population includes more breeding males than females due to the fact that males reach sexual maturity earlier (Smith et al. 2009). We highly recommend further study on detection rates to determine more conclusive results on the effect of sex on detection.

The effect of time on detection may be an artifact of an unexplained environmental phenomenon or due to tag loss. Previous work on tag loss found that tags are being lost at a rate that changes over time as an increasing linear trend (Butler et al., Chapter 1 of this thesis). If tags are becoming worn or lost over time it would adversely influence the probability of an individual being detected. Though this analysis did not account for tag loss with time directly, it would be of value for future studies to incorporate tag loss with each time step to reduce any uncertainty within this model and help determine why detection rates were found to vary over time. Future studies would also benefit with the inclusion of more covariates as alternative hypothesis to help explain the variation of time.

Further analyses on the effects of agency on detection would be of value to the improvement of current management methods. Individuals tagged by a biomedical agency had a statistically significant and slightly negative association on detection ( $\beta_{\text{Biomed}} = -0.3336$ , S.E. = 0.0558, 95% C.I. = -0.4430 – -0.2241) compared to that of non-biomedical agencies. Although this association may be statistically significant, more research on detection by specific agency would be beneficial to the improvement of mark-recapture techniques. Early tag loss due to misapplication may contribute to a decrease in detection probability if those individuals essentially become removed from the study population and is one hypothesis as to why individuals tagged by biomedical companies would have a lower probability of detection. However, there is also concern that individuals have increased mortality rates due to the bleeding process by biomedical agencies compared to non-bled horseshoe crabs (Rudloe 1983; Walls and Berkson 2003; Hurton and Berkson 2006; Leshcen and Correia 2010). If individuals are dying and, like tag loss, are becoming removed from the study then this would also show a negative effect on detection. While our hypothesis to represent the immediate effect of a biomedical agency over the first three years on detection were not as strong of models as our top model, they

were all within a difference in AICc values of only 23. Further studies with increased detection rates may help to reduce the uncertainty of these models and elucidate this effect.

This analysis supports an effect of sex, time and agency on survival. As with detection, although the effect of sex was found to be statistically significant, the large variance and confidence intervals between the actual estimates of survival make it hard to interpret the biological significance with confidence. The larger size of females and longer time spent in the water during spawning may make them more likely to be susceptible to predation in the water. Known predators of the horseshoe crab include the loggerhead turtles (*Caretta caretta*) (Botton et al. 2009). Survival may be effected by harvest by fishermen as female horseshoe crabs are preferred over males as bait (Kreamer and Michels 2009). Alternatively, this effect may be a result of a bias in detection. If it was determined that females do have a lower probability of detection, survival could appear lower if their resightings are not reported.

An effect of time can represent an assortment of hypotheses about environmental variation or trends that have not yet been explained (Williams et al. 2002). There are several aspects of horseshoe crab ecology that suggest weather and climate may have an effect on demographics and including a covariate as an index of annual weather patterns may help to explain the effect of time. High wave action, caused by wind and storms, has been shown to delay or even suppress spawning in horseshoe crabs as turbulent weather may increase the risk of stranding and death (Botton and Loveland 1989; Smith et al. 2002). The current predictive model makes the assumption that the mean rate of adult survival is the same for all individuals once the individual reaches sexual maturity (McGowan et al. 2011). Future studies may also benefit from the inclusion of an effect of age as it could greatly alter population projects under the current management model.

Knowledge of the effect of agency on survival would be of interest to the conservation of this species as well as the improvement of management models. This analysis found a statistically significant difference between the effect of the two agencies and interestingly, found that individuals tagged by biomedical agencies had slightly higher probability of survival ( $\beta_{\text{Biomed}} = 0.2361$ , S.E. = 0.06737, 95% C.I. = 0.1040 – 0.3681) compared to the survival of individuals tagged by a non-biomedical agency. While this trend is not consistent with previous studies (Rudloe 1983; Walls and Berkson 2003; Hurton and Berkson 2006; Leshcen and Correia 2010), it is of interest that a statistical difference was found between the two agencies.



Biomedical companies favor individuals with no visible signs of injury and little or few epibionts on their carapace so it would be reasonable to conclude that this set of individuals were younger and healthier compared to a truly random sample of the population. However, this study was not specifically developed with the intent of analyzing an effect of bleeding and so more detailed studies on the effect of bleeding by a biomedical company on adult survival would be of great biological interest and necessary to illuminate accurate hypotheses as to why this difference may be occurring.

### *Management Implications*

To help the efficiency of monitoring and estimation in the future we recommend an increase in sampling effort. We attempted to include a covariate of reporter type on detection probability but this resulted in the inestimability of all Phi parameters. With the current detection rates, the inclusion of a detection covariate of reporter type did not help to explain this data set. Knowledge of who is reporting resight data would help to improve the economic efficiency of mark-recapture work and population monitoring programs for this managed species. Conducting monitoring efforts, such as trawl or other research surveys, can be expensive and time consuming and so many agencies rely on the public to help report data. If more data can be gathered to assess the effectiveness of public resight rates, future studies may be able to simply rely on the public.

Higher rates in detection may result in greater support for the effect of gender and agency on both detection and survival. If conclusive support can be found to favor the detection of one sex over the other, we recommend the tagging of just one sex to improve the effectiveness of field studies. More certainty of the effect of gender on survival would be crucial to the improvement of the accuracy of the current management models. The effect of biomedical agencies on both detection and survival brings about many interesting biological questions. Improved detection rates would help to reduce the uncertainty of the results presented in this study and lead to a better understanding of this effect and how it should be incorporated into future management models.

With this work, the current predictive management models proposed by McGowan et al. 2011 can be updated to incorporate a survival estimate specific to Delaware Bay. Updated and site-specific demographic parameters estimates will reduce uncertainty in models thereby improving the accuracy of model predictions. Site specific and updated parameter estimates also

increase confidence in the accuracy of the management models underlying decision making for horseshoe crab harvest. Additionally our efforts provide estimates of the variability in survival which can now be incorporated into the management models. Heretofore, Sweka et al. (2007) and McGowan et al. (2011) used reasonable and expert elicited coefficients of variation to incorporate annual variation in horseshoe crab survival because direct estimates of variation, such as we have estimated here, were not available. This research will have valuable significance for future actions of conservation and management goals with the ambition of being used as a stepping stone for which more wide scale work on horseshoe crabs can be conducted. From an accurate model of a population, characteristics about that population can be confidently assessed and with that knowledge, predictive models can be formed to more effectively accomplish conservation and management objectives.

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Table 1: Model selection criteria for apparent survival ( $\Phi$ ) and detection ( $p$ ) for the Atlantic horseshoe crab in Delaware Bay. The effect of agency is denoted by Biomed for models where agency was applied to all years, Biomed 1 when agency was applied to only the first year, Biomed 2 when applied to the first two years and Biomed 3 when applied to the first three years.

Model	AICc <sup>a</sup>	$\Delta$ AICc <sup>b</sup>	AICc Weights <sup>c</sup>	Model Likelihood <sup>d</sup>	Num. Par <sup>e</sup>	Deviance
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed})$	58805.85	0	0.98	1.00	46.00	58713.82
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 1})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 1})$	58814.05	8.20	0.02	0.02	46.00	58722.02
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed})$	58819.12	13.27	0	0	45.00	58729.09
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 3})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 3})$	58825.84	20.00	0	0	46.00	58733.81
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 2})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed 2})$	58829.48	23.63	0	0	46.00	58737.45
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t})$	58833.37	27.53	0	0	44.00	58745.34
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t}+\text{Biomed})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t})$	58834.77	28.92	0	0	45.00	58744.74
$\Phi(\text{sex}+\text{time}+\text{sex}^*\text{t})$ $p(\text{sex}+\text{time}+\text{sex}^*\text{t})$	58838.13	32.28	0	0	44.00	58750.10
$\Phi(\text{sex}+\text{time}+\text{Biomed})$ $p(\text{sex}+\text{time}+\text{Biomed})$	58940.24	134.39	0	0	26.00	58888.23
$\Phi(\text{sex}+\text{time}+\text{Biomed 1})$ $p(\text{sex}+\text{time}+\text{Biomed 1})$	58949.69	143.85	0	0	26.00	58897.68
$\Phi(\text{sex}+\text{time}+\text{Biomed 3})$ $p(\text{sex}+\text{time}+\text{Biomed 3})$	58955.70	149.86	0	0	26.00	58903.69
$\Phi(\text{sex}+\text{time}+\text{Biomed 2})$ $p(\text{sex}+\text{time}+\text{Biomed 2})$	58962.02	156.17	0	0	26.00	58910.01
$\Phi(\text{sex}+\text{time})$ $p(\text{sex}+\text{time})$	58967.42	161.58	0	0	24.00	58919.42
$\Phi(\text{time})$ $p(\text{sex}+\text{time})$	58976.44	170.59	0	0	23.00	58930.43
$\Phi(\text{sex}+\text{time})$ $p(\text{time})$	59094.38	288.53	0	0	23.00	59048.37
$\Phi(\text{time}+\text{Biomed})$ $p(\text{time}+\text{Biomed})$	59564.58	758.73	0	0	24.00	59516.57
$\Phi(\text{time})$ $p(\text{time})$	59615.02	809.17	0	0	22.00	59571.01
$\Phi(\text{sex}+\text{Biomed})$ $p(\text{sex}+\text{Biomed})$	59705.55	899.71	0	0	6.00	59693.55
$\Phi(\text{time})$ $p(\cdot)$	59912.54	1106.70	0	0	12.00	59888.54
$\Phi(\cdot)$ $p(\cdot)$	60485.17	1679.32	0	0	2.00	60481.17

<sup>a</sup>Akaike's Information Criterion

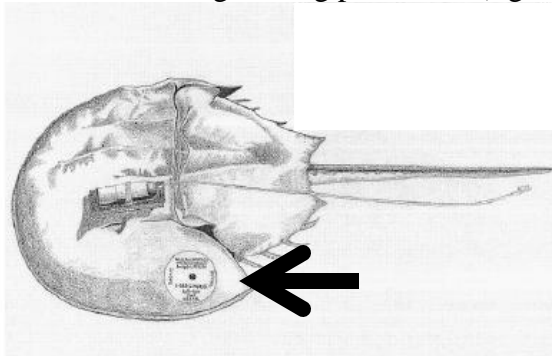
<sup>b</sup>Difference between the AICc value of the selected model and the minimum AICc value

<sup>c</sup>Strength of evidence for selected model / strength of evidence for the competing previous model

<sup>d</sup>The likelihood of the model  $i$  given the data is  $= \exp(-1/2\Delta_i)$

<sup>e</sup>Number of parameters estimated in selected model

Figure 1: Detail of tag and tag placement (figure from Brousseau et al. 2004)



## Chapter 3: A multistate model for the estimation of survival of the Atlantic horseshoe crab confounded by tag loss

### Introduction

Ordinary Cormack-Jolly-Seber models are adequate to estimate survival but the addition of multiple state and transition parameters can reduce uncertainty and answer more complex questions about a population (Schwarz et al. 2003). The transition parameters of a multistate model provide the flexibility and complexity necessary for analyzing a wide variety of questions in population biology beyond the simple estimation of survival (Lebreton and Pradel 2002; Conn et al. 2004). Often in mark-recapture studies, partial observability or imperfect detection is a concern and the use of a multistate model with hidden states can help explore data sets with this uncertainty (Conn and Cooch 2009; Kendall et al. 2012). The inclusion of a hidden state provides a natural framework to model tag loss. Individuals who lose a tag during a mark-recapture study become unintentionally removed from the study and are inaccurately treated as if they have emigrated or died (Pollock et al. 1990; Alisauskas and Lindberg 2002). This bias can be avoided by the addition of an unobservable state to represent the loss of a tag. Expanding upon models these designs, we propose a unidirectional multistate model with forward movement and an unobservable state to improve upon prior studies of the estimation of survival of the Atlantic horseshoe crab, *Limulus polyphemus*.

Recent concerns and new management paradigms of the Mid-Atlantic population of *Limulus polyphemus* have increased the need for further analysis of the current demographic data to achieve a broader, more in depth understanding of the economic and ecological significance of this prehistoric creature. This species has a significant socioeconomic impact on the communities of the mid-Atlantic Coast due to their value as bait for the eel and whelk harvest, as a resource for biomedical companies, and ecotourism during their spring spawning (Walls et al. 2002). Horseshoe crabs are utilized by biomedical companies for their blood which can be made into a produce called *Limulus* Ameobocyte Lysate (LAL). LAL has the ability to detect nanogram quantities of endotoxin, which is a pyrogen that can be found within the cell wall of gram-negative bacteria (Levin et al. 2003). However, each biomedical company maintains its own methods and guidelines for procuring the blood of individuals and so mortality rates must be

determined independently by each company and the overall effect of bleeding of horseshoe crabs is not well understood.

The horseshoe crab also plays a vital role in their ecosystem because of their relationship with shorebirds. The Spring, northern migration of an assortment of shorebirds along the Atlantic coast coincides with the annual spawning of horseshoe crabs as many shorebirds utilize the surplus of horseshoe crab eggs on the beach as a main food source to refuel during their stopover (Baker et al. 2004; Morrison et al. 2004; Karpanty et al. 2006; Mizrahi and Peters 2009; Niles et al. 2009; Smith et al. 2009). However, concerns for shorebird population declines made it apparent that management and harvest regulations of horseshoe crabs were necessary to take into account the interaction of these two species in and around Delaware Bay (Walls et al. 2002; McGowan et al. 2011). While current management actions are underway, the most recent predictive model by McGowan et al. (2011) suggested recommendations to improve their multi-species model with updated and site specific life history parameters, such as adult survival (ASMFC 2009; McGowan et al. 2011). Updated and site specific estimates of adult survival will help to reduce uncertainty within the current management models making the predictive models more effective in accomplishing conservation and management objectives (McGowan et al. 2011).

Previous studies on the horseshoe crab indicate that tag loss is occurring (Butler et al. unpublished, Chapter 1 of this thesis). One of the assumptions of a Cormack-Jolly-Seber model is that tags are not being lost as it would result in the underestimation of survival (Pollock et al. 1990; Williams et al. 2002). Mark-recapture studies that show even a minimal potential for tag loss should take into account this bias to ensure that survival is estimated accurately (Williams et al. 2002). Nichols et al. (1992) advocates the use of a single estimation model to account for tag loss over other multistep processes (see Arnason and Mills 1981). A single estimation model has the advantage of producing survival estimates directly which reduces uncertainty and allows for the ability to easily test and compare alternative models.

The use of a multistate model to simultaneously estimate tag loss and survival has, in recent years, proved to be a successful alternative to multistep approaches (e.g., Alisauskas and Lindberg 2002; Joe and Pollock 2002; Conn et al. 2004; Kendall et al. 2006). In many cases, it appears more logical that tags are lost as a function of time since tagging due to misapplication or physical wear as opposed to following patterns of a calendar year (Nichols et al. 1992;



Nichols and Hines 1993; Conn et al. 2004). This can make the incorporation of a bias by tag loss difficult when survival follows an effect of time by calendar year. The addition of transition parameters allows for the flexibility necessary to develop a model that can handle this complexity. In a multistate model, parameters are estimated using a maximum likelihood approach in the same fashion as a single state model, such as a Cormack-Jolly-Seber approach, except that all parameters become state specific so that multistate survival,  $\phi$ , becomes a parameter that incorporates both apparent survival,  $S$ , and the added transition probabilities,  $\psi$  (Equation 1, Williams et al. 2002).

$$\phi_i^{rs} = S_i^r \psi_i^{rs} \quad (1)$$

If transition is defined as the probability of tag loss then both survival and tag loss may be estimated within the same model yet each parameter may be defined by distinct constraints. As defined in this manner, apparent survival,  $S_i$ , becomes the probability that an individual survives at time  $i$  and remains within the study population. Transition,  $\psi_i$ , is the probability that an individual in one state at time  $i$  moves to another state at time  $i+1$  and is conditional on an individual being alive at time  $i$ . This separation of survival and transition allows us to address many interesting biological hypothesis about a system (Williams et al. 2002).

In this paper we used a multistate modeling approach to simultaneously estimate adult survival and the rate of tag loss to test competing hypotheses about the population of Delaware Bay. We included the covariates of sex and time in the estimation of the parameters of apparent survival ( $S$ ), detection ( $p$ ), and transition rates ( $\Psi$ ). In addition we included a covariate of agency which was divided into two groups, individuals tagged by a research agency and those tagged by a biomedical agency, to test if there is an effect of bleeding by a biomedical agency on adult survival.

## Methods

### *Study Design*

Independent tagging studies conducted by 37 different agencies were combined into a single data set for this research. These agencies were composed of a variety of different fields including of biomedical companies, independent research or academic institutions, and

government agencies. We chose to run this analysis on data collected between 1999 and 2010 which gave us a total of 164,415 released individuals. In May and June of 2003, 3,098 of these individuals were released with two different tags by the U.S. Fish & Wildlife Service, Maryland Fisheries (MFRO) and the U.S. Geological Survey in Delaware (USGS). We removed individuals of unknown sex from this data set. The identification of sex was performed visually in the field. Males and females can be distinguished by size, as females are usually larger in size than males (Carmichael et al. 2003; Swan 2005). Males also possess claspers which are a modified version of the first pair of walking legs used to aid in attachment to females during spawning (Swan 2005). No other individual covariates were measured consistently enough across each independent study to be included in this analysis.

All individuals were given a standard button tag. This type of tag is attached by inserting a “Christmas Tree” pin through a 4mm diameter hole that was drilled into the lower left region of the carapace along the edge of the shell in the field using a rechargeable drill (Swan 2005; Smith et al. 2010). A standard button tag consists of circular metal tag that displays a unique number and methods for reporting a resighting (Brousseau et al. 2004; Swan 2005; Smith et al. 2006). It is expected that mortality is not affected by the presence of a tag (Smith et al. 2006; Mattei et al. 2011). For the double tagged individuals in 2003, a second flat auxiliary tag was also glued onto the dorsal surface of the carapace.

Due to the individual objectives of each agency’s study, the methodology, duration and effort of resighting individuals varied. Data was collected through survey efforts of the agency, in conjunction with harvesting or blood collecting efforts or by beach goers and beach comers who reported tagged horseshoe crab encounters. Many agencies offered a way for the general public to report a tag online or by phone, while some states required fishermen to report tags. Some agencies offered a reward for reporting a tag to encourage help from the general public (Swan 2005). The U.S. Fish and Wildlife Service maintains a central database which we used for this study. Resight data that did not explicitly state that an individual was captured or resighted alive was discarded for this analysis to ensure that their exposure was not biasing results since there was no way to determine for how long an individual had been dead.

### *Model Development*

All statistical modeling was done using the program MARK (White and Burnham 1999). Akaike's Information Criteria (AIC) was used for model selection of the results of this analysis (Anderson et al. 2000; Burnham and Anderson 2002; Johnson 1999). Models were ranked from lowest to highest AICc value and the model with the lowest AIC value was considered most supported by the data (Anderson et al. 2000). The multistate model is defined by 4 strata; A (both tags present), B (main tag only), C (aux tag only), and D (no tags present) (Figure 1).

A set of models were chosen *a priori* to represent plausible biological hypothesis that included the covariates of sex, strata, agency, and time. The covariate of sex, male or female, was defined as a group in Program MARK. This covariate was applied to the estimation of survival, detection, and transition rates to test varying hypothesis about if gender contributes to any or all of these parameters. Agency was used an individual covariate to differentiate between individuals initially tagged by a research agency and those initially tagged by a biomedical agency. This covariate was included in the estimation of all three parameters like sex to test the hypothesis that stress due to bleeding of a biomedical agency may adversely affect survival of individuals and the hypothesis that due to the consistency of tagging, tags applied by a biomedical agency may last longer and thereby be more easily detected compare to other agencies. The effect of being bled and tagged by a biomedical agency was applied to either all years (Biomed), only the first year after being tagged (Biomed 1), the first two years after being tagged (Biomed 2), or the first three years after being tagged (Biomed 3). The covariate of what stratum an individual was resighted in was included in the estimation of detection to test the hypothesis that the main tag is easier to detect, being seen in strata A or B, compared to the auxiliary tag, being seen in strata C. As it was previously determined that the presence of any tag does not affect survival (Smith et al. 2006) the covariate of stratum was not used in the estimation of survival.

The covariate of time was included in the estimation of survival, detection, and transition rates to gain a better understanding of how individuals are moving through this multistate system. The parameters of survival and detection were modeled with a time dependency (t), a linear trend (T), or held constant to represent no change with respect to time (.). Transition rates were modeled with an age structure to give us estimates that reflect the life span of a tag although the notation was kept the same as survival and detection for consistency (t, T, or ., Table 1).

To reflect the reality of this system, many constraints had to be imposed on the multistate model. It is physically impossible to gain a tag after the initial tagging process and so the majority of statistically possible transitions could be constrained to equal zero. Intuitively, it is impossible to ever detect an individual in stratum D, a state of having no tags, and so the probability of detection of this state was also constrained to equal zero. Stratum D is classified as an absorbing state meaning that once an individual moves into this state, it cannot leave. This constraint assumes that the probability of an animal losing both tags and being subsequently recaptured to have a new tag applied is negligibly small and can be ignored and also allowed us to constrain all transition parameters of this stratum to be zero. An individual may only enter into this system through stratum A or stratum B and so constraints also had to be made manually to the Parameter Index Matrices in Program MARK. Survival of all strata were set to vary by time and constrained to be equal to one another assuming survival is not affected by the presence of either tag. However, since an individual could not start in stratum C or D, the first year of survival for these strata were set to zero. Transition parameters were set to vary as a function of age of the tag to derive estimates that would tell us the life span of the tag. Like with survival, an individual cannot transition from stratum C or D until the second time period and so the first year of each stratum was set to zero.

There is no indication to suggest that either tag should be affected by the presence of the other allowed for further simplification of this system by setting corresponding transitions equal to one another. Moving from stratum A to stratum C and from stratum B to stratum D were both scenarios in which an individual has lost a main tag. Since there was no reason to believe that these two scenarios should be any different, we constrained  $\psi_{BD}$  to be equal to  $\psi_{AC}$ , reducing the number of parameters that were estimated. Similarly,  $\psi_{CD}$  was constrained to equal  $\psi_{AB}$  to represent the probability of losing an auxiliary tag. While not possible to observe, an individual could also transition from stratum A to D by losing both tags over a year so that there were only three total transition parameters that needed to be estimated. As transition parameters must sum to one, the remaining transitions of staying in a stratum were not explicitly calculated (Williams et al. 2002).

## Results

Of the 164,415 tagged individuals, 59,018 individuals were tagged by government affiliated agencies, 48,801 individuals were tagged by biomedical industries, and the remaining 56,596 individuals were tagged by research or academic institutions. Overall, 63,080 were females and 101,335 were males. Of the 3,098 individuals given two tags, 1,749 were tagged by the USGS, 1349 were tagged by MFRO, 2,027 of the tagged individuals were females, and 1,071 were males. The USGS tagged only females with the exception of one male while MFRO tagged both males and females. Over the twelve years in our data set, there were 5,648 resightings of individuals. Overall there were 4,321 resighting of males and 1,227 resightings of females. 2,442 of the resightings were initially released from a government agencies, 1,708 from a biomedical company and 1,398 were from other research or academic institutions.

There were 17 models that represented plausible biological scenarios for this data set (Table 1). One top model, a hypothesis that included all covariates possible of sex, strata, agency and time for the three parameters, held all of the AICc weight within this candidate set with an AICc value of 64,309.60 and a deviance of 64,279.59. Specifically, the effect of agency was the model where the covariate of being tagged by a biomedical company was applied to the first two years of data (Biomed 2). Since all subsequent models carried no model weight we based all of the following results from the top model.

Using estimates from the top model, the mean rate of annual survival was 69.61% (S.E. = 0.0417, 95% C.I. = 0.5139 - 0.7348). The model selection analysis supported an effect of sex on survival ( $\beta_{\text{Female}} = -0.4069$ , S.E. = 0.0624, 95% C.I. = -0.5292 - -0.2846). Males had a higher probability of annual survival at an average of 73.63% (S.E. = 0.0346, 95% C.I. = 0.5593 – 0.7686). Females had a mean probability of annual survival of 65.59% (S.E. = 0.0487, 95% C.I. = 0.4686 – 0.7010). The data did not support an effect on survival resulting from tagging by a biomedical company ( $\beta_{\text{Biomed}} = 7.3464$ , S.E. = 8.6387, 95% C.I. = -9.5855 – 24.2785).

The mean annual rate of loss of the main tag from the top model was 5.43% (S.E. = 0.0143, 95% C.I. = 0.0328 – 0.0940). For the auxiliary tag, the mean annual rate of tag loss was 17.17% (S.E. = 0.0344, 95% C.I. = 0.1184 – 0.2645). This data set did not have sufficient data to estimate the rate of losing both tags and all of these individual parameters were 0. The data does support an effect of sex on all transition parameters and females were found to have a higher probability of transitioning on average ( $\beta_{\text{Female}} = 0.3114$ , S.E. = 0.1129, 95% C.I. = 0.0802 – 0.5227). For the loss of a main tag only, females had a mean rate of loss of 6.09% (S.E. =

0.0161, 95% C.I. = 0.0369 – 0.1056) while males had a rate of 4.76% (S.E. = 0.0126, 95% C.I. = 0.0287 – 0.0825). Females had a mean annual rate of auxiliary tag loss of 18.68% (S.E. = 0.0376, 95% C.I. = 0.1288 – 0.2887) while males had a rate of 15.67% (S.E. = 0.0312, 95% C.I. = 0.1080 - .2403).

As expected, the mean annual probability of detection of stratum A was higher than any other stratum at 7.76% (S.E. = 0.0239, 95 % C.I. = 0.0418 – 0.1392). Stratum B had a mean probability of detection of 2.39% (S.E. = 0.0010, 95% C.I. = 0.0220 – 0.261) and stratum C had a mean of 4.08% (S.E. = 0.0135, 95% C.I. = 0.0212 – 0.0770). All strata had a higher probability of detection for males than females and the effect of sex on detection was statistically significant ( $\beta = -0.5279$ , S.E. = 0.0513, 95% C.I. = -0.6286 - -0.4273).

## Discussion

Updated and site-specific life history parameters are essential to the accuracy of future management models of the Atlantic horseshoe crab population within Delaware Bay (McGowan et al 2011). This analysis found a rate of adult survival to be higher and sex specific compared to previous best estimates. Current management models utilize an average rate of adult survival of 66.0% (C.V. = 0.15) for both sexes, a value determined by a study conducted in Pleasant Bay, Massachusetts in 2003 (Carmichael et al. 2003; Sweka et al. 2007; McGowan et al 2011). The Atlantic horseshoe crab can be found across a very broad range and there is genetic and morphological evidence to suggest that there are distinct populations of horseshoe crabs (King et al. 2005). We would expect to see differing rates of survival due to contrasting climates along such a broad range.

In conjunction with updated and site specific life history parameters McGowan et al. (2011) recommends looking into the level of annual variation of these parameters since management decisions are made regarding the harvest of horseshoe crabs on a regular basis (McGowan et al. 2011). Initial analyses using this data set found that explicitly modeling time resulted in the inestimability of more than half of the survival parameters and so final candidate set had to be composed using a trend. The ability to include a model of time would be of great interest to future studies. We also recommend the consideration of a model of time to look at if there is any effect of harvest on these parameters. While future studies would benefit greatly

from more data to allow for time to be modeled, there are several aspects of horseshoe crab ecology that suggest weather or climate may have an effect on demographics. With this result supporting that Delaware Bay does have a distinct value of survival compared to other regions, including a covariate as an index of annual weather patterns could be advantageous to understanding this trend. Turbulent weather may increase the risk of stranding and death while high wave action, caused by wind and storms, has been shown to delay or even suppress spawning in horseshoe crabs (Botton and Loveland 1989; Smith et al. 2002).

The data supports a significant effect of sex on survival of the Atlantic horseshoe crab which may be explained by biased harvest ratios, true differences in survival, or as an artifact of a bias in detection probability. On average males were found to have a higher probability of survival compared to females. Female horseshoe crabs, specifically females carrying eggs, are preferred over males as bait by fishermen and are harvested in a greater quantity (Kreamer and Michels 2009). Survival may also be effected by gender due to behavioral differences during spawning. Fertilization is external by free swimming sperm and multiple males can succeed in fertilization of a female's eggs (Shuster 1982). Consequently, females spend less time on the beach than males on average leaving them less likely to become stranded and become desiccated. In the vulnerable position of being flipped onto their back, stranded individuals also face a significant chance of mortality due to predation by large gulls (Botton and Loveland 1993).

On average, females were found to have a lower probability of detection compared to males. Spawning surveys tend to find more males on the beach than females because more males congregate on the beach during spawning. Evidence suggests that many beaches become overwhelmed with males due to the fact that males reach sexual maturity earlier and therefore the population includes more breeding males than females. As fertilization is external by free swimming sperm, multiple males can succeed in fertilization of a female's eggs and so during spawning there are often multiple satellite males for each female. (Shuster 1982).

Further analyses on the effects of agency on detection and survival would be of value to the improvement to current monitoring methods. Individuals tagged by a biomedical agency had a statistically significant and positive association on detection ( $\beta = 0.0756$ , S.E. = 0.0796, 95% C.I. = 0.6003 – 0.9127) compared to that of non-biomedical agencies for our top model. Although this association may be statistically significant, more research on detection by specific agency would be beneficial to the improvement of mark-recapture techniques. Agencies that

employ long term mark-recapture efforts may have increased probabilities of detection during resight efforts due to experience or greater sampling effort. Alternatively, early tag loss due to misapplication by less experienced researchers may contribute to a decrease in detection or survival probability if those individuals inadvertently become removed from the study population.

Concern for an effect of agency on survival would also be of interest to improve management models. Previous studies (see Rudloe 1983; Walls and Berkson 2003; Hurton and Berkson 2006; Leshcen and Correia 2010) suggest that bleeding by biomedical industries increases mortality. Although the effect of agency on survival was not statistically significant, the difference in AICc values between models with and without this individual covariate was substantial enough to warrant further research. The top model within this candidate set had the individual covariate of agency applied to only the first two years and proved a stronger model than models that had agency applied to one, three, or all years of data suggesting that there is some effect of biomedical agencies on adult survival for only an extended period of time. James-Pirri et al. (2012) observed measurable physiological effects on individuals who had been bled by a biomedical agency over a period of at least two weeks. More research would be valuable to fully understand this phenomenon and how any effect of a biomedical agency should be incorporated into future estimates of survival and management models.

The added complexity of the multistate model we used here provides an efficient framework for assessing movement of individuals between states and estimating survival and tag loss. The incorporation of the unobservable state, stratum D, can be easily accommodated under a multistate framework (Kendall et al. 2012) to further reduce any uncertainty within this model. This ability to directly estimate tag loss parameters and survival simultaneously greatly increases the precision of the results as it has previously been established that tag loss is occurring for this population (see Butler et al. unpublished, Chapter 1 of this thesis). Our analysis provides updated sex and site specific demographic parameters that can be directly incorporated into current management models. From an accurate model of a population, characteristics about that population can be confidently assessed and with that knowledge, predictive models can be formed to more effectively accomplish conservation and management objectives. To help the efficiency of monitoring and models in the future we advocate an increase in sampling efforts to find stronger support for differences between gender and agency.



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Figure 1: Movement of the system of tag loss across four stratum.

Stratum A: An individual who possesses both a main tag and an auxiliary tag.

Stratum B: An individual who has a main tag and no auxiliary tag.

Stratum C: An individual who has an auxiliary tag and no main tag.

Stratum D: An individual who has no tags.

$\Psi$ : The probability of an individual transitioning from one stratum to another stratum. The probability of remaining in a stratum was set as the reference and is not shown.

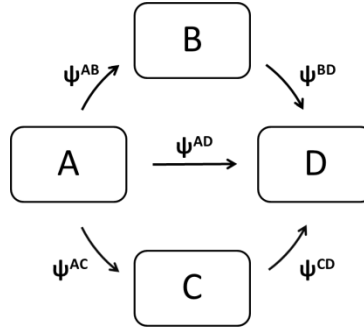


Table 1: Model selection criteria for apparent survival (S), detection (p), and transition (Psi) for the Atlantic Horseshoe crab in Delaware Bay.

Model	AICc <sup>a</sup>	$\Delta$ AICc <sup>b</sup>	AICc Weights <sup>c</sup>	Model Likelihood <sup>d</sup>	Num. Par <sup>e</sup>	Deviance
S(sex+T <sup>f</sup> +Biomed 2) p(strata+sex+T+Biomed 2) Psi(main <sup>g</sup> +aux <sup>h</sup> +both <sup>i</sup> +sex+T+Biomed 2)	64309.60	0.00	1	1	15	64279.60
S(sex+T+Biomed 3) p(strata+sex+T+Biomed 3) Psi(main+aux+both+sex+T+Biomed 3)	64384.65	75.05	0	0	16	64352.64
S(sex+T+Biomed 1) p(strata+sex+T+Biomed 1) Psi(main+aux+both+sex+T+Biomed 1)	64436.39	126.79	0	0	15	64406.39
S(sex+T+Biomed) p(strata+sex+T+Biomed) Psi(main+aux+both+sex+T+Biomed)	64694.45	384.85	0	0	15	64664.45
S(T+Biomed) p(strata+sex+T+Biomed) Psi(main+aux+both+sex+T+Biomed)	64703.71	394.11	0	0	15	64673.70
S(sex+T) p(strata+sex+T) Psi(main+aux+both+sex+T)	64708.85	399.25	0	0	13	64682.84
S(sex+T+Biomed) p(sex+T+Biomed) Psi(main+aux+both+sex+T+Biomed)	64714.11	404.51	0	0	13	64688.10
S(sex+T+Biomed) p(strata+sex+T+Biomed) Psi(main+aux+both+T+Biomed)	64722.35	412.75	0	0	14	64694.35
S(sex+Biomed) p(strata+sex+Biomed) Psi(main+aux+both+sex+Biomed)	64778.50	468.90	0	0	12	64754.50
S(sex+T+Biomed) p(strata+T+Biomed) Psi(main+aux+both+T+Biomed)	64801.64	492.04	0	0	13	64775.63
S(sex+T+Biomed) p(.) Psi(main+aux+both)	64873.06	563.46	0	0	8	64857.06
S(T+Biomed) p(strata+T+Biomed) Psi(main+aux+both+sex+T+Biomed)	65040.57	730.97	0	0	14	65012.57
S(.) p(.) Psi(main+aux+both+sex+T+Biomed)	65119.29	809.69	0	0	7	65105.29
S(T+Biomed) p(strata+T+Biomed) Psi(main+aux+both+T+Biomed)	65450.86	1141.26	0	0	13	65424.86
S(.) p(strata) Psi(main+aux+both)	65576.64	1267.04	0	0	7	65562.64
S(sex+Biomed) p(strata+sex+T+Biomed) Psi(main+aux+both+sex+T+Biomed)	116651.43	52341.83	0	0	14	116623.43
S(sex+Biomed) p(strata+sex+Biomed) Psi(main+aux+both+sex+T+Biomed)	116675.01	52365.41	0	0	14	116647.01

<sup>a</sup> Akaike's Information Criterion

<sup>b</sup> Difference between the AICc value of the selected model and the minimum AICc value

<sup>c</sup> Strength of evidence for selected model / strength of evidence for the competing previous model

<sup>d</sup> The likelihood of the model  $i$  given the data is  $= \exp(-1/2\Delta_i)$

<sup>e</sup> Number of parameters estimated in selected model

<sup>f</sup> Covariate of time modeled as a linear trend

<sup>g</sup> Transition of main tag loss ( $\psi^{AC}$  and  $\psi^{BD}$ )

<sup>h</sup> Transition of auxiliary tag loss ( $\psi^{AB}$  and  $\psi^{CD}$ )

<sup>i</sup> Transition of loss of both tags ( $\psi^{AD}$ )

## Conclusions

Our preliminary analysis of tag loss found the mean annual rate of tag loss for the main tag to be 8.07% (0.02 SE, 0.05-0.14 95% CI). The results also determined a statistically significant difference between tag loss of males and females ( $\beta_{\text{Females}}=0.62$ , 0.17 SE, 0.29-0.94 95% CI) finding that females had a higher probability on average of tag loss compared to males. The final multistate model of both survival and tag loss found the mean annual rate of loss of the main tag from the top model to be 5.43% (0.01 SE, 0.03–0.09 95% CI). This analysis also found that females had a higher probability of tag loss compared to males with a statistically significant effect of sex ( $\beta_{\text{Female}} = 0.31$ , 0.11 SE, 0.08–0.52 95% CI). The multistate analysis that estimated adult survival and tag loss simultaneously was found to have smaller standard error compared to the initial multistate model of tag loss.

Our estimates of survival in both analyses, Cormack-Jolly-Seber and multistate, were found to be similar to the current estimate used in McGowan et al. 2011 of 66.00% (CV = 0.15). The CJS analysis found a mean annual rate of adult survival of 65.81% (0.07 SE, 0.39-0.76 95% CI). With a post hoc correction for a constant rate of tag loss, this estimate becomes 71.07%. The multistate analysis to determine adult survival with the bias of tag loss simultaneously determined a mean rate of annual survival of 69.61% (0.04 SE, 0.51-0.73 95% CI). The reduced standard error on the estimate produced from the multistate models suggests that, as expected, the multistate model was the best method for incorporating a bias due to tag loss.

With these analyses we were also able to gain a more detailed understanding of how gender and being bled by a biomedical company affected adult survival. For both the CJS and multistate models, males were found to have a higher probability of adult survival compared to females. For males, the mean annual rate of survival was found to be 73.03% (0.04 SE, 0.45-0.78 95% CI), 77.31% with the post hoc bias of constant tag loss, using the CJS model and 73.63% (0.03 SE, 0.55–0.76 95% CI) using the multistate model. Females were found to have a mean annual rate of survival of 58.60% (0.09 SE, 0.33-0.73 95% CI), 64.62% with the post hoc bias of constant tag loss, and 65.59% (0.05 SE, 0.45–0.70 95% CI) using the multistate model. Both models supported a statistically significant effect of sex on adult survival. Again, while both models produced similar results, the multistate model provided smallest standard errors on the parameter estimates compared to the CJS model.

Both the CJS and multistate model found a positive effect of being bled by a biomedical company on adult survival. For the CJS model, our analysis found a statistically significant difference between individuals tagged by a biomedical company compared to a research agency. Individuals tagged by biomedical agencies, meaning that they had been bled before initial tagging, had a slightly higher probability of survival ( $\beta_{\text{Biomed}} = 0.23, 0.07 \text{ SE}, 0.10\text{--}0.37 \text{ 95\% CI}$ ) compared to the survival of individuals tagged by a non-biomedical agency. The multistate analysis found a similar trend although the results did not support statistical significance on the effect of being bled on survival ( $\beta_{\text{Biomed2}} = 7.35, 8.64 \text{ SE}, -9.59\text{--}24.28 \text{ 95\% CI}$ ). Interestingly, the top model of the multistate analysis had the effect of being bled by a biomedical company applied to only the first two years after initial tagging while the CJS analysis had the effect of being bled applied to all years. This trend is not consistent with results found in Rudloe 1983 and Walls and Berkson 2003 and so would be of value to the future management of this species to gain a better understanding of the effect that bleeding has on the health of horseshoe crabs.

The overall goals of this analysis were to; 1) gain an understanding of, if and at what rate (constant or over time) main tags were being lost and 2) to estimate adult survival using updated and site specific data. This research will be valuable for future objectives and decisions of management actions and brings about many interesting questions about horseshoe conservation. The results of the initial multistate model of tag loss (Chapter 1) found evidence to support that not only is tag loss occurring, but that tag loss changes over time. Now that we have support to conclude that tags are being lost, all future models will need to incorporate a bias of tag loss when estimating life history parameters of the horseshoe crab. In Chapter 2, we estimated the rate of adult survival and found a rate similar to that of previous studies of *Limulus*. Tag loss was accounted for *a posteriori* using a constant rate of tag loss in this analysis. However, because the results of our initial tag loss analysis showed that tag loss changes over time a multistate model was developed to estimate tag loss and survival simultaneously (Chapter 3). This final multistate model proved to be the most effective method of estimating parameters since all of the results had smaller standard errors. This work can be used to improve the confidence of the current predictive management models so that researchers can more effectively accomplish conservation and management objectives.