A HORSESHOE CRAB (*Limulus polyphemus*) DEMOGRAPHIC STUDY

by:

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(**ABSTRACT**)

We examined various aspects of horseshoe crab populations in conjunction with BioWhittaker, a biomedical company that bleeds horseshoe crabs (*Limulus polyphemus*) and utilizes their blood for biomedical purposes. We examined mortality rates of bled and unbled crabs by holding crabs in tanks for 2-week periods. The mean differential mortality between bled and unbled horseshoe crabs was 7.5% (95% CI: 0.14% - 38.1%), significant with $P < 0.001$. We examined the range in amounts of blood extracted from 98 male horseshoe crabs. Mean mass of blood extracted was 78.3 grams (*95% CI:* 70.5g - 86.0g) and ranged from 8.2g to 212.3g.

We compared sex-, size-, and stage-class distributions of crabs caught in trawls by BioWhittaker in Chincoteague, Virginia and Ocean City, Maryland during the years 1999 – 2000. Sex distribution ($P = 0.0062$), size distribution ($P = 0.0002$), and stage-class distribution ($P < 0.001$) were significantly different between locations. Chincoteague, Virginia's population was comprised of smaller and younger crabs, with greater proportions of females than the population in Ocean City, Maryland. Between years 2000 and 2001, overall sex distributions were also significantly different ($P = 0.0109$), with greater proportions of females present in 2000 than in 2001.

We tagged 7,500 bled, adult horseshoe crabs to gain information on horseshoe crab population dynamics. From resight reports (N=121), we examined movement patterns and found average distance traveled was 47.7 km and maximum distance traveled was 312 km, suggesting mixing along the Atlantic coast. We found a 1.6% recovery rate of tagged crabs and tags found detached from crabs. We document tag loss, as 11.6% of our resights consisted of tags found detached from crabs. We use information gained in our study to suggest improvements for future tagging efforts that could lead to further knowledge of horseshoe crab population dynamics.

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CHAPTER 1 – INTRODUCTION AND JUSTIFICATION

INTRODUCTION

Management of the American species of horseshoe crab (*Limulus polyphemus*) is currently surrounded by controversy. The horseshoe crab is a unique marine invertebrate important to various stakeholders. While there have been numerous studies concerning the general biology and life history of the horseshoe crab, information concerning its status and population dynamics is lacking. As the demand for horseshoe crabs intensifies from each of its user groups, information about its status becomes increasingly necessary. Concern has arisen that horseshoe crab populations are declining in abundance. However, the traditional population and harvest data needed to design appropriate management strategies has not been collected for this species. Without accurate population data, future management of this species will become increasingly difficult due to the conflicting needs of its various user groups.

Horseshoe crabs, the closest living relatives of the trilobites (Shuster 1982a), have existed for at least 60 million years, as far back as the Cretaceous Period of the Mesozoic Era (Reeside and Harris 1952). Unmistakable horseshoe crab types have been found as far back as 500 million years ago, in the latter half of the Paleozoic era (Sekiguchi 1988). Today, horseshoe crabs are found in only two regions of the world. Three species occupy coastal waters of Asia from India to Japan, including waters around the Dutch East Indies and the Philippine Islands: *Tachypleus tridentatus*, *T. gigas*, and *Carcinoscorpius rotundicauda.* One species is found along the Atlantic coastline of North America from Maine to the Yucatan, from about 19°N to 42°N (Shuster 1982a). This "American" species of horseshoe crab, *Limulus polyphemus*, is the focus of this study.

The largest population of *Limulus* is found in Delaware Bay, and the life history of the population there is generally characteristic of the species (Shuster 1982b). Horseshoe crabs are considered both ecological and behavioral generalists, tolerant of a broad range of conditions, and capable of reacting to their environment in many ways. However, Shuster (1982b) suggested that characteristics, including behavior, of any one population are not identical to those of another. This alludes to a central question facing managers: How are horseshoe crabs distributed along the Atlantic coast? Are there distinct subpopulations of horseshoe crabs with genetic differences? Is there one large, homogeneously-distributed population? Is there one large, heterogeneously-distributed population along the Atlantic coast? The answer to these questions will help managers target their efforts to managing individual subpopulations or to managing the coast as a whole. There is evidence of genetic variability (Shuster 1982b), including evidence of gene flow (Saunders et al. 1986, Selander et al. 1970), as well as morphological variation in both size and shape (Riska 1981). In one study, horseshoe crabs appeared to exhibit marked population subdivision even over a relatively small geographic range (Pierce et al. 2000).

Horseshoe crabs molt numerous times as they grow from their larval stage, shedding their exoskeleton at least 16 to 17 times before reaching sexual maturity (Shuster 1982b). The molting process occurs during the warm-water months, and becomes more difficult and time consuming at each growth stage (Shuster 1982a). Horseshoe crabs require 9 to 10 years to reach sexual maturity (Shuster 1958), and at this time, apparently cease to molt and grow (Shuster 1982a). They are fairly long-lived, and some may reach a maximum age of 20 (Shuster 1958). It is difficult to determine adult

age directly, so it must be approximated using indirect methods. This can be accomplished through tagging, from the age of any epibionts (symbiotic organisms) present on the shell (Botton and Ropes 1988) or by examining the type and site of carapace wear and the size and kind of epibionts. Using the appearance of the carapace as a general indicator of the age of an individual is based on a simple rationale: If adults do not molt, their exposure to a sand-abrasive environment, the larvae of epibionts, and diseases of the shell increase with age. Three categories are commonly used to describe the ages of adult crabs. These categories include: Young Adults (1 to 3 years after maturity), Middle-aged Adults (3 to 7 years after maturity), and Old Adults (7 plus years after maturity) (Shuster 1999).

Diet:

An adult horseshoe crab diet consists of several bivalve mollusks (*Mulina lateralis, Mya arenaria, Macoma* spp. and *Ensis* spp.), and worms (polychaete *Nereis* spp. and nemertean *Cerebratulus* spp.) (Botton 1984a, Botton 1984b, Shuster 1982a). To eat, *L. polyphemus* digs after its food, grasping its prey with pincer-tipped legs (Shuster 1982a). The food is then crushed between the legs and pushed forward toward and into the mouth (Shuster 1982a).

Predators:

Horseshoe crabs face dangers from a variety of predators throughout their lifecycle. These include mollusks, crustaceans, fish, leopard sharks, eels, birds, sea turtles, and humans (Table 1.1; deSylva et al. 1962, Keinath et al. 1987, Shuster 1982a).

Stakeholders:

There are 3 main groups that utilize the horseshoe crab as a resource and depend on its availability for their livelihood. The first of these groups are migratory shorebirds and their bird-watching enthusiasts. Each spring, at least 11 species of shorebirds (Charadriiformes: Charadridae and Scolopacidae) feed on eggs deposited by spawning horseshoe crabs along the shorelines in the Delaware Bay area (Shuster 1982a). These species include red knots (*Calidris canutus*), ruddy turnstones (*Arenaria interpres*), sanderlings (*C. alba*), and semipalmated sandpipers (*C. pusilla*) (Shuster 1982a), as well as dunlins (*C. alpina*), *Limnodromus* spp.(Castro and Myers 1993), and laughing gulls (*Larus atricilla*) (Botton 1984b). The birds use the Delaware Bay region as a staging area during their migration. Staging areas serve as intermediate stopover points for the birds to feed before continuing their migration (Botton et al. 1994). An estimated 425,000 to 1,000,000 shorebirds stop here during May and June (Clark et al. 1993, Myers 1981, Myers 1986, Myers et al. 1987, Shuster 1982a), as they travel from their South American wintering grounds to their Arctic breeding grounds (Myers 1986). The timing of their migration north is critical, as they need to reach the Arctic while the snow is melting, to ensure their eggs will hatch in time for the annual insect hatch, which comprise the primary diet of the young shorebirds (Clark 1996).

The migrating shorebirds arrive in the Delaware Bay area simultaneously as hundreds of thousands of horseshoe crabs emerge from the Delaware Bay to lay their eggs in the sandy beaches. Peaks in both shorebirds migrating through the Bay area and horseshoe crab spawning tend to occur during the third or fourth week in May (Clark 1996). This phenomenon attracts thousands of bird-watchers each spring, contributing to

the local economies of the Delaware Bay area, as the birders purchase recreation-related goods and services, including food, lodging, and equipment.

The second stakeholder group is biomedical companies that use horseshoe crabs to test for sterility in biomedical products. Five biomedical companies on the Atlantic coast bleed horseshoe crabs for the production of Limulus Amoebocyte Lysate (LAL). Limulus Amoebocyte Lysate is a clotting agent used to detect the presence of endotoxins pathogenic to humans in injectible and implantable medical and dental devices (Novitsky 1984, Mikkelsen 1988). The LAL test is derived from the blue, copper-based blood of the horseshoe crab. Biomedical companies catch horseshoe crabs, extract their blood, and through a series of chemical processes, develop the LAL substance. While alternate tests exist for the detection of endotoxin, the LAL test is the most effective, as it is capable of detecting as little as one millionth of a billionth of a gram of endotoxin (Mikkelsen 1988). The LAL test is now a standard used to protect human health around the world, and horseshoe crabs are the sole source of LAL.

Finally, the third stakeholder group includes the commercial fishermen who harvest horseshoe crabs for use as bait. During the second half of the 20th century, a commercial fishery on horseshoe crabs developed to provide bait for use in catching American eel (*Anguilla rostrada)* and whelk (commonly referred to as "conch" (*Busycon* spp.)) (Horseshoe Crab Technical Committee [HCTC] 1998). Female crabs are preferred for use in the American eel pot fishery due to the eel's preference for certain chemical odors unique to the egg laden females (HCTC 1998). This has obvious consequences for the sex distribution of the population (Loveland et al. 1996). Both male and female crabs are used in the conch pot fishery (HCTC 1998). In addition, crabs serve as bait for the

catfish (*Ictaluridae*) fishery, although to a lesser extent. A variety of methods have been employed by the fishers to obtain horseshoe crabs. Horseshoe crabs are caught by trawl, dredge, hand, and gillnet (HCTC 1998). An entire spawning beach of horseshoe crabs can easily be harvested in the hand fishery simply by gathering up all of the crabs that have congregated in the shallow waters and sandy beaches. Because of the species prevalence in the waters in the mid-Atlantic region, fishery effort is concentrated within the mid-Atlantic coastal and surrounding federal waters (HCTC 1998). However, a significant fishery has been growing in the New England area as well (Atlantic States Marine Fisheries Commission [ASMFC] 1998).

Fishery Management and Population Information:

There has been concern among managers, biomedical companies, and environmental groups that with all of the demands for horseshoe crabs, horseshoe crab populations may be declining in abundance. However, traditional population data and fishery-management data that could support or refute this concern are lacking (Berkson and Shuster 1999). Historically, the responsibility for monitoring and regulating horseshoe crab fisheries was up to the states, causing existing data to be fragmented and highly variable (ASMFC 1998). In fact, the Horseshoe Crab Peer Review Panel (a panel of 3 experts appointed by the ASMFC (the commission formed by the 15 Atlantic states to assist in managing and conserving shared coastal fishery resources) to evaluate available stock assessment data) found no single data set reliable enough for use in coastwide horseshoe crab stock assessment (ASMFC 1998). Although there are data from a number of trawl surveys, it was judged by the Peer Review Panel to have little to no value for assessing the status of horseshoe crabs (ASMFC 1998). The trawl surveys

were multi-species finfish surveys not designed for horseshoe crabs, and some even utilized devices to exclude horseshoe crabs from the survey (ASMFC 1998). Horseshoe crabs were considered bycatch in these surveys, as they were not a targeted species. In 1990, an annual survey of number of spawners began, but since it was a volunteer effort, it lacked the manpower necessary to produce sufficient data (ASMFC 1998). In addition, the periodicity of the surveys did not correspond to the periodicity of horseshoe crab concentrations on the spawning grounds (ASMFC 1998). There have been some attempts at collecting egg count data, but these are insufficient for a comprehensive population analysis due to insufficient sampling and exclusion of needed sampling strata (ASMFC 1998). One important stock assessment report concluded that no increasing or decreasing trend existed in horseshoe crab abundance (HCTC 1998). However, the Horseshoe Crab Peer Review Panel reviewed that report and found that no trend was identified, not because no trend was present, but because of the uninformative nature of the data (ASMFC 1998). Thus, the need for statistically sound, quality data is imperative to investigate the supposed decline in population size.

PROJECT PURPOSE AND OBJECTIVES

In response to concerns of declining horseshoe crab populations, unanswered questions necessary for proper management, and the need for population information, Virginia Tech conducted a 3-year study in conjunction with BioWhittaker, the largest producer of LAL. The main purpose of the project was to investigate various aspects of the horseshoe crab issue that could lead to a better understanding of horseshoe crab populations.

The project had 3 main objectives. In response to an initial mandate by the Atlantic States Marine Fisheries Commission, the study's first objective was to estimate post-bleeding mortality rates of horseshoe crabs undergoing BioWhittaker's blood extraction process and to quantify the amount of blood extracted. The second objective of the study was to collect demographic information on horseshoe crabs obtained in trawls for BioWhittaker, and to compare the sex, age-class, and size distributions both spatially and temporally. Finally, also in response to the mandate, the study's third objective was to tag horseshoe crabs bled by BioWhittaker in an attempt to gather population information.

The following chapters describe in detail the results of each objective of this study. This study begins to answer questions hampering sound management of horseshoe crabs. First, by quantifying the post-bleeding mortality rate of horseshoe crabs involved in biomedical processing, management efforts now have estimates of the degree of impact of biomedical processing on horseshoe crab populations. Second, the demographic analyses performed in this study give further insights to questions involving population structure, adding to the discussion of subpopulation distribution. Finally, the results of the tagging performed in this study provide a basis for an improved tagging study that can be used to quantify population size. In addition, this tagging study shows movement patterns of horseshoe crabs and quantifies the degree of mixing of horseshoe crabs among locations, allowing management efforts to target the scale of management. Invariably, this study should facilitate further horseshoe crab research that continues to answer questions and ensures future availability to user groups.

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Table 1.1. Organisms known to prey upon the horseshoe crab, *Limulus polyphemus*.

Group:	Species:	Group:	Species:
Birds	Semipalmated plover (*Charadrius semipalmatus*) [eggs]	Fish	Silver Perch (*Bairdiella chrysura*) [eggs and larvae]
	Black-Bellied plover (*Pluvialis squatarola*) [eggs]		Weakfish (*Cynoscion regalis*) [eggs and larvae]
	Pectoral sandpiper (*Calidris melanotos*) [eggs]		Northern kingfish (*Menticirrhus saxatilis*) [eggs and larvae]
	Least sandpiper (*Calidris minutilla*) [eggs]		Atlantic silverside (*Menidia menidia*) [eggs and larvae]
	Semipalmated sandpiper (*Calidris pusilla*) [eggs]		Summer flounder (*Paralichthys dentatus*) [eggs and larvae]
	Sanderling (*Calidris alba*) [eggs]		Winter flounder (*Pseudopleuronectes americanus*) [eggs and larvae]
	Laughing Gull (*Larus atricilla*) [eggs]		Leopard Shark (*Triakis semifasciata*) [Adults]
	Boat-tailed grackle (*Cassidix major*) [eggs]		Catfish [eggs]
	Red knots (*Calidris canutus*) [eggs]		Puffers (*Tetraodontidae*) [juveniles]
	Ruddy turnstones (*Arenaria interpres*) [eggs]		Devil Ray (*Mobula hypostoma*)
Arthropods	Sand Shrimp (*Crangon septemspinosa*) [eggs]		Swordfish (*Xiphiidae*)
	Fiddler crab (*Uca pugnax*) [first and second tailed stages]		Mullet *(Mugilidae*) [eggs and larvae]
	Blue crab (*Callinectes sapidus*) [juveniles]		Striped bass (*Morone saxatilis*) [eggs]
	Green crab (*Carcinides maenus*) [juveniles]		White perch (*Morone americana*) [eggs]
	Spider crab (*Libinia* spp.) [juveniles]		American eel (*Anguilla rostrata*) [eggs and larvae]
	Amphipods [larvae]		
Mollusks	(*Melongena* spp.) [Adults]		
Reptiles	Loggerhead sea turtle (*Caretta caretta*)		

CHAPTER 2 – BLOOD EXTRACTION EFFECTS ON HORSESHOE CRABS

ABSTRACT

Biomedical companies extract blood from the horseshoe crab, *Limulus polyphemus*, for the production of Limulus Amoebocyte Lysate (LAL). This compound is used worldwide for detecting endotoxins in injectible and implantable medical devices. We estimated mortality associated with blood extraction for horseshoe crabs bled by BioWhittaker, the largest producer of LAL. During summers 1999, 2000, and 2001, bled (N = 200) and unbled (N = 200) horseshoe crabs were transported from BioWhittaker's bleeding facility in Chincoteague, Virginia to the Virginia Seafood Agricultural Research and Extension Center in Hampton, Virginia. At the facility, they were held in a tank and their mortality was monitored for 2 weeks. We found a 7.5% increase in mortality in bled crabs compared to unbled crabs ($P < 0.001$). In addition, we examined the amount of blood extracted from 98 male horseshoe crabs to estimate the range of extracted amounts. Mean weight of blood extracted was 78.3 grams and ranged from 8.2 grams to 212.3 grams. Due to horseshoe crabs' ecological and economic importance, proper management requires that any human-induced impact on mortality be precisely monitored.

INTRODUCTION

Horseshoe crabs, *Limulus polyphemus* (Müller), are important to a variety of user groups for a variety of purposes (Berkson and Shuster 1999). They are caught by commercial fishermen for use as bait in eel and whelk fisheries. Migratory shorebirds rely on horseshoe crab eggs for food as they journey from South American wintering grounds to Arctic breeding grounds (Clark 1996). Birdwatchers come to feeding areas as shorebirds pause in their migration to consume horseshoe crab eggs. Biomedical companies rely on horseshoe crabs for the production of an important endotoxin detectant.

Horseshoe crabs are bled by 5 biomedical companies on the Atlantic coast for the production of Limulus Amoebocyte Lysate (LAL). Limulus Amoebocyte Lysate is a clotting agent used to detect the presence of endotoxins pathogenic to humans in injectible and implantable medical and dental devices (Novitsky 1984, Mikkelsen 1988). While alternate tests exist for the detection of endotoxin, the LAL test is the most effective, as it is capable of detecting as little as one millionth of a billionth of a gram of endotoxin (Mikkelsen 1988). The LAL test is now a standard used to protect human health around the world, and horseshoe crabs are the sole source of LAL.

The LAL test is derived from the blue, copper-based blood of the horseshoe crab. Each biomedical company maintains its own procedures for harvesting horseshoe crabs, extracting the crabs' blood, and releasing the bled crabs while developing the LAL substance. The amount of blood extracted varies from crab to crab, as blood will continue to be extracted until a clot forms. Precise estimates of the total amount of blood in a horseshoe crab are not known at this time, so it is unclear what percentage of the

total blood volume is extracted during the biomedical process. Novitsky (1984) estimated that up to 30% of the blood volume is extracted, while Brown and Clapper (1981) reported total blood volume in a 1-foot diameter female horseshoe crab was 300 mL. At the time this study was undertaken, the Food and Drug Administration (FDA) mandated that biomedical companies release horseshoe crabs alive after the bleeding process.

Proper management of the horseshoe crab resource requires estimates on all significant human-induced sources of mortality. In 1998, the Atlantic States Marine Fisheries Commission (ASMFC) mandated that all biomedical companies actively bleeding horseshoe crabs estimate mortality rates resulting from their bleeding process. Prior to the ASMFC mandate, 2 studies examined the mortality associated with blood extraction. Each estimated resultant mortality to be 15% or less (Rudloe 1983, Thompson 1998). However, due to the unique methods of the different biomedical companies, each company was required to quantify its own rate of mortality.

BioWhittaker, a CAMBREX company, is the largest producer of LAL. In response to the ASMFC mandate, BioWhittaker requested that Virginia Tech conduct the mortality study for their company. Our objectives were to determine horseshoe crab mortality for a 2-week period following the bleeding process and to quantify the range of blood extracted.

METHODS

We compared mortality rates between crabs that underwent the bleeding process (bled) and crabs that were suitable to undergo the bleeding process, but did not (unbled). Throughout the 1999, 2000, and 2001 bleeding seasons (June through August),

BioWhittaker obtained horseshoe crabs by trawling in the Atlantic Ocean off the coasts of Chincoteague, Virginia and/or Ocean City, Maryland. After capture, the horseshoe crabs were brought to BioWhittaker's bleeding facility in Chincoteague, Virginia. At the bleeding facility, we randomly selected a predetermined number (10 in 1999, 30 in 2000 and 2001) of newly-matured, male horseshoe crabs (identified by pristine shell condition and boxing-glove lower claws (Shuster 1999)) from all of the crabs obtained in that day's trawls. We selected newly-matured, male crabs to minimize covariance in our study. These crabs were not bled and served as a control in the experiment. They were packed in coolers labeled "unbled," and set aside. The same number of newly-matured, male crabs were then randomly selected from the remaining crabs and underwent BioWhittaker's normal bleeding process. Upon completion of the bleeding process, the crabs were packed in coolers labeled "bled."

All coolers containing horseshoe crabs, both bled and unbled, were immediately packed in an air-conditioned vehicle, and transported to the Virginia Seafood Agricultural Research and Extension Center in Hampton, Virginia. The horseshoe crabs were removed from the coolers and the unbled crabs were marked with external tags to distinguish them from the bled crabs. All of the crabs were placed in 4 replicated, flow-through holding tanks, with equal numbers of bled and unbled crabs in each tank. The horseshoe crabs remained in the tank system at Hampton for 2 weeks. Crabs were maintained in appropriate conditions (Brown and Clapper 1981), and monitored daily. Crabs that died during the 2-week period were removed at the time of their death.

At the conclusion of each 2-week period, the status of each crab (dead or alive) was recorded. All surviving horseshoe crabs were removed from the tank, placed in

coolers, packed in an air-conditioned vehicle, returned to BioWhittaker's bleeding facility in Chincoteague, Virginia, and returned to the Atlantic Ocean in accordance with BioWhittaker's standard operating procedures. This procedure was repeated 8 times during summers 1999, 2000 and 2001. The results from each of the replicates were combined due to an absence of time effects, and the overall percentage mortality was calculated for the bled and the unbled groups.

In addition to monitoring the post-bleeding survival of horseshoe crabs, the amount of blood extracted from male horseshoe crabs during standard operating procedures at BioWhittaker was measured. This involved extracting blood from a randomly selected, male horseshoe crab using BioWhittaker's standard operating procedures, and weighing the collection bottle after the flow of blood had ceased.

Analyses

Using Fisher's Exact Test, we evaluated statistical significance of differences in mortality between the bled and unbled crabs (Mehta and Patel 1999). We then calculated a 95% confidence interval for average differential mortality using the Common Odds Ratio in the statistical program StatXact (Mehta and Patel 1999). In addition, we tested for normality in the distribution of amount of blood extracted using Stephens' modification of the Kolmogorov-Smirnov Test for Normality (Hollander and Wolfe 1999).

RESULTS

A Fisher's Exact Test for statistical significance showed significant differences between mortality rates in bled and unbled crabs with P = 2.085E-04. Bled crabs (N = 200) had an overall mortality rate of 8% compared to the 0.5% mortality rate of the unbled crabs (N = 200; P < 0.001) (Table 2.1). Thus, this study estimates average differential mortality between bled and unbled horseshoe crabs to be 7.5%. The 95% confidence interval for this average differential mortality ranges from 0.14% to 38.1% as calculated using the Common Odds Ratio (Mehta and Patel 1999).

Mean mass of blood extracted from 98 male crabs was 78.3 grams (*95% CI:* 70.5g - 86.0g; range: 8.2g - 212.3g) (Figure 2.1). Volume of blood extracted did not differ significantly from a normal distribution (P > 0.150).

DISCUSSION

Our results indicate that horseshoe crab mortality due to bleeding is relatively low. Rudloe (1983) found that bleeding increased mortality by 10% during the first year after bleeding, and 11% during the second year, by observing bled and unbled horseshoe crabs in a penned cove in Florida. Thompson (1998) estimated mortality associated with LAL processing was 15% during the first week following blood extraction by observing bled and unbled crabs in tanks in South Carolina.

Each LAL producer has a unique operation in terms of horseshoe crabs. They each have unique bleeding methods, methods of captures, distances and methods of travel to the bleeding lab, holding times and conditions, and methods of return most appropriate to their own setting and situation. The results found in this study reflect those of BioWhittaker and may not be reflective of other companies' procedures.

This study examined the survival of the crabs in a controlled, tank environment, as opposed to their natural environment. This may not be reflective of the mortality rate of crabs returned to the wild. Transfer and holding induce stress on the crabs. Thus, the survival of the bled crabs could be compromised in translocation and confinement in the tank. However, the tank environment may also provide protection for the crabs when they may be weakened or more susceptible to predation following blood-extraction.

Further, this study looked only at newly matured, male crabs in an attempt to minimize variation of external influences, so that the only difference between the 2 groups was whether or not they underwent the blood extraction process. Additional studies should examine differences in mortality in other age and sex classes.

Estimates of amount of blood extracted show a great range. This could also affect post-bleeding mortality, as greater proportions of blood extracted to total blood volume may cause greater rates of mortality. An average blood loss of 78.3 grams as found in this study represents a substantial blood loss by any standard. Given the distribution of blood extraction found, it is quite interesting to observe only a 7.5% increased post-bleeding mortality rate. Further studies estimating total blood volume of horseshoe crabs and relating amount of blood extracted to post-bleeding survival are imperative.

The Food and Drug Administration estimates that 260,000 horseshoe crabs were caught, bled, and returned by biomedical companies when last reported in 1997 (HCTC 1998). The commercial fishery reported landings of 5,542,497 pounds in 1999 with a 100% mortality rate, down from the 6,835,305 reported in 1998 (National Marine Fisheries Service [NMFS] 2001). When comparing the magnitude of crabs caught and the associated mortality rates, it is evident that the bleeding process has a substantially

smaller impact than the commercial fishery on the horseshoe crab population. However, information on both biomedical and commercial fishery-induced mortality are necessary to determine total harvest mortality of horseshoe crabs.

Because horseshoe crabs are a valuable necessity to numerous interests, any impact on their mortality needs to be carefully monitored. With this information, managers can regulate the resource, ensuring sustained yields.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Carl N. Shuster, Jr. and Dr. William McCormick for their helpful advice on the design of this study. Michael Schwarz, Ryan Cool, and Dr. Michael Jahnke of the Virginia Seafood Agricultural Research and Extension Center, a unit of Virginia Tech, provided the facilities for holding the crabs and maintained them. Funding for this study was provided by BioWhittaker, a CAMBREX Company. A special thanks is extended to Dr. Tammy Newcomb and Dr. Michael Vaughan for their helpful advice throughout all stages of this study.

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Table 2.1. Comparison of mortality rates between bled and unbled groups of horseshoe crabs captured near Chincoteague, Virginia and Ocean City, Maryland, 1999-2001.

	Unbled Horseshoe Crabs			Bled Horseshoe Crabs		
Dates monitored	# of crabs monitored	# of crabs that died	% dead at study end	# of crabs monitored	# of crabs that died	% dead at study end
07/08/99 - 07/22/99	10	0	0%	10	0	0%
07/22/99 - 08/05/99	10	0	0%	10	3	30%
6/19/00 – 07/03/00	30	0	0%	30	0	0%
7/7/00 – 7/21/00	30	0	0%	30	0	0%
08/01/00 - 08/15/00	30	1	3.3%	30	6	20%
06/06/01 - 06/20/01	30	0	0%	30	0	0%
06/20/01 - 07/04/01	30	0	0%	30	2	6.7%
8/15/01 – 8/29/01	30	0	0%	30	5	16.7%
Totals:	200	1	0.5%	200	16	8%

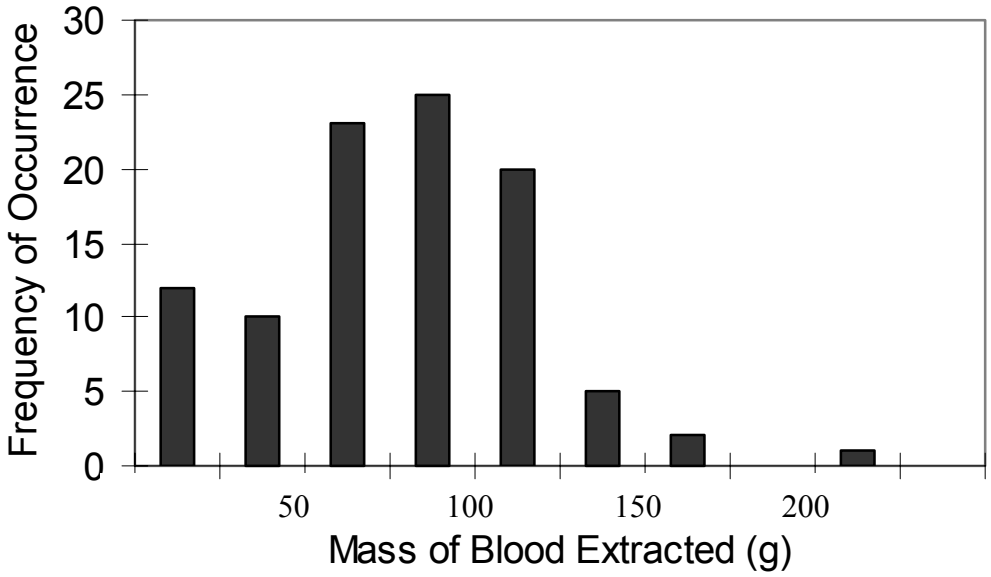

Figure 2.1. Amount of blood extracted (g) from male horseshoe crabs (N = 98) by BioWhittaker, Chincoteague, Virginia, 1999.

CHAPTER 3 - COMPARING SPATIAL AND TEMPORAL DISTRIBUTIONS OF HORSESHOE CRABS IN MARYLAND AND VIRGINIA

ABSTRACT

The structure and dynamics of horseshoe crab (*Limulus polyphemus*) populations along the Atlantic coast is poorly documented. One of the many questions facing managers involves how horseshoe crabs are distributed. It is not certain whether or not horseshoe crabs are found in genetically distinct subpopulations, nor how they are spatially distributed demographically. During the summers of 1999, 2000, and 2001, we collected demographic data for horseshoe crabs obtained in trawls in the Atlantic Ocean off the coasts of Chincoteague, Virginia and Ocean City, Maryland. We compared sex, stage-class, and size distributions of crabs caught in each location throughout the 3 years and examined spatial and temporal trends. We observed significant differences in sex distribution ($P = 0.0062$), size distribution ($P = 0.0002$) and stage-class distribution ($P < 0.001$) between locations, with Chincoteague, Virginia's population comprised of smaller, younger crabs, and greater proportions of females as compared with Ocean City, Maryland. In addition, more females ($P = 0.0109$) were present in 2000 than in 2001. Our analyses give further insight into horseshoe crab population structure, suggesting spatially stage-distributed populations that require spatial management. In addition, our results suggest overfishing in both the decline in percentage of females and the decline of older stage-classes in the catch. Additional years of data are required to verify if this is true.

INTRODUCTION

The structure and dynamics of horseshoe crab populations along the Atlantic coast are poorly understood. A potential decline in horseshoe crab population numbers has become an increasing concern to a number of user groups, including commercial fishermen, biomedical companies, and bird watching enthusiasts (Berkson and Shuster 1999). Commercial fishermen harvest horseshoe crabs for use as bait in eel and whelk fisheries, biomedical companies extract blood from horseshoe crabs for pharmaceutical purposes, and migratory shorebirds rely on horseshoe crab eggs to fuel their migration. Current attempts to manage horseshoe crabs are hampered by huge gaps in knowledge (Berkson and Shuster 1999).

One of the many questions facing managers involves how horseshoe crabs are distributed. It is not certain whether crabs are homogeneously distributed as one extensive population along the Atlantic coast, if there is one extensive population with heterogeneous spatial distribution, or if there are several smaller geographic subpopulations. Some studies support the possibility of local populations (Shuster 1982, Widener and Barlow 1999), but genetic evidence may show some discrepancies (Selander et al. 1970, Saunders et al. 1986, Mitton 1994, Pierce et al. 2000).

In this study, we examine demographic characteristics of horseshoe crabs found in 2 relatively close locations, Chincoteague, Virginia and Ocean City, Maryland. The purpose of our project is to examine the sex, size, and stage-class structures of the populations of horseshoe crabs captured during trawling. With this information, we ask the following questions:

- Do sex, size, and stage-class distributions change over time?

- Do sex, size, and stage-class distributions differ among the various locations where the trawler obtains crabs?

The results of this analysis will add to the study of population distribution and structure, and will provide a framework for further studies examining the effects of commercial exploitation on population dynamics of horseshoe crabs.

METHODS

During the summers of 1999, 2000, and 2001, we collected demographic data for horseshoe crabs obtained in trawls in the Atlantic Ocean off the coasts of Chincoteague, Virginia and Ocean City, Maryland. These locations are separated by 56 kilometers. Samples were taken from trawls for the biomedical company BioWhittaker, which extracts blood from horseshoe crabs in their Chincoteague, Virginia laboratory. Trawls are targeted in areas of the ocean where horseshoe crabs are known to inhabit. Following the captain and crew's normal procedure, the boat's trawl nets were brought in and emptied onto the deck, forming a mound of horseshoe crabs. Before any culling took place to select appropriate crabs for the biomedical company, an apparatus (Figure 3.1) was placed on top of this mound, and set to delineate a 15° sector of the mound. This offset a random sample of $1/24^{th}$ of the crabs obtained in the trawl. All crabs that lay in this sector were collected and placed in special totes on board the trawler, and then the boat captain and crew continued their normal culling of the remaining crabs and continued trawling. Once the necessary numbers of horseshoe crabs for daily processing at BioWhittaker were obtained, all crabs were returned to the laboratory and demographic information was collected and recorded on the random sample of crabs collected that day. This information consisted of:

- Stage-class (juvenile, newly matured, young adult, middle aged adult, or old adult),
- Sex (male or female),
- Width measurements (intraocular width),
- Overall condition of the crab.

Determining Stage-class

The age of horseshoe crabs cannot be determined directly. Thus, we used the appearance of the carapace as a general indicator of the stage-class of an individual, based on a simple rationale: If adults do not molt (Shuster 1982), their exposure to a sand-abrasive environment, the larvae of epibionts, and diseases of the shell increase with time. We used the following categories to describe the stage-classes of adult crabs:

- Newly-Matured Adults (year of maturity): Virgin males can be identified by the atrophied non-moveable chela, which break off upon mating. Virgin females can be identified by a pristine shell with no mating scars.
- Young Adults: Carapace is lustrous with few, if any, scratches or epibionts. Chela of males have broken off.
- Middle-aged Adults: Lustrous sheen of carapace is being eroded away, as a black layer of shell becomes exposed. Both sexes exhibit increasingly extensive scratches on their carapace. Female crabs have large black areas on the middle and posterior portions of their abdomens, which are mating scars resulting from abrasion caused by sand during mating. "Pressure spots" are evident where the claspers of the male attach to the trailing

edges of the females abdomen during spawning. Epibionts are usually present on the carapace.

- Old-aged Adults: Carapace tends to be almost completely blackened, and in the case of extreme erosion, the black layer is also worn away, exposing a brownish-colored layer that is often tinged with green. The shell of the crab is thin and can be easily depressed. Epibionts are almost always present and may have reached large sizes.

(Source: Carl Shuster, "Carapace Appearance and Age of Adult *Limulus polyphemus*" unpublished)

Determining Sex

We determined the sex of adult horseshoe crabs through observation of their lower claws. Adult males have a modified pincer as their lower claw, which adult females lack. We distinguished between male and female juvenile crabs through visual analyses under their genital operculum. Juvenile males have small, raised testes, while juvenile females have larger, flat ovaries.

Measuring Intra-Ocular Width

Using a metric measuring stick, we measured the distance between eye-slits of the crabs' compound eyes to the nearest millimeter, and recorded this as the intra-ocular (I/O) width. Intra-ocular width is the most common method for measuring horseshoe crab size.

Analyses

We compared sex distributions between locations and among years of study using a logistic classification model with likelihood-ratio tests for main effects, interactions, and factor level comparisons at the $\alpha = 0.05$ significance level. Using tests for main effects and interactions in an analysis of variance model, we compared size distributions between locations and among years of study at the $\alpha = 0.05$ significance level. Finally, to compare stage-class distributions between locations and among years of study, we used likelihood-ratio tests in a McCullagh proportional odds model at the $\alpha = 0.05$ significance level (McCullagh 1980). We used a McCullagh proportional odds model to determine predicted probabilities of crab distribution in each stage-class.

RESULTS

No data were collected in Chincoteague, VA during summer 1999 due to logistical complications. Thus, our results exclude any comparisons that would involve a data set from Chincoteague, 1999.

Sex Ratios

Analyses of between-location and among-year comparisons indicated a higher proportion of female horseshoe crabs in Chincoteague, VA than in Ocean City, MD ($P = 0.0062$; Figure 3.2*a*) and a higher proportion of female horseshoe crabs in 2000 than 2001 ($P = 0.0109$; Figure 3.2*b*). The proportion of females in Ocean City, MD in 2000 was greater than in 2001 ($P = 0.001$; Figure 3.2*c*), but we detected no differences in sex ratios in Ocean City between 1999 and 2000 ($P = 0.1056$), or 1999 and 2001 (P =

0.5397). Sex ratios between 2000 and 2001 in Chincoteague, VA were not different (P = 0.4861).

Size Distribution

In our analyses of between-location and among-year comparisons of size distribution, we found horseshoe crabs in Chincoteague, VA were smaller than those in Ocean City, MD (P = 0.0002; Figure 3.3). The size distribution of horseshoe crabs did not differ by year in either Chincoteague, VA (2000 vs. 2001 P = 0.3394) or Ocean City, MD (1999 vs. 2000 P = 0.0605; 2000 vs. 2001 P = 0.5343; 1999 vs. 2001 P = 0.1543).

Stage-class Distribution

In our analyses of between-location and among-year comparisons of stage-class distribution, we found horseshoe crabs in Chincoteague, VA were younger than those in Ocean City, MD (P < 0.001; Figure 3.4). We found a higher proportion of juvenile horseshoe crabs in Chincoteague, VA in 2000 than in 2001 (P = 0.0007; Figure 3.5). In Ocean City, we detected a decrease over time in the percentage of older crabs, as there were more older crabs in 1999 than 2000 (P < 0.001; Figure 3.5), more older crabs in 2000 than 2001 (P = 0.0147; Figure 3.5), and, therefore, more older crabs in 1999 than 2001 (P < 0.001; Figure 3.5).

DISCUSSION

The distribution and structure of horseshoe crabs along the Atlantic coast has been puzzling to researchers, as pressures to more effectively manage the horseshoe crab population increase. The presence or absence of localized subpopulations as well as the distribution of these populations are important considerations when designing effective

fishery management strategies. If the distribution of horseshoe crabs is spatially segregated by stage, it may be important to enable management on a finer spatial scale. Demographic analyses performed in this study give further insights into population structure, adding to the discussion of population distribution.

Historically, much of the investigation into horseshoe crab population structure has involved genetic studies. Genetic studies examining allozyme frequency showed modest, but significant differences between horseshoe crabs from the Gulf of Mexico and horseshoe crabs from the Atlantic Coast (Selander et al. 1970, Mitton 1994). Similarly, Random Fragment Length Polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) showed substantial differentiation between horseshoe crabs from these two regions (Saunders et al. 1986). However, the RFLP analysis of mtDNA did not detect population subdivision *within* the Atlantic region, only *between* the Atlantic and Gulf of Mexico (Saunders et al. 1986). The location of the break between the Gulf of Mexico population and the Atlantic populations was a well-established zoogeographic boundary for many species (Avise 1992).

Other examples of studies suggesting discrete populations include morphometric data analysis (Shuster 1978, Shuster 1982). Larger animals and larger populations are found in the mid-portion of the horseshoe crabs' range, between Georgia and New York (Shuster 1978). Smaller animals and populations can be found north of Cape Cod, along the gulf coast of Florida, and the entrances to the Gulf of Mexico (Shuster 1982).

Most recently, a new mtDNA study has shown population subdivision within the Atlantic region (Pierce et al. 2000). Using mtDNA sequence analysis, Pierce et al. (2000) demonstrated that populations of horseshoe crabs in the Delaware Bay and the

Chesapeake Bay are genetically distinct, suggesting localized population subdivision. However, their Random Amplification of Polymorphic DNA (RAPD) fingerprinting was *not* able to differentiate between the two locations (Pierce et al. 2000).

Localized differences in sex, size, and stage-class distribution as seen in our study suggest that the horseshoe crab populations are spatially distributed in patches with different demographic characteristics. Our study shows the population in Chincoteague, VA is comprised of smaller crabs, a greater proportion of females, and many juveniles combined with very few old-aged crabs. The large proportion of juveniles suggests that Chincoteague, VA may serve as a nursery area for the crabs. Our study shows the population in Ocean City, MD is comprised of larger crabs, a greater proportion of males, many old-aged crabs with few younger crabs. The small proportion of juveniles could signify that Ocean City, MD may lack appropriate spawning conditions, causing limiting recruitment in this population. With heterogeneous spatial distribution, a large fishing effort in one location could adversely affect the numbers of crabs in a critical stage. For example, if a commercial fishing fleet were to focus their efforts on areas near Chincoteague, it could dramatically impact the juvenile population, and thus, the overall population. Effort must therefore be distributed among areas using knowledge of demographic structure. Thus, our results suggest that individual locations should possibly be managed separately, although movement between populations should be taken into consideration.

The non-random nature of the trawls obtaining these crabs likely influences our results. BioWhittaker targets areas in each trawling location where crabs are known to reside. Thus, our samples do not represent truly random samples of horseshoe crabs in

each location. Future studies should examine population distribution using a randomized design.

We used a subjective, categorical aging key to determine stage-classes of individuals based on carapace appearance. Inaccuracies from this subjective aging may influence our conclusions as we compare stage-distributions spatially and temporally. Thus, an aging method with increased precision is necessary to facilitate increased accuracy in management.

In addition, our study gives limited information as to the effects of time on population distribution, due to the short duration of our study. Continuing this study would be beneficial to increase the time series of data available.

We do see an overall decrease in the percentage of females in each location between years 2000 and 2001. This could be related to the preference for female crabs for use in the eel and conch fishery. In addition, we see younger crabs increasing in prevalence in Chincoteague between years 2000 and 2001, as well as in Ocean City between 1999 and 2000 and between 1999 and 2001. Again, this could be related to the preference for adult horseshoe crabs for bait in the eel and conch fishery. These results may be due to overfishing, but additional years of data would be required to reach that conclusion.

Future studies should continue to monitor temporal changes in population distribution, as they may be increasingly noticeable over longer periods of time. This is the typical kind of data that is collected to manage fisheries, but has not been collected during the more than 100-year history of the horseshoe crab fishery. Our study shows the potential for collecting these data for managing the horseshoe crab fishery. Our brief

time study suggests the possible existence of spatially stage-distributed populations, and hints at possible evidence of overfishing in both the decline in percentage of females and the decline of older stage-classes in the catch.

ACKNOWLEDGEMENTS

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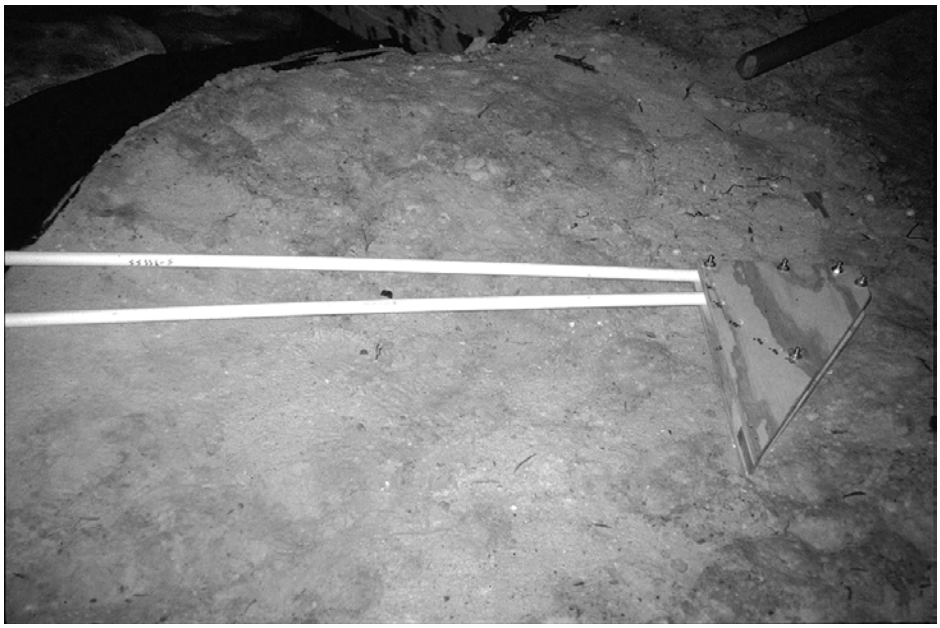


Figure 3.1. Apparatus placed upon mound of horseshoe crabs brought in during BioWhittaker's trawling processes in Chincoteague, VA and Ocean City, MD during 1999-2001. The apparatus was set to delineate a random sample sector of the mound of horseshoe crabs.

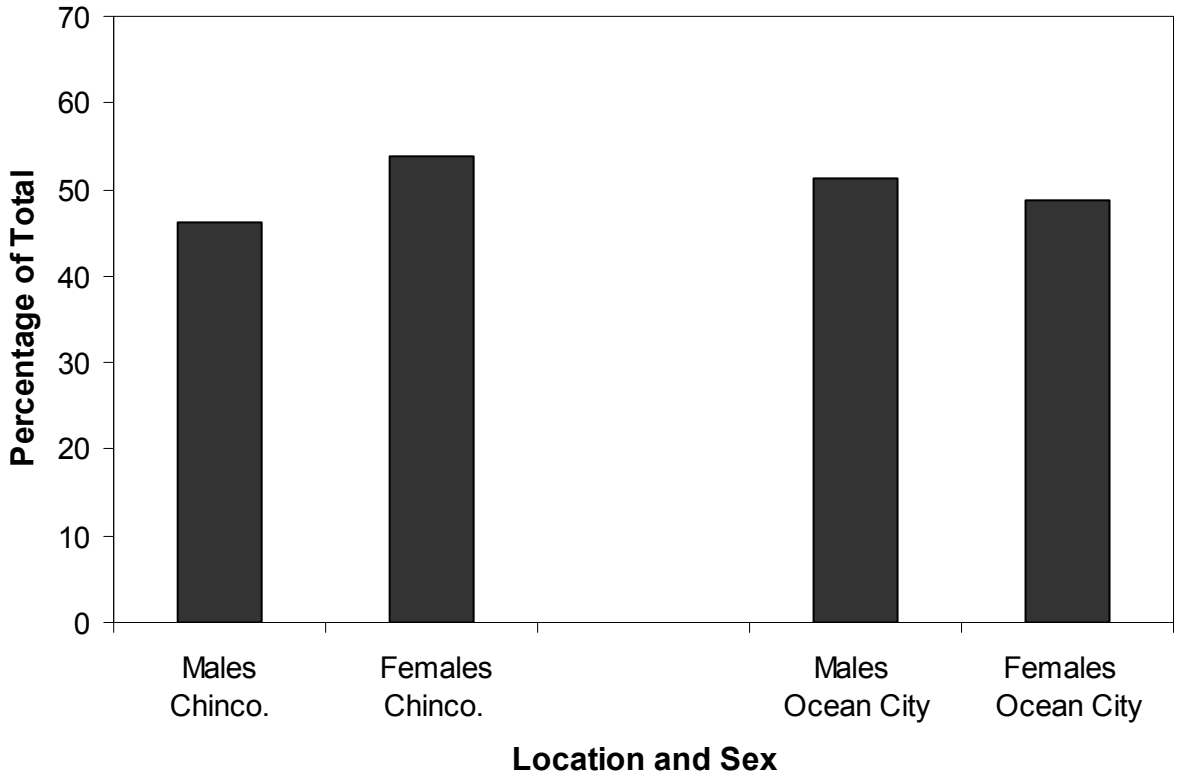


Figure 3.2(*a*-*c*). Comparison of sex ratios of horseshoe crabs obtained in trawls by BioWhittaker in Ocean City, MD and Chincoteague, VA (2000-2001). Figure 3.2*a*. Overall sex distribution for locations Ocean City, MD and Chincoteague, VA combining years.

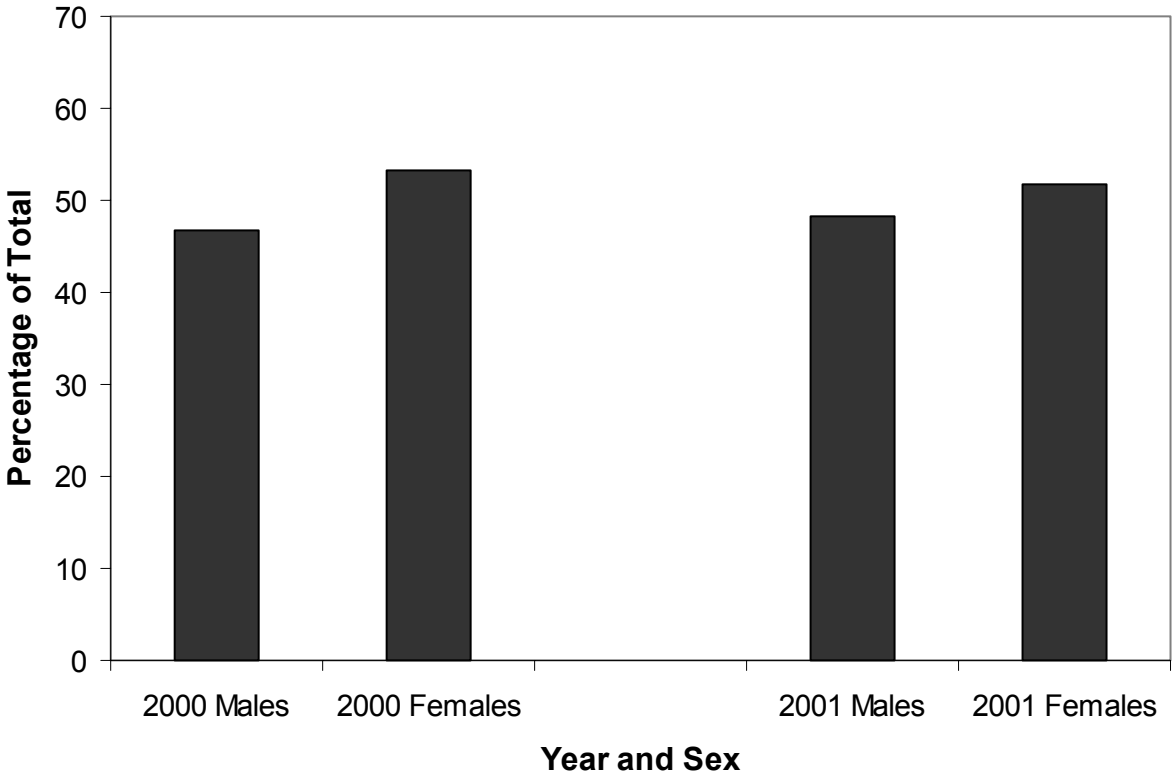


Figure 3.2*b*. Overall sex distribution for horseshoe crabs during years 2000 and 2001 combining locations Chincoteague, VA and Ocean City, MD.

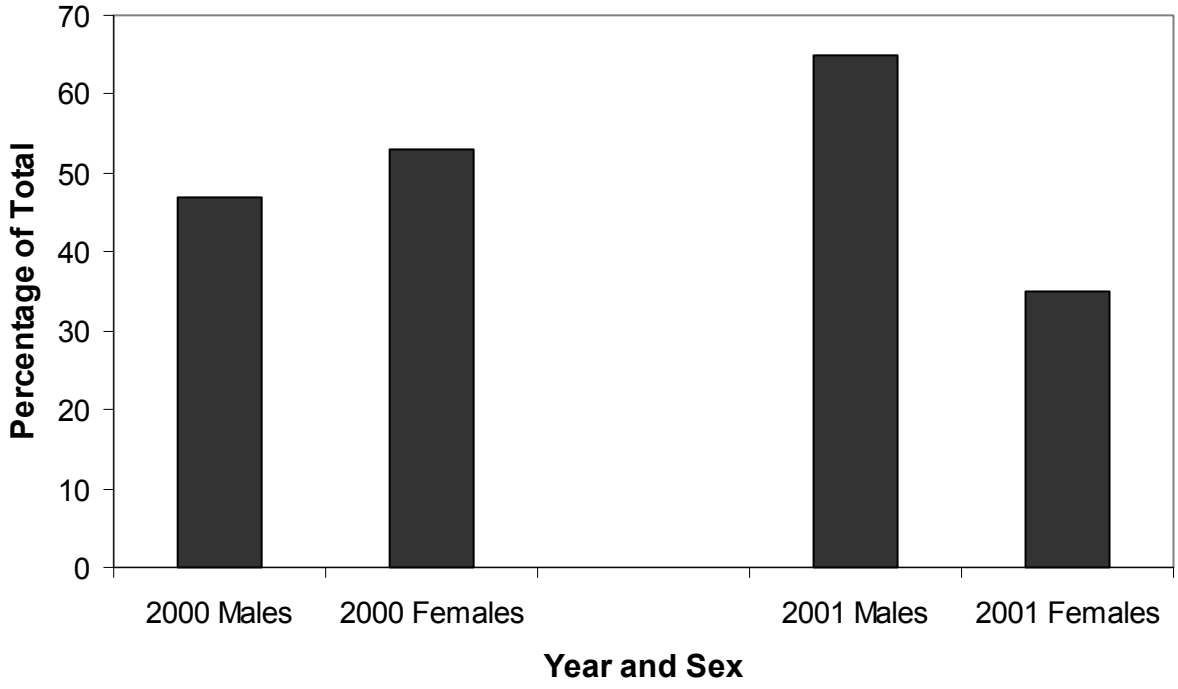


Figure 3.2*c*. Sex distribution for horseshoe crabs during years 2000 and 2001 in Ocean City, MD.

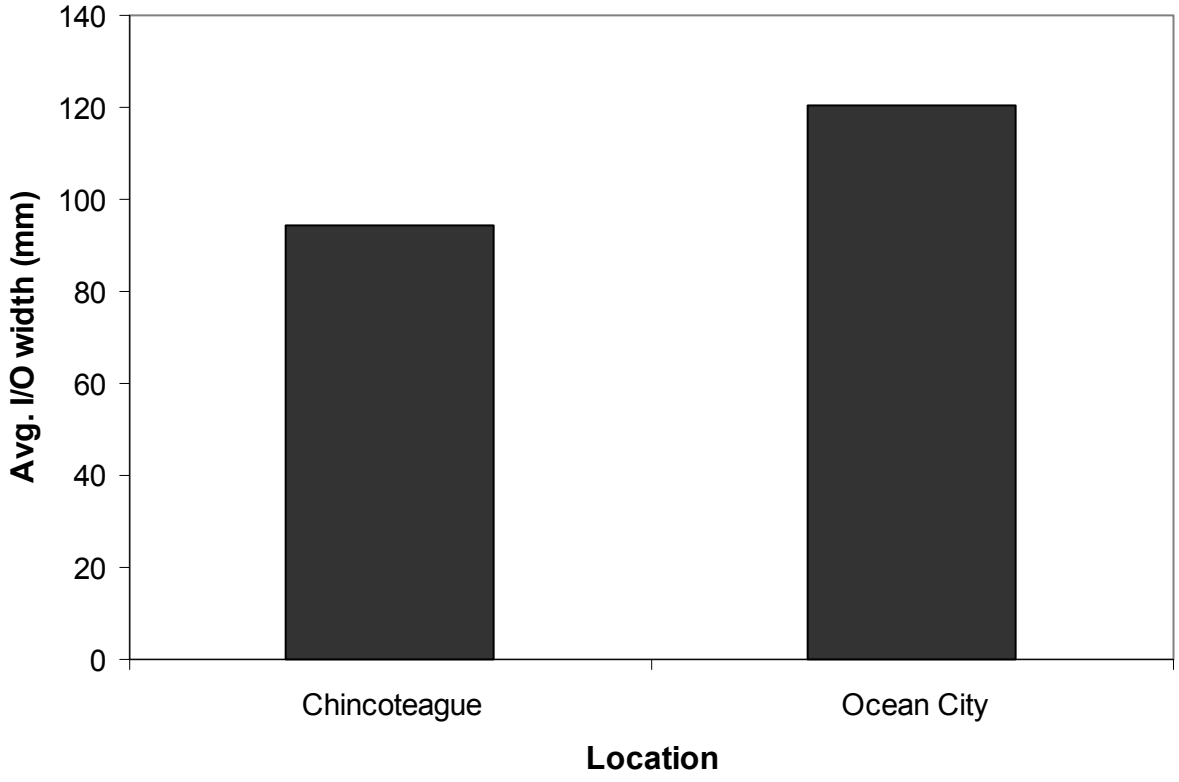
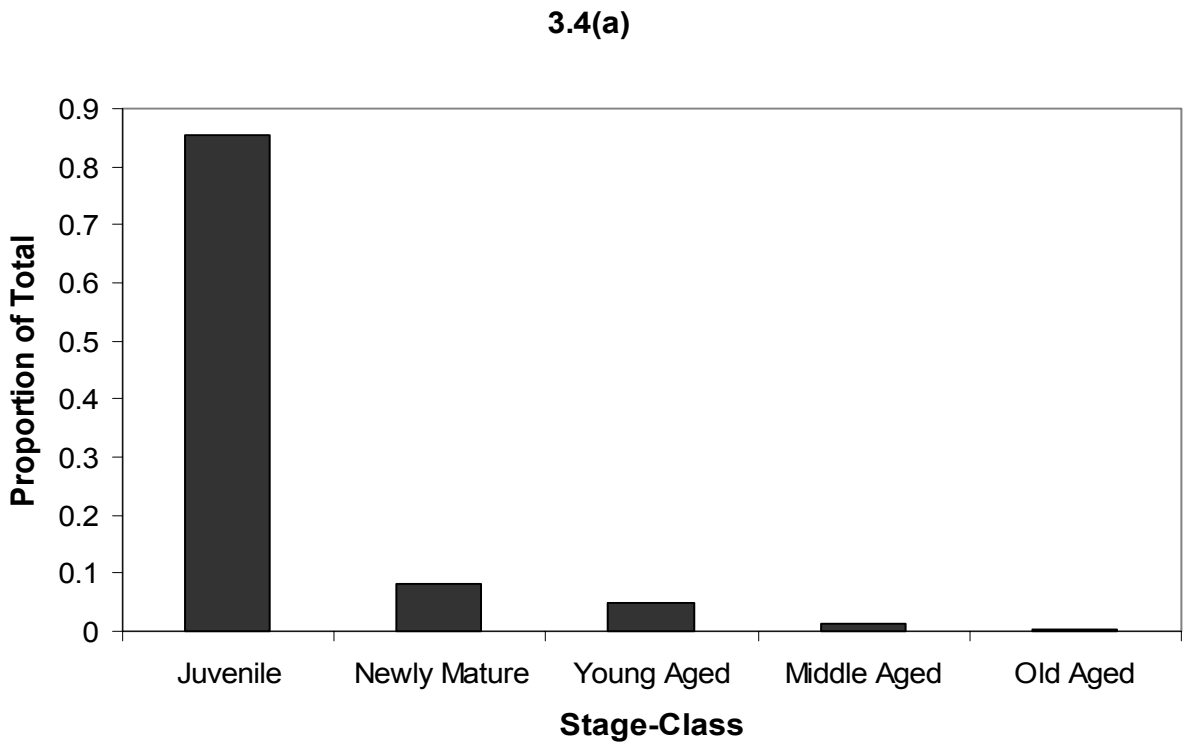


Figure 3.3. Comparison of average size of horseshoe crabs obtained in trawls by BioWhittaker in Chincoteague, VA (2000 – 2001; N = 1,027), and in Ocean City, MD (1999 – 2001; N = 438).



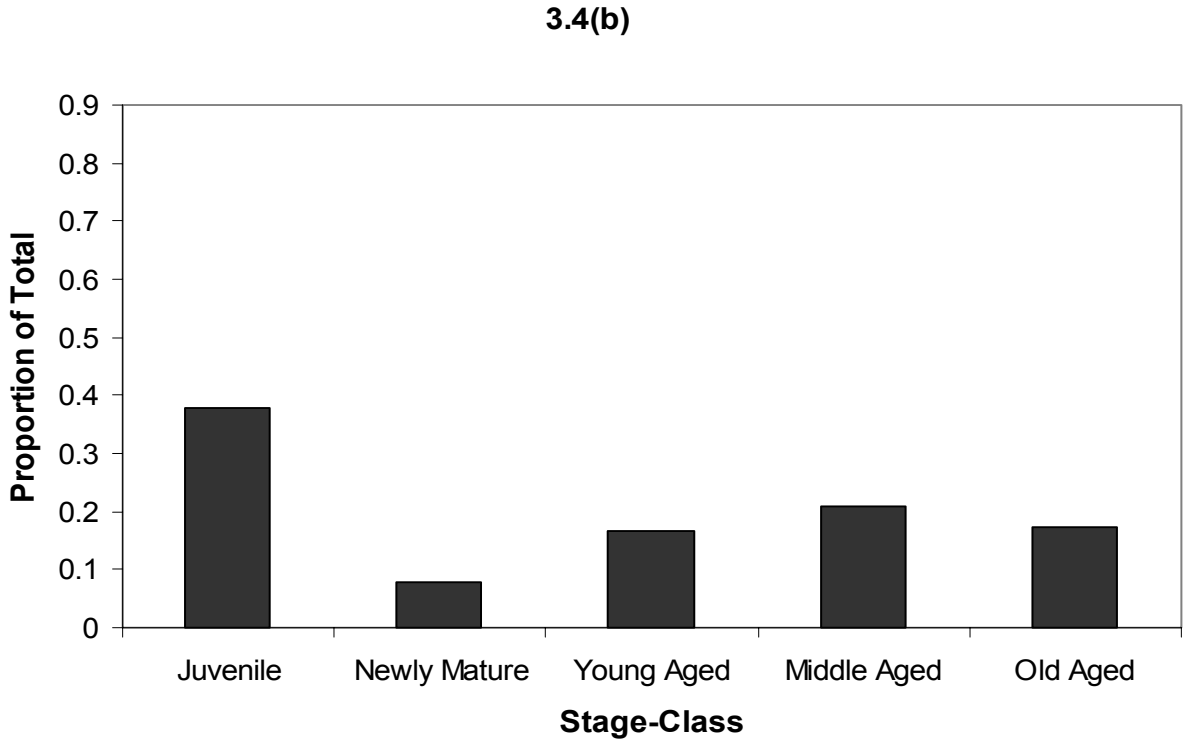


Figure 3.4(*a*-*b*). Comparison of population distribution in each stage-class for horseshoe crabs obtained in trawls by BioWhittaker during 2000 and 2001. Figure 3.4*a*. Stage-class distribution in Chincoteague, VA. Figure 3.4*b*. Stage-class distribution in Ocean City, MD.

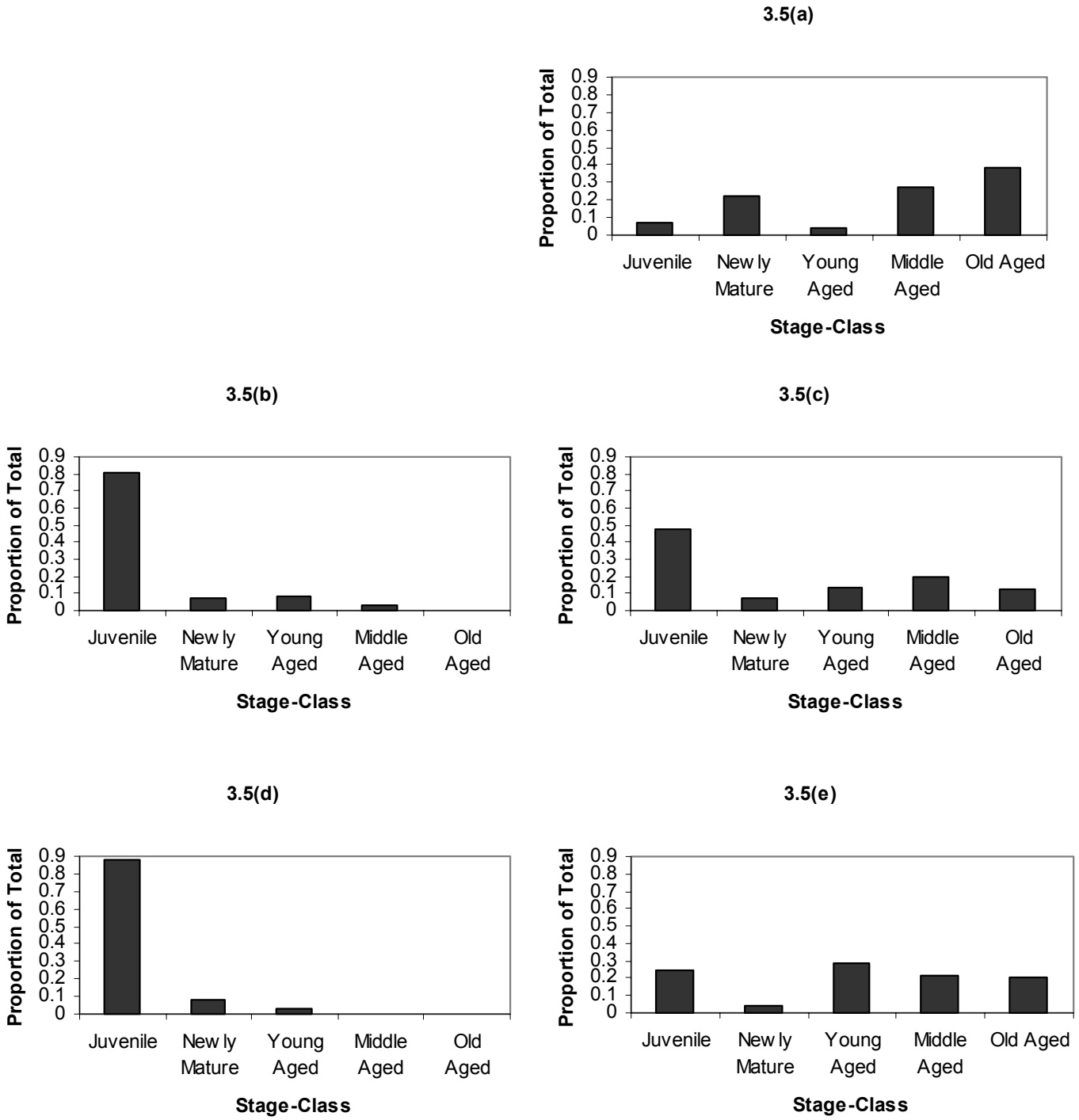


Figure 3.5 (*a*-*e*). Comparison of population distribution in each stage-class for horseshoe crabs obtained in trawls by BioWhittaker. Figure 3.5*a*. Stage-class distribution in Ocean City, MD, 1999. Figure 3.5*b*. Stage-class distribution in Chincoteague, VA, 2000. Figure 3.5*c*. Stage-class distribution in Ocean City, MD, 2000. Figure 3.5*d*. Stage-class distribution in Chincoteague, VA, 2001. Figure 3.5*e*. Stage-class distribution in Ocean City, MD, 2001.

CHAPTER 4 – TAGGING STUDY: MONITORING SURVIVAL AND MOVEMENT OF BLED HORSESHOE CRABS

ABSTRACT

There is a lack of information regarding horseshoe crab population dynamics, and growing concern of population decline among various user groups. Thus, we undertook a horseshoe crab tagging study in conjunction with the Atlantic States Marine Fisheries Commission to provide information useful for the management of horseshoe crabs. During summers 1999, 2000, and 2001, we tagged a portion of the adult horseshoe crabs bled by the biomedical company, BioWhittaker, and released the horseshoe crabs off the coasts of Ocean City, Maryland and Chincoteague, Virginia. We tagged 7,500 horseshoe crabs with Petersen disc tags; the Horseshoe Crab Tag Recovery Center of the United States Fish and Wildlife Service in Annapolis, Maryland maintained corresponding release and recapture data. One hundred twenty-one of the 7,500 tagged horseshoe crabs have been resighted as of October 15, 2001, constituting 1.61% recovery. Fifty-three percent of the 121 resighted crabs were alive at the time of resight. Average distance traveled between release and recapture was 47.7 kilometers, while maximum distance traveled of all resighted crabs was 312 kilometers. Information gained in this study can be used to design an improved tagging study that will lead to further knowledge of horseshoe crab population dynamics.

INTRODUCTION

A potential decline in horseshoe crab population numbers has become an increasing concern to a number of user groups, including commercial fishermen, biomedical companies, and bird watching enthusiasts. Commercial fishermen harvest horseshoe crabs for bait in eel and whelk fisheries, biomedical companies extract blood from horseshoe crabs for pharmaceutical purposes, and migratory shorebirds rely on eggs of horseshoe crabs to fuel their migration (Berkson and Shuster 1999). Management of horseshoe crabs is currently surrounded by controversy, as demands for them have increased and fear that horseshoe crab and migratory shorebird populations may be declining has arisen. Information on population status, distribution, and structure is needed to develop effective management plans.

In an effort to gather some much-needed population data and to move towards coordinated, coastwide management of horseshoe crabs, the Atlantic States Marine Fisheries Commission (ASMFC) initiated a tagging program with biomedical companies in 1999. Initially, all biomedical companies bleeding horseshoe crabs were required to tag a portion of the horseshoe crabs they bleed. Weighing the needs for data with manpower constraints, the ASMFC decided arbitrarily that biomedical companies defined as "large" by the ASMFC would tag 2,500 horseshoe crabs per year. However, due to various other demands and priorities, the tagging program was de-emphasized in 2000, and made strictly voluntary. The biomedical company BioWhittaker continued tagging horseshoe crabs after the ASMFC mandate was lifted.

Ideally, the goal of the tagging program was to ascertain information regarding movement patterns and long-term survival following blood extraction, estimates of tag

recovery, and estimates of horseshoe crab abundance. Realistically, due to the hurried design of the study, results were useful, but much more modest.

METHODS

During bleeding seasons 1999, 2000, and 2001, we tagged a portion of bled horseshoe crabs at BioWhittaker's bleeding facility in Chincoteague, Virginia. The tagging was performed using the same basic protocol employed by biomedical companies coast-wide, with some minor modifications. Crabs were stratified by age, and only adult crabs were selected, as juvenile crabs would lose their tag upon molting. Adults were selected in existing sex ratios from totes of bled crabs stored in a climate-controlled room designed to maintain gill moisture. Selected horseshoe crabs were placed on a table, and any epibionts or other growths present on the crab were removed from the surface of the crab where the tag was to be placed. The terminal portion of the carapace on the right hand side was then sprayed with Isopropanol for disinfectant purposes. A Ryobi™ cordless, battery-powered drill was used to drill a hole 5/32 of an inch in diameter and 1/4 inch deep in the rear prosoma. A white Petersen disc-tag was then inserted into the crab through the drilled hole. Tags were individually numbered and listed a toll-free phone number for the central Horseshoe Crab Tag Recovery Center operated by the U.S. Fish and Wildlife Service (1-888-LIMULUS). Information recorded for each tagged crab included sex, life-history stage, intra-ocular width, prosoma width, as well as the date, waterbody, and nearest town of capture and release. Life-history stage was determined through the examination of the carapace of the crab, with lustrous carapaces indicating mature, young aged crabs and extremely scratched carapaces indicating old aged crabs (Shuster 1999). Data were entered into a spreadsheet and submitted to the Horseshoe

Crab Tag Recovery Center. This procedure was followed for 2,500 horseshoe crabs during each bleeding season. Following the tagging procedure, the horseshoe crabs were returned to the ocean in close proximity to their original point of capture in accordance with BioWhittaker's standard operating procedures.

Individuals spotting a tagged horseshoe crab could call the number on the tag to the Horseshoe Crab Tag Recovery Center. They were asked to provide the following information: date of resight, waterbody and nearest town of resight, and condition of the crab (dead, alive, or tag found only). No rewards were offered for reporting resighted crabs, nor was any publicity used to encourage reporting of resighted crabs.

Analyses

From the resight reports, estimates of percent recovery, percent found alive, percent of tags found detached, and movement patterns were obtained. Percent recovery was determined by dividing the number of horseshoe crabs resighted by the total number of crabs tagged. Percent alive was the number of horseshoe crabs reported alive at time of resight divided by the total number of resighted horseshoe crabs. Percent of tags found detached was the number of resights reported as "Tag Found Only" divided by the total number of resights. Distance of travel was calculated as the straight-line distance between the release location and the resight location. Using Wilcoxon Rank Sum tests at the $\alpha = 0.05$ significance level (Hollander & Wolfe 1999), we examined differences in distance traveled between male and female horseshoe crabs and between crabs released in Ocean City, Maryland and Chincoteague, Virginia.

RESULTS

Tag Returns

During the 3 field seasons, we tagged 2,500 bled, adult horseshoe crabs each year for a total of 7,500 crabs (Table 4.1). By the end of the tagging study, October 16, 2001, the Horseshoe Crab Tag Recovery Center had received 121 reported resights of tags and tagged crabs, overall, a 1.61% recovery rate (Tables 4.2 and 4.3). Forty-six of the horseshoe crabs tagged in 1999 were recovered (1.8%), 70 of the horseshoe crabs tagged in 2000 were recovered (2.8%), and 5 of the horseshoe crabs tagged in 2001 were recovered (0.2%). Condition of the crab when resighted ("Alive," "Dead," "Tag Found Only," "Not Reported") was reported to the Horseshoe Crab Tag Recovery Center. Overall, 52.9% of the resights were tagged crabs reported "Alive" at the time of the resight (Table 4.4). Fourteen of the 121 resights (11.6%) were reported as "Tag Found Only" (Table 4.4). In 3 cases (0.02%), the condition of the crab was “Not Reported” to the Horseshoe Crab Tag Recovery Center.

Movement Patterns

The average distance traveled by the 121 resighted horseshoe crabs was 47.7 kilometers (*95% CI:* 37.8 – 57.6 kilometers), while the maximum distance traveled was 312 kilometers by a female crab tagged in Chincoteague Virginia on 7/14/99 and resighted at Sandy Hook, New Jersey on 12/15/00 (Table 4.5, Appendix 1). Forty-eight of the 121 resights (39.7%) were crabs resighted in the same location as their original release. Further, 28 (23.1%) of the resighted crabs moved from Chincoteague, Virginia to Ocean City, Maryland or vice versa. Twenty-one horseshoe crabs (17.4%) tagged and

released by BioWhittaker were resighted in the Delaware Bay region. Overall, locations of reported resights stretched from Sandy Hook, New Jersey to Virginia Beach, Virginia (Figure 4.1)

Male horseshoe crabs traveled greater distances (N = 78; mean = 52.3 km) than female horseshoe crabs (N = 43; mean = 39.5 km; P = 0.0264). No differences in travel distances were detected between crabs released in Chincoteague, VA (N = 44; mean = 64.5 km) and those released in Ocean City, MD (N = 77; mean = 38.1 km; P = 0.1813).

DISCUSSION

This study was initiated to provide population information on horseshoe crabs. With concerns arising about declining populations and lack of informative population data for management, the ASMFC quickly designed this study with more consideration given to resource constraints than to statistical design. As a result, this study cannot provide information about population abundance or estimates of mortality as a traditional mark/recapture study might. The non-random sampling stemming from the biomedical trawling process coupled with the non-random method of resight does not meet the assumptions required to estimate population abundance. However, this tagging study does provide initial estimates of tag recovery rates and movement patterns, as well as sufficient information to design an improved tagging study.

Tag Recovery

In our study, we found a 1.61% tag recovery rate. This rate includes the resights of 14 tags found detached from crabs due to tag loss. Negating these detached tags yields a 1.42% recovery rate of tagged crabs. It should be noted that this tag recovery rate is not

a true resight rate, but more of an encounter rate based on opportunistic resightings and nonrandom sampling.

Movement Patterns

Previously, it has been unclear whether horseshoe crabs exist in geographically-isolated subpopulations with little to no movement between subpopulations, if there is one large, homogeneously distributed population with considerable mixing, or if there is one extensive population with a heterogeneous spatial distribution. The type of distribution would influence which management strategies would be most effective. If horseshoe crabs are distributed as several isolated subpopulations along the Atlantic coast, the subpopulations should be managed as separate populations. Different productivities, age structures, and subpopulation sizes might require different harvest regulations for the separate subpopulations. In contrast, movement of horseshoe crabs up and down the coast would result in panmixis and genetic heterozygosity. This would require management attention focused on the population as a whole.

Our study suggests that in a 2-year period, most horseshoe crabs do not migrate great distances. However, we did document mixing beyond the geographic areas where crabs were released after tagging. Horseshoe crabs released in Ocean City were reported 56 kilometers south in Chincoteague and vice versa. In addition, we documented movement of horseshoe crabs from the Maryland and Virginia coasts into the Delaware Bay. The majority of resighted crabs (96.6%) were observed within the area stretching from the northern Delaware Bay south to Virginia Beach, Virginia, illustrating that the population of horseshoe crabs off the eastern shore of Virginia and those in the Delaware Bay have some degree of mixing.

Pierce et al. (2000) recently compared the genetic makeup of horseshoe crabs in the Delaware Bay to those found in the upper Chesapeake Bay. They showed extremely limited gene flow between these areas based on mtDNA analyses, supporting the existence of at least 2 subpopulations along the east coast. Other genetic studies comparing populations along the Atlantic coast are in progress. Several instances of large-scale movements as seen in our tagging study, along with the number of resights in the Delaware Bay suggest that mixing along the Atlantic coast does occur, at least between horseshoe crabs found in the Chincoteague, Ocean City, and Delaware Bay areas. The results of tagging studies combined with independent genetic studies will provide the clearest picture of population structure of the horseshoe crab population.

Assumptions

Any horseshoe crab tagging study should meet the assumptions of mark-recapture studies including: all crabs have an equal probability of capture and recapture, tagged crabs are representative of the population of interest, all crabs retain their tags or tag-loss rate can be calculated, tagging does not alter the behavior of crabs, tagged crabs equally mix with unmarked crabs following release, and, re-sighted crabs are reported (Ricker 1975, Seber 1982).

In contrast, in our study designed by ASMFC, crabs did not have an equivalent probability of capture and recapture. The trawler did not use random search patterns in searching for crabs for the initial capture, and recaptures consisted of opportunistic resightings. Tagged crabs were representative only of crabs used by one of the biomedical companies in terms of size and life-history stage, not of the entire population as needed. We saw evidence of tag loss, as 11.6% of the resights were tags found

detached from crabs. It was also common knowledge that fishermen were not reporting tags they recaptured, because there was no reward for doing so (Eyler, personal communication).

Recommendations for future tagging efforts

Tagging studies are utilized to estimate a variety of population parameters, including population abundance, survival, and movement patterns, all of which are critical to the development of effective management policies (Ricker 1975). An improved horseshoe crab tagging study would not be a preferred method for estimation of population abundance due to logistical constraints, stringent assumptions, and the wide confidence intervals that would likely result. Other procedures for estimating population abundance (a random trawl survey, for example (ASMFC 1998)) would be more efficient and more cost-effective. In order to use an improved tagging study to estimate survival, we would need to obtain an accurate estimate of rate of resight, and could then only estimate adult survival as juvenile crabs would shed tags when molting. Thus, an improved tagging study would be most useful for improving our understanding of horseshoe crab movement and population structure.

If we go through the steps of designing an improved horseshoe crab tagging study, we must first define the population of interest in both a spatial and temporal sense. Given the mixing found in our study together with the absence of analyses showing subpopulations (with the exception of Chesapeake Bay), a closed mark-recapture study would require horseshoe crabs along the entire Atlantic coast to be designated as the population of interest. Due to the non-random nature of the tagging and resighting in our study, we are not able to estimate the precise tagging levels required of any new study, so

tags should be placed on as many horseshoe crabs as possible to enhance recovery numbers.

Future tagging studies should involve a series of multiple marking and recapturing periods (Seber 1982, Quinn and Deriso 1999). The horseshoe crabs marked in the study should be obtained through random samples of the ocean from random trawl surveys. Tag loss must be reduced through an improved tagging protocol, and further quantified by double-tagging or multiple-marking of individual horseshoe crabs. Incentives must be provided to commercial fishermen for reporting resighted crabs, as shown in other fisheries tagging studies (Matlock 1981, Jenkins et al. 2000). Resightings of marked crabs would be considered valid only if: 1) the crabs obtained in the resight are representative of the population of interest, and, 2) in the area sampled, there is an equal probability of encountering a marked crab compared with an unmarked crab. In addition, sufficient financial support must be guaranteed for the study to last for an extended time-period.

Effective management of this ecologically, economically, and medically essential species is dependent on knowledge of population structure. This study provides useful evidence of population mixing which, if true coastwide, eliminates the need for managers to develop subpopulation-specific management plans. The study also provides evidence of potentially high tag-loss, important because these same tags are used by many groups up and down the coast. Most importantly, we can use the results of this study to design a statistically valid mark/recapture study for the future. Tagging studies provide important information to managers and, because of this, likely will continue to play a role in future horseshoe crab research and management.

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Table 4.1. Sex, age, and number of bled horseshoe crabs tagged in Chincoteague, Virginia during each year of a 3-year study (1999-2001), as originally mandated by the Atlantic States Marine Fisheries Commission.

	Males					Females					
Year	Newly Matured	Young Aged	Middle Aged	Old Aged	Total	Newly Matured	Young Aged	Middle Aged	Old Aged	Total	Grand Totals
1999	1319	107	127	150	1703	632	40	72	51	797*	2500
2000	561	517	278	166	1522	211	321	270	176	978	2500
2001	969	296	45	49	1359	791	216	92	42	1141	2500
Totals	2849	920	450	365	4584	1634	577	434	269	2916*	7500

*2 female juvenile crabs were mistakenly tagged during the first field season

Table 4.2. Number recovered and percent recovery of horseshoe crabs tagged in Chincoteague, Virginia during 1999-2001. These figures represent recovery of tagged horseshoe crabs as well as tags found detached from horseshoe crabs.

Year Tagged	Number Tagged	Number Recovered From Year's Tagged Crabs	Percent Recovery
1999	2500	46	1.8%
2000	2500	70	2.8%
2001	2500	5	0.2%
Totals:	7500	121	1.61%

Table 4.3. Number of resights per year of horseshoe crabs tagged in Chincoteague, Virginia during 1999-2001. These figures represent recovery of tagged horseshoe crabs as well as tags found detached from horseshoe crabs. (N/A - Not Applicable, that is, a horseshoe crab tagged in 2000 could not be recovered in 1999, et cetera.)

	Year Recovered		
Year Tagged	1999	2000	2001
1999	17	18	11
2000	N/A	39	31
2001	N/A	N/A	5

Table 4.4. Status of horseshoe crabs tagged at Chincoteague, Virginia during 1999-2001 as reported at time of resight. Status at time of resight was not reported for 3 of the 121 resighted horseshoe crabs (0.02%).

Year Tagged	Number of Tags Recovered	Number Reported "Alive"	Number Reported "Tag Only"	Percent Reported "Alive"	Percent Reported "Tag Only"
1999	46	24	6	52.2%	13.0%
2000	70	40	8	57.1%	11.4%
2001	5	0	0	0.0%	0.0%
Total:	121	64	14	52.9%	11.6%

Table 4.5. Average and maximum distances traveled by horseshoe crabs tagged in Chincoteague, Virginia during 1999-2001 and released in Chincoteague, VA or Ocean City, MD. Distances (km) are based on point of release and point of resight of tagged crabs or tags. Distances were determined by calculating straight-line distances between point of release and point of resight.

Year Tagged	Average Distance Traveled by Crabs Released in Chincoteague, VA	Maximum Distance Traveled of Crabs Released in Chincoteague, VA	Average Distance Traveled by Crabs Released in Ocean City, MD	Maximum Distance Traveled of Crabs Released in Ocean City, MD	Overall Average Distance Traveled	Overall Maximum Distance Traveled
1999	78.7 (n = 33)	312	49.8 (n = 14)	110	70.1 (n = 47)	312
2000	39.7 (n = 6)	176	35.7 (n = 64)	120	36 (n = 70)	176
2001	0.8 (n = 5)	0.8	N/A (n = 0)	N/A	0.8 (n = 5)	0.8
Overall	64.5 (n = 44)	312	38.2 (n = 77)	120	47.7 (n = 121)	312

Figure 4.1. Resight locations for horseshoe crabs bled by BioWhittaker, and tagged in Chincoteague, Virginia during 1999-2001. Horseshoe crabs released in Chincoteague, Virginia are symbolized by a cross (+), while horseshoe crabs released in Ocean City, Maryland are symbolized by an asterisk (*).

APPENDIX – RELEASE AND RESIGHT INFORMATION FOR HORSESHOE CRABS BLED BY BIOWHITTAKER, CHINCOTEAGUE, VIRGINIA, 1999–2001.

Tag No.	Tagging Date	Tagging Location	Resight Date	Resight Location	Status at Resight	Distance Traveled
17756	7/19/99	Ocean City, MD	5/29/01	Milford, DE	Tag Found Only	91
17765	7/19/99	Ocean City, MD	5/8/00	Prime Hook Beach, Milton, DE	Alive	72
17819	7/20/99	Ocean City, MD	9/4/99	Chincoteague, VA	Tag Found Only	56
17835	7/20/99	Ocean City, MD	10/13/99	Ocean City, MD	Alive	2
17835	7/20/99	Ocean City, MD	12/15/99	Ocean City, MD	Alive	0.8
17956	7/20/99	Ocean City, MD	9/12/00	Fenwick, MD	Alive	19
18005	7/20/99	Ocean City, MD	5/13/01	Milton, DE	Alive	72
18011	7/20/99	Ocean City, MD	8/1/99	Ocean City, MD	Alive	0.8
18024	7/20/99	Ocean City, MD	7/12/00	Green Creek, Little Township, NJ	Dead	50
18050	7/20/99	Ocean City, MD	7/29/00	Roosevelt Inlet, Lewis, DE	Alive	56
18095	8/4/99	Ocean City, MD	11/2/99	Assawoman Bay, Ocean City, MD	Dead	2
18161	8/4/99	Ocean City, MD	7/14/01	Milton, DE	Dead	0.8
18310	7/12/99	Chincoteague, VA	8/26/00	Indian River Bay, Rehobeth, DE	Alive	85
18334	7/12/99	Chincoteague, VA	5/6/00	Holgate, Beach Haven, NJ	Alive	216
18390	7/12/99	Chincoteague, VA	8/6/00	Indian River Bay, Ocean View, DE	Not Reported	85
18376	7/12/99	Chincoteague, VA	6/1/01	Little Creek, DE	Dead	160
18430	7/14/99	Chincoteague, VA	12/15/00	Sandy Hook, NJ	Dead	312
18470	7/13/99	Chincoteague, VA	6/24/00	Assawoman Bay, Ocean City, MD	Alive	56
18593	7/14/99	Chincoteague, VA	5/10/01	Port Norris, NJ	Alive	155
18619	7/14/99	Chincoteague, VA	7/29/99	Assateague Island, VA	Dead	2
18696	7/19/99	Ocean City, MD	5/13/00	Broadkill Beach, DE	Dead	64
18744	7/19/99	Ocean City, MD	6/17/00	Fortescue, NJ	Alive	110
18958	6/29/99	Chincoteague, VA	8/27/00	Barnegat Light, NJ	Alive	251
18990	6/29/99	Chincoteague, VA	12/4/99	Metomkin Island, Accomac, VA	Tag Found Only	35
19048	6/29/99	Chincoteague, VA	8/28/00	Ocean City, MD	Tag Found Only	56
19106	6/29/99	Chincoteague, VA	5/16/01	Milton, DE	Alive	128
19234	6/28/99	Chincoteague, VA	6/15/00	Maurice River, Port Norris, NJ	Alive	155
19235	6/28/99	Chincoteague, VA	7/3/99	Assateague Island, MD	Dead	32
19402	6/24/99	Chincoteague, VA	1/21/00	Ocean City, MD	Alive	56
19431	6/23/99	Chincoteague, VA	5/6/01	Cape May, NJ	Alive	120
19437	6/23/99	Chincoteague, VA	9/5/99	Chincoteague, VA	Tag Found Only	0.8
19461	6/21/99	Chincoteague, VA	5/15/01	Bethany Beach, DE	Alive	83
19550	6/22/99	Chincoteague, VA	2/25/00	Virginia Beach, VA	Alive	131
19559	6/21/99	Chincoteague, VA	7/9/99	Assateague Beach, VA	Dead	0.8
19569	6/21/99	Chincoteague, VA	9/14/00	Ocean City, MD	Alive	56
19588	6/21/99	Chincoteague, VA	10/1/01	Rock Hall, MD	Alive	154
19626	6/18/99	Chincoteague, VA	9/8/00	Ocean City, MD	Alive	56
19657	6/17/99	Chincoteague, VA	10/16/01	Ocean City, MD	Alive	56

19679	6/14/99	Chincoteague, VA	6/30/99	North End of Wallops Island, VA	Dead	3
19714	6/18/99	Chincoteague, VA	7/1/99	Assateague Island, MD	Dead	32
19826	6/14/99	Chincoteague, VA	7/18/99	Assateague Channel, Chincoteague, VA	Dead	0.8
19871	6/9/99	Chincoteague, VA	7/4/99	Assateague Wildlife Refuge, VA	Dead	0.8
19873	6/9/99	Chincoteague, VA	4/2/01	Wallops Island, Chincoteague, VA	Dead	3
20021	6/14/99	Chincoteague, VA	11/29/99	Quimby, VA	Alive	51
20025	6/18/99	Chincoteague, VA	6/30/99	North End of Wallops Island, VA	Dead	3
24089	6/6/00	Chincoteague, VA	1/28/01	Wallops Island, Chincoteague, VA	Dead	3
20141	6/17/99	Chincoteague, VA	10/19/99	Wallops Island, Chincoteague, VA	Tag Found Only	3
24044	6/6/00	Chincoteague, VA	6/29/00	Chincoteague, VA	Alive	0.8
24226	6/14/00	Chincoteague, VA	5/23/01	Chincoteague, VA	Alive	0.8
24261	6/14/00	Chincoteague, VA	5/28/01	Dover, DE	Dead	176
24265	6/14/00	Chincoteague, VA	10/17/00	Ocean City, MD	Alive	56
24449	6/15/00	Chincoteague, VA	6/26/00	Pelagin Island, Chincoteague, VA	Dead	2
25007	7/11/00	Ocean City, MD	7/14/00	Ocean City, MD	Dead	0.8
25011	7/11/00	Ocean City, MD	6/1/01	Ocean City, MD	Tag Found Only	0.8
25013	7/11/00	Ocean City, MD	8/1/00	Ocean City, MD	Alive	0.8
25045	7/11/00	Ocean City, MD	9/15/00	Ocean City, MD	Alive	0.8
25049	7/11/00	Ocean City, MD	9/15/00	Ocean City, MD	Alive	0.8
25052	7/12/00	Ocean City, MD	10/17/00	Ocean City, MD	Dead	0.8
25056	7/12/00	Ocean City, MD	8/3/00	Ocean City, MD	Dead	0.8
25087	7/12/00	Ocean City, MD	6/5/01	Little Creek, DE	Alive	104
25090	7/12/00	Ocean City, MD	9/12/00	Ocean City, MD	Alive	0.8
25109	7/12/00	Ocean City, MD	9/12/00	Fenwick, MD	Alive	19
25118	7/12/00	Ocean City, MD	3/12/01	Toms Cove, Chincoteague, VA	Tag Found Only	56
25121	7/12/00	Ocean City, MD	1/3/01	Assateague Island, MD	Tag Found Only	10
25125	7/12/00	Ocean City, MD	8/17/00	Ocean City, MD	Tag Found Only	0.8
25200	7/14/00	Ocean City, MD	7/6/01	Rehobeth, DE	Tag Found Only	91
25203	7/13/00	Ocean City, MD	7/17/00	Ocean City, MD	Alive	0.8
25230	7/13/00	Ocean City, MD	8/4/00	Ocean City, MD	Alive	0.8
25235	7/13/00	Ocean City, MD	8/1/00	Ocean City, MD	Alive	0.8
25239	7/13/00	Ocean City, MD	7/16/00	Ocean City, MD	Dead	0.8
25262	7/14/00	Ocean City, MD	6/3/01	Milton, DE	Alive	72
25307	7/17/00	Ocean City, MD	3/13/01	Wallops Island, Chincoteague, VA	Dead	59
25367	7/17/00	Ocean City, MD	4/3/01	Wachapreague, VA	Alive	104
25417	7/17/00	Ocean City, MD	10/7/00	Wallops Island, Chincoteague, VA	Dead	59
25431	7/17/00	Ocean City, MD	8/10/01	Chincoteague, VA	Dead	120
25494	7/17/00	Ocean City, MD	5/5/01	Assateague Beach, VA	Dead	56
25496	7/17/00	Ocean City, MD	5/1/01	Chincoteague, VA	Alive	56
25570	7/17/00	Ocean City, MD	10/3/00	Toms Cove, Chincoteague, VA	Dead	56
25574	7/17/00	Ocean City, MD	8/1/00	Ocean City, MD	Alive	0.8
25609	7/17/00	Ocean City, MD	8/3/00	Ocean City, MD	Alive	0.8

25615	7/17/00	Ocean City, MD	9/12/00	Ocean City, MD	Alive	0.8
25659	7/17/00	Ocean City, MD	8/3/00	Ocean City, MD	Alive	0.8
25678	7/17/00	Ocean City, MD	7/1/00	Ocean City, MD	Not Reported	0.8
25686	7/17/00	Ocean City, MD	7/1/00	Ocean City, MD	Not Reported	0.8
25697	7/17/00	Ocean City, MD	8/21/00	Ocean City, MD	Alive	0.8
25718	7/17/00	Ocean City, MD	4/28/01	Wallops Island, Chincoteague, VA	Dead	59
25751	7/18/00	Ocean City, MD	8/1/00	Wallops Island, Chincoteague, VA	Dead	59
25815	7/18/00	Ocean City, MD	11/2/00	3 miles offshore, Chincoteague, VA	Alive	64
25925	7/17/00	Ocean City, MD	4/16/01	Chincoteague, VA	Alive	56
26074	7/18/00	Ocean City, MD	9/1/00	Assateague Beach, VA	Dead	56
26032	7/18/00	Ocean City, MD	5/27/01	Chincoteague, VA	Alive	56
26042	7/18/00	Ocean City, MD	6/4/01	Lewes, DE	Alive	56
26130	7/19/00	Ocean City, MD	5/7/01	Chincoteague, VA	Alive	56
26161	7/19/00	Ocean City, MD	9/14/00	Ocean City, MD	Dead	0.8
26254	7/19/00	Ocean City, MD	4/6/01	Assateague Island, MD	Alive	16
26282	7/19/00	Ocean City, MD	10/4/00	Assateague Beach, VA	Dead	56
26293	7/19/00	Ocean City, MD	8/10/00	Ocean City, MD	Alive	0.8
26295	7/19/00	Ocean City, MD	5/24/01	Dover, DE	Alive	120
26302	7/19/00	Ocean City, MD	7/5/01	Milford, DE	Dead	29
26330	7/19/00	Ocean City, MD	7/30/00	Wallops Island, Chincoteague, VA	Tag Found Only	59
26364	7/19/00	Ocean City, MD	9/11/00	Ocean City, MD	Alive	0.8
26401	7/19/00	Ocean City, MD	9/11/00	Ocean City, MD	Alive	0.8
26406	7/19/00	Ocean City, MD	6/26/01	Rehobeth, DE	Alive	35
26427	7/19/00	Ocean City, MD	8/2/00	Ocean City, MD	Alive	0.8
26472	7/19/00	Ocean City, MD	9/13/00	Fenwick, MD	Tag Found Only	19
26483	7/19/00	Ocean City, MD	5/25/01	Little Heaven, DE	Alive	112
26488	7/19/00	Ocean City, MD	11/15/00	Cape Henlopen, DE	Alive	59
26500	7/19/00	Ocean City, MD	8/17/00	Ocean City, MD	Alive	0.8
26544	7/20/00	Ocean City, MD	7/30/01	Dover, DE	Dead	0.8
26612	7/20/00	Ocean City, MD	8/12/00	Metomkin Island, Accomac, VA	Dead	91
26627	7/20/00	Ocean City, MD	6/4/01	Chincoteague, VA	Alive	56
26679	7/31/00	Ocean City, MD	5/16/01	Fenwick, DE	Tag Found Only	19
26706	7/31/00	Ocean City, MD	5/24/01	Cape May, NJ	Alive	56
26732	7/31/00	Ocean City, MD	6/25/01	Oyster, VA	Alive	0.8
26727	7/31/00	Ocean City, MD	5/1/01	Chincoteague, VA	Alive	64
26772	7/31/00	Ocean City, MD	6/7/01	Chincoteague, VA	Alive	56
27131	5/30/01	Chincoteague, VA	7/7/01	Chincoteague, VA	Dead	29
27156	5/30/01	Chincoteague, VA	10/6/01	Chincoteague, VA	Dead	0.8
27317	6/4/01	Chincoteague, VA	7/15/01	Chincoteague, VA	Dead	72
27508	6/7/01	Chincoteague, VA	6/17/01	Chincoteague, VA	Dead	56
27719	6/8/01	Chincoteague, VA	7/28/01	Chincoteague, VA	Dead	0.8

VITA

Elizabeth Walls was born in Baltimore, Maryland in 1977 and lived in Bel Air, Maryland until graduating from C. Milton Wright High School in 1995. Her parents, Bruce and Carmella Walls, still live in Bel Air, Maryland. Elizabeth attended college at the University of Maryland at College Park and received a B.S. in Biology with a concentration in Animal Behavior, Ecology, Evolution and Systematics in May of 1999. She spent the summers of 1999, 2000, and 2001 in Chincoteague, Virginia collecting data for this thesis. In December of 2001, she completed her M.S. in Fisheries and Wildlife Science in the Department of Fisheries and Wildlife Science at Virginia Polytechnic Institute and State University in Blacksburg, Virginia.

Elizabeth A. Walls