A MID-ATLANTIC STUDY OF THE MOVEMENT PATTERNS AND POPULATION DISTRIBUTION OF THE AMERICAN HORSESHOE CRAB (*Limulus polyphemus*)

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(**ABSTRACT**)

In conjunction with Cambrex, a biomedical company that utilizes horseshoe crabs for the production of *Limulus* Amoebocyte Lysate (LAL), a study was conducted to investigate movement patterns and population distributions of horseshoe crabs to increase understanding of mid-Atlantic horseshoe crab populations. In addition, areas of the shoreline of Tom's Cove, Assateague Island, Virginia were investigated as possible locations for annual spawning surveys. Twelve thousand five hundred horseshoe crabs were tagged and released in Chincoteague, Virginia and Ocean City, Maryland as part of a movement study; 431 (3.45%) were reported as resights. The mean distance between site of release and site of recapture for all resighted crabs was 68.3 km; maximum distance moved was 493.7 km. During 1999-2004, demographic data were collected from horseshoe crabs harvested in Chincoteague, VA and Ocean City, MD. The proportion of females ($p < 0.0001$) and juveniles ($p < 0.0001$) sampled varied from year-to-year, but no trends were observed. This study also showed that a greater proportion of females were observed in the juvenile cohort sampled compared to the adults sampled ($p < 0.0001$). The spawning survey revealed that spawning activity in Tom's Cove varied between years. On May 30th, 2003, 1,192 horseshoe crabs were observed spawning on the northern shoreline of Tom's Cove. The maximum number of horseshoe crabs observed spawning in the same area in 2004 was 94. This study provides no evidence for isolated subpopulations in Chincoteague, VA or Ocean City, MD. It also shows that horseshoe crab sex and age ratios fluctuate annually, therefore requiring a long time series of data to detect trends.

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CHAPTER 1 – INTRODUCTION AND JUSTIFICATION

INTRODUCTION

The American horseshoe crab, *Limulus polyphemus* (L.), is a multiple-use resource with both ecological and economic value. Environmentalists are interested in the fate of this species because of the important function it serves as a food source for migrating shorebirds. Commercial fishermen rely on the horseshoe crab primarily as bait for eel and whelk fisheries. Perhaps most vital is the role the horseshoe crab plays in the biomedical industry, mainly as a source of *Limulus* Amoebocyte Lysate (LAL), a substance derived from the blood of horseshoe crabs for testing sterility in pharmaceutical products.

Approximately one million horseshoe crabs are harvested annually by the commercial fishery. Since horseshoe crabs require 10 years to reach sexual maturity (Shuster 1958; Berkson and Shuster 1999), overexploitation of the population could have severe consequences. An overfished population would take at least a decade to recover. In response to concerned stakeholder groups, management of the horseshoe crab resource has come to the forefront in recent years. The development of a management plan has been difficult because of the lack of information regarding the status of the horseshoe crab population. Therefore, the Atlantic States Marine Fisheries Commission (ASMFC) has developed a horseshoe crab management plan that calls for reductions in the overall catch of horseshoe crabs. It also calls for the development of better monitoring techniques, including the establishment of pilot programs to survey spawning horseshoe crabs (ASMFC 1998b). As more information becomes available, ASMFC can modify management strategies to better meet the needs of all interested groups.

In conjunction with Cambrex, a leading producer of LAL, Virginia Polytechnic Institute and State University conducted a 3-year study to investigate demographic distributions and movement patterns of horseshoe crabs to increase understanding of mid-Atlantic horseshoe crab populations (Walls 2001). That study found significant differences in sex distribution, size distribution, and stage-class distribution between horseshoe crabs captured in waters surrounding Chincoteague, Virginia and those captured in waters surrounding Ocean City, Maryland. A tagging component of that study revealed that significant mixing occurred among horseshoe crabs in the Chincoteague, VA, Ocean City, MD and Delaware Bay areas. A continuation of this tagging study will increase sample size of resighted horseshoe crabs, leading to more robust conclusions. Since horseshoe crabs have a long life cycle, it is also important to continue collecting demographic data over many years to identify changes in population structure that may be related to exploitation. For these reasons, Cambrex renewed its contract with Virginia Polytechnic Institute and State University to continue studying horseshoe crabs in the mid-Atlantic.

The following literature review will describe the basic natural history of the horseshoe crab as well as human uses of this species. Current management of the horseshoe crab will also be described, followed by a description of the objectives of this study. This study will help provide information regarding the status of horseshoe crab populations in the waters surrounding Chincoteague, VA and Ocean City, MD by further examining movement patterns and the demographics associated with these populations. In addition, in response to the Atlantic States Marine Fisheries Commission's call to establish pilot programs to survey spawning horseshoe crabs, a spawning survey will be developed to monitor beach use at Tom's Cove located on Assateague Island, VA. Changes in the spawning population at this location may be reflective of harvest levels in waters surrounding Chincoteague, VA.

NATURAL HISTORY

The American or Atlantic horseshoe crab, *Limulus polyphemus* (Linnaeus), is one of 4 species of horseshoe crabs in existence today. *L. polyphemus* currently inhabits Atlantic waters from Maine to Yucatan (Shuster 1953). The remaining 3 species (*Carcinoscorpius rotundicauda* (Latr.), *Tachypleus tridentatus* (Leach), and *T. gigas* (Müller)) are endemic to the Indo-Pacific region (Shuster 1992). These 4 species dispersed some 60 million years ago when all horseshoe crabs left European waters; one group moving westward, the others eastward (Shuster 1953).

L. polyphemus is the most widely studied species. In addition to American or Atlantic horseshoe crab, other common names include: horsefoot, horsefeet, pan crab, piggy back crab, and king crab, although the actual king crab is *Paralithodes camtschatica* (Shuster 1992). These common names likely resulted from the misclassification of *L. polyphemus* as a crustacean (Lockwood 1870; Ives 1891). Horseshoe crabs are more closely related to ancient sea scorpions and modern scorpions and spiders than they are to true crabs (Shuster 1953; Mikkelsen 1988). Some consider the horseshoe crab a "living fossil" as it has remained relatively unchanged for 200 million years (Botton and Ropes 1987b; Walls et al. 2002). The taxonomic classification of the Atlantic horseshoe crab is as follows (Mikkelsen 1988; Walls et al. 2002):

Kingdom: Animalia

Phylum: Arthropoda

Class: Merostomata

Subclass: Xiphosura

Order: Xiphosurida

Suborder: Limulina

Superfamily: Limulacea

Family: Limulidae

Subfamily: Limulinae

Genus: *Limulus*

Species: *L. polyphemus*

Distribution

The American horseshoe crab ranges from northern Maine to the Yucatan peninsula from about 42°N to 19°N (Shuster 1982a; Pierce et al. 2000), although some reports indicate a specimen being found as far north as Nova Scotia (Mikkelsen 1988). *Limulus* is eurythermal, voluntarily occupying a 15-40°C temperature range with a preference for temperatures between 25°C and 30°C (Shuster 1979). Temperature appears to be the limiting factor for the northern range of *Limulus* and may also be a factor in the south (Shuster 1982a). Although the largest population of American horseshoe crabs is found in the Delaware Bay (Shuster 1992; Pierce et al. 2000), each of the major estuaries along the Eastern coast of the United States has a *Limulus* population (Shuster 1979). Some studies provide evidence for local populations that interact little with neighboring populations (Widener and Barlow 1999; Ehlinger et al. 2003). Data suggest that *Limulus* along the Atlantic Coast can be characterized into distinct populations by range in size of adults (Shuster 1957). Coastal surveys during spawning seasons from 1949 to 1954 concluded that these populations were relatively discrete based on differences in prosomal widths of adults, with the largest prosomal widths found in adults inhabiting estuaries from Georgia to New Jersey and smaller individuals both north and south of this range (Shuster 1979). Differences in external anatomy also distinguish discrete populations of *Limulus* (Shuster 1979). In addition, genetic analyses suggest there are distinct populations along the Atlantic coast even over a relatively small geographic range (Saunders et al. 1986; Pierce et al. 2000). Pierce et al. (2000) found that Delaware Bay and Chesapeake Bay populations of horseshoe crabs are genetically distinct. However, more recently, King et al. (2003) suggested significant gene flow between these 2 populations. Through examination of microsatellite DNA markers, King et al. (2003) clustered populations of *Limulus* into 4 regional management units within the United States: Gulf of Maine, mid-Atlantic, Florida-Atlantic, and Florida-Gulf. They also identified a fifth management unit in waters surrounding Mexico's Yucatan Peninsula (King et al. 2003). Interestingly, Walls' (2001) tagging study showed that mixing does occur among horseshoe crabs along the Atlantic coast, at least among horseshoe crabs in the Chincoteague, VA, Ocean City, MD and Delaware Bay areas.

Horseshoe crabs are benthic creatures, using both estuarine and continental shelf habitats. Early life stages usually inhabit intertidal flats and shallow water areas near their natal beach. As they grow older, they move to deeper estuarine and continental shelf waters (Shuster 1979). Horseshoe crabs are generalists, tolerant of a wide range of environmental conditions (Shuster 1990). Shuster (1957) found 11 to 30.6 ppt (parts per thousand) salinity to be the usual range; however *Limulus* encounters higher salinities when on the continental shelf (Shuster 1979). The majority of horseshoe crabs inhabit shallow waters up to 30 meters, although some have been found at depths as great as 290 meters (Botton and Ropes 1987b).

Spawning

Many adult horseshoe crabs spend most of the winter on the continental shelf and spring and summer months on intertidal mudflats feeding on various marine animals (Shuster 1953). Each spring, as temperature and length of daylight increase, adult horseshoe crabs migrate from deep waters to spawn on intertidal sandy beaches protected from wave action (Shuster 1979). Normally, male horseshoe crabs arrive to the spawning area before the females. As the females arrive, one or more males may attach to a single female's opisthosoma or to each other's with boxing glove-like pincers (Mikkelsen 1988).

Not all spawning habitats are the same or are equally utilized. Preference for specific sites is based on long-term environmental conditions as well as short-term weather changes (Shuster 1992; Berkson and Shuster 1999). Low-energy environments are preferred as they reduce the chance of stranding during spawning events (Gibson and Olszewski 2001). The Delaware Bay contains many low-energy beaches and hosts the largest numbers of spawning horseshoe crabs, which are widely distributed throughout the area (Botton and Harrington 2003). The height of waves has a significant impact on spawning activity and large groups can assemble only when waters are calm (Shuster 1958). For example, during a prevailing northeasterly wind in the Cape May, New Jersey region of the Delaware Bay, horseshoe crabs will remain offshore until it becomes calm

(Shuster 1953) or they will move to the more protected Delaware shore to spawn (Shuster 1958).

Spawning generally occurs from March through July, with peak spawning corresponding to the new and full moon high tides of May and June. However, peak times vary latitudinally (Shuster 1992). For example, in the Indian River Lagoon in Florida, horseshoe crabs were observed spawning year round, peaking in late winter-early spring, with no significant correlation to the lunar cycle (Ehlinger et al. 2003).

Just above the mean high tide line where the development of her eggs is maximized (Penn and Brockman 1994), the female will dig a nest approximately 5-30 cm deep (Brockman 1990) where she will deposit approximately 3,650 eggs in a cluster (Shuster and Botton 1985). Wave action immediately covers the eggs with sand (Lockwood 1870). The placement of egg clusters at depths greater than 10 cm offers protection from the predominately short-billed shorebirds that feed on horseshoe crab eggs (Botton et al. 1994). Wave action washes the male's spermatozoa over the eggs to fertilize them (Shuster 1992).

Over one or more tidal cycles, the female will continue laying eggs until 88,000-100,000 eggs are deposited (Shuster 1999). Throughout this time, males generally remain attached to a single female (Shuster 1999). Afterwards, the female will move back out to deeper waters while males remain inshore to continue spawning with other females (Shuster 1999). Currently, it is not known if a second clutch of eggs ripens rapidly enough to allow the female to spawn twice a year, although both male and female horseshoe crabs appear to bear mature gametes most of the year (Shuster 1999). Horseshoe crabs may remain reproductively active for at least 8 years (Shuster 1999).

Development

Most eggs develop in 2-4 weeks, depending on temperature, moisture, and oxygen content of the nest environment (Sekiguchi et al. 1982; Shuster 1982a). However, some eggs overwinter and do not hatch until the following spring (Botton et al. 1992). The larval horseshoe crabs are liberated when wave action uncovers the nest and ruptures the outer membrane surrounding the embryo. Tidal currents carry tailless larvae down to intertidal mudflats. The larvae continue to receive nourishment from the remnants of the egg yolk. Once this food source is depleted, the animal molts for the first time. At this point the immature horseshoe crab has a telson and its body fully resembles that of an adult. It feeds on minute organisms living in the mudflat (Shuster 1953).

As the horseshoe crab grows it continues to molt. The number of molts per year decreases with age. In its first year, the horseshoe crab will molt 5 times (Shuster 1979), followed by 2 to 3 times in its second year, 2 times in its third year, and 1 time each year thereafter (Shuster and Sekiguchi 2003). The timing of the annual molt changes with size; the larger the juvenile the later in the summer it molts. Before molting, a soft new skin forms under the old shell, the front edge of the shell splits and the horseshoe crab emerges from the opening 25% larger than before (Shuster 1953). Internal pressure, created by an increase of water in the horseshoe crab's tissues, aids in this process. Soon after, the outer shell hardens (Shuster 1953). Exuviation takes only a few minutes for larvae and several hours for nearly full-grown horseshoe crabs (Shuster 1990). If there is a terminal molt it occurs in the fall (Shuster 1999), at which time the horseshoe crab becomes an adult and males emerge with modified claws (Lockwood 1870; Koons 1883).

Carmichael et al. (2003) suggest that there is a possibility that some horseshoe crabs may continue to molt as often as once per year after reaching adulthood.

Aging

It is suspected that males reach sexual maturity between ages 9 and 10, with females becoming adults at age 10 or 11 (Mikkelsen 1988; Carmichael et al. 2003) after an additional molt, which is thought to account for the larger size of females (Shuster 1990; Carmichael et al. 2003). There is no known way to age horseshoe crabs; however, several indirect aging methods suggest that horseshoe crabs may live up to 20 years (Shuster 1958). Indirect methods of aging include tagging, aging of epibionts (encrusting organisms) present on the carapace (Botton and Ropes 1988), and examination of carapace wear. It is assumed that if adults do not molt, their exposure to a sand-abrasive environment, epibionts, and diseases of the carapace increases with age. Shuster (1999) identifies 3 categories to describe the ages of adult horseshoe crabs (Walls et al. 2002). These are young adults, middle-aged adults, and old-aged adults. However, if Carmichael et al. (2003) are correct in their assessment that horseshoe crabs may molt annually after reaching adulthood, this method of aging would not be reliable.

Sexual Dimorphism

Horseshoe crabs exhibit sexual dimorphism (Shuster 1982a). The female is generally larger than the male. In addition, males possess a modified boxing-glove shaped pincer for grasping the female's opisthosoma (Lockwood 1870). Male and females can also be differentiated by examination of genital pores (Koons 1883). Beneath the genital operculum, males possess a pair of hard, conical papillae, while females have a pair of soft oval openings (Shuster 1990). Sex ratios on spawning beaches can range from 5 males for every female to 3 males for every female (Shuster and Botton 1985). Offshore trawl surveys suggest a reversed sex ratio, with females outnumbering males from 3:2 to 2:1 (Rudloe 1980). Therefore the overall sex ratio may be close to 1:1 (Rudloe 1980; Thompson 1999). A more recent fishery-independent survey of the Delaware Bay region found that females make up approximately 66% of immature horseshoe crabs but only 37% of mature animals (Hata and Berkson 2003). In addition, the sex ratio for juvenile horseshoe crabs in Pleasant Bay, Cape Cod was nearly equal while the ratio for adult horseshoe crabs in that area was male-dominated (Carmichael et al. 2003). This is possibly the result of the bait industry's preference for gravid females (HCTC 1998), though Carmicheal et al. (2003) suggest that a greater number of molts among female horseshoe crabs as compared to male horseshoe crabs may increase mortality and account for the skewed sex ratio. They also propose that spatial segregation of male and female horseshoe crabs may account for the greater number of males in the embayment (Carmichael et al. 2003).

Ecology

Horseshoe crabs play an important role in the marine ecosystem. Ectocommensal organisms, including various species of bryozoans, annelids, and mollusks are prevalent on adult horseshoe crabs (Mikkelsen 1988). Parasites may also be present, including flatworms that lay eggs on the leaves of book gills (Hall 1995).

The horseshoe crab is also an important predator species. It is a dietary generalist (Botton and Haskin 1984; O'Connell et al. 2003). Larval horseshoe crabs feed on a variety of small polychaetes and nematodes. Larger juvenile and adult horseshoe crabs are regarded as shellfish predators, capable of crushing the shells of mollusks (Novitsky

1991). In addition to mollusks, juvenile and adult horseshoe crabs feed upon various species of polychaetes, nematodes, annelids, arthropods and vascular plant material (Botton 1984; Botton and Haskin 1984). The horseshoe crab digs after its food grasping its prey with its pincer-tipped legs. It then crushes the prey item between its other legs forcing it into its mouth (Shuster 1982a).

Horseshoe crabs are also important prey items for various marine organisms, including sea turtles, finfish, rays, sharks, and a variety of invertebrates (Mikkelsen 1988). Although all stages of horseshoe crabs are preyed upon, the greatest mortality occurs in the egg stage of the horseshoe crab's life. Several species of finfish, crabs, shrimp and other invertebrates feed on them at this time (Shuster 1982a).

Shorebird Connection

In addition horseshoe crab eggs are a vitally important food source for migrating shorebirds (Castro and Myers 1993). Several species of migratory shorebirds, including some endangered species, fly as many as 5,000 miles (USFWS 1998) non-stop from South America to Delaware beaches in May to eat for 2 to 3 weeks to regain up to 70-80% of their body weight lost during the flight. Afterwards they head north to their Arctic breeding grounds (Dutton 1998; Tsipoura and Burger 1999). The rest and food the shorebirds receive at Delaware Bay are critical to their survival (Harrington and Shuster 1999).

Horseshoe crab eggs constitute the majority of food consumed by these birds (Castro and Myers 1993); however, they are not taken to the exclusion of other items (Tsipoura and Burger 1999). Since hundreds of thousands of shorebirds utilize these stopovers, it is likely that collective energy consumption is high, requiring a large number of horseshoe crab eggs (Castro and Myers 1993). Castro and Myers (1993) estimate that, if all shorebirds fed exclusively on horseshoe crab eggs, 539 metric tons of eggs would be consumed annually. Further calculations suggest that at least 1,820,000 female horseshoe crabs are required to produce this mass of eggs (Castro and Myers 1993). These data are important for determining the worst-case scenario. The actual number of horseshoe crab eggs consumed is likely fewer than this estimate, requiring a smaller number of horseshoe crabs to provide the actual amount consumed by migratory birds. However, these risk-averse estimates support the belief that the conservation of horseshoe crabs and their spawning habitats may be crucial to the protection of migratory shorebirds (Castro and Myers 1993).

There is concern that the current density of horseshoe crabs is not sufficient to provide nourishment to migratory shorebirds (Widener and Barlow 1999). Biologists and birdwatchers believe that the Delaware Bay horseshoe crab population has decreased and that the harvest of horseshoe crabs should be reduced (Dutton 1998; Widener and Barlow 1999). Since horseshoe crabs do not reach sexual maturity for 10 years, reproduction may not be able to keep up with the increased loss.

Most migrating shorebirds are incapable of digging deep enough to reach buried eggs. Large numbers of horseshoe crabs occupying the same spawning areas result in many nests being unearthed. Few of the displaced eggs develop further; instead they become food for thousands of shorebirds (Harrington and Shuster 1999). Therefore, a surplus of horseshoe crabs is needed to unearth each others' eggs making them available for food. Otherwise, birds may not have enough energy to complete their journey and successfully reproduce (Harrington and Shuster 1999). For successful management, it is important to understand how shorebirds use horseshoe crab eggs during this critical stage and what other prey items are found in the shorebird diet (Tsipoura and Burger 1999).

In addition to being ecologically important to the survival of shorebirds, the horseshoe crabs provide an economic benefit to the Delaware Bay area. Each year, thousands of birders visit the Delaware Bay for the primary purpose of observing the horseshoe crab-shorebird phenomenon. The Cape May region accrues specific benefits because birders purchase recreation-related goods and services in the area. Annual economic activity resulting from horseshoe crab dependent eco-tourism in Cape May is estimated to be between $7 and $10 million (1999 dollars) (Manion et al. 2000).

Mortality

There is no current estimate of survival between hatching and sexual maturity for horseshoe crabs coastwide (ASMFC 1998b). However, Carmichael et al. (2003) determined that only 0.06% of horseshoe crab eggs deposited in Pleasant Bay, Cape Cod, Massachusetts survived to hatchling stage and only 0.001% of eggs produced juveniles that survived to year 1. Of the horseshoe crabs surviving to year 1, 78% reached adulthood (Carmichael et al. 2003). They also found that percent mortality increased with size among adult horseshoe crabs (Carmichael et al. 2003). Botton et al. (2003) estimated that only 0.00003% of horseshoe crab larvae on a beach in Delaware Bay, New Jersey remained as fourth instar juveniles at the end of their first summer. They note that both mortality and emigration could be responsible for the observed decrease (Botton et al. 2003). Sources of natural mortality for adult horseshoe crabs include predation, old age, and excessive energy expenditure during spawning (ASMFC 1998b). However, humans probably account for the most significant amount of adult mortality. Around 1 million horseshoe crabs are harvestsed each year by commercial fishermen (ASMFC 2004a). In addition, approximately 282,000 (Michels et al. 2000) horseshoe crabs were used in 1998 by the biomedical industry for production of Limulus Amoebocyte Lysate (LAL), of which an estimated 7.5% to 15% do not survive (Rudloe 1983; Walls and Berkson 2003). Additional mortality may be attributed to entrapment in man-made structures, such as bulkheads and jetties (ASMFC 1998b).

BIOMEDICAL USES

Horseshoe crabs have been used for biomedical research since the early 1900s (Shuster 1962). Three Nobel prizes have been awarded to scientists who conducted their research on various aspects of the horseshoe crab's physiology (Hall 1995). Scientists learned much about how the human eye functions after studying the horseshoe crab's large compound eyes. Because of the size of these eyes, their relatively simple construction and easy accessibility to the optic nerve, the horseshoe crab is the ideal animal for eye research (Hall 1995). Studies of electric impulses from the optic nerve, conducted by Dr. H. Keffer Hartline among others, have revealed many fundamental physiological principles, which can be transferred directly to all visual systems (Mikkelsen 1988).

Other researchers have found uses for chitin, a cellulose-like component found in the shells of horseshoe crabs. All arthropods have some chitin, but that found in the shells of horseshoe crabs is a very pure type (Hall 1995). Since the mid-1950s, scientists have known that chitin-coated sutures enhanced healing time by 35-50%. By the 1970s, scientists had developed a method to spin pure chitin filaments for suturing (Hall 1995).

Currently, chitin is also being used in wound dressings for burn victims, on surface wounds, and on skin-graft donor sites, which dramatically reduces pain and accelerates healing (Hall 1995). Horseshoe crab blood is also beneficial in cancer research (ASMFC 1998b).

Perhaps the most important use of horseshoe crabs is in the development of the clotting agent, *Limulus* Amoebocyte Lysate, or LAL (Hall 1995). The clotting of *Limulus* blood was first described by W. H. Howell in 1885. Shortly thereafter, the Marine Biological Laboratory in Woods Hole, Massachusetts was created to serve as the center of horseshoe crab research (Novitsky 1991). Here, Leo Loeb continued Howell's work, describing in great detail the blood and circulation in the horseshoe crab (Novitsky 1991). In 1956, Frederick Bang revealed the presence of the coagulation agent responsible for clotting in *Limulus* blood (Mikkelsen 1988). This agent, which is wholly contained within the amoebocytes and is extremely sensitive to endotoxin, was termed LAL after Bang and Jack Levin were able to extract it and develop a simple in-vitro test to detect endotoxins (Mikkelsen 1988). Using the LAL test, one millionth of a billionth of a gram of endotoxin can be detected in less than one hour (Mikkelsen 1988).

In the 1970s, the effort of 3 scientists, Donald Hochstein, Edward Seligmann, Jr., and James Cooper, initiated acceptance of LAL by the Food and Drug Administration as an alternative to the rabbit pyrogen test for detecting endotoxin (Novitsky 1991). Since then, LAL has become the required standard test for all injectable and implantable medical devices approved by the FDA (ASMFC 1998b). In addition to detecting sterility of pharmaceutical products, LAL is useful for detecting endotoxemia in conjunction with cirrhosis, cancer, meningitis, eye disease, dental problems, gonorrhea, and urinary tract infections (Rudloe 1983; Novitsky 1991). It has also been shown useful in detecting bacterially contaminated meat, fish, and dairy products, including frozen items (Novitsky 1991). If LAL became unavailable, it could take years to find a suitable replacement (ASMFC 1998b).

Currently there are 5 biomedical companies producing LAL (Manion et al. 2000). These companies are required to hold special permits for collecting horseshoe crabs (ASMFC 1998b). Horseshoe crabs collected for bait purposes may be used by the biomedical industry first. These horseshoe crabs are counted against the state's annual quota for commercial harvest (ASMFC 2004b). Any horseshoe crabs released alive must be returned to the waters from which they were collected (ASMFC 1998b). Monthly reports are required on harvest numbers and percent mortality up to the time of release (ASMFC 1998b). The world market for LAL generates annual revenues of approximately $60 million (1999 dollars) and creates between 440 and 540 jobs (Manion et al. 2000).

There is an upward trend in the number of horseshoe crabs being used by the biomedical industry, although growth has slowed in recent years (Berkson and Shuster 1999). In 1983 only 30,000 horseshoe crabs were used for the production of LAL (Rudloe 1983). By 1998, a reported 281,663 were harvested for biomedical purposes (Michels et al. 2000). Between 100 and 300 ml of blood are usually taken from a horseshoe crab, while it is estimated that the maximum available volume is only between 200 and 300 ml (Rudloe 1983). This leads to questions concerning mortality associated with the bleeding process. Current estimates show that 7.5% to 15% do not survive bleeding (Rudloe 1983; Walls 2001; Walls and Berkson 2003). However, mortality rates associated with capture and handling are still unknown (Berkson and Shuster 1999). Regardless, mortality associated with the biomedical industry has a substantially smaller impact on the horseshoe crab population than the commercial fishery, which has 100% mortality.

COMMERCIAL FISHERY

Human use of horseshoe crabs dates back to at least the 1500s, when European settlers observed Native Americans eating the meat of horseshoe crabs and keeping the carapace for use as a vessel for food and drink (Hall 1995; Mikkelsen 1988). Native Americans also used the telson as spear tips to kill fish (Mikkelsen 1988). Colonial fishermen used the empty carapaces to bail out their boats (Shuster 1953). Settlers began using dried, crushed horseshoe crabs as fertilizer for fields and feed for livestock (Mikkelsen 1988). In 1856, along a few kilometers of the Cape May beach, Delaware Bay, more than 1.2 million were caught for use as fertilizer and feed. By 1930, at least 5 million were harvested along the East coast for these purposes (Mikkelsen 1988). Eventually, the fertilizer industry based on horseshoe crabs ceased because of a decline in the horseshoe crab population and competition from other commercial fertilizer producers (Berkson and Shuster 1999). In addition, the demand for horseshoe crabs as feed for livestock ended after complaints concerning the foul aftertaste of meat and eggs produced by horseshoe crab-fed animals (Mikkelsen 1988). Horseshoe crab populations rebounded in the 1950s and 1960s when the commercial fishery was at a minimum (Berkson and Shuster 1999).

In the latter part of the 20th century, horseshoe crabs started being harvested again, primarily for use in the bait industry for American eel (*Anguilla rostrata*), whelk (*Busycon* spp.) and to a lesser extent catfish (Ictaluridae) and killifish (*Fundulus spp.)* fisheries (HCTC 1998; ASMFC 2004a). Fishing effort for horseshoe crabs, which can be caught by trawl, dredge, hand, and gillnet, is concentrated within the mid-Atlantic coastal waters and adjacent federal waters because of high abundance in these areas (HCTC 1998). An entire spawning beach of horseshoe crabs can easily be harvested (Botton and Ropes 1987a; HCTC 1998). In the 1980s the estimated catch ranged from 348,000 to 817,000 horseshoe crabs (Botton and Ropes 1987a). However, in 1998 reported coastwide landings were 2,756,949 horseshoe crabs (Michels et al. 2000). Although commercial landings data of horseshoe crabs are collected by the National Marine Fisheries Service (NMFS), before mandatory reporting their records were often incomplete and conversion between pounds and numbers landed was variable (HCTC 1998). An increase in reported landings during the 1990s has been attributed to both improvements in reporting requirements and increases in actual catch. Landings reported in Delaware for the years 1995 to 1997 shows a 2-fold increase during this period (HCTC 1998). Effort in Delaware, measured as the number of hand permits issued, showed a 6-fold increase from 1991 to 1997 (HCTC 1998).

Increase in horseshoe crab harvest is due to increased demand in the eel and whelk fisheries. Eels are sold as bait primarily for striped bass and as seafood (Dutton 1998). Juvenile eels are used as live bait, while larger eels are cut for long-line and trotline bait (Harrington and Shuster 1999). Eels are also sold throughout the United States and in foreign markets where they are a popular food (Harrington and Shuster

1999). Female horseshoe crabs are preferred for use in the eel pot fishery, as eels are attracted to chemical odors unique to gravid females (Botton and Ropes 1987a; HCTC 1998). This preference has obvious consequences for the sex distribution of the population (Loveland et al. 1996) and could threaten horseshoe crab reproductive success (Botton and Ropes 1987a). Whelks are sold for food primarily to a U.S. domestic Asian market (Harrington and Shuster 1999). Both male and female horseshoe crabs are used in the whelk pot fishery (HCTC 1998). Economic benefits associated with the commercial fishery include an estimated $2 million (1999 dollars) and 70 jobs for the eel pot fishery and an estimated $11-$15 million (1999 dollars) and about 440-540 jobs for the whelk fishery (Manion et al. 2000).

Environmentalists have identified several concerns regarding the horseshoe crab fishery and the sustainability of current harvest levels. Horseshoe crab populations declined during the middle of the century at fishing levels comparable to current harvest levels. Horseshoe crabs are easily harvested with minimal financial investment, which makes them highly vulnerable to overharvesting. Life history of the horseshoe crab is another factor increasing the susceptibility to overharvest. It takes 10 years for horseshoe crabs to reach sexual maturity, which creates a delay in population recovery (Berkson and Shuster 1999). These concerns led to the development of the Horseshoe Crab Fishery Management Plan.

HORSESHOE CRAB MANAGEMENT

In 1997, a possible decline in horseshoe crab abundance and a decline in the migrating shorebird census totals in Delaware Bay prompted the Atlantic States Marine

Fisheries Commission (ASMFC) to begin work on a management plan for horseshoe crabs (Wenner and Thompson 2000). The Horseshoe Crab Fishery Management Plan (FMP) was adopted by the ASMFC on October 22, 1998 (ASMFC 2000). Before this time only a few states had management regulations regarding horseshoe crabs (ASMFC 1998b). States with regulations prior to 1998 included New Jersey, Delaware, and Maryland who began requiring harvesting permits in the early 1990s (Walls et al. 2002). These states also placed restrictions on when and where horseshoe crabs could be harvested. In 1996, Maryland required that all harvest be reported and restricted harvest during the spawning season (Walls et al. 2002).

The original Horseshoe Crab FMP outlined coastwide requirements for monitoring and reporting, and identified research needs (Wenner and Thompson 2000). The lack of good fishery and population data prohibited the development of a comprehensive scientific management strategy (Walls et al. 2002). Instead the plan called for mandatory monthly reporting of all harvest, a continuation of existing benthic sampling programs, evaluation of post-bleeding mortality associated with biomedical usage, and identification of potential horseshoe crab habitat (ASMFC 1998b). The plan also required New Jersey, Delaware, and Maryland to maintain their harvest regulations (ASMFC 2000). Remaining states were encouraged to place a cap on landings, but specific requirements were not established (ASMFC 1998b). The Management Board did state their intention to develop a cap on landings for commercial bait fisheries to be enforced in 2000 (ASMFC 1998b).

Restrictions placed on landings in New Jersey, Delaware, and Maryland caused a shift in landings toward states with little to no regulations (Walls et al. 2002). From 1995 to 1997, the average number of horseshoe crabs landed in Virginia was 200,000. This number grew to over 1.2 million in 1998 (Walls et al. 2002). Pennsylvania, with no prior history of horseshoe crab landings and no coast line, reported 75,000 horseshoe crabs harvested in 1998 (ASMFC 2000). It is alleged that most of the horseshoe crabs landed came from coastal waters surrounding the Delaware Bay and Maryland (Walls et al. 2002). Coastwide landings for 1998 were 2,756,949, negating the management efforts in New Jersey, Delaware, and Maryland (ASMFC 2000).

Addendum I of the Horseshoe Crab FMP was approved on February 9, 2000 (ASMFC 2000). This established a state-by-state cap of 25% below the harvest average caught during a designated reference period of known harvest levels. Once the cap is reached, the fishery is closed for the year. Any overages are deducted from the next year's quota (ASMFC 2000). *De minimis* criteria were established for states that harvest less than one percent of coastwide horseshoe crab landings (ASMFC 2000). This reduction is considered to be a risk-averse approach to management until more quantitative data are available to formulate more appropriate guidelines (HCSAC 2000). Addendum I also recommended that the National Marine Fisheries Service (NMFS) prohibits the harvest of horseshoe crabs in federal waters within a 30-nautical-mile radius of the mouth of Delaware Bay (ASMFC 2000). This reserve was approved on March 7, 2001 (ASMFC 2001). Then on April 24, 2001, Addendum II to the FMP was approved to allow voluntary transfer of harvest quotas between states to alleviate bait shortages. These transfers require approval by the Management Board (ASMFC 2001). To be approved, information regarding the genetics of the populations, population status, and other data regarding the horseshoe crab stock must be considered. Since this information is not currently available it is unlikely that quota transfers will occur (Walls et al. 2002). Then in May, 2004, in response to recommendations of the United States Fish and Wildlife Service's Shorebird Technical Committee, Addendum III was put into effect to further reduce landings in Delaware, Maryland, and New Jersey. Additionally, Addendum III prohibits landings of horseshoe crabs for bait from May 1st through June 7th in these states. Addendum III also calls for stricter monitoring of all horseshoe crab harvest (ASMFC 2004b).

Current Status

The status of the coastwide stock remains unknown. In 2004, the Atlantic States Marine Fisheries Commission concluded that there was a lack of information for coastwide stock assessment. Information was not available to establish biological reference points, fishing mortality rates, or recruitment estimates (ASMFC 2004a). However based on trend analysis, they did conclude that the Delaware Bay population of horseshoe crabs has been declining for years (ASMFC 2004a). This decline is cause for concern; however, sufficient data were not available to set appropriate harvest thresholds (ASMFC 2004a).

The Horseshoe Crab Stock Assessment Committee recommended the implementation of fishery-independent survey to detect trends in relative stock size over time, with a long-term goal to form management criteria and develop biological reference points (HCSAC 2000). After 5 to 10 years of implementation, the coastwide benthic survey and harvest statistics from mandated reporting will provide data to construct a population dynamics model (HCSAC 2000). The Stock Assessment Committee will also rely on the redesigned, statistically valid Delaware Bay spawning survey, the Delaware

Bay trawl survey, and other appropriate surveys to evaluate trends in spawning biomass and fishing mortality (HCSAC 2000).

In the fall of 2001, the recommended fishery-independent benthic trawl survey was conducted in the Delaware Bay area (Hata and Berkson 2003). Data from this stratified random survey estimated the total number of horseshoe crabs available to the survey gear. Night sampling produced greater numbers with a mean abundance estimate of 11.4 million horseshoe crabs in the 2,912-km^2 study area. A 95% confidence interval produced estimates for the lower bound of horseshoe crab abundance equal to 2.29 million for day sampling and 5.95 million for night sampling. These lower limits provide useful reference points for conservative, risk-averse management (Hata and Berkson 2003).

Hata and Berkson's (2003) survey also provided estimates of new recruits, with a mean of 1.07 million. However, the lower confidence limit of 259,000 leads to concern regarding sustainability of the harvest (Hata and Berkson 2003), since in 2000 coastwide horseshoe crab landings exceeded 1.9 million (ASMFC 2002). In addition, based on night sampling, Hata and Berkson (2003) noted that females make up approximately 66% of immature horseshoe crabs, but only 37% of mature animals. This is possibly the result of the bait industry's preference for gravid females (HCTC 1998). A reduction in the number of mature females would reduce fecundity of the population (Hata and Berkson 2003).

Hata and Berkson's (2003) survey provides information unavailable in the past (ASMFC 2004a). It has been conducted for the past 3 years, with its study area increasing annually (ASMFC 2004a). However, it is unlikely that a few years of data from new monitoring programs will provide enough information to clearly assess horseshoe crab stock. The horseshoe crab's long maturation time and lifespan prevent quick assessment of population status (Berkson and Shuster 1999). But, over the long term this information will be critical for the development of management strategies that best meet the needs of all user groups (Berkson and Shuster 1999).

PROJECT PURPOSE AND OBJECTIVES

In response to the lack of information identified by the ASMFC, Cambrex, a leading producer of LAL, renewed a contract with Virginia Polytechnic Institute and State University to continue gathering information regarding this species. The Horseshoe Crab Stock Assessment Committee recognized tagging programs, harvest sampling, and spawner surveys as highly valuable to the stock assessment process (HCSAC 2000). The first objective of this study utilizes a tagging program to give us insight concerning movement patterns of horseshoe crabs in Atlantic waters surrounding Chincoteague, VA and Ocean City, MD. A continuation of the 1999-2001 tagging study conducted by Walls (2001) will increase sample size of resighted horseshoe crabs, leading to more robust conclusions. Information concerning movement patterns of horseshoe crabs is useful in the identification of stock structure. Movement information combined with genetic analysis is necessary for defining the scale of management appropriate for horseshoe crabs.

A second objective, concerning harvest sampling, requires the collection of demographic data, including age class, sex, and size of horseshoe crabs harvested for Cambrex's bleeding program. This information is useful in identifying differences in the population between locations and among years. Since horseshoe crabs have a long life cycle, it is important to continue collecting demographic data beyond Walls' (2001) 3-year study to identify changes in the population that may be related to exploitation. Identifying these changes is necessary for properly managing the species.

The final objective, developed in response to the ASMFC's call to establish pilot programs to survey spawning horseshoe crabs, is development of a spawner survey to monitor numbers of horseshoe crabs using Tom's Cove, located on Assateague Island, as a spawning site. Tom's Cove is adjacent to coastal waters where horseshoe crabs are harvested. Changes in the spawning population from year to year may be reflective of harvest levels in waters surrounding that area.

The following chapters describe in detail the methods and results of each objective. At a time when limited information is available regarding the population dynamics and stock structure of horseshoe crabs, this study provides information that will allow more insight into the horseshoe crab resource and how to better manage it for all user groups. Each chapter also includes suggestions for future research to further increase understanding of this species.

REFERENCES

ASMFC (Atlantic States Marine Fisheries Commission). 1998a. Horseshoe crab stock assessment report for peer review. Stock Assessment Report No. 98-01 of the Atlantic States Marine Fisheries Commission. Richmond, VA. 57pp.

_____. 1998b. Interstate fishery management plan for horseshoe crab. Fishery management report No.32 of the Atlantic States Marine Fisheries Commission. Washington, D.C. 58pp.

_____. 2000. Addendum I to the interstate fishery management plan for horseshoe crab. Fishery management report No. 32a of the Atlantic States Marine Fisheries Commission. Washington, D.C. 9pp.

_____. 2001. Addendum II to the interstate fishery management plan for horseshoe crab. Fishery management report No. 32b of the Atlantic States Marine Fisheries Commission. Washington, D.C. 5pp.

_____. 2004a. Horseshoe crab 2004 stock assessment report of the Atlantic States Marine Fisheries Commission. Washington, D.C. 87pp.

_____. 2004b. Addendum III to the interstate fishery management plan for horsesehoe crab. Fishery management report No. 32c of the Atlantic States Marine Fisheries Commission. Washington, D.C. 14pp.

Berkson, J. and C. N. Shuster, Jr. 1999. The horseshoe crab: the battle for a true multiple-use resource. Fisheries Management 24(11):6-11.

Botton, M. L. 1984. Diet and food preferences of the adult horseshoe crab *Limulus polyphemus* in Delaware Bay, New Jersey, USA. Marine Biology 81:199-207.

_____. and B. A. Harrington. 2003. Synchronies in migrations: shorebirds, horseshoe crabs, and Delaware Bay. Pages 5-26 *in* C. N. Shuster, Jr., R. B. Barlow, and H. J. Brockmann, eds. The American horseshoe crab. Harvard University Press, Cambridge, Massachusetts.

_____. and H. H. Haskin. 1984. Distribution and feeding of the horseshoe crab, *Limulus polyphemus*, on the continental shelf off New Jersey. Fishery Bulletin 82(2):383 389.

_____., R. E. Loveland, and T. R. Jacobsen. 1992. Overwintering by trilobite larvae of the horseshoe crab, *Limulus polyphemus*, on a sandy beach of Delaware Bay (New Jersey, USA). Marine Ecology Progress Series 88:289-292.

_____. 1994. Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. The Auk 111(3):605-616.

Botton, M. L., R. E. Loveland, and A. Tiwari. 2003. Distribution, abundance, and survivorship of young-of-the-year in a commercially exploited population of horseshoe crabs *Limulus polyphemus*. Marine Ecology Progress Series 265:175-184.

Botton, M. L. and J. W. Ropes. 1987a. The horseshoe crab, *Limulus polyphemus*, fishery and resource in the United States. Marine Fisheries Review 49(3):57-61.

_____. 1987b. Populations of horseshoe crabs, *Limulus polyphemus*, on the northwestern Atlantic continental shelf. Fishery Bulletin 85(4):805-812.

_____. 1988. An indirect method for estimating longevity of the horseshoe crab (*Limulus polyphemus*) based on epifaunal slipper shells (*Crepidula fornicata*). Journal of Shellfish Resources 7:407-412.

Brockman, H. J. 1990. Mating behavior of horseshoe crabs, *Limulus polyphemus*. Behavior 114:206-220.

Carmichael, R. H., D. Rutecki, and I. Valiela. 2003. Abundance and population structure of the Atlantic horseshoe crab *Limulus polyphemus* in Pleasant Bay, Cape Cod. Marine Ecology Progress Series 246:225-239.

Castro, G. and J. P. Myers. 1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. The Auk 110(4):927-930.

Dutton, J. 1998. Of horseshoe crabs, eels, conch, and red knots. Underwater Naturalist, Bulletin of the American Littoral Society 24(2):10-12.

Ehlinger, G. S., R. A. Tankersley, and M. B. Bush. Spatial and temporal patterns of spawning and larval hatching by the horseshoe crab, *Limulus polyphemus,* in a microtidal coastal lagoon. Estuaries 26(3):631-640.

Gibson, M. and S. Olszewski. 2001. Stock status of horseshoe crabs in Rhode Island in 2000 with recommendations for management. Rhode Island Division of Fish and Wildlife. Wakefield, Rhode Island. 13pp.

Hall, W. R., Jr. 1995. The horseshoe crab. MAS Bulletin. University of Delaware Sea Grant College Program. Newark, Delaware,

Harrington, B. and C. N. Shuster, Jr. 1999. Crab crisis at Delaware Bay - commercial bait collectors are robbing migratory shorebirds of essential food. Defenders 74(3): 31-35.

Hata, D. and J. Berkson. 2003. Abundance of horseshoe crabs, *Limulus polyphemus*, in the Delaware Bay area. Fishery Bulletin 101:933-938.

HCSAC (Horseshoe Crab Stock Assessment Committee). 2000. A conceptual framework for the assessment of horseshoe crab stocks in the mid-Atlantic region. Report to the Horseshoe Crab Technical Committee of the Atlantic States Marine Fisheries Commission. Washington, D.C. 19pp.

HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast. Report to the Atlantic States Marine Fisheries Commission. Washington, D.C. 48pp.

Ives, J. E. 1891. Crustacea from the northern coast of Yucatan, the Harbor of Vera Cruz, the west coast of Florida and the Bermuda islands. Proceedings of the Academy of Natural Sciences of Philadelphia 43:176-280.

King, T. L., M. S. Eackles, A. P. Spidle, and H. J. Brockmann. 2003. Microsatellite DNA reveals regional differentiation among populations of the horseshoe crab (*Limulus polyphemus*). Final Report submitted to the Atlantic States Marine Fisheries Commission.

Koons, B. F. 1883. Sexual characters of Limulus. American Naturalist 17: 1297-1299.

Lockwood, S. 1870. The horse foot crab. American Naturalist 4(5):257-274.

Loveland, R. E., M. L. Botton and C. N. Shuster, Jr. 1996. Life history of the American horseshoe crab (*Limulus polyphemus* L.) in Delaware Bay and its importance as a commercial resource. Proceedings of the horseshoe crab forum: status of the resource. J. Farrell and C. Martin. Lewes, Delaware, University of Delaware Sea Grant College Program: 15-22.

Manion, M., R. West and R. Unsworth. 2000. Economic assessment of the Atlantic coast horseshoe crab fishery. Cambridge, MA, Prepared for the Division of Economics, U.S. Fish and Wildlife Service, Arlington, VA, by Industrial Economics, Inc.

Michels, S., T. O'Connell, P. Perra, and E. Schrading. 2000. 1999 Review of the fishery management plan for horseshoe crab, Horseshoe Crab Plan Review Team.

Mikkelsen, T. 1988. The Secret in the Blue Blood. Beijing, China, Science Press.

Novitsky, T. J. 1991. Discovery to commercialization: the blood of the horseshoe crab. Oceanus 27(1):13-18.

O'Connell, C. W., S. P. Grady, A. S. Leschen, R. H. Carmichael, and I. Valiela. 2003. Stable isotopic assessment of site loyalty and relationships between size and trophic position of the Atlantic horseshoe crab, *Limulus polyphemus*, within Cape Cod estuaries. Biological Bulletin 205:254-255.

Penn, D. and H. J. Brockman. 1994. Nest-site selection in the horseshoe crab, *Limulus polyphemus*. Biological Bulletin 187(3):373-384.

Pierce, J. C., G. Tan, and P. M. Gaffney. 2000. Delaware Bay and Chesapeake Bay populations of the horseshoe crab (*Limulus polyphemus*) are genetically distinct. Estuaries 23(5):690-698.

Rudloe, A. 1980. The breeding behavior and patterns of movement of horseshoe crab, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. Estuaries 3:177-183.

_____. 1983. The effect of heavy bleeding on mortality of the horseshoe crab, *Limulus polyphemus*, in the natural environment. Journal of Invertebrate Pathology 42: 167-176.

Saunders, N. C., L. G. Kessler and J. C. Avise. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics 112:613-627.

Sekiguchi, K., Y. Yamamichi and J. D. Costlow. 1982. Horseshoe crab development studies I. Normal embryonic development of *Limulus polyphemus* compared with *Tachypleus tridentatus*. Pages 53-73 *in* J. Bonaventura, C. Bonaventura, and S. Tesh, eds. Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals. Alan R. Liss, New York.

Shuster, C. N., Jr. 1953. Odyssey of the horseshoe crab. Audubon Magazine. 55:162-163.

_____. 1957. Xiphosura (with special reference to *Limulus polyphemus*). Geological Society of America. 67(1):1171-1174.

_____. 1958. On morphometric and seriological relationships within the Limulidae, with particular reference to Limulus polyphemus (L.). Dissertation Abstracts 18: 371-372.

_____. 1962. Serological correspondences among horseshoe "crabs" (Limulidae). Zoologica 47(1):1-9.

_____. 1979. Distribution of the American horseshoe crab, (*Limulus polyphemus* (L.)). Pages 3-26 *in* E. Cohen, ed. Biomedical applications of the horseshoe crab (Limulidae). Alan R. Liss, New York.

_____. 1982a. A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. Pages 1-

52 *in* J. Bonaventura, C. Bonaventura, and S. Tesh, eds. Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals. Alan R. Liss, New York.

_____. 1990. The American horseshoe crab, *Limulus polyphemus*. Pages 15-25 *in* R. B. Prior, Ph.D, ed. Clinical Applications of the Limulus Amoebocyte Lysate Test. CRC Press, Boca Raton, Florida.

_____. 1992. Xiphosurida. Pages 590-594 *in* McGraw-Hill Encyclopedia of Science and Technology. 7th edition. McGraw-Hill Book Company, Inc, New York.

_____. 1999. Managing horseshoe crab resources: it's the adult age that counts. Symposium on Horseshoe Crab: Biology and Opportunities for Integrated Resource Management. American Fisheries Society Annual Meeting, Charlotte, North Carolina. 47pp.

_____. and M. L. Botton. 1985. A contribution to the population of horseshoe crabs, *Limulus polyphemus* (L.), in Delaware Bay. Estuaries 8(4):363-372.

_____. and K. Sekiguchi. 2003. Growing up takes about ten years and eighteen stages. Pages 5-26 *in* C. N. Shuster, Jr., R. B. Barlow, and H. J. Brockmann, eds. The American horseshoe crab. Harvard University Press, Cambridge, Massachusetts.

Thompson, M. 1999. Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in the South Atlantic Bight. Master's thesis, University of Charleston, Charleston, South Carolina.

Tsipoura, N. and J. Burger. 1999. Shorebird diet during spring migration stopover on Delaware Bay. The Condor 101:635-644.

USFWS (United States Fish and Wildlife Service). 1998. The horseshoe crab: A living fossil, U.S. Fish and Wildlife Service: 2pp.

Walls, B. A. 2001. A horseshoe crab (*Limulus polyphemus*) demographic study. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 70 pp.

_____. and J. M. Berkson. 2003. Blood extraction effects on horseshoe crabs (*Limulus polyphemus*). Fishery Bulletin 101(2):457-459.

_____., J. M. Berkson and S. A. Smith. 2002. The Horseshoe Crab, Limulus polyphemus: 200 Million years of Existence, 100 Years of Study. Reviews in Fisheries Science 10(1):39-73.

Wenner, E. and M. Thompson. 2000. Final report to the National Oceanographic and Atmospheric Administration. Evaluation of harvesting impacts and population trends for *Limulus polyphemus* in South Carolina. Charleston, Marine Resources Research Institute, South Carolina Department of Natural Resources. 96pp.

Widener, J. W. and R. B. Barlow. 1999. Decline of a horseshoe crab population on Cape Cod. Biological Bulletin 197:300-302.

CHAPTER 2 – MONITORING TAG RECOVERY AND MOVEMENT PATTERNS OF BLED HORSESHOE CRABS IN THE MID-ATLANTIC REGION

ABSTRACT

To gain information on movement patterns and stock structure of horseshoe crabs a tagging study was conducted on horseshoe crabs harvested for biomedical use in the waters surrounding Chincoteague, Virginia and Ocean City, Maryland. Twelve thousand five hundred adult horseshoe crabs were tagged and released during the summers of 1999-2002 and 2004. As of November 1, 2004, 431 resighted tags had been reported to the United States Fish and Wildlife Service Horseshoe Crab Tag Recovery Center. This constitutes a resight rate of 3.45%. Fifty-seven percent of the resighted horseshoe crabs were alive at the time of resight. The mean distance between site of release and site of recapture for all resighted horseshoe crabs was 68.33 kilometers. The maximum distance traveled was 493.74 kilometers. The majority of crabs reported were found in the Delaware Bay. The location of resighted horseshoe crabs ranged in latitude from Bristol, Rhode Island (41.7°N) to Corolla, North Carolina (36.4°N). Information from this study, together with genetic analyses of horseshoe crabs along the Atlantic coast, will provide a clearer picture of horseshoe crab stock structure in Atlantic waters, better enabling us to manage the horseshoe crab fishery.

INTRODUCTION

The American horseshoe crab, *Limulus polyphemus,* is valuable both ecologically and economically. Environmentalists are concerned about the status of this species primarily because of the important role it serves as a food resource for migrating shorebirds that utilize the Delaware Bay as a stopover point during their spring migration (Castro and Myers 1993; Harrington and Shuster 1999). A commercial fishery harvests horseshoe crabs for use as bait in the American eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries (HCTC 1998). In addition, the biomedical industry harvests over 280,000 horseshoe crabs annually for the production of a clotting agent, *Limulus* Amoebocyte Lysate (LAL) (Michels et al. 2000). LAL is the required standard for detection of endotoxins in all injectable drugs and implantable medical devices approved by the Food and Drug Administration (ASMFC 1998). If LAL became unavailable, it could take years to find a suitable replacement (ASMFC 1998). For these reasons, it is important that the horseshoe crab resource be properly managed to meet the needs of all user groups.

In spite of the importance of horseshoe crabs to a number of user groups, many characteristics of the Atlantic horseshoe crab population are still uncertain. The degree to which horseshoe crab populations are discrete or open is unclear (Botton and Loveland 2003). Some studies suggest that horseshoe crabs exist in localized subpopulations that may require separate management (Shuster 1979; Widener and Barlow 1999; Ehlinger et al. 2003). However, recent genetic findings suggest that the Atlantic horseshoe crab stock is composed of large regional subpopulations, and therefore large-scale management may be more appropriate (King et al. 2003). Prior to a formal assessment of horseshoe crabs, stocks must be defined and delineated (HCSAC 2000). Genetic population structure, spatial and seasonal distribution, and movement information are necessary for defining horseshoe crab stocks (HCSAC 2000).

In 1999 the Atlantic States Marine Fisheries Commission initiated a tagging program with biomedical companies to evaluate post-release mortality of horseshoe crabs (ASMFC 1998). Initially, biomedical companies were required to tag a portion of the horseshoe crabs bled, with large biomedical companies tagging 2,500 horseshoe crabs per year. In 2000, the tagging program was made strictly voluntary. The biomedical company Cambrex, formerly BioWhittaker, has continued tagging horseshoe crabs. Cambrex has contracted with Virginia Polytechnic Institute and State University to conduct its tagging study. The initial years of this study, 1999-2001, were conducted by Elizabeth Walls (2001). The U.S. Fish and Wildlife Service (USFWS) is responsible for collecting and compiling resight data for the tagging program (USFWS 1999).

In addition to evaluating post-release mortality, data from this tagging study can be used to acquire information about resight rates and movement patterns of tagged horseshoe crabs. Tagging studies are a primary tool for discovering migration patterns of marked animals (Nielsen 1992). Monitoring movement can be useful for stock identification (Dizon et al. 1992), identifying what scale is appropriate for the management of the horseshoe crab resource.

In her tagging study, Walls (2001) found that in a 2-year period, most horseshoe crabs do not migrate great distances. However, she did find mixing among horseshoe crabs found in Chincoteague, VA and Ocean City, MD, which are 56 kilometers apart. Additional movement was also observed between the Maryland and Virginia coasts and the Delaware Bay. Walls found the average distance traveled between release and resight was 47.7 kilometers, while the maximum distance observed among all resighted horseshoe crabs was 312 kilometers. Over two and one-half years, Walls (2001) received 121 reported resights of tags and tagged horseshoe crabs, 1.61% of the total number of horseshoe crabs tagged. A greater number of resighted tagged horseshoe crabs would lead to a better understanding of horseshoe crab movement patterns and stock structure. This chapter will include findings from the study conducted during 2002 - 2004, along with updates on horseshoe crabs tagged during the 1999 - 2001 study.

METHODS

From May 22nd until August 8th in 2002 and from June 22nd until September 9th in 2004, a portion of horseshoe crabs bled by Cambrex was tagged using the methods of Walls (2001). Each year 2,500 adult horseshoe crabs were selected for tagging. The tagged horseshoe crabs were caught and released in Atlantic waters surrounding Chincoteague, Virginia or Ocean City, Maryland. Only adult horseshoe crabs were used, as juvenile horseshoe crabs would lose their tags upon molting. Adult males were identified by the presence of a modified boxing glove-like pincer. Adult females were identified by the presence of mating scars, or if newly matured, the presence of eggs. If any epibionts or other growths were present, they were removed from the surface of the horseshoe crab where the tag was to be placed. Using a 9/64” drill bit, equipped with a guard to allow only ¼ inch penetration, a hole was drilled into the posterior of the left prosoma in an area where the tag would lay flat. A white Petersen disc tag, which displayed a toll-free phone number for the USFWS Horseshoe Crab Tag Recovery Center and an identification number, was then inserted using a plastic attachment pin. Tag number, sex, date of tagging, waterbody of release, and nearest town of release were recorded for each tagged horseshoe crab. After this information was recorded, horseshoe crabs were released at approximately the same point of original capture in accordance with Cambrex's standard operating procedures.

Release information was given to the USFWS Horseshoe Crab Tag Recovery Center bi-monthly. When resighted tag numbers were reported to the USFWS Horseshoe Crab Tag Recovery Center, information regarding their location and condition (alive, dead, or "tag found only") was recorded. Information was downloaded from the USFWS monthly, and from it movement patterns were examined. Data collected from horseshoe crabs released in 2002 and 2004 were combined with data from horseshoe crabs released in the summers of 1999 through 2001 as part of Walls' (2001) study. In the 5 years of tagging (1999 – 2002, 2004) 12,500 horseshoe crabs were tagged and released. Of these, 5,246 were females and 7,254 were males (Table 2.1). A total of 6,234 were released in Chincoteague, Virginia and 6,266 were released in Ocean City, Maryland (Table 2.1).

ANALYSES

From resight reports, estimates of percent recovery, percent of tags found on living horseshoe crabs, percent of tags found on dead horseshoe crabs, percent of tags found detached, and distances traveled were calculated. Movement patterns were also observed. Percent recovery was calculated as the number of resighted tags reported divided by the total number of tagged horseshoe crabs released. All other percentages were calculated as proportions of reported tag resights. Program SAS (Statistical

Analysis System, Version 8) was used to perform chi-square analyses to compare differences among proportions reported from living, dead, and "tag found only" resights, as well as proportions of males resighted versus females resighted.

To calculate distance traveled, geographical coordinates were obtained for each resight location using the web-based database, topozone.com (TopoZone 2004). These coordinates were then used to calculate the straight-line distance from point of release to point of resight of all reported tags. From these distance estimates, the mean minimum distances traveled by horseshoe crabs of each sex and from each release location were calculated. The proportion of horseshoe crabs that did not move from their location of release was also calculated. Since distances traveled were not normally distributed, all distances greater than zero were log-transformed then entered into program SAS (Statistical Analysis System, Version 8) where a 2-way analysis of variance (ANOVA) was performed to test for differences among the distances traveled between male and female horseshoe crabs released from Chincoteague, Virginia and Ocean City, Maryland. Since the movement of dead horseshoe crabs and detached tags may be affected by ocean currents, movement of tagged horseshoe crabs reported as alive at resight was also examined separately.

RESULTS

Tag Returns

As of November 1, 2004, 431 tags had been reported as resights to the USFWS Horseshoe Crab Tag Recovery Center (Table 2.2 and Table 2.3). This equates to an overall recovery rate of 3.45%. The greatest recovery rate for any given year was for horseshoe crabs tagged in 2000 ($p < 0.0001$; df = 4). One hundred and fifty-three resights were reported for tags released in that year, constituting a 6.12% recovery rate (Table 2.2). In addition, the recovery rate for tagged male horseshoe crabs (3.85%) was greater than that of tagged female horseshoe crabs (2.90%) for every year ($p = 0.0041$; df = 1; Table 2.2).

There were significant differences in the number of horseshoe crabs reported for each condition category ($p < 0.0001$; df = 2; Table 2.4). Two hundred forty-eight (57.5%) of the resights were reported as horseshoe crabs found alive. One hundred and thirty-four (31.1%) were reported as being found on dead horseshoe crabs. Forty-nine (11.4%) of the resights were reported as "tag found only". A larger proportion of female than male horseshoe crabs were reported dead upon recapture (40.13% versus 26.16% respectively; $p = 0.0008$; df = 2).

Movement

The locations of 2 resighted tagged horseshoe crabs were not reported and therefore excluded from movement analyses. The average minimum distance traveled by 429 of the reported resighted horseshoe crabs and tags found detached was 68.33 ± 5.69 (mean ± 1.96 SE) kilometers (Table 2.5). The maximum distance traveled was 493.74 kilometers by a female horseshoe crab found dead in Bristol, Rhode Island on June 20, 2003 after being released in Ocean City, Maryland on July 12, 2000. One hundred eleven of the resights (25.9%) were reported in the same location as release. Fifty-five (12.8%) of the resights were reported as moving from Chincoteague, Virginia to Ocean City, Maryland or vice versa. The majority of reported resights however, were in the Delaware Bay where 184 (42.9%) were reported. The remainder of the resights ranged from as far north as Bristol, Rhode Island to as far south as Corolla, North Carolina (Figure 2.1 and Figure 2.2). Additionally, one horseshoe crab was reported to have been found in the northeastern waters of the Chesapeake Bay (Figure 2.1).

Differences in distances traveled existed between sexes and between release sites. On average males who moved from their point of release ($n = 221$; mean = 95.11 ± 5.81 (mean ± 1.96 SE) km) traveled significantly farther than females who moved from their point of release ($n = 97$; mean = 83.09 ± 13.68 (mean ± 1.96 SE) km; $p < 0.0001$). In addition, horseshoe crabs who moved from their point of release in Chincoteague, VA ($n = 148$; mean = 117.72 ± 8.10 (mean ± 1.96 SE) km) traveled farther than horseshoe crabs who moved from their point of release in Ocean City, MD ($n = 170$; mean = 68.57 ± 6.62 (mean ± 1.96 SE) km; $p = < 0.0001$).

The average distance traveled by the 246 horseshoe crabs reported as living at the time of resight was 85.23 ± 6.89 (mean ± 1.96 SE) kilometers (Table 2.6). The maximum distance traveled by a living horseshoe crab was 363.7 kilometers by a male horseshoe crab released from Chincoteague, Virginia on June 24, 1999 and resighted on June 24, 2003 in Long Island Sound, New York. When comparing the average distances traveled by living male ($n = 156$; mean = 104.13 ± 6.41 km) and female ($n = 49$; mean = 95.66 ± 14.7 (mean ± 1.96 SE) km) horseshoe crabs who moved from their point of release, there was no significant difference ($p = 0.1531$). There was a significant difference in the distance traveled by living horseshoe crabs who moved from their point of release in Chincoteague, VA ($n = 111$; mean = 121.65 ± 8.49 (mean ± 1.96 SE) km) as compared to those who moved from their point of release in Ocean City, MD ($n = 94$; mean = 79.03 ± 5.62 (mean ± 1.96 SE) km; $p < 0.0001$).

DISCUSSION

Tag Recovery

As expected, due to the longer duration of this study, the overall resight rate of all horseshoe crabs calculated in year 6 of the study was much greater than that reported in year 3 of the previous study (Walls 2001). Horseshoe crabs are a long-lived species; the longer duration of this study increased the chance that an individual horseshoe crab would be encountered (Table 2.3).

Mark-recapture studies were first used to study movements and migrations of marked individuals, but C. G. J. Petersen, a Danish fisheries biologist, realized that tagging could be used to estimate population size and mortality rates (Krebs 1999). However, for these estimates to be accurate they must adhere to a strict set of assumptions (Krebs 1999). The assumptions are as follows: every animal has an equal probability of capture, every marked animal has the same probability of surviving between sampling periods, marked animals do not lose their marks, and all marks are reported upon recovery (Seber 1982). This study does not meet these assumptions. All horseshoe crabs did not have an equal probability of capture, because the trawling process is nonrandom, targeting areas horseshoe crabs are known to inhabit. In addition, the reported tag recovery rate for both studies is not a true resight rate, rather it is an encounter rate based upon opportunistic resightings and non-random sampling. Furthermore, bled horseshoe crabs were used for marking. These individuals likely have different probabilities of survival based on age and amount of blood loss. Of the reported resights, 11.4% were reported as tags found detached from horseshoe crabs. Therefore, the assumption that marked animals do not lose their marks has been violated. However, it is not known whether these tags were lost from living or dead horseshoe crabs. Finally, many tagged horseshoe crabs may be resighted but never reported to the USFWS Horseshoe Crab Tag Recovery Center.

For 367 of the reported resights the reporter type was recorded. Of these 367, 194 (52.9%) were reported by beachcombers, 83 (22.6%) were reported by commercial fishermen, and 63 (17.2%) were reported by researchers. Commercial fisherman likely encountered more than 83 tagged horseshoe crabs since they harvest more than one million adult horseshoe crabs annually. However, it is well known that many commercial fishermen do not report resighted tagged horseshoe crabs because they see no benefit in doing so (Sheila Eyler, USFWS, pers. comm.).

Lack of reporting by commercial fishermen is not unexpected. Many tagging studies rely on the voluntary reporting of resighted tagged animals but the number of actual resights is often underreported (Matlock 1981; Green et al 1983; Schmalz et al 2004). Based on returns of tags inserted into already captured fish (without the anglers knowledge), Green et al. (1983) directly estimated the tag reporting rate of anglers as only 29%. Additional research has shown that a reward system greatly increased the number of reported resights for tagged fish (Jenkins et al. 2000; Pollock et al. 2001; Denson and Jenkins 2002). Some studies suggested that a 100% reporting rate could be reached if the reward was high enough (Jenkins et al. 2000; Pollock et al. 2001; Denson et al. 2002). Denson et al. (2002) also found that anglers who caught fewer tagged fish were more likely to report the finding regardless of the reward. Similarly, Smalz et al. (2004) found that recreational fishermen were more likely to report tags even without rewards because of an interest in the species and the tagging program. Meanwhile, commercial fishermen required an additional incentive to report tags. A low encounter rate per individual and interest in the species and tagging program could explain why beachcombers made up the largest proportion of reporters. Pollock et al. (2001) believe that it is important to have a reward system for any tagging study and that rewards be granted in a timely manner to better influence tag reporting.

Movement

Intraspecific structure of a population can range from complete panmixia to isolation so complete that speciation occurs (Dizon et al. 1992). It is important to understand the degree of structure in a harvested population in order to determine what level of management is appropriate (Allendorf et al. 1987). Stock structure is often determined through examination of genetic differentiation (Allendorf et al. 1987; Dizon et al. 1992; Shaklee and Currens 2003). However, neutral markers may not always show differentiation due to selection pressures, and therefore may be a poor proxy for demonstrating variation between populations (Dizon et al. 1992). Dizon et al. (1992) recommend that for most species, research on movement patterns be given high priority for its usefulness in evaluating levels of reproductive isolation. Dispersal estimates from mark-recapture studies are limited in space and time and may differ from genetic findings which are averaged over thousands of generations (Bohonak 1999). For this reason, it is important to consider both when determining scale of management for a harvested population.

Currently, there is much debate concerning the amount of gene flow among horseshoe crabs along the Atlantic coast. Some have suggested that local subpopulations of horseshoe crabs exist, which may require localized management (Shuster 1982;

Widener and Barlow 1999; Ehlinger et al. 2003). Widener and Barlow (1999) are concerned that harvesting large numbers of horseshoe crabs from localized populations, such as the one they studied at Mashnee Dike, would have a significant impact on local population size.

Contrastingly, several genetic studies have provided evidence for larger population subdivisions along the Atlantic coast, which would suggest a need for management on a larger scale (Saunders et al. 1986; King et al. 2003). King et al. (2003) examined microsatellite DNA markers in 892 horseshoe crabs collected from 21 sites ranging from Franklin, Maine to the Yucatan Peninsula, Republic of Mexico. Their survey revealed a high degree of genetic diversity and heterozygosity among horseshoe crabs sampled and found substantial gene flow between each population and its nearest neighbors. Through the examination of genetic distances between populations sampled, they identified 4 regional management units within the United States: Gulf of Maine, mid-Atlantic, Florida-Atlantic, and Florida-Gulf. They also identified a fifth management unit in waters surrounding Mexico's Yucatan Peninsula (King et al. 2003). Further, the correlation of genetic distances with geographic distances among populations sampled suggests isolation by distance as the mechanism behind population structure of horseshoe crabs in Atlantic waters (King et al. 2003). However, King et al. (2003) note the potential for substructure within these larger subdivisions. For example, they suggest that horseshoe crabs from Hog Bay, Maine are a geographically isolated population with little gene flow between it and its nearest neighbors. In all cases, tagging studies are necessary to document the amount of possible gene flow among these regional or localized populations (Dizon et al. 1992).

The movement patterns of horseshoe crabs in this study fit into the framework of King et al.'s (2003) definition of regional management units. All resighted horseshoe crabs were resighted within the mid-Atlantic management unit defined as coastal waters from Massachusetts to South Carolina (King et al. 2003). Also the mean minimum distance traveled by all resighted living horseshoe crabs was only 85.23 kilometers. This supports the current theory of isolation by distance as the mechanism behind horseshoe crab stock structure along the Atlantic coast.

The large number of tagged horseshoe crabs resighted in the Delaware Bay suggests that horseshoe crabs harvested in Atlantic waters surrounding Chincoteague, Virginia and Ocean City, Maryland are part of the larger spawning population in the Delaware Bay and are not members of isolated subpopulations. Therefore horseshoe crabs harvested in Chincoteague and Ocean City do not need separate management. Instead they should be managed through a coordinated effort at least among the states of Virginia, Maryland, and Delaware. Overharvesting horseshoe crabs in Virginia and Maryland could negatively impact the spawning population in Delaware Bay, reducing the number of eggs available as a food resource to migrating shorebirds.

RECOMMENDATIONS

Future tagging studies should strive to meet the assumptions of mark-recapture studies required to allow the calculation of population and survival estimates. Horseshoe crabs should be captured and recaptured randomly. Double marking should be used to estimate and account for tag loss (Krebs 1999). Handling time should be kept at a minimum to reduce stress and minimize possible mortalities due to the tagging process.

If the population of interest is the entire Atlantic population of horseshoe crabs, tagging should be conducted randomly coastwide. However, it may not be possible to meet these assumptions. Therefore other methods of population estimation may be more appropriate for such a widespread species.

The tagging study would benefit from a reward system. It is likely that an advertised reward would increase the number of tags reported, especially by commercial fishermen who likely encounter the greatest number of tagged horseshoe crabs and who are less likely to report a tag without some incentive. If a reward system is put into place, the disc tags used should be modified to include the word "Reward" on them. Commercial fishermen should be interviewed to determine what type of reward system would increase their likelihood of reporting tagged horseshoe crabs.

The findings of this study may only be indicative of horseshoe crabs harvested in waters surrounding Chincoteague, Virginia and Ocean City, Maryland. Similar studies would be useful especially for populations that are considered to be isolated. The Chesapeake Bay population may be a population of interest. Through mtDNA sequencing, Pierce et al. (2000) found that Delaware Bay and Chesapeake Bay populations of horseshoe crabs are genetically distinct. However, King et al. (2003) suggested significant gene flow between these 2 populations. In addition one horseshoe crab released from Chincoteague, Virginia was found in the Chesapeake Bay, leading to questions regarding the level of isolation of horseshoe crabs in that area. A similar tagging study conducted in the Chesapeake Bay, would give more insight into the stock structure of horseshoe crabs found in that area.

Together additional genetic analyses and tagging studies will provide the clearest picture of horseshoe crab stock structure in Atlantic waters. Current findings suggest that coordinated efforts among states are necessary to manage the horseshoe crab fishery. However, it may be necessary to manage smaller isolated populations separately if little movement and gene flow is seen between them and neighboring populations.

REFERENCES

Allendorf, F.W., N. Ryman, and F. M. Utter. 1987. Genetics and fishery management. Pages 1-19 *in* N. Ryman and F. Utter editors. Population genetics and fishery management. Washington Sea Grant Program. University of Washington Press, Seattle, Washington.

ASMFC (Atlantic States Marine Fisheries Commission). 1998. Interstate fishery management plan for horseshoe crab. Fishery management report No.32 of the Atlantic States Marine Fisheries Commission. Washington, D.C. 58pp.

Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. The Quarterly Review of Biology 74(1):21-45.

Botton, M. L. and R. E. Loveland. 2003. Abundance and dispersal potential of the horseshoe crab (*Limulus polyphemus*) larvae in the Delaware estuary. Estuaries 26:1472-1479.

Castro, G. and J. P. Myers. 1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. The Auk 110(4):927-930.

Denson, M. R., W. E. Jenkins, A. G. Woodward, and T. I. J. Smith. 2002. Tag-reporting levels for red drum (*Sciaenops ocellatus*) caught by anglers in South Carolina and Georgia estuaries. Fishery Bulletin 100:35-41.

Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster, and J. Sisson. 1992. Rethinking the stock concept: a phylogeographic approach. Conservation Biology 6(1):24-36.

Ehlinger, G. S., R. A. Tankersley, and M. B. Bush. Spatial and temporal patterns of spawning and larval hatching by the horseshoe crab, *Limulus polyphemus,* in a microtidal coastal lagoon. Estuaries 26(3):631-640.

Eyler, S. United States Fish and Wildlife Service. Annapolis, Maryland. Personal communication.

Green, A. W., G. C. Matlock, and J. E. Weaver. 1983. A method for directly estimating the tag-reporting rate of anglers. Transactions of the American Fisheries Society 112:412-415.

Harrington, B. and C. N. Shuster, Jr. 1999. Crab crisis at Delaware Bay - commercial bait collectors are robbing migratory shorebirds of essential food. Defenders 74(3):31 35.

HCSAC (Horseshoe Crab Stock Assessment Committee). 2000. A conceptual framework for the assessment of horseshoe crab stocks in the mid-Atlantic region. Report to the Horseshoe Crab Technical Committee of the Atlantic States Marine Fisheries Commission. Washington, D.C. 19pp.

HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast. Report to the Atlantic States Marine Fisheries Commission. Washington, D.C. 48pp.

Jenkins, W. E., M. R. Denson, and T. I. J. Smith. 2000. Determination of angler reporting level for red drum (*Sciaenops ocellatus*) in a South Carolina estuary. Fisheries Research 44:273-277.

King, T. L., M. S. Eackles, A. P. Spidle, and H. J. Brockmann. 2003. Microsatellite DNA reveals regional differentiation among populations of the horseshoe crab (*Limulus polyphemus*). Final Report submitted to the Atlantic States Marine Fisheries Commission.

Krebs, C. J. 1999. Ecological methodology. 2nd edition. Addison-Wesley Educational Publishers, Inc. Menlo Park, California.

Matlock, G. C. 1981. Non-reporting of recaptured tagged fish by saltwater recreational boat anglers in Texas. Transactions of the American Fisheries Society 110:90-92.

Michels, S., T. O'Connell, P. Perra, and E. Schrading. 2000. 1999 Review of the fishery management plan for horseshoe crab, Horseshoe Crab Plan Review Team.

Nielson, L. A. 1992. Methods of marking fish and shellfish. American Fisheries Society Special Publication 23, Bethesda, Maryland.

Pierce, J. C., G. Tan, and P. M. Gaffney. 2000. Delaware Bay and Chesapeake Bay populations of the horseshoe crab (*Limulus polyphemus*) are genetically distinct. Estuaries 23(5):690-698.

Pollock, K. H., J. M. Hoenig, W. S. Hearn, and B. Calingaert. 2001. Tag reporting rate estimation: 1. An evaluation of the high-reward tagging method. North American Journal of Fisheries Management 21:521-532.

Saunders, N. C., L. G. Kessler, and J. C. Avise. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics 112:613-627.

SAS (Statistical Analysis System). 1999. The SAS/STAT user's guide, version 8. SAS Institute, Cary, North Carolina.

Schmalz, P. J., M. J. Hansen, M. E. Holey, P. C. McKee, and M. L. Toneys. 2004. Effect of rewards on lake trout tag returns in Northwestern Lake Michigan. North American Journal of Fisheries Management 24:1-6.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. 2nd edition. Edward Arnold, London.

Shaklee, J. B. and K. P. Currens. 2003. Genetic stock identification and risk assessment. Pages 291-328 *in* E. M. Hallerman, editor. Population genetics: principles and Applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Shuster, C. N., Jr. 1979. Distribution of the American horseshoe crab, (*Limulus polyphemus* (L.)). Pages 3-26 *in* E. Cohen, ed. Biomedical applications of the horseshoe crab (Limulidae). Alan R. Liss, New York.

_____. 1982. A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. Pages 1-52 *in* J. Bonaventura, C. Bonaventura, and S. Tesh, eds. Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals. Alan R. Liss, New York.

_____. 1990. Tracking horseshoe crabs. Underwater Naturalist, Bulletin of the American Littoral Society 19:22-23.

TopoZone. 2004 [Online]. TopoZone.com © 1999-2004 Maps a la carte, Inc. Available: http://www.topozone.com [11/27/2004].

USFWS (United States Fish and Wildlife Service). 1999. Biomedical horseshoe crab tagging protocol. Annapolis, MD, U. S. Fish and Wildlife Service:2.

Walls, B. A. 2001. A horseshoe crab (*Limulus polyphemus*) demographic study. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

_____., J. M. Berkson and S. A. Smith. 2002. The Horseshoe Crab, Limulus polyphemus: 200 Million years of Existence, 100 Years of Study. Reviews in Fisheries Science 10(1):39-73.

Widener, J. W. and R. B. Barlow. 1999. Decline of a horseshoe crab population on Cape Cod. Biological Bulletin 197:300-302.

	Chincoteague, Virginia		Ocean City, Maryland		
Year of Release	**Female**	**Male**	**Female**	**Male**	**Totals**
1999	581	1303	216	400	2500
2000	112	388	866	1134	2500
2001	1013	1187	128	172	2500
2002	835	815	300	550	2500
2004	0	0	1195	1305	2500
Totals	2541	3693	2705	3561	12500

Table 2.1 Sex and number of horseshoe crabs tagged and released in Chincoteague, Virginia and Ocean City, Maryland after being bled by Cambrex in the years 1999 - 2002 and 2004.

	Chincoteague, Virginia		Ocean City, Maryland		
Year of Release	**Female**	**Male**	**Female**	**Male**	**Totals**
1999	18 (3.10%)	56 (4.30%)	5 (2.31%)	16 (4.00%)	95 (3.80%)
2000	1 (0.89%)	16 (4.12%)	52 (6.00%)	84 (7.40%)	153 (6.12%)
2001	17 (1.68%)	41 (3.45%)	2 (1.56%)	4 (2.33%)	72 (2.88%)
2002	17 (2.04%)	29 (3.56%)	9 (3.00%)	20 (3.64%)	73 (2.92%)
2004	n/a	n/a	31 (2.59%)	13 (1.00%)	44 (1.76%)
Totals	53 (2.09%)	142 (3.85%)	99 (3.66%)	137 (3.85%)	431 (3.45%)

Table 2.2 Sex, number, and percentage of horseshoe crab tags resighted, as of November 1, 2004 after being released from Chincoteague, VA or Ocean City, MD during the years 1999-2004

Year of Release	Year of Resight					
	1999	**2000**	**2001**	**2002**	**2003**	**2004**
1999	16	19	14	20	19	7
2000	-	38	38	39	28	10
2001	-	-	8	23	25	8
2002	-	-	-	27	33	15
2004	-	-	-	-	-	44

Table 2.3 Number of tag resights per year, as of November 1, 2004, of tagged horseshoe crabs released in Chincoteague, Virginia and Ocean City, Maryland. These figures include resights of tagged horseshoe crabs as well as tags found detached from horseshoe crabs.

	Year of Release	Total Tags Resighted	Reported "Alive"	Reported "Dead"	Reported "Tag Only"
Female	**1999**	23	12 (52.2%)	8 (34.8%)	3 (13.0%)
	2000	53	30 (56.6%)	14 (26.4%)	9 (17.0%)
	2001	19	9 (47.4%)	4 (21.1%)	6 (31.5%)
	2002	26	14 (53.8%)	9 (34.6%)	3 (11.6%)
	2004	31	4 (12.9%)	26 (83.9%)	1 (3.20%)
Male	**1999**	72	55 (76.4%)	13 (18.1%)	4 (5.50%)
	2000	100	68 (68.0%)	23 (23.0%)	9 (9.00%)
	2001	45	31 (68.9%)	10 (22.2%)	4 (8.90%)
	2002	49	24 (49.0%)	15 (30.6%)	10 (20.4%)
	2004	13	1 (7.70%)	12 (92.3%)	0 (0.00%)
Totals		431	248 (57.5%)	134 (31.1%)	49 (11.4%)

Table 2.4 Condition at resight of tagged horseshoe crabs released in Chincoteague, Virginia and Ocean City, Maryland as reported to the USFWS Horseshoe Crab Tag Recovery Center as of November 1, 2004.

	Chincoteague, Virginia		Ocean City, Maryland		Overall	
	Mean Distance	**Maximum Distance**	**Mean Distance**	**Maximum Distance**	**Mean Distance**	**Maximum Distance**
Female	74.11 km (n = 53)	303.27 km	42.84 km (n = 99)	493.74 km	53.74 km (n = 152)	493.74 km
Male	96.84 km (n = 141)	363.70 km	55.07 km (n = 136)	154.67 km	76.33 km (n = 277)	363.70 km
Overall	90.64 km (n = 194)	363.70 km	49.92 km (n = 235)	493.74 km	68.33 km (n = 429)	493.74 km

Table 2.5 Average and maximum straight-line distances traveled by resighted horseshoe crabs tagged and released in Chincoteague, Virginia and Ocean City, Maryland after being bled by Cambrex in the years 1999 - 2002 and 2004.

	Chincoteague, Virginia		**Ocean City, Maryland**		**Overall**	
	Mean Distance	**Maximum Distance**	**Mean Distance**	**Maximum Distance**	**Mean Distance**	**Maximum Distance**
Female	99.91 km (n = 26)	264.17 km	49.09 km (n = 43)	184.55 km	68.24 km (n = 69)	264.17 km
Male	114.86 km (n = 95)	363.70 km	65.19 km (n = 82)	154.67 km	91.85 km (n = 177)	363.70 km
Overall	111.65 km (n = 121)	363.70 km	59.65 km (n = 125)	184.55 km	85.23 km (n = 246)	363.70 km

Table 2.6 Average straight-line distances traveled by resighted living horseshoe crabs tagged and released in Chincoteague, Virginia and Ocean City, Maryland that moved from their original site of release after being bled by Cambrex in the years 1999 - 2002 and 2004.

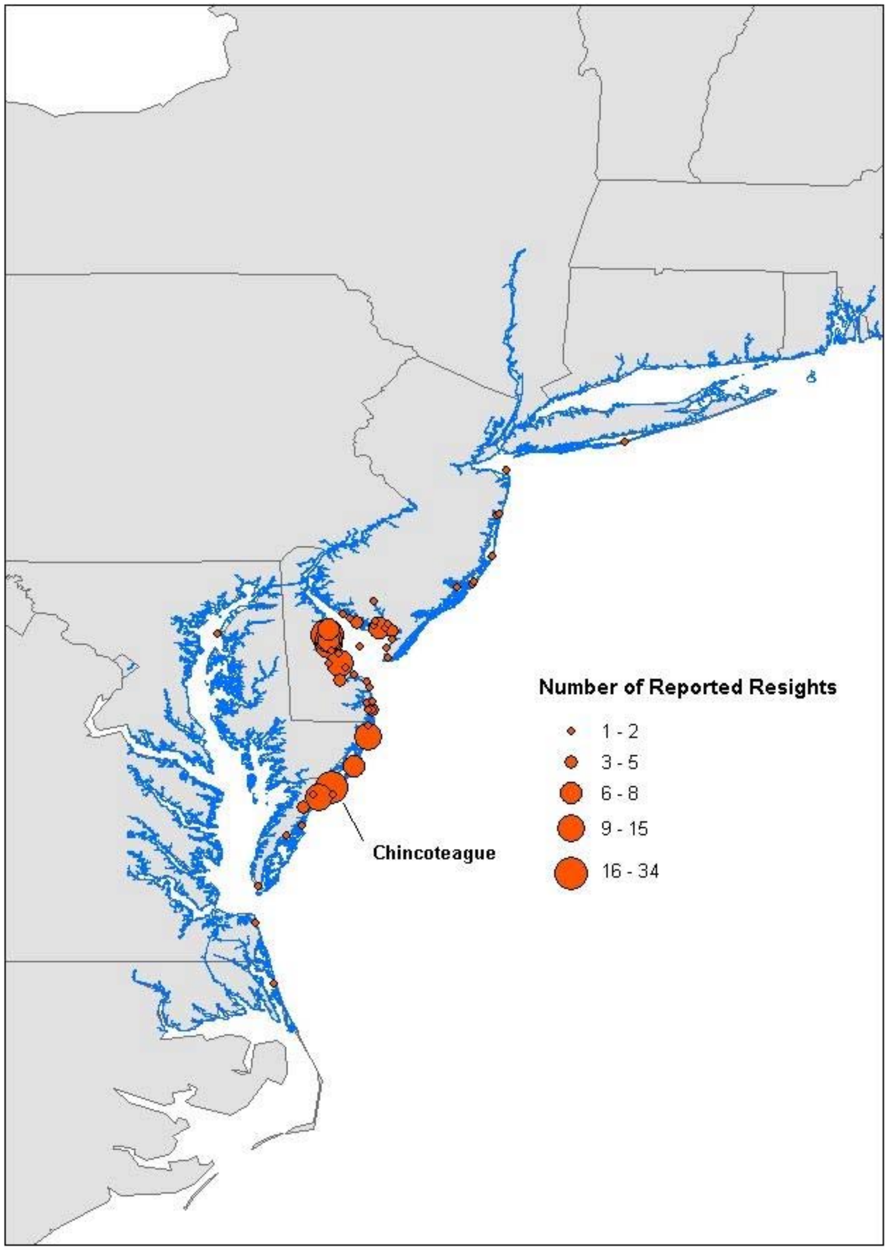

Figure 2.1 Resight locations for horseshoe crabs tagged and released from Chincoteague, Virginia after being bled by Cambrex in the years 1999-2002.

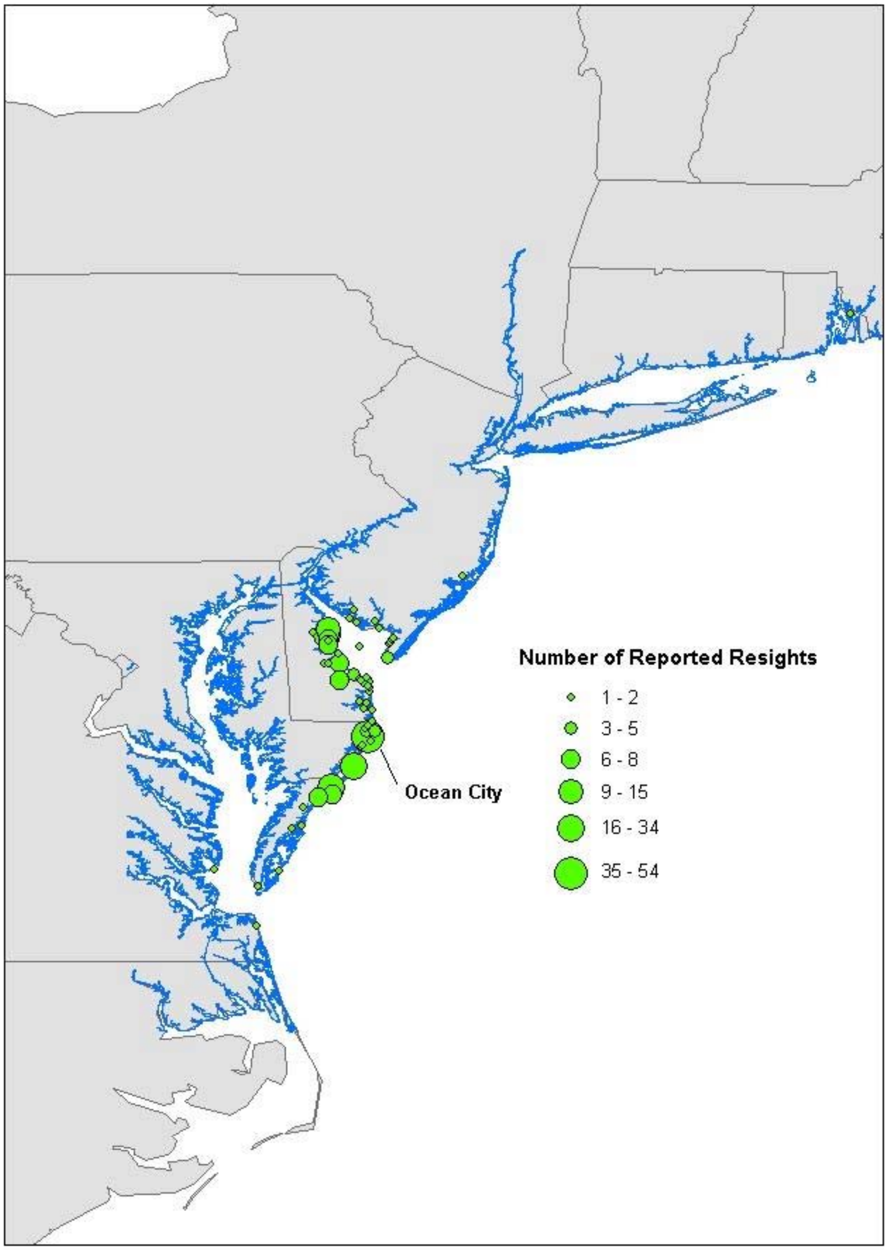

Figure 2.2 Resight locations for horseshoe crabs tagged and released from Ocean City, Maryland after being bled by Cambrex in the years 1999-2002 and 2004.

CHAPTER 3 – COMPARING SPATIAL AND TEMPORAL DISTRIBUTIONS OF HORSESHOE CRABS IN CHINCOTEAGUE, VIRGINIA AND OCEAN CITY, MARYLAND

ABSTRACT

Increased harvest of horseshoe crabs throughout the 1990s has led to concerns regarding the status of the population. In response to these concerns, the Atlantic States Marine Fisheries Commission adopted the Horseshoe Crab Fisheries Management Plan in 1998. However, lack of good fishery and population data prohibited the development of a comprehensive scientific management strategy. At a time when little population information was available, Virginia Polytechnic Institute and State University contracted with the biomedical company Cambrex to compare spatial and temporal distributions of horseshoe crabs harvested for biomedical use in Virginia and Maryland from 1999-2004. This study showed there were no differences in interocular widths of adult male and female horseshoe crabs harvested in Chincoteague, Virginia and Ocean City, Maryland. Annual comparisons revealed that both proportion of females ($p < 0.0001$) and proportion of juveniles ($p < 0.0001$) sampled varied significantly from year to year, but no increasing or decreasing trends were observed. This study also showed that a greater proportion of females were observed in the juveniles sampled as compared to the adults sampled ($p < 0.0001$). Demographic data such as those examined in this study should continue to be collected from the biomedical and commercial harvest to increase understanding of horseshoe crab stocks coastwide.

INTRODUCTION

The American horseshoe crab, *Limulus polyphemus*, is a multiple-use resource with both ecological and economic value. Ecologically, it is important as a food resource to many marine organisms including migrating shorebirds that utilize the Delaware Bay as a stopover point during their spring migration (Mikkelsen 1988; Castro and Myers 1993; Harrington and Shuster 1999). The horseshoe crab is economically important to a commercial fishery that harvests them for use as bait primarily for the American eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries (HCTC 1998). In addition, blood collected from horseshoe crabs is used to produce an invaluable substance, *Limulus* Amoebocyte Lysate (LAL), which is the standard for detecting the presence of endotoxin in pharmaceutical products (ASMFC 1998).

Increased harvest of horseshoe crabs throughout the 1990s has led to concerns regarding the status of the population (Berkson and Shuster 1999). In October, 1998, the Atlantic States Marine Fisheries Commission (ASMFC) adopted the Horseshoe Crab Fishery Management Plan (ASMFC 2000). However, a lack of good fishery and population data prohibited the development of a comprehensive scientific management strategy (Walls et al. 2002). Instead, the management plan outlined coastwide requirements for monitoring and reporting, and identified research needs (Wenner and Thompson 2000). Addendums to the original plan have been implemented to reduce horseshoe crab harvest (ASMFC 2000; ASMFC 2004a).

Demographic data are critical to developing improved horseshoe crab management (Berkson and Shuster 1999). Currently it is not evident whether localized subpopulations exist, which would require localized management. For example, if a small local population, such as the one described by Widener and Barlow (1999) at Mashnee Dike, Cape Cod, Massachusetts, was isolated from other horseshoe crab populations it would need to be managed separately. Otherwise, if the size of this local population was smaller than the annual quota for the state of Massachusetts, the possibility exists that the local population at Mashnee Dike could be extirpated. If immigration into the area was limited, the population may never rebuild. Therefore, if isolated populations with little immigration exist and need to be preserved, they must be managed separately. Appropriate harvest levels would need to be set for each area inhabited by a localized population. Walls (2001) observed significant differences in sex distribution, size distribution, and stage class distribution between horseshoe crabs captured in Chincoteague, VA and those captured in Ocean City, MD. These findings suggest these may be subpopulations that could possibly benefit from separate management (Walls 2001).

It is also unclear what effect the commercial harvest of horseshoe crabs is having on the structure of the population. Walls noted a decline in percentage of females and a decline of older stage classes in the catch, suggesting the possibility of overexploitation of horseshoe crabs in these areas (Walls 2001). To see if these trends continue, Walls recommended additional years of data to determine if sex, size, and stage class distributions vary temporally and spatially. A decrease in females or older stage classes in horseshoe crab populations may suggest a decline in the health of populations, signifying reduced survival which would lead to reduced fecundity of the overall population. The long life cycle of horseshoe crabs requires at least 10 years for the population to recover if overharvested. In addition, this long life cycle requires many years of data collection before changes in the population structure are realized.

METHODS

Data Collection

During the summers of 2002, 2003, and 2004 demographic data were collected for horseshoe crabs harvested in Atlantic waters off the coast of Ocean City, Maryland. Horseshoe crabs were sampled on board trawling vessels harvesting horseshoe crabs for use by Cambrex, a biomedical company that extracts blood from horseshoe crabs for the production of LAL. Harvesters for Cambrex harvest horseshoe crabs non-randomly. They schedule their trips at various times of day to harvest horseshoe crabs and trawl in locations horseshoe crabs are known to inhabit. Sampling was conducted from a 16.8-meter commercial fishing vessel. A flounder trawl equipped with a Texas sweep was used to collect horseshoe crabs (Hata and Berkson 2003). The sweep consisted of a chain line instead of rope, which is considered more effective in digging up crabs buried in the sediment. Duration of the tow varied among tows. Horseshoe crabs collected during each tow were emptied onto the deck of the vessel. Before any culling took place to select crabs for bleeding, a random subsample ranging from 19 to 50 crabs was collected and held in containers on board the vessel. These crabs were selected without preference to size, sex, or stage class.

The subsample was then transported to Cambrex's bleeding facility where demographic information was collected and recorded. Sex, life history stage (stage class), prosomal width (width at the widest point of the horseshoe crab's shell), interocular width (distance between eyes), date of capture, waterbody of capture, and nearest town of capture were recorded for each crab. For juvenile horseshoe crabs, sex was determined by the examination of genital pores (Walls 2001; Hata and Berkson 2003). Male horseshoe crabs were identified by a pair of hard conical testes, while the presence of flat round ovaries was indicative of a female horseshoe crab. Adult males were identified by the presence of a modified boxing glove-like pincer. Adult females were identified by the presence of mating scars, or if newly matured, the presence of eggs. Life history stage of adults was determined by examination of the carapace using the protocol developed by Carl Shuster (Walls et al. 2002). Horseshoe crabs were identified to one of five stage classes: juvenile, newly matured, young adult, middle-aged adult, or old-aged adult (Walls et al. 2002). Newly-matured males were identified by the presence of an atrophied non-moveable chela, which breaks off upon mating. Newly-matured females were identified by a pristine shell with no mating scars. A horseshoe crab was considered to be a young adult (1 to 3 years after maturity) if its carapace was lustrous with few, if any, scratches or epibionts. Middle-aged adults (3 to 7 years after maturity) were identified by extensive scratches on their carapaces. Horseshoe crabs with almost completely blackened carapaces were considered to be old-aged adults (6 to 10 years after maturity). Calipers were used to measure prosomal and interocular width to the nearest millimeter. After this information was recorded, horseshoe crabs were released at approximately the same point of original capture in accordance with Cambrex's standard operating procedures.

Data Analyses

Data collected from this 3-year study were combined with data collected in Chincoteague, Virginia and Ocean City, Maryland by Walls (2001) (Table 3.1). All horseshoe crabs sampled from Chincoteague, VA were collected mid-June through early-July, while horseshoe crabs sampled from Ocean City, MD were collected mid-July through mid-August. Data were analyzed using program SAS (Statistical Analysis System, Version 8). Walls (2001) analyzed her data using a logistic classification model with likelihood-ratio tests at the $\alpha = 0.05$ significance level. In this study, sex distribution, age distribution, and stage class distribution were compared between locations and among years using Chi-Square analysis at the $\alpha = 0.05$ significance level. To eliminate observer effects, stage-class analysis was separated for the years 1999 through 2001 and 2002 through 2004. Size distribution differences between locations and among years were evaluated using tests for main effects and interactions in an analysis of variance (ANOVA) model at the $\alpha = 0.05$ significance level. All comparisons between locations were conducted only for the years when data were collected for both locations to eliminate any variation due to year.

RESULTS

Spatial Comparisons

Sex Distribution

In both locations the proportion of females in the juvenile catch was higher than the proportion in the adult catch ($p < 0.0001$; df = 1; Figure 3.1). Overall, there were no significant differences in the sex ratios of horseshoe crabs harvested in Chincoteague, Virginia as compared to those harvested in Ocean City, Maryland. Of all the horseshoe crabs sampled in Chincoteague, Virginia, 53.7% were females. This was not different from the samples obtained in Ocean City, Maryland, which had a female composition of 48.7% ($p = 0.09$; df = 1; Figure 3.2). When examining the sex ratios in the adult and juvenile portions of the samples separately, there were no differences between locations. Of Chincoteague, Virginia's adult horseshoe crabs, 34.4% were females as compared to Ocean City, Maryland's 37.5% ($p = 0.55$; df = 1). The proportion of females in the juvenile catch in Chincoteague, Virginia was 56.7% as compared to 64.8% in Ocean City, Maryland ($p = 0.06$; df = 1).

Stage Class Distribution

When examining stage class distributions, differences ($p < 0.0001$; df = 4) were found between the distributions in Chincoteague, Virginia and Ocean City, Maryland for every stage class except newly-matured horseshoe crabs ($p = 0.26$; df = 1; Figure 3.3) during the years 2000 and 2001. Samples collected in Chincoteague, from mid-June through early-July, were comprised mostly of juvenile and newly-matured horseshoe crabs. In Chincoteague, 86.7% of the horseshoe crabs sampled were juvenile horseshoe crabs as compared to Ocean City's 41.1% juvenile composition ($p < 0.0001$; df = 1). Samples collected in Ocean City, from mid-July through mid-August, had greater proportions of young adults, middle-aged, and old-aged horseshoe crabs. Of the horseshoe crabs sampled in Ocean City, 14.7% were classified as old-aged, as compared to only 0.20% of the horseshoe crabs sampled in Chincoteague.

Size Distribution

Least squares mean size of horseshoe crabs of the same age (adult or juvenile) and the same sex, in Chincoteague, Virginia and Ocean City, Maryland were not different for adult females ($p = 0.82$), adult males ($p = 0.46$), or juvenile males ($p = 0.41$). However, juvenile females from Ocean City (interocular width = 96.4 mm) were larger than juvenile females from Chincoteague (interocular width = 89.35 mm) ($p < 0.0001$; df = 27; Figure 3.4).

Annual Comparisons

Sex Distribution

Sex ratios of horseshoe crabs collected in Chincoteague, Virginia in the years 2000 and 2001 did not differ. Overall, 53.0% of horseshoe crabs from Chincoteague in 2000, and 54.7% in 2001 were females ($p = 0.4103$; df = 1). Separated by age, 30.5% in 2000 and 37.5% in 2001 were adult females ($p = 0.4018$; df = 1), while 56.1% and 57.0% in 2000 and 2001 respectively, were juvenile females ($p = 0.7970$; df = 1).

When comparing annual changes in the sex ratios of all horseshoe crabs sampled in Ocean City, Maryland, during the years 1999 through 2004, which ranged from 35.7% to 64.3% females, there were differences ($p < 0.0001$; df = 5; Appendix 1, Table A1.1). However, no increasing or decreasing trend was observed (Figure 3.5). When only analyzing the sex ratios of adults sampled in Ocean City, which ranged from 28.2% to 63.5% females, there were differences with more females captured in 2002 than other years ($p < 0.0001$; df = 5; Figure 3.6; Appendix 1, Table A1.2). When the data were analyzed without samples from 2002, there were still significant among sex ratios for the remaining years ($p = 0.0012$; df = 4). Sex ratios, ranging from 52.3% to 77.8% females, were also different among years when analyzing only the juveniles sampled ($p = 0.0249$; df = 5; Appendix 1, Table A1.3).

Stage Class Distribution

Overall, there were differences among stage class distributions of horseshoe crabs sampled in Chincoteague, Virginia between the years 2000 and 2001 ($p = 0.0005$; $df = 4$; Appendix 1, Table A1.4), but no difference in the proportion of juvenile horseshoe crabs in 2000 and 2001 (84.0% and 88.2% respectively; $p = 0.0595$; $df = 1$). There were differences among stage class distributions of horseshoe crabs sampled in Ocean City, Maryland among the years 1999 through 2001 ($p < 0.0001$; $df = 8$; Appendix 1, Table A1.5) and also among the years 2002 through 2004 ($p < 0.0001$; $df = 8$; Appendix 1, Table A1.6), with differences in the proportion of juvenile horseshoe crabs among all 6 years ranging from 5.05% to 53.3% juvenile composition ($p < 0.0001$; $df = 5$; Figure 3.7; Appendix 1, Table A1.7). However, no increasing or decreasing trend was observed.

Size Distribution

Mean interocular width of adult females was much more consistent among years than for other demographic groups ($df = 27$; Appendix 1, Table A1.8); mean interocular widths ranged from 161.02 mm in 2004 to 167.96 mm in 1999. Seven of the 15 pair-wise comparisons of adult male interocular width during 1999 – 2004 were significant ($df = 27$; Appendix 1, Table A1.9). Juvenile females had the greatest variability in mean interocular width. The mean interocular width of juvenile females was significantly different for 14 of the 15 pair-wise comparisons during 1999 - 2004 ($df = 27$; Appendix 1, Table A1.10). Juvenile males had significantly different mean interocular widths for 6 of the 15 pair-wise comparisons for the years 1999 - 2004 ($df = 27$; Appendix 1, Table A1.11). Overall means for each demographic group are reported in Table 3.2.

DISCUSSION

Spatial Comparisons

Information regarding the population structure of the American horseshoe crab is lacking. It is important to identify spatial segregation to define the appropriate scale for management of the horseshoe crab. The most recent genetic analyses suggest that the entire mid-Atlantic portion (from Massachusetts to South Carolina) of the horseshoe crabs' distribution should be considered as one management unit (King et al. 2003).

Methods other than genetic analyses have also been used to identify population structure. While genetic analyses compare genetic similarities to determine relatedness and population structure, morphometric analyses determine relatedness based on quantitative measurement of physical features, the outward expression of genetic relatedness. Morphometrics are commonly used to identify population structure and were often the primary tool used before genetic analyses became more common (Shaklee and Currens 2003; Elewa 2004). Morphometrics have been used as one means of classifying populations of *Limulus*. Coastal surveys have shown that populations of horseshoe crabs in major estuaries were relatively discrete based on differences of widths of adults (Shuster 1979). In this study, comparisons of mean interocular widths of male and female adult horseshoe crabs between Chincoteague and Ocean City showed no significant differences between locations. Therefore, horseshoe crabs from these two areas cannot be distinguished based on this morphometric evidence.

However, differences in stage class distribution did exist between Chincoteague, Virginia and Ocean City, Maryland. Chincoteague had a greater proportion of juvenile horseshoe crabs as compared to Ocean City. It should be noted that the stage class distributions could possibly be an artifact of temporal differences in sampling. However another possibility is that Chincoteague may serve as a nursery area for horseshoe crabs (Walls 2001). There may be a greater availability of spawning habitat around Chincoteague as compared to Ocean City, leading to greater recruitment (Walls 2001). If this is true, horseshoe crabs in Chincoteague may require special management to allow for protection of the juveniles. Another possible explanation for the greater proportion of juveniles sampled in Chincoteague is that harvest pressure is greater in that area. If a larger number of adult horseshoe crabs are harvested from waters surrounding Chincoteague, it would result in an increase in the proportion of juveniles relative to the adults in the catch. Only 0.20% of the horseshoe crabs sampled in Chincoteague were classified as old aged as compared to 14.72% in Ocean City. A low number of older stage classes may be a result of increased harvest. In addition, the smaller mean size of juvenile females in Chincoteague as compared to Ocean City could be evidence for increased harvest in Chincoteague. Large juvenile females may be harvested in Chincoteague, thereby reducing the overall mean size of juvenile females in that area.

Annual Comparisons

This study also examined annual changes in sex distribution, stage class distribution, and size distribution of horseshoe crabs. There were significant differences among the 6 years of this study for each demographic parameter. However, no increasing or decreasing trends were evident. These year-to-year fluctuations may be due to differential survival among year classes and between sexes. Regular fluctuations in environmental conditions may affect larval survival from year to year. In addition, harsh environmental conditions for some years may have a greater impact on survival of one sex as compared to the other. If there is differential survival between sexes, it would have an effect on the proportion of females from year to year.

Horseshoe crab life histories and environmental stochasticity lead to high variability of horseshoe crab population structure from year to year. For this reason, the Horseshoe Crab Stock Assessment Subcommittee concluded that for the majority of available horseshoe crab indices, 10 - 15 years of data would be required to detect small changes in the population size (ASMFC 2004b). Annual changes in proportion of females and proportion of juveniles in the catch of the magnitude observed in this study would not be appropriate indices for making management decisions. It would be difficult to manage horseshoe crabs for maximum sustainable yield (MSY) because of the high year-to-year variability of horseshoe crab populations observed in this study.

Sex Ratios

For each year of the study, the proportion of females in the juveniles sampled was greater than the proportion of females in the adults sampled. This shift in sex ratio has been reported in other studies (Carmichael et al. 2003; Hata and Berkson 2003). Some would suggest that this may be due to a commercial fishery that historically has targeted adult female horseshoe crabs (Hata and Berkson 2003) and may be the first sign of a population decline (Swan et al. 1994). However, the observed sex ratios may be an artifact of the life history of the horseshoe crab. It is thought that female horseshoe crabs undergo an additional molt before becoming adults (Shuster 1990; Carmichael et al. 2003), one year later than male horseshoe crabs. Assuming equal survival of males and females, a simple model can confirm that the proportion of females would be greater among juveniles as compared to adults, if females remain in the juvenile stage class one year longer than males. Consequently, assuming equal survival, it would be expected that a greater number of adults are males since male horseshoe crabs are recruited into the adult population one year earlier than females. Caution should be taken when interpreting a reduced proportion of females in the adult harvest. It should not be assumed that this is a result of overfishing. Multiple years of data should be collected to determine if sex ratios are changing annually.

Limitations and Recommendations

The findings of this study were likely affected by the way in which the horseshoe crabs were sampled. At a time when little information regarding the population dynamics of horseshoe crabs is known (Carmichael 2003), this study provides information on the catch composition of horseshoe crabs harvested in 2 separate locations. However, it is important to consider the non-random nature of sample collection in this study and the effect it may have on the results.

Trawls were targeted at areas where horseshoe crabs were known to be present at various times of day. Distance from shore varied among trawls, possibly affecting catch composition. Juvenile horseshoe crabs remain inshore (Shuster 1979). Therefore, it is likely that the proportion of juveniles in the catch is reduced the further the vessel moves offshore. In addition, topography, depth, and time of day have been found to affect horseshoe crab sampling (Hata and Berkson 2004). These factors could affect both sex ratios and stage class distributions. Furthermore, the degree to which sampling times overlap with horseshoe crab spawning times may also affect the demographics of the catch. If sampling was conducted while adults were inshore spawning, the juvenile composition of the catch would be increased. The proportion of adult females as compared to adult males may also be increased, because males tend to remain inshore spawning for longer periods of time (Rudloe 1980; Swan et al. 1994). All horseshoe crabs sampled from Chincoteague, Virginia were collected mid-June through early-July, while horseshoe crabs sampled from Ocean City, Maryland were collected mid-July through mid-August. This sampling schedule could be the reason a larger proportion of juveniles were observed in the samples taken from Chincoteague, Virginia. The data collected in this study were not appropriate for examination of seasonal differences in catch composition.

Future studies should take into account time of year (especially as it relates to spawning season), distance from shore, topography, depth, and time of day when determining how and when to sample horseshoe crabs for spatial and temporal comparisons. Ideally, spatial and temporal distributions of horseshoe crab populations would be examined as part of a randomized fishery independent survey, such as the pilot trawl survey conducted by Hata and Berkson (2003). A random survey would be more appropriate for year-to-year comparisons and comparisons between locations. Random surveying would allow for annual population estimations for each demographic group which would better enable managers to set annual harvest quotas.

In addition to fishery independent data regarding horseshoe crab populations, fishery dependent data such as those collected in this study are required for comprehensive stock assessment. The stock assessment technique based on the catch-survey method that has been developed by the Horseshoe Crab Stock Assessment Committee (HCSAC) requires an estimate of mature and newly mature females in the harvest. The HCSAC also noted that harvest sampling programs that provide demographic information from the commercial harvest are of high value and high priority to the stock assessment process (HCSAC 2000). For this reason, the biomedical industry and commercial fishery should continue collecting demographic data regarding horseshoe crab catch composition.

REFERENCES

Atlantic States Marine Fisheries Commission (ASMFC). 1998. Interstate fishery management plan for horseshoe crab. Fishery management report No.32 of the Atlantic States Marine Fisheries Commission. Washington, D.C. 58pp.

_____. 2000. Addendum I to the interstate fishery management plan for horseshoe crab. Fishery management report No. 32a of the Atlantic States Marine Fisheries Commission. Washington, D.C. 9pp.

_____. 2004a. Addendum III to the interstate fishery management plan for horsesehoe crab. Fishery management report No. 32c of the Atlantic States Marine Fisheries Commission. Washington, D.C. 14pp.

_____. 2004b. Horseshoe crab 2004 stock assessment report of the Atlantic States Marine Fisheries Commission. Washington, D.C. 87pp.

Berkson, J. and C. N. Shuster, Jr. 1999. The horseshoe crab: the battle for a true multiple-use resource. Fisheries Management 24(11):6-11.

Carmichael, R. H., D. Rutecki, and I. Valiela. 2003. Abundance and population structure of the Atlantic horseshoe crab *Limulus polyphemus* in Pleasant Bay, Cape Cod. Marine Ecology Progress Series 246:225-239.

Castro, G. and J. P. Myers. 1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. The Auk 110(4):927-930.

Elewa, A. M. T., editor. 2004. Morphometrics: Applications in Biology and Paleontology. Springer, Berlin, Germany.

Harrington, B. and C. N. Shuster, Jr. 1999. Crab crisis at Delaware Bay - commercial bait collectors are robbing migratory shorebirds of essential food. Defenders 74(3): 31-35.

Hata, D. and J. Berkson. 2003. Abundance of horseshoe crabs, *Limulus polyphemus*, in the Delaware Bay area. Fishery Bulletin 101:933-938.

Hata, D. and J. Berkson. 2004. Factors affecting horseshoe crab *Limulus polyphemus* trawl survey design. Transactions of the American Fisheries Society 133:292-299.

HCSAC (Horseshoe Crab Stock Assessment Committee). 2000. A conceptual framework for the assessment of horseshoe crab stocks in the mid-Atlantic region. Report to the Horseshoe Crab Technical Committee of the Atlantic States Marine

Fisheries Commission. Washington, D.C. 19pp.

HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast. Report to the Atlantic States Marine Fisheries Commission. Washington, D.C. 48pp.

King, T. L., M. S. Eackles, A. P. Spidle, and H. J. Brockmann. 2003. Microsatellite DNA reveals regional differentiation among populations of the horseshoe crab (*Limulus polyphemus*). Final Report submitted to the Atlantic States Marine Fisheries Commission.

Mikkelsen, T. 1988. The Secret in the Blue Blood. Beijing, China, Science Press.

Rudloe, A. 1980. The breeding behavior and patterns of movement of horseshoe crab, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. Estuaries 3:177-183.

Shaklee, J. B. and K. P. Currens. 2003. Genetic stock identification and risk assessment. Pages 291-328 *in* E. M. Hallerman, editor. Population genetics: principles and Applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Shuster, C. N., Jr. 1979. Distribution of the American horseshoe crab, (*Limulus polyphemus* (L.)). Pages 3-26 *in* E. Cohen, ed. Biomedical applications of the horseshoe crab (Limulidae). Alan R. Liss, New York.

_____. 1990. The American horseshoe crab, *Limulus polyphemus*. Pages 15-25 *in* R. B. Prior, Ph.D, ed. Clinical Applications of the Limulus Amoebocyte Lysate Test. CRC Press, Boca Raton, Florida.

Swan, B. L., W. R. Hall, Jr., and C. N. Shuster, Jr. 1994. *Limulus* spawning activity on Delaware Bay shores on June 5, 1993. Delaware Estuary Program:flyer. 4pp.

Walls, B. A. 2001. A horseshoe crab (*Limulus polyphemus*) demographic study. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 70 pp.

_____., J. M. Berkson and S. A. Smith. 2002. The Horseshoe Crab, Limulus polyphemus: 200 Million years of Existence, 100 Years of Study. Reviews in Fisheries Science 10(1):39-73.

Wenner, E. and M. Thompson. 2000. Final report to the National Oceanographic and Atmospheric Administration. Evaluation of harvesting impacts and population trends for *Limulus polyphemus* in South Carolina. Charleston, Marine Resources Research Institute, South Carolina Department of Natural Resources. 96pp.

	Chincoteague, Virginia						Ocean City, Maryland					
		Adult Stage Class						Adult Stage Class				
	Juvenile	Newly Matured	Young Adult	Middle Aged	Old Aged	Total	Juvenile	Newly Matured	Young Adult	Middle Aged	Old Aged	Total
1999	n/a	n/a	n/a	n/a	n/a	n/a	3	10	2	12	17	44
2000	310	25	28	6	0	369	134	19	38	54	34	279
2001	540	52	16	2	2	612	28	5	33	25	24	115
2002	n/a	n/a	n/a	n/a	n/a	n/a	9	5	28	92	45	179
2003	n/a	n/a	n/a	n/a	n/a	n/a	251	52	145	200	27	675
2004	n/a	n/a	n/a	n/a	n/a	n/a	1133	104	343	409	134	2123
Total	850	77	54	8	2	981	1558	195	589	792	281	3415

Table 3.1 Numbers of horseshoe crabs sampled in each stage class from Chincoteague, Virginia and Ocean City, Maryland during 1999-2004.

	Minimum	Maximum	Mean	Standard Deviation
Adult Females	132 mm	202 mm	162.22 mm	11.725 mm
Adult Males	65 mm	199 mm	121.501 mm	10.324 mm
Juvenile Females	51 mm	171 mm	97.886 mm	23.285 mm
Juvenile Males	49 mm	130 mm	90.773 mm	15.082 mm

Table 3.2 Interocular width measurements of all horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland during 1999 - 2004.

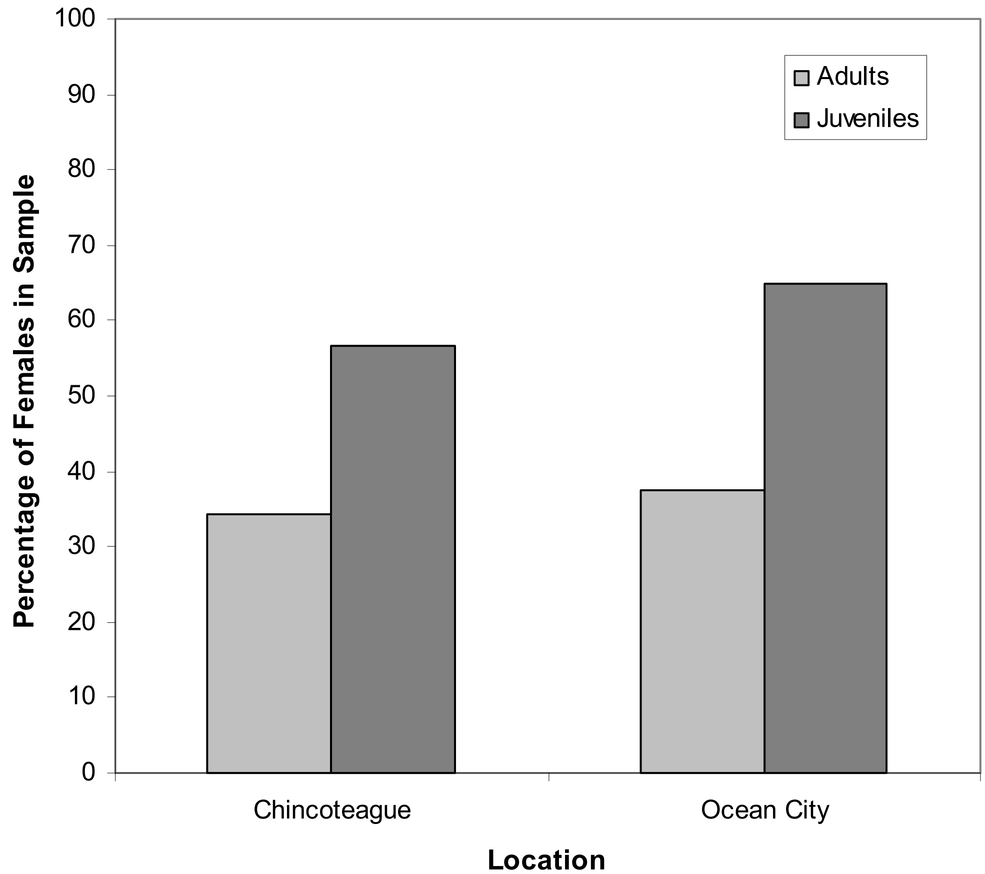

Figure 3.1 Proportion of females in the adult sample versus the proportion of females in the juvenile sample of horseshoe crabs collected in Chincoteague, Virginia and Ocean City, Maryland for the years 2000 - 2001 combined.

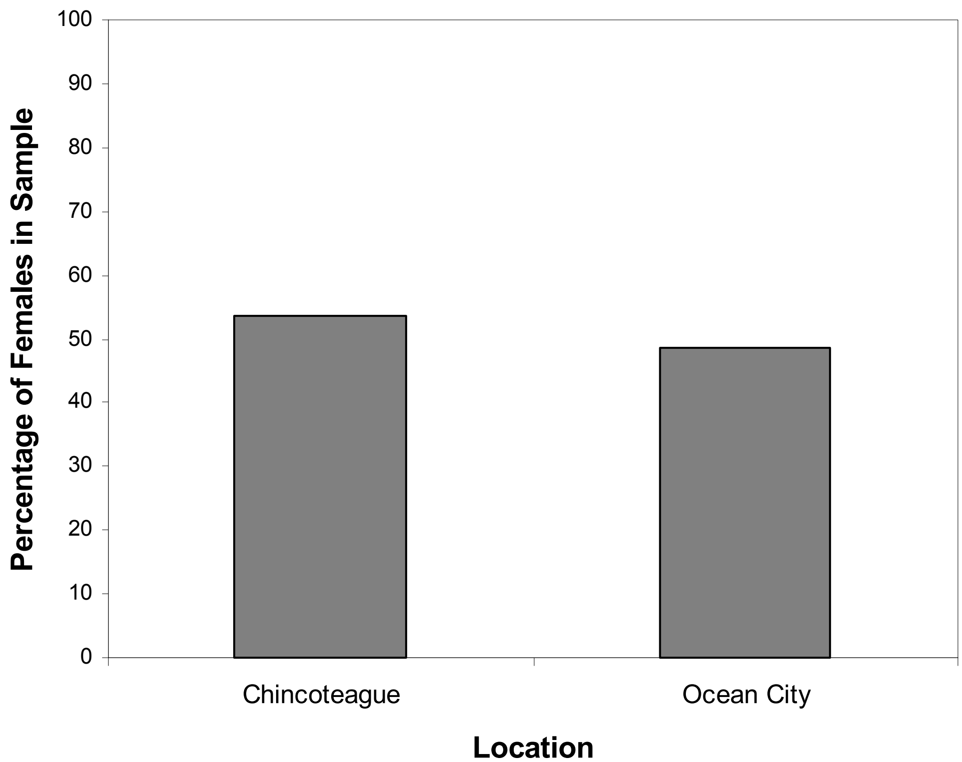

Figure 3.2 Proportion of female horseshoe crabs captured in Chincoteague, Virginia and Ocean City, Maryland during the years 2000 - 2001.

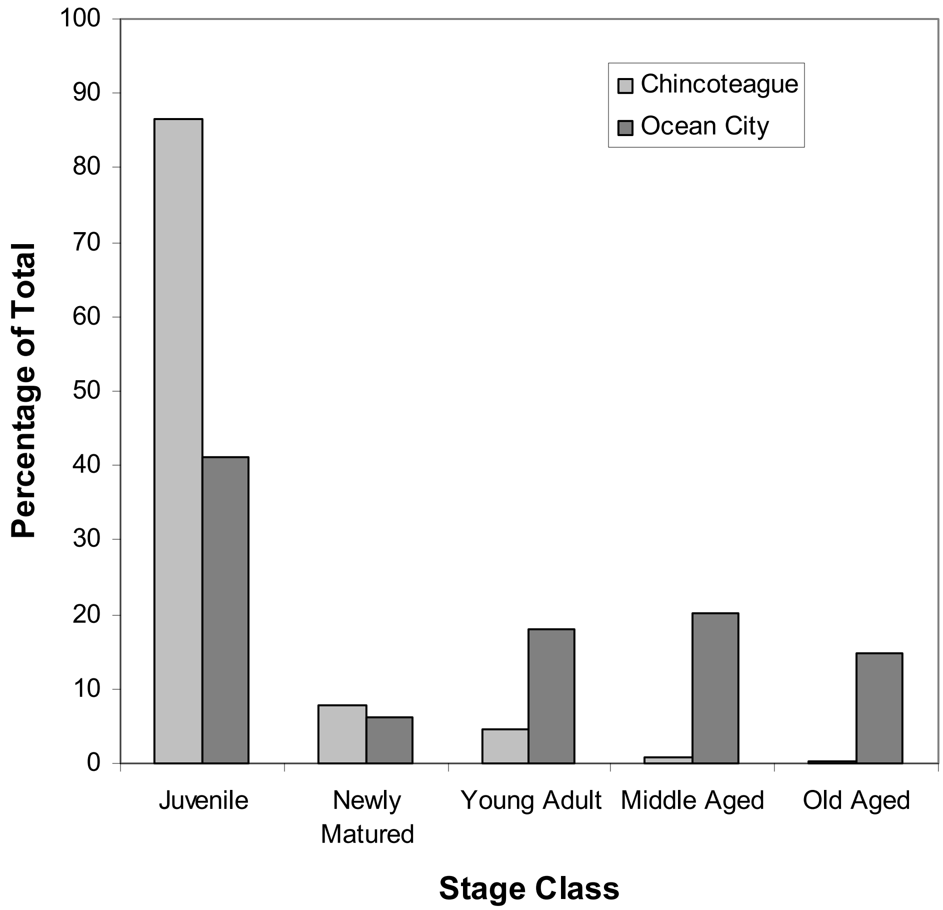

Figure 3.3 Proportion of each stage class of horseshoe crabs captured in Chincoteague, Virginia and Ocean City, Maryland in the years 2000 - 2001.

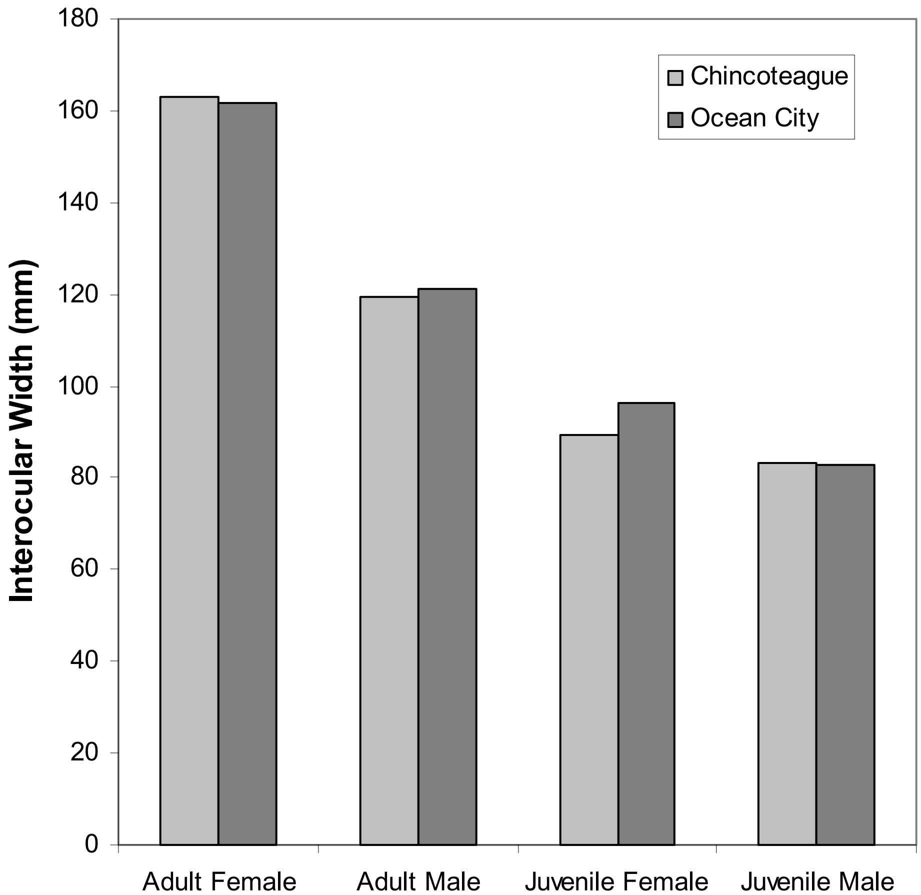

Figure 3.4 Mean interocular width of horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland in 2000 - 2001.

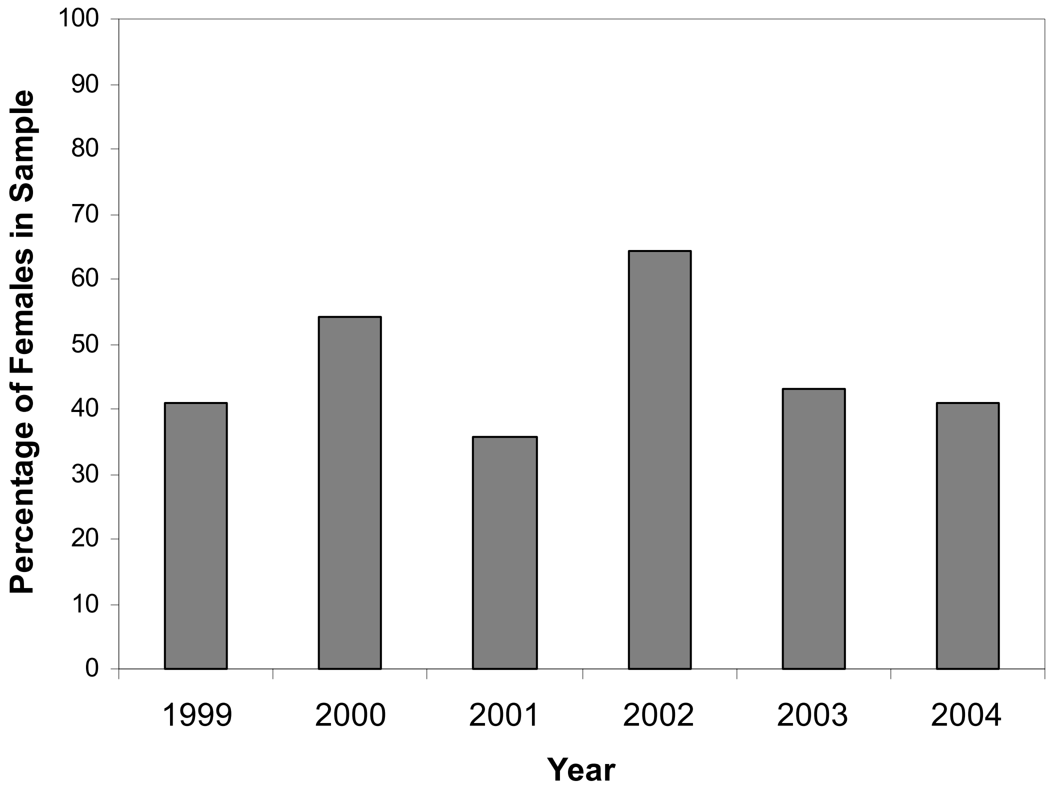

Figure 3.5 Proportion of females in a sample of the total catch of horseshoe crabs captured in Ocean City, Maryland during the years 1999 - 2004.

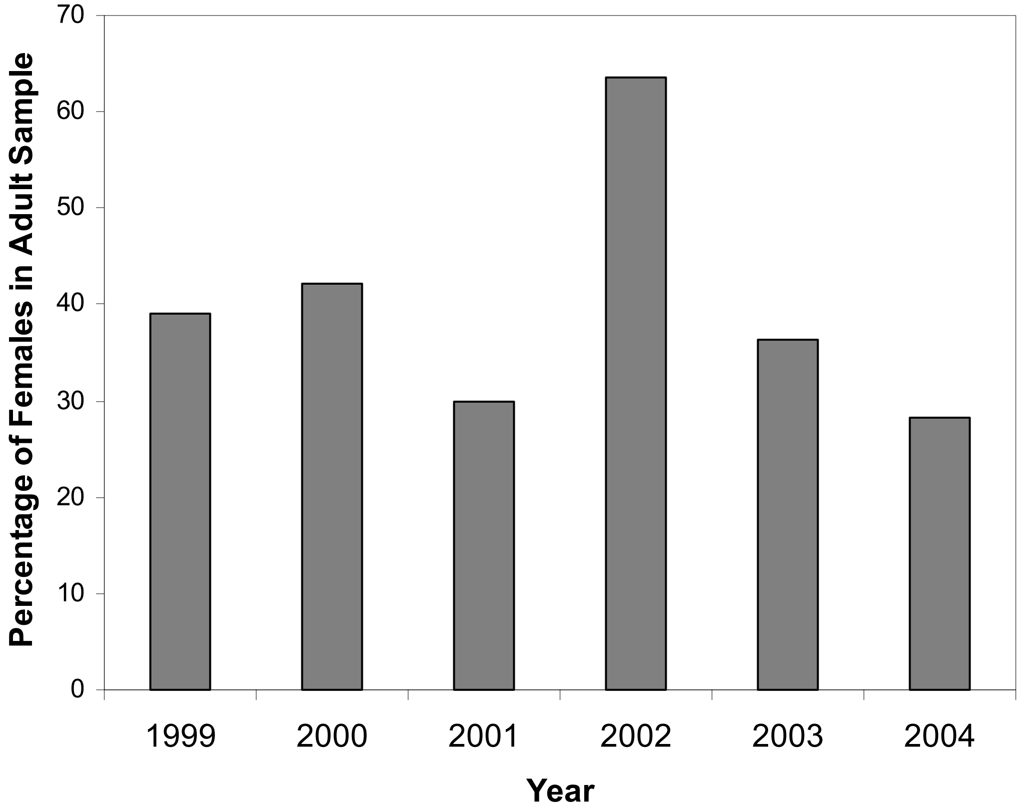

Figure 3.6 Proportion of females in a sample of the adult catch of horseshoe crabs captured in Ocean City, Maryland during the years 1999 - 2004.

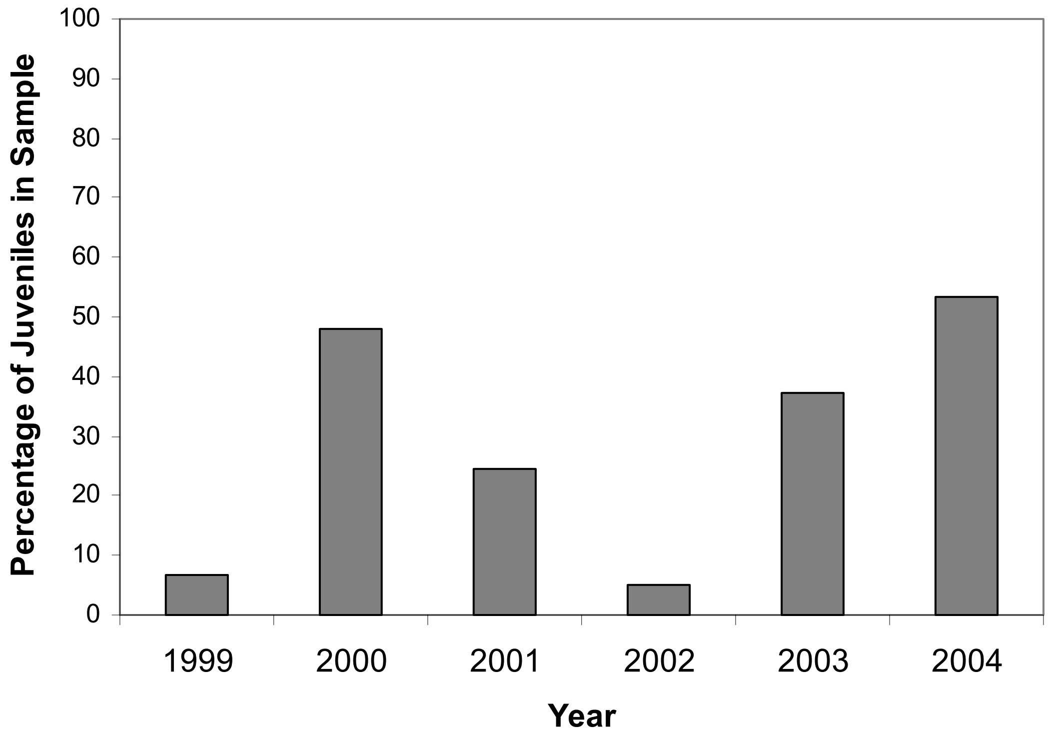

Figure 3.7 Proportion of juveniles in a sample of the total catch of horseshoe crabs captured in Ocean City, Maryland during the years 1999 - 2004.

CHAPTER 4 – DESIGNING A HORSESHOE CRAB SPAWNING SURVEY FOR TOM'S COVE, ASSATEAGUE ISLAND, VIRGINIA

ABSTRACT

In response to the Atlantic States Marine Fisheries Commission's call to identify spawning areas coastwide and develop monitoring programs, 2 portions of the shoreline of Tom's Cove, Assateague Island, Virginia were investigated as possible areas to conduct annual horseshoe crab spawning surveys. This study showed that spawning occurs on both the northern and southern shorelines of Tom's Cove. This study also showed a minimum of 1,192 horseshoe crabs utilized Tom's Cove as a spawning area on one night in 2003 and a minimum of 621 horseshoe crabs utilized the area in 2004 over 10 nights. The reduction in observed horseshoe crab spawning activity in 2003 was likely due to natural variability in both horseshoe distribution and environmental factors that could not be examined in the scope of this study. Future spawning surveys in this region should include simultaneous surveying of both study sites at Tom's Cove over a greater time scale than was permitted in this study. In addition, similar surveys should be conducted in other suitable areas to identify the scale of spawning habitat usage in the mid-Atlantic region.

INTRODUCTION

The American horseshoe crab, *Limulus polyphemus*, is a multiple-use resource with both ecological and economic value. Ecologists are interested in the fate of this species because of the important function it serves as a food source for migrating shorebirds (Castro and Myers 1993; Harrington and Shuster 1999). Commercial fishermen rely on the horseshoe crab primarily as bait for the American eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries (HCTC 1998). The biomedical industry relies on the horseshoe crab for the vital role it plays as the only source of *Limulus* Amoebocyte Lysate (LAL), a substance derived from the blood of horseshoe crabs for testing sterility in pharmaceutical products (ASMFC 1998b).

Approximately one million horseshoe crabs are harvested annually by the commercial fishery (ASMFC 2004). Since horseshoe crabs require 10 years to reach sexual maturity (Shuster 1958; Berkson and Shuster 1999), overexploitation of the population could have severe consequences. An overfished population would take at least a decade to recover. In response to concerned stakeholder groups, management of the horseshoe crab resource has become an important conservation issue in recent years.

The development of a management plan has been difficult due to the lack of information regarding the status of the horseshoe crab population (Berkson and Shuster 1999). However, the Atlantic States Marine Fisheries Commission (ASMFC) has developed a management plan that calls for reductions in the overall catch of horseshoe crabs (ASMFC 1998b). It also calls for the development of better monitoring techniques.

As more information becomes available, ASMFC will be able to modify management strategies to better meet the needs of all interested groups.

In its Fishery Management Plan (FMP) for Horseshoe Crab, the ASMFC calls for the establishment of pilot programs to survey spawning horseshoe crabs (ASMFC 1998b). In addition, the Horseshoe Crab Peer Review Panel recommends, as a high priority, coastwide standardized data collection of spawning surveys to provide an index of spawning population size (ASMFC 1998a). Optimal spawning beaches, which are considered essential habitat for adult horseshoe crabs, may be a limiting reproductive factor for the coastwide horseshoe crab population (ASMFC 1998b). One goal of the horseshoe crab FMP is the identification of potential spawning habitat within each state (ASMFC 1998b). Tom's Cove, Assateague Island, Virginia is an area of interest, since horseshoe crabs are harvested in surrounding coastal waters. In addition, it is thought that this area may serve as a nursery ground for horseshoe crabs (Walls 2001). Over time, effects of the fishery may be observed with annual monitoring of this spawning habitat.

A decline in the number of horseshoe crabs in Tom's Cove could have negative effects on shorebirds that may rely on the horseshoe crab eggs as a major food source. The Master Plan of the Chincoteague National Wildlife Refuge (NWR) identifies Assateague Island as important habitat for shorebirds, providing premium feeding and resting areas (CNWR 1993). Horseshoe crab eggs, found on the intertidal sand and mud flats on the cove side of Tom's Cove Hook, are an important high quality food for shorebirds (CNWR 1993). The Chincoteague NWR ranks 4^{th} in the number of shorebirds utilizing the area during their migration, among 454 sites in the United States east of the

Rocky Mountains and 2nd in diversity of shorebird species from among all 450 sites in the International Shorebird Survey network (CNWR 1993). A spawning survey of Tom's Cove to monitor horseshoe crab numbers could alert us to possible declines in the abundance of spawning horseshoe crabs that may prove harmful to the shorebird community. In response to the ASMFC's call to establish pilot programs to survey horseshoe crabs, this study addressed the following objectives: 1) determine what specific areas in Tom's Cove are being used for horseshoe crab nesting, 2) estimate the number of horseshoe crabs spawning there, and 3) design a more thorough spawning survey which could be used for annual monitoring purposes.

METHODS

Pilot Study - 2003

During the summer of 2003, 2 sections of shoreline along Tom's Cove were investigated as possible areas to conduct a horseshoe crab spawning survey (Figure 4.1). In the Delaware Bay, peak spawning generally occurs in conjunction with the evening full moon and new moon high tides in May and June (Shuster and Botton 1985; D. Smith, U.S. Geological Survey (USGS), pers. comm.). Therefore lunar cycle was considered when determining survey dates. Unfortunately, due to bad weather and safety issues not all scheduled dates could be sampled. An approximately 950-meter portion of the southern shoreline of Tom's Cove, known as Tom's Cove Hook, was surveyed with the evening high tides of May 13th, 14th, and 28th (with a new moon occurring on May 15th). Coordinates for the starting point of this study site are 37° 52.579'N and 75° 23.260'W. Coordinates for the ending point of this study site are 37° 52.350'N and 75° 22.667'W.

An approximately 900-meter portion of the northern shoreline, accessible by the Woodland Trail on Chincoteague NWR, was surveyed with the evening high tides of May 29th and 30th and the daytime high tides of May 31st and June 2nd, 3rd, and 14th (with a full moon occurring on May 31st and a new moon on June 14th). Coordinates for the starting point of this study site are 37° 53.605'N and 75° 22.286'W. Coordinates for the ending point of this study site are 37° 53.596'N and 75° 22.901'W.

Sampling began at the predicted evening or daytime high tide. A coin toss was used to determine from which end of the study site sampling would begin. Instead of using a systematic random sampling technique as implemented at the Delaware Bay (Smith et al. 2002), a total count, by sex, of all spawning horseshoe crabs was taken. The study area was small and horseshoe crabs were present in much lower densities than in the Delaware Bay. This allowed a census of observable spawning horseshoe crabs to be taken, a method that is preferred but not always practical in areas of high density. On 2 sampling occasions, May 29th and May 30th, a second total count of all spawning horseshoe crabs was taken beginning one hour after high tide to identify differences in spawning crab density relative to time after high tide.

Mark-Recapture – 2004

Data Collection

From May 17th through May 21st and from June 1st through June 5th, 2004, a mark-recapture study was conducted on horseshoe crabs spawning over a 900-meter section of the northern shoreline of Tom's Cove. The sampling site was the same as the northern site surveyed in 2003. As compared to the site on Tom's Cove Hook, this site was preferable because of ease of access. In addition, surveying of the northern shoreline eliminated concerns of interrupting shorebird nesting on the Overwash area adjacent to Tom's Cove Hook (CNWR 1993). Dates were chosen based on the predicted peak of spawning for horseshoe crabs in the Delaware Bay (D. Smith, USGS, pers. comm.). A full moon occurred on May 19th, with a new moon occurring on June 3rd.

The study site was divided into 3 300-meter sections for surveying. Teams of 2 or more people were assigned to each section. Beginning at the predicted time for the evening high tide and ending one hour later, all horseshoe crabs observed spawning in each section were captured and tagged. Using a 9/64" drill bit, equipped with a guard to allow only ¼-inch penetration, a hole was drilled into the posterior of the left prosoma in an area where the tag would lay flat. A uniquely numbered Petersen disc tag, with a toll-free telephone number to the Horseshoe Crab Tag Recovery Center, was then inserted. The tag number was recorded along with the sex of the tagged horseshoe crab. Tagging was repeated for each of the 10 nights of the study. In addition, for each night after the first, all previously tagged horseshoe crabs that were observed spawning were recorded; they were not retagged.

For comparison, a portion of the southern shoreline of Tom's Cove known as Tom's Cove Hook was surveyed with the evening high tide of May 19th. Coordinates for the starting point of this approximately 1400-meter study site are 37° 51.963'N and 75° 22.129'W. Coordinates for the ending point of this study site are 37° 52.476'N and 75° 22.906'W. At this site a total count of horseshoe crabs was taken and sex was recorded for each individual. No animals at the second site were tagged and there were no recaptures of horseshoe crabs that had been tagged on the northern shoreline.

Data Analyses

Mean and standard error of the number of horseshoe crabs observed spawning on the northern shoreline were calculated for each 5-day sampling period and for all 10 days combined. The sex and number of each horseshoe crab tagged and recapture information for each individual were entered into Program MARK for analyses (White and Burnham 1999). Program CAPTURE (Rexstad and Burnham 1992) was run through program MARK to develop population estimates. To better meet the assumption of equal catchability, recapture information for male and female horseshoe crabs was analyzed separately. Mark-recapture data were analyzed for each week of sampling and for both weeks combined. In addition, data from both weeks combined, for each sex, were analyzed using the Jolly-Seber method for estimating abundance in open populations (Krebs 1999).

RESULTS

In both areas investigated, horseshoe crabs were observed spawning. Table 4.1 shows the total number and sex ratios of horseshoe crabs observed in these areas during each sampling period. The maximum number of horseshoe crabs observed during a single sampling period was 1,192. These horseshoe crabs were observed spawning on the northern shoreline of Tom's Cove, one hour after the evening high tide of May 30th, 2003. The ratio of males to females during that sampling period was 1.24:1. The maximum number of horseshoe crabs observed spawning on the northern shoreline on a single night in 2004 was 94, observed with the new moon evening high tide of May 19th. The ratio of males to females during that sampling period was 1.09:1. The mean number of horseshoe crabs observed from May 17th – 21st, 2004 was 73.2 ± 11.8 (mean ± SE). The mean number of horseshoe crabs observed from June 1st – 5th, 2004 was 68.2 ± 12.9 (mean ± SE). The mean for all ten days surveyed in 2004 was 70.7 ± 8.3 (mean ± SE).

In total, 621 horseshoe crabs were individually marked in 2004 while spawning on the northern shoreline of Tom's Cove. Of these, 281 were females and 340 were males for a sex ratio of males to females equal to 1.21:1. Thirty-three (13%) females were resighted one or more times during the survey. Thirty-nine (13%) males were resighted one or more times during the survey. Population estimates from Program CAPTURE are reported in Table 4.2. Ninety-five percent confidence intervals are reported for population estimates from the most appropriate model with an available estimator as predicted by Program CAPTURE (Table 4.2). Using data from both weeks of sampling, the population of female horseshoe crabs utilizing the northern shore of Tom's Cove for spawning was estimated to be 1,282. The population of male horseshoe crabs was estimated to be 1,913. The Jolly-Seber method for estimating open populations estimated 597 female horseshoe crabs and 685 male horseshoe crabs utilized the northern shore of Tom's Cove as a spawning area. In addition, resight information from 9 horseshoe crabs in this study has been reported to the Horseshoe Crab Tag Recovery Center (Table 4.3). One horseshoe crab was reported as being found alive at Fowler's Beach, Delaware Bay 3 months after being tagged at Tom's Cove.

DISCUSSION

Both the northern and southern shorelines of Tom's Cove are utilized by horseshoe crabs for spawning. Unfortunately these areas could not be surveyed simultaneously to determine the relative degree at which these sites were being used. Data from this study show the minimum number of horseshoe crabs that utilized the northern shoreline for spawning in 2003 was 1,192. However, this observation came from only one night of sampling. In 2004, the minimum number utilizing the same shoreline was 621, observed over 10 nights of sampling. In 2003, 34 horseshoe crabs were observed spawning on the southern shoreline and in 2004, 135were observed there. Each of these observations only encompassed one night of sampling. The actual number of spawners is likely much higher. Sampling time was restricted to one hour during each sampling period; therefore only horseshoe crabs spawning within that one-hour timeframe were observed. In addition, observability of horseshoe crabs was affected by tide height. Tide height exceeded the vegetation line, thus forcing horseshoe crabs to spawn under water during the week of the full moon (June 1st-5th) in 2004. These horseshoe crabs were much harder to locate and capture than horseshoe crabs spawning at tide level.

Population Estimation

The feasibility of using information from this study to estimate the population size of horseshoe crabs that utilize Tom's Cove as a spawning area was considered. Population size estimation is an important aspect of the conservation of many species (Menkens and Anderson 1988) and mark-recapture studies can be used to estimate population size and mortality rates (Krebs 1999). For population estimates to be accurate they must adhere to a strict set of assumptions (Krebs 1999). The assumptions are as follows: every animal has an equal probability of capture, every marked animal has the same probability of surviving between sampling periods, marked animals do not lose their marks, and all marks are reported upon recovery (Seber 1982). For many population estimators, the assumption of a closed population (no births, deaths, immigration, or emigration) must also be met (Boulanger and Krebs 1996; Kendall 1999). In addition, for both open and closed population estimation techniques, random sampling is crucial in obtaining accurate estimates (Krebs 1999).

This study does not adhere to the required assumptions. All horseshoe crabs did not have an equal probability of capture. An animal observed spawning at the beach early in the sampling schedule may have a lower probability of returning to the beach at a later date (Thompson 1999). Horseshoe crabs of different sizes and ages may also have different propensities to spawn at certain times. However, these types of variability in capture probability may be accounted for using Program CAPTURE (Rexstad and Burnham 1991). Three primary sources of variation that can cause changes in capture probabilities have been identified and are considered when making population estimates in CAPTURE (Boulanger and Krebs 1996; Krebs 1999). There are 8 possible models that CAPTURE may use to make population estimates. These include: a null model (o), which assumes that all animals have equal catchability; a time (t) model, which assumes that the probability of capture is a function of time; an heterogeneity (h) model, which assumes that each animal has a constant individual capture probability, which may differ between individuals; a behavior (b) model, which assumes that an individual animal changes its capture probability after it is captured the first time; and 4 models that are combinations of all 3 sources of variation (th, tb, bh, and tbh; Boulanger and Krebs 1996; Krebs 1999). Presently there is not an estimator for the full model that incorporates time, heterogeneity, and behavior (Krebs 1999). CAPTURE uses a series of goodness-of-fit tests and discriminant analysis to determine which model is most appropriate for a given set of data (Otis et al. 1978). However, high capture probabilities are typically needed to obtain reliable data from mark-recapture studies for population estimation (White et al. 1982; Krebs 1999). In this study, capture probabilities, as estimated in CAPTURE, ranged from 0.0301 to 0.0947. Therefore the population estimates from CAPTURE are probably inaccurate, but may be useful for trend detection.

In addition, the assumption of closure may have been violated. It has been suggested that multiple groups of spawning horseshoe crabs possibly utilize the same shoreline, arriving at different times within the season (Shuster and Botton 1985). If this is the case in Tom's Cove immigration and emigration would occur throughout the sampling period. If so, an open population model would be more appropriate for population estimations. The Jolly-Seber estimate of 1,282 horseshoe crabs may be a more accurate estimate of the actual numbers utilizing the northern shoreline of Tom's Cove as a spawning area in 2004. However, the Jolly-Seber model is very sensitive to violations of the assumption of equal catchability and therefore likely produces a biased estimate (Krebs 1999). For this reason, estimates from Jolly-Seber are more appropriate for trend detection than for predicting the actual population size. Assuming the biases present in estimates from Program CAPTURE and the Jolly-Seber model do not change over time, the biased estimates of population size could be reliable indicators of population change over time (Krebs 1999). Information from this study cannot assess whether the spawning population at Tom's Cove is an open or closed population. Additionally, because all assumptions were not met, data from this study are not appropriate for determining abundance of horseshoe crabs spawning at Tom's Cove.

Considerations for Future Surveys

Monitoring spawning horseshoe crab numbers is difficult because distribution of spawning horseshoe crabs can change considerably from year-to-year based on weather and other factors (Berkson and Shuster 1999). It has been suggested that if spawning conditions are less than ideal throughout the spawning season, then all horseshoe crabs may be forced to spawn over a shorter time period (Shuster and Botton 1985). If this occurs, then the density of horseshoe crabs observed on the beach during this shorter time span would be greater than the densities observed each night over a longer spawning period (Shuster and Botton 1985). This type of behavior may explain why 1,192 horseshoe crabs were observed spawning on one night in 2003, while a maximum of 94 horseshoe crabs was observed spawning on one night over the same area in 2004. In addition, the maximum number observed in 2003 was observed one hour after high tide. To reduce variability, all sampling in 2004 was conducted at high tide. It is possible that peak spawning activity in Tom's Cove does not occur at high tide (as predicted for the Delaware Bay) but occurs when the tide is receding. At high tide in Tom's Cove the water level is often above the vegetation line. It may be more beneficial for horseshoe crabs to delay spawning until the tide is receding to increase access to more suitable nesting areas.

High winds and low temperatures may prevent horseshoe crab spawning. Thompson (1999) found that in South Carolina spawning activity rarely occurred at water temperatures less than 20°C. Low water temperatures may be the cause of low spawning numbers observed in the 2004 survey. In addition, wind may affect wave height which has a significant impact on spawning activity (Shuster 1958; Smith et al. 2002). For example, during a prevailing northeasterly wind in the Cape May, New Jersey region of the Delaware Bay, horseshoe crabs will remain offshore until it becomes calm (Shuster 1953) or they will move to the more protected Delaware shore to spawn (Shuster 1958). Similarly, wind conditions may affect which shoreline horseshoe crabs utilize for spawning in Tom's Cove. In 2003, the maximum number of horseshoe crabs spawning on a 950-meter portion of the southern shoreline at Tom's Cove Hook was 34. In 2004, 135 horseshoe crabs were observed spawning over a 1400-meter section of shoreline of Tom's Cove Hook. These differences may be due to timing of the survey. However, they could also be reflective of spatial variation in horseshoe crab spawning preferences from year to year.

Currently it is not evident if horseshoe crabs spawning in Tom's Cove are members of a localized population that utilize Tom's Cove exclusively as a spawning area. It may be possible that changes in observed spawning numbers at Tom's Cove are indicative of migration between Tom's Cove and other spawning areas. A male horseshoe crab tagged in Tom's Cove on May 19th, 2004 was resighted alive on August 21st, 2004 at Fowler's Beach, Delaware Bay. This suggests that horseshoe crabs spawning in Tom's Cove may not remain in that area. In addition to utilizing Tom's Cove as a spawning area, they may also be part of the larger spawning population in the Delaware Bay. Annual changes in environmental conditions or random migration with ocean currents may affect which estuaries horseshoe crabs utilize for spawning.

RECOMMENDATIONS

The ASMFC states that spawning surveys should initially include as much of the temporal and spatial extent of spawning usage as possible to better characterize the distribution of spawning horseshoe crabs and identify key spawning locations (ASMFC 1998a). Once horseshoe crab spawning distribution and activity are well understood, more extensive surveys can be developed to monitor key areas for the purpose of identifying trends in abundance (ASMFC 1998a). In addition to monitoring horseshoe crab spawning in Tom's Cove, other possible areas for spawning habitat along the mid-Atlantic coastline should be investigated. If horseshoe crabs are migrating between Tom's Cove and other areas to spawn, these alternate locations need to be identified to increase understanding of year-to-year differences in spawning counts.

This study provides information on horseshoe crab usage in areas of Tom's Cove. Sex ratios of spawning horseshoe crabs in this area are much lower than reported in other surveys. Shuster and Botton (1985) found that sex ratios on spawning beaches ranged from 3 males for every female to 5 males for every female. In the Delaware Bay spawning survey, the median sex ratio was reported as 3.5 males for every female (Smith et al. 2002). At Tom's Cove, sex ratios never exceeded 1.56 males per female on any night. In addition, Brockmann and Penn (1992) found in their spawning survey of a site along the Gulf Coast of Florida that over half of the males marked were resighted a second time within the same year, while only a third of the females returned. In this study, the resight rates for males and females were equal. These differences may indicate that horseshoe crab spawning activity in Tom's Cove is unique and should be studied further.

Given the uncertainty regarding horseshoe crab spawning behavior in this area, more comprehensive surveying should be conducted in the future. With a longer time series of data, understanding of spawning activity in the area will be improved. Ideally, equal portions of the northern and southern shorelines should be monitored simultaneously to observe spatial variation in spawning activity within the cove. For at least the new and full moon high tides of May and June, the sampling period should be lengthened to include a minimum of 2 hours before and 2 hours after high tide to increase understanding of peak spawning times as related to tide height in Tom's Cove. Days surrounding and including the new and full moon high tides in late June and July should also be surveyed to investigate the possibility of delayed spawning in certain years. Finally, weather conditions, especially wind speed and direction and water temperature, should also be considered and recorded when conducting the spawning survey (Smith et al. 2002) .

The mark-recapture component of this survey should also be continued. Population estimates, though biased, may be useful in trend detection, which could alert us to possible declines in the horseshoe crab population in the Tom's Cove area. However, if population estimates are found to be of little use, marking should still be continued. Marking of horseshoe crabs will identify individuals, which will increase understanding of horseshoe crab behavior as it relates to spawning. With individually marked horseshoe crabs, it is possible to determine whether different horseshoe crabs are spawning each night or if the same individuals are being surveyed over multiple nights.

An additional research need identified by ASMFC is the determination of beach site fidelity by horseshoe crabs (ASMFC 1998b). Through marking, movement patterns of horseshoe crabs spawning in Tom's Cove can be tracked to help determine whether they are members of a localized population or part of a larger population of horseshoe crabs in the mid-Atlantic region. This type of information is useful in determining what scale of management is necessary in the mid-Atlantic region. Over time, examination of movement patterns will help us understand whether changes in the numbers of horseshoe crabs at Tom's Cove are due to actual changes in the population size of horseshoe crabs or movement of horseshoe crabs between Tom's Cove and other spawning areas.

REFERENCES

ASMFC (Atlantic States Marine Fisheries Commission). 1998a. Horseshoe crab stock assessment report for peer review. Stock Assessment Report No. 98-01 of the Atlantic States Marine Fisheries Commission. Richmond, VA. 57pp.

_____. 1998b. Interstate fishery management plan for horseshoe crab. Fishery management report No.32. Richmond, VA, Atlantic States Marine Fisheries Commision**:** 58pp.

_____. 2004. Horseshoe crab 2004 stock assessment report of the Atlantic States Marine Fisheries Commission. Washington, D.C. 87pp.

Berkson, J. and C. N. Shuster, Jr. 1999. The Horseshoe Crab: The Battle for a True Multiple-use Resource. *Fisheries Management* 24(11):6-11.

Boulanger, J. G. and C. J. Krebs. 1996. Robustness of capture-recapture estimators to sample biases in a cyclic snowshoe hare population. The Journal of Applied Ecology 33(3):530-542.

Brockmann, H. J. and D. Penn. 1992. Male mating tactics in the horseshoe crab, *Limulus polyphemus*. Animal Behaviour 44:653-665.

Castro, G. and J. P. Myers. 1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. The Auk 110(4):927-930.

CNWR (Chincoteague National Wildlife Refuge). 1993. Chincoteague National Wildlife Refuge Master Plan. Assateague Island, Virginia, U.S. Fish and Wildlife Service, U.S. Dept. Interior**:** 125 pp.

Harrington, B. and C. N. Shuster, Jr. 1999. Crab crisis at Delaware Bay - commercial bait collectors are robbing migratory shorebirds of essential food. Defenders 74(3): 31-35.

HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast. Report to the Atlantic States Marine Fisheries Commission. Washington, D.C. 48pp.

Kendall, W. L. 1999. Robustness of closed capture-recapture methods to violations of the closure assumption. Ecology 80(8):2517-2525.

Koper, N. and R. J. Brooks. 1998. Population-size estimators and unequal catchability in painted turtles. Canadian Journal of Zoology 76:458-465.

Krebs, C. J. 1999. Ecological methodology. 2nd edition. Addison-Wesley Educational Publishers, Inc. Menlo Park, California.

Menkens, G. E., Jr. and S. H. Anderson. 1988. Estimation of small-mammal population size. Ecology 69(6):1952-1959.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:7-135.

Rexstad, E. and K. P. Burnham. 1991. User's guide for interactive Program CAPTURE. Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University, Fort Collins, Colorado.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. 2nd edition. Edward Arnold, London.

Shuster, C. N., Jr. 1958. On morphometric and seriological relationships within the Limulidae, with particular reference to *Limulus polyphemus* (L.). *Dissertation Abstracts* 18:371-372.

_____. 1992. Xiphosurida. Pages 590-594 *in* McGraw-Hill Encyclopedia of Science and Technology. 7th edition. McGraw-Hill Book Company, Inc, New York. Boston, CRC Press, Boca Raton, Florida.

_____. and M. L. Botton. 1985. A contribution to the population of horseshoe crabs, *Limulus polyphemus* (L.), in Delaware Bay. Estuaries 8(4):363-372.

Smith, D. R., P. S. Pooler, B. L. Swan, S. F. Michels, W. R. Hall, P. J. Himchak, and M. J. Millard. 2002. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: implications for monitoring. Estuaries 25(1):115-125.

Thompson, M. 1999. Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in the South Atlantic Bight. Master's thesis, University of Charleston, Charleston, South Carolina.

Walls, B. A. 2001. A Horseshoe Crab (*Limulus polyphemus*) Demographic Study. Fisheries and Wildlife Sciences. Blacksburg, Virginia Polytechnic Institute and State University**:** 70 pp.

White, G. C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, 120-138.

White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory Publication LA-8787-NERP 235 pp.

Location	Date	Time	Total Number of Horseshoe Crabs	Number of Females	Number of Males	Sex Ratio M:F
Tom's Cove Hook	5/13/2003	1838	1	0	1	n/a
Tom's Cove Hook	5/14/2003	1928	9	4	5	1.25
Tom's Cove Hook	5/28/2003	1919	34	16	18	1.13
Woodland Trail	5/29/2003	1956	438	186	252	1.35
Woodland Trail	5/29/2003	2056	593	259	334	1.29
Woodland Trail	5/30/2003	2031	836	358	478	1.34
Woodland Trail	5/30/2003	2131	1192	533	659	1.24
Woodland Trail	5/31/2003*	0902	36	16	20	1.25
Woodland Trail	6/02/2003	1021	2	1	1	1.00
Woodland Trail	6/03/2003	1104	0	0	0	n/a
Woodland Trail	6/14/2003*	0827	3	2	1	0.50
Woodland Trail	5/17/2004	1954	93	42	51	1.21
Woodland Trail	5/18/2004	2030	73	33	40	1.21
Woodland Trail	5/19/2004*	2105	94	45	49	1.09
Tom's Cove Hook	5/19/2004*	2105	135	60	75	1.25
Woodland Trail	5/20/2004	2139	77	34	43	1.26
Woodland Trail	5/21/2004	2214	29	13	16	1.23
Woodland Trail	6/01/2004	1921	21	10	11	1.10
Woodland Trail	6/02/2004	2011	91	40	51	1.28
Woodland Trail	6/03/2004*	2101	61	28	33	1.18
Woodland Trail	6/04/2004	2154	86	38	48	1.26
Woodland Trail	6/05/2004	2251	82	32	50	1.56
* New moons occurred on May 15th and June 14th, 2003 and May 19th, 2004 Full moons occurred on May 31st, 2003 and June 3rd, 2004						

Table 4.1 Numbers of horseshoe crabs observed spawning on the northern (Woodland Trail) and southern (Tom's Cove Hook) shorelines of Tom's Cove, Assateague Island, Virginia in 2003 - 2004.

Table 4.2 Selection criteria, population estimates, and standard errors of models in Program CAPTURE for estimates of horseshoe crabs spawning over a 900-meter section of the northern shoreline of Tom's Cove, Assateague Island, Virginia from May 17-21 and June 1-5, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.13	0.06	0.00	0.13	0.79	1.00	0.27	0.32
Population Estimate	1067	1015	704	482	1053	1282	9583	n/a
Standard Error	150.65	66.70	223.90	183.72	147.80	233.42	35178	n/a
95% Confidence Interval	n/a	n/a	n/a	n/a	n/a	919-1852	n/a	n/a

Table 4.2a Population estimates of spawning female horseshoe crabs based on mark-recapture data from May 17-21 and June 1-5, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.38	0.00	0.02	0.49	0.35	1.00	0.98	0.74
Population Estimate	794	438	244	171	984	1328	842	n/a
Standard Error	193.72	29.65	40.97	8.788	296.63	469.08	2048.5	n/a
95% Confidence Interval	n/a	n/a	n/a	n/a	n/a	707-2651	n/a	n/a

Table 4.2b Population estimates of spawning female horseshoe crabs based on mark-recapture data from May 17-21, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.75	0.81	0.04	0.00	0.27	0.40	0.44	1.00
Population Estimate	457	324	n/a	343	440	482	n/a	n/a
Standard Error	100.2	25.66	n/a	210.79	94.76	131.12	n/a	n/a
95% Confidence Interval	n/a	280-381	n/a	n/a	n/a	n/a	n/a	n/a

Table 4.2c Population estimates of spawning female horseshoe crabs based on mark-recapture data from June 1-5, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.29	0.39	0.09	0.35	0.70	1.00	0.00	0.69
Population Estimate	1182	1280	954	2047	1166	1913	11424	n/a
Standard Error	141.86	73.02	330.51	2435.1	139.2	338.35	30704	n/a
95% Confidence Interval	n/a	n/a	n/a	n/a	n/a	1378-2726	n/a	n/a

Table 4.2d Population estimates of spawning male horseshoe crabs based on mark-recapture data from May 17-21 and June 1-5, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.38	0.00	0.06	0.50	0.31	0.93	1.00	0.74
Population Estimate	1310	538	298	214	1278	1949	1993	n/a
Standard Error	354.28	32.91	46.79	13.40	343.4	732.54	6908.4	n/a
95% Confidence Interval	n/a	n/a	n/a	n/a	n/a	260-46770	n/a	n/a

Table 4.2e Population estimates of spawning male horseshoe crabs based on mark-recapture data from May 17-21, 2004.

Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Selection Criteria	0.75	0.99	0.02	0.57	0.00	0.86	0.03	1.00
Population Estimate	382	385	n/a	n/a	367	644	38465	n/a
Standard Error	56.10	27.73	n/a	n/a	52.52	152.40	n/a	n/a
95% Confidence Interval	n/a	337-445	n/a	n/a	n/a	n/a	n/a	n/a

Table 4.2f Population estimates of spawning male horseshoe crabs based on mark-recapture data from June 1-5, 2004.

Tag Number	Sex	Release Date	Resight Date	Condition	Resight Location
88532	F	5/21/2004	9/5/2004	Dead	Tom's Cove
88539	F	5/21/2004	5/30/2004	Dead	Tom's Cove
88828	M	6/2/2004	8/28/2004	Unknown	Tom's Cove
88835	M	6/3/2004	8/8/2004	Dead	Assateague Bay, MD
89124	M	5/19/2004	8/21/2004	Alive	Fowler's Beach, DE
89125	M	5/20/2004	9/10/2004	Dead	Assateague Bay, MD
89224	M	5/17/2004	7/29/2004	Alive	Tom's Cove
89226	M	6/2/2004	7/26/2004	Alive	Assateague Beach, VA
89240	M	6/2/2004	10/3/2004	Alive	Gargetha, VA

Table 4.3 Release and resight information for horseshoe crabs tagged and released in Tom's Cove, Assateague Island, Virginia.

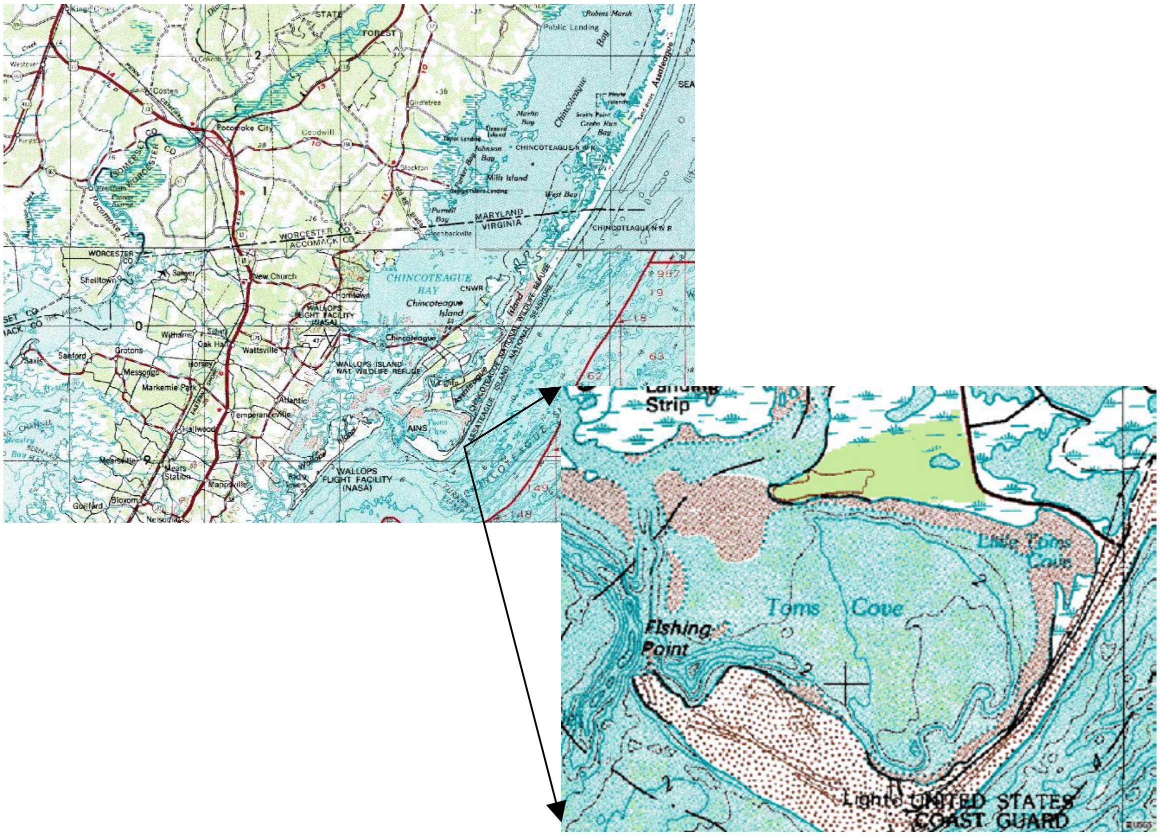

Figure 4.1 Map of Tom’s Cove, Assateague Island, Virginia, USA.

APPENDIX 1 – Significance of between year comparisons of sex distribution, age distribution, stage class distribution, and mean interocular widths of horseshoe crabs sampled from 1999-2004. (* indicates p-values < 0.05 and ** indicates p-values < 0.01)

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.1029	-				
2001	p = 0.5393	p = 0.0009**	-			
2002	p = 0.0047*	p = 0.0322*	p < 0.0001**	-		
2003	p = 0.7897	p = 0.0017**	p = 0.1419	p < 0.0001**	-	
2004	p = 0.9824	p < 0.0001**	p = 0.2492	p < 0.0001**	p = 0.3856	-

Table A1.1 Significance of differences observed between sex ratios of all horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.7267	-				
2001	p = 0.3042	p = 0.0635	-			
2002	p = 0.0042*	p < 0.0001**	p < 0.0001**	-		
2003	p = 0.7314	p = 0.2178	p = 0.2523	p < 0.0001**	-	
2004	p = 0.1340	p = 0.0007**	p = 0.7420	p < 0.0001**	p = 0.0025**	-

Table A1.2 Significance of differences observed between sex ratios of adult horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.9855	-				
2001	p = 0.6649	p = 0.1707	-			
2002	p = 0.7003	p = 0.5094	p = 0.1982	-		
2003	p = 0.6661	p = 0.0137	p = 0.9509	p = 0.1621	-	
2004	p = 0.6198	p = 0.0011**	p = 0.8977	p = 0.1280	p = 0.5973	-

Table A1.3 Significance of differences observed between sex ratios of juvenile horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 1999 through 2004.

Juvenile	Newly Matured	Young Adult	Middle Aged	Old Aged
p = 0.0595	p = 0.3314	p = 0.0003**	p = 0.0284*	p = 0.2717

Table A1.4 Significance of differences observed in stage class distributions between the years 2000 and 2001of all horseshoe crabs sampled in Chincoteague, Virginia.

Stage Class		1999	2000	2001
Juvenile	1999	-		
	2000	$p < 0.0001$**	-	
	2001	$p = 0.0126$*	$p < 0.0001$**	-
Newly Matured	1999	-		
	2000	$p = 0.0006$**	-	
	2001	$p = 0.0004$**	$p = 0.3529$	-
Young Adult	1999	-		
	2000	$p = 0.0894$	-	
	2001	$p = 0.0010$**	$p = 0.0004$**	-
Middle Aged	1999	-		
	2000	$p = 0.2260$	-	
	2001	$p = 0.4601$	$p = 0.5910$	-
Old Aged	1999	-		
	2000	$p < 0.0001$**	-	
	2001	$p = 0.0220$*	$p = 0.0270$*	-

Table A1.5 Significance of differences observed in stage class distributions of all horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 1999 through 2001.

Stage Class		2002	2003	2004
Juvenile	2002	-		
	2003	$p < 0.0001$**	-	
	2004	$p < 0.0001$**	$p < 0.0001$**	-
Newly Matured	2002	-		
	2003	$p = 0.0193$*	-	
	2004	$p = 0.2028$	$p = 0.0057$**	-
Young Adult	2002	-		
	2003	$p = 0.0840$	-	
	2004	$p = 0.8575$	$p = 0.0015$**	-
Middle Aged	2002	-		
	2003	$p < 0.0001$**	-	
	2004	$p < 0.0001$**	$p < 0.0001$**	-
Old Aged	2002	-		
	2003	$p < 0.0001$**	-	
	2004	$p < 0.0001$**	$p = 0.0247$*	-

Table A1.6 Significance of differences observed in stage class distributions of all horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 2002 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p < 0.0001**	-				
2001	p = 0.0126*	p < 0.0001**	-			
2002	p = 0.6373	p < 0.0001**	p < 0.0001**	-		
2003	p < 0.0001**	p = 0.0019**	p = 0.0078**	p < 0.0001**	-	
2004	p < 0.0001**	p = 0.0990	p < 0.0001**	p < 0.0001**	p < 0.0001**	-

Table A1.7 Significance of differences observed between age ratios of all horseshoe crabs sampled in Ocean City, Maryland between each pair of years from 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.1173	-				
2001	p = 0.4041	p = 0.3235	-			
2002	p = 0.4198	p = 0.1566	p = 0.8550	-		
2003	p = 0.2642	p = 0.3293	p = 0.8236	p = 0.5388	-	
2004	p = 0.0841	p = 0.9450	p = 0.2818	p = 0.0442*	p = 0.1326	-

Table A1.8 Significance of least squares mean comparisons of interocular width of adult female horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland among the years 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.3955	-				
2001	p = 0.1085	p = 0.1779	-			
2002	p = 0.6908	p = 0.0788	p = 0.0065**	-		
2003	p = 0.6529	p = 0.0153*	p = 0.0003**	p = 0.9987	-	
2004	p = 0.0447*	p = 0.0409*	p = 0.7387	p = 0.0001**	p < 0.0001**	-

Table A1.9 Significance of least squares mean comparisons of interocular width of adult male horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland among the years 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p < 0.0001**	-				
2001	p < 0.0001**	p = 0.0488*	-			
2002	p = 0.0074**	p = 0.0004**	p = 0.0027**	-		
2003	p < 0.0001**	p < 0.0001**	p = 0.0018**	p = 0.0617	-	
2004	p < 0.0001**	p < 0.0001**	p = 0.0490*	p = 0.0137*	p = 0.0252*	-

Table A1.10 Significance of least squares mean comparisons of interocular width of juvenile female horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland among the years 1999 through 2004.

	1999	2000	2001	2002	2003	2004
1999	-					
2000	p = 0.5471	-				
2001	p = 0.3289	p = 0.0002**	-			
2002	p = 0.0799	p = 0.0327*	p = 0.1098	-		
2003	p = 0.2165	p = 0.0001**	p = 0.1596	p = 0.2050	-	
2004	p = 0.1163	p < 0.0001**	p = 0.0003**	p = 0.4185	p = 0.0013**	-

Table A1.11 Significance of least squares mean comparisons of interocular width of juvenile male horseshoe crabs sampled in Chincoteague, Virginia and Ocean City, Maryland among the years 1999 through 2004.

VITA

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