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Movement Patterns and Population Genetics of the American Horseshoe Crab in Relation to Long Island Sound Conservation Strategies

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ABSTRACT

The Connecticut Department of Environmental Protection (CTDEP) established three no-harvest zones for the horseshoe crab (*Limulus polyphemus*) population as part of a conservation plan for the species. Data from a long-term mark/recapture study of horseshoe crabs in conjunction with a microsatellite-based genetic survey of the population were analyzed to determine if this plan was appropriate to conserve genetic diversity and broaden our knowledge of movement patterns of *Limulus* in Long Island Sound (LIS). To date, ~53,000 crabs have been tagged over a 10 year period through the Project *Limulus* program with an annual average recapture rate of 12 to 15%. In addition to the ongoing tagging study, 187 horseshoe crabs collected from 5 distinct sites spanning the geographic extent of Long Island Sound (Rye and Mt. Sinai, NY; Milford, New Haven, and Groton, CT) were genotyped for 11 microsatellite loci to determine the overall genetic health of the LIS population and determine if regional genetic differentiation was sufficient to identify sub-populations within this region. The genetic data indicates that the LIS *Limulus* population is genetically homogenous with no signs of inbreeding and substantially similar to other Mid-Atlantic populations. Data from the mark-recapture study indicate significant migration east and west along the north shore of LIS relative to the original tag site and in addition cross LIS migrations have also been observed. Therefore, the locations of the established no-harvest zones are appropriate to conserve genetic diversity. However, based on their tri-state migration patterns, a multi-state management strategy is needed for the LIS horseshoe crab population.

INTRODUCTION

The American Horseshoe Crab, *Limulus polyphemus,* lives along the Atlantic coast of the United States from Maine to the Yucatan Peninsula (Anderson and Shuster, 2003). *L. polyphemuss* is an economically and ecologically important species. Economically, *L. polyphemus* hemolymph is harvested for the multi-million dollar biomedical industry that uses the blood-clotting compound *Limulus* Amoebocyte Lysate (LAL). This product is mainly used to detect pathenogenic endotoxins in vaccines (Berkson and Shuster 1999). Adult horseshoe crabs are commercially harvested for use as bait in the eel and whelk fisheries (Manion et al., 2000; Ferari and Targett, 2003). Ecologically, horseshoe crabs are important members of food webs up and down the coast of the eastern United States and are biologically linked to many different species. They provide habitat for more than 20 epibiont species, one is only found on horseshoe crabs (Turner et al., 1988; Dietl et al., 2000; Grant, 2001). Of particular importance is their tight ecological link to shorebirds. *Limulus* eggs are a major food source for migrating shorebirds (Castro and Myers, 1993; Clark et al., 1993; Botton et al., 1994). While there have been extensive studies conducted on the populations of horseshoe crabs which live along the mid-Atlantic coast, there has been a lack of research conducted on the horseshoe crabs which inhabit the New England region, and specifically the crabs which live in the Long Island Sound.

Project *Limulus* is a broad scale research initiative which is focused on the horseshoe crabs which inhabit Long Island Sound. The overall goals of Project *Limulus* are to understand the basic population dynamics and genetics of the crabs, to understand their links to other species, and to develop management plans for the conservation of *Limulus* (Beekey et al., 2008). Project Lim-molecular is a sub-branch that focuses on the molecular analysis of the

population, with goals to establish the structure of the population genetics of the horseshoe crabs as well as to determine the effectiveness of the established conservation plans in place.

The harvest of horseshoe crabs is permitted within Long Island Sound by New York and Connecticut state laws. However, in 2006, the Connecticut Department of Environmental Protection (CTDEP) established three no-harvest zones within LIS in an attempt to provide horseshoe crabs with the opportunity to increase their population size. These no-harvest zones are located at Milford Point Beach in Milford, Sandy Point Beach in New Haven, and Menunketesuck Island in Westbrook, CT. The Connecticut shoreline is a total of 110 miles long, not including near-shore islands. Milford Point and Sandy Point are located 11 miles apart, and Menunketesuck Island is another 25 miles from Sandy Point. Due to the proximity of the no-harvest zones, we investigated whether they would be effective in maintaining the genetic diversity of the horseshoe crabs in LIS.

METHODS

Mark Recapture study:
Project *Limulus* researchers and volunteers have been tagging horseshoe crabs in LIS since 2000. From 2000–2007 yellow Floy Cinch-tags (model FT-4, 8": http://www.floytag.com) and from 2008-2010, U.S. Fish and Wildlife Service issued white disc tag were attached to crabs using a #2 Yellow scratch awl to make a hole in the lower rear of either the right or left posterior side of the prosoma. The tag number, sex (based on the morphology of the pedipalps), and mating behavior were recorded (Mattei et al., 2010). Recapture data were obtained by researchers and volunteers who reported organisms to the USFW.

Microsatellite genotype determination:
Tissue samples were collected from Rye (39) and Mt. Sinai (37), NY; Milford (38), New Haven (35), and Groton (38), CT. The distal segment of the 3rd walking leg of each individual was excised, stored on ice in the field and subsequently frozen at -80 degrees C until further processing. Frozen muscle tissue dissected from stored samples was ground in liquid nitrogen without thawing, and stored at -80 degrees C. DNA was isolated from ground tissue with the Gentra Puregene Cell Kit (Quiagen, Valencia, CA) following the manufacturers protocols.. Microsatellite Loci (D60, A315, A37, A67, D3, A42, A40, A5, A64, A52, A38) were genotyped by fluorescent primer PCR using primer pairs and amplification conditions previously described in King and Eackles (2004). Amplified Microsatellite Loci were sized by capillary electrophoresis at the DNA Analysis Facility on Science Hill (Yale University) using an Applied Biosystems 3730xl 96-Capillary Genetic Analyzer (DS31 5 Color Dye Family, Liz-500 size standard). Electrophoretogram output was analyzed for determination of fragment size and allele assignment using GeneMarker (Softgenetics, State College, PA). Allele Identity and Frequency Statistics and Analysis of Molecular Variance (AMOVA) between populations were calculated using GENEALEX (Peakall and Smouse, 2006). Fixation indices and Hardy-Weinberg Equilibrium analyses were calculated with GenePop (Rousset, 2008).

RESULTS

The tagging effort has increased throughout the past 10 years (Figure 1). From 1997-2000 a few hundred crabs were tagged, and in 2000 the first 6 recaptures were found. In 2010, with the help of hundreds of volunteers, over 14,000 crabs were tagged and 3295 were recaptured. According to the mark recapture data 95% of horseshoe crabs tagged in LIS are recaptured within LIS. Horseshoe crabs migrate all over the Sound; this includes crossing state boundaries and moving from Connecticut to New York and Rhode Island. 87% of the horseshoe crabs recaptured within 30 days of their tag date are found at the site that they were tagged at. With increasing time after tagging there is an increase in the number of crabs that are found further away from their original tag site. Around 33% of the crabs recaptured after 200 days after tagging were found at the same location (Figure 2).

To date a total of 187 individuals from 5 different beach locations have been amplified across 11 different microsatellite loci. There is no evidence showing any regional genetic differences within the Long Island Sound population. A pairwise population comparison test was performed in order to detect any subpopulation division. There was no significant differentiation between any of the beach locations we tested, significance defined by $P > 0.05$ (Table 1). The populations of horseshoe crabs within the LIS also are in a state of good relative genetic health. The population is in Hardy-Weinberg equilibrium, and there is no evidence of inbreeding (F_{is} -0.015 value is close to zero). Thirdly, the population is comparable to other Mid-Atlantic populations of horseshoe crabs. The number of effective alleles and heterozygosity of the populations are not significantly different from published data of populations in the Greater Delaware Bay Region (King, 2005).

DISCUSSION

The mark/recapture data shows that 95% of the horseshoe crabs tagged within the Long Island Sound stay there, although they migrate all through the Sound. The horseshoe crabs show site affinity within a breeding season, although as time goes on more crabs are found further away from their original tagging site.

The genetic data show that the horseshoe crabs are one population and can be managed as a unit. It also suggests that the established no harvest zones are appropriately located to conserve the genetic diversity of the horseshoe crabs in the LIS. Due to the observed movement patterns of the crabs a multi-state management plan should be developed to further conserve the LIS horseshoe crab population.

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	Rye	Milford	New Haven	Groton
Rye			No Significant Differentiation P > 0.05	
Milford	0.012			
New Haven	0	0.019		
Groton	0.011	0.001	0.011	
Mt Sinai	0.002	0.008	0 0	

TABLE 1. Estimates of genetic differentiation between populations (Amova-Rst). Probability values based on 99 permu.

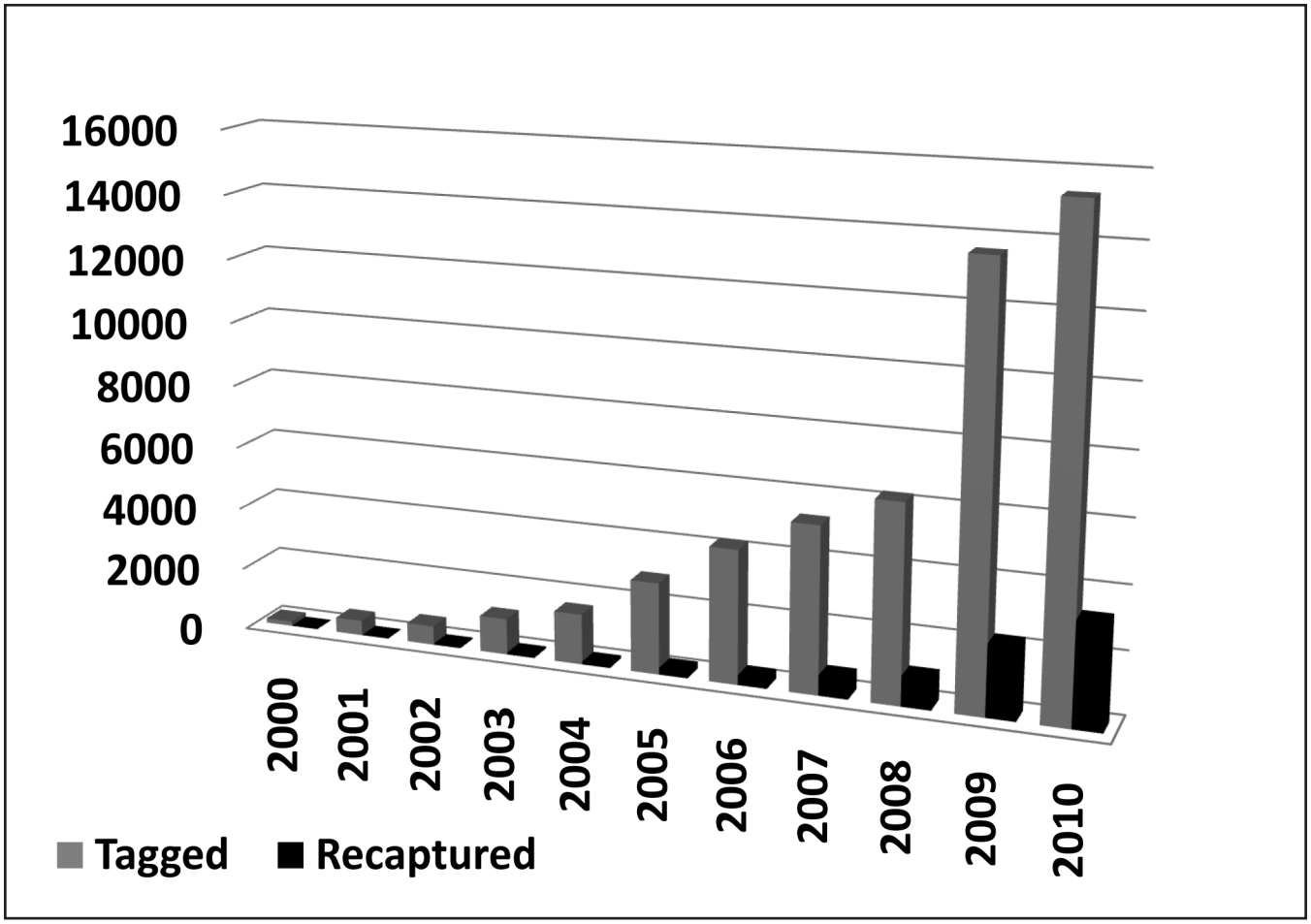

FIGURE 1. Number of tagged and recaptured horseshoe crabs per year across spawning beaches throughout Long Island Sound.

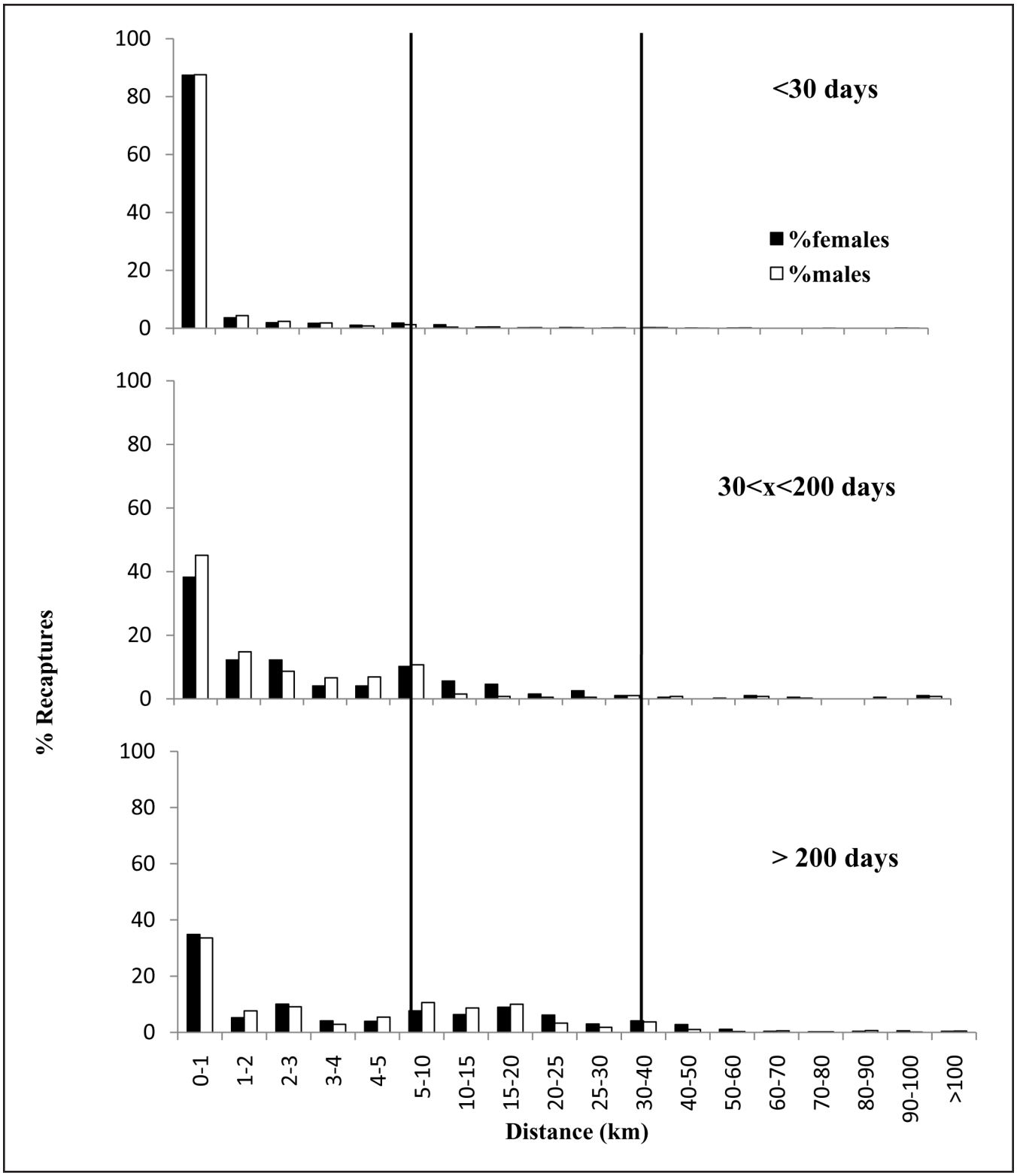

FIGURE 2. Number of recaptured female and male horseshoe crabs plotted by distance and time (less than 30 days, between 30 and 200 days, and greater than 200 days) from when they were originally tagged.