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The Breeding Behavior and Patterns of Movement of Horseshoe Crabs, *Limulus polyphemus*, in the Vicinity of Breeding Beaches in Apalachee Bay, Florida

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ABSTRACT: **Breeding activity of the horseshoe crab, *Limulus polyphemus*, was quantitatively monitored in Apalachee Bay, Florida, throughout one breeding season. Breeding peaked at times of full and new moon at the hour of high tide. Breeding activity was heavier on night tides than on corresponding day tides of the same date. Males routinely outnumbered females and indications of sperm competition were present. Many horseshoe crabs buried in the intertidal zone throughout the subsequent low tide and returned to the beach to breed again on the following high tide.**

A tagging study of the horseshoe crab indicated that male animals return to breeding beaches more frequently than females. Most animals tagged at breeding beaches did not move away from the tagging site during a breeding season and were recovered at the point of release. No long-range movements were noted. The sex ratio of animals tagged near breeding beaches was predominately male while it was predominately female for animals collected and tagged 3–6 miles offshore. A nine percent recovery rate was achieved.

Existing localized populations are potentially subject to depletion due to heavy collecting pressure on breeding beaches.

Introduction

The sand beach serves as a breeding site for marine animals as phylogenetically diverse as fish (the grunion *Leuresthes tenuis*, for instance), the seven extant species of sea turtles and the horseshoe crab, *Limulus polyphemus*. The use of this narrow zone between land and sea for breeding demands precise orientation in space as well as accurate coordination with tidal cycles. Once this precision is achieved, however, the eggs are well protected from most predators and survival to hatching is correspondingly enhanced.

The massive emergence of horseshoe crabs on beaches of the Atlantic and the Gulf of Mexico to breed is one of the most spectacular phenomena of the sandy shoreline. While the role of wave surge in guiding crabs to and from the beach has been reported (Rudloe and Herrnkind 1976), the phenomenon itself has not been quantitatively described until the present study.

Males move parallel to the shoreline in a meter or more of water until they intercept and attach themselves to females heading directly to the beach (Schuster 1958). The female with attached male proceeds to the beach, and the male fertilizes eggs as they are laid. More than one male may be associated with each female, and a predominance of male crabs is common on breeding beaches. Animals appear at the beaches in large numbers and emerge from the water only in conjunction with breeding activities (Schuster 1958; Sokoloff 1978; Rudloe 1978, 1979a).

The present study quantitatively describes breeding of *Limnulus polyphemus* as it takes place in the field in terms of breeding rhythmicity, sex ratios and post-breeding movements, as indicated by tagging, and

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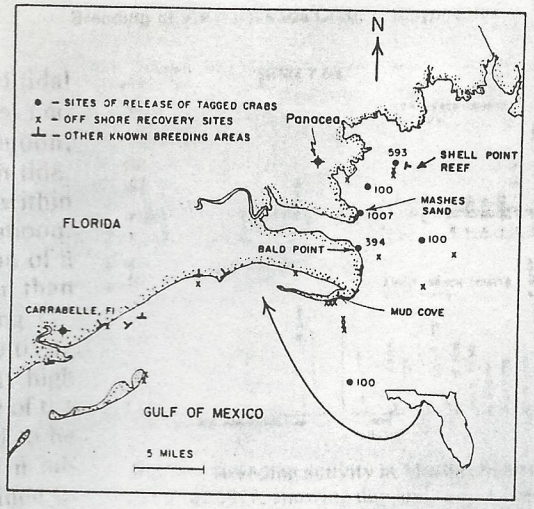

Fig. 1. Chart of Apalachee Bay, Florida, showing study sites, points and number of tag releases, recoveries and other known breeding sites.

examines this behavior pattern for clues to the stimuli controlling its occurrence in both time and space.

Methods and Materials

Mashes Sands, a heavily used breeding beach near the town of Panacea, in Wakulla County, Florida, was monitored throughout several breeding cycles between 1976 and 1978. Animals were counted over a marked 170-meter section of the beach by day from the date of first appearance of breeding in March 1977, to their last appearance the following November. Night tides were monitored from mid-May until November. Water and air temperature, salinity, wave height, wind speed and level of surf on the beach were recorded for each tide as well as sex ratio and number of males associated with each female. All animals on the beach at those times were engaged in breeding or were actively attempting to breed.

To ascertain how far offshore crabs move after leaving the breeding beach, breeding adults were marked over a three-day period at the peak of spawning with one-meter lengths of survey tape as they left the beaches. The tape was attached to a wire inserted through a small hole drilled in the left genal spine with an ice pick. The exposed intertidal area was then searched at the subsequent low tide. Although the animals commonly buried in the sand, they were easily located by the survey tape streamers on the surface. A total of 25 females and 64 males were marked with color coded streamers.

During the fall of 1973 and in April 1974, 144 tagged adult *Limulus polyphemus* were released at various points between Panacea and Carrabelle, Florida, in conjunction with other experiments. In March and April of 1976, and in September of 1977, an additional 2,294 marked animals were released. Animals were tagged with a yellow plastic tag, 2.5 cm in length manufactured by Howitt Plastics of Mollala, Oregon. Tags were attached to the left genal spine (at the rear margin of the prosoma) using stainless steel fishing tackle leader wire fastened with connector sleeves of nickle manufactured by the Berkeley Co. The wire was inserted through a small hole made with an ice pick.

The animals disbursed and/or buried rapidly upon release, so that there was minimal likelihood of premature recovery. In only one instance were animals picked up immediately after release and these data were discarded. Tag loss could easily be recognized if it occurred due to the hole in the prosoma and did not prove to be a significant problem.

Of these crabs, 1,401 were collected from and released at two major breeding beaches: Mashes Sands, on the northern side of Ochlockonee Bay (1,007); and Bald Point, Franklin County, Florida, on the southern side of Ochlockonee Bay (394). In addition, 593 were captured, marked and released at Shell Point Reef, a subtidal sand bar located approximately 3 miles offshore from Panacea, Florida. This sand bar regularly attracts large numbers of *Limulus* during the breeding season despite the fact that it is not emergent. Another 300 were obtained in the Apalachee Bay area from shrimp trawlers, tagged and released at several points several miles offshore. Figure 1 is a charge of the Apalachee Bay area, indicating these points of release as well as locations where crabs were recovered.

Results

Field Breeding Activity

Figure 2 indicates breeding activity at Mashes Sands over a total of 128 tides between March 17 and November 24, 1977.

e breeding showed strong lunar and tidal thmicities, with animals appearing at and hin a few days of the new and full moon, d within 2 hours of the hour of high tide. e absolute peak was nearly always within hours of the actual full or new moon. tivity associated with the full moon of a en month was consistently higher than t on the new moon. No breeding oc- red on neap high tides. Breeding activity s consistently higher on the night high e for each date than on the day tide of the me date, although day tides tended to be ghtly higher in predicted height. In ad- tion, breeding was essentially confined to e night tides from mid-June through the d of August. Thus, a circadian activity ythm with nocturnal peaks of activity is parent in the field in addition to the lunar d tidal breeding periodicity.

The lunar rhythmicity of breeding is es- ecially striking. However, a number of ariables are present in addition to lunar nase and time of day that could conceiv- bly affect the number of animals appearing n the beach. In an effort to identify other actors that might be of significance, a mul- ple regression analysis was performed on e beach count data. Five variables were onsidered as potential sources of influ- nce—lunar cycle, day of the year, wave eight at the beach, predicted tide height, nd the bearing of waves relative to the each. Only lunar cycle, day of year and ave height correlated significantly with the umber of crabs on the beach.

Sex Ratios on Breeding Beaches

Sex ratios over the 66 tides for which reeding activity occurred ranged from 1 to 4 males per female, with a mean of 3.56 nales/female. Higher proportions of males were associated with more individuals which in turn coincided with the lunar cycle. Similarly, when the sex ratio for night tides was compared to that for day tides, night ides were found to have a significantly igher sex ratio with a night mean of 3.98 nales/female and a day mean of 2.87 males/ emale ($t = 4.12$, $p < 0.01$).

Movements Following Breeding

Of the crabs marked with survey tape as hey left the breeding beaches, 11 of 25 fe-

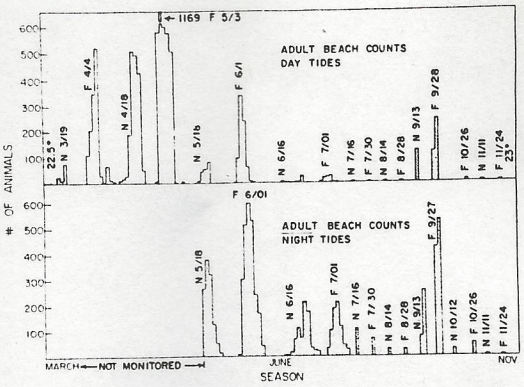

Fig. 2. Breeding activity at Mashes Sands. March–November 1977, showing day and night tides. F = full moon, N = new moon; dates are given below the lunar phases. Day counts were initiated in March, with the first appearance of animals, while night collections were initiated in May. Counts terminated in November when breeding ceased.

males (44%) and 18 males (28%) were relocated buried in the intertidal zone at low tide. Forty-eight percent of the marked females and 39% of the marked males reappeared on the breeding beach at the next high tide, 12 hours after being tagged. The remainder of the sample was presumed to have moved into the subtidal zone. Tag loss was not a factor. Animals returning to the beach after having lost the streamer tag could easily have been recognized as such due to the small hole in the margin of the prosoma. No such animals were found.

Tagging

Of 2,438 tagged adult *Limulus polyphemus*, 788 were females and 1,732 were males, giving a ratio of 2.2 males per female. This reflects the predominance of males to females on the breeding beaches. A total of 211 returns were made, giving a 9% recovery rate.

Of these, 34 were females and 177 were males for a ratio of 5.2 males/females for recovered crabs. Thus, females represented 31% of all tagged animals but only 16% of recovered crabs. The high recovery rate and the increased proportion of males may both be attributed to the fact that 171 or 81% of all recoveries were made at the point of release on the 2 major breeding areas. Of animals recovered at the point of release, only 15 or 9% were female.

TABLE 1. Time elapsed between recoveries for tagged *Limulus* recovered two times or more.

Elapsed Time	# Recoveries	% of Total
≤7 days (same tide series)	106	41%
2 weeks (next spring tide)	54	21%
1 month (next lunar month)	48	19%
6 weeks (spring tide of next month)	8	3%
2–12 months	13	11%
≥1 year (next spring breeding season)	28	11%

TABLE 2. Comparison of animals tagged during spring and in fall at Mashes Sands. No significant difference in size occurred for males or females between spring and fall.

	Spring	Fall
Sample size	395	295
Recovery rate	61 (15%)	36 (12%)
Sex ratio	1.8 M/F	2.9 M/F
Free of fouling	133 (34%)	199 (67%)
Prosomal width, cm	17.1 ± 1.1 (male)	16.7 ± 1.3 (male)
	22.9 ± 1.8 (female)	22.6 ± 1.8 (female)

Only 40 crabs or 19% of those recovered had moved away from the point of release before being recovered. Of those 40, 18 moved to some non-breeding area, usually being recovered by trawlers offshore, while 22 or 10% of the recovered crabs moved to another breeding site between release and recovery. Fifty-two percent were recovered during the same breeding season while 48% were recovered during the following year's season. This is in contrast to animals recovered at the site of release. Of these, 97% were recovered during the same breeding season, and only 3% were recovered during the next year's season. Only 10 or 5% of all recoveries moved between breeding areas within a breeding season.

Of the 300 animals released offshore, 11 were recovered; 5 at Mashes Sands, 3 at Bald Point and 3 offshore after periods ranging from 6 weeks to 21 months. This 4% recovery rate is well below that for the sample as a whole, reflecting both the high site specificity of animals tagged at a breeding site and the smaller sample size employed.

Thirty-eight crabs were recovered more than once, and 87% of observed repeat breeders were males. In most cases, they remained at the point of release. Of the 4 that moved, 3 had been released at offshore sites, then came ashore at Mashes Sands and remained there.

Of 257 intervals between observations of these 38 crabs 41% were less than 7 days indicating repeat breeding within the same series of spring tides (Table 1). Twenty-one percent occurred after a 2-week interval, reflecting repeat breeding on the following spring tide. Eleven percent were recovered after periods of 1 year or more, reflecting the breeding season of the following year. Of these, 15% were females and 85% were males. This is in contrast to recoveries made within the same breeding season, of which only 7% were females.

The maximum time interval for a recovery was 26 months for a male crab, at the end of which the animal was found approximately two miles from its release point. The mean distance travelled for the 40 animals recovered away from the point of release was 4.1 nautical miles (SD 4.1) while the maximum distance travelled was 22 miles, a distance covered in 13 days. The shortest distance was 1.9 miles, the distance between the 2 major breeding sites of Mashes Sands and Bald Point.

A comparison was made of animals tagged at Mashes Sands for spring vs. fall months (Table 2). The sex ratio was more predominantly male in the fall, and approximately twice as many crabs had fouled shells as in the spring. For all crabs tagged in 1976–1977, 32% of the males had clean unfouled shells when tagged as opposed to fifty-two percent of the females, suggesting that females molt more frequently than males. In view of the larger size reached by females, this seems likely.

Four-hundred sixty-five animals were collected offshore by trawling between December 1976 and February 1977, and 100 animals were collected offshore in April and May 1978 (Table 3). Away from the breeding areas the sex ratio is reversed with females outnumbering males up to 2:1 in all collections. Likewise, the 300 animals collected offshore for the tagging study were predominantly female, by 3:2.

. Population characteristics of adult *Limulus* collected offshore in winter and in spring months. Size ntimeters.

Month	#	Mean Size	Size Range	Sex Ratio (F/M)	% Recently Molted	% Soft
er	67	18.5	10.7–26.4	2.0/1	24%	3%
	24	17.8	12.8–24.6	1.7/1	52%	17%
(Apalachee Bay)	125	18.8	9.2–25.4	1.4/1	23%	17%
(St. Joe Bay)	249	17.3	9.4–26.0	1.4/1	23%	17%
av	100	20.1	11.5–25.0	2.5/1	3%	0%

there was so little movement be- breeding beaches, the Lincoln Peter- dex was used to arrive at a rough tion estimate for those animals com- Mashes Sands to breed. More so- ated methods of population estima- ch as those recently used in sea turtle ng beach studies[1] require a saturation effort and were therefore not fea- this study. The total number count- ween September and November 1977, 524 of which 333 were tagged and 18 ecovered. Therefore:

$$\frac{\text{rked animals present}}{\text{ber animals present}} = \frac{\text{total tags released}}{\text{total population}}$$

$$18/1{,}524 = 33/x$$

$$x = 33{,}300$$

nsufficient data precludes such es- for Bald Point and Shell Point Reef, oint typically had three to four times mber of animals as Mashes Sands for ven time. In any case, since the ani- the beach were predominantly male males appear to remain offshore to a extent than do males, this estimate better be viewed as an estimate of the tion size of males using this breeding rather than as an estimate of total tion size.

Discussion and Conclusions

is the first quantitative study of ng behavior in this species. It has revealed striking lunar, as well as tidal rhythmicities, in *Limulus*' breeding activity, associated with clear nocturnal peaks of activity. Nocturnal activity is also characteristic of *Limulus* larvae, but is in contrast to the diurnal activity patterns of juvenile *Limulus* in the intertidal zone (Rudloe 1978, 1979b).

The functional value of these rhythmicities is apparent since eggs are left well above the high tide line and secure from aquatic predators for all but a few days of each month. Subtidal breeding of *Limulus* adjacent to beaches in depths of 1 meter or less has also been observed in Massachusetts (K. French, pers. commun.) and in South Carolina (F. Pearson, pers. commun.). Subtidal breeding was never observed during this study, however, despite frequent monitoring of the offshore zone of the beach to a depth of 1 meter.

Limulus experiences a wide range of tidal patterns and temperature ranges between Maine and Yucatan. Breeding seasons are reported to be more concentrated in time in the more northerly parts of the range (C. Shuster and R. Barlow, pers. commun.). Whether or not the predominance of nocturnal breeding is as strong during mid-summer on the more northerly beaches would also be of interest. Animals from regions having diurnal rather than semi-diurnal tidal frequencies and smaller tidal amplitudes may show significant differences in tidal rhythms of breeding behavior from those reported herein. Comparative quantitative studies of breeding periodicity of *Limulus* in other parts of its range on the Atlantic coast would be most desirable to answer these and other questions.

Sekiguchi, et al. (1977) report comparable lunar breeding patterns for the closely related horseshoe crabs, *Tachypleus gigas*

ardson, J. I., T. H. Richardson, and M. W. . Population estimates for nesting female log- sea turtles (*Caretta caretta*) in the St. Andrew rea of Southeastern Georgia, U.S.A. Unpub- anuscript.

and *Carcinoscorpus rotundicauda*. While *T. gigas* breeds on exposed sand beaches comparable to those preferred by *Limulus polyphemus*, *C. rotundicauda* prefers mud substrates in the mangroves along river shorelines and often breeds on ½ to 1 meter of water at high tide.

The multiple regression analysis showed that wave height had some significant, albeit subordinate, effect on the level of breeding activity on the beach. This is consistent with earlier work (Rudloe and Herrnkind 1976) that indicated *Limulus* uses wave surge as an orientation cue in the vicinity of breeding beaches. This analysis tends to support that conclusion since wave surge is proportional to wave height. Wave surge might conceivably represent a releasing as well as an orienting stimulus for movement of animals to breeding beaches.

There are far more males than females on a beach, reducing the probability of any individual male successfully fertilizing eggs. Under this condition, a male should hypothetically return repeatedly in order to maximize its chances of successful reproduction. The female, however, is assured of reproductive success whenever she comes to the beach and as a result should come less often than does the male. While some females did return after being marked in this study, laying eggs on successive visits, the tagging data showed the preponderance of repeat breeding by male individuals. While females may return several times to breed, the return rate of tagged males greatly exceeded that of females in general.

Parker (1970), in a discussion of sperm competition among insects and its potential behavioral consequences, described several potential strategies of use to males competing for reproductive success. These include extended coupling during breeding to block access to later males to the eggs; a prolonged precopulatory amplexus in which the male remains joined to the female during non-reproductive periods, thus ensuring access at the time of spawning; and sperm plugs with internal fertilization. Guarding of females by males is also a suggested indication of male competition as is avoidance of other males by a mated male.

Limulus polyphemus, with breeding sex ratios as high as 14 to 1 in some instances, provides a good test of this hypothesis. B prolonged breeding and extended ample are prominent features of *Limulus*'s bre ing behavior. Remaining coupled af breeding assures that a given male reta access to the female should she return the beach on the next tide. Animals rem on the beach for up to two hours as the t falls, and coupled pairs are routinely c lected in depths up to 90 feet, far from breeding area. In addition, animals kep large aquaria form attached pairs at all s sons of the year, with the male taking initiative and attaching to the female. other criteria are not appropriate to *Li lus*, since spawning is external and the male controls the movements of the p However, this species does appear to Parker's model and further investigation this point is desirable.

Perhaps one of the most significant res of the tagging program was the great dominance of males among repeat breed This is not surprising in view of the h proportion of males to females on bree beaches and resultant male/male comp tion for access to females. Conversely, s a female is assured of reproductive succe she derives no apparent benefit from peated visits to the breeding beach.

The fact that offshore collections of mals were predominantly female furt suggests that some spatial segregation of sexes may exist along an offshore/onsh gradient, at least during the breeding s son. If this is the case, the overall sex r may be 1:1.

The lack of long-range recoveries and high proportion of recoveries at or near point of release suggests a local rather t a migratory population with a high deg of site specificity during breeding. An ear tagging study of *Limulus* in Massachus (Baptist, et al. 1957) also indicated a l population with little if any long-ra movement of animals. The small numbe recoveries of animals released offshore d not permit any serious conclusions reg ing offshore movements. Similarly, the on the between-year movements must considered as preliminary due to the s number of animals recovered and the imp of any molt that might have occurred dur the intervening winter months.

ag loss was not a major problem in this y. Animals that had lost tags could be gnized upon their reappearance due to persistence of the small hole through ch the tag is fastened. A total of only such animals were noted during the encourse of the study. It is doubtful that ed animals could successfully molt and n the tags. Unlike Crustacea, there is oint where a tag could be placed to ve a molt. However, adult *Limulus* ear to molt with a frequency of once a or less (Rudloe 1978). This molting urs during the winter months, so that the act of molting losses on within breeding son tag returns would not be excessive. Currently, heavy use of *Limulus* in medical research (Cohen 1979) and as in the commercial eel fishery of the ntic coast is occurring. It is estimated discussions with seafood dealers and rmaceutical spokesmen that thousands nimals are being bled and/or destroyed ually. These activities could lead to popion depletion of the horseshoe crab oughout its range, particularly in view of use of breeding beaches as collecting and the preference for large animals. ects to manage *Limulus* populations as arine resource should be initiated if the seshoe crab, famous as a living Paleozoic is to continue its 400 million years of ence into the future.

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