

Breeding Biology of *Oryzomys Palustris*, the Marsh Rice Rat, in Eastern Virginia

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ABSTRACT

The objectives of our study were to determine the age of maturity, litter size, and the timing of the breeding season of marsh rice rats (*Oryzomys palustris*) of coastal Virginia. From May 1995 to May 1996, monthly samples of rice rats were live-trapped in two coastal tidal marshes of eastern Virginia, and then necropsied. Sexual maturity was attained at 30-40 g for both sexes. Mean litter size of 4.63 (n = 16) did not differ among months or in mass or parity classes. Data from two other studies conducted in the same county, one of them contemporaneous, also were examined. Based on necropsy, rice rats bred from March to October; breeding did not occur in December-February. By contrast, rice rats observed during monthly trapping on nearby live-trap grids were judged, using external indicators, to be breeding year-round except January. Compared to internal examinations, external indicators of reproductive condition were not reliable for either sex in predicting breeding status in the marsh rice rat.

INTRODUCTION

Oryzomys palustris (Harlan), the marsh rice rat, has the northernmost range in the genus *Oryzomys*, extending from southern Texas and Florida as far north as southern New Jersey. Apart from *O. couesi* Alston, found in two counties in southern Texas, other *Oryzomys* species are distributed from Mexico to South America (Hall 1981). (NOTE: A recent paper [Hanson et al. 2010], which explores molecular divergence in *Oryzomys*, suggests the possibility that *Oryzomys* in parts of Mississippi and Louisiana should be assigned to *O. texensis*. But even after a possible revision of the taxonomy, *O. palustris* lives in the most seasonal environments for this tropical genus.)

Studies of the reproductive biology of the marsh rice rat have not provided a consensus on the timing and duration of the breeding season. In Mississippi, using assessments from live-trapped animals only, Wolfe (1985) observed breeding year round with the major breeding period from late spring to late autumn. By contrast, in Louisiana, breeding was not observed during two of three winters in which rice rats were trapped (Negus et al. 1961). Edmonds and Stetson (1993) report the breeding season in Delaware usually extends from March to September, but can last into late autumn when environmental conditions are favorable. Svihla (1931) and Worth (1950) report February-October breeding seasons in eastern Texas and Florida, respectively.

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Laboratory studies provide a similar picture of occasional year-round breeding, with Conaway (1954) reporting year-round breeding for captive rice rats from Tennessee.

Whereas small mammals in the tropics often breed year round, in temperate latitudes they commonly cease or reduce breeding in the winter. To compensate for a shorter breeding season, they frequently increase reproduction with larger or more frequent litters or by maturing more quickly. We sought to learn how *O. palustris*, living far from its tropical origins, has adjusted its breeding season near the northern limits of its distribution. We also were interested to learn if litter sizes are similar to southern populations and whether sexual maturity is reached at 30-40 g, as in other regional populations (Conaway 1954, Negus et al. 1961) or if maturity is reached earlier to compensate for a shorter breeding season. The objectives of our study were to determine the age of maturity, litter size, and the timing of the breeding season of marsh rice rats of coastal Virginia. We examined whether rice rats mature at 30-40g and tested the null hypotheses that litter size does not vary throughout the breeding season and that *O. palustris* populations in coastal Virginia have equal breeding intensity for each bimonthly period throughout the year.

MATERIALS AND METHODS

We trapped rice rats monthly from May 1995 through May 1996 from tidal marshes at two sites near Oyster and Townsend in Northampton County on the Eastern Shore of Virginia. Marshes in this region are dominated by *Spartina alterniflora* Loisel, salt marsh grass, *S. patens* Muhl., salt meadow hay, *Juncus roemerianus* Scheele, black needlerush, and *Baccharis halimifolia* L., saltbush. Traplines were placed at the wrack line, i.e., the transition from the *Spartina* spp. and needlerush to saltbush, so all vegetation types were trapped.

We set traplines consisting of 50-60 Fitch live traps (Rose 1994) at 2-3 m intervals in transects on the wrack line just above mean high water at each site. We baited traps with birdseed in the evening and checked them in the early morning. Potentially mature rice rats (≥ 30 g) were euthanized in the field with chloroform and stored in a -20 C freezer until necropsy; smaller rice rats and other species were released. Our methods followed the 1987 guidelines of the American Society of Mammalogists for the use of mammals in research (*ad hoc* Committee 1987).

Necropsy methods and variables measured followed those of Keller and Krebs (1970). We recorded mass (g), total length (mm), and lengths (mm) of the tail, ear, and hind foot and assigned rice rats to sub-adult (31-50 g) and adult (≥ 51 g) age classes following the criteria of Wolfe (1985). We also recorded a fat index for each rice rat with 1 designating no fat, 2 for gonadal fat only, 3 with the addition of inguinal or scapular fat, 4 with the addition of mesenteric fat, and 5 for obese rats. Additional information recorded for females included perforate or imperforate vagina, open or closed pubic symphysis, uterine mass (mg), numbers of embryos, resorbing embryos, and placental scars, and numbers of corpora lutea and corpora albicantia. We classified females as nulliparous (having no reproductive experience) if the pubic symphysis was closed and there was no evidence of placental scars or ovarian corpora. Primiparous females had pubic bones separated and embryos or placental scars and corpora of similar size and appearance. Multiparous females had separated pubic bones combined with both embryos and placental scars (or numerous scars of two color classes) and corpora that differed in appearance. For males, we recorded the position of the testes

(abdominal or scrotal), masses of testes and seminal vesicles (mg), and looped or convoluted epididymis. If the cauda epididymides are convoluted, then sperm are present and the male is fertile (Jameson 1950). We defined winter as Dec-Feb, summer as Jun-Aug, and spring and autumn as the intervening months.

We also used data from two other studies of rice rats from the same county in the analysis. Rice rats from Fisherman's Island, 7 kmsouth of our field sites, were trapped and necropsied in January and February 1982; these are the only necropsy results from January and February. Additional information on breeding condition was gleaned from rice rats captured during a contemporaneous population study (Bloch and Rose 2005), which used capture-mark-release (CMR) methods on two grids located within 2 km of our removal transects and provided information during the same months that we collected our rice rats for necropsy. Bloch and Rose (2005) provides external information on reproduction: males with scrotal testes were considered to be breeding and for females, vaginal patency, nipple size, and pregnancy were recorded, whereas necropsied animals yielded both internal and external reproductive information.

The hypothesis that rice rats breed throughout the year was tested using Chi-square analysis. Differences in litter size between age classes were analyzed using ANOVA.

We examined differences in litter size by parity with ANOVA after the data had been square-root transformed. A Kruskal-Wallis test was used to analyze differences in litter sizes by month because the assumptions of normality for ANOVA tests were not met even after data transformations. Pearson's correlations were used to examine the relationship between fat index and breeding condition. Finally, logistic regression was conducted to determine how effectively external indicators can predict breeding condition.

RESULTS

We trapped 85 males for necropsy during May 1995-May 1996. However, due to a sharp mid-winter decline in population density (also observed on Bloch's CMR grids), no males were trapped during January or February. We necropsied 44 females, but 0 were captured during May 1995 or the next January, February, or March. Rice rats reached sexual maturity close to the 30-40 g expectation. The smallest pregnant female was 34 g and the smallest male with convoluted cauda epididymides was 43 g.

Sixteen pregnant females were captured during the study: in April, July, August, September, and October (Table 1). When these differences in litter size among months, parity, and age classes were examined, litter sizes did not differ among months ($H = 0.09$, NS). After data for litter size by month were square-root transformed to meet the assumptions of ANOVA, litter size did not vary between primiparous ($\bar{x} = 4.89$) and multiparous ($\bar{x} = 4.00$) females ($F = 1.61$, NS) or between subadults ($\bar{x} = 3.83$) and adults ($\bar{x} = 5.10$; $F = 3.86$, NS).

For the analysis of breeding season, all necropsied rice rats ($n = 170$), including the 41 from Jan-Feb 1982 from Fisherman's Island, were combined into bimonthly periods to compensate for small sample sizes. Males were in breeding condition from March to November (Figure 1), but both testes and seminal vesicles showed substantial regression with the approach of winter. The mean mass of paired testes, expressed as

TABLE 1. Mean litter size for *Oryzomys palustris* based on embryo counts during the year.

Month	Mean	n
April 6.001	6.00	1
July4.502	4.50	2
August4.673	4.67	3
September4.889	4.88	9
October	1.00	1
Totals	4.63	16

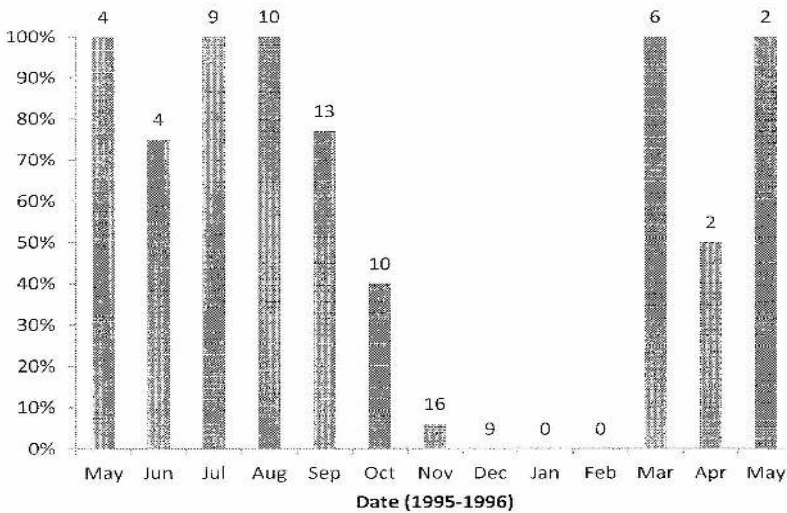


FIGURE 1. The percent of male rice rats from eastern Virginia in breeding condition from May 1995 through May 1996. Males were considered to be breeding if the cauda epididymides were convoluted. Numbers above bars indicate sample sizes.

mg/10 g of body mass, was greater in spring and summer (50.9-113.7) than during November (14.1) and December (7.3). The mean mass of seminal vesicles (also in mg/10 g body mass) also was greater in spring and summer (36.7-139.6) than in November (13.9 mg) and December (2.5). The mean testes masses, expressed as mg/10 g body mass, were 11.73 ± 8.2 for January 1982 and 51.17 ± 4.6 for February 1982, compared to 103.4 ± 5.5 in July and 113.7 ± 3.4 in August, the period of peak breeding. Mean body masses of male rice rats trapped in January 1982 ($\bar{x} = 57.8 \pm 2.7$ g; n = 15) and in February 1982 ($\bar{x} = 60.8 \pm 3.1$ g; n = 11) indicated that winter males were large adults. The mean mass of females for January 1982 was 38.5 ± 3.2 g (n = 4) and for February 1982 was 49.6 ± 2.0 g (n = 11).

Rice rats in breeding condition were found from March-April to November-December (Figure 2), with males having a longer breeding season on each end than

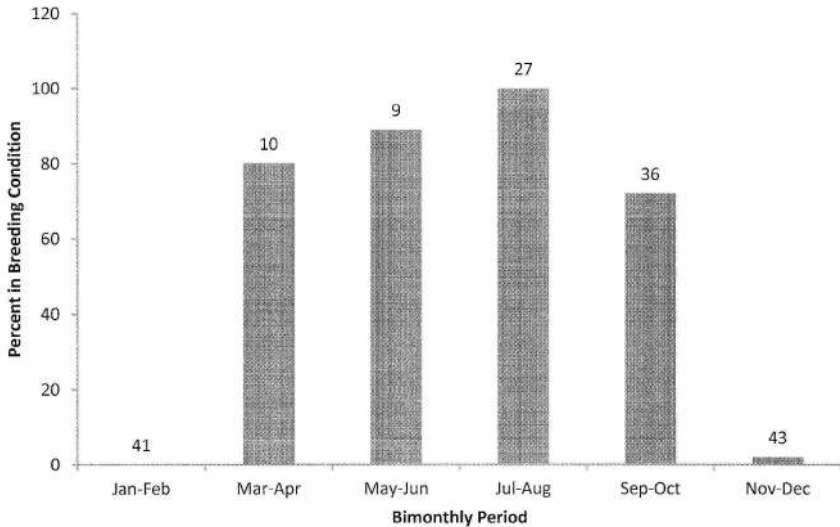


FIGURE 2. The percent of breeding rice rats from eastern Virginia for bi-monthly periods, when breeding condition is defined as descended testes in males and pregnant, lactating, or recently littered for females. Results for both sexes combined and including animals collected from Fisherman’s Island in January and February 1982; no rice rats were caught in January or February 1996.

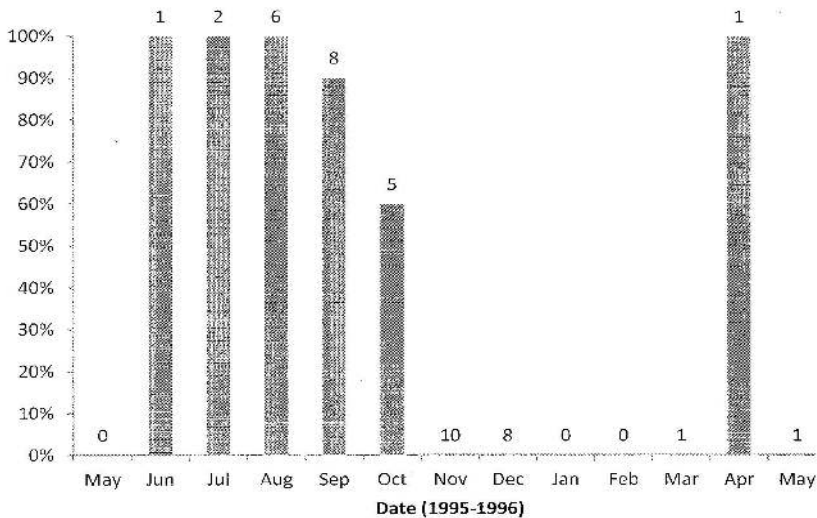


FIGURE 3. The percent of female rice rats from eastern Virginia in breeding condition from May 1995 through May 1996. Females were considered to be in breeding condition if they were pregnant, lactating, or recently had litters.

TABLE 2. Percent correct of external indicators in predicting breeding condition in marsh rice rats from eastern Virginia, as determined by logistic regression. Regressions using nipple size and nipple size + mass were unsuccessful due to small sample sizes.

Model	Percent correct	G	df	r	SE	p
Scrotal testes	73.2	77.01	1	0.98	0.7979	0.0001
Testes + mass	92.6	67.62	2	0.86	0.0268	0.0066
Perforate vagina	61.5	54.05	1	0.92	0.7585	0.0001
Vagina + nipple size	68.5	42.32	2	0.83	1.1853	0.1294
Vagina + mass	92.9	34.14	2	0.86	0.0573	0.0039

females. The null hypothesis that rice rats breed at uniform rates throughout the year was rejected ($X^2 = 86.91$, $df = 5$, $p < 0.001$). Breeding females (pregnant, lactating, or recently littered) were recorded during June-October and the next April (Figure 3).

Fat index and breeding condition were not correlated for either males ($r = 0.1188$, NS) or females ($r = 0.0320$, NS). Fat index was negatively correlated with percent breeding in the month for males ($r = -0.2177$, $p < 0.05$) but not for females ($r = -0.1063$, NS). Males trapped in January-February 1982 (midwinter) had higher fat indices than those trapped in July-August 1995 (midsummer) ($t = 3.69$, $df = 14$, $p < 0.05$). However, fat indices in a similar comparison of females did not differ significantly. Rice rats with high fat indices (large fat stores) were trapped during winter, whereas those trapped in spring had lower fat indices.

Logistic regression was used to analyze external predictors of breeding condition (Table 2). For males, the best predictor (highest r-value) was descended testes ($r = 4.5462$, $df = 1$, $p < 0.001$). When breeding condition is defined as pregnancy, lactation, or recently littered, the best predictor for females was perforate vagina ($r = 3.1781$, $df = 1$, $p < 0.001$). For both sexes, mass was also a useful predictor variable.

DISCUSSION

Based on necropsied specimens collected at monthly intervals throughout the year, female rice rats bred from April to October, and males were fertile a few weeks earlier and later than females. However, neither sex was fertile during the winter months of December, 1995 and January and February, 1982, although males rapidly regained fertility in February. Litter sizes and masses at maturity were similar to those of other southern populations of rice rats.

Litter size did not differ among months, between age classes, or with parity. Placental scar counts were not used to assess litter size because to be accurate, the time of parturition should be known (Martin et al. 1976). Also, previous studies of rice rats have used only embryo counts (Negus et al. 1961, Edmonds and Stetson 1993) or counts of young at parturition in the laboratory (Conaway 1954). The latter method may underestimate litter size because recently parturient females may eat their stillborn or other young without those losses being detected. All studies of litter size have small sample sizes of pregnant females: Conaway (1954, $n = 5$), Edmonds and Stetson (1993:

n = 20), and we examined 16 pregnant females. Although these sample sizes are small, there is little variation among populations.

Many small mammals show a trend of increased litter size in northern populations (Sadleir 1969). The mean litter size of 4.63 young for our study was comparable to that of 4.8 young reported for the 3-year study of Negus et al. (1961) in Louisiana but was much smaller than the mean of 6.7 young of the Delaware populations (Edmonds and Stetson 1993). The larger litter size of Delaware rice rats may be an adaptation to increase reproductive output in response to a shorter reproductive season; Delaware is near or at the northern limit of the distribution of *Oryzomys palustris*. Such responses by geographic populations are illustrated by *Sigmodon hispidus* Say and Ord (hispid cotton rat), for which litter size ranges from 2.5 in Isla del Toro, Mexico, 4.8 in Houston, Texas, 6.0 in Stillwater, Oklahoma, to 9.0 in Lawrence, Kansas (Cameron and McClure 1988). These authors conclude that variation in litter size in geographic populations of *S. hispidus* is attributable to greater seasonality of resources and to abiotic factors in populations with larger litter sizes. The increased litter size of northern populations probably is a means to increase reproductive output in a shortened breeding season (Sadleir 1969). Given the absence of breeding during the winter, we would have expected larger litter sizes in the Virginia population.

External and internal indicators showed some agreement in the age at which rice rats reached sexual maturity. The smallest necropsied pregnant female, 34 g, was only slightly smaller than the 39-g female judged to be pregnant on the CMR grids (Bloch and Rose 2005). However, the mass of males at maturity differed between the studies because the smallest necropsied males with convoluted epididymides were 43 g whereas one male under 20 g had scrotal testes on the CMR grids. Although several juvenile males on the CMR grids had descended testes, most did not. Overall, there was modest agreement in the external and internal indicators of reproduction that rice rats mature at 30-40 g. However, based on external features, males appear to have reached maturity before females. By contrast, the common pattern seen in studies of other genera of small mammals is for males to mature days or weeks after females (Sadleir 1969).

In our necropsy study, breeding males were observed from March to November, but by November the percent in breeding condition was declining and none was seen in December. Therefore, the transition from breeding to non-breeding status was documented before the population crash (no rice rats were trapped in January or February 1996). The males trapped on Fisherman's Island in January-February 1982 were not in breeding condition, which further supports that Virginia rice rats do not breed in winter.

The mass of testes declined during late autumn and in winter, which corresponded to the cessation of breeding. The gonads of small mammals regress to minimal levels during periods of non-breeding (e.g., Sadleir 1969). For example, the testes of adult hispid cotton rats from southeastern Virginia regress during late autumn to about 1/25 (2000 vs 80 mg) that of values during the breeding season (Rose and Mitchell 1990). Although the male rice rats trapped in Jan-Feb 1982 were adults based on body mass, the mean testes mass (expressed as mg/10 g body mass) were 10-49% of mean testes mass of males from July and August. Thus, with body masses near 60 g, these winter males were full adults with highly regressed testes.

Pregnant females were recorded in spring, summer, and autumn but none in

November or December 1996. If defined by pregnant females alone, the breeding season is April-October. The females trapped in January and February 1982, with body sizes sufficiently large to reproduce, were not breeding either, contributing additional support that rice rats do not breed in winter in eastern Virginia.

An inverse relationship between breeding season and fat index was expected; however, only males showed a significant correlation between fat index and percent breeding in the month. Males with greater fat stores were trapped in winter; all had fat deposits around the testes, which is the site of the first-laid fat for males. Although females showed the same pattern of greater fat stores in winter, the correlation was not significant. Lacking the brown adipose tissue that many temperate small mammals use during winter to make heat by non-shivering thermogenesis, the storage of fat in winter may represent an adaptation in rice rats for winter conditions. Laboratory mice, *Mus musculus*, which also lack brown fat, when kept in cool conditions (11 C) die within hours of missing one feeding period because they have a high metabolism and small energy stores (Bronson 1989). Bronson and Perrigo (1987) conclude that due to their energy-related constraints, small mammals usually allocate energy to fat storage rather than reproduction when thermoregulatory costs are high, as in winter at most locations in the southeastern US. If so, then rice rats may have sufficient energy to breed in Louisiana during mild winters but most winters in Virginia or Delaware are substantially longer and colder, making winter breeding unlikely or rare there.

In order to compare estimates of reproduction with contemporaneous populations from nearby CMR grids (Bloch and Rose 2005), we excluded rice rats < 30 g to conform to necropsied animals. Position of testes and vaginal patency were used to assess the breeding condition of rice rats trapped on the CMR grids. Males in breeding condition were recorded throughout the year but the percent of males in breeding condition was low during the months of winter (25-50%) and highest in spring (67-100%) and summer (91-100%). Although perforate females were found throughout the year, the percent was highest in spring and summer and declined in winter (Bloch and Rose 2005) and the percent of females judged to be pregnant (0% in winter) was highest in spring ($\geq 50\%$) and summer ($\geq 42\%$) and declined in autumn months ($\leq 46\%$).

Thus, in contrast to the results of necropsied specimens, marsh rice rats on the CMR grids were judged to be in breeding condition throughout the year (Bloch and Rose 2005). The percentage of mature rice rats in breeding condition based on external examination did decline in autumn, but a substantial number remained in breeding condition in December (14-68%), January (19-25%), and February (8-100%). Wolfe (1985) also concluded marsh rice rats bred year round in Mississippi, but he, like Bloch and Rose (2005), evaluated only CMR animals. However, none of the necropsied rice rats were in breeding condition in winter based on the internal indicators evaluated in our study.

We conclude that external indicators are unreliable in predicting the real breeding status of marsh rice rats. The results of our necropsy study and those of the contemporaneous CMR study differed due to the subjectivity and relative inaccuracy of external indicators. For example, six of 16 necropsied pregnant females were classified as non-breeders based on external indicators; they were not perforate, had small nipples, closed pubic symphyses, and were not visibly or heavily pregnant. McCravy and Rose (1992) also found external indicators to be less accurate for females

than for males. The inaccuracy of external indicators may be the reason that rice rats studied on the CMR grids (Wolfe 1985, Bloch and Rose 2005) show signs of breeding throughout the year whereas breeding during the winter months was not observed for the rice rats examined by necropsy. Thus, the breeding season of the rice rat in Virginia, based on the time for the first to last conception, was from March to October. Large overwintering males regain fertility in February with rapid testicular recrudescence and a smaller fraction of males remains fertile for part of November. Although no necropsied male was judged to be fertile in December or January, testicular regression was less pronounced than that seen in male hispid cotton rats of the region, perhaps enabling a rapid return to fertility should benign winter conditions persist, as they sometimes do in coastal Virginia.

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