A C T A T H E R I O L O G I C A

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Estimation of the Reproduction of the Bank Vole Under Field Conditions [1])

[With 4 Tables & 2 Figs.]

Investigations were made in three areas covered by mixed forest. By taking vaginal smears from individually marked females caught in live-traps duration of pregnancy was determined as 22 days. The number of pregnant females varied from 10% to 70% in different months of the reproduction season. The average size of litter, determined by dissection, was 4.9. The percentage of females in the population varied from 36% to 47% on different stations. Calculation was made of how many animals remained in the different areas throughout the whole study period, on the basis of analysis of captures of marked bank voles. The above data enabled the number of voles born to be estimated by two methods, and also permitted of estimating what part of all individuals living in the study area is formed by individuals born in that area.

The aim of the study was to estimate the reproduction of the bank vole under field conditions on the basis of methods of examining live animals (vaginal smears) and to assess what percentage of all individuals living in the study area is formed by individuals born there.

I. REVIEW OF LITERATURE ON THE REPRODUCTION OF *CLETHRIONOMYS GLAREOLUS* (SCHREBER, 1780)

The study by Stockard & Papanicolou describing the connections between the cyclic changes in the ovary and changes in the epithelium of the vagina of female guinea pigs initiated a series of investigations of the oestral cycle of rodents, carried out chiefly on laboratory animals (Allen, 1922; Long & Evans, 1922 etc.). These observations were concerned primarily with definition of the length of the oestral cycles and of the factors which modify them. The results of these investigations were summed up by Snell (1956).

[1]) This study was carried out under the Rodent Project of the International Biological Programme in Poland.

Examination of the reproduction of *Clethrionomys glareolus* was made under laboratory conditions, using the method of observations of live animals and autopsy (Wrangel, 1940) or of live animals only (Drożdż, 1963). Analysis was also made of killed animal taken from their natural habitat (Baker, 1930; Brambell & Rowlands, 1936; Naumov, 1948; Popov, 1960 and others).

In the first place it was found that the picture of histological changes in the reproductive organs of females of the species *Clethrionomys glareolus* is identical to *Mus musculus* (Brambell & Rowlands, 1936).

Duration of pregnancy was investigated in the first place. In the case of the bank vole this lasts from 17.5 (Drożdż, 1963) to 18 days (Wrangel, 1940). This agrees with the observations made by Popov (1960), who defined duration of pregnancy as 17—18 days. During lactation pregnancy may be considerably prolonged, due to the delayed implantation of the eggs (Brambell & Rowlands, 1936). In such cases pregnancy lasts from 21.5 (Wrangel, 1940) to 25 (Naumov, 1948) or even to 30 days (Drożdż, 1963). Prolonged pregnancy most often lasts, however, from 19—22 days (Drożdż, 1963).

The average size of the litter varies from 4 (Baker, 1930; Wrangel, 1940) or 4.05—4.11 (Drożdż, 1963; Brambell & Rowlands, 1936) to 6.1 (Naumov, 1948; Popov, 1960).

After parturition and oestrus occurs in the bank vole during which the female may be fertilized (Brambell & Rowlands, 1936). Sex proportions are subject to some fluctuation. Analysis of embryos gave the ratio as 1:1, while prodominance of males was found in nests (52%), which is even more distinct in adult animals (56%), (Popov, 1960). A similar predominance of males (respectively 57.2% and 56%) was observed by Brambell & Rowlands (1936) and Wrangel (1940). Baker (1930) also observed seasonal variations in sex ratio and stated that the predominance of males is even more marked in spring.

The seasonal variations in the percentage of pregnant females have also been described; they reached a figure of 90% only in June and July (Brambell & Rowlands, 1936). The only study analysing the oestral cycle on the basis of vaginal smears under field conditions is that by Larina & Golikova (1960). They do not limit only to determining the duration of the different phases of the oestral cycle, but describe the increase of home range of the females in the pro-oestrus phase. But there are no data concerning the percentage of pregnant females in the area, or data on duration of pregnancy.

II. MATERIAL AND METHODS

1. Description of the study area and methods of catching the voles. Investigations were carried out near the Field Station of the Institute of Ecology, Polish Academy of Sciences, at Dziekanów Leśny near Warsaw.

The study area was covered by a forest of the following type: *Pineto-Quercetum, Vaccinio-myrtylli-Pineto subass. molinetosum, Tilio-Carpinetum, Carici elongatae-Alnetum* (Traczyk, *in litt.*).

Material for studies on the reproduction of *Clethrionomys glareolus* (Schreber, 1780) was collected from three areas: *A* and *B* in 1963, and *C* in 1964, on which several other methodical experiments were carried out simultaneously.

The animals were caught in live-traps. Cylinders sunk into the ground were used on area *B* in addition to live traps. Oats were used as bait. During the

periods between captures the animals were prebaited by placing oats into blocked traps and cylinders. The captured voles were marked individually, according to Naumov's method (1948), then weighed, sex determined, vaginal smears taken from adult females and then the animals were released on the spot on which they had been captured.

The study areas differed from each other as to size, period during which the investigations were carried out on them, spacing of traps and frequency of trap-setting.

Study area *A* (3.5 ha, April 18th—October 5th). The traps were spaced in a grid, one trap on each point. They were left set for the whole of a 24-hour period twice a week and during this time inspected every 12 hours. During the periods from July 6th — Aug. 7th and Sept. 9th — Oct. 8th the traps were inspected daily (once and twice a day by turns).

Study area *B* (5.4 ha, May 15th — Oct. 1st). The traps were arranged at intervals of 15 m. in a grid. Two traps, or one trap and one cylinder, were placed on every second point alternately. The traps and cylinders were set twice a week and inspected after 24 hours.

Study area *C* (2.7 ha, April 10th — Oct. 18th). The traps were spaced at intervals of 7.5 m. in a grid, two traps on each point. During the whole observation period (except for the period from April 15th to June 16th when they were set for a 24-hour period twice a week, and examined every 12 hours) the traps were inspected every 12 hours. The distance between the traps was changed during this time, setting them in turn on successive days on all points, then every second and every third point.

2. Examination of vaginal smears. Smears were taken from the vagina under field conditions, next the female's number and the date was noted, then the smear was stained with methyl blue. When taking the smear attention was paid to: (1) degree of development of teats (presence of milk noted), (2) presence of developed embryos (perceptible by palpation), (3) presence of copulation plug. In addition the observed cases of sexual inactivity (closed vagina) were recorded.

The following phases of the oestral cycle were distinguished on the basis of microscopic examination of the smear: Prooestrus, oestrus, metoestrus and dioestrus.

The secretion from the vagina in the early periods of pregnancy does not, strictly speaking, differ from the secretion observed in the dioestrus phase. The surest indicator of pregnancy is the presence of bloody mucus (about 8th day of pregnancy). The pictures from the later periods of pregnancy are characterised by the gradual increase in the amount of mucus. The additional information described above, and also information as to increase in body weight and births of young in the traps were also used to determine pregnancy.

The data obtained in the above way were entered on the calendar of catches (Petrusewicz & Andrzejewski, 1962). This method permits of tracing the histories of individuals by comparing all the information available, which enabled gaps to be filled in cases in which the female concerned was not captured, e.g. the appearance of bloody mucus made it possible to foresee the date the young would be born.

A total of 934 smears from 225 females were taken on all the areas.

III. RESULTS OF INVESTIGATIONS

1. Estimation of population numbers. The numbers of the bank voles were estimated, on the basis of a calendar of catches, separately for each study area. Distinction was made between migrating individuals (caught during the course of one day only) and settled individuals (spending at least two days in the study area) (Andrzejewski & Wierzbowska, 1961; Petrusewicz & Andrzejewski, 1962). Only settled individuals were examined in the present study. The mean period of stay was defined by dividing the total sum of all periods of stay by the number of settled individuals.

In order to describe variations in numbers in the study areas calculation was made of turnover, dividing the period during which investigations were made by the mean period of stay. Turnover is therefore an indicator of how many times during the study period a complete exchange of individuals takes place in a given study area.

By calculating the mean numbers for the whole study period, and multiplying this value by the size of turnover, an estimate was obtained of the total number of individuals living in the study areas (Table 1).

Table 1.

Number of individuals staying in the study area estimated from turnover.

Area	Duration of investigations in days	Mean number per 1 ha	Mean period of stay in days	Turnover	Total number of individuals per 1 ha for the whole study period
A	163	10.4	27.3	5.9	61.3
B	136	5.7	31.2	4.3	24.5
C	156	8.8	20.6	7.5	66.0

2. Estimation of the mean size of litter. Information was obtained as to the average size of litter under field conditions on the basis of an analysis of pregnant females which died or were killed on neighbouring areas, and also of determination of the number of young born in traps. Litter size was calculated on the basis of 52 cases, as the mean value for the whole reproduction period (from the beginning of April to the end of September), in 1963—1964.

On account of the possibility of resorbtion the embryos were divided into two classes of size: (1) 2—10 mm; (2) over 10 mm. Four cases of birth in traps were also taken into consideration. The two classes of size were examined separately. The average litter calculated for the first class was 5.6 (29 cases examined), and the second 4.9 (23 cases examined).

The average litter size of 4.9 young was accepted for estimation of reproduction.

3. Duration of pregnancy. Only those pregnant females in the area for which accuracy in determining duration of pregnancy did not exceed ± 2 days (in the case of, for instance, a gap of two days in captures towards the end of pregnancy) were taken into consideration. Data for 6 females were obtained from the whole material.

Successive lengths of pregnancy were: 17, 21, 21, 22, 23, 28 days, hence the mean duration of pregnancy was 22 days.

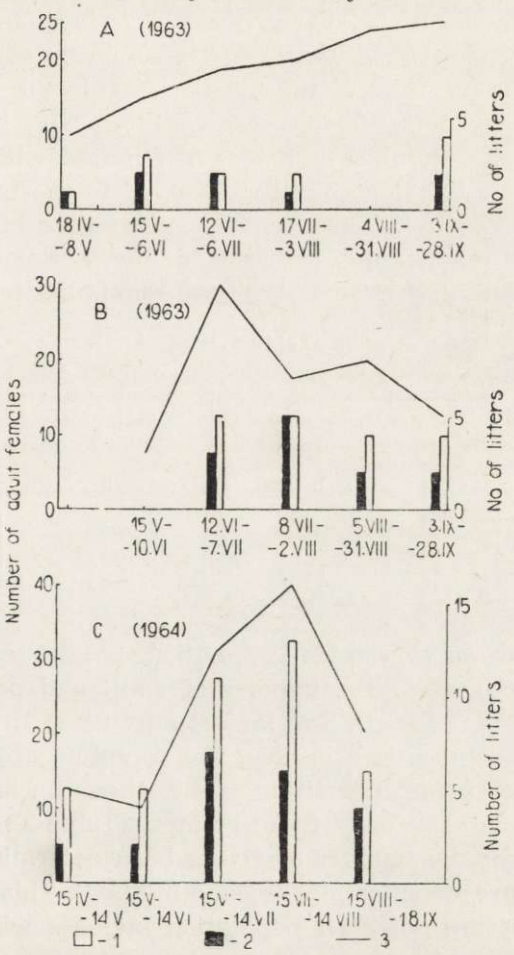

Fig. 1. Relation between number of adult females and number of litters 1 — "a" degree of accuracy in estimating number of litters, 2 — "b" degree of accuracy in estimating number of litters, 3 — ——— number of adult females.

4. Sex ratio. A lower percentage of females was found on all the study areas. Throughout the whole study period the following average percentages were found on the different areas: *A* — 36%, *B* — 44%, *C* — 47% of females.

5. Estimation of the number of young born. Two methods of calculation were used for estimating the number of young born during the study period.

5.1. Estimation of the number of young born was made on the basis of the number of litters (first method). This method consisted in direct calculation of the number of litters recorded in the calendar of captures and multiplying them by the size of the average litter. Date of birth was determined by means of the criteria described in the section on determination of pregnancy. Two degrees of accuracy of estimation connected with the duration of the females stay in the study area were distinguished: (a) when the female was captured both before and after giving birth, (b) the number of litters of females which ceased to be captured about 5 days before birth was added to the number of litters according to estimate "a". These females might give birth to young in the study area, or might either emigrate or die. Estimate "a" therefore defines the minimum and estimate "b" the maximum number of births.

Table 2.

Number of young born determined on the basis of number of litters.

Area	Number of litters acc. to degree of accuracy:		Number of young born per 1 ha acc. to degree of accuracy:	
	a	b	a	b
A	8	12	11.2	16.8
B	12	18	10.8	16.3
C	21	40	38.1	72.5

Differences were observed in estimation of the course taken by reproduction (expressed in the number of adult and pregnant females) on the different areas (Fig. 1). The largest number of litters, determined according to both degrees of accuracy, was found on area *C*. Taking the average number of young per litter as 4.9, then the number of young born on area *C* was also found to be greatest (Table 2).

5.2. Estimation of the number of young born determined on the basis of percentage of pregnant females (second method). This method is based on calculations of the potential population increase when the value of an average litter, duration of pregnancy, percentage of pregnant females in the population and average numbers for the whole study period are all known. These parameters are, according to Golley (1961) interconnected in the following way:

$$PR = \frac{\log (M\,p + 1)\,T\,N}{(t : f) \log e}$$

where *PR* — potential reproduction during the whole study period, *M* — avg. litter, *p* — percentage of females in the adult population, *T* —

study period (in days), N — average numbers, t — average duration of pregnancy, f — mean percentage of pregnant females, e — basis of natural logarithms.

In this formula the size of litter (M) is calculated per adult female. Value $t:f$ indicates the average time after which each adult female in the population examined produces young.

Differences were found in the percentage of pregnant females during different periods (Fig. 2). The average percentage of pregnant females

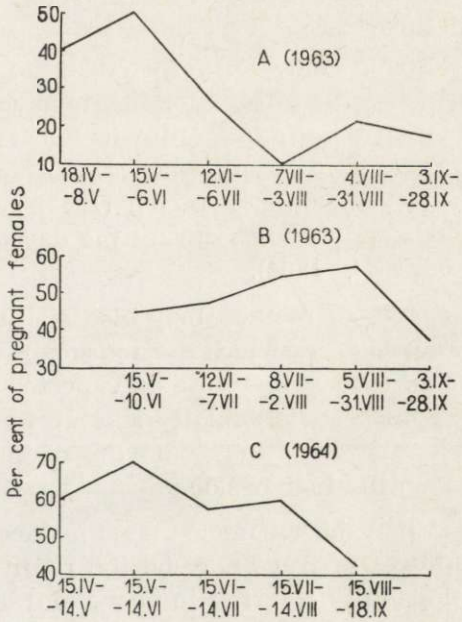

Fig. 2. Variations in percentage of pregnant females in different periods of the investigations.

Table 3.

Number of young born determined on basis of percentage of pregnant females (after Golley's method, 1961).

Area	Duration of investigations in days (T)	Mean number per 1 ha (N)	Mean % of pregnant females (f)	Percentage of females in population	Potential fecundity per 1 ha
A	163	10.4	0.29	0.36	23.3
B	136	5.7	0.47	0.45	19.1
C	156	8.8	0.58	0.47	43.1

for the whole study period was calculated for the purposes of a general description of reproduction. Taking the average duration of pregnancy as 22 days and size of the average litter as 4.9, calculation was made of potential increase according to the Golley formula described above, converting the values obtained to value per hectare (Table 3).

IV. COMPARISON OF NUMBER OF YOUNG BORN WITH THE NUMBER OF INDIVIDUALS STAYING IN THE STUDY AREA

Variations in the numbers of bank voles in the study areas are due to reproduction and mortality, and also to the immigration and emigration of individuals. The mean period of stay of adult individuals in the area, defined by means of the calendar of catches, would appear to be the resultant of their mean length of life, also the different reaction of bank voles to the method used, and the error of estimates due to errors of method.

Among the voles' reactions to the method we may mention, for instance, the different estimate of sex proportions obtained by the means of snap-traps and capture cylinders placed in trenches (Popov, 1960) differences in trap proneness or trap shyness, as the studies by Kikkawa (1964) and Tanton (1965) suggest, and different trappability of males and females during the day and at night (Bujalska & Ryszkowski, 1964).

Errors of method may also result from estimating the period of stay in study areas which are too small (Chełkowska & Ryszkowski, 1966). It may therefore be expected that the average time of stay which we observed is actually shorter than the mean length of life of adult individuals and thus estimates of their number presented in table 3 may err on the higher side.

It may be assumed that the sex proportions found on the basis of captures in traps may be the result not only of the different degree of trappability referred to above, but also of actual differences in sex ratio due to the different mortality rate among males and females (Wrangel, 1940; Popov, 1960). Since it was not possible to distinguish the two causes affecting estimation of sex proportions in the material we analysed, the proportions determined by trapping were accepted.

Estimation of the number of pregnant females was also uncertain in our materials on account of the difficulty in diagnosing the early stages of pregnancy (particularly when the number of captures was small).

Bearing the above reservations in mind the estimates of the number of young born, made by the first and second method, were compared (Table 2 and 3) with the variations in the total numbers estimated by turnover (Table 1). In order to calculate the number of individuals which appear on the study areas during the whole study period those individuals which initiated reproduction were subtracted from total numbers. Calculation was next made of the share of young born among individuals appearing, estimated by the different methods, i.e. the numbers of

voles born, calculated by different methods, was divided by the number of animals estimated on the basis of turnover (Table 4).

The table of the number born in relation to those staying in the area, according to the method of determination of number of litters (first method), with "a" degree of accuracy, proved to be the lowest on all the areas.

Table 4.

Number of young born in relation to those appearing on sampling area.

Area	Share of young born in relation to those appearing			Number of individuals appearing per 1 ha
	Estimated no. of young born on basis of no. of litters Degrees of accuracy:		Estimated no. of young born on basis of Golley's (1961) method	
	a	b		
A	0.22	0.33	0.56	50.9
B	0.57	0.86	1.10	18.8
C	0.67	1.27	0.79	57.2

On account of the difficulty in determining the early stages of pregnancy better results are obtained when captures are more frequent. On area *C* where daily captures were made and the intervals between traps were the smallest (in comparison with areas *A* and *B*) estimates of the number of young born are closest to the number of animals appearing (Table 4).

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OCENY ROZRODU NORNICY RUDEJ W WARUNKACH TERENOWYCH

Streszczenie

W oparciu o dane dotyczące długości trwania ciąży, wielkości miotu, udziału samic ciężarnych oraz proporcji płci i liczebności określono przyrost młodych w wolnożyjącej populacji *Clethrionomys glareolus* (Schreber, 1780).

Badania prowadzono w Dziekanowie Leśnym koło Warszawy, w kilku biotopach lasu mieszanego. Stosowano pułapki żywołowne i cylindry.

Oznaczono fazy cyklu płciowego przy pomocy rozmazów pochwowych. Ciężarność nornic określano również przy pomocy badań dodatkowych jak wymacywanie embrionów, czy ciężar ciała. Uzyskane dane nanoszono na kalendarz złowień (Petrusewicz & Andrzejewski, 1962). Ogółem przebadano 225 sa-

mic. Wielkość średniego miotu oceniono analizując padłe zwierzęta na powierzchniach badawczych lub zabijane na terenach sąsiednich. Oparto się na średniej wielkości miotu obliczonej dla embrionów powyżej 10 mm.

Ocenę liczebności populacji przeprowadzono na podstawie kalendarza złowień, biorąc pod uwagę jedynie osobniki osiadłe (Petrusewicz & Andrzejewski, 1962). Zmiany liczebności populacji scharakteryzowano obliczając turnover (dzieląc okres w którym przeprowadzono badania przez średni czas przebywania). Mnożąc wartość turnover przez średnią liczebność dla całego okresu badań otrzymano ocenę całkowitej liczebności osobników przebywających na badanych powierzchniach (Tabela 1).

Na badanych powierzchniach stwierdzono mniejszy udział samic w populacji. Wahał się on od 0,36—0,47.

Liczbę urodzonych oszacowano przy pomocy dwóch metod: pierwsza polegała na bezpośrednim przeliczeniu liczby miotów i pomnożeniu jej przez wartość średniego miotu (Tabela 2), druga metoda natomiast oparta jest o obliczenia potencjalnego przyrostu populacji według metody Golley'a (1961) — Tabela 3.

Przeprowadzono dyskusję wyników i porównano zmiany liczebności określone na podstawie turnover z liczbą młodych urodzonych na powierzchni (obliczoną dwoma metodami). Ocena liczby urodzonych najbliższa jest liczbie pojawiających się na powierzchni C (Tabela 4). Wynika to z większej częstości połowów, a tym samym z dokładniejszego określenia wczesnych stadiów ciąży.