ABSTRACT: A method was proposed for studying population density, microhabitat preference, daily activity and seasonal activity in small rodents. In this method, artificial tunnels (PVC pipes) imitating a natural system of burrows were applied. Their use by rodents was recorded by means of the electronic counters provided with a photoelectric cell. The study was conducted in an alder swamp 110 years old, located in the Kampinos National Park (52°25'N, 20°53'E) during 2003–2005. In this area, the bank vole Clethrionomys glareolus (Schreber, 1780) accounted for 90–99% of the captured rodents. The use of the tunnels by rodents (the number of passages) did not depend on their length within a range of 1–8 m applied in the experiment \( P = 0.22 \). The tunnels were used by day and night, at the highest rate in the evening and at night. The use of tunnels increased when a bait was exposed in the vicinity \( P = 0.001 \). It was positively correlated with population density (estimated with the CMR technique), and varied from season to season (in spring, summer, and autumn). The formula: 
\[
\text{density} = 0.1717 + 0.0304 \times \text{mean number of passages per day}
\]
enabled the estimation of population density based on the number of passages through the tunnels. It has been found that a single tunnel was typically used by 5 individuals (mean 4.6 individuals, SD = 1.8). The location of tunnels had a significant effect on their use. Tunnels connecting fallen logs or bases of alders (hummocks) were more frequented than those leading to shrubby areas \( P < 0.000 \) or to microhabitats covered with herbaceous vegetation \( P < 0.001 \). This method enabled a multisided analysis of the behaviour of rodents at a minimum interference in the life of animals. Thanks to the application of electronic counters of passages, it was possible to obtain easily a large number of data. It is proposed to mark rodents with electronic transponders in the future studies to identify the individuals using artificial tunnels. This method could replace the methods used so far in the studies of small rodents, requiring trapping (CMR) or radio-telemetry.

KEY WORDS: artificial tunnels, rodents, field technique, population

1. INTRODUCTION

As it is difficult to observe the behaviour of small rodents directly under natural conditions, most often indirect methods are applied such as various traps (CMR technique) or devices enabling radio recording (radio-telemetry) (Mazurkiewicz 1981, Korn 1986, Douglass 1989). These methods are commonly applied to estimate population density, or to examine various aspects of the spatial activity of rodents (Mazurkiewicz and Rajska-Jurgiel 1998, Bujalska and Saitoh 2000). They have, however, serious
drawbacks such as temporary removal of individuals from the population, or dependence upon the trappability of a species in the case of the trapping method, or restriction of the locomotor activity of an individual, and a small number of observed animals in the case of radio-telemetry.

There is no commonly applied alternative method for studying the natural behaviour of small mammals without catching them. The application of fluorescent powder (Lemen and Freeman 1985) or introduction of artificial requisites for nesting (Kaufman and Kaufman 1989) did not find a wider approval.

The group of alternative methods also includes the introduction of so called track stations or track tubes to the environment (Drennan et al. 1998, Glennon et al. 2002). With this technique it is possible to record the presence of animals, or evaluate population density and species composition of rodents without trapping them (Raphael et al. 1986). For this purpose we need short 20–30 cm sections of PVC pipes or PVC profiles provided with a bait. The pipes are usually provided with a substance dyeing the feet of animals and with a strip of paper on which they leave footprints (Glennon et al. 2002, Nams and Gills 2003, Gotrat et al. 2004). The kind and numbers of footprints enable the identification of species, and possibly the calculation of indices of population density. A drawback of this method is a need for frequent visits and limited resistance to weather conditions. Nonetheless, the method of exposure pipe sections in the field creates wide research prospects. This is due to the fact that rodents searching the area move along trails on the ground surface or in the system of underground burrows (Andrzejewski 2002). Fragments of pipes exposed in the area, imitating natural corridors, are attractive requisites for rodents, readily visited and used for moving. Movements of rodents along such artificial tunnels can readily be traced not only by footprints but also by means of simple electronic counters. This method negligibly interferes in the life of small mammal populations, and it does not depend on weather conditions.

The objective of this study was to develop a method for studying populations of small rodents (population density, daily activity, microhabitat preference) by using artificial tunnels (PVC pipes) provided with electronic counters of passages.

2. STUDY AREA

The study was conducted in the Kampinos National Park, central Poland (52°25′–52°15′N and 20°17′–20°53′E) during 2003–2005. The study area supported a Ribo nigri-Alnetum (Solinska-Gornicka, 1975) swamp forest 110 years old, covering about 8 km². The tree stand was dominated by the black alder Alnus glutinosa (L.), with admixture of the common birch Betula pendula (L.), and the European ash Fraxinus excelsior (L.). The undergrowth comprised the alder buckthorn Frangula alnus (Mill.), hazel Coryllus avellana (L.), and European black current Ribes nigrum (L.). Bank voles Clethrionomys glareolus (Schreber, 1780) were dominant among small rodents, accounting for 90–99% of the number of small rodents captured in this area (Owadowska 1999, Mróz 2007).

3. MATERIAL AND METHODS

The method used in this study is based on taking measurements of the utilization of artificial tunnels by small rodents under field conditions.

Artificial tunnels were made of PVC pipes 40 mm in diameter (Photo 1). The number of passages was counted by electronic counters placed in the central section of each tunnel (Photo 2). The counter consisted of a 10 × 8.5 × 5 cm box attached to a 20 cm section of a PVC pipe also 40 mm in diameter. Infrared light beam crossing the tunnels from the emitter to the receiver was applied to measure the number of passages. When rodents moving through the tunnels crossed the light beam, their passage was recorded. The counter was loaded with four R6 batteries working for about two weeks. The number of passages was recorded in the memory of the counter. It could be seen after switching on the projector showing the number of passages in a given period. The tunnels were exposed for least four months before starting the experiment – rodents had opportunity to get accustomed to new elements in their habitat.
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Photo. 1. Entry to the PVC tunnel situated on the forest bottom (photo M. Badaszek).

Photo. 2. Electronic counter (without cover) fixed on PVC tunnel covered by forest litter (photo M. Badaszek).
The following issues were addressed:

- **The use of tunnels of different lengths.** Tunnels 1, 3, 5, and 8 metres long were applied, five of each length, in sum 20 tunnels. Readings were taken twice a day, at 7:00 and 19:00, for 5 days. These tests were conducted in late August and early September of 2003. In total, 833 passages were recorded. One-way ANOVA and post hoc Fisher LSD test were used for statistical analyses.

- **The daily cycle of activity in the utilisation of artificial tunnels.** Ten tunnels were selected (2 one metre long, 2 three metres long, 3 five metres long, and 3 eight metres long). Readings of the number of passages were taken every three hours for three days. The observations were conducted in September of 2003. In total, 313 passages were recorded.

- **The effect of food on the intensity of the use of tunnels.** In this test, ten tunnels were used, the same as previously used for testing the daily activity of rodents, and this test was made immediately after testing the daily cycle of activity. The number of passages recorded during the test for daily activity was considered as the control. To test the effect of food on the intensity of utilization of artificial tunnels, a piece of bread was attached to the ground 1 m from the entrance to the tunnels (from one side). When the bait was eaten, a new one was provided. The readings of the number of passages were taken twice a day (at 7:00 and 19:00) over three days. The test was conducted in September 2003. In total, 780 passages were recorded. One-way ANOVA and post hoc Fisher LSD test were used for statistical analyses.

- **Seasonal variation in the use of artificial tunnels in relation to population density.** The test was conducted with the use of 20 artificial tunnels exposed in lines 15 m apart. All tunnels were 2 m long. Readings of passages were taken once a day for 6 days. The tests were conducted in three seasons of two years: in spring (April 2005), summer (August/September 2004 and 2005) and autumn (October 2004 and 2005). When this test was ended, the density of rodents was assessed in the same area by using live traps (CMR technique). A line of 20 traps placed every 15 m and baited with oat grains was run for 5 days (20 traps x 5 days = 100 trap-days). The traps were visited once a day. The captured individuals were marked by fur clipping. 112 individuals of *C. glareolus* and 10 individuals of *A. flavicollis* were captured in 2004 and 133 individuals of *C. glareolus* and 8 individuals of *A. flavicollis* in 2005. One-way ANOVA and post hoc Fisher LSD test were used for statistical analyses. In addition, Pearson’s correlation coefficient was calculated.

- **Assessment of the number of individuals using a single tunnel.** The test was conducted in 16 tunnels 2 m long. One live trap was placed near the entrance to each tunnel (ca 15 cm). The traps were visited everyday, and readings of the number of passages through the artificial tunnels were taken. The traps were not baited with oat grains that rodents can smell from a large distance, but with a piece of apple to supply them with a portion of water and food. The captured individuals were identified to species, sexed, and taken to the laboratory. Trapping was conducted until the number of passages dropped to zero on all counters. The test was conducted in summer and autumn 2005. In summer ~ 85 and in autumn ~ 51 individuals were captured.

- **The effect of the location of tunnels on their use by rodents.** Twenty 2-m tunnels were set out in such a way that each of them connected two chosen sites (microhabitats) on the forest floor. Four categories of connections were used:

  - (a) tree-tree: a tunnel connecting two live trees (alders *A. glutinosa*) with a well developed root system in the form of a hummock,

  - (b) tree-log: a tunnel connecting a live tree (alder) with a fallen log in different stages of decay,

  - (c) tree-shrubs: a tunnel connecting a live tree with shrubbery (alder buckthorn, hop, black cherry) and luxuriant herbs,

  - (d) tree-open space: a tunnel connecting a live tree with a place covered with a forest litter and sparse herbaceous vegetation.

  There were 5 tunnels of each category. The tests were conducted in late August and early September of 2004 and 2005. The tunnels were visited once a day. In 2004, the experiment was run for 6 days, and 2579 passages were recorded. In 2005, these were 12 days, and 3043 passages.
4. RESULTS

To test whether bank voles show preference for artificial tunnels of a specified length, mean numbers of passages were compared in 12-h periods. No difference was found in the use of tunnels applied in the experiment (F = 1.49; \( P = 0.22, N = 200 \)) (Fig. 1). This means that the shortest tunnels (1 m long) and the longest ones (8 m) were used by rodents. It should be noted, however, that the use of 1 m tunnels was lower, close to significance, as compared with the tunnels 3 \( (P = 0.065) \) and 5 m long \( (P = 0.064) \).

To assess changes in the use of artificial tunnels by bank voles within the daily cycle of activity, mean numbers of passages were calculated in three-hour periods. As the number of passages did not depend on the day of the experiment \( (F = 0.35; \ P = 0.71, N = 30) \), the results from the three successive days of the experiment were pooled (Fig. 2). The results showed that the tunnels were used during the day and at night, with the highest intensity in the evening and at night.

To test the effect of the bait on the intensity of use of tunnels, the mean number of passages on days without baiting (mean 5.21; SD = 4.5) was compared with that on the days with baiting (mean 13; SD = 13.3). It has been found that the activity of bank voles in artificial tunnels significantly increased when the bait was exposed in vicinity \( (F = 20.82; \ P = 0.001; N = 120) \).

To test the effect of population density on the use of artificial tunnels, the intensity of their use in different seasons of the year was compared with population densities estimated in the same periods by using the trapping technique (Fig. 3). The highest number of passages through tunnels was recorded in late summer of both 2004 and 2005, and the lowest one in the spring of 2005. In autumn 2004 and 2005, the number of passages was at a medium level. The number of passages was positively correlated \( (r = 0.94, \ P = 0.02) \) with population density in these seasons of the year (Fig. 4). This correlation was used to calculate a formula for estimating population density (that is, the mean number of individuals per trap per day) from the number of passages through a tunnel: density = 0.1717 + 0.0304 × the mean number of passages per day.

The experiment testing for the number of individuals passing through a single tunnel showed that tunnels were used by 1–10 individuals, mean 4.6 individuals (SD = 1.8).

To test the effect of the location of tunnels on their use by forest rodents, a mean number of passages per day was calculated for each category of microhabitats connected by the tunnels (Fig. 5). As the test was conducted in two years that significantly differed from each other \( (F = 28.27, P < 0.0001) \), the results are calculated separately for each of them. In 2004, tunnels connecting two trees were most frequently visited, whereas tun-
nels of the category “tree-open space” were least frequently visited ($P < 0.001$). Tunnels of the other categories were used at a similar rate. In 2005, a different pattern of the use of tunnels was observed. The tree-tree, tree-log, and tree-open space categories were used at a similar level ($P > 0.89$), and the tunnels connecting trees with shrubs were significantly less used as compared with the other categories ($P < 0.000$).

5. DISCUSSION

The method proposed in this study is based on exposing artificial tunnels in the environment so that small rodents could find and use them as walking trails. All artificial pathways were discovered by rodents and utilised to varying degrees.

The experiments have shown that the use of artificial tunnels depends on several fac-
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tors. The most important factor is the place of exposure. Tunnels leading to fallen logs and mounds at tree bases, that is, places used by rodents for movements or nesting sites (Olszewski 1968, Montgomery 1980) are more frequently used. Tunnels with at least one end facing places less frequented by rodents are also less frequently used, or used with variable intensity (Fig. 5, categories tree-shrubs or tree-open space). Seasonal variation in the use of these places can result, for example, from variation in the available food supply in successive years.

Tunnels are used by a specified group of individuals occurring in a given area, and the intensity of the use is positively correlated with population density. This relationship enables the application of the method of artificial tunnels to estimating population density without trapping and marking of individuals. Based on the number of passages, the density of rodents in a given area can be estimated from the base curve (Fig. 4). With the method of artificial tunnels it is thus possible to estimate population density without baiting (unlike with the previously used method of track stations: Drennan et al. 1998, Glennon et al. 2002). This is an important advantage in view of the fact that the bait has a significant effect on visiting the site by rodents, accounting for a local increase in density, as shown in one of the experiments.

Also the time of the day affects the number of passages through the tunnels. The pattern of activity, as measured by the number of passages through the tunnels at different times of the day and night, is similar to that previously described in the literature on the 24-hour activity, with preference for the evening and night hours (Wójcik and Wołk 1985).

The length of artificial tunnels was of lower importance for their use by bank voles. They used 1-m tunnels as well as 8 m tunnels. The fact that 1 m tunnels were used at a lower rate suggests that rodents show preference for tunnels in which they can move larger distances. Presumably, short sections of the pipes previously used (Drennan et al. 1998, Gortat et al. 2004) are not so attractive requisites as are tunnels that enable movements several-metres long. The optimum length of tunnels for future experiments seems to be 2–5 m. In some experiments, 2 m long tunnels were doing well. In future investigations, however, longer tunnels can be used if needed. So far, a maximum length of the tunnels that rodents can use is unknown.

To sum up the advantages of the proposed method, its non-invasive character should be emphasised. There is no need for baiting and trapping rodents when applying this method. As a result, we can obtain data about their natural behaviour undisturbed by trapping devices or by radio-telemetry (Andrzejewski 2002). An additional advantage of the method of artificial tunnels is a low labour consuming. Tunnels once exposed can be left in the field for several years, and the counter of passages can be installed only for some periods. The extension of this method should concern an improvement of the counter of passages so that individuals passing through the tunnels could be identified, the direction of their movements could be recorded, and the exact time of passages could be recorded. The identification of individuals could be based on electronic transponders implanted under the skin of animals living in a given area. That kind of identification was used with success for Heterocephalus glaber (Braude and Ciszek 1998) and Microtus oeconomus (Korslund and Steen 2006). These improvements of artificial tunnel method can enable conducting short and long-term studies even over large areas and could be one of the main methods for studying small rodents, precluding the artefacts involved, for example, in the traditional trapping method.

6. REFERENCES


Roman Andrzejewski et al.

Mazurkiewicz M. 1981 – Spatial organization of bank vole population in years of small or large numbers – Acta theriol. 26: 31–45.
Mongomery W.I. 1980 – The use of arboreal runways by the woodland rodents, Apodemus sylvaticus (L.), A. flavicollis (Melchior) and Clethrionomys glareolus (Schreber) – Mammal Rev. 4: 189–195.
Mróz I. 2007 – Response of the bank vole Clethrionomys glareolus (Schreber) to the odour and presence of heterospecifics as measured by scent marking behaviour and trapping in double traps in an alder forest – Pol. J. Ecol. in press.
Owadowska E. 1999 – The range of olfactory familiarity between individuals in a population of bank voles – Acta theriol. 44: 133–150.

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