J. Zool., Lond. (2002) 256, 159–163 © 2002 The Zoological Society of London Printed in the United Kingdom DOI:10.1017/S0952836902000195

# Marking methods in small mammals: ear-tattoo as an alternative to toe-clipping

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(Accepted 21 February 2001)

**Abstract**

In a field study on the behavioural response of grey-sided voles *Clethrionomys rufocanus* to predator odour ear tattoos were used for individual marking of the voles in the field. The study was conducted over three summer seasons in the tundra of northern Norway. In this paper we report our experience with ear-tattooing in order to compare it with other methods used for marking small mammals. Methods should be compared for their different influences on physiology and behaviour and to find alternatives to the widely used toe-clipping. Looking for alternatives becomes mandatory because in many countries this method needs a special permit or is totally prohibited by law. Marking a vole with ear-tattoos took us 2 min on average. The rate for the first recapture after marking voles was 87%. This is much higher than reported recapture rates for toe-clipped voles. From all recaptured individuals we were able to identify > 89.9% of the codes. The time lag between marking and first recapture was higher than the lag between second and third recapture, which indicates a trauma caused by the marking procedure. However, there was no evidence of any weight loss as reported for other marking methods, and most of the tattooed animals did not show any behaviour indicating irritation after being marked. It is concluded that ear-tattooing, as an alternative to other methods of marking small mammals is useful even in the field. However, to assess different advantages and disadvantages in other circumstances, the chosen method should be examined critically before use.

**Key words**: *Clethrionomys rufocanus*, recapture rates, live trapping, marking, ear-tattoo

## INTRODUCTION

Marking individual animals is an essential part of the behavioural and demographic investigation of field populations and can undoubtedly influence the physical and behavioural patterns of the studied animal. Besides minimizing this influence, all studies require cheap, easy-to-use and long-lasting markings. Furthermore, ethical concerns impose increasing pressure for marking methods to be non-invasive and adoption of such methods is becoming mandatory because, in some countries (e.g. U.S.A., Germany and EU conventions), current methods require special permits or are totally prohibited by law. Therefore, it is necessary to provide information about a marking method used in the field to allow a comparison.

Different techniques have different effects on the animals marked, but the type and the intensity of these effects are widely debated (Fullagar & Jewell, 1965; Fairley, 1982; Pavone & Boonstra, 1985; Korn, 1987; Hugo, 1990; Wood & Slade, 1990; Salamon & Klettenheimer, 1994; Braude & Ciszek, 1998). As a contribution to this debate and to provide useful information to compare different techniques, the use and evaluation of marking small mammals by ear-tattoos during a large field study is described. The ear-tattoo technique is a relatively new method in field biology (Boye & Sondermann, 1992). Therefore details are given here of the reliability of this technique, the handling time required, and possible impacts on the marked animals.

There are some reports about the impact on the physiology and behaviour of other marking methods. Pavone & Boonstra (1985) showed in experiments with the meadow vole *Microtus pennsylvanicus* that survival in these voles when toe-clipped was low, especially in males. Other studies have found evidence for a decreased recapture rate after toe-clipping (Fairley, 1982; Wood & Slade, 1990). In contrast, Ambrose (1972) and Korn (1987) could neither find increased predation nor

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decreased body weight of individuals marked with the toe-clipping method. Braude & Ciszek (1998), however, found a higher survival rate in toe-clipped mole rats *Heterocephalus glaber* than in animals with implanted transponders. Other marking methods such as leg rings (Fullagar & Jewell, 1965; Fairley, 1982), colouring the tail base (Hugo, 1990) or ear tags from metal or plastic (Salamon & Klettenheimer, 1994) caused occasional infections or trap shyness. As far as we know, only a few observations that have examined the influence of ear-tattooing on the animals have been reported (Boye & Sondermann, 1992). The changes produced by any new method should at least be no greater than those caused by existing methods, and they should be clearly tested and explicitly compared with the alternatives. In this study, the effects of tattooing on recapture time and weight loss were tested and the results compared with literature data on other methods, in particular the widely used toe-clipping technique, in order to effectively judge the strengths and weaknesses of these methods. Comments are also made on the cost and ease of use.

## MATERIAL AND METHODS

A study on the behavioural responses of *Clethrionomys rufocanus* to predator odour was conducted from 1997 to 1999 in the Finnmarksvidda of northern Norway. In this study an ear tattooing method was used for individual marking of the voles, and our experience and data from this study form the basis of this paper. Every year, 6 plots of 1 ha were investigated: 3 treatment plots and 3 control plots; 100 Ugglan multiple-capture live-traps were distributed over each plot. The traps were baited with oats and covered to give protection against the weather. The 6 plots were checked twice within 3 days and 3 nights. On the first day, the traps on plot 1a and 1b were checked alternately every 2 h from 5:00 to 17:00. With this schedule, captured animals remained in a trap for < 4 h. On the following 2 days, plots 2a/2b and 3a/3b were checked in the same way. On the fourth day we changed to night captures and then checked the plots every 2 h beginning at 17:00 for a further 3 days resulting in 400 trap-checks/day. The next trapping round was started after breaks of 2 or 3 days without trapping. The different length of these breaks affected the measurement of recapture intervals, which then had to be accounted for in our statistical analysis. During the field season of 1998, 8 rounds (day and night) were conducted and all subsequent data used for statistics come from this year. Individuals were categorized by their age at first capture. The category 'juvenile' contained all individuals weighing < 22 g, caught only at 1 or 2 trap sites. The category 'sub-adults' contained all dispersers with body weights of > 22 g. Adults were identified as animals showing gender-specific reproduc-

**Table 1.** Capture and recapture numbers of *Clethrionomys rufocanus* in 1998

| Kind of captures | Total | Proportion | (%) |
|---|---|---|---|
| Total no. of capures | 1123 | 100.0 | |
| Captures – | | | |
| Marked new | 274 | 24.4 | |
| Unmarked[a] | 74 | 6.6 | |
| Recaptures | 775 | 69.0 | 100.0 |
| Identified | 697 | 62.1 | 89.9 |
| Recently marked[b] | 50 | 4.4 | 6.5 |
| Unidentified | 28 | 2.5 | 3.6 |

[a] Animals remained unmarked because they died in the trap or were too stressed to handle or were too small to mark (the latter could be marked later).
[b] Animals with fresh tattoo ink on their ears. These animals could be identified later if recaptured.

6 plots. It was not possible to identify the individual code of all recaptured animals; therefore, 775 recaptures were used for the statistical analysis. Individuals too small to mark, too stressed to mark, dead or with a marking from the previous year were not considered (Table 1).

The study was conducted by OF who had *c.* 10 years experience in handling small mammals and marking with ear-tattoos. The 2 assistant field workers of 1998 (the year we obtained the data for this study) had no previous experience. However, 1 spent 3 months of summer field work on the project and the other one (EL) joined the team for the last 6 weeks. Both were trained in handling and marking voles during the field work. The different skill levels of the field workers allowed us to determine whether there was any significant effect of skill on animal processing and so strengthened the comparative value of our study.

For marking tattoo pliers (by EBECO) with a revolving head for numbers were used for 1 ear. On the other ear a second pair of pliers (by Hauptner) with letters that had to be changed piecewise were used. Black tattooing ink was used on experimental plots and green on control plots. The ink (by Hauptner) was a special dye for marking small mammals. To mark the voles, they were held in the left hand with the hind legs fixed between the fourth and the fifth fingers and the head showing between the thumb and first finger. Thus the voles could not use their strong hind legs to push themselves out of the hand and the grip around the voles body could be loose enough to allow them to breath freely. The right hand of the operator was free to handle the tattoo pliers. First, the ear of the vole was penetrated with the pins of the pliers before the tattoo colour was applied with the fingertips. Existing codes were identified by shining light from a small torch through the ear. The tattoo pliers were developed by Klimisch (1986) to mark small mammals in laboratories and their use under field conditions was first described

plot. Interchanging the use of numbers and letters between ears increased the number of code combinations available to 520. To avoid confusion between plot animals and occasional wide-ranging animals from neighbouring plots distinct code combinations and different colours were used on adjacent plots. Similar letters were not used at the same time on any plot (e.g. F-E, P-R, O-Q) to reduce possible confusion.

The data of the voles caught in 1998 were used to test the hypothesis that adult *C. rufocanus* growth rates were unaffected by marking. For this Wilcoxon's signed-rank test was used. Assuming that growth rates are linear inside an age category, only individuals that stayed in the same age category for the observed trapping events were considered.

As a second hypothesis whether inter-capture intervals were unaffected by tattooing was tested, the length of time between capture/marking and first recapture with the time between the second and third recapture was compared. Due to the irregular times between trapping sessions, the requirements for Wilcoxon's signed-rank test, a continuous distribution of data that is symmetric (Stahel, 2000), were not met and therefore, our hypothesis was tested by a paired samples randomization test (Manly, 1997) of the statistic package S-PLUS 4.5 specifying 5000 repetitions in every test. Individuals were only included in the test if they had been recaptured > 3 times and if they fell into the same age group classification at both marking and third recapture. These stipulations reduced the total number

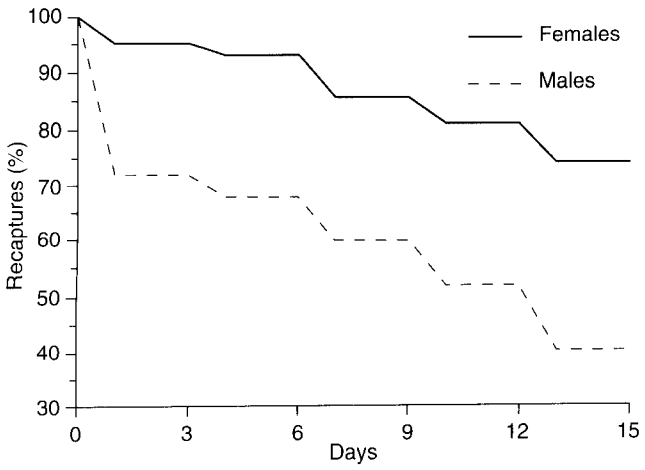


**Fig. 1.** Percentage of recaptures for adult males and adult females

re only caught once whereas 7%
n the third and fourth trapping day
the loss of males was much higher,
captured after marking but only 8%
n the third and fourth trapping day.
examined, 89.9% had readable codes
ed individually and 3.6% could not.
% were recaptures marked on the
too much tattoo ink covering the
s had had some days cleaning their
d usually be identified (Table 1).

of the females wer
disappeared betwee
(Fig. 1). In contrast,
28% could not be re
disappeared betwee
Of all marked voles
and could be identifi
The remaining 6.5%
same day with still
code. After the vole
ears these codes coul

**Table 2.** Wilcoxon signed rank test for differences in change of weight of *Clethrionomys rufocanus* over time. There were no differences for the several age classes in change of weight between the first capture and first recapture, and between the second and the third recapture. Splitting in age classes was done by the age at the first recapture. a, Interval between the first capture and the first recapture; b, interval between the second recapture and the third recapture

| Age classes | Time interval | *n* | Mean | ±SD | Min | Max | *Z* | *P* |
|---|---|---|---|---|---|---|---|---|
| Juvenile | a | 24 | 0.0590 | 0.0773 | −0.0092 | 0.2500 | −1.286 | 0.199 |
| | b | 24 | −0.0200 | 0.3724 | −1.0000 | 0.7500 | | |
| Sub-adult | a | 17 | 0.0170 | 0.0616 | −0.0161 | 0.2500 | −0.698 | 0.485 |
| | b | 17 | 0.0910 | 0.3176 | −0.5000 | 0.7500 | | |
| Adult | a | 17 | 0.0055 | 0.1155 | −0.2500 | 0.2500 | −0.308 | 0.758 |
| | b | 17 | −0.0015 | 0.4424 | −1.0000 | 1.2500 | | |

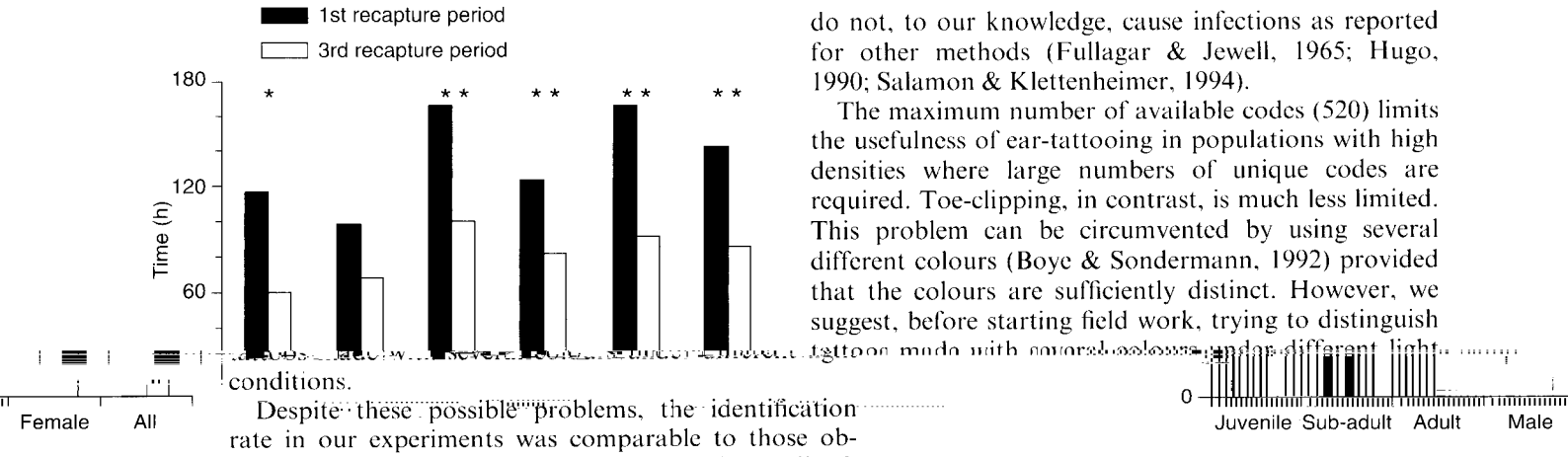


**Fig. 2.** Paired samples randomization tes
time lags between recaptures of *Clethr*
Difference for juveniles ($P < 0.001$; = 35),
$n = 17$), males ($P < 0.001$; $n = 57$), females
and all age categories including age tr
($P < 0.001$; $n = 100$) were significant w
($P = 0.238$; $n = 13$) did not show significance

t for differences in
*ionomys rufocanus*.
adults ($P = 0.017$;
($P < 0.001$; $n = 43$)
ansient individuals
whereas sub-adults
e.

-marking factors,
atly disadvantage
clipping or other

luals were identi-
failure were badly
les with wrinkled
ink remaining on
l information on
d weight, can be
and which could
re cleaned by the

some extent by
ver, tattooing the
l because the risk
This, of course,
toos for marking
important. Ear-
sulting from mis-
ge resulting from
ut or fall off and

do not, to our knowledge, cause infections as reported for other methods (Fullagar & Jewell, 1965; Hugo, 1990; Salamon & Klettenheimer, 1994).

The maximum number of available codes (520) limits the usefulness of ear-tattooing in populations with high densities where large numbers of unique codes are required. Toe-clipping, in contrast, is much less limited. This problem can be circumvented by using several different colours (Boye & Sondermann, 1992) provided that the colours are sufficiently distinct. However, we suggest, before starting field work, trying to distinguish tattoos made with several colours under different light conditions.

Despite these possible problems, the identification rate in our experiments was comparable to those obtained for toe-clipping and other methods (Weile & Sohler, 1996; S. Halle & J. Jacob, pers. comm.) and re-identification rates of tattooed animals close to just 100% are possible as reported by Boye & Sondermann (1992).

It is self-evident that tattooing hurts the animal and this is seen in some of the behaviour reported. However, most voles showed little immediate effect of tattooing. There were no subsequent infections caused by the tattooing procedure in the recaptured animals. Ear tattoos cannot, furthermore, affect locomotion nor do they become snagged in debris or vegetation.

Ear-tattoos had no effect on vole body weight, although minor effects could have been masked by natural increase in body weight during non-adult stages. This contrasts with toe-clipping, which can significantly reduce body weight (Korn, 1987) and suggests that tattooing is less stressful than clipping.

In our study, individuals of all age groups showed a greater delay of recapture after marking than after subsequent simple recapture implying that tattooing trauma reduces recapture rate. Similar results have been reported for toe-clipped voles (Wood & Slade, 1990; Fairley, 1992), however, and the size of the effect in our study is comparable with that for toe-clipping.

Jacob (2000) reported a recapture rate of 70–80% for the first recapture after toe-clipping. He found a higher recapture rate in females. This data, based mainly on *Microtus arvalis* captures from agricultural areas in Germany, with a loss of 20–30%, seems to be high as our loss rate was only 13%. The proportion of adult

and the importance of abiotic, non-
the longer handling time does not gre
ear-tattooing in comparison to toe-c
techniques.

Using ear-tattoos, 89.9% of indivic
fied. The major cause of identification
applied markings, especially on juveni
ears and, occasionally, excess tattoo
the ears. In these animals, additiona
place of capture, species, sex, age an
used to clarify their identification
be confirmed later when the ears whe
voles.

Most errors could be reduced to
increased training of operators. Howe
smallest juveniles is not recommended
of incomplete marking is too high.
greatly limits the suitability of ear-tat
in studies where identifying juveniles is
tattoos are, however, free of errors res
cutting, cannot be confused with dama
natural loss or fighting, do not grow o

females in our study that disappeared after tattooing was within the range of losses of subsequent recaptures. This indicates that tattooing had no negative effect on these animals. Recapture rates of adult males showed a different pattern. Their recapture rates after marking were much smaller than subsequent recapture rates. Nevertheless, we cannot imagine that the handling had that much of a different effect on males and females. The observed pattern might be explained by the fact that males roamed around whereas females were resident on the trapping plots. Therefore the chance of catching an individual only a single time should be much higher for males.

Ear-tattoos are consequently a fully appropriate method for marking small mammals in field studies. There were no major effects on behaviour or survival greater than those associated with other techniques and the low proportion of voles lost after marking and the lack of negative influence on growth rates implies that tattooing is less disruptive to normal behaviour. Such reductions in behavioural disruption are to be welcomed, although many ecological studies need to mark individual animals and every method unavoidably has some effect. Although deciding to avoid some techniques (e.g. toe-clipping) for ethical reasons must be the decision of individual researchers, it is felt that ear-tattooing is a less invasive technique. Even if toe-clipping, or other techniques, are practically necessary, animal protection legislation may force researchers to find alternatives like ear-tattooing. There is, however, a growing need for adequate formal comparative study with respect to field practicability, effects on the animals, and ethical and legal considerations, of all the marking methods available for small mammals (e.g. toe-clipping, ear-tattoos, ear-tags, transponders and tail marks) as without such work a properly informed decision cannot be made.

**Acknowledgements**

This study would have been impossible without the hospitality of Tarja and Lauri Oksanen and their team who allowed us to work in their study area, for which we are especially grateful. We also thank A. Davis, J. McNamee and S. Pfeiffer for much help with the English, J. Gutsell and J. Schumacher for support with the statistics, S. Halle for his help with the Delta Graph package, and A. Davis, S. Halle, M. Nilsen, Ø. Varpe and G. Wagner for useful comments on the manuscript. This project was financed by the Deutsche Forschungs Gemeinschaft (DFG Project HA 1513 / 9–1).

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