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**Reference:**

Borremans Benny, Sluydts Vincent, Makundi Rhodes H., Leirs Herwig.- *Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent*

**Wildlife research** - ISSN 1035-3712 - (2014), p. 1-6

DOI: <http://dx.doi.org/doi:10.1071/WR14109>

**Pre-publication version for archiving.**

**Published version:** <http://dx.doi.org/10.1071/WR14109>; You can also request a pdf by emailing the corresponding author.

## **Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent**

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### **Abstract**

**Context.** Toe clipping is a widely used method for permanent marking of small mammals, but its effects are not well known, despite the ethical and scientific implications. Most studies do not find any clear effects, but there is some indication that toe clipping can affect survival in specific cases. Although effects on survival are arguably the most important, more subtle effects are

also plausible, yet very few studies have included body condition and none have investigated effects on mobility.

**Aims.** We analysed the effects of toe clipping on free-living *Mastomys natalensis*, a common, morphologically and behaviourally intermediate small rodent.

**Methods.** Using a 17-year capture-mark-recapture dataset, we compared movement, body weight and survival between newly and previously clipped animals, and tested whether any of these parameters correlated with the number of clipped toes.

**Key results.** No evidence for a correlation between total number of clips and any of the variables was found. Newly clipped animals had a slightly smaller weight change and larger travel distance than those that were already clipped, but we show that this is most likely due to stress caused by being captured, clipped and handled for the first time than to the actual clipping.

**Conclusions.** The combination of trapping, handling and marking has a detectable effect on multimammate mice, but there is no evidence for a clear effect of toe clipping.

**Implications.** Our study suggests a re-evaluation of ethical guidelines on small mammal experiments in order to reach a rational, fact-based decision on which marking method to use.

**Additional keywords:** animal welfare, capture-mark-recapture, animal ethics, individual identification, *Mastomys natalensis*, permanent marking, survival, toe-clipping

## **Introduction**

Many field studies on small mammals need permanently marked animals for individual identification. Several permanent marking methods exist, each with their own advantages and disadvantages, the choice of which will depend on ethical considerations and the needs and logistical possibilities of the study. Because a marking procedure may affect an animal's behaviour and physiology, care must be taken when extrapolating study results to unmarked animals, which can only be done if the effect of marking has been quantified. In order to make a well-informed decision about which marking method to use, it is necessary to have a reliable assessment of the effects of each method. Commonly used identification methods include implanted microchips (PIT-tags), tattooing, ear punching, ear tags, dyes, radio transmitters, and toe clipping (Fullagar and Jewell 1965; Fagerstone and Johns 1987; Leclercq and Rozenfeld 2001; Metzgar 1967; Ostfeld and Heske 1993; Stockdale 1932; Hamley and Falls 1975). For a thorough overview of animal identification methods see Murray and Fuller (2000). The main advantages of toe clipping are low costs, short handling times, and the possibility to simultaneously collect tissue samples for genotyping. Disadvantages are the stress and pain suffered by the animal, and possible consequences on survival and behaviour.

Although many institutional welfare guidelines and scientific journals advise against the use of toe clipping and require justification for its use (e.g. National Research Council of the National Academies 2011; Sikes and Gannon 2011), little research has actually been done on the possible effects of toe clipping on small mammals, especially in field studies (Murray and Fuller 2000). Pavone and Boonstra (1985), who investigated the effects of toe clipping on survival using an unclipped control group and a clipped group of meadow voles, found no effect on body weight, but did observe that toe-clipped males (but not females) had a lower survival in summer. This interaction between sex and season was attributed to changes in behavioural interactions that only occurred in males. A study by Tamarin and Krebs (1969) found that a combination of blood

sampling and toe clipping lowered vole survival in some cases, but their study design did not allow quantification of the uncombined effects. Owl predation rates and health of *Microtus pennsylvanicus* do not seem to be affected by toe clipping (Fullagar and Jewell 1965; Ambrose 1972). When comparing toe-clipped and PIT-tagged free-living naked mole-rats, Braude and Cizek (1998) found a slightly lower survival of the latter group, and suggested the use of toe clipping as the most preferable marking technique. The few other studies on small mammal species that exist (see Murray and Fuller 2000) were usually not able to detect any effects of clipping.

The effects of toe clipping are clearly not the same for all small mammals, and in fact the only unambiguous effect found so far is a lower survival on male meadow voles in summer (Pavone and Boonstra 1985). Most existing studies investigated survival, and although this is arguably the most critical effect, it is quite plausible that the consequences are not severe enough to reduce survival probability, but rather assert themselves more subtly, and perhaps only temporarily, as a decrease in mobility and/or lowered body condition. No field studies have looked at mobility, but some indirect evidence for a possible morphological effect has been observed in laboratory studies in the form of decreased suspension bar grip strength, although this was found in some (Iwaki *et al.* 1989; Schaefer *et al.* 2010), but not all (Castelhano-Carlos *et al.* 2010) studies, and only in newborns. Although laboratory studies have the advantage of a controlled experimental design, their relevance for natural conditions is debatable, and should be followed by evaluations in a field setup (Murray and Fuller 2000).

In this study, we assessed the effect of toe clipping on movement, body condition and survival of the Natal multimammate mouse *Mastomys natalensis*, a common and widely distributed Sub-Saharan Africa rodent. *M. natalensis* is a ground-dwelling omnivore, without specialised or exceptional morphological traits and a body size (30-70 g) intermediate for commonly studied small rodents, and can therefore be considered to be a suitable representative for a wide range of small mammal species.

## Materials and Methods

### *Trapping and marking*

Analyses were done using an existing capture-mark-recapture (CMR) dataset. Between March 1994 and January 2011, trapping was conducted monthly for three consecutive nights in a rectangular 300 x 100 m grid of Sherman live traps (76 x 89 x 229 mm, Sherman Live Trap Co., Tallahassee, FL, USA) spaced evenly at 10 m intervals, on the campus of the Sokoine University of Agriculture (Morogoro, Tanzania). This trapping effort resulted in 36 425 captures of 15 471 individuals during 212 700 trap nights in 223 trapping sessions. At first capture, animals were weighed and sexed, breeding status was determined, and they were marked using a unique toe clipping code. Because of high abundances (Sluydts *et al.* 2009), combinations of up to six toes per animal were used, with a maximum of one toe per front paw and two per hind paw. Around 2800 unique codes were used in rotation across seasons. A toe was clipped by cutting the upper phalanx using ethanol-cleaned sharp dissection scissors. In 1987, at the start of the CMR studies on rodents in Morogoro, toe clipping was chosen as the marking method because it allows the use of a large number of unique codes, it is easy, fast and cheap to apply and does not use electronic equipment which at the time was an advantage in Tanzania (Leirs *et al.* 1989). Moreover, the clipped toes provided tissue samples for population genetics research. The same marking technique was used for all follow-up CMR studies in Morogoro. The work was conducted under the animal ethics regulations of Sokoine University of Agriculture and in retrospect followed the guidelines in Sikes & Gannon (2011).

The effect of toe clipping was tested with respect to four response variables: survival, home range, travel distance and body weight, chosen because they represent relevant ecological traits on different temporal scales. The absence of a proper control group, i.e., non-clipped recaptured individuals, complicated the analyses. Instead, we used an approach in which the four variables

were compared between newly and previously marked animals. Because the effect of marking may be confounded by stress caused by being trapped and handled the first time, we also tested for a correlation between the variable of interest and the number of clipped toes, which would indicate a direct effect of toe clipping. Data were divided into four classes according to the number of clipped toes: two, three, four, and five/six toes (the latter category pooled because only 18 individuals had six clips).

### *Survival*

Monthly survival was evaluated as a proxy for mid- to long-term clipping effects. The dataset consisted of 223 trapping sessions that were analysed using a standard Cormack-Jolly-Seber approach to survival analysis (Lebreton *et al.* 1992). Because this dataset was too large for the available computational power, it was divided into two subsets, the first containing sessions 1-111, and the second sessions 112-223. A goodness-of-fit (GOF) test was conducted to evaluate possible confounding factors such as trap-dependence and an excess of transient animals (Pradel *et al.* 2003). Model selection was based on the AICc (Burnham and Anderson 2002), and the effect of toe clipping was evaluated by comparing the fit of models including and excluding the effect of clipping in survival and recapture probability.

### *Home range*

Long-term effects were assessed by testing whether home range size correlated with the number of clipped toes. Home range estimation was done as described in Borremans *et al.* (2014). Briefly, home ranges (using the definition of Burt 1943) were estimated using the minimum convex polygon (MCP) method based on all capture locations, with an inclusive 5 m boundary strip (Stickel 1954). Only animals that were trapped in at least two different months were used for MCP estimation, as these animals were assumed to be resident. In order to account for an edge effect on MCP size, home ranges with a centroid less than 12 m from the trapping grid edge were removed from the

dataset (Dice 1938; Stickel 1954; Borremans *et al.* 2014). We tested for a correlation between home range size and number of clipped toes, and for an effect of the presence/absence of two clipped toes on the same hind paw using ANOVA of a linear model including these variables as well as sex and number of recaptures.

### *Travel distance and weight change*

Short- and mid-term effects were investigated through the use of travel distance and weight change between captures. Each time an animal was captured on two consecutive days or months, weight change (body weight at the second capture divided by that at the first, multiplied by 100 to obtain a percentage value) and distance between the two trap locations were calculated. These variables were compared between newly and previously clipped animals, taking into account weight and temporal/environmental effects in the linear mixed model by adding body weight category (10 g interval categories: 0-10 g, 10-20 g, etc., up to 90 g) and period of the year (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec) as random effects, and sex and reproductive status as fixed effects. Using the same model variables, we tested for an effect of having two clipped toes on a hind paw and for a correlation with the total number of clipped toes. Models including and excluding variables of interest were compared using likelihood ratio testing. Pregnant females were excluded because sudden weight loss due to birth could influence results.

### *Software*

Most data manipulation, statistical analyses and plotting was done using R (R Core Team 2013), with functions available in R packages RMark 2.1.6-1 (Laake 2013), adehabitatHR (Calenge 2006), gpclib (Peng and Murta 2012), maptools (Lewin-Koh and Bivand 2012), lme4 (Bates *et al.* 2012), Matrix (Bates and Maechler 2012) and plotrix (Lemon 2006). U-Care v 2.3.2 was used for GOF calculations (Choquet *et al.* 2009). Error margins denote standard errors unless stated otherwise. Statistical significance was assumed for *P*-values lower than 0.05.

## Results

### *Survival*

The global GOF test indicated a strong effect of both an excess of transient animals ( $N(0,1) = 8.55$ , d.f. = 896,  $P$ -value < 0.001) and “trap-happiness” ( $N(0,1) = -18.02$ , d.f. = 896,  $P$ -value < 0.001). These confounding effects were therefore taken into account using the methodology described in Pradel (1993) and Pradel *et al.* (1997). Following the notation in Lebreton *et al.* (1992) we compared five models:  $\Phi_{(a2+toe)}p_{(m+toe)}$ ,  $\Phi_{(a2+trend)}p_{(m+toe)}$ ,  $\Phi_{(a2)}p_{(m+toe)}$ ,  $\Phi_{(a2+toe)}p_{(m)}$  and  $\Phi_{(a2)}p_{(m)}$  (shown in Supplementary Material Tables S1 and S2). Both datasets supported a model including an effect of clipped toes in both survival and recapture probability (Supplementary Material Tables S1 and S2), but there were no obvious linear trends (Figure 1 and Supplementary Material Figures S1 and S2). Given the confounding effect of transients and the fact that the proportion of transients is higher in the newly clipped category, survival estimates in this category are lower than those of the previously marked group. In the first dataset the survival estimates for the category of four clipped toes were lower, while this was not the case in the second data set. The most likely reason for this drop appeared to be that, in the first dataset, most individuals with four clips were concentrated in the annual period of population decrease that occurs in the late dry season (Leirs 1994), which was not the case in the second dataset.

### *Home range*

There was no significant correlation between an animal’s number of clipped toes and its home range size (F-value = 0.08, d.f. = 1,4164,  $P$ -value = 0.78), and there was no significant interaction between sex and number of toe clips (F-value = 0.25, d.f. = 1,4164,  $P$ -value = 0.61). Mean home range sizes per toe clip category were  $637 \pm 48 \text{ m}^2$  (two clips),  $680 \pm 30 \text{ m}^2$  (three clips),  $626 \pm 21 \text{ m}^2$  (four clips) and  $651 \pm 16 \text{ m}^2$  (five/six clips).

### *Travel distance and weight change*

One-day travel distance was higher for newly clipped animals ( $13.5 \pm 0.4$  m) than for previously clipped animals ( $11.9 \pm 0.2$  m), but this difference disappeared for one-month travel distance ( $18.6 \pm 0.4$  m and  $18.1 \pm 0.4$  m for newly and previously clipped animals, respectively) (Table 1). Adults had a larger one-day, but not one-month, travel distance than juveniles, while males had a larger one-month, but not one-day, travel distance than females. There were no interactions between clipping category and sex or reproductive status. For newly clipped animals, travel distance was unaffected by the total number of toe clips or the number of hind paw clips (Figure 2a).

One-day as well as one-month weight change was significantly affected by clipping category, sex and reproductive status. Newly clipped animals had a lower weight change ( $93.5 \pm 0.2$  % and  $102.7 \pm 0.3$  % one-day and one-month weight change, respectively) than those that were clipped previously ( $94.5 \pm 0.2$  % and  $103.7 \pm 0.3$  % one-day and one-month weight change, respectively); i.e. over one day, newly clipped animals lost more weight than previously clipped animals, while over one month they gained less weight. Regardless of clipping category, females had a smaller weight change than males, while that of adults was larger than that of juveniles. No correlations were observed between weight change and total number of toe clips or the presence of hind paw clips (Figure 2b).

### **Discussion**

Despite our large dataset that should allow the statistical detection of even very small effects, no evidence was found for a correlation between the number of clipped toes and any of the studied variables. We did observe that newly clipped animals lost more (one day) or gained less (one month) body weight than those that were already clipped. These differences were small however: 3.3% in one month and 0.7% in one day, which, for an animal with a body weight of 30 g, would translate into 0.9 g (3.3%) and 0.21 g (0.7%). So although statistically significant, the biological

significance of this difference is debatable. Movement was affected similarly by recent clipping, resulting in a larger travel distance from the last trap location, but this was only the case for the first day (and not one month) after clipping. As these effects on weight change and movement were small and unaffected by the number of clipped toes, they were not necessarily due to wounding.

Instead, our findings suggest that the observed effects are transient and more likely caused by stress, due to being captured and handled for the first time. Similar conclusions were reached by Korn (1987), who investigated the effects of toe clipping on five rodent species and found no biologically relevant effects but did observe small weight losses due to trapping-related stress. If true, this means these effects can be expected regardless of which marking method is used, as they all require stressing and wounding the animal in some way. Indeed, when using ear-tattooing for permanent marking of grey-sided voles, the time interval between the first and the second capture has been observed to be longer than that between consecutive captures (Lindner and Fuelling 2002).

In order to inform and improve ethical guidelines on animal experiments and field study methods, it is necessary to evaluate the effects of marking methods in a range of settings and for multiple species. Our study on the multimammate mouse, a morphologically and behaviourally intermediate small rodent, found no evidence for any direct effects of toe clipping, but did observe a stress effect most likely caused by being captured and handled for the first time, which disappears in consecutive captures. This calls into question the current recommendations against the use of toe clipping, and encourages a critical re-evaluation in order to make a rational, fact-based decision on which method to use for the permanent identification of small mammals.

## **Acknowledgements**

We thank the staff at the Pest Management Center of the Sokoine University of Agriculture (Morogoro, Tanzania) for years of support. Major contributions to the original data collection were

made by Solveig Vibe-Petersen, Saskia Mercelis, Christopher A. Sabuni, Loth Mulungu, Apia Massawe, the SUAPMC technical staff and several undergraduate students. Funding was provided over the years by several projects under the European Union Framework Programmes, VLIR-UOS (the Flemish University Development Cooperation), DANIDA-Rådet for U-landsforskning and the University of Antwerp. BB is currently a research assistant of Research Foundation Flanders (FWO). No conflicts of interest exist.

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**Table 1** Travel distance statistics

Variable	Travel distance – 1 day					Travel distance – 1 month				
	Est (m)	SE (m)	Chisq	df	P	Est (m)	SE (m)	Chisq	df	P
Intercept	12.2	3.6	–	–	–	21.8	5.2	–	–	–
Newly clipped	1.9	0.5	15.1	1	<0.001	1.1	0.6	3.63	1	0.057
Male	0.5	0.5	1.27	1	0.26	1.5	0.7	4.76	1	0.029
Juvenile	-2.9	0.7	16.3	1	<0.001	-1.4	1	2.03	1	0.15
Newly clipped *										
Male	1.3	1	1.79	1	0.18	-0.3	1.2	0.09	1	0.77
Newly clipped *										
Juvenile	-0.03	1.3	0	1	0.97	2.9	1.6	3.15	1	0.076
Number of clips	1.6	0.9	3.39	1	0.065	-0.01	0.87	0	1	0.99
Hindclips (1)	2.9	6.5	2.62	2	0.27	-5.8	6.1	1.14	2	0.56
Hindclips (2)	0.5	6.8	2.62	2	0.27	-5.2	6.4	1.14	2	0.56

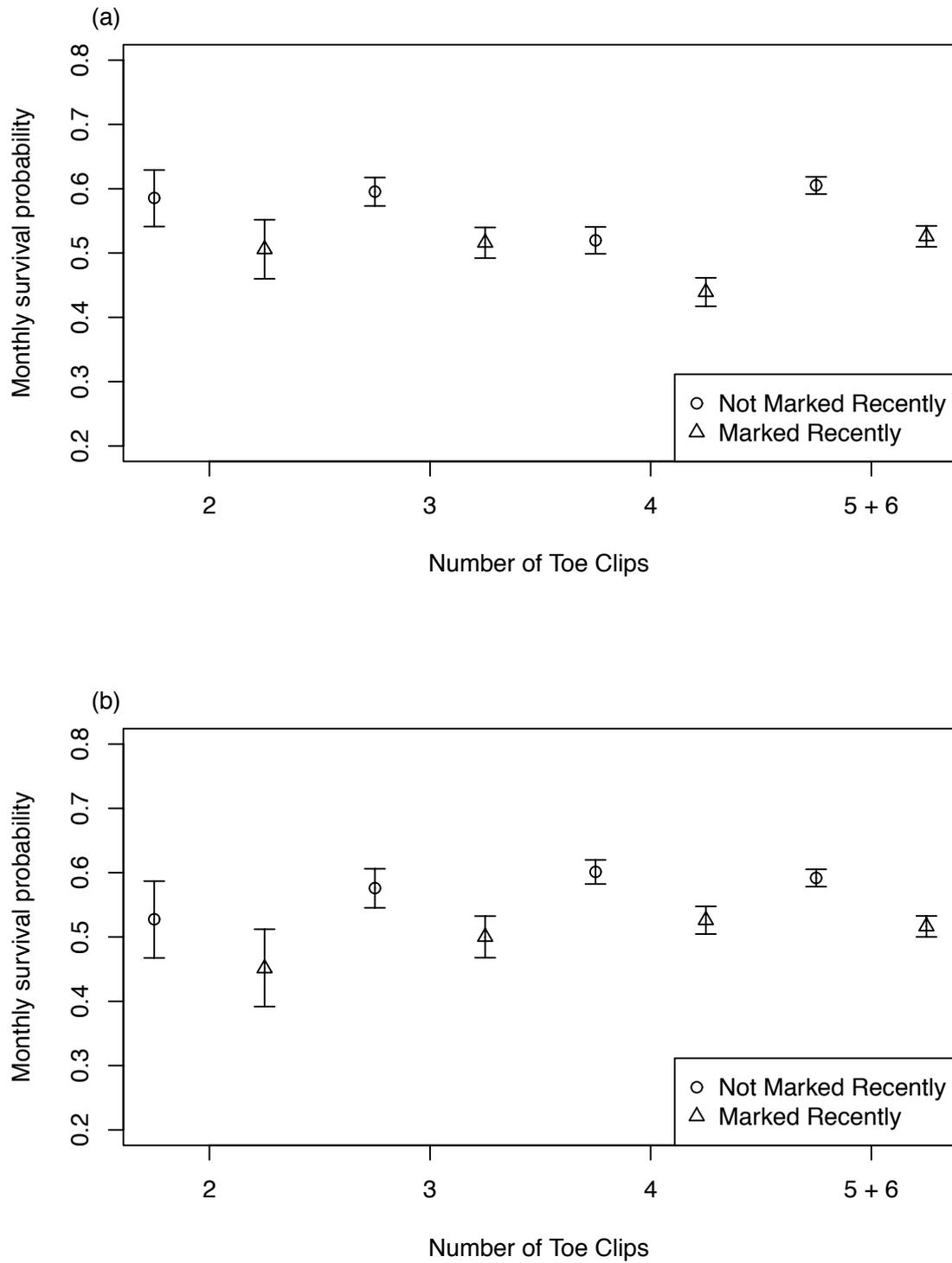
Test statistics of likelihood ratio tests of linear mixed models, testing the effect of different variables on travel distance. Effect estimates (Est) are shown with standard errors (SE). Variables are clipping category (newly/previously clipped), sex, reproductive status (juvenile/adult), number of clips (two, three, four or five/six) and the maximum number of hind paw clips (none, one or two). The latter two variables were tested for newly clipped individuals. Statistics for the variable hind clips are the same for both levels (one and two), but effect estimates were estimated for each level.

**Table 2** Weight change statistics

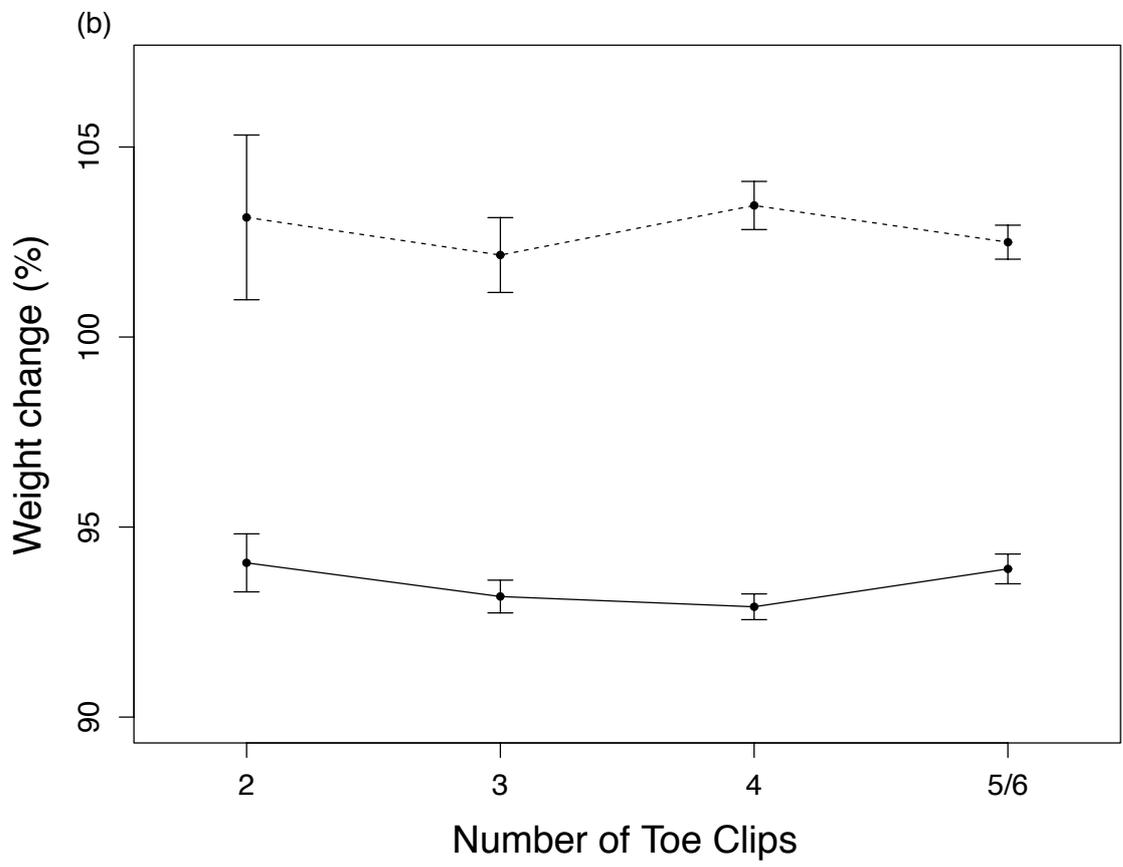
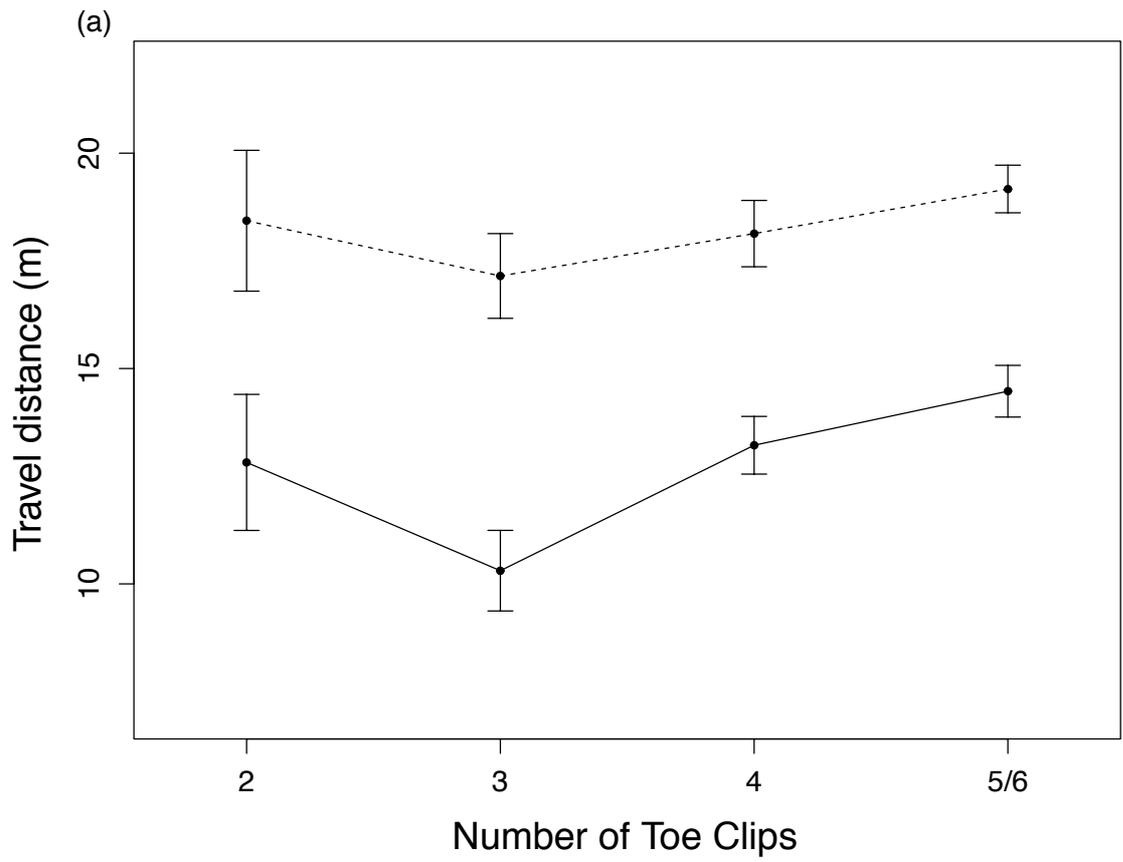
Variable	Weight change – 1 day					Weight change – 1 month				
	Est (%)	SE (%)	Chisq	df	P	Est (%)	SE (%)	Chisq	df	P
Intercept	96.1	2.9	–	–	–	105.5	8.1	–	–	–
Newly clipped	-0.7	0.3	5.62	1	0.018	-3.3	0.5	48.3	1	<0.001
Male	0.8	0.3	6.3	1	0.012	4.2	0.4	102	1	<0.001
Juvenile	-1.1	0.4	6.03	1	0.014	-1.8	0.6	7.44	1	0.006
Newly clipped *										
Male	0.5	0.6	0.55	1	0.46	-0.5	0.8	0.39	1	0.53
Newly clipped *										
Juvenile	-0.2	0.8	0.03	1	0.86	2.1	1.2	3.3	1	0.069
Number of clips	-0.1	0.6	0.03	1	0.87	-1	0.7	2.04	1	0.15
Hindclips (1)	-2.4	3.9	1.07	2	0.59	2.1	3.9	0.35	2	0.84
Hindclips (2)	-1.7	4.1	1.07	2	0.59	2.4	4.1	0.35	2	0.84

Test statistics of likelihood ratio tests of linear mixed models, testing the effect of different variables on travel distance. Effect estimates (Est) are shown with standard errors (SE). Variables are clipping category (newly/previously clipped), sex, reproductive status (juvenile/adult), number of clips (two, three, four or five/six) and the maximum number of hind paw clips (none, one or two). The latter two variables were tested for newly clipped individuals. Statistics for the variable hind clips are the same for all levels (one and two), but effect estimates were estimated for each level.

## Figures



**Fig. 1.** Survival probability (point estimates with 95% confidence intervals) versus number of clipped toes (mean  $\pm$  standard error), for the first (a) and second (b) half of the dataset.



**Fig. 2.** One-day (full line) and one-month (dotted line) travel distance (a) and weight change (b) versus number of clipped toes (mean  $\pm$  standard error).