Evaluation of Methods for

Permanently Marking Kangaroo Rats

(*Dipodomys*: Heteromyidae)

DANIEL F. WILLIAMS, WALTER TORDOFF III, AND DAVID J. GERMANO

Abstract

We analyzed the loss and failure rates, misreading errors, mortality during applying and reading marks, and relative costs of marking kangaroo rats, *Dipodomys heermanni*, *D. ingens*, and *D. nitratoides*, during an 8-year study. Tattooing was considered to be an unacceptable method because of the high rates of misread marks (3.9%) and mortality during handling (0.5%). Cheek-pouch tags showed unacceptable problems with infections and injuries from the tags. Ear tags were the least expensive, but had the highest rates of loss (a range of 1-58% for time intervals of 6 days to > 1 year) and a relatively high rate of reading errors (0.5%). Infections often were associated with tags after they had been attached for several weeks or months. Passive integrated transponders (PIT tags) had the highest equipment and supply costs, but required the least amount of time to identify marked animals. They had lower loss rates (1-7%) than ear tags and a very low misreading rate (0.005%). Readers that store numbers can eliminate all misreading and transcription errors. No infections or other pathology were noted for PIT tags. The rate of mortality during handling for applying and reading ear and PIT tags (most were marked by both methods) was 0.04%; all mortalities involved the smallest-sized species, *D. nitratoides*.

INTRODUCTION

Most long-term population studies require that individual animals have a unique, permanent identifying mark. Statistical models for estimating population size assume that marks are read correctly and the animals do not lose their marks during a study (Pollock et al., 1990). Different marking methods are prone to different rates of loss and reading error. Further, some methods of marking may risk injury to the animal during handling and cause infection, reducing fitness of marked individuals for these or unforeseen reasons.

Methods of marking small mammals have been analyzed and reviewed by several researchers. These include reports by Fullagar and Jewell (1965), Twigg (1975), Bazhenov et al. (1984), and Genoud (1984). Currently, the most common methods of marking small mammals are toe-clipping, attaching ear tags, and injecting passive integrated transponders (PIT tags). The *ad hoc* Committee on Acceptable Field Methods in Mammalogy (ASM; 1987) included tattooing, ear tagging, and toe clipping among the acceptable methods. Despite the ASM endorsement, there is a virtual lack of information on the potential problems and efficacy of tattooing small mammals (Twigg, 1975). The California Department of Fish and Game does not permit toe-clipping of small mammals with protected status, so this method was unavailable to us. Though we do not review toe clipping in detail, comparative information can be found in Stoddart (1970), Fairley (1982), Pavone and Boonstra (1985), Korn (1987), Wood and Slade (1990), and Bias et al. (1993).

Ear tags are lost at rates that may be unacceptably high, depending upon the species, duration, and objectives of the

study (Twigg, 1975), but are inexpensive and easily applied by one person in the field. Metal tags also have been attached to the edge of the opening of cheek pouches of pocket gophers (Geomyidae; J.L. Patton, pers. comm.), but the effects of the tag on manipulation and transport of food and rate of tag loss are unknown. For pocket gophers and other species such as pocket mice (*Chaetodipus, Perognathus*), tags cannot be secured to the ears because they are too small.

Plastic- and glass-encased PIT tags, implanted under the skin or intra-abdominally, have been used to mark a variety of vertebrates. Transponders are programmed with a unique code during manufacture. The battery-free device is activated by a low-frequency electromagnetic signal with an external reader. The signal energizes the transponder and causes it to transmit its code (Fagerstone and Johns, 1987). Since its first use for marking wild vertebrates, the technology has been modified several times, and there are different transponder frequencies and designs available. Yet data on field use and failure are mostly unavailable (Fagerstone and Johns, 1987; Camper and Dixon, 1988; Rao and Edmondson, 1990; Ball et al., 1991; Germano and Williams, 1993; Schooley et al. 1993).

Also of concern, but seldom discussed or analyzed, are the personnel time to apply and read marks, equipment costs, and the number of errors made in reading marks. Error in reading codes from toe-clipped animals was mentioned by Le Boulenge–Nguyen and Le Boulenge (1986). Their and our (D.F. Williams, unpubl. observ.) experiences are that both novices and veteran field workers may make errors in marking and reading toe-clip codes at rates that could compromise the objectives of the study. Also, natural amputations occur at unknown rates in different populations of small mammals, which introduces possible additional error in identifying animals.

Rates of tag loss and reading error, effects on fitness, ethical issues such as minimizing pain to the animal, and costs should be considered when selecting a marking method. Yet, surprisingly little information is available to evaluate marking methods for most groups of small mammals. Without adequate information, researchers and agency personnel who issue permits are likely to select traditional or low-cost marking methods. Cost often is the overriding factor.

During a long-term demographic study of endangered giant kangaroo rats (*Dipodomys ingens*), we were initially required by permit-issuing agencies to mark by tattooing, but we changed methods of marking as accumulating experience showed unacceptable problems with tattooing and tagging cheek pouches. Because we lacked information on the failure rates for ear- and PIT-tagging, we simultaneously used two tagging methods to reduce the number of individuals with lost identity, and to gather information on tag losses for both methods. In a subsequent demographic study of Tipton (*D. nitratoides nitratoides*) and Heermann's (*D. heermanni*) kangaroo rats, we continued to double tag. While this paper was in review, we continued to collect data and updated values from two sites to better compare loss rates, but did not update other statistics. Herein we report and discuss data and observations on four methods of marking kangaroo rats: tattooing, attaching metal tags to cheek pouches and ears, and injecting passive integrated transponders.

MATERIALS AND METHODS

Field studies were conducted on the Carrizo Plain Natural Area, San Luis Obispo Co., California (*D. ingens, D. nitratoides*), and Pixley National Wildlife Refuge, Tulare Co., California (*D. heermanni, D. nitratoides*). Animals were captured between 6 July 1987 and 25 February 1995 on 12 100- to 196-trap grids and from trap stations at burrow systems spread over an area of about 40 ha. A total of 2,884 kangaroo rats were marked; they were captured a total of 26,707 times. Some animals were marked by all four methods. Cost of equipment and supplies are 1993 dollars and do not include shipping or taxes. Manufacturers and suppliers are listed where appropriate, but this does not denote endorsement of a particular supplier or product. Marking methods changed over the course of the studies and are described below in the sequence of their adoption.

Tattooing

In the field, 148 kangaroo rats (97 *D. ingens* and 51 *D. nitratoides*) were tattooed between July and October 1987. The tattooing machine was custom-made. Power to operate the device in the field was generated by a 110-V portable, gasoline-powered generator. A 25-V transformer with an in-line rheostat controlled power to the magnets. Two carbon-based black inks, India and Higgins Eternal, and Higgins Waterproof Red, No. 4085 (Faber Castell Corp., Newark, NJ 07107 USA), were used. One hundred animals were tattooed with India, 34 with Eternal, and 50 with Waterproof Red ink.

Two *D. heermanni* and one *D. nitratoides* were tattooed in the laboratory to practice techniques and find the best site for tattooing. These three laboratory animals and 14 field-collected *D. nitratoides* first were anesthetized with halothane (2-bromo-chloro-1, 1,1-trifluoro-ethane; Halocarbon Laboratories, Inc.), a fast-acting respiratory anesthetic. During the period when we were tattooing animals, all animals captured in the field were brought to a central point for weighing and reading or applying tattoos.

Efficient handling of both anesthetized and unanesthetized animals for tattooing required that one person hold the animal while a second tattooed the ear pinna. Because animals recovered quickly from effects of halothane, firm, two-handed restraint was necessary—one hand holding the animal by the skin on the neck, the other holding the legs and thighs. Unanesthetized animals were difficult to restrain for tattooing without causing them injury.

Tattooed marks were the letters A-Z, applied singly to the left or right ear, then letters on both ears. A few whose tattoos became illegible due to damaged ears were retattooed on the pads at the base of the digits of the manus. Toe pads were marked with a large dot, using a numbering sequence commonly used for toe clipping (DeBlase and Martin, 1981).

Cheek-pouch Tags

Size-1, monel, numbered, self-piercing tags (style 4-1005-1, National Band and Tag Co., Newport KY 41072 USA) were attached to the anterior edge of the cheek pouch with applica-tor pliers (style 4-1005S-1). Tags were applied in the field at the site of capture.

Ear Tags

Size-1, self-piercing tags were applied to ear pinnae using applicator pliers. Initially, a single tag was applied to one ear in the field at the site of capture. Subsequently, from approximately January 1989 through September 1992, an identically numbered tag was applied to each ear. After September 1992, only a single ear was tagged.

PIT Tags

Passive integrated transponders were injected subdermally using a 2.54-cm long, 12-gauge, Luer-Lok stainless-steel needle (Jorgensen Laboratories, Inc., 2198 W. 15th Street, Loveland, CO 80538 USA) in a modified, plastic, disposable, Luer–Lok, 3-cc syringe (Fig. 1). Modifications of the syringe included removing the flexible gasket at the tip of the plunger from its stalk and reinserting the gasket in the barrel of the syringe; cutting a straight slot through one side of the barrel of the syringe and inserting a plunger formed from brass welding rod approximately 90 mm long. The plunger was made by heating and bending it as shown in Fig. 1. The rod was inserted into the syringe through the slot cut in the barrel. Its tip was forced through the gasket and out the opening at the tip of the syringe.

Transponders were glass-encapsulated, approximately 11 mm long and 2.1 mm in diameter and activated at 400 kHz. Transponders and readers were manufactured by Destron Identi-
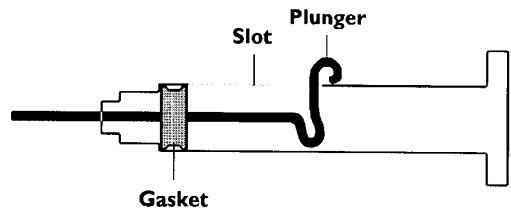

Fig. 1. Diagram of 3-cc, Luer-Lok plastic syringe, modified to inject passive integrated transponders (PIT tags). A slot about 2 mm wide and 20 mm long was cut in the barrel of the syringe from the gasket to behind the position of the plunger. The plunger was made by heating and bending a brass welding rod 1.62 mm in diameter.

fication Devices, Inc. and obtained from Bio-sonics, Inc. (3670 Stone Way N., Seattle, WA 98103 USA).

PIT tags and needles were sterilized by soaking in 70-100% isopropyl alcohol for several minutes. Tags were then coated with triple-antibiotic ointment and inserted into the tip of the needle. The coating of ointment kept the PIT tag from falling out of the needle. Loaded needles were capped with a segment of tygon tubing a few mm longer than the shaft and stored in sterilized plastic 35-mm film canisters. Capping and storing needles kept them clean and prevented their points from being dulled. Needles were sterilized and reused repeatedly until they became dull.

Three *D. heermanni* and four *D. nitratoides* were injected with PIT tags in the laboratory. They were sacrificed and autopsied one month later to determine effects of tagging.

A single worker injected animals with PIT tags in the field. Animals were tagged at their site of capture. They were removed from traps into a cloth bag, then restrained by grasping the loose skin on their back with one hand so that the skin of the animal's rump area was held between the thumb and forefinger (the reverse of the normal grip). The needle was slipped through the skin being held taut by the fingers of the gripping hand. As the PIT tag was injected, it was gripped under the skin and pushed forward and to the side of the injection site. The needle was then withdrawn as the fingers of the gripping hand held the animal's skin against the shaft of the needle. The skin at the site of injection then was squeezed tightly for a few seconds while the PIT tag was being read.

Error Detection, Failures and Statistical Analysis

Tattoos that became indecipherable because of damage to ears or disappearance of ink were scored as marking failures. Animals with a torn cheek pouch but no tag were scored as having lost a tag. Some of these animals also had been tattooed, so the lost tag was verifiable and the animal's identity was retained. Animals with a torn ear were scored as having lost a tag if the damage was consistent with having been tagged. Ears with tags that had been in place for a few weeks before loss typically had a rounded hole

at the medial edge of the tear. Since most ear-tagged individuals initially had a tag on each ear and a PIT tag, almost all losses were verifiable and the identity of the animal was retained. During the first year of use, we attempted to determine if the PIT was lost or inoperable by carefully feeling over the animal's body for a tag. Because almost all cases of "failure" within about 30 days of injection were from lost tags, we ceased spending much time feeling for an inoperable tag before retagging the animal. All lost cheek, PIT, and ear tags were scored as marking failures. Inoperable PIT tags also were scored as marking failures, though the nature of failure was recorded whenever it was determined.

Misread tattoo marks, cheek, or ear tags were identified by comparing data on field forms with permanent records for an animal. When discrepancies in data for a marked animal were found, they were reviewed to find the erroneous datum. Often, tattooed animals had not yet been released, so the source of error could be identified. Because tagged animals were released after examination at the site of capture, there was no opportunity to immediately identify the source of error. For animals with both ear and PIT tags, misread or mistranscribed ear or PIT tags were identified by discrepancy between the two. Because the PIT readers electronically stored the numbers read, these records were uploaded to a computer, printed, and checked against field forms to resolve the source and type of error.

Loss rates of PIT and ear tags were compared by species overall and temporally: recaptures within six days of marking (most census sessions lasted five or six days); recapture between 6 and 42 days ("monthly" censuses varied from 4-6 weeks apart depending on weather); recaptures between six weeks and one year of marking and recapture more than one year from marking. Animals that were not recaptured within an interval were not included in the tallies. Because most animals were double or triple-tagged (one or two ear tags, one PIT tag), few animals with a lost tag were unidentifiable. Error and loss rates were compared statistically using the Row by Column G-test for independence (Pimentel and Smith, 1990). Significant values were $p \leq 0.05$.

RESULTS AND DISCUSSION

Tattooing

Animals were captured and handled a total of 781 times (*D. ingens* 542; *D. nitratoides* 229) between 6 July 1987 and 10 October 1987, when we stopped tattooing. Some problems associated with tattooing and handling animals apparent in the first months after adopting this marking method are summarized in Table 1.

The inner surface of the ear was sparsely covered with small hairs, had relatively little dark pigment, and the skin was backed by a layer of cartilage, making this the best site for a tattoo. Tattooing the inner surface of the ears of the three captive individuals with a single letter on each ear was easy when the animals were anesthetized first and held by a second person. The marks, using suspended carbon-particle inks, were permanent and easily read four years after tattooing. After tattoo wounds had healed, reading tattoos was easier when the ear first was swabbed with water or dilute ethanol.

Applying a tattoo that did not damage ear tissue, yet was deep enough to be permanent, was more difficult. Penetration of the needles through the dermis into the cartilage layer resulted in wounding of the ear and, often, subsequent loss of the pigment when the scab fell off. These tattoos were usually legible as scars, however. On a few animals, ink moved into cracks and channels in the cartilage creating unintentional marks and causing difficulty reading tattoos. For *D. nitratoides*, wounding often was more serious than for *D. ingens*, resulting in loss of portions of the pinna and illegible marks. The ears of many kangaroo rats were also cut and torn by fighting. Kangaroo rats with extensive damage to ears that precluded retattooing the ear were tattooed on the front foot pads (the soles and pads of the hind feet are too densely furred for tattooing, so only a few individuals could be uniquely marked in this way).

The carbon-based inks were viscous and dried too quickly in field use. They also were messy and obscured the position of tattooed marks during application. If an animal moved and interrupted tattooing, the ear had to be cleaned before resuming. The red ink was less opaque and tattoos could be distinguished from surrounding ink, so cleaning the ear was not required when a subject moved during tattooing. Both carbon-based inks were permanent, but tattoos of animals marked with Higgins Waterproof Red ink began to disappear after about a week. These animals had to be retattooed with black ink when they were recaptured. Not all red marks remained legible and some letters had to be deciphered from scars. Tattoos of three individuals could not be read and they had to be assigned new letters. Because red ink was

Table 1. Summary of problems associated with marking *Dipodomys* spp. with tattoos. Individuals with damaged ears had tears or scars that occurred after marking and include injuries due to fighting and tattooing that rendered the marks illegible or difficult to read. Values in parentheses are number of times animals were handled and tattoos read and numbers of individuals. Individuals were retattooed if original marks were partly or wholly illegible, or if first tattooed with red ink. All data are from field-marked animals.

	Species		
Problem	*D. ingens*	*D. nitratoides*	Pooled Species
Illegible marks leading to lost data	0.5% (2 of 443)	1.7% (3 of 176)	0.8% (5 of 619)
Misread marks	2.9% (13 of 443)	6.3% (11 of 176)	3.9% (24 of 619)
Individuals with damaged ears	9.3% (9 of 97)	24.5% (12 of 49)	14.4% (21 of 146)
Individuals retattooed	33.0% (32 of 97)	36.7% (18 of 49)	34.2% (50 of 146)
Mortality from anesthetic	—	14.3% (2 of 14)	14.3% (2 of 14)
Mortality during handling	0.4% (2 of 542)	0.9% (2 of 227)	0.5% (4 of 769)

used relatively late in the field studies in 1987, 28 of the 50 animals marked with red ink were not recaptured in time to decipher their marks. For the next trapping sessions in February and March 1988, only 9 of the 28 marked individuals were identified. Probably less than half the other individuals were still present on plots, though, if the proportion of recaptures of these individuals was similar to the proportion of animals with black, carbon-particle ink tattoos.

Halothane proved to be too toxic to use in the field on kangaroo rats. The first two *D. nitratoides* died of exposures of less than 20 s. Subsequent administration of Halothane to 13 *D. nitratoides* was successful, but the animals typically recovered prior to completion of tattooing. Due to these problems, use of Halothane was terminated and all other kangaroo rats were marked without benefit of anesthetic.

Mortality during tattooing was relatively high (Table 1). Handling time was relatively long for previously marked animals because many tattoos were difficult to read and records had to be checked to verify identity of animals with ambiguous marks. Efficient processing of animals so that they could be released before daytime ambient temperatures approached a lethal level for *D. ingens* (about 35°C) required three or four workers to handle an average of 40 captives from two 100-trap grids.

The 0.8% loss from illegible tattoos (Table 1) was less than the 2-11% loss of ear tags reported in various studies of small mammals (Krebs et al., 1969; Le Boulenge–Nguyen and Le Boulenge, 1986; Stoddart, 1970; this study). However, because these data were based only on a 4-month period, additional losses and errors from illegible tattoos from damage to ears from fighting could be expected. The rate of errors from misreading tattoos exceeded errors for numbered tags. (see Tag Reading and Recording Errors).

Cheek-pouch Tags

A total of 112 kangaroo rats were tagged in a cheek pouch (107 *D. ingens*, 5 *D. nitratoides*) and 2.8% of them lost the tags within three months (three *D. ingens*, no *D. nitratoides*). Some animals retained cheek-pouch tags for about three to four years, when they disappeared from plots, but we discontin-

ued attaching tags to cheek pouches after using this marking method for about three months in 1988. Problem tags (i.e., numbers turned inside; infections or tears in the skin) were removed and replaced by ear tags. Thus, we are unable to measure a loss rate over a longer period.

Problems associated with cheek-pouch tags were apparent soon after adopting this method. Though the tags were attached so that the numbered side was outside, many tags soon turned so that the numbered side was inside the pouch. Reading these tags required careful manipulation of the skin a few mm from the incisors. Of greater concern was massive infections in three animals at the site where the tag pierced the double layer of skin of the cheek pouch. We drained these abscesses and removed the tags with wire cutters, replacing them with ear tags. One animal tore a 2-cm opening in the skin on the side of the junction between head and neck, exposing its salivary glands and muscles. The tear probably resulted from catching a toe in the tag.

Ear Tags

Rates of ear tag loss differed significantly among the species (Table 2; G = 44.35, p < 0.01). The loss rates for *D. ingens* (10.6%) and *D. nitratoides* (8.0%; Table 3), captured at the same sites on the Elkhorn Plain, did not differ significantly (G = 1.41, p = 0.23), but the difference in their overall loss rates were highly significant (G = 44.23, p < 0.01). The loss rate for *D. nitratoides* was about twice that of *D. ingens*. The overall loss rate for *D. ingens* was significantly lower than that of *D. heermanni* (G = 4.13, p = 0.04; Table 2).

When loss rates were compared temporally, those of *D. nitratoides* were significantly greater than for the other species for all time intervals for which comparisons were appropriate (Table 4). About 38% of all individuals of *D. nitratoides* lost ear tags between 7 and 42 days of marking, and almost 58% lost ear tags between 42 days and one year from marking. All rates of loss between *D. nitratoides* and *D. ingens* were highly significant (p < 0.01), as were the loss rates for *D. nitratoides* and *D. heermanni*. In stark contrast, none of the loss rates differed significantly for *D. ingens* and *D. heermanni*.

Table 2.   Numbers of tags applied and lost for three species of *Dipodomys* over all studies. Total Applied is the total of all tags applied, including retagged animals.

Species	Ear Tags					PIT Tags		
	Single Tagged	Double Tagged	Individuals Tagged	Lost[1]	Total Applied	Tagged	Lost	Total Applied
D. ingens	537	920	1,439	191 (9.1%)	2,532	1,278	38 (2.9%)	1,316
D. heermanni	301	5	306	39 (11.1%)	350	306	8 (2.6%)	314
D. nitratoides	767	137	904	225 (15.0%)	1,497	865	63 (8.7%)	928

[1] Animals with two ear tags were not retagged when one was lost.

Table 3.   Numbers of tags applied and lost during specific studies of *Dipodomys* spp.

Species	Ear Tags			PIT tags		
	Applied	Lost	Loss	Applied	Lost	Loss
Elkhorn Plain **1 Jan. 1988–3 Sep. 1992**						
D. ingens	1,043	111	10.64%	532	16	2.84%
D. nitratoides	263	21	7.98%	117	0	0%
Soda Lake **14 July 1989–25 Feb. 1995**						
D. heermanni	26	0	0%	25	0	0%
D. ingens	1,089	73	6.70%	665	18	2.71%
Pixley National Wildlife Refuge **3 Dec. 1992–7 Nov. 1994**						
D. heermanni	340	39	11.47%	309	8	2.59%
D. nitratoides	1,155	435	37.66%	783	63	8.05%

D. nitratoides is the smallest kangaroo rat—adults weigh about 32-45 g; *D. heermanni* is intermediate in size (mean of about 72 g), and *D. ingens* is the largest (mean weight about 135-140 g). The much higher loss rate for *D. nitratoides* compared to *D. heermanni* and *D. ingens* (Table 3) suggests that the small, thin pinnae of *D. nitratoides* are not suited to retaining ear tags. The small ears of *D. nitratoides* make it difficult to properly attach a tag to them, especially if the tips of the applicator pliers are not ground off so that they can fit properly behind the ear. Yet, even with this modification and despite careful application, the number of lost tags was unacceptably high for this species.

For animals that were properly tagged (Fig. 2), most losses were caused by necrosis of tissue around the area where the tag pierced the

Table 4.   Tag loss of *Dipodomys* spp. during four time periods. Values in parentheses are the number of animals that lost a tag and the total number of animals captured during that period. Values are pooled for all studies of > than one year duration.

Period	*D. heermanni* Tag		*D. nitratoides* Tag		*D. ingens* Tag	
(Days)	Ear	PIT	Ear	PIT	Ear	PIT
1-6	0% (0/132)	3.03% (4/132)	5.92% (17/287)	6.97% (20/287)	0.78% (2/255)	1.18% (3/255)
7-42	3.11% (5/161)	0.62% (1/161)	38.43% (191/497)	6.64% (33/497)	3.64% (12/330)	1.82% (6/330)
42-365	18.97% (33/174)	1.15% (2/174)	57.61% (227/394)	2.54% (10/394)	15.99% (51/319)	2.19% (7/319)
>365	6.25% (1/16)	6.25% (1/16)	0% (0/26)	0% (0/26)	11.43% (8/70)	2.86% (2/70)

pinna. The movement of the tag at this site sometimes caused a necrotic callous to build. This callous apparently occluded normal blood circulation, causing edema, infection, and death of additional tissue. Eventually, a large plug of tissue was lost from the site, freeing the tag to be easily ripped from the ear. The larger, thicker ears of *D. ingens* appeared to be more prone to this syndrome than those of *D. nitratoides* and *D. heermanni*, but its rate of lost tags was significantly lower than either of the latter species, suggesting their smaller, thinner ears were more prone to having loose tags ripped out.

Overall rates of loss of ear tags for the two larger kangaroo rats (Table 2) were within the range reported for other small mammals, but on the high side. These other studies were of shorter duration and some did not exclude ani-

mals marked but not recaptured, lowering the apparent loss rates. Reported loss rates were 2.2% and 5.1% for *Microtus ochrogaster* and *M. pennsylvanicus*, respectively (Krebs et al., 1969), 5% for *Arvicola terrestris* (Stoddart, 1970), 9.5% for *Mus musculus* (Bias et al., 1993), and 16% for *M. ochrogaster* (Wood and Slade, 1990). All these studies used the Style-1 tags manufactured by National Tag and Brand Co. Le Boulenge–Nguyen and Le Boulenge (1986) found losses of 4.1% for *Apodemus sylvaticus* and 8.1% for *Clethrionomys glareolus* for a tag made from metal surgical wound clips.

These studies also did not partition loss rates temporally. When this is done, kangaroo rats have significantly higher loss rates for individuals that persist on plots for more than a single 5-6 day census, and rates increase significantly for animals that are present six weeks after marking (Table 4). Whether these rates are inordinately high compared to other small mammals cannot be determined, but these values at least strongly suggest that some objectives of long-term studies of kangaroo rats that rely solely on ear tags for marking individuals risk being compromised by tag loss.
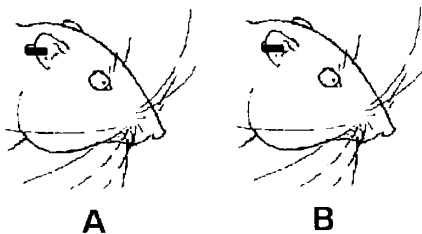

Fig. 2.   Heads of kangaroo rats (Dipodomys), illustrating: A—improper; and B—proper attachment of ear tags.

PIT Tags

On autopsy of animals tagged in captivity, the tag of one *D. nitratoides* was found project-

ing partly out of the opening made by the needle and adhering to a scab that had formed around the edges. Tags of three of five other kangaroo rats had become loosely bound in connective tissue 30 days after injection, while tags of two still were freely mobile. There was no evidence of infection or inflammation either at the injection sites or in the subdermal region where the tags were located. Ball et al. (1991) and Rao and Edmondson (1990) also found no adverse tissue reactions to implanted transponders for laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*), respectively. Ball et al. (1991) did not detect effects on normal body weight gain nor food consumption. For field-tagged animals, no infection or other problems were noted in our study.

The overall 4.3% rate of loss of PIT tags from kangaroo rats (Table 2) was much less than the 30.4% failure rate for PIT tags reported by Fagerstone and Johns (1987) for captive domestic ferrets (*Mustela putorius furo*). PIT tags used in their study were encased in plastic. Failures were from moisture leaking through the plastic. Rao and Edmondson (1990) reported 5% (three lost and four failed tags) of 140 implanted in laboratory mice, though the tags used were from a different supplier and the glass casing was capped with polypropylene to elicit a mild tissue reaction so that it would be immobilized by connective tissue at the site of implantation.

The total failure rate (from losses and failures of all kangaroo rats combined) of PIT tags (4.3%) was significantly lower than that of ear tags (20.8%; Table 2; G = 76.67, p < 0.01; Table 2) and generally similar to within-year rates for *Spermophilus townsendii* (Schooley et al., 1993). The loss rates of the two larger kangaroo rats, however, were lower than the pooled loss rate (3.4%) for *S. townsendii*, while that of *D. nitratoides* was almost twice as great. The rates of loss of PIT tags between kangaroo rat species were significantly different when data for all sites were pooled (G = 19.81, p < 0.01). The rates of loss for *D. ingens* and *D. heermanni* were lower than that of *D. nitratoides* (p < 0.01 for both comparisons), but the rates of the two larger species did not differ (G = 0.10, p = 0.75). Yet, loss rates did not differ for *D. ingens* and *D. nitratoides* in the Elkhorn Plain study (G = 0.235, p = 0.633; Table 3).

Only three PIT tags were known to have become inoperative in animals at the Elkhorn Plain Ecological Reserve, though we did not attempt to find the source of "failure" of about half the tags. In contrast, later batches of tags, made by a different unit of the supplier, were much more prone to failure. Eighteen of the latter were known to have failed, though we suspect many more failures were not distinguished from lost tags. Inoperative tags probably failed by leaking moisture, either by the glass cover being cracked or because of improper sealing of the ends after inserting the transponder during manufacture.

Losses can be minimized by taking care to inject the tag sufficiently posterior so that it can be pushed far forward and to the side of the injection site. We believe that firmly squeezing the injection site for several seconds after withdrawing the needle also reduces losses. Clipping the hair close to the skin at the injection site and applying liquid suture to the wound left by the needle probably would further reduce losses (Patrick A. Kelly, pers. comm.). This technique, however, greatly increases handling and the time to apply a tag, rendering it impractical when large numbers must be marked by each field worker. Soaking the tags for several days in an alcohol solution, then reading before use should identify bad transponders.

When battery power to readers became weak, the reader would fail to detect the tag on the first few passes. Thus, 10-12 animals (two were not recaptured for verification) were tagged more than once. The simultaneous signals from two or more PIT tags caused the reader to fail to respond. In at least five cases, this was interpreted as a lost or failed PIT, leading to injection of yet another tag. The two animals that were not recaptured for verification probably had three and four tags injected, but this was not noticed until data were being transcribed to permanent records. The extra PIT tags were located and removed from the other individuals.

Tag Reading and Recording Errors

The size-1 tags applied to ears and edges of cheek pouches were prone to reading errors. For 1988 and 1989, when cheek-pouch and ear tags were read every time the animal was captured, the misreading/recording error rate was

0.5% (23 of 4,771). This rate was significantly lower than the 3.9% misread tattoos (Table 1; $G = 43.742$, $p < 0.01$). The small size, font, and the deep stamping of numbers combined to make it difficult to distinguish readily some numbers (i.e., 4, 7, and 9), especially when the tag had a veneer of dirt. The greatest number of mistakes was due to tags being read upside down, resulting in errors for some number combinations (e.g., 66-99, 68-89, 606-909, 106-901). Other reading errors were caused by failing to record the thousand digit, which was on the folded edge of the tag, in a different plane from the last three digits. Having all personnel attach tags to the ear on the same side and in the same orientation should minimize the former type of error.

Reading and transcription errors were few for PIT tags, despite the complex, 10-digit hexadecimal numbers. Only five errors were detected out of 10,208 tags read (0.005%), a rate two orders of magnitude lower than for ear tags (G $= 30.114$, $p < 0.01$). Errors were readily identified and corrected when the ear tag number also was recorded. By ensuring the PIT tag readers are set to scan and store numbers and regularly uploading stored files to a computer, all such errors should be easily corrected. Having both ear and PIT tags provides the greatest margin of safety against loss of identity of marked animals and the need to repeat a study or trapping session because of the occasional failure of a reader's power supply.

Mortalities While Tagging and Reading Tags

Mortalities during tagging and reading tags were few—five animals died while tagging or reading tags out of 12,905 times animals with ear or PIT tags were handled. All involved *D. nitratoides*, which was handled 2,714 times—only 21% of the total for all kangaroo rats. Only the difference in mortality rates for *D. ingens* and *D. nitratoides* was significant in species-pair comparisons (G $= 15.792$, $p < 0.01$). The rate of mortality during applying and reading of tattoos (Table 1) was significantly greater than that for ear and PIT tags for all species (G $= 11.229$, $p < 0.01$).

Costs

Though we did not precisely measure time for each method, applying and reading tattoos and returning animals to the site of capture required three-four people and took the longest amount of time (typically 4-5 h) to operate two grids with a combined daily capture of about 20-60 animals. Two people could operate two grids with a combined daily capture of 120-160 animals in less time (2-4 h) using PIT and ear tags. The tattoo device cost $200, but this price would vary widely, as would a power supply to tattoo in the field. The transformer for converting electricity from AC to DC was made from spare parts and was estimated to cost about $30 if the parts were purchased new. The cost of ink was negligible.

When animals only had to be handled to read their mark, reading PIT tags required less than half the time needed for reading ear tags, reducing personnel costs. The newer model PIT tag reader (HS5105L118K110) could search stored records and immediately signal (by a double beep from the scanner) recaptures, reducing time to process animals to about half that when using the old style readers. Ear tags were $0.08 each in lots of 500 and applicator pliers cost $13.50; no other costs were associated with ear tagging. PIT tags were $5.00 each in lots of 500. Reader prices varied: the newer model cost $1,130; the larger, bulkier model (HS5101) cost $1,888. Operating costs included electricity to recharge batteries (unmeasured) and periodic replacement of batteries. Batteries for model HS5101 (two lead-acid batteries) cost $60.00; batteries had to be replaced every 2-3 years. The newer model's battery life varied from about 1 to 18 months and averaged about 12 months. The small lead-acid battery was welded with epoxy to the electronic circuit board. Readers had to be returned to the manufacturer for replacement, which cost $150 and took from 10 days to more than three months. Needles cost $0.45 each and had to be replaced after 20-50 uses. Syringes cost $0.11 in lots of 100; a syringe was used hundreds of times. Other minor costs associated with PIT-tagging included costs of alcohol, tygon tubing, and containers for storing and sterilizing tags and equipment.

CONCLUSIONS

Tattooing required excessive handling of animals, was slow, resulted in unacceptable errors in reading marks, and required more time and a larger field crew to process animals and read their marks, compared to other marking methods. Mortality from handling during applying and reading marks was relatively high compared to handling during applying and reading ear and PIT tags. We consider tattooing to be unacceptable for marking kangaroo rats.

Cheek-pouch tags had a low short-term rate of loss, but this method also is unacceptable because of infections and injuries associated with the attached tag. Error rates for cheek-pouch tags were not distinguished from rates for the identical tags attached to ears.

Infections at the site of application of ear tags often were seen after the tags had been in place for several weeks or months. Infection sometimes led to loss of the tag. We have no data on other consequences of infection, but are concerned by the relatively common occurrence in *D. ingens*. No infections or other problems were found to be caused by PIT tagging.

Ear tags had a lower rate of misreading errors than tattoos, but much higher loss and misreading rates than PIT tags. Reading errors for PIT tags were correctable when the readers were set to store scanned numbers (not all models do). Loss of ear tags varied from about 7.5 to 15.0% without regard to duration. Time-dependent rates, however, showed increasing loss rates between the first six days and one year, such that about 58% of the smallest kangaroo rat (*D. nitratoides*) lost ear tags between six weeks and a year from marking. Though short-term loss rates for ear and PIT tags did not differ significantly, greater loss rates for ear tags were highly significant compared to PIT tags for periods longer than six days (Table 4).

For one time, short-duration population censuses, marking with a single ear tag is probably sufficient, especially for all but the smallest species of kangaroo rats (*D. nitratoides* and its relatives). Attaching a tag to each ear is advisable for all longer studies that do not simultaneously use two marking methods. The rates of ear tag loss for all three species for periods over six weeks may be unacceptably high for long-term demographic studies. For studies longer than about a week, we recommend double-marking with an ear and a PIT tag. If an ear tag is lost, a second tag should be applied to the opposite ear. If the second tag is lost and the PIT tag still is functional, applying a third ear tag is not advisable because both ears probably already have been torn and the third tag likely will be lost more quickly.

The cost of PIT tags and readers may make this marking method too expensive for many studies, though other manufacturers and models with lower costs are available now. The occasional failure of reader batteries requires that a backup reader always be available or that ear tags also be applied, else information can be lost or studies may have to be repeated. Because PIT-tags can be read without holding and inspecting the animal, however, the time required to operate a trapping plot is significantly less for studies where the animal does not have to be physically inspected each time it is captured. Savings in personnel costs can more than offset the expense of PIT tags and readers for large and long-term studies. Less handling also is desirable because it minimizes adverse effects to the animals and lessens the potential for biasing results.

ACKNOWLEDGMENTS

John Brode, Wendy Duron, Rosalie Faubion, Bill Lehman, Jim Lidberg, Gail Kobetich, Ted Rado, Richard Reiner, Andy Robinson, Larry Saslaw, Ron Schlorff, and George Sheppard contributed logistic support in obtaining equipment, funding, or permits. The PIT-tag injector was modeled after a design described by Galen Rathbun (pers. comm.). Work with the endangered *D. ingens* and *D. nitratoides* was authorized by USDI Fish and Wildlife Service permit number WILLDF-9 and a Memorandum of Understanding between the California Department of Fish and Game and California State University, Stanislaus.

LITERATURE CITED

ad hoc Committee on Acceptable Methods in Mammalogy. 1987. Acceptable field methods in mammalogy: Preliminary guidelines approved by the American Society of Mammalogists. Journal of Mammalogy, 68(4), supplement:1-18.

Ball, D.J., G. Argentieri, R. Krause, M. Lipinski, R.L. Robinson, R.E. Stoll, and G.E. Visscher. 1991. Evaluation of a microchip implant system used for animal identification in rats. Laboratory Animal Science, 41:185-186.

Bazhenov, A.V., V.N. Bol'shakov, and O.F. Sadykov. 1984. New method for marking small mammals. Soviet Journal of Ecology, 15:96-98 [English translation of Russian journal].

Bias, M.A., N.L. Breuner, and M. L. Morrison. 1993. House mice as indicators of potential marking effects on salt marsh harvest mice. 1992 Transactions of the Western Section of the Wildlife Society, 28:34-37.

Camper, J.D., and J.R. Dixon. 1988. Evaluation of a microchip marking system for amphibians and reptiles. Texas Parks and Wildlife Department, Research Publication, 7100-159:1-22.

DeBlase, A.F., and R.E. Martin. 1981. A manual of mammalogy with keys to families of the world. Second ed. W.C. Brown Company Publishers, Dubuque, IA, 436 pp.

Fagerstone, K.A., and B.E. Johns. 1987. Transponders as permanent identification markers for domestic ferrets, black-footed ferrets and other wildlife. Journal of Wildlife Management, 51:294-297.

Fairley. 1982. Short-term effects of ringing and toe-clipping on the recapture of wood mice (*Apodemus sylvaticus*). Journal of Zoology (London), 197:295-297.

Fullagar, P.J., and P.A. Jewell. 1965. Marking small rodents and the difficulties of using leg rings. Journal of Zoology (London), 147:224-228.

Genoud, M. 1984. Activity of *Sorex coronatus* (Insectivora, Soricidae) in the field. Zeitschrift für Säugetierekunde, 49:74-78.

Germano, D.J., and D.F. Williams. 1993. Field evaluation of using passive integrated transponder (PIT) tags to permanently mark lizards. Herpetological Review, 24: 54-56.

Korn, H. 1987. Effects of live-trapping and toe clipping on body weight of European and African rodent species. Oecologica, 71:597-600.

Krebs, C.J., B.L. Keller, and R.H. Tamarin. 1969. *Microtus* population biology: Demographic changes in fluctuating populations of *Microtus ochrogaster* and *M. pennsylvanicus* in southern Indiana. Ecology, 50:587-607.

Le Boulenge–Nguyen, P.Y., and E. Le Boulenge. 1986. A new ear-tag for small mammals. Journal of Zoology (London), 209:302-304.

Pavone, L.V., and R. Boonstra. 1985. The effect of toe-clipping on the survival of meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology, 63:499-501.

Pimentel, R.A., and J.D. Smith. 1990. BioΣtat I. A univariate statistical toolbox version 2 tutorial manual. Sigma Soft, Placentia, CA, 392 pp.

Pollock, K.H., J.D. Nichols, C. Brownie, and J.E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs, 107:1-97.

Rao, G.N., and J. Edmondson. 1990. Tissue reaction to an implantable identification device in mice. Toxicologic Pathology, 18:412-416.

Schooley, R.L., B. Van Horne, and K.P. Burnham. 1993. Passive integrated transponders for marking free-ranging Townsend's ground squirrels. Journal of Mammalogy, 74:480-484.

Stoddart, D.M. 1970. Individual range, dispersion and dispersal in a population of water voles (*Arvicola terrestris* (L.)). Journal of Animal Ecology, 39:403-425.

Twigg, G.I. 1975. Marking mammals. Mam-
  mal Review, 5:101-116.
Wood, M.D., and N.A. Slade. 1990. Compari-
  son of ear-tagging and toe-clipping in prairie
  voles, *Microtus ochrogaster*. Journal of Mam-
  malogy, 71:252-255.

Daniel F. Williams and Walter Tordoff III, Department of Biological Sciences, California State Uni-
versity, Stanislaus, Turlock, California 95382; David J. Germano, Department of Biology, Califor-
nia State University, Bakersfield, California