



Mark my words: experts' choice of marking methods used in capture-mark-recapture studies of small mammals

THOMAS S. JUNG,* RUDY BOONSTRA, AND CHARLES J. KREBS

Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2H1, Canada (TSJ)

Department of Environment, Government of Yukon, Whitehorse, Yukon Y1A 2C6, Canada (TSJ)

Department of Biological Sciences, University of Toronto, Scarborough, Ontario M1C 1A4, Canada (RB)

Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada (CJK)

* Correspondent: thomas.jung@gov.yk.ca

Crucial to the success of studies based on capture-mark-recapture (CMR) designs is the retention (permanency) and recognition (readability) of marks to identify individuals. Several marking methods for small mammals (< 60 g) are available, but their efficacy and use is not well known. We implemented a targeted survey of experts to gather their experiences and opinions regarding marking small mammals. Respondents ($n = 114$) stated their beliefs, perceptions, and current and future use, of marking methods, as well as factors influencing their choices, based on Likert and rank order scale questions. We compared responses based on where researchers' studies occurred, their level of experience, and their subfield of mammalogy. Most respondents (73%) had > 5 years experience marking small mammals, with 60% each marking > 1,000 individuals. Respondents believed that ear-tagging was most preferable in terms of efficiency, impact to affected animals (survival, pain), and personal ethics, whereas passive integrated transponder (PIT)-tagging was the most preferable with regard to retention and recognition, and toe-clipping with respect to cost. Most respondents plan to use ear-tagging (78%) or PIT-tagging (70%) in the future. PIT- and genetic-tagging are expected to increase, and toe-clipping to decline, in the future. The factors influencing which marking method respondents used were ranked—in order of decreasing preference—as impact, retention, recognition, cost, efficiency, and ethics. There were few differences in the mean response or consensus among respondents, regardless of their experience, location, or subfield. Most respondents (66%) agreed that additional studies on the performance and impact of various marking methods are needed to assess their costs and benefits for CMR-based studies. Ultimately, choice of marking method will depend on the species, research question, available resources, and local legislation and permitting. Our study, however, illustrates that collective insights by experienced mammalogists may aid individual researchers in deciding on study designs and protocols, particularly early career scientists.

Key words: animal welfare, capture-mark-recapture, ear-tagging, expert opinion, PIT-tagging, Potential for Conflict Index (PCI₂), small mammals, toe-clipping

Capture-mark-recapture (CMR)-based approaches are widely used to estimate the abundance, density, and demography of various species (Williams et al. 2002; Lindberg 2012). Paramount to the success of CMR-based studies is the retention (permanency) and recognition (readability) of marks applied to individuals. Two key assumptions that underlie CMR analyses are that marks are retained (not lost) and recognized (not misread—Otis et al. 1978; Pollock et al. 1990; Amstrup et al. 2005). Violation of these assumptions may result in abundance or density estimates that are unreliable (McDonald et al. 2003),

which ultimately may lead to flawed ecological inferences. As such, the method chosen to mark individuals in CMR-based studies is of utmost importance (McDonald et al. 2003; Fokidis et al. 2006). Unfortunately, there is often a lack of information on the performance and effect of marking methods available to researchers about to embark on CMR-based studies (Murray and Fuller 2000).

Small mammals (≤ 60 g; e.g., *Apodemus*, *Microtus*, *Mus*, *Myodes*, *Peromyscus*, etc.) have long been used as model species to investigate basic questions regarding behavior, ecology,

evolution, physiology, and diseases, probably because they are excellent experimental models to ask fundamental questions, and it can be relatively efficient and inexpensive to obtain a sufficient number of individuals to permit robust analyses. Additionally, some species that reach high densities may be pests that are involved in human–wildlife conflicts, particularly alien and invasive species such as house mice (*Mus musculus*—Singleton 1989; Krebs et al. 2004; Singleton et al. 2005). Several other species have been driven to low densities due to human activities and are thus of conservation concern (i.e., several species of hopping mice, *Notomys* spp.—Moseby et al. 1999; Dietsch et al. 2016). Both pest and threatened species evoke a strong management need to monitor populations. CMR-based designs are frequently used to answer both basic and applied questions (Anderson et al. 1983; Krebs and Boonstra 1984; Hammond and Anthony 2006; Gerber and Parmenter 2015; Romairone et al. 2018; Torre et al. 2018), with some studies spanning nearly 50 years (Krebs et al. 2019).

Research on the performance of methods used to capture and mark small mammals is needed to inform the development of robust experimental designs aimed at gaining reliable knowledge (Parmenter et al. 2003; Gerber and Parmenter 2015; Bovendorp et al. 2017; Romairone et al. 2018; Torre et al. 2018). Several studies have investigated the relative performance of various types of traps (e.g., Boonstra and Krebs 1978; Beacham and Krebs 1980; Boonstra and Rodd 1984; Lambin and MacKinnon 1997; Tasker and Dickman 2002; Anthony et al. 2005; Jung 2016; Torre et al. 2016; Korslund 2018) and trapping protocols (e.g., Parmenter et al. 2003; Bovendorp et al. 2017; Torre et al. 2018; Harkins et al. 2019) to capture small mammals. Marking small mammals, however, is challenging because of the constraints that small body size places on the size of marks that can be used without altering behavior or survival, and while ensuring adequate retention and recognition.

Individually marking small mammals for scientific study dates to at least the 1930's (e.g., Stockdale 1932; Chitty 1937; Blair 1941; Scott 1942; Manville 1949). Since then, a variety of methods have been used, with the most common probably being ear-tagging and toe-clipping. Other methods have been used less frequently (e.g., ear-notching–punching, fur-clipping, fur-dyeing, and radiotagging; reviewed by Twigg 1975). Passive integrated transponder (PIT)-tags (e.g., Fokidis et al. 2006; Korslund and Steen 2006; Lebl and Ruf 2009), genetic-tagging (e.g., Miller et al. 2005; Ferreira et al. 2018), tattoos (Lindner and Fuelling 2002; Petit et al. 2012), and visible implant elastomers (VIEs; e.g., Grant 2008) are relatively new methods that may be increasingly used in future studies. Several studies have focused on the impact of various marking methods (particularly toe-clipping) on the survival (Ambrose 1972; Fairley 1982; Pavone and Boonstra 1985; Braude and Ciszek 1998), growth (Fisher and Blomberg 2009), body condition (Korn 1987; Wood and Slade 1990), movements (Borremans et al. 2015), and ectoparasite loads (Ostfeld et al. 1993) of affected small mammals. Mixed effects have been reported from these studies. However, little attention has been given to the retention or recognition of marks used in small mammal studies even though these traits are a crucial consideration in CMR-based designs (Murray and

Fuller 2000; but see Le Boulenger-Nguyen and Boulenger 1986; Salamon and Klettenheimer 1994; Leclercq and Rozenfeld 2001; Kuenzi et al. 2005; Fokidis et al. 2006; Lebel and Ruf 2009). Despite a variety of marking methods being employed in CMR-based studies of small mammals, a review of their performance and use by researchers is lacking.

Our aim was to assess the use of various marking methods for small mammals based on researchers' perceptions of them and factors influencing their choices. We surveyed small mammal researchers with expertise in marking individuals to collect and collate their experiences and beliefs. Expert opinion may be a useful means of exploring important questions for which field data are not available and may be otherwise difficult to obtain (e.g., Petit and Waudby 2012; Rode et al. 2018). Indeed, consulting colleagues on their evaluation of marking methods has been recommended, given gaps in available information (Murray and Fuller 2000). Our central questions were: what marking method(s) small mammal researchers used in their previous studies, which they envisioned using in future, and what factors influenced their choices. We predicted that most researchers previously used toe-clipping and ear-tagging but anticipated using PIT-tags or genetic marking in the future, possibly as a means of increasing tag retention and recognition. We also asked them a series of questions to probe deeper into their beliefs and perceptions about, as well as factors influencing their choice of, various marking methods. We predicted that given the critical importance of mark retention and recognition in CMR-based studies that these factors would be among the most important when researchers choose a marking method. Finally, we used a Potential for Conflict Index approach (PCI₂—Manfredo et al. 2003; Vaske et al. 2010; Vaske 2018) to compare the degree of consensus among groups of respondents, based on the relative experience researchers had marking small mammals, where they primarily did field research (e.g., North America, Australia, Asia, etc.), as well as their general field of study (e.g., behavioral ecology, population ecology, etc.). The intent of our study was to harness the collective beliefs, perceptions, and choices of experts regarding small mammal marking methods so that they could provide a resource to individual researchers when deciding which marking method(s) to use in their work.

MATERIALS AND METHODS

Survey of practitioners.—We developed and implemented a questionnaire with 23 questions. Our target sample for the survey was researchers who were experienced marking small mammals. The survey was designed to ask respondents about 1) their background capturing and individually marking small mammals (Questions [Q1]–[6]), 2) their beliefs and perceptions about marking methods, using a 5-point Likert scale (Q9–[20]), 3) their opinions on the efficacy of three common marking methods (ear-tagging, toe-clipping, and PIT-tagging; Q7–[8]) in terms of relative cost, efficiency, reliability, and impact to the animals, and 4) which marking methods they planned to use in the future (Q21) and factors influencing their choice (Q22). Finally, we added an opportunity to provide additional comments (Q23).

Our survey was implemented online using SurveyMonkey as a platform (<https://surveymonkey.com>). We delivered the questionnaire by distributing a link to the online survey via Mammal-L, the Smithsonian Institution's listserver for mammalogists, and through social media websites of the IUCN–SSC Small Mammal Specialist Group (i.e., Twitter and Facebook). By targeting these forums, we sought to engage the global small mammal research community. The online survey was open from 21 March 2018 to 10 April 2018 (21 days) and reminders of the closing date were sent out via Mammal-L on 27 March 2018 and 6 April 2018.

Data analyses.—Responses based on a Likert scale (Q9–20) were coded numerically with 1 = strongly disagree and 5 = strongly agree. We report descriptive statistics (median and mean \pm SD) for each question, as well as the frequency distribution of responses that agreed, disagreed, or were neutral to the question. When calculating the frequency distributions for these questions we lumped “strongly agree” and “agree” as well as “disagree” and “strongly disagree” into two discrete choices (“agree” or “disagree”) to improve the interpretation of the results. We also calculated PCI_2 scores to quantify the degree of consensus among respondents (Manfredo et al. 2003; Vaske et al. 2010; Vaske 2018) for each question based on a Likert scale (Q9–20). Calculated PCI_2 values range from 0 to 1, with 0 representing complete agreement, and 1 representing no agreement, among respondents (Manfredo et al. 2003). We recoded Likert scale data from -2 (strongly disagree) to 2 (strongly agree) in order to use the equations from Vaske et al. (2010) for calculating PCI_2 from a 5-point bipolar scale with a neutral response.

To further examine variation in responses, we categorized respondents based on their comparative experience trapping small mammals (“High” or “Low”), the location (North America [“NA”] or “Other”) and field of study (population ecology [“Pop”] or “Other”) of most of their small mammal research. Respondents that indicated they had > 5 years of experience and marked $> 1,000$ individual small mammals were classified as having a High level of experience. All other respondents were classified in the Low experience group. We then repeated the same analyses as above for the Likert scale questions (Q9–20) that we used for the full data set. We tested for differences in the mean responses among groups (groups of three pairs based on trapping experience, location, and field of study) with pairwise *t*-tests. Further, we tested for differences in consensus among respondent groups by comparing PCI_2 values and simulated SD values between groups with difference tests (*d*), where if $d \geq 1.96$ then $P \leq 0.05$ (Vaske et al. 2010; Vaske 2018).

Differences in the types of marks previously used by respondents (Q5) compared to those that they planned to use in the future (Q21) were evaluated with likelihood-ratio chi-square tests. We used aggregate scores to assess rank preferences by respondents on their perceived performance of three commonly used marking methods (ear-tagging, toe-clipping, and PIT-tagging), as well as factors influencing their choice of marking method in the future. Six traits of marking methods

were used for the ranking exercises, including mark retention and recognition, impact on the animal (i.e., survival, pain), personal ethics, cost, and efficiency in the field.

RESULTS

Survey respondents.—We received 115 responses to the online survey, although one respondent was discounted because they had no experience marking small mammals. Most respondents (73%) had > 5 years of experience, with 60% of respondents each marking $> 1,000$ individual small mammals. Notably, 22 respondents (19%) indicated that they had > 25 years of experience marking small mammals. Assuming mid-range values of the four experience classes in Q1, respondents represented about 1,223 (range 859–1,586) person-years of experience capturing and marking small mammals. Our sample was skewed toward those that primarily conducted their research in North America (65%) compared to other continents (35%; $G^2_1 = 10.140$; $P = 0.001$) and researchers focusing broadly on questions related to population ecology (60%) compared to other areas of inquiry (40%; $G^2_1 = 4.246$; $P = 0.039$; Supplementary Data SD1). Respondents were close to equal in terms of having categorically high (56%) or low (44%) levels of experience trapping small mammals, with those having high experience each trapping $> 1,000$ small mammals and > 5 years of experience.

Expert's perceptions and beliefs.—Respondents varied in their perceptions and beliefs of methods for marking small mammals. Based on medians, means, and frequency distributions of responses, most respondents agreed that radiotagging has limited applications in CMR-based designs (74% of respondents agreed; Q9), using two ear-tags increases retention (67%; Q11), toe-clipping offers excellent mark retention (67%; Q13) but is antiquated (72%; Q14) and poses personal ethical issues (80%; Q15), and PIT-tagging has many advantages (63%; Q16) but is too costly (67%; Q17; Table 1). Most respondents also agreed that more studies are needed on the efficacy and impact of marking methods (66%; Q19) and improved marking methods are also needed (55%, Q20). Median responses regarding whether mark recognition was a concern when using ear-tags (Q12), or that genetic CMR will be important in the future, were neutral (Q18; Table 1). Most respondents disagreed that ear-tag retention was not permanent enough for their research (55%; Q10).

The degree of consensus among respondents on their beliefs and perceptions—as indicated by PCI_2 scores—was variable among questions, with a 2-fold difference observed (range = 0.18–0.39; Fig. 1). The highest PCI_2 scores (least consensus) were reported from questions regarding ear-tag retention (Q10; $PCI_2 = 0.39$), errors in recognition with ear-tags (Q12; $PCI_2 = 0.39$), and whether PIT-tagging was too expensive (Q17; $PCI_2 = 0.32$; Fig. 1). Conversely, the lowest PCI_2 scores (highest consensus) were reported from questions regarding whether PIT-tagging had many advantages compared to other marking methods (Q18; $PCI_2 = 0.18$) or new marking methods were needed (Q20; $PCI_2 = 0.18$), followed closely by the need

Table 1.—Likert-style questions posed in the online survey and the sample size (*n*), median, mean (\pm *SD*), and frequency distribution of responses by respondents. The “agree” category includes “strongly agree” and “agree”, and the “disagree” category includes both “strongly disagree” and “disagree” responses by respondents to the questions. CMR = capture-mark-recapture; PIT = passive integrated transponder.

Survey question	<i>n</i>	Median	Mean \pm <i>SD</i>	Frequency distribution		
				Agree	Neutral	Disagree
Q9. Radiotagging has limited applications for marking individuals and is only an option in the case of studies marking a small number of animals	91	Agree	3.8 \pm 1.1	74%	8%	18%
Q10. Ear-tagging is generally not permanent enough to employ in most of my small mammal research	96	Disagree	2.7 \pm 1.3	31%	12%	57%
Q11. Placing an ear-tag in both ears significantly increases the reliability of ear-tagging as an individual marking method in terms of mark retention	93	Agree	3.7 \pm 1.1	67%	14%	19%
Q12. Mark recognition and data entry (i.e., correctly reading and recording an ear-tag) is a major concern when using ear-tagging	101	Neutral	3.0 \pm 1.2	42%	16%	42%
Q13. Toe-clipping offers excellent mark retention (permanency) for long-term studies of individually marked small mammals	87	Agree	3.7 \pm 1.2	67%	16%	17%
Q14. Toe-clipping is an antiquated method of individually marking small mammals	95	Agree	4.0 \pm 1.2	72%	14%	24%
Q15. For me personally, toe-clipping poses ethical concerns	101	Agree	4.1 \pm 1.2	80%	5%	15%
Q16. PIT-tagging has many advantages compared to other methods currently used for individually marking small mammals	95	Agree	3.6 \pm 1.3	63%	16%	21%
Q17. PIT-tagging is currently too costly to employ in most of my small mammal research	98	Agree	3.8 \pm 1.2	67%	12%	21%
Q18. Marking of individual small mammals based on genetic (DNA)-tagging will be an important method in future CMR-based studies of small mammals	87	Neutral	3.2 \pm 1.1	46%	31%	23%
Q19. More published studies on the efficacy and impact of various small mammal marking methods are needed to help researchers assess the costs and benefits of choosing a marking method	101	Agree	3.6 \pm 1.0	66%	17%	17%
Q20. Improved methods for individually marking small mammals are needed	102	Agree	3.5 \pm 1.0	55%	26%	19%

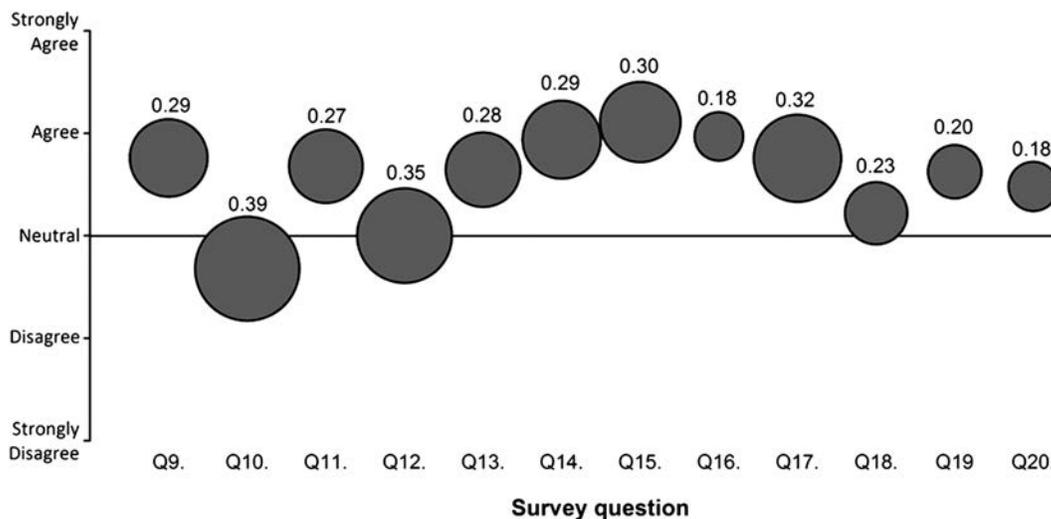


Fig. 1.—Bubble plots of the mean and degree of consensus of survey respondents (*n* = 114) on questions regarding beliefs and perceptions about various small mammal marking methods. Relative sizes of the bubbles indicate the degree of consensus among respondents (larger bubbles equal less consensus). Values above each bubble are PCI₂ scores for that question. Specific questions can be found in Table 1. PCI₂ = Potential for Conflict Index.

for more studies on the efficacy and impact of marking methods (Q19; PCI₂ = 0.18; Fig. 1).

Group comparisons of perceptions and beliefs.—Overall, there were few significant (*P* ≤ 0.05) differences in either the mean response or degree of consensus (PCI₂ scores) by

different groups of respondents regarding their beliefs about marking methods (Fig. 2; Supplementary Data SD2). Level of experience, location (North America or elsewhere), or primary research interest made little difference in either group means or PCI₂ scores.

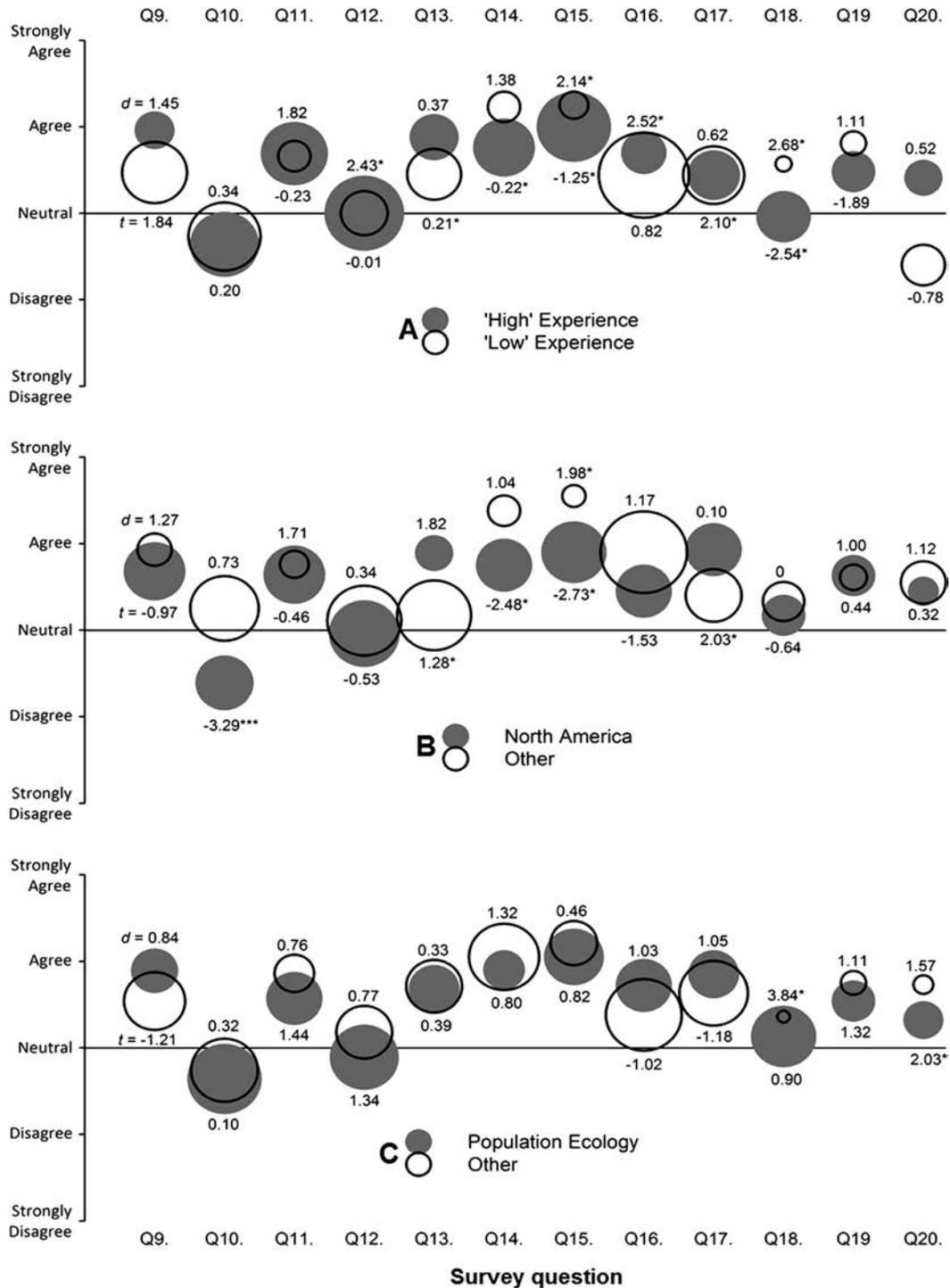


Fig. 2.—Bubble plots of the mean and degree of consensus of survey respondent groups on questions regarding beliefs and perceptions about various small mammal marking methods. Respondent groups were based on (A) level of experience marking small mammals (High versus Low; see “Materials and Methods” for more details), (B) location where most of their research occurred (North America or Other), and (C) their primary field of study (Population Ecology versus Other). Relative sizes of the bubbles indicate the degree of consensus among respondents (larger bubbles equal less consensus). Values above each bubble are *d* statistic, while those below each bubble are the *t* statistic. *d* and *t* values followed by an asterisk are significantly different (**P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001). Specific questions can be found in Table 1.

Researchers’ level of experience (HIGH versus LOW) resulted in different mean responses, and different PCI₂ scores, for five and four of 12 questions, respectively (Fig. 2). In four of five instances where the mean response was significantly

different among groups based on experience level, respondent groups did not differ in whether they agreed or disagreed with the statement; rather, the difference was a matter of how much they agreed with the statement. For example, both groups agreed

that toe-clipping offers excellent mark retention (Q13) and that PIT-tagging was too costly to include in their research programs (Q17; Fig. 2). However, in both instances, the degree with which respondents agreed with these statements was slight but statistically significant (Supplementary Data SD2). In the fifth instance, the mean response was neutral and agree for the High and Low groups, respectively, regarding whether they believed that genetic CMR will be important in the future (Q18; Fig. 2). When considering responses within these groups on the question of the future role of genetic-tagging, however, consensus differed significantly ($d_1 = 2.68$, $P < 0.05$). Respondents in the Low group were much more consistent in their response than those in the High group (Q18; Fig. 2). With one exception, significant differences in the degree of consensus between High and Low groups were caused by a higher PCI_2 score (less consensus) among respondents within the High group: significantly higher PCI_2 score for the Low, compared to the High, group regarding whether PIT-tagging has many advantages (Q18; Fig. 2).

When grouped by where respondents primarily conducted their research (NA versus Other), significant differences in the mean responses and degree of consensus between groups were found for five and one of 12 questions, respectively (Supplementary Data SD2; Fig. 2). Most of these differences (three of five) regarded toe-clipping. The Other group was significantly less in agreement that toe-clipping offered excellent mark retention (Q13) and in greater agreement that it is an antiquated method (Q14) and poses ethical concerns (Q15; Fig. 2). These groups of respondents also differed regarding whether ear-tagging provided adequate retention for their research (Q10), with the mean response for the NA group being disagree, whereas the Other group mean was neutral. Conversely, the Other group was approaching neutral, while the NA group more firmly agreed, regarding whether genetic CMR will be important in the future (Fig. 2). Finally, PCI_2 scores differed significantly ($d_1 = 1.98$, $P < 0.05$) among groups when considering responses to whether toe-clipping poses ethical concerns (Q15). Respondents in the Other group had a significantly higher degree of consensus in their responses than those in the NA group (Fig. 2).

There were fewer significant differences between respondents when grouped by their field of study (Pop versus Other), compared to their level of experience or where they primarily conducted their small mammal research. Mean response and degree of consensus differed for one question each between groups (Supplementary Data SD2; Fig. 2). The mean response to the question of whether improved marking methods were needed (Q20) differed significantly between the two groups ($t_{100} = 2.03$, $P = 0.046$), with the Pop group being close to neutral and the Other group in more agreement (Fig. 2). Consensus in the Pop group was significantly less than in the Other group when considering the future use of genetic CMR ($d_1 = 3.84$, $P < 0.05$; Fig. 2).

Comparison of ear-tagging, toe-clipping, and PIT-tagging.—Most respondents believed that ear-tagging and PIT-tagging were the most preferable with respect to their personal ethics and having the least impact (pain, survival) to affected individuals, whereas toe-clipping was the least preferred for these two traits (Fig. 3). PIT-tagging was ranked as the most preferable method in terms of both mark retention and recognition by

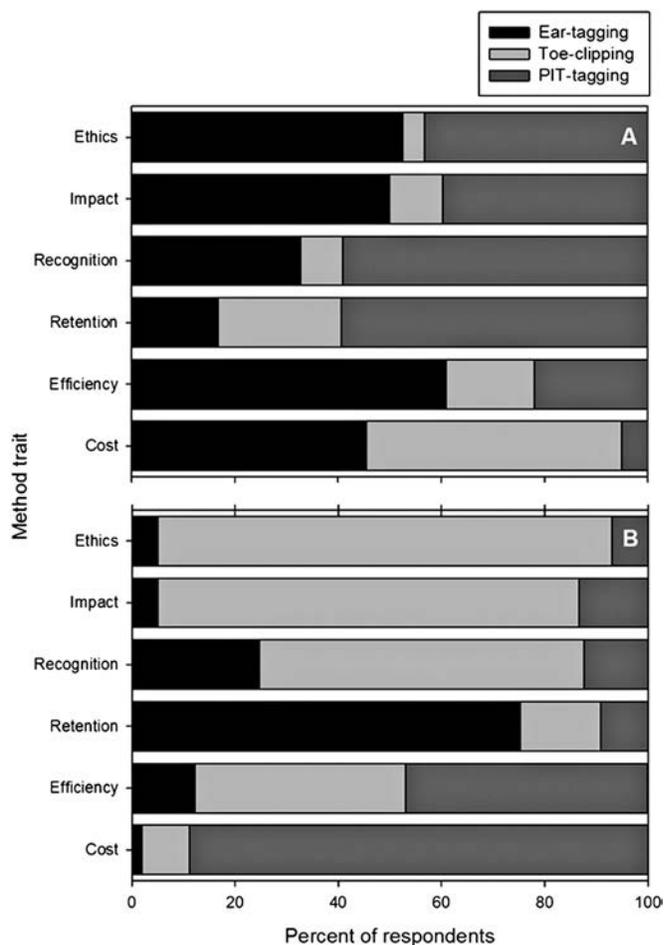


Fig. 3.—Perceptions of small mammal researchers' ($n = 114$) choice of the most preferable (top panel, A) and least preferable (bottom panel, B) method (ear-tagging, toe-clipping, or PIT-tagging) in terms of its comparative performance with respect to personal ethics, impact to affected animals, mark recognition and retention, efficiency in the field, and cost. PIT = passive integrated transponder.

about 60% of respondents. Ear-tagging and toe-clipping were ranked as the least preferable methods in terms of retention and recognition, respectively (Fig. 3). Indeed, 75% of respondents believed that ear-tagging was the least preferred option in terms of mark retention, and 63% agreed that toe-clipping was the least preferred regarding mark recognition. Most respondents (60%) believed that ear-tagging was the most efficient method to employ, whereas an almost equal percentage of respondents reported toe-clipping and PIT-tagging as the least efficient (Fig. 3). Respondents reported in almost equal measure that either ear-tagging or toe-clipping was the preferred choice based on cost, and that PIT-tagging was overwhelmingly reported (89%) as the least preferred marking method based on cost (Fig. 3).

Past and future choices in marking methods.—Ear-tagging, followed by PIT-tagging, toe-clipping, fur-dyeing, and radiotagging were the methods used most frequently in the past by survey respondents (Fig. 4). Ear-punching, fur-clipping, and other were used by $\leq 11\%$ of respondents, and none reported the use of genetic mark-recapture for small mammals. The frequency that respondents reported use of ear-tagging or radiotagging did not

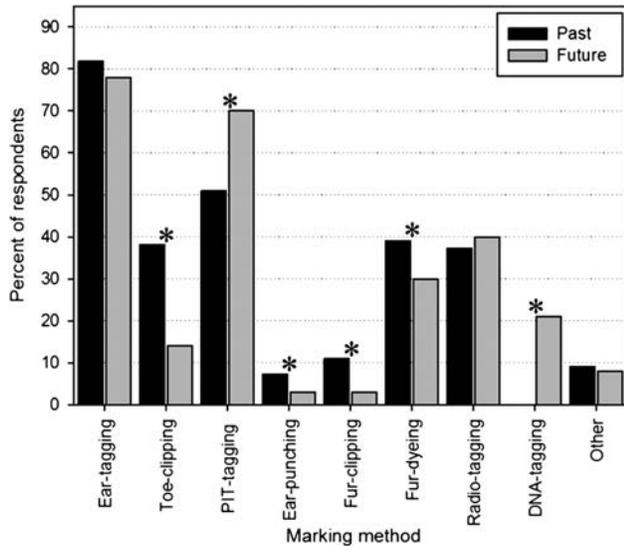


Fig. 4.—Percent of respondents ($n = 114$) to an online survey that reported that they had previously used various methods for individually marking small mammals and those they plan to employ in their future studies. Methods with an asterisk (*) above were significantly different ($P \leq 0.05$) in terms of the percent of respondents using the method in the past and that in future studies, based on contingency table analyses (see “Results”). “Other” methods included leg-ringing, ear-tattooing, and visible implant elastomers (VIEs).

significantly change between the past and future. Going forward, however, respondents reported that they would make significantly ($P \leq 0.05$) greater use of PIT-tagging and genetic-tagging, and significantly ($P \leq 0.05$) less use of toe-clipping, ear-punching, fur-clipping, and fur-dyeing, in the future (Fig. 4).

Factors influencing choice of marking method.—The factors influencing respondents’ decision on which marking method to use were ranked—in decreasing order—as impact on affected animals, retention, recognition, cost, efficiency, and personal ethics. Impact on the affected animals was the highest or second highest ranked factor influencing choice of marking method by 53% of respondents, whereas ethics was the lowest or second lowest ranked factor by 41% (Fig. 5). Retention and recognition were ranked as one of the top three factors influencing choice by > 50% of respondents. Conversely, efficiency and cost were ranked as the lowest factor by > 20% respondents (Fig. 5).

Open-ended responses.—We received additional, open-ended comments by 36 respondents (32%) at the end of the survey (Q23). No attempt was made to qualitatively analyze these responses. Verbatim comments added by these respondents are available in [Supplementary Data SD3](#).

DISCUSSION

Our primary aim was to evaluate which marking methods small mammal researchers previously used compared to those that they envision using in the future. Additionally, we aimed to understand the factors influencing their choices, and assessing what future directions or work is needed to improve upon marking methods. We explored these questions

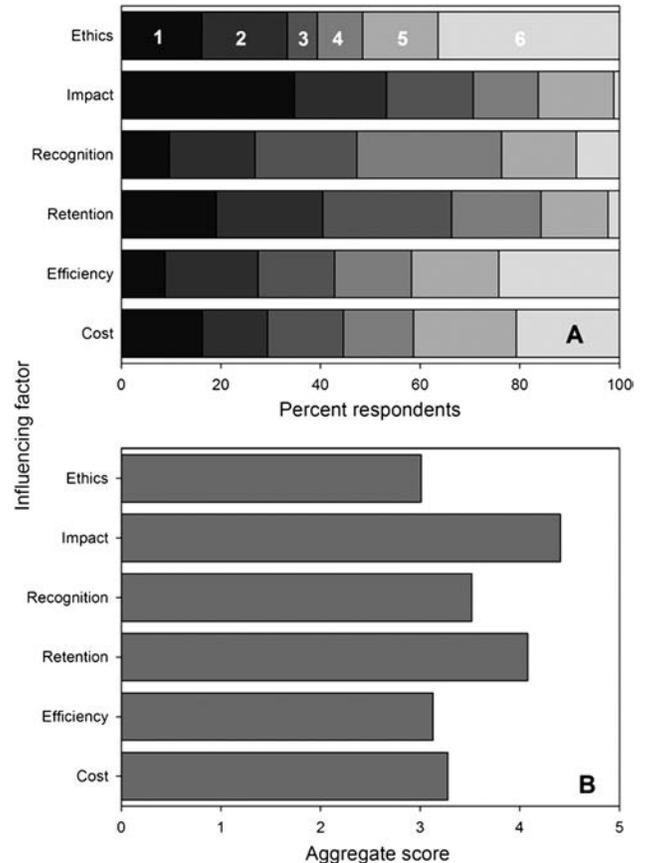


Fig. 5.—Relative role of personal ethics, impact to affected animals, mark recognition, mark retention, efficiency to employ in the field, and cost, in terms of influencing the decisions of researchers ($n = 114$) choice of which method to use to individually mark small mammals. Top panel (A) is the percent of respondents that gave each factor a rank of 1–6, with 1 being the most, and 6 being the least, influential factor in their choice of which method to use. (See the top bar [ethics] for a legend to the color coding for each rank.) Bottom panel (B) is the aggregate score for each influencing factor, with higher scores indicating factors ranked higher.

by harnessing the beliefs, perceptions, and experiences of experts in the field. In summary, our main finding was that the marking methods respondents used in past studies were not necessarily what they plan to use in the future, with one exception. Toe-clipping and a variety of other methods (e.g., fur-dyeing) were used widely in the past; however, respondents reported that they planned to use these methods significantly less in future studies of small mammals. On the other hand, PIT-tagging and genetic mark-recapture were reported to have significantly increased use by respondents in the future. The exception was that ear-tagging, which respondents indicated as used by most in the past, will likely to continue to be the most used in the future.

Respondents believed that retention and recognition of marks was not perfect when using ear-tagging, PIT-tagging, or toe-clipping. Ear-tags and PIT-tags can become dislodged, resulting in a lack of mark retention. While toe-clipping does offer excellent retention, additional toes can be subsequently lost or hair may grow over and conceal clipped toes,

complicating mark recognition. Regardless of the marker chosen, researchers concerned about retention or recognition may wish to apply two marks on each animal to ensure assumptions of mark retention in CMR-based designs are met. For example, this may include using ear-tags on both ears, which 67% of survey respondents agreed may help with retention. Alternatively, it may include ear-tagging individuals that are also PIT-tagged, given that retention of PIT-tags may be questionable depending on how they are applied (Fokidis et al. 2006; Lebel and Ruf 2009; Rigby et al. 2012). Loss of PIT-tags by deer mice (*Peromyscus maniculatus*), for example, has been as high as 32% (Kuenzi et al. 2005). Additionally, it is vital to ensure that the proper size of marker is used, and that field staff are trained in applying marks, so that impacts are minimized and retention is maximized. For example, some respondents commented (Q23; Supplementary Data SD3) that ear-tags commonly used for mice and voles may be too large, and there is a need to manufacture appropriately sized tags for mammals this small. Respondents also commented on the need to ensure that ear-tags need to be inserted properly at the base of the ear to ensure retention. Similarly, inserting PIT-tags into small mammals requires experience to maximize retention and minimize mortalities. Researchers evaluating the use of 12-mm PIT-tags on Daubenton's bat (*Myotis daubentonii*) noted that it took three field seasons before tag retention rates were > 90%, which they attributed to increased experience and confidence inserting the tags (Rigby et al. 2012).

Eventually, noninvasive methods may reduce the need to physically capture and mark small mammals in studies aimed at estimating density or abundance alone. Approaches such as camera trapping (De Bondi et al. 2010; Villette et al. 2016) or genetic-tagging (Ferreira et al. 2018) using appropriately sized hair snares (Pocock and Jennings 2006; Pocock and Bell 2011) may reduce the need for livetrapping studies. Overall, survey respondents were neutral on their thoughts about genetic CMR being an important technique in the future (Q18), and there were differences in mean responses and consensus within groups on this question. It will likely take some time, however, until these noninvasive methods are widely applied in small mammal studies, if ever given their cost. Even so, many questions will continue to require CMR-based studies to collect data that are best assessed by examining captured animals, such as body condition and reproductive status. As such, researchers will need to continue to assess the advantages and disadvantages of marking methods that may be employed in their study designs.

Expert-based surveys can be useful for informing decisions made by researchers or managers where data are not available (Petit and Waudby 2012; Rode et al. 2018), but they have limitations. A limitation of our study was the relatively small sample sizes, particularly from experts outside of North America. We sought to maximize our sample size through distribution of the online link to our survey on social media sites and e-mail listservers that targeted small mammal researchers. Future surveys such as ours should seek means to increase sample size and ensure participation by a large group of experts. For instance,

allowing respondents more time to complete the survey than we did may help improve sample sizes. Perhaps a more conventional approach would have been to conduct a literature review of published studies. Clearly, larger sample sizes would have resulted, and several biases addressed (see below), via a literature review. Our expert-based survey, however, provided several advantages compared to a literature review. Specifically, our survey was able to probe the beliefs and perceptions of a sample of experts, and respondents were asked what they plan to use in the future and which factors influenced their choices. Deeper questions such as these would not be possible to address through a literature review of use in past studies alone.

Another limitation of our study was observed biases in terms of the location where respondents do their studies and their field of study. Our data were biased toward researchers working in North America and those focused on population ecology. We tested for these respondent biases in our data and the analyses indicated that there were some significant differences among respondent groups that should be kept in mind when interpreting our results. Many of our observed differences, however, did not have a substantial impact on the overall response, likely because of variability within respondent groups as indicated by PCI₂ scores. Nonetheless, similar surveys with a greater response rate from other continents or fields of study may report different results. In particular, substantial information gaps remain on choices made by small mammal experts working in Latin America, Asia, and Africa. Researchers from these continents may differ, collectively, in their preferred marking method or factors influencing their choices compared to each other or those in North America. Future studies such as ours should consider means to more thoroughly engage mammalogists by targeting other regional forums to increase global representation.

Ultimately, the choice of marking method will depend on the species, the question, available resources, as well as individual preferences (Murray and Fuller 2000). Importantly, local legislation (Loretto et al. 2013; Powell and Proulx 2003), permit authorizations and conditions, and standard operating procedures (Petit and Waudby 2012) dictate what methods are locally permissible. For example, toe-clipping is banned in some countries (e.g., much of the European Union), under consideration of being banned in others (e.g., Loretto et al. 2013), and elsewhere generally acceptable only when no other marking method is feasible or biological samples (e.g., DNA) are also required (Powell and Proulx 2003; Petit et al. 2012; Sikes et al. 2016). Additionally, some scientific journals may not accept manuscripts that include toe-clipping as a marking technique. Regardless, as several survey respondents aptly noted "one size does not fit all" (Supplementary Data SD3), meaning that no marking method is perfect for all species of small mammals and all questions—they each have their advantages and disadvantages, and this was evident in our data. Within the bounds of local legislation and permit authorizations, researchers will need to continue to make personal choices on which marking method to employ in their studies. In doing so, they need to consider assumptions regarding mark retention and recognition

when using CMR-based designs (Otis et al. 1978; McDonald et al. 2003; Fokidis et al. 2006), and reducing their impact (pain, survival) on marked individuals (Powell and Proulx 2003; Petit and Waudby 2012; Wilson et al. 2019). It is encouraging that these were the three factors that respondents most frequently reported influencing their choice of marking method.

Many survey respondents (66%) indicated that additional studies on the efficacy and impact of various marking methods are needed. As such, we echo the earlier comments by Murray and Fuller (2000) that more studies along these lines are needed to allow researchers to make better informed decisions. For instance, there is concern regarding retention of PIT-tags in small mammals (Fokidis et al. 2006; Lebel and Ruf 2009; Rigby et al. 2012). Given that our data suggest that PIT-tagging small mammals may increase substantially in the future, assessing the performance and impacts (e.g., pain, survival) of this method on target species should be paramount. The same is true for improving methods to apply existing marks to small mammals (Lebel and Ruf 2009) and adequate training of field staff in applying them (Rigby et al. 2012). Moreover, 55% of survey respondents—particularly those with more experience—indicated that there is a need for the development of new marking methods. It is encouraging that researchers continue to explore the use of new marking methods such as tattoos (Petit et al. 2012) and genetic-tagging (Ferreira et al. 2018). Additional exploration of new marking methods that aim to reduce impact to affected animals, as well as cost and time to apply in the field, while increasing mark retention and recognition, are encouraged. Added to this is the improvement of existing marks currently available to small mammal researchers. For example, the development of ear-tags and PIT-tags more appropriately sized for small mammals may alone be a key advance.

Finally, we conclude by suggesting that collective insights on the perceptions and factors influencing the decisions of mammalogists may help researchers (especially early career scientists) develop their study designs and protocols. It may also serve as a catalyst to spur or guide the assessment and improvement of field methods and protocols. To support greater knowledge transfer and discourse among mammalogists, we encourage other expert opinion surveys on methodological issues within the research community.

ACKNOWLEDGMENTS

D. Jolkowski, M. McCaw, and S. van Delft participated in the review of the questionnaire as part of an undergraduate course in mammalogy taught by the senior author. P. Kukka also kindly reviewed a draft of our questionnaire. Foremost, we heartily thank the small mammal researchers that took the time to complete the online survey and openly share their experience, knowledge, and perspectives about marking small mammals. C. Dickman, R. Kennerly, and I. Torre are thanked, in particular, for kindly helping to disseminate our online survey within the global community of small mammal researchers. We thank C. Pavey, H. Henttonen, and two anonymous reviewers for kindly providing comments that improved this manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Continent where most of the field studies on small mammals is conducted by (top panel, A), and broad field of study of (bottom panel, B), respondents ($n = 114$) to an online survey about methods for individually marking small mammals.

Supplementary Data SD2.—Median, mean ($\pm SD$), and range of responses for Likert scale-based questions in a survey of small mammal researchers ($n = 114$) about marking methods. Comparisons are made for respondents with 1) comparatively High versus Low levels of small mammal trapping experience, 2) that work in North America or Other continents, and 3) with a focus on Population Ecology or Other subfields of biology. Means between groups are compared with *t*-tests. Questions can be found in Table 1.

Supplementary Data SD3.—Verbatim (unedited) responses ($n = 36$) to the final, open-ended question in the survey (Question 23) regarding respondents experiences, beliefs, and perceptions about individually marking small mammals or the survey.

LITERATURE CITED

- AMBROSE, H. W. 1972. Effect of habitat familiarity and toe-clipping on rate of owl predation in *Microtus pennsylvanicus*. *Journal of Mammalogy* 53:909–912.
- AMSTRUP, S. C., T. L. McDONALD, AND B. F. J. MANLY (EDS.). 2005. Handbook of capture–recapture analysis. Princeton University Press. Princeton, New Jersey.
- ANDERSON, D. R., K. P. BURNHAM, G. C. WHITE, AND D. L. OTIS. 1983. Density estimation of small-mammal populations using a trapping web and distance sampling methods. *Ecology* 64:674–680.
- ANTHONY, N. M., C. A. RIBIC, R. BAUTZ, AND T. GARLAND. 2005. Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Society Bulletin* 33:1018–1025.
- BEACHAM, T. D., AND C. J. KREBS. 1980. Pitfall versus live-trap enumeration of fluctuating populations of *Microtus townsendii*. *Journal of Mammalogy* 61:486–499.
- BLAIR, W. F. 1941. Techniques for the study of mammal populations. *Journal of Mammalogy* 22:148–157.
- BORREMANS, B., V. SLUYDTS, R. H. MAKUNDI, AND H. LEIRS. 2015. Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent. *Wildlife Research* 42:143–148.
- BOONSTRA, R., AND C. J. KREBS. 1978. Pitfall trapping of *Microtus townsendii*. *Journal of Mammalogy* 59:136–148.
- BOONSTRA, R., AND F. H. RODD. 1984. Efficiency of pitfalls versus live traps in enumeration of populations of *Microtus pennsylvanicus*. *Canadian Journal of Zoology* 62:758–765.
- BOVENDORP, R. S., R. A. MCCLEERY, AND M. GALETTI. 2017. Optimising sampling methods for small mammal communities in Neotropical forests. *Mammal Review* 47:148–158.
- BRAUDE, S., AND D. CISZEK. 1998. Survival of naked mole-rats marked by implantable transponders and toe-clipping. *Journal of Mammalogy* 79:360–363.
- CHITTY, D. 1937. A ringing technique for small mammals. *Journal of Animal Ecology* 6:36–53.
- DE BONDI, N., J. G. WHITE, M. STEVENS, AND R. COOKE. 2010. A comparison of the effectiveness of camera trapping and live

- trapping for sampling terrestrial small-mammal communities. *Wildlife Research* 37:456–465.
- DIETE, R. L., P. D. MEEK, C. R. DICKMAN, AND L. K. P. LEUNG. 2016. Ecology and conservation of the northern hopping-mouse (*Notomys aquilo*). *Australian Journal of Zoology* 64:21–32.
- FAIRLEY, J. S. 1982. Short-term effects of ringing and toe-clipping on the recapture of wood mice (*Apodemus sylvaticus*). *Journal of Zoology* (London) 197:295–297.
- FERREIRA, C. M., ET AL. 2018. Genetic non-invasive sampling (gNIS) as a cost-effective tool for monitoring elusive small mammals. *European Journal of Wildlife Research* 64:46.
- FISHER, D. O., AND S. P. BLOMBERG. 2009. Toe-bud clipping of juvenile small marsupials for ecological field research: no detectable negative effects on growth or survival. *Austral Ecology* 34:858–865.
- FOKIDIS, H. B., C. ROBERTSON, AND T. S. RISCH. 2006. Keeping tabs: are redundant marking systems needed for rodents? *Wildlife Society Bulletin* 34:764–771.
- GERBER, B. D., AND R. R. PARMENTER. 2015. Spatial capture-recapture model performance with known small-mammal densities. *Ecological Applications* 25:695–705.
- GRANT, E. H. C. 2008. Visual implant elastomer mark retention through metamorphosis in amphibian larvae. *Journal of Wildlife Management* 72:1247–1252.
- HAMMOND, E. L., AND R. G. ANTHONY. 2006. Mark-recapture estimates of population parameters for selected species of small mammals. *Journal of Mammalogy* 87:618–627.
- HARKINS, K. M., D. KEINATH, AND M. BEN-DAVID. 2019. It's a trap: optimizing detection of rare small mammals. *PLoS One* 14:e0213201.
- JUNG, T. S. 2016. Comparative efficacy of Longworth, Sherman, and Ugglan live-traps for capturing small mammals in the Nearctic boreal forest. *Mammal Research* 61:57–64.
- KORN, H. 1987. Effects of live-trapping and toe-clipping on body weight of European and African rodent species. *Oecologia* 71:597–600.
- KORSLUND, L. 2018. Relative efficiency of two models of Ugglan Special live-traps for capturing small rodents in boreo-nemoral forest. *Annales Zoologici Fennici* 55:247–256.
- KORSLUND, L., AND H. STEEN. 2006. Small rodent winter survival: snow conditions limit access to food resources. *The Journal of Animal Ecology* 75:156–166.
- KREBS, C. J., AND R. BOONSTRA. 1984. Trappability estimates for mark-recapture data. *Canadian Journal of Zoology* 62:2440–2444.
- KREBS, C. J., ET AL. 2004. Can outbreaks of house mice in south-eastern Australia be predicted by weather models? *Wildlife Research* 31:465–474.
- KREBS, C. J., R. BOONSTRA, B. S. GILBERT, A. J. KENNEY, AND S. BOUTIN. 2019. Impact of climate change on the small mammal community of the Yukon boreal forest. *Integrative Zoology* in press.
- KUENZI, A. J., M. M. ZUMBRUN, AND K. HUGHES. 2005. Ear tags versus passive integrated transponder (PIT) tags for effectively marking deer mice. *Intermountain Journal of Sciences* 11:66–70.
- LAMBIN, X., AND J. MACKINNON. 1997. The relative efficiency of two commercial live-traps for small mammals. *Journal of Zoology* (London) 242:400–404.
- LEBEL, K., AND T. RUF. 2009. An easy way to reduce PIT-tag loss in rodents. *Ecological Research* 25:251–253.
- LE BOULENGE-NGUYEN, P. Y., AND E. LE BOULENGE. 1986. A new ear-tag for small mammals. *Journal of Zoology* (London) 209:302–304.
- LECLERCQ, G. C., AND F. M. ROZENFELD. 2001. A permanent marking method to identify individual small rodents from birth to sexual maturity. *Journal of Zoology* (London) 254:203–206.
- LINDBERG, M. S. 2012. A review of designs for capture–mark–recapture studies in discrete time. *Journal of Ornithology* 152:355–370.
- LINDNER, E., AND O. FUELLING. 2002. Marking methods in small mammals: ear-tattoo as an alternative to toe-clipping. *Journal of Zoology* (London) 256:159–163.
- LORETTO, E., ET AL. 2013. On the practice of toe clipping for small mammal studies in Brazil. *Boletim da Sociedade Brasileira de Mastozoologia* 66:12–14.
- MANFREDO, M. J., J. J. VASKE, AND T. L. TEEL. 2003. The potential for conflict index: a graphic approach to practical significance of human dimensions research. *Human Dimensions of Wildlife* 8:219–228.
- MANVILLE, R. H. 1949. Techniques for capture and marking of mammals. *Journal of Mammalogy* 30:27–33.
- MCDONALD, T. L., S. C. AMSTRUP, AND B. F. J. MANLY. 2003. Tag loss can bias Jolly-Seber capture-recapture estimates. *Wildlife Society Bulletin* 31:814–822.
- MILLER, C. R., P. JOYCE, AND L. P. WAITS. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. *Molecular Ecology* 14:1991–2005.
- MOSEBY, K. E., R. BRANDLE, AND M. ADAMS. 1999. Distribution, habitat and conservation status of the rare dusky hopping-mouse, *Notomys fuscus* (Rodentia: Muridae). *Wildlife Research* 26:479–494.
- MURRAY, D. L., AND M. R. FULLER. 2000. A critical review of the effects of marking on the biology of vertebrates. Pp 15–64 in *Research techniques in animal ecology: controversies and consequences* (L. Boitani and T. K. Fuller, eds.). Columbia University Press. New York.
- OSTFELD, R. S., M. C. MILLER, AND J. SCHNURR. 1993. Ear tagging increases tick (*Ixodes dammini*) infestation rates of white-footed mice (*Peromyscus leucopus*). *Journal of Mammalogy* 74:651–655.
- OTIS, D. L., K. P. BURNHAM, G. C. WHITE, AND D. R. ANDERSON. 1978. Statistical inference from capture data on closed animal populations. *Wildlife Monographs* 62:1–35.
- PARMENTER, R. R., ET AL. 2003. Small-mammal density estimation: a field comparison of grid-based vs. web-based density estimators. *Ecological Monographs* 73:1–26.
- PAVONE, L. V., AND R. BOONSTRA. 1985. The effects of toe clipping on the survival of the meadow vole (*Microtus pennsylvanicus*). *Canadian Journal of Zoology* 63:499–501.
- PETIT, S., AND H. P. WAUDBY. 2012. Standard operating procedures for aluminum box, wire cage, and pitfall trapping, handling, and temporary housing of small wild rodents and marsupials. *Australian Journal of Zoology* 60:392–401.
- PETIT, S., H. P. WAUDBY, A. T. WALKER, R. ZANKER, AND G. RAU. 2012. A non-mutilating method for marking small wild mammals and reptiles. *Australian Journal of Zoology* 60:64–71.
- POCOCK, M. J. O., AND S. C. BELL. 2011. Hair tubes for estimating site occupancy and activity-density of *Sorex minutus*. *Mammalian Biology* 76:445–450.
- POCOCK, M. J. O., AND N. JENNINGS. 2006. Use of hair tubes to survey for shrews: new methods for identification and quantification of abundance. *Mammal Review* 36:299–308.
- POLLOCK, K. H., J. D. NICHOLS, C. BROWNIE, AND J. E. HINES. 1990. Statistical inference for capture-recapture experiments. *Wildlife Monographs* 107:1–97.

- POWELL, R. A., AND G. PROULX. 2003. Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. *ILAR Journal* 44:259–276.
- RIGBY, E. L., J. AEGERTER, M. BRASH, AND J. D. ALTRINGHAM. 2012. Impact of PIT tagging on recapture rates, body condition and reproductive success of wild Daubenton's bats (*Myotis daubentonii*). *The Veterinary Record* 170:101.
- RODE, K. D., ET AL. 2018. Survey-based assessment of the frequency and potential impacts of recreation on polar bears. *Biological Conservation* 227:121–132.
- ROMAIRONE, J., J. JIMÉNEZ, J. J. LUQUE-LARENA, AND F. MOUGEOT. 2018. Spatial capture-recapture design and modelling for the study of small mammals. *PLoS One* 13:e0198766.
- SALAMON, M., AND B. KLETTENHEIMER. 1994. A new technique for marking and later recognizing small mammals in the field. *Journal of Zoology (London)* 233:314–317.
- SCOTT, T. G. 1942. Ear tags on mice. *Journal of Mammalogy* 23:339.
- SIKES, R. S., AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97:663–688.
- SINGLETON, G. R. 1989. Population dynamics of an outbreak of house mice (*Mus domesticus*) in the mallee wheatlands of Australia—hypothesis of plague formation. *Journal of Zoology (London)* 219:495–515.
- SINGLETON, G. R., P. R. BROWN, R. P. PECH, J. JACOB, G. J. MUTZE, AND C. J. KREBS. 2005. One hundred years of eruptions of house mice in Australia—a natural biological curio. *Biological Journal of the Linnean Society* 84:617–627.
- STOCKDALE, L. G. 1932. Technique for marking rats numerically with dye. *Journal of Comparative Psychology* 14:237–240.
- TASKER, E. M., AND C. R. DICKMAN. 2002. A review of Elliot trapping methods for small mammals in Australia. *Australian Mammalogy* 23:77–87.
- TORRE, I., L. FREIXAS, A. ARRIZABALAGA, AND M. DÍAZ. 2016. The efficiency of two widely used commercial live-traps to develop monitoring protocols for small mammal biodiversity. *Ecological Indicators* 66:481–487.
- TORRE, I., A. RASPALL, A. ARRIZABALAGA, AND M. DÍAZ. 2018. SEMICE: an unbiased and powerful monitoring protocol for small mammals in the Mediterranean Region. *Mammalian Biology* 88:161–167.
- TWIGG, G. I. 1975. Techniques in mammalogy. Chapter 3: marking mammals. *Mammal Review* 5:101–116.
- VASKE, J. J. 2018. Visualizing consensus in human dimensions data: the potential for conflict index₂. *Human Dimensions of Wildlife* 23:83–39.
- VASKE, J. J., J. BEAMAN, H. BARRETO, AND L. B. SHELBY. 2010. An extension and further evaluation of the potential for conflict index. *Leisure Studies* 32:240–254.
- VILLETTE, P., C. J. KREBS, T. S. JUNG, AND R. BOONSTRA. 2016. Can camera trapping provide reliable estimates of small mammal (*Myodes rutilus* and *Peromyscus maniculatus*) density in the boreal forest? *Journal of Mammalogy* 97:32–40.
- WILLIAMS, B. K., J. D. NICHOLS, AND M. J. CONROY. 2002. Analysis and management of animal populations. Academic Press. San Diego, California.
- WILSON, R. P., ET AL. 2019. Towards informed metrics for examining the role of human-induced animal responses in tag studies on wild animals. *Integrative Zoology* 14:17–29.
- WOOD, M. D., AND N. A. SLADE. 1990. Comparison of ear-tagging and toe-clipping in prairie voles, *Microtus ochrogaster*. *Journal of Mammalogy* 71:252–255.

Submitted 4 July 2019. Accepted 4 November 2019.

Associate Editor was Chris Pavey.