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Reappraising the use of forearm rings for bat species

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A R T I C L E I N F O

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A B S T R A C T

Long-term mark-recapture studies are essential for bat conservation. Over the last decades, millions of bats across Europe and America have been marked with forearm rings for this purpose. Although it is considered a cost-effective method compared to Passive Integrated Transponders (PIT) tags, direct injuries from using forearm rings have been reported since their very first use. Yet, their impact on bats' welfare has not been systematically evaluated and remains a highly controversial issue among the scientific community and policymakers. Here we assess the impact of forearm rings and PIT tags on the health of different bat species. We reviewed 12 years of the existing recapture data of free-ranging bats from NE Spain and evaluated the impact of both marking tools in a captive colony of *Carollia perspicillata*, by assessing the development of skin lesions and levels of cortisol metabolites in guano (CG) after marking. We report that 55.1 % (435/790) of the recaptured free-ranging bats with forearm rings presented skin lesions. All banded *C. perspicillata* (n = 22, 100 %) developed skin lesions, whereas none of the PIT-tagged (n = 21) presented lesions. Levels of CG were significantly higher after marking with forearm rings only for one group. Banded *C. perspicillata* exhibited discomfort-associated behaviours due to forearm rings. Under the "precautionary principle", we recommend the ban of forearm rings for all bat species until species-specific studies under controlled conditions are performed and approved by a legally constituted ethics committee. Consideration of other long-term marking tools is mandatory to align with global bat conservation strategies.

1. Introduction

Bat populations have experienced significant declines over the past century, leading the International Union for Conservation of Nature (IUCN) to classify nearly half of the species as threatened or near threatened globally (IUCN, 2022). In Europe, bat populations have shown downward trends for the past 50 years, especially throughout Western Europe (Hutson et al., 2001). At present, 22 % of the European bat species are classified as Endangered (EN) or Vulnerable (VU) by the IUCN (Hutson et al., 2001; IUCN, 2022) but strict species and habitat protection, accompanied by investments in research to improve conservation strategies, have stabilized populations for a number of species

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(Browning et al., 2021; Haysom et al., 2013). However, population trends and key threatening processes are not fully understood for the vast majority of species.

The Agreement on the Conservation of Populations of European Bats (UNEP/EUROBATS) was set up in 1991 to cooperate towards the conservation of bats throughout Europe. The Agreement provides guidelines to be implemented by European institutions and conservationists on diverse topics, including the capture and marking of bats (Hutson et al., 2001; UNEP/EUROBATS, 2003). For decades, the capture-mark-recapture technique has been used in wildlife for research and conservation (Cody and Smallwood, 1996; Gaisler and Chytil, 2002; Shenbrot et al., 2010) Particularly, the recognised challenge of providing information about bat ecology, population dynamics and social behaviour identifies long-term mark-recapture studies as an important tool for bat conservation (Locatelli et al., 2019). At present, most of these long-term bat monitoring studies are undertaken using two marking tools: forearm rings (also dubbed bands) and Passive Integrated Transponder (PIT) tags (Kunz and Parsons, 2009; Mitchell-Jones and McLeish, 2004). Tagging with forearm rings is a cost-effective method for marking bats, compared to PIT tags, and no technical device is required to read it. These attributes have given bands a rising popularity among bat researchers and conservationists (Godinho et al., 2015; van Harten et al., 2019; van Harten et al., 2022a).

Millions of bats have been marked with forearm rings worldwide, especially in Europe and North America, over the last decades (Ellison, 2008; Hutson et al., 2001; Hutterer et al., 2005). However, direct injuries from using bands have been reported, suggesting that this marking technique could silently compromise the conservation efforts of bat populations worldwide (Baker et al., 2001; Balmori and Quetglas, 2000; Ellison, 2008; Hutterer et al., 2005). This issue has become highly controversial among the scientific community and policymakers. The recommended ring sizes and designs in Europe are based on field recapture experience from Bulgaria, Germany, The Netherlands, Portugal, Ukraine, and the United Kingdom (UNEP/EUROBATS, 2003). However, the UNEP/EUROBATS Agreement recognises that banding is an invasive procedure and that the degree of tolerance to this marking tool varies among species and populations of the same species (UNEP/EUROBATS, 2018). For this reason, some countries (i.e. USA, Australia and France) have implemented moratoriums on the use of forearm rings in bat research based on field studies that reported direct physical injuries in bat wings produced by this marking tool (Baker et al., 2001; Ellison, 2008; Hutterer et al., 2005). Currently, the knowledge of the impact of marking tools on bats' health is mainly based on field studies with low recapture rates (Ellison, 2008; Hutterer et al., 2005; Steffens et al., 2007). As a consequence, the direct effects and the magnitude of the impact might be largely underestimated. Furthermore, few studies have examined the impact of marking tools on survival rates and sublethal effects (e.g. reduction in body condition) in free-ranging bats (Baker et al., 2001; Locatelli et al., 2019; Mellado et al., 2022). These field studies, although valuable, are unable to explore other collateral effects such as alterations in social or reproductive behaviour, or increased stress and susceptibility to disease.

Measurement of cortisol metabolites in faeces has been broadly used to evaluate stress in animals, including bats (Hernández-Arciga et al., 2020; Kelm et al., 2016). Sustained high cortisol concentrations have been associated with poor body condition, reduced immunocompetence, decreased reproductive success, and ultimately fitness disruption (Cain and Cidlowski, 2017; Edwards et al., 2019). Research on bat marking tools under controlled conditions to document and understand these acute and long-term effects on the health and survival of these species is crucial. Insights from such controlled studies can help to develop evidence-based national and international policies to guarantee animal welfare and support current bat conservation strategies.

The aim of the present study was to assess the impact of forearm rings and Passive Integrated Transponder (PIT) tags on the health of a selected group of bat species by 1) reviewing the existing recapture data of wild insectivorous bats from Catalonia (NE Spain) from 12 years (2009 to 2021) and 2) monitoring skin injuries, assessing cortisol metabolites in guano and observing alterations in the behaviour of captive *Carollia perspicillata*, derived from both marking tools, under captive conditions in a zoological institution.

2. Materials and methods

2.1. Assessment of the impact of forearm rings in free-ranging recaptured bats from Catalonia, NE Spain

We analysed data from bat recaptures in NE-Spain, from the last 12 years (2009–2021), on forearm rings and PIT tags (Table A1, Appendix A) collected by two institutions for the research of bats in Catalonia (BiBio Research Group - Natural Sciences Museum of Granollers and Centre de Ciència i Tecnologia Forestal de Catalunya).

We calculated the proportion of bats detected with injuries from the total recaptured per species and marking method, with exact confidence intervals of 95 %. First, considering all the recaptured bats, we tested whether there was a statistically significant difference in the frequency of lesions between bats marked with forearm rings and bats marked with PIT tags using the Fisher's exact test. Then, for recaptured bats marked with forearm rings, we also evaluated whether there were statistically significant differences in the frequency of lesions between the different species of bats. To do that, we used pairwise Fisher's exact tests with the Holm's correction to account for multiple comparisons. Only those species for which there were at least seven individuals were included in the comparison.

In a small subset of the bats marked with forearm rings ($n = 304$), besides the data on the species, there was supplementary information collected on the age, sex and reproductive state. The effect of age could not be evaluated because all animals included in the study were adults. To evaluate whether the other factors had a significant effect on the probability of developing a lesion, a logistic regression model was built with the variables species, sex, and reproductive state.

2.2. Assessment of the impact of tagging microbats in captivity

We tested the impact of forearm rings and PIT tags on a captive colony of adult Seba's short-tailed bats (*Carollia perspicillata*), housed at Butterfly Park (license number G25-00119), a small zoo established in Empuriabrava (NE Spain). These animals were already required to be individually identified for the zoo collection register. All bats were born in captivity and adapted to the routine institutional management strategies: daily cleansing of the facilities between 9:00 and 10:00 and food supply between 18:00 and 19:00 h.

The Seba's short-tailed bat is a widespread *Phyllostomidae* bat species inhabiting Central and South America. This species is classified as Least Concern by the IUCN Red List (Barquez et al., 2015) and is exhibited in many zoological institutions due to its small size, diet specifications (mainly frugivorous species) and its gregarious behaviour, roosting in groups up to hundreds of individuals (Rasweiler IV et al., 2009). Roosts can be organised as a harem (1 adult male with large aggregations of females) and as a bachelor (large aggregations of adult and subadult males without females) (Martínez-Medina et al., 2018). The phenotypic characteristics of this species (similar in size and morphology to several species of European bats) and its adaptation to a captive environment (Rasweiler IV et al., 2009) made it suitable for the purpose of this study.

An intentional sampling method was used to select 32 adult females and 11 adult males from the captive colony of Seba's short-tailed bats ($n = 150$), excluding individuals with scars, solved fractures or any other integument abnormalities. Bats were captured with a telescopic net bag (BTHK Tree-Roost net bag, NHBS). Best practices for the care and handling of bats (H. Miller, 2016) were followed throughout the study and at least one European Diplomate in Veterinary Medicine (Wildlife Population Health and/or Veterinary Dermatology) and a bat specialist were present during all procedures. Bats were distributed in four groups: group 1 (one male and eight females) and group 2 (one male and 12 females) were marked with forearm rings, and group 3 (one male and 12 females) and group 4 (8 males) were fitted with PIT tags. Each group was tested independently and housed at different times in a separate enclosure of 50 square meters and 3 m high, meeting the environmental requirements of *C. perspicillata* (relative humidity of 80 % and average minimum and maximum temperature of 20–26 °C) (Bat Taxonomic Advisory Group, 2004; Martínez-Medina et al., 2018). The enclosure provided a green semi-opened area and an artificial cave for light insulation during daylight hours. Additionally, bats were isolated from external stressors such as visitors and educational activities for the whole duration of the study. The adaptation period for each group spanned four weeks, after which a marking tool (forearm ring or PIT tag) was implemented and tested for six consecutive weeks. Forearm rings were withdrawn at the end of the sixth week in groups 1 and 2 and then those bats were PIT-tagged and returned to their original enclosure.

Forearm rings used for groups 1 and 2 were distributed by the SECEMU Association (Spanish Association for the Conservation and Research of Bats, Spain) and approved for their use in Spain. They contained a unique alpha-numerical code, were omega-shaped, 4.2 mm in diameter and made of a light alloy of aluminum and magnesium (model 3X, 0.103 g of weight). Bats were banded (Fig. A1, Appendix A) by highly experienced personnel. Fingers were used to gently close the ring, maintaining their round shape and leading to ~1 mm wide opening between the tips so that it was free to slide up and down the forearm but prevented finger bones from becoming trapped.

Bats from groups 3 and 4 were PIT-tagged using a sterilised 10 mm × 1.4 mm transponder of 0.036 g of weight (FrenChip Mini, Avid®, California, USA), injected subcutaneously in the dorsum so that the tag rested between the shoulder blades, as described previously in the literature (van Harten et al., 2020). Next, a drop of surgical glue (3M™ VetBond™) was applied to the injection site to minimise tag loss, and the animal was restrained for a few seconds until the complete drying of the adhesive.

2.2.1. Physical impact assessment

All bats were physically examined prior to the study and at the end of the adaptation period to ensure they were physically fit and healthy before any tag was implanted. Particular attention was given to fur and patagia. The evaluation of skin lesions caused by both marking tools was carried out by a Diplomate specialist from The European College of Veterinary Dermatology (ECVD) at the end of the six-week period in all four groups. Cutaneous lesions found after marking with forearm rings were classified in order of increasing severity in Stage 1, Stage 2, and Stage 3 (Table A2, Appendix A). Stage 1 was assigned to any mild lesion in the sense that the epidermis was not damaged, and no treatment was required after the ring withdrawal. Stage 2 referred to moderate cutaneous lesions associated with a superficial breakdown in the continuity of the epidermis. Topical treatment may improve and boost the patient's recovery time assigned to this stage. Stage 3 included injuries with significant skin tissue and fluid loss, exposure of the dermis and subcutis, and epidermal ischemia. Topical, parenteral, and surgical treatment may be necessary for some presentations of Stage 3 to avoid the development of sepsis or loss of extremity function (Miller et al., 2012).

2.2.2. Complementary animal welfare monitoring

All bats were continuously monitored by two infrared video cameras (Foscam FI9926P®) located at the entrance of the artificial cave of the experimental facility and in front of the feeding plates, respectively. Video cameras allowed the live view of the animals and stored a continuous recording for delayed observation and assessment of behavioural indicators of welfare. Furthermore, zoo caretakers reported any abnormal conduct detected during feeding and cleansing times.

Additionally, to explore the indirect effects of forearm rings and PIT tags on the welfare of bats, we measured cortisol metabolites in guano (CG) from each group, twice a week during the adaptation and marking period. Hormone metabolites were extracted following a methanol extraction protocol previously described (Palme et al., 2013; Tallo-Parra et al., 2014) (Cortisol analysis, Appendix B).

2.2.3. Statistical analyses

A Fisher's exact test was applied to compare the frequencies of lesions between groups marked with forearm rings and PIT tags.

To evaluate whether the marking tool (forearm ring or PIT tag) influenced the levels of CG from the animals in the different groups, a linear regression model was built considering CG as the response variable, and the time point (sampling time) and the interaction between period (adaptation or marking) and group (1–4) as the covariates. The comparison between the estimated marginal means was computed. We hypothesised that CG might be modulated by the stress response produced by being tagged. Therefore, intragroup CG concentrations after tagging were expected to be significantly higher than those during the adaptation period, particularly in the two groups with forearm rings.

All the analyses were performed using R programming (R Core Team, 2022) language and RStudio (RStudio Team, 2022) environment. We use the package lsmeans (Least-Squares Means) for the analyses (Russell V, 2016) and ggplot2 (Wickham, 2016) and tidyverse (Wickham et al., 2019) for the figures.

3. Results

3.1. Physical impact of tagging free-ranging bats with forearm rings

Information regarding 1236 recaptured free-ranging bats was analysed, 790 of which had forearm rings and 446 had PIT tags (Table 1). Recaptured bats included the following species: *Miniopterus schreibersii*, *Myotis capaccinii*, *M. daubentonii*, *M. myotis*, *Nyctalus lasiopterus*,

Table 1
Percentage of injured bats due to forearm rings and PIT tags. Data obtained during the last 12 years (2009–2021), from bats recaptured in Catalonia (NE-Spain).

Species	Marking tool	Bats recaptured	Bats with injuries	Injuries occurrence % ($CI_{95\%}$)
M. schreibersii	Forearm ring	445	262	58.9 (54.2–63.5)
	PIT tag	116	0	0.0 (0.0–3.1)
M. capaccinii	Forearm ring	57	8	14.0 (6.3–25.8)
M. daubentonii	Forearm ring	3	1	33.3 (0.8–90.6)
M. myotis	Forearm ring	7	2	28.6 (3.7–71.0)
N. lasiopterus	Forearm ring	12	0	0.0 (0.0–26.5)
	PIT tag	5	0	0.0 (0.0–52.2)
N. leisleri	Forearm ring	105	56	53.3 (43.3–63.1)
	PIT tag	79	0	0.0 (0.0–4.6)
N. noctula	Forearm ring	2	0	0.0 (0.0–84.2)
P. pygmaeus	Forearm ring	159	106	66.7 (58.8–74.0)
	PIT tag	246	0	0.0 (0.0–1.5)
Total	Forearm ring	790	435	55.1 (51.5–58.6)
	PIT tag	446	0	0.0 (0.0–0.8)

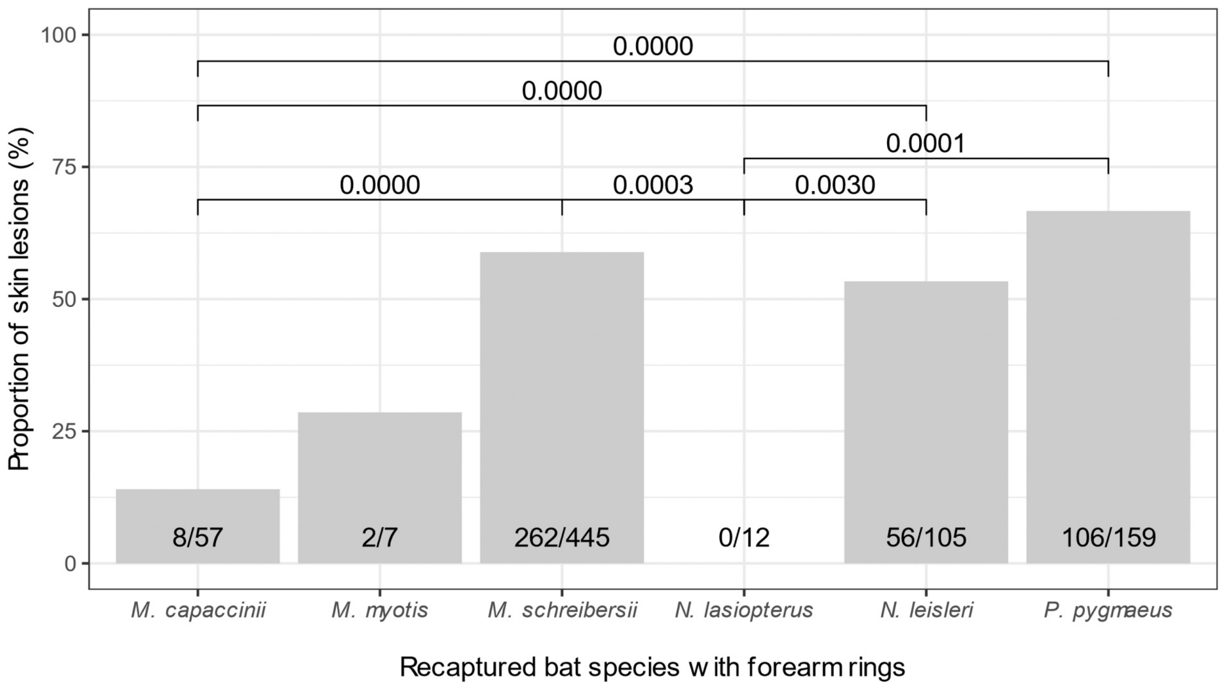

Fig. 1. Differences between proportions of skin lesions found in free-ranging recaptured bats with forearm rings (pairwise Fisher's exact tests with Holm's correction, p-value <0.05). The number of bats detected with skin lesions from the total recaptured is depicted in each species box.

N. leisleri, *N. noctula* and *Pipistrellus pygmaeus*. A total of 435 recaptured bats (55.1 %, $CI_{95\%}$: 51.5–58.6) showed skin lesions derived from the forearm ring, whereas no skin lesions (0 %, $CI_{95\%}$: 0.0–0.8) were detected in any of the 446 bats recaptured with PIT tags (Table 1). The frequency of lesions was significantly higher in bats marked with forearm rings compared with bats marked with PIT tags (p-value < 0.001). Statistically significant differences between species are shown in Fig. 1.

The presence or absence of skin lesions was reported for all recaptured species, but the classification or description of the lesions was only provided for the following four species. The most reported injury in *M. schreibersii* and *N. leisleri* was a callus formation (55.2 % and 66.7 % respectively) (Fig. 2A), followed by an ulcerated callus (21.2 % and 14.6 % respectively). Ulcerated callus (Fig. 2B) was the most described lesion (29.4 %%) in *P. pygmaeus*, followed by callus formation (20.3 %), ulcers and extended erosions (19.6 %), and ring embedding (13.1 %) (Fig. 2C). The recovered *M. daubentonii* with skin lesions showed an extended area of skin thinning and depigmentation, and an erythematous plaque in the distal carp (Fig. 2D).

Additional information on sex and reproductive state (for the latter see Fig. A2, Appendix A) was available only for a subset of 304 bats marked with forearm rings: *M. schreibersii* (n = 163), *N. leisleri* (n = 17), and *P. pygmaeus* (n = 124). The results of the logistic regression model using the previous subset of the dataset showed that the sex of the bats did not have a significant effect on the probability of lesions. In contrast, the reproductive state did, showing that bats in a passive reproductive state were less likely to develop skin lesions than those in an active reproductive state (OR = 0.09, p-value = 0.018) (Table 2). The logistic regression model showed that *P. pygmaeus* had a significantly lower risk of developing skin lesions derived from the use of forearm rings than *M. schreibersii* (OR = 0.38, p-value = 0.003) (Table 2).

3.2. *Impact of tagging C. perspicillata in captivity*

3.2.1. *Skin lesions*

All banded bats (n = 22, 100 %) developed skin lesions of varying degrees of severity. Forearm rings were found displaced, piercing the dorsal and ventral aspects of the distal forearm in most banded *C. perspicillata* (n = 15, 68.2 %). The most frequently found lesions were skin depigmentation and thinning (n = 19, 86.4 %), erythema (n = 16, 72.7 %) (Fig. 3A) and skin neovascularisation (n = 13, 59.1 %) (Fig. 3). The area of skin depigmentation and thinning coincided with the location of ring displacement along the forearm ring, dorsally and ventrally to the radius and ulna. Erythematous plaques (n = 12, 54.5 %) (Fig. 3B) were visible along the same area of skin depigmentation and thinning, presumably in the most proximal area where the ring could be displaced. These erythematous plaques were commonly surrounded by neovascularisation, newly formed capillaries arising from the brachial vein (Fig. 3B). Erosions (Fig. 3C) were present in six bats, mostly on the edges of the propatagium close to the carp and in the same area of the erythematous plaques, due to forearm ring rubbing. Four (18.2 %) bats developed severe cutaneous lesions, corresponding with ulcers (Fig. 3D) located dorsally and ventrally to the radius and ulna, and also in the most proximal area where the ring could be displaced. One forearm ring was found displaced from the propatagium, completely closed around the radius and ulna, and embedded in the skin (Fig. 3E). After the removal, granulomatous tissue and inflammation were evidenced, and the subcutis and muscles from the radius and ulna were exposed (Fig. 3F). Following the previous classification, 22 (n = 22, 100 %) bats showed cutaneous lesions derived from the use of forearm rings, of which six (27.3 %) had at least one mild lesion (Stage 1), 12 (54.5 %) presented at least one moderate lesion (Stage 2), and four (18.2 %) showed at least one severe lesion (Stage 3). All cutaneous lesions (Table A2, Appendix A) were treated as needed and monitored until their resolution. We examined all forearm rings (n = 22) after their removal and recorded any signs of biting. Sixteen (72,7 %) forearm rings showed clear marks of bites (Fig. A3, Appendix A).

All tags were read correctly by the microchip scanner and found by palpation and skin transparency between the shoulder blades (Fig. A4, Appendix A) at the end of the study. None of the PIT-tagged bats (n = 21, 0 %) showed cutaneous lesions by the end of the study. Bats marked with forearm rings presented a significantly higher frequency of lesions than PIT-tagged bats (p-value < 0.0001).

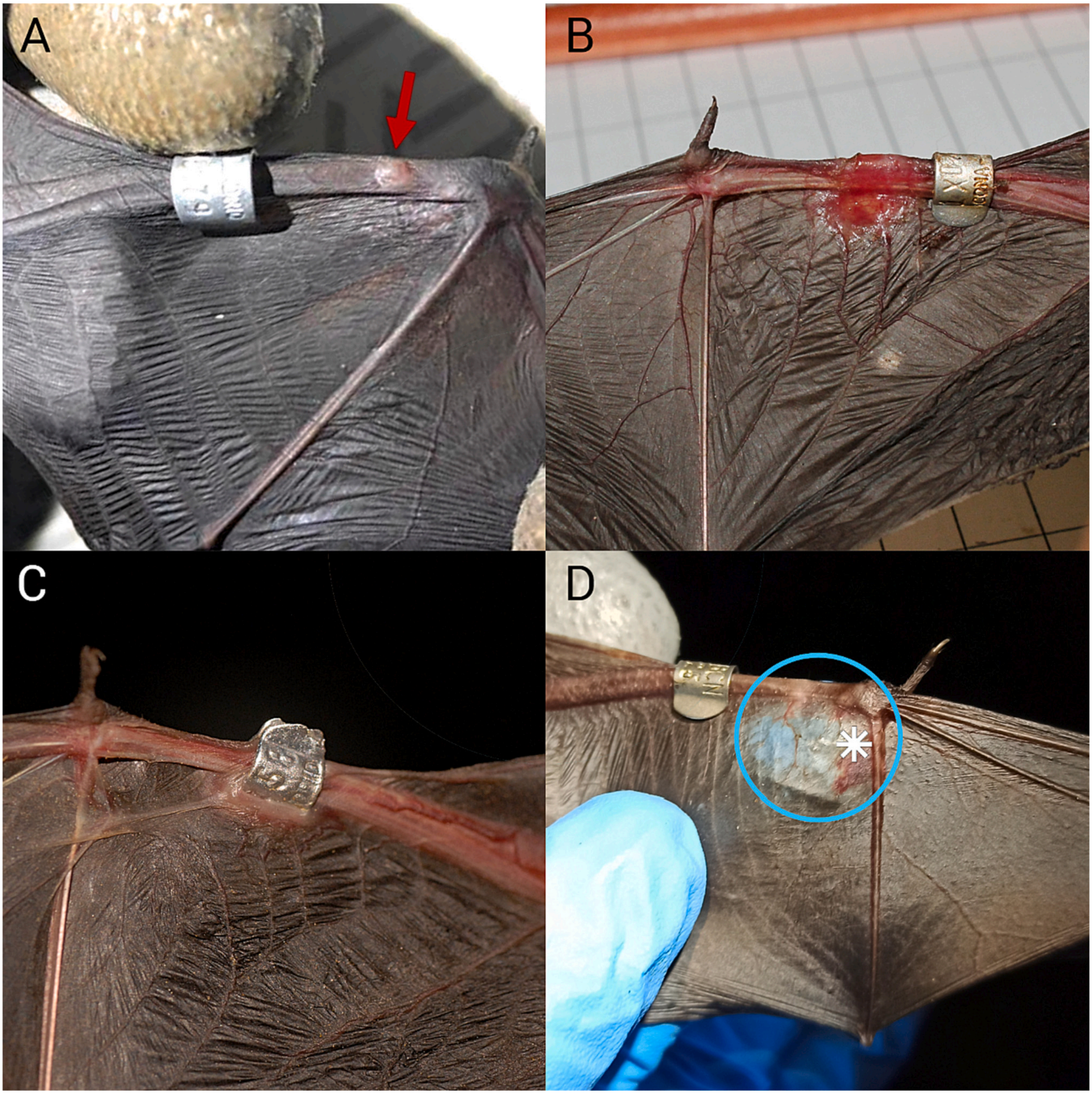

Fig. 2. Skin lesions found in free-ranging bats recovered with forearm rings in Catalonia. A. Callus formation (red arrow) in the dorsal aspect of the forearm of a *Nyctalus leisleri*. B. Ulcerated callus found in the forearm of a *Pipistrellus pygmaeus*. C. Marks of ring bite and ring embedding in the forearm of a *Pipistrellus pygmaeus*. D. Extended area of skin thinning and depigmentation (encircled), and an erythematous plaque (white star), in the distal carp of a banded *Myotis daubentonii*.

Table 2
Summary of the logistic regression model with presence of lesion as the response and, species and reproductive state as predictors. The coefficients of the model are presented in terms of odds ratio along with their 95 % confidence interval. *Miniopterus schreibersii* and active reproductive state are the reference categories for species and reproductive state, respectively. Statistically significant values are indicated with *.

Variable	OR	95%CI	p-Value	
Intercept	72.87	[14.31, 1342.91]	0.0000	*
Nyctalus leisleri	0.97	[0.15, 18.77]	0.9763	
Pipistrellus pygmaeus	0.38	[0.20, 0.71]	0.0026	*
Passive reproductive state	0.09	[0.00, 0.43]	0.0182	*

3.2.2. Behavioural changes and levels of cortisol metabolites in guano

We observed some of the banded bats biting the ring (Supplementary Video C1, Appendix C) and scratching it with their hindlimbs in an attempt to remove it (Supplementary Video C2, Appendix C). In contrast, no behaviours indicative of tagging-related discomfort were observed in PIT-tagged bats.

A statistically significant difference between the levels of CG before and after the intervention (Table B1, Appendix B) was found for group 1 (forearm ring, p-value = 0.013). In the remaining groups, the differences were not statistically significant (Fig. B1, Appendix B).

4. Discussion

Decades of individually marking bats with forearm rings have helped

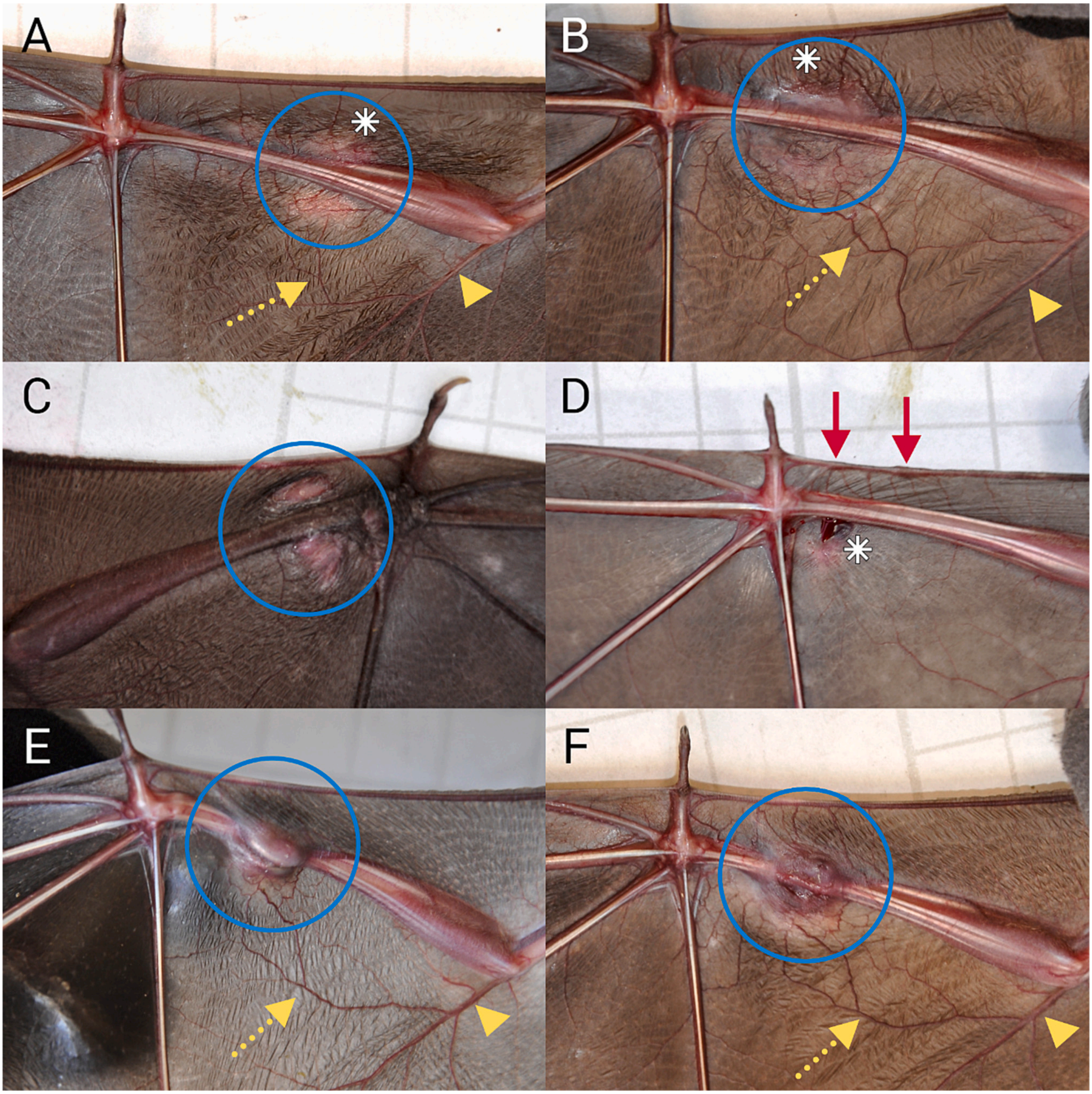

Fig. 3. Skin lesions found in captive *Carollia perspicillata* due to marking with forearm rings. Areas of neovascularisation are indicated with yellow dashed arrows, and their respective brachial veins marked with arrow heads. A Skin depigmentation and thinning, dorsally and ventrally to the radius and ulna (encircled). An area of erythema is indicated with a white star. B. Skin depigmentation and thinning, dorsally and ventrally to the radius and ulna (encircled). An erythematous plaque is indicated with a white star. C. Dorsal cutaneous erosions, dorsally and ventrally to the radius and ulna (encircled). D. Cutaneous ulcer ventrally to the radius and ulna with active bleeding (white star) manifested after the forearm ring removal. Additionally, two punctiform erosions (red arrows) were present on the edges of the propatagium. E. Forearm ring displaced from the propatagium, firmly closed, and embedded in the skin (encircled). F. The same individual than E, after the forearm ring removal and disinfection of the wound. The subcutis and muscles from the radius and ulna were exposed and the cutaneous tissue around the ulcer was swollen and erythematous (encircled).

us to understand many aspects of their ecology and biology (Ellison, 2008; Hutterer et al., 2005). However, the proportion of recovered bats is usually <1 % (Steffens et al., 2007), except for studies that work in roosting sites with high-fidelity species (Gaisler and Chytil, 2002). Therefore, recapture data accuracy relies on capturing and marking a relatively large proportion of the population (Ellison, 2008; Hutterer et al., 2005).

Knowledge of the physical impact of forearm rings has been obtained by banding reports (Baker et al., 2001; Ellison, 2008; Hutterer et al., 2005; Steffens et al., 2007), systematic studies in the wild (Mellado et al., 2022; Zambelli et al., 2009), and other studies using bat bands whose authors noticed negative associated consequences and decided to report them (Balmori and Quetglas, 2000; Dietz et al., 2006; O'Shea et al., 2004; Sendor and Simon, 2003). Our study confirms the health impact of forearm rings in free-ranging bats, showing that a high percentage (55.1 %) of bats recaptured in Catalonia over the last decade had injuries derived from this marking tool. Moreover, considering the overall low recapture rate in bat mark-recapture studies, we must be cautious in extrapolating the results to the whole population of bats. For instance, it is likely that some of the non-recaptured bats had suffered severe injuries from marking tools and died. This assumption is reinforced by the high percentages of *C. perspicillata* from our study in captivity that presented moderate (54.5 %) and severe (18.2 %) lesions derived from the use of forearm rings.

Factors reported to affect the level of injury caused by forearm rings are the type of metal used, the accuracy of banders when placing the ring and the size of the ring (Baker et al., 2001; Dietz et al., 2006; Zambelli et al., 2009). Zambelli et al. (2009) reported skin lesions caused by forearm rings in *N. leisleri*. They concluded that an incorrect band size was the most likely reason for the injuries and suggested the use of slightly larger band sizes in case of doubt or in the absence of species-specific recommendations. Although we used a larger band size for *N. leisleri* bats, we still detected a high injury rate (53.3 %), even larger than the 11.2 % reported by Zambelli et al. (2009). Furthermore, certain morphological factors, such as the shape and width of the propatagium, have been targeted as potential defining features for sensibility to bands (Dietz et al., 2006). However, these factors have not been accurately validated for European bats using long-term studies under captivity conditions and conclusions were based on field observations. Notably, a high injury rate was reported by Dietz et al. (2006) in banded *Rhinolophus ferrumequinum*, *R. euryale* and *R. mehelyi*, regardless of the size of the band or the existence of well-established recommendations. The authors of the study concluded that the higher incidence of injuries in these three species compared with those of banded vespertilionids resulted from increased ring rubbing along the frontal margin of the propatagium due to a wider propatagium. In our study, only vespertilionid species were assessed, but still, we found a concerning proportion of injured bats. Since some of them are classified as cave-roosting species (e.g., *M. schreibersii*) and others as crevice-dwelling species (e.g., *P. pygmaeus*), a larger impact was expected to be found in the latter (Palomo et al., 2007). However, when the proportions of skin lesions were compared among our bat species, we found three species, *N. leisleri*, *P. pygmaeus* and *M. schreibersii*, exhibiting significantly worse responses to bands than the rest. For some of the species evaluated the sample sizes were really small, so larger studies are needed. The logistic regression model accounting for the effect of sex and reproductive state showed that *P. pygmaeus*, our smallest crevice-dwelling species assessed, was 62 % less likely to develop skin lesions due to forearm rings than *M. schreibersii*. This result contrasts with the lack of a statistically significant difference between the two species in the study using the complete dataset. This contrast can be attributed to the different methodologies employed and/or differences between the complete dataset and the subpopulation used in the logistic regression model, involving factors that influence the risk of developing lesions. A comprehensive understanding of the factors and variables influencing the development of skin lesions is essential for a nuanced interpretation of results. Future studies should incorporate larger datasets with a comprehensive range of variables. Unlike males, female *N. leisleri* exhibit long-distance migratory behaviour (Tomás Alcalde et al., 2013), a trait that was previously assumed to cause worse consequences than sedentary habits. However, our results showed that sex does not seem to have a significant effect on the probability of developing lesions. In contrast, we found that bats in an active reproductive state were more likely to develop skin lesions than those in a passive state. All these findings emphasize that forearm rings have a significant impact on the health of insectivorous bats, regardless of their ecology, size, or sex. Furthermore, our findings highlight that during the reproductive season, bats may be particularly vulnerable to banding activities. Skin lesion development due to forearm rings may be influenced by specific reproductive behaviour, hormonal fluctuations, immune system changes, or energy allocation during the reproductive season (Ruoss et al., 2019). Bats have a low annual reproductive output, compensated by a significantly longer life span than other mammal species (O'Shea et al., 2004). Accordingly, any reduction in their reproductive success resulting from the consequences of being banded may risk the bat population's survival (Davy et al., 2022; Schorcht et al., 2009). Overall, we found much larger proportions of lesions compared to previous studies involving *P. pygmaeus* (Dietz et al., 2006; Sendor and Simon, 2003), *M. schreibersii* (Baker et al., 2001) and *N. leisleri* (Zambelli et al., 2009). Variable dimensions of injury perception may lead to different conclusions between researchers, particularly if their studies omit to report injuries. However, from a welfare point of view, no skin lesion regardless of its severity should be neglected but recognised as a source of physical discomfort to bats.

Carollia perspicillata is one of the most studied bat species in the laboratory and other captive settings due to its adaptability to captivity. Furthermore, it is an abundant species in its indigenous habitat, so it is also widely used in ecological studies (Rasweiler IV et al., 2009). Because these studies may need to identify the bats individually, several externally attached tools have been employed, including forearm bands (Alviz and Pérez-torres, 2020; Mellado et al., 2022; Monteiro et al., 2019; Sánchez et al., 2007), plastic splits (Knörnschild et al., 2013; Rex et al., 2011), and collars (Mellado et al., 2022; Monteiro et al., 2019; Rodríguez-Posada and Santa-Sepúlveda, 2013). However, despite the large body of scientific studies where the individual marking of *C. perspicillata* is witnessed, there is often a lack of information regarding the design or manufacturer reference (Alviz and Pérez-torres, 2020; Sánchez et al., 2007) and size (Alviz and Pérez-torres, 2020; Monteiro et al., 2019; Rex et al., 2011); and their physical impact or absence of injuries is hardly ever reported. All these factors significantly hinder making an optimal choice in marking tools for *C. perspicillata* studies. Similarly to Dietz et al. (2006), our study with captive *C. perspicillata* suggests that a wider propatagium may interfere with a proper slide of the band along the forearm. Slightly splitting the plagiopatagium and passing the ring through it has been used as an alternative for bats with large propatagia, such as *C. perspicillata* (Monteiro et al., 2019). However, this marking method is highly harmful, as bats are essentially left with an open wound, susceptible to bacterial colonisation. Recently, Mellado et al. (2022) reported a significant occurrence of skin lesions in *C. perspicillata* resulting from this method. The healing process (inflammatory, proliferative and remodeling phases) may be prolonged and easily complicated when splitting the plagiopatagium, as friction between the wound edges and the ring is expected to be maintained as long as the ring is fitted (Schultz et al., 2011).

In our study with *C. perspicillata*, we found a significant difference in the levels of CG between the adaptation and the marking period in group 1, which was marked with forearm rings. However, the higher levels of CG after the marking period cannot be explained by the marking tool alone because bats from group 2 (also marked with forearm rings) did not show the same tendency as group 1. Because cortisol secretion is a dynamic process (Busch and Hayward, 2009) and is individually modulated by intrinsic factors such as age, reproductive state, or social status (Palme, 2019), many factors may have jeopardised correct data interpretation of the pooled group samples. We collected all faecal samples at the same hour (in the morning) and CG in each sample represented an average of the defaecating period (12 h). During this period, different stressful events, such as social disputes, may have randomly happened. Moreover, the temporal stability of cortisol metabolites shows significant variations among species (Donini et al., 2022). For instance, the fast intestinal transit of *C. perspicillata* (Laska, 1990), coupled with differences in time deposition of faeces or low stability of cortisol metabolites after deposition, could explain the absence of correlation in the remaining groups (Donini et al., 2022). Therefore, as a first step, we recommend investigating the species-specific stability of cortisol metabolites in faeces before any physiological study is performed. Additionally, increasing the sample size and testing cortisol metabolites levels individually -if possible- may be helpful to ameliorate the problem of high data variance in longitudinal studies where cortisol changes in relation to an event are evaluated.

Remarkably, there is no upper limit on the acceptable amount of physical damage and pain that bats may experience due to marking tools. EUROBATS recognises that banding bats is an invasive procedure that can seriously harm or even kill the bat if performed incorrectly. While recommendations of ring sizes by species are given, the Agreement also states that sensitivity to bands may vary between species and also between individuals of the same species or population (UNEP/EUROBATS, 2018). Nonetheless, the decision of banding or not banding bats rests on the researcher, as the establishment of a tolerable damage limit does. If we would follow the recommendation of Baker et al. (2001) to discontinue banding bats if injury rates surpass 2 %, banding activities would need to be terminated in most, if not all, insectivorous bat species. However, clinging to the principles of ethical research with animal subjects (*Ethical Guidelines for the Use of Animals in Research,* 2018), bat banding activities continue regardless of the evidence of physical injuries because of the expected benefit obtained by this technique (UNEP/EUROBATS, 2018). However, assumptions from these activities such as that marks are not lost with time or do not interfere with survival may introduce significant biases in bat population studies. Furthermore, it is imperative that researchers minimise the risk of disturbance and suffering of bats and be responsible for considering options that may improve the animal's welfare while still obtaining the same benefit from the research (*Ethical Guidelines for the Use of Animals in Research,* 2018; UNEP/EUROBATS, 2018). In our results and the reviewed literature, the band injury rate for several species exceeded 10 % (Baker et al., 2001; Dietz et al., 2006; Mellado et al., 2022; Zambelli et al., 2009), which points to considering other ethically acceptable alternatives.

In contrast, recent studies using PIT tags in free-ranging bats have proved to be suitable for individual recognition (Locatelli et al., 2019; van Harten et al., 2020), assessing bat movements (van Harten et al., 2019; van Harten et al., 2022a), reproductive success (Rigby et al., 2012), body condition (Locatelli et al., 2019; Rigby et al., 2012), and survival estimates (Ellison et al., 2007; van Harten et al., 2022b). This method has several advantages in comparison with the use of other traditional and long-term marking techniques. For instance, it provides higher encounter probabilities with no handling or physical disturbance for species roosting in caves or bat boxes (Godinho et al., 2015; van Harten et al., 2019; van Harten et al., 2022b). Furthermore, there is no evidence linked to detrimental effects on bats' health, reproductive or survival success (Edmonds et al., 2017; Locatelli et al., 2019; Rigby et al., 2012; van Harten et al., 2020). This is supported by the results obtained in the present study. The use of a sterilised small-sized PIT tag injected subcutaneously in the dorsum caused no physical impact in any of our captive *C. perspicillata* bats. Four free-ranging vespertilionid species from Catalonia, including *P. pygmaeus*, whose body size does not exceed 4 g, neither showed lesions associated with this marking tool. The present study highlights the potential of this technique to be used in bat species regardless of their morphological differences or body size. Nevertheless, appropriate theoretical and practical training is crucial before implementing any marking method.

5. Conclusions

Due to the high injury rates found in free-ranging and captive bats, banding activities are at odds with conservation goals and call into question the scientific value of recapture data obtained with this method. Our study concludes that the use of forearm rings should be banned under the 'precautionary principle' for all bat species until systematic studies under controlled conditions are performed. Particularly, efforts should be made to include indicators of indirect effects such as changes in behaviour or reduced fitness. While decisions on marking tools for bat species must be on a case-by-case basis, new technological advances may provide efficient solutions and tools, such as PIT tags, to achieve multi-species tolerance.

Better coordination at the international level among institutions is needed to implement evidence-based strategies on bat research without compromising the provision and exchange of information, particularly for migratory bat species. Decisions on the acceptable rate of injuries derived from marking tools should be standardised following ethical principles and evaluated by competent authorities and recognised institutions. We recommend that policymakers restrict the authorisation of the use of forearm rings in bat species until adequate studies to evaluate their impact have been performed under controlled conditions. In any case, studies carrying out marking activities on bats should be submitted to a legally constituted ethics committee for consideration, guidance, and approval.

CRediT authorship contribution statement

Lourdes Lobato-Bailón: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Data curation, Writing – original draft, Writing – review & editing. **Adrià López-Baucells:** Conceptualization, Methodology, Investigation, Supervision, Data curation, Writing – review & editing. **David Guixé:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Carles Flaquer:** Investigation, Data curation, Writing – review & editing. **Jordi Camprodon:** Investigation, Data curation, Writing – review & editing. **Xavier Florensa-Rius:** Investigation, Data curation, Writing – review & editing. **Maria Mas:** Investigation, Data curation, Writing – review & editing. **Laura Torrent:** Investigation, Data curation, Writing – review & editing. **Laura Ordeix:** Investigation, Writing – review & editing. **Oriol Tallo-Parra:** Supervision, Writing – review & editing. **Maria P. Ribas:** Investigation, Writing – review & editing. **Ignasi Marco:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Annaïs Carvajal:** Formal analysis, Writing – review & editing. **Manel López-Bejar:** Formal analysis, Supervision, Writing – review & editing. **Sebastian Napp:** Formal analysis, Writing – review & editing. **Lola Pailler-García:** Formal analysis, Writing – review & editing. **Johan Espunyes:** Investigation, Writing – review & editing. **Oscar Cabezón:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2023.110268.

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