Marking Great Tit *Parus major* nestlings: identifying sources of paint loss and assessing an effective marking effort

Javier Quesada & Juan Carlos Senar

The aim of this study was (i) to evaluate which variables determine the disappearance of paint marks on chicks of the Great Tit *Parus major*, (ii) to provide a predictive tool for designing marking experiments and (iii) to assess the negative consequences of such techniques on the nestlings of similar-size passerines. We marked the toenails, tarsi and wings of Great Tit nestlings with a new type of paint. We first evaluated which part of the body (toenail, tarsus or wing) retained most paint and then which other factors were correlated to paint loss. To assess the invasiveness of the marking method, we analyzed the differences in weight and in the proportion of fledglings between marked and unmarked chicks from the same nests, and between manipulated and control nests. Results showed that the best place to mark nestlings was the toenails, followed by wings and the tarsi. For toenails, only nestling age and time elapsed since the last marking affected paint permanence. Although nine- or 16-day-old nestlings retain markings for over a week, two-day-old nestlings should be re-marked within two days. We found no deleterious effects of paint on body weight or brood survival. In order to improve useful marking effort, studies in which nestlings have to be marked should therefore take into account the differences in marking permanence between different body parts and variation due to nestling age.

Key words: Great Tit, *Parus major,* nestling marking, nestling age, multivariate analysis, predictive marking model

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Received: 24.04.12; Accepted: 17.09.12 / Edited by P. Laiolo

In most studies in behavioural ecology there is a need to recognize individually the different subjects under study (Lebreton & North 1993). Numerous techniques have been used for marking birds, from coloured rings (metal or plastic), tags applied to the back, wings or necks, collars, nasal marks, colour dyes or paints, to radio transmitters and electronics tags (North 1969, Marion & Shamis 1977, Lehner 1979, Calvo & Furness 1992, González-Solís *et al.* 2000, Nicolaus *et al.* 2008, Bonter & Bridge 2011).

The individual identification of chicks is essential for early-age studies such as partial cross-fostering experiments (Roulin *et al.* 1999, 2000) or studies on begging behavior (Kölliker *et al.* 1998, Redondo 2000). In these cases, most of the marking techniques used with adult birds are ill-suited for the recognition of individual altricial nestlings. For instance, metal or coloured rings are widely used in chick recognition, but cannot be used until the chick has reached a minimum age; otherwise the rings are liable to fall off or chicks' feet may be damaged by the ring as they grow (Pinilla 2000). Consequently, a number of alternative methods have been described. Harper & Neill (1990) developed a successful technique

whereby chicks were ringed with elastic rings. Velcro leg-tags have also been used for gull chicks (Willsteed & Fetterolf 1986) and Brewer's Blackbirds *Euphagus cyanocephalus* (Balph 1975). Fluorescent powder applied to feathers has also been used in precocial birds (Steketee & Robinson 1995), although this procedure is presumably not suitable for altricial species.

Some of these methods, however, may be difficult to apply in very small passerine species due to the small size of recently hatched chicks. In these species, one of the most widely applied techniques is the use of dyes and paints on different parts of the body including the claws (Oniki 1981), head (Roulin *et al.* 1999, 2000, Buechler *et al.* 2002) and legs (Balph 1975, Redondo 2000).

However, individualizing with paint requires the re-marking of each chick several times during their development since, otherwise, the original markings are lost. The loss of marks in altricial nestlings are presumably related to: 1) environmental conditions such as temperature, which can alter the properties of the paint; 2) nestling behaviour in the nest since friction between chicks could speed up paint loss; 3) the dermal renovation of the skin. Moreover, different body parts may lose or retain marks at different rates due to different amounts of in-nest chafing and friction. For instance, we would expect differences in the speed of mark loss between marks on naked skin (epithelium) and on nails or feathers (keratin). More exact knowledge of the most important parameters causing marks to disappear could save research time and money, make marking efforts more efficient and avoid causing nestlings unnecessary stress due to 'over-visiting'.

Despite the number of published studies on dyeing or painting techniques in birds, surprisingly little work has been done to evaluate its efficiency or the possible deleterious consequences of marking animals (Calvo & Furness 1992, Gaunt & Oring 1999, Murray & Fuller 2000).

The aims of this study were (i) to evaluate which factors determine the disappearance of the paint applied to the chicks of the Great Tit, a small altricial passerine species, (ii) to provide a predictive tool that will be useful in the design of marking experiments and (iii) to assess the possible negative consequences of the use of such a technique on nestlings.

Material and methods

The study was carried out during the 2003 breeding season on a population of Great Tits at the field station at Can Catà (Barcelona, NE Spain) in a forest dominated by oaks and pines [for more details see Senar *et al.* (1997)]. The Great Tit is an altricial species weighing 17 g that breeds in cavities but also readily nests in nest-boxes (Gosler 1993, Cramp 1998). In our study population females lay 3–11 eggs (authors' unpublished data).

Marking experiment

A total of 171 nest-boxes distributed throughout the study area were visited every four days in order to obtain data on the reproductive status of birds (nest building and laying and hatching dates). On day 1–3 post-hatching (taken as day 1), half of the chicks of 48 pairs were randomly marked as soon as possible with red paint (Eco-marker®, Germany). When there was an odd number of chicks we always marked the additional unpaired chick. Eco-marker® paint is used in the construction industry; it is particularly durable in harsh weather conditions, dries very quickly and is sticky, three reasons that make it a good candidate for marking birds. Nestlings (N_{nests} = 48) were marked from day 1–3 on the toenails of a single foot, on one tarsus and on one wing (above the carpus). Unlike the head, these body parts are not (or only very poorly) visible to parents and so the chance of parents reacting abnormally to marked chicks was lessened.

We evaluated the permanence of the paint on the chicks each time nests were revisited (2–7 days) by establishing an index of mark permanence, expressed in the form of a qualitative code: 0= No evidence of mark; 1= Few spots (remainders) of red paint still visible; 2= Mark obviously smaller but still clearly visible; 3= Mark practically intact. Although this index is qualitative, it does express a continuum of variation of increasing paint permanence. For this reason, we considered that we could calculate its mean value and perform regressions (see below) as if it were a quantitative variable.

We considered the mean index value of each brood to avoid any possible pseudoreplication effect. The same observer (JQ) obtained all the data. In order to avoid a possible effect of

observer manipulation, both marked and unmarked nestlings were removed from the nest each time the broods were marked. Broods were re-visited and re-marked every 2–7 days during the nestling phase. We randomly considered only one 're-marking event' (which implies marking, re-visiting and evaluation) per nest in order to maintain the independence of the observations in the sample.

The evaluation of the deleterious effects of marking

In order to evaluate any possible deleterious effects of paint on nestlings, we examined chicks every time that the nests were revisited and noted any visible anomalies. Furthermore, and in order to evaluate any possible negative effects on nestling survival, we analyzed whether or not there were any differences in the weight (as a surrogate of body condition) of marked and unmarked chicks. Birds were weighed with a pocket balance (Tanita®, Japan) on day 16 and the mean weight for each group was used in the analyses. In addition, we tested whether there were any differences between the percentage of marked and unmarked fledglings in the same nest (% Marked fledged Nestlings = [$No._{\text{marked fledged nestlings}}/No._{\text{initially marked nestling}}$] x 100, the same formula being used for non-marked nestlings). We also evaluated an external control by comparing our sample with 14 broods that were not manipulated.

Statistical analysis

To evaluate the variables that influence marking loss we first conducted a Repeated Measures ANOVA where the dependent variables (levels) were the values (0–3) of the permanence of paint on toenails (P_{nail}), tarsi (P_{tarsus}) and wings (P_{wing}). Combined with a Newman-Keuls Post-hoc analysis, this ANOVA enabled us to determine which part of the body retained paint the most effectively. Afterwards, we focused on which part of the body retained the greatest amount of paint cover. We then conducted a multiple stepwise backward regression whereby 'the paint permanence of this selected body part' (the average index of permanence per brood) was the dependent variable, and the following variables were the predictors: 'Hatching date' (1= 1st April), as an indicator of environmental conditions (given that temperature increases in the breeding season), 'Brood size', as the number of nestlings in the nest when chicks were marked, 'Nestling age' (in days), which is related to dermal renovation and chick mobility, and 'Time of re-marking', as the time elapsed since the last marking. We then used a multiple regression model to explore how paint retention evolved by studying predicted values when predictors changed. We were particularly interested in seeing how the paint was lost at different nestling ages in relation to the time elapsed between each visit. We considered three nestling ages in which chick behaviour (e.g. mobility) in the nest changes clearly (Redondo 1991) and may have an effect on the rubbing of body parts. These three ages were 2-, 9- and 16-days old out of a total nestling period of 19–22 days (Cramp 1998).

We used paired-*t* tests to examine differences in weight between marked and unmarked fledglings (at 16-days old), and between manipulated and control nests. We also evaluated differences in survival between marked and unmarked nestlings by means of a Wilcoxon Matched Pairs Tests. Mean and standard deviations are given; we used two-tailed tests with a significance level of $\alpha = 0.05$.

Results

Variables affecting paint disappearance

Paint permanence depended on the area of the body on which the paint was applied and the best place to mark altricial chicks was found to be the toenails followed by the wings and tarsi (Figure 1) (Repeated measures ANOVA $F_{2,94} = 57.4$, $P < 0.001$; $n = 48$). A Newman-Keuls post-hoc analysis showed that all of the body locations differed significantly from each other (see Figure 1). Marked nails retained high visibility on subsequent visits (P_{nail}: mean index ± SD = 2.26 ± 0.75), while the other body parts only had the remainders of paint marks or no signs of the paint at all (P_{tarsus}: mean index ± SD = 0.98 ± 1.00; P_{wing}: mean index ± SD = 1.26 ± 1.10). It is worth noting that an index value of less than 1 means that at least one chick had lost its paint marks. Thus, we focused on P_{nail}

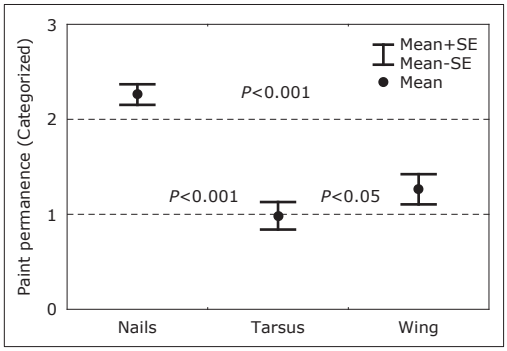

Figure 1. Differences in paint retention between different nestling body parts that were marked. Toenails are the best candidate location for marking nestlings (points and whiskers: mean index value ± SE). P values refer to one-way ANOVAs comparing retention for different body parts: differences between toenails and wings, and between toenails and tarsi: P<0.001; differences between tarsi and wings: P<0.05.
Diferències en la retenció de la pintura entre les diferents parts del cos dels polls que van ser marcats. Les ungles són el millor lloc de marcatge per als polls (punts i bigotis: valor índex mitjà ± ES). Els valors de p es refereixen a ANOVAS unidireccionals que comparen retenció per diferents parts del cos: les diferències entre ungles dels peus i les ales, i entre les ungles dels peus i tarsos: P <0,001; diferències entre els tarsos i ales: P <0,05.

to analyse which variables affect paint loss and to develop our predictive model.

When looking at the effects of our explanatory variables on marking permanence on toenails we found that 'Nestling age' and the time elapsed after marking ('Time of re-marking') significantly affected marking permanence on toenails. However, we found no relationship between P_{nail} and 'Brood size' or 'Hatching date' (Table 1). The positive relationship between nestling age and paint permanence implied that the older the nestling is, the longer the paint mark remains intact.

In a further step, we analyzed how 'Nestling age' and 'Time of re-marking' influenced paint retention. Since our regression model only explained 50% of variance and in order to obtain conservative results, we also considered both the lower predicted value of the 95% likelihood interval and its mean value. In Figure 2 we define these two explorations as 'Standard' and 'Conservative prediction', respectively.

As Figure 2a shows, two-day-old nestlings should be re-marked within the next five days or, otherwise, they lose the paint marks as they continue to grow. The more conservative approach predicts that they should be re-marked within two days. However, when nestlings are 9- and 16-days old (Figure 2b,c) paint marks take longer to disappear and so re-marking sessions can be delayed for a week.

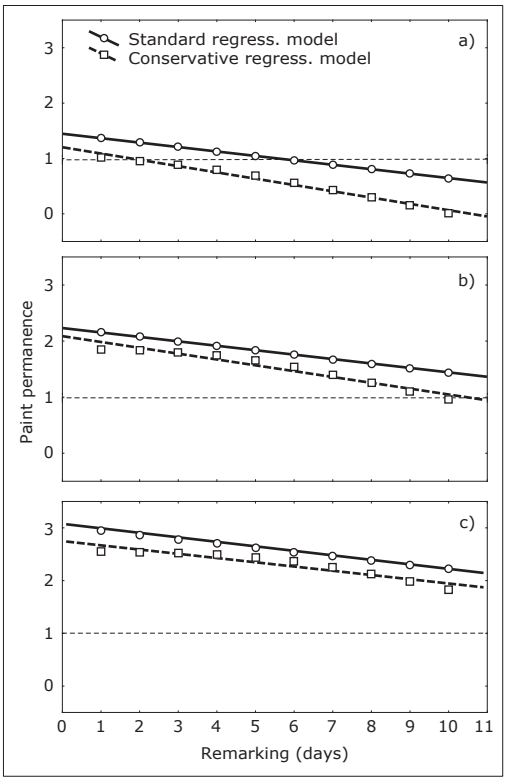

Figure 2. Plot depicting variation in the paint retention index after re-marking obtained using a multiple regression model that explores how the time elapsed before the next marking (in days) influences the loss of paint from the nestling's toenails at different ages (2, 9, 16 days). The continuous line represents the fitted line according to model (Standard model), while the dotted line shows the lowest predicted value (95% likelihood interval) (Conservative model). A value of < 1 implies that the marking may have disappeared in at least one chick.
Representació gràfica que mostra la variació en l'índex de retenció de pintura després de tornar a marcar, obtingut utilitzant un model de regressió múltiple que explora com el temps transcorregut abans de la marca (en dies) influeix en la pèrdua de la pintura d'ungles dels peus dels polls en diferents edats (2, 9, 16 dies). La línia contínua representa la línia ajustada segons el model (model estàndard), mentre que la línia de punts mostra el menor valor previst (95% interval de probabilitat) (model conservador). Un valor de <1 implica que el marcatge pot haver desaparegut en almenys un poll.

Table 1. Multiple regression analysis testing for variation in the paint permanence index on toenails in relation to hatching date, nesting age, time of remarking and brood size.
Anàlisi de regressió múltiple per testar la variació en l'índex de permanència de la pintura en les ungles dels peus en relació amb la data d'eclosió, l'edat dels polls, el temps de remarcatge i mida de la llocada.

Multiple backward regression / *Regressió multiple pas a pas enrere*					
Dependent var: Permanence of paint on toenails (P_{nail}) / *Var. dependent: Permanència de la pintura a les ungles dels peus.*					
	β	B	B- S.E.	t_{48}	*P*-level
r =0.72; r^2 = 0.52; P < 0.0001; n = 48					
Initial model / *Model inicial*					
Intercept / *Interceptar*		0.98	0.43	2.3	0.02
Hatching date / *Data d'eclosió*	0.20	0.02	0.01	1.2	0.22
Nestling age / *Edat dels poll*	0.63	0.09	0.02	3.7	<0.001
Time of remarking / *Temps de remarcatge*	-0.23	-0.08	0.04	-2.1	0.04
Brood size / *Mida de posta*	-0.03	-0.02	0.05	-0.3	0.78
R=0.71; R^2=0.50; p<0.0001					
Final model (step 3) / *Model final (pas 3)*					
Intercept / *Interceptar*		1.22	0.21	5.8	<0.001
Nestling age / *Edat dels polls*	0.78	0.11	0.02	7.1	<0.001
Time of remarking / *Temps de remarcatge*	-0.23	-0.08	0.04	-2.1	0.04

The deleterious effects of paint on chicks

We detected no deleterious effects of paint on either body condition or chick survival into the nest. The body mass of marked and unmarked nestlings was similar (Paired t-test: t_{30} = -0.14, P = 0.89; n = 32; $mean_{Marked}$ ± SD = 15.64 ± 1.55 g; $mean_{Non\text{-}marked}$ ± SD = 15.68 ± 1.63 g); likewise, the mean weight of chicks from the manipulated nests did not differ from that of chicks from the untouched nests (control) (Unpaired t-test: t_{45} = -1.35, P = 0.18; mean $_{Manipulated\ nests}$ ± SD = 15.62 ± 1.42 g $n_{Manipulated\ nests}$ = 32; $mean_{Control\ nests}$ ± SD = 15.99± 1.39 g; $n_{Control\ nests}$ = 14). We found no differences between the percentage of marked or unmarked fledglings that left the nest (Wilcoxon Matched Pairs Test: T=97.50, Z = 0.28, P = 0.78; n = 31; $median_{Marked\ fledglings}$ [25-75% quartiles] = 75.00; [66.00-100.00]; $median_{Non\text{-}marked\ fledglings}$ [25-75% quartiles] = 100.00 [60.00-100.00]. Neither did we find any differences between the percentage of nestlings fledged in manipulated and in control nests (U- Mann-Whitney: U=289.00; Z = -0,17, P = 0.86; $median_{manipulated\ nest}$ [25-75% quartiles] = 71.42; [0.00-88.23], $n_{manipulated\ nests}$ = 47; median $_{control}$ [25-75% quartiles] = 62.50 [50.00-75.00]; $n_{Control\ nests}$ = 13)

Nevertheless, we did observe slight damage to four chicks caused by the marking. When the chicks were young, the stickiness of the paint caused toes to stick together such that a small deformation appeared when nestlings were older. This problem could, however, be easily solved by separating chicks' toes when they are marked and by leaving the paint to dry before placing the nestlings back in the nest.

Discussion

The effective and long-lasting marking of nestlings is a critical part of many ecological and behavioural studies. Our study shows that the correct choice of body part to be marked is crucial in ensuring that the marking period is effective and enduring and thus must be taken into account in experiment design.

Here we first evaluated which parameters affect mark retention in Great Tit nestlings through the use of a new type of paint. The predictive model we obtained allowed us to evaluate which variables determined mark disappearance

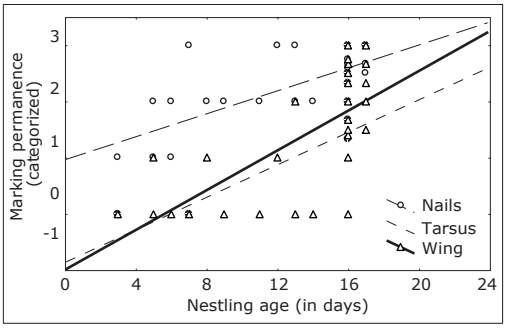

Figure 3. Paint permanence on different parts of nestlings' bodies in relation to nestling age (in days). A value of < 1 implies that the marking may have disappeared in at least one chick.
Permanència de la pintura en diferents parts del cos dels polls "en relació amb l'edat dels polls (en dies). Un valor de <1 implica que el marcatge pot haver desaparegut en almenys un poll.

and when marking should be repeated. Our data suggest that marking toenails – as opposed to any other body part – is the best way of increasing marking efficiency. Paint was retained longer in our marked nestlings when we marked toenails than when we marked tarsi or wings. Unlike skin, nails are not renewed and grow continuously and so marks remain on the young birds. Tarsi and wings are poor candidate surfaces for marking, above all when chicks are young (Figure 3). Great Tit chicks hatch with naked tarsi and wings and the continuous renovation of the skin in these body parts probably leads to a rapid loss of paint. Wings would seem to be a better location than tarsi because feathers appear in the middle of the nestling phase and retain the paint better than the tarsus, which is still naked at that point. This would explain why wings retain more paint than the tarsi as the chicks grow older (Figure 3).

Our multiple regression model suggests that marking effort (e.g. the frequency of re-marking) should be greater when nestlings are younger. However, we found no relationship between P_{nail} and 'Brood size' or 'Hatching date', possibly because in-nest friction between chicks did not affect the retention of dye; neither did the progress of the season influence paint disappearance. For small passerines with similar nestling development to Great Tits, we propose that recently hatched chicks should be repainted every two days (as a conservative criteria). However, when the chicks have reached an age of nine days or older, re-marking should be delayed for one week. Since the time span between re-marking depends on the developmental characteristics of the species and on the type of paint used, we suggest that similar studies should be carried out on other altricial species to confirm the pattern we have identified in our study.

In most animal marking studies it is generally assumed that marking has no effect on the individuals under experiment or that the effects are unappreciable (Murray & Fuller 2000). However, in spite of several warnings and recommendations, few attempts have ever been made to test for the possible deleterious effects of marking, either in with dyes or with other techniques (Calvo & Furness 1992, Gaunt & Oring 1999, Murray & Fuller 2000). Our results indicate that the paint we used is not harmful – provided it is used properly (see below) – and enables altricial birds to be marked safely. Since there was no negative or positive variation between the body mass of marked and unmarked nestlings, marking would appear not to alter parental feeding behaviour. However, we only tested short-term effects and potential long-term toxic effects on birds after fledglings were not taken into account.

Specifically, colour marks have been shown to influence conspecific behaviour (Frankel & Baskett 1963, Calvo & Furness 1992). For instance, red colour can attract an adult bird's attention when applied to chicks (Oniki 1981) and may therefore have an important effect on parental behaviour (Griggio *et al.* 2009). Therefore, we believe that it is advisable not to paint exposed feathers or the nestlings' heads in order to avoid any alteration to parental care behaviour. Given that birds – but not humans – can see UV, particular attention should be paid to this aspect. In fact, it has been recently demonstrated that chicks' UV coloration may alter parental feeding allocation (Galvan *et al.* 2008, Parejo *et al.* 2010). For these reasons and as a precautionary measure, it is advisable to use paint and dyes on parts of the nestlings' bodies that are hidden so as to minimise possible abnormal parental behaviour.

In conclusion, we suggest that the best place to paint altricial nestlings to ensure that marks are visible for the longest period of time is the toenails. Re-marking should be done every two

days when chicks are recently hatched, although this time period can be extended to a week once chicks are nine-days old or older. We consider that this finding could be applied to birds that are similar in size to the Great Tit. We encourage ornithologists to perform similar studies on other species to establish a standard marking method and optimize marking effort, thereby saving time and money and avoiding unnecessary stress to chicks.

Acknowledgements

We would especially like to thank Cristina Cabrera for her assistance with the fieldwork and the Gil family for allowing us to work on their property. Marta Arenas kindly provided the paint and Lluïsa Arroyo helped us with the laboratory work. We also wish to thank two referees and Paola Laiolo for the comments that improved an earlier version of this manuscript. Research projects BOS 2003-09589 to JCS and FPI Grant FP2000-6439 to JQ from the Spanish Research Council, Ministerio de Ciencia y Tecnología, funded this work.

Resum

Marcatge de polls de Mallerenga Carbonera *Parus major*: identificació de les causes de la pèrdua de la pintura i avaluació de l'esforç efectiu de marcatge

L'objectiu d'aquest estudi és avaluar les variables que determinen la desaparició del marcatge físic amb pintura de polls de Mallerenga Carbonera *Parus major*, per proporcionar una eina predictiva de disseny d'experiments de marcatge i per avaluar les conseqüències perjudicials d'aquesta tècnica en polls de passeriformes de mida similar. Es van marcar polls de Mallerenga Carbonera amb una nova pintura a les ungles, tars i ala. En primer lloc, vam avaluar quina part del cos (ungles, tars i ala) conservava millor la pintura i vam estudiar quins factors es van correlacionar amb la pèrdua d'aquesta. Per avaluar els efectes deleteris del marcatge, es van analitzar diferències de pes i de proporció dels polls marcats i no marcats d'un mateix niu, i entre nius marcats i no marcats. El millor lloc per marcar van ser les ungles, seguit per l'ala i el tars. Per a les ungles, l'edat dels polls i el temps transcorregut des de l'últim marcatge afecta la permanència de la marca. Per evitar la pèrdua del marcatge, els polls de dos dies d'edat s'haurien de marcar abans de dos dies entre cada remarcatge, mentre que els polls de més edat (9 a 16 dies) poden mantenir la marca durant més d'una setmana. No es va trobar cap efecte deleteri de la pintura en el pes corporal o la supervivència dels polls. Els estudis en els quals s'han de marcar els polls, haurien de tenir en compte les diferències en la permanència de la marca entre les diferents parts del cos i l'edat dels polls per fer un millor esforç de marcatge.

Resumen

Marcaje de pollos de Carbonero Común *Parus major*: identificación de las causas de la pérdida de la pintura y evaluación del esfuerzo efectivo de marcaje

El objetivo de este estudio es evaluar las variables que determinan la desaparición del marcaje físico con pintura de pollos de Carbonero Común *Parus major*, para proporcionar una herramienta predictiva de diseño de experimentos de marcaje y para evaluar las consecuencias perjudiciales de esta técnica en pollos de paseriformes de tamaño similar. Se marcaron pollos de Carbonero Común en las uñas, tarso y ala con una nueva pintura. En primer lugar, evaluamos qué parte del cuerpo (uñas, tarso y ala) conservaba en mayor grado la pintura y estudiamos qué factores se correlacionaron con la pérdida de ésta. Para evaluar el perjuicio del marcaje, se analizaron diferencias de peso y de proporción de pollos marcados y no marcados de un mismo nido, y entre nidos marcados y no marcados. El mejor lugar para marcar fueron las uñas, seguido por el ala y el tarso. Para las uñas, y para evitar la pérdida del marcaje, los pollos de dos días de edad se deberían marcar antes de dos dias, mientras que pollos de mayor edad (9 a 16 días) pueden mantener la marca durante más de una semana. No se encontró ningún efecto deletéreo de la pintura en el peso corporal o la supervivencia de los pollos. Los estudios en los que se deben marcar pollos, deberían tener en cuenta las diferencias en la permanencia de la marca entre las diferentes partes del cuerpo y la edad de los pollos para realizar un mejor esfuerzo de marcaje.

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