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Effects of capture and video-recording on the behavior and breeding success of Great Tits in urban and forest habitats

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ABSTRACT. Behavioral research often involves capturing and video-recording birds, but these procedures may have undesired effects on the behavior of birds that have rarely been quantified. In addition, birds in urban and more natural areas may differ in their sensitivity to disturbance. We examined the possible effects of both capturing, weighing and measuring, and taking a blood sample, and the presence of video-cameras on the behavior of male and female Great Tits (*Parus major*) breeding in urban and forest habitats. Using a 2 × 2 block design, we compared the behavior and breeding success of parents that either were or were not captured on their nests a few days before behavioral observations, and of parents that either were or were not habituated to the presence of a concealed video-recorder mounted on nest boxes. We found no significant effects of habituation to the camera on bird behavior, but males captured in their nest boxes were more vigilant and hesitated longer before entering nest boxes, and also had slightly lower provisioning rates than males that had not been captured. Captured females also tended to be more vigilant than females that had not been captured, but their provisioning rates were not affected. Capturing males also influenced the behavior of their non-captured mates, but capturing females had no effect on the behavior of their non-captured mates. We found no difference in the effects of capture on Great Tits in urban and forest habitats, and our treatments also had no effect on the mass, size, and survival of nestlings until fledging. Our results suggest that, for Great Tits, being captured results in sex-dependent behavioral effects that can last for at least several days. As such, we suggest that the possibility of similar effects in other species of birds should be considered in behavioral studies where birds must be captured, and recommend either that behavioral data be collected before capturing birds or that all birds in a study should be captured and handled in a standardized way.

RESUMEN. **Efecto de la captura y la toma de videos en la conducta y éxito reproductivo de *Parus major* en hábitats urbanos y naturales**

Los estudios de conducta muchas veces incluyen la captura y la toma de video de aves. Pero estos procedimientos pudieran tener efectos, no deseados, en la conducta de las aves que raras veces es cuantificado. Además las aves en hábitats urbanos y áreas más naturales pudieran diferir en su susceptibilidad al disturbio. Examinamos el posible efecto de la captura, pesaje, toma de medidas y de muestras de sangre y la presencia de cámaras de video en la conducta de hembras y machos de *Parus major*, reproduciéndose en hábitats urbanos y naturales. Utilizando un diseño de "bloque" 2 × 2, comparamos la conducta y el éxito reproductivo de individuos que eran capturados o no capturados en sus nidos días antes de llevarse a cabo observaciones de conducta, y de individuos que estaban o no estaban habituados a la presencia de cámaras de video escondidas, pero montadas, en cajas de anidamiento. Encontramos que no hubo efecto significativo en la conducta de las aves habituadas a la cámara. Sin embargo los machos capturados en la caja donde anidaban fueron más cuidadosos y precavidos para entrar en sus cajas. Además se redujo, ligeramente, el número de viajes para llevar comida al nido, en comparación con machos que no fueron capturados. Las hembras capturadas también fueron más precavidas que las no capturadas, pero su tasa de llevar alimentos a los pichones no fue afectada. La captura de los machos también influyó en la conducta de su pareja, que no fue capturada, aunque no hubo efecto en la conducta, de la pareja, en hembras capturadas. No encontramos diferencias en el efecto de la captura de estas aves en ambientes urbanos y forestados. Nuestra manipulación no tuvo efecto en la masa, tamaño y sobrevivencia de los pichones hasta el momento de dejar el nido. Los resultados sugieren que el efecto de la captura, en la conducta de *Parus major*, depende del sexo del ave y que dicha conducta puede durar por varios días. A tales efectos advertimos que existe la posibilidad de efectos similares en otras especies

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de aves que debe considerarse en estudios de conducta en donde las aves sean capturadas. Recomendamos que los datos sobre conducta sean tomados previos a la captura de las aves, o que todas las aves en el estudio sean capturadas y manejadas en una forma estandarizada.

Key words: disturbance, handling, nest trapping, nestling provisioning, risk taking, video camera, vigilance

In ecological and behavioral research, capturing, measuring, and marking free-living animals is a common practice. When studying breeding birds, for instance, catching and marking specific individuals, e.g., parents of a certain brood, are often necessary. However, capturing individuals may have negative impacts, including injuries and increased mortality (Fair and Jones 2010), nest desertion (Kania 1996, Dubiec 2011, Cole et al. 2012), and stress-related responses such as hormonal, physiological, and behavioral changes (Duarte 2013) that could influence breeding success (Uher-Koch et al. 2015, Ledwoń et al. 2016). Some of these detrimental effects have received considerable attention. For example, Kania (1996) compiled examples of nest desertion by birds captured on nests for more than 80 species, and other investigators have found higher baseline (Love et al. 2004) or stress-induced (Ouyang et al. 2012) levels of corticosterone in birds that abandoned nests after being captured on nests. Few investigators, however, have examined how trapping birds on their nests might affect nest success (Uher-Koch et al. 2015, Ledwoń et al. 2016) and behaviors other than nest desertion (Hill and Talent 1990, Burger et al. 1995, Gregory et al. 2002, Ellenberg et al. 2009, Angelier et al. 2011, Dubiec 2011). In addition, most such previous studies were designed with other objectives so did not include experiments specifically designed to examine the possible effects of being captured on bird behavior. Among the few studies to date, Uher-Koch et al. (2015) found lower nest survival for Pacific (*Gavia pacifica*) and Yellow-billed (*G. adamsii*) loons that had been captured, whereas Ledwoń et al. (2016) found no significant effect of being captured on the hatching success of eggs of Whiskered Terns (*Chlidonias hybrida*).

The use of video recorders at nests could also negatively impact birds and alter their behavior. Although video-recording can reduce the possible effects of investigator disturbance around nests, use of video recorders can also introduce undesired bias into the data if the presence of cameras affects the behavior of focal birds.

A further complication is that birds in different populations may differ in their responses to being captured and/or video-recorded. For example, investigators have found significant habitat-related intraspecific variability in neophobia (Miranda et al. 2013, Sol et al. 2013), fearfulness of humans (Møller 2008, Sol et al. 2013, Geffroy et al. 2015), rates of habituation to human disturbance (Vincze et al. 2016), and hormonal stress-responses (Bonier 2012). These differences can influence the ability of birds to either recover from handling-induced stress or to tolerate research activities and the proximity of sampling devices such as cameras. Ultimately, this can either mask existing differences or generate artificial or exaggerated habitat-related differences in behavioral responses that do not exist in natural, undisturbed situations. For example, if birds are less neophobic in urban areas than in natural habitats, as reported for Great Tits (*P. major*; Riyahi et al. 2017), then studies involving the use of nest cameras may reveal higher provisioning rates in urban areas than in natural habitats when, in the absences of cameras, no differences actually exist. As such, determining how birds in different habitats might respond to being video-recorded is important.

Our objective was to examine the potential effects of capturing and video-recording on the behavior of adult Great Tits, and the potential effect of any change(s) in adult behavior on the development and survival of nestlings. First, we tested whether capturing, banding, and sampling parent birds influenced their subsequent behavior, measured several days after the procedure. We predicted that if being captured sensitizes birds to human disturbance, they will be more alert and approach nest boxes more cautiously than control (i.e., not captured) birds and, as a result, capturing birds may also have negative impacts on provisioning rates and the development and survival of nestlings. Second, we examined possible differences in the behavior of birds allowed to habituate to the presence of a video camera before video-recording and birds that were not habituated. We predicted that, if the sudden presence of video-recorders disturbed parents, non-habituated birds would be more reluctant to enter nest boxes than habituated birds and, as a result, provisioning rates of non-habituated birds may be lower, potentially having an effect on nestling development and breeding success. We also examined possible interactions between these two treatments, i.e., whether the effect of trapping influences the effect of habituation to the presence of a video-recorder or vice versa. Additionally, we compared the behavioral responses of males and females because the sexes may differ in their susceptibility to disturbance (Ellenberg et al. 2009, Pipoly et al. 2011, Bonier 2012). Finally, we compared the effects of capturing and video-recording on Great Tits in urban and forest habitats. Because urban birds may be more tolerant of human disturbance (Geffroy et al. 2015, Vincze et al. 2016) and sometimes less neophobic than birds in natural areas (Sol et al. 2011), we expected reduced treatment effects in urban populations.

METHODS

Experimental design. We studied Great Tits at two forest and two urban sites in Hungary in 2014. Forest study sites were located in deciduous woodlands near Szentgál (47°06′39.75″N, 17°41′17.94″E, 10.1 ha), characterized mainly by European beech (*Fagus sylvatica*) and European hornbeam (*Carpinus betulus*), and in Vilma-puszta (47°05′02.74″N, 17°52′01.28″E, 12.8 ha), characterized mainly by downy oak (*Quercus cerris*) and South European flowering ash (*Fraxinus ornus*). Our urban study sites were in the cities of Veszprém (47°05′17.29″N, 17°54′29.66″E, 9.4 ha) and Balatonfüred (46°57′30.82″N, 17°53′34.47″E, 6.3 ha), mostly in public parks, a cemetery, and university campuses where vegetation consisted of both native and introduced ornamental species. All urban locations were strongly influenced by various anthropogenic disturbances, including frequent human presence and activity, high building density, and much traffic within or around the study sites, whereas humans and vehicles were rarely present at the two forest sites.

We monitored nest boxes (inner dimensions = 12.5 × 12.5 × 23 cm) at least twice a week from March to May to record laying and hatching dates and the number of eggs and nestlings. When more than one egg was found during a nest check, we assumed that one egg was laid per day to calculate the laying date of the first egg. To avoid inducing nest desertion, we never removed incubating females or parents brooding nestlings during these checks (Dubiec 2011). We followed the same monitoring procedure for each treatment group (see below). The present experiment included only the first annual brood of each studied pair.

To study the effects of capture and camera-habituation on adult behavior, we conducted an experiment using a 2 × 2 block design (Table 1). The two treatments were (i) capturing and banding one of the parents, and (ii) equipping nest boxes with a dummy camera to let birds habituate to its presence. With this combination of two treatments, we had four treatment groups: no capture + no

Table 1. Treatment combinations and disturbances in the treatment groups.

	Non-captured	Captured
Camera-habituated	– Response to short human disturbance at start of video-recording	– Captured a few days before video-recording – Response to short human disturbance at start of video-recording
Not camera-habituated	– Presence of a novel camera in a familiar camera-hiding box – Response to short human disturbance at start of video-recording	– Captured a few days before video-recording – Presence of a novel camera in a familiar camera-hiding box – Response to short human disturbance at start of video-recording

dummy camera, no capture + dummy camera (habituated), capture + no dummy camera, and capture + dummy camera (habituated). In both treatments, half of the active nests were chosen to receive the treatment whereas the other half served as controls (i.e., no capture or no dummy camera). At each study site, we chose the treatment combination for the first nest randomly, after which we allocated the further treatment combinations uniformly throughout the season to ensure the similar number of broods in every treatment combinations. We applied each treatment combinations in each study site. Note that the final sample sizes differ between the four treatment groups due to the failure of some nests or disappearance of parents, see below. In the capture treatment group, we also randomized the sex of the captured parent for the first nest and then captured males and females in alternating order at nests at each study site.

Adults were captured in nest boxes using a trap door operated manually by a string. After installing the trap, we hid in either a car or small tent (typically 30–40 m from nests), and observed the nest box using binoculars. When the parent selected for capture entered the nest box, the trap that closed the entrance of the nest box was triggered. The trap was removed immediately after capture. Adults were captured when their nestlings were 6–8 d old (mean = 7.3 ± 0.1 [SE]), and from 1 to 5 d (mean = 2.4 ± 0.2) before we recorded their behavior. Due to logistical constraints, we were not able to video-record every nest on the same day after capture, but the number of days elapsed between capturing and video-recording was similar in the compared groups (in the captured treatment: 2.4 ± 0.3 d for camera-habituated and 2.4 ± 0.2 d for non-habituated treatments, 2.6 ± 0.3 d in urban and 2.2 ± 0.2 d in forest habitats, and 2.6 ± 0.3 d for males and 2.2 ± 0.2 d for females).

In the camera-habituation treatment, we placed a dummy camera on the nest box during incubation so parents had an average of 17.4 ± 0.3 (SE) d to habituate to its presence before video-recording. Dummy cameras were the same size and color as the video cameras used when video-recording and were placed in the same position (Fig. S1). We recorded parental behavior using a small video camera (HD Hero, GoPro, San Mateo, CA), with the dummy camera replaced with a real one in the camera-habituated group. We hid the camera (dummy or real) in a small non-transparent plastic box for concealment (~15 cm from the entrance; Fig. S2) so the only parts of the camera that were visible were the front lens and the back LCD display, the latter of which was turned off during video recording. Camera boxes were permanent accessories of our nest boxes at all of our study sites so birds were already familiar with them. Thus, camera-habituated birds experienced little change in the appearance of the camera box during video-recording, whereas the non-habituated group was faced with an unfamiliar object instead of the familiar empty box (Fig. S2). We acknowledge that installing cameras at the beginning of the video recording might have posed a brief disturbance to the birds, but, because each treatment groups received the same level of human disturbance (Table 1), this would not have affected our results.

We weighed (± 0.1 g) and measured the length of the left tarsus (± 0.1 mm) and right wing (from the bend of the wing to the tip of the longest primary; ± 1 mm) of each captured adult and collected a small drop of blood from the brachial vein (for purposes not related to this study). For individual identification, each bird received a unique combination of a numbered metal band and three plastic color bands. After capture, individuals were handled at least 50 m from nest boxes, either in a car or in the open. The handling procedure took ~10 min, and birds were then released. Some birds in both the captured and non-captured treatment groups had been nest-trapped either 1 or 2 yr previously (2 males and 3 females in the captured group, and 14 males and 19 females in the non-captured group). To ensure that a difference in capture history of the birds did not bias our results, we compared the behavior of birds captured in different years in an additional analysis (see the section Data analysis). For the captured group, we followed the same protocol, but did not replace their original bands. When reaching the near-fledging age of 14–16 d post-hatching (mean = 15.1 ± 0.1 [SE] d), nestlings also received a metal band, and we recorded their mass, tarsus length, and wing length as described above.

Behavioral data collection and variables. We recorded parental behavior when nestlings were 9–11 d old (mean = 9.6 ± 0.1 [SE] d old). We collected one video sample per pair during a continuous 60-min period because this observation length was suggested to be adequate for sampling the parental behavior of Great Tits (Pagani-Núñez and Senar 2013). Recording started between 6:34 and 16:56, with about 80% of recordings between 8:00 and 15:00. We did not record during adverse weather conditions, e.g., heavy rain or strong wind. Each recording started with a brief disturbance where we approached the nest to either place the camera in the camera box or replace the dummy camera. Nests were then left undisturbed during the recordings. The number of nestlings was determined when video cameras were removed (i.e., right after the video recording). Parents attended their offspring during most of the 60-min recordings, as reflected by their latency from the experimenter's departure until the time of the individual's first arrival to the nest box (mean ± SE, males = 378 ± 41 s [range = 11–2642 s] and females = 499 ± 40 s [range = 7–1776 s]).

For each 60-min video, we recorded each parental visit to nest boxes. To describe their behavior, we used hesitation time, vigilance, and provisioning rates. Hesitation time was calculated as the time (in seconds) from the first appearance of a bird at the nest box until it first entered. This variable describes the reluctance of a bird to enter nest boxes for the first time after nest disturbance and in the presence of the real camera. Vigilance was measured by scoring the response of birds toward the camera on a four-point scale each time they entered nest boxes. This score was 0 if a bird spent < 1 s on the nest box before entering; in most cases, a score of 0 meant that a bird entered the nest box immediately upon arrival. A score of 1 was assigned if a bird spent > 1 s time on the nest box before entering while the camera was in a bird's potential field of sight; this value was typically given when a bird paused and briefly scanned its environment before moving on. We assigned a score of 2 if a bird was clearly moving or leaning toward the camera or landed on the slat holding the camera box. Finally, we assigned a score of 3 if a bird physically touched the camera box, pecked it, or landed on it. From these individual vigilance values, we calculated a mean vigilance score for the whole 60-min sample for each parent. The number of provisioning visits was determined for the 60-min video-recording as the number of times a parent entered a nest box with food divided by the number of nestlings. The rare occasions when a parent entered its nest obviously without food, or when we could not determine whether it carried any food items, were not counted as chick-feeding events.

Data analysis. To analyze the responses of Great Tits to the treatments, we used linear mixed-effects (LME) models (using package 'nlme' in R 3.3.1, R Core Team 2016). We analyzed the above described three behaviors (hesitation, vigilance, and number of provisioning visits) as dependent variables in three separate models. Because the distributions of hesitation and number of provisioning visits were left-skewed, we transformed them before analyses as $\log_e(x+1)$. Each initial model included the following predictors: capture treatment (yes/no), camera-habituation treatment (yes/no), date of video recording (number of days since 1 April), time of the day at the start of the recording (categorized into three intervals with similar sample sizes: before 10:00, 10:00–13:00, and after 13:00), habitat type (forest/urban), and brood size (number of nestlings at the time of video recording; this variable was excluded from analyses of provisioning visits). Each initial model contained all two-way interactions between capture, camera-habituation, and habitat, and also the three-way capture × camera-habituation × habitat interaction. Study site was included as a random factor to control for the non-independence of birds breeding at the same site. We analyzed males and females separately to avoid interactions between more than three variables. Because parental behavior was recorded during the same short window of nestling age at every nest, we did not include nestling age in the models. We did not include the number of days elapsed between capturing and video-recording as a predictor to our models because no values could be assigned to non-captured birds. However, we note that, for captured birds, this variable had little variation and did not differ by sex or any of our experimental treatments as detailed above (see the section Experimental design).

In total, we recorded parental behavior at 103 nests at our four study sites. For these video recordings, we determined the sex of visiting parents either by plumage traits or color bands. We excluded two nests from our analyses of males and females because we could not reliably distinguish the two parents in the videos. In analyses of male behavior, we also excluded three additional nests where males did not appear in videos and were not observed before or later during nest-monitoring. Additionally, for the analyses of hesitation and vigilance of males, we excluded three nests where males (although they attended their nestlings on other occasions) did not appear in the video so we could not measure their behavior. Thus, we had 95 males for the hesitation and vigilance analyses and 98 for provisioning rate analysis. For females, for the same reasons, we had 97 nests for the analyses of hesitation and vigilance and 100 for provisioning rate analysis (Table 2a).

We also determined the total number of provisioning visits by summing the visits of both parents and dividing by the number of nestlings. We did this because we assumed that overall feeding rates are biologically relevant from the point of view of nestlings, whose main interest is to obtain food regardless of which parent delivers it. In this analysis, capture was used as a two-level variable with the following levels: "yes" if one parent was nest-trapped or "no" if neither was nest-trapped. For the total number of provisioning visits, our sample size was 99 nests; we only excluded pairs where either the male ($N = 3$) or female ($N = 1$) was never observed.

Because a bird's behavior may not be independent of that of its mate, we also examined the behavior of adults that were not captured relative to the capture status of their mate. We used separate models for males and females and analyzed the hesitation and vigilance behavior of 70 males and 73 females, and the number of provisioning visits for 73 males and 76 females (Table 2b). We applied LME models with the same predictors and random factors as described above, except that now we included the capture status of the birds' mate (yes / no) instead of the capture status of the bird itself (because the latter was non-captured in each case).

Finally, we also analyzed the possible effects of the capture and/or camera-habituation treatments on breeding success as reflected by nestling survival, and on nestling body size as reflected by body mass and size shortly before fledging. We used mean body mass, mean tarsus length, and mean wing length of entire broods as dependent variables separately in three models containing the above-described main effects and interactions between the two treatments and habitat, date, and, in models of body mass, time of the day when nestlings were banded. We analyzed the effects of male and female treatments separately ($N = 99$ and 101 broods, respectively), and also analyzed pairs as units ($N = 98$ broods); in the latter

Table 2. Sample sizes in treatment groups for testing the effects of capture and camera-habituation on the behavior of (a) focal birds and (b) their mates (M = males, F = females).

(a)	Not captured	Captured	Total
Camera-habituated	Urban: 10 pairs (17 M, 14 F) Forest: 13 pairs (18 M, 21 F)	Urban: 11 pairs (5 M, 7 F) Forest: 13 pairs (7 M, 6 F)	47 pairs (47 M, 48 F)
Not camera-habituated	Urban: 10 pairs (16 M, 14 F) Forest: 20 pairs (22 M, 27 F)	Urban: 11 pairs (5 M, 7 F) Forest: 11 pairs (8 M, 4 F)	52 pairs (51 M, 42 F)
Total	53 pairs (73 M, 76 F)	46 pairs (25 M, 24 F)	–

(b)	Mate not captured	Mate captured	Total
Camera-habituated	Urban: 10 M, 10 F Forest: 12 M, 14 F	Urban: 7 M, 4 F Forest: 6 M, 7 F	35 M, 35 F
Not camera-habituated	Urban: 10 M, 10 F Forest: 19 M, 19 F	Urban: 6 M, 4 F Forest: 3 M, 8 F	38 M, 41 F
Total	51 M, 53 F	22 M, 23 F	–

case, capture status was categorized as "yes" if one parent was captured and "no" if neither was captured. Because nestling mortality was rare (see below), we did not analyze all treatment effects, interactions, and predictors on nestling survival in a single linear model because the model would have been overparameterized. Instead, we used separate generalized linear mixed-effects models with quasi-binomial error and logit link function to compare nestling survival for captured and non-captured pairs and also for camera-habituated and non-habituated pairs. For the capture treatment, we calculated the proportion of nestlings surviving for the period from the time of a parent was captured (or, for non-captured pairs, 3 d before the video recording) to when nestlings were banded (for this period, mortality occurred in only seven of the 99 nests, 17 nestlings in total). For the camera-habituation treatment, nestling survival was calculated from the day of video recording to the day nestlings were banded (mortality occurred in six of 99 nests, 16 nestlings in total). In both models, study site was included as a random factor and treatment was the only fixed effect.

Each initial model was reduced by backwards stepwise model selection, excluding the term (interaction or main effect) with the highest P value in each step until only either significant ($P < 0.05$) or marginally non-significant ($P < 0.08$) terms remained (we never omitted the random factor). We report the results of the final models in the Results section, and present the initial models (i.e., before model selection) in Tables S1–S3. For significant interactions, post hoc tests were conducted by calculating linear contrasts from the final model using the R package "glht" and correcting the P-values for the false discovery rate. Values are presented as means ± SE.

To explore the possibility that capturing birds in previous years affected their behavior, we re-ran models for males and females that included the significant predictors and three levels for the capture-treatment variable: "never trapped", "trapped previously" (but not in the present breeding season), and "trapped in the present study" (including the five birds captured in previous years). Use of this latter variable did not affect our main conclusions because we found no difference between "never trapped" versus "trapped previously" groups in hesitation, vigilance, or provisioning rate (Table S4).

RESULTS

Captured males hesitated more, taking a significantly longer time to enter nest boxes after their first appearance on the video (Table 3a, Fig. 1A), and had higher vigilance scores when approaching nest entrances than control males (Table 3a, Fig. 1B). Captured males also tended to make fewer provisioning visits than non-captured males (0.96 ± 0.14 for captured, 1.23 ± 0.09 for non-captured males, but see Table 3a). Male behavior was not affected by camera-habituation, and we found no interactions among the effects of capture, camera-habituation, and habitat type for any of the three studied behaviors (Table S1). Date had a significant negative effect on the hesitation behavior of males (Table 3a), with males tested early in the breeding season hesitating more than those tested later in the season.

Female hesitation behavior was not affected by either capture or camera habituation (Table 3b). Captured females tended to have higher vigilance scores while approaching nest entrances than non-captured females (Table 3b, Fig. 2A). For female provisioning visits, we found a significant interaction between the effects of camera-habituation and habitat type (Table 3b), indicating that habituated females tended to have lower provisioning rates per nestling than non-habituated females at urban sites (difference = 0.20 ± 0.09, $P = 0.057$), but not in forests (difference = 0.08 ± 0.08, $P = 0.31$; Fig. 2B). Date also had a significant negative effect on the hesitation behavior and provisioning rates of females (Table 3b).

The capture status of mates had no significant effect on the behavior of non-captured males (Table 4a); hesitation decreased with increasing date and brood size was negatively associated with male vigilance (Table 4a). For non-captured females, vigilance scores were higher if their mate had been captured (Fig. 3B), and there was a similar tendency in hesitation (Table 4b, Fig. 3A). The number of provisioning visits by non-captured females was not affected by the capture status of their mates, but was negatively affected by date (Table 4b).

Table 3. Final linear mixed-effects models of the effects of capture and camera-habituation on hesitation behavior, vigilance, and provisioning rates of (a) males and (b) females, and on (c) the two parents' summed provisioning rate. Hesitation and provisioning rates were log-transformed using the formula $\log_e(x+1)$. Table S1 contains the results of the initial (full) models.

	b ± SE	df	*t*	*P*
(a) Males				
Hesitation behavior				
Intercept	7.48 ± 1.50	89	4.5	< 0.001
Capture[a]	−2.50 ± 0.56	89	−4.5	< 0.001
Date	−0.13 ± 0.05	89	−2.4	0.02
Vigilance				
Intercept	0.68 ± 0.07	90	9.4	< 0.001
Capture[a]	−0.43 ± 0.08	90	−5.6	< 0.001
Provisioning rate				
Intercept	0.65 ± 0.14	93	6.1	< 0.001
Capture[a]	0.14 ± 0.07	93	1.9	0.061
(b) Females				
Hesitation behavior				
Intercept	4.18 ± 1.24	92	3.4	0.001
Date	−0.09 ± 0.05	92	−2.1	0.039
Vigilance				
Intercept	0.35 ± 0.06	92	5.8	< 0.001
Capture[a]	−0.13 ± 0.07	92	−2.0	0.054
Provisioning rate				
Intercept	1.11 ± 0.23	93	4.9	< 0.001
Camera habituation[b]	0.08 ± 0.08	93	1.0	0.31
Habitat[c]	0.20 ± 0.13	2	1.5	0.27
Date	−0.02 ± 0.01	93	−2.7	0.008
Camera habituation × habitat	−0.28 ± 0.12	93	−2.3	0.022
(c) Male and female combined				
Provisioning rate				
Intercept	2.49 ± 0.34	93	7.3	< 0.001
Date	−0.05 ± 0.01	93	−4.6	< 0.001
Habitat[c]	−1.62 ± 0.42	2	−3.9	0.06
Date × habitat	0.06 ± 0.01	93	4.5	< 0.001

[a]Capture refers to non-captured compared to captured birds.
[b]Camera habituation refers to habituated compared to non-habituated birds.
[c]Habitat refers to urban compared to forest birds.

The number of provisioning visits by both parents was not significantly affected by either capture or camera-habituation, but we found a significant interaction between date and habitat type (Table 3c). Similarly, in all but one case, neither capture nor habituation treatments had significant effects on the mean body mass, tarsus length, or wing length of fledglings. The only exception was a significant capture × habitat interaction for males, i.e., in urban habitat, the tarsus length of fledglings was shorter if their father had been captured (difference between captured versus non-captured groups = 0.34 ± 0.14 mm, $P = 0.028$), but there was no such difference in the forest habitat (0.04 ± 0.11 mm, $P = 0.76$; Table 5). Date negatively influenced nestling body mass regardless of whether the male, female, or neither parent was captured (Table 5a, b and c). Nestling survival rates for captured and non-captured pairs did not differ, whereas, for camera-habituated nests, we found a marginally non-significant trend for greater nestling survival than in non-habituated nests (Table 5d).

DISCUSSION

Our first prediction was that capturing Great Tits on their nests for banding, measuring, and blood sampling would make them

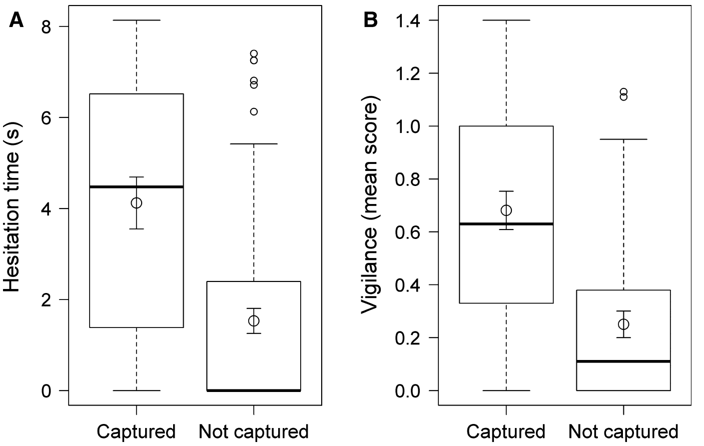

Fig. 1. The effect of capture on the (A) hesitation and (B) vigilance behavior of male Great Tits. Captured birds were trapped and banded before behavioral observations. Hesitation refers to the time elapsed (in seconds) between when a bird first appeared at its nest box and when it entered the nest box. Vigilance was the response of birds to video-recorders scored on a four-point scale when entering the nest (see the Methods section for details). Medians and interquartile ranges are indicated by the thick middle lines and boxes, respectively, whereas open circles with associated whiskers show means ± SE.

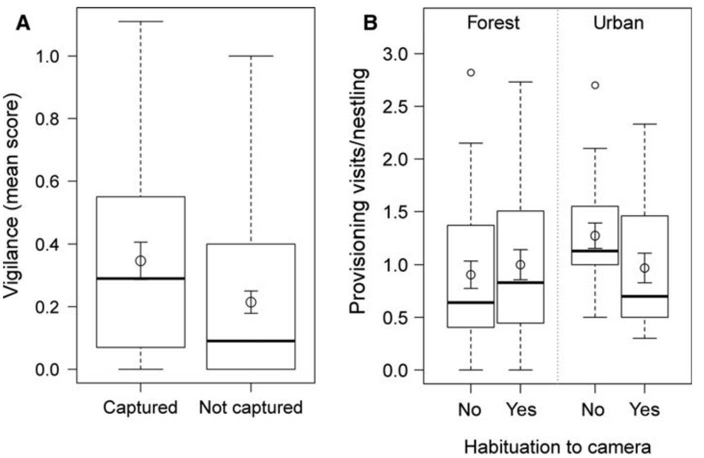

Fig. 2. The effects of (A) being captured on vigilance behavior and (B) camera-habituation on the number of provisioning visits (during 1-h video-recordings) by female Great Tits. Captured birds were trapped and banded before behavioral observations. Vigilance was the response of birds to the presence of a video-recorder scored on a four-point scale when entering their nest boxes (see Methods section for details). Camera-habituation refers to birds that were habituated to the presence of a concealed camera on their nest box. Medians and interquartile ranges are indicated by the thick middle lines and boxes, respectively, whereas open circles with associated whiskers show means ± SE.

more alert and they would approach nests more cautiously than non-captured conspecifics. Indeed, we found that capturing Great Tits had detectable behavioral effects, especially for males, even several days after capture. Compared to non-captured males, captured males needed more time to enter nest boxes after nest disturbance. They were also more vigilant, often pausing to scan the environment before entering nest boxes, and provisioning nestlings less often. The only difference between control and captured

Table 4. Final linear mixed-effects models of the effects of partner's capture status on the behavior of their mates, i.e., non-captured (a) males and (b) females. Hesitation and chick-feeding rates were log-transformed using the formula $\log_e(x+1)$. In the analysis of the provisioning rates of non-captured males, all included variables were non-significant ($P > 0.20$) so are not shown. Table S2 contains the results of the initial (full) models.

	b ± SE	df	*t*	*P*
(a)				
Non-captured males' hesitation behavior				
Intercept	5.60 ± 1.51	65	3.7	< 0.001
Date	−0.15 ± 0.06	65	−2.7	0.008
Non-captured males' vigilance				
Intercept	0.62 ± 0.22	65	2.9	0.005
Brood size	−0.04 ± 0.02	65	−1.8	0.08
(b)				
Non-captured females' hesitation behavior				
Intercept	1.98 ± 0.39	68	5.1	< 0.001
Mate captured[a]	−0.91 ± 0.46	68	−2.0	0.055
Non-captured females' vigilance				
Intercept	0.30 ± 0.05	68	5.7	< 0.001
Mate captured[a]	−0.13 ± 0.07	68	−2.1	0.043
Non-captured females' chick-feeding rate				
Intercept	1.15 ± 0.26	69	4.3	< 0.001
Camera-habituation[b]	0.08 ± 0.09	69	0.9	0.40
Habitat[c]	0.31 ± 0.17	2	1.8	0.22
Date	−0.02 ± 0.01	69	−2.6	0.011
Camera-habituation × habitat	−0.37 ± 0.15	69	−2.5	0.014

[a]Mate captured refers to captured compared to non-captured mates.
[b]Camera-habituation refers to habituated compared to non-habituated birds.
[c]Habitat refers to urban compared to forest birds.

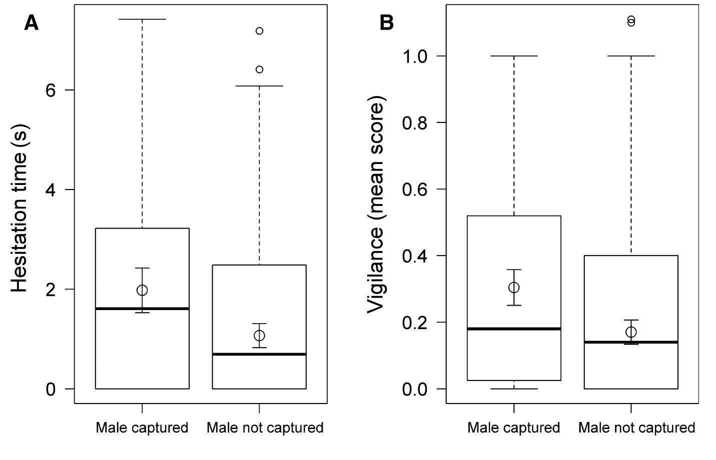

Fig. 3. Effect of capturing male Great Tits on the (A) hesitation time and (B) vigilance of their mates. Captured birds were trapped and banded before behavioral observations. Hesitation time was the time (in seconds) between the first appearance of a bird at its nest box and when it entered its nest box. Vigilance was the response of birds to the presence of a video-recorder scored on a four-point scale when entering their nest box (see the Methods section for details). Medians and interquartile ranges are indicated by the thick middle lines and boxes, respectively, whereas open circles with associated whiskers show means ± SE.

Table 5. Final linear mixed-effects models of the effects of capture and camera-habituation on the body mass, tarsus length, and wing length of nestling Great Tits, using the capture status of (a) males, (b) females and (c) at the pair level; (d) shows the survival of nestlings in relation to the pair level capture status. In the pair level analyses (c, d), a pair's capture-status was "yes" if one of the parents was captured and "no" if neither was captured. In the analyses of mean wing length of nestlings for males, mean tarsus length and wing length of nestlings for females, and mean tarsus length and wing length of nestlings at the pair level, all of the included variables were non-significant ($P > 0.13$) so are not shown. Table S3 contains the results of the initial (full) models.

	b ± SE	df	*t*	*P*
(a) Males				
Nestling mean body mass				
Intercept	19.09 ± 0.92	94	20.7	< 0.001
Date	−0.06 ± 0.02	94	−2.5	0.013
Nestling mean tarsus length				
Intercept	19.84 ± 0.22	93	91.1	< 0.001
Capture[a]	−0.04 ± 0.11	93	−0.3	0.76
Habitat[b]	−0.22 ± 0.32	2	−0.7	0.56
Capture × habitat	0.38 ± 0.18	93	2.1	0.039
(b) Females				
Nestling mean body mass				
Intercept	19.02 ± 0.91	96	21.0	< 0.001
Date	−0.06 ± 0.02	96	−2.4	0.018
(c) Pairs				
Nestling mean body mass				
Intercept	18.97 ± 0.90	93	21.1	< 0.001
Date	−0.06 ± 0.02	93	−2.4	0.02
(d) Nestling survival (pairs)				
Effect of capture on nestling survival				
Intercept	3.59 ± 0.49	93	7.3	< 0.001
Capture[a]	1.31 ± 0.95	93	1.4	0.17
Effect of camera-habituation on nestling survival				
Intercept	3.59 ± 0.50	93	7.2	< 0.001
Camera-habituation[c]	2.66 ± 1.37	93	1.9	0.056

[a]Capture refers to non-captured compared to captured birds.
[b]Habitat refers to urban compared to forest birds.
[c]Camera-habituation refers to habituated compared to non-habituated birds.

females was that captured females showed a tendency of increased vigilance when entering nest boxes, suggesting that they were less affected by being captured than males. These results are important because, although some of the more evident effects (e.g., nest desertion) of nest disturbance or capture and handling have received considerable attention (see the cited literature in the introduction), the more subtle impacts of capture and handling on bird behavior have rarely been quantified (but see Schlicht and Kempenaers 2015).

In parallel with our study, Schlicht and Kempenaers (2015) found similar patterns in Blue Tits (*Cyanistes caeruleus*), although they only studied the immediate responses of birds. After applying different field protocols of capture, handling, marking, and sampling, these authors found that the more stressful the handling protocol an individual received, the longer they took to return to their nests after release. The handling protocols used by Schlicht and Kempenaers (2015) took more time (~30 min vs. 10 min in our study) and, for certain treatment groups, the handling consisted of more invasive procedures (e.g., insertion of a small, subcutaneous transponder, and collection of feathers samples, wax from preen glands, and sperm), supposedly evoking a more substantial physiological stress response. Thus, our results suggest that even supposedly less stressful experiences can alter bird behavior and that these impacts are detectable even over a longer period of time. Despite use of different methods, our results and those of Schlicht and Kempenaers (2015) suggest that capturing and handling protocols can have a greater effect on bird behavior (e.g., vigilance and wariness) than is sometimes assumed (Duarte 2013). Additional studies are needed, however, to determine how long the behavior of birds is affected by being captured and how this might vary among species.

Although birds in areas with more frequent anthropogenic disturbances are often more tolerant of such disturbances (Sol et al. 2013, Geffroy et al. 2015), we consistently found, contrary to our prediction, no differences in the behavioral responses of Great Tits in urban and forest habitats to capture and handling. However, to confirm the generality of this conclusion, further studies are needed with more species in more habitats.

Our results suggest a greater effect of capture and sampling procedures on male Great Tits than females. In addition, non-captured females were more vigilant and tended to hesitate more when entering nest boxes if their mate had been captured. Because captured males behaved more warily, females might have noted the behavior of their mate and responded by adjusting their own behavior. At least two factors may contribute to differences between the sexes in their responses to capture and handling. One possibility is that the motivation to provide parental care differs between the sexes. For example, although Great Tits are typically socially monogamous with biparental care, extra-pair paternity is also frequent (15–50% of broods have at least one extra-pair young in our study populations; Bókony et al. 2017), resulting in uncertainty in male paternity. Thus, males may reduce parental care more than females as a behavioral response to stressful events (Wingfield et al. 1998, Wingfield and Sapolsky 2003) and prioritize their own survival because their fitness gain from a given brood may be smaller than that of females. In addition, the different susceptibilities of males and females to stress may be mediated by sex differences in the stress-induced hormonal response that have been reported in some species of birds (O'Reilly and Wingfield 2001, Grace and Anderson 2014).

The changes in behavior that resulted from capture and video-recording did not affect the reproductive success of Great Tits in our study. Although males made slightly fewer provisioning visits after capture, the number of visits made by both parents combined was not affected. We also found no consistent differences between captured and non-captured groups in the body mass, size, and survival of nestlings. The only exception was the reduced tarsus length in urban nestlings if the male parent had been captured. However, the difference was small (0.34 mm, ~2% of average fledgling tarsus length), and nestling mass and wing length were unaffected. Collectively, these results suggest that, although being captured can influence parental behavior over several days, these effects do not necessarily manifest at the level of breeding success. Similarly, Schlicht and Kempenaers (2015) found that capturing and sampling, as described above, had pronounced immediate effects on the behavior of Blue Tits, but, once captured parents returned to their nests, they resumed normal parental activities, with no significant effect on provisioning rates and breeding success.

Contrary to our expectation, we found that the behavior of Great Tits exposed to a dummy camera for several weeks before video-recording did not differ from that of those that were unfamiliar with the camera and encountered it only during the recording session. The only behavioral effect of camera habituation involved urban females where, surprisingly, the camera-habituated group had somewhat lower provisioning rates than non-habituated females. Regarding reproductive success, we found a trend in the opposite direction, i.e., lower nestling mortality in habituated than non-habituated nests. However, this latter difference was based on a small number of mortalities and contrasts with the general lack of camera-habituation effects in all other analyses of nestling traits. This general lack of effect may be attributed to the fact that we used a "familiar" plastic box to hide the cameras (as a permanent accessory of our nest boxes), so even the non-habituated birds might have perceived little change in their environment during video-recording.

We have demonstrated that a common capture and sampling protocol can have detectable, sex-dependent effects on bird behavior for several days, but not on breeding success. These results are relevant for all of investigators quantifying bird behavior in the field and who intend to mitigate or control for the potential disturbance effects of capturing birds on their nests and/or when using video-recorders. We recommend the investigators consider the effects of capturing and handling birds in their study designs because of the possible undesired, significant and, at least in some species, sex-dependent effects on bird behavior. If not standardized, such effects can influence the quality of data collected. Therefore, we suggest either not capturing and blood sampling shortly before collecting behavioral data or delaying capture until after data collection. Alternatively, all studied individuals should be captured and handled in a standardized way. If these options are not feasible, we recommend at least statistically controlling for individual capture status in the data analysis. Also, for Great Tits, to minimize the possible effects on parental behavior and breeding success, we recommend that females be captured first (e.g., 6–7 d after hatching), and males only during the later phases of the nestling period because the increased response of males and their influence on the behavior of their non-captured mates may have less of an effect on older nestlings than younger ones (e.g., due to less developed abilities to thermoregulate).

Our results also suggest that properly concealed video-recorders can be used at nests without needing to habituate birds to their presence. Although our camera setup (i.e., pre-existing shelters for cameras) was specific to our study, and the placement and use of video-recorders can vary among studies, our results suggest that, by concealing video-recorders, any effects on bird behavior can be minimized even when deployed close to nests (e.g., 15 cm in our study).

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REFERENCES

Angelier, F., H. Weimerskirch, and O. Chastel. 2011. Capture and blood sampling do not affect foraging behaviour, breeding success and return rate of a large seabird: the Black-browed Albatross. Polar Biology 34: 353–361.

Bókony, V., I. Pipoly, K. Szabó, B. Preiszner, E. Vincze, S. Papp, G. Seress, T. Hammer, and A. Liker. 2017. Innovative females are more promiscuous in Great Tits (*Parus major*). Behavioral Ecology 28: 579–588.

Bonier, F. 2012. Hormones in the city: endocrine ecology of urban birds. Hormones and Behavior 61: 763–772.

Burger, J., I. C. Nisbet, J. M. Zingo, J. A. Spendelow, and C. Safina. 1995. Colony differences in response to trapping in Roseate Terns. Condor 97: 263–266.

Cole, E. F., J. Morand-Ferron, A. E. Hinks, and J. L. Quinn. 2012. Cognitive ability influences reproductive life history variation in the wild. Current Biology 22: 1808–1812.

Duarte, L. M. G. 2013. Impact of capture and handling on wild birds. Ph.D. dissertation, Cardiff University, Cardiff, UK.

Dubiec, A. 2011. Condition-dependent clutch desertion in Great Tit (*Parus major*) females subjected to human disturbance. Journal of Ornithology 152: 743–749.

Ellenberg, U., T. Mattern, and P. J. Seddon. 2009. Habituation potential of Yellow-eyed Penguins depends on sex, character and previous experience with humans. Animal Behaviour 77: 289–296.

Fair, J. M., and J. Jones (eds.). 2010. Guidelines to the use of wild birds in research. Ornithological Council, Washington, D.C.

Geffroy, B., D. S. M. Samia, E. Bessa, and D. T. Blumstein. 2015. How nature-based tourism might increase prey vulnerability to predators. Trends in Ecology & Evolution 30: 755–765.

Grace, J. K., and D. J. Anderson. 2014. Corticosterone stress response shows long-term repeatability and links to personality in free-living Nazca Boobies. General and Comparative Endocrinology 208: 39–48.

Gregory, M. J. P., A. G. Gordon, and R. Moss. 2002. Impact of nest-trapping and radio-tagging on breeding Golden Eagles *Aquila chrysaetos* in Argyll, Scotland. Ibis 145: 113–119.

Hill, L. A., and L. G. Talent. 1990. Effects of capture, handling, banding, and radio-marking on breeding Least Terns and Snowy Plovers. Journal of Field Ornithology 61: 310–319.

Kania, W. 1996. Safety of catching adult British birds at the nest. Ringers' Bulletin 9: 24–27.

LEDWOŃ, M., J. BETLEJA, AND G. NEUBAUER. 2016. Different trapping schemes do not affect hatching success of Whiskered Terns *Chlidonias hybrida*. Bird Study 63: 136–140.

LOVE, O. P., C. W. BREUNER, F. VÉZINA, AND T. D. WILLIAMS. 2004. Mediation of a corticosterone-induced reproductive conflict. Hormones and Behavior 46: 59–65.

MIRANDA, A. C., H. SCHIELZETH, T. SONNTAG, AND J. PARTECKE. 2013. Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? Global Change Biology 19: 2634–44.

MØLLER, A. P. 2008. Flight distance of urban birds, predation, and selection for urban life. Behavioral Ecology and Sociobiology 63: 63–75.

O'REILLY, K. M., AND J. C. WINGFIELD. 2001. Ecological factors underlying the adrenocortical response to capture stress in Arctic-breeding shorebirds. General and Comparative Endocrinology 124: 1–11.

OUYANG, J. Q., M. QUETTING, AND M. HAU. 2012. Corticosterone and brood abandonment in a passerine bird. Animal Behaviour 84: 261–268.

PAGANI-NÚÑEZ, E., AND J. C. SENAR. 2013. One hour of sampling is enough: Great Tit *Parus major* parents feed their nestlings consistently across time. Acta Ornithologica 48: 194–200.

PIPOLY, I., V. BÓKONY, AND A. LIKER. 2011. Hogyan befolyásolja az id ő járás a házi verebek (*Passer domesticus)* fiókaetetési viselkedését? Állattani Közlemények 96: 97–111.

R CORE TEAM [online]. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org (Accessed 2 February 2017).

RIYAHI, S., M. BJÖRKLUND, F. MATEOS-GONZALEZ, AND J. C. SENAR. 2017. Personality and urbanization: behavioural traits and DRD4 SNP830 polymorphisms in Great Tits in Barcelona city. Journal of Ethology 35: 101–108.

SCHLICHT, E., AND B. KEMPENAERS. 2015. Immediate effects of capture on nest visits of breeding Blue Tits, *Cyanistes caeruleus*, are substantial. Animal Behaviour 105: 63–78.

SOL, D., A. S. GRIFFIN, I. BARTOMEUS, AND H. BOYCE. 2011. Exploring or avoiding novel food resources? The novelty conflict in an invasive bird. PLoS ONE 6: e19535.

———, O. LAPIEDRA, AND C. GONZÁLEZ-LAGOS. 2013. Behavioural adjustments for a life in the city. Animal Behaviour 85: 1101–1112.

UHER-KOCH, B. D., J. A. SCHMUTZ, AND K. G. WRIGHT. 2015. Nest visits and capture events affect breeding success of Yellow-billed and Pacific loons. Condor 117: 1–28.

VINCZE, E., S. PAPP, B. PREISZNER, G. SERESS, V. BÓKONY, AND A. LIKER. 2016. Habituation to human disturbance is faster in urban than rural House Sparrows. Behavioral Ecology 27: 1304–1313.

WINGFIELD, J. C., D. L. MANEY, C. W. BREUNER, J. D. JACOBS, M. RAMENOFSKY, AND R. D. RICHARDSON. 1998. Ecological bases of hormone-behavior interactions: the "Emergency life history stage." American Zoologist 38: 191–206.

———, AND R. M. SAPOLSKY. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15: 711–724.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Fig. S1. (A) The video camera and the dummy camera. (B) Column 1, front view: the video camera and the dummy camera, respectively, hidden in the plastic box for concealment, and the plastic box attached emptily to the nest box. Column 2, back view: the plastic box with the camera and the dummy camera, respectively, and attached emptily to the nest box.

Table S1. Initial full linear mixed-effects models of (A) males' and (B) females' hesitation behavior, vigilance and chick-feeding rate, and (C) parents' total chick-feeding rate (where a pair's capture status was "yes" if one of the parents was captured and "no" if none of them were captured).

Table S2. Initial linear mixed-effects models of the effects of partner's capture status on non-captured (A) males' and (B) females' behavior.

Table S3. Initial linear mixed-effects models of the effects of capture and camera-habituation on nestlings' body mass, tarsus length and wing length, using the capture status of (A) males, (B) females and (C) at the pair level (i.e., one or none of the parents captured).

Table S4. Final linear mixed-effects models of the effects of capture and camera-habituation on hesitation behavior, vigilance and chick-feeding rate of (A) males and (B) females, using "capture treatment" as a three-level variable to describe birds' nest-trapping experience: "never captured", "captured in previous years" (but not in the present breeding season), "captured in the present study" (i.e., in the present breeding season).