Research disturbance negatively impacts incubation behaviour of female Great Tits

Léanne Clemencin[1], Emilio Barba[2] & David Diez-Méndez[3,4]*

[1] Université Claude Bernard Lyon 1, 69622 Villeurbanne Cedex, France

[2] "Cavanilles" Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain

[3] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic.

[4] University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic.

* Corresponding author

Abstract

Human-induced disturbance is perceived by avian species as a predation risk. However, the anti-predatory behaviour triggered by these non-lethal events can have negative impacts on reproduction and offspring survival. Research on breeding birds often imply visiting their nests and likely disrupt parental behaviour, but nest visits where females are not directly handled have previously been overlooked as important disturbance events. This study focuses on the impacts of short visits to the nest of incubating Great tit (*Parus major*) females. We investigated how long they stay away from the nest (off-bout) after a disturbance, their possible compensatory behaviour once they resume incubation (longer on-bouts), and the effects on daily incubation rhythms. We used three years of data from two breeding populations to assess the consequences of disturbance in two scenarios: when the female is present in the nest and flushed out, and when the female is absent. We found that following a disturbance, the immediate off-bout was longer, both when the female was present and absent, with the magnitude of the disturbance being greater when females were flushed. The females did not compensate by longer on-bouts afterwards, therefore research

disturbance altered daily incubation behaviour by decreasing the total time spent on the nest as a function of the number of daily disturbance events. Females that alter their behaviour in response to the perception of predation risk would perform longer incubation periods that result in lower hatching rates. These effects of research on female behaviour should be considered when planning field experiments.

Keywords: Incubation behaviour, anti-predator response, off-bouts, *Parus major*, human disturbance

Introduction

Human-induced disturbance events are perceived similarly to predation risk ("risk-disturbance hypothesis", Frid & Dill, 2002), although they are usually non-lethal by nature (Cresswell, 2008). In response, animals may adjust their behaviour (Beale & Monaghan, 2004; Perona et al., 2019), physiology (Eggers et al., 2006; Coslovsky & Richner, 2011) or life-history traits (Hutfluss & Dingemanse, 2019) to the magnitude of perceived predation risk (Lima & Bednekoff, 1999). Anti-predatory behavioural responses can have a negative impact on reproduction and offspring survival, even if they contribute to a breeding individual not being predated. These effects can be both indirect, by facilitating nest predation in the absence of parents (Stien & Ims, 2016) or via maternal effects on offspring (Coslovsky & Richner, 2011), and direct, by altering incubation behaviour (Baudains & Lloyd, 2007) or offspring feeding (Verhulst et al., 2001). Direct effects may impair offspring growth (Remacha et al., 2016) and influence their survival and subsequent recruitment (Blas et al., 2007). Such non-consumptive impacts are often associated with human recreation, although research activities during the breeding season may have analogous effects.

Nest disturbance is inevitably associated with avian reproductive research (Lenington, 1979). It can be assumed that research activities are more disruptive than recreational activities, as they usually involve handling individuals, visiting nests or manipulating eggs, but only temporarily. Attracting predators to nest sites has traditionally been the main impact that researchers have been concerned about, although an early review showed no clear patterns overall (Götmark, 1992). Later studies not only found no measurable negative effects (Schaub et al., 1992; Lloyd et al., 2000; Weidinger, 2008; Hu et al., 2020) but even positive effects of research activities (Ibáñez-Álamo & Soler, 2010, as the presence of humans near nests may deter mammal predators rather than attracting them (reviewed in Ibáñez-Álamo et al., 2012). However, as with recreational activities, nest predation is only one possible indirect effect, and other direct effects of research activities on breeding individuals may also be at play.

Breeding passerines that are the subject of research activities are regularly visited to record their reproductive phases from nest building to offspring feeding (e.g., Cresswell & McCleery, 2003; Andreasson et al., 2018; Arct et al., 2022). Nest visits while incubating have been dismissed as important disturbance events as there are almost no previous assessments of their impact. The incubating period is of particular interest because females, in gyneparental species, spend up to 80% of their active time in the nest to incubate (on-bouts), leaving it only briefly for foraging or other self-care activities (off-bouts) (e.g., Ardia et al., 2010; Johnson et al., 2013; Ospina et al., 2018; Diez-Méndez et al., 2021a). Regular incubation rhythms facilitate researchers to encounter females in the nest. Females are constraint to maintain an adequate clutch temperature for embryonic development (Nord & Nilsson, 2011; DuRant et al., 2013), but show certain plasticity adjusting the number and length of their bouts to external conditions (Conway & Martin, 2000a; Diez-Méndez et al., 2021a). Incubating females undergo high energy costs (Nord & Williams, 2015) and may abandon their nest when predation risk is high, especially in the first half of the period when, according to life-history theory, the trade-off between current and future reproduction favours the latter (Clutton-Brock, 1991; Stearns, 1992). For this reason, assessments of research activity in this period have only been conducted when visiting a nest implies high desertion rates, like after handling incubating females (up to 41% of desertions, Kania, 1989; Kilgas et al., 2007; Dubiec, 2011) or after experimental drastic reductions in clutch size (e.g., simulating partial predation, Verboven & Tinbergen, 2002; Johnston, 2011; Zieliński, 2020).

Visiting a nest and flushing the female to determine clutch size, a common practise, may also affect incubation behaviour, but to a lesser extent than the above-mentioned disturbances. Simply visiting a nest is perceived by females as a predation risk and triggers anti-predatory behaviours such as hissing (Krams et al., 2014; Zub et al., 2017; Dutour et al., 2020; Van Iersel et al., 2023). Following previous studies using stuffed predators, we may expect an effect on incubation behaviour if females reduce their activity in the nest. Nevertheless, lengthening both off- and on-bouts, ends up with having no negative impacts in nest attendance (Conway & Martin, 2000b; Ghalambor & Martin,

2002; Kovařík & Pavel, 2011; Basso & Richner, 2015a; Tkaczyk et al., 2023, but see Basso & Richner, 2015b). However, similar predation experiments resulted in females reducing their activity by returning later after being exposed to the predator (longer off-bouts), and limiting the time spent in the nest (shorter on-bouts), resulting in an overall decrease in nest attendance (Zanette et al., 2011; Ibáñez-Álamo & Soler, 2012). Repeated prolonged absences of the incubating female may have dramatic consequences on embryonic development due to increased temperature variability (Olson et al., 2006) and longer incubation period that lead to reduced egg hatchability (Nord & Nilsson, 2011; Macdonald et al., 2013; Diez-Méndez et al., 2020) and survival rates after fledging (Hepp et al., 2006; Berntsen & Bech, 2016; Ospina et al., 2018, reviewed in DuRant et al., 2013).

Here we investigated the effects of research disturbance during the incubation period in two Great tit (*Parus major*) breeding populations. We assessed the effects of disturbance and potential recovery time in two common scenarios when conducting nest visits: 1) the incubating female is present in the nest (an incubating on-bout) and is consequently flushed, and 2) the female is absent (during an off-bout). Female flushing has been dismissed as an important disturbance because, to our knowledge, its effects have not been considered so far, despite Great tits are one of the most common model species in the wild. We hypothesized that visiting a nest-box would have a negative effect, albeit limited, on incubation behaviour in both scenarios, i.e., either when the female is present and flushed or when she is absent. A limited negative effect would mean that females lengthen the immediate off-bout after a disturbance event, followed by a longer on-bout to compensate. This limited effect would not cause overall changes in daily incubation behaviour. We hypothesised that the effect of research disturbance in the absence of a female would still elicit a noticeable behavioural response because foraging territories are small (Naef-Daenzer & Keller, 1999) and females can still detect intruders. We also expected that flushing a female triggers a stronger response than when females are absent as it is perceived as a riskier encounter.

Material and Methods

Study area and species

Great tits are small (12.5 – 15 cm) secondary hole-nesting passerines that naturally breed in cavities but also readily accept nest-boxes (Perrins, 1979). They lay one egg per day and usually start incubating when the clutch is complete, although there is a great variation depending on external conditions (Diez-Méndez et al., 2021b). They are assisted gyneparental incubators (Nord & Williams, 2015), which means that only the females incubate and the male may assist by bringing food to the nest (Bambini et al., 2019), yet the female regularly leaves the nest to forage and perform other self-care activities (intermittent incubation).

Breeding data was collected in two Mediterranean nest-box populations during three consecutive breeding seasons (2015-2017) as part of ongoing long-term projects (e.g., (Álvarez et al., 2013)): Sagunto (39.70°N, 0.25°W) is part of an extensive monoculture of orange trees (*Citrus aurantium*) at sea level (30 m a.s.l.), while Pina (40.02°N, 0.63°W) is part of a mountain range (1200 m a.s.l.) which contains a mixed forest dominated by maritime pines (*Pinus pinaster*) with scattered stands of Portuguese oaks (*Quercus faginea*).

Field procedures

We monitored the first clutches of breeding Great tits in both populations to determine when the first egg was laid in each nest and to initiate the protocol for measuring incubation behaviour (see Diez-Méndez et al., 2021b, 2021a for further details). In brief, after the first egg was laid, we attached a probe (30-gauge wire, accuracy ± 1.0°C; Onset Computer Corporation, USA) to the centre of the clutch, which was connected to a thermocouple data logger (HOBO UX100-014M Single Channel, Onset Computer Corporation, USA), and recorded the temperature every 10s until the first egg hatched. The probe was inserted into a plastic egg (Factory Direct Craft Supply, USA), filled with a lubricant (Clear Glide, Ideal industries, USA), that was similar in shape and colour to the Great tit eggs but slightly smaller (15.9 × 11.5 mm versus 18.1 × 13.3 mm; average egg size from Sagunto; Encabo et al. 2001). In addition, we attached a Thermochron iButton data logger (accuracy ± 0.5°C,

model DS1922L-F5, Maxim Integrated) to the upper inner part of each nest-box wall to record the local ambient temperature every 520 s.

We visited each nest-box at least three times during the incubation period: 1) when we first found an incubating female or warm eggs during a regular morning check, 2) five days into the incubation period, when we visited the nest to check the probe and clutch size, and 3) eleven days into the incubation period, when we began monitoring egg hatching. Each time we visited a nest, we noted the time and whether the female was present or absent from the nest. If the female was present, we always flushed her out to check the contents of the nest and experimental materials. The nest-boxes of these populations allow the front wall to be removed during checkings, so the female usually left the nest as soon as we opened the nest-box. For the few more stubborn females we carefully push them out. The incubating females were never handled in order to be as unobtrusive as possible. The standard visits to the nest-boxes lasted less than one minute (i.e., a short disturbance), but depending on the year and ongoing experiments (e.g., measuring and weighing individual eggs), these visits lasted longer (i.e., 1-5 min, long disturbance events).

Incubation behaviour and disturbance measurements

Incubation behaviour of Great Tit females was assessed using recorded nest-cup temperatures in each nest during the incubation period, We defined the onset of incubation as the first time nest attentiveness exceeded 50% of the active day (incubation day 1), and the incubation period lasted to the day before the first egg hatched (following Cresswell & McCleery, 2003; Simmonds et al., 2017; Diez-Méndez et al., 2021b). A female's active day was considered as the period between the first off-bout in the early morning and the last on-bout in the evening to overnight.

To identify off- and on-bouts, we used the software *Rhythm* (Cooper & Mills, 2005) and followed the criteria of setting off-bouts to last at least 2 min and indicating a temperature drop of 2.0°C. We then visualized the Rhythm output in Raven (Cornell Laboratory of Ornithology) and modified off-

bouts when we detected a misidentification or visualized clear off-bouts that did not meet the criteria. Only off-bouts with a duration of less than 120 minutes were taken into account, as longer off-bouts are rare and considered outliers (~1.5%, Diez-Méndez et al., 2021a). A total of 46 nests, 29 from Sagunto and 17 from Pina, were suitable for assessing the effects of research visits on female incubation behaviour. We recorded 273 disturbances and analysed the on- and off-bout duration before, during and after an event, totalling 1,638 bouts (see below, Suppl. Mat. Table S1).

Statistical analyses

We performed statistical analyses with the software R 4.3.1 (R Core Team, 2023). Six linear (LMMs) and one generalised linear mixed-effects model (GLMMs) were created using the package *lme4* (Bates et al., 2015). We built a model to assess the off-bout duration when a female was absent during a nest-box visit (disturbance) using the off-bout temporal sequence (before, during and after disturbance) as a fixed factor together with day of incubation, ambient temperature (quadratic term, poly- function), time of the day (quadratic term, poly- function), calendar date, population and year, including nest-box as a random effect. A second model was built to assess the off-bout duration when a female was present in the nest during a visit, using off-bout categorization (before, immediately after the disturbance, and the next one) as a fixed factor. The third model looked at the duration of the on-bouts when a female was absent, using a fixed factor categorizing on-bouts before (one) and after the disturbance (two on-bouts). The fourth model dealt with the duration of the on-bouts when the incubating female was present during a visit to the nest, using a fixed effect to categorize on-bouts before (one) and after the disturbance (two on-bouts). We did not consider the on-bout that abruptly finished with the disturbance because its duration was shortened by the visit itself. Bout duration was measured in minutes and log transformed in every model. Two more LMMs were built to determine whether the duration of the disturbance influenced the subsequent off-bout duration. Off-bout duration when a female was present or absent was used as the dependent variable, and disturbance duration was an independent factor with two levels: short and long. The rest of the fixed and random factors were similar to the previous LMMs. Our final model

(GLMM, binomial family) addressed daily nest attentiveness and added disturbance intensity as a fixed factor. The number of disturbance events per day could be up to two if the experiments required it. For each day on which at least one disturbance event occurred, a preceding or subsequent day on which no disturbance occurred was also selected. In addition, day of incubation, mean daily ambient temperature (quadratic, poly- function), calendar date, population and year were included as fixed factors, and nest- box as a random factor in the model. All numerical variables were centred. Normality, homogeneity of variance and collinearity were visually checked for the LMMs models using the *performance* package (Lüdecke et al., 2021). The resulting figures were created with *ggplot2* (Wickham, 2016) after calculating the estimates ± 95%CI with the package *Effects* (Fox & Hong, 2009).

Results

Our data showed the general pattern of average off-bouts being three times shorter than on-bouts (~10 vs. ~30 min, Supp. Mat. Table S1). When the female was absent from the nest during a nest-box visit, the ongoing off-bout lasted longer than both the previous and following off-bouts (Table 1, Fig. 1a). However, we found no detectable effect on the first on-bout after a disturbance compared to the previous and the following on-bouts (Table S2, Fig. 1c). When the female was present in the nest during a nest-box visit, the immediate off-bout also lasted longer than the previous and following off-bouts (Table 1, fig 1b), and the two on-bouts following the disturbance event tended to be longer than before, but the effect was not clear (Table S2, Fig. 1d).

Finding a female in the nest, and flushing her out, led to a subsequent longer off-bout compared to off-bouts from females that were out of the nest. While in the latter females extended their off-bout by ~35%, the former performed ~75% longer off-bouts (Table S1). The duration of the disturbance also affected how long a female stayed off the nest, but only if she was present in the nest during the visit: a long disturbance caused a longer off-bout (Table 2, see Table S3 for absent females). Moreover, only when females were absent, the off-bouts after a disturbance event tended to be

shorter in the Sagunto population (an anthropized orange plantation) than in Pina (a mixed forest) (Table S3).

Disturbances in the nest were reflected in daily nest attentiveness. Females spent less time incubating when they underwent two disturbance events in a single day (estimate = 67.4, CI = 64.1 – 70.6) than when there was only one (estimate = 71.3, CI = 68.7 – 73.7), and less time when there was only one daily event than when there was no disturbance at all (estimate = 76.1, CI = 74.0 – 78.1) (Table 3. Fig 2).

We also found in our data subset clear effects of both the incubation day and ambient temperature. As incubation progressed, females, either absent or present in the nest, shortened their off-bouts (Table 1) and increased their daily incubation effort (Table 3). Females also lengthened their off-bouts at increasing ambient temperatures, but only if they were present in the nest during a visit (Table 1 & 2). Lastly, females decreased their daily nest attendance at increasing daily ambient temperatures (Table 3).

Discussion

In this study, we found evidence that research disturbance negatively affects Great tit incubation behaviour. Our initial hypothesis was that females reacted to a disturbance event by staying away from the nest longer than expected given their incubation rhythms, but we also expected that this anti-predatory behaviour, observed in previous studies (Kovařík & Pavel, 2011; Ibáñez-Álamo & Soler, 2012; Croston et al., 2021; Tkaczyk et al., 2023), was compensated with longer on-bouts that ended up balancing any negative effect on nest attendance. However, we found that 1) the response of females was restricted to the ongoing (absent females) or first (present females) off-bout after a disturbance, i.e., a short recovery period; 2) females did not clearly compensate for their prolonged absence from the nest by lengthening subsequent on-bouts; and consequently,

and despite the limited anti-predator response, 3) daily incubation rhythms were negatively affected by the disturbance events (i.e., lower nest attentiveness).

Breeding females are flexible in their response to different perceived predation risks (Lima, 2009; Martin & Briskie, 2009). We found that females that were present in the nest at the time of a disturbance stayed away from the nest longer than absent females, presumably because they perceived being flushed as a greater risk than simply recognising an intruder from a distance. Flushed females also stayed away from the nest longer if the researcher performed additional activities during a visit (e.g., measuring eggs), in contrast to absent females, which seemed unaffected by prolonged visits. Experiments simulating the presence of predators near the nest generally result in incubating females leaving the nest for longer periods (Kovařík & Pavel, 2011; Ibáñez-Álamo & Soler, 2012; Croston et al., 2021; Tkaczyk et al., 2023), although the duration and magnitude of the response is species-specific (Ghalambor & Martin, 2002; Zanette et al., 2011; Ghalambor et al., 2013) and/or dependent on predator type (Basso & Richner, 2015a). Absent females appeared to evaluate the disturbance caused by the researchers differently. While flushed females remained near the nest-box, called and displayed mobbing behaviour before flying away (DDM pers. obs.), absent females may encounter a researcher in their nest when they return to incubate or while already foraging nearby. Flushed females may refrain from foraging or other self-care activities during part of their off-bout to increase vigilance (Morosinotto et al., 2013; Croston et al., 2021), at least until the researcher has left the nest, which could partly explain their longer off-bouts. An unexpected trend also emerged between populations when females were absent from the nest, although more data would be needed to assess a clear effect. In Sagunto, off-bouts of absent females tended to be shorter than in Pina. Given the anthropogenic nature of Sagunto, an extensive orange tree plantation, Great tit females could either be partially habituated to humans near the tree in which they built the nest, or the structure of the trees (short trees with crowns that generally reach the ground) would partly conceal the researcher from the foraging the bird.

Females were still constrained by a clutch that requires a suitable thermal range for embryo development (Drent, 1975; DuRant et al., 2013). In elicited long absences from the nest, after being flushed, higher air temperature allowed females to stay off the nest for longer, pointing towards thermal constraints in the magnitude of the anti-predatory response, a trade-off between their own risk of being predated and the embryo survival that air temperature ameliorates. This is in line with a milder response when females were absent, where no association between air temperature and off-bout duration was found. Additionally, the general relationship between day of incubation and off-bout duration was expected (Diez-Méndez et al., 2021a) because when embryos develop their circulatory system, eggs cooling rates increase, causing a shortening in off-bouts (Cooper & Voss, 2013).

Reduced activity near the nest, either by prolonged or less frequent off-bouts is the expected default behaviour when predation risk increases (Skutch, 1957; Conway & Martin, 2000b). However, longer absences are usually compensated by longer incubation bouts, levelling out overall nest attendance (Kovařík & Pavel, 2011; Ibáñez-Álamo & Soler, 2012; Basso & Richner, 2015b; Tkaczyk et al., 2023). In our study, despite the limited females' response to a disturbance event, their daily incubation rhythms were altered, reducing their time on the nest. Reduced nest attendance may lengthen incubation period (Nord & Nilsson, 2012) and reduce egg hatchability (Diez-Méndez et al., 2020) in the short term and reduce offspring survival in the long-term (Berntsen & Bech, 2016; Ospina et al., 2018). Furthermore, Great tit females did not show a habituation response (see also Croston et al., 2021), and a second disturbance event within a day maintained the decline in daily nest attentiveness. Based on our results, it can be assumed that field experiments requiring regular, repeated visits during the incubation period may result in at least a decrease in egg hatchability compared to completely undisturbed nests. However, we were unable to link offspring productivity and egg hatchability to disturbance in our population, as all the nests were involved in different experiments and each of them was visited at least three times during the incubation period.

Our models showed a certain amount of variance that was not explained by the variables we measured. Physiological conditions at the individual level, which were not considered in this study, may explain some of the observed variability. For example, prolactin concentration following a disturbance may regulate nest attentiveness (Hope et al., 2020), and poorer body condition could lead to higher nest desertion rates (Dubiec, 2011). Female personality could also explain the observed variability, as shy individuals take longer to return to the nest after being exposed to a novel object (Cole & Quinn, 2014), although a long-term study on the effects of human recreation on Great tit breeding parameters showed no measurable within-population differences given their risk-taking personality (Hutfluss & Dingemanse, 2019). Overall, Great tits are a resilient species that tolerates high degree of manipulation in the nest compared to other passerines (e.g., shrikes Tryjanowski & Kuźniak, 1999; Golawski & Zduniak, 2022). However, we must not lose sight of the importance of reducing disturbance from nest building to egg incubation. Visiting a nest while the female is absent is desirable, although less likely if it is not intentional, because incubating Great tit females sit on the nest for about 75% of their active day (Bueno-Enciso et al., 2017; Schöll et al., 2019; Diez-Méndez et al., 2021a). Handling females in the nest during incubation can lead to nest abandonment (Dubiec, 2011) but nest visits also generate disturbances that disrupt incubation rhythms, a problem that should be taken into account when planning experimental approaches in the breeding populations.

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Data availability statement

Data will be available from the Dryad Digital Repository

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552 **Table 1.** Estimates of linear mixed-effect models analysing the effect of a disturbance event on the
553 ongoing off-bout (female absent in the nest-box) compared to the previous and next off-bouts, or
554 the immediate off-bout after a disturbance (female present and flushed out of the nest-box)
555 compared to the previous and the next off-bouts. Incubation day, ambient temperature, time of the
556 day, population and year were also considered. Nest-box is a random effect. Significant effects P
557 < 0.05 are highlighted in bold.

558

559

	Estimate	**se**	***t***	***P***
Off-bout (female absent)				
($R^2_{\text{fixed factors}}$ = 0.18, R^2_{model} = 0.23)				
Intercept	2.74	0.200	12.46	
Previous	**-0.27**	**0.098**	**-2.80**	**0.006**
Next	**-0.42**	**0.096**	**-4.40**	**<0.001**
Incubation day	**-0.21**	**0.059**	**-3.58**	**<0.001**
Temperature	0.87	0.806	1.07	0.284
Temperature^2	-0.49	0.736	-0.67	0.503
Time	0.27	0.803	0.33	0.739
Time^2	-0.71	0.857	-0.83	0.408
Date	0.01	0.130	0.05	0.963
Population: Sagunto	-0.24	0.244	-0.99	0.330
Year 2016	0.18	0.177	1.01	0.319
Year 2017	0.03	0.171	0.19	0.851
Off-bout (female present)				
($R^2_{\text{fixed factors}}$ = 0.20, R^2_{model} = 0.22)				
Intercept	2.75	0.207	13.32	
Previous	**-0.61**	**0.069**	**-8.84**	**<0.001**
Next	**-0.54**	**0.069**	**-7.84**	**<0.001**
Incubation day	**-0.11**	**0.039**	**-2.91**	**0.004**
Temperature	**2.72**	**0.856**	**3.18**	**0.002**
Temperature^2	0.52	0.718	0.73	0.466
Time	0.30	0.802	0.37	0.709
Time^2	0.13	0.807	0.16	0.872
Date	0.08	0.084	1.00	0.323
Population: Sagunto	-0.03	0.178	-1.17	0.867
Year 2016	0.24	0.152	1.59	0.117
Year 2017	0.12	0.149	0.83	0.409

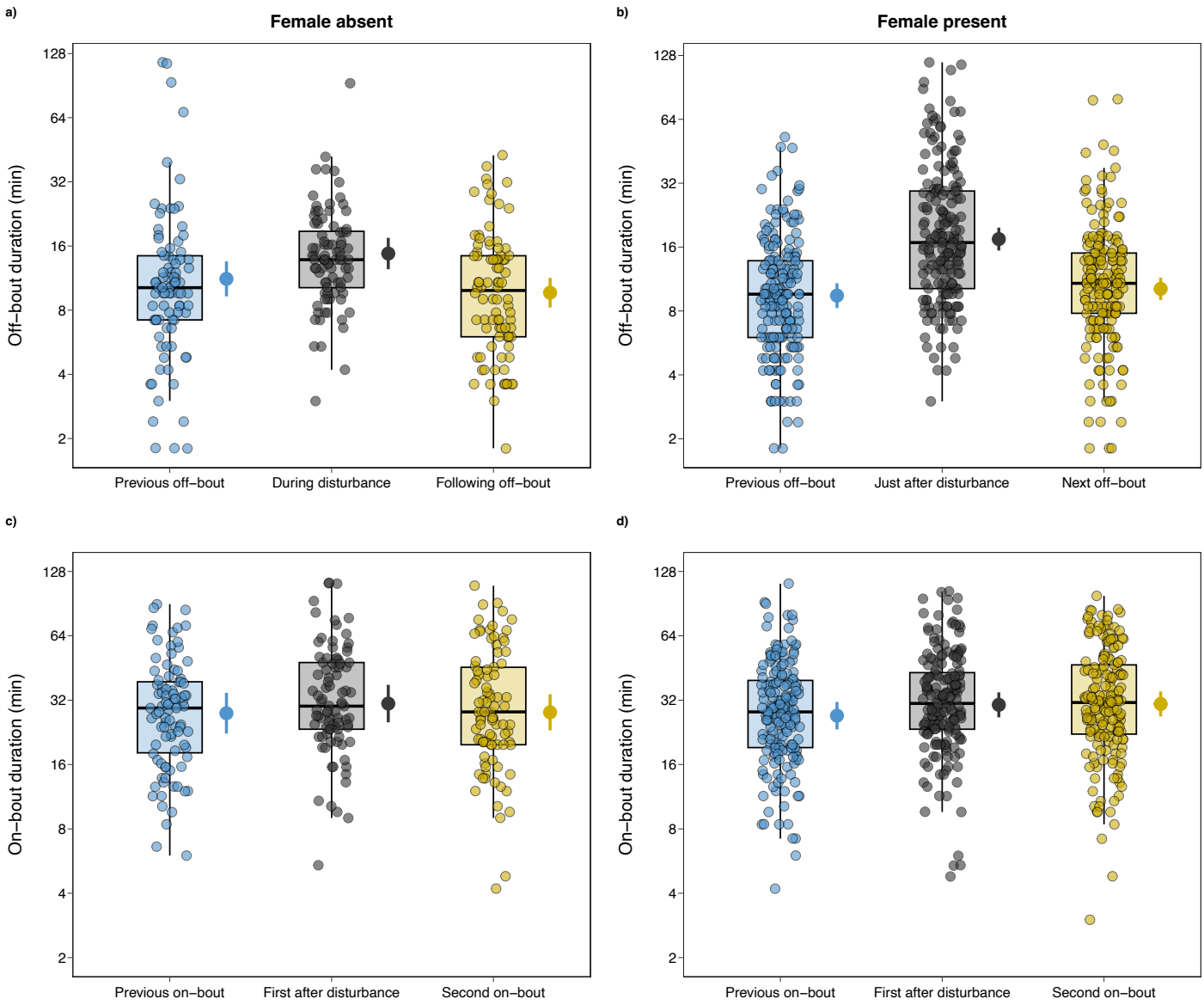
Table 2. Estimates of a linear mixed-effect model analysing the effect of the duration of a disturbance event (short vs. long disturbance) on the duration of the subsequent off-bout when a female was present and flushed out of the nest-box. Incubation day, ambient temperature, time of the day, population and year were also considered. Nest-box is a random effect. Significant effects $P < 0.05$ are highlighted in bold.

	Estimate	**se**	***t***	***P***
Off-bout duration (female present)				
($R^2_{\text{fixed factors}} = 0.16$, $R^2_{\text{model}} = 0.32$)				
Intercept	2.40	0.385	6.24	
Long disturbance	**0.37**	**0.138**	**2.69**	**0.008**
Incubation day	0.03	0.074	0.36	0.718
Temperature	**2.00**	**0.828**	**2.42**	**0.017**
Temperature^2	0.27	0.710	0.38	0.702
Time	0.69	0.755	0.92	0.359
Time^2	-0.26	0.811	-0.32	0.748
Date	0.20	0.161	1.21	0.234
Population: Sagunto	0.27	0.339	0.80	0.431
Year 2016	0.41	0.286	1.45	0.154
Year 2017	0.12	0.279	0.45	0.658

Table 3. Estimates of a generalized linear mixed-effect model analysing the effect of the number of disturbance events on the daily nest attentiveness. Incubation day, daily ambient temperature, time of the day, population and year were also considered. Nest-box is a random effect. Significant effects $P < 0.05$ are highlighted in bold.

	Estimate	**se**	***z***	***P***
Daily nest attentiveness ($R^2_{\text{fixed factors}} = 0.33$, $R^2_{\text{model}} = 0.68$)				
Intercept	1.03	0.234	4.40	
One event	**-0.25**	**0.047**	**-5.36**	**<0.001**
Two events	**-0.43**	**0.063**	**-6.81**	**<0.001**
Incubation day	**0.19**	**0.047**	**3.97**	**<0.001**
Temperature	**-0.91**	**0.340**	**-2.68**	**0.007**
Temperature^2	-0.31	0.318	-0.98	0.327
Time	-0.17	0.151	-1.10	0.273
Time^2	-0.02	0.316	-0.05	0.961
Date	0.15	0.129	1.20	0.229
Population: Sagunto	0.15	0.221	0.66	0.513
Year 2016	**1.03**	**0.234**	**4.40**	**<0.001**
Year 2017	**-0.25**	**0.047**	**-5.36**	**<0.001**

575 **Figure 1.** Comparison of the duration of the ongoing/first bout after a disturbance event and the
576 bout before the event and the next one. The incubating female may be present or absent during
577 such events. Box-plots represent the median and 50% of the values, and upper and lower whiskers
578 represent values > 75th and < 25th percentile. Semi-transparent circles represent the bout duration
579 raw data. Full circles and associated bars show the model estimates and 95% confidence intervals.
580



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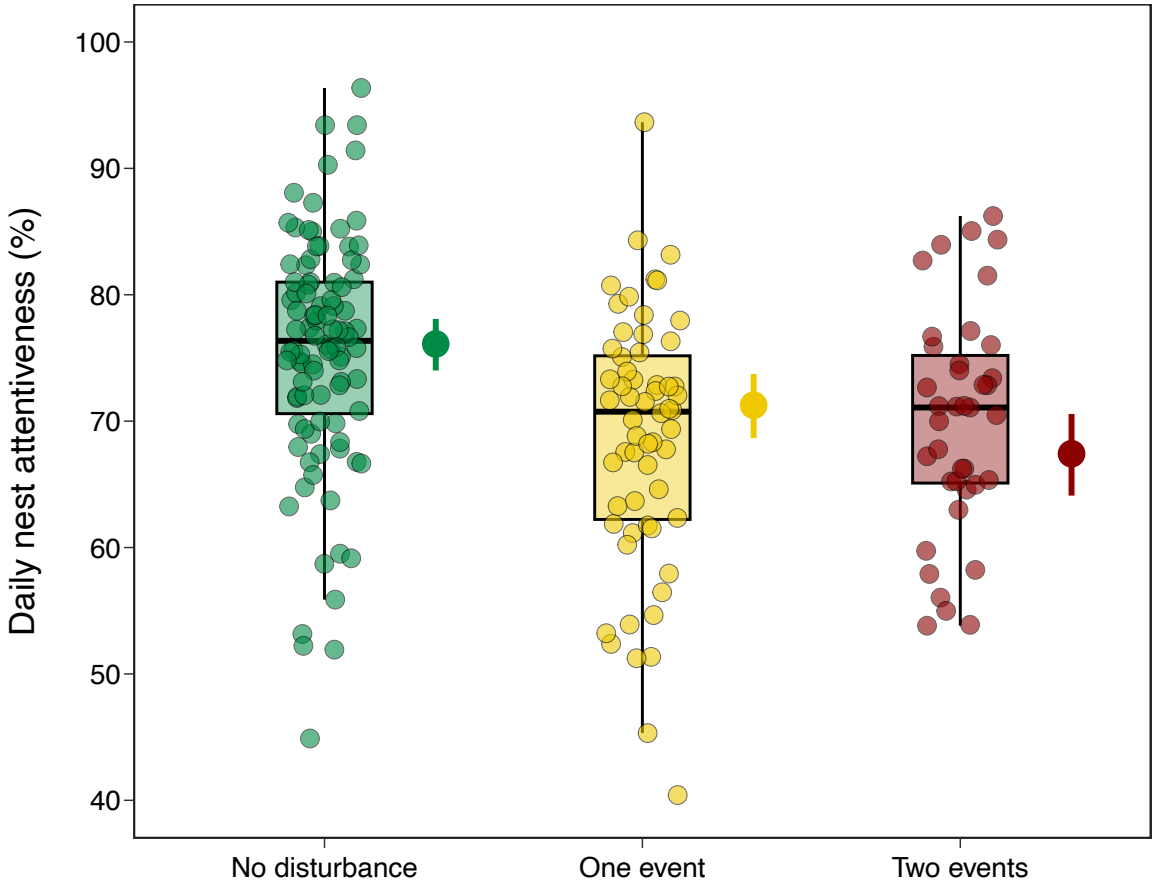
Figure 2. Daily nest attentiveness (proportion of time a female spends incubating compared to the time spent out of the nest on self-care related activities) depending on the number of disturbance events in a day. Box-plots represent the median and 50% of the values, and upper and lower whiskers represent values > 75th and < 25th percentile. Semi-transparent circles represent the bout duration raw data. Full circles and associated bars show the model estimates and 95% confidence intervals.