The immediate impact of ringing, blood sampling and PIT-tag implanting on the behaviour of Blue Tits *Cyanistes caeruleus*

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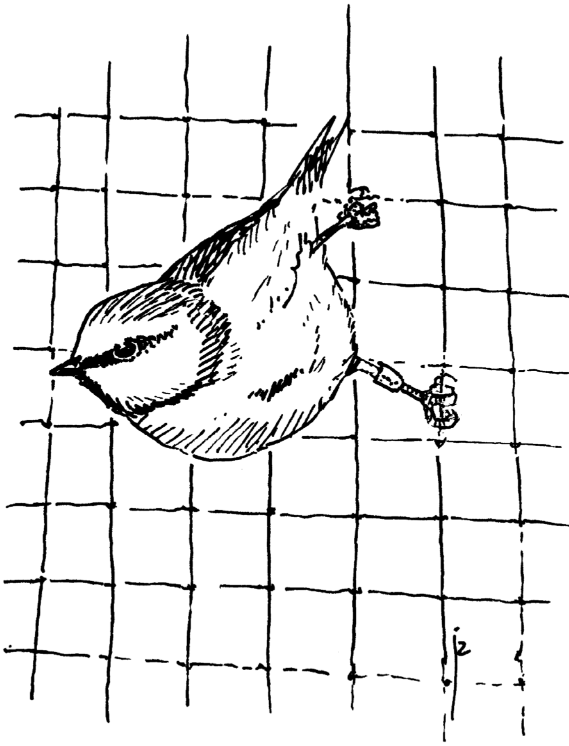
Schlicht E. & Kempenaers B. 2018. The immediate impact of ringing, blood sampling and PIT-tag implanting on the behaviour of Blue Tits *Cyanistes caeruleus*. Ardea 106: 39–50. doi:10.5253/arde.v106i1.a8



Blood sampling from the brachial vein and sub-cutaneous implantation of PIT-tags ('passive integrated transponders') are techniques widely practiced in ornithological research. Longer-term consequences of these procedures (across months or years) have been studied in detail. However, it remains largely unknown how blood sampling and PIT-tagging affect birds during and immediately following the procedure. Here, we test the impact of these procedures on respiration rate and on behavioural correlates of avian pain, stress, and discomfort in the Blue Tit *Cyanistes caeruleus*. Ten wild-caught Blue Tits were divided in two groups: five were measured, ringed, blood sampled and implanted with a PIT tag ('treatment birds'); the other five were handled in the same way, but blood sampling and PIT-tagging were conducted as a sham-procedure ('control birds'). Treatment and control birds did not differ in respiration rates during handling, but treatment birds showed behaviours indicative of an acute stress event associated with brief and moderate pain. Following release in an aviary, treatment and control birds did not differ in behaviour. Birds showed no indication of pain or stress. Instead, they foraged, preened and explored the aviary immediately after handling. Individuals spent much of their time pecking at their new rings. Our results suggest that blood sampling and implantation of PIT-tags have limited short-term effects on Blue Tits. However, the process of handling and ringing itself may have substantial behavioural consequences, which may be relevant for animal welfare and the quality of collected data.

Key words: Blue Tit, *Cyanistes caeruleus*, blood sampling, banding, ringing, welfare, pit tag, respiration rate, distress

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In the majority of ornithological field studies birds are captured and handled to obtain measurements, to ring them, and often also to take a blood sample and tag them with passive integrated transponders (PIT) or other devices. In particular, with the expansion of techniques in endocrinology and molecular genetics in the late 20th century, the number of birds sampled for blood has increased dramatically. Long-term effects of blood sampling on birds have received considerable attention in the literature (e.g. Stangel 1986, Hoysak & Weatherhead 1991, Redmond & Murphy 2011, Bowers *et al.* 2016, Smith *et al.* 2017). The majority of these studies found no adverse effects on short- or long-term mortality (reviewed in Sheldon *et al.* 2008, Fair *et al.* 2010, Owen 2011, but see Brown & Brown 2009).

Similarly, the spread of PIT-tagging techniques in the last ten years has led to studies inspecting the potential impact of this method. PIT-tags are unpowered electronic microchips that allow automatized individual identification of animals carrying them. Studies on several bird species showed that effects of the implantation of PIT-tags on survival, reproduction or growth are generally absent or small (Clarke & Kerry 1998, Carver *et al.* 1999, González-Solís *et al.* 1999, Applegate *et al.* 2000, Jamison *et al.* 2000, Low *et al.* 2005, Nicolaus *et al.* 2008, Tóth *et al.* 2010, Schroeder *et al.* 2011, Bridge & Bonter 2011, Brewer *et al.* 2011). Reported cases of infections, tissue changes or PIT-tag encapsulation are restricted to isolated cases (Clarke & Kerry 1998, Carver *et al.* 1999, Low *et al.* 2005, Dugger *et al.* 2006).

Longer-term behavioural consequences of PIT-tagging have already been studied. Lab studies conducted in poultry found no behavioural changes one to 84 days after tag implantation (Jackson & Bünger 1993, Carver *et al.* 1999, Jamison *et al.* 2000, but see anecdotal result in Applegate *et al.* 2000). In wild birds, PIT-tagging did not affect homing behaviour of Dark-eyed Juncos *Junco hyemalis* (Keiser *et al.* 2005) and foraging behaviour of Adélie Penguins *Pygoscelis adeliae* (Ballard *et al.* 2001, Dugger *et al.* 2006) and Southern Rockhopper Penguins *Eudyptes chrysocome* (Ludynia *et al.* 2012) were unaffected by tagging. Also, PIT-tagging had no effect on changes in body mass in Pied Flycatchers *Ficedula hypoleuca* (Ratnayake *et al.* 2014). Recently, Oswald *et al.* (2017) studied the impact of PIT-tagging on behaviour and physiology within 24 hours after implantation. They observed negative effects only when small birds were implanted in the peritoneum, but not when implanted between the scapulae. In contrast, it remains largely unknown how birds react to blood sampling, PIT-tagging and other procedures during and immediately after handling. This is unfortunate, both from an animal welfare and a methodological perspective, because these procedures may cause immediate distress and this may in turn affect the well-being of the bird and the behaviours under study.

Here, we present results of an experiment on Blue Tits *Cyanistes caeruleus* designed to assess physiological effects, pain, and distress accompanying blood sampling and PIT-tagging. We focus on blood sampling from the brachial vein and sub-cutaneous PIT-tag implantation between the scapulae, because these are the procedures predominantly used in small songbirds (Nicolaus *et al.* 2008, Owen 2011). Our control birds ($n = 5$) received sham bleeding and PIT-tagging treatments and were otherwise exposed to exactly the same capture and handling procedures as treatment birds ($n = 5$). Therefore, we are able to explicitly separate effects of bleeding/PIT-tagging from the effects of capture, measuring, ringing and handling. This allows a meaningful interpretation of data collected during and immediately after manipulation of the birds. We expect that physiological effects, pain and distress are the most likely impacts of blood sampling and PIT-tagging and those most important for behavioural alterations following these procedures. They should also be most relevant for animal welfare considerations. Birds show active avoidance/escape reactions (excessive movement, jumping, flapping, defence behaviour, distress calls) in response to brief pain, while intense or ongoing pain is associated with reduced food and water intake, reduced grooming, reduced activity and alertness, immobility with crouched position, squinting/blinking and ptiloerection ('fluffing up'; Gentle 1992, Machin 2005, Lierz & Korbel 2012). Behavioural indicators of distress are similar (Carstens & Moberg 2000). Pain and distress in birds are rarely assessed quantitatively, but studies in poultry have employed tonic immobility, the catatonic state characteristic of intense or prolonged pain or fear (Gentle 1992, Machin 2005), as a measure (Gallup 1979, Jones 1986, Forkman *et al.* 2007). Pain is also associated with an increase in heart rate, blood pressure and respiration rate (Woolley & Gentle 1987, Gentle & Hunter 1991, Carstens & Moberg 2000, Sneddon *et al.* 2014).

METHODS

Ten Blue Tits were captured in mist nets in upper Bavaria (47°58'16"N, 11°14'09"E), while approaching feeding stations, on 24–25 or 28–31 October 2013, between 07:55 and 12:09. Each day, we caught two birds, except on 28 and 29 October when only one bird was caught. Immediately after capture, we extracted the bird from the net (mean ± SE time spent in net: 1.6 ± 0.5 min, range: 0–5 min) and carried it in a cotton bag inside for processing. We fitted each bird with one metal ring and with two colour rings, one on each leg, to allow verification of identity from video recordings. One Blue Tit had been ringed the previous year with a metal ring on one leg and a colour ring on the other leg and we added another colour ring on the leg with the metal ring. Birds were sexed based on morphology (6 females, 4 males) and aged (4 yearlings, 6 adults) based on plumage (Svensson 1992). We measured tarsus, third distal primary and body mass. For each two birds caught, we had beforehand randomly assigned the first-caught individual to the treatment ($n = 3$) or to the control group ($n = 2$). The next Blue Tit was then assigned to the other group (control: $n = 3$; treatment: $n = 2$). Treatment birds were blood sampled (c. 5 μl) by puncturing the brachial vein as described in Owen (2011; venipuncture with 30G-needle, 20μl-mini-capillary to collect emerging blood, application of cotton wool and closing wing) and afterwards received a PIT-tag (EM4102 ISO animal tag 134.2 kHz ISO, 8.5 mm×1.35 mm, 0.067 g), which was inserted between the scapulae under the skin on the back following (Nicolaus *et al.* 2008; puncture and insertion with 12G-needle and syringe, closing of wound with Epiglu tissue glue, Meyer-Haake Medical Innovations). Control birds were subjected to a sham procedure with equivalent steps in the exact same order (e.g. fixation of bird, duration and direction of exposure to needle), except that no punctures were made and the subcutaneous/intravenous parts were simulated and carried out directly above the skin. After measuring, birds were positioned such that the remaining procedures could be video-taped from below with a camera (GZ-MG77E, JVC; 25 frames/s). All birds were held for two minutes ('before recording phase') in the position used for bleeding (back down, one wing extended) before proceeding with (true or sham) venipuncture. After blood sampling was completed, the bird was kept fixed in the position for another minute ('between recording phase'). It was then turned around for (true or sham) subcutaneous PIT-tag injection. After this, the bird was kept in the position used for implantation for another two minutes ('after recording phase'). Handling of birds started between 08:00 and 12:13 and took 9–13 minutes (mean ± SE: 11.6 ± 0.4 min), including the five minutes from the recording phases. All handling procedures were performed by the same two persons, one of which implanted the PIT-tag while the other carried out the remaining tasks.

Video recordings during handling were inspected for signs of distress during blood sampling and tag-implantation, i.e. we scored the presence of calls, opened beak, crown erection, twitching or blinking/squinting (eye squeeze; Langford *et al.* 2010). We did not include struggling or wing flapping, because each bird was held tight. The scored responses allowed an assessment of to what extent birds assumed a catatonic state. We extracted the respiration rate from the video recordings during handling by tracking (using frames made every 0.04 s) the positional coordinates of a fixed point on the breast, rump or tail using the software Tracker 4.83 (Brown & Christian 2013). In this way we obtained the precise respiration movements (Figure S1). From these data we extracted (1) the number of breaths per recording phase and (2) the number of breaths in every second for the 10 s before and 10 s after venipuncture and the 10 s before puncture for tag-implantation and the 10 s after tag-insertion. The 10-s interval was chosen because Woolley & Gentle (1987) found that a comb pinch in Domestic Chicken *Gallus gallus domesticus* induced an immediate 25% increase in respiration rate, which returned to normal after 10 s. As a validation of our method we counted for each recording phase the number of rhythmic movements of breast, rump or tail (Carere & van Oers 2004, Fucikova *et al.* 2009, Class *et al.* 2014). This was performed by a student naïve to the purpose of the project. The phase-wise rates obtained from these count data (mean ± SE: 3.1 ± 0.1, range: 2.0–5.0) and from the tracked movements (mean: 2.9 ± 0.1, range: 2.0–5.0) were highly correlated ($r = 0.93$, $n = 50$) and we only report measurements based on tracked movements (results were similar when using the count data).

After completing all procedures described above, we carried the bird in a cotton bag approximately 60 m to an outdoor aviary (floor: 2×3 m, height: 2.5 m) and released it. The sides and top of the aviary were made from solid wood, but the rear side consisted of wire mesh (Supplementary Video S1). Branches and four suet food balls were attached to the mesh and water and a standard insect-seed-mix were provided in bowls on the floor. Immediately after release, we observed each bird through a one-way glass window for 60 min (three observers watched ($n = 5$), 3 and 2 birds, respectively). Observers were blind to the treatment status of the bird. During each session, the observer scored the presence or absence of the following behaviours every 30 s. (1) Activity: bird is alert, as shown by attentive visual investigation, and moves head, beak, etc., but does not change position. (2) Movement: bird changes position by hopping or short flight (<1.5 m). (3) Long flight: bird changes position by flight of at least 1.5 m. (4) Lethargy: bird is inactive and not alert and/or sits in crouched posture with head and tail lowered. (5) Squinting/blinking: eyes partially or completely closed. (6) Ptiloerection: bird sits still and 'puffs up' feathers. (7) Excessive movements: bird shows agitation and/or tumultuous flights across the aviary. Different from (1–3) the sequence of movements is extremely rapid and without an obvious target. (8) Irritation rings: bird pecks at rings. (9) Irritation back: bird pecks at or is occupied with the back area relevant for PIT-tag implantation. (10) Irritation wing: bird pecks at or is otherwise occupied with the location relevant for blood sampling on either wing. (11) Grooming: feather shakes, scratching, preening at sites other than those under (8–10). (12) Feeding: pecking at food or drinking. (11) Vocalization: calls. We selected these behaviours because they cover the known responses of birds to stress and acute or ongoing pain (see above).

Upon release in the aviary, we also video-taped each bird for six hours (including the observation session) using a dome camera (25 frames/s) mounted at the ceiling of the aviary. The camera was set up to film the wire mesh so that recordings covered only the rear half of the aviary (Video S1). Scoring of video-taped behaviour was performed blind to the treatment and in the same way as during the live observations, excluding vocalizations. Due to light conditions, for two birds the last 28 and 108.5 min, respectively, had to be excluded from analysis. Thus, 720 30-s intervals were scored for 8 birds, and 664 and 503 intervals for the remaining two birds, respectively. Because birds were not visible all the time, we also noted whether the bird was visible or not during all or part of each 30-s interval. A total of 6927 intervals were scored. Birds were visible on the video in 6505 (94%) of the scoring intervals and only these are considered in further analysis. In these, birds often (54% of intervals) flew briefly to the front of the aviary, where they were temporarily out of vision to the camera. During these flights they usually returned immediately to the back of the aviary (Video S1). Therefore, the time when no behaviour could be scored was limited and at least three behaviours were scored for 98% of such intervals. Overlap between observation and video data from the first hour was high (for all behaviours scored at least five times: mean ± SE: 82 ± 3%, range: 72–91%), despite the fact that the start of 30-s-intervals was not synchronized. Therefore, in the Results, we only report the data based on the video recordings, with the exception of vocalizations. Results are qualitatively similar when restricting all analyses to the data from the one hour direct observation of each bird. After video-taping was completed, we released the bird.

For statistical analysis we used R v. 3.1.3 (R Core Team 2015). For all tests, we constructed generalized linear mixed effect models with bird identity as random factor and experimental group (treatment or control) as explanatory variable. Given the small sample size, we did not include covariates such as capture order, measurements, age or sex. All measurements were within two standard deviations of the mean for our study population (1617 adults; e.g. Schlicht & Kempenaers 2015), except for one tarsus measurement (for which only 21 birds had a smaller measurement). Both age and sex groups were present in the treatment and control group (treatment group: 1 adult male, 1 adult female, 1 yearling male, 2 yearling females; control group: 2 adult males, 2 adult females, 1 yearling female). We performed the following tests. (1) Respiration rates (Gaussian error structure, package MCMCglmm; Hadfield 2010): first, we conducted pairwise comparisons of respiration rates between the 'before', 'between' and 'after' recording phase for treatment and control birds. Secondly, we compared respiration rates before and after venipuncture as well as before puncture for tag-implantation and after tag-insertion for treatment and control birds, using an interval of either 10 s or 2 s before and after. We included the 2-s interval, because inspection of Figure 2 suggested that changes primarily occurred at this temporal scale. Thirdly, we inspected the change in respiration rate in the 10 s after venipuncture and after tag-insertion for treatment and control birds. (2) Behaviour in the aviary (binomial error structure, packages lme4 (Bates *et al.* 2014) and lmerTest (Kuznetsova *et al.* 2016)): we compared the frequency of behaviours between treatment and control birds. We only analysed behaviours that had summed across all birds in total at least 50 intervals with occurrence and at least 50 intervals without occurrence: vocalization, movement, long flight, grooming, feeding, ring pecking. For these models, we calculated 95% confidence intervals (CI) by inference from the general linear hypothesis of the model (significance level 0.05; package multcomp; Hothorn *et al.* 2008). Mean values are shown with standard errors.

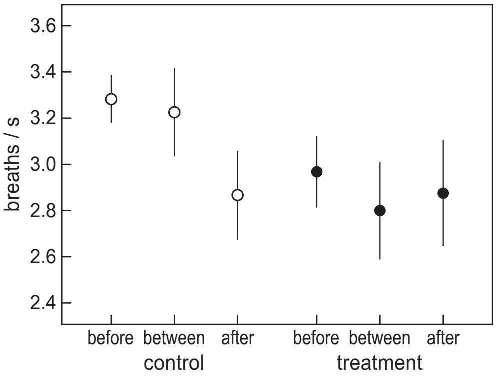

Figure 1. Comparison of average respiration rates during the three recording phases (120 s before venipuncture, 60 s between venipuncture and tag-implantation, 120 s after tag-implantation) for control (white circles, n = 5) and treatment birds (filled circles, n = 5). Circles and whiskers show means and standard errors. For results of statistical analysis see Table S1.

RESULTS

Behaviour during handling

During (true or sham) venipuncture, one treatment and one control bird continuously raised their crown feathers, and another treatment bird twitched when punctured. During (true or sham) implantation, one treatment and two control birds blinked once, one treatment bird blinked once and opened and closed its beak twice, and one treatment bird blinked twice and opened and closed its beak once. No birds vocalized. Frequency of distress behaviours was not significantly different between treatment and control birds, but power of this test is low (Fisher's exact test; venipuncture: $P = 1.0$, power = 0.03; implantation: $P = 0.5$, power = 0.14; power calculated based on 100,000 simulated data sets at significance level 0.05).

Respiration rate

Treatment and control birds did not differ in respiration rates (Figure 1, Table S1). Furthermore, respiration rates did not differ in either group between the 'before', 'between', or 'after'-procedure phase (Figure 1, Table S1). For both treatment and control birds, respiration rate was higher in the 10 s before than in the 10 s after venipuncture (Figure 2A, B, Table S2), and the same was true for the 10 s before the puncture for tag-implantation and the 10 s after the insertion of the PIT-tag (Figure 2C, D; Table S2). When the before-after comparison was restricted to two seconds before and after, groups again did not differ (Table S2), but respiration rate declined slightly after (true or sham) tag-insertion in both groups (Table S2). This decline was not explained by a decline in respiration rates after the procedure was finished: respiration rates did not change over the 10 s following (true or sham) tag-insertion (Table S2). However, for both treatment and control birds respiration rates declined slightly over the 10 s following (true or sham) venipuncture. Thus, respiration rates were not affected by the treatment, but declined,perhaps due to the paused handling movements.

Behaviour in aviaries

Distress behaviours were extremely rare: lethargic behaviour and ptiloerection were not observed at all and squinting was only seen during two 30-s intervals (one from a treatment and one from a control bird). Furthermore, only one bird (from the treatment group) showed excessive movements during one 30-s interval. The vocalizations scored during life observations did not include any distress calls.

The frequency of contact calls showed high individual variation (range: 5 to 113 of the 120 observation intervals; mean: 49 ± 13). There were no differences between treatment and control birds (Figure 3, Table S3). Birds were almost continuously alert and exploratory. Activity was absent for only 13 intervals across all 10 birds (number of intervals and bird category: 2 (treatment), 1 (treatment), 6 (control), 2 (control), 1 (control), 1 (control)). Across birds, the proportion of intervals with movements or long flights was 0.83 ± 0.03 and 0.62 ± 0.04, respectively. Neither probability of movement nor probability of long flights differed between treatment and control birds (Figure 3, Table S3).

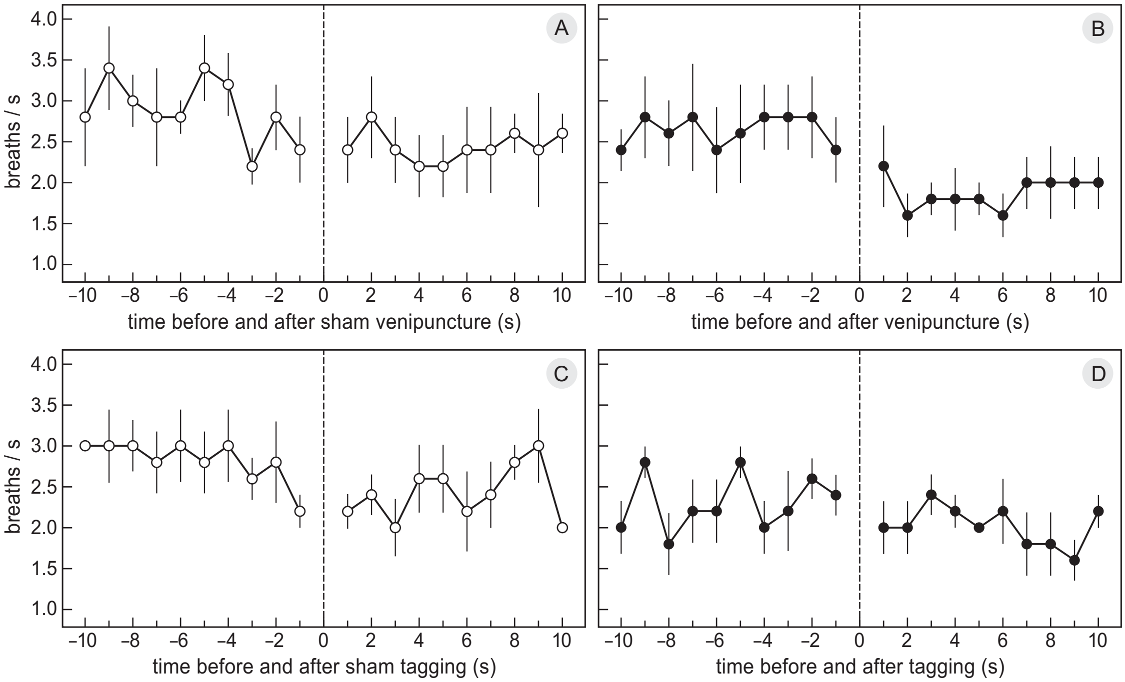

Figure 2. Change of respiration rate over the 10 s before and after venipuncture (A, B) and the 10 s before puncture for tag-implantation and after tag-insertion (C, D) for control (A, C; white circles; $n = 5$) and treatment (B, D; filled circles; $n = 5$) birds. Circles and whiskers show means and standard errors. For results of statistical analysis see Table S2.

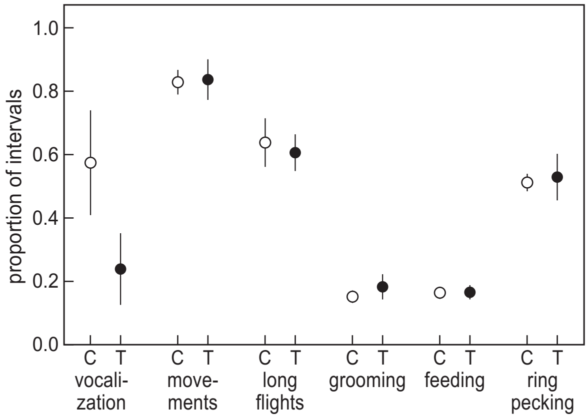

Figure 3. Occurrence of behaviours in aviaries as the proportion of 30-s intervals visible during a 6 h observation period for control ('C', white circles, *n* = 5) and treatment birds ('T', filled circles, *n* = 5). Circles and whiskers show means and standard errors. For ring pecking we estimated the mean and standard error excluding one individual of the control group that did not show this behaviour. Note that for vocalizations the number of intervals is limited to 120 (1-h observation).

Birds showed maintenance behaviour soon after release and throughout the recording period. The first occurrence of grooming was within the first 11 minutes of the recording period for all birds (mean: 2.6 ± 1.0 min) and the last occurrence was within the last hour of the recording period (mean: 14.9 ± 5.8 min before end of recording). Across all birds, the proportion of intervals with grooming behaviour was 0.17 ± 0.02. The probability of grooming did not differ between treatment and control birds (Figure 3, Table S3). The first occurrence of feeding was within the first 20 minutes of the recording period for all birds (mean: 4.1 ± 1.9 min) and the last occurrence was within 9 minutes of the end of the recording period (mean: 4.8 ± 0.9 min). Across all birds, the proportion of intervals with feeding was 0.16 ± 0.01. Birds fed briefly (mean duration of 388 feeding events: 2.7 ± 0.1 30-s intervals, median: 2 intervals) and regularly (on average every 9.2 ± 1.7 min). The probability of feeding increased slightly with ongoing recording (average change in probability from one 30-s interval to the next: 0.1%; estimate on binomial scale: 0.001; 95% CI: 0.00004 to 0.002; *P* = 0.01), but did not differ between treatment and control birds (Figure 3, Table S3).

Irritation at the site of venipuncture or of tag-implantation seemed negligible. 'Irritation back' was scored for 0.1% of intervals (4 birds; number of 30-s intervals and bird group: 5 (treatment), 2 (treatment), 2 (treatment), 1 (control)). 'Irritation wing' was scored for 0.6% of intervals (4 birds; number of intervals: 12 (treatment), 14 (control), 9 (control), 9 (control)). For the only treatment bird that showed this behaviour, the cotton wool used during venipuncture had been accidentally left under the wing and was removed by the bird, which accounted for 6 of the 12 intervals. Therefore, this parameter was not inspected statistically.

In contrast to blood sampling and PIT-tagging, we found clear evidence that ringing itself causes irritation. Nine birds showed a high rate of ring pecking throughout the recording period (Figure 4, Figure S2, Video S1 and S2). For each of these birds, the first occurrence was within 6 min after the start of the recordings (mean: 2.7 ± 0.8 min) and the last occurrence within 8 minutes of the end of the recordings (mean: 1.9 ± 0.9 min), respectively. The proportion of intervals with ring pecking was 0.52 ± 0.04 (range: 0.39–0.77). The probability of ring pecking did not

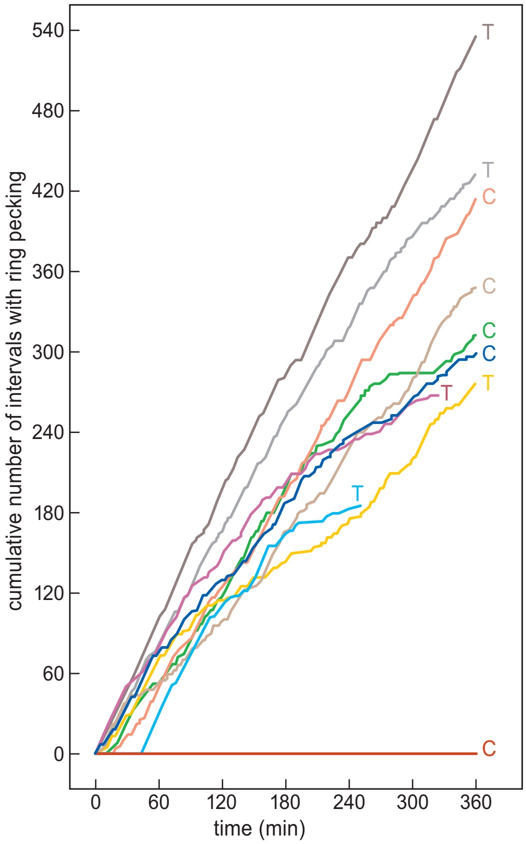

Figure 4. Temporal pattern of ring pecking across the 6 hour observation period (720 observation intervals of 30 s each). Labels 'C' and 'T' denote control and treatment birds, respectively. Note that one bird of the control group did not exhibit any ring pecking (in red). Colours code individuals.

differ between treatment and control birds (Figure 3, Table S3). The only bird that did not peck at its rings at all was the one that had been ringed prior to the experiment.

DISCUSSION

We performed a small-scale experiment to assess the immediate impact of standard procedures in ornithological field studies on bird behaviour associated with stress, pain or irritation. We collected three types of data: respiration rate during handling, behaviour during handling and behaviour during the six hours following handling. Overall, the only variable significantly affected by the procedures was ring pecking.

Direct effects of venipuncture and tag-implantation

Both blood sampling and implantation of PIT-tags involve local skin lesions of 0.07 and 3–4 mm^2, respectively. We do not expect injuries of such size to affect the physiological function of the integument. However, they may be accompanied by pain (see below). Apart from skin lesions, direct effects of subcutaneous implantation should be limited, because the tag is too small to significantly affect energetics of a bird the size of a Blue Tit (0.7% of body mass; Wilson & McMahon 2006, Fair *et al.* 2010). The back implantation method also allows for a relatively loose fit, where the tag does not place pressure on the skin or impair tissue below the integument.

Blood sampling directly affects physiology in two ways: (1) the reduction in the volume of circulatory fluid causes a decrease in systemic blood pressure; (2) the decrease in the number of circulating red blood cells reduces oxygen- and glucose-carrying capacity (Brown & Brown 2009, Fair *et al.* 2010, Voss *et al.* 2010). These effects depend strongly on the amount of blood that is sampled. The general rule of thumb assumes that 10% of a bird's blood volume can be drawn without causing adverse effects (Fair *et al.* 2010) and blood volume (in ml) can be estimated as 6% of lean body mass (in grams; Gold & Dahlsten 1983, Fair *et al.* 2010, Owen 2011). For Blue Tits, this implies that c. 50 μl can be drawn (assuming 5.5–7.5% fat, Woodburn & Perrins 1997, and a minimal body weight of 9.3 g), ten times the volume sampled here. This suggests that in our case physiological impacts of blood sampling are mild. Still, even slight physiological effects may be sufficient to cause some fatigue and reduced physical endurance, which may carry over to the bird's observed behaviour. Fatigue may also arise as consequence of struggling, agitation and blood loss, as well as through the interruption of foraging during capture (Laiolo *et al.* 2009).

We found little evidence that this is the case in the Blue Tit. All birds were alert and active immediately after release and engaged in behaviours that are at odds with a state of fatigue. Birds showed no signs of 'catch-up-feeding': feeding rate increased slightly with ongoing observation. Also, birds fed briefly, but regularly, throughout the recording period. Importantly, we found no behavioural differences between treatment and control birds.

Indirect effects of venipuncture and tag-implantation

Indirect physiological effects of venipuncture and tag-implantation arise from the stress induced by the procedures. Our data suggest that Blue Tits are able to deal well with this and the required recovery period is very short. During processing, the majority of birds at one point showed stress-related behaviours in the form of beak opening, blinking, crown erection or – in one instance – twitching. However, blinking was not continued and beak opening did not occur in the form of 'panting', but instead reflected situations in which the bird attempted to peck the hand that held it. None of the birds went into a catatonic state (Gallup 1979, Jones 1986, Forkman *et al.* 2007). If anything, respiration rates tended to decline during the recording phases. During these phases the pause in processing alone may have been sufficient to prompt some recovery. Thus, Blue Tits showed symptoms of going through an acute stress event of limited severity (Gentle 1992, Machin 2005). In line with this, after release the birds exhibited behaviours indicative of distress only in rare instances. Birds never 'puffed up' or went into a lethargic state (Machin 2005). Pronounced arousal was absent as indicated by the lack of distress calls and (except for a single interval) excessive movements. Most birds took only few minutes before they engaged in behaviours such as grooming and feeding that are inconsistent with continued high stress levels (Carstens & Moberg 2000).

Pain during venipuncture and tag-implantation

Venipuncture activates nociceptors located both in the skin and the wall of the blood vessel, while pain of subcutaneous PIT-tag implantation is restricted to nociception of the skin. The skin of birds is only locally attached and lies loosely on the subcutis at the site of injection (Stettenheim 2000, Weir & Lunam 2011), which reduces nociceptive activation when the PIT-tag moves. In several avian species, signs of pain or distress during tag-implantation are anecdotally reported as either absent or limited to a twitch (Renner & Davis 2000, Low *et al.* 2005, Brewer *et al.* 2011).

Our data suggest that acute pain during venipuncture and tagging is limited. Some of the behaviours characterizing acute pain (see above) could not be assessed, because birds were physically confined during the procedures. However, eight of ten birds exhibited defence behaviour (beak opening, crown erection) or behaviours which could reflect instantaneous responses to pain (twitching, blinking). These behaviours were also more common in treatment (5/5) than in control birds (3/5). Birds did not assume an immobile state following painful stimuli, indicating that pain was not severe or ongoing (Gentle 1992, Machin 2005, Lierz & Korbel 2012). Birds also did not vocalize.

Furthermore, our Blue Tits did not change their respiration rate before and after the procedures and respiration rate did not differ between treatment and control birds (Figure 1, 2). A sharp increase in respiration rate is expected during acute pain (Woolley & Gentle 1987, Carstens & Moberg 2000, Landa 2012, Lierz & Korbel 2012, Sneddon *et al.* 2014). Taken together, our results suggest that pain during venipuncture and tag-insertion was brief and moderate.

Pain and distress following venipuncture and tag-implantation

Short-term behavioural consequences of blood sampling are rarely reported (Murray & Fuller 2000). Several studies that implanted PIT-tags in birds anecdotally reported normal behaviour (Becker & Wendeln 1997, Carver *et al.* 1999, González-Solís *et al.* 1999, Applegate *et al.* 2000, Brewer *et al.* 2011). We also found no evidence for any negative effect. Blue Tits showed no signs of alarm and immediately started exploring the aviary, interspersed with feeding and preening. Thus, neither the preceding procedures nor being in an aviary per se appeared to impact birds. They also showed no signs of pain or distress and they did not behave as expected if they felt irritation at the venipuncture or tag-insertion site. However, all newly ringed birds spent a surprisingly high proportion of their time manipulating their rings. The birds' irritation with their rings also suggests that they were not experiencing substantial pain, stress or physiological strain. The ring pecking was not merely a displacement behaviour, because it was consistent among all previously unringed birds, but was not exhibited by the bird that had been ringed earlier.

Effects of ringing

Ringing is the most longstanding and common technique for marking birds individually and is generally considered safe (Marion & Shamis 1977, Calvo & Furness 1992, Murray & Fuller 2000). Studies considering impacts of leg rings on birds primarily deal with the occurrence and consequences of leg injuries (e.g. Sedgwick & Klus 1997, Berggren & Low 2004, Splittgerber & Clarke 2006, Pierce *et al.* 2007, Griesser *et al.* 2012, Broughton 2015, Hach *et al.* 2016). Further, coloured leg rings may affect intra- and interspecific signals (perception by mates, competitors, predators, and prey; Burley *et al.* 1982, Brodsky 1988, Tinbergen *et al.* 2013, Song *et al.* 2017). Although rarely studied, there is no evidence that rings impede performance of everyday activities (e.g. foraging or flight; Weiss & Cristol 1999, Cresswell *et al.* 2007). However, rings may impact behaviour, if the attachment of a foreign object to the leg is not readily accepted. Indeed, many songbird species have been observed manipulating their rings (House finch *Carpodacus mexicanus*, Stedman 1990, Hill 1992; Green Finch *Carduelis chloris*, Kosinski 2004; Northern Cardinal *Cardinalis cardinalis*, Lovell 1948; House Sparrow *Passer domesticus*, pers. obs.; Black-capped Chickadee *Poecile atricapillus*, Carpenter 1981), sometimes to a degree that metal rings become illegible (Tits, *Parus* ssp., Harris 1980; House Sparrow, Harris 1980).

The Blue Tits in our study spent much of the 6 hours observation time vigorously pecking at their rings, presumably in an attempt to remove them. Ringing thus substantially affected their time budget. This may have been an artefact of captivity: the birds did not have as many behavioural alternatives as in the wild and food was readily available. However, anecdotal observations suggest that birds in the wild show similar behaviour. Recently captured birds should be especially wary of predators, while pecking at the rings requires considerable attention and prevents scanning the environment. In addition, resting would have clearly saved energy and additional foraging would have increased energy reserves. Vigorous pecking at rings also demands substantial force, adding to energetic losses.

Apparently, there is marked variation between species and individuals in how well they accept rings (Ludwig 1967, Marion & Shamis 1977, Spear 1980, Carpenter 1981), which may also be influenced by sex and age (Spear 1980, Kosinski 2004). Sometimes individuals react violently to the attachment of rings (Reese 1980), even to the extent that they lose balance, fall to the ground, bleed, or are completely exhausted (Young 1941, Lovell 1948, Ludwig 1967, Carpenter 1981). In our study, individuals were observed toppling over (Video S2) or losing grasp of their perch. Further, Blue Tits sometimes quickly drew back their leg in an obvious reaction to pain resulting from having missed the ring and pecked at the skin. Reviews indicate that habituation to newly attached rings takes a short time (Marion & Shamis 1977, Calvo & Furness 1992), but birds may continue to manipulate and remove rings for days (Stedman 1990, Burton 2001, Kosinski 2004, Griesser *et al.* 2012), months (Reese 1980, Hill & Talent 1990), or even years after attachment (Young 1941, Poulding 1954, McCollough 1990, Hauff 1995).

In our study, the lack of ring pecking in the previously ringed bird and observations from the wild suggest that Blue Tits ultimately habituate to rings. In an earlier study, where birds were immediately released after procedures were completed, we observed that previously captured ‘known’ birds returned 4.4 h earlier to feed young at the nest than unknown birds and 3.1 h earlier than local recruits (Schlicht & Kempenaers 2015). Return latencies for birds in all three categories varied widely (from return after 20 min to return on the following day). Once they had returned to tending the young, birds resumed normal visit patterns. Known birds were captured, handled and measured, while unknown birds additionally received a metal ring, three colour rings and a PIT-tag, and were blood sampled. Recruits had already received a metal ring on one leg as nestlings, but otherwise were treated like unknown birds. The results of this experiment suggest that neither PIT-tagging nor blood sampling were the primary source of the delayed return of the parents. Instead, irritation caused by the newly attached rings may have played a role. Recruits returned to the nest faster than unknown birds, perhaps because they were habituated to wearing a ring on one leg and accepted new rings faster. In our experiment, the previously ringed bird was already wearing one ring on each leg. Here, the attachment of an additional colour ring on the leg with the metal ring induced no ring pecking. Interestingly, while there was no obvious weakening in ring pecking over the six hours of observation, the level of ring pecking varied considerably between individuals (Figure 4). Taken together, our results suggest that Blue Tits react strongly to the attachment of leg rings and commonly need more than half a day to habituate to their presence. Once habituation has occurred, obvious behavioural effects of rings appear absent (Schlicht & Kempenaers 2015).

Implications

We found that effects of PIT-tagging and blood sampling on bird behaviour or indices of pain or stress were restricted to an acute reaction during procedures, which was indicative of limited pain and stress and ceased immediately after completion. While the small sample size of our study clearly calls for replication and extrapolation to other species has its limitations (e.g. Brown & Brown 2009), this result is reassuring for scientists using these techniques. In addition to the already reported absence of effects on survival, reproduction, growth, condition or longer-term behaviour (Jackson & Bünger 1993, Clarke & Kerry 1998, Carver *et al.* 1999, González-Solís *et al.* 1999, Applegate *et al.* 2000, Jamison *et al.* 2000, Keiser *et al.* 2005, Low *et al.* 2005, Sheldon *et al.* 2008, Nicolaus *et al.* 2008, Tóth *et al.* 2010, Fair *et al.* 2010, Schroeder *et al.* 2011, Bridge & Bonter 2011, Brewer *et al.* 2011, Owen 2011, Ludynia *et al.* 2012, Ratnayake *et al.* 2014, Oswald *et al.* 2017), these procedures also appear to have limited immediate impact.

Our finding that ring attachment affects bird behaviour substantially over several hours suggests that these effects deserve more attention. Irritation with new rings may be underdocumented, because the bills of many species do not allow mutilation of rings to a degree that it becomes apparent at recapture. Behaviourally, discomfort with rings may be difficult to observe in species that cannot reach their rings as well as Blue Tits or that do not show pecking as a prominent component of their behavioural repertoire.

When birds spend much of their time manipulating their rings, this has important consequences. First, discomfort with new rings is a concern for animal welfare. This is especially true if individuals suffer falls, bruises, or exhaustion in their attempts to remove their rings (Young 1941, Lovell 1948, Ludwig 1967, Carpenter 1981, this study). However, high agitation is itself undesirable from an animal welfare perspective. Manipulating the rings also reduces attention to the environment, and may thus increase predation risk. Further, birds may forgo opportunities to perform alternative behaviours such as foraging, with potentially negative effects. For example, if birds are ringed during nestling feeding – a commonly employed procedure – this may have negative effects on breeding success (but see Schlicht & Kempenaers 2015).

Second, behavioural alterations due to the attachment of rings may affect the data collected by researchers. Behavioural measurements are clearly distorted by the effect ringing can have on the time budget of birds. Effects on foraging patterns, offspring feeding rates, or competitive interactions may also alter fitness estimates. These problems can be avoided to some extent by collecting data after birds have habituated to wearing rings.

Obviously, we do not question the importance of bird ringing for ornithology (Bairlein 2001, Anderson & Green 2009, Fiedler 2009, Newton 2014). It has proven an invaluable tool and has been practiced for over a century without overt negative impact on populations. However, we show here that unobtrusive effects can be substantial enough to require attention and awareness.

ACKNOWLEDGEMENTS

We are very grateful to Jonas Geurden, Leonard Scharfenberg, Lotte Schlicht, Agnes Türk and Andrea Wittenzellner for help with field work, data collection and data analysis and to Rosemarie Kentie and two anonymous reviewers for valuable comments that improved the manuscript. This work was funded by the Max Planck Society.

REFERENCES

Anderson G.Q.A. & Green R.E. 2009. The value of ringing for bird conservation. Ringing Migr. 24: 205–212.

Applegate R.D., Jamison B.E., Robel R.J. & Kemp K.E. 2000. Effects of passive integrated transponders on Ring-necked Pheasant chicks. Trans. Kansas Acad. Sci. 103: 150–156.

Bairlein F. 2001. Results of bird ringing in the study of migration routes. Ardea 89: 7–19.

Ballard G., Ainley D., Ribic C. & Barton K. 2001. Effect of instrument attachment and other factors on foraging trip duration and nesting success of Adélie Penguins. Condor 103: 481–490.

Bates D., Maechler M,. Bolker B. & Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. http://CRAN.R-project.org/package=lme4

Becker P. & Wendeln H. 1997. A new application for transponders in population ecology of the Common Tern. Condor 99: 534–538.

Berggren Å. & Low M. 2004. Leg problems and banding-associated leg injuries in a closely monitored population of North Island Robin (*Petroica longipes*). Wildl. Res. 31: 535–541.

Bowers E.K., Sakaluk S.K. & Thompson C.F. 2016. No effect of blood sampling or phytohaemagglutinin injection on post-fledging survival in a wild songbird. Ecol. Evol. 6: 3107–3114.

Brewer L.W., Redmond C.A., Stafford J.M. & Hatch G.E. 2011. Marking Ruby-throated Hummingbirds with radio frequency identification tags. J. Wildl. Manage. 75: 1664–1667.

Bridge E.S. & Bonter D.N. 2011. A low-cost radio frequency identification device for ornithological research. J. Field Ornithol. 82: 52–59.

Brodsky L.M. 1988. Ornament size influences mating success in male Rock Ptarmigan. Anim. Behav. 36: 662–667.

Broughton R.K. 2015. Low incidence of leg and foot injuries in colour-ringed Marsh Tits *Poecile palustris*. Ringing Migr. 30: 37–42.

Brown D. & Christian W. 2013. Tracker video analysis and modeling tool version 4.83. http://physlets.org/tracker/

Brown M.B. & Brown C.R. 2009. Blood sampling reduces annual survival in Cliff Swallows (*Petrochelidon pyrrhonota*). Auk 126: 853–861.

Burley N., Krantzberg G. & Radman P. 1982. Influence of colour-banding on the conspecific preference of Zebra Finches. Anim. Behav. 30: 444–455.

Burton N.H.K. 2001. Reaction of Redshank *Tringa totanus* to colour rings. Ringing Migr. 20: 213–215.

Calvo B. & Furness R.W. 1992. A review of the use and the effects of marks and devices on birds. Ringing Migr. 13: 129–151.

Carere C. & van Oers K. 2004. Shy and bold Great Tits (*Parus major*): body temperature and breath rate in response to handling stress. Physiol. Behav. 82: 905–12.

Carpenter T.W. 1981. Observations of a Black-capped Chickadee attempting to remove a band. Inl. Bird Band. 53: 10.

Carstens E. & Moberg G.P. 2000. Recognizing pain and distress in laboratory animals. ILAR J. 41: 62–71.

Carver A.V., Burger Jr. L.W. & Brennan L.A. 1999. Passive integrated transponders and patagial tag markers for Northern Bobwhite chicks. J. Wildl. Manage. 63: 162–166.

Clarke J. & Kerry K. 1998. Implanted transponders in penguins: implantation, reliability and long-term effects. J. Field Ornithol. 69: 149–159.

Class B., Kluen E. & Brommer J.E. 2014. Evolutionary quantitative genetics of behavioral responses to handling in a wild passerine.. Ecol. Evol. 4: 427–40.

Cresswell W., Lind J., Quinn J.L., Minderman J. & Whitfield D.P. 2007. Ringing or colour-banding does not increase predation mortality in Redshanks *Tringa totanus*. J. Avian Biol. 38: 309–316.

Dugger K.M., Ballard G., Ainley D.G. & Barton K.J. 2006. Effects of flipper bands on foraging behavior and survival of Adélie Penguins (*Pygoscelis adeliae*). Auk 123: 858–869.

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

Fiedler W. 2009. New technologies for monitoring bird migration and behaviour. Ringing Migr. 24: 175–179.

Forkman B., Boissy A., Meunier-Salaün M.-C., Canali E. & Jones R.B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92: 340–374.

Fucikova E., Drent P.J., Smits N. & van Oers K. 2009. Handling stress as a measurement of personality in Great Tit nestlings (*Parus major*). Ethology 115: 366–374.

Gallup G.G. 1979. Tonic Immobility as a measure of fear in Domestic Fowl. Anim. Behav. 27: 316–317.

Gentle M. 1992. Pain in birds. Anim. Welf. 1: 235–247.

Gentle M.J. & Hunter L.N. 1990. Physiological and behavioural responses associated with feather removal in *Gallus gallus* var *domesticus*. Res. Vet. Sci. 50: 95–101.

Gold C. & Dahlsten D. 1983. Effects of parasitic flies (*Protocalliphora* spp.) on nestlings of Mountain and Chestnut-backed Chickadees. Wilson Bull. 95: 560–572.

González-Solís J., Becker P. & Wendeln H. 1999. Divorce and asynchronous arrival in Common Terns *Sterna hirundo*. Anim. Behav. 58: 1123–1129.

Griesser M., Schneider N.A., Collis M.-A., Overs A., Guppy M., Guppy S., Takeuchi N., Collins P., Peters A. & Hall M.L. 2012. Causes of ring-related leg injuries in birds – evidence and recommendations from four field studies. PLoS One 7: e51891.

Hach S., Bertrand P., Fiola M.-A., Thériault S., Bayne E.M. & Villard M.-A. 2016. Band-related foot loss does not prevent successful return and reproduction in the Ovenbird (*Seiurus aurocapilla*). Wilson J. Ornithol. 128: 913–918.

Hadfield J.D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J. Stat. Softw. 33: 1–22.

Harris M.P. 1980. Loss of weight and legibility of bird rings. Ringing Migr. 3: 41–49.

Hauff P. 1995. Seeadler (*Haliaeetus albicilla*) entfernen Aluminium-Ringe. Vogelwarte 38: 113–116.

Hill G.E. 1992. An inexpensive source of colored leg bands. J. Field Ornithol. 63: 408–410.

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

Hothorn T., Bretz F. & Westfall P. 2008. Simultaneous inference in general parametric models. Biometrical J. 50: 346–363.

Hoysak D. & Weatherhead P. 1991. Sampling blood from birds: a technique and an assessment of its effect. Condor 93: 746–752.

Jackson D.H. & Bünger W.H. 1993. Evaluation of passive integrated transponders as a marking technique for turkey poults. J. Iowa Acad. Sci. 100: 60–61.

Jamison B.E., Beyer R.S., Robel R.J. & Pontius J.S. 2000. Passive integrated transponder tags as markers for chicks. Poult. Sci. 79: 946–8.

Jones R.B. 1986. The tonic immobility reaction of the Domestic Fowl: a review. World's Poult. Sci. J. 42: 82–96.

Keiser J.T., Ziegenfus C.W.S. & Cristol D. a. 2005. Homing success of migrant versus nonmigrant Dark-Eyed Juncos (*Junco hyemalis*). Auk 122: 608–617.

Kosinski Z. 2004. The removal of colour rings by Greenfinches *Carduelis chloris*. Ringing Migr. 22: 4–6.

Kuznetsova A., Brockhoff P.B. & Christensen R.H.B. 2016. lmerTest: tests in linear mixed effect models. R package version 2.0-30. http://CRAN.R-project.org/package=lmerTest

Laiolo P., Banda E., Lemus J.A., Aguirre J.I. & Blanco G. 2009. Behaviour and stress response during capture and handling of the Red-billed Chough *Pyrrhocorax pyrrhocorax* (Aves: Corvidae). Biol. J. Linn. Soc. 96: 846–855.

Landa L. 2012. Pain in domestic animals and how to assess it : a review. Vet. Med. 57: 185–192.

Langford D.J., Bailey A.L., Chanda M.L., Clarke S.E., Drummond T.E., Echols S., Glick S., Ingrao J., Klassen-Ross T., Lacroix-Fralish M.L., Matsumiya L., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wong D., van den Maagdenberg A.M.J.M., Ferrari M.D., Craig K.D. & Mogil J.S. 2010. Coding of facial expressions of pain in the laboratory mouse. Nat. Methods 7: 447–449.

Lierz M. & Korbel R. 2012. Anesthesia and analgesia in birds. J. Exot. Pet Med. 21: 44–58.

Lovell H.B. 1948. The removal of bands by Cardinals. Bird-Banding 19: 71–72.

Low M., Eason D. & McInnes K. 2005. Evaluation of passive integrated transponders for identification of Kakapo *Strigops habroptilus*. Emu 105: 33–38.

Ludwig J. 1967. Band loss – its effect on banding data and apparent survivorship in the Ring-billed Gull population of the Great Lakes. Bird-Banding 38: 309–323.

Ludynia K., Dehnhard N., Poisbleau M., Demongin L., Masello J.F. & Quillfeldt P. 2012. Evaluating the impact of handling and logger attachment on foraging parameters and physiology in Southern Rockhopper Penguins. PLoS One 7: e50429.

Machin K.L. 2005. Avian pain: physiology and evaluation. Compend. Contin. Educ. Pract. Vet. 27: 98–109.

Marion W.R. & Shamis J.D. 1977. An annotated bibliography of bird marking techniques. Bird-Banding 48: 42–61.

McCollough M.A. 1990. Evaluation of leg markers for Bald Eagles. Wildl. Soc. Bull. 18: 298–303.

Murray D. & Fuller M. 2000. A critical review of the effects of marking on the biology of vertebrates. In: Boitani L. & Fuller T.K. (eds) Research techniques in animal ecology. Controversies and consequences. Columbia University Press, New York NY pp. 15–64.

Newton I. 2014. Is bird ringing still necessary? Br. Birds 107: 572–574.

Nicolaus M., Bouwman K. & Dingemanse N. 2008. Effect of PIT tags on the survival and recruitment of Great Tits *Parus major*. Ardea 96: 286–292.

Oswald K.N., Evlambiou A.A., Ribeiro Â.M. & Smit B. 2017. Tag location and risk assessment for passive integrated transponder-tagging passerines. Ibis doi:10.1111/ibi.12558

Owen J.C. 2011. Collecting processing and storing avian blood: a review. J. Field Ornithol. 82: 339–354.

Pierce A.J., Stevens D.K., Mulder R. & Salewski V. 2007. Plastic colour rings and the incidence of leg injury in Flycatchers (*Muscicapidae monarchidae*). Ringing Migr. 23: 205–210.

Poulding R.H. 1954. Loss of rings by marked Herring Gulls. Bird Study 1: 37–40.

R Core Team. 2015. R: a language and environment for statistical computing. Vienna, Austria. www.R-project.org

Ratnayake C.P., Morosinotto C., Ruuskanen S., Villers A. & Thomson R.L. 2014. Passive integrated transponders (PIT) on a small migratory passerine bird: absence of deleterious short and long-term effects. Ornis Fenn. 91: 1–12.

Redmond L. & Murphy M. 2011. Multistate mark-recapture analysis reveals no effect of blood sampling on survival and recapture of Eastern Kingbirds (*Tyrannus tyrannus*). Auk 128: 514–521.

Reese K.P. 1980. The retention of colored plastic leg bands by Black-billed Magpies. North Am. Bird Bander 5: 136–137.

Renner M. & Davis L.S. 2000. Marking penguins with implanted transponders. Notornis 47: 163–165.

Schlicht E. & Kempenaers B. 2015. Immediate effects of capture on nest visits of breeding Blue Tits *Cyanistes caeruleus* are substantial. Anim. Behav. 105: 63–78.

Schroeder J., Cleasby I., Nakagawa S., Ockendon N. & Burke T. 2011. No evidence for adverse effects on fitness of fitting passive integrated transponders (PITs) in wild House Sparrows *Passer domesticus*. J. Avian Biol. 42: 271–275.

Sedgwick J.A. & Klus R.J. 1997. Injury due to leg bands in Willow Flycatchers. J. Field Ornithol. 68: 622–629.

Sheldon L.D., Chin E.H., Gill S.A., Schmaltz G., Newman A.E.M. & Soma K.K. 2008. Effects of blood collection on wild birds: an update. J. Avian Biol. 39: 369–378.

Smith R.J., Benson T.J. & Carey M. 2017. Blood sampling does not negatively affect apparent survival, recapture probability, or within-season site fidelity in Field Sparrows (*Spizella pusilla*). Auk 134: 240–246.

Sneddon L.U., Elwood R.W., Adamo S.A. & Leach M.C. 2014. Defining and assessing animal pain. Anim. Behav. 97: 201–212.

Song Z., Liu Y., Booksmythe I. & Ding C. 2017. Effects of individual-based preferences for colour-banded mates on sex allocation in Zebra Finches. Behav. Ecol. 28: 1228–1235.

Spear L. 1980. Band loss from the Western Gull on southeast Farallon Island. J. Field Ornithol. 51: 319–328.

Splittgerber K. & Clarke M.F. 2006. Band-related leg injuries in an Australian passerine and their possible causes. J. Field Ornithol. 77: 195–206.

Stangel P.W. 1986. Lack of effects from sampling blood from small birds. Condor 88:244–245.

Stedman S.J. 1990. Band opening and removal by House Finches. North Am. Bird Bander 15: 136–138.

Stettenheim P.R. 2000. The integumentary morphology of modern birds – an overview. Am. Zool. 40: 461–477.

Svensson L. 1992. Identification guide to European passerines. Fingraf, Stockholm.

Tinbergen J.M., Tinbergen J. & Ubels R. 2013. Is fitness affected by ring colour? Ardea 101: 153–163.

Tóth Á., Gaál K.K., Turcsán Z., Ásványi-Molnár N., Ásványi B., Szigeti J. & Fébel H. 2010. Tracking possibilities in the poultry sector – a review. Arch. Tierzucht 53: 328–336.

Voss M., Shutler D. & Werner J. 2010. A hard look at blood sampling of birds. Auk 127: 704–708.

Weir K. & Lunam C. 2011. The structure and sensory innervation of the integument of ratites. In: Glatz P., Lunam C. & Malecki I. (eds) The welfare of farmed ratites. Springer, Berlin, Heidelberg, pp. 131–145.

Weiss V.A. & Cristol D.A. 1999. Plastic color bands have no detectable short-term effects on White-breasted Nuthatch behavior. Condor 101: 884–886.

Wilson R. & McMahon C. 2006. Measuring devices on wild animals: what constitutes acceptable practice? Front. Ecol. Environ. 4: 147–154.

Woodburn R. & Perrins C. 1997. Weight change and the body reserves of female Blue Tits *Parus caeruleus* during the breeding season. J. Zool. Lond. 243: 789–802.

Woolley S. & Gentle M. 1987. Physiological and behavioural responses in the Hen (*Gallus domesticus*) to nociceptive stimulation. Comp. Biochem. Physiol. 88A: 27–31.

Young J. 1941. Unusual behavior of a banded Cardinal. Wilson Bull. 53: 197–198.

SAMENVATTING

Het nemen van een bloedmonster via de vleugelader en het onderhuids implanteren van zogenaamde PIT-tags (*Passive Integrated Transponders*) zijn veelgebruikte technieken in het hedendaags ornithologisch onderzoek. De langetermijneffecten van deze handelingen op in het wild levende vogels zijn al in detail bestudeerd, maar het blijft onduidelijk welke invloed ze hebben gedurende en meteen na de behandeling. Hier testen we de impact van deze procedures op de ademhalingsfrequentie en op gedragingen die samenhangen met pijn, stress en ongemak bij Pimpelmezen *Cyanistes caeruleus*. Tien in het wild gevangen Pimpelmezen werden in twee groepen verdeeld: vijf vogels werden gemeten, geringd en geïmplanteerd met een PIT-tag; daarnaast werd een bloedmonster afgenomen (verder aangeduid als 'behandelde groep'). De andere vijf vogels werden op exact dezelfde manier behandeld, maar zonder in de ader van de vleugel te prikken en zonder een PIT-tag te implanteren (verder aangeduid als 'controlegroep'). De ademhalingsfrequentie tijdens de behandeling verschilde niet tussen de behandelde en de controlegroep, maar de vogels in de behandelde groep toonden wel gedrag dat wijst op een acute stressgebeurtenis geassocieerd met een korte en matige pijn. Nadat ze waren vrijgelaten in een volière toonden de behandelde en de controlegroep geen verschil in gedrag. Bovendien toonde geen enkel individu gedrag dat kan wijzen op pijn of stress. In plaats daarvan foerageerden ze, streken ze hun verenkleed glad en verkenden ze de volière. Ze brachten wel veel tijd door met het pikken aan de aangelegde ringen. Onze resultaten suggereren dat bloedafname en implantatie van PIT-tags slechts geringe kortetermijneffecten hebben op Pimpelmezen. Het hanteren en ringen van de vogels kan echter aanzienlijke gedragsveranderingen veroorzaken, die mogelijk relevant kunnen zijn voor het dierenwelzijn en voor de kwaliteit van de verzamelde gegevens.

Corresponding editor: Roos Kentie
Received 17 November 2017; accepted 5 March 2018

Supplimentary Material is available online
www.ardeajournal.nl/supplement/s106–39–50

SUPPLEMENTARY MATERIAL

Table S1. Changes in respiration rate (respirations/s) during procedures and differences between experimental groups (treatment vs.control). Respiration rates were recorded during three phases: before bleeding, between bleeding and implantation, after implantation. Results from linear mixed-effects models (package MCMCglmm) with bird identity as a random factor (variance explained by random factor < 0.01%). Effect sizes and *P*-values were similar in a model without the interaction term (not shown).

	Estimate (95% CI)	***P* (MCMC)**
Intercept: experimental group = control, recording phase = before	3.29 (2.93–3.68)	<0.001
Experimental group (treatment vs. control)	–0.33 (–0.85–0.17)	0.21
Recording phase (between vs. before)	–0.06 (–0.55–0.48)	0.82
Recording phase (after vs. before)	–0.42 (–0.94–0.11)	0.12
Recording phase (after vs. between)*	–0.35 (–0.82–0.21)	0.18
Experimental group × recording phase (between vs. before)	–0.10 (–0.84–0.55)	0.79
Experimental group × recording phase (after vs. before)	0.33 (–0.40–0.99)	0.35
Experimental group × recording phase (after vs. between)*	0.42 (–0.30–1.08)	0.25

* This result was obtained from a model where factor levels were shifted so as to include this comparison (i.e. intercept is not identical).

Table S2. Effect of experimental treatment on respiration rate (respirations/s). Linear mixed-effects models (package MCMCglmm) with bird identity as random factor were used to compare periods before and after a particular procedure or to investigate changes in respiration rate after a particular procedure was finished. Unless otherwise indicated, all *P*-values and effect sizes remained similar when interactions were removed from the models.

	Estimate (95% CI)	*P* (MCMC)
Venipuncture – 10 s before and after[1]		
Intercept	2.22 (1.66–2.83)	<0.001
Interval (before vs. after)	0.71 (0.4–0.99)	<0.001
Experimental group (treatment vs. control)	–0.52 (–1.5–0.18)	0.17
Interval × experimental group	0.12 (–0.34–0.52)	0.56
Venipuncture – 2 s before and after[2]		
Intercept	2.63 (1.90–3.32)	<0.001
Interval (before vs. after)	–0.07 (–0.65–0.53)	0.81
Experimental group (treatment vs. control)	–0.76 (–1.94–0.23)	0.16
Interval × experimental group	0.58 (–0.21–1.45)	0.18
Venipuncture – changes during 10 s after[3]		
Intercept	2.73 (2.06–3.37)	<0.001
Time after treatment (s)	–0.09 (–0.15—0.01)	0.02
Experimental group (treatment vs. control)	–0.56 (–1.46–0.36)	0.20
Time × experimental group	0.01 (–0.09–0.10)	0.83
PIT-tagging – 10 s before and after[4]		
Intercept	2.42 (1.96–2.87)	<0.001
Interval (before vs. after)	0.43 (0.22–0.65)	<0.001
Experimental group (treatment vs. control)	–0.43 (–1.13–0.14)	0.14
Interval × experimental group	–0.09 (–0.41–0.21)	0.58
PIT-tagging – 2 s before and after		
Including interaction[5]		
Intercept	2.25 (1.8–2.72)	<0.001
Interval (before vs. after)	0.28 (–0.14–0.72)	0.20
Experimental group (treatment vs. control)	–0.32 (–0.99–0.29)	0.32
Interval × experimental group	0.25 (–0.36–0.82)	0.40
Without interaction[6]		
Intercept	2.19 (1.74–2.64)	<0.001
Interval (before vs. after)	0.41 (0.14–0.71)	0.01
Experimental group (treatment vs. control)	–0.21 (–0.76–0.41)	0.46
PIT-tagging – changes during 10 s after[7]		
Intercept	2.29 (1.7–2.76)	<0.001
Time after treatment (s)	0.02 (–0.03–0.08)	0.39
Experimental group (treatment vs. control)	–0.19 (–0.99–0.53)	0.59
Time × experimental group	–0.04 (–0.11–0.03)	0.29

[1] Percent variance explained by random factor 'bird ID': 36% (95% CI: 12–50%)
[2] Percent variance explained by random factor 'bird ID' : 54% (95% CI: 12–86%)
[3] Percent variance explained by random factor 'bird ID': 31% (95% CI: 7–47%)
[4] Percent variance explained by random factor 'bird ID': 38% (95% CI: 14–54%)
[5] Percent variance explained by random factor 'bird ID': <0.01%
[6] Percent variance explained by random factor 'bird ID': 41% (95% CI: 0–53%)
[7] Percent variance explained by random factor 'bird ID': 43% (95% CI: 16–57%)

Table S3. Comparison of behaviour after release in the aviary between treatment and control birds (see Methods for descriptions). For vocalizations, data are available only for the first 60 minutes. Results are from generalized linear mixed-effects models (packages lme4 and lmerTest) with binomial error structure (logit-link function) and bird identity as random factor.

	Estimate (95% CI)	***P*-value**
Vocalization[1]		
Intercept	0.3 (–1.3 to 1.9)	0.67
Experimental group (treatment vs. control)	–1.8 (–4.1 to 0.5)	0.09
Movements[2]		
Intercept	1.7 (0.6 to 2.8)	<0.001
Experimental group (treatment vs. control)	0.5 (–1.0 to 2.1)	0.45
Long flights[3]		
Intercept	0.7 (0.04 to 1.3)	0.02
Experimental group (treatment vs. control)	–0.2 (–1.0 to 0.7)	0.64
Grooming[4]		
Intercept	–1.7 (–2.2 to –1.3)	<0.001
Experimental group (treatment vs. control)	0.1 (–0.5 to 0.8)	0.62
Feeding[5]		
Intercept	–1.7 (–1.9 to –1.4)	<0.001
Experimental group (treatment vs. control)	0.004 (–0.4 to 0.4)	0.98
Ring pecking[6]		
Intercept	–1.3 (–3.1 to 0.6)	0.14
Experimental group (treatment vs. control)	1.4 (–1.2 to 4.0)	0.25

[1] Percent variance explained by random factor 'bird ID': 62%
[2] Percent variance explained by random factor 'bird ID': 53%
[3] Percent variance explained by random factor 'bird ID': 38%
[4] Percent variance explained by random factor 'bird ID': 32%
[5] Percent variance explained by random factor 'bird ID': 20%
[6] Percent variance explained by random factor 'bird ID': 66%

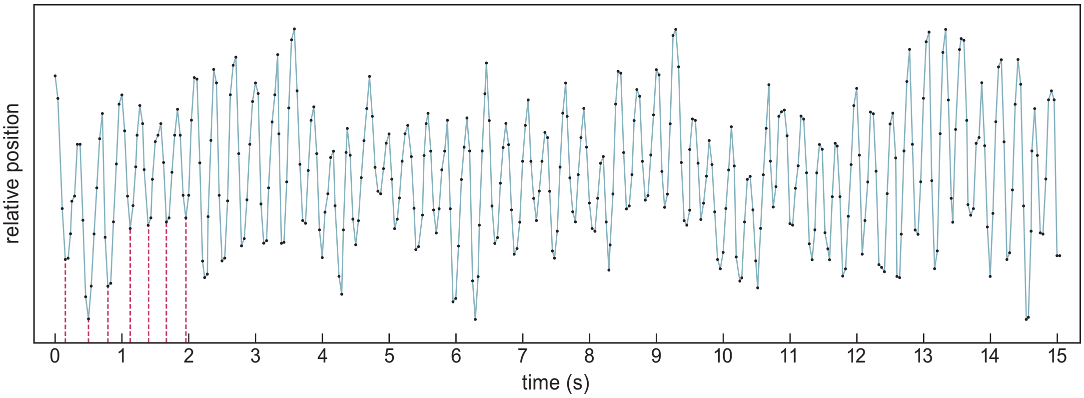

Figure S1. Example for tracked movements during respiration for one bird over 15 s. Black dots indicate the registered position, connected by blue lines. One up and down movement represents one respiration. For example, there were five breaths in the first two s (indicated by red broken lines).

Figure S2. Photographs illustrating ring pecking in the Blue Tit. These pictures were taken as part of another experiment. The presentation of the behaviour is representative also for this study (Videos S1 and S2).

Video S1. Example sequence showing aviary setup and recording situation. The bird is primarily pecking at its rings. At 3:11 minutes the bird is briefly out of sight. https://youtu.be/z_2DjubelkU

Video S2. Example sequence showing bird toppling over while pecking at its rings. https://youtu.be/vHuf9ZNm7ac