 doi: 10.1111/ibi.12558

Short communication

Tag location and risk assessment for passive integrated transponder-tagging passerines

KRISTA N. OSWALD,[1*†] ANTHONY A. EVLAMBIOU,[1] ÂNGELA M. RIBEIRO[2] & BEN SMIT[1†]
[1]*Department of Zoology, Nelson Mandela University, Summerstrand, 6031 Port Elizabeth, South Africa*
[2]*Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark*

Understanding changes in body temperature is central to several fields in biology, but determining these changes accurately without harming or restraining individuals can be challenging, particularly for small species. We tested first whether body temperature readings differed between passive integrated transponder (PIT) tags injected subcutaneously inter-scapulae (i.e. solely through the skin) and intra-peritoneally (through the skin and abdominal muscle wall) and, secondly, whether intra-peritoneal tag injuries differed among three weight classes of passerines. We found no significant difference in body temperature readings between subcutaneous inter-scapulae and intra-peritoneal PIT-tags, and observed that the intra-peritoneal injection of PIT-tags may cause adverse effects among smaller (<25 g) birds. Our findings suggest a reduced gradient between core and peripheral body temperature in small species, which to the best of our knowledge has not yet been quantified. We further show that the risk of detrimental injury was greatest in small species, and thus recommend implanting PIT-tags subcutaneously between the scapulae for smaller birds.

Keywords: body temperature, PIT-tagging, risk assessment, tag location.

Body temperature (T_b) is considered one of the most influential biological traits; in endotherms, it can vary in relation to climate, circadian cycle, metabolic status and pathological states, among others (McNab 2002, Bicudo *et al.* 2010). Therefore, being able accurately to measure T_b has been fundamental for behavioural and eco-physiological studies, especially of birds (e.g. Swanson & Olmstead 1999, McKechnie & Lovegrove 2002, Tieleman *et al.* 2002, McKechnie *et al.* 2015).

Several approaches have been used to measure body temperature. Until the early 2000s, T_b measurements were taken from restrained animals by inserting a thermocouple into the cloaca or rectum (e.g. Maloney & Dawson 1998, McKechnie & Wolf 2004), inserting a thermocouple into the brainstem (Hammel *et al.* 1967), or surgically implanting temperature-sensitive loggers and transmitters (Meerlo *et al.* 1996, Luheshi *et al.* 1999). Recently, researchers have been opting for implanting temperature-sensitive passive integrated transponders (PIT-tags) to obtain measures of T_b (e.g. Milne *et al.* 2015, McKechnie *et al.* 2016a, Nord *et al.* 2016). This change was prompted by multiple concerns regarding thermocouples. First, cloacal/rectal thermocouples can increase stress levels when secured to the animal's integument (McKechnie *et al.* 2016b) and hence lead to stress-induced hyperthermia (Bouwknecht *et al.* 2007, McKechnie *et al.* 2016b). Secondly, risk of lacerating internal organs has been recorded in Black Rockfish *Sebastes melanops* (Parker & Rankin 2003) and observed in multiple Kalahari passerines (B. Smit pers. obs.). Thirdly, the data obtained may not be indicative of core T_b if thermocouple wires loosen or fall out during measurements. Indeed, Wacker *et al.* (2012) compared T_b via a rectally inserted thermocouple with subcutaneously inserted PIT-tags in the Striped-faced Dunnart *Sminthopsis macroura* and found that PIT-tags were more accurate at temperatures above 24 °C.

Although the above experience suggests that the move from thermocouple to PIT-tag implantation has reduced many negative handling effects, PIT-tag implantation may also cause harm to individuals and the T_b values may be subject to bias depending on tag location within the body of the animal. Although several studies have reported no significant deleterious effects of PIT-tags injected subcutaneously in mammals (Fagerstone & Johns 1987), reptiles (Keck 1994, Jemison *et al.* 1995), large non-passerine birds (Jamison *et al.* 2000, Gauthier-Clerc *et al.* 2004) and, more recently, small passerines (Nicolaus *et al.* 2008, Schroeder *et al.* 2011, Ratnayake *et al.* 2014), there are no comparative data regarding the effect of PIT-tag placement on animal condition and measured values of T_b.

Our aim was to evaluate whether PIT-tags injected subcutaneously inter-scapulae (SC) are a lower-risk alternative to intra-peritoneal (IP) implants. This involved two main experiments: in the first we tested whether core and subcutaneous T_b (determined through SC and IP implants) vary in their response to varying air temperatures in a small (~15 g) bird. Additionally, we tested whether short-term survival differed between three

†Present address: Department of Zoology and Entomology, Life Sciences Building, Rhodes University, African Street Grahamstown 6139, South Africa.

*Corresponding author.
Email: knoswald@gmail.com

passerine species ranging from ~15 to ~55 g in body mass, and whether short-term survival differed between SC and IP implants in the ~15 g species. We predicted that SC implants would be less harmful than IP implants because SC tags are injected solely through the skin wall, whereas IP tags are injected through the skin and abdominal muscle wall.

METHODS

PIT-tags

Temperature-sensing passive integrated transponders are electronic microchips with unique identification codes capable of measuring temperature. These chips are glass-encased and do not require a power source; they are activated electromagnetically when a transceiver is placed nearby and hence have an unlimited lifespan allowing for multiple discrete measurements (Gibbons & Andrews 2004, McCafferty *et al.* 2015). We used BioThermo13 tags in conjunction with a standard 13 × 2.12 mm FDX-B 134.2 kHz PIT-tag (Biomark, Boise, ID, USA), hereafter referred to as PIT-tags.

Study species

Zebra Finches *Taeniopygia guttata*, native to Australia with an average wild-living body mass (M_b) of ~12 g (Meijer *et al.* 1996), were our small-sized passerine. Domesticated captive-living Zebra Finches (generally with greater M_b than wild-living counterparts, see below) were donated by a local aviculturist and tested between May and June 2016. Karoo Scrub-robins *Cercotrichas coryphaeus*, endemic to southern Africa, had an average M_b of ~20 g (Hockey *et al.* 2005) and were our medium-sized species. Wild-living Karoo Scrub-robins were captured between November 2015 and July 2016 from six different sites in South Africa – two in Western Cape province and four in Northern Cape province. Cape Rockjumpers *Chaetops frenatus*, endemic to the Cape Fold Mountains of South Africa with an average M_b of ~57 g (Hockey *et al.* 2005), were the largest species we tested. Cape Rockjumpers were captured between July 2015 and January 2016 at Blue Hill Nature Reserve in the Western Cape of South Africa.

In the context of this study, we henceforth refer to these species as 'finches', 'scrub-robins' and 'rockjumpers', respectively.

Species body mass and implant location

After cleansing the skin with 96% ethanol we injected sterilized PIT-tags using a plastic syringe with a 3.17-mm non-replaceable sterile needle. After implantation, the puncture hole was sealed with cyanoacrylate adhesive. The whole procedure took less than 1 min.

Finches were alternately implanted subcutaneously inter-scapulae (SC, n = 10) or intra-peritoneally (IP, n = 11) (following Nord *et al.* 2016). Their body mass was: (mean ± sd): males = 15.31 ± 1.48 g, females = 15.15 ± 2.0 g. Of the 11 finches given IP tags, one female died within 2 days before experimentation due to PIT-tag implantation and another female died during experimentation, also as a consequence of implantation, reducing finch IP sample size to nine individuals.

Scrub-robins and rockjumpers had only IP implantation of PIT-tags. The sample sizes were 73 scrub-robins (41 males, 31 females, one unknown; mean M_b ± sd: males = 19.40 ± 1.41 g, females = 18.70 ± 1.19 g), and 35 rockjumpers (16 males, 14 females, five immature hence sex unknown; mean M_b ± sd: males = 57.7 ± 3.3 g, females = 52.1 ± 3.5 g, immature = 48.59 ± 2.01 g).

Risk assessment: studies using IP implants

Eleven finches (six females, five males) were injected IP and housed in outdoor aviaries (200 × 150 × 200 cm) with food and water available *ad libitum*.

Scrub-robins had IP PIT-tags injected as part of a study examining physiological change across an environmental gradient at two time points – summer and winter. Scrub-robins were kept in cages (15 × 12 × 22 cm) with food provided *ad libitum* for no longer than 48 h after capture, after which 53 individuals were released at the point of capture. Twenty birds were killed and then examined. In these birds we looked for evidence of internal injuries that PIT-tags could have caused. One individual (female) was re-captured during summer with its winter PIT-tag still present and active.

Rockjumpers had IP PIT-tags injected as part of a separate seasonal physiological study. Rockjumpers were kept in temporary cages (30 × 30 × 40 cm) with food provided *ad libitum* for no longer than 48 h after capture before being released at the point of capture. Four individuals trapped in winter were re-trapped in summer, with three given new tags in summer (n = two males, one female) as palpation showed their previous PIT-tags had been lost, possibly due to improperly sealed punctures. In five of the nine territories defended by rockjumpers that were given PIT-tags, we performed *ad hoc* observations at Blue Hill Nature Reserve from January 2016 to October 2016, recording any re-sighted individuals (n = 20 of a possible n = 27).

Experimental protocol to compare IP and SC approaches

In addition to the 11 finches that were IP tagged we also injected 10 birds by the SC method (five females, five males). Finches were housed in outdoor aviaries (200 × 150 × 200 cm) with food and water available *ad libitum*. After experimentation, we returned them to the aviculturist who had donated them initially.

After insertion of PIT-tags, finches were placed in a respirometry chamber consisting of a 1.5-L bird chamber constructed from airtight plastic fitted with a wire-mesh platform to ensure normal posture (Smit & McKechnie 2010) and placed in an environmental chamber constructed from a 100-L cool-box lined with copper tubing where temperature-regulated water circulated to control air temperature (T_{air}). A small fan was used to ensure air circulation within the chamber. Each bird was subjected to three temperatures (T_{air} ≈ 5, 30 and 40 °C) for either 1 h (T_{air} ≈ 5 and 30 °C) or 30 min (T_{air} ≈ 40 °C) each. Body temperature was recorded every minute using a portable PIT-tag reader (Biomark HPR plus; Biomark) placed inside the environmental chamber. Sample sizes were 10 (five male, five female) for the T_{air} ≈ 40 °C treatment and nine (five male, four female) for the T_{air} ≈ 5 and 30 °C treatments.

Data analysis

All analyses were performed in R version 3.1.2 (R Core Team, 2014). Data were checked for normality using Levene's test with package *car* (Fox & Weisberg 2011). Linear mixed-effects models, as implemented in package *nlme* (Pinheiro *et al.* 2014), were fitted to the response variable (T_b) using predictor variables T_{air}, treatment, sex and individual identification as a random factor; as we only tested the finches at three T_{air} we did not include interaction effects for the variables. We assumed an alpha value of 0.05 to assess significance, with data presented as means ± sd.

Short-term survival analysis

We assessed short-term survival based on whether an individual showed signs of physical distress: lethargy, abstaining from feeding, erratic movement or mortality within a 24-h period after PIT-tag implantation. If any of these signs were observed, individuals were considered to have sustained an 'injury'. For scrub-robins that were subject to necropsy, we considered the bird to have sustained an injury if any internal organ was damaged, namely perforation of intestine, liver or pancreas, or when an internal haemorrhage was found (performed by A.M.R.)

RESULTS

We found no significant effect of sex ($\chi^2_{1,58}$ = 0.07, P = 0.80) or treatment ($\chi^2_{1,58}$ = 0.26, P = 0.61) on finch T_b (Fig. 1). The only significant predictor of T_b in our models was T_{air}; T_b was significantly positively related to T_{air} ($\chi^2_{1,58}$ = 54.7; P < 0.001).

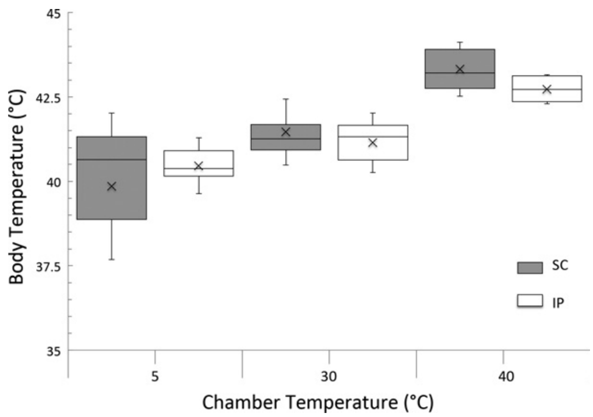

Figure 1. Variation of body temperature of Zebra Finches *Taeniopygia guttata* with PIT-tags inserted either intra-peritoneally (IP) or subcutaneously inter-scapulae (SC) at air temperatures of ≈5, 30 or 40 (n = 19, 19 and 20 respectively). Boxplots show 1st quartile, median and 3rd quartile, 'x' indicates mean, and whiskers are standard deviation.

The largest injury percentage, 18.2%, was recorded for the IP treatment in finches, our small-sized passerine, with some injuries, 2.7%, also recorded for IP implants among the scrub-robins, the medium-sized passerine (Fig. 2). No injuries were recorded for either the SC finches or the IP rockjumpers (Fig. 2).

DISCUSSION

A shortcoming in thermal biology research has been the ability accurately to quantify T_b while minimizing the potential impact of the method used on the individual's condition. Selecting the placement location of thermo-sensitive PIT-tags by invasive procedures is thus critical to minimize risk and maximize the validity of the T_b measures obtained. We compared the placement of PIT-tags subcutaneously to demonstrate that estimated body temperature in a small bird (finch) was similar whether PIT-tags were placed underneath the skin or implanted in the abdominal cavity. This contrasts with previous studies showing more variable external skin temperature measurements as compared with core or subcutaneous estimates of body temperature (Körtner *et al.* 2000, Dausmann 2005, Boyles *et al.* 2010). Differences between peripheral and core T_b seem particularly large in larger species. For example, Körtner *et al.* (2000) found that T_b differed from 27.2 °C for skin to 29.1 °C for core T_b in ~500 g Tawny Frogmouths *Podargus strigoides*. Our results in finches (~15 g) therefore suggest a reduced gradient between core and peripheral T_b in small species. To the best of our knowledge the effect of

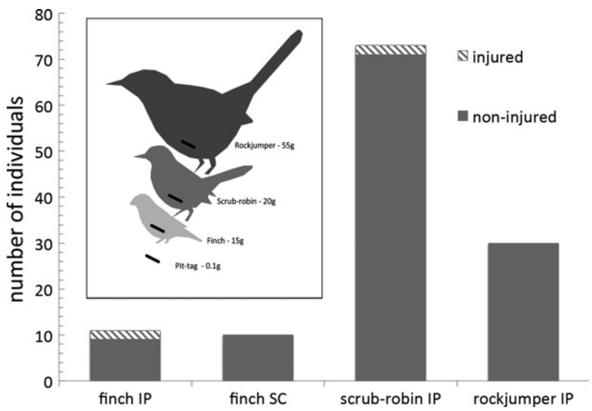

Figure 2. Number of individuals given PIT-tags for three different species of bird: Zebra Finch *Taeniopygia guttata*, 'finch'; Karoo Scrub-robin *Cercotrichas coryphaeus*, 'scrub-robin' and Cape Rockjumper *Chaetops frenatus*, 'rockjumper'. Inset depicts the relative size of the birds and PIT-tag. IP, intra-peritoneal; SC, inter-scapulae.

animal size on the difference between subcutaneous and core T_b has not yet been quantified.

We further show that the risk of detrimental injury was greatest in small species. Moreover, risk of injury and mortality was only observed when injecting PIT-tags into the abdominal cavity of the finches we studied (Fig. 2). In addition to the four injuries previously noted (two finches and two scrub-robins), two scrub-robins had liver damage discovered during necropsy. This suggests that there may have been additional injuries in non-euthanized individuals that went unnoticed. No protocol was set in place to measure long-term survival (i.e. post-release), but our *ad hoc* observations of rockjumpers indicated that most individuals (74% re-sighted) survived and were actively breeding up to 1 year after experimentation.

We propose that while IP PIT-tagging may be suitable for slightly larger birds illustrated here by the rockjumpers, the lack of significant T_b differences between IP and SC implantation in finches suggests the less invasive SC method should be preferentially chosen for smaller species. Indeed, we also suggest that a similar study should be performed among other taxa to test whether similar conclusions are reached. We conclude that there seems to be no disadvantage to injecting PIT-tags following the SC method instead of IP in small bird species and urge researchers to consider SC implants in future studies of avian physiology and behaviour to minimize the risk of harming the study animals.

All experimental procedures were approved by the Animal Research Ethics Committee at Nelson Mandela University: Finches (A16-SCI-ZOO-003), Scrub-robins (A15-SCI-ZOO-005) and Rockjumpers (A15-SCI-ZOO-007). For Scrub-robins, bird capture and sacrifice permits were issued by Cape Nature, Western Cape, South Africa (0056-AAA008-00057) and the Department of Environment and Nature Conservation, Northern Cape, South Africa (1611/2015). For Rockjumpers, bird capture authorization was issued by Cape Nature, Western Cape, South Africa (0037-AAA041-00060). Funding was provided to B.S. by the National Research Foundation Thuthuka Grant (Ref. no. SFH150708124412) and Nelson Mandela Metropolitan University Research Themes Grant. A.M.R. was supported by a Marie Sklodowska-Curie Individual fellowship (European Union Horizon 2020 Research and Innovation Programme, grant agreement No. 655150 - BARREN project). We are grateful to the Lee family, Alan Lee, Gavin Emmons, Alacia Welch, Audrey Miller, Jenny Tartini, Christina Ebneter, Nicholas Pattinson, Cuen Muller, Maxine Smit, George Koutsoudis, Michael Koutsoudis, Daniel Evlambiou, Sibley Levack, Jerry Molepo, Shene van der Westhuizen and Megan Smith for their help both in the field and in the laboratory. We would also like to thank Mark Brigham for his valuable comments on an early draft of the manuscript.

REFERENCES

Bicudo, J.E.P., Buttemer, W.A. & Chappell, M.A. 2010. *Ecological and Environmental Physiology of Birds.* Oxford: Oxford University Press.

Bouwknecht, J.A., Olivier, B. & Paylor, R.E. 2007. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. *Neurosci. Biobehav. Rev.* **31**: 41–59.

Boyles, J.G., Smit, B. & McKechnie, A.E. 2010. A new comparative metric for estimating heterothermy in endotherms. *Physiol. Biochem. Zool.* **84**: 115–123.

Dausmann, K.H. 2005. Measuring body temperature in the field – evaluation of external vs. implanted transmitters in a small mammal. *J. Therm. Biol.* **30**: 195–202.

Fagerstone, K.A. & Johns, B.E. 1987. Transponders as permanent identification markers for domestic ferrets, black-footed ferrets, and other wildlife. *J. Wildl. Manage.* **51**: 294–297.

Fox, J. & Weisberg, S. 2011. An {R} Companion to Applied Regression,. Thousand Oaks, CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gauthier-Clerc, M., Gendner, J.-P., Ribic, C., Fraser, W.R., Woehler, E.J., Descamps, S., Gilly, C., Le Bohec, C. & Le Maho, Y. 2004. Long-term effects of flipper bands on penguins. *Proc. R. Soc. Lond. B* **271**: S423–S426.

Gibbons, W.J. & Andrews, K.M. 2004. PIT tagging: simple technology at its best. *Bioscience* **54**: 447–454.

Hammel, H., Caldwell, F.T. & Abrams, R.M. 1967. Regulation of body temperature in the blue-tongued lizard. *Science* **156**: 1260–1262.

Hockey, P., Dean, W.R.J. & Ryan, P. 2005. *Roberts Birds of Southern Africa.* Cape Town, South Africa: Trustees of the John Voelcker Bird Book Fund.

Jamison, B., Beyer, R., Robel, R. & Pontius, J. 2000. Research notes: passive integrated transponder tags as markers for chicks. *Poult. Sci.* **79**: 946–948.

Jemison, S.C., Bishop, L.A., May, P.G. & Farrell, T.M. 1995. The impact of PIT-tags on growth and movement of the rattlesnake, *Sistrurus miliarius*. *J. Herpetol.* **29**: 129–132.

Keck, M.B. 1994. Test for detrimental effects of PIT tags in neonatal snakes. *Copeia* **1994**: 226–228.

Körtner, G., Brigham, R.M. & Geiser, F. 2000. Metabolism: winter torpor in a large bird. *Nature* **407**: 318–318.

Luheshi, G.N., Gardner, J.D., Rushforth, D.A., Loudon, A.S. & Rothwell, N.J. 1999. Leptin actions on food intake and body temperature are mediated by IL-1. *Proc. Natl Acad. Sci. USA* **96**: 7047–7052.

Maloney, S.K. & Dawson, T.J. 1998. Changes in pattern of heat loss at high ambient temperature caused by water deprivation in a large flightless bird, the emu. *Physiol. Zool.* **71**: 712–719.

McCafferty, D.J., Gallon, S. & Nord, A. 2015. Challenges of measuring body temperatures of free-ranging birds and mammals. *Anim. Biotelem.* **3**: 33.

McKechnie, A.E. & Lovegrove, B.G. 2002. Avian facultative hypothermic responses: a review. *Condor* **104**: 705–724.

McKechnie, A.E. & Wolf, B.O. 2004. Partitioning of evaporative water loss in White-winged Doves: plasticity in response to short-term thermal acclimation. *J. Exp. Biol.* **207**: 203–210.

McKechnie, A.E., Noakes, M.J. & Smit, B. 2015. Global patterns of seasonal acclimatization in avian resting metabolic rates. *J. Ornithol.* **156**: 367–376.

McKechnie, A.E., Whitfield, M.C., Smit, B., Gerson, A.R., Smith, E.K., Talbot, W.A., McWhorter, T.J. & Wolf, B.O. 2016a. Avian thermoregulation in the heat: efficient evaporative cooling allows for extreme heat tolerance in four southern Hemisphere columbids. *J. Exp. Biol.* **219**: 2145–2155.

McKechnie, A.E., Smit, B., Whitfield, M.C., Noakes, M.J., Talbot, W.A., Garcia, M., Gerson, A.R. & Wolf, B.O. 2016b. Avian thermoregulation in the heat: evaporative cooling capacity in an archetypal desert specialist, Burchell's Sandgrouse (*Pterocles burchelli*). *J. Exp. Biol.* **219**: 2137–2144.

McNab, B.K. 2002. *The Physiological Ecology of Vertebrates: a View from Energetics*. Ithaca, NY: Cornell University Press.

Meerlo, P., De Boer, S., Koolhaas, J., Daan, S. & Van den Hoofdakker, R. 1996. Changes in daily rhythms of body temperature and activity after a single social defeat in rats. *Physiol. Behav.* **59**: 735–739.

Meijer, T., Rozman, J., Schulte, M. & Stach-Dreesmann, C. 1996. New findings in body mass regulation in Zebra Finches (*Taeniopygia guttata*) in response to photoperiod and temperature. *J. Zool.* **240**: 717–734.

Milne, R., Cunningham, S., Lee, A. & Smit, B. 2015. The role of thermal physiology in recent declines of birds in a biodiversity hotspot. *Conserv. Physiol.* **3**: 1–17.

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

Nord, A., Lehmann, M., MacLeod, R., McCafferty, D.J., Nager, R.G., Nilsson, J.Å. & Helm, B. 2016. Evaluation of two methods for minimally invasive peripheral body temperature measurement in birds. *J. Avian Biol.* **47**: 417–427.

Parker, S.J. & Rankin, P.S. 2003. Tag location and retention in black rockfish: feasibility of using PIT tags in a wild marine species. *N. Am. J. Fish. Mana.* **23**: 993–996.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. 2014. R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1-117. Available at: http://CRAN. R-project. org/package= nlme (accessed 10 January 2016)

R Core Team. 2014. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

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**: 244–255.

Schroeder, J., Cleasby, I.R., 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.

Smit, B. & McKechnie, A.E. 2010. Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**: 330–339.

Swanson, D.L. & Olmstead, K.L. 1999. Evidence for a proximate influence of winter temperature on metabolism in passerine birds. *Physiol. Biochem. Zool.* **72**: 566–575.

Tieleman, B.I., Williams, J.B. & Buschur, M.E. 2002. Physiological adjustments to arid and mesic environments in larks (Alaudidae). *Physiol. Biochem. Zool.* **75**: 305–313.

Wacker, C.B., Rojas, A.D. & Geiser, F. 2012. The use of small subcutaneous transponders for quantifying thermal biology and torpor in small mammals. *J. Therm. Biol* **37**: 250–254.

Received 27 July 2017;
revision accepted 8 November 2017.
Associate Editor: Jesus Martínez-Padilla.