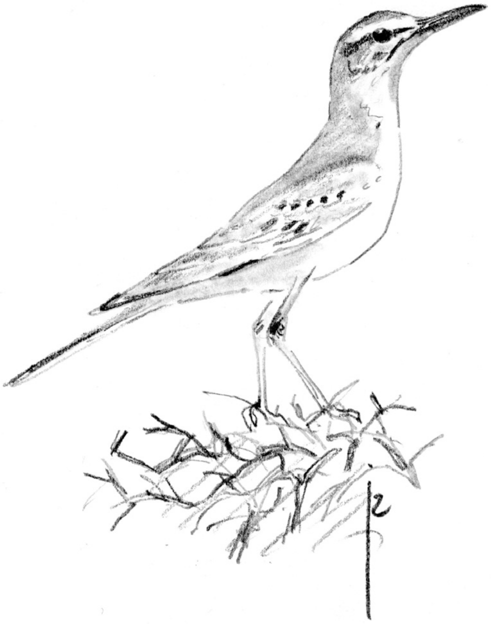
Validation of temperature-sensitive radio transmitters for measurement of body temperature in small animals

Joseph B. Williams[1,*], B.I. Tieleman[2] & Mohammed Shobrak[3]



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As part of a study on the core body temperature (T_b) of desert birds, we purposed to use temperature-sensitive implantable radio transmitters. Because of the difficulty in recapturing these birds, we needed to know if these electronic devices held their calibration over the duration of normal battery life (21 days). Our initial calibration at 40°C was consistently less than that of the manufacturer, although in some cases differences were small: deviations in temperature as predicted by the company's calibration and our own ranged from 0.1 to 1.7°C. We tested for drift in calibration and found that for the first 9 days of operation, most radio transmitters deviated by less than 0.5°C from the initial calibration. Differences between predicted and actual temperature became progressively larger with time, sometimes exceeding 2.0°C. If possible, we suggest that transmitters be calibrated, implanted, and then after taking data on T_b of the free-living bird, the transmitters should be recovered and recalibrated. If it is not possible to recapture birds, then our data suggest that T_b predicted by pulse periods are reasonable up to 9–12 days after the initial calibration but data recorded beyond this time period are potentially suspect.

Key words: temperature sensitive radio transmitters, body temperature, thermoregulation

[1]Dept. EEOB, Ohio State University, Columbus, OH 43210, USA; [2]Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 Haren, The Netherlands; [3]Biology department, Science College, Taif University, P.O. Box 888, Taif, Saudi Arabia;
*corresponding author (Williams.1020@osu.edu)

Core body temperature (T_b) is a complex parameter, the result of heat gain from metabolism and heat loss to the environment (King & Farner 1961, Tieleman & Williams 1999). In endotherms, T_b is the centerpiece of the concept of thermoregulation, the ability of an animal to maintain its T_b within narrow limits despite changes in its environmental temperature. A relatively low average T_b in a species may result from low rates of metabolism or a high rate of heat loss, which in turn may have evolutionary and ecological implications (McNabb 1966, Lane *et al.* 2004, Ostrowski *et al.* 2003, McKechnie *et al.* 2006). Because of their higher rates of metabolism, birds have a higher T_b than mammals, on average 1.87°C higher during rest and 2.4°C higher during their active phase (McNab 1966).

Historically the study of T_b of birds began with the advent of small, quick-registering, accurate, clinical thermometers, such as the Schultheiss thermometer (e.g. Wetmore 1921, Dawson & Bennett 1981). Animals were hand-held and a thermometer inserted into the cloaca to measure T_b. However the 'Grab and Stab' method did not provide information about thermoregulation of animals in their natural environment because this was a single point measurement made when animals were being handled (Avery 1982, Taylor *et al.* 2004). Advancement in understanding thermoregulation of free-living animals came when small temperature-sensitive radio transmitters were developed, consisting of an oscillating circuit that used a temperature-sensitive resistor as the sensing element. An increase in temperature altered resistance such that the frequency of oscillation of the pulse emitted increased, and concomitantly pulse period, the time from initiation of one pulse to the initiation of a second pulse, decreased (Mackay 1970). A number of researchers have used implantable radio transmitters to measure T_b of free-living birds (Dolby *et al.* 2004, McKechnie *et al.* 2006), or to measure skin temperature, an index of T_b (Brigham 1992, Kenow *et al.* 2003, Lane *et al.* 2004, McKechnie *et al.* 2007). Investigators sometimes calibrated the pulse period of transmitters against a standard laboratory thermometer, then implanted them into the peritoneal cavity, sub-dermally, or as an external harness, and released the bird (Fletcher *et al.* 2004, Fortin *et al.* 2000, McKechnie *et al.* 2007). Thereafter pulse period was measured at a distance, sometimes by counting the number of pulses over a given time period as measured with a stopwatch, but more often by an electronic measurement device. Rarely were transmitters recovered from the bird because of the difficulty in recapturing individuals, and therefore one assumption in data gathered in this way was that radio transmitters do not drift in calibration. Some researchers employed calibration curves provided by the manufacturer rather than calibrate individual transmitters against a standard precision thermometer prior to implanting them (Dolby *et al.* 2004, Lane *et al.* 2004, Fletcher *et al.* 2004). The level of error introduced in measurement of T_b using such a procedure is unknown.

As part of a study on thermoregulation of desert birds in Saudi Arabia, we used temperature-sensitive implantable radio transmitters. From past experience, we were aware that it would be difficult to recapture individuals which had a radio transmitter implanted in them, and therefore re-calibration of the transmitters would be difficult. Hence we deemed it important to ascertain if transmitters held their calibration over the duration of normal battery life while immersed in body fluids. Moreover, because we implanted transmitters over several months, we wanted to assure that storage of transmitters in the refrigerator did not affect calibration.

Methods

Initially we purchased temperature-sensitive radio transmitters of two different types, ten BD-2 (14 x 6.5 x 3.5 mm) with a helix antenna and ten BD-2 with external whip antennae from Holohil Systems Ltd, Ontario, Canada. We purchased these two types of transmitters at the recommendation of the manufacturer given our set of field circumstances. The transmitters with a helix antenna are completely encapsulated, whereas the transmitters with a whip antenna has a wire that extends from the body of the radio. The bodies of both radio types were encapsulated in inert waterproof epoxy by the manufacturer. After we received transmitters, we ensured that they were not emitting a signal, as suggested by the company, and then stored them in a refrigerator during our field season, March–June. In early July 2007, after our experiments on wild birds in Saudi Arabia were completed, we calibrated 9 remaining transmitters, 4 with helix antennae and 5 with whip antennae. However, one of the transmitters with a whip antenna failed after 4 days of operation, so we excluded it from analyses.

At the end of our initial calibration of transmitters, in July, we sent two transmitters back to the company to have new batteries retrofitted. We received these transmitters in March 2008, and re-calibrated them for 21 days, at 35 and 40°C; we began our calibration procedure immediately when we received radio transmitters. Hence these transmitters were not stored in a refrigerator.

We calibrated radio transmitters against a precision thermometer (±0.05°C) with a calibration certificate traceable to the National Institutes of Standards and Technology (ErTco, Precision Thermometer, serial 5101, range 25–50°C, USA). Transmitters were submerged in a digital Neslab circulating water bath, model RTE 7, and their pulse period determined at 37, 40, 43, and 46°C using a Telonics TR-5 telometry-scanning receiver to monitor the radio signal and pulse period (±1 msec). Transmitters remained in the water bath at each temperature for 30 min to assure equilibration prior to reading of the pulse period. After the initial calibration, we placed the radio transmitters in physiological saline in a temperature-controlled incubator (Fisher Isotemp Digital Incubator) at 40°C to mimic ionic conditions in the intraperitoneal cavity of birds. Every third day we removed transmitters from the incubator, blotted them dry, and weighed them with a Mettler electronic balance (±0.0001 g) to assess water uptake. Then we placed them in the Neslab water bath, and determined their pulse period, again at 37, 40, 43, and 46°C. We continued this process for 21 days, the minimum life-expectancy of these transmitters as suggested by the manufacturer. For each determination of pulse period at a given temperature, we recorded 3 consecutive readings. Variation around readings was small, typically 0.1%, and hence SE that we calculated were also small, and are contained within symbols in our graphs.

We used least-squares regression to determine the relationship between pulse period and temperature for all radio transmitters. In all cases $R^2 = 0.999$. The relationship between pulse period and temperature is curvilinear over a large temperature range. We also calculated regressions using log transformed values for pulse period, but over the narrow range of temperatures that we measured, this did not improve the goodness of fit. Therefore we ran linear regressions on untransformed data using General Linear Models of SPSS 14.0. Means are presented ±1 SD.

Results

Manufacturer’s calibration

We compared the graph provided to us, which included a regression fitted to data from 30–45°C, for each transmitter with our own initial calibration in the laboratory. We present a comparison of the manufacturer’s calibration with our own at 40°C only; results were similar for other temperatures (Fig. 1). Our initial calibration at 40°C was consistently less than that of the manufacturer, although for 5 transmitters differences were less than 0.3°C. The largest differences in initial calibration were for transmitters 86, 79, and 76, which deviated from actual temperature by 1.7, 1.4 and 1.2°C, respectively. For all 8 transmitters, the average difference between the calibration of the manufacturer and actual temperature was 0.62 ± 0.6°C.

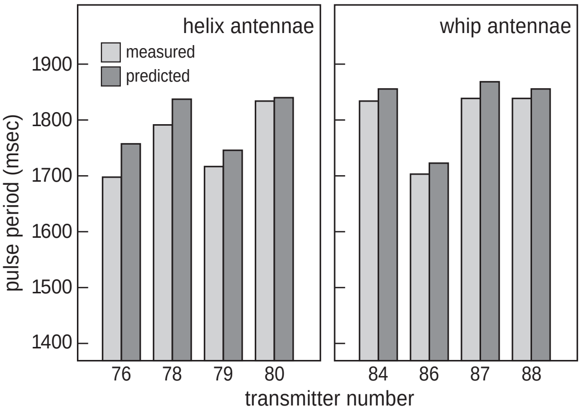

Figure 1. A comparison of the pulse period of temperature-sensitive radio transmitters at 40°C provided by the manufacturer and the pulse period measured in this study.

Change in pulse period with time

For transmitters with helix antennae, pulse period decreased at the same temperature over time, in a non-linear fashion, for 3 of 4 transmitters (Fig. 2). As radio transmitters continued to emit a signal, the magnitude of the deviation in calibration increased. Transmitter 76 failed after 16 days, whereas the other 3 transmitters with helix antennae functioned for 21 days. Transmitter 79 predicted temperature within ±0.3°C for the entire measurement period. For transmitters with whip antennae, we found a general trend in most transmitters of a reduction in pulse period over time. Transmitter 87 failed after 16 days (Fig. 3).

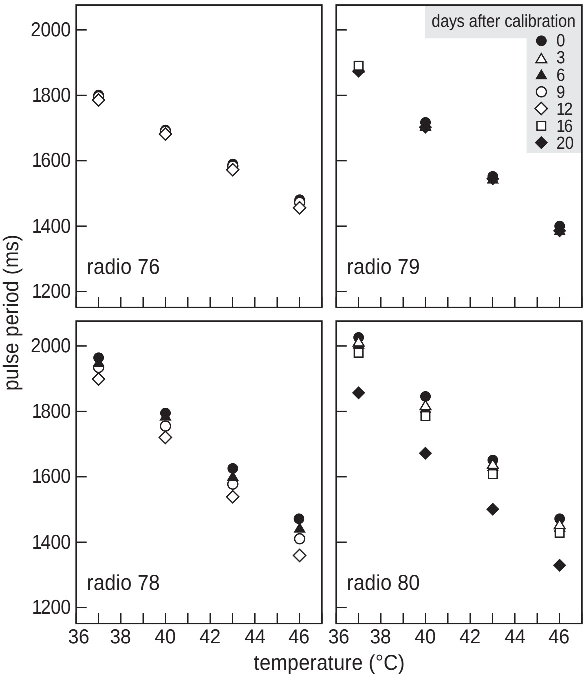

Figure 2. Pulse periods of temperature-sensitive radio transmitters with helix antennae at 37, 40, 43, and 46°C as a function of time. Error bars are within symbols. Each symbol represents a different day of calibration. Extreme values of transmitter 78 on the last two days are not indicated in the figure.

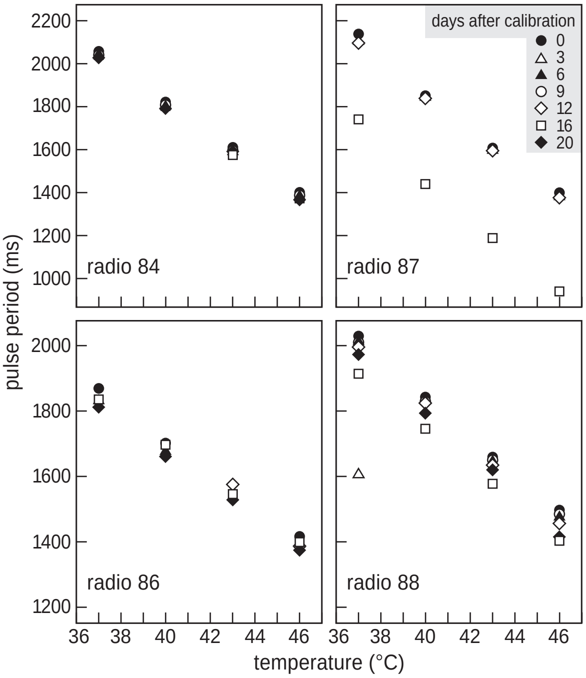

Figure 3. Pulse periods of temperature-sensitive radio transmitters with whip antennae at 37, 40, 43, and 46°C as a function of time. Error bars are within symbols. Each symbol represents a different day of calibration.

Change in prediction of temperature with time

We tested the idea that our initial calibration of transmitters was invariant during the life of the battery by comparing the temperature given by the equation for our initial calibration with the actual temperature that the radio was experiencing on that day. For the first 9 days of operation, 6 of 8 radio transmitters deviated by less than 0.5°C from our initial calibration, but differences between predicted and actual temperature became progressively larger with time (Fig. 4). Transmitter 78 had altered the predicted temperature by 1.5°C after 12 days of functioning. Radio transmitters with whip antennae performed reasonably well up to 12 days after start of signal output, but two of the transmitters deviated significantly from predictions after 12 days of operation, 43% of the way through normal battery life.

Mass increases to transmitters

Initially mass increases attributable to water influx were large, presumably as the outer layer of epoxy became hydrated, but influx of water slowed with time. After three days, transmitters gained an average mass of 39.4 ± 2.0 mg, but after 21 days mass increases averaged only 1.2 ± 0.4 mg.

Recalibration

After the manufacturer placed a new battery in radio transmitters no. 78 and 80, we calibrated them for 21 days. Transmitter 78 showed a small but persistent decline at both 35 and 40°C in pulse period, as it had done previously, and quit after 16 days of operation (data not shown). It predicted temperature within ±0.5°C for 12 days, and thereafter deviations increased markedly. Radio 80 predicted temperature within ±0.5°C for 12 days and then deviations from actual temperature increased, again consistent with the previous pattern for this radio. The fact that the refurbished radio transmitters showed similar patterns of deviations from actual temperature as the original transmitters suggests that our findings cannot be attributed to ageing of the batteries.

Discussion

We evaluated the performance of small temperature-sensitive radio transmitters commonly used in studies of T_b on free-living animals. We have shown that trusting the calibration of the manufacturer can lead to errors as much as 1.7°C in measurement of T_b, even if the transmitters do not drift during their operation. We recommend that these radio transmitters be calibrated by the investigator prior to use.

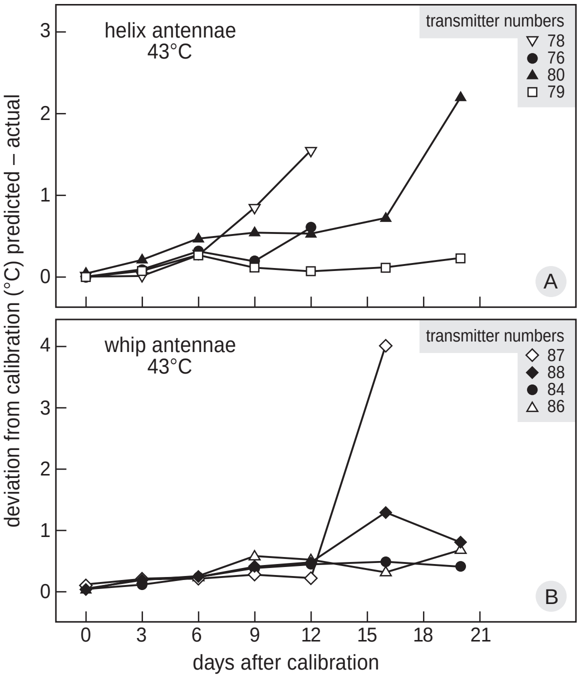

Figure 4. Graph representing the deviation in temperature predicted by pulse periods of radio transmitters with a helix antennae (A) and with a whip antennae (B). Error bars are within symbols. Transmitter numbers are indicated. Extreme values of transmitter 78 on the last two days are not indicated in the figure.

Our data indicate that some temperature-sensitive radio transmitters can decrease pulse period with time, although one of our transmitters held its calibration for the entire 21 days of battery life. Several of our transmitters failed before the 21 day-battery life, as designated by the company, for reasons that are unclear. We suggest that, where possible, transmitters be calibrated, implanted, and then after taking data on T_b of the animal, we recommend removing the transmitter and recalibrating it to assure that they have not drifted from the initial calibration. When it is not possible to recapture birds, then our data suggest that T_b predicted by pulse periods are reliable up to 9–12 days after the initial calibration but data recorded beyond this time period are potentially suspect. Alternatively, if the magnitude of drift is known, as predicted by calibrations of a series of transmitters simultaneously run during the period when other transmitters are implanted, then data can perhaps be corrected.

Our data on water influx into radio transmitters do not allow us to directly link water influx and drift in pulse period. The epoxy coating hydrated early during the experiment, but this was not the time when the most serious drift in transmitters was occurring. Only half of the radio transmitters increased in mass after the first 6 days of submersion in physiological saline, and those increases were small, suggesting that water uptake was minimal after initial hydration of the epoxy coat. Hence, the reason(s) for drift in performance of these radio transmitters remains ambiguous.

Suppose that we had used the manufacturer's calibration for our measurements of temperature using these temperature-sensitive transmitters. On July 27, our last day of calibration, transmitter no. 78, 79, 80, 84, 86, and 88, all at 39.95°C, would be predicted to be at –18.7, 41.8, 42.32, 40.94, 42.39, 40.87 by calibration curves from the company. This yields an average difference of 1.71 ± 0.32°C if we eliminate transmitter no. 78, which was far from the calibration curve. These small transmitters varied by as much as 2.44°C than the company's original calibration curve.

Finally, it was not our aim to test all temperature-sensitive transmitters available on the market, but we suspect that the problems we encountered may be applicable to all small temperature-sensitive implantable radio transmitters. We have not found any documentation on the limitations of the performance of these transmitters to measure T_b when implanted, and think it is important for the scientific community to be aware of the potential for these problems.

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Samenvatting

De lichaamstemperatuur is een belangrijke maat bij eco-physiologisch onderzoek maar is bij vrijlevende vogels lastig te meten. Zendertjes die klein genoeg zijn om geïmplanteerd te worden en die de lichaamstemperatuur registreren lijken uitkomst te bieden. In dit onderzoek werd nagegaan hoe nauwkeurig dergelijke registraties zijn door de zendertjes aan een test te onderwerpen. Ze werden daartoe in een waterbad met wisselende temperatuur gelegd. De afwijkingen tussen de voorspelling van de fabrieksijking en de werkelijke temperatuur liepen op tot 1,7°C. Daarnaast bleek een verloop in gevoeligheid op te treden waardoor na enige tijd de zendertjes een te hoge temperatuur aangaven – als ze al werkten want vier van de negen zendertjes begaven het binnen 2 weken. Gedurende de eerste negen dagen waren de registraties van de vijf beste zendertjes redelijk met afwijkingen van minder dan 0,5°C. Gemaand wordt tot voorzichtigheid bij de interpretatie van gegevens die met dergelijke zendertjes zijn verzameld. (JS)

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