



RESEARCH ARTICLE

Using radiotelemetry to study behavioural thermoregulation in insects under field conditions

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Abstract

1. Thermoregulation is a central aspect of animal physiology. Mobile ectotherms have the potential to influence their temperature through their location and orientation. Behavioural thermoregulation has been extensively studied in insects, particularly in the migratory locust *Locusta migratoria*. However, most field studies are confined to daytime observations typically using invasive thermocouples with obvious potential to disrupt natural behaviour.
2. We demonstrate that miniature radiotransmitters represent an alternative and less invasive method to study insect thermoregulation. We discuss how this method can be used to study the thermal behaviour of free-ranging animals for extended periods. Specifically, we show that there is a close correlation between temperature recordings from implanted thermocouples in locusts *L. migratoria* and externally mounted radiotransmitters on the same animals.
3. Our experiments match earlier observations of locust thermoregulatory behaviour confirming that the locusts with transmitters exhibit 'normal' thermoregulatory responses to feeding and to infections (behavioural fever).
4. Finally, we demonstrate the practicality of a radiotransmitter-based system by recording natural thermoregulatory behaviour of locusts in a semi-field setting. Our field study showed locusts actively chose warm microclimates during the day and cold microclimates at night. We conclude that the use of radiotelemetry in studies of behavioural thermoregulation in wild insects could provide unique continuous recordings of body temperature over several days. Such data will provide researchers with a more complete understanding of how insects use behavioural thermoregulation in nature.

KEYWORDS

behaviour, fungi, insects, monitoring, non-invasive, nutrition, telemetry, temperature

1 | INTRODUCTION

Temperature is important for all biological processes and most organisms, including insects, have a particular set of temperatures they prefer over others (Angilletta, 2009; Cossins & Bowler, 1987). The preferred temperature of an insect may vary depending on the animal's condition and it is possible that different physiological

systems, such as the digestive system (Clissold, Coggan, & Simpson, 2013; Coggan, Clissold, & Simpson, 2011; Yang & Joern, 1994) and the immune system (Inglis, Johnson, & Goettel, 1996; Ouedraogo, Goettel, & Brodeur, 2004) benefit from different thermal optima. To accommodate these different requirements, insects have evolved physiological and behavioural adaptations to control their body temperature. A few insect species, including flies (Gomes, Köberle,

Zuben, & Andrade, 2018), bees (Southwick & Heldmaier, 1987) and moths (Heinrich, 1971), regulate body temperature physiologically through endothermy or evaporation. However, insects are generally small and characterized by poor insulation and a large relative surface area which results in high rates of heat transfer (Stevenson, 1985). Combined with limited metabolic heat production this means that the body temperature of insects is typically close to that of their surroundings when the animal is not in direct sunlight. Most insects therefore rely on variation in environmental temperature and solar radiation to regulate their body temperature (May, 1979). Accordingly insects thermoregulate through behavioural adjustments such as posturing and/or microhabitat selection. These, postures include *crouching* close to the surface, *flanking* towards the sun or *stiling* above the surface to allow the insect to achieve a body temperature that differs from the average air temperature (Chappell & Whitman, 1990; May, 1979). However, the most effective way for insects to regulate body temperature is to seek microenvironments that differ in solar radiation, humidity and wind speed (May, 1979).

Behavioural thermoregulation has been studied in many insect species and in particular in orthopterans. A search on 'Google Scholar' using 'behavioural thermoregulation insects' resulted in 24 relevant studies of insect thermoregulation. Seventeen of these studies were conducted on orthopterans, 22 were performed during daytime, 20 were conducted with the use of thermocouples attached to individuals and 14 out of 19 studies reported body temperatures close to or above 20°C (Table S1). This small literature search indicates that little is known regarding behavioural thermoregulation during colder parts of the season and particularly at night. It also illustrates that the use of invasive thermocouples and a grab-and-stab method (where individuals are caught, and their internal temperature is measured immediately using a thermocouple which pierces the cuticle) are dominant in thermoregulatory studies of insects. Such an approach can only give a single observation of an individual, highlighting the general absence of continuous recordings from individuals (Blanford & Thomas, 2000; Chappell, 1983; Harrison & Fewell, 1995).

The use of temperature sensitive radiotransmitters to study behavioural thermoregulation in ectotherms is not novel, but here we examine whether small temperature sensitive radiotransmitting tags also represent an appropriate method to study behavioural thermoregulation in large insects like the migratory locust *Locusta migratoria*. Insect telemetry studies have been increasing over recent years, but most studies have concerned positional tracking where it is possible to use very lightweight passive tags which do not require a battery (Sword, Lorch, & Gwynne, 2008; Vinatier et al., 2010). However, telemetric monitoring of physiological parameters or *bio-telemetry* (Cooke et al., 2004) requires a battery and has therefore traditionally been restricted to vertebrates. Recent advances in the miniaturization of batteries and the tags themselves have now made it possible to manufacture radiotransmitters capable of monitoring the temperature of insects. Compared to the techniques mentioned earlier, such methods are less invasive and can monitor the temperature of the insect continuously including during the night or when

animals are hiding. However, before this method can be applied to field studies, it must be demonstrated that the tags allow for "normal" behaviour. To examine this, we designed experiments to:

1. Test whether transmitters reliably record the body temperature of a locust in an environment where it is free to choose its preferred temperature.
2. Test whether locusts displayed normal behaviour when fitted with a transmitter by replicating existing studies on locust thermoregulatory behaviour during feeding/fasting and when challenged with a pathogenic infection (Coggan et al., 2011; Inglis et al., 1996).
3. Test whether locusts fitted with a transmitter show thermoregulatory behaviour when exposed to variable field conditions.

2 | MATERIALS AND METHODS

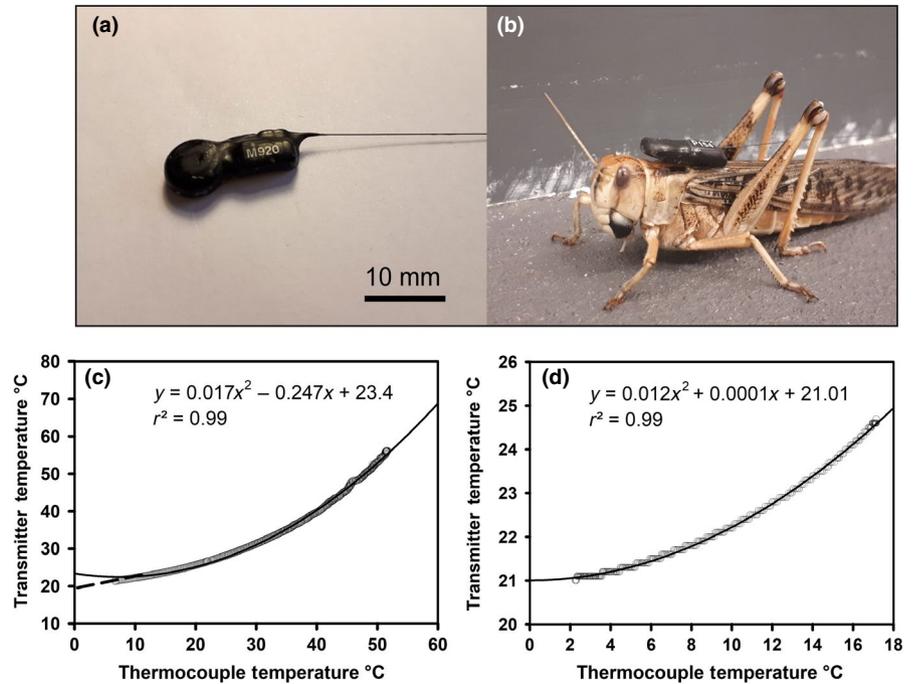
2.1 | Experimental animals

All tests were performed on adult female *L. migratoria* at least 7 days post adult emergence (supplied by Peter Andersen, Aps). Danish law and University of Aarhus ethical regulations do not place any restrictions on experiments involving insects but we designed our study to minimize the number of insects used and all insects were euthanized at the end of the experiments. Animals arrived as 5th instar nymphs and were housed in ventilated plastic tanks of approximately 0.45 m³ under a 16:8 hr light:dark cycle. They had free access to wheat bran and water and were also fed daily with fresh wheat sprouts. Each tank contained a metal grid that allowed animals to bask when a 150 W lamp was turned on during the light cycle. The lamp produced a thermal gradient within the tank with temperatures ranging from 25 to 45°C, night-time temperature was set at 25°C.

2.2 | Thermal gradient and temperature recording

Radiotransmitters or 'PicoPip tags' were purchased from Biotrack Ltd. (www.biotrack.co.uk) along with an SRX-800D receiver and Yagi antenna. The 14 transmitters used in this study varied slightly in size depending on battery size with the largest transmitters weighing 0.7 g and the smallest weighing 0.3 g (Figure 1a). The difference in weight was due to availability at the time of purchase. The transmitters contain a thermistor that changes the interval between signals depending upon its temperature. By calibrating each transmitter (see below) the temperature of the transmitter can be recorded by monitoring time between the signals (unit: signals per minute (SPM)) received from the transmitter. Each transmitter broadcasts at a specific radiofrequency, allowing the receiver to be programmed to alternately monitor several simultaneously broadcasting transmitters. We programmed the receiver to monitor each transmitter for 10 s (3–6 signals) in turn. In this experiment, we continuously track a maximum of four individuals simultaneously. The Yagi antenna can

FIGURE 1 (a) PicoPip tag used in the experiments. The antenna is 10 cm long and the tag itself weighs 0.3 g. (b) An adult female *Locusta migratoria*, fitted with the same type of tag. (c) Example of a calibration curve from one of the tags. The signal-per-minute (SPM) value of the tag as a function of temperature and a fitted regression curve for later estimation of temperature from the SPM value of the tag. Inconsistency between the regression curve and the data arises below 12°C as illustrated by the broken line. (d) In vivo calibration of the same tag at low temperatures for maximum accuracy at temperatures below 12°C. These curves represent calibrations of a single tag and so this was done for every tag used in the following experiments (14 different tags in total)



be programmed to track hundreds of transmitters, but recording from each transmitter requires time, so the number of transmitters limits the frequency of temperature estimates from each one (using 4 transmitters we received an estimate of body temperature every 40 s. from each animal).

To standardize the data collected from each transmitter we calculated the SPM using only the interval between the last two signals within each 10 s period. The program 'SRX-800 HOST' (supplied with the purchase of the SRX-800D receiver) automatically stored these values as: transmitter number; time of measurement; SPM. The transmitters contain a switch that allows the battery to be disconnected in the presence of a magnet. This allowed us to extend the 2-week continuous broadcasting time of the transmitters, as indicated by the manufacturer, to a period of several months of occasional use.

2.3 | Calibration of transmitters

For calibration of the system all reference temperatures were measured with K-type thermocouples connected to a TC-08 picolog (www.picotech.com). Data from the thermocouples were recorded with the program PicoLog. Before use, the thermocouples were calibrated to 0°C by placing them in an ice-water slurry. In some experiments, thermocouples were used to record body temperature of locusts fitted with transmitters and in others the thermocouples were placed directly on the radiotransmitters (see details below).

Calibration of each individual transmitter was performed by attaching a thermocouple directly to the transmitter and placing the transmitter on a temperature adjustable Peltier element. Simultaneous measurements of temperature (thermocouple) and SPM from transmitter were then obtained while increasing or

decreasing the temperature of the transmitter in the range 5°C–55°C at 1°C/min. The data were then fitted to a second-order polynomial (Figure 1c). This relation was obtained for each individual transmitter and because the polynomial fit was different at temperatures below and above 12°C (Figure 1c,d), we made two polynomial fits for each transmitter (one used above and one used below 12°C). If the SPM value of the 'high temperature' polynomial (Figure 1c) was below that representing 12°C we shifted to the 'low temperature' polynomial (Figure 1d).

3 | EXPERIMENTS

3.1 | Do the transmitters accurately report body temperature?

We performed a series of experiments where we recorded temperature (using SPM) from a tag placed on the locust while simultaneously recording temperature from thermocouples placed in the body cavity of the same locust. This was done by introducing a thin thermocouple approximately 2–3 mm into the thorax of a locust and fixing it with a cyanoacrylate adhesive. After this, a transmitter was glued to the top of the pronotum using the same adhesive (See supplementary information for information validating that the transmitters had negligible effects on locust behaviour). The experiments to compare temperature recordings from thermocouples and transmitters were performed in a thermal gradient in which locusts were either constrained to remain in one position or were free to move along the gradient. The thermal gradient consisted of a wooden box measuring 155 × 60 × 22 cm (L, W, H) with a 4-cm-thick aluminium floor. The aluminium plate was covered with a thin coat of paint with sand grains embedded in it to create a rough surface that provides traction for walking.

During experiments the gradient was sealed above with a transparent Perspex roof (with air holes for ventilation). Both ends of the aluminium plate had a hole to allow the plate to be perfused with water allowing us to use temperature baths at either end of the aluminium plate to create a 155 cm long gradient from 26 to 60°C.

3.1.1 | Stationary insects

Locusts were placed in an open-bottomed box (10 × 10 × 8 cm) to confine them to specific locations within the thermal gradient. The box was moved to a new location every 10 min until the subjects had covered the whole range of the gradient. This experiment was performed on 6 individuals divided equally between 2 runs.

3.1.2 | Free moving insects

We investigated whether the thermocouple and the transmitter reported similar temperatures in freely roaming locusts. The locusts were fitted with thermocouple and tag as described above and were placed at the physical mid-point of the thermal gradient. Body temperature was recorded simultaneously from transmitter and thermocouple in the freely roaming locusts for 5 hr. This experiment was conducted on 15 individuals divided equally over 5 runs.

3.2 | Do transmitters accurately report thermal behaviour?

To examine if locusts display normal thermoregulatory behaviour when fitted with a radiotransmitter we designed two experiments to replicate previous studies. Clissold et al. (2013) demonstrated that locusts choose different temperatures depending on the amount and/or quality of their food and Ouedraogo et al. (2004) demonstrated that locusts infected with a pathogenic fungus exhibit 'behavioural fever' where they select higher temperatures than uninfected controls.

3.2.1 | Effects of nutritional state on thermal preference in *L. migratoria*

Prior to the experiment, adult female locusts were acclimated at 31°C for 24 hr to ensure that all locusts had a similar recent thermal history. Locusts were divided into two groups of six each, one with *ad lib.* access to dry wheat, wheat sprouts and water, and one group without access to food or water. After 24 hr the locusts were removed from the acclimation treatment, fitted with a transmitter (but no invasive thermocouple) and placed in the middle of the thermal gradient. Body temperature was then recorded for 5 hr, but data from the first 2 hr were excluded to ensure that the behavioural thermoregulation of the locust was not influenced by initial exploration or handling stress. To limit interaction between conspecifics, we never placed more than

three locusts in the gradient at the same time. The temperatures of individuals within the 'fasted' group were compared to the 'fed' group using a *t* test.

3.2.2 | Behavioural fever

Eighteen locusts were used in this experiment. Nine were untreated controls and 9 were inoculated with the pathogenic fungus *Beauveria bassiana* (supplied by Borregaard Bioplant, www.bioplant.dk). Treated locusts were inoculated by spraying 2 ml of a solution containing approximately 10⁸ colony-forming units of *B. bassiana* per animal which is ~1,000 times the dose necessary to kill a locust within a week (pers. corr. Steen Brock, MuscaTech Biosystems which was later confirmed in a separate test). After inoculation the locusts were placed in a small box (28 × 17 × 17 cm) where the humidity could be kept at 90%–95% RH to promote fungal growth. The locusts were kept for 72 hr at 31°C with *ad lib.* access to wheat bran under the 16:8 light:dark cycle before they were fitted with a transmitter and placed inside the thermal gradient. Again, no more than three locusts were in the gradient simultaneously and the selection of temperature was monitored for the subsequent 6 hr, excluding data from the first 2 hr. The temperature of individuals over the last 4 hr from all infected individuals was compared to that of non-infected individuals using a *t* test.

3.3 | Can transmitters record body temperature under semi-natural conditions?

To test the transmitter system in the field we used a wood-framed cage (120 × 90 × 120 cm) with insect net on the sides. The cage was situated in a private garden in Aarhus, Denmark, during a warm week in late June (daytime high temperatures from 25 to 30°C in the shade). A wooden stool, some twigs and leaves were placed within the cage to allow for spots of sun and shade and the locusts were provided with *ad lib.* water, wheat bran and fresh wheat sprouts. Thermal heterogeneity in the cage was monitored by placing eight thermocouples at varying positions inside the cage. Each thermocouple was placed inside a black Eppendorf tube to record the 'black bulb' temperature that includes heat gain from solar radiation. The temperature of each thermocouple was recorded every 20 s throughout the experiment. This allowed us to approximate minimum and maximum temperature within the cage by taking the highest or lowest temperature from the eight thermocouples respectively. There were periods during which parts of the cage were in full sun, but all of the thermocouples were shaded. During such periods our maximum recorded thermocouple temperature is likely to underestimate the maximum available temperature within the cage.

For the semi-field experiment we fitted four adult female locusts with a transmitter and placed them inside the cage on 26/06 2018 at 3p.m. The body temperature of the locusts was monitored continuously for 45.5 hr at 40 s intervals. To analyse the thermoregulatory behaviour relative to the available environmental

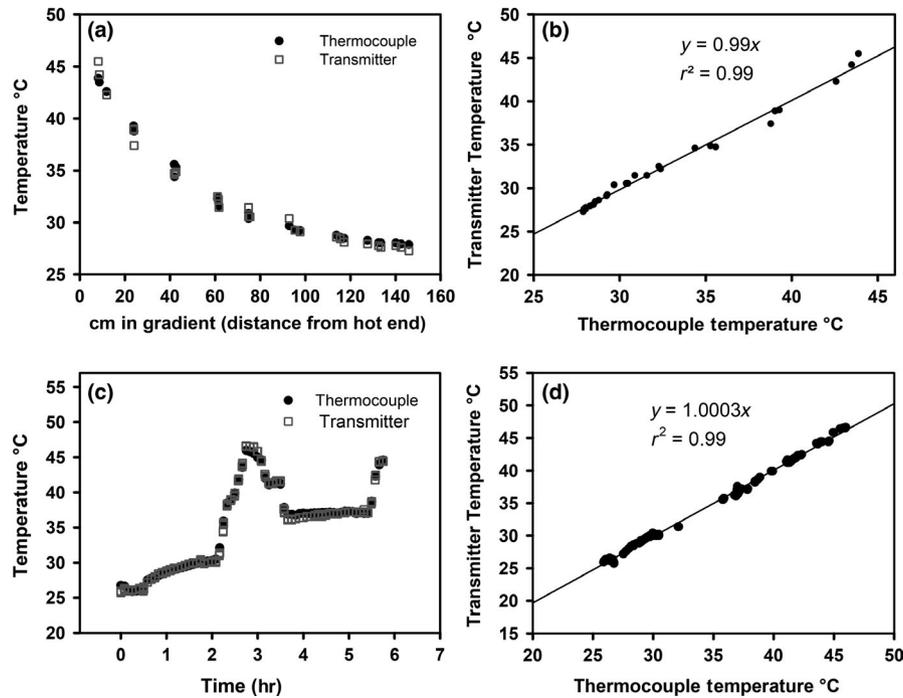


FIGURE 2 (a) Example of the internal temperature of a locust (black circles) and the temperature of the tag fitted to its pronotum (white squares), in stationary locusts at varying distances from the hot end of the thermal gradient (b) The correlation between the internal temperature of the locust and the temperature of the tag, from the same data as in (a). This experiment was performed twice on three separate transmitters ($n = 6$), and this is one example from transmitter 081. (c) Example of the temperature of a mounted tag (white squares) and implanted thermocouple (black circles) in locusts roaming freely throughout the thermal gradient. (d) Regression of the temperature recorded by the thermocouple and the temperature recorded by the transmitter, from the same data as in (c). This experiment was performed three times on five transmitters ($n = 15$)

temperatures (microhabitat temperatures) we divided the 45.5 hr into four different phases: Night (from sunset to sunrise); Dawn (from sunset until parts of the cage were exposed to direct sunlight); Sun (the time when direct sun was available); Dusk (The afternoon period when direct sunlight became unavailable in the cage and until sunset).

4 | RESULTS

4.1 | Transmitters accurately report body temperature

As seen in Figure 2a there is an excellent correspondence between the temperature of a thermocouple inserted into the thorax (black circles) and the temperature reported by the transmitter mounted on top of the same locust (white squares). This correlation was found in both stationary (Figure 2a,b) and freely roaming locusts (Figure 2c,d). In six stationary locusts the relationship between thermocouple temperature (i.e. internal body temperature) and transmitter temperature had a mean slope of 1.002 ± 0.02 (SE) and a mean r^2 of $.97 \pm .02$ (SE). Similar relationships were also found when the temperature was recorded from 15 locusts moving freely in the gradient (slope mean = 1.007 ± 0.004 [SE] and $r^2 = .95 \pm .015$ [SE]). There was a small tendency for the transmitters to be slightly colder than the body temperature at low temperatures and slightly warmer than body temperature at the warm temperatures. However, this temperature difference was typically less than 1°C and

therefore not cause of great concern (See Figures S3 and S4 in supplements for further discussion).

4.2 | Do locusts fitted with transmitters display normal thermoregulatory behaviour?

4.2.1 | Effects of feeding and fasting

As seen in Figure 3a,b fasted locusts (black bars) preferred a mean temperature of $35.9^\circ\text{C} \pm 0.9$ SE, whereas the fed locusts (grey bars) preferred a mean temperature of $39.5^\circ\text{C} \pm 0.6$ SE. An independent sample t test revealed a significant difference between the two groups. ($t = 3.106$, $p = .013^*$, $n = 6$).

4.2.2 | Behavioural fever

As predicted, infected locusts (black bars) preferred a temperature that was higher than control locusts (grey bars) Figure 3c,d. The infected locusts had a mean body temperature of $41.0^\circ\text{C} \pm 0.7$ SE which was significantly higher than the healthy locusts which preferred a mean temperature of $38.7^\circ\text{C} \pm 0.6$ SE ($t = -2.28$, $p = .03^*$, $n = 9$).

4.3 | Telemetry under semi-natural conditions

Recordings from the eight 'black bulb' thermocouples were used to estimate the range of thermal microhabitats available to the animals.

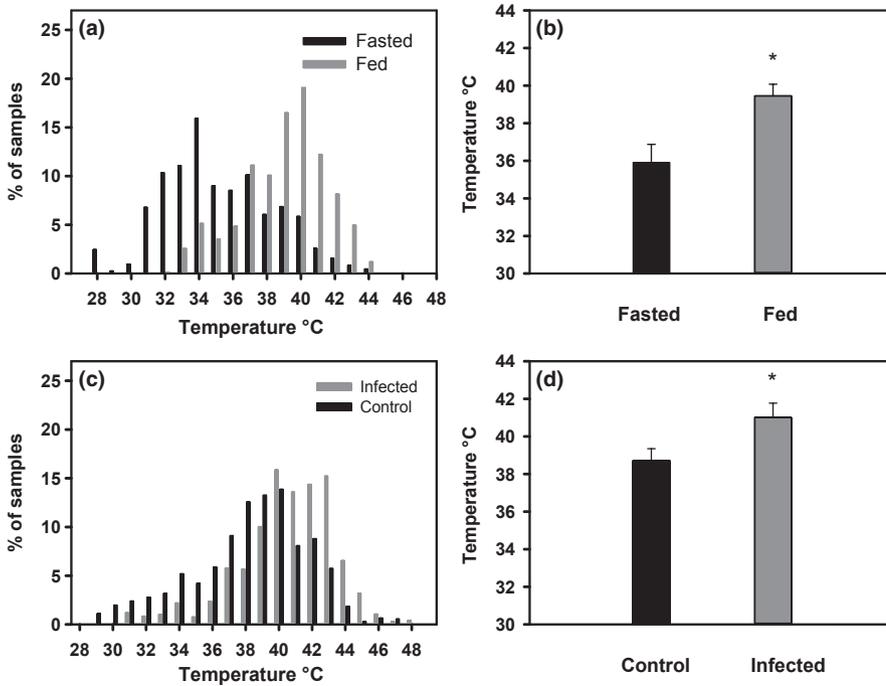


FIGURE 3 (a) Temperature preference of all fasted (black bars) and Fed (grey bars) locusts from all samples. (b) The mean temperature of all fasted (black bar) and Fed (grey bar) locusts, $t = -2.81$, $p = .03^*$, $n = 6$. Error bars indicate standard error. (c) Temperature preference of all Infected (grey bars) and Control (black bars) locusts from all samples. (d) The mean temperatures of all Control (black bar) and Infected (grey bar) locusts, $t = -3.29$, $p = .013^*$, $n = 9$. Error bars indicate standard error

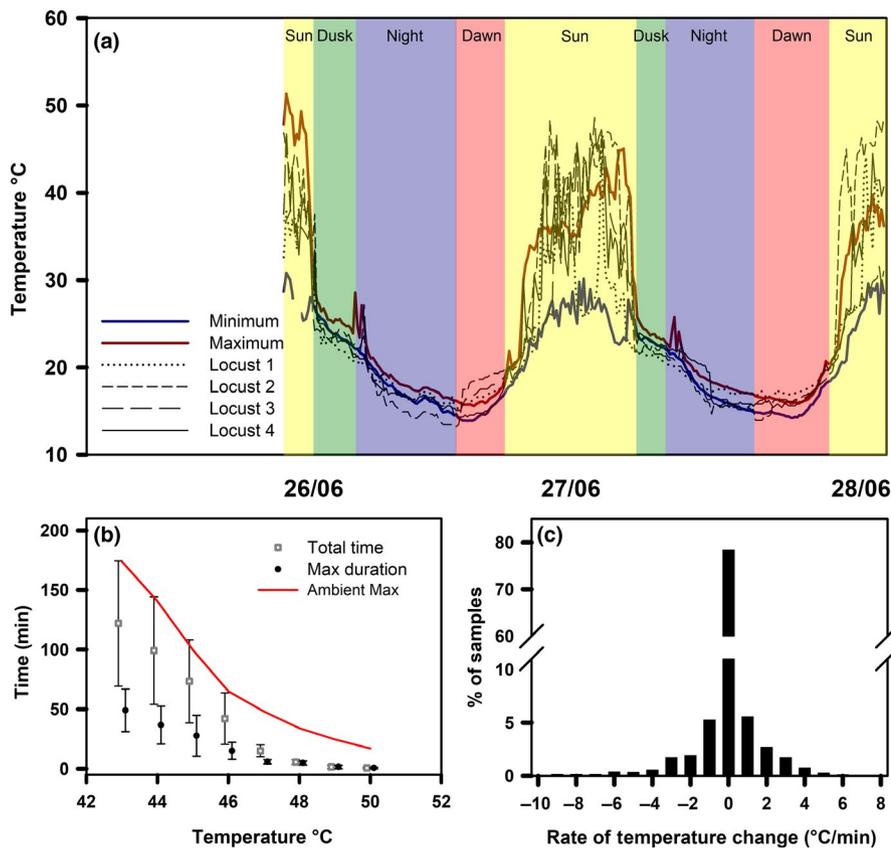


FIGURE 4 (a) The Minimum (blue line) and Maximum (red line) temperature inside the cage throughout the 3-day period. The 4 black lines represent the temperatures of the four locusts inside the cage. (b) The mean time spent by the locusts above the temperatures. The white squares represent the total amount of time spent at or above the temperatures, whereas the black circles represent the longest stretch of time the locusts could endure at or above the temperatures. The difference becomes non-existent above 48°C. The red line illustrates how long the maximum ambient temperature inside the cage was at or above the indicated temperatures. Error bars indicate standard error. (c) The distribution of temperature change rates among all four locusts. A positive number indicates an increase in body temperature, whereas a negative number indicates a decrease in body temperature

Figure 4a shows the minimum (blue line) and maximum (red line) temperatures within the cage. At night (blue areas in Figure 4a) there was generally little difference between minimum and maximum temperature inside the cage (difference between minima and maxima <4°C), whereas during the day, the range of temperatures occasionally spanned >25°C when the cage was exposed to direct sun (yellow

areas). During the night and at dawn and dusk when direct sunlight was unavailable, the locusts had body temperatures close to the average available temperature in the environment (Table 1). As seen in Figure 4 and Table 1 there was a tendency for locusts to track the minimum estimated available temperatures during dusk before the cooler night, while they would generally track the maximum available temperature

TABLE 1 The average minimum, mean and maximum ambient temperature inside the cage, at the four different time periods sun, dusk, night and dawn, and the mean temperature of the average locust at the same time periods

	Thermocouple			Animal
	Minimum	Mean	Maximum	Mean Tb
Sun	24.3°C ± 4.2 SD	29.4°C ± 6.7 SD	33.9°C ± 8.7 SD	32.1°C ± 9.0 SD
Dusk	22.9°C ± 2.2 SD	23.5°C ± 3.3 SD	26.4°C ± 6.4 SD	23.4°C ± 4.5 SD
Night	16.1°C ± 1.2 SD	16.4°C ± 1.0 SD	17.5°C ± 1.0 SD	16.8°C ± 1.0 SD
Dawn	14.7°C ± 0.6 SE	15.7°C ± 1.0 SD	16.8°C ± 0.3 SD	16.8°C ± 0.7 SD

during dawn before the sun was available. As soon as solar radiation was available the locusts chose the high temperature locations created by patches of direct sunlight. Thus, average body temperature of the four locusts was higher than the average of the maximum available temperature in the periods of sun (presumably because the 8 thermocouples underestimate the maximum available temperature of the entire cage). During periods of sun the locusts would sometimes choose very warm temperatures (>43°C). As seen in Figure 4b the total time spent at or above 43°C (white squares) by the average locust was 125 min over the 2-day period with no single period longer than 50 min. Locusts generally avoided temperatures above 46°C even though such temperatures were occasionally available. This indicates a preference for high temperatures during the sunny parts of day, but also behavioural avoidance of very high temperatures.

The telemetry system we used has some limitations in terms of temporal resolution as body temperature is only recorded every 40 s. Nevertheless, this allowed us to detect locusts shifting their body temperature very rapidly (in some cases heating was >7°C/min and cooling by >9°C/min) in periods when they moved from sun to shade (Figure 4c).

5 | DISCUSSION

Our goal was to test and potentially validate the use of biotelemetry in studies of insect thermoregulation. We chose to use a large insect for these studies because the size and mass of the transmitters would be excessively burdensome on a smaller insect. We anticipate that transmitter size will continue to decrease as technology advances, allowing this approach to be applied to smaller species.

5.1 | Transmitter calibration

By dividing temperatures into two ranges and fitting independent second-order polynomials we were able to achieve very satisfactory calibration of all transmitters (Figure 1). Individual calibration curves needed to be constructed for each transmitter, but once established transmitters consistently reported precise temperature recordings even several months later.

5.2 | Correlation between body temperature and transmitter temperature

The premise of our method is that the temperature of the transmitter can be used as a proxy for the internal temperature of the insect

because the transmitter is expected to acquire ambient and radiant energy in the same fashion as the insect to which it is attached. In the absence of radiant heat, the body temperature of most insects is assumed to be almost identical to the surrounding temperature (Angilletta, 2009; Cossins & Bowler, 1987; Stevenson, 1985). Our simultaneous recordings of body temperature (thermocouple inside the locust) and transmitter temperature reveal that in both stationary and freely roaming locusts, internal body temperature was a very powerful predictor of the transmitter temperature (r^2 -value between .91 and .98) with an almost exact 1:1 relationship between the measures (slopes between [0.97–1.04] [Figure 2]). These measurements confirm that the transmitters can accurately monitor the body temperature of our locusts. Nevertheless, we emphasize that transmitter recordings of body temperature may be problematic for endothermic insect species such as moths or bumblebees. Future studies should also confirm that body temperature and transmitter temperature are similar when the animals are placed under a strong radiant heat source as there could be a difference in heat gain between the insect body and the transmitter (See also Figures S3 and S4 for further discussion).

5.3 | Behavioural thermoregulation

To validate biotelemetry as a viable method to study behavioural thermoregulation it is also important to ensure that transmitters do not drastically change behaviour. We addressed this by examining whether locusts fitted with a transmitter exhibited thermoregulatory behaviour that was consistent with earlier studies using other methods. The first experiment was inspired by Clissold et al. (2013) and Coggan et al. (2011) who demonstrated that well fed locusts preferred 38°C, whereas fasted locusts preferred 32°C. Consistent with these findings we found that locusts fed wheat bran *ad lib.* preferred 40°C, whereas fasted locusts preferred 34°C (Figure 3a). Although the absolute temperature preference was slightly higher in our study compared to that of Clissold et al. (2013) and Coggan et al. (2011) the locusts fitted with transmitters display the same temperature difference between fasted and fed animals as the previous studies. The higher temperature preferences in this study could be a result of a lower acclimation temperature as our subjects were acclimated to 31°C and unable to thermoregulate in the 24 hr prior to the experiment. The difference could also be due to differing life stages of the locusts used in the two experiments. Our second experiment was inspired by Inglis et al. (1996) and Ouedraogo et al. (2004) who demonstrated that locusts infected with a pathogenic fungus exhibit

behavioural fever such that inoculated locusts prefer a higher temperature than healthy controls. We repeated this experiment using locusts fitted with a transmitter. Consistent with the earlier studies we found that infected locusts preferred temperatures that were 2–3°C hotter than healthy locusts (Figure 3c,d) which is a similar difference in preferred temperature as found by the Desert locust *Schistocerca gregaria* infected with the fungus *Metarhizium anisopliae* (Bundey et al., 2003). Again, this experiment confirms that locusts fitted with a transmitter exhibit behaviour that is consistent with earlier studies using laboratory based and invasive methods (Bundey et al., 2003; Inglis et al., 1996).

In addition to these experiments, we showed in two separate experiments (see Figures S1 and S2) that locusts fitted with transmitters display 'normal' mating behaviour, and chose similar placement in a thermal gradient as un-instrumented animals. Locusts with transmitters jumped and flew with similar frequency to un-instrumented animals and it is our subjective assessment that the transmitters do not restrict their movement in any identifiable way (See supplement).

5.4 | Using transmitters in the field

We tested the applicability of the transmitter-based system in a semi-natural setting, inside a large cage in which locusts were able to move around freely among branches, leaves and a stool which served as a basking platform on top and source of shade below. All four locusts were recorded in several places, and sometimes while recording their temperature, we could not find them by eye. Apart from the fact that this species is not native to Denmark, we did not release locusts into the wild because we were not confident that the animals would stay within the 10–15 m range of the transmitters. The latter point highlights that these transmitters are probably best suited to flightless insects that reside in a particular locality (for instance species of burrow-digging mole crickets and field crickets (Bretman, Rodríguez-Muñoz, Walling, Slate, & Tregenza., 2011), or to studies where subjects' movement can be constrained as in our experiment.

During the night we found the thermal heterogeneity to be modest inside the cage (3–4°C difference between the warmest and coldest positions at any given time point). However, when solar radiation was available the range of available temperatures increased dramatically, giving the locusts a wider selection of temperatures to choose from (compare blue and red lines in Figure 4). As shown in Figure 4 and Table 1 the mean temperature of the 8 thermocouples inside the cage was 29.4°C during the hours of sun. During this time the locusts would generally select warmer microclimates achieving an average body temperature of 32.1°C. This illustrates that the locusts exhibit typical behavioural thermoregulation (Blanford & Thomas, 2000; Harrison & Fewell, 1995; O'Neill & Rolston, 2007) under natural conditions when fitted with a transmitter. We found that locusts chose relatively cold temperatures at night, with average body temperatures close to the minimum environmental temperature. It is possible that this choice represents an adaptation to keep metabolism

low during the non-feeding part of the day, but the behaviour could also be related to seeking shelter. We were also able to identify evidence of behavioural thermoregulation during the dusk and dawn measurements, when locusts moved from minimum towards maximum temperature locations during dawn and conversely from maximum to minimum temperature locations during dusk (Figure 4 and Table 1). Because we sample body temperature from the same individual many times we were also able to observe that locusts were able to increase their body temperature by 7°C/min which is very fast compared to many other insect species. Dragonflies (spp.) (May, 1976) and bees *Anthophora plumipes* (Stone, 1993) have shown thoracic heating rates of 1.9°C/min and 5.4°C/min during warm up, whereas honeybees *Apis mellifera* are able to heat their thorax at a rate of 16°C/min prior to flight (Heinrich, 1980). It should, however, be noted that the heat up we are measuring is mainly facilitated by choice of microhabitat (solar radiation), whereas that of dragonflies, moths and bees primarily represents physiological thermoregulation. We found that locusts can endure body temperatures above 43°C for extended times (Figure 4b). There was considerable variation between our four specimens where one individual was relatively cold seeking and spent only 30 min at or above 43°C, whereas a conspecific spent 260 min at or above the same temperature over the course of the experiment. In a separate experiment, we had previously estimated lethal time in *L. migratoria* to 152 min at a body temperature of 48°C and 32 min at 50°C (O'Sullivan, MacMillan, & Overgaard, 2017). In the present study we found that all individuals avoided spending time at these very high and noxious temperatures (> 48°C), and that no locust experienced body temperatures above 48°C more than once throughout the whole experiment, even though these high temperatures were readily available.

5.5 | Critique of method

Our laboratory experiments clearly demonstrate that the transmitter reports a reliable estimate of the true body temperature and that locusts fitted with transmitters exhibit normal thermoregulatory behaviour under some conditions. Nevertheless, there are some potential pitfalls with this method that should be acknowledged. Active tags that report physiological parameters like temperature, pressure etc., are typically bigger than passive tags because they include measuring devices and processors. The temperature sensitive element of our transmitters is not very heavy, but the repeated signal emission requires a larger battery than transmitters that only emit signals to position the animal. For our transmitters the battery is the heaviest part of the transmitter and any reduction in battery size could only be achieved at the expense of battery life or detection range. Any increase in battery size will on the other hand add weight to the animal and therefore potentially inhibit normal behaviour. The extent to which the extra weight of tags influences the metabolic rate of an insect appears to be highly species specific and depends on the tag in question. For instance weight loss and lower rates of flower visits have been observed in scarabid beetles *Osmoderma eremita* and bumblebees (*Bombus* spp) fitted with tags weighing 30% and 66%–100% of the body weight of

the insects respectively (Dubois & Vignon, 2008; Hagen, Wikelski, & Kissling, 2011). Conversely, Watts and Thornburrow (2011) found no significant effect of transmitter mass/body mass on the distance travelled by a weta *Deinacrida heteracantha* during night-time foraging using a tag with much lower relative mass (ca. 3%–5% of body weight). Finally, Fischer and Kutsch (2000) found no negative effects on the flight-speed or wing-beat frequency of desert locust *S. gregaria* fitted with a transmitter weighing 10%–27% of the body mass. Based on this small set of studies it is difficult to generalize the effects of transmitter size across insect species but as mentioned above we could not identify any restriction in movement or mating (See Figures S1 and S2). A rule-of-thumb for vertebrate bio-telemetry states that the tag should never exceed 5% of the weight of the animal, however, the applicability of this rule to insects is a matter of guesswork. Our tags ranged from 15% to 35% of the insect weight, a ratio that is in accordance with other studies on insect telemetry (Boiteau & Colpitts, 2001; Dubois & Vignon, 2008; Fischer & Kutsch, 2000). For some insects this ratio might be problematic, whereas other species that can carry weights hundreds of times heavier than themselves are unlikely to be troubled by this extra weight (Lailvaux, Hathway, Pomfret, & Knell, 2005). The magnitude of this problem should therefore be critically evaluated before such biotelemetry studies are initiated. Another aspect of fitting a device to the thorax of a locust could be related to the movements of the animal. Navigation through dense vegetation or burrows will be more difficult (Kissling, Pattermore, & Hagen, 2014) and the fitted transmitter might also affect intraspecific interactions towards individuals with a transmitter. To our knowledge, there are no studies of altered insect behaviour towards conspecifics with radiotransmitters which was also confirmed by our mating experiment (Figure S1). However, for some species it is possible that males with transmitters might be perceived as being larger, or that an individual fitted with a transmitter could be deemed undesirable by the opposite sex. Determining the extent of such effects and finding ways to minimize them will be an important aspect of developing the application to specific species.

6 | CONCLUSION

Our study successfully validates the use of biotelemetry as a powerful method to study behavioural thermoregulation in large insects. We established that the internal temperature of a locust is accurately reported by an externally mounted transmitter over a wide range of temperatures. The behaviour of locusts fitted with a transmitter was indistinguishable from that observed in previous studies using other techniques. The application of the technique to a multi-day study of thermoregulation in a semi-natural environment demonstrates that multiple individuals can be monitored and that they exhibit expected patterns of behavioural thermoregulation in a heterogeneous thermal environment. Further studies are still needed to understand the effects of transmitter attachment to wild insects, including studies of increased physiological strain or altered behaviour towards conspecifics. However, this method clearly has enormous potential, opening up the possibility of a range of studies in which

thermoregulation is studied in unrestrained insects both day and night, even when they conceal themselves or when interacting with conspecifics or other features of their variable thermal environment.

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CONFLICT OF INTERESTS

The authors declare no competing or financial interests.

AUTHOR CONTRIBUTIONS

Conceptualization: J.O. and C.K.Ø. Formal analysis: C.K.Ø., J.O.; Investigation: C.K.Ø.; Writing C.K.Ø, J.O. and T.T.; Supervision: J.O. and T.T.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.1ch4264> (Ørskov, Tregenza, & Overgaard, 2019).

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