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Non-Contact Infrared Thermometers Can Accurately Measure Amphibian Body Temperatures

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Body temperature affects almost all biochemical and physiological processes in ectothermic organisms (Hutchinson and Dupré 1992), and thus affects movement, habitat selection and thermoregulatory behavior (Bartelt and Peterson 2005). A variety of devices have been used to determine the body temperatures of amphibians in the field. The most typical is a thermocouple probe connected to a quick-reading thermometer, which has been used to measure skin (Brattstrom 1963; Heath 1975; Lillywhite 1970; Navas 1996; Tracy 1976), oral (Brattstrom 1963; Lillywhite et al. 1973; Lillywhite et al. 1998), or cloacal (Brattstrom 1963; Cabanac and Cabanac 2004; John-Alder et al. 1988; Passmore and Malherbe 1985; Thorson 1955; Vences et al. 2002) temperature. These methods require manual handling of each individual, which may artificially elevate its body temperature (due to conduction; Navas and Araujo 2000), or may alter the behaviour of individuals in studies of a longer duration (i.e., by capture stress).

To date, non-invasive methods of measuring amphibian body temperature that do not require the manual handling of individuals have included the attachment of thermally sensitive radio-transmitters (Bradford 1984; Heath 1975; Seebacher and Alford 2002) and the use of physical models (Bartelt and Peterson 2005; Hasegawa et al. 2005; Navas and Araujo 2000; O'Connor and Tracy 1987; Seebacher and Alford 2002). More recently, an additional method of measuring amphibian body temperature has become available, via the use of hand-held, non-contact, infrared thermometers (e.g., Young et al. 2005). These thermometers have been widely adopted in the medical sciences, and have been shown to accurately measure body temperature in humans (Koçak et al. 1999; Rotello et al. 1996; Terndrup et al. 1997). Despite their promise as a rapid, non-invasive method of measuring amphibian body temperature, their accuracy for measuring amphibian body temperature has not been quantified. We designed this study to determine whether a non-contact infrared thermometer can be used to accurately measure the body temperatures of amphibians.

Materials and Methods.—Our experiment was designed to allow us to measure body temperatures of frogs allowed to experience a wide range of thermal environments in a laboratory setting. We set up three opaque plastic containers (60 cm × 40 cm × 40 cm), each with a small water bowl in the center and a metal fly-screen lid. The containers were housed in a constant temperature room, which maintained ambient temperature between 19.5 and 21.5°C. Relative humidity fluctuated between 64 and 96% (mean 74%). A 150-watt heat lamp was provided at one end of the container during the day (0930–2130 h) in order to provide a heat gradient within the normal temperature range of the species.

Twelve adult Common Green or White's Tree Frogs (*Litoria caerulea*) were captured near Townsville, Queensland, Australia. They ranged from 74.1–91.8 mm SVL and 26–65 g body mass. Prior to experiments, they were maintained in the constant temperature room in which the experiments were carried out, but in smaller containers with no access to heat lamps. Frogs were fed crickets *ad libitum*. Each frog was used in a single run of the experiment. We ran four temporal replicates of the experiment, creating a total of twelve sets of measurements of frog and model temperatures for comparison. Each replicate ran for three days.

Body temperatures of frogs were recorded five times per day (0900, 1100, 1300, 1500, and 1700 h), producing 15 measurements for each of the 12 frogs. The first time (0900 h) was chosen because at that time the frogs had received no source of heat for almost 12 h, and their body temperature should have been similar to a nocturnal reading in the field. Each run of the experiment was set up at least 60 minutes before the first temperature reading was taken, allowing frogs to reach a thermal steady state.

At each reading, frog body temperature was recorded using three techniques. Firstly, temperature was recorded by holding a Raytek ST80 Pro-Plus Non-contact thermometer (RAYST80; "IR thermometer") approximately 5 cm away from the frog and aiming at the lower dorsal area near the thigh. The model of IR thermometer used in this study had a distance to spot ratio of 50:1, and the area measured is delineated by a circle of laser diodes. Emissivity was set on the IR thermometer at 0.95, as it is generally accepted that amphibians have a long-wave emissivity of approximately 0.95–0.97 (Carroll et al. 2005; Tracy 1976), regardless of their color (Nussear et al. 2000; Tracy 1979). In initial trials, we determined that measured frog body temperatures did not vary by more than 0.1°C when the emissivity setting of the IR thermometer was varied between 0.95–1.0.

After taking a reading using the IR thermometer, we then measured skin and cloacal temperatures (in that order) using a small, chromel-alumel "K" type thermocouple (diameter approx. 1 mm) with the tip coated in plastic, attached to a digital thermometer type 90000. To measure skin temperature, the thermocouple was held firmly against the skin on the lower dorsal area near the thigh while the frog remained in its original position in the container. During cloacal temperature measurement, each frog was held by a single leg, while still in the container, and the thermocouple was inserted 10–20 mm inside the cloaca and the reading was taken when the indicated temperature stablized.

Frogs were usually in the water conserving posture immediately prior to temperature measurement, except for several instances at 0900 h. It is probable that there are small differences among individual frogs, related to body size or individual behaviour, that affect how temperatures measured using the three techniques are correlated, so that the measurements for each individual frog are not entirely statistically independent. We therefore did not carry out any hypothesis tests, but concentrated on modelling the relationships between measurements taken using the three techniques, and determining how well our models fit the data. Because all three variables are measured with error, we constructed models of their relationships using major axis regression (Sokal and Rohlf 1995). We determined how well our regression models fit the data by calculating coefficients of determination, using standard correlation analysis.

Room temperatures were recorded every 30 minutes using thermal data loggers (Thermochron iButtons by Dallas Semicondictor, Dallas, Texas USA). Data loggers and thermocouples were calibrated against a high precision mercury thermometer in a magnetically stirred water bath.

Results.—Body temperatures measured using the IR thermometer and both skin and cloacal temperatures measured using the thermocouple probe were highly correlated. The major-axis regression lines relating each type of temperature measurement to the others were all very similar to the line of equality (Fig. 1). The major-axis regression lines never predict a mean difference greater than approximately 0.5°C between any two temperatures in the range of 18–34°C for any pair of measurements.

Discussion.—The surface of basking animals may reach slightly higher temperatures faster and decrease more rapidly after basking ceases than the body core (Remmert 1985). However, we found that skin temperatures measured either by contact thermocouple or IR thermometer were almost always within 0.5°C of cloacal temperatures (Fig. 1); this appears to be relatively common in small ectotherms such as frogs (Wygoda 1984), and other small ectotherms such as lizards (<10–20 g; Jones and Avery 1989). It is likely that the small number of points which depart to a larger-than-usual extent from the lines of equality and regression lines in Fig. 1 were measured on animals that had recently changed from basking to non-basking or the reverse.

We found that cloacal temperature was slightly better predicted by surface temperature as measured by the IR thermometer than it was by skin temperature measured using a thermocouple. This indicates that surface temperatures measured using the IR thermometer should provide accurate indicators of internal body temperatures in most amphibians.

Good quality IR thermometers have long-range optical resolution, allowing measurement of small targets at long distances. As the distance from the object increases, the spot size of the area measured by the unit becomes larger. Therefore, the smaller the target, the closer you must be to it in order to avoid measuring a combination of amphibian and background temperatures. Especially in the field, it is necessary to take the distance to spot ratio into account. When studying small frogs, it may only be possible to measure temperatures at short ranges (< 0.5 m). As the laser is located above the sensor in many models, it is also important to take parallax effects into account when aiming the sensor, as at near distances the point of aim of the sensor will be displaced from the point of aim of the laser diode.

It is likely that IR thermometers will be useful in measuring other small animals, such as reptiles, in the field. As all plants and animals act almost as black-bodies in the middle infrared (Sustare 1979), having an emissivity nearing 1.0, no major changes in the technique will be necessary when used on different species. Indeed, we are presently using IR thermometers successfully for measuring the body temperatures of a number of amphibian species in the field (Rowley and Alford, unpubl. data).

We have shown that non-contact infrared thermometers can be used to accurately determine the body temperatures of amphibians. Benefits of this technique include relatively low cost (approximately US $340), small size and therefore high portability, and the ability to rapidly record the temperature of a large number

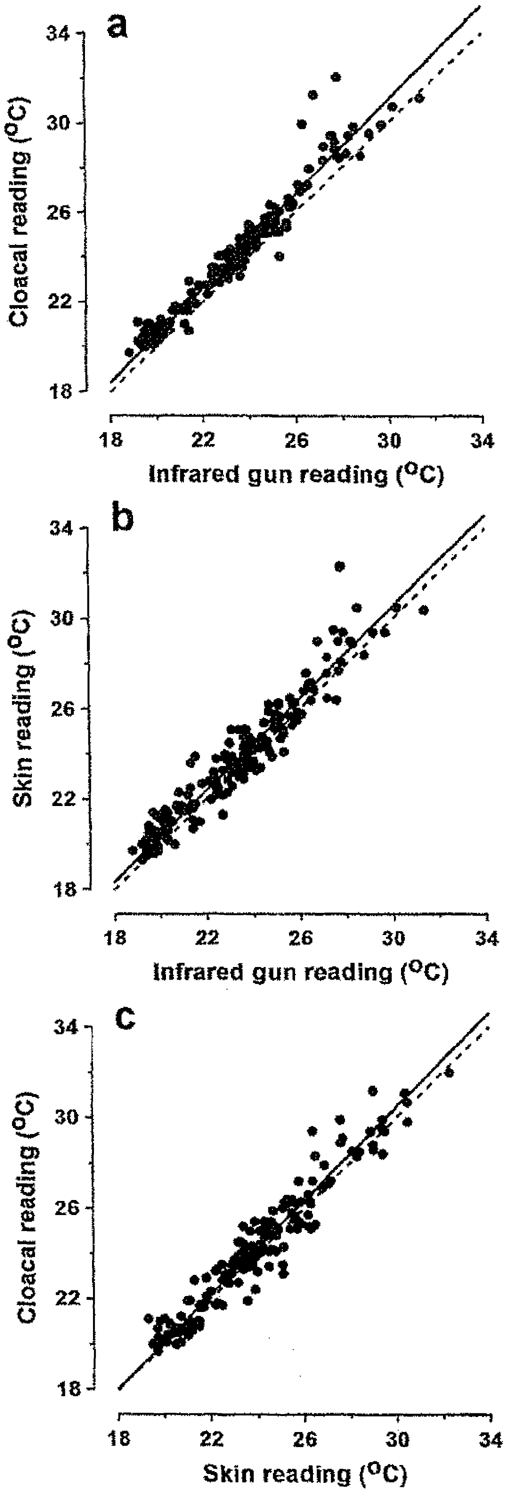

FIG. 1. (a) Measured cloacal temperature as a function of infrared gun reading. Dashed line is at $y = x$, solid line is the major axis regression $y = 1.052x - 0.535$ with $r^2 = 0.949$. (b) Measured skin temperature as a function of infrared gun reading. Dashed line is at $y = x$, solid line is the major axis regression $y = 1.014x + 0.1049$, with $r^2 = 0.922$. (c) Measured cloacal temperature as a function of skin temperature. Dashed line is at $y = x$, solid line is the major axis regression $y = 1.038x - 0.657$, with $r^2 = 0.932$.

of individuals. Perhaps the most important feature of the technique, however, is its ability to record the temperature of amphibians without handling them. This reduces disturbance, which can be important when the same individual is to be measured repeatedly, or when disturbance may cause an animal to abandon a retreat or basking site, exposing it to increased risks of predation or desiccation. In addition, such non-invasive methods of determining amphibian body temperature are likely to be increasingly important due to the need to minimise handling stress and the possibility of disease transmission, particularly when studying species of conservation concern.

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Correct Orientation of a Hand-held Infrared Thermometer is Important for Accurate Measurement of Body Temperatures in Small Lizards and Tuatara

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Measurement of cloacal temperature using a thermocouple (TC) is a standard and long-established method for determining body temperature (T_b) in reptiles. There are some disadvantages, however, to this method. The animal must first be captured and handled, and the thermocouple must be inserted into the cloaca; such manipulations potentially raise the animal's breathing rate (Langkilde and Shine 2006) and, if prolonged, could initiate a stress response involving elevated plasma corticosterone (e.g., Moore et al. 1991). Furthermore, the animal's retreat to a different microenvironment when chased, and/or its subsequent handling, potentially result in an unrepresentative measurement of T_b. An experienced researcher is able to minimize these outcomes in most cases; however, alternative non-invasive methods are desirable.

Useful alternatives for long-term studies of T_b include radiotelemetry and intra-coelomically implanted temperature loggers. While these methods do not require frequent recapture of the animal, they still require the initial attachment of the device, which itself sometimes requires surgery (Charland 1995; Forsythe et al. 2004; Taylor et al. 2004). Consequently, these methods are not suitable for very small animals.

The advent of infrared technology potentially offers a non-invasive alternative for on-the-spot measurement of T_b in small reptiles. Both infrared imaging systems and hand-held thermometers are now available. Imaging systems have proved useful in controlled laboratory environments where relatively large systems can be set up for a long period of time, and if properly calibrated produce highly accurate results (Jones and Avery 1989). Unfortunately these systems are too bulky for use in the field. However, hand-held infrared thermometers (IRT) are available, at much lower cost. These devices consist of a handle, a sensor, and a laser sight. The sensor beneath the laser sight measures infrared radiation of the surface at which the IRT is aimed, and produces a temperature reading precise to about 0.1°C (see Methods), comparable to many thermocouples. Studies using IRT are currently being evaluated for medical purposes, and show promising results for use in some non-critical applications, although some reservations have been expressed (Banitalebi and Bangstad 2002; Hoffman et al. 1999; Leon et al. 2005).

An IRT potentially offers several advantages for measuring T_b in small reptiles. The method has already been used to study temperatures selected by small geckos on a thermal gradient (Christian et al. 1998; Werner et al. 2005) but no rigorous comparison of