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A TEST OF THE ACCURACY OF OPERATIVE TEMPERATURE THERMOMETERS FOR STUDIES OF SMALL ECTOTHERMS

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Abstract—1. Various devices have been used to estimate the equilibrium body temperature of ectotherms occupying natural environments. We tested the accuracy of such devices under a range of conditions.

2. We measured body temperatures of lizards (*Sceloporus magister*) exposed to short-wave radiation under varying convective conditions and compared these to temperatures of hollow metal casts duplicating the animal's shape and reflectivity, as well as to the temperatures of cylinders similar to those used by other workers.

3. Casts equilibrated within 2–3°C of live animals, yielding errors of 14–37% of the radiation-produced elevation of body temperature.

4. Various cylinders differed from animal body temperature more than lizard casts did, producing errors equally 33–53% of the radiation-produced elevation.

5. It is imperative that workers using operative-temperature thermometers experimentally confirm the adequacy of the devices they use for the range of conditions encountered within a specific analysis. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Thermoregulatory requirements importantly affect site selection and activity patterns in terrestrial ectotherms such as typical lizards, and a large fraction of the extant literature dealing with these organisms focusses on the interactions of behavior and physiology with the thermal environment. A major complication in such analyses is that terrestrial environments are physically complex and their critical thermal properties vary with space and time. This complexity of natural environments has led to increased emphasis on techniques that simplify their analysis (Bakken, 1992).

Commonly used tools in studies of the thermal ecology and physiology of terrestrial ectotherms are devices that are expected to equilibrate at a temperature near the equilibrium body temperature of the species under study. Bakken and Gates (1975) and Bakken (1976) formalized biophysical theory supporting the use of such 'operative environmental temperature (T_E) thermometers'. Bakken and Gates (1975) noted drawbacks of some devices thought to approximate T_E-thermometers and concluded that "Most of these limitations are eliminated if an object that exactly duplicates the animal except for metabolism and water loss is used as a thermometer and placed in the same position in the environment as the animal under study". They concluded that under these conditions the equilibrium temperature of such T_E-thermometers should equal that of a live animal.

Given that 'exactly duplicating' the animal except for metabolic heat production and water loss is unachievable, what approximation is sufficient to yield acceptable results? Bakken and Gates (1975) and Bakken (1976, 1992) recommended the use of hollow metal casts of small animals such as lizards, with the casts painted to match the animal's solar absorptivity. Such casts do not, of course, subsume effects such as temporal and spatial variations in animal surface temperature that might be produced by animal properties other than metabolic heat production or evaporative water loss. These include, for example, variations in blood flow within and between regions of the animal (Cowles, 1958; Heath, 1964; Morgareidge and White, 1969a, b; Weathers and Morgareidge, 1971; Baker *et al.*, 1972; Smith and Adams, 1978; Rice and Bradshaw, 1980). Bakken (1976) argues, however, that these processes are not likely to be of major importance and several workers have taken the approach of using hollow metal casts (e.g. Crawford *et al.*, 1983; Grant and Dunham, 1988, 1990; Grant, 1990; Hertz, 1992). Others have concluded that even simpler constructions are adequate. The range of devices used as T_E-thermometers for ectotherms include, for example, mummified lizards (e.g. Beuchat, 1986, 1989) and closed cylinders constructed of plastic (e.g. Van Berkum *et al*., 1986; Adolph, 1990) or metal (e.g. Peterson, 1987; Huey *et al*., 1989; Plummer, 1993). These devices typically are painted in an attempt to match the solar absorptivity of the live animal. In some cases, the match of animal to T_E-thermometer is ensured through precise measurements using reflecting spectrophotometers (e.g. Hertz, 1992). In other cases, the similarity of paint and animal absorptivity is unstated, as is the method by which the match was achieved (e.g. Adolph, 1990, Plummer, 1993). Given the spectral sensitivity of the human eye, simply visually matching colors is not a safe guide to achieving similar values of reflectivity across the range from ultraviolet to infrared radiation represented in sunlight (Coulson, 1975).

Are such devices adequate in their primary role of estimating the equilibrium body temperature of an ectotherm in a particular microclimate? Determining this clearly is a critical step (Bakken, 1992), and some workers have tested this hypothesis before proceeding to analyses relying upon T_E-thermometers. Grant and Dunham (1988), for example, constructed hollow metal casts of the lizard *Sceloporus merriami*, and painted these to match the typical solar reflectivity of live animals, as measured with a reflecting spectrophotometer. Under conditions representative of those in which data were later collected for ecological analyses, body temperatures of lizards and mounts were simultaneously measured and found to correspond closely. Unfortunately, such validations are rare and T_E-thermometers often are used apparently without meaningful calibration.

Previously, we explored the utility of a related technique, which is the use of heated and unheated taxidermic mounts to estimate metabolic power consumption by endotherms with insulating coats (Walsberg and Wolf, 1996). Our results demonstrated that the adequacy of taxidermic mounts varied widely with species, type of mount, and environmental conditions. Often, predictions using data from mounts produced large errors.

Given such results as well as the widespread use of various types of T_E-thermometers, our goal is to explore the extent to which errors might be accrued through the use of devices similar to those commonly employed. In this analysis, we therefore measure equilibrium body temperatures of live lizards (*Sceloporus magister*) exposed to simulated solar radiation under a range of convective conditions. We then compare these values to the equilibrium temperatures of hollow metal shells duplicating the shape and posture of live lizards as well as to the temperatures of four different closed cylinders similar to those used as T_E-thermometers by other workers. In our analysis, the range of error is constrained by examining a restricted set of physical environments and because the unknown errors that might be introduced by visually matching the solar absorptivity of T_E-thermometers to live animals is eliminated by accurately measuring reflectivity to short-wave radiation.

MATERIALS AND METHODS

Animal capture and maintenance

Desert spiny lizards, *Sceloporus magister*, were captured in September and October in Maricopa County, Arizona. Lizards were housed in $0.5 \times 0.5 \times 0.5$ m cages, two animals per cage. Air temperature was maintained at 23–29°C and a heat lamp was provided to allow behavioral thermoregulation. Lizards were fed crickets and provided with water *ad libitum*.

Environmental simulation

Measurements were made within a closed-circuit wind tunnel described by Walsberg and Wolf (1995), with air temperature held at 15°C $\pm$ 0.2°C. Wind speeds were maintained at either 0.5, 1.0, or 2.0 m s^{-1}. Wind speed was measured with a Omega HHF52 thermoanemometer, calibrated using the method of Walsberg (1988). Turbulence intensity was less than 5% (Walsberg and Wolf, 1995). Simulated solar radiation was produced by a Spectral Energy Corp. Series II solar simulator, which filters light produced by a xenon arc lamp to simulate direct solar radiation. Radiation passed through a 4.8 mm flint glass window in the upper portions of test chambers that blocked intense ultraviolet radiation which would have burned the animals' skin and eyes. Irradiance in the test chambers was measured with a LiCor LI200sz pyranometer that had been calibrated against an Oriel pyroelectric radiometer. Simulated solar irradiance in the center of the test chamber was maintained within 1% of 935 W m^{-2} and varied less than 5% across the chamber floor. The long-wave radiant environment within the chambers was held nearly constant by painting the walls with flat-black enamel and maintaining their temperature at 15°C $\pm$ 2°C. Assuming an emissivity of 0.98 and calculating long-wave irradiance by the Stefan–Boltzmann relation, long-wave emission therefore varied less than 6%.

Body temperature and reflectivity measurements

Lizards were tethered to a base plate of polystyrene 'foam board', consisting of a composite of 6 mm rigid foam laminated between two sheets of white paper. At both wrists and both ankles, a rubber band was passed through 5 mm holes in the base plate and around the respective joint. These bands prevented the lizard from moving but allowed it to maintain a normal posture. A 0.6 mm diameter, vinyl-coated thermocouple was inserted 2–3 cm into the cloaca and secured by taping the lead to the ventral portion of the tail. The lizard and base plate were then placed in the wind tunnel such that the lizard was aligned parallel to air flow and faced into it. Body temperatures reported are equilibrium values achieved after 20–30 min and when lizard body temperature is not changing more than 0.1°C in 10 min.

Reflectivity was recorded simultaneously with equilibrium body temperature by collecting light using a 5 mm diameter fused-silica optical bundle that was directed at an approximately 10 × 20 mm area of the lizard's dorsum. The optical bundle conducted light to an Oriel model 7080 pyroelectric radiometer. A plate coated with 2 mm of Kodak Total Reflectance Paint™, which has a reflectance ranging from 0.96 to 0.99 over the waveband of 300–1300 nm (average reflectance = 0.98), was used as a standard by substituting it in the position of the lizard.

Construction of operative temperature thermometers

Two mounts were manufactured, representing a large and small *Sceloporus magister*. The large mount had a snout-vent length of 96 mm and a maximum torso diameter of 29 mm. The small mount had a snout-vent length of 68 mm and a maximum torso diameter of 20 mm. Mounts were prepared by the method of Bakken and Gates (1975) and thus consisted of hollow copper duplicates of lizards in postures mimicking that of live animals during measurement of body temperature. Mount temperature was sensed with a 26 GA thermocouple inserted into the mid-abdominal region of the mount.

We also constructed a series of cruder T_E-thermometers essentially similar to those in common use. These consisted of two pairs of large and small cylinders made from contrasting materials. One pair of cylinders was constructed of plastic (polyvinylchloride) pipe. The large cylinder was 120 mm long and 27 mm wide; the small cylinder was 118 mm long and 16 mm wide. The second pair was constructed of copper pipe with dimensions similar to that of the plastic cylinders (large cylinder: 22 mm wide and 120 mm long, small cylinder: 16 mm wide and 112 mm long). All cylinders were sealed with caps of material matching that of the pipe. Temperature was sensed with 26 GA thermocouples inserted into the center of each device.

All cylinders and casts were painted so that their absorptivity closely matched that of live lizards during these experiments. Using the same apparatus as used to measure average reflectivity to simulated solar radiation of live lizards, reflectivity of the commercial paint chosen (Dunn-Edwards Vin-L-Tex "Smokestone") equaled 16.9%, similar to the average measured for lizards during this analysis (17.2%; see Results). Cylinders and casts were placed in the wind tunnel such that air flow was parallel to their long axis, and mounts were placed so that they faced into the wind.

Data analysis

The Kruskal–Wallis test followed by a non-parametric Tukey-type test was used for multiple contrasts examining lizard body temperature or reflectivity as a function of wind speed (Zar, 1984). Correlations between lizard body mass and body temperature or reflectivity were tested by analysis of variance (ANOVA). Statistical significance was accepted at $P < 0.05$. Values for lizard body temperature and reflectivity are reported as means ± 95% confidence intervals, with $n = 8$.

RESULTS

Lizard body temperature and reflectivity.

At the end of the 20–30 min period required for lizards to reach thermal equilibrium, reflectivity was 18.5% ± 4.5% at 0.5 m s^{-1}, 17.1% ± 4.3% at 1 m s^{-1}, and 16.0% ± 4.2% at 2 m s^{-1} None of these values differs significantly ($P > 0.5$). If a single value is computed for each individual by averaging data collected at each wind speed, overall mean reflectivity equals 17.2% ± 4.2% ($n = 8$). There was no significant correlation of body size with reflectivity at any wind speed ($P > 0.05$).

At the lowest wind speed of 0.5 m s^{-1}, lizard body temperatures equilibrated at temperatures averaging 12.9°C above air temperature (Fig. 1). This $T_B - T_{AIR}$ difference declined with increasing wind speed and averaged only 5.9°C at 2 m s^{-1} wind. At no wind speed was there a significant correlation of body mass with the $T_B - T_{AIR}$ difference (Fig. 1).

T_E-thermometer temperatures

Heating of metal casts of lizards by simulated solar radiation showed similar wind speed dependence as

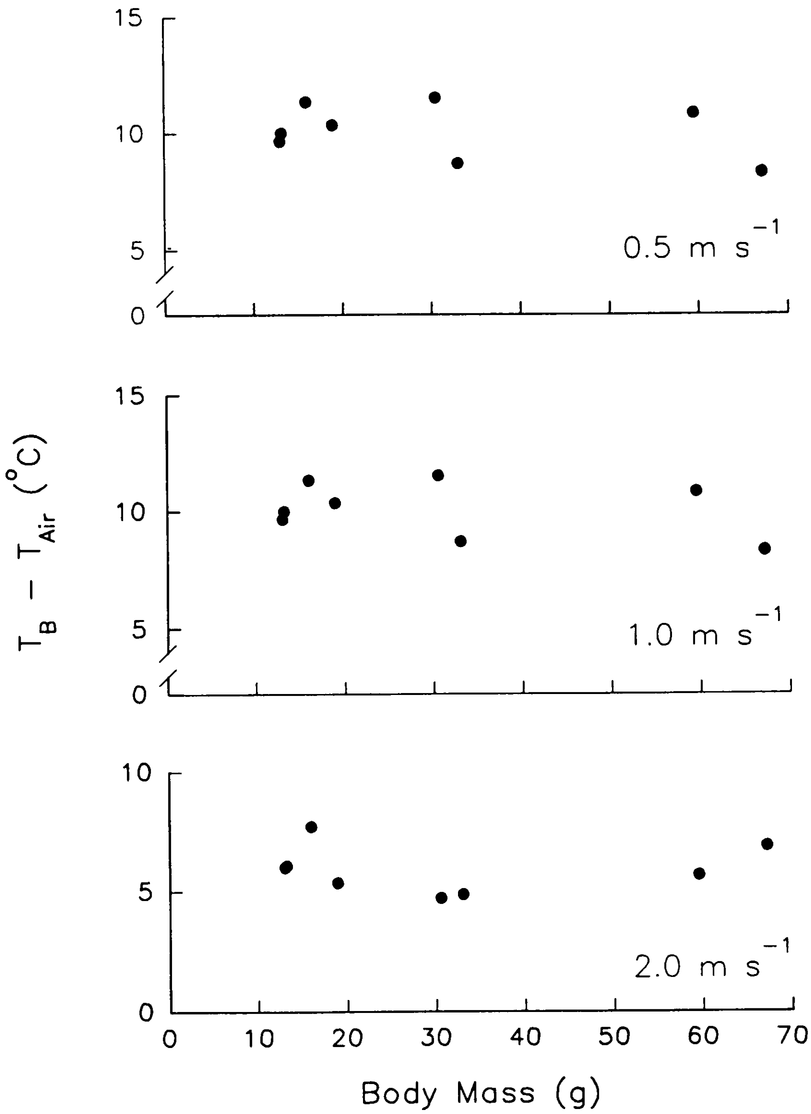

Fig. 1. Lack of effect of body mass upon the elevation of body temperature over air temperature. Values are equilibrium temperatures for individual lizards. At no wind speed is elevation of body temperature significantly correlated with body mass ($P > 0.6$).

observed for live animals, although cast temperatures averaged 1.8°C–2.5°C lower than those of lizards exposed to identical conditions (Fig. 2). These differences equal 14–37% of the radiation-produced elevation of lizard body temperature over air temperature. The large cast achieved temperatures 0.3–1.1°C above that of the small cast. Cylindrical models diverged farther from the thermal response of live animals, with temperatures 2.5°C–5.7°C below that characteristic of lizards. Large metal and plastic cylinders produced similar errors (Fig. 1), and achieved elevations over air temperature of 58–67% of those observed in live animals. Small plastic cylinders exhibited the greatest errors, and exhibited radiation-produced elevations over an air temperature of only 47–56% of those achieved by live animals exposed to identical conditions.

DISCUSSION

Unexpected independence of body temperature from body size

Body size is generally viewed as an important determinant of the thermoregulatory abilities of ectotherms, and increased body size in heliothermic forms should facilitate maintenance of elevated body temperatures (Bartholomew, 1982; Stevenson, 1985). Data for inanimate mounts and cylinders conform to these expectations; for any particular mount type, larger devices equilibrated at higher temperatures

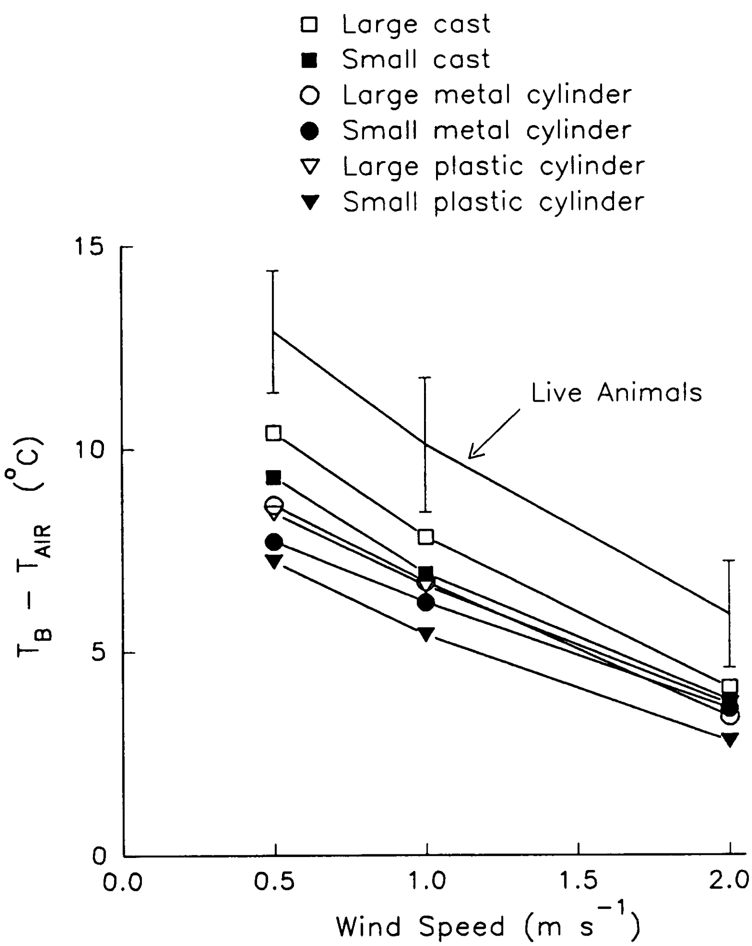

Fig. 2. Equilibrium temperatures of lizards and various types of T_E-thermometers. Values for live animals are means and 95% confidence intervals ($N = 8$).

(Fig. 2). In contrast, no such tendency was observed in live *Sceloporus* despite a five-fold range in body mass (Fig. 1). This suggests that these lizards possess mechanisms to uncouple them from simple size-induced effects. The nature of these mechanisms is unknown, but could involve a variety of processes, such as alterations in peripheral circulation. Changes in coloration apparently are not involved, as there is no correlation between body mass and short-wave reflectivity.

Failure of T_E-thermometers to mimic lizard body temperatures

The devices that functioned best as operative-temperature thermometers were painted metal casts of lizards, with larger errors accruing from use of metal or plastic cylinders. The magnitude of errors resulting from use of the latter (up to 6°C) is such that, under some conditions, results would be more accurate if workers simply relied upon air temperature as an index of animal body temperature. This is not a recommendation to do so, but does demonstrate that these major errors could well vitiate an analysis relying upon uncritical use of such devices.

In addition, note that for three reasons our analysis of potential error is limited. First, one source of uncertainty was eliminated because animal and mount reflectivity were closely matched. Second, only a single body posture and orientation was employed; smaller or larger errors might result in other positions. Third, we used only a single range of environmental conditions. Changes in factors such as substrate conductivity, level of irradiance, or wind speed are likely to alter the amount of error. Lower wind speeds, for example, are common in nature and probably would produce larger differences between T_B and T_E estimates (Fig. 2). Finally, we emphasize that workers should not assume that the relations and degree of error we observed will hold for other species and environmental conditions.

Concluding comments

A fundamental tenet of experimental science is that techniques must be validated, and instruments calibrated, within the range of conditions in which they will be used. It is particularly striking, therefore, that such validation tests are rare for forms such as ectothermic lizards, given that these animals have received extensive attention and that the physiological parameter of interest is body temperature. Quantifying the latter is relatively simple, for example, compared to the measurements of metabolic heat production needed to calibrate operative-temperature devices used for analyses of endothermic homeotherms (e.g. Bakken *et al.*, 1981; Walsberg and Wolf, 1996). Failure to clearly demonstrate the accuracy of techniques for a particular species and set of environmental conditions under study is a critical lapse, however, that can importantly lessen confidence in the validity of data subsequently collected as well as the conclusions drawn from those data.

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