AN ASSESSMENT OF THE EVAPORATIVE RELEASE OF HEAT FROM THE BUCCOPHARYNGEAL, CUTANEOUS, AND CLOACAL EPITHELIA OF BIRDS AND REPTILES

by

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ABSTRACT

Evaporation can simultaneously subject an animal to a detrimental loss of physiologically essential water and to a beneficial loss of life-threatening heat. Buccopharyngeal evaporation occurs from the mouth and pharynx, and it is only one component of an animal's total evaporation. For tetrapods other than mammals, non-buccopharyngeal evaporation (the remainder of total evaporation) occurs despite an incapacity for sweating. High rates of non-buccopharyngeal evaporation have been measured in many bird species, and rates of non-buccopharyngeal evaporation have been shown to change gradually during acclimation to changes in temperature or aridity. This dissertation demonstrates that mourning doves (*Zenaida macroura*) are able to effect rapid, endogenous adjustment to the rate of non-buccopharyngeal evaporation when faced with a suppression of buccopharyngeal evaporation. This implies that non-buccopharyngeal evaporation can serve as a transient mechanism for thermoregulation. However, the adjustment of non-buccopharyngeal evaporation shown in mourning doves prompts the question of how that non-buccopharyngeal evaporation is apportioned among the non-buccopharyngeal epithelia.

Historically, researchers have assumed that all non-buccopharyngeal evaporation occurs from the skin (cutaneous evaporation). This research demonstrates that the cloaca can be the site of much of an animal's total evaporation and that cloacal evaporation sheds enough heat to be important for thermoregulation. Both Gila monsters (*Heloderma suspectum*) and Inca doves (*Columbina inca*) underwent a transition from negligible to significant rates of cloacal evaporation as ambient temperature increased beyond a

critical point. Cloacal evaporation accounted for 82% of Gila monsters' total evaporation at 40°C and for 21% of Inca doves' total evaporation at 42°C. Heat dissipation by cloacal evaporation could allow these species to inhabit hotter microclimates for longer time periods, potentially increasing time allocated to foraging and reproductive behaviors.

Evidence that cloacal evaporation is not a universal feature of animals possessing a cloaca is provided by results from Eurasian quail (*Coturnix coturnix*) and ball pythons (*Python regius*). Both exhibited negligible cloacal evaporation even when heat-stressed. These negative results, especially from the ball python, a tropical snake unlikely to require cloacal evaporative cooling, serve as preliminary evidence that cloacal evaporation is an adaptive mechanism for thermoregulation.

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Evaporative Heat-Loss and Evaporative Water-Loss: The Importance of Retaining and of Releasing Water

On a physicochemical level, organisms can be described as self-regulating sets of chemical reactions and physical processes. Water serves as the biological solvent in the various fluid solutions in which these chemical reactions occur, and water is therefore essential to organismal life. The temperature of an organism's fluids has profound effects on the rates of life's myriad chemical reactions (Kleiber, 1961). Organisms therefore must remain within physiological limits of viability with respect to both hydration and temperature. Among organisms, these limits are comparatively narrow for animals in general and for homeotherms in particular (Prosser, 1991).

Interestingly, water plays vitally important but potentially conflicting roles in an animal's maintenance of hydration (hydrostasis) and of temperature (thermostasis). In a normally hydrated animal, hydrostatic feedback mechanisms trigger effectors that reduce or minimize the rate of loss of water. Because water contains heat, any loss of water will shed heat from the animal (Gates, 1962). However, if the water that is lost from that animal is allowed to evaporate from the body's surface, then considerably more heat is shed than if water is lost simply as liquid (Monteith and Unsworth, 1990). That extra heat is the latent heat of vaporization of water, or the amount of heat required for the change in state from liquid to gas. Evaporation, therefore, can serve as an effective thermostatic mechanism when body temperature is elevated, in which case thermostatic feedback mechanisms trigger effectors that increase or maximize the rate of evaporation.

The potential conflict between the dual roles played by water is epitomized by an animal exposed to wind, sunlight, and high ambient temperature. Under such

environmental conditions, the animal will gain heat by convection, radiation, and conduction (Gates, 1962), in addition to the heat gained as a by-product of metabolism (Prosser, 1991). Therefore, the animal is faced with two options with respect to thermoregulation. If the animal escapes the prevailing meteorological conditions, it must retreat to a different microclimate to eliminate or reduce the convective, radiative, or conductive gain of heat (Porter and Gates, 1969). This can be done by seeking shelter from the wind by seeking shade, by reducing the fraction of the body's surface exposed to the substrate, or by moving to a cooler substrate (Gates, 1962). Any of these behavioral adjustments will either reduce or reverse the gain of environmental heat. Such a reduction or reversal will, in turn, reduce the need for evaporative cooling, which will stave off dehydration. Obviously, these behavioral adjustments require the availability of such microclimates. That availability is constrained by habitat, season, time of day, body size, and locomotory ability. If cooler microclimates are available, an animal opting for behavioral thermoregulation, though reaping benefits in terms of hydration status, will nonetheless be forced to contend with any detrimental consequences of moving to the new microclimate. These consequences can include reduction or even preclusion of foraging ability, increased risk of attack by predators, and increased costs to reproductive or parental behavior (Martín et al., 2003). If, on the other hand, the animal remains in the microclimate and face the convective, radiative, and conductive heat-loads, then evaporation, as the only remaining mode of heat transfer, becomes the only possible mechanism for heat-loss under those conditions (Monteith and Unsworth, 1990). Keeping body temperature below the lethal limit will thus require the loss of body water, a

hydrostatic cost. Nevertheless, favoring evaporative thermoregulation over behavioral thermoregulation could benefit the animal with respect to foraging, reproduction, depredation, or parental care (Martín et al., 2003).

The hottest microclimates in the biosphere coincide largely with the driest microclimates. Animals inhabiting hot deserts therefore face the competing demands of thermostasis, which calls for the evaporative loss of water from the body to the environment, and of hydrostasis, which calls for the bodily retention of water that is environmentally scarce.

Because hydrostatic demands directly oppose thermostatic demands, it is reasonable to expect that evaporation is a tightly controlled process. One way that evaporation can be controlled is by adjusting the apportionment of evaporation among its possible routes. Birds and reptiles, the subjects of this dissertation, possess three anatomically distinct epithelial surfaces from which water can evaporate. These occur in the mouth and pharynx, on the skin, and in the cloaca. Hereafter, I describe evaporation occurring from these respective epithelia as ‘buccopharyngeal’, ‘cutaneous’, and ‘cloacal’, and I use ‘non-buccopharyngeal’ to refer to the sum of cutaneous evaporation and cloacal evaporation.

Birds exposed to high environmental temperatures can often be seen panting, which is a way to convectively enhance buccopharyngeal evaporation (Bouverot et al., 1974; Calder and Schmidt-Nielsen, 1966; Larcombe et al., 2003; Richards, 1970). Whether or not panting is employed, however, birds must evaporate some amount of water buccopharyngeally, as a result of ventilation. While birds do not possess sweat

glands, some birds nevertheless show high rates of non-buccopharyngeal evaporation (Arieli et al., 2002; Marder and Gavrieli-Levin, 1987; Tieleman and Williams, 2002; Wolf and Walsberg, 1996). Several studies have shown that rates of non-buccopharyngeal evaporation can exceed rates of buccopharyngeal evaporation (Hoffman and Walsberg, 1999; Marder et al., 1989; McKechnie and Wolf, 2004; Webster and King, 1987; Withers and Williams, 1990). Those findings prompted questions about possible mechanisms of control of non-buccopharyngeal evaporation. Subsequent studies revealed that rates of non-buccopharyngeal evaporation can be affected by habitat (Tieleman and Williams, 2002; Williams and Tieleman, 2005) and by acclimation and acclimatization over long time periods (Marder and Gavrieli-Levin, 1987; McKechnie and Wolf, 2004; Ophir et al., 2003). Rates of non-buccopharyngeal evaporation in birds have been shown to quickly increase in response to artificial blockade of β-adrenergic receptors (Arieli et al., 1999; Marder and Raber, 1989; Ophir et al., 2004). However, prior to the work described in this dissertation, short-term, physiological (i.e., endogenous) adjustments to rates of non-buccopharyngeal evaporation had not been demonstrated in birds (Hoffman and Walsberg, 1999). In Chapter 2, I investigate whether the mourning dove (*Zenaida macroura* Linnaeus), a bird that is capable of high rates of buccopharyngeal evaporation and tolerates high environmental temperatures, is able to rapidly adjust non-buccopharyngeal evaporation.

For decades, biologists have distinguished between buccopharyngeal evaporation and non-buccopharyngeal evaporation by employing methods that separately measure each (Bernstein, 1971; Hoffman and Walsberg, 1999; Lahav and Dmiel, 1996; Richards,

1976; Tracy and Walsberg, 2000; Webster and King, 1987; Wolf and Walsberg, 1996). However, prior to the experiments I conducted for this dissertation, the cloaca has not been considered as a possible route for evaporative loss of heat (DeNardo et al., 2004). Chapters 3 through 5 present results of experiments designed to test the efficacy of cloacal evaporation as a thermoregulatory mechanism in birds and reptiles. In Chapter 3, I discuss the effects of temperature and dehydration on rates of cloacal, cutaneous, and buccopharyngeal evaporation in the Gila monster (*Heloderma suspectum* Cope), a lizard of the Sonoran Desert. Chapter 4 builds on the results from Gila monsters and investigates the apportionment of evaporation among its three routes in two avian species, the Inca dove (*Columbina inca* Lesson) and the Eurasian quail (*Coturnix coturnix* Linnaeus). Finally, in Chapter 5, I test for use of cloacal evaporation by a tropical snake, the ball python (*Python regius* Shaw) and compare the results for this mesic-adapted reptile to those for the arid-adapted Gila monster.

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Inhibiting Buccopharyngeal Evaporation Produces an Adaptive Increase in Non-buccopharyngeal Evaporation in Mourning Doves (*Zenaida macroura*)

Summary

I tested the hypothesis that birds can rapidly change the conductance of water vapor at the skin surface in response to a changing need for evaporative heat loss. Mourning doves (*Zenaida macroura* Linnaeus) were placed in a two-compartment chamber separating the head from the rest of the body. The rate of non-buccopharyngeal evaporation was measured in response to dry, head-compartment inflow at three ambient temperatures and in response to vapor-saturated, head-compartment inflow at two ambient temperatures. At 35°C, non-buccopharyngeal evaporation increased by 72% when evaporation from the mouth was prevented, but no increase was observed at 45°C. For both dry and vapor-saturated treatments, non-buccopharyngeal evaporation increased significantly with increased ambient temperature. Changes in skin temperature made only a minor contribution to any observed increase in non-buccopharyngeal evaporation. This indicates that *Z. macroura* can effect rapid adjustment of evaporative conductance at the skin in response to acute change in thermoregulatory demand.

Introduction

Homeothermy requires continual adjustment of one or more of five possible heat fluxes (conduction, convection, radiation, evaporation and metabolism), such that the sum of these fluxes remains at or near zero. Metabolism always represents a heat gain of appreciable magnitude for an endotherm. On hot sunny days, when environmental conditions are such that each of the heat fluxes due to conduction, convection and radiation is positive, homeothermy can persist only if evaporative heat flux is sufficient to dissipate all the heat added to the body *via* the other four routes.

Water has a high latent heat of vaporization that is only weakly dependent on the temperature of the water being evaporated (Harrison, 1963). The evaporation of water is therefore a highly endergonic process that is well-suited to the demands of heat dissipation (Calder and Schmidt-Nielsen, 1967; Crawford and Schmidt-Nielsen, 1967; MacMillen and Trost, 1967; Dawson and Bartholomew, 1968; Moldenhauer, 1970; Schleucher et al., 1991). Biologists discriminate two spatially and physiologically distinct routes of evaporative heat loss. Evaporation from the external surface of an animal is variously called ‘cutaneous evaporation’, ‘transepidermal evaporation’ or ‘peripheral evaporation’; that from the pharyngeal or buccal epithelia is usually called ‘respiratory evaporation’ or ‘pulmonary evaporation’. I use ‘non-buccopharyngeal’ to describe the former evaporative route and ‘buccopharyngeal’ to describe the latter route, which encompasses evaporation by normal breathing, hyperventilation, panting and gular flutter.

The capacity for thermally significant evaporation from the skin of birds has long been disputed (Menon et al., 1986) because of the lack of avian sweat glands. However, it is now firmly established that many bird species are able to dissipate substantial amounts of heat by non-buccopharyngeal evaporation of water (Bernstein, 1971a,b; Lasiewski et al., 1971; Dawson, 1982; Marder and Ben-Asher, 1983; Marder et al., 1989). Depending on ambient temperature (T_a), non-buccopharyngeal evaporation can even account for the majority of evaporation (Webster and King, 1987).

On energetic grounds, it seems logical that non-buccopharyngeal evaporation could be important in birds, because all modes of buccopharyngeal evaporation entail some gain of heat in the forms of friction in the musculature involved and of the metabolism required to power the muscles. Non-buccopharyngeal evaporation appears to be a passive diffusional process, wherein the rate at which vapor escapes from the external surface is simply a function of the vapor density gradient and the resistance to diffusion. Considered in this light, non-buccopharyngeal evaporation would occur at no energetic cost and should, therefore, be favored over buccopharyngeal evaporation.

Such an assessment of these processes is, however, overly simplistic. First, both routes of evaporative heat loss are ultimately passive, diffusional processes. They differ only in the site of the epithelium from which vapor escapes. Also, both evaporative routes might entail energetic costs. Whereas buccopharyngeal evaporation requires muscular work for the convective flow of air across the normally moist pharyngeal and buccal epithelia, non-buccopharyngeal evaporation requires the delivery of water to the stratum corneum. The mechanism of such delivery might, in itself, be energetically costly. In

addition, the circulatory shunt presumably required for delivery of water to the stratum corneum must involve a convective redistribution of heat within the body. This could have appreciable thermal or physiological consequences.

With the importance of non-buccopharyngeal evaporation for heat dissipation well-established in many avian species, the question arises whether birds can regulate non-buccopharyngeal evaporative heat flux. Regulation could be accomplished, for instance, by changing the rate of water delivery to the stratum corneum, the non-buccopharyngeal resistance to diffusion, or both. Nevertheless, a direct test of the ability of birds to adjust non-buccopharyngeal evaporation in response to change in heat load is lacking. Directly addressing this question entails experimentally changing the rate of non-buccopharyngeal evaporation required for thermostasis, while keeping conductive, convective and radiative conditions constant. Increasing the thermostatic need for non-buccopharyngeal evaporation can be accomplished by decreasing buccopharyngeal evaporation. In this study, I minimized the ability of mourning doves (*Zenaida macroura* Linnaeus) to evaporate water from the pharyngeal and buccal epithelia by placing them in a two-compartment respirometry chamber and sending water-saturated air into the compartment containing the head (thereby drastically reducing or eliminating the head-compartment vapor pressure gradient), while sending dry air into the torso-compartment.

Materials and methods

Adult mourning doves (*Zenaida macroura* Linnaeus) of undetermined sex were captured in late January 1998 in Tempe, Arizona, USA, and subsequently housed in a temperature-controlled room on the campus of Arizona State University. The room was maintained at 30°C under a 12 h:12 h L:D photoperiod. For 15 min prior to and following full illumination, a low-output light source was turned on to graduate the artificial day/night transitions. Birds were housed in pairs in metal mesh cages (61 cm × 42 cm × 61 cm) and given food and water *ad libitum*.

Measurements were made using a 10.9 L, two-compartment, respirometry chamber (28.6 cm × 20.4 cm × 18.7 cm), constructed on five sides of aluminum and on the sixth of acrylic to allow constant monitoring of the subject. An aluminum partition separated the head-compartment volume (3583 ml) from the torso-compartment volume (7327 ml). An 8 cm × 8 cm opening in the aluminum partition was spanned with latex sheeting (#07315 Heavy Dental Dam, Hygenic, Akron, OH, USA), into which a hole was cut to accommodate the neck of the experimental subject. The size of the hole was such that the neck very slightly stretched the latex, thus sealing the head-compartment volume from the torso-compartment volume while allowing unimpeded breathing. An aluminum stock placed above the latex sheet prevented the head from being pulled through the latex. Unless the subject struggled, the stock did not restrain it. If a subject struggled for more than a few seconds, the trial was terminated and the data were discarded. Subjects stood in a natural posture, with the feet on a stainless-steel grid that allowed for the

passage of excreta into a bath of non-volatile mineral oil, precluding the contribution of excreta to measurements of vapor density.

The respirometry chamber was placed in a temperature-controlled room that also served to isolate the subjects sonically. Measurements were made in total darkness, and subjects were monitored under infrared light using a CCD camera (#18MC205T, Magnavox, Marietta, GA, USA). Separate head-compartment and torso-compartment ambient temperatures were continuously measured using copper-constantan thermocouples.

With the subject in place, the head-compartment was sealed from the torso-compartment. Each chamber was equipped with its own influx and efflux ports. The influx ports were fitted with diffusion plates to facilitate mixing of chamber air.

Measurements were made at 35, 45 and 50°C for trials in which dry, acapnic influent was sent to both chambers (‘dry’ trials). At 35 and 45°C, measurements were also made using vapor-saturated, acapnic head-compartment influent and dry, acapnic torso-compartment influent (‘wet’ trials). I deemed 50°C wet trials to be too dangerous for the subjects, but I collected data for 50°C dry trials to test whether rates of evaporation during 45°C wet trials represented absolute maxima.

Influent air was scrubbed of CO_2 and dried by an air purifier (#PCDA11129022, Puregas, Denver, CO, USA) before being sent through rotameters (#FL3405ST, Omega Engineering, Stamford, CT, USA, calibrated against a soap-film flowmeter). For the dry trials, air exiting the rotameters was sent through 3.2 mm i.d. tubing (Bev-A-Line, Thermoplastic Processes Inc., Stirling, NJ, USA) directly to the chamber inlets. During

wet trials, air exiting the rotameters was diverted to a sealed, plastic cylinder (206 cm, 7.6 cm i.d.) serving as a hydration chamber. The air line entering the hydration chamber branched to terminate in several porous aquarium aerators at the floor of the hydration chamber. These were used to increase the number and decrease the size of bubbles introduced into the water column. Air was bubbled through a 165 cm column of distilled water before exiting the hydration chamber, and the water was continuously circulated through an external metal coil immersed in a water bath equipped with a temperature controller (Dyna-Sense 2156, Scientific Instruments Inc., Skokie, IL, USA). Both the hydration chamber and the water bath were placed in the temperature-controlled room housing the test chambers to ensure that influent air was vapor-saturated at the ambient temperatures encountered in the experiment. In case hydration-chamber temperature exceeded head-compartment temperature, air exiting the hydration chamber was sent to a glass vessel to collect any condensed water before being sent to the head-compartment. Preliminary measurements were made on wet-trial influent, using a dew-point hygrometer (EG&G 911, EdgeTech, Marlborough, MA, USA), yielding dew-point temperatures equaling head-compartment ambient temperatures. These measurements, and the appearance of condensate in the glass vessel, leave me confident that wet trials were conducted with vapor-saturated head-compartment influent. Since the rotameters were upstream of the hydration chamber, head-compartment airflow was corrected to reflect the addition of vapor to the air stream.

Effluent from both test chambers was sent to a capacitance hygrometer (#PC2101, Thunder Scientific, Albuquerque, NM, USA) before passing through a desiccant and then

into an infrared CO_2 analyzer (#LI6252, Li-Cor, Lincoln, NE, USA). The CO_2 analyzer was used only to supplement my visual assessment of quiescence in the subjects; data were collected when subjects appeared to stand still and when the CO_2 analyzer indicated a relatively flat and low-level output. A glass vessel was interposed between the head-compartment and the hygrometer to collect condensate from the saturated, head-compartment effluent during wet trials. This protected the hygrometer from water that would have condensed because gas analysis was conducted in a room much cooler than the test chambers.

I calibrated the hygrometer by passing vapor-saturated air at 23°C through a thermocouple-equipped copper coil immersed in calcium chloride brines of various concentrations and cooled to slurry with frozen CO_2. Emerging air was brought to room temperature and sent to the hygrometer. Hygrometric readings were thus measurements of vapor density, not relative humidity. The CO_2 analyzer was calibrated daily using pure nitrogen and a CO_2/N_2 mixture of known composition as zero and span gases, respectively.

I tried unsuccessfully to monitor core temperature (Minimitter transmitter implant) and cloacal temperature (thermocouple probe); both procedures proved too injurious to the birds. Skin temperature was measured continuously during 45 and 50°C trials using a 40 AWG copper-constantan thermocouple soldered to a rectangle of copper foil (approximately 0.25 cm^2) attached using cyanoacrylic glue to the ventral apterium immediately posterior to the sternum. Unfortunately, I did not develop the technique for cutaneous thermometry until all but one of the 35°C trials were completed.

The order of treatment (dry or wet) for any one temperature was randomized. No bird was subjected to more than one trial in a single day. Most trials were conducted at a particular temperature before proceeding to the next temperature, because the test room and the water in the hydration chamber required hours for equilibration following a change of temperature.

Subjects were placed in the thermally equilibrated respirometry chamber and monitored for at least 30 min before any data were collected. Transient and infrequent twitches were permitted but, in general, data were collected after the subject had been still for at least 8 min (the time for 99% chamber-air turnover, after Lasiewski et al., 1966).

Data for temperatures (air, skin and water), vapor density and CO_2 content were sampled every second and averaged every 60 s while being recorded on a datalogger (#CR23X, Campbell Scientific, Logan, UT, USA). The data were continuously monitored on a computer running data-acquisition software (Campbell Scientific PC208W). Effluent from either test compartment was serially shunted to the gas analysis system by solenoid valves. Head-compartment measurements were thus temporally displaced (by approximately 5 min) from torso-compartment measurements, although all measurement sequences were made during the same near-steady state with respect to CO_2 and vapor density.

Statistical analyses

All data were subjected to paired *t*-tests to determine the significance of differences between treatments, with $P<0.05$ being considered significant. Each *t*-test was conducted twice: once with all available data, and once with outliers removed. An outlier was defined, using a two-sample *t*-test, as any datum differing at the 0.01 significance level from the remaining data taken as a group. In all but one of the *t*-tests, removal of outliers made no difference to the significance. However, in comparing evaporative conductance (g_v) between 45 and 50°C dry trials, removal of outliers indicated a change from no significance ($P>0.082$) to significance ($P<0.014$). Although less frequently reported in the literature than resistance, I report values for conductance to indicate ease of evaporative flux. For any given skin temperature (T_s), conductance varies directly with flux density, whose variance is assumed to be normally distributed. Therefore, variance in conductance (and not its reciprocal, resistance) is normally distributed, and I retain the power of the parametric, paired *t*-test, which assumes normality. All values are presented as means ± the standard error of the mean (S.E.M.). All values for *P* are results of paired *t*-tests.

Results

Evaporation

At an ambient temperature (T_a) of 35°C, non-buccopharyngeal evaporation underwent a significant (P<0.006) increase of 72% between dry trials and wet trials (Fig. 2.1). At T_a=45°C, however, there was no significant increase from dry trials to wet trials (P>0.057). Ambient temperature strongly affected non-buccopharyngeal evaporation (Fig. 2.1). For dry trials, it increased by 135% (P<0.002) from T_a=35°C to T_a=45°C. Similarly, it increased by 68% (P<0.004) from T_a=45°C to T_a=50°C. For wet trials, non-buccopharyngeal evaporation increased by 83% (P<0.004) from T_a=35°C to T_a=45°C.

In dry trials, the effect of T_a on total evaporation was just as strong. At T_a=35°C, total evaporation was 10.72±1.16 mg min^{-1}. At T_a=45°C, this increased by 99% to 21.30±1.69 mg min^{-1}(P<0.0005), and a further 61% increase occurred at T_a=50°C when total evaporation reached 34.34±2.58 mg min^{-1}(P<0.007).

The relative contribution of non-buccopharyngeal evaporation to total evaporation in dry trials changed only slightly with changes in T_a, accounting for 49.6±1.2% of the total at T_a=35°C, 40.3±4.3% at T_a=45°C and 44.9±1.6% at T_a=50°C. Only values for T_a=35°C and T_a=50°C differed significantly (P<0.012, Fig. 2.2B).

Non-buccopharyngeal compensatory capacity (NBCC) was calculated as the wet-trial non-buccopharyngeal evaporation divided by the dry-trial total evaporation and expressed as a percentage (Fig. 2.2A). Defined in this way, NBCC represents the degree to which a bird can increase its non-buccopharyngeal evaporation to make up for a decrease in buccopharyngeal evaporation. At T_a=35°C, NBCC was between 74.0±8.3%

and 86.4±9.4% (see explanation in Discussion). At T_a=45°C, the NBCC fell to 62.5±12.1%, which is a decrease of between 15.5% (P<0.007) and 27.7% (P<0.0008) compared with the range at T_a=35°C.

Skin temperature

Mean skin temperatures (T_s) are reported in Fig. 2.3. For T_a=45°C (the only trials for which T_s was measured for both treatments of head-compartment influent), no significant change (P>0.23) in T_s occurred between dry (44.9±0.094°C) and wet (44.9±0.30°C) trials. In dry trials for which T_s was measured, values for T_s increased significantly (P<0.002) from 44.9±0.094°C at T_a=45°C to 45.6±0.32°C at T_a=50°C. The single animal for which T_s was measured at T_a=35°C had a dry-trial T_s of 40.3°C and a wet-trial T_s of 43.0°C.

Evaporative conductance

The conductance of water vapor (g_v) is defined as the ratio of evaporative flux density (mass of water per unit surface area of skin per unit time, g m^{-2} s^{-1}) to vapor-density gradient (difference in absolute humidity between skin and air, g m^{-3}). Therefore, by cancellation, g_v takes units of m s^{-1}. This is in keeping with the fact that g_v is the reciprocal of resistance to water-vapor diffusion (r_v), which is usually expressed in s m^{-1}.

The calculation of g_v requires knowledge of T_s and, therefore, values of g_v are reported only for the 45 and 50°C trials (Fig. 2.4). At T_a=45°C, there was no significant change (P>0.058) in g_v between dry trials (245±30 μm s^{-1}) and wet trials (316±37

μm s^{-1}). Among dry trials, g_v increased by 71% (P<0.014) from 245±30 μm s^{-1} at T_a=45°C to 420±8.0 μm s^{-1} at T_a=50°C.

Discussion

These data clearly indicate a substantial increase in the rate of non-buccopharyngeal evaporation in mourning doves when ambient temperature is 35°C and buccopharyngeal evaporation is greatly reduced, compared with non-buccopharyngeal evaporation at the same ambient temperature but with uninhibited buccopharyngeal evaporation. However, there are two peculiarities of the 35°C trials that must be addressed: the absence of skin temperature measurements and the possibility that buccopharyngeal evaporation was not eliminated during wet trials.

Possibility of buccopharyngeal evaporation during 35°C wet trials

While the incurrent air during wet trials had a dew-point of 35°C, this may have been insufficient to eliminate the buccopharyngeal vapor-density gradient. If portions of the pharyngeal epithelium had temperatures in excess of 35°C during these wet trials (and therefore vapor densities exceeding that of the influent), then some buccopharyngeal evaporation could have occurred. Since condensation from the excurrent air precluded head-compartment hygrometry during wet trials, I was unable to measure how much buccopharyngeal evaporation (if any) was occurring. I assumed that the birds attained a maximal lung temperature of 40°C. This would allow for buccopharyngeal evaporation across an 11.5 $\mu g\ ml^{-1}$ vapor-density gradient (the saturation vapor density of 40°C air minus that of 35°C air). Thus, non-buccopharyngeal compensatory capacity at T_a=35°C was calculated as a range from 74% (no buccopharyngeal evaporation) to 86% (buccopharyngeal evaporation across an 11.5 $\mu g\ ml^{-1}$ vapor-density gradient), as shown

in Fig. 2.2A. My estimate of 40°C for lung temperature is based on measurements of body temperature of columbiforms at an ambient temperature of approximately 35°C (Lasiewski and Seymour, 1972; Dawson and Bennet, 1973; Webster and King, 1987; Withers and Williams, 1990; Prinzinger et al., 1991) coupled with the assumption that lung temperature is at least slightly reduced, by evaporation, compared with body temperature. A regression (Schmidt-Nielsen et al., 1970) of exhaled air temperature on T_a for pigeons yields an exhaled air temperature slightly above 35°C at T_a=35°C. If this is typical of columbiforms, then the low end of the range (74%) may be the most realistic estimate of non-buccopharyngeal compensatory capacity.

Absence of empirical data for skin temperature during 35°C trials

Except for one bird, skin temperatures were not measured during 35°C trials. This precludes calculation of g_v at T_a=35°C and allows for the proposal that the 72% increase in non-buccopharyngeal evaporation in response to curtailment of buccopharyngeal evaporation is due to a change in skin temperature rather than to a change in conductance of water vapor. Indeed, this proposal seems appealing in the light of the fact that non-buccopharyngeal evaporation in 45°C wet trials was not significantly increased over that of 45°C dry trials. Several lines of evidence exist, however, to suggest that most of the increase in non-buccopharyngeal evaporation during wet trials at 35°C must have been due to a substantial shift in g_v.

First, the single dove for which T_s was measured during the 35°C dry trial had a skin temperature of 40.3°C. Although this unique measurement carries little statistical weight,

a dry-trial T_s of 40.3°C is unlikely to be atypically high. Mourning doves kept at 4°C overnight have been shown to maintain skin temperatures of approximately 39°C (Bartholomew and Dawson, 1954). The same study demonstrated that skin temperature is independent of ambient temperature (remaining between 39 and 40°C) up to approximately 30°C. Above this, skin temperature rises slightly. It is unlikely, therefore, that the doves in the present study, held by a restraint in a chamber at an ambient temperature of 35°C, had skin temperatures below 39°C during dry trials.

Second, at least three independent studies of columbiforms (Bartholomew and Dawson, 1954; Randall, 1943; von Saalfeld, 1936) have shown that panting does not occur until body temperature reaches a threshold of between 42 and 43°C. In the present study, doves were monitored continuously, and no panting occurred during any 35°C trials. Thus, it is reasonable to assume that body temperature was less than 43°C throughout these trials. Since, in the absence of irradiance, body temperature must exceed skin temperature when body temperature is higher than ambient temperature, wet-trial skin temperatures could not have exceeded 43°C and were probably considerably lower. The overall range of skin temperature at an ambient temperature of 35°C (wet and dry trials combined) was almost certainly, therefore, within the 39–43°C range (Fig. 2.5).

Change in non-buccopharyngeal evaporation decoupled from change in skin temperature

The range of probable skin temperatures (39–43°C) is much too narrow to account for the observed increases in non-buccopharyngeal evaporation between dry

trials and wet trials, assuming a constant evaporative conductance. An analysis of superlative scenarios reveals why.

Assuming that skin temperature for 35°C wet trials is 43°C (above which panting would occur), then evaporative conductance can be calculated as:

$$g_{v,35} = \frac{NBE_{35,W}}{\rho'_v(43°C)}, \tag{1}$$

where $g_{v,35}$ is the evaporative conductance at T_a=35°C, $NBE_{35,W}$ is the wet-trial, non-buccopharyngeal evaporation at T_a=35°C and ρ'_v(43°C) is the saturation vapor density of air at 43°C. The latter equals the vapor-density gradient, since the influent is dry. Assuming that g_v at T_a=35°C is the same for dry trials and wet trials, then the dry-trial skin temperature necessary for the increase in non-buccopharyngeal evaporation to depend entirely on a change in skin temperature is:

$$\begin{aligned} T_{s,D} &= T_D(\Delta\rho_v) \\ &= T_D\left(\frac{NBE_{35,D}}{g_{v,35}}\right), \end{aligned} \tag{2}$$

where $T_{s,D}$ is the dry-trial skin temperature, $\Delta\rho_v$ is the vapor-density gradient, $T_D(\Delta\rho_v)$ is the dew-point for a vapor density equal to $\Delta\rho_v$, and $NBE_{35,D}$ is the dry-trial, non-buccopharyngeal evaporation at T_a=35°C. The calculation predicts a dry-trial skin temperature of 33°C when ambient temperature is 35°C. This is not credible for two reasons. First, a T_s of 33°C would mean that doves are undergoing a full 10°C shift in skin temperature (with no change in T_a) to compensate for a reduction in buccopharyngeal evaporation. Furthermore, this 10°C shift would occur without any

attempt to shed heat by panting. Second, a T_s of 33°C is lower than any skin temperature reported by Bartholomew and Dawson (1954), even for mourning doves exposed to ambient temperatures as low as 3°C.

It is also possible, given a dry-trial T_s, to calculate the wet-trial skin temperature required for the increase in non-buccopharyngeal evaporation to be due entirely to a change in skin temperature (based solely on data for T_a=35°C). Fig. 2.5 shows that none of the required temperatures falls within the region of likelihood, based on the absence of panting.

Finally, although it is possible for an animal to have a skin temperature below both ambient temperature and body temperature, maintaining such a skin temperature would involve both of the following: (1) that the animal is gaining heat across the thermal gradient from environment to skin, and (2) that the animal is evaporating water non-buccopharyngeally at a rate sufficient to maintain both the thermal gradient from environment to skin and the thermal gradient from skin to body core. This is, at best, an unlikely scenario at T_a=35°C.

Components of water-vapor conductance

A change in evaporative conductance need not be entirely due to a change in skin conductance, because g_v is a measure of total water-vapor conductance. Total conductance is a combination of constituent conductances at the skin, at the plumage and at the boundary layer. While depth of plumage was not measured, I observed no change in the appearance of the plumage of any animal, whether from dry trial to wet trial at any

given ambient temperature or between different ambient temperatures. Moreover, Webster et al. (1985) made direct measurements of constituent water-vapor resistances in pigeons and found boundary-layer resistance to be negligible compared with those at the skin and plumage. In addition, for ambient temperatures between 10 and 40°C, they showed that plumage resistance to water-vapor diffusion is only approximately 5–20% of total vapor resistance. This means that vapor conductance at the skin constrains g_v over this range of ambient temperatures, and that plumage conductance probably only becomes important when skin conductance is high (i.e. at high rather than moderate ambient temperatures).

Relative effects of skin temperature and evaporative conductance on non-buccopharyngeal evaporation

Skin temperatures were measured for all 45 and 50°C trials, which enabled me to make empirical evaluations of the relative contributions made by changes in g_v and T_s to the significant increase in non-buccopharyngeal evaporation found between the 45 and 50°C dry trials. For all dry trials, influent vapor density, ρ_v, was zero, so the vapor-density gradient, $\Delta\rho_v$, was just the saturation vapor density of air at skin temperature, $\rho'_v(T_s)$. The change in non-buccopharyngeal evaporation between 45°C dry trials and 50°C dry trials can therefore be calculated as:

$$\begin{aligned} \Delta NBE &= NBE_{50} - NBE_{45} \\ &= \left(\Delta\rho_{v,50} g_{v,50}\right) - \left(\Delta\rho_{v,45} g_{v,45}\right) \\ &= \left[\rho'_v\left(T_{s,50}\right) g_{v,50}\right] - \left[\rho'_v\left(T_{s,45}\right) g_{v,45}\right] \end{aligned} \quad , \qquad (3)$$

where symbols are defined as before, with numbers in subscripts indicating the ambient temperature of the trial. Using individual values for Δg_v and ΔT_s (i.e. measured differences, within individual birds, in values for g_v and T_s, between 45 and 50°C trials), I calculated two predicted values for ΔNBE, for comparison with empirical values for ΔNBE. One predicted value (ΔNBE_1) assumed no change in g_v (i.e. $g_{v,50}=g_{v,45}$); the other (ΔNBE_2) assumed no change in T_s (i.e. $T_{s,50}=T_{s,45}$). This gives:

$$\begin{aligned}\Delta NBE_1 &= \left[\rho'_v\left(T_{s,50}\right)g_{v,50}\right]-\left[\rho'_v\left(T_{s,45}\right)g_{v,45}\right]\\ &= \left[\rho'_v\left(T_{s,50}\right)g_{v,45}\right]-\left[\rho'_v\left(T_{s,45}\right)g_{v,45}\right]\\ &= g_{v,45}\left[\rho'_v\left(T_{s,50}\right)-\rho'_v\left(T_{s,45}\right)\right]\end{aligned} \tag{4}$$

and

$$\begin{aligned}\Delta NBE_2 &= \left[\rho'_v\left(T_{s,50}\right)g_{v,50}\right]-\left[\rho'_v\left(T_{s,45}\right)g_{v,45}\right]\\ &= \left[\rho'_v\left(T_{s,45}\right)g_{v,50}\right]-\left[\rho'_v\left(T_{s,45}\right)g_{v,45}\right]\\ &= \rho'_v\left(T_{s,45}\right)\left(g_{v,50}-g_{v,45}\right).\end{aligned} \tag{5}$$

The mean change in evaporation assuming no change in conductance (from equation 4) was only 12% of the observed change; the mean change in evaporation assuming no change in skin temperature (from equation 5) was 88% of the observed change. This means that most of the increase in non-buccopharyngeal evaporation between the 45 and 50°C dry trials was caused by an increase in g_v rather than an increase in T_s, despite the fact that both g_v and T_s changed significantly between those ambient temperatures. The change of less than 1°C in T_s from T_a=45°C to T_a=50°C was significant because variance was low. However, despite the steeply increasing relationship between saturation vapor density and ambient temperature, a temperature increase from 44.7 to 45.6°C allows only

a 4.4% increase (Flatau et al., 1992) in saturation vapor density (from approximately 64 $\mu g\ ml^{-1}$ to approximately 67 $\mu g\ ml^{-1}$). Since the influent was dry for all trials, the vapor-density gradient was simply the saturation vapor density of air at a temperature equal to skin temperature, and any increase in non-buccopharyngeal evaporation that was driven wholly by an increase in T_s must have been directly proportional to the increase in the vapor-density gradient, which was relatively small. In contrast, mean dry-trial conductance increased by approximately 48% from T_a=45°C to T_a=50°C, thereby accounting for the overwhelming majority of the 49% increase in non-buccopharyngeal evaporation.

In the present investigation, the overall range of mean values for non-buccopharyngeal evaporation is from 40 $\mu g\ cm^{-2} min^{-1}$ (at T_a=35°C) to 158 $\mu g\ cm^{-2} min^{-1}$ (at T_a=50°C). This is an increase of 295%. The highest individual value for T_s was 46.6°C at T_a=50°C. The vapor density gradient at this skin temperature is 70.3 $\mu g\ ml^{-1}$, which is a 295% increase from a gradient of 17.8 $\mu g\ ml^{-1}$. The dew-point of air containing 17.8 $\mu g\ ml^{-1}$ of water vapor, and therefore the dry-air T_s required for that gradient, is 19.6°C. Birds with such low skin temperatures at T_a=35°C would have been noticeably cool to the touch. Thus, it is quite unlikely that changes observed in non-buccopharyngeal evaporation with changes in T_a are largely due to changes in T_s.

Despite the change in conductance across ambient temperatures, g_v did not increase at T_a=45°C from dry trials to wet trials. This is somewhat surprising, because the values for g_v at T_a=50°C clearly indicate that g_v was not maximized at T_a=45°C, despite the need (based on observations of panting) for doves at that T_a to shed more heat during

wet trials than they actually did. A definitive explanation must await elucidation of the mechanism by which birds are able to adjust g_v to acute changes in T_a.

Mechanisms for adjusting water-vapor conductance

Previous studies by other investigators have examined possible ways in which birds can adjust non-buccopharyngeal evaporation to meet changing needs for heat loss. Menon et al. (1988) observed that the increase in non-buccopharyngeal evaporation of zebra finches (*Poephila guttata*) from nestling to adult could be explained by a comparative abundance of lipid bodies (vacuoles and multigranular bodies) in nestling epidermis. This finding is bolstered by the results of a separate study (Menon et al., 1989) on zebra finches, in which deprivation of water caused both an increase in intercellular deposition of the contents of multigranular bodies in adult epidermis and a change in composition of epidermal lipids, with a concomitant decrease in non-buccopharyngeal evaporation. Rehydration served to reverse both effects of dehydration. These studies are, however, concerned with comparatively long-term changes (ontogeny or acclimation) rather than acute changes in non-buccopharyngeal evaporation, as observed in mourning doves.

Marder and Raber (1989) elicited very large changes in skin resistance to water-vapor diffusion in pigeons. Oral administration of a β-receptor blocker (propranolol) caused an increase in non-buccopharyngeal evaporation *via* a global decrease in skin resistance. Similarly, intradermal injection of propranolol caused a local reduction in skin resistance. Changes took effect within 1–5 min of injection. Marder and Raber (1989)

suggest that endogenous chemical transmitters, whether neural or humoral, control avian non-buccopharyngeal evaporation by reversing the vasoconstrictive effect of tonic stimulation of β-receptors in the cutaneous smooth vasomusculature.

While long-term changes in g_v might be effected by changes in structure and function of epidermal lipids and multigranular bodies (Elias and Menon, 1991; Menon et al., 1991, 1996), short-term control of g_v might be exercised by displacing the constraint on diffusion of water. In this way, evaporative conductance would usually be constrained by the rate of delivery of water to the epidermis, a physiological property of the animal dependent on the current state of cutaneous vasoconstriction, which is under neural and hormonal control. During prolonged heat-stress, g_v would be constrained by conductance of water vapor at the skin surface, an anatomical property of the epidermis, under acclimatory control.

Concluding comments

In conclusion, this study and others (e.g. Marder and Ben-Asher, 1983; Webster and King, 1987) demonstrate that non-buccopharyngeal evaporation is an important means of thermoregulatory heat dissipation in birds. These results provide the first evidence that birds are able adaptively to adjust their rates of non-buccopharyngeal evaporation. This previously unappreciated capacity for physiological adjustment of non-buccopharyngeal water-vapor conductance represents an expansion of the known set of thermoregulatory strategies used by birds.

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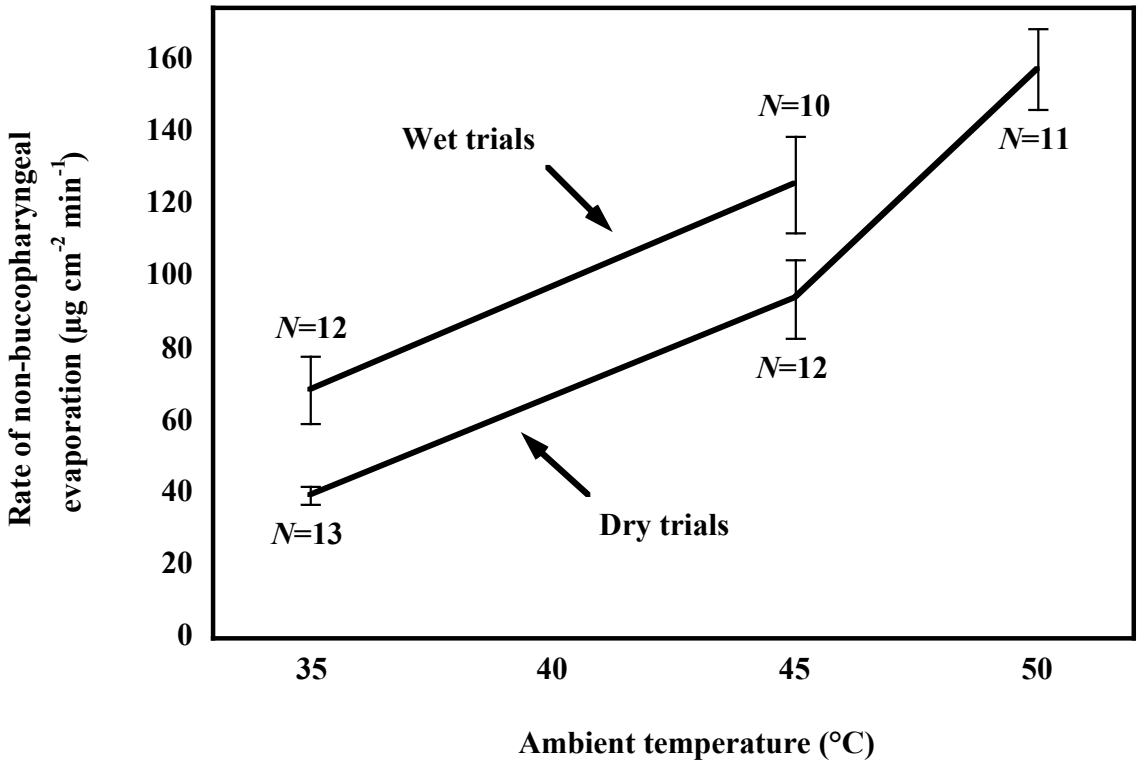

Fig. 2.1. Non-buccopharyngeal evaporative flux-density of mourning doves at three ambient temperatures. Values are means ± S.E.M. The difference in non-buccopharyngeal evaporation at T_a=35°C between wet and dry trials is significant (P<0.006).

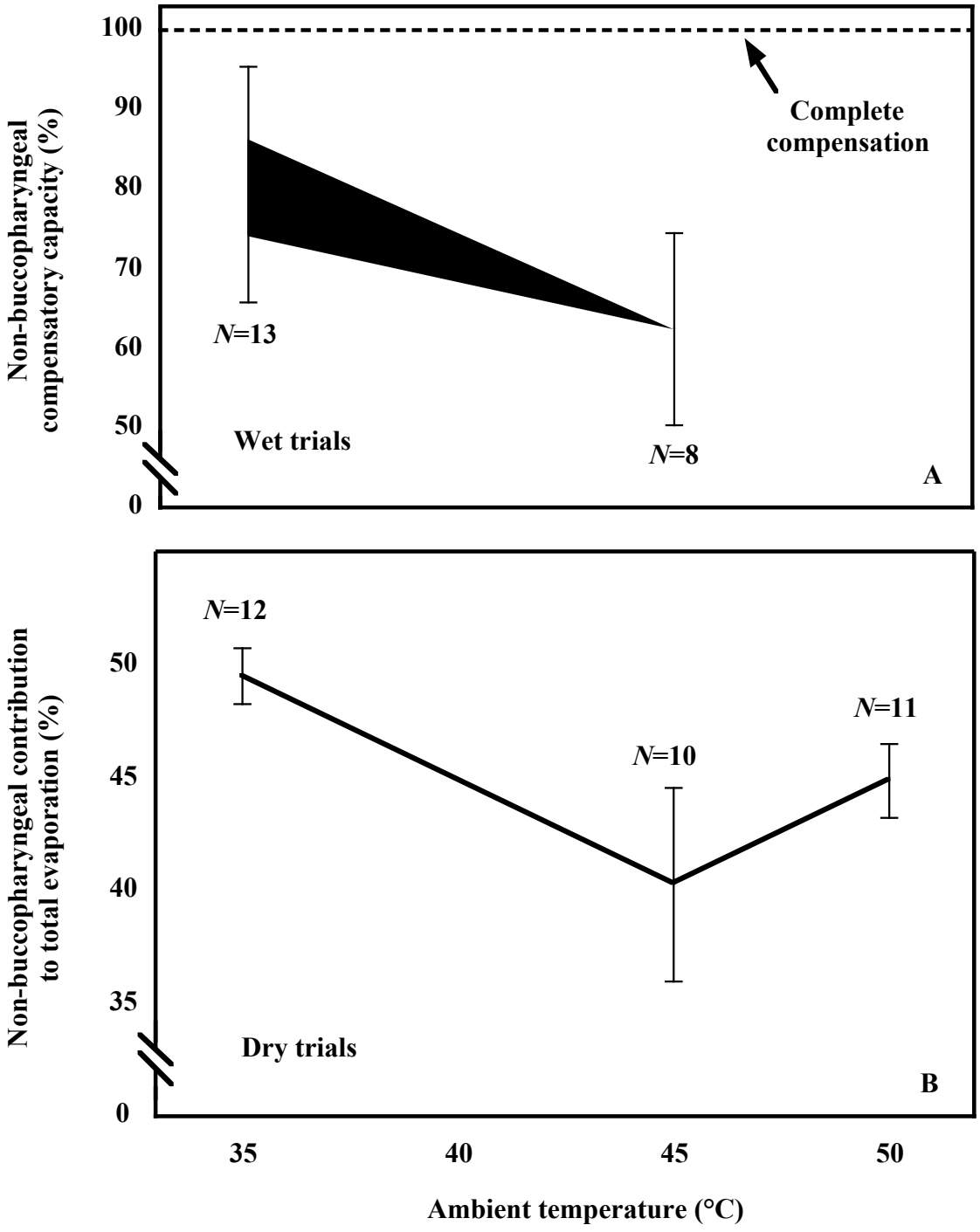

Fig. 2.2. (A) Non-buccopharyngeal compensatory capacity of mourning doves. Wet-trial non-buccopharyngeal evaporation expressed as a percentage of dry-trial total evaporation indicates the degree of compensation, *via* increased non-buccopharyngeal evaporation,

for the elimination of a buccopharyngeal contribution to total evaporation during wet trials. The range of means at T_a=35°C reflects the possibility that buccopharyngeal evaporation was not completely eliminated at that ambient temperature (see text). (B) Non-buccopharyngeal contribution to total evaporation. Dry-trial non-buccopharyngeal evaporation expressed as a percentage of dry-trial total evaporation indicates normal apportionment of buccopharyngeal and non-buccopharyngeal components of evaporation. Values are means ± S.E.M.

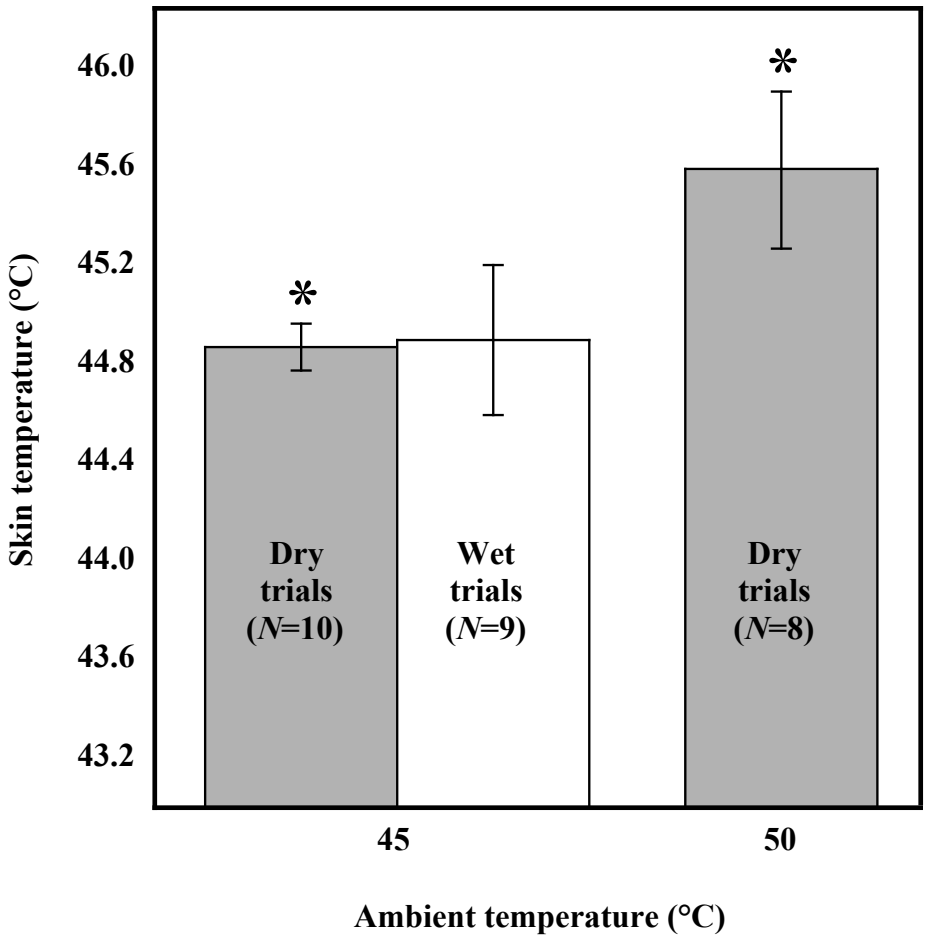

Fig. 2.3. Effects of ambient temperature on skin temperature of mourning doves. Values are means ± S.E.M. Asterisks indicate significantly different values (P<0.002).

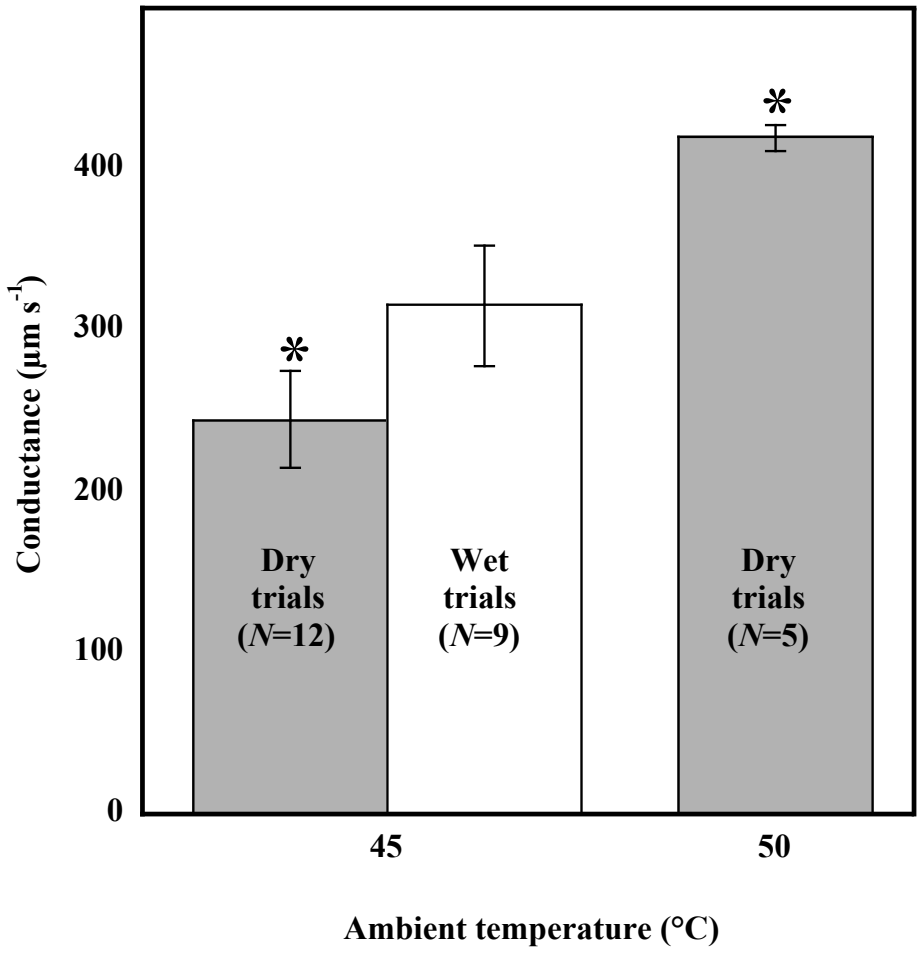

Fig. 2.4. Effects of ambient temperature on evaporative conductance of mourning doves. Values are means ± S.E.M. Asterisks indicate significantly different values ($P<0.014$).

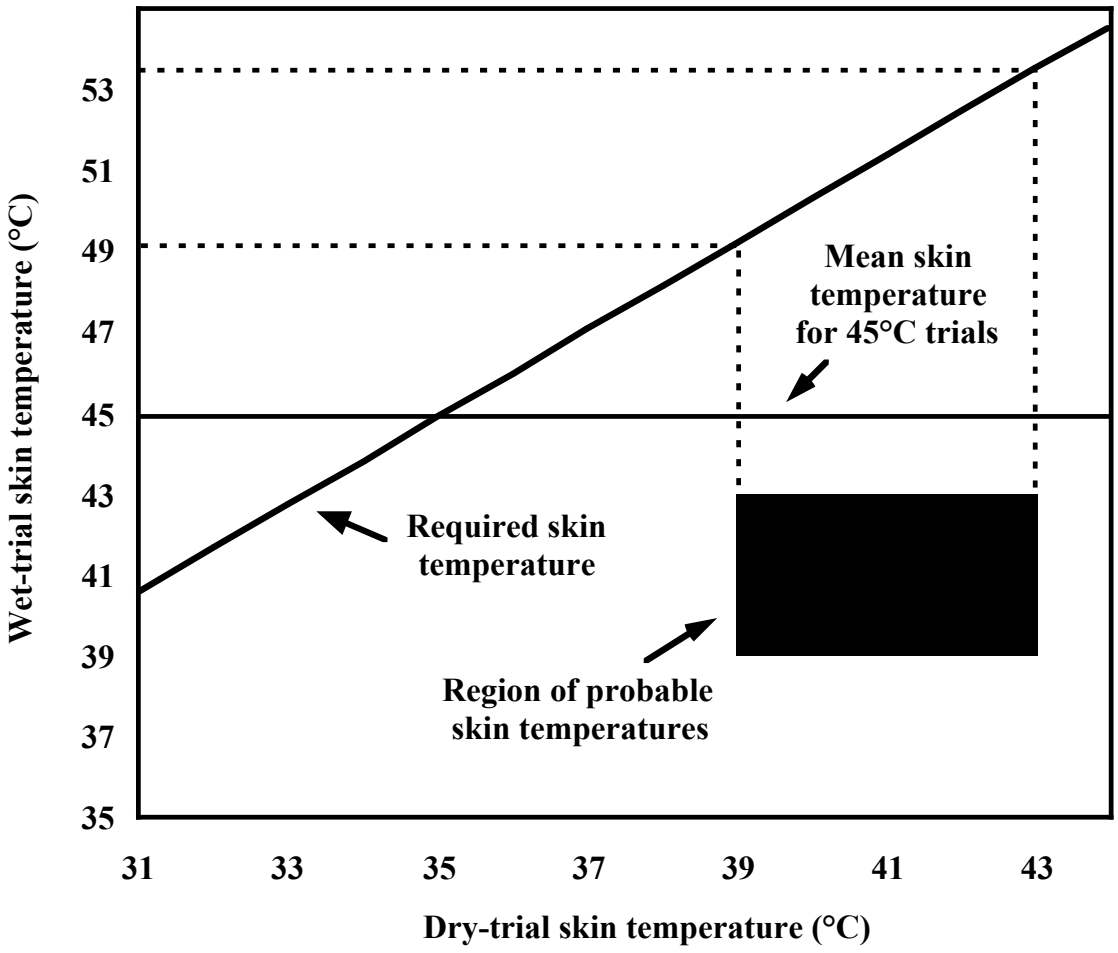

Fig. 2.5. Wet-trial skin temperatures of mourning doves necessary for observed evaporation if conductance did not change. Values along the curve were calculated from data for evaporation at T_a=35°C (N=12) assuming the dry-trial skin temperatures indicated on the abscissa. The filled region indicates the boundaries of probable skin temperatures (see text). Dashed lines indicate the range of required wet-trial skin temperatures assuming that dry-trial skin temperatures were between 39° and 43°C.

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Cloacal Evaporative Cooling: A Previously Undescribed Means of Increasing Evaporation at Higher Temperatures in a Desert Ectotherm, the Gila Monster (*Heloderma Suspectum*)

Summary

The Gila monster, *Heloderma suspectum* Cope, is an active forager in an environment that, at times, can be extremely hot and arid. Thus, Gila monsters face extreme thermostatic and hydrostatic demands. For a desert ectotherm routinely risking dehydration, evaporative water loss (EWL) is typically viewed as being detrimental. Yet evaporation simultaneously dehydrates and cools an animal. I explored EWL in Gila monsters by measuring cutaneous, buccopharyngeal, and cloacal EWL at five ambient temperatures between 20.5°C and 40°C. My results show that Gila monsters have high EWL rates relative to body mass. Cutaneous EWL underwent a consistent, temperature-dependent increase over the entire range of test temperatures (Q_{10} = 1.61, with EWL ranging from 0.378 to 0.954 mg g^{-1} h^{-1}). Buccopharyngeal EWL did not show a significant temperature-dependent response, but ranged from 0.304 to 0.663 mg g^{-1} h^{-1}. Cloacal EWL was extremely low and relatively constant between 20.5°C and 35°C, but rose dramatically above 35°C (Q_{10} > 8.3 X 10^7, from 0.0008 at 35°C to 7.30 mg g^{-1} h^{-1}at 40C). This steep rise in cloacal EWL coincided with an increasing suppression of body temperature relative to ambient. Dehydration to 80% of initial body mass led to a delay in the onset and an attenuation of the dramatic increase in cloacal EWL. These results emphasize the potential value of EWL for thermoregulation in ectotherms and demonstrate for the first time the role of the cloaca in this process.

Introduction

One of the fundamental physiological dichotomies among vertebrates is that of endothermy and ectothermy (Henzel et al., 1973; Hayes and Garland, 1995). While ectothermy is metabolically inexpensive, it allows for little independence from the thermal vagaries of the environment (Crompton et al., 1978; McNab, 1978). Ectotherms have insufficient heat production to elevate body temperature (T_b) above ambient temperature (T_a), and therefore must obtain heat from the environment *via* radiation, conduction, and convection.

In addition to having limited capabilities for internal heat production, ectotherms are thought to have limited physiological mechanisms for significantly reducing T_b when environmental temperatures are high (Schmidt-Nielsen, 1964). Evaporative water loss (EWL) represents the predominant means by which any organism can cool its body when T_a exceeds T_b. Not surprisingly, then, EWL has been shown to be critical to thermoregulation in endotherms (see Dawson and Batholomew, 1968; Calder and King, 1974 for reviews). However, EWL of ectotherms is rarely investigated in terms of its potential for suppressing body temperature. Instead, it is widely accepted that the only means for reptiles to lower T_b is by moving to a cooler environment such as a burrow.

While EWL could provide a means for reptiles to reduce T_b when T_a exceeds T_b, EWL is typically viewed merely as a detriment to water balance (see Mautz, 1982a for review). Reptiles living in arid environments tend to have reduced EWL compared to more mesic species, and this low EWL rate is considered to be an adaptive response to xeric conditions (Cohen, 1975; Mautz, 1982a, b; Dmi'el, 1998, 2001). While seldom

studied, the use of EWL for thermoregulation by ectotherms has been reported in several species. Cicadas can effectively reduce T_b below T_a by actively increasing cutaneous EWL (Toolson, 1987). Additionally, some arid-environment lizards increase EWL by panting at thermally challenging temperatures (Templeton, 1960; Dawson and Templeton, 1963; Warburg, 1965; Crawford and Kampe, 1971).

Gila monsters, *Heloderma suspectum* Cope, are relatively large active foraging lizards of the Sonoran Desert of Arizona and northern Mexico. Single foraging bouts can cover considerable distances (in excess of 1 km) over an extended period of time (12 hours or more) in search of vertebrate nests, the contents of which comprise their diet (Bogert and Del Campo, 1956; Beck, 1990). The Sonoran Desert summer consists of two distinct climatic seasons. From mid-April through mid-July, the Sonoran Desert is hot (daytime high temperatures of 35-45°C) and dry (no rainfall). However, a summer rainy season commences approximately in mid-July and extends into mid-September. During this summer rainy season, temperatures remain high but there is a relatively reliable, albeit limited, rainfall (approximately 10cm). While Gila monsters are predominantly crepuscular or nocturnal during the summer to avoid peak temperatures, air temperatures frequently exceed 40°C at sunset and remain warm throughout much of the night. Requiring lengthy surface activity in an environment that is hot and dry for several consecutive months suggests that Gila monsters should have low cutaneous evaporation to benefit water balance. Nevertheless, Gila monsters are said to have ‘leaky skin’ (Lowe et al., 1986; Brown and Carmony, 1991), though published data in support of this contention are lacking. From the perspective of water balance, leaky skin in a xeric

environment is maladaptive because it increases the rate of desiccation (Brown and Carmony, 1991). Consequently, the purported existence of leaky skin is used as evidence to support the hypothesis of a tropical origin for Gila monsters (Lowe et al., 1986).

Since EWL rates of Gila monsters have physiological, ecological, and even evolutionary implications, I examined evaporative water flux in this species. In addition to measuring total EWL, I investigated the relative contribution by the skin and by other potential routes of water loss. I designed a means by which I could partition cutaneous, buccopharyngeal, and cloacal EWL. I use the term 'buccopharyngeal' to refer to evaporation occurring from the mouth and pharynx, whether or not such evaporation is being enhanced by breathing. While cloacal EWL has not previously been described, I considered it a viable means by which water could be evaporated from the body. While usually confined within the body cavity, the mucous membranes of the cloaca can be exposed to the environment through the vent (with or without eversion; DeNardo, personal observation). Furthermore, water permeability of the lizard cloaca has been demonstrated in the context of post-renal concentration of urine (Braysher and Green, 1970). While previous studies of EWL in reptiles have neglected or intentionally prevented cloacal EWL (see Mautz, 1982a for review), I chose to examine this mucosal surface as a possible route for EWL.

I hypothesized that EWL could provide thermal advantages to an actively foraging ectotherm that inhabits a hot, arid environment. Evaporation would be especially advantageous if there were a fairly predictable water resource. In fact, despite living in an arid environment, cicadas are able to invest large volumes of water into EWL because of

their high tolerance of desiccation (Toolson, 1987) and their ability to regularly obtain water from the xylem of bushes (Cheung and Marshall, 1973). The predictable late summer rains of the Sonoran Desert provide a water resource to replenish water expended during the dry spring and early summer months. Additionally, the Gila monster possesses a large urinary bladder that might serve as a water reservoir during extended dry periods, as it does in the Desert Tortoise, *Gopherus agassizii* (Dantzler and Schmidt-Nielsen, 1966; Minnich, 1976). Therefore, I predicted that Gila monsters would have a relatively high EWL rate and that elevated water flux would be especially apparent at thermally challenging temperatures, when hyperthermia would be more of an immediate physiological threat than dehydration. I further predicted that, by providing a mechanism for shedding body heat, high EWL rates would allow the animal to maintain sub-ambient T_b, at least in the short term. Lastly, since water is especially critical during dehydration, I predicted that dehydration would lead to a reduction in EWL.

Materials and methods

Animals

Eighteen adult (432-691g) Gila monsters from a colony acquired from the Arizona Game and Fish Department (AZ G&F holding permit #SP689454), were housed individually in 91 cm × 71 cm × 46 cm cages with a basking light at one end of the cage to provide a thermal gradient. The room was maintained at 25 ± 1°C with a 12:12 light:dark cycle. This thermal regime (25°C room temperature with a basking lamp provided for 12 hours of the day) allowed the Gila monsters to maintain selected body temperature (29.4°C, Bogert and Del Campo, 1956) during the day, but drop to a typical active season nighttime temperature (D. F. DeNardo, unpublished data). Except during the dehydration experiment, animals were provided water *ad libitum* and fed one dead adult mouse approximately biweekly (however, animals were deprived of food for at least one week prior to any experimental trial).

Body surface area was estimated by representing the animal as a collection of simple geometric volumes. I made actual body measurements that allowed me to calculate the surface areas of the geometric constituents: a square pyramidal frustum (the head), five right regular cylinders (the torso and four limbs), and a right circular cone (the tail). Estimated whole body surface areas were between 622 and 958 cm^2.

Experimental Apparatus

Ambient temperature was maintained throughout trials by housing the test chamber in an environmental chamber fitted with an electronic temperature controller

(Omega Engineering CN2011, Stamford, CT, USA). The test chamber was thus a chamber within a chamber, and it experienced very little thermal oscillation throughout trials (T_a was maintained ± 0.2°C during a given trial and ± 0.5°C among trials).

I partitioned EWL into two components (hereafter referred to as 'head' and 'torso', though the latter includes the torso, the four limbs, and the tail) by placing Gila monsters individually into a two-compartment test chamber fitted for separate flows of air into and out of the compartments. The test chamber was custom made to fit the test species, thereby minimizing the time for turnover of air and maximizing the temporal resolution of hygrometric measurements. The chamber was constructed almost entirely of borosilicate glass (Pyrex), because glass is minimally hygroscopic, and it allowed for continuous visual monitoring of the test animal using an infrared camera connected to a remote monitor. The overall geometry of the test chamber was a horizontally placed, right circular cylinder (overall length = 52 cm; inside diameter = 9.5 cm) with closed, flat ends. To allow for partitioning, the main cylinder consisted of two open-ended cylinders of unequal length (torso compartment: 39.5 cm long, 2800 ml volume; head compartment: 12 cm long, 850 ml volume). A two-part neck stock composed of aluminum plate was attached perpendicularly to a horizontal base and served to safely hold the venomous lizard in place while the investigator installed the compartments and thereafter during trials. Attached to the open end of the head compartment was a latex sheet (#07315 Heavy Dental Dam, Hygenic, Akron, OH, USA) perforated with an elliptical hole (17 mm × 21 mm) through which the head was passed. With the animal in place, the cylinders were clamped against the stock using a bar clamp. A closed-cell foam

gasket formed a seal between the torso compartment and the stock. The compliant latex sheet sealed the head compartment and prevented mixing of air between compartments even if the animal moved. The lack of mixing of air between the head and torso chamber was verified during test trials that delivered 100% saturated air into one chamber without causing any change in dewpoint in the other chamber.

Each compartment was fitted with three threaded, borosilicate glass hose connectors (#7 Chem-Thread, Chemglass, Vineland, NJ, USA). Two connectors accepted non-hygroscopic tubing (Bev-A-Line, Thermoplastic Processes Inc., Stirling, NJ, USA) for both influent and effluent air. The third hose connector served as a port for passage of type T (copper-constantan) thermocouple cables, permitting continuous recording of animal temperatures and ambient temperatures. A small, outward leak at the thermocouple port, required to allow for play in the cables, allowed for equalization of pressures between compartments (a higher flow rate was used in the torso compartment). The leak did not affect the sub-sampled effluent in the positive-pressure setup, and equalization of pressures further reduced the chance of mixture of air between compartments.

Influent air was first passed through an industrial purifier (#PCDA11129022, Puregas, Denver, CO, USA) that removed carbon dioxide and water vapor. Dried air was sent through a manifold to supply separate air lines for each of the compartments. Mass-flow controllers (#FMA-A2406 & #FMA-A2409, Omega Engineering, Stamford, CT, USA) were placed in the air lines upstream of the compartments to maintain separate and constant influxes (head: 1000 ml min^{-1}; torso 4000 ml min^{-1}). I calibrated the mass-flow

controllers for the experimental air mixture (dry and CO_2-free) using soap film flow meters, and I generated calibration curves describing STP (standard temperature and pressure) mass flow (ml min^{-1}) as a function of electrical potential difference (mV). At the flow rates selected, the air in the head and torso compartments underwent 99% turnover (Lasiewski et al., 1966) every 3.4 and 3.2 minutes, respectively.

Each compartment's effluent was sent to its own hygrometer (#RH100, Sable Systems, Las Vegas, NV, USA) and then vented to the room. The hygrometers were calibrated with bottled nitrogen (zero gas) and experimental air that was bubbled through three serially-placed columns of distilled water, each approximately 150 cm deep, before being sent individually to the hygrometers (span gas). A copper-constantan thermocouple measured the water temperature in the columns, and each hygrometer was individually heated to be warmer than the water, thus preventing condensation. The hygrometers were set to output dewpoint and were adjusted so that the dewpoint reading equaled the water temperature. I verified the linearity of the hygrometers and the veracity of the calibrations by later sending air through the columns when the water was comparatively cooler, and the hygrometers indicated the correct (and lower) dewpoints. The hygrometers remained powered throughout the entire experiment to minimize calibration drift, and calibrations were checked occasionally and readjusted when necessary. While the hygrometers showed little or no drift, I minimized the effects of any drift by calculating evaporative fluxes based on elevations in dewpoint above separate baseline values obtained by flowing air through the sealed, empty compartments before each trial. Measurements were sampled every second and averaged every minute by a computer-interfaced

datalogger (CR23x, Campbell Scientific, Logan, UT, USA) that received inputs from five thermocouples, two mass-flow controllers, and two hygrometers.

Experiment 1: Effects of T_a on EWL

In order to monitor T_b throughout the experimental trials, each of six lizards (mean body mass 606 ± 26.8 g) was implanted with a thermocouple array. Each array consisted of three 30 gauge, type T thermocouple cables (#TT-T-30-SLE, Omega Engineering, Stamford, CT, USA) extending from three (two male, one female) subminiature connectors (#SMP-W, Omega Engineering, Stamford, CT, USA). To prevent injury to the animal, the thermocouples terminating the long cable and one of the short cables were thinly covered with pourable rubber coating (Plasti-Dip, PDI Inc., Circle Pines, MN, USA).

With the animal under isoflurane anesthesia, an approximately 1 cm incision was made ventro-laterally in the abdominal region. From the incision site, a metal trocar was routed subcutaneously until it was exteriorized on the dorsum at mid-body. The two thermocouples coated with Plasti-Dip were inserted from the dorsum retrograde into the trocar. The trocar was removed, leaving the short thermocouple situated subcutaneously at the back. The body wall was punctured at the superficial ventro-lateral incision site, and the long thermocouple was placed 1 cm deep into the body cavity and sutured to the body wall. The array was triply sutured to the skin where it emerged on the dorsum to keep it in place and reduce tension at the dorsal incision site. Both the dorsal and ventro-lateral incisions were closed with everting mattress sutures (3-0 Vicryl, Ethicon,

Somerville, NJ, USA). The third thermocouple was glued to the skin surface directly superficial to the subcutaneous thermocouple using cyanoacrylate and then covered with a thin coating of Plasti-Dip. When connected to the datalogger, these three thermocouples could provide continuous measurements of core body, subcutaneous, and skin temperatures. Because of the failure of several subcutaneous and skin thermocouples, only core T_b results are reported here. Each animal was given at least three days to recover from surgery prior to participating in the experiment trials.

Each Gila monster was tested once at each of five ambient temperatures (approximately 20.5, 30.0, 35.0, 37.5, and 40.0°C). The 30°C T_a approximates the body temperature selected by Gila monsters in a laboratory thermogradient (29.4°C, Bogert and del Campo, 1956) as well as the mean T_b obtained from free-ranging Gila monsters (29°C, Lowe et al., 1986; 28.5°C, Beck, 1990), while the other temperatures lie near or beyond the extremes of the species' active T_b range (24-37°C, Beck, 1990). Animals were used only when they were not undergoing or about to undergo ecdysis, as ecdysis can impact EWL. Trials for an individual were separated by at least 24 hours, and the five treatment temperatures were randomized. Animals were moved from the housing room to the environmental chamber and allowed at least two hours to adjust to the trial temperature. Based on pilot tests, this time was sufficient for T_b to stabilize while the animal was kept in the new thermal environment. During this stabilization time, air was flowed through the sealed but empty compartments to obtain baseline compartment air temperatures and dewpoints. Compartment vapor densities calculated from the baseline dewpoints were subtracted from vapor densities calculated from dewpoints during the

experimental trial, and the resulting differences (along with flow rates and body mass) were used to determine EWL (see *Calculations* below).

Animals were then placed in the partitioned chamber for at least 40 minutes to allow them to adjust to the new environment and for stabilization of dewpoints and body temperatures. The three body temperatures, ambient temperatures of the two compartments, and separate dewpoints of air flowing over the head and air flowing over the rest of the body were recorded for 20 minutes while the animal was at rest. Upon collection of these data, a cotton wad was placed in the cloaca, and an H-shaped piece of latex was tied around the hind limbs to cover the vent. This 'diaper' prevented moisture from leaving the cloaca, while minimally impeding cutaneous evaporation (the diaper covered approximately 1% of the animal's total surface area). After being fitted with the diaper, the animal was returned to the test chamber and a second set of data was collected in the same fashion as the original set. For any trial in which moisture (e.g. urine) was visible on the animal or the walls of the chamber during or at the end of the trial, the data were discarded and the trial was repeated at a later time. The presence of such liquid was also easily detectable as a rapid rise on the plot of torso chamber dewpoint.

Experiment 2: Effects of dehydration on EWL

I recorded the mass of six adult Gila monsters not used in experiment 1 (mean body mass = 520 ± 27.9 g) and then deprived them of food and water for 6 to 10 weeks, until they reached approximately 80% of initial mass. Six additional adult Gila monsters (mean body mass = 523 ± 29.5g) were provided water *ad libitum* but no food for 10

weeks. I was thereby able to assess the fraction of mass-loss attributable to energetic demands (catabolism), rather than to dehydration. To assess the effect of dehydration on serum osmolality, a blood sample was collected from the caudal vein of each animal after the 6 to 10 week period. I centrifuged the samples and stored the serum in sealed tubes at -80°C for later analysis. I measured serum osmolality twice for each sample with a vapor pressure osmometer (#5500, Wescor, Logan, UT, USA) that was calibrated immediately prior to measurements using standard solutions (290 mmol kg^{-1} and 1000 mmol kg^{-1}, Wescor, Logan, UT, USA).

The six dehydrated Gila monsters underwent experimental trials similar to that of experiment 1, except that animals were not implanted with thermocouple arrays, and trials were limited to 37.5°C and 40°C. Imposing these limitations allowed for much faster completion of the trials (to minimize the duration of the dehydrated state) while still providing valuable data for assessing the effect of dehydration on EWL at the most thermally challenging temperatures.

Calculations

For each trial, I determined values for dewpoint and temperature by calculating the mean values over a five minute period near the end of the trial when values were nearly constant. I used dewpoints to calculate ambient-temperature vapor pressures using an 8^{th} order polynomial describing saturation vapor pressure as a function of air temperature (Flatau et al., 1992). Vapor pressures were used to calculate ambient-temperature vapor densities using the Ideal Gas Law (Campbell and Norman, 1998).

Finally, evaporative fluxes (mg min^{-1}) were calculated by multiplying vapor density (mg ml^{-1}) by ATP (ambient temperature and pressure) rate of flow of air (ml min^{-1}) for each of the two compartments. I calculated absolute evaporative flux (mg H_2O h^{-1}) as well as fluxes relative to both mass (mg H_2O g^{-1} h^{-1}) and surface area (mg H_2O cm^{-2} h^{-1}) to account for variation in size between individuals. I assumed that a portion of the water vapor appearing in the head compartment was attributable to evaporation from the cranial integument (skin and conjunctivae) and that evaporative flux from the skin of the head equaled that from the skin of the torso. I further assumed that, despite the probably greater evaporative flux from the moist eyes than from the dry skin, the small size of the eyes compared to the head made the absolute increase negligible. I therefore estimated the non-buccopharyngeal component of the flux occurring in the head chamber based on the surface area of the head and on the area-specific value for evaporative flux from the skin in the torso compartment during the diapered trial. The resulting non-buccopharyngeal, head-chamber component was then subtracted from the total head-chamber flux to yield buccopharyngeal flux, and it was added to the torso-chamber flux to yield non-buccopharyngeal flux. Finally, non-buccopharyngeal flux during the diapered trial was subtracted from non-buccopharyngeal flux during the non-diapered trial to yield cloacal flux, and non-buccopharyngeal flux during the diapered trial was taken to be cutaneous flux. Lastly, for experiment 1 I assessed the ability of Gila monsters to physiologically thermoregulate by subtracting the mean air temperature of the torso compartment from the mean core T_b during each trial.

Statistical Analysis

I used StatView (version 5, SAS Institute, Cary, NC, USA) for all statistical analyses. For experiments 1 and 2, I used repeated measures analyses of variance (RMANOVA), with T_a and cloacal patency as within-subjects factors, and either T_b or water flux as the dependent variable. To compare EWL rates of hydrated animals in experiment 1 with dehydrated animals in experiment 2, I used RMANOVA with hydration as the between-subjects factor, T_a as the within-subjects factor, and water flux as the dependent variable. *Post-hoc* comparisons were made with paired Student's *t*-tests adjusted for an experimentwise Type 1 error rate of 0.05. The adjusted alpha for controlling Type 1 experimentwise error was $0.05/N$, where N = the number of sampling periods (i.e. $a = 0.01$ and $a = 0.025$ for experiments 1 and 2, respectively). Osmolality results were analyzed using a Student's *t*-test. All values are presented as means ± S.E.M.

Results

Experiment 1

EWL from both the head and torso compartments increased with increasing T_a (head: $F_{4,5} = 10.07$, $P < 0.0001$; torso: $F_{4,5} = 25.83$, $P < 0.0001$). The head compartment showed a linear increase across all temperatures, while the increase in the torso compartment was linear between 20.5 and 35°C, and then showed a dramatic increase above 35°C. Applying the diaper significantly reduced EWL in the torso compartment (cloacal patency main effect: $F_{1,5} = 30.27$, $P = 0.0003$), and this effect was temperature dependent (cloacal patency × T_a effect: $F_{1,5} = 22.42$, $P < 0.0001$). *Post-hoc* analyses indicate the diaper significantly reduced torso chamber EWL only at 40°C [mean reduction = 7.30 mg g^{-1} h^{-1} (89%), $P = 0.0033$], although the mean reduction in EWL at 37.5°C was also substantial [mean reduction = 2.14 mg g^{-1} h^{-1} (75%), $P = 0.013$]. Contrary to the suppressive effect on torso-compartment EWL, applying the diaper had a positive effect on EWL in the head compartment (cloacal patency main effect: $F_{1,5} = 10.54$, $P = 0.0088$), but this effect was not temperature dependent (cloacal patency × T_a: $F_{1,5} = 0.80$, $P = 0.53$). *Post-hoc* analyses showed that the increase was only significant at 37.5°C [mean increase = 0.229 (48%), $P = 0.0065$], although a considerable increase also occurred at 40°C [mean increase = 0.209 (28%), $P = 0.054$].

Increasing T_a led to a significant increase in both cutaneous and cloacal, but not buccopharyngeal, evaporative fluxes (cutaneous: $F_{4,5} = 10.27$, $P = 0.0001$; cloacal: $F_{4,5} = 21.34$, $P < 0.0001$; buccopharyngeal: $F_{4,5} = 2.38$, $P = 0.086$, Fig. 3.1, Table 3.1). Cutaneous flux showed a relatively constant increase throughout all trial temperatures

(Q_{10} = 1.61), while cloacal flux was low and relatively constant between 20.5°C and 35°C, but rose dramatically above 35°C (Q_{10} = 8.3 X 10^7).

Trial temperature affected the difference between chamber temperature and T_b, with increasing chamber temperatures leading to a greater suppression of T_b below chamber temperature ($F_{4,5}$ = 27.90, P < 0.0001; Fig. 3.2). While applying the diaper consistently reduced the degree of temperature suppression at all higher temperature, the lack of an effect at lower temperatures led to no overall effect of diaper application on temperature suppression ($F_{1,5}$ = 2.57, P = 0.14). However, the interaction between chamber temperature and diaper application approached, but failed to reach, statistical significance ($F_{1,5}$ = 2.39, P = 0.067).

Experiment 2

Restricting food and water to six experimental animals for 6-10 weeks led to a significantly greater loss in body mass compared to animals provided no food but free access to water (water-deprived: 78 ± 1% of initial body mass, range 75-81%; *ad libitum* water: 95 ± 2%, range 87-101%; P < 0.0001). Furthermore, serum osmolality of the water-deprived Gila monsters was significantly higher than that of the animals provided water *ad libitum* (water-deprived: 603 ± 7 mOsm kg^{-1} H_2O; *ad libitum* water: 487 ± 37 mOsm kg^{-1} H_2O; P = 0.013). Combined, these results demonstrate that the majority of mass lost in the experimental group was water, and, although the degree of dehydration is not quantifiable, the water-deprived animals were considerably dehydrated.

As in experiment 1, T_a had a significant effect on EWL in both the head and torso compartments for the dehydrated Gila monsters (head: $F_{1,5} = 51.50$, $P < 0.0001$; torso: $F_{1,5} = 14.30$, $P = 0.0036$). Also, similar to the results of experiment 1, applying the diaper had a significant effect on EWL in the torso compartment (cloacal patency main effect: $F_{1,5} = 8.44$, $P = 0.016$; cloacal patency × T_a: $F_{1,5} = 10.03$, $P = 0.010$), but not the head compartment (cloacal patency main effect: $F_{1,5} = 0.13$, $P = 0.72$; cloacal patency × T_a: $F_{1,5} = 0.37$, $P = 0.56$). *Post-hoc* tests indicate that significant results from the torso compartment were due to a diaper-induced reduction in EWL at 40°C ($P = 0.021$).

Increasing T_a had a positive effect on all fluxes (cutaneous: $F_{1,5} = 7.56$, P = 0.040; cloacal: $F_{1,5} = 10.67$, $P = 0.022$; buccopharyngeal: $F_{1,5} = 15.54$`, $P = 0.011$, Table 5.2). Comparing results from experiments 1 and 2 reveals that dehydration had a significant effect on cloacal and buccopharyngeal fluxes, but not on cutaneous flux (cutaneous: $F_{1,5} = 0.75$, $P = 0.41$; cloacal: $F_{1,5} = 19.74$, $P = 0.0012$; buccopharyngeal: $F_{1,5} = 8.08$; $P = 0.018$; Fig. 3.3). Dehydration suppressed cloacal flux relative to that of hydrated animals at both temperatures tested ($P = 0.0038$ and $P = 0.0082$ at 37.5 and 40°C, respectively). While the effect of dehydration was negative for cloacal flux, it was positive for buccopharyngeal flux (i.e. buccopharyngeal flux in dehydrated animals was higher than that of hydrated animals).

Discussion

Like that of many reptiles, EWL of Gila monsters is highly sensitive to temperature, with Q_{10} values for cutaneous EWL comparable to other lizard species (Crawford and Kampe, 1971). Even at the cooler temperatures tested, Gila monsters have a high total EWL relative to other lizards from arid environments (see Mautz, 1982a for a comparative summary of EWL in reptiles). The finding that EWL in Gila monsters compares most closely with that of lizards from mesic rather than arid environments might be viewed as support for the contention that Gila monsters evolved in a more tropical environment than they now inhabit (Lowe et al., 1986). However, when considering body size, which is inversely related to EWL rate (Mautz, 1982a), EWL of Gila monsters is considerably higher than that of other lizards regardless of habitat type. Therefore, the high evaporation rate of Gila monsters more likely exists for physiological reasons (i.e. thermal homeostasis) rather than simply as a relic of this lizard's more tropical ancestry.

The current trend is to evaluate EWL simply for its negative impact on water balance (e.g. Eynan and Dmi'el, 1993; Dmi'el, 1998, 2001; Winne et al., 2001), but high EWL has the potential to be a major contributor to thermostasis, assuming sufficient water availability. This is especially true at higher temperatures, where EWL can help maintain T_b below the thermal maximum temperature (i.e. below those temperatures where locomotory activity is substantially impaired). Several lizard species have been shown to dramatically increase EWL at higher temperatures (Table 3.2). In each of the previously studied species, the increase in EWL is a result of increases in

buccopharyngeal flux (predominantly a reflection of panting). Gila monsters are apparently unique among studied species in that EWL rates at high temperatures are considerably higher than even those of panting lizards and in that the source of this dramatic increase in EWL is the cloaca. Previous studies either ignored (e.g. Dawson and Templeton, 1963) or prevented (e.g. Templeton, 1960; Warburg, 1965; Crawford and Kampe, 1971) any EWL occurring from the cloaca. Cloacal EWL might be a unique physiological adaptation of Gila monsters, or it might be more widely spread among lizard taxa. It is worth noting that none of the Gila monsters in the current study panted during the trials, even at the highest T_a. This observation is reinforced by the small change in buccopharyngeal EWL as temperature increased. While panting is common among lizards, other species also fail to pant at thermally challenging temperatures (Dawson, 1960). Cloacal evaporation might simply be an alternative mechanism by which lizards can decrease T_b. Lastly, the temperature at which Gila monsters exhibit extreme elevations in EWL is somewhat lower (37.5°C) than that of other species studied (typically ≥ 40°C). For some species this difference could be a result of a lack of data for temperatures between 37 and 40°C, but this difference might also be attributable to the relatively low selected body temperature of Gila monsters. While it might have been advantageous to examine the response of Gila monsters at temperatures higher than 40°C, such temperatures are extremely risky to the health of this species, especially when cloacal EWL is prevented.

Data regarding the ability of EWL to reduce T_b of lizards to below T_a are mixed. Evaporation seems to be of marginal importance in decreasing T_b in some species

(Templeton, 1960; Crawford and Kampe, 1971), but it can significantly reduce T_b in others (Dawson and Templeton, 1963; Warburg, 1965; DeWitt, 1967; this study). Regardless of its effectiveness, EWL is probably not used to extend activity bouts for long durations at high T_a, because of the relative scarcity of water in habitats with such high temperatures. Nevertheless, it might allow the lizard to slightly extend the duration of activity (Dawson and Templeton, 1963), and even a slight extension could be important in an extreme environment.

The Sonoran Desert is characterized by an extended period of high temperature. Maximum daily temperature often exceeds 40°C from mid spring until early fall (i.e. April – October). Despite living in a hot environment, Gila monsters have a relatively low selected body temperature of approximately 29°C (Bogert and del Campo, 1956; D. F. DeNardo, unpublished data). Furthermore, Gila monsters are active foragers, preying on the contents of bird, mammal, and reptile nests. Relying on such widely dispersed resources requires Gila monsters to forage over long distances (Beck, 1990). To regulate T_b during the summer, Gila monsters restrict activity to the cooler periods of the day. However, EWL might allow extension of the activity period to complete critical activities (e.g. locating shelter, consuming prey, or engaging in combat) without reaching temperatures that approach their critical thermal maximum.

While water is a limited resource in all deserts, much of the Sonoran Desert has a reliable summer monsoon season (mid-July to mid-September) that provides relatively frequent access to water for at least the latter half of the hot summer. Therefore, the length of time during which Sonoran Desert residents cope with arid conditions (typically

mid-April through mid-July) is reduced compared to many desert environments. The periodic availability of water and the concomitant increase in food availability associated with the summer monsoon season might underlie the predominant restriction of Gila monsters to these areas of the Sonoran Desert. Additionally, Gila monsters possess extremely large urinary bladders that potentially act as reservoirs for water during the dry periods. Previous studies support such a role for the bladder in other desert lizards (Beauchat et al., 1986; Cooper and Robinson, 1990), but water storage in the bladder remains unexplored in Gila monsters.

Despite experiencing a summer rainy season and perhaps possessing a water reservoir, Gila monsters are vulnerable to dehydration during the dry summer months (Bogert and Del Campo, 1956; Beck and Jennings, 2003), and high EWL rates at this time would be costly and possibly fatal. Therefore, it is not surprising that Gila monsters reduce cloacal EWL rates when dehydrated by increasing the minimum temperature at which significant cloacal EWL occurs and by reducing evaporative flux at higher temperatures. I recognize that the decrease in evaporative flux during dehydration is almost certainly due in part to the physical effect that the increased osmotic pressure of the blood has on the vapor-pressure gradient driving the evaporation. However, the direct effect of increased osmolality is unlikely to account for the full magnitude of the reduction in EWL. Instead, it is likely that much of the reduction in EWL is due to physiological adjustments made to minimize loss of body water. For example, alteration in cloacal perfusion and or vent gape could substantially affect the rate of cloacal EWL. While vent gape has been anecdotally observed in Gila monsters at high environmental

temperatures, possible regulatory mechanisms remain to be tested. Similarly unknown yet interesting and deserving of future study are the regulatory parameters for cloacal EWL. In dehydrated desert iguanas, *Dipsosaurus dorsalis*, an increase in plasma osmolality delays the onset and extent of panting, which induces a 'right shift' and blunting of the EWL-temperature response curve (Dupré and Crawford, 1985). While serum osmolality increased significantly in the dehydrated Gila monsters, it is unknown whether cloacal EWL in Gila monsters is similarly regulated by osmolality. However, since the presence of water in the urinary bladder might allow for water expenditure without changing plasma osmolality, regulation of cloacal EWL in Gila monsters might also be influenced by urinary bladder volume. While the results presented here do not provide insight into the underlying mechanisms or regulatory parameters involved in cloacal EWL, this study presents a previously undescribed means for controllable evaporative water loss and points out the possible importance of EWL for thermoregulation in ectotherms. The degree to which EWL can serve as a thermoregulatory mechanism depends on the availability of water (within both the organism and the environment) and on the ability of the organism to regulate water loss. Further studies of this and other species are warranted to better understand how desert organisms trade off between thermostasis and hydrostasis.

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Table 3.1. *Mean evaporative water flux of (A) six normally hydrated Gila monsters, each tested at five ambient temperatures, and (B) six dehydrated Gila monsters, each tested at two ambient temperatures*

	Evaporative water flux (mg g^{-1} h^{-1})			
T_a (°C)	Total	Cutaneous	Cloacal	Buccopharyngeal
(A) Normally hydrated animals				
20.0	0.748 ± 0.117	0.378 ± 0.041 (51%)	0.066 ± 0.053 (9%)	0.304 ± 0.053 (41%)
30.0	1.345 ± 0.315	0.785 ± 0.107 (58%)	0.164 ± 0.262 (12%)	0.397 ± 0.061 (29%)
35.0	1.302 ± 0.159	0.923 ± 0.170 (71%)	0.001 ± 0.062 (0%)	0.378 ± 0.072 (29%)
37.5	3.316 ± 0.588	1.008 ± 0.172 (30%)	1.930 ± 0.514 (58%)	0.378 ± 0.104 (11%)
40.0	8.921 ± 1.31	0.954 ± 0.107 (11%)	7.303 ± 1.39 (82%)	0.663 ± 0.114 (7%)
(B) Dehydrated animals				
37.5	1.374 ± 0.095	0.719 ± 0.102 (52%)	0.008 ± 0.059 (0%)	0.663 ± 0.096 (48%)
40.0	4.176 ± 0.764	0.964 ± 0.104 (23%)	2.221 ± 0.679 (52%)	0.991 ± 0.108 (25%)

T_a, ambient temperature.
Values are means ± S.E.M.
Numbers in parentheses represent the percentage of total water flux at that temperature.
Note that temperature differentially affects cutaneous, cloacal, and buccopharyngeal water flux, leading to changes in the relative contribution of each, and that dehydration significantly decreased cloacal water flux.

Table 3.2. *Total evaporative water fluxes (EWL) of various arid and semi-arid lizards at moderate and thermally challenging air temperature (T_a)*

Genus and Species	Body Mass (g)	T_a (°C)	EWL (mg g^{-1} h^{-1})	Reference
Crotaphytus collaris	30	32	0.46	Dawson & Templeton, 1963
		40	0.73	
		44	4.70	
Dipsosaurus dorsalis	48	32	0.86	Templeton, 1960
		40	2.08	
		44	3.64	
Sauromalus obesus	140	26	0.22	Crawford & Kampe, 1971
		40	0.67	
		43.5	2.36	
Pogona barbatus (*Amphibolurus barbatus*)	241	30	0.47	Warburg, 1965
		40	1.04	
Trachydosaurus rugosus *(Tiliqua rugosa)*	315	30	0.67	Warburg, 1965
		40	1.12	
Heloderma suspectum	606	30	1.35	This study
		40	8.92	

All species, except Gila monsters *Heloderma suspectum*, are known to pant at higher temperatures, thus explaining the substantial increase in EWL at those temperatures. The dramatic elevation in EWL of Gila monsters at 40°C is attributable to cloacal water flux.

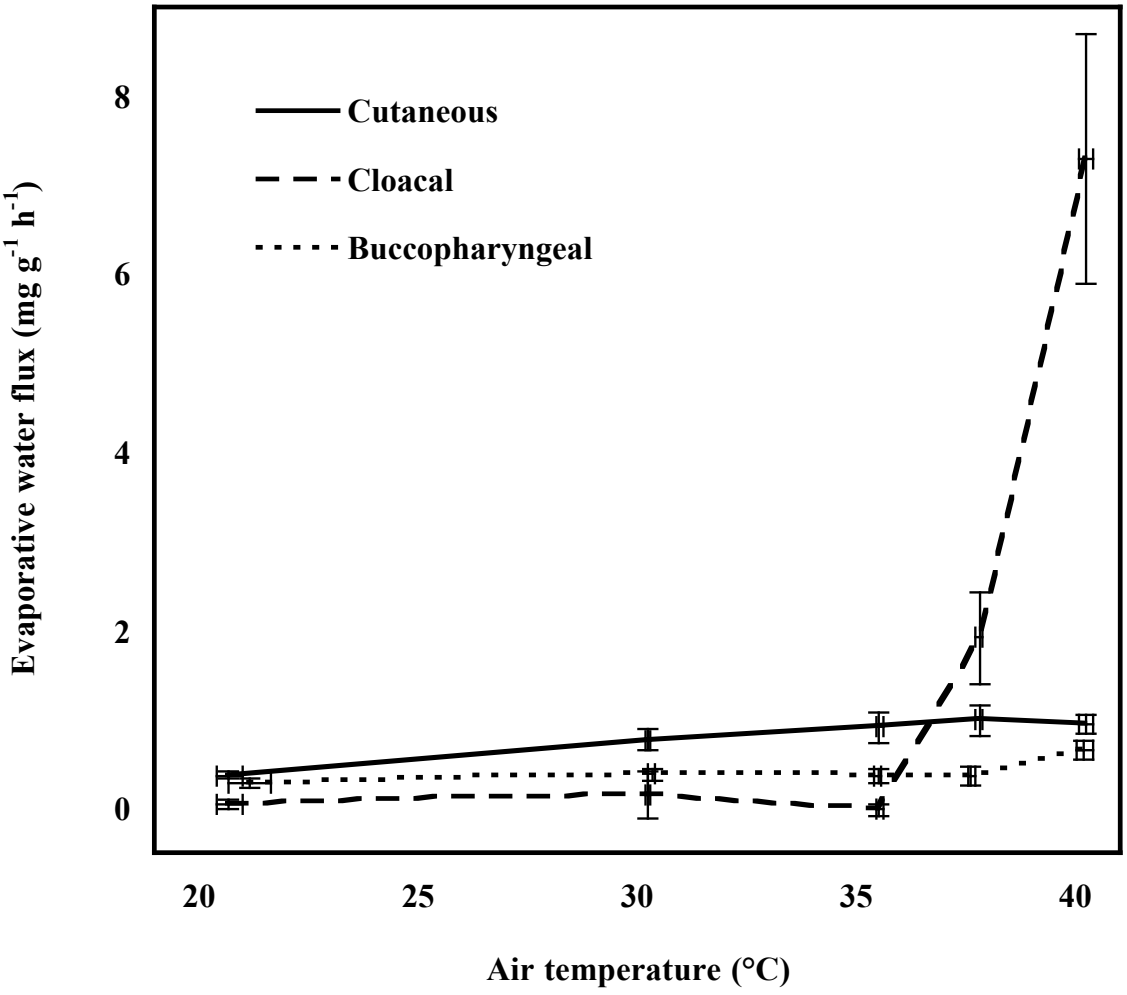

Fig. 3.1. Mean cutaneous, buccopharyngeal, and cloacal water loss rates in six Gila monsters at various experimental temperatures. Note that buccopharyngeal EWL shows little temperature sensitivity, while cutaneous EWL increases gradually as T_a increases, and cloacal EWL shows a dramatic increase at T_a greater than 35°C. Vertical and horizontal error bars represent ± 1 standard error for water loss rates and chamber temperature, respectively.

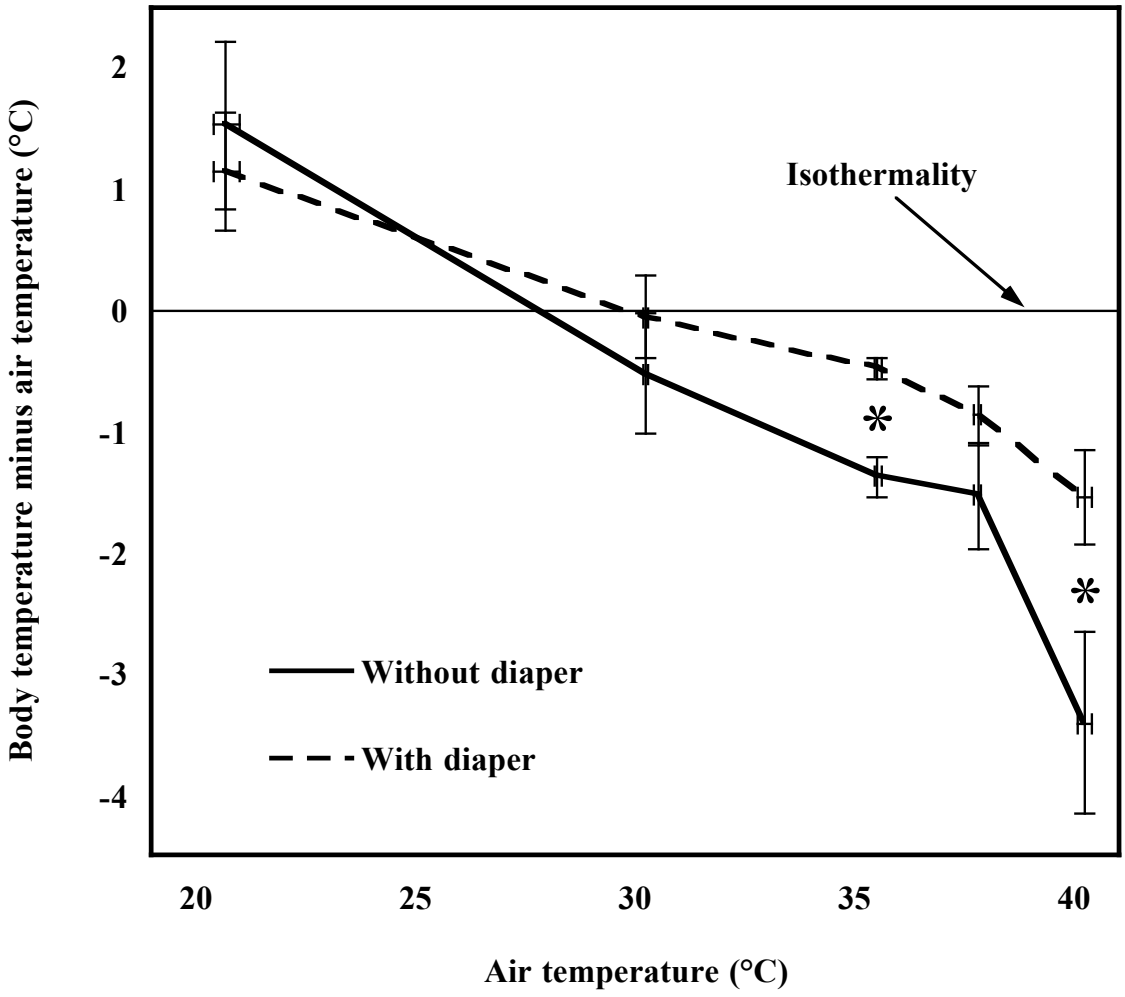

Fig. 3.2. Mean differences between T_a and T_b of six Gila monsters at five experimental temperatures. Increasing T_a led to increasing suppression of T_b ($P < 0.0001$), and T_b was consistently lower than T_a at higher temperatures. Asterisks represent significant differences ($P < 0.01$) between the control and diaper run values. Vertical and horizontal error bars represent ± 1 standard error for temperature suppression and chamber temperature, respectively.

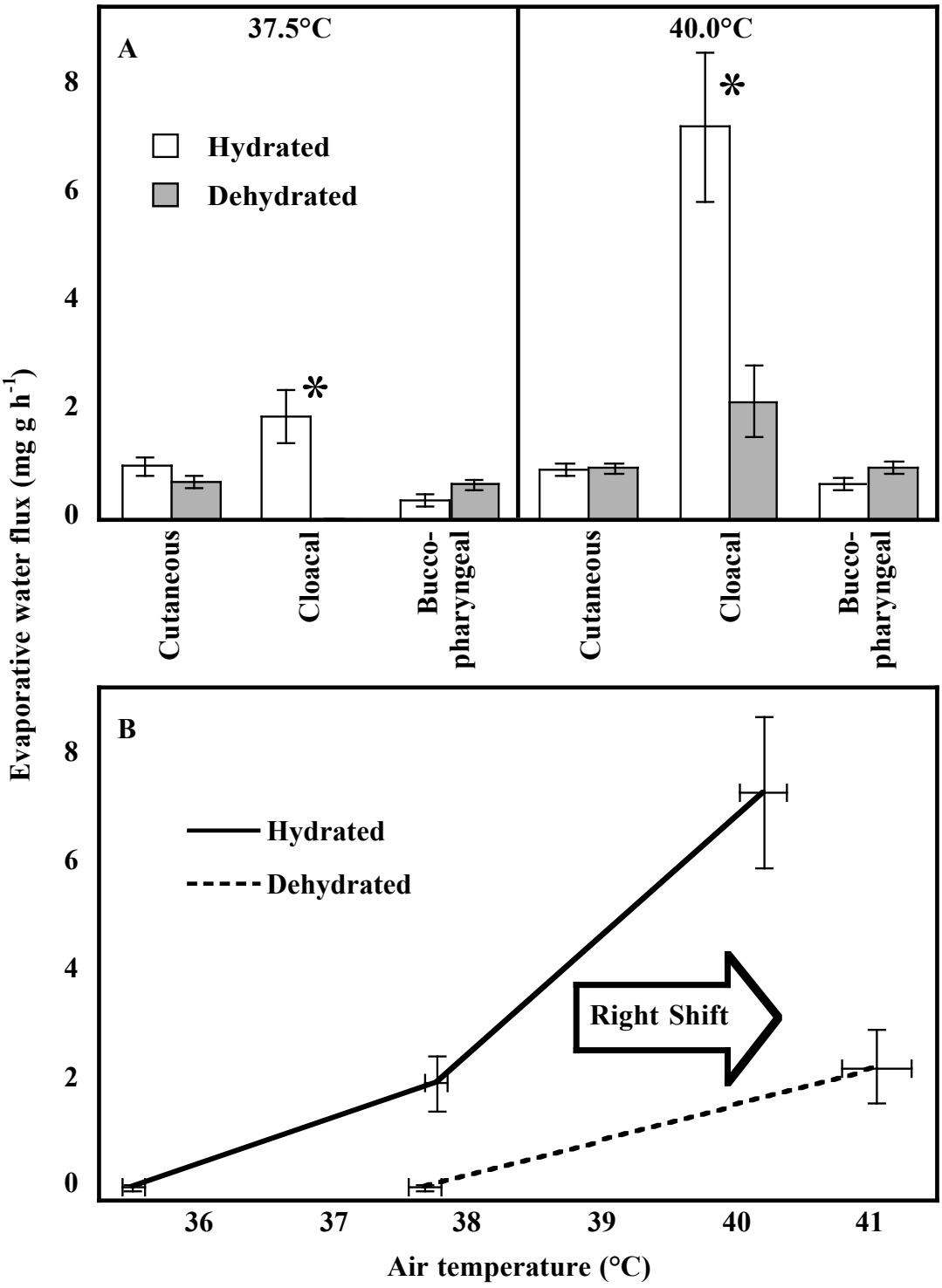

Fig. 3.3. The effect of dehydration on EWL by Gila monsters at thermally challenging temperatures. Each symbol indicates the mean value for six Gila monsters; error bars

indicate ± 1 standard error. A: Effect on cutaneous, buccopharyngeal, and cloacal EWL at 37.5°C and 40°C. An asterisk indicates a statistically significant ($P < 0.025$) difference between values from hydrated and dehydrated animals. B: Cloacal EWL of hydrated animals and dehydrated animals. Note the lack of elevated cloacal EWL at 37.5°C and attenuation of cloacal EWL at 40°C for dehydrated animals (i.e. a right shift in the EWL-temperature response curve).

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Cloacal Evaporation: An Important and Previously Undescribed Mechanism for Avian Thermoregulation

Summary

I present the first experimental evidence that a bird is capable of evaporating enough water from the cloaca to be important for thermoregulation. I measured rates of evaporation occurring from the mouth, the skin, and the cloaca of Inca doves (*Columbina inca* Lesson) and Eurasian quail (*Coturnix coturnix* Linnaeus). Inca doves showed no significant increase in cutaneous evaporation in response to curtailment of buccopharyngeal evaporation. Cloacal evaporation in doves was negligible at ambient temperatures of 30°, 35°, and 40°C. However, at 42°C, the apportionment of total evaporation in doves was 53.4% cutaneous, 25.4% buccopharyngeal, and 21.2% cloacal, with cloacal evaporation shedding, on average, 150mW of heat. In contrast, the evaporative apportionment in quail at 32°C (the highest ambient temperature tolerated by this species) was 58.2% cutaneous, 35.4% buccopharyngeal, and 6.4% cloacal. These results suggest that, for some birds, cloacal evaporation can be controlled and could serve as an important emergency tactic for thermoregulation at high ambient temperatures.

Introduction

Organisms are able to exchange heat with the environment *via* four modes: conduction, convection, radiation, and evaporation (Porter and Gates, 1969). Of these modes, evaporation holds a place of peculiar ecological interest. First, evaporation from an organism always results in a decrease in the temperature of the surface from which evaporation takes place. Evaporation is therefore a one-way transfer, always representing a loss of heat from the organism. In contrast, heat can be lost or gained either conductively, convectively, or radiatively, depending on the direction of the gradient for each respective mode of transfer. Second, biological evaporation always involves the loss of water, a vital resource on which nearly all biochemical processes depend. Evaporation, then, is loss of heat *via* loss of mass. Among the four modes of heat transfer, evaporation is unique in its coupling of heat loss with resource loss. These fundamental differences underlie an important biological conflict of interests: the animals with the least access to water for hydrostasis (such as desert forms) are the animals with the greatest need to lose water for thermostasis. The competing needs for water retention and water evaporation lead one to expect that many desert animals adjust the rate of evaporation as a tradeoff between avoidance of overheating and avoidance of dehydration.

Adjustment of evaporation can be made either by changing the evaporative conductance of (and therefore the rate of evaporation from) any specific epithelium or by changing the surface area of exposed epithelia. Experimental partitioning of total evaporation into components, or evaporative routes, has been done for many species using various methods in studies that have used a variety of terms to describe those

evaporative routes (e.g. Bernstein, 1971a; Richards, 1976; Maloney and Dawson, 1998; Webster and Bernstein, 1987; Taylor et al., 1971; Arieli et al., 1999; Menon et al., 1986; Lee and Schmidt-Nielsen, 1971; McKechnie and Wolf, 2004; Tieleman and Williams, 2002). Birds possess three anatomically distinct epithelia from which evaporation can occur: the mouth and pharynx, the dry skin, and the cloaca. I therefore categorize avian evaporative routes as either buccopharyngeal, cutaneous, or cloacal. The present study is the first to measure avian rates of cloacal evaporation. Buccopharyngeal evaporation includes gular fluttering and evaporation due to breathing, whether by panting or not. For simplicity, I include ocular evaporation within cutaneous evaporation. Because previous studies did not discriminate between evaporation from the dry skin and from the cloaca, I describe the sum of cutaneous and cloacal evaporation as non-buccopharyngeal evaporation.

Despite the lack of sweat glands, several bird species have been shown to exhibit rates of non-buccopharyngeal evaporation that rival or exceed buccopharyngeal rates (e.g. Hoffman and Walsberg, 1999; McKechnie and Wolf, 2004; Marder et al., 1989; Webster and King, 1987; Arad et al., 1987; Wolf and Walsberg, 1996; Withers and Williams, 1990; Marder and Gavrieli-Levin, 1987; Smith, 1969). Historically, workers (Bernstein, 1969; Smith and Suthers, 1969) have assumed that all but a negligible portion of this non-buccopharyngeal evaporation occurs from the skin or from the conjunctivae. Terms such as ‘cutaneous’ (Lasiewski et al., 1971; Bernstein, 1969; Smith and Suthers, 1969), ‘peripheral’ (Dawson, 1982), and ‘transepidermal’ (Hattingh, 1972; Menon et al., 1989; Muñoz-Garcia and Williams, 2005) were thus used to describe the remainder of a

bird's evaporative output, after evaporation due to ventilation and gular fluttering were subtracted. Though some workers (Cade and Dybas, 1962) have conducted hygrometric measurements in which the avian cloaca was occluded, the rationale for such experimental treatment was to prevent urination and defecation, either of which would render a hygrometric measurement unusable in analyses of evaporation from the skin. A recent study of a desert reptile, the Gila monster, *Heloderma suspectum* Cope (DeNardo et al., 2004), demonstrated for the first time in any an animal that cloacal rates of evaporation can rid the body of enough heat to be important for thermoregulation. Those results raised the possibility that birds (which, like reptiles, possess cloacae) are similarly able to exploit this previously undescribed evaporative route.

Columbiform species, which can tolerate high ambient temperatures without panting (Arieli et al., 1988; Marder and Arieli, 1988; Ophir et al., 2002), show some of the highest non-buccopharyngeal rates of evaporation for any bird (Hoffman and Walsberg, 1999; Marder and Ben-Asher, 1983; McKechnie and Wolf, 2004). I have demonstrated previously (Hoffman and Walsberg 1999) that mourning doves (*Zenaida macroura* Linnaeus) are able to make rapid adjustments to the rate of non-buccopharyngeal evaporation in response to an experimental suppression of evaporation from the mouth. Here, to add insight regarding the generality of the results observed in mourning doves, I investigate the response to suppression of buccopharyngeal evaporation in a different columbiform, the Inca dove (*Columbina inca* Lesson). In addition, I refine the experimental technique to quantify the apportionment of non-buccopharyngeal evaporation into its cutaneous and cloacal components. For comparison,

I present values for all three evaporative rates in a gallinaceous bird, the Eurasian quail (*Coturnix coturnix* Linnaeus). Both of the test species are easily obtained and are widely distributed, occurring in arid and semiarid habitats, but they represent distinct taxonomic orders.

Materials and Methods

Animals

Adult Inca doves of undetermined sex were captured using drop traps in Phoenix, Arizona, USA in June 2004. Adult male Eurasian quail were purchased (Pratt's Feed and Supply) in Phoenix in January 2005. The birds were housed in wire cages (1-5 doves or 1-2 quail per cage) in a temperature-controlled room on the campus of Arizona State University in Tempe, Arizona, and the room provided a 12h:12h L:D artificial photoperiod. Ambient temperature (T_a) was maintained at 25°C. All birds had continuous access to water and food (seed for doves and game bird feed for quail), except during trials. A few downy feathers around the cloaca were trimmed from each bird to allow for safe and consistent access for cloacal manipulation and to prevent retention of wet feces during trials. Feather trimming did not differ between types of trials, and the removal of such a small fraction of plumage is unlikely to have made any appreciable change to evaporative conductance (Webster et al., 1985).

Respirohygrometry

1. Inca doves

I used the flow-through method to measure evaporative rates, which allowed me also to measure rates of change in oxygen and carbon dioxide. To minimize hygroscopicity, I constructed the test chamber of plate glass with aluminum corner supports. The chamber included two compartments - one for the head and one for the torso - separated by an aluminum partition that supported a thin sheet of latex (4 × 4 cm)

into which a hole was cut to allow for passage of the head and neck. With the bird in place the latex was stretched slightly, forming a barrier between the two compartments while not interfering with the bird's breathing. The head compartment (426 ml) was contained by a borosilicate bell jar fitted with borosilicate ports that accepted minimally hygroscopic tubing (3 mm i.d., Bev-a-Line IV, Thermoplastic Processes, Inc., Stirling, NJ, USA) for both influent and effluent. Identical ports were attached to the plate glass of the torso compartment (17.72 L) using epoxy, and the influent port was equipped with a copper-constantan (type T) thermocouple for measurement of ambient temperature. A steel rod hanging from the aluminum partition was equipped to support a removable polypropylene shackle that was placed on the bird's legs prior to placement into the chamber. An aluminum neck stock positioned immediately below the latex sheet prevented the bird from pulling its head through the neck hole. An illustration of a similar chamber appears elsewhere (Wolf and Walsberg, 1996).

Air entering the two compartments was first passed through an industrial air purifier (#PCDA11129022, Puregas, Denver, CO, USA) that removed carbon dioxide and water vapor. Flux through each of the two influent lines was controlled and measured by separate mass flow controllers (#FMA-A2406 and #FMA-A2409, Omega Engineering, Stamford, CT, USA) positioned upstream of the compartments. Flux into the head compartment and torso compartment was maintained at ca. 1300 ml min^{-1} and ca. 6700 ml min^{-1}, respectively. A borosilicate U-tube containing mineral oil was interposed between tubes connecting the compartments. The U-tube served as a manometer to allow for minimization of any intercompartmental pressure gradient due to unequal flow rates,

thus minimizing the possibility of a gas leak from one compartment to the other. I occasionally verified that leaking was not occurring by sending air subsampled from the torso compartment to the CO_2 analyzer and ensuring that the air was virtually free of carbon dioxide. To avoid any appreciable increase of chamber air pressure beyond barometric pressure, both effluent lines were kept short and allowed to empty into spill tubes from which separate subsampling pumps drew air and delivered it to the downstream instruments.

Sample air from the two compartments was pumped to separate dewpoint hygrometers (#RH100, Sable Systems International, Las Vegas, NV, USA). Effluent from the torso-compartment hygrometer was vented to the temperature-controlled room in which the test chamber sat. Effluent from the head-compartment hygrometer was sent through anhydrous calcium sulfate to rid it of water vapor, and the dried air then passed through a carbon dioxide analyzer (#LI-6252, Li-Cor Biosciences, Lincoln, NE, USA) and an oxygen analyzer (#FC-1B, Sable Systems International, Las Vegas NV, USA). Prevailing barometric pressure was continuously measured by an electronic manometer.

For half of the trials, the acapnic air supplying the head compartment was diverted to a series of three copper water columns through which it was bubbled to saturate the air with water vapor. Condensate, visible in the tubing that exited the water columns, assured me of saturation. The water-saturated air was then sent to the test chamber, just as for dry air in all other trials. To avoid condensation in the mass flow controller, and because I calibrated the controller for dry air, it was placed upstream of the water columns. I used the value for saturation vapor density at the temperature of the water to calculate the

volumetric rate at which water vapor was added to the air stream, and I added that rate to the flux through the mass flow controller to determine head-compartment influx for those trials.

Measurements from all sensors were sampled every second by a datalogger (#CR23X, Campbell Scientific, Logan, UT, USA) and then averaged for output every minute. The effective volumes (Lasiewski et al., 1966) of the compartments were calculated as 1960 ml (head) and 81.5 L (torso), yielding 99% equilibration periods of 1.5 min and 12.2 min, respectively.

2. Eurasian quail

Because of the size and body geometry of Eurasian quail, I was not able to conduct trials in the compartmentalized chamber. Instead, quail were placed in a cylindrical chamber made of borosilicate glass (5.1 L) with an aluminum lid and a glass floor. A cylindrical, polycarbonate mask (open on one end) was placed over the bird's head and secured at the neck by nylon twine. The distal (closed) end of the mask was attached to a flexible tube connected to a miniature air swivel that allowed the bird to move about the cage without tangling the air line. The effluent line from the swivel was attached to a pump that drew air from the chamber, through the mask, and into a dewpoint hygrometer (Sable Systems RH100), from which it was sent through anhydrous calcium sulfate and then through a carbon dioxide analyzer (Li-Cor 6252) and an oxygen analyzer (Sable Systems FC-1B).

The cylindrical chamber was fitted with three borosilicate ports, each of which connected to minimally hygroscopic tubing (Bev-a-Line IV). Thus, there were separate air lines for chamber influent, chamber effluent, and mask effluent. The influent line was equipped with a copper-constantan thermocouple for measurement of ambient temperature. Negative-pressure flux through the mask was maintained by a mass flow controller (Omega Engineering FMA-A2406) at ca. 630 ml min^{-1}, sufficient to capture the expired air and disallow it from escaping at the junction between the mask and the neck. Positive-pressure flux into the chamber was maintained at ca. 6730 ml min^{-1} by a separate mass flow controller (Omega Engineering FMA-A2409), resulting in a 3.5 min period for gaseous equilibration (Lasiewski et al., 1966). Collecting all of the expired air at the mask served to effectively partition the chamber into torso and head compartments. The baseline gas for the torso compartment was dry, acapnic air as described above for the Inca dove experiment. The chamber effluent provided for measurement of non-buccopharyngeal evaporation. In addition, this effluent served as the baseline gas for the mask, because air drawn through the mask included water vapor added to the chamber from the bird's torso. As for the Inca dove experiment, the chamber effluent line was allowed to empty into a spill tube from which air was subsampled and sent to a dewpoint hygrometer (Sable Systems RH100). This chamber effluent was also able to be routed to the carbon dioxide and oxygen analyzers. By ensuring that there was a negligible change in the dried fractions of respiratory gases sampled from the body compartment, I was assured that leaking from the mask did not occur.

The specifics of data acquisition for Eurasian quail were the same as for Inca doves.

Experimental Protocol

1. Inca doves

The experiment included three treatment variables: ambient temperature, ventilatory humidity, and cloacal patency (*N*=8 to 13; see Table 4.1). Trials were conducted at four ambient temperatures (30°, 35°, 40°, and 42°C), two levels of ventilatory humidity (‘dry trials’ and ‘humid trials’), and two levels of cloacal patency (‘unsealed trials’ and ‘sealed trials’). For humid trials, the torso compartment was supplied with dry air, and the head compartment was supplied with air saturated at the respective ambient temperature with water vapor. Immediately prior to placement of the bird into the chamber for sealed trials, the cloaca was occluded with cyanoacrylic glue. The resulting cloacal cap remained in place throughout the trial and was removed using acetone immediately after the trial. Any feces released during unsealed trials fell into a layer of mineral oil on the floor of the chamber, thereby eliminating fecal water from hygrometric measurements.

During unsealed trials, the hygrometers directly measured buccopharyngeal and non-buccopharyngeal evaporation; during sealed trials, they directly measured buccopharyngeal and cutaneous evaporation. These direct measurements allowed me to calculate cloacal evaporation as the difference between non-buccopharyngeal and cutaneous evaporation. During humid trials, buccopharyngeal evaporation was eliminated

(or at least severely reduced), because the influent was already saturated with water vapor. This required the bird to either store that extra heat or dissipate it by increasing evaporative flux elsewhere. The bird remained in the test chamber for two hours. For the first 60 minutes, dry air was delivered to both compartments. A remote switch then triggered a re-routing of the influent without disturbing the bird, thereby delivering water-saturated air to the head chamber for an additional 60 minutes, before the bird was removed from the chamber. Data used in analyses were averages of measurements taken over the last 10 minutes of each portion (dry or wet) of the overall time spent in the chamber. All trials were conducted during daylight hours, but in darkness to promote quiescence.

2. Eurasian quail

The experiment included two treatment variables: ambient temperature and cloacal patency (N=8). Trials were conducted at two ambient temperatures (30° and 32°C) and two levels of cloacal patency ('unsealed trials' and 'sealed trials'). I did not conduct trials at T_a>32°C, because in pilot tests quail became distressed at higher temperatures, as evidenced by observation of persistent struggling. Because quail stood on the floor of the test chamber, no mineral oil was used; consequently, data were discarded from six trials during which defecation occurred, and those trials were repeated at a later date. Except for differences in the method of partitioning evaporative routes and in the ambient temperatures of trials, the protocol for the Eurasian quail experiment was

the same as for the dry trials using Inca doves. All trials were conducted in darkness during daylight hours.

Calculations

Evaporation represents an input of gas into the chamber, so that the efflux and influx differ. Similarly, oxygen consumption, $\dot{V}_{O_2}$, and carbon dioxide production, $\dot{V}_{CO_2}$, alter the flux. To incorporate these changes into the data, I derived the following equations for calculating evaporative rates. A key to all symbols is provided in Table 4.2.

$$\dot{V}_A = \dot{V}'_A + \dot{V}_{H_2O} + \dot{V}_{CO_2} - \dot{V}_{O_2} \tag{1}$$

$$\dot{V}_{H_2O} = \frac{\dot{V}'_A(F_{H_2O} - F'_{H_2O}) + F_{H_2O}(\dot{V}_{O_2} - \dot{V}_{CO_2})}{1 - F_{H_2O}} \tag{2}$$

$$\begin{aligned} \dot{V}_A &= \dot{V}'_A + \frac{\dot{V}'_A(F_{H_2O} - F'_{H_2O}) + F_{H_2O}(\dot{V}_{O_2} - \dot{V}_{CO_2})}{1 - F_{H_2O}} + \dot{V}_{CO_2} - \dot{V}_{O_2} \\ &= \dot{V}'_A\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right) \end{aligned} \tag{3}$$

$$\begin{aligned} \dot{M}_{H_2O} &= \dot{V}_A\rho_V - \dot{V}'_A\rho'_V \\ &= \left\{\dot{V}'_A\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right)\right\}\rho_V - \dot{V}'_A\rho'_V \\ &= \dot{V}'_A\left(\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right]\rho_V - \rho'_V\right) + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right)\rho_V \end{aligned} \tag{4}$$

$$F_{H_2O} = \frac{P_V}{P_B} \tag{5}$$

$$F'_{H_2O} = \frac{P'_V}{P_B} \tag{6}$$

$$\dot{M}_{H_2O} = \left\{ \dot{V}_A' \left[1 + \frac{\left(\frac{P_V}{P_B} - \frac{P_V'}{P_B} \right)}{\left(\frac{P_B}{P_B} - \frac{P_V}{P_B} \right)} \right] + (\dot{V}_{O_2} - \dot{V}_{CO_2}) \left(\frac{\left(\frac{P_V}{P_B} \right)}{\left(\frac{P_B - P_V}{P_B} \right)} - 1 \right) \right\} \rho_V - \dot{V}_A' \rho_V' \quad (7)$$

$$= \dot{V}_A' \left(\left[1 + \frac{P_V - P_V'}{P_B - P_V} \right] \rho_V - \rho_V' \right) + (\dot{V}_{O_2} - \dot{V}_{CO_2}) \left(\frac{P_V}{P_B - P_V} - 1 \right) \rho_V$$

For non-buccopharyngeal evaporation, I assumed $\dot{V}_{O_2}$=0 and $\dot{V}_{CO_2}$=0, thereby simplifying Eqn. 7 as:

$$\dot{M}_{H_2O} = \dot{V}_A' \left(\left[1 + \frac{P_V - P_V'}{P_B - P_V} \right] \rho_V - \rho_V' \right) \quad (8)$$

For respirometric measurements, I used the following:

$$\dot{V}_{O_2} = \dot{V}_A' \left[F_{O_2}' - F_{O_2} \frac{(1 - F_{O_2}' - F_{CO_2}' - F_{H_2O}')}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})} \right] \quad (9)$$

$$\dot{V}_{CO_2} = \dot{V}_A' \left[F_{CO_2} \frac{(1 - F_{O_2}' - F_{CO_2}' - F_{H_2O}')}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})} - F_{CO_2}' \right] \quad (10)$$

The derivation of Eqns. 9 and 10 can be found elsewhere (Walsberg and Hoffman, 2006).

I calculated sampled water vapor pressure from the measured dewpoint, using the eighth-order polynomial of Flatau et al. (1992), and I calculated vapor density from vapor pressure using the Ideal Gas Law (Campbell and Norman 1998).

Analysis of Data

I used SAS (Version 9.1, SAS Institute, Cary, NC, USA) to perform all statistical tests. For Inca doves, the MIXED procedure was used to perform repeated-measures analyses of variance (RMANOVA) and Tukey-Kramer *post-hoc* comparisons. I chose the

MIXED procedure, because it allows for analysis of data with missing values, and because it is more robust than the GLM procedure with respect to violations of homoskedasticity. Non-buccopharyngeal evaporation (*NBE*), buccopharyngeal evaporation (*BE*), oxygen metabolism ($\dot{V}_{O_2}$), and carbon-dioxide metabolism ($\dot{V}_{CO_2}$) were separately defined as dependent variables. For each of these tests, the within-subjects factors were ambient temperature, humidity of the head-chamber influent, and cloacal patency. The same tests were performed for Eurasian quail, but humidity was not included as a within-subjects factor, because humidity was not adjusted in trials using quail. In all tests for both species, I specified the Compound Symmetry covariance structure, because it yielded the lowest values for both Akaike's Information Criterion and Schwartz' Bayesian Criterion.

Results

Inca doves

Table 4.1 provides means and standard errors for hygrometric and respirometric measurements, along with numbers of individuals on which measurements were made. Values for non-buccopharyngeal evaporation (*NBE*) and buccopharyngeal evaporation (*BE*) are plotted in Fig. 4.1. There was a significant effect of T_a on *BE* (F=15.30, P<0.0001) and on *NBE* (F=88.88, P<0.0001); but the effect of temperature on the two measures differed dramatically. Over the range of experimental ambient temperatures, *NBE* changed by 219.5 μg g^{-1} min^{-1} during dry, unsealed trials, 254.9 μg g^{-1} min^{-1} during wet, unsealed trials, 125.5 μg g^{-1} min^{-1} during dry, sealed trials, and 146.8 μg g^{-1} min^{-1} during wet, sealed trials. These changes in *NBE* represent increases by 235.3%, 279.6%, 83.3%, and 127.6%, respectively. The large differences in these percentages between unsealed trials and sealed trials reflect the magnitude of cloacal evaporation, which is a component of *NBE*. In contrast, the corresponding changes in *BE* were much smaller (dry, unsealed trials: 27.5 μg g^{-1} min^{-1} change, 43.5% increase; dry, sealed trials: 58.3 μg g^{-1} min^{-1} change, 91.3% increase).

The overall fixed effect of cloacal patency was not significant for either *BE* (F=3.16, P=0.0988) or *NBE* (F=3.09, P=0.1020). However, there was a significant interaction between cloacal patency and ambient temperature (F=6.09, P=0.0052), and *post-hoc* analysis revealed that cloacal patency significantly affected *NBE* at T_a=42°C (t=-4.29, adjusted P=0.0091). This is clearly indicated in Fig. 4.1, in which the values for sealed trials diverge from those for unsealed trials at T_a=42°C. All other interactions

(temperature × patency for *BE* and *NBE*; humidity × patency, humidity × temperature, and humidity × temperature × patency for *NBE*) were non-significant. The overall effect of humidity on *NBE* was significant (F=5.61, P=0.0308). However, *post-hoc* tests could not identify a significant effect at any fixed level of temperature or patency. This is illustrated in Fig. 4.1, in which values for wet trials appear only marginally greater than values for dry trials.

Cloacal evaporation (*CloE*) was negligible at $T_a \leq 40$°C. However, at T_a=42°C, mean values for *CloE* were 91.3 μg g^{-1} min^{-1} during dry trials and 85.0 μg g^{-1} min^{-1} during wet trials. These values are similar to mean *BE* at T_a=42°C during dry trials (90.7 μg g^{-1} min^{-1}) and slightly less than half of mean cutaneous evaporation (*CutE*, 222.4 μg g^{-1} min^{-1} during dry trials, 256.6 μg g^{-1} min^{-1} during wet trials). That is, for trials at 42°C, total evaporation was apportioned as 25.4% buccopharyngeal, 21.2% cloacal, and 53.4% cutaneous (Fig. 4.2). This indicates that cloacal evaporation was thermoregulatorily important at the highest experimental temperature, on a par with buccopharyngeal evaporation. The heat liberated by cloacal evaporation at T_a=42°C averaged 3.7 mW g^{-1}, or 27.6% of mean metabolic heat (13.4 mW g^{-1}) at that temperature.

I separately calculated the volumetric rate (μl g^{-1} min^{-1}) of *BE*, so I could relate buccopharyngeal evaporation to oxygen metabolism as the dimensionless evaporespiratory ratio, $BE : \dot{V}_{O_2}$. A temperature-dependent change in this ratio indicates an uncoupling of the rate of buccopharyngeal evaporation from the rate of ventilation. This, in turn, can be partially caused by an attempt to increase evaporation from the rate that would occur just as a result of breathing. The evaporespiratory ratio increased with

ambient temperature more than threefold from 30° to 42°C (F=62.9, P<0.0001), and the ratio at each temperature differed significantly from that at all other temperatures (P≤0.0011 at all temperatures). This reflects the significant decrease in $\dot{V}_{O_2}$ as T_a increased from 30° to 35°C (t=8.69, adjusted P<0.0001) and the temperature-dependent increase in *BE*, along with the birds' use of panting or gular fluttering that I observed at the higher temperatures.

Eurasian quail

Table 4.1 provides means and standard errors for hygrometric and respirometric measurements, along with numbers of individuals on which measurements were made. There were no significant effects of treatment variables on *NBE* (T_a: F=0.01, P=0.9215; patency: F=3.57, P=0.1009; T_a × patency: F=0.02, P=0.8908). Similarly, *BE* did not change with treatment (T_a: F=1.16, P=0.3163; patency: F=0.36, P=0.5689; T_a × patency: F=1.79, P=0.2226), nor did the evaporespiratory ratio (T_a: F=3.77, P=0.0932; patency: F=0.15, P=0.7075; T_a × patency: F=1.00, P=0.3513). Despite frequent observations of panting, cloacal evaporation remained comparatively low, accounting for only 8.3% (T_a=30°C) and 6.4% (T_a=32°C) of total evaporation, and *CloE* did not change with T_a (F=0.01, P=0.9151). Evaporation from the cloaca was about one-fifth to one-third of *BE*, the latter of which accounted for 26.2% (T_a=30°C) and 35.4% (T_a=32°C) of total evaporation. Thus, the majority of evaporation from Eurasian quail was cutaneous (65.4% and 58.2% at 30° and 32°C, respectively). The relatively constant rate of cloacal evaporation liberated 330 μW g^{-1} of heat at T_a=30°C and 283 μW g^{-1} at T_a=32°C,

corresponding to presumably negligible portions (2.8% and 2.5%) of metabolic heat at the respective ambient temperatures.

Discussion

These results indicate for the first time that the rate of evaporation from the avian cloaca can be high enough to be important for thermoregulation, accounting for the loss of more than one quarter of metabolic heat at 42°C. Moreover, I have demonstrated that at least Inca doves are able to control the rate of cloacal evaporation, greatly increasing evaporative heat loss at high ambient temperatures, while virtually preventing cloacal evaporation at lower temperatures. The results of the Inca dove study show that, at 42°C, as much water can be evaporated from the cloacal epithelium as from the buccal epithelium. Yet, buccopharyngeal evaporation has always been recognized as being important for thermoregulation, while cloacal evaporation has always been assumed to be negligible. I view these results as the foundation of a major revision of our knowledge of hydric and thermal relations in birds.

The earliest accepted standard view of avian evaporation was driven by the anatomical discovery that birds do not possess sweat glands; workers therefore assumed that a lack of sweat glands indicated a corresponding lack of evaporation from the avian integument, and that effectively all of the water lost evaporatively from a bird's body was lost from its mouth (Bartholomew and Cade, 1963; Bartholomew and Dawson, 1953; Bartholomew et al., 1962; Cowles and Dawson, 1951; Schmidt-Nielsen et al., 1969; Calder and Schmidt-Nielsen, 1966; Lasiewski and Dawson, 1964). This assumption was challenged by subsequent studies in which separate hygrometric measurements were made from the head and from the rest of the body (Bernstein, 1971a; Bernstein, 1971b; Smith and Suthers, 1969; Lasiewski et al., 1971; Lee and Schmidt-Nielsen, 1971; Marder

and Ben-Asher, 1983; Taylor et al. 1971). These newer results threw into question the original assumption of negligible evaporation from the skin of birds, and they prompted microanatomical investigations (Arieli et al. 1999; Menon et al., 1989; Menon et al., 1986; Menon et al., 1996) that revealed major differences between mammalian and avian epidermis, helping to explain the observed rates of cutaneous evaporation in the absence of sweating. Yet with cutaneous evaporation having been clearly established as occurring in birds, researchers continued to assume that evaporation from the cloaca was negligible (Marder and Ben-Asher, 1983; Marder, 1983; Crawford and Lasiewski, 1968). That is, any measurement of avian evaporation that was not occurring from the mouth was assumed to be a measurement of cutaneous evaporation. Our results demonstrate that non-buccopharyngeal evaporation in birds can be subdivided into cutaneous and cloacal components, and that avian evaporation should now be considered on a tripartite basis.

Rates of cloacal evaporation in Eurasian quail and Inca doves differed markedly. Unfortunately, quail became thermally stressed in the test chamber at ambient temperatures much lower than I anticipated, and I was forced to restrict my measurements to two, relatively low and closely spaced temperatures. This did not afford me the experimental resolution necessary for determining whether these birds make any thermally driven adjustment to the rate of cloacal evaporation. Nevertheless, two interesting findings emerge. First, evaporation is dominated just as strongly by the cutaneous route in Eurasian quail as it is in Inca doves, despite the fact that the Eurasian quail is a non-columbiform bird. Second, cloacal evaporation accounts for about 7% of total evaporation in Eurasian quail. This fraction is lower than the cloacal fraction

observed in Inca doves, despite the large anatomical difference between the cloacae of these species. The Eurasian quail has a cloaca appearing as a semilunar slit, the orifice of which is much larger in relation to the body than that of the small, circular sphincter occurring in the Inca dove.

For Inca doves at all four experimental temperatures, the majority of total evaporation was non-buccopharyngeal, ranging from 58.9% of total evaporation at 30°C to 76.8% at 42°C. Below 42°C, virtually all of the non-buccopharyngeal evaporation was cutaneous. However, at 42°C, cloacal evaporation accounted for over one-quarter of non-buccopharyngeal evaporation and over one-fifth of total evaporation. These results suggest that Inca doves could employ a three-stage approach toward evaporative thermoregulation. At lower temperatures, at which breathing might rid the body of sufficient heat for thermostasis, cutaneous evaporation is minimized and cloacal evaporation is virtually eliminated by constricting the cloacal sphincter. As temperature increases beyond a point at which buccopharyngeal evaporation is inadequate, cutaneous evaporation is increased to make up for the thermoregulatory deficit. At still higher temperatures, when evaporation by panting and from the skin might be maximized, the cloacal epithelium is exposed to provide for increased latitude with respect to the range of survivable microenvironments.

Hoffman and Walsberg (1999) previously showed that another columbiform, the mourning dove, is able to make temperature-dependent adjustments to rates of non-buccopharyngeal evaporation, and that those adjustments are larger than any that could be explained passively, or simply on the basis of a change in skin-surface temperature.

Because that experiment did not discriminate between cloacal and cutaneous evaporation, it is uncertain how much of the observed change in non-buccopharyngeal evaporation resulted from a change in cutaneous evaporation. The present study of Inca doves is intriguing in light of those earlier results for mourning doves, because suppression of buccopharyngeal evaporation in Inca doves did not significantly increase cutaneous evaporation at any individual temperature, though cutaneous evaporation increased greatly with increasing temperature. Whether mourning doves possess a greater capacity than Inca doves for adjusting rates of cutaneous evaporation or whether the adjustment of evaporation in mourning doves was largely due to adjustment of cloacal evaporation remains to be tested.

It is interesting to note that the direction (and, to a lesser extent, the magnitude) of the response of cloacal evaporation to increase in ambient temperature is similar in Inca doves and Gila monsters (DeNardo et al., 2004), the two species for which cloacal evaporation has been demonstrated at magnitudes sufficient for thermoregulation. Both of these species are able to tolerate very high temperatures, and in both of these species cloacal evaporation remains negligibly low until a critically high ambient temperature prompts a steep rise in cloacal evaporation. This is in keeping with the notion that cloacal evaporation might be used by some animals as a last resort, when the only alternatives are an immediate change of microenvironment or a potentially life-threatening increase in body temperature.

These novel observations of avian cloacal evaporation raise several interesting questions. Perhaps most obvious is the question of how cloacal evaporation is controlled.

Apart from simply relaxing the cloacal sphincter, is the bird everting the cloaca? If so, then how much of the cloacal surface is exposed? Whether or not the cloaca is everted, the rate of evaporation therefrom could be altered by changes in such properties as the surface temperature and degree of perfusion of the cloacal epithelium. Independent of all of these factors, a rhythmic ventilation of the cloaca could increase the rate of evaporation, as could postural adjustments that take advantage of the convective air currents to which the bird is exposed.

A second set of important questions raised by these findings involves possible tradeoffs that might occur. Traditionally, the cloaca has been viewed as a fairly simple repository for excretory, digestive, and reproductive products. Given its additional function of serving as an evaporative organ, perhaps the cloaca will prove to possess unforeseen complexities. Since avian urine can undergo postrenal processing, how might the resorption of water into the hindgut interfere with cloacal evaporation, and how quickly can changes be made to these seemingly competing processes? Similarly, how might the demands for cloacal evaporation affect (and be affected by) the digestive and reproductive functions of the cloaca?

Indeed, because such high rates of cloacal evaporation have now been observed in Inca doves and Gila monsters, most of these questions apply to both birds and reptiles. Further refinement of measurement techniques and testing of other taxa will provide much needed insight.

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Table 4.1. *Hygrometric and respirometric measurements*

Species	T_a	Humidity	Cloacal Patency	*BE* (μg g^{-1} min^{-1})	*NBE* (μg g^{-1} min^{-1})	*CutE* (μg g^{-1} min^{-1})	*CloE** (μg g^{-1} min^{-1})	$\dot{V}_{O_2}$ (μl g^{-1} min^{-1})	$\dot{V}_{CO_2}$ (μl g^{-1} min^{-1})	$BE:\dot{V}_{O_2}$
Columbina inca Lesson	30	Dry	Unsealed	63.2 ±6.2 (10)	93.3±10.6 (10)	120.3±19.3	-27.1±17.7	65.3±3.9 (10)	61.8±4.0 (10)	1.72±0.07 (10)
			Sealed	63.9±3.4 (10)	120.3±19.3 (10)	(10)	(10)	71.0±4.1 (9)	66.4±3.2 (9)	1.64±0.11 (9)
		Wet	Unsealed	n/a	91.1±8.6 (10)	113.0±18.4	-21.84±15.0	62.8±3.2 (10)	55.8±2.7 (10)	n/a
			Sealed	n/a	113.0±18.4 (10)	(10)	(10)	63.6±3.2 (9)	55.4±2.9 (9)	n/a
	35	Dry	Unsealed	69.9±7.0 (13)	117.3±18.8 (13)	90.8±38.1	7.3±35.6	43.1±3.1 (12)	45.1±3.4 (12)	3.05±0.20 (12)
			Sealed	73.1±6.6 (9)	95.1±33.9 (9)	(8)	(8)	44.3±3.5 (9)	41.7±3.0 (9)	3.03±0.24 (9)
		Wet	Unsealed	n/a	113.2±15.8 (13)	108.8±42.7	-8.2±33.8	42.1±3.0 (12)	42.0±2.9 (12)	n/a
			Sealed	n/a	110.3±37.6 (9)	(8)	(8)	43.8±3.6 (9)	40.9±3.4 (9)	n/a
	40	Dry	Unsealed	83.6±5.4 (10)	201.2±25.3 (10)	194.3±21.5	7.9±27.7	41.9±4.5 (10)	38.8±3.5 (10)	4.48±0.42 (10)
			Sealed	83.4±5.8 (11)	189.3±18.3 (11)	(9)	(9)	37.5±2.0 (11)	35.7±1.6 (11)	4.53±0.23 (11)
		Wet	Unsealed	n/a	236.6±26.9 (10)	242.2±25.9	-6.9±30.0	47.1±4.1 (10)	40.9±3.6 (10)	n/a
			Sealed	n/a	256.7±24.0 (11)	(9)	(9)	45.4±3.6 (11)	41.0±3.3 (11)	n/a
	42	Dry	Unsealed	90.7±5.1 (8)	312.8±28.5 (8)	222.4±33.4	91.3±28.4	38.9±3.1 (8)	36.5±2.5 (8)	5.44±0.41 (8)
			Sealed	122.1±13.0 (9)	220.6±26.1 (9)	(7)	(7)	38.1±3.7 (9)	35.9±3.0 (9)	6.25±0.71 (9)
		Wet	Unsealed	n/a	346.0±38.3 (8)	256.6±23.9	85.0±34.6	48.0±3.4 (8)	45.0±3.7 (8)	n/a
			Sealed	n/a	257.1±19.7 (9)	(7)	(7)	45.2±5.3 (9)	42.8±6.7 (9)	n/a
Coturnix coturnix Linnaeus	30	Dry	Unsealed	18.7±1.4 (8)	56.9±7.4 (8)	48.7±4.7	8.1±5.8	27.0±2.4 (8)	20.9±1.3 (8)	1.39±0.17 (8)
		Dry	Sealed	33.2±1.4 (8)	48.7±4.7 (8)	(8)	(8)	33.4±3.7 (8)	24.4±2.7 (8)	1.55±0.20 (8)
	32	Dry	Unsealed	36.8±12.0 (8)	55.9±5.6 (8)	48.9±4.5	7.0±5.5	27.4±2.8 (8)	21.4±1.5 (8)	2.17±0.46 (8)
		Dry	Sealed	31.3±4.9 (8)	48.9±4.5 (8)	(8)	(8)	29.2±2.4 (8)	21.7±1.7 (8)	1.80±0.22 (8)

Values shown are means ± S.E.M.
Numbers in parentheses indicate numbers of individuals used in analyses.
Symbols are described in Table 4.2.
*Calculation of *CloE* can yield negative values when *CloE* is negligible, because of extensive overlap of variances in *NBE* means for unsealed and sealed trials.

Table 4.2. *Key to symbols*

BE	Buccopharyngeal evaporation
$CloE$	Cloacal evaporation
$CutE$	Cutaneous evaporation
F_X'	Fractional content of Gas X in influent
F_X	Fractional content of Gas X in effluent
$\dot{M}_{H_2O}$	Mass rate of water evaporation
NBE	Non-buccopharyngeal evaporation
P_B	Barometric pressure
P_V'	Water-vapor pressure of influent
P_V	Water-vapor pressure of effluent
T_a	Ambient temperature
$\dot{V}_A'$	Volumetric flux of influent air
$\dot{V}_A$	Volumetric flux of effluent air
$\dot{V}_{O_2}$	Volumetric rate of oxygen metabolism
$\dot{V}_{CO_2}$	Volumetric rate of carbon-dioxide metabolism
ρ_V'	Water-vapor density of influent
ρ_V	Water-vapor density of effluent

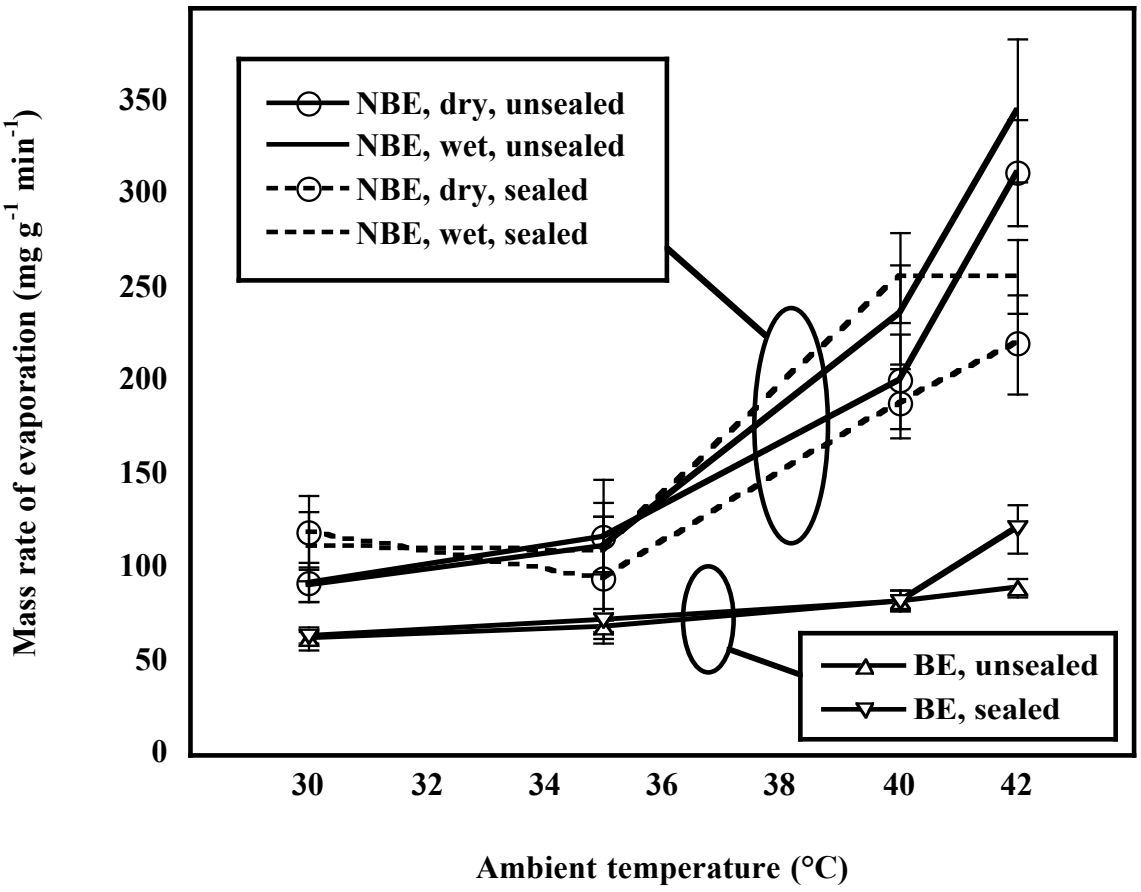

Fig. 4.1. Rates of evaporation measured in Inca doves at four ambient temperatures. During 'Sealed' trials cloacae were occluded with cyanoacrylic glue; during 'Unsealed' trials cloacae were not occluded. Relative humidity of the head-compartment influent was near 0% during 'Dry' trials and near 100% during 'Wet' trials. The differences between non-buccopharyngeal traces for 'Unsealed' and 'Sealed' trials indicate rates of cloacal evaporation. Those differences (and therefore the rates of cloacal evaporation) were negligible at 40°C and significant at 42°C. The differences between traces for 'Dry' and 'Wet' trials indicate compensatory adjustment of cutaneous evaporation; the differences were non-significant at all four ambient temperatures. Values shown are means ± S.E.M.

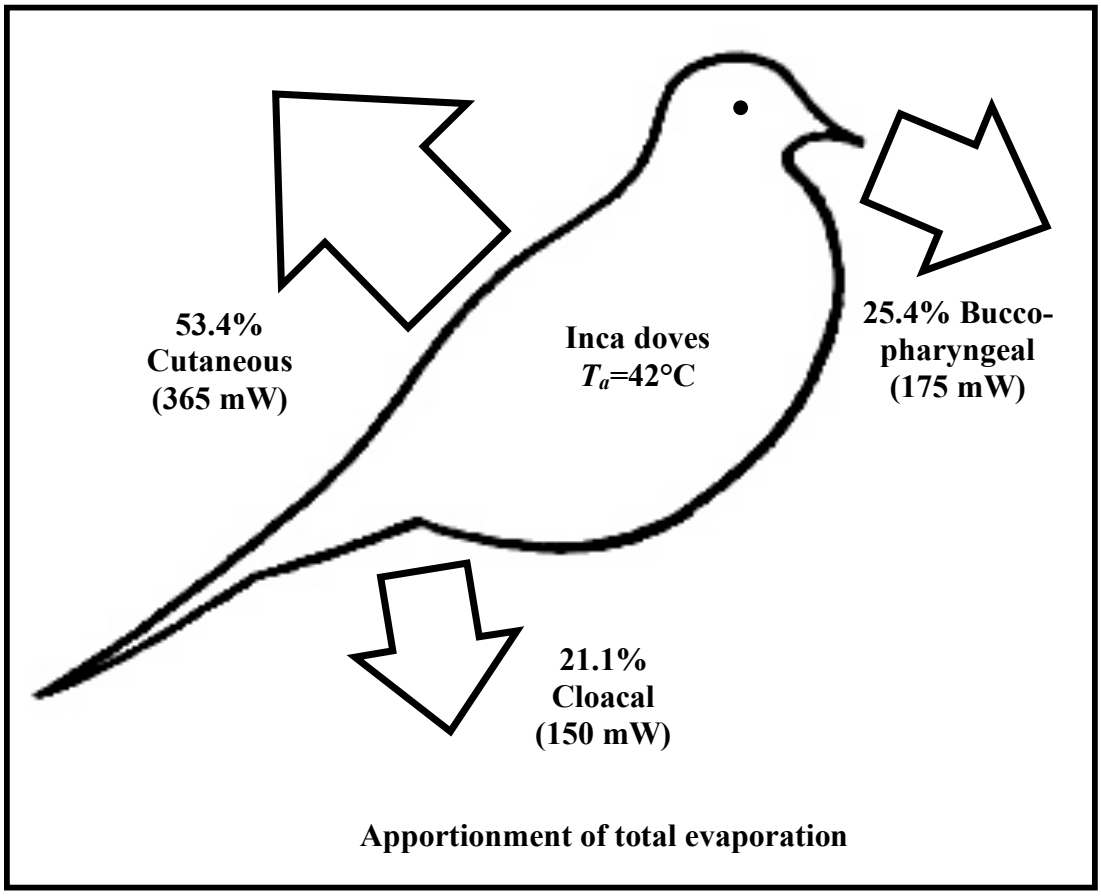

Fig. 4.2. Average apportionment of total evaporation in Inca doves at 42°C. Buccopharyngeal and non-buccopharyngeal evaporation were directly and separately measured. Cutaneous evaporation was defined as the whole of non-buccopharyngeal evaporation during 'Sealed' trials, in which cloacae were occluded. Cloacal evaporation was calculated as non-buccopharyngeal evaporation during 'Unsealed' trials minus non-buccopharyngeal evaporation during 'Sealed' trials. Parenthetic values indicate average rates of evaporative heat loss.

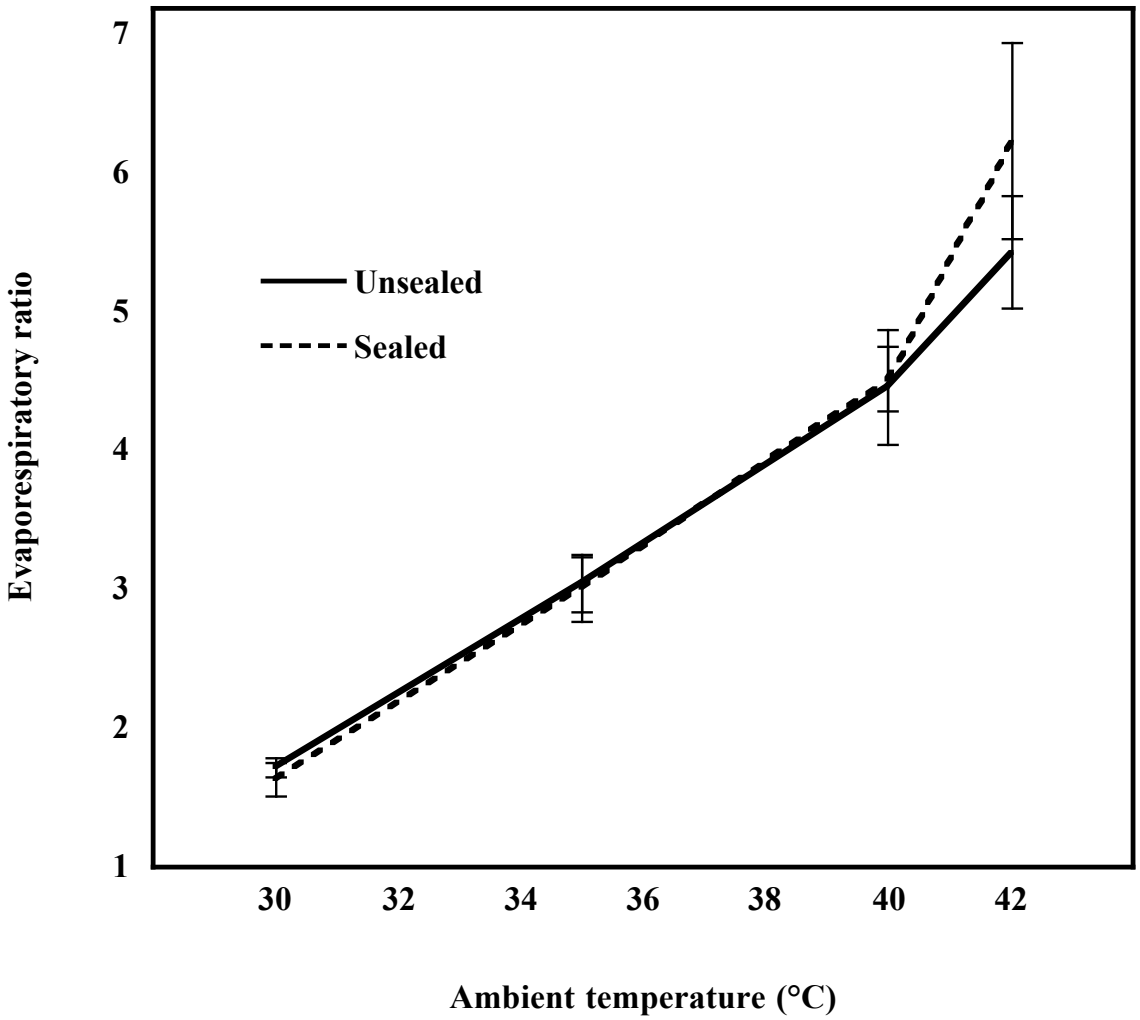

Fig. 4.3. The ratio of volumetric rate of buccopharyngeal evaporation to volumetric rate of oxygen metabolism in Inca doves at four ambient temperatures. This evaporespiratory ratio was nearly quadrupled as ambient temperature increased from 30° to 42°C, indicating that birds were elevating buccopharyngeal evaporation above rates that would occur just as a result of breathing. There is no statistical difference between traces for 'Unsealed' and 'Sealed' trials. Values shown are means ± S.E.M.

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Apportionment of Whole-body Evaporation among its Buccopharyngeal, Cutaneous, and Cloacal Components in the Ball Python (*Python regius*)

Summary

Most studies of evaporation from reptiles have focused on the detrimental loss of water that evaporation causes, ignoring potential benefits of evaporative cooling. Recently, however, cloacal evaporation from the desert-dwelling Gila monster (*Heloderma suspectum* Cope) was shown to provide thermoregulatory benefits at high temperatures. It is unknown whether Gila monsters are unusual in this respect, or whether cloacal evaporation is universally available to reptiles as a thermoregulatory mechanism. To address that uncertainty, I measured evaporation from the ball python (*Python regius* Shaw), a tropical species for which thermal benefits of cloacal evaporation are less likely to be important than for an arid-adapted species. I partitioned evaporation into buccopharyngeal and non-buccopharyngeal components at 25, 40, and 42°C, and into buccopharyngeal, cutaneous, and cloacal components at 42°C, because cloacal evaporation, if it does occur, should be maximized at the highest tolerated temperatures. Ball pythons evaporated virtually no water from their cloacae, demonstrating that cloacal evaporation is not a universal response to thermal stress among reptiles. Though non-buccopharyngeal evaporation accounted for the majority (66%) of evaporation at 25°C, this fraction is lower than that in other snake species not adapted to arid conditions. At 42°C, buccopharyngeal evaporation predominated, accounting for 57% of total.

Introduction

Over the last six decades, there have been several studies on the rates of evaporation from reptiles of various taxa. These have ranged from in vitro measurements of skin resistance (Agugliaro and Reinert, 2005) to whole-body hygrometry (Gans et al., 1968; Neilson, 2002; Walsberg and Hoffman, 2006) to experiments in which evaporation from the mouth was measured separately from evaporation occurring elsewhere (Bentley and Schmidt-Nielsen, 1966; Bennett and Licht, 1975; Davis et al., 1980; Eynan and Dmi'el, 1993; Lahav and Dmi'el, 1996; Dmi'el, 1998). In nearly all of these studies, evaporation of water vapor from a reptile's body was viewed simply as a detrimental but inevitable loss of body-water. All reptiles must ventilate the lungs, and the convection of air across the buccal and pharyngeal epithelia must entail the evaporative loss of some amount of water in the expired breath (Spotila and Berman, 1976). Similarly, because the reptilian integument is an imperfect barrier against transcutaneous movement of water (Lillywhite, 2006), some amount of vapor is inevitably lost to the surrounding air. This classic view is reflected in the frequent use of the phrase ‘evaporative water loss’ to describe reptilian evaporation (Bennett and Licht, 1975; Davis et al., 1980; Mautz, 1980; Dunson and Bramham, 1981; Kobayashi et al., 1983; Perry et al., 2000; Dmi'el, 2001), ignoring the always thermal - and potentially thermoregulatory - implications of such evaporation. In addition to eschewing consideration of the thermal aspects of evaporation, nearly all past studies of reptilian evaporation have ignored the cloaca as a site of potentially elevated evaporative flux.

To date, cloacal evaporation has been measured in only one reptile species, the Gila monster, *Heloderma suspectum* Cope (DeNardo et al., 2004). That study demonstrated that the Gila monster is able to use the cloaca as an evaporating organ when exposed to challengingly high ambient temperatures. Moreover, the increase in evaporative flux from that lizard's cloaca was such that cloacal evaporation came to vastly predominate at 40°C ambient temperature, accounting for 82% of total evaporation and causing a significant suppression of body temperature (DeNardo et al., 2004).

The startling revelation of thermoregulatory rates of cloacal evaporation in the Gila monster prompts the question of whether cloacal evaporation is a universal feature of reptiles. Here, I attempt to answer that question by conducting the second study to employ triple partitioning of reptilian evaporation. I present rates of evaporative flux in an equatorial African snake, the ball python, *Python regius* Shaw, with total evaporation partitioned into buccopharyngeal, cutaneous, and cloacal components. In addition, I provide respirometric results obtained simultaneously with hygrometric measurements.

Studying ball pythons provides for an excellent contrast with Gila monsters. Most obvious is the taxonomic difference: Gila monsters are lizards (Suborder Lacertilia); ball pythons are snakes (Suborder Serpentes). Also, whereas Gila monsters inhabit hot, arid environments (Beck, 1990; Beck and Jennings, 2003; Gienger and Tracy, 2003; Sullivan et al., 2004), ball pythons are found in tropical grasslands or forests (Kreger and Mench, 1993; Aubret et al., 2003) and are not adapted to arid conditions. I predicted, therefore, that evaporation is relatively unimportant for thermoregulation in ball pythons, as compared to Gila monsters. But if, rather than being a thermoregulatory mechanism,

cloacal evaporation in Gila monsters is just an incidental result of exposure to high test temperatures, then ball pythons should be just as likely to exhibit substantial cloacal evaporation when thermally stressed.

Materials and Methods

Animals

I used 11 captive-bred, adult ball pythons (8 females, 3 males; mean mass of 719 ± 52 g) randomly selected from the collection of D. F. DeNardo. Snakes were housed in individual cages at Arizona State University in Tempe, Arizona, USA. All work was approved by the Arizona State University Institutional Animal Care and Use Committee. Cages remained in a room maintained at 25°C on a 12 h:12 h L:D artificial photoperiod, and each cage featured a subsurface heating element (Flexwatt, Flexwatt Corp., West Wareham, MA, USA) at one end of the cage that provided the snake with a thigmothermic gradient. Each snake was fasted for at least 14 days prior to any experimental trial. Water was provided *ad libitum*. To avoid any confounding effects on cutaneous evaporation, snakes were not used in trials if they showed signs of imminent ecdysis or if they had shed in the previous 7 days. External loops of polypropylene monofilament (Prolene, Ethicon, Somerville, NJ, USA) were sutured on the sides of each python's neck to provide a means for attaching a mask (see below).

Respirohygrometry

Pythons were tested in an open-flow system that included a circular, cylindrical test chamber (19 cm dia., 18 cm height, 5.1 L) constructed of borosilicate glass, with a plate glass floor and an aluminum lid. Air entered and exited the test chamber *via* threaded, borosilicate ports (Chem-Thread, Chemglass, Vineland, NJ, USA) that accepted minimally hygroscopic tubing (Bev-A-Line, Thermoplastic Processes, Inc., Stirling, NJ,

USA). Water vapor and carbon dioxide were removed from the influent by an industrial air purifier (#PCDA11129022, Puregas, Denver, CO, USA), and positive-pressure flux of dry, acapnic air into the test chamber was maintained (ca. 1800 - 2300 ml min^{-1}, depending on snake mass) by a mass flow controller (#FMA-A2409, Omega Engineering, Stamford, CT, USA). A second mass flow controller (Omega Engineering #FMA-A2406) was interposed between the test chamber and an air pump to maintain negative-pressure flux (range of ca. 280 - 1100 ml min-1 over all trials) through a second efflux port connected in the chamber's interior to an air swivel. Pliable tubing inside the test chamber connected the swivel to a cylindrical, polycarbonate mask that could be attached to the polypropylene loops on the snake's neck, holding the mask in place. The two distinct effluents (one from the overall test chamber, one from the mask) were delivered to separate dewpoint hygrometers (#RH100, Sable Systems, Las Vegas, NV, USA). After exiting the hygrometer, the mask effluent was dried by a column of anhydrous calcium sulfate and then delivered to a carbon dioxide analyzer (#LI-6252, Li-Cor, Lincoln, NE, USA) and an oxygen analyzer (Sable Systems #FC-1B). Air temperature (T_a) in the test chamber was measured by a copper-constantan (type T) thermocouple. Prevailing barometric pressure (P_B) was measured by an electronic manometer.

The mass flow controllers were calibrated for dry, acapnic air, using soap-film flow meters. The hygrometers were calibrated for dewpoint (because it is independent of air temperature) by water-saturating air at various temperatures by bubbling it through three, serially arranged columns of water (each ca. 100 cm deep) maintained at each of

the calibration temperatures. The carbon dioxide analyzer was calibrated using bottled gases of known concentrations. The oxygen analyzer was calibrated using atmospheric air. The manometer was calibrated against a mercury-standard barometer. All calibrations were adjusted to provide STP values.

To ensure that the pump removed air through the mask at a rate sufficient to collect all of the snake's breath, I occasionally directed the effluent from the overall chamber to the carbon dioxide and oxygen analyzers. Negligible differences between influent and effluent indicated that no breath was escaping the mask.

Outputs from all sensors were connected to an electronic datalogger (#CR23X, Campbell Scientific, Logan, UT, USA), which sampled values at 1 Hz and recorded average values once per minute. The period of 99% equilibration of gases (Lasiewski et al., 1966) in the test chamber depended on the influx and ranged from 10 to 13 minutes. The corresponding equilibration period for the mask ranged from 15 seconds to 1 minute. However, because the influent to the mask was air from the test chamber, which varied in composition, equilibration periods for both the chamber and the mask were taken to be 10 to 13 minutes.

Experimental Protocol

For each individual, I conducted trials at three ambient temperatures: 25, 40, and 42°C. I chose these temperatures, because 25°C represented a thermally neutral temperature, while 40°C represented a thermally challenging temperature at which the snakes reliably remained calm, and 42°C represented an extreme thermal challenge that

induced periodic episodes of distress (e.g. escape behavior). At each of these temperatures, I conducted 'unsealed trials', in which the snake was placed into the test chamber and fitted with the mask, whereupon I separately measured buccopharyngeal evaporation (*BE*) and non-buccopharyngeal evaporation (*NBE*). The latter of those measures is the sum of cutaneous evaporation (*CutE*) and cloacal evaporation (*CloE*). In addition, I measured consumption of oxygen ($\dot{V}_{O_2}$) and production of carbon dioxide ($\dot{V}_{CO_2}$). At 42°C, each individual underwent an additional trial ('sealed trials') in which the cloacal vent was sealed with cyanoacrylic glue immediately prior to placement of the snake into the test chamber. Hygrometry of the overall (i.e. non-mask) effluent represented direct measurement of *NBE* in all unsealed trials. However, during trials in which the cloaca was sealed, hygrometry of the overall effluent represented direct measurement of *CutE*. I then calculated *CloE* as the difference between *NBE* (as measured during unsealed trials) and *CutE* (as measured during sealed trials). At the conclusion of sealed trials, I verified that the cloacal seal had not broken, and I removed the seal with acetone. I chose to measure *CloE* only at 42°C, because *CloE* is most likely to approach its maximum at thermally challenging temperatures.

All trials were conducted in darkness during daylight hours, and I used remote, infrared surveillance to ensure that snakes did not become strenuously active. Six trials were aborted because of incorrigibility, and those data were discarded. Trials lasted 90 to 120 minutes, and data from the last 10 minutes of each trial were used in analyses. Computing means in this manner standardized the measurements with respect to both the smoothing of any transient changes and the time of exposure of the snakes to the

experimental conditions. No individual was used for more than one trial on any single day.

Calculations and Analysis of Data

Measured dewpoints were used to calculate vapor pressures (P_V) using the eighth-order polynomial of Flatau et al. (1992), and vapor densities (ρ_V) were calculated from vapor pressures using the Ideal Gas Law (Campbell and Norman, 1998). I used the following equations (Hoffman et al., 2006) to calculate rates of metabolism:

$$\dot{V}_{O_2} = \dot{V}'_A \left[F'_{O_2} - F_{O_2} \frac{(1 - F'_{O_2} - F'_{CO_2} - F'_{H_2O})}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})} \right] \tag{1}$$

$$\dot{V}_{CO_2} = \dot{V}'_A \left[F_{CO_2} \frac{(1 - F'_{O_2} - F'_{CO_2} - F'_{H_2O})}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})} - F'_{CO_2} \right] \tag{2}$$

Buccopharyngeal rates of evaporation were calculated (Hoffman et al., 2006) as:

$$\begin{aligned} \dot{M}_{H_2O} &= \left\{ \dot{V}'_A \left[1 + \frac{\left(\frac{P_V}{P_B} - \frac{P'_V}{P_B} \right)}{\left(\frac{P_B}{P_B} - \frac{P_V}{P_B} \right)} \right] + (\dot{V}_{O_2} - \dot{V}_{CO_2}) \left(\frac{\left(\frac{P_V}{P_B} \right)}{\left(\frac{P_B - P_V}{P_B} \right)} - 1 \right) \right\} \rho_V - \dot{V}'_A \rho'_V \\ &= \dot{V}'_A \left(\left[1 + \frac{P_V - P'_V}{P_B - P_V} \right] \rho_V - \rho'_V \right) + (\dot{V}_{O_2} - \dot{V}_{CO_2}) \left(\frac{P_V}{P_B - P_V} - 1 \right) \rho_V \end{aligned} \tag{3}$$

For non-buccopharyngeal evaporation, I assumed $\dot{V}_{O_2}$=0 and $\dot{V}_{CO_2}$=0, thereby simplifying Equation. 3 as:

$$\dot{M}_{H_2O} = \dot{V}'_A \left(\left[1 + \frac{P_V - P'_V}{P_B - P_V} \right] \rho_V - \rho'_V \right) \tag{4}$$

A description of all variables is found in Table 5.1. I calculated Q_{10}, the coefficient of change in a measured rate in response to a 10°C change in temperature, as follows.

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (5)$$

Here, R denotes the measured rate, and T denotes centigrade temperature.

I used SAS (Version 9.1, SAS Institute, Cary, NC, USA) to perform all statistical tests. I used the MIXED procedure to perform repeated-measures analyses of variance (RMANOVA) and Tukey-Kramer *post-hoc* comparisons, because the MIXED procedure allows for analysis of data with missing values, and because it is more robust than the GLM procedure with respect to violations of homoskedasticity. Non-buccopharyngeal evaporation (*NBE*), buccopharyngeal evaporation (*BE*), oxygen consumption ($\dot{V}_{O_2}$), and carbon-dioxide production ($\dot{V}_{CO_2}$) were separately defined as dependent variables. For each of these tests, T_a was defined as a within-subjects factor. For trials conducted at 42°C, cloacal patency was defined as an additional within-subjects factor. In all tests, I specified the Compound Symmetry covariance structure, because it yielded the lowest values for both Akaike's Information Criterion and Schwartz' Bayesian Criterion.

Results

Snake mass ranged from 477 to 954 g. Because there are both advantages and disadvantages to expressing hygrometric and respirometric data as either whole-body values or mass-specific values, Table 5.2 provides both measures (along with results from respective statistical tests) for total evaporation (*TE*), non-buccopharyngeal evaporation (*NBE*), buccopharyngeal evaporation (*BE*), oxygen consumption ($\dot{V}_{O_2}$), and carbon dioxide production ($\dot{V}_{CO_2}$). Statistical tests on whole-body measures yielded qualitatively identical results to those for mass-specific measures. That is, there was no change in significance at the P=0.05 level for any test. I also present in Table 5.2 the respiratory exchange ratio (*RER*), evaporespiratory ratio ($BE:\dot{V}_{O_2}$), and numbers of individuals used in experimental trials.

The effect of T_a on rates of evaporation (*TE*, *NBE*, and *BE*) is shown in Fig. 5.1 for unsealed trials at three ambient temperatures. Total evaporative flux (μg g^{-1} h^{-1}) increased 104% as T_a rose from 25° to 42°C, with a 67% increase between 25° and 40°C and a 22% increase between 40° and 42°C (Table 5.2). The rate of the non-buccopharyngeal component (μg g^{-1} hr^{-1}) of total evaporation did not change significantly as T_a increased (Table 5.2). In contrast, there was a highly significant, temperature-dependent increase in *BE* (Table 5.2). Furthermore, *post-hoc* analysis revealed that both temperature increments had a significant effect on *BE* (Table 5.2), which increased 139% between 25° and 40°C and increased 41% between 40° and 42°C. The overall increase in *BE* between 25° and 42°C was 238%. The Q_{10} values corresponding to the increase in ambient temperature from 25° to 40°C were consistent with the ANOVA (*NBE*:

Q_{10}=1.19; *BE*: Q_{10}=1.79). Since *TE* is the sum of *NBE* and *BE*, the significant temperature dependence of *BE* was largely responsible for the overall significance of the temperature dependence observed for *TE* (Table 5.2). However, for *TE*, *post-hoc* tests indicated significance of temperature dependence only for the lower increment (Table 5.2; 25° to 40°C: Q_{10}=1.41). This is not surprising given the relatively small sample size and a temperature difference of only 2°C.

The combination of a relative thermal independence of *NBE* and a strong thermal dependence of *BE* resulted in a significant change in the non-buccopharyngeal percentage of total evaporation. Average values for the apportionment of *NBE* were 65.8±4.0% at 25°C, 51.2±2.1% at 40°C, and 43.2±2.2% at 42°C (Overall ANOVA: F=12.97, P=0.0004; 25° to 40°C: adjusted P=0.0141; 40° to 42°C: adjusted P=0.2512).

At T_a=42°C, cloacal evaporation (*CloE*, μg g^{-1} hr^{-1}) was calculated for each individual as the difference between *NBE* during the unsealed trial and *NBE* during the sealed trial, because *NBE* during a sealed trial is just cutaneous evaporation (*CutE*). There was no significant effect of cloacal patency at 42°C on *NBE* (F=0.32, P=0.5908). Similarly, sealing the cloaca did not affect *BE* at 42°C (F=0.32, P=0.5871). In the case of an appreciable rate of cloacal evaporation, non-buccopharyngeal evaporation will exceed cutaneous evaporation by a difference that indicates the magnitude of cloacal evaporation (Hoffman et al., 2006). In the present study, this did not occur. Cutaneous evaporation (i.e. *NBE* during sealed trials) measured 346±47 μg g^{-1} hr^{-1} at 42°C, an average slightly larger than - but well within the standard error of - that for *NBE* during unsealed trials at 42°C (Table 5.2). In other words, cloacal evaporation at 42°C was negligible.

The temperature dependence of both oxygen consumption ($\dot{V}_{O_2}$, μl g^{-1} hr^{-1}) and carbon dioxide production ($\dot{V}_{CO_2}$, μl g^{-1} hr^{-1}) is shown in Fig. 5.2 for unsealed trials at three ambient temperatures. The overall effect of temperature was significant for both measures (Table 5.2). Though the averages differed for $\dot{V}_{O_2}$ between 25° and 40°C, the increase was not shown by *post-hoc* analysis to be significant for this sample size (Table 5.2; 25° to 40°C: Q_{10}=1.48). However, as T_a was increased from 40° to 42°C, $\dot{V}_{O_2}$ underwent a significant, 91% increase (Table 5.2). Carbon dioxide production ($\dot{V}_{CO_2}$) qualitatively showed the same response to ambient temperature (Table 5.2; 25° to 40°C: Q_{10}=1.45). Neither of the two measures of metabolic rate were affected by cloacal patency at 42°C ($\dot{V}_{O_2}$: F=0.01, P=0.9214; $\dot{V}_{CO_2}$: F=0.00, P=0.9821). The similarity of temperature dependence between $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ resulted in *RER* being independent of ambient temperature, but *RER* measured unexpectedly high (McLean and Tobin, 1987, Blaxter, 1989, Walsberg and Hoffman, 2005) in all trials (i.e. averaging up to 1.01; Table 5.2).

The evaporespiratory ratio (BE:$\dot{V}_{CO_2}$) is a dimensionless measure when *BE* is expressed volumetrically, as I have done here (Table 5.2). Stasis of the evaporespiratory ratio can indicate a tight coupling between evaporation from the mouth and ventilation to meet metabolic demand. There was no significant change in BE:$\dot{V}_{CO_2}$ as temperature increased (Table 5.2).

Discussion

In stark contrast to Gila monsters, which were shown to employ cloacal evaporation to reduce the rate of increase in body temperature as air temperature was increased beyond a critical point (DeNardo et al., 2004), cloacal evaporation in ball pythons was negligible at 42°C. Nevertheless, ball pythons clearly were thermally stressed at that temperature. One individual had to be rescued by cooling it with water after its rate of gas exchange suddenly declined at the end of a trial, and all individuals exhibited escape behavior after having been in the test chamber for an hour or more at 42°C.

The lack of cloacal evaporation in ball pythons is not surprising considering that, in its natural habitat, this species is not expected to require supplemental evaporative cooling from the cloaca. Nevertheless, my results with respect to cloacal evaporation are interesting for a couple of reasons. First, because this is only the second study of cloacal evaporation in a reptile, I have demonstrated that cloacal evaporation, as seen in the Gila monster (DeNardo et al., 2004), is not a universal feature of reptiles. Ball pythons in this study were thermally stressed at 42°C, a temperature that they are extremely unlikely to encounter in nature. In addition, the test temperature of 42°C is 2°C higher than the maximum temperature to which Gila monsters were exposed and nearly 5°C higher than the temperature at which Gila monsters made the transition from negligible to substantial rates of cloacal evaporation (DeNardo et al., 2004). I believe that, if cloacal evaporative cooling were available as an adaptive response in ball pythons, surely it would be employed at my unnaturally high test temperature. Second, because ball pythons and Gila

monsters have anatomically similar cloacal vents, any opening of that vent that might occur simply as a result of activity in response to thermal stress would be expected to be similar in the two species. If, instead of cloacal evaporation being a thermoregulatory response, it were simply an artifact of thermally induced struggling that opens the cloacal vent, then ball pythons would be expected to exhibit at least measurable rates of cloacal evaporation, if not rates similar to those measured in Gila monsters. I therefore believe that my negative results bolster the notion that the transition from negligible to substantial rates of cloacal evaporation observed in Gila monsters (DeNardo et al., 2004) represents an adaptive, thermoregulatory response in that reptile.

Because cloacal evaporation was negligible at 42°C, effectively all of the non-buccopharyngeal evaporation in ball pythons was cutaneous evaporation at that temperature. Furthermore, because cloacal evaporation (if it occurs) should be maximized at thermally stressful temperatures, I assume that cloacal evaporation in ball pythons is negligible at all temperatures, and that ‘non-buccopharyngeal evaporation’ and ‘cutaneous evaporation’ are synonymous for this species.

Several studies have demonstrated that non-buccopharyngeal evaporation accounts for the majority of total evaporation in both lizards (Mautz, 1982; Eynan and Dmi'el, 1993; Dmi'el et al., 1997; Perry et al., 1999) and snakes (Dmi'el and Zilber, 1971; Dmi'el, 1972; Bennett and Licht, 1975; Dmi'el, 1985). The apportionment of non-buccopharyngeal evaporation is similar in the two taxa. Published values for lizards range from 27% (Dawson et al., 1966) to 87% (Bentley and Schmidt-Nielsen, 1966) of total evaporation. However, these extreme values were obtained before the advent of much

more accurate hygrometric methods, and most of the more recent studies report apportionment of *NBE* in lizards close to 75% (e.g. Eynan and Dmi'el, 1993; Dmi'el et al., 1997; Perry et al., 1999). Published values for snakes range from 61% (Dmi'el, 1998) to 88% (Prange and Schmidt-Nielsen, 1969). My measurements of *NBE* in ball pythons yielded apportionment values of 66% at 25°C, 51% at 40°C, and 43% at 42°C. The latter two values were driven low, because buccopharyngeal evaporation accounted for most of the total evaporation as increasing air temperature caused large increases in ventilation (Fig. 5.2). Also, my test temperatures of 40° and 42°C exceed those employed in published experiments, making the value of 66% *NBE* at 25°C the best suited for comparisons with other studies.

While the apportionment of *NBE* in ball pythons is similar to that measured in other snake species, it is perhaps lower than expected for a snake that is not adapted to arid habitats. The correlation between habitat aridity and *NBE* (or skin resistance, which constrains *NBE*) is well documented in reptiles (Bogert and Cowles, 1947; Bentley and Schmidt-Nielsen, 1966; Dawson et al., 1966; Prange and Schmidt-Nielsen, 1969; Mautz, 1982; Dmi'el, 1985). Species living in dry, hot environments show relatively lower rates of *NBE* than those living in moister and more temperate habitats. These lower rates of *NBE* then account for comparatively smaller fractions of total evaporation in arid adapted species. The tropical ball python, therefore, is expected to exhibit *NBE* apportionment values near the high end of the range observed. Instead, the ball python's apportionment of *NBE* is near the low end. Rather than interpreting this finding as evidence of a departure of the ball python from the norm, I suspect that my seemingly aberrant

observations reflect my choice to allow the pythons to coil while being tested rather than testing them in a tubular chamber that would have forced the snakes to remain elongated. Not surprisingly, the magnitude of *NBE* is affected by changes in the fraction of the total integumentary surface that is exposed to air (Cohen, 1975). In addition, the level of activity can partially determine the rate of *NBE* in reptiles (Gans et al., 1968). These complications underscore several difficulties that arise in hygrometry of animals. On the one hand there is the need to conduct hygrometric measurements in the laboratory, with its many artificial conditions, including stress of handling, exposure to unnaturally dry air, and restraint of the animal. On the other hand there is the bewildering variety of techniques employed in such laboratory experiments. The compounding of these problems doubtless clouds, at least to some degree, comparisons drawn between various studies.

The snakes used in this experiment were allowed to change posture during trials, and a mask (rather than a rigidly held septum) was used for partitioning of evaporation. I believe that both of these techniques reduced the level of stress experienced by the snakes, compared to the more frequently used technique of placing snakes in long cylinders, thereby disallowing them to coil and greatly restricting movement. While the postural dependence of non-buccopharyngeal evaporation probably resulted in an increase in the variances seen in my results, I believe that my experimental setup more closely reflected natural conditions.

The effect of air temperature on rates of gas exchange (Fig. 5.2) is fairly typical at and below 40°C. At these lower temperatures, ball pythons were nearly or completely

inactive, and the increase in metabolism between 25° and 40°C is likely attributable to the effect of an increase in ambient temperature (Q_{10}=1.45 to 1.48). Above 40°C, the steep rise in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Fig. 5.2) is better explained as the result of the escape behavior I observed.

To date, there are only two species that have been shown to employ cloacal evaporation as a thermostatic cooling mechanism. These are the Gila monster (DeNardo et al., 2004) and the Inca dove (Hoffman et al., 2006). I have previously demonstrated (Hoffman et al., 2006) that appreciable cloacal evaporation does not occur in all birds. The present study provides evidence that cloacal evaporation is not a universal feature of reptiles, either. These preliminary studies have sought to answer only the most basic questions regarding this previously unappreciated mechanism of heat exchange. Many questions remain. For instance, is the rate of cloacal evaporation regulated by adjusting the degree of exposure of the cloacal mucosa, by adjusting the rate of perfusion, or by some other mechanism? How do the excretory, digestive, and reproductive demands on the cloaca affect the ability to evaporate, and do tradeoffs occur as a result of potential conflicts between the cloaca's disparate functions? How does the rate of cloacal evaporation (or the temperature at its onset) correlate with such factors as phylogeny, habitat, body size, or acclimatization? Surely, the list of questions will expand as further work is completed.

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Table 5.1. *Key to symbols*

BE	Buccopharyngeal evaporation
$CloE$	Cloacal evaporation
$CutE$	Cutaneous evaporation
F'_X	Fractional content of Gas X in influent
F_X	Fractional content of Gas X in effluent
$\dot{M}_{H_2O}$	Mass rate of water evaporation
NBE	Non-buccopharyngeal evaporation
P_B	Barometric pressure
P'_V	Water-vapor pressure of influent
P_V	Water-vapor pressure of effluent
RER	Respiratory exchange ratio
T_a	Ambient temperature
$\dot{V}'_A$	Volumetric flux of influent air
$\dot{V}_A$	Volumetric flux of effluent air
$\dot{V}_{O_2}$	Volumetric rate of oxygen exchange
$\dot{V}_{CO_2}$	Volumetric rate of carbon-dioxide exchange
ρ'_V	Water-vapor density of influent
ρ_V	Water-vapor density of effluent

Table 5.2. *Evaporation and respiration in ball pythons*

	Mean ± S.E.M.			Overall ANOVA		Tukey-Kramer Adjusted *P*	
	25°C Trial (*N*=11)	40°C Trial (*N*=9)	42°C Trial (*N*=9)	*F*	*P*	25° to 40°C	40° to 42°C
Whole-body measures, unsealed trials							
TE (mg hr^{-1})	254±29	404±19	507±44	19.50	<0.0001	0.0054	0.0715
NBE (mg hr^{-1})	172±27	204±8.1	222±24	1.51	0.2499	0.5243	0.8380
BE (mg hr^{-1})	81.1±8.0	200±17	286±24	39.46	<0.0001	0.0003	0.0080
$\dot{V}_{O_2}$ (ml hr^{-1})	20.9±3.9	35.7±2.0	72.9±16	7.83	0.0042	0.5309	0.0432
$\dot{V}_{CO_2}$ (ml hr^{-1})	19.3±3.2	33.0±4.6	70.3±15	10.79	0.0011	0.4497	0.0152
RER	1.01±0.11	0.82±0.03	1.01±0.05	1.99	0.1692	0.2062	0.2377
$BE{:}\dot{V}_{O_2}$	6.47±0.69	8.52±0.72	7.60±1.0	1.60	0.2333	0.2104	0.7444
Mass-specific measures, unsealed trials							
TE (μg g^{-1} hr^{-1})	354±30	590±51	721±55	19.05	<0.0001	0.0035	0.1330
NBE (μg g^{-1} hr^{-1})	234±28	303±32	316±34	1.77	0.2023	0.3406	0.9651
BE (μg g^{-1} hr^{-1})	120±16	287±24	406±26	52.78	<0.0001	<0.0001	0.0025
$\dot{V}_{O_2}$ (μl g^{-1} hr^{-1})	29.5±4.7	52.9±5.9	101±20	8.98	0.0024	0.3872	0.0400
$\dot{V}_{CO_2}$ (μl g^{-1} hr^{-1})	26.8±3.7	46.6±4.8	98.8±19	12.86	0.0005	0.3745	0.0090

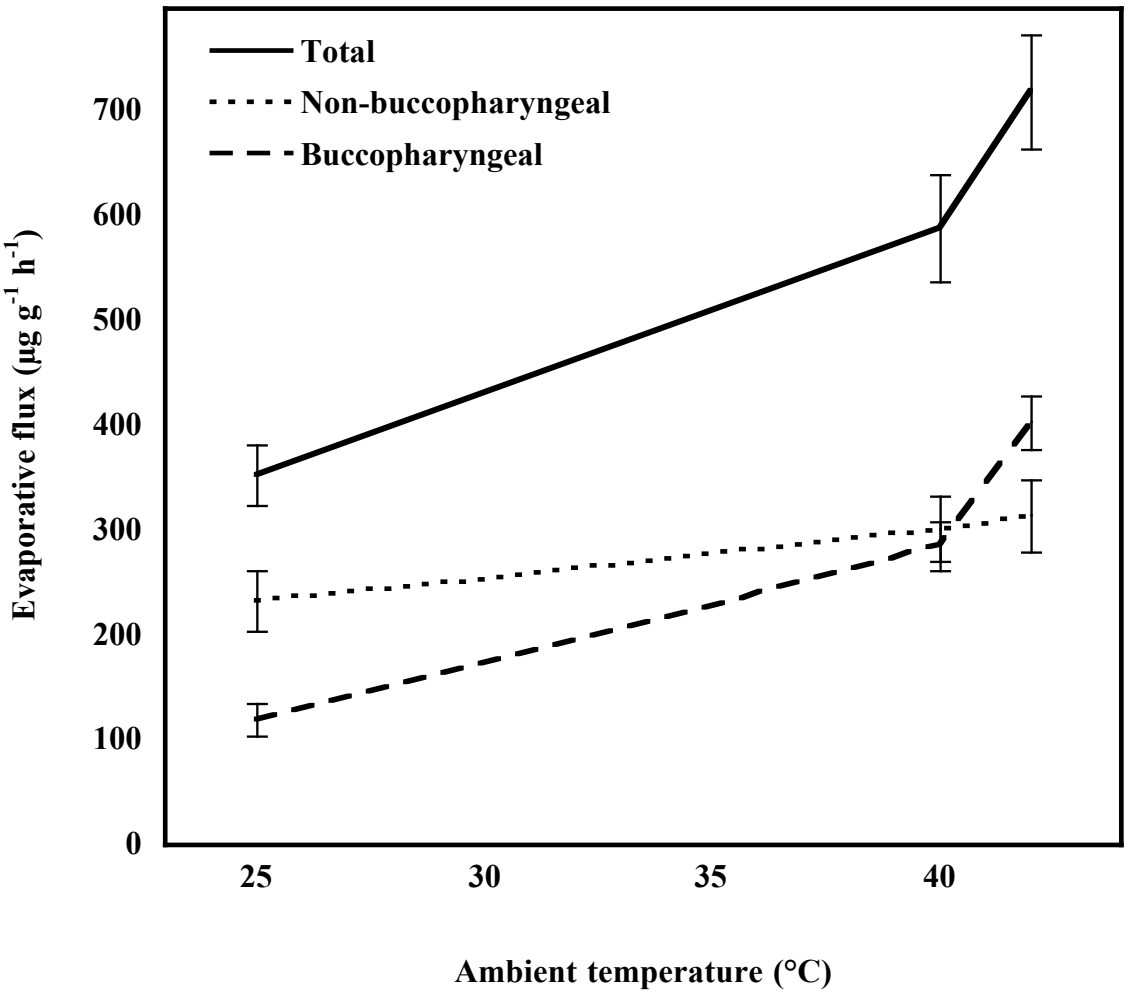

Fig. 5.1. The effect of air temperature on rates of evaporation from the mouth (buccopharyngeal) and from the rest of the body (non-buccopharyngeal) in ball pythons. Total evaporation (solid line) is the sum of the two components (hashed lines). There is no significant change in non-buccopharyngeal evaporation. The change in buccopharyngeal evaporation is significant at both temperature increments. Values are means ± S.E.M. At 25°C, *N*=11; at 40° and 42°C, *N*=9.

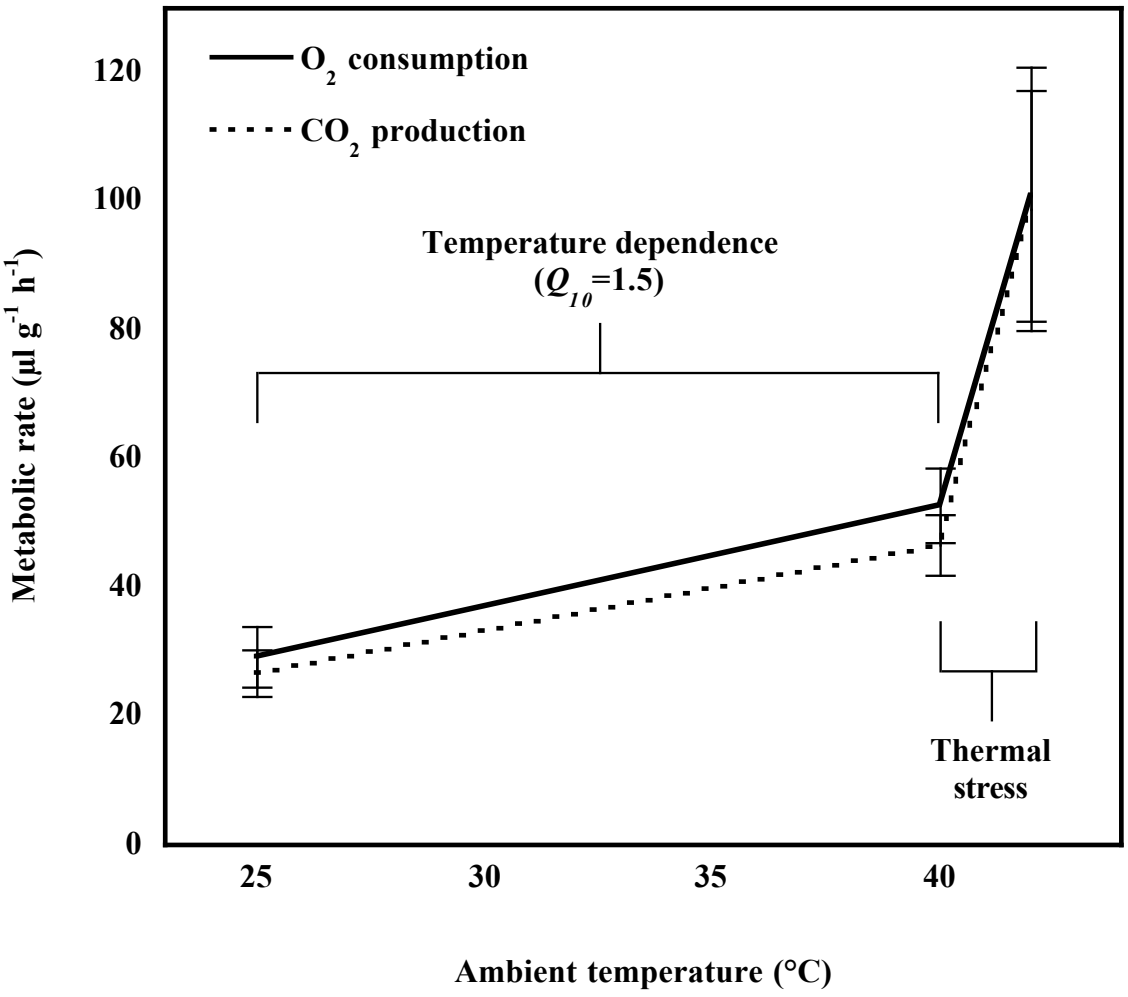

Fig. 5.2. The effect of air temperature on rates of exchange of oxygen and carbon dioxide in ball pythons. At 25° and 40°C, snakes remained calm, and Q_{10} values between those temperatures were 1.48 (O_2) and 1.45 (CO_2). There was no significant change in rates of metabolic gas exchange between 25° and 40°C. At 42°C, snakes were thermally stressed and exhibited escape behavior, significantly increasing metabolism. Values are means ± S.E.M. At 25°C, *N*=11; at 40° and 42°C, *N*=9.

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Conclusion

This dissertation presents a number of novel findings with respect to thermoregulatory evaporation in birds and reptiles. It has long been known that evaporation from the mouth (i.e. buccopharyngeal evaporation) constitutes just one part of an animal's total evaporation (e.g. Bernstein, 1971; Lasiewski et al., 1971; Marder and Ben-Asher, 1983; Webster and Bernstein, 1987; Wolf and Walsberg, 1996), and that in several birds the buccopharyngeal fraction of total evaporation can sometimes be a minor fraction (e.g. Marder and Ben-Asher, 1983; Marder and Gavrieli-Levin, 1987; Arieli et al., 2002; McKechnie and Wolf, 2004). Yet, in studies prior to those presented here, all evaporation occurring elsewhere (i.e. non-buccopharyngeal evaporation) was assumed to occur from the skin. This assumption has ignored the cloacal epithelium as a potential site for evaporation. Part of the present work was devoted to testing the assumption that all non-buccopharyngeal evaporation is cutaneous in origin. For Gila monsters and Inca doves, the results are remarkable. Not only does cloacal evaporation occur in both of these disparate species, but enough water is evaporated from their cloacae to be important for thermoregulation (DeNardo et al., 2004; Hoffman et al, 2006). In heat-stressed Gila monsters, cloacal evaporation is the vastly predominate route of evaporation, shedding eleven times as much heat as buccopharyngeal evaporation, and nearly eight times as much heat as cutaneous evaporation (DeNardo et al., 2004). In heat-stressed and vigorously panting Inca doves, nearly as much heat is dissipated by cloacal evaporation as by panting (Hoffman et al, 2006). Clearly, the assumption that cloacal evaporation is negligible can no longer stand. Moreover, the large relative contribution of cloacal evaporation to total evaporation in Inca doves and Gila monsters suggests that cloacal

evaporation has the thermoregulatory potential to allow some animals to extend the time that they are able to spend in microclimates that would otherwise push body temperatures to lethal limits. This would have important ecological implications. For example, cloacal evaporation might suppress body temperature enough to allow an incubating Inca dove to remain on her eggs when air temperature reaches a point that would otherwise endanger both the incubating female and her clutch. Gila monsters, for which cloacal evaporation at high temperatures is the overwhelming majority of total evaporation, might be able to use cloacal evaporation to extend periods of active foraging. Certainly, other ecologically important activities could be affected as well by an increased thermal latitude provided by cloacal evaporation.

The revelation that cloacal evaporation constitutes a large fraction of non-buccopharyngeal evaporation in some reptiles and birds prompts many questions for future research. How phylogenetically widespread is cloacal evaporation? How much of the non-buccopharyngeal evaporation measured by others in past studies was actually cloacal evaporation that was assumed to be cutaneous evaporation? How does cloacal evaporation affect other behaviors, such as foraging, reproduction, and avoidance of predators? Mechanistic questions abound, as well. This dissertation has shown that dehydration in Gila monsters results in a decrease in the rate of cloacal evaporation and an increase in the temperature above which cloacal evaporation occurs (DeNardo et al., 2004). Future studies should address how cloacal evaporation can be controlled and how it is affected by the excretory, digestive, and reproductive functions of the cloaca.

It is interesting to compare the results presented herein for the two dove species that were studied. Mourning doves were studied to address the question of whether birds are able to make rapid, endogenous adjustment to the rate of non-buccopharyngeal evaporation. When faced with an immediate suppression of buccopharyngeal evaporation, mourning doves responded by increasing the rate of non-buccopharyngeal evaporation (Hoffman and Walsberg, 1999). This novel finding demonstrates that some birds are able to exert acute control of non-buccopharyngeal evaporation, but the apportionment of that controlled, non-buccopharyngeal evaporation remains to be resolved. The Inca dove study prompts questions about *all* past avian experiments involving hygrometric partitioning, including the mourning dove study. Additional testing is required to determine whether mourning doves responded to suppressed buccopharyngeal evaporation by increasing cutaneous evaporation, by increasing cloacal evaporation, or both.

Though much more detailed phylogenetic testing is needed, the results from Eurasian quail and ball pythons provide an interesting contrast to those from Inca doves and Gila monsters. Eurasian quail occur in many habitats similar to those in which Inca doves are found, but the species belong to separate avian orders. Eurasian quail are similar to Inca doves in their predominant reliance on cutaneous evaporation (Hoffman et al, 2006). This weakens the contention that columbiforms possess especially high evaporative conductance at the skin (Marder and Gavrieli-Levin, 1987; Arieli et al., 2002; Ophir et al., 2003). The two species differ dramatically, however, with respect to cloacal evaporation. On a purely anatomical level, one would expect cloacal evaporation

to occur to a greater extent in Eurasian quail, which have comparatively much larger cloacal vents that often appear to form a poor seal. Nevertheless, Eurasian quail exhibited negligible cloacal evaporation, despite panting (Hoffman et al, 2006). In contrast, Inca doves underwent a transition from negligible evaporation from the cloaca at moderate temperatures to rates of cloacal evaporation at higher temperature that shed as much heat as did panting (Hoffman et al, 2006). The contrast in the results from these two species is strong evidence that cloacal evaporation is not a necessary consequence of having a cloaca, nor is it simply an artifact of experimental exposure to challengingly high temperatures. Rather, it is a controlled process used for thermoregulatory dissipation of heat.

Similarly, the results of the ball python study indicate that cloacal evaporation clearly is not a universal feature of reptiles. Rates of cloacal evaporation were negligible in ball pythons that were subjected to nearly the maximum experimental temperature that this snake species can survive (Hoffman et al, 2006). The fact that the mesic-adapted ball python does not employ cloacal evaporation, whereas the arid-adapted Gila monster shows rates of cloacal evaporation that can far exceed rates of all other evaporative routes, has important implications for cloacal evaporation. Tests on other taxa are required to determine whether cloacal evaporation occurs generally in lizards and does not occur generally in snakes. Nevertheless, the contrast in the results from ball pythons and Gila monsters suggests that, for an animal that is naturally exposed to microclimatic conditions under which evaporation is the only available mode of heat loss, cloacal evaporation is an adaptive mechanism by which the animal is able to conserve body

water by preventing cloacal evaporation until it is absolutely necessary to employ that evaporative mechanism to maintain body temperature below a lethal limit.

Accurate hygrometric measurements can be difficult to make even under ideal laboratory conditions, and meaningful field measurements of evaporative apportionment in animals remain prohibitively impractical, if not impossible. Future research on evaporative apportionment will therefore continue to be conducted in the laboratory. Recent improvements to hygrometric technology have vastly increased both the precision and the temporal resolution available to researchers. These improvements, along with the refined techniques used in the present studies for partitioning non-buccopharyngeal evaporation into its cutaneous and cloacal components, make possible the experimental designs required to address the many questions that this dissertation evokes.

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