The effect of heat transfer mode on heart rate responses and hysteresis during heating and cooling in the estuarine crocodile *Crocodylus porosus*

Craig E. Franklin[1] and Frank Seebacher[2,*]

[1]*Department of Zoology and Entomology, University of Queensland, St Lucia, Qld 4072, Australia* and [2]*School of Biological Sciences A08, University of Sydney, Sydney, NSW 2006, Australia*

*Author for correspondence (e-mail: fseebach@bio.usyd.edu.au)

Summary

The effect of heating and cooling on heart rate in the estuarine crocodile *Crocodylus porosus* was studied in response to different heat transfer mechanisms and heat loads. Three heating treatments were investigated. *C. porosus* were: (1) exposed to a radiant heat source under dry conditions; (2) heated *via* radiant energy while half-submerged in flowing water at 23°C and (3) heated *via* convective transfer by increasing water temperature from 23°C to 35°C. Cooling was achieved in all treatments by removing the heat source and with *C. porosus* half-submerged in flowing water at 23°C. In all treatments, the heart rate of *C. porosus* increased markedly in response to heating and decreased rapidly with the removal of the heat source. Heart rate during heating was significantly faster than during cooling at any given body temperature, i.e. there was a significant heart rate hysteresis. There were two identifiable responses to heating and cooling. During the initial stages of applying or removing the heat source, there was a dramatic increase or decrease in heart rate ('rapid response'), respectively, indicating a possible cardiac reflex. This rapid change in heart rate with only a small change or no change in body temperature (<0.5°C) resulted in Q_{10} values greater than 4000, calling into question the usefulness of this measure on heart rate during the initial stages of heating and cooling. In the later phases of heating and cooling, heart rate changed with body temperature, with Q_{10} values of 2–3. The magnitude of the heart rate response differed between treatments, with radiant heating during submergence eliciting the smallest response. The heart rate of *C. porosus* outside of the 'rapid response' periods was found to be a function of the heat load experienced at the animal surface, as well as on the mode of heat transfer. Heart rate increased or decreased rapidly when *C. porosus* experienced large positive (above 25 W) or negative (below –15 W) heat loads, respectively, in all treatments. For heat loads between –15 W and 20 W, the increase in heart rate was smaller for the 'unnatural' heating by convection in water compared with either treatment using radiant heating. Our data indicate that changes in heart rate constitute a thermoregulatory mechanism that is modulated in response to the thermal environment occupied by the animal, but that heart rate during heating and cooling is, in part, controlled independently of body temperature.

Key words: thermoregulation, reptiles, heart rate, hysteresis, heat transfer, body temperature, crocodiles, *Crocodylus porosus*.

Introduction

The cardiovascular system of reptiles can play a significant role in the transfer of heat between the body core and the environment (Grigg et al., 1979; Bartholomew, 1982; Seebacher, 2000; Dzialowski and O'Connor, 2001). It has been shown for several species, including crocodiles and lizards, that during basking (warming environment) heart rate increases and, conversely, heart rate decreases when animals enter a cooling environment. Hence, at any given body temperature, heart rate during heating is significantly faster than during cooling; a phenomenon known as heart rate hysteresis (Bartholomew and Tucker, 1963; Grigg and Alchin, 1976; Grigg and Seebacher, 1999). The thermoregulatory advantages conferred by the heart rate hysteresis, and associated changes in cardiac output and peripheral blood flow, allow a reptile to spend longer per day with body temperatures within its preferred thermal range (O'Connor, 1999; Seebacher, 2000).

Rapid changes in heart rate during the initial stages of heating and cooling in the heliothermic lizard *Pogona barbata* (instantaneous changes of 10 beats min^{-1}) indicate a reflex-like response that is at least partly mediated by the autonomic nervous system (Seebacher and Franklin, 2001). This rapid response augmented the heart rate hysteresis seen during heating and cooling in *P. barbata* and appears to be an important component of the physiological control of body temperature in this lizard (Seebacher and Franklin, 2001). A reflex-like response of heart rate also occurred after local application of radiant heat to the dorsal surface of the

freshwater crocodile *Crocodylus johnstoni*, although this was recorded in one animal only (Grigg and Alchin, 1976).

During thermoregulation, crocodiles utilise microhabitats that encompass both terrestrial and aquatic environments and a variety of behavioural postures (Seebacher and Grigg, 1997; Seebacher, 1999; Grigg and Seebacher, 2001). Regulation of body temperature can be achieved by basking on land, shuttling between land and water, and changing postures while in the water so that varying proportions of surface area are exposed to the sun (Seebacher, 1999). The amphibious lifestyle of crocodiles has a significant influence on rates of heat gain and loss and thermoregulation due to the markedly different thermal characteristics of water and air. It is possible, therefore, that cardiac responses of crocodiles during heating and cooling are different for different heat transfer mechanisms experienced by the animals. Additionally, it has been suggested that thermoregulation in reptiles is facilitated by the light-sensitive pineal gland and parietal eye (Tosini and Menaker, 1996; Tosini, 1997; Cagnacci et al., 1997), which may indicate that responses to basking (i.e. exposure to high light intensity) may be fundamentally different to heating or cooling in the absence of radiation. Note that crocodilians were considered for a long time to lack a pineal gland (Tosini, 1997), but the recent discovery of a pineal gland in the American alligator *Alligator mississippiensis* (Daphne Soares, personal communication) dispels that notion.

The aim of this study was to investigate the heart rate response of the estuarine crocodile *Crocodylus porosus* to different heat transfer mechanisms (radiation and convection) and to varying heat loads.

Materials and methods
Animals

Juvenile estuarine crocodiles (*Crocodylus porosus* Schneider 1801; body mass 533±44 g, mean ± S.D., *N*=6) were obtained from the Cairns Crocodile Farm, North Queensland and transported to The University of Queensland. They were housed outdoors in a large fibreglass tank (4 m diameter) supplied with filtered and re-circulated freshwater at 31°C. Crocodiles had access to a basking platform and were fed a mixture of chopped chicken necks and ox hearts. All experiments were conducted during summer 2002.

Experiments were approved by the University of Queensland animal ethics and experimentation committee, Permit No. ZOO/ENT/266/01/URG, and crocodiles were held under the Queensland Parks and Wildlife scientific purposes permit, No. W4/002709/01/SAA.

Experimental setup

Heart rate in *C. porosus* was measured from electrocardiograms (ECGs). Pacemaker stainless steel electrodes (Medtronic, Fourmes, France) were placed under the skin (after application of the local anaesthetic lignocaine) on the ventral surface just anterior to the heart and at the base of the tail. The insulated ECG leads were sutured to the skin and secured with tape at the tail. A small drop of superglue was used to waterproof the holes in the skin from which the leads exited. Body temperature was measured with a K-type thermocouple, which was inserted 5–6 cm into the cloaca. The experimental animal was then transferred to a custom-built, Perspex chamber (10 cm×60 cm×60 cm, width × length × height), which allowed the animal to sit comfortably on the bottom but did not permit it to turn around. Crocodiles were heated with an infra-red heat lamp suspended above the Perspex chamber. Radiation from the heat lamp was measured with a pyranometer (Sol Data, Silkeborg, Denmark) connected to a data logger (Data Electronics, Melbourne, Australia), and the height of the lamp above the animal was adjusted so that it delivered $800\,kW\,m^{-2}$ to the surface of the animal. The heat from the lamp was similar to the solar irradiation during basking on a summer morning (F.S., unpublished data). For control treatments, a cold, fibreoptic light covered with red cellophane was also positioned above the chamber and directed onto the surface of the crocodile. Water flow through the experimental chamber could be adjusted remotely and, when water was used as a treatment, depth was adjusted to half of the height of the crocodile in a lying position, and flow rate was set at $3\,cm\,s^{-1}$. A thermocouple was also placed in the water in the chamber to record water temperature. The experimental chamber (and animal) was located in an isolated controlled temperature room set at 23°C, which was monitored by a remote video camera. Physiological data collection, lamps and water flow were controlled from an adjoining room, preventing disturbance to the animals.

The ECG and thermocouple leads were directed to a computer data acquisition system. ECG leads were connected to a high-gain AC amplifier (BioAmp; AD Instruments, Sydney, Australia) that was coupled to a four-channel PowerLab (AD Instruments). The signals from the thermocouples (body and water temperatures) were also directed to the PowerLab. The PowerLab was connected to a Toshiba laptop computer and its output was displayed using Chart software (AD Instruments). Sampling rate was set at 100 Hz, and Chart software calculated heart rate in real-time. Heart rate, body temperature and water temperature were recorded continuously during experimentation.

Treatments

The effects of heating and cooling on the heart rate and body temperature of *C. porosus via* a radiant heat source (lamp) and by convective transfer from flowing water were investigated. Five treatments (see below) were applied in random order to each of the six experimental animals. Heart rate, body temperature and ambient temperature were recorded for 10 min prior to the treatments to obtain baseline resting values.

Cold light control (CLC)
This treatment examined the potential effects of light, rather than heat, on heart rate. A fibreoptic light emitting red light
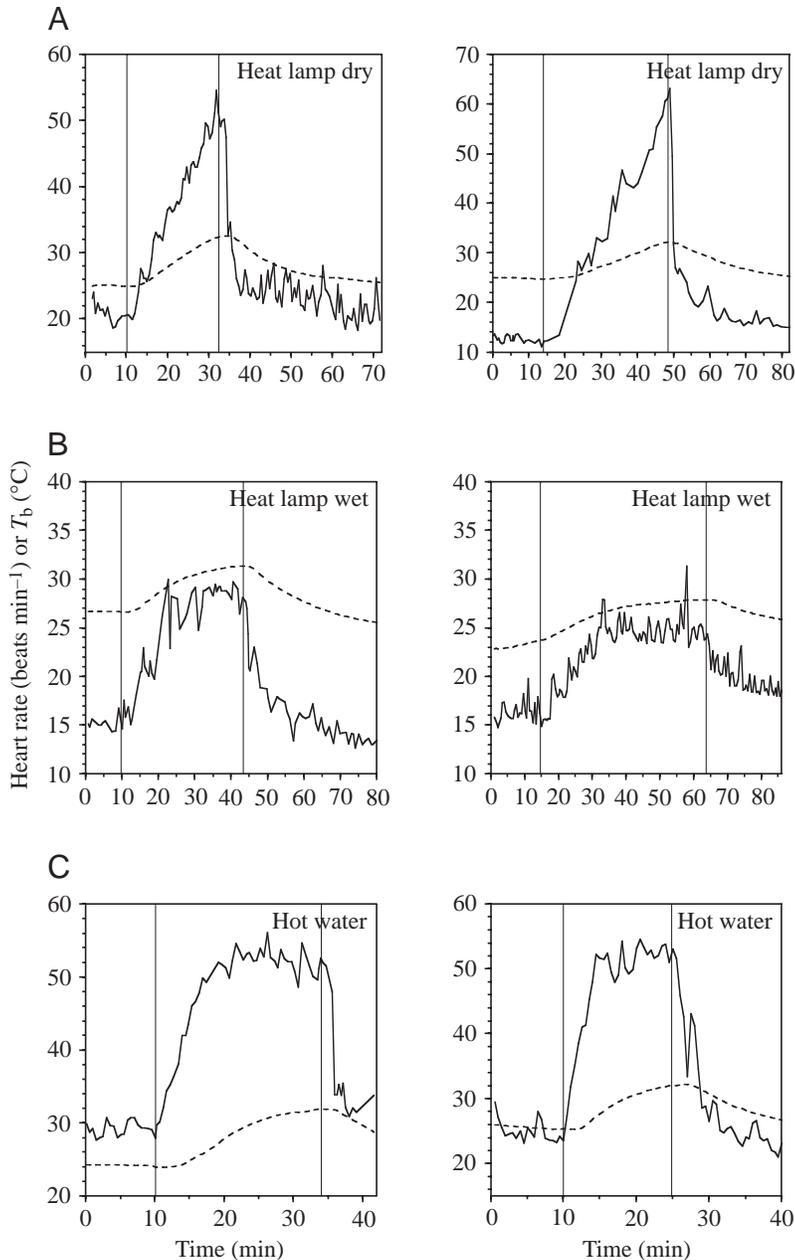
A

B

C

Fig. 1. Representative examples of the heart rate (solid line) and body temperature (broken line) response to the different treatments: (A) heat lamp dry; (B) heat lamp wet; (C) hot water. The vertical lines indicate the time when the treatment commenced (i.e. when the heat lamp was switched on or hot water was introduced; line on the left) and when it was concluded (right-hand line). Note the almost instantaneous increase and decrease in heart rate when the heat was applied and removed, respectively, which occurred while body temperature change was negligible. Note that values on the *y*-axis denote both heart rate and body temperature (T_b).

was directed past *C. porosus* in the experimental chamber, and heart rate was recorded for 10 min before the water temperature was increased to 35°C. When body temperature reached 31–33°C, the water was switched back to 23°C until body temperature returned to its initial value (approximately 23°C).

Heat lamp dry (HLD)

This treatment tested the effect of irradiation from a heat lamp under dry conditions (i.e. no water in the chamber). The heat lamp was switched on until body temperature reached 32–33°C and then, simultaneously, the heat lamp was switched off and water flow to the chamber (at 23–25°C) was turned on.

Heat lamp wet (HLW)

This treatment investigated the effect of irradiation from a heat lamp while *C. porosus* was half-immersed in flowing water (at 23–25°C). Heating was applied until body temperature reached equilibrium (typically 26–28°C), after which the heat lamp was switched off and animals were allowed to cool to their initial body temperature.

Statistical analysis

To eliminate short-term variation in heart rate resulting from breathing bradycardia, for example, heart rates used in statistical analyses were averaged within 1°C body temperature bins. Additionally, in order to eliminate intrinsic differences between individual study animals, heart rate data used in statistical analyses (but not in figures) were transformed by dividing heart rates during heating and cooling by resting heart rates measured prior to the treatments.

Treatments were compared by a three-factor analysis of variance (ANOVA) with body temperature used as a co-variate; the factors were 'treatment' (three levels: HLD, HLW and HW), 'heating/cooling' (two levels) and 'crocodile' (six levels). The error d.f. for the co-variate was 171 (including heart rate measurements at different body temperatures), but, for comparisons between 'treatments' and 'heating/cooling', probabilities were calculated with 'crocodile' as the level of

was switched on for 10 min, then switched off, and recording of heart rate continued for another 10 min.

Cold water control (CWC)

This treatment examined the effect of water flow on heart rate. The experimental chamber was emptied of water and the animal allowed to rest undisturbed for 30–60 min before water flow to the chamber was turned on. Water temperature was equal to body temperature, and recording of heart rate was continued for 10 min.

Hot water (HW)

This treatment examined the effect of convective heat transfer from heated water flowing past *C. porosus*. Water at 23–25°C

replication, i.e. error d.f. = 30. In the CLC treatment, heart rates measured while the cold light was on were compared with the periods preceding and following this interval by one-way ANOVA with 'crocodile' as the level of replication. Similarly, in the CWC treatment, heart rates measured during the period while the water was on were compared with resting heart rates in the preceding period by one-way ANOVA. The effect of surface heat loads on changes in heart rate was compared among treatments by a one-way analysis of co-variance (ANCOVA) with 'treatment' as factor and 'surface heat load' as co-variate.

Note that in all statistical analyses we assumed that heart rates between temperature bins were independent. This was warranted because temperature had only a very minor effect on heart rate, and 'body temperature' was used as a co-variate.

Calculations of heat load

Heat loads experienced at the animal surface were calculated by solving heat transfer equations for heat rate (see Incropera and DeWitt, 1996). The experimental treatments represent step function changes in steady-state thermal conditions. In the different treatments, the relative importance of heat transfer mechanisms (convection in air, convection in water, radiation and conduction) varied so that the heat load experienced by the animals differed, resulting in either heating or cooling. In order to estimate heat transfer by the different mechanisms, the total animal surface areas were calculated by the 'polynomial' method (Seebacher et al., 1999; Seebacher, 2001), and the relative surface areas exposed to different heat transfer mechanisms in each treatment were estimated from direct observations. In all water treatments (i.e. all cooling episodes, CWC, HLW and HW treatments), the water level was adjusted so that half the crocodile's body was submerged and, therefore, half the animal surface area experienced convective heat exchange with water; note that, while in water, the ventral surface of the animals was never firmly pressed against the substrate so that it was assumed to exchange heat by convection. The upper half of the body would exchange heat by free convection with air; there was no air flow in the constant temperature room, and the animals were more or less motionless during the treatments so that free convection conditions were assumed. Radiation from the heat lamp is absorbed by the silhouette area of the animal, which is 33% of the total surface area exposed (Muth, 1977). Hence, in the HLW treatment, 50% of the animal surface was exposed to air, and, of this proportion, 33% would have absorbed radiation. In the HLD treatment, 33% of the animal surface was in contact with the ground while 67% exchanged heat by convection with air, and, of this 67%, 33% (the silhouette area) absorbed radiation from the heat lamp. Moreover, the total exposed area would emit and absorb thermal radiation with the environment.

Convection coefficients (h) for free convection conditions can be estimated by:

$$h = k/D \times \mathrm{Nu}, \qquad (1)$$

where k is thermal conductivity of air, D is diameter of the crocodile, and Nu is the Nusselt number. The Nusselt number can be estimated as:

$$\mathrm{Nu} = \{0.6 + (0.387\mathrm{Ra}^{1/6}) / [1 + (0.559/\mathrm{Pr})^{9/16}]^{8/27}\}^2, \qquad (2)$$

where Ra is the Raleigh number and Pr is the Prandtl number (Churchill and Chu, 1975). The Raleigh number is calculated as:

$$\mathrm{Ra} = \mathbf{g}\beta(T_s - T_e)D^3/\nu\alpha, \qquad (3)$$

where $\mathbf{g}$ is gravitational force, β is the thermal volumetric expansion coefficient (air), T_s is surface (body) temperature, T_e is operative temperature, α is kinematic viscosity (air) and ν is thermal diffusivity (air) (Incropera and DeWitt, 1996).

Coefficients for forced convection in water were calculated for a cylinder with cross flow (Churchill and Bernstein, 1977). Reynolds numbers describe the ratio of inertial and viscous forces (Re=$\mathbf{v}$L/ν, where $\mathbf{v}$ is fluid velocity and L is the characteristic dimension of solid), and the Prandtl number represents the ratio of the momentum and thermal diffusivities (Pr=ν/α). For cylinders with cross flow, the following single comprehensive equation exists, which relates dimensionless numbers for a wide range of flow patterns, i.e. for a wide range of Re and Pr (Churchill and Bernstein 1977):

$$\mathrm{Nu} = 0.3 + (0.62\mathrm{Re}^{0.5}\mathrm{Pr}^{0.33}) / [1 + (0.4/\mathrm{Pr})^{0.67}]^{0.25}[1 +$$
$$(\mathrm{Re}/282\,000)^{0.625}]^{0.8}. \qquad (4)$$

Convection coefficients were calculated from the above equation and the definition of Nu.

The heat rate at the animal surface can be calculated from the surface energy balance (Incropera and DeWitt, 1996):

$$q_{\mathrm{rad}} - q_{\mathrm{cv}} - q_{\mathrm{cd}} = 0, \qquad (5)$$

where q_{rad} is the energy received at the animal surface by thermal and short-wave radiation, q_{cv} is the heat exchanged by convection, and q_{cd} is the heat exchanged by conduction. Radiation heat transfer is defined as:

$$q_{\mathrm{rad}} = \varepsilon\sigma A_{\mathrm{therm}}(T_s^4 - T_a^4) + \acute{\alpha}A_{\mathrm{sw}}Q, \qquad (6)$$

where ε is emissivity, σ is the Stefan–Boltzmann coefficient, T_s is the surface temperature, which under steady-state equilibrium conditions is equal to body temperature (T_b), T_a is the air temperature in the constant temperature room, $\acute{\alpha}$ is the absorptivity to short-wave radiation, Q is the short-wave radiation intensity (800 W m^{-2}, as measured with the pyranometer), A_{therm} is the surface area exchanging heat by thermal radiation, and A_{sw} is the surface area absorbing short-wave radiation. q_{cv} represents the energy exchanged by convection according to:

$$q_{\mathrm{cv}} = hA_{\mathrm{cv}}(T_b - T_{a/w}), \qquad (7)$$

which is calculated separately for convection in water and air [for either T_a or water temperature (T_w), using the convection coefficients described above]. A_{cv} is the surface area exchanging heat by convection. q_{cd} is conductive heat transfer experienced by the ventral surface during the HLD treatment

and was estimated by calculating heat transfer through the ventral skin:

$$q_{cd} = kA_{cd}/l(T_b - T_g),\qquad(8)$$

where k is thermal conductivity, A_{cd} is the surface area exchanging heat by conduction, l is skin thickness (assumed to be 10% of the animal radius) and T_g is the ground temperature, which was the same as T_a in the constant temperature room.

In comparisons with heat load, heart rates were expressed as the change in heart rate with temperature, i.e. the second derivative. Conceptually, these units resemble Q_{10}, although the advantage is that they can be expressed as both positive and negative numbers. In addition, the 'rapid response' periods during which body temperature remained stable (see below) were not included in the analysis of surface heat loads.

Results

In all treatments, heart rate increased sharply in response to heat, either provided by the heat lamp or hot water (Fig. 1). Conversely, heart rate decreased instantaneously on removal of the heat source (Fig. 1). During the hot water (HW) and heat lamp wet (HLW) treatments, heart rate formed a plateau before the heat source was removed, whereas heart rate continued to increase until the removal of the heat source in the heat lamp dry (HLD) treatment. All crocodiles showed similar responses to the treatments, and heart rate increased significantly with increasing body temperature ($F_{1,171}=14.37$, $P<0.0001$). There were significant differences in the magnitude of the heart rate response between treatments, with the HLW treatment eliciting the least response ($F_{2,30}=4.13$, $P<0.03$). Heart rate during heating was significantly faster than during cooling at any body temperature in all treatments ($F_{1,30}=31.80$, $P<0.0001$; Fig. 2).

In the control treatments, heart rate was not significantly different while the cold light was on (CLC) compared with the preceding or following periods ($F_{2,15}=0.46$, $P=0.64$; Fig. 3). Similarly, the cold water (CWC) treatment, i.e. exposing the crocodiles to water at the same temperature as their body temperature, did not elicit a significant change in heart rate ($F_{1,10}=0.02$, $P=0.89$; Fig. 3).

Although heart rate changed significantly with body temperature, Q_{10} values associated with the heart rate response varied considerably with time during the heating or cooling phases (Fig. 4A). In fact, Q_{10} values were exceedingly high when the heat source was switched on or off. For example, when the heat lamp was switched off in the HLD treatment (Fig. 4A), heart rate changed with a Q_{10} of 4627 – this is, of course, a nonsensical value that reflects that heart rate changed while body temperature remained nearly stable. During these 'rapid response' periods when heat was first applied or removed, heart rate changed dramatically while body temperature remained nearly constant (Fig. 4B,C). In the later phases of heating and cooling, heart rate changed with body temperature, representing a Q_{10} of 2–3 (Fig. 4A).

Changes in heart rate outside the 'rapid response' periods are a function of the heat load experienced at the animal
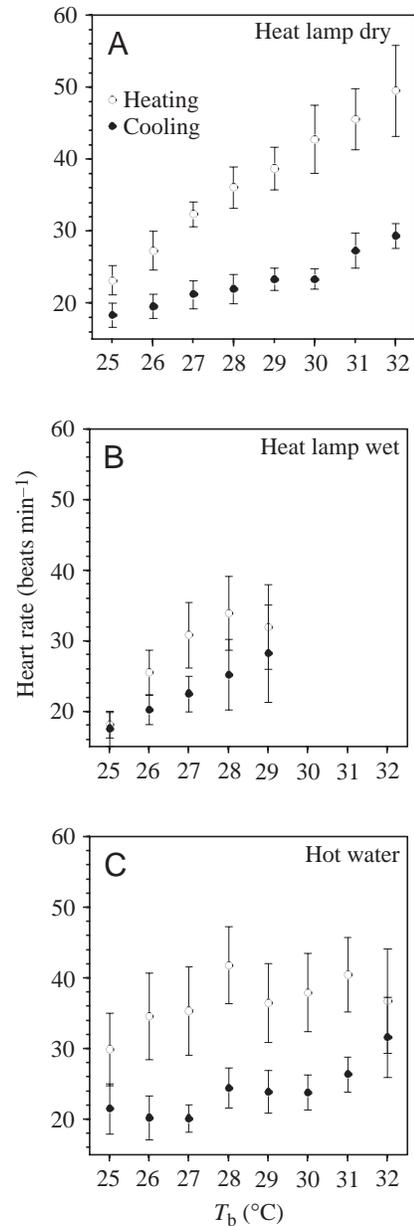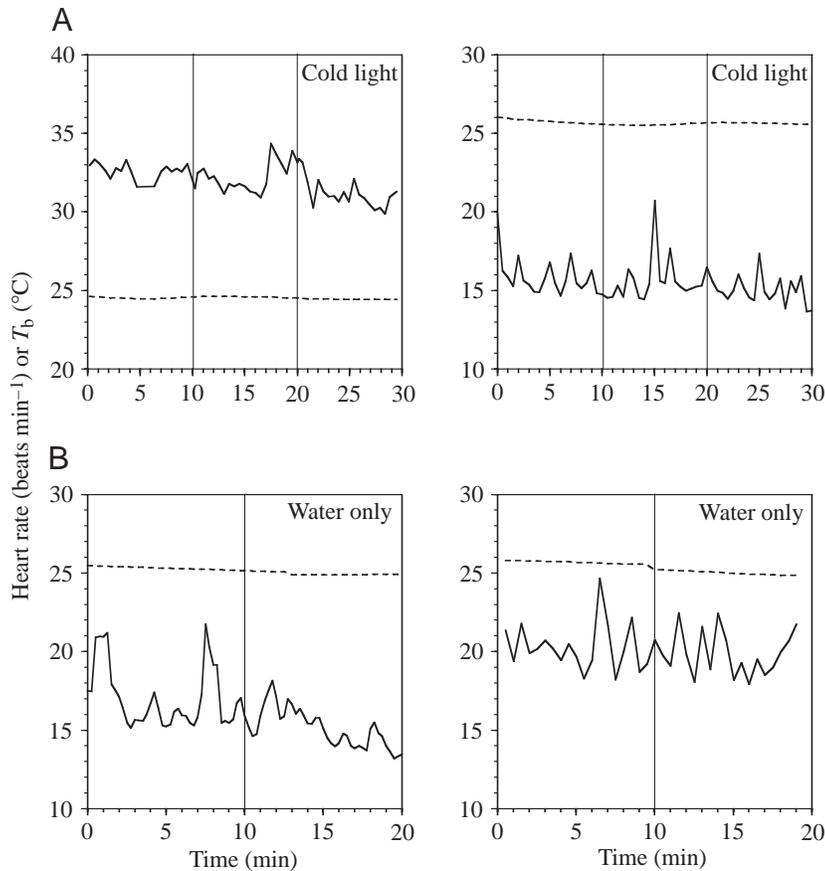

Fig. 2. Mean heart rate (averaged over 1°C body temperature intervals, ±S.E.M.) was significantly faster during heating than during cooling at any given body temperature in all treatments – (A) heat lamp dry, (B) heat lamp wet, (C) hot water – although the difference was least pronounced in the heat lamp wet treatment.

surface. In all three treatments, changes in heart rate per °C body temperature (ΔHR; measured in beats min^{-1} $deg.^{-1}$) changed sigmoidally with heat load [$\Delta HR=(0.575+0.203W)/(1+0.0168W-0.00108W^2)$; $r^2=0.92$; Fig. 5A]. Hence, heart rate increased or decreased very rapidly when the animal experienced large positive (above 25 W) or negative (below –15 W) heat loads, respectively. Between –15 W and 25 W, the increase in heart rate with increasing heat load was linear (Fig. 5B). Over this linear range, heart rate increased significantly with increasing heat load (ANCOVA $F_{1,31}=23.31$, $P<0.0001$), but changes during the HW treatment were

A

B

Fig. 3. Representative examples of body temperature (broken lines) and heart rate (solid lines) responses to the control treatments: (A) cold light and (B) cold water. The solid vertical lines indicate when the cold light was switched on and off (in A) or when water flow was commenced (in B). None of the control treatments elicited a significant heart rate response. Note that values on the y-axis denote both heart rate and body temperature (T_b).

distinct performance peak that coincides with a narrow range of optimal body temperatures. Performance may cease altogether outside the boundaries of acceptable body temperatures, which are defined by the critical thermal minima and maxima (Huey, 1982; Huey and Bennett, 1987; Angiletta et al., 2002). Such thermal dependence is presumably a function of underlying biochemical processes whose rate is temperature dependent, although their thermal sensitivity may change as a result of acclimatisation (phenotypic changes) or adaptation (genotypic changes) (St Pierre et al., 1998; Crawford et al., 1999; Guderley and Leroy, 2001). If performance were directly related to fitness, it could be expected that temperature-sensitive physiological functions proceed at optimal rates at those body temperatures that are achievable by thermoregulating animals (Bennett et al., 1992; Leroi et al., 1994), and adaptive changes in thermal optima within and between species have been shown to occur along altitudinal and latitudinal gradients (Crawford and Powers, 1992; Pierce and Crawford, 1997; Ståhlberg et al., 2001). Our data, however, indicate that despite their importance in controlling rates of heating and cooling (Seebacher, 2000; Seebacher and Franklin, 2001), changes in heart rate are to a large extent independent of body temperature. It seems more plausible that the mechanisms controlling heart rate during heating and cooling evolved as a correlated response to selection pressures favouring optimal performance of temperature-sensitive rate function. Hence, commonly accepted models used to explain evolutionary relationships between body temperature and physiological performance (Huey and Bennett, 1987; Angiletta et al., 2002) may not be applicable to cardiac function in heliothermic reptiles.

The heart rate response appears to be elicited, at least in part, by the heat load experienced at the animal surface, which indicates that, rather than being an on–off response, the control mechanisms act in an analogue manner and their magnitude depends on environmental stimuli. Interestingly, in 'unnatural' heating situations (e.g. hot water), heart rate hysteresis was evident, but the patterns of heart rate were different from the heat lamp treatments. Heat loads experienced during the HW treatment were similar to those during the HLD treatment, but the mechanisms by which heat was exchanged differed. These data indicate that there exists a heat-sensitive control
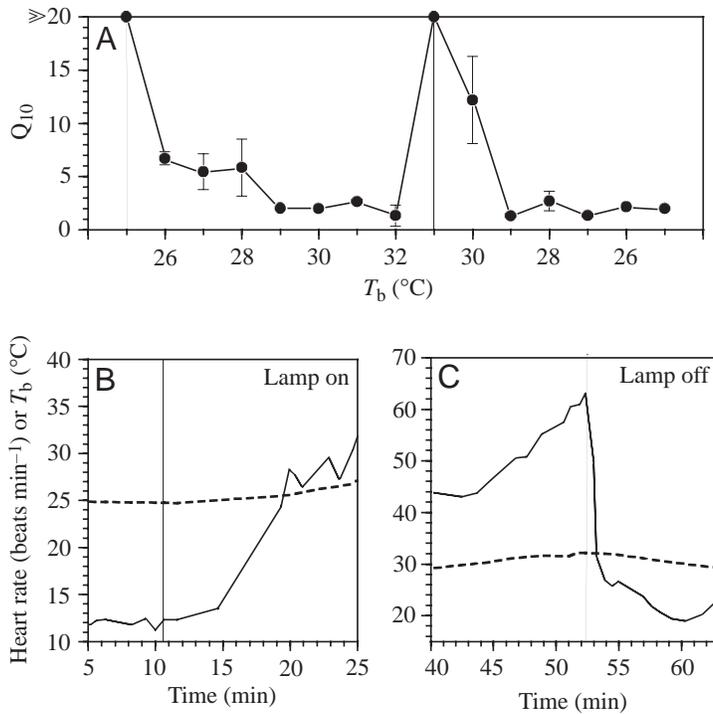
significantly less than during the two heat lamp treatments, which did not differ from each other ($F_{2,31}$=6.43, P<0.01; Fig. 5B). During the HW treatment, changes in heart rate increased according to ΔHR=–1.07+0.11W (r^2=0.43), and during the HLW and HLD treatments (data from both treatments combined) ΔHR=1.90+0.38W (r^2=0.70; Fig. 5B).

Discussion

Our data document the exceptional nature of heart rate control during heating and cooling in reptiles. Exceptional, because the widely applicable and accepted concept of Q_{10} is not sufficient in explaining changes in heart rate with body temperature in crocodiles. During the initial period after heat was applied or removed (the 'rapid response' period), heart rate changed dramatically despite the fact that body temperature remained stable. In other words, heart rate during this period is controlled independently of body temperature. A similar pattern, although less pronounced, has been reported for a lizard (*Pogona barbata*; Seebacher and Franklin, 2001), where it could be explained, as least in part, by the action of the cholinergic and β-adrenergic nervous systems. Given the influence of the autonomic nervous system on heart rate in a lizard, it would be of interest to determine whether or not the 'rapid response' period constitutes a neural reflex arc.

It is generally accepted that physiological performance is sensitive to changes in body temperature and that there is a

Fig. 4. (A) Q_{10} values for the change in heart rate during heating and cooling in the heat lamp dry treatment (means ± S.E.M.). During the initial 'rapid cardiac response' to the application or removal of heat, heart rate changed dramatically while body temperature (T_b) remained nearly stable. (B,C) Close-up views of representative examples for the rapid response periods are shown. Q_{10} values for the heart rate change during the 'rapid response' periods were extremely high (>4000, represented by ≫20 on the *y*-axis), indicating the temperature independence of heart rate during those periods. The times when the heat lamp was switched on and off are indicated by the solid vertical lines. Note that values on the *y*-axis in B and C denote both heart rate and body temperature.

control cardiac response during heating and cooling (Robleto and Herman, 1988) *via* the baroreflex, by contraction or dilation of capillary beds, and/or by directly stimulating the heart. The baroreflex is likely to play a role in modulating heart rate, particularly in crocodiles (Altimiras et al., 1998); it has been demonstrated that peripheral blood flow changes in response to heat (Grigg and Alchin, 1976; Smith et al., 1978), and this response in blood flow may precede the response in heart rate (Morgareidge and White, 1972).

The difference between heat lamp and hot water treatments indicates, however, that there may be additional control mechanisms operating. In many reptiles, thermoregulatory responses are thought to be controlled by the light-sensitive pineal gland (Tosini, 1997). Crocodilians were long believed not to possess a pineal gland (Tosini 1997), but a pineal-like organ was recently discovered in the American alligator (Daphne Soares, personal communication). The fact that, in our study, the heart rate response differed between radiant heating and convective heating in water (although the hysteresis effect was apparent in all experimental treatments) indicates that light-sensitive mechanisms may play a role in controlling cardiac response during heating and cooling. Furthermore, the body surface/region over which heat is transferred may also modulate the response of the heart. During radiant heating, the dorsal surface of the crocodile was chiefly responsible for the transfer of heat,
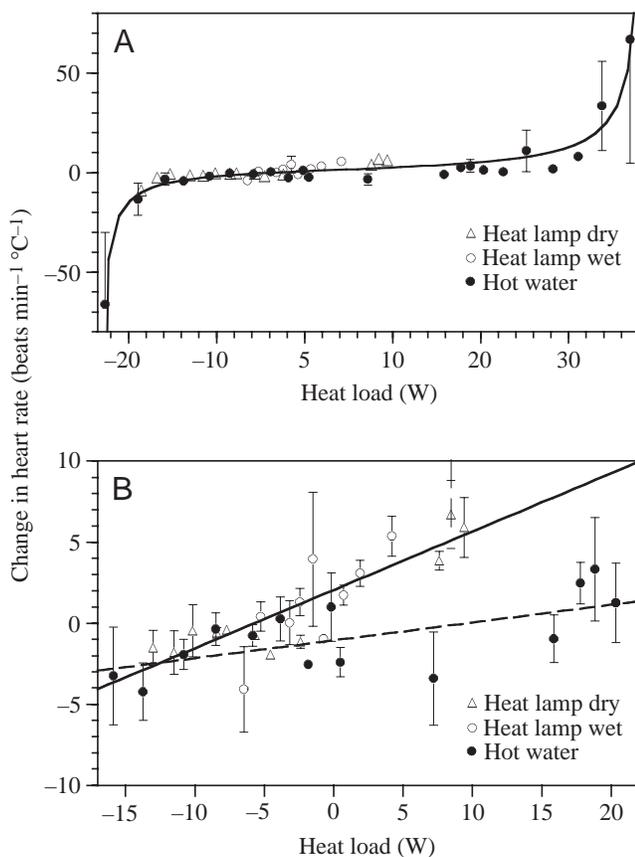
mechanism that triggers a heart rate response before body temperature changes. It seems likely, therefore, that the heart rate response is at least partly controlled locally at the animal surface. Prostaglandins are a possible mechanism that may

Fig. 5. Changes in heart rate during heating and cooling changed in proportion to the heat load experienced at the animal surface (means ± S.E.M.). (A) The heart rate response to heat load was sigmoidal in shape, with rapid decreases and increases below –15 W and above 25 W, respectively. The range of heat loads experienced by the animals was greatest during the hot water (HW) treatment and least in the heat lamp wet (HLW) treatment. The fitted line shows a rational function. (B) Changes in heart rate during the linear portion of the sigmoidal curve (–16 W to 20 W) increased with increasing heat load. Over this linear range, there were no differences between the heat lamp dry (HLD) and HLW treatments, but rates of change were significantly less in the HW treatment. Linear regression lines are shown for the HW treatment (broken line) and for the HLD and HLW treatments combined (solid line).

whereas during convective heating, it was the ventral and lateral surfaces of the crocodile that were involved in the transfer of heat. Along with the involvement of the pineal gland, our results also suggest that the heat transferred across the dorsal surface (as opposed to the ventral and lateral surfaces) could augment the cardiac response to heating and cooling.

Many reptiles use an array of postures while thermoregulating behaviourally. In particular, crocodiles in the wild alter the relative proportions of body surface area exposed to different heat transfer mechanisms to regulate body temperature (Seebacher, 1999; Grigg et al., 1998; Seebacher et al., 1999). Our experimental treatments mimicked some typical thermoregulatory postures observed in thermoregulating crocodiles (Seebacher, 1999), and our data indicate that the placement of the animal within its biophysical environment determines the magnitude of the physiological mechanisms used to control rates of heating and cooling.

There were marked 'rapid responses' in heart rate of *C. porosus* to the initial stages of heating and cooling. Similar responses were recorded by Seebacher and Franklin (2001) in *Pogona barbata*. However, a number of studies investigating changes in heart rate in reptiles with heating and cooling have failed to show this initial rapid response period (Dzialowski and O'Connor, 2001). We believe that this reflex response could have been masked by the methods used by previous investigators, where animals were often restrained when heated and cooled. For example, Dzialowski and O'Connor (2001) tied their lizards to doweling. It is well known that restraint activates the adrenergic (release of catecholamines) and cholinergic systems in reptiles (Lance and Elsey, 1999) and, given that Seebacher and Franklin (2001) identified a role of the adrenergic and cholinergic systems in the control of heart rate during thermoregulation in *Pogona barbata*, activation of the stress axes may override or mask the rapid cardiac response. We recommend that in future studies where the heart rate responses of reptiles to heating and cooling are investigated, experiments are conducted with unrestrained animals.

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