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The relationship between heart rate and rate of oxygen consumption in Galapagos marine iguanas (*Amblyrhynchus cristatus)* at two different temperatures

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Summary

To enable the use of heart rate (f_H) for estimating field metabolic rate (FMR) in free-ranging Galapagos marine iguanas *Amblyrhynchus cristatus*, we determined the relationships between f_H and mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) in seven iguanas before and during exercise on a treadmill and during the post-exercise period. The experiments were conducted at 27 and 35 °C, which are the temperatures that represent the lowest and highest average body temperatures of these animals in the field during summer. There were linear and significant relationships between f_H and $s\dot{V}_{O_2}$ at both temperatures (r^2=0.86 and 0.91 at 27 °C and 36 °C, respectively). The slopes of the two regression lines did not differ, but there were significant differences in their intercepts. Thus, while heart rate can be used to predict FMR, the effects of temperature on the intercept of the regression must be taken into account when converting f_H to $s\dot{V}_{O_2}$. On the basis of our data, this can be achieved by applying the following formula:

$$s\dot{V}_{O_2} = 0.0113 f_H - 0.2983 Q_{10}^{(T_b-27)/10} .$$

The increase in $s\dot{V}_{O_2}$ with elevated body temperature results from an increase in f_H, with no significant change in mass-specific oxygen pulse (sO_2 pulse; cardiac stroke volume times the difference in oxygen content between arterial and mixed venous blood). However, during exercise at both temperatures, increases in f_H are insufficient to provide all of the additional O_2 required and there are also significant increases in the sO_2 pulses. This creates the situation whereby the same f_H at the two temperatures can represent different values of $s\dot{V}_{O_2}$.

Key words: heart rate, rate of oxygen consumption, exercise, Galapagos marine iguana, *Amblyrhynchus cristatus*.

Introduction

The maximisation of net energy gain during a particular activity, such as foraging, has been used to explain some behavioural patterns of animals in their natural environment (Charnov, 1976). However, as pointed out by Speakman (1997), the energetics explanation of observed traits must be supported by measurements of rate of energy expenditure. Also, many models that estimate the food consumption of populations are based on energetics (Weins, 1984; Lavigne et al., 1985). Thus, accurate estimates of the rates of energy expenditure of animals in the field are essential components of many behavioural and ecological studies. Field metabolic rate (FMR) of many species of air-breathing vertebrates has been determined by the doubly labelled water method (DLW; see Nagy et al., 1999). This method estimates the rate of CO_2 production ($\dot{V}_{CO_2}$) and is relatively easy to use in the field. However, the DLW method only provides an average value for $\dot{V}_{CO_2}$ over the duration of the experiment, which is itself constrained by the biological half-life of ^{18}O (Nagy, 1983). Thus, it is not easy to obtain estimates of rates of energy expenditure for specific activities such as foraging, breeding, etc. Heart rate (f_H) may also be used as an indicator of metabolic rate (Butler, 1993). This method is based on the relationship between rate of oxygen consumption ($\dot{V}_{O_2}$) and f_H, as described by Fick's convection equation for the cardiovascular system:

$$\dot{V}_{O_2} = f_H \times V_S(C_{aO_2} - C_{\bar{v}O_2}) , \quad (1)$$

where V_S is cardiac stroke volume, C_{aO_2} is the oxygen content of arterial blood and $C_{\bar{v}O_2}$ is the oxygen content of mixed venous blood. If $V_S(C_{aO_2}-C_{\bar{v}O_2})$, the oxygen pulse ($sO_2$ pulse), remains constant or varies systematically, there is a linear relationship between $\dot{V}_{O_2}$ and f_H. Clearly, the usefulness of the f_H method for estimating FMR depends on the relationship between $\dot{V}_{O_2}$ and f_H being similar under different conditions,

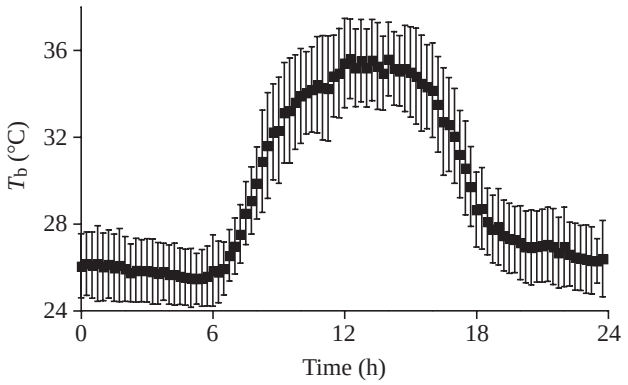

Fig. 1. Daily body temperatures T_b (means ± S.D.) of Galapagos marine iguanas measured in the field by radiotelemetry during the summers of 1991/92 and 1992/93. The data were collected every 15 min and are from 27 animals in 91/92 and 10 animals in 92/93. The transmitters were implanted for 14–81 days (M. Wikelski and F. Trillmich, unpublished data).

or necessitates any differences to be quantified and taken into account. Ideally, therefore, the relationship should be determined for animals experiencing different physiological conditions (see, for example, Green et al., 2001; Froget et al., 2001). Studies on birds have demonstrated that this method is at least as accurate as the DLW method (Nolet et al., 1992; Bevan et al., 1994, 1995a; Hawkins et al., 2000) and recent advances in the miniaturisation of electronic circuits have made it possible to record f_H in free-ranging birds and mammals over long periods (Woakes et al., 1995; Bevan et al., 1995b; Butler et al., 1998; Boyd et al., 1999).

Heart rate has not previously been used to predict FMR of reptiles. In these animals, the relationship between $\dot{V}_{O_2}$ and f_H may be complicated by variations in metabolic rate associated with changes in ambient temperature and the possible variation in shunting between the left and right sides of the heart. Thus, the aim of the present study was to determine the relationship between $\dot{V}_{O_2}$ and f_H in a reptile at two different temperatures that represent the extremes of its average daily range during summer (Fig. 1). Using implantable heart rate data loggers (HRDL; Woakes et al., 1995), this relationship will be used in ongoing field studies employing HRDLs to determine FMR and the energetic costs of specific behaviours (see Fig. 4).

The reptile chosen was the Galapagos marine iguana, *Amblyrhynchus cristatus*. This is the only lizard that dives beneath the sea to feed and whose food is primarily marine algae. There appears to be a size limit to these animals that is related to the availability of food, with smaller animals out-competing larger ones when food availability declines (Wikelski et al., 1997). During particularly lean (El Niño) years, some animals 'shrink' (reduction in body length as well as in body mass), and those that 'shrink' the most survive the longest (Wikelski and Thom, 2000). On top of this is the cost of reproduction. Females actively choose mates (Wikelski et al., 2001) and suffer a cost of reproduction in terms of a lower probability of survival during the following season (Laurie, 1989). Thus, there are many potential applications for the f_H method in this species, which occupies an unusual niche for a lizard, in order to determine the energy costs of specific behaviours.

Materials and methods

This study was performed on seven marine iguanas *Amblyrhynchus cristatus* Bell at the Darwin Research Station, Santa Cruz Island (SC iguanas), and on four iguanas on board the vessel *Quest* anchored off the Island of Santa Fé, Galapagos Islands, Ecuador (SF iguanas). The iguanas were captured by noosing or by hand and held in captivity for no longer than 48 h before being used. The body mass (mean ± S.D.) of the SC animals was 1.39±0.31 kg (see Table 2 for individual values), while that of the SF animals was 1.35±0.55 kg. Heart rate data loggers were implanted into the abdominal cavity of the iguanas while they were anaesthetised with a mixture of Isoflurane (Abbott Laboratories, USA) and air. Upon exposure to the mixture, the iguanas usually stopped breathing for several minutes. However, once they started to inhale the Isoflurane, they immediately became deeply anaesthetised. They were then positioned upside down between two foam pads. The skin and underlying muscles were opened along a 3.5–4 cm long incision, which was approximately 2 mm off the ventral midline, in order to avoid a small vein.

The sterilised loggers were inserted with one electrode close to the heart and the other laying along the side of the HRDL. The body of the HRDL was fixed in place with two sutures of surgical silk (#2, Dexon, Germany) through the body wall. The logger incorporated a low-power radio frequency transmitter which emitted a short pulse on each QRS wave of the electrocardiogram (ECG). Detection of this signal by a radio receiver indicated when the electrodes were in the correct position. The muscle layer and skin were individually closed together with dissolvable surgical suture (#1, Dexon, Germany) and an antibiotic/antifungal spray (Chloromycetin, Parke Davis, USA) was used on the wound after surgery. The animal was then taken off Isoflurane and woke within 5 min. The iguanas were left for at least 24 h before they were used in an experiment.

To determine whether iguanas suffered from post-surgical stress after the implantation of data loggers, we took a blood sample from the tail vein of four implanted females approximately 24 h after surgery and of six control females that had not undergone surgery. Plasma corticosterone levels were determined using a standard radioactive immuno-assay procedure (Romero and Wikelski, 2000). There was no difference in the levels of corticosterone between the two groups (implanted, 6.3±1.8 ng ml^{-1}; control, 5.4±1.4 ng ml^{-1}; means ± S.D., *t*-test, P=0.39). Thus, we conclude that iguanas do not suffer post-surgical trauma that would be indicated by an increased corticosterone level compared to that of controls. None of the animals showed signs of infection or discomfort and all data loggers were removed 3 days after implantation. All the animals survived the surgical procedures and four of the SC animals were seen 9 months later at the location where they had been caught and released.

The animals were studied either in the early morning or early afternoon when their body temperatures were at approximately 27 °C or 36 °C, respectively. When required, the use of a refrigerator or an infrared lamp enabled us to maintain the animals at these temperatures. Body temperature was determined by a thermocouple placed 3–4 cm into the cloaca. Once body temperature had been maintained close to the required value for at least an hour, the animal was fitted with a loop aerial on the top of its body. This enabled the transmitted heart beat signal from the implanted data logger to be detected by a radio receiver and the output from the receiver to be sent to a pre-amplifier (Isleworth, Electronics, England, model A101) and the signal appropriately filtered.

A transparent mask constructed from a plastic water bottle was placed over the head of the animal and held in place with a rubber collar around the neck. An airtight seal between the collar and the skin of the iguana was achieved with a layer of quick-setting, non-toxic polyether material (Impregum, ESPE Dental AG, Germany). The mask was fitted with inlet and outlet tubes through which air was drawn at a rate of approximately 2.6 l min^{-1} STPD by a pump (Reciprotor, Denmark, model 506R) on the outlet side. The air flow rate was set and monitored by a mass flow meter and controller (Sierra, models 840L and 902C). A subsample of the air leaving the pump was passed through a drying column (Drierite, Hammond) and analysed for the fractional content of O_2 and CO_2 by a gas analyser (ADInstruments, model ML205). The gas analyser was calibrated with room air and was accurate to 0.01 % for both gases. Outputs from the ECG pre-amplifier and gas analyser were collected at 1 kHz (Powerlab 800, ADInstruments) and displayed on a computer using Chart software (ADInstruments) as heart rate and rate of oxygen consumption. Rate of oxygen consumption was determined from the airflow through the mask and the difference between incurrent and excurrent fractional concentrations of dry air following consideration of respiratory quotient (RQ)-related errors (see Appendix in Frappell et al., 1992).

After instrumentation, the iguanas were placed on a variable-speed treadmill (1.2 m long and 0.5 m wide) and allowed to settle for at least 30 min, when f_H and $\dot{V}_{O_2}$ had reached steady (pre-exercise) values. They were then run at the maximum speed that they could comfortably maintain for a few minutes (maximum exercise). Although this included bursts of locomotion, the animals were not run to exhaustion. When an iguana no longer wanted to run, the treadmill was stopped and recordings continued for approximately 60 min during the recovery phase (see Gleeson, 1980, for recovery times of $\dot{V}_{O_2}$ after exhaustive exercise in marine iguanas). Rate of oxygen consumption and f_H data were obtained from each animal during the pre-exercise period, at maximum exercise (one datum point at each) and at four approximately equally spaced points during recovery. Data were averaged over 30–60 s. At the Darwin Station, each iguana was run, in random order, at body temperatures of 27 °C and 36 °C. On board the *Quest*, the animals were run only at a body temperature of 36 °C and values of f_H and $\dot{V}_{O_2}$ were recorded only during the pre-exercise period and at maximum exercise. All values of $\dot{V}_{O_2}$ are at standard temperature and pressure, dry (STPD).

Least-squares regressions were used to determine the relationships between f_H and $\dot{V}_{O_2}$ for individuals and for the group data at the two different temperatures for the SC animals. Regression equations were compared using an analysis of variance (ANOVA) general linear model (GLM; Zar, 1984) and, after testing for normality (Kolomogorov–Smirnov test), a Student's *t*-test was used to compare the significance of any difference between the means of two populations. When more than two means were compared, a repeated-measures ANOVA was used with two grouping factors (location and level of exercise). *Post-hoc* modified *t*-tests with Bonferroni corrections were used to test for differences between the various factors.

Table 1. *Mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$), heart rate (f_H) and mass-specific oxygen pulse (sO_2 pulse) of marine iguanas during pre-exercise and maximum exercise at 27 °C and 36 °C*

	Body temperature T_b			
	27 °C		36 °C	
	Pre-exercise	Maximum exercise	Pre-exercise	Maximum exercise
Santa Cruz animals				
$s\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	0.09±0.02	0.47±0.07†	0.14±0.03*	0.68±0.06*,†
f_H (beats min^{-1})	32.3±4.8	60.4±4.2†	62.7±5.5*	106±3.7*,†
sO_2 pulse (μl g^{-1} beat^{-1})	0.047±0.010	0.130±0.018†	0.037±0.008	0.107±0.007*,†
Santa Fé animals				
$s\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	–	–	0.15±0.04	0.60±0.09†
f_H (beats min^{-1})	–	–	57.4±1.0	99.3±7.6†
sO_2 pulse (μl g^{-1} beat^{-1})	–	–	0.044±0.011	0.102±0.016†

Values are means ± S.D.

N=7 marine iguanas from Santa Cruz Island, Galapagos, *N*=4 for iguanas from Santa Fé Island, Galapagos.

*Denotes a significant difference between comparable variables at 27 °C and 36 °C.

†Denotes a significant difference between pre-exercise and maximum exercise at a given temperature.

Two means were considered to be significantly different when $P<0.05$ and are quoted at the level at which they were found to be significant. All mean values are given ± S.D.

Results

The mean values of f_H, mass-specific $\dot{V}_{O_2}$ ($s\dot{V}_{O_2}$) and mass-specific oxygen pulse (sO_2 pulse, see equation 1) for animals during the pre-exercise period and at maximum levels of exercise at the two different body temperatures (T_b) are given in Table 1. In all animals, both f_H and $s\dot{V}_{O_2}$ exhibit significant increases over their pre-exercise values at maximum levels of exercise. For the SC animals at 27 °C, $s\dot{V}_{O_2}$ increased 5.2-fold, whereas there was only a 1.9-fold increase in f_H. This means that there was a 2.8-fold increase in sO_2 pulse. The comparable factorial increases at 36 °C were: $s\dot{V}_{O_2}$, 4.9-fold; f_H, 1.7-fold and sO_2 pulse, 2.9-fold. Similar factorial increases were seen in the SF animals at 36 °C.

In SC animals, pre-exercise f_H was 94 % higher at a T_b of 36 °C compared with that at 27 °C, which is equivalent to a Q_{10} of 2.1. The comparable pre-exercise values for $s\dot{V}_{O_2}$ were 55 % higher, equivalent to a Q_{10} of 1.6. The maximum f_H value recorded during exercise was 75 % higher at a T_b of 36 °C compared with that at 27 °C, which yields a Q_{10} of 1.9. The comparable value for maximum $s\dot{V}_{O_2}$ during exercise was 45 % higher, equivalent to a Q_{10} of 1.5. Mass-specific oxygen pulses during the pre-exercise period were not significantly different at 27 °C and 36 °C (P=0.11) whereas sO_2 pulse during maximum exercise at 27 °C was significantly (21 %) greater than that at 36 °C (P=0.02). There was no significant difference between the values obtained from the SC and SF animals at 36 °C during the pre-exercise period and at maximum exercise for f_H, $s\dot{V}_{O_2}$ and sO_2 pulse.

Heart rate and $s\dot{V}_{O_2}$ were well correlated in each individual SC iguana (Table 2) and the relationships were well described by a linear function. Analysis of covariance (ANCOVA) was used to compare the values of the intercepts (a) and slopes (b) of the individual regressions within each group (i.e. at 27 °C and at 36 °C). While there was no significant difference between the slopes, there were significant differences between the intercepts. Thus, the intercepts were regarded as a random sample from a distribution of intercept values and a random-effects model was adopted (see Green et al., 2001). The group regression equations derived in the present study are:

$$s\dot{V}_{O_2} = (0.013\pm0.0009)f_H - 0.361\pm0.048 \quad (2)$$

for animals at 27 °C (N=7, r^2=0.86, P=0.018) and

$$s\dot{V}_{O_2} = (0.011\pm0.0006)f_H - 0.561\pm0.058 \quad (3)$$

for the same animals at 36 °C (N=7, r^2=0.91, P=0.015), where

Table 2. *Individual regression equation variables for mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) against heart rate (f_H) for seven marine iguanas from Santa Cruz Island, Galapagos, at 27 °C and 36 °C*

Iguana number	Mass (kg)	N	Variable							
			a	b	r^2	S_b	S_a	$S_{y.x}$	$\bar{X}$	P
Iguanas at 27 °C										
Ig1	1.35	6	–0.5644	0.0175	0.8131	0.0042	0.2038	0.0965	47.667	0.0014
Ig2	1.73	6	–0.1479	0.0090	0.9600	0.0009	0.0399	0.0249	42.167	0.0143
Ig3	1.15	6	–0.3824	0.0136	0.9931	0.0006	0.0263	0.0138	45.333	0.0004
Ig4	1.18	6	–0.2945	0.0122	0.9552	0.0013	0.0658	0.0351	48.667	0.0022
Ig5	1.38	6	–0.5175	0.0142	0.8672	0.0028	0.1563	0.0546	55.667	>0.0001
Ig6	1.90	6	–0.2883	0.0116	0.8389	0.0026	0.1128	0.0542	43.333	>0.0001
Ig7	1.05	6	–0.4526	0.0145	0.8930	0.0025	0.1302	0.0624	51.000	0.0229
Pooled regression		42	–0.2983	0.0117	0.7945	0.0009	0.0459	0.0651	47.690	>0.0001
Group regression		7	–0.3610	0.0130	0.8598	0.0009	0.0480	0.0571	47.690	0.0180
Iguanas at 36 °C										
Ig1		6	–0.5908	0.0117	0.9397	0.0015	0.1277	0.0622	84.167	0.0140
Ig2		5	–0.3777	0.0093	0.8979	0.0018	0.1493	0.0841	80.000	0.0006
Ig3		6	–0.5613	0.0110	0.9680	0.0010	0.0855	0.0443	83.167	>0.0001
Ig4		6	–0.7883	0.0138	0.9240	0.0020	0.1791	0.0664	89.333	0.0008
Ig5		6	–0.6607	0.0127	0.9742	0.0010	0.0885	0.0331	84.500	>0.0001
Ig6		6	–0.5712	0.0112	0.9906	0.0005	0.0432	0.0174	78.167	0.0103
Ig7		6	–0.5549	0.0099	0.7633	0.0028	0.2499	0.1073	89.000	0.0045
Pooled regression		41	–0.5390	0.0108	0.8608	0.0007	0.0599	0.0737	84.146	>0.0001
Group regression		7	–0.5606	0.0111	0.9060	0.0006	0.0577	0.0639	84.146	0.0150

The form of the equation is: $s\dot{V}_{O_2} = a + f_H \times b$.

N=number of data points for each regression, a=intercept, b=slope of the regression (regression coefficient), r^2=coefficient of determination, S_b=standard error of b, S_a=standard error of a, $S_{y.x}$=standard error of estimate, $\bar{X}$=mean value of f_H (heart rate).

The pooled and group regressions for the data at each temperature are also given.

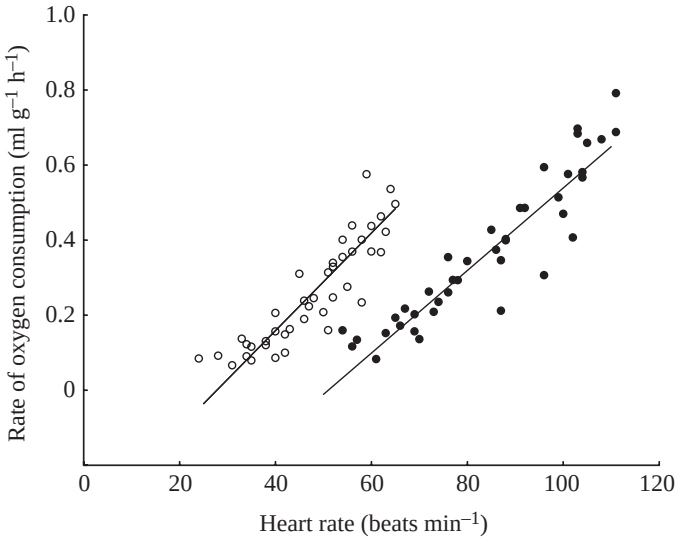

Fig. 2. The relationship between mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) for seven marine iguanas from Santa Cruz Island, Galapagos, at 27 °C (open circles) and 36 °C (filled circles). The group regression equations are: $s\dot{V}_{O_2}=0.013f_H-0.361$, $r^2=0.86$, and $s\dot{V}_{O_2}=0.011f_H-0.561$, $r^{2=}0.91$, respectively.

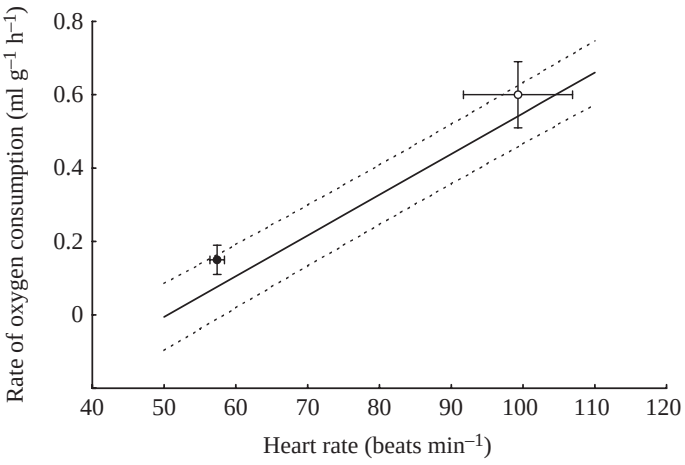

Fig. 3. The regression line for mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) plotted against heart rate (f_H) for seven marine iguanas from Santa Cruz Island, Galapagos, at 36 °C (see Fig. 2) together with the 95 % prediction intervals when $s\dot{V}_{O_2}$ is estimated from four measurements of f_H from four animals. Also included are the values (mean ± S.D.) for $s\dot{V}_{O_2}$ and f_H from four marine iguanas from the island of Santa Fé, Galapagos, during the pre-exercise period (filled circle) and at maximum exercise (open circle) at 36 °C.

$s\dot{V}_{O_2}$ is in ml g^{-1} h^{-1} and f_H is in beats min^{-1}. These regression lines with their individual data points are illustrated in Fig. 2. The slopes of these lines are not significantly different from each other (P=0.10), but the intercepts are (P=0.003).

The intercept is a random factor, so if one of the above equations is to be used to estimate $s\dot{V}_{O_2}$ from an average value of f_H measured in the field, the usual method for estimating the standard deviation (σ) of an estimate using regression equations (see equation 17.28 in Zar, 1984) has to be modified, as indicated in equation 11 of Green et al. (2001):

$$\sigma = \sqrt{d^2\left(\frac{1}{n_1}+\frac{1}{n_3}\right)+e^2\left[\frac{1}{n_2}+\frac{1}{n_4}+\frac{(X_i-\bar{X})^2}{\sum x^2}\right]}, \quad (4)$$

where d^2 is the error associated with the variation between the intercepts of the individual regression lines for the calibration (SC) iguanas, n_1 is the number of iguanas used in the calibration process, n_3 is the number of iguanas from which the field value of heart rate was obtained, e^2 is the error associated with the scatter around the regression line (residual mean square), n_2 is the total number of data points in the regression, n_4 is the number of data points from which the field value of heart rate was obtained, $\bar{X}$ is the mean value of all the heart rates used in the regression, X_i is the average value of heart rate from the field and $\sum x^2$ is the sum of squares (SS_x) of heart rate values used in the regression. If the values of n_3 and n_4 for the data obtained at 36 °C are set to 4, which represents the number of animals and of data points, respectively, from the SF iguanas, then equation 4 can be used to produce the 95 % prediction intervals for the regression under these conditions (Fig. 3). Also in Fig. 3 are the values (mean ± S.D.) of f_H and $\dot{V}_{O_2}$ for the four SF animals during the pre-exercise period and at maximum exercise, and it can be seen that they fall within the 95 % prediction intervals of the regression line.

Discussion

In order to be able to use f_H for the estimation of $\dot{V}_{O_2}$ in the field, it is important that the calibrations are performed under conditions that are representative of those in the natural environment. Fig. 1 shows mean daily variation in T_b from animals in the field during two consecutive summers and the two values of temperature that we used in the present experiments were chosen on the basis of these data. Iguanas, like many other species of lizards, often use relatively short bursts of locomotion. For example, most (>95 %) marine iguanas forage in intertidal areas and make repeated short forays during which they run to particular foraging sites, take a few bites of algae, and run back to safe places to escape huge waves (Wikelski and Trillmich, 1994). Similarly, iguanas walk in short bouts from coastal resting areas towards foraging sites. Each walking bout consists of approximately 20–40 fast steps, with interspersed resting pauses (seconds to minutes; Wikelski and Hau, 1995). Likewise, iguana males engage in relatively fast head-bob walking bouts during territorial activities, again with intermittent resting phases. During the mating season, females are constantly harassed by satellite males and have to run away and struggle away from males several times every hour (Wikelski et al., 1996, 2001; Wikelski and Bäurle, 1996). Nesting females engage in bouts of intense digging activities lasting several minutes at a time. Even during diving bouts,

marine iguanas are only active for a short time while grazing under water for an average of 2–5 min. Animals then resurface and remain floating largely motionless before diving again or swimming back to shore (Wikelski and Trillmich, 1994; Drent et al., 1999). Thus, the inclusion of data during the recovery period after activity is important if the energy cost of a particular behaviour is to be determined in the field (Scholnick and Gleeson, 2000).

Perhaps because of their propensity for short bursts of locomotion, we did not find it easy to persuade all the animals to walk/run at speeds below the maximum speed they could maintain for a few minutes. Nonetheless, we were successful in achieving this with three animals at both temperatures and there was no significant difference between the slopes of the regression lines of data obtained from animals during the pre-exercise period and when walking/running at different speeds, and data obtained during the recovery period (P=0.67 at 27 °C and 0.07 at 36 °C, Fig. 4). Thus our experimental procedures simulated as closely as possible what is known for marine iguanas exercising in the wild and we are confident that the regression lines given in Fig. 2 represent both exercise at different levels and recovery from maximum exercise.

The $s\dot{V}_{O_2}$ data that we obtained are similar to those obtained by other workers studying the Galapagos marine iguana (Bennett et al., 1975, who electrically stimulated the animals in order to obtain activity; Bartholomew and Vleck, 1979; Gleeson, 1979, 1980), with the exception of pre-exercise $s\dot{V}_{O_2}$ at 27 °C, where our mean value is approximately twofold greater than the 'resting' values obtained by the above workers. On the other hand, our mean value for pre-exercise f_H at 27 °C is within the range given by Bartholomew and Lasiewski (1965), but our mean value for pre-exercise f_H at 36 °C is approximately 75 % of that reported by the latter authors while the iguanas were being heated and cooled. As far as we can determine, there are no values in the literature for f_H of marine iguanas during exercise.

It would appear from the present study that it should be possible to use f_H as an indicator of $s\dot{V}_{O_2}$ for iguanas in the field, as between 86 and 91 % of the variation in the latter could be explained by the fitted regressions from the calibration experiments. The utility of the f_H method for the estimation of $s\dot{V}_{O_2}$ in the marine iguana was further supported by the fact that the mean values of $s\dot{V}_{O_2}$ from four animals from a population different from that involved in producing the calibration equations were within the 95 % prediction intervals of the regression. However, the effect of temperature is to vary the intercept of the relationship between the two variables, rather than to extend a single regression line (see Fig. 2).

It is clear from the field data that have been obtained so far (M. Wikelski and A. J. Woakes, unpublished data) that temperature can change by between 6–12 °C or so within 1–3 h, particularly when the animals are foraging so, during such periods estimations of $s\dot{V}_{O_2}$ from f_H will have to involve adjustments to equations (2) and (3) based on the Q_{10} values determined from the data from the SC animals (Table 1). A

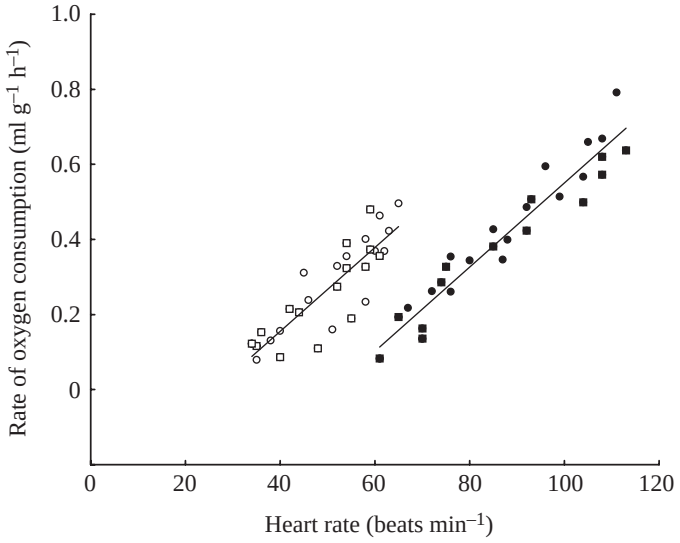

Fig. 4. The relationship between mass-specific rate of oxygen consumption and heart rate for three marine iguanas from Santa Cruz, Galapagos at 27 °C (open symbols) and 36 °C (filled symbols). The squares represent data obtained from animals during the pre-exercise period and while walking/running at different speeds, while the circles represent data obtained from the animals during recovery from the preceding exercise (see text for further details). The lines represent the least-squares regressions through all of the data points. At 27 °C, $s\dot{V}_{O_2}$=0.011f_H–0.240, r^2=0.76. At 36 °C, $s\dot{V}_{O_2}$=0.011f_H–0.572, r^2=0.92.

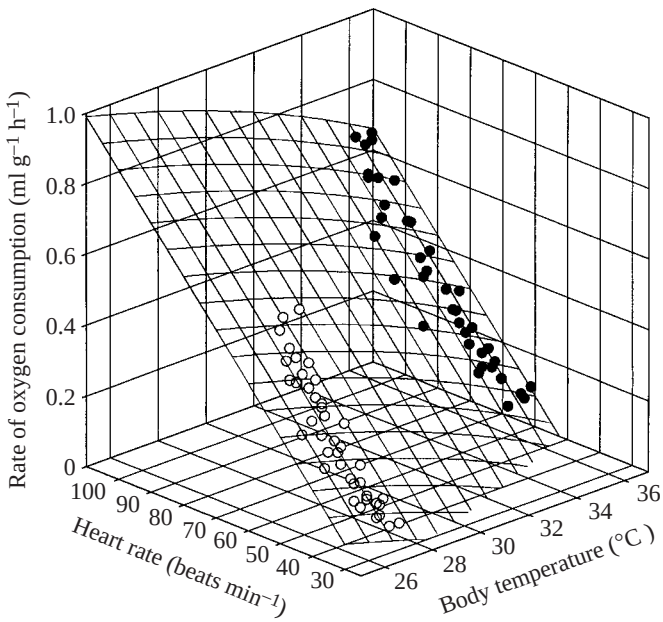

Fig. 5. The relationships between mass-specific rate of oxygen consumption, heart rate and body temperature for marine iguanas (N=7). Data are from Fig. 2. The mesh is described by the equation: $s\dot{V}_{O_2}$=0.0113f_H–0.2983$Q_{10}^{(T_b-27)/10}$ (see text for further details).

convenient way of incorporating temperature as an influence on $s\dot{V}_{O_2}$ is to include Q_{10} in the regression. There are linear relationships between $\log\dot{V}_{O_2}$ and T_b for resting and active *Sauromalus hispidus* (an iguanid) and the marine iguana between 25 and 35 °C (Bennett, 1972; Bennett et al., 1975) and between $\log f_H$ and T_b for resting and active *S. hispidus* between the same temperatures (Bennett, 1972). Thus, it is assumed that there are also linear relationships between $\log f_H$ and T_b for resting and active marine iguanas between 27 and 36 °C. Given that there is no difference between the slopes of the pooled regression equations (Table 2), the relationship between the two lines can be ascribed to a Q_{10} effect, where the Q_{10} can be determined from the intercepts. In the present case Q_{10}=1.93, which is similar to the Q_{10} values derived from the pre-exercise and maximum f_H values in Table 1. If the average slope of the pooled regression equations for the two temperatures is used (see Table 2), the relationship between $s\dot{V}_{O_2}$, f_H and T_b is shown in Fig. 5 and is described by the following equation:

$$s\dot{V}_{O_2} = 0.0113 f_H - 0.2983 Q_{10}^{(T_b-27)/10}. \quad (5)$$

The standard error of the estimate can be determined from equation 17.28 in Zar (1984).

An interesting aspect of the present data is the fact that the increase in $s\dot{V}_{O_2}$ in response to an increase in T_b is the result of an increase in f_H, with no significant change in sO_2 pulse. However, during exercise at both temperatures, the increases in f_H are insufficient to provide all of the additional O_2 required and there are significant increases in the sO_2 pulses. Consequently, the situation arises (as illustrated in Fig. 2) whereby an f_H value of around 60 beats min^{-1} is related to an $s\dot{V}_{O_2}$ value of approximately 0.4 ml g^{-1} h^{-1} at 27 °C, when the animal is exercising maximally, and to an $s\dot{V}_{O_2}$ value of approximately 0.1 ml g^{-1} h^{-1} at 36 °C during the pre-exercise period. This means, of course, that the sO_2 pulse is fourfold greater during the former than during the latter. It is apparent from equations 1–4 and 6–9 and Fig. 9 of Bennett (1972) that a similar phenomenon occurs in *S. hispidus* and in *Varanus gouldii*, when activity is the result of electrical stimulation, although in the latter species, the values of f_H during 'exercise' at 27 °C and while at 'rest' at 36 °C do not actually overlap. On the basis of a study on *Iguana iguana* and *Varanus exanthematicus* at 35 °C, it would seem that the major contribution to the increase in sO_2 pulse during exercise is a twofold increase in Ca_{O_2}–$C\bar{v}_{O_2}$ (Gleeson et al., 1980).

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