RESEARCH ARTICLES

Effect of husbandry manipulations on respiratory rates in captive Bearded Dragons (*Pogona vitticeps*)

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ABSTRACT — The behaviour, body temperatures and respiratory rates of captive Bearded Dragons (*Pogona vitticeps*) were recorded during periods with handling and non-handling intervals. Respiratory rates increased at approximately equal rates with increasing body temperatures irrespective of whether the animals were handled or not. Respiratory rates increased significantly after handling but there were no significant differences in behaviour before and after handling. The implications of the results are discussed with regard to use of animals in captive husbandry and education programmes.

REPTILE behaviour is shaped by many factors; their genetic heritage, environments, and experiences among others, and these may interlink to define what are easy or difficult species to deal with in a husbandry context. Progress in reptile husbandry has now gone beyond basic techniques and in recent years has extended to asking questions about behavioural and physiological problems in captive conditions (Warwick et al, 1995). As captive breeding and rearing of reptiles becomes more common and perceived as both an aid to conservation and education, there is a need for an increased understanding of the subtler requirements of their maintenance in captivity and the frequency of their usage in demonstrations for educational purposes.

In this respect it is interesting that the response to captive conditions are varied and often surprising. For example the discovery of elevated body temperatures after husbandry manipulations in the teiid lizard *Callopistes maculatus* (Cabanac & Gosselin, 1993) which has been termed emotional fever, does not necessarily apply to all lizards; a recent study on the Australian Bearded Dragon (*Pogona vitticeps*) was unable to detect any significant influence of handling on thermoregulation (Cannon et. al., 2002). However the effects of handling on lizards may concern factors other than thermoregulation and we have expanded the question to test for the effects on respiratory rates in *P. vitticeps*. One of the benefits of this approach is that although respiratory frequency is irregular in lizards (Avery & D'Eath, 1986), it is readily observed and often elevates in response to external stimuli (Milsom, 1984; Avery, 1993) and hence it is a convenient, albeit indirect, method for determining stress as direct measurement requires the examination of hormone levels such as adrenalin and corticosteroids. We simultaneously measured behaviour and body temperatures to test for any possible influences they may also have had on respiration. The results are based on data collected by K.M. for a second year HND research assignment at Huddersfield Technical College supervised by R.M. but with additional data gathered by T.W.

METHODS AND MATERIALS

The observations were made between September 2002 and February 2003 on five adult *P. vitticeps* aged 3–4 years consisting of one male and four females. All animals were captive bred at the

	Before handling		No handling at all		After handling	
	Median	Q1 - Q3	Median	Q1 – Q3	Median	Q1 – Q3
Basking	37.5	25.0 - 70.0	20.0	3.3 - 53.5	66.6	20.0 - 73.3
Perching	40.0	28.4 - 52.5	73.3	34.0 - 79.9	40.0	13.3 - 80.0
Shade	5.0	1.25 - 27.5	13.3	6.6 - 23.3	1.0	0 - 10.4

Table 1. Behaviours of *P. vitticeps* with the results shown as the grand medians (with interquartile ranges Q1 - Q3) which have been calculated from the median percentage frequencies of individual lizards, i.e. *n* = 5.

college and had previously been employed in teaching programmes including demonstrations in animal handling. The data were usually collected once a week between 12:30 and 15:00 hours on a single enclosure measuring 2.24 m long and 0.94 m wide with a height of 1.77 m. The cage was exposed to natural sunlight as well as light provided by UV lamps and infrared heating lamps. The latter produced a temperature range of between 28 and 49°C. Body temperature, here determined as skin surface temperature, was measured using an Omega OS204 non-invasive digital thermometer by directing the instrument about 1– 5 cm from the surface of the skin. Skin surface temperature is in good agreement with cloacal temperature in lizards particularly in medium sized animals (Meek, 1999).

The observational conditions were 1) 30 minutes of continuous observation with no handling at any time, 2) 15 minutes of no handling with a 5 minute handling interval followed by 3) a further 15 minute period of no handling after the handling interval. The lizards were handled on a 1-week rotational basis, two females were handled during one session followed by a week of no handling with the other two females handled on the weeks when the others were not handled. The male was handled on alternate weeks. The

behaviours were: *basking*, positioned either in the sun or under a heat lamp which could be either on tree stumps or rocks; *perching*, positioned in an open area but not in sun or under a lamp; *hiding*, located down an under ground retreat area; *locomotory activity*, engaged in movement in any area of the enclosure. Counts of respiratory frequency were based on each complete cycle of ventilatory movements of the anterior rib cage (Milsom, 1984) lasting 1 minute at 5-minute intervals over the 15 or 30-minute observational periods.

The total data collected were 320 observations each on behaviour and body temperature and 320 one-minute observations periods of respiration. However, because of the degree of scatter in respiratory frequency when the animals were active these data have been eliminated from the main analysis and used only in calculating behaviour frequencies. Therefore observations of respiratory frequencies were 309. The method of analysis used here however was to consider each individual lizard as one data point based on the means of respiratory rates, body temperatures or the medians of individual percentage behaviours (Hurlbert, 1984). This considerably reduces the degrees of freedom and hence the possibility of committing a type II statistical error (Wilson & Dugatin, 1996) but it avoids overestimating the degrees of freedom, as would be the case if each measurement had been treated as an independent event (Lombardi & Hurlbert, 1996). Plus or minus values given are standard deviations.

RESULTS

Body temperature effects on respiration. To test for possible influences of body temperature on respiration it was found that the simplest method was to mathematically model the data sets and compare the rates of change. Since most physiological processes increase exponentially with temperature, body temperature (T_b) was treated as the independent variable x and the logarithmic transforms of respiratory rate as the dependent variable y giving an equation of the form:

$$y = ae^{bx}$$

where e is the base natural logarithm, b the exponential and a the y-intercept. Standard errors for b have been calculated using the method described by Bailey (1981). Figure 1 shows plots of the data on semi logarithmic coordinates and indicates increased respiratory frequency with increasing body temperatures, irrespective of whether the animals were handled or not. When the lizards were not handled (both no handling data sets pooled) the relationship was defined by:

$$Respiration = 1.548e^{0.06 \pm 0.01 Tb} \ n = 250 \ (1)$$

and after handling by:

$$Respiration = 6.594e^{0.034 \pm 0.02 Tb} \ n = 59 \ (2)$$

Equation (1) indicates a higher rate of increase in respiration with temperature but the standard errors attached to the exponentials are high in both equations particularly in equation (2) reflecting the amount of scatter. A test for differences between exponentials showed that they were not significantly different $t = 0.16$, $p > 0.05$ (Bailey, 1981) and therefore respiration increased with increasing temperature at similar rates.

Respiration rates. The mean respiratory rates of resting lizards are shown in Fig. 2 as box plots. The highest rates were from the animals after they were handled (grand mean = 20.6±1.0), before they were handled, grand mean = 14.1±1.2 and when they were not handled at any time 13.2±1.1. A test using ANOVA, $F_{(2,12)} = 64.61$, $p < 0.0001$ showed differences in the data sets. A two sample t-test with variances assumed unequal indicated
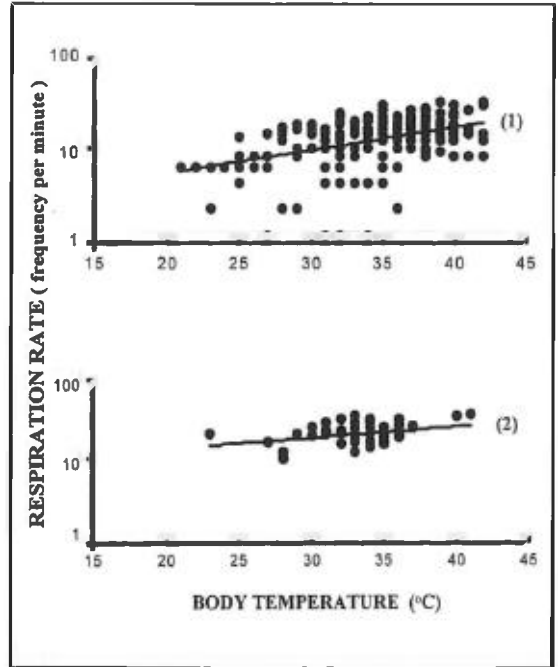

Figure 1. Graphs on semi logarithmic coordinates showing the relationship between respiratory rate and body temperature after handling (lower graph) and when not handled. The lines taken through the data were fitted using equations (1) and (2) given in the text. See text for a further explanation of these results.

that the differences were significant between respiration after the lizards were handled and the other two samples respectively: before they were handled, $t = 9.09$, $p < 0.0001$, $d.f. = 8$, not handled at any time, $t = 11.07$, $p < 0.0001$, $d.f. = 8$. These results support the validity of pooling both no handling data sets for calculating equation (1).

Behaviour related respiratory rates. To determine whether there were purely behaviour aspects to respiratory rates, the data were analysed in their behaviour subsets. We could examine only for the principal behaviours as these had the only useful sample sizes. The results showed that the mean was greater in perching after handling (18.4± 3.9 per minute) compared to perching before handling (12.8±1.1per minute) and not handled at any time (11.7±1.8 per minute). ANOVA detected differences in the data sets $F_{(2,12)} = 10.71$, $p =$
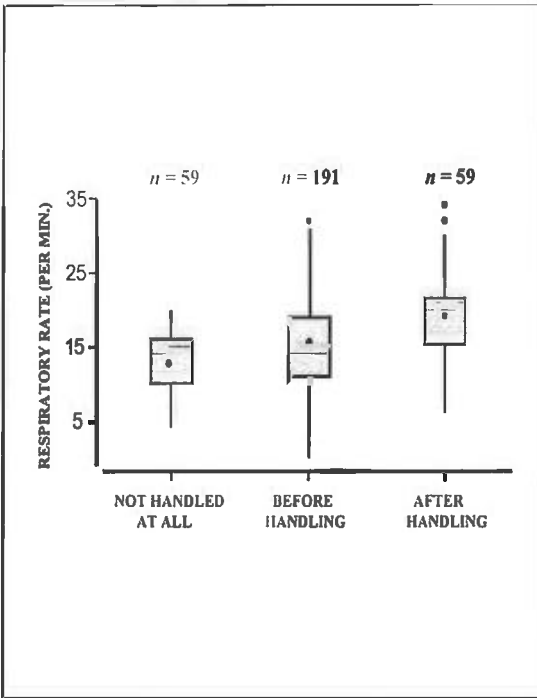
Figure 2. Box plots of respiratory rates of *P. vitticeps* when they were not handled at any time (left) immediately before (centre) and after they were handled. The boxes represent the interquartile ranges with the means shown as solid circles and medians as horizontal bars. The vertical lines either side of the interquartile ranges represent the general ranges of the data but solid circles indicate outliers – data that are between 1.5 to 3 times from the interquartile ranges.

0.002 and two sample *t*-tests with the variances assumed unequal showed the differences were between respiration rates after handling and the other two data sets: before handling, $t = 3.28$, $p = 0.03$, $d.f. = 4$, not handled at any time, $t = 3.65$, $p = 0.015$, $d.f. = 5$. Respiratory rates were greater in basking lizards after they were handled (mean = 20.2 ± 2.3) followed by before handling (16.3 ± 2.0) and when they were not handled at any time (14.4 ± 1.0). Again ANOVA detected differences in the data sets, $F_{(2,12)} = 12.18$, $p = 0.001$ and two sample *t*-tests showed the differences in respiratory rates were between after handling and immediately before handling ($t = 2.79$, $p = 0.027$, $d.f. = 7$) and after handling and not handled at any time ($t = 5.06$, $p = 0.004$, $d.f. = 5$).

Behaviour. We compared behaviour differences to determine if changes here could have been influencing respiratory rates. The data were converted to percentage frequencies of total behaviours and the results are shown in Table 1 as median percent values. Kruskal Wallis non –parametric tests showed no significant differences between the data sets; *Basking*, $H = 3.96$, *perching*, $H = 4.74$, shade, $H = 3.04$, all $p>0.05$ and at $3n = 15$. Activity levels were low during the study (from 1.6 to 4.5% of total behaviours) and not considered viable for statistical testing.

DISCUSSION

The present findings show a strong association between lizard handling and increased respiratory rates in *P. vitticeps*. The statistical methods employed, including the obvious differences in *y*-intercepts between equations (1) and (2) satisfactorily eliminated behaviour and body temperature as contributing effects. The results therefore indicate that the increased respiration rates were due to handling with the expectation that adrenalin secretion followed. Why should the findings be a consideration in reptile husbandry? Previous research has shown that elevated respiratory rates, through what has been described as handling stress, initiated dramatic hormone changes even in reptiles that are habituated to humans (Lance, 1992) resulting in reduced growth, suppressed reproductive capacity and susceptibility to infection (Stephens, 1980; Lillywhite & Gatten, 1995; Guillette et. al., 1995). Irrespective of the view that animals must be at some time be subject to some stress in their natural environments (Greenberg & Wingfield, 1987) and is an evolutionary mechanism for adaptation to changing environments (Guillette, 1985), it would appear nevertheless that elevated respiratory rates, particularly if handling is a frequent normal practice, is an important issue not only for animal welfare but also captive breeding programmes. What was perhaps unexpected was the failure of our *P. vitticeps* to habituate to frequent handling, in the sense that subjectively they appeared to be little disturbed by the experience. However, increased respiratory rates associated with sudden

stimuli – loud noises among other things, were observed in Lacertid lizards that had been in captivity for long periods (Avery, 1999) and prey capture probability also decreased with increasing respiratory frequency with a total absence of feeding at high respiratory movements (Avery, 1993).

A theoretical consideration is that being picked up and benignly handled is something that reptiles are unlikely to have experience of in natural conditions and therefore may have no inbuilt behavioural mechanisms, determined by evolutionary processes, that enable them to respond positively to the experience. Indeed contact, particularly with larger species, is something they would be expected to avoid based on evidence from observations on animals in both field and captive conditions. For example, free-living lizards that were awoken at night in their regular resting place avoided these spots on the following nights (Bowers & Burghardt, 1992) and captive Australian Treefrogs (*Litoria caerulea*) living in large naturalistic enclosures abandoned regular resting places after being handled (R. Meek, pers. obs.). This indicates the evolution of behaviours designed to avoid contact with other species, which must be adaptive in most circumstances – antipredation is one obvious example. Many reptiles held in private and education establishments are handled on an almost daily basis which may physiologically equate with frequent predator encounters, but there is no real evidence that reptiles are naturally subject to such high intensities of predator interaction and as such may be detrimental to long term well being.

It would appear then that the evidence suggests some species appear to be more affected by husbandry manipulations than others; an example is the different effects on thermoregulatory behaviour (e.g. Cabanac & Gosselin, 1993; Cannon et. al., 2002). Handling effects may very well depend on, among other things, the animal's natural lifestyle – species that employ flight as an escape tactic rather than crypsis could be more susceptible to stress through human contact. This could be a useful area for further investigation and a key factor to be taken into account in respect to research or educational programmes and relevant since practical husbandry courses on reptiles are a high growth area in UK education.

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