Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12004

ORIGINAL ARTICLE

Gut and intestinal passage time in the Rainbow Skink (*Trachylepis margaritifer*): implications for stress measures using faecal analysis

A. K. Miller, B. F. N. Erasmus and G. J. Alexander

School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

Keywords
corticosterone metabolites, conservation physiology, barium meal

Correspondence
A. K. Miller, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, PO Box 324, Jukskei Park, Johannesburg 2153, South Africa. Tel: +27 (0)82 375 2205; Fax: +27 (0)11 704 2358
E-mail: ashadee.k.miller@gmail.com

Received: 17 April 2012;
accepted: 20 August 2012

Summary

Stress levels in organisms provide a rapid measure for assessing population health. Handling and capture stress, however, cause error in blood measures, so this method is rapidly being replaced by assessing levels of stress metabolites in faeces. This eliminates the source of error because there is a lag period between stress perception and the resultant stress metabolite accumulation within faeces. This lag period is correlated with specific intestinal passage time, a measure that can vary greatly between taxa, particularly amongst ectotherms. Due to two deleterious consequences associated with extended exposure of the metabolites to the intestinal environment, species that exhibit long and variable intestinal passage times are not good candidates for metabolite studies. We measured gut and intestinal passage times in *Trachylepis margaritifer* to ascertain whether it would be an appropriate candidate for stress metabolite studies. We first tested if barium sulphate in the meal had an effect on gut passage time at three ambient temperatures (25, 27 and 32 °C). Barium sulphate had no effect; however, temperature had a significant effect with an unexpected pattern: gut passage time was fastest at 32 °C but was slower at 27 °C than at 25 °C. We then used X-ray technology and barium sulphate-loaded meals to measure gut and intestinal passage times at 25 and 27 °C. This allowed us to observe which parts of the digestive process were responsible for increased passage times at 27 °C: the faster passage time at 25 °C was due to faster intestinal passage time; there was no difference in gastric emptying time. We assess the species to be a suitable candidate for studies using faeces to measure stress. It is imperative however, that the effect of temperature on passage rates is known and taken into account in such studies.

Introduction

Stress metabolite studies are growing in popularity as a result of the advantages of being able to measure stress levels using only faecal samples (Stevenson et al., 2005). The method is non-invasive, so sample collection is relatively easy, with minimal disturbance to the subject. Even in situations where samples are actively collected from captured animals (e.g. through abdominal massage), the stress of capture and handling will not immediately reflect in samples due to the time that the stress metabolites take to accumulate in the faecal matter. Handling stress can affect experimental results (Halberg et al., 1960; Quirce and Maickel, 1981; Riley, 1981) and is a common source of error when sampling blood (Hennessy and Levine, 1976; Gärtner et al., 1980; Armario et al., 1986; Haemisch et al., 1999). Blood

samples are also less immune to episodic fluctuations in hormone levels than faecal samples are; blood samples reflect an organism's hormonal status at a single point in time (acute stress).

Stress metabolites such as glucocorticoids are ever-present at baseline levels within all vertebrates (Wingfield et al., 1997; Moore and Jessop, 2003; Wikelski and Cooke, 2006), and only deviations from these species-specific norms indicate stress. In addition to this, glucocorticoids levels may exhibit regular or episodic changes over time. However, the use of metabolite measures made from faecal samples avoids this complication by acting as a proxy for stress levels over a particular window period (Touma et al., 2004). As stress metabolites continue to accumulate in the faeces, whilst the faeces remain in the gut, intestinal passage time determines this window period and this may vary greatly between species (Möstl and Palm, 2002). Thus, without information on passage time, the potential to categorically identify stressors through matching increased metabolite levels to a particular event is greatly impaired.

Intestinal passage time is therefore a necessary metric for data interpretation and can also determine whether a species is a suitable candidate for faecal sampling for stress metabolites. Ideal candidates are those that have relatively short and invariant intestinal passage times because there are two problems associated with extended and highly variable passage times. First, metabolites continue to accumulate within faeces for as long as the faeces are present within the intestinal tract. Thus, metabolite levels within samples collected from subjects with extended and variable passage times may have accumulated over several stress-inducing events and the relative production of stress metabolites cannot be apportioned separately to these events. Second, with extended exposure to anaerobic bacteria within the gut, the structural integrity of these metabolites becomes increasingly threatened (Möstl and Palm, 2002). Therefore, the metabolite levels can not only reflect multiple stressful events, but may also degrade through time.

Endotherms generally have relatively short and consistent intestinal passage times across taxa; in mice, it is 9–10 h (Touma et al., 2004), whilst sheep, ponies and pigs have intestinal passage times of 12, 24 and 48 h respectively (Möstl and Palm, 2002). Ectotherm physiological performance is greatly affected by external factors (especially temperature) and life history (Dawson, 1975; Stevenson et al., 1985; Dorcas et al., 1997; Shine, 2005; Pafilis et al., 2007), and there is thus great variation in gut passage times (Lillywhite et al., 2002). Gaboon Adders (*Bitis gabonica*) have gut passage times of up to 183 days, whilst Burmese Pythons (*Python bivittatus*; as *Python molurus*), Western Ratsnakes (*Pantherophis obsoletus* as *Elaphe obsoleta*), Madagascan Speckled Hognose Snakes (*Leioheterodon geayi*) and Emerald Tree Boas (*Corallus caninus*), all under the same standard conditions, have gut passage times of 35.4, 2.6, 11.6 and 25.4 days respectively (Lillywhite et al., 2002). Alexander et al. (2012) report gut passage times of between 3.9 and 5.2 days, depending on temperature, for Rinkhals (*Hemachatus haemachatus*). In addition to this, the long gut passage times of stocky, terrestrial snakes also tend to be very variable (Lillywhite et al., 2002). This variation emphasizes that care must be taken when selecting reptilian candidates for stress metabolite studies.

To our knowledge, no literature on gut or intestinal passage times exists for any scincid lizard; the majority of studies on lizards focus on herbivorous species (i.e. Iguanas), with fewer than twenty studies focusing on carnivorous and insectivorous species. The digestion rates of herbivorous lizards are known to be much slower and their intestine length longer in comparison with carnivorous and insectivorous species (Secor, 2005; Diaz-Figueroa and Mitchell, 2006). The few studies that have focused on insectivorous species are reviewed by Pafilis et al. (2007). In summary, the gut passage rates of 14 insectivorous species varied greatly even when exposed to the same temperature. For example, at 30 °C, the shortest passage time was 11.2 h for *Zootoca vivipara* (as *Lacerta vivipara*), whilst the longest was 4 days for *Pseudocordylus melanotus melanotus* (as *Cordylus melanotus melanotus*) (Avery, 1971; Van Damme et al., 1991; McConnachie and Alexander, 2004). Pafilis et al. (2007) concluded that gut passage times are not only profoundly affected by temperature but also profoundly affected by life history, corroborating other findings. This emphasizes the need for focused gut and intestinal passage studies.

As part of a larger study, we measured gut and intestinal passage time in *Trachylepis margaritifer* (Rainbow Skink), a predominantly insectivorous species, to evaluate its suitability for stress metabolite studies. Gut passage time was taken as the time from ingestion of a food item to the resultant defecation, whilst intestinal passage time was from the start of gastric emptying to when faeces can be massaged from the individual. We used barium sulphate ($BaSO_4$) and X-ray measures to track the progress of food through the intestine. First, we measured the effect of barium sulphate on passage times, because

it has been suggested that its presence may slow the passage of digesta through the gut (Schumacher and Toal, 2001). Next, we tested the effect of temperature on both gut and intestinal passage times using barium sulphate-loaded food items to track the progression of food through the gut.

Materials and methods

Study animal

Trachylepis margaritifer is a large, colourful southern African skink with a snout-vent length (SVL) of 85–110 mm. It occurs on rocky outcrops throughout mesic and arid savannas in the north-eastern parts of southern and East Africa (Branch, 1998) in relatively dense colonies on exposed rock faces. Rainbow Skinks are active, territorial insectivores but are known to include the fruits of *Lantana camara* and the flowers of *Erythrina* spp. in their diet (personal observation). For a species description, see Broadley (2000).

Experimental design

Adult skinks were wild-caught from a population in Mpumalanga Province, South Africa (25°34′26.5″ S; 31°11′05.9″ E) using non-toxic Catchmaster® 90 × 120 mm Mouse Glue Traps (Atlantic Paste and Glue Co., Brooklyn, NY, USA). Set traps were monitored continuously, and trapped lizards were removed from traps immediately using cooking oil (Whiting and Alexander, 2001). Lizards were transported to the University of Witwatersrand, Johannesburg, South Africa, where all laboratory experiments were conducted. Individuals were weighed and their SVL recorded prior to experimentation. The same skinks were used in the two experimental trials.

Experiment A: the effect of barium sulphate and temperature on gut transit rates

Rainbow Skinks (n_{total} = 31) were randomly assigned to three temperature-controlled rooms set at 25, 27 and 32 °C ($n_{25\ °C}$ = 11, $n_{27\ °C}$ = 9, $n_{32\ °C}$ = 11). During this experiment, they were individually housed in glass terraria and were acclimated for a period of 3 months. Terraria were fitted with slate tiles to best approximate the rocky substrate of their habitat. The tile arrangement provided skinks with two retreats so as to minimize stress (Fig. 1). Water was provided *ad libitum* and the photoperiod was from 6 to 18 h.

Dead European House Crickets (*Acheta domestica*; mass 0.41 ± 0.08 g) were marked through the implantation of two small, coloured glass beads.

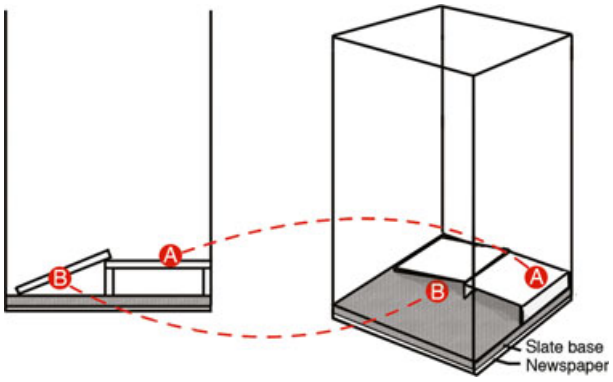

Fig. 1 Terraria were fitted with slate tiles arranged in such a manner that two retreats were available to the skinks; one closed on three sides (A) and the other open on two (B).

When allocated to treatment trials, each skink was fed one marked, dead cricket containing 0.15 ml barium sulphate liquid suspension (constituted at a 1:1 $BaSO_4/H_2O$ ratio). Although previous reptilian studies have utilized a dosage of 10–15 ml/kg (Taylor et al., 1996), we found during calibrations that this volume was three times the amount necessary to produce an adequate visual on X-ray images. Control trials differed in one aspect only – crickets were marked with beads but were barium-free. The order of trial sequence (i.e. control then treatment or treatment then control) was randomized amongst individuals and between experimental temperatures. Trials were conducted one week apart.

Experiment B: temperature effect on gut and intestinal transit time

A total of 30 (17 females; 13 males) Rainbow Skinks (mass 37.3 ± 1.9 g) were used in this experimental component. To facilitate measures of intestinal passage time, dead crickets (*A. domestica*; mass 0.41 ± 0.08 g) were each injected with 0.15 ml of a barium sulphate liquid suspension (constituted at a 1:1 $BaSO_4/H_2O$ ratio) and force-fed to the skinks. We fed each skink a single barium sulphate-loaded cricket.

During barium meal trials, subjects were individually housed in well-ventilated, opaque, two-litre plastic containers and only temporarily removed and transferred into smaller, transparent perspex containers for the duration of their scheduled X-ray measures. X-rays were captured on a mobile X-ray unit (Shimadzu MobileArt Plus MUX-100H, Kyoto, Japan) set at an exposure of 49 KV and 0.56 mAS per exposure. Barium meals were conducted at two ambient temperatures: 25 and 27 °C. We selected these two temperatures due to the unexpected

patterns observed in the first set of experiments (gut passage times were slower at 27 °C than at 25 °C). Due to the risks associated with multiple X-ray exposures, each skink (n_t = 30) was used for only one feeding trial ($n_{25\ °C}$ = 20; $n_{27\ °C}$ = 10). The unequal allocation of skinks to the two temperature trials was due to logistical constraints; access to the X-ray machine was limited. Skinks were X-rayed every 2–4 h, depending on the progress of each individual meal. Individuals were removed from trials once their respective meal was contained within the large intestine. At this stage, samples were collected by abdominal massage.

In order to better interpret the X-ray films with regards to anatomical position of the barium meal, three museum specimens of Rainbow Skinks (SVL = 95 ± 13 mm) were dissected to reveal the normal position of the digestive viscera. This step is regarded as critical when using barium sulphate in X-ray studies (Valente et al., 2008), so that X-ray images can be interpreted in the light of this information (Fig. 2).

Gut passage was divided into five phases:

(i) Phase I – in the stomach,
(ii) Phase II – gastric emptying initiated,
(iii) Phase III – gastric emptying completed,
(iv) Phase IV – movement in the small intestine and entry into the large intestine,
(v) Phase V – movement into the large intestine completed.

The time from when gastric emptying was initiated until movement into the large intestine was complete (phase II through V) was defined as the intestinal passage time.

Statistical treatment of results

Differences in mass and SVL of skinks between treatments were tested for using ANOVA. We used time-to-event (survival) analyses to detect differences in gut and intestinal passage times: Kaplan–Meier cumulative proportions were plotted to visualize the differences in transit times (effect of barium sulphate and temperature) detected by Gehan's Wilcoxon tests or chi-squared analysis (for multiple comparisons). Weibull estimates were plotted to test goodness-of-fit. The influence of covariates was tested through Proportional Harzard (Cox) regressions. All analyses were performed using STATISTICA v. 8 (STATISTICA Data Analysis Software System, http://www.statsoft.com, 2001). Results were considered statistically significant for $p < 0.05$.

Results

There were no significant differences in mass and SVL of skinks between temperature treatments for both experiments A and B (Table 1).

Experiment A: the effect of barium sulphate and temperature on gut transit rates

Individual, sex, body condition and order of experimental sequence had no significant effect on gut passage times across all temperature trials (Proportional Hazard Regression, $p > 0.05$). Barium sulphate had no significant effect on gut passage times across all three temperatures (individually or pooled; Table 2). When data from temperature trials were pooled, mean gut passage times for treatment and control components were 40.10 ± 21.20 and 45.25 ± 23.21 h respectively (Fig. 3).

Gut passage time was significantly different (χ^2 = 41.60, df = 2, $p < 0.001$) at different temperatures. The gut passage times overall (control and treatment data combined) were fastest in the individuals exposed to 32 °C (19.34 ± 10.44; 3.31 h: mean ± SD; SEM). The slowest rates were recorded

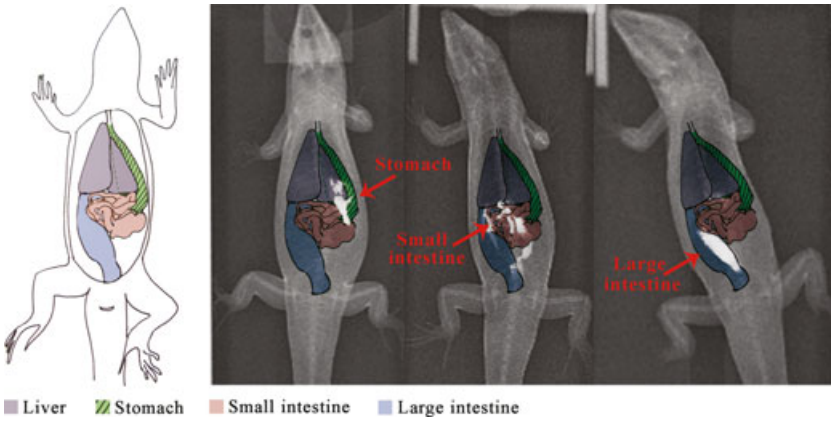

Fig. 2 Schematic showing the anatomical position of the liver, stomach, small and large intestine in *Trachylepis margaritifer* (left) and subsequent schematic overlays onto X-ray images (ventral view) to aid in interpreting the position of barium sulphate (white).

Table 1 Details of the experimental groups of Rainbow Skinks (*Trachylepis margaritifer*) used to test the effect of barium sulphate on gut passage times

Experiment	Temperature treatment (°C)	Number of skinks (♂:♀)	Mass (g ± SD)	SVL (mm ± SD)	ANOVA Mass	SVL
A	25	4:6	32.14 ± 6.64	101.00 ± 8.29	$F_{2,28}$ = 2.51	$F_{2,28}$ = 0.84
A	27	5:4	37.50 ± 5.93	104.75 ± 6.93	p = 0.10	p = 0.44
A	32	4:7	31.06 ± 7.43	105.32 ± 9.59		
B	25	8:12	31.50 ± 5.23	103.00 ± 8.29	$F_{1,28}$ = 1.21	$F_{1,28}$ = 0.66
B	27	5:5	32.42 ± 5.21	104.08 ± 8.48	p = 0.28	p = 0.42

SVL, snout-vent length.
Differences between treatments were tested for using ANOVA.

Table 2 Mean gut passage times measured at three different ambient temperatures in the Rainbow Skink (*Trachylepis margaritifer*; Experiment A)

Ambient temperature (°C)	Treatment ($BaSO_4$-loaded) Mean ± SD; SEM (h)	Control ($BaSO_4$-free) Mean ± SD; SEM (h)	Gehan's Wilcoxon *w*; p
25	49.47 ± 7.76; 4.26	53.51 ± 21.00; 4.26	−37.00; 0.23
27	56.46 ± 13.00; 4.91	61.89 ± 10.69; 4.91	−7.00; 0.79
32	16.21 ± 7.76; 4.43	22.47 ± 12.04; 4.43	−43.00; 0.13
Pooled	40.10 ± 21.20; 3.72	45.25 ± 23.13; 4.15	−138.00; 0.33

Skinks were fed both barium sulphate-loaded and barium sulphate-free European House Crickets (*Acheta domestica*) in two separate trials to test the effect of barium sulphate on these times. Differences between groups were tested for using Gehan's Wilcoxon tests.

for lizards at 27 °C (59.18 ± 11.92; 3.69 h: SD; SEM), which are significantly different (Gehan's Wilcoxon, $w = -183.00$, p = 0.012) to the observed rates in the 25 °C treatment (51.49 ± 16.52; 3.06 h: SD; SEM) (Fig. 4).

Experiment B: temperature effect on gut and intestinal transit time

Intestinal passage times were significantly different at different temperatures (Gehan's Wilcoxon: $w = 105$, p = 0.009). As with measures of gut passage times in the first set of experiments, lizards at 25 °C had significantly faster passage times than did lizards at 27 °C (Table 3). On average, digesta took 10 h less to move from the stomach into the large intestine in the skinks maintained at 25 °C than it did in the skinks maintained at 27 °C. For more than 75% of the skinks maintained at 25 °C, intestinal passage time fell within 26 h, whilst the same percentage of skinks maintained at 27 °C have intestinal passage times that fell closer to 42 h (Fig. 5).

The temporal deficit appears to originate primarily at the stage where digesta move into the large intestine (phase IV). Once established, this deficit is not recovered and experimental groups remain significantly different from each other in phase V (Table 4). The differences in phases IV and V between experimental groups (faster in skinks maintained at 25 °C)

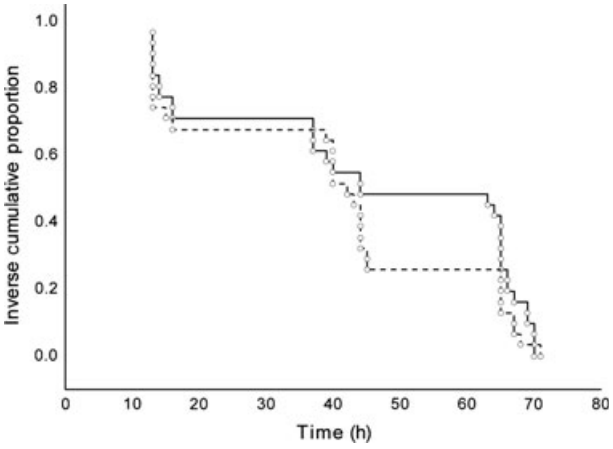

Fig. 3 Kaplan–Meier curve for Rainbow Skinks (*Trachylepis margaritifer*) showing completed gut passage over time (Experiment A). Inverse cumulative proportions indicate the proportions of skinks showing incomplete gut passage (i.e. at 0.6, 60% of the skinks show incomplete gut passage, whilst 40% show completed gut passage). The dashed line represents measures made from individuals that were fed barium sulphate-loaded European House crickets (*Acheta domestica*) (i.e. treatment; all temperatures combined), whilst the solid line represents measures made from skinks that were fed barium sulphate-free crickets (i.e., control; all temperatures combined). Mean gut passage times (mean ± SD) for the treatment and control groups were 40.10 ± 21.20 and 45.25 ± 23.21 h, respectively, which are not significantly different from each other (Gehan's Wilcoxon, $w = -138.00$, p = 0.33).

are highly significant (Gehan's Wilcoxon test: $w = 129$, p = 0.002; $w = 170$, p = 0.0001 respectively). There was no significant difference observed

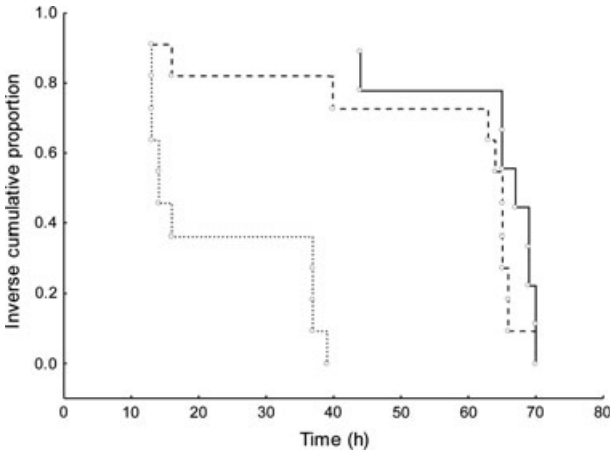

Fig. 4 Kaplan–Meier curve for Rainbow Skinks (*Trachylepis margaritifer*), maintained at three different environmental temperatures: 25 °C (dashed line), 27 °C (solid) and 32 °C (dotted), showing completed gut passage over time (Experiment A). Inverse cumulative proportions indicate the proportions of skinks showing incomplete gut passage (i.e. at 0.6, 60% of the skinks show incomplete gut passage, whilst 40% show completed gut passage). Temperature had a highly significant effect on gut passage times ($\chi^2 = 41.60$, df = 2, $p < 0.001$). Transit times recorded at 25 °C and 27 °C are significantly different (Gehan's Wilcoxon, $w = -183.00$, $p = 0.012$).

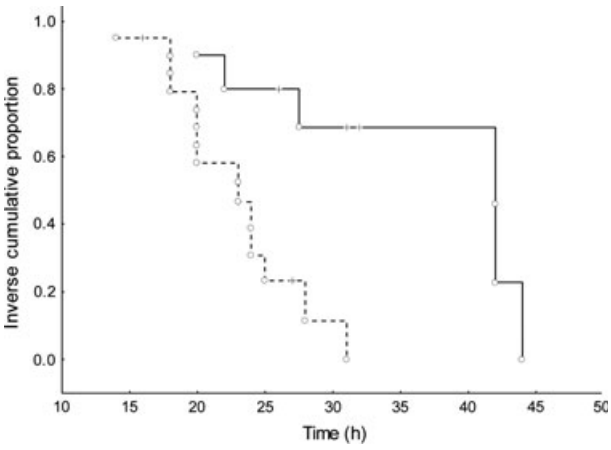

Fig. 5 Kaplan–Meier curve for Rainbow Skinks (*Trachylepis margaritifer*) showing completed intestinal passage over time (Experiment B). Inverse cumulative proportions indicate the proportions of skinks showing incomplete intestinal passage (i.e. at 0.6, 60% of the skinks show incomplete intestinal passage, whilst 40% show completed intestinal passage). The dashed line represents data collected from skinks at 25 °C, whilst the solid line is for skinks at 27 °C. Points marked + indicate data points which have been censored (i.e. no event recorded). Uncensored (i.e. event recorded) are marked °. Intestinal passage times are significantly different between temperatures trials (Gehan's Wilcoxon: $w = 105$, $p = 0.009$).

Table 3. Mean intestinal passage times of two experimental groups of Rainbow Skinks (*Trachylepis margaritifer*; Experiment B)

Experimental group	Intestinal transit time (h ± SD)	Censored (complete: censored)	Gehan's Wilcoxon (*w*; p)
25 °C	21.75 ± 4.17	14:6	105; 0.009*
27 °C	31.85 ± 8.57	5:5	

Censorship data represent the number of skinks in which intestinal passage times were available for calculation (complete) versus the number of skinks in which intestinal passage times were not available for calculation (censored). Differences between experimental groups were tested for using Gehan's Wilcoxon.
*Significant.

in phase I or II between groups (Gehan's Wilcoxon test: $w = 66$, $p = 0.142$; $w = -10.00$, $p = 0.764$ respectively). These trends are shown in Kaplan–Meier curves for each phase (Fig. 6).

Finally, least-squared event-avoidance function estimates (Weibull model) were calculated for each temperature trial to test goodness-of-fit (Fig. 7). This provides a weighted estimate, which performs accurately with small sample sizes. For both experimental groups, the theoretical distribution of weight $1/V$ shows the best fit, where V is the variance of the hazard estimate.

Discussion

In Rainbow Skinks, passage times were dependent on temperature but were not affected by the presence of barium sulphate in the meal. As expected, gut passage times were fastest at the warmest trial temperature (32 °C). However, passage times (both gut and intestinal) were slowest at the intermediate temperature (27 °C) and were intermediate at the lowest trial temperature (25 °C). Differences in passage time were due mainly to differences in the length of time that the digesta spent in phase IV (movement in the small intestine and entry into the large intestine), during which time, digested food is absorbed across the gut wall (Karasov and Diamond, 1983). Gut passage time was generally fast for an ectotherm, ranging from 19 h at 32 °C to 59 h at 27 °C.

The faster passage time at lower temperatures was an unexpected finding. This trend was evident for lizards in both of our experiments (gut and intestinal passage times) and is thus unlikely to be an experimental artefact. The majority of digestion studies on reptiles report a simple positive relationship between temperature and passage rate (e.g. Skoczylas, 1970a; Jiang and Claussen, 1993; Alexander et al., 2001; Angilletta et al., 2002; Wang et al., 2003; McConnachie

Table 4 Mean duration post-ingestion of barium sulphate-loaded meals for the phases of digestion involved in intestinal transit (i.e., phase II–V) identified from X-ray (Experiment B)

Phase	Experimental group	Hours post-ingestion (h ± SD)	Censorship (complete: censored)	*w*; p
II Gastric emptying initiated	25 °C	10.5 ± 3.17	16:4	66.00; 0.142
	27 °C	16.85 ± 8.70	10:0	
	Combined	12.61 ± 6.27	26:4	
III Gastric emptying completed	25 °C	22.60 ± 8.70	14:6	−10.00; 0.764
	27 °C	25.70 ± 11.18	8:2	
	Combined	23.20 ± 9.52	22:8	
IV Movement in the small intestine and entry into the large intestine	25 °C	29.80 ± 3.24	20:0	129.00; 0.002*
	27 °C	39.35 ± 10.53	8:2	
V Movement into the large intestine completed	25 °C	32.25 ± 4.62	18:2	170.00; 0.0001*
	27 °C	48.70 ± 7.63	6:4	

Censorship data represent the number of individuals in which each phase was categorically identified (complete) from the X-rays captured versus the number of skinks in which the phased was not identified (censored). Means of combined data are reported for phases in which no statistical difference was observed between the experimental groups.
*Significant.

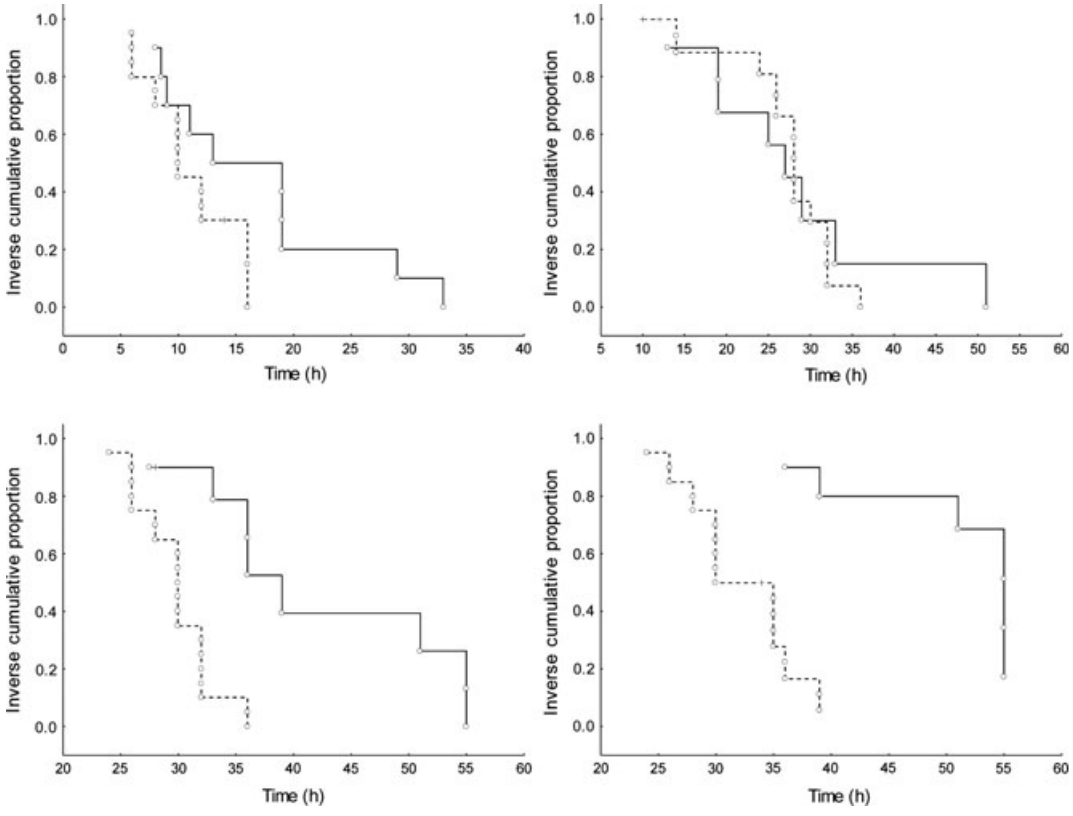

Fig. 6 Kaplan–Meier curves for two experimental groups (maintained at 25 and 27 °C) of Rainbow Skinks (*Trachylepis margaritifer*) showing phase completion over time (Experiment B). Inverse cumulative proportions indicate the proportions of skinks showing incomplete phase (i.e. at 0.6, 60% of skinks have not undergone phase completion, whilst 40% of skinks have). The dashed line represents data collected from skinks at 25 °C, whilst the solid line is for skinks at 27 °C. Points marked + indicate data points which have been censored (i.e. no event recorded). Uncensored (i.e. event recorded) are marked. Graphs represent four phases of digestion; phase II: gastric emptying initiated (top left), phase III: gastric emptying complete (top right), phase IV: movement in the small intestine and entry into the large intestine (bottom left) and phase V: movement into the large intestine completed (bottom right). Temperature had a highly significant effect on Phase IV (Gehan's Wilcoxon: $w = 129.00$, $p < 0.01$) and V (Gehan's Wilcoxon: $w = 170.00$, $p < 0.001$).

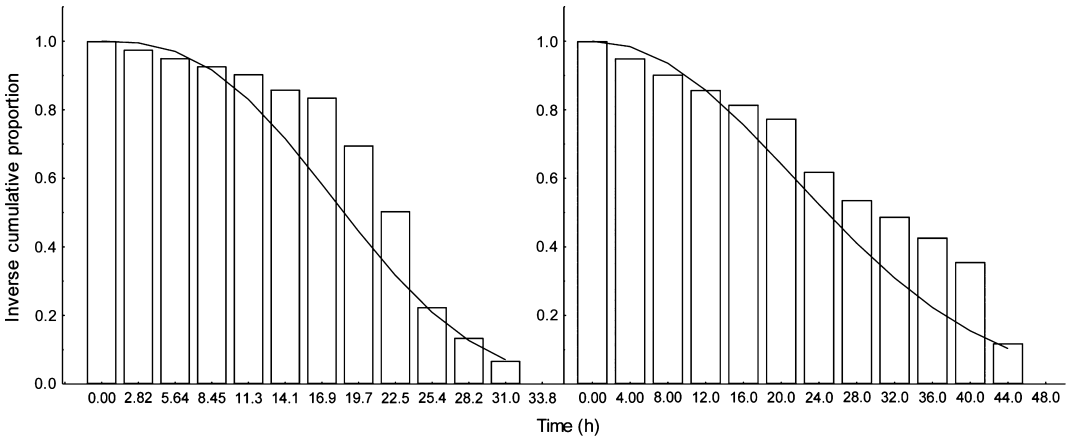

Fig. 7 Weibull estimates of time-to-event function for the Rainbow Skink (*Trachylepis margaritifer*) based on data collected from skinks maintained at environmental temperatures of 25 °C (left) and 27 °C (right; Experiment B). The inverse cumulative proportion represents the proportion of the population that has avoided the event, in this case – completing intestinal passage. The bars represent observed values, whilst the solid lines represent the theoretical distribution of weight $1/V$, where V is the variance of the hazard estimate.

and Alexander, 2004; Pafilis et al., 2007), whilst a few report temperature independence of passage rates (Karasov and Diamond, 1985; Zimmerman and Tracy, 1989; Mckinon and Alexander, 1999). Only Sadeghayobi et al. (2011) have previously reported a negative relationship between passage rate and temperature. They recorded retention times of digesta of 4 days longer at 25.5 °C than 23.3 °C in Galápagos Tortoises (*Chelonoidis nigra*), but the significance of this trend was not considered. Cases of a negative relationship between passage rate and temperature may be due to cold reptiles voiding gut contents more quickly because temperatures are too low for digestion or assimilation, resulting in a 'cut your losses' strategy.

We suggest that at low temperatures, risk associated with temperature-induced digestive suppression cause some reptiles to adopt mechanisms that prevent digesta rotting in the digestive tract. Some snakes avoid putrefaction of food in the stomach by regurgitating meals at low temperatures (Tsai et al., 2008). As reptiles generally do not allow meals to putrefy in their digestive systems, the consequences of this are difficult to measure. Thus, there are no documented cases of septicaemia stemming from undigested food within the gut in reptiles, although Tsai et al. (2008) do report increased mortality rates in recently fed *Viridovipera stejnegeri* (as *Trimeresurus stejnegeri stejnegeri*) at high (35 °C) and low (10 °C) temperatures. Because lizards generally feed on small prey items, a 'cutting your losses' strategy would entail decreasing gut passage time and foregoing the potential nutritional gain associated with an increased one. Thus, it would be expected that at 25 °C, the faster passage time seen in *T. margaritifer* would correlate with reduced apparent assimilation efficiency (AAE; see McConnachie and Alexander, 2004). This relationship is worthy of further study.

We can infer that septicaemia would be the likely outcome in cases were putrefaction occurred, because deleterious effects of low temperature on gut chemistry and enzymatic function have been well documented (Skoczylas, 1970b; Low et al., 1973). Additionally, appetite suppression at low temperatures is also known for many ectothermic taxa (Alexander et al., 2001; Wang et al., 2003; McConnachie and Alexander, 2004), and a sudden postprandial drop in temperature will elicit an emetic response in some species (Stevenson et al., 1985; Van Damme et al., 1991; Beaupre et al., 1993). This suggests that there are severe physiological consequences associated with a loaded gut at low temperatures.

Despite Schumacher and Toal's (2001) concerns that barium sulphate might slow intestinal passage times, our study found no such effect. It is possible that this is due to the fact that we used only one-third the usual quantity of barium sulphate in our experiments (see Taylor et al., 1996). This finding suggests that barium sulphate can be used in passage time measures, at least at the concentration that we used in our study. Thus, we were able to use X-ray technology to measure the position of the digesta within the digestive tract of our lizards and observe the progression of the food bolus along the digestive tract. This information could then be used to detect where differences in passage rate occurred under various conditions. For example, we found that

lizards at 27 and 25 °C had similar rates of gastric emptying, but differed significantly in intestinal passage times.

Relative to other insectivorous lizards at equivalent temperatures (Pafilis et al., 2007), Rainbow Skinks have fast gut and intestinal passage times. We recorded gut passage times as fast as 16 and 49 h at 32 and 25 °C respectively. In fact, intestinal passage times recorded in our skinks were even faster than those documented for some endotherms such as pigs, and faster than those recorded for ponies (Möstl and Palm, 2002) when skinks were at 25 and 32 °C. From this perspecitive, they are ideal subjects for studies using measures of stress metabolites in faecal samples.

Although the gut passage times that we measured in Rainbow Skinks were far less variable than gut pasage times reported for most snake species (see Lillywhite et al., 2002 for several examples), they were slightly more variable than passage times reported for some other lizards species (e.g. Van Damme et al., 1991; Zhang and Ji, 2004). An important source of variability in the gut passage times of our lizards stemmed from the fact that they are strictly diurnal, remaining inactive in crevices during the night. They did not defecate during this time, holding faeces in the gut until the following morning. Because our measures of intestinal passage times were not as greatly affected by night time inactivity (faeces were massaged from the lizard as soon as they entered the colon), they tended to be faster and less variable than gut passage times (21.75 ± 4.17 h rather than 49.47 ± 7.76 for 25 °C).

Overall, our study showed that *T. margaritifer* is an appropriate candidate species for stress metabolite studies using faecal analysis. The intriguing result of shorter gut passage times at 25 °C than at 27 °C emphasizes that, for stress metabolite studies on ectothermic species, the effects of temperature on specific intestinal passage times should be known.

Acknowledgements

We thank the University of the Witwatersrand's Animal Unit staff who assisted during barium meal procedures, particularly Mary-Ann Costello whose involvement also included assistance in animal husbandry. Bryan Maritz is thanked for his assistance in interpreting X-ray images and for commenting on draft versions of this manuscript. Mimmie Kgaditse and Mankurwana Mahlase assisted in data collection. All experimental protocols were cleared by the University of the Witwatersrand's Animal Ethics Committee under permit number 2009/19/2A. Skinks were collected under permit number MPB. 5300 (Mpumalanga Tourism and Parks Agency).

References

Alexander, G. J.; van Der Heever, C.; Lazenby, S. L., 2001: Thermal dependence of appetite and digestion rate in the Flat Lizard, *Platysaurus intermedius wilhelmi*. *Journal of Herpetology* **35**, 461–466.

Alexander, G. J.; Hanrahan, S. A.; Mitchell, D., 2012: Assimilation efficiency and gut passage time in an African elapid snake, *Hemachatus haemachatus*. *African Journal of Herpetology* **61**, 3–13.

Angilletta, M. J. Jr; Niewiarowski, P. H.; Navas, C. A., 2002: Invited review: the evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**, 249–268.

Armario, A.; Montero, J. L.; Balasch, J., 1986: Sensitivity of corticosterone and some metabolic variables to graded levels of low intensity stresses in adult male rates. *Physiology and Behavior* **37**, 559–561.

Avery, R. A., 1971: Estimates of food consumption by the lizard, *Lacerta vivipara* Jaquin. *Journal Animal Ecology* **40**, 351–365.

Beaupre, S. J.; Dunham, A. E.; Overall, K. L., 1993: The effects of consumption rate and temperature on apparent digestibility coefficient, urate production, metabolizable energy coefficient and passage time in the canyon lizards (*Sceloporus merriami*) from two populations. *Functional Ecology* **7**, 273–280.

Branch, W. R., 1998: *Field Guide to the Snakes and Other Reptiles of Southern Africa*, 3rd edn. Struik, Cape Town.

Broadley, D. G., 2000: A review on the genus *Mabuya* in southeastern Africa (Sauria: Scincidae). *African Journal of Herpetology*, **49**, 87–100.

Dawson, W. R., 1975: On the physiological significance of the preferred body temperatures of reptiles. In: D. M. Gates, R. B. Schmerl (eds), *Perspectives in Ecological Ecology*. Springer, New York, pp. 443–473.

Diaz-Figueroa, O.; Mitchell, M. A., 2006: Gastrointestinal anatomy and physiology. In: D. R. Mader (ed.), *Reptile Medicine and Surgery*. Elsevier, Philadelphia, PA, pp. 145–162.

Dorcas, M. E.; Peterson, C. R.; Flint, M. E., 1997: The thermal biology of digestion in rubber Boas (Charina *bottae):* physiology, behavior, and environmental constraints. *Physiological Zoology* **70**, 292–300.

Gärtner, K.; Büttner, D.; Döhler, K.; Friedel, R.; Lindena, J.; Trautschold, I., 1980: Stress response of rats to handling and experimental procedures. *Laboratory Animals* **14**, 267–274.

Haemisch, A.; Guerra, G.; Furkert, J., 1999: Adaptation of corticosterone – but not ß-endorphin – secretion to repeated blood sampling in rats. *Laboratory Animals* **33**, 185–191.

Halberg, F.; Albrecht, P. G.; Bittner, J. J., 1960: Corticosterone rhythm of mouse adrenal in relation to serum corticosterone and sampling. *American Journal of Physiology* **197**, 1083–1085.

Hennessy, M. B.; Levine, S., 1976: Sensitive pituitary-adrenal responsiveness to varying intensities of psychological stimulation. *Physiology and Behavior* **21**, 295–297.

Jiang, S.; Claussen, D. L., 1993: The effects of temperature on food passage time through the digestive tract in *Notophthalmus viridescens*. *Journal of Herpetology* **27**, 414–419.

Karasov, W. H.; Diamond, J. M., 1983: Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *The American Physiological Society* **245**, G443–G462.

Karasov, W. H.; Diamond, J. M., 1985: Digestive adaptations for fueling the cost of endothermy. *Science* **228**, 202–204.

Lillywhite, H. B.; de Delva, P.; Noonan, B. P., 2002: Patterns of gut passage time and the chronic retention of fecal mass in viperid snakes. In: G. W. Schuett, M. Höggren, M. E. Douglas, H. W. Greene (eds), *Biology of the Vipers*. Eagle Mountain Publishing, Utah, pp. 497–506.

Low, P. S.; Bada, J. L.; Somero, G. N., 1973: Temperature adaptation of enzymes: roles of the free engery, the enthalpy and the entropy of activation. *Proceedings of the National Academy of Science of the United States of America* **70**, 430–432.

McConnachie, S.; Alexander, G. J., 2004: The effect of temperature on digestive and assimilation efficiency, gut passage time and appetite in an ambush foraging lizard, *Cordylus melanotus melanotus*. *Journal of Comparative Physiology B* **174**, 99–105.

Mckinon, W.; Alexander, G. J., 1999: Is temperature dependence of digestive efficiency an experimental artifact in lizards? A test using the common flat lizard (Platysaurus intermedius) *Copeia* **1999**, 299–303.

Moore, I. T.; Jessop, T. S., 2003: Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior* **43**, 39–47.

Möstl, E.; Palm, R., 2002: Hormones as indicators of stress. *Domestic Animal Endocrinology* **23**, 67–74.

Pafilis, P.; Foufopoulos, N.; Poulakakis, P.; Lymberakis, P.; Valakos, E., 2007: Digestive performance in five Mediterranean lizard species: effects of temperature and insularity. *Journal of Comparative Physiology B* **177**, 49–60.

Quirce, C. M.; Maickel, R. P., 1981: Alterations of biochemical parameters by acute and repetitive stress situations in mice. *Psychoneuroendocrinology* **6**, 91–97.

Riley, V., 1981: Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science* **212**, 1100–1109.

Sadeghayobi, E.; Blake, S.; Wikelski, M.; Gibbs, J.; Mackie, R.; Cabrera, F., 2011: Digesta retention time in the Galápagos tortoise (*Chelonoidis nigra*). *Comparative Biochemistry and Physiology, Part A* **120**, 493–497.

Schumacher, J.; Toal, R. L., 2001: Advanced radiography and ultrasonography in reptiles. *Seminars in Avian and Exotic Pet Medicine* **10**, 162–168.

Secor, S. M., 2005: Evolutionary and cellular mechanisms regulating intestinal performance of amphibians and reptiles. *Integrated Comparative Biology* **45**, 282–294.

Shine, R., 2005: Life-history evolution in reptiles. *Annual Review of Ecology, Evolution and Systematics* **36**, 23–46.

Skoczylas, R., 1970a: Influence of temperature on gastric digestion in the grass snake, *Natrix natrix* l. *Comparative Biochemistry and Physiology* **33**, 793–804.

Skoczylas, R., 1970b: Salivary and gastric juice secretion in the grass snake, *Natrix natrix* l. *Comparative Biochemistry and Physiology* **35**, 885–903.

Stevenson, R. D.; Peterson, C. R.; Tsuji, J. S., 1985: The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiological Zoology* **58**, 46–57.

Stevenson, R. D.; Tuberty, S. R.; de Fur, P. L.; Wingfield, J. C., 2005: Ecophysiology and conservation: the contribution of endocrinology and immunology – introduction to the symposium. *Integrative and Comparative Biology* **45**, 1–3.

Taylor, S. K.; Citino, S. B.; Zdziarski, J. M.; Bush, R. M., 1996: Radiographic anatomy and barium sulfate transit time of the gastrointestinal tract of the leopard tortoise (*Testudo pardalis*). *Journal of Zoo and Wildlife Medicine* **27**, 180–186.

Touma, C.; Palme, R.; Sachser, N., 2004: Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. *Hormones and Behavior* **45**, 10–22.

Tsai, T.; Lee, H.; Tu, M., 2008: Specific dynamic action, apparent assimilation efficiency, and digestive rate in an arboreal pitviper, *Trimeresurus stejnegeri stejnegeri*. *Canadian Journal of Zoology* **86**, 1139–1151.

Valente, A. L.; Marco, I.; Parga, M. L.; Lavin, S.; Alegre, F.; Cuenca, R., 2008: Ingesta passage and gastric emptying times in loggerhead sea turtles (*Caretta caretta*). *Research in Veterinary Science* **84**, 132–139.

Van Damme, R.; Bauwens, D.; Verheyen, R. F., 1991: The thermal dependence of feeding behaviour, food consumption and gut-passage time in the lizard *Lacerta vivpara* Jacquin. *Functional Ecology* **5**, 507–517.

Wang, T.; Morten, Z.; Arvedsen, S.; Vedel-Smoth, C.; Overgaard, J., 2003: Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comparative Biochemistry and Physiology Part A* **133**, 519–527.

Whiting, M. J.; Alexander, G. J., 2001: Oil spills and glue: a comment on a sticky sampling problem for lizards. *Herpetological Review* **32**, 78–79.

Wikelski, M.; Cooke, S. J., 2006: Conservation physiology. *Trends in Ecology and Evolution* **21**, 38–46.

Wingfield, J. C.; Hunt, K.; Breuner, C.; Dunlap, K.; Fowler, G. S.; Freed, L.; Lepson, J., 1997: Environmental stress, field endocrinology, and conservation biology. In: J. R. Clemmons, R. Buchholz (eds), *Behavioral Approaches to Conservation in the Wild*. Cambridge University Press, Cambridge, pp. 95–131.

Zhang, Y. P.; Ji, X., 2004: The thermal dependence of food assimilation and locomotor performance in southern grass lizards, *Takydromus sexlineatus* (Lacertaidae). *Journal of Thermal Biology* **29**, 45–53.

Zimmerman, L. C.; Tracy, C. R., 1989: Interactions between the environment and ectothermy and herbivory in reptiles. *Physiological Zoology* **62**, 374–409.