Thermal plasticity in young snakes: how will climate change affect the thermoregulatory tactics of ectotherms?

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SUMMARY

Climate change will result in some areas becoming warmer and others cooler, and will amplify the magnitude of year-to-year thermal variation in many areas. How will such changes affect animals that rely on ambient thermal heterogeneity to behaviourally regulate their body temperatures? To explore this question, we raised 43 captive-born tiger snakes *Notechis scutatus* in enclosures that provided cold (19–22°C), intermediate (19–26°C) or hot (19–37°C) thermal gradients. The snakes adjusted their diel timing of thermoregulatory behaviour so effectively that when tested 14 months later, body temperatures (mean and maximum), locomotor speeds and anti-predator behaviours did not differ among treatment groups. Thus, the young snakes modified their behaviour to compensate for restricted thermal opportunities. Then, we suddenly shifted ambient conditions to mimic year-to-year variation. In contrast to the earlier plasticity, snakes failed to adjust to this change, e.g. snakes raised at cooler treatments but then shifted to hot conditions showed a higher mean body temperature for at least two months after the onset of the new thermal regime. Hence, thermal conditions experienced early in life influenced subsequent thermoregulatory tactics; the mean selected temperature of a snake depended more upon its prior raising conditions than upon its current thermoregulatory opportunities. Behavioural plasticity thus allows snakes to adjust to suboptimal thermal conditions but this plasticity is limited. The major thermoregulatory challenge from global climate change may not be the shift in mean values (to which our young snakes adjusted) but the increased year-to-year variation (with which our snakes proved less able to deal).

Key words: climate change, temperature, growth, plasticity, snake.

INTRODUCTION

The potential consequences of global climate change on biodiversity are a growing concern (Sala et al., 2000; Thomas et al., 2004; Malcolm et al., 2006; Deutsch et al., 2008; Kearney et al., 2009). Although predictions vary, many models suggest that large areas of the planet will probably experience shifts not only in mean temperature (some areas hotter, some colder) but also in variance [unusual heat waves or cold snaps and strong year-to-year variation in thermal regimes and precipitation (Houghton et al., 2001; IPCC, 2007)]. Thus, many animal populations will be exposed to both of these forms of climate change (Parmesan and Yohe, 2003; Root et al., 2003). Terrestrial ectotherms may be at particular risk because they are less effective at buffering body temperature against ambient temperature using physiological mechanisms, and instead rely heavily on ambient thermal heterogeneity to regulate their temperature behaviourally (Deutsch et al., 2008; Kearney et al., 2009). Further, growth, locomotion and reproduction are strongly dependent on body temperature (Seigel et al., 2001).

Understanding the consequences of global thermal shifts for body-temperature regimes of ectotherms is a complex task (Kearney et al., 2009). Ectotherms can, to a degree, behaviourally escape changes in the ambient thermal environment, an ability that allows them to maintain a 'preferred' temperature over a wide range of conditions (e.g. Lillywhite, 1970; Lillywhite, 1980; Slip and Shine, 1988; Kelsch, 1996; Kemp and Krockenberger, 2002; Seebacher and Shine, 2004). This buffering reduces the degree to which a change in ambient temperature causes a resultant change in body temperature. Mechanistic models have incorporated this buffering capacity into models of how ectotherms will respond to climate change (Kearney and Porter, 2004; Kearney et al., 2009).

Although models that incorporate this behavioural plasticity represent a major advance in predicting impacts of climate change on ectotherm body temperatures, the models omit another type of flexibility: developmental plasticity (Seebacher, 2005; Franklin and Seebacher, 2009). The thermal environment in which a reptile lives can modify its thermoregulatory tactics (Glanville and Seebacher, 2006). For example, lizards given only a brief basking opportunity each day select higher-than-usual temperatures when they do bask (Shine, 2006). If such modified preferenda become canalised (Angilletta et al., 2002; Glanville and Seebacher, 2006), then the body temperatures that an ectotherm selects may depend upon its thermal history, as well as on the current conditions that it experiences. If so, the temperatures selected by a reptile would depend not only on the animal's own characteristics (sex, size, health, etc.) and on current ambient thermal regimes but also on the thermal regimes that the animal had experienced earlier in its life [i.e. irreversible acclimation (Glanville and Seebacher, 2006)]. Such long-lasting effects of developmental plasticity have been documented for other traits, such as relative head size (Aubret et al., 2004), growth rates (Madsen and Shine, 2000) and reproductive allocation tactics (Doughty and Shine, 1998).

To understand how shifting ambient temperatures might affect the body temperatures exhibited by an ectotherm, we need to know: (1) do heliotherms modify basking patterns to maintain similar mean temperatures over a wide range of ambient thermal conditions? (2) If so, can this 'buffering' effect of behavioural thermoregulation

eliminate negative effects of cooler ambient conditions on organismal function? (3) Do such adjustments eventually become rigid, such that early experience continues to influence thermoregulatory tactics even if ambient conditions change?

Experimental manipulation will provide the most robust answers to these questions. Therefore, we raised three groups of young heliothermic ectotherms (juvenile tiger snakes) under different ambient thermal conditions (cold, intermediate and hot). After 14 months, we quantified thermoregulatory tactics of the young snakes as well as other fitness-relevant traits (growth rates, anti-predator responses, locomotor performance). We then abruptly changed the thermal conditions, to assess the possibility that prior experience continued to influence thermoregulatory tactics.

MATERIALS AND METHODS
Study species
Tiger snakes (*Notechis scutatus* Peters 1861) are highly venomous elapid snakes that are widely distributed throughout southern and eastern Australia (Cogger, 1992), ranging from sub-tropical Queensland to cold temperate Tasmania and southwestern Western Australia. This extensive range encompasses a wide range of climatic conditions and a diverse array of habitat types; this species has been particularly successful on many offshore islands (Keogh et al., 2005). Despite this huge range, there is little genetic divergence among populations across Australia [less than 0.3% variation from east to west (Scott et al., 2001; Keogh et al., 2005)].

Experimental procedure
The 43 neonate tiger snakes used for this study were born to five pregnant female snakes captured on Carnac Island (32°07′S; 115°39′E), a small (16 ha) limestone plateau 12 km off the coast of Fremantle. All females were transferred to a controlled-temperature room and individually housed in plastic boxes (80 cm × 30 cm × 20 cm), featuring a heating lamp, water dish and wood shavings as substratum. Females were fed dead mice weekly. Birth occurred between 13 March and 24 April 2007. Less than 24 h after parturition, we recorded neonates body mass (M_b) with a digital scale (±0.01 g, Nutriflo Hydroponic Systems, West Gosford, NSW, Australia) and snout–vent length (SVL) to the nearest 5 mm. For the purpose of the experiment, we selected 11, 15 and 17 animals from the total offspring of the five litters (split-clutch design) and allocated them among three treatments: hot-, intermediate- or cold-rearing conditions. Neonates from each litter were allocated evenly to each treatment group to balance potential maternal (litter-of-origin) effects. The rearing conditions were designed to be ecologically relevant, in allowing snakes the opportunity to select their body temperatures from among a biologically meaningful range; the treatments varied only in basking opportunities and hence in the availability of relatively high temperatures. We maintained the animals under the following conditions for 16 months.

Hot, intermediate and cold thermal gradients
Snakes were housed in individual wooden enclosures (100 cm long × 15 cm wide × 50 cm high), including a water dish and with pine shavings as substratum. Enclosures were open-topped, and each was fitted with a 40 W incandescent light located at one end, 40 cm above the floor of the enclosure. Each globe was on from 06:00 h to 21:00 h. All enclosures were located in a controlled-temperature room (using two air conditioning systems located at opposite sides of the room) set at 19°C by day and 16°C by night (night temperature was slightly modified by outside variations). Two wire thermostats were located directly below the globes at ground level: one in the cold-treatment

group set to turn off the globe if the temperature rose above 22°C, and one in the intermediate-treatment group with a shut-down temperature set to 26°C. There was no thermostat fitted in the hot-treatment enclosures to allow for a hotter basking spot. We used thermal data loggers (Dallas Thermochron iButton, Maxim Integrated Products, Sunnyvale, CA, USA; model DS1922L-F50; precision 0.063°C) on the cage floor to quantify the temperature gradients created by this system. Thermal data were recorded for 13 h a day (from 07:00 h to 20:00 h; one record every minute) for two to five consecutive days, in replicate enclosures of each treatment (7 hot, 9 intermediate and 11 cold enclosures). Mean and maximum temperatures (averaged across the recording period) at the cool end of the gradients were similar between treatments (reflecting ambient room temperature) but differed strongly at the heated ends of the enclosures (Fig. 1).

All snakes were offered pre-killed lab mice (between 1.5 g and 3.0 g) weekly. We measured M_b and SVL of each snake every two months to describe rates of growth. We also recorded sloughing events and amounts of food consumed per individual snake throughout the experiment.

Experimental design
The experiment included four stages of thermal data gathering: (1) after 14 months in the respective gradients, we determined the mean and maximum selected temperatures for each animal (eight days recording of 'Routine temperature records'). (2) One week later, all gradients were set to hot mode for a week (five days recording of 'Short-term shift into hot conditions'). Then, all gradients were returned to routine functioning. (3) Three weeks later, all gradients were set to cold conditions for a week (five days recording of 'Short-term shift into cold conditions'). (4) Finally, all snakes were returned to hot conditions for two months, after which period we recorded body temperatures for another week (five days recording of 'Long-term shift into hot conditions').

Snake body-temperatures
To record the thermal regimes selected by snakes, we directly taped an ibutton temperature data logger onto each snake's body (three-
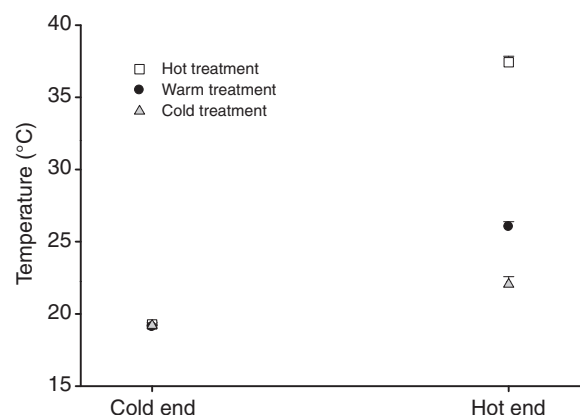

Fig. 1. Thermal data for ambient conditions experienced by three groups of neonatal tiger snakes that were reared in individual enclosures under three experimental conditions: access to cold (11 snakes), intermediate (15 snakes) or hot (17 snakes) thermal gradients. Temperatures at cold ends of the enclosures were similar between the cold, intermediate and hot groups, both in mean ($F_{2,24}=0.42$; $P=0.66$) and maximum temperature ($F_{2,24}=0.61$; $P=0.55$). At the heated end of the enclosures, however, the three treatment groups experienced very different mean ($F_{2,24}=138.31$; $P<0.0001$) and maximum temperatures ($F_{2,24}=87.52$; $P<0.0001$). Means ± standard errors are plotted.

quarters of the way along the body), in direct contact to the animal's skin (hereafter referred to as 'snake body-temperature'). This procedure was standardised to minimise error (i.e. position of the logger on the body, amount of attaching tape) and temperatures obtained using this method accurately follow internal body temperatures variations (Shine et al., 2001a; Shine et al., 2003).

For both the hot- and cold-treatment groups, we calculated, for each hourly period from 07:00h to 20:00h, a grand mean of the hourly mean body temperature from multiple days (between four and eight days) in order to describe typical basking patterns of the snakes. Temperature data were recorded outside of shedding events and feeding events. If a snake was in the process of sloughing, its test was postponed to the following week. Data for the day when data loggers were fitted were discarded from the analysis to eliminate short-term effects of handling disturbance on snake behaviour.

Defensive behaviour

We quantified defensive behaviour at body temperatures of 18°C and 30°C in all three treatment groups (following at least 6h acclimation to the test temperature in each case). For the test, each snake was lifted from its enclosure with a small hook and placed on an open bench top. The defensive behaviour exhibited at this time was recorded as 'neck flattening', 'biting attempt' or 'no response'. As the snake crawled across the open bench top, it was then suddenly restrained mid-body by a gloved hand. We recorded the number of bites directed either to the glove or to a large pen brought in front of the snake's head, as well as defensive displays, such as neck-flattening or the adoption of a raised ('cobra like') threat posture.

Swimming performance

In order to record swimming performance, we used a standard procedure adopted in previous studies of snakes (Shine and Shetty, 2001; Aubret, 2004; Aubret and Shine, 2007) consisting of a swimming track (220cm × 20cm, 35cm high, with water 15cm deep). Tiger snakes often inhabit swampy areas and swim in the course of their daily activities (Mirtsin and Bailey, 1990; Shine, 1977; Shine, 1987). The bottom of the pond liner was painted with a white (non-toxic) paint and line markers drawn every 30cm. Snakes were individually dropped from a few centimetres above the water at one end of the track and encouraged to swim back-and-forth by gently tapping the tail with a paintbrush. Trials were videotaped (JVC hard disk camcorder, Gosford, NSW, Australia; 25 frames s^{-1}) and digitally processed on a computer. For each snake, swimming speed was recorded five to eight times, and the fastest speed was retained for analysis. Each snake was tested once in warm water (30°C) and once in cold water (20°C). Water temperature was monitored throughout the trials and frequently readjusted to testing temperature. To match snake body-temperature with water temperature (and limit thermal shock), heating lamps were turned off in all enclosures and air temperature was brought close to water temperature several hours before testing.

RESULTS
Snake growth rates

At the beginning of the experiment, all three groups were similar in mean M_b (cold 5.57±0.80g; intermediate 5.44±0.75g; hot 5.74±0.77g; $F_{2,40}=0.56$; $P=0.58$), SVL (cold 19.00±0.74cm; intermediate 19.03±1.20cm; hot 19.38±0.96cm; $F_{2,40}=0.67$; $P=0.52$) and body condition [cold 5.65±0.80; intermediate 5.50±0.75; hot 5.60±0.77; analysis of covariance (ANCOVA) with M_b as the factor and SVL as the covariate; $F_{2,39}=0.22$; $P=0.81$]. Although all three groups were fed similar amounts of food throughout the experiments

(grams of food: cold 55.55±0.97g; intermediate 55.89±2.32g; hot 55.91±2.57g; $F_{2,40}=0.88$; $P=0.42$), growth rates in M_b [repeated-measures analysis of variance (RM ANOVA) with treatment group as the factor and M_b as the repeated measure through time] and SVL (RM ANOVA with SVL as the repeated measure) differed significantly among treatments ($F_{12,240}=4.11$; $P<0.0001$; $F_{12,240}=5.30$; $P<0.0001$, respectively; see Fig.2). However, body condition at the end of the experiment did not differ significantly among the three groups: cold 17.58±1.69; intermediate 17.25±2.45; hot 16.41±1.70 ($F_{2,39}=0.22$; $P=0.81$).

In all three treatment groups, snakes shed their skin about three times during the experiment (cold 2.81±0.60 times; intermediate 3.20±0.56 times; hot 3.24±0.66 times; $F_{2,40}=1.99$; $P=0.15$). However, the first slough occurred later in the cold group (after 223.81±56.21 days) than in the intermediate group (139.60±41.93 days) or hot group (143.47±42.23 days; comparing the three groups, $F_{2,40}=10.22$; $P<0.0003$). Finally, the among-group differences in average intervals between successive sloughing events were close to statistical significance (cold 152.07±19.54 days; intermediate 136.99±19.04; hot 129.60±26.41; $F_{2,40}=3.02$; $P<0.060$).

Mean selected body temperatures and maximum body temperatures
Routine temperature records

Statistical analyses revealed no significant difference in either mean body temperature or maximum body temperature among the three treatment groups when we measured these variables 14 months after
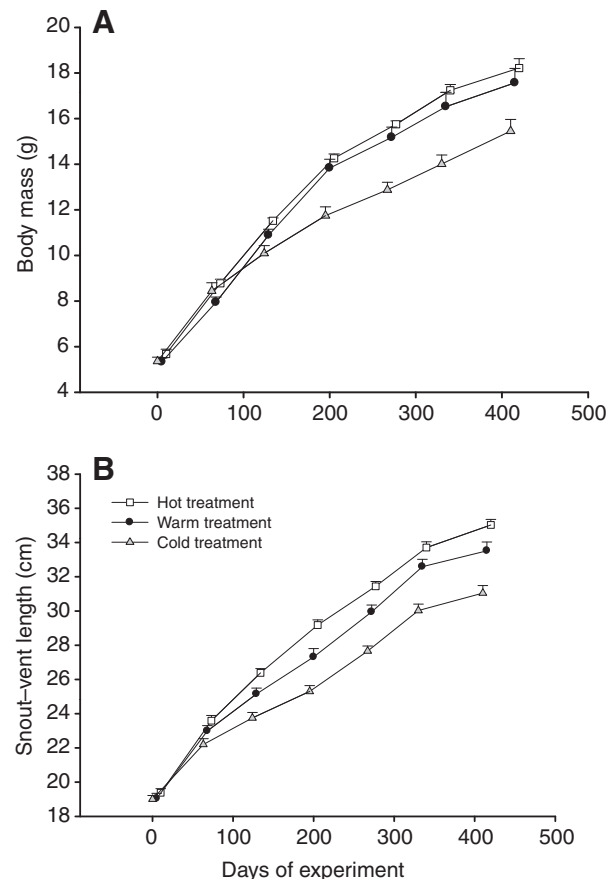

Fig. 2. Rates of growth in body mass (A) and snout–vent length (B) in juvenile tiger snakes raised in three alternative treatments gradients [access to cold (11 snakes), intermediate (15 snakes) or hot thermal gradients (17 snakes)] for 14 months. Mean ± standard errors are plotted.

initiation of the study (see Table 1 and Fig. 3). Nonetheless, diel patterns of basking diverged strongly between the cold- and hot-treatment groups (see Fig. 4; RM ANOVA with groups as the factor and mean hourly temperature as the repeated measure; $F_{12,312}$=18.75; P<0.0001); cold-treatment snakes initiated basking earlier in the day than did hot-treatment animals (Fig. 4).

Short-term shift into hot conditions
During the week following the acute shift into a hot gradient, mean body temperatures did not differ significantly among the three groups overall (P=0.11 – see Table 1 and Fig. 3). If the analysis is restricted to the two extremes, however (hot 24.81±1.65°C *versus* cold 26.58±2.49°C), the difference in mean body temperature is statistically significant ($F_{1,26}$=4.96; P<0.035). Mean maximum temperatures differed among the two treatment groups, with cold-raised animals selecting higher maximum temperatures than did the hot-raised group (31.96±2.08°C *versus* 29.21±2.28°C, respectively). Following the shift to hot conditions, the cold-raised group increased their mean body temperature by an average of 2.1°C (Wilcoxon matched pair test; N=11; Z=2.58; P<0.001) and their mean maximum body temperature by 2.2°C (P<0.008). Similarly, the intermediate-raised group increased their mean (by 1.62°C; N=15; Z=2.50; P<0.012) and maximum (by 1.3°C) temperatures (P<0.041). Basking patterns of these animals also changed (see Fig. 4); the cold-raised snakes maintained high body temperatures throughout the day, continuing to bask at higher body temperatures than did the hot-raised snakes exposed to the same conditions (RM ANOVA $F_{12,312}$=2.28; P<0.008).

Short-term shift into cold conditions
Mean body temperatures differed between the cold-raised group and both of the other groups (higher by almost a degree on average in the former group) whereas maximum temperatures did not (see Table 1 and Fig. 3). The intermediate group did not shift significantly either in mean (Wilcoxon matched pair test; N=15; Z=1.25; P=0.21) or maximum temperatures (P=0.86) whereas the hot group lowered their mean temperature by 1.5°C (N=17; Z=3.62; P<0.0003; their mean maximum temperature did not change significantly, P=0.76). Basking patterns differed strongly between snakes from the cold-raised *versus* hot-raised groups (RM ANOVA; interaction term $F_{12,312}$=1.67; P=0.073; effect of treatment $F_{1,26}$=11.20; P<0.0025; see Fig. 4).

Long-term shift into hot conditions
When tested two months after their shift to hot conditions, the snakes exhibited body-temperature regimes similar to those they had
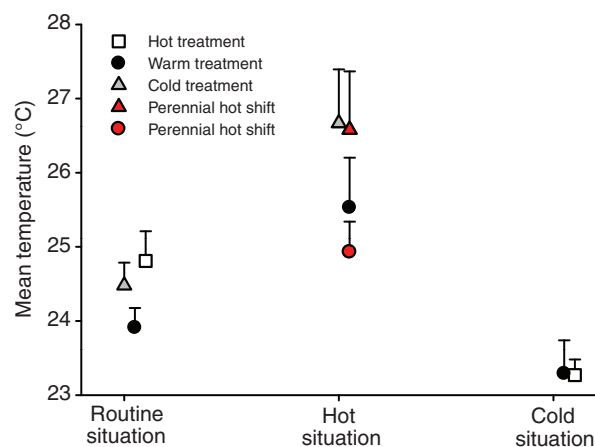

Fig. 3. Changes in mean body temperature in juvenile tiger snakes acclimated to three alternative thermal regimes, and then subjected to a short-term shift to either hot or cold conditions (see text for details on procedure). Changes in mean temperature after a long-term shift into hot conditions are plotted for both cold and warm treatment groups. Means ± standard errors are plotted.

shown immediately after a short-term shift into these conditions. For example, body-temperature records for the cold-raised group after two months in hot conditions were similar to those after one week (from the earlier experiment) in terms of mean body temperature (26.58±2.49°C *versus* 26.71±3.16°C, respectively; Wilcoxon matched paired test: N=10; Z=0.051; P=0.95 – see Fig. 2) and maximum body temperature (31.96±2.07 *versus* 31.02±1.24°C; P=0.28). A similar pattern was recorded for the intermediate group for both mean (24.93±1.42°C; P=0.64) and maximum body temperatures (29.66±2.68°C; P=0.34).

Defensive behaviour
Defensive behaviour at 18°C body temperature
The thermal regimes under which snakes had been raised for 14 months prior to testing had no significant influence on defensive responses, such as the percentage of snakes displaying a flattened neck (Pearson χ^2 test; χ^2=3.37; d.f.=2; P=0.19; 53% of snakes overall) or attempting to bite (P=0.18; 13% of snakes overall) upon capture. The same was true of responses exhibited when the snakes were restrained by hand (% of snakes flattening the neck=27% overall, P=0.39; raising the forebody=10% overall, P=0.19; mean number of bites 8.73±8.75; $F_{2,37}$=0.34; P=0.71).

Table 1. Neonate tiger snakes were raised for 14 months in three alternative thermal gradients: cold, intermediate or hot gradients

Body temperatures	Cold gradient N=11	Intermediate gradient N=15	Hot gradient N=17	d.f.; F	P
Routine conditions:					
Mean body temperature (°C)	24.48±1.02	23.91±0.88	24.81±1.65	2,40; 1.97	0.15
Maximum (°C)	29.76±1.22	29.22±1.77	29.20±2.28	2,40; 0.39	0.68
Short-term shift to hot conditions:					
Mean body temperature (°C)	26.58±2.49	25.53±2.23	24.81±1.65	2,40; 2.32	0.11
Maximum (°C)	31.96±2.08	30.56±2.46	29.20±2.28	2,40; 4.69	**0.015**
Short-term shift to cold conditions:					
Mean body temperature (°C)	24.48±1.02	23.29±1.49	23.27±0.87	2,40; 4.52	**0.017**
Maximum (°C)	29.76±1.22	29.33±2.90	29.17±1.76	2,40; 0.30	0.74

We recorded the snakes' mean and maximum body temperatures in their home cages ('routine conditions') at the end of this 14-month period. We then shifted the snakes to 'hot' conditions and measured their body temperatures as before; and a few weeks later did the same with cold conditions. The table shows mean values ± s.d.
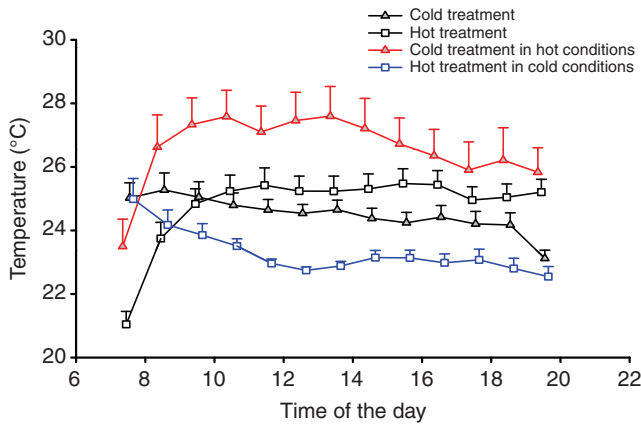
Fig. 4. Mean body temperatures over each hour of the day (from 07:00h to 20:00h; averaged across several days) for tiger snakes raised in the cold and hot treatments. The graphs show means ± standard errors for body-temperatures of snakes in (1) routine conditions, prior to the shift; (2) after a shift to hot conditions; and (3) after a shift to cold conditions (see text for details on procedure).

Defensive behaviour at 30°C body temperature

Again, no significant difference was detected among the groups for any of the defensive traits tested, either when first taken out of their home cage (displays involving body-flattening=8% of snakes, P=0.94; biting attempt=9% of snakes, P=0.17) or when seized by a hand (flattening=9% of snakes, P=0.47; raised posture=14% of snakes, P=0.27; mean number of bites 11.73±8.28, $F_{2,37}$=0.54; P=0.59).

Locomotor performance

In all three treatments, swimming speeds were higher when the snakes were tested in warm water than in cold water, in absolute terms (cm s^{-1}; RM ANOVA; $F_{1,34}$=34.61; P<0.0001) as well as in relative speed (body lengths s^{-1}; $F_{1,34}$=34.84; P<0.0001). However, mean swimming speeds were unaffected by prior raising conditions, regardless of whether the snakes were tested in cold water (absolute terms – hot 42.99±7.07 cm s^{-1}; intermediate 44.01±6.54 cm s^{-1}; cold 39.40±7.52 cm s^{-1}; $F_{2,34}$=1.43; P=0.25; relative to SVL – hot 1.23±0.19; intermediate 1.31±0.19; cold 1.27±0.24 SVL swum s^{-1}; $F_{2,34}$=0.53; P=0.59) or warm water (absolute terms – hot 53.91±7.20 cm s^{-1}; intermediate 49.61±8.96 cm s^{-1}; cold 51.16±5.46 cm s^{-1}, $F_{2,34}$=1.43; P=0.25; relative to SVL – hot 1.54±0.21; intermediate 1.47±0.24; hot 1.65±0.24, $F_{2,34}$=1.80; P=0.19).

DISCUSSION

The impact of climate change on terrestrial ectotherms will depend upon the effectiveness of behavioural buffering mechanisms, i.e. to what degree does a change in ambient thermal conditions translate into a change in the body temperatures (and thus fitness) of an ectotherm? That simple question is difficult to answer. For example, the relationship between body temperature and fitness is complex; some taxa (e.g. tropical species) may be less able to deal with thermal variation than are others (Deutsch et al., 2008); and plasticity at several levels allows ectotherms to reduce the costs of sub-optimal body temperatures [e.g. by adjusting anti-predator tactics to thermally-imposed constraints of locomotor ability (Smith, 1997; Shine et al., 2000; Mori and Burghardt, 2004)]. Nonetheless, there

are clear limits to such plasticity, and our long-term study identifies two such limits.

First, although the young snakes were capable of modifying their thermoregulatory tactics enough that their body-temperature regimes (see also Hertz and Nevo, 1981), anti-predator responses and locomotor performance were virtually unaffected by the differing levels of basking availability that we imposed, the snakes nonetheless exhibited one clear fitness-related effect. Animals in the cold treatment grew less rapidly than did those in the hot treatment (Fig. 2), despite their similar mean body temperatures. We have no data on the mechanisms generating that growth-rate differential but suspect that it relates either to metabolic costs of frequent movement to fine-tune thermoregulatory behaviour to exploit all ambient opportunities; or a thermal difference among treatments that we did not detect because we measured body temperatures only on unfed snakes. Many snakes select higher temperatures post-feeding to accelerate digestion and perhaps increase its efficiency (Regal, 1966; Harwood, 1979; Naulleau, 1983; Christian et al., 1984; Sievert and Andreadis, 1999; Blouin-Demers and Weatherhead, 2001; Tattersall et al., 2004). If our hot-treatment snakes exploited the availability of high temperatures in this way (and we have no evidence that they did), it might explain the higher growth rates of these animals (i.e. their overall temperatures were higher, even though their non-feeding temperatures were not). Alternatively, the growth-rate effect might be adaptive, i.e. snakes benefit from smaller size under cool conditions because they thereby lower their thermal inertia and can heat more quickly (Grigg et al., 1979; Carrascal et al., 1992; Martín and López, 2003; Angilletta et al., 2007; Herczeg et al., 2007).

Given the considerable differences in available maximum temperatures among our three experimental treatments (19–37°C: see Table 1), the young animals' ability to maintain similar mean and maximum body temperatures is impressive. This result reflects behavioural buffering, where cool-treatment snakes emerged to bask earlier in the day, and kept basking for longer as well as closer to the heating lamp (F. A., personal observation). Comparable buffering behaviour has been described in lizards where sub-optimal temperatures led to longer basking periods – but these lizards often foraged at sub-optimal temperature with lower sprint speeds and predatory efficiency (Bennett, 1980; Christian and Tracy, 1981; Avery et al., 1982; Herczeg et al., 2008). In our study, body-temperature regimes were unaffected by our experimental manipulations (at least outside of feeding periods); thus, showing that lower growth rates [as also seen in lizards kept at unfavourable thermal regimes (Avery, 1984; Sinervo and Adolph, 1994; Martin and Lopez, 1999)] may be elicited by ambient thermal challenges even if body-temperatures are unaffected.

Second, we saw no evidence that the young snake's plasticity in thermoregulatory tactics (behavioural buffering) carried over to the period after temperature regimes were switched (i.e. when the snakes were 14 months of age). In strong contrast to the earlier results, neither a short-term nor a long-term change in thermal conditions led to a readjustment of snake thermoregulatory tactics (at least not within two months). That is, the animals continued to thermoregulate in the same way they had done previously, creating a mismatch between their behaviour and ambient thermal opportunities. Both at two weeks and two months after the change, these older snakes exhibited behaviours that had been fashioned by their exposure to specific thermal regimes earlier in life. Logistical constraints prevented us from continuing the study longer to see whether the animals would eventually exhibit plasticity similar to that seen in their first year of life. Regardless, our data provide a striking example

of how an ectotherm's thermoregulatory tactics (and thus its mean selected body temperature) can depend more upon previously encountered conditions than upon current thermal challenges. This 'historical' effect is not included in current methods of evaluating the thermoregulatory tactics of ectotherms (e.g. Christian and Weavers, 1996; Hertz et al., 1993; Martin and Huey, 2008).

Our study was designed to mimic potential changes in ambient thermal regimes as predicted under climate-change models. We presented young snakes with thermoregulatory opportunities (*via* a thermal gradient that provided a range of attainable temperatures during daylight hours) but the treatments differed in the degree of difficulty associated with maintaining high body temperatures. The young snakes proved adept at meeting this challenge, suggesting that a long-term shift into cooler or warmer conditions need not greatly affect mean selected body temperatures of an ectotherm. Nonetheless, growth rates were reduced by that increased thermal challenge. Lower growth rates might well impair fitness: larger body size can increase reproductive success (Bonnet et al., 2000; Brown and Shine, 2007) and maximum ingestible prey sizes [and thus the number of available prey (King, 2002; Vincent et al., 2006)] and reduce vulnerability to predation (Jayne and Bennett, 1990; Janzen 1993; Shine et al., 2001b).

The second shift (when the snakes were 14 months old) was designed to mimic another situation predicted to become common under climate change, i.e. a change from one year to the next in ambient thermal regimes (Houghton et al., 2001; IPCC, 2007). Importantly, changes at this temporal scale can occur at any time in the animal's life – and thus it becomes critical as to whether or not developmental plasticity in thermoregulatory tactics is retained into this later stage [*versus* being lost earlier in ontogeny, as occurs with many developmentally plastic traits (Stearns, 1989; Grünbaum et al., 2007; Scotti and Foster, 2007; Bonte et al., 2008)]. Our data hint that the plasticity is indeed lost (or, at least, reduced). Although our data relate only to a two-month period after the thermal shift, this period presumably is long enough for fitness costs of suboptimal thermoregulatory tactics to be significant (i.e. predation exposure, foraging success, etc.).

In summary, our long-term study suggests that behavioural plasticity in thermoregulatory tactics may be manifested at two timescales: immediate and developmental. Immediate plasticity buffers body temperatures against changes in ambient temperature but long exposure to a consistent set of thermal conditions may result in canalised thermoregulatory tactics. Such developmental canalisation may create problems in a world of sudden climatic shifts, with increased year-to-year variation in climate creating mismatches between an animal's tactics and the thermal challenges that it currently faces. Ideally, our models to predict climate-change effects should incorporate not only short-term behavioural flexibility (Kearney et al., 2009) but also developmental plasticity. We will need additional experimental work to more fully characterise that plasticity, the factors that limit its expression, and the consequences of such processes for organismal fitness.

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