THE INFLUENCE OF INCUBATION TEMPERATURE ON THE MORPHOLOGY, BEHAVIOUR AND PHYSIOLOGY OF A TROPICAL COLUBRID SNAKE (*TROPIDONOPHIS MAIRII*)

Introduction

Temperature has a profound effect on the biological processes of all animals (Birchard, 2004). As ectothermic organisms, reptiles maintain their body temperatures within limits predominantly by behavioural means, and are particularly sensitive to environmental conditions (Bogert, 1949). Reptile embryos require appropriate levels of temperature, moisture and gas exchange for successful development (Shine and Thompson, 2006). Since early-stage embryos are unable to control these factors, lacking both the physiological means and the behavioural responses of post-hatching animals, it is likely that reptiles are most vulnerable to adverse thermal conditions at early stages of development (Birchard, 2000). Thus, a warming climate has potentially significant implications for embryogenesis in reptiles.

Many oviparous snake species lay their eggs underground (Booth, 2006), presumably to attain protection from predation and avoid thermal extremes. The thermal buffering properties of soil attenuate heat flux, typically causing a complete absence of diel fluctuations in temperature at depths of between 30 to 50 cm and deeper (Ackerman and Lott, 2004). Despite little variation in temperatures at even shallow soil depths, it is often assumed that a wide range of thermal regimes are available for reptiles, from which they can select suitable nest sites. Testing of this assumption is required if we are to understand the potential susceptibility of oviparous reptiles to climate change.

The effects of climate change on reptiles could potentially be mitigated by acclimation responses. Thermal acclimation is the response of organisms to environmental temperatures (Angilletta, 2009). Several studies have found that hatchling snakes prefer or respond positively to ambient temperatures that correspond with the temperature experienced during incubation (Blouin-Demers *et al.*, 2000; Bronikowski, 2000), suggesting acclimation may pre-adapt individuals to post-hatching conditions. Most current studies examining the effects of incubation regime on reptiles test hatchlings at one temperature (Deeming, 2004). However, the significance of differential temperature-performance relationships (temperature reaction norms) cannot be accurately tested without accounting for the effects of phenotypic acclimation (Seebacher, 2005).

To address the aforementioned issues, I used a two-stage approach that aimed:

1. to provide information on the degree of thermal heterogeneity at different soil depths, and thus to determine the availability of suitable nest sites to oviparous reptiles. To achieve this, soil temperature profiles were measured in a tropical, open eucalypt woodland habitat where keelback snakes are locally abundant.
2. to use the temperature profiles, together with current projections for climate over the next century, as a basis for incubation treatments to test the morphological, behavioural and phenotypic responses of hatchling keelbacks to incubation temperatures. Evidence of acclimation responses were examined by testing the performance of hatchlings at three test temperatures, which were the same as the three incubation temperatures.

Main findings

- The maximum difference in average temperatures from all locations at the Townsville Town Common, regardless of depth or shade level, was 2.8°C.
- Soil temperatures at a depth of 2cm were warmer and varied more than at either 10cm or 20cm.
- Incubation temperature significantly affected 13 of the 15 traits examined.

- Incubation temperature was more important on hatchling performance than test temperature, including locomotor ability and willingness to swim.
- Hot incubated hatchlings had a lower hatching success rate, were smaller and had more developmental abnormalities than hatchlings from the mid and cold incubation treatments.
- There was only one significant interaction between incubation temperature and test temperature (anti-predator response), suggesting developmental acclimation does not always pre-adapt individuals to post-hatching environments.

Significance and future research suggestions

The results of this study reinforce the importance of thermal regime on the developmental plasticity of snakes. There were very few differences in traits between cold and mid treatments, thus supporting previous findings suggesting a surprisingly low thermal optimum in this tropical reptile (Brown and Shine, 2006a). Adaptation to relatively cool temperatures may have arisen due to covariation between thermal and hydric attributes of nest sites, with keelbacks preferring moister environments which are generally cooler (Brown and Shine, 2006a). Timing of breeding in keelbacks is dependent on abiotic factors, rather than biotic factors such as maximising food availability and minimizing predation (Brown and Shine, 2006b). The strong influence of season on the timing of breeding suggests suitable nest sites are already in sufficiently short supply, with little redundancy should global temperatures continue to rise as predicted.

There was a significant interaction among incubation and test temperatures in anti-predator responses, with hatchlings from the hot incubation treatment displaying relatively large increases in intensity of response to anti-predatory stimuli as test temperature increased. Given the size of hatchling keelbacks, this response appears maladaptive. However, it is unclear why reversible acclimation should occur in anti-predator behaviour but not performance traits. High incubation temperatures can disrupt normal development of the vertebral column (Yntema, 1960; Bustard, 1969). Perhaps shorter incubation durations associated with warmer incubation treatments prevent complete osteogenesis but over-develop neurological functions, resulting in heightened responses to stimuli but reduced locomotor ability. Further investigation into the mechanisms causing such large morphological and physiological differences between incubation temperatures would allow for a greater understanding of the persistent influence of incubation temperature on traits.

Whilst this study found little evidence for favourable developmental acclimation, individuals may, over time, be able to compensate for the negative effects of sub-optimal development temperatures through reversible acclimation (Angilletta, 2009). Compensation responses may permit some hatchlings to develop at a greater rate and effectively 'catch-up' to other hatchlings, achieving the same genetic potential but following a different developmental path. Thus far, evidence for compensation in reptiles has been mixed, with some studies finding differences in phenotypes remain stable for at least a few months (Elphick and Shine, 1998; Caley and Schwarzkopf, 2004), whilst others do not (Qualls and Shine, 1998). A study on *Liasis fuscus* found that differences in morphological traits among incubation temperature treatments *increased* over the month proceeding hatching (Shine *et al.*, 1997), suggesting a widening of differences in phenotypic traits. Such a response would have considerable ecological and evolutionary consequences should it be a widespread trait in snakes. Compensation responses are highly pertinent to the severity of impacts of incubation temperature, and further investigation into the prevalence and nature of these responses is critical.

Little is known about whether effects caused by different incubation temperatures lead to significant differences in post-hatching survival, and ultimately, fitness. Survivorship can be negatively, positively or uncorrelated to incubation temperatures within turtles, lizards and crocodiles (Deeming, 2004). Limited evidence from mark-recapture studies on keelbacks suggests that large sizes at hatching enhance survival (Brown and Shine, 2004). However, despite a lack of information on long-term compensation effects, and the relationship between specific traits and survivorship, a relatively small increase in incubation temperatures can considerably reduce viability and performance of hatchling keelbacks. Further research on the fitness effects of incubation temperature in the field is vital to allow for effective management decisions and mitigation measures, such as *ex-situ* conservation programmes, and *in-situ* habitat manipulation and enrichment initiatives (Deeming, 2004).