

Short Notes

Ontogenetic differences in the preferred body temperature of the European adder *Vipera berus*

Gábor Herczeg^{1,2}, Abigél Gonda³, Jarmo Perälä², Jarmo Saarikivi², Aino Tuomola² & Juha Merilä²

¹Behavioural Ecology Group, Department of Systematic Zoology and Ecology, Eötvös Loránd University, Budapest, Hungary

²Ecological Genetics Research Unit, Department of Bio- and Environmental Sciences, University of Helsinki, Finland

³Department of General Zoology, Eötvös Loránd University, Budapest, Hungary

We tested the hypothesis that the costs of thermoregulation (e.g. predation) can affect the preferred body temperature (T_p , the “target” body temperature in thermoregulation) of reptiles. In European adders (*Vipera berus*), juveniles face higher predation risks than adults. We compared T_p between adult and juvenile adders and found that the T_p of juveniles was approximately 5 °C lower than that of adults, while adult males and females did not differ. All groups were characterized by narrow T_p ranges. Our results suggest that reptiles may change their T_p in response to the high ecological costs of thermoregulation. Alternative explanations for the reported pattern are also discussed.

Key words: behaviour, predation, snake, thermoregulation

For ectotherms, the maintenance of body temperature (T_b) within a thermally optimal range is a necessity for the efficient maintenance of physiological processes (Stevenson, 1985; Angilletta et al., 2002a,b). Almost all aspects of physiological or behavioural performances in reptiles have shown to be T_b dependent (e.g. Van Damme et al., 1991; Shine & Harlow, 1993; Bauwens et al., 1995). The cost-benefit model of ectotherm behavioural thermoregulation predicts variation within and between species in their thermoregulation strategy, with respect to the local costs and benefits of the thermoregulatory behaviour and the thermal quality of the given environment (Huey & Slatkin, 1976). The two extreme strategies are thermoconformity (a lack of behavioural regulation) and active thermoregulation. It seems that maintaining T_b within the preferred range has a high priority for reptiles even in cool habitats (Huey et al., 2003; Blouin-Demers &

Nadeau, 2005), suggesting that the physiological benefits of optimal T_b are higher than the costs imposed by thermally challenging habitats (Gilchrist, 1995). On the other hand, when faced with the immediate risk of predation, reptiles trade the benefits of thermoregulation for safety by avoiding the thermally superior but hazardous basking sites (Downes & Shine, 1998; Downes, 2001; Webb & Whiting, 2005). According to the general theory, the latter behavioural pattern should be an indicator of shifting the behavioural thermoregulatory strategy towards thermoconformity (Huey & Slatkin, 1976), but other explanations cannot be excluded. For example, another possible mechanism connected to the avoidance of the hazardous basking sites can be a decrease in the preferred body temperature (T_p , the “target” T_b of behavioural thermoregulation).

We investigated the hypothesis that high predation pressure at a certain life stage is coupled with decreased T_p in reptiles. To test this hypothesis, we required a model organism displaying considerable within-population differences in predation risk during a short time interval while inhabiting the same area. Juvenile European adders, *Vipera berus*, are susceptible to very high predation pressure compared to the adults, resulting in approximately 75–90% mortality before maturity (Prestit, 1971; Madsen & Shine, 1994). Therefore, it is not surprising that more than 50% of the variance in lifetime reproductive success of *V. berus* is due to juvenile mortality (Madsen & Shine, 1994). Previous long-term studies reported that juveniles of both *V. berus* and the closely related *V. aspis* are secretive and rarely bask, probably as a result of predator avoidance (Madsen et al., 1999; Lourdaï et al., 2002). Hence, adders offer a model to study if differences in predation pressure (juveniles vs. adults) would result in within-population variation in T_p .

We collected nine male, six female and 11 juvenile adders (representing the nominate subspecies *Vipera berus berus*) in the spring of 2004. All adders were released at the site of capture after the experiments. Our study site was a rocky outcrop about 50 m long acting as a common hibernaculum, located in a young open coniferous/deciduous forest in the Vantaa area near Helsinki, southern Finland. The first male emerged from hibernation on 30 March, whereas the first female and juvenile appeared on 8 April. Individual snakes were captured and measured for snout–vent length (SVL; males: range = 41–53 cm, mean = 45.5 cm; females: range = 44–57 cm, mean = 48.5 cm; juveniles: range = 13.5–19 cm, mean = 15.1 cm), and the experiments were initiated within 10 days of the appearance of the first individual. We note that in our study population no melanistic individuals, which might differ in their thermal ecology from the zigzag morph (Forsman, 1995), were present.

We measured T_p (usually measured in a controlled thermal gradient where ecological costs of behavioural thermoregulation are assumed to equal zero, e.g. Hertz et al., 1993) in three rectangular glass terraria (150 cm long x 60 cm wide x 50 cm high). The terraria were kept in a room

with adjustable temperature fixed at 10 °C. One 250W reflector bulb was suspended at one end of each container to provide a photothermal gradient. We measured the operative temperature distribution (T_e , actually an estimate of T_b for an object that does not behaviourally or physiologically thermoregulate, and that has reached thermal equilibrium; Bakken et al., 1985) in the terraria with a fresh road-killed carcass of an adult adder (stored prior to the experiment in a freezer; the injuries were minimal) using the thermometer described below. The T_e gradients were established as 12.5–60 °C for an average adult adder. Although this relatively steep gradient would allow adult snakes to have regional T_b differences (e.g. between the head and the cloaca), we believe that this effect was minimal because 1) most snakes were basking in the typical coiled position, 2) adult body temperatures were measured 4 cm deep in the cloaca and 3) we can assume the temperature homogenising effect of blood flow. We note that the gradients would mean something different for juvenile snakes due to their small size and thus smaller thermal inertia, but this potential difference was negligible from our point of view (assuming a zero cost thermal environment with available temperatures both beyond and below the preferred range). Water was provided *ad libitum*. The individuals were not fed because, firstly, a one-day starvation is totally natural for a snake, secondly, adders do not feed early in the season (Prestit, 1971) and thirdly, digestion might influence T_p (Blouin-Demers & Weatherhead, 2001). We used a 5 cm layer of sand as substrate in each terrarium, and evenly distributed rocks were provided as shelters. We changed the substrate and washed the rocks between experiments. Due to logistic constraints, we housed a maximum of three adults (sexes separately) or six juveniles (separated from adults) in a single terrarium. However, as 1) a lack of social interactions has been reported for adders in the period after hibernation and before the mating season (e.g. Prestit, 1971), 2) we observed that snakes sometimes basked together in the field and 3) we did not observe any social interactions (which could have limited accessibility to the heat sources) during the experiments, we assumed that snakes were thermoregulating independently from their conspecifics. We applied paint codes for individual recognition of the snakes. Adders were transported from the field to the laboratory during late afternoon, and housed in the experimental terraria for acclimation to the setting. Each experiment started on the following day. We measured the cloacal T_b of the experimental animals at hourly intervals (1000–1800) with a K-type thermocouple connected to a digital thermometer (TESTO 925, TESTO GmbH, Lenzkirch, Germany). The heating bulb was switched on 1 h prior to the first measurements. This period is sufficient for adders to reach high T_b (Forsman, 1995). We only measured T_b of active adders (that is, basking or moving; not hiding under rocks).

As repeated body temperature measurements of the same individuals are unlikely to be independent (e.g. Angilletta & Werner, 1998), we used a General Linear Mixed Model (GLMM) with individual as a random factor and group (male, female, juvenile) as a fixed factor to account for the lack of independence in our data. This

approach was also able to handle the problem imposed by the missing data caused by inactive individuals (those hiding under rock shelters). We compared least squares group means with Tukey–Kramer post hoc tests. Activity patterns during the experiments were analysed with χ^2 tests, using the frequency data. We quantified activity as the ratio between the potential maximal activity (the number of measurements) and the actual activity (number of times when individuals of a given group were not hiding). Statistical analyses were performed using STATISTICA 4.5 for Windows (StatSoft Inc. Tulsa, Oklahoma) and SAS 8.02 for Windows (SAS Institute Inc., Cary, NC).

We found significant differences in T_p of the different age/sex groups ($F_{2,19}=76.13$; $P<0.001$; Fig. 1). The individual effect was nonsignificant ($z=1.53$; $P>0.05$). Post hoc tests revealed that adult males and females did not differ in their T_p (Tukey–Kramer test: $P=0.24$), while both differed from juveniles (Tukey–Kramer tests; all $P<0.001$). Percentage of activity was 100% for males and 96.3% for females, but only 57.6% for juveniles; the juveniles were found to differ significantly from both males and females after Bonferroni correction (male–female: $\chi^2_1=3.05$, $P=0.08$; male–juvenile: $\chi^2_1=44.82$, $P<0.001$; female–juvenile: $\chi^2_1=25.57$, $P<0.001$).

Juvenile adders maintained considerably lower body temperatures (approximately 5 °C; Fig. 1) in the zero cost thermal gradient than their adult conspecifics did, while adult males and females had similar thermal preferences. One could argue that juveniles simply avoided the hot area, as their smaller size resulted in an increased heating rate (e.g. Grigg et al., 1979), making them more vulnerable to overheating. However, even the much smaller sympatric lizard *Zootoca vivipara* thermoregulates accurately, reaching high body temperatures in similar enclosures (Gvozdk & Castilla, 1999; Carretero et al., 2005; Herczeg et al., 2006). The narrow T_p ranges pertaining to both adults and juveniles suggest a drive towards precise thermoregulation, although T_b maintained in a laboratory zero cost thermal environment does not necessarily reflect thermoregulatory behaviour in the field (Fitzgerald et al., 2003).

The preferred body temperature of reptiles can vary between seasons (Christian & Bedford, 1995), with photoperiod (Rismiller & Heldmaier, 1988), with food intake (Blouin-Demers & Weatherhead, 2001) or with reproductive state (Mathies & Andrews, 1997) within populations, and interactions between different external and internal factors are conceivable (Rock et al., 2000). However, we sampled one adder population from a small area during a short time interval. Furthermore, adult adders remain within the close vicinity of the hibernacula entrances without feeding or involvement in social interactions after spring emergence and before the mating season (e.g. Viitanen, 1967; Prestit, 1971; Nilson, 1980). Although juveniles might start foraging soon after hibernation, we checked them after capture by palpation, and their stomachs were found to be empty. Hence, the most obvious factors affecting T_p can be ruled out in our study (we note that the pattern reported here may be different in other seasons). It is noteworthy that shifts in T_p do not neces-

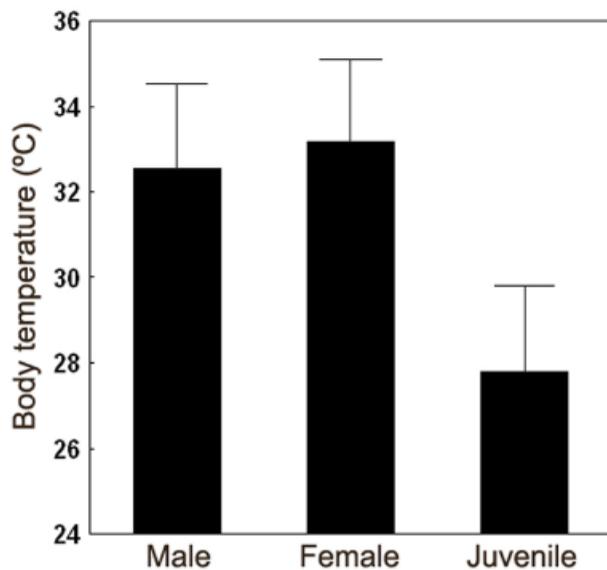


Fig. 1. Preferred body temperatures (mean \pm SD) of adult male, female and juvenile European adders (*Vipera berus*) after spring emergence.

sarily represent shifts in the physiologically optimal body temperature, even on an evolutionary time scale (Huey & Bennett, 1987).

The difference between juveniles and adults can be interpreted from the point of view of one of the main costs of behavioural thermoregulation, namely increased exposure to predation. Various potential adder predators, such as ravens (*Corvus corax*), crows (*Corvus corone cornix*), magpies (*Pica pica*), hedgehogs (*Erinaceus europaeus*), weasels (*Mustela nivalis*) and, given the close proximity of the city of Helsinki, feral dogs and cats occur on our study site. Hence, basking in conspicuous places far from refuges might be too costly for the vulnerable juvenile adders irrespective of the sites' thermal quality. On the other hand, with their low T_p and small size (faster heating), juveniles might be able to thermoregulate accurately and efficiently (with respect to their lower T_p) *sensu* Hertz et al. (1993), using more hidden, although cooler sites. This strategy would reduce the physiological costs of low T_b when compared to a hypothetical thermoconformer. Moreover, maximum sprint speed and endurance were found to be far less temperature dependent than digestive performance in reptiles (Angilletta et al., 2002a), suggesting that juvenile adders might not lose too much from their ability to escape by selecting lower T_b than the adults. However, costs of low digestive performance and probably lost foraging opportunities in terms of slow development and thus late maturity seem to be considerable (Webb & Whiting, 2005).

However, based on our approach (i.e. lack of experimental manipulation of predation pressure), we cannot exclude other explanations for the reported T_p differences than the effect of size-dependent predation risk. In theory, differences in T_p can reflect either "real" physiological differences between the groups (e.g. differences in the thermal optima of physiological functions, thermal reaction norms or metabolism) or different ecological constraints on thermoregulation, especially on thermal

microhabitat choice. For example, permanent differences between adder age groups in their prey, feeding frequency, microhabitat selection, moving rates, etc. could all influence the thermal optima of the physiological functions over an evolutionary time scale or alter T_p without changing the thermal optima. Further, in theory, the T_p of juveniles could be interpreted as the norm, with T_p of adults being viewed as an increase over this, but considering diurnal heliotherms, this is unlikely to be the case.

Juveniles hid under rock shelters during almost half the time available for basking, while adults – with no sex difference – exploited close to 100% of the time for basking. This behaviour is in accordance with the secretive habit of vulnerable juvenile snakes in nature, which has been reported for several species, including *V. berus* (Madsen et al., 1999; Lourdais et al., 2002; Webb & Whiting, 2005), and is generally interpreted as a mechanism of predator avoidance.

In summary, we found considerable differences in T_p between adult and juvenile *V. berus* constrained by different levels of predation risk. Our results suggest that the costs of thermoregulation can not only shift thermoregulatory behaviour from active thermoregulation towards thermoconformity, but decrease the "target" T_b of behavioural thermoregulation as well. In this way, maintaining T_b at a predictable level with successful thermoregulatory behaviour (with regard to the lower T_p), and decreasing the physiological costs of low T_b (compared to thermoconformity) would be possible. Besides the reported plasticity in T_p , further investigations are needed to explore whether 1) the thermal optima of the different physiological functions, and 2) the thermoregulatory behaviour of adders – in terms of accuracy and effectiveness of thermoregulation (as defined by Hertz et al., 1993) – is labile or not.

Acknowledgements. We thank M. Angilletta, G. Nilson, M. Fowler and two anonymous reviewers for useful suggestions on a previous draft. G.H. was supported by the Hungarian Eötvös State Fellowship (25/2004) and a CIMO Fellowship (TM-04-2140) during the research. The experiments were done under the license of the Helsinki University Animal Experimentation Committee (HY-205-04).

REFERENCES

- Angilletta Jr, M.J. & Werner, Y.L. (1998). Australian geckos do not display diel variation in thermoregulatory behaviour. *Copeia* 1998, 736–742.
- Angilletta Jr, M.J., Hill, T. & Robson, M.A. (2002a). Is physiological performance optimised by thermoregulatory behavior? A case study of the eastern fence lizard, *Sceloporus undulatus*. *Journal of Thermal Biology* 27, 199–204.
- Angilletta Jr, M.J., Niewiarowsky, P.H. & Navas, C.A. (2002b). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27, 249–268.
- Bakken, G.S., Santee, W.R. & Erskine, D.J. (1985). Operative and standard operative temperature: tools for thermal energetics studies. *American Zoologist* 25, 933–943.

- Bauwens, D., Garland Jr, T., Castilla, A.M. & Van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* **49**, 848–863.
- Blouin-Demers, G. & Weatherhead, P.J. (2001). An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. *Journal of Animal Ecology* **70**, 1006–1013.
- Blouin-Demers, G. & Nadeau, P. (2005) The cost-benefit model of thermoregulation does not predict lizard thermoregulatory behaviour. *Ecology* **86**, 560–566.
- Carretero, M.A., Roig, J.M. & Llorente, G.A. (2005). Variation in preferred body temperature in an oviparous population of *Lacerta (Zootoca) vivipara*. *Herpetological Journal* **15**, 51–55.
- Christian, K.A. & Bedford, G.S. (1995). Seasonal changes in thermoregulation by the frillneck lizard, *Chlamydosaurus kingii*, in tropical Australia. *Ecology* **76**, 124–132.
- Downes, S. (2001). Trading heat and food for safety: costs of predator avoidance in a lizard. *Ecology* **82**, 2870–2881.
- Downes, S. & Shine, R. (1998). Heat, safety or solitude? Using habitat selection experiments to identify a lizard's priorities. *Animal Behaviour* **55**, 1387–1396.
- Fitzgerald, M., Shine, R. & Lemckert, F. (2003). A reluctant heliotherm: thermal ecology of the arboreal snake *Hoplocephalus stephensii* (Elapidae) in a dense forest. *Journal of Thermal Biology* **28**, 515–524.
- Forsman, A. (1995). Heating rates and body temperature variation in melanistic and zigzag *Vipera berus*: does colour make a difference? *Annales Zoologici Fennici* **32**, 365–374.
- Gilchrist, G.W. (1995). Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *American Naturalist* **146**, 252–270.
- Grigg, G.C., Drane, C.R. & Courtice, G.P. (1979). Time constants of heating and cooling in the eastern water dragon, *Physignatus lesuerui* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology* **4**, 95–103.
- Gvozdík, L. & Castilla, A.M. (2001). A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient. *Journal of Herpetology* **35**, 486–492.
- Herczeg, G., Gonda, A., Saarikivi, J. & Merilä, J. (2006). Experimental support for the cost-benefit model of lizard thermoregulation. *Behavioral Ecology and Sociobiology* **60**, 405–414.
- Hertz, P.E., Huey, R.B. & Stevenson, R.D. (1993). Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist* **142**, 796–818.
- Huey, R.B. & Bennett, A.E. (1987). Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* **41**, 1098–1115.
- Huey, R.B., Hertz, P.E. & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *American Naturalist* **161**, 357–366.
- Huey, R.B. & Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology* **51**, 363–384.
- Lourdais, O., Bonnet, X., DeNardo, D. & Naulleau, G. (2002). Do sex divergences in reproductive ecophysiology translate into dimorphic demographic patterns? *Population Ecology* **44**, 241–249.
- Madsen, T. & Shine, R. (1994). Components of lifetime reproductive success in adders, *Vipera berus*. *Journal of Animal Ecology* **63**, 561–568.
- Madsen, T., Shine, R., Olsson, M. & Wittzell, H. (1999). Restoration of an inbred adder population. *Nature* **402**, 34–35.
- Mathies, T. & Andrews, R.M. (1997). Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: Why do pregnant females exhibit low body temperatures? *Functional Ecology* **11**, 498–507.
- Nilson, G. (1980). Male reproductive cycle of the European adder, *Vipera berus*, and its relation to annual activity periods. *Copeia* **1980**, 729–737.
- Prestt, I. (1971). An ecological study of the viper *Vipera berus* in southern Britain. *Journal of Zoology* **164**, 373–418.
- Rismiller, P.D. & Heldmaier, G. (1988). How photoperiod influences body temperature selection in *Lacerta viridis*. *Oecologia* **75**, 125–131.
- Rock, J., Andrews, R.M. & Cree, A. (2000). Effects of reproductive condition, season and site on selected temperatures of a viviparous gecko. *Physiological and Biochemical Zoology* **73**, 344–355.
- Shine, R. & Harlow, P. (1993). Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122–127.
- Stevenson, R.D. (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *American Naturalist* **126**, 362–386.
- 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 vivipara* Jacquin. *Functional Ecology* **5**, 507–517.
- Viitanen, P. (1967). Hibernation and seasonal movements of the viper, *Vipera berus* (L.), in southern Finland. *Annales Zoologici Fennici* **4**, 472–546.
- Webb, J.K. & Whiting, M.J. (2005). Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. *Oikos* **110**, 515–522.

Accepted: 20 August 2006