

# Contrasted thermal preferences translate into divergences in habitat use and realized performance in two sympatric snakes

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## Keywords

ectotherm; thermoregulatory strategy; cost-benefit model of thermoregulation; microhabitat selection; performance.

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Editor: Mark-Oliver Rödel

Received 30 August 2010; revised 31 January 2011; accepted 31 January 2011

doi:10.1111/j.1469-7998.2011.00802.x

## Abstract

Temperature influences ectotherm fitness by affecting physiological performance. Terrestrial reptiles behaviourally regulate their body temperature by selecting particular microhabitats or activity periods. In temperate climates, thermal constraints make precise thermoregulation costly. Theoretical models of thermoregulation predict that species in cool environments should exhibit lower optimal temperature for performance and lower thermal preferences to minimize thermoregulatory costs. Empirical data in support of this prediction remain equivocal because several species maintain high and constant body temperatures, even in cool environments. We studied two largely sympatric colubrid snakes, *Hierophis viridiflavus* and *Zamenis longissimus* that share numerous morphological and ecological similarities, but differ markedly in thermal preference. Our objective was to quantify their thermoregulatory strategies in the field to determine how thermal preferences translate in habitat use and performance gain. The thermophilic species, *H. viridiflavus*, selected open microhabitats, whereas *Z. longissimus*, which prefers cooler temperatures, used a greater diversity of microhabitats. The two species differed markedly in their exposure levels. *Hierophis viridiflavus* was constrained to shuttle between sun and shade to maintain preferred body temperatures rendering it very exposed, while covered microhabitats were usually thermally compatible with the requirements of *Z. longissimus*. High exposure was apparently counterbalanced by higher locomotor performances in *H. viridiflavus*. The divergence in thermal ecology between *Z. longissimus* and *H. viridiflavus* likely reflects different trade-offs between energy gain and predator avoidance.

## Introduction

Ectotherms depend on environmental thermal conditions to optimize physiological performances (Huey & Stevenson, 1979; Stevenson, Peterson & Tsuji, 1985). The body temperature achieved by reptiles in the field depends on environmental conditions as well as intrinsic factors such as morphological (colouration, thermal inertia), physiological (metabolic rate, cardiovascular adjustments) and behavioural (microhabitat selection, activity patterns, posturing) characteristics (Huey, 1991). Behavioural thermoregulation is often the most important determinant of habitat use in terrestrial reptiles, particularly in temperate climates (Reinert, 1984; Adolph, 1990; Blouin-Demers & Weatherhead, 2002; Heard, Black & Robertson, 2004; Carfagno & Weatherhead, 2006).

Under cool thermal conditions, active thermoregulation usually implies basking. Basking entails high costs, both ecological, in terms of predation risk and time allocation

(Huey & Slatkin, 1976; Downes, 2001; Martin & Lopez, 2001; Daly, Dickman & Crowther, 2008) and physiological in terms of energy expenditure (Bedford & Christian, 1998; Dubois, Blouin-Demers & Thomas, 2008). Many species inhabiting cool environments are precise thermoregulators with relatively high thermal preferences (Martin & Lopez, 1999; Blouin-Demers & Nadeau, 2005). This partly contradicts one prediction of the cost-benefit model of thermoregulation (Huey & Slatkin, 1976), which proposed that coadaptation between environmental temperature, thermal preference and optimal temperature for performance (Huey & Bennett, 1987; Angilletta *et al.*, 2006) should produce a decrease in preferred temperature in cool environments to reduce the costs associated with thermoregulation. Indeed, lower thermal needs are likely to be associated with shorter basking time and reduced exposure to predators. Major ecological traits such as activity capacities (Bennett, Hicks & Cullum, 2000), energy budget (Secor & Nagy, 1994) or level of exposure are directly linked to thermal requirements.

Therefore, contrasted thermal preferences in species living in the same environment may illustrate different ecological orientation and thermoregulatory trade-off (Belluire, Carrascal & Diaz, 1996).

Here, we studied two largely sympatric colubrid snakes, *Hierophis viridiflavus* and *Zamenis longissimus* that are appropriate models to investigate variation in thermoregulatory strategies, in spite of the limitations inherent to two species comparisons (Garland & Adolph, 1994). The two species are sympatric over most of their distribution, and syntopic situations are common (Luiselli & Capizzi, 1997; Naulleau, 1997*a,b*). Both species are similar in major morphological and ecological traits including mean body size, macrohabitat use, reproductive mode, hibernation and egg-laying dates (Naulleau, 1984). Previous results on diet indicate major dietary overlap (Luiselli & Capizzi, 1997; Naulleau, 1997*a,b*) between the two species and this is also true in our study site (H. Lelièvre, unpubl. data). Both species exhibit the same metabolic reaction norms, but have contrasted thermal preferences and coadapted optima for performance (Lelièvre *et al.*, 2010). *Hierophis viridiflavus* is faster and selects higher temperatures compared with *Z. longissimus*. These two colubrids sharing major ecological traits, but differing in their thermal requirements, provide an opportunity to study two contrasted thermoregulatory strategies within the same temperate environment, thus holding general thermal conditions and predation level constant.

We tested the following predictions: (1) environmental conditions should be more constraining for the species with higher thermal preferences (*H. viridiflavus*); (2) higher thermal preferences in *H. viridiflavus* should translate in stronger selection for open habitats and more exposure; (3) higher thermal requirements should be counterbalanced by larger gains in physiological performance in *H. viridiflavus*, in accordance with the 'hotter is better' hypothesis (Huey & Kingsolver, 1989).

To test our predictions, we used detailed radio-telemetry data to characterize microhabitat selection in *H. viridiflavus* and *Z. longissimus*. We simultaneously measured the thermal quality of the various microhabitats available to both species. We used thermoregulation indices (Hertz, Huey & Stevenson, 1993; Christian & Weavers, 1996) to describe thermoregulatory strategies. Finally, we used information on thermal reaction norms (Lelièvre *et al.*, 2010) to quantify the respective performance improvement accrued through differential thermoregulation (Blouin-Demers & Weatherhead, 2008).

## Methods

### Study species and study site

*Hierophis viridiflavus* and *Z. longissimus* are sympatric colubrid snakes distributed in central Europe (Naulleau, 1997*a,b*; Rugiero, Capizzi & Luiselli, 2002). *Hierophis viridiflavus* is a typical racer according to its morphological (long tail, large eyes), behavioural (fast, diurnal, terrestrial) and

ecological (high levels of activity and exposure) characteristics (Shine, 1980; Luiselli, 2006). *Zamenis longissimus* shows strong morphological and behavioural similarities with other ratsnakes (constrictor, semi-arboreal, slow moving and secretive; Lenk, Joger & Wink, 2001; Burbrink & Lawson, 2007). Both species have approximately equal body size and are active foragers showing important overlap in their diets (mostly composed of small mammals; Naulleau, 1984; Capizzi *et al.*, 1995). These two species, however, differ markedly in preferred body temperature ( $T_{set}$ ): *Hierophis viridiflavus* is a thermophilic snake ( $T_{set}$  27.5–31 °C), whereas *Z. longissimus* prefers cooler temperatures ( $T_{set}$  21.5–25.5 °C; Lelièvre *et al.*, 2010).

We conducted this study at the Centre d'Études Biologiques de Chizé in western France (46°07'N; 00°25'O) between 2006 and 2008 during the snake activity season (May to September). The study site is a 2600 ha biological reserve managed by the Office National des Forêts. The forest is dominated by deciduous trees (*Fagus*, *Quercus*, *Carpinus* and *Acer*), regeneration areas characterized by scrub species (e.g. *Rubus*, *Clematis*) and grasslands. Climatic conditions are temperate oceanic with annual precipitations between 800 and 1000 mm, annual mean temperature of 12 °C and an average of 2000 h of sunshine per year.

### Field surveys

Snakes were caught under concrete boards disposed throughout the study area for this purpose (Bonnet, Naulleau & Shine, 1999). We followed 78 snakes via radio-telemetry for 6–116 days between July and September in 2006, and between May and September in 2007 and 2008. A temperature data logger (miniaturized 8 kb iButton thermochron DS1922, Dallas Semiconductor, Dallas, TX, USA; see Robert & Thompson (2003) for details on miniaturization) and a radio-transmitter (R1650, Advanced Telemetry Systems, Isanti, MN, USA) sterilized in diluted benzalkonium chloride were surgically implanted in the abdominal cavity of the snakes under the influence of isoflurane anaesthesia (see Reinert & Cundall (1982), Whitaker & Shine (2003) for details). Total mass of logger and transmitter represented at most 2.2% of snake body mass. We kept snakes under observation for 6 days and then released them at their exact point of capture. Snakes were located during the day every 48 h. We systematically changed relocation order to avoid always locating the same individuals at the same time of day. Upon locating a snake, we recorded its precise position using GPS (eTrex, Garmin, Olathe, KS, USA), its posture and its behaviour (concealed, under ground, under concrete boards, basking or moving). We delayed habitat characterization until the individual had moved to another location to avoid disturbance.

### Habitat characterization

Habitat use was characterized for every other snake location (i.e. every 4 days) in 2007 and 2008 following the protocol of Blouin-Demers & Weatherhead (2001*a*). For each snake

location characterized, we replicated the habitat description at a random location determined by walking 60 m (the mean distance travelled per day by the snakes) from the snake location in a randomly selected direction. We measured 27 structural variables within circular plots centred on the snake or random location (See Table S1 in the electronic appendices). To evaluate per cent ground cover and canopy closure, we used a wide-angle digital camera (Panasonic Lumix DMC-TZ2, Panasonic Corporation, Osaka, Japan, 28 mm). For canopy, the camera was placed on the ground and at 1.5 m height. For ground cover, the camera was placed on a 2 m perch. Pictures were analysed with GIMP 2.6.7 (<http://www.gimp.org>). When snakes did not move between successive habitat characterizations (on average 22% of the time), data from the first description of the snake location were duplicated and we characterized a new random location. Although this procedure induces some pseudoreplication, it does reflect biological reality as snakes actively chose to stay at the same location.

### Thermoregulation

Body temperature ( $T_b$ ) was measured in 30 *H. viridiflavus* (21 males and 9 females; SVL =  $89.5 \pm 7.3$  cm; BM =  $234.1 \pm 64.9$  g) and 27 *Z. longissimus* (18 males and 9 females; SVL =  $95.4 \pm 8.4$ ; BM =  $249.3 \pm 62.1$  g) every 30 min for 4–103 days (mean = 42 days) between May 2006 and September 2008. Simultaneously, we measured operative environmental temperatures ( $T_e$ ) in the various microhabitats available to the snakes (Bakken, 1992). We used 15 physical models made from rubber tubes filled with water, reproducing the thermal characteristics of the study species. We ensured the realism of our models by calibrating them against two fresh snake carcasses placed in variable thermal conditions during 10 h (linear regression between mean temperature from models and snakes:  $y = 0.9793x + 1.5234$ ,  $R^2 = 0.97$ ). We placed the models in each of four microhabitats: on the ground in forest ( $n = 3$ ), on the ground under scrubs ( $n = 4$ ), in an underground retreat ( $n = 4$ ) and on the ground in the open ( $n = 4$ ).

Days were classified as hot, medium or cold (henceforth day category) based on the amount of time with maximal  $T_e$  ( $T_{e \max}$ ) above the lower bound of  $T_{set}$  ( $T_{set \ low}$ ) for each species. Days were cold when  $T_{e \max}$  did not reach  $T_{set \ low}$ , medium when  $T_{e \max}$  was above  $T_{set \ low}$  for 1–6 h (set according to the median for time above  $T_{set \ low}$ , which was 6 h in *Z. longissimus* and 4.5 h in *H. viridiflavus*) and hot when  $T_{e \max}$  was above  $T_{set \ low}$  during more than 6 h. Each day was separated into two periods: daytime between 8:00 and 18:00 h and night-time between 18:00 and 8:00 h.

We used indices developed to measure both the accuracy and the effectiveness of thermoregulation in reptiles (Hertz *et al.*, 1993; Christian & Weavers, 1996; Blouin-Demers & Weatherhead, 2001b). Because active thermoregulation is essentially diurnal under temperate climates, indices were calculated for daytime only and for each type of day. We calculated the accuracy of  $T_b$  as the mean of the deviations of  $T_b$  from  $T_{set}$  (individual deviation,  $d_b$ ) in a restricted

daytime period (between 12:00 and 18:00 h) when radiant heat is maximal and behavioural thermoregulation is possible. We measured the extent of thermoregulation as the time spent with  $T_b$  above  $T_{set \ low}$  during daytime. We measured thermal exploitation ( $Ex$ ) using a modification of the index proposed by Christian & Weavers (1996): the proportion of time spent above  $T_{set \ low}$  during daytime expressed as a percentage of the time available to do so ( $T_{e \max} > T_{set \ low}$ ). We measured the thermal quality of each habitat by the daily duration when  $T_e$  was above  $T_{set \ low}$  and within  $T_{set}$  of each species in each habitat.

### Performance gain estimates

Thermal reaction norms for locomotor performance and digestion transit time have been measured in both species (Lelièvre *et al.*, 2010). We used general equations to predict crawling speed and digestion speed based on body temperature. Snakes consistently regurgitate their meals at 10 °C (Naulleau, 1983; Stevenson *et al.*, 1985; Hailey & Davies, 1987; Tsai, Lee & Tu, 2008). We fixed the lower thermal limit at 15 °C for both species, as it corresponds to the lowest experimental temperature that enables complete digestion. We assumed that the snakes that selected habitats randomly with respect to their  $T_e$  would realize a  $T_b$  distribution equivalent to the available  $T_e$ . We estimated overall available  $T_e$  by weighing each habitat by its mean availability within an area containing all home ranges of radio-tracked snakes (total area from MCP calculation = 420 ha). Excluding road surface that is not used by snakes and underground retreat-sites (not quantifiable), the main habitats were open field (19%), scrub (59%) and forest (18%). Because scrub is a heterogeneous habitat with many sun patches, we divided  $T_e$  in scrub as 50% open field and 50% scrub. We then randomly sampled 10 000  $T_e$  weighed by availability and the same number of daytime snake  $T_b$  in each species across the whole  $T_b$  dataset. Random samples were bootstrapped 100 times with replacement, and mean sample sizes ( $\pm$  SD) were calculated for 1 °C intervals. Applying the equations of the thermal reaction norms to those temperatures, we obtained distributions of performance observed in snakes and expected performance of hypothetical snakes using their habitat randomly with respect to thermal quality.

### Statistical analyses

All data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett test), and transformed when necessary to achieve the conditions for using parametric tests. All statistical comparisons were performed in R software (R Development Core Team, 2007). For thermoregulation, we used mixed models (NLME package) with individuals as random effect (log-likelihood maximization). We used five models to test for the effects of species and type of day with thermoregulation indices as dependent variables ( $Y$ ) from constant model ( $Y \sim 1$ ) to complete model ( $Y \sim \text{Species} + \text{Type Day} + \text{Interaction}$ ). The effect of sex was tested, but it accounted for a very small portion of the

variation. We thus pooled the sexes in our presentation of the results. We used stepwise model selection based on corrected Akaike information criterion ( $AIC_c$ ) to select the most appropriate model (Burnham & Anderson, 2002). Then, we used the best model to estimate parameters.

To analyse habitat selection, we performed principal component analyses (PCA) on microhabitat variables. We calculated the correlation coefficients between the variables and most were below 0.70 (only 5/325 combinations > 0.70). Thus, multicollinearity was not an issue. Factor scores derived from PCA were used in logistic mixed models (LME4 package) with the presence/absence data (random vs. snake locations) as the dependent variable. Habitat surveys were conducted in four areas that differ in their proportions of available habitat types. Hence, we used the area with individuals nested within the area as random effects in our model. Snake behaviour at location (exposed vs. concealed) was examined considering only individuals monitored

across the active season and located at least on 30 occasions ( $n = 10$  for *Z. longissimus* and  $n = 8$  for *H. viridiflavus*). For each individual, we calculated a mean proportion of concealed locations. Means are provided as  $\pm 1$  SE. We accepted significance at an  $\alpha$  level of 0.05.

## Results

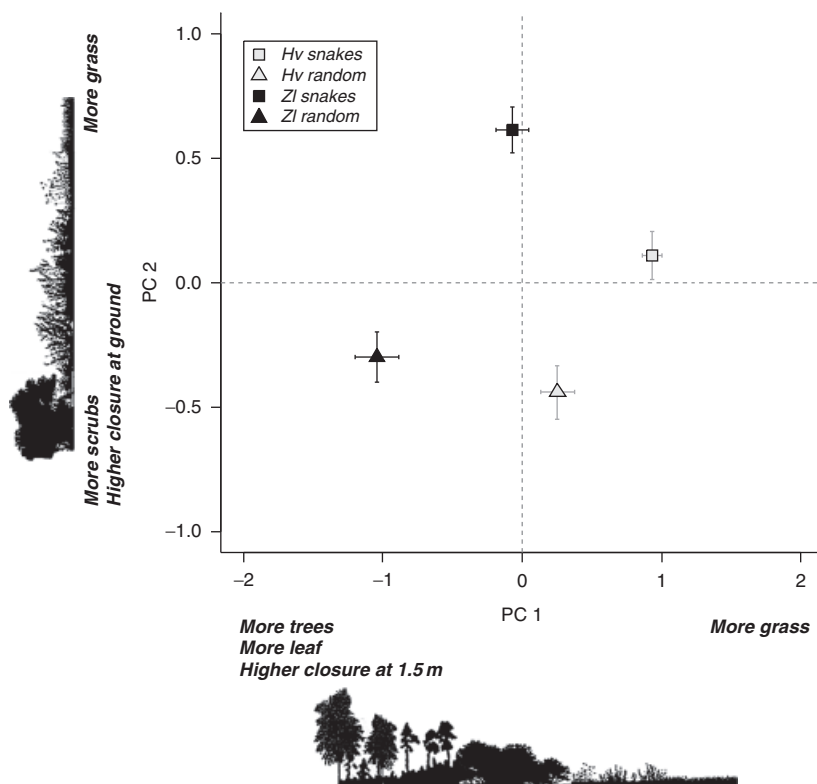
### Environmental operative temperatures

We calculated monthly mean daily maximal  $T_e$  for each habitat type during the activity season of snakes. In all habitat types,  $T_e$  increased from May to July before decreasing until September. Open-field habitat had higher thermal quality (daily time above  $T_{set, low}$ ) than closed habitats for the two species (Table 1). For daily time within  $T_{set}$ , scrub provided a better thermal environment than open field for *Z. longissimus*, but not for *H. viridiflavus*. All four

**Table 1** Thermal quality of the four main habitats available during activity season for *Zamenis longissimus* (Zl) and *Hierophis viridiflavus* (Hv)

| Habitat (n)     | Maximum $T_e$ (°C) | $T_e > T_{set, low}$ duration (h) |                 | $T_e$ within $T_{set}$ duration (h) |                 |
|-----------------|--------------------|-----------------------------------|-----------------|-------------------------------------|-----------------|
|                 |                    | Zl                                | Hv              | Zl                                  | Hv              |
| Open Field (4)  | 35.49 $\pm$ 0.18   | 8.69 $\pm$ 0.15                   | 5.28 $\pm$ 0.21 | 2.46 $\pm$ 0.13                     | 1.63 $\pm$ 0.18 |
| Scrub (4)       | 23.51 $\pm$ 0.13   | 4.11 $\pm$ 0.11                   | 0.64 $\pm$ 0.15 | 2.91 $\pm$ 0.09                     | 0.50 $\pm$ 0.13 |
| Forest (3)      | 21.24 $\pm$ 0.18   | 2.84 $\pm$ 0.15                   | 0.24 $\pm$ 0.22 | 2.31 $\pm$ 0.13                     | 0.18 $\pm$ 0.18 |
| Underground (4) | 18.60 $\pm$ 0.17   | 1.35 $\pm$ 0.15                   | 0.00 $\pm$ 0.21 | 1.33 $\pm$ 0.12                     | 0.00 $\pm$ 0.17 |

Thermal quality is assessed by three parameters: the maximal daily environmental temperature ( $T_e$ ), the time when  $T_e$  is above the lower bound of the range of preferred body temperature selected in laboratory thermal gradient ( $T_{set, low}$ ) and the time when  $T_e$  is within  $T_{set}$ . Means are provided  $\pm$  SE.



**Figure 1** Differences in microhabitat selection of *Zamenis longissimus* (Zl; black colour) and *Hierophis viridiflavus* (Hv; grey colour) based on principal component analyses of all observations (both species, both random sets). Squares (snake location) and triangles (random location) represent means for each group and bars are SE.

sampled habitat types were more thermally challenging for *H. viridiflavus* because they provided less time available above  $T_{\text{set low}}$  and within  $T_{\text{set}}$  (Table 1).

### Habitat use and behaviour

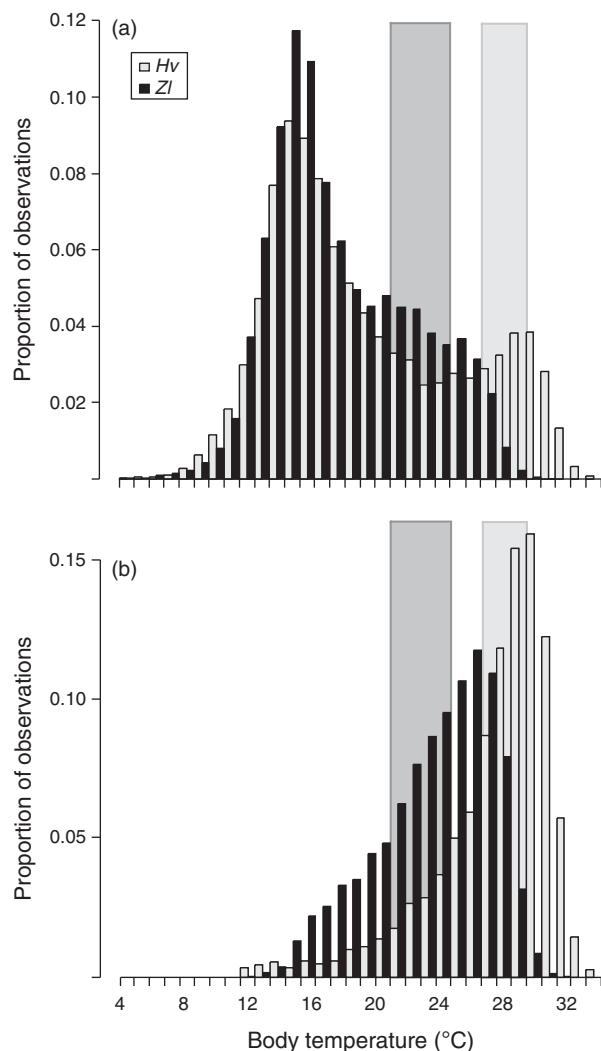
We characterized habitat use at 828 locations used by snakes and at 828 random locations. PCA indicated that the 27 habitat variables could be synthesized by PC that accounted for 43.9, 37.8 and 38.3% of the total variation among comparisons (see Table S2 in the electronic appendices). Random locations for both species differed significantly, so we kept them separate for comparisons. The first PC was negatively correlated with the coverage of trees and the maximum height of vegetation. The second PC was correlated significantly with coverage of scrub for comparisons between *H. viridiflavus* and random locations as well as between the two snake species, but was negatively correlated for the comparison between *Z. longissimus* and random locations. Based on  $AIC_c$ , both species selected habitat non-randomly. PC1 and PC2 significantly influence the presence of the two species (model with PC1 and PC2,  $w_i = 1$  for each case). *Hierophis viridiflavus* avoided forested habitat (more trees, higher vegetation and closed canopy) more than *Z. longissimus* who was more generalist in its habitat use (Fig. 1). Indeed, comparisons between species showed a significant effect of PC1 and PC2, and an interaction between PC1 and species (difference in forest gradient), but not between PC2 and species (no difference in scrub gradient) ( $w_i = 1$  for the best model).

On average, *Z. longissimus* was more often located concealed ( $67.3 \pm 3.4\%$ ) undercover, underground or under concrete boards) compared with *H. viridiflavus* ( $42.3 \pm 3.4\%$ ; Student's *t*-test,  $t_{2, 19} = 5.41$ ,  $P < 0.0001$ ).

### Thermoregulation

We collected 114 503  $T_b$  measurements (58 320 for *Z. longissimus* and 56 183 for *H. viridiflavus*) from 57 individuals (among the 78 radio-tracked) on 511 snake  $\times$  days (215 for *Z. longissimus* and 296 for *H. viridiflavus*). We collected 45 314 (39.6%)  $T_b$  values during the day (08:00–18:00 h) and the remaining 69 189 (61.4%) during the night. Following our classification, only 3 days were cold, 24 were medium and 188 were hot for *Z. longissimus* (1.4, 11.2 and 87.4%, respectively, of the total) while 27 were cold, 93 medium and 194 hot for *H. viridiflavus* (9.1, 31.4 and 65.5%, respectively of the total).

On average, *H. viridiflavus* exhibited a higher  $T_b$  than *Z. longissimus* [ $20.69 \pm 0.10$  °C (range 4–36) vs.  $18.65 \pm 0.21$  °C (4–33); linear mixed effects model,  $t_{55, 114447} = 19.52$ ,  $P < 0.0001$ ; averages for the whole day; Fig. 2]. Only a small proportion of  $T_b$  was within  $T_{\text{set}}$  (25.0% in *Z. longissimus* and 16.4% in *H. viridiflavus*, Fig. 2). The model with species, day category and their interaction was the best to explain mean  $T_b$  variation during the day (Table 2). Both species exhibited higher  $T_b$  when thermal conditions were hotter. *Hierophis viridiflavus* maintained higher  $T_b$  than *Z. long-*



**Figure 2** Frequency distribution of field body temperatures ( $T_b$ ) in *Zamenis longissimus* (*Zl*) and *Hierophis viridiflavus* (*Hv*). (a) for all measurements, (b) considering only hot days between 12:00 and 18:00 h (see “Methods” section for day classification). Preferred  $T_b$  selected in a laboratory thermal gradient ( $T_{\text{set}}$ ) is represented by grey boxes (dark for *Z. longissimus* and light for *H. viridiflavus*).

*issimus* for all day categories, but this divergence was particularly marked during hot days ( $25.66 \pm 0.60$  vs.  $21.68 \pm 0.56$  °C; Fig. 3). During hot days between 8:00 and 12:00 h, a time window that corresponds to the basking phase after emergence from nocturnal retreat-site, we observed a strong correlation with a slope close to 1 between  $T_b$  and  $T_e$  in the sun in *H. viridiflavus*, whereas  $T_b$  was more correlated with  $T_e$  under vegetation in *Z. longissimus* (spline interpolation; Fig. 4). Model selection for *Ex* indicated effects of species and type of day without interaction (Table 2). When thermoregulation was possible during medium and hot days, *H. viridiflavus* showed higher *Ex* than *Z. longissimus* ( $49.16 \pm 2.14$  vs.  $39.90 \pm 1.89\%$ , and  $78.68 \pm 2.35$  vs.  $69.41 \pm 2.08\%$  for medium and hot days respectively). For time spent above  $T_{\text{set low}}$ , we selected the model

**Table 2** Model selection used to describe thermoregulation of *Hierophis viridiflavus* and *Zamenis longissimus* using corrected Akaike information criterion (AIC<sub>c</sub>)

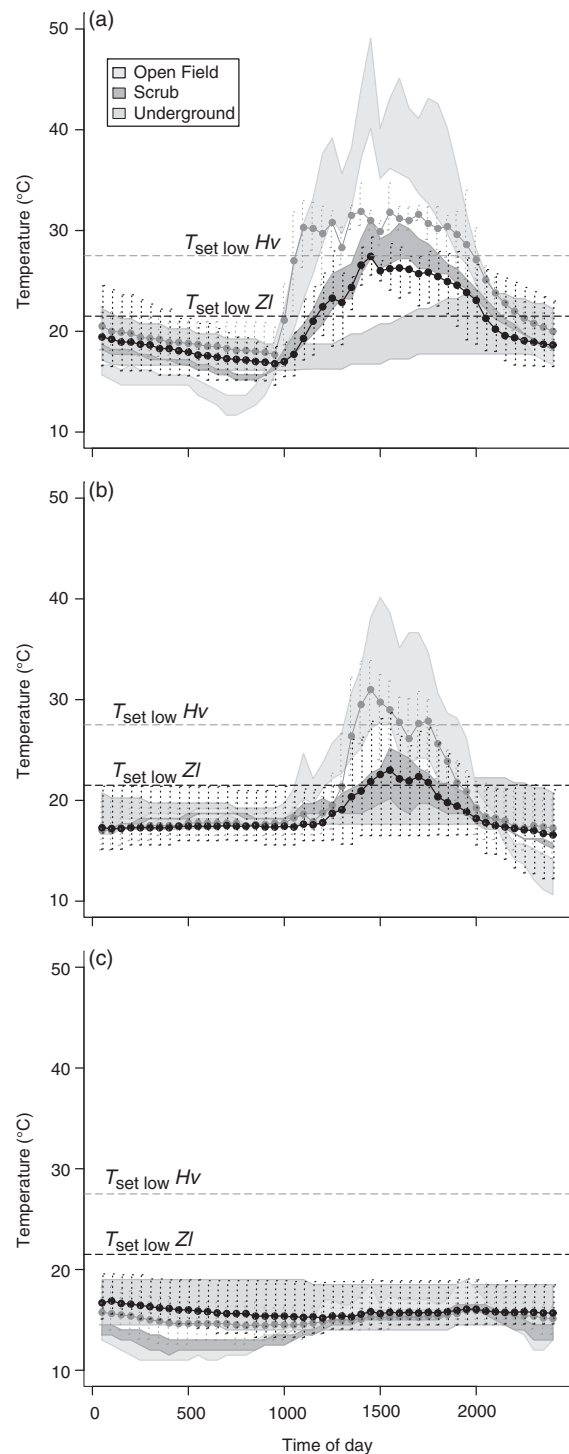
|   | K | AIC <sub>c</sub> | ΔAIC <sub>c</sub> | w <sub>i</sub> AIC <sub>c</sub> |
|---|---|------------------|-------------------|---------------------------------|
| Mean $T_b$                                  |   |                  |                   |                                 |
| <b>Y ~ Species + Type Day + Interaction</b> | 8 | 11306.34         | 0.00              | 0.990                           |
| Y ~ Species + Type Day                      | 6 | 11315.48         | 9.14              | 0.010                           |
| Ex  |   |                  |                   |                                 |
| <b>Y ~ Species + Type Day</b>               | 5 | 19480.06         | 0.00              | 0.675                           |
| Y ~ Species + Type Day + Interaction        | 6 | 19481.55         | 1.49              | 0.321                           |
| Y ~ Type Day                                | 4 | 19489.98         | 9.92              | 0.005                           |
| Time above $T_{set\ low}$                   |   |                  |                   |                                 |
| Y ~ Species + Type Day + Interaction        | 6 | 9760.81          | 0.00              | 0.452                           |
| <b>Y ~ Type Day</b>                         | 4 | 9761.40          | 0.59              | 0.336                           |
| Y ~ Species + Type Day                      | 5 | 9762.33          | 1.52              | 0.211                           |
| $d_b$                                       |   |                  |                   |                                 |
| <b>Y ~ Species + Type Day + Interaction</b> | 8 | 9580.31          | 0.00              | 1.000                           |

The function with the lowest AIC<sub>c</sub> is the one that best describes the data.  $K$ , number of parameters in the function;  $w_i$ , Akaike weight. Models in boldface were selected for analyses. Only the most reliable models are presented. See text for details on models and definitions of index.

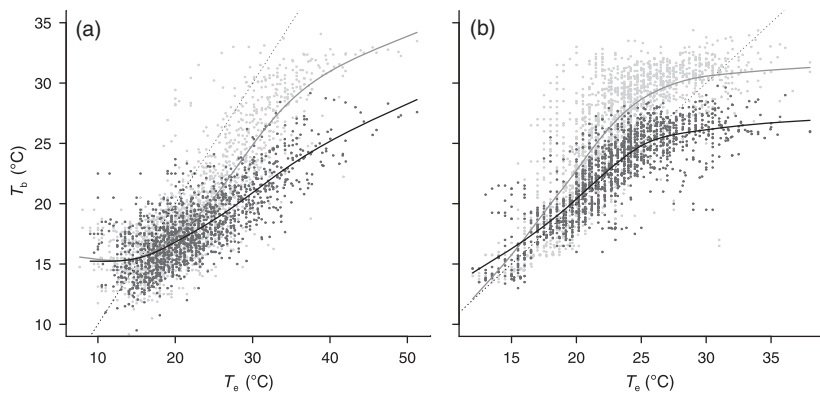
with only type of day (Table 2) despite a lower AIC<sub>c</sub> because it was more parsimonious (fewer parameters). Time spent above  $T_{set\ low}$  was strongly linked to thermal conditions for both species, but no difference between species was detected ( $5.76 \pm 0.12$  h in hot days vs.  $2.32 \pm 0.13$  h in medium days). Thermoregulation accuracy was highly dependent on daily thermal conditions and species with a significant interaction (Table 2). *Zamenis longissimus* showed more precise thermoregulation than *H. viridiflavus* (lower  $d_b$ ) under constraining conditions ( $6.88 \pm 0.39$  vs.  $10.87 \pm 0.42$  °C for cold days, and  $3.75 \pm 0.40$  vs.  $4.58 \pm 0.43$  °C for medium days), but *H. viridiflavus* became as precise as *Z. longissimus* during hot days ( $1.59 \pm 0.41$  and  $1.52 \pm 0.39$  °C, respectively). Over the active season, the precision of thermoregulation was the highest during June and July in *H. viridiflavus*, but was less variable in *Z. longissimus* (Fig. 5).

### Performance gain accrued through thermoregulation

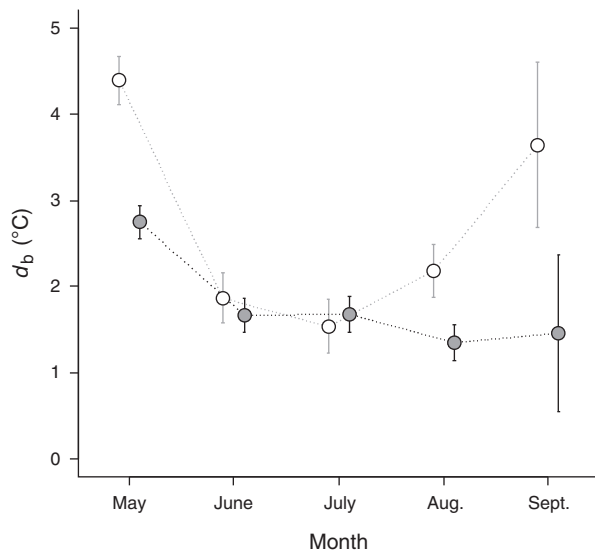
Applying the equations of the locomotory and digestive thermal reaction norms to randomly selected, but weighted by habitat availability,  $T_e$  produced a distribution with a substantial proportion of zeros for crawling speed ( $9.3 \pm 1.3\%$  in *H. viridiflavus* and  $18.6 \pm 1.9\%$  in *Z. longissimus*; Fig. 6) and digestion transit time ( $28.8 \pm 2.8\%$  in *H. viridiflavus* and  $38.6 \pm 3.5\%$  in *Z. longissimus*; Fig. 6). The distribution of randomly sampled  $T_b$  resulted in highly skewed distributions of crawling speed and digestion transit time that were significantly different from the distributions generated from the  $T_e$  ( $\chi^2$ -tests,  $P < 0.0001$ ; Fig. 6). *Hierophis viridiflavus* appeared to maximize both crawling speed and digestion transit time ( $41.5 \pm 1.4\%$  of observations above 95% of maximal performance for crawling speed and  $38.7 \pm 1.2\%$  of observations below 3 days of transit).



**Figure 3** Field thermoregulation in *Zamenis longissimus* (Zl; black symbols) and *Hierophis viridiflavus* (Hv; grey symbols) during 3 contrasted days (a) hot, (b) medium and (c) cold; see "Methods" section for day classification). Points represent mean body temperature ( $T_b$ ) and lines represent 95% confident intervals. Shaded lines represent the lower bound of the range of preferred body temperature selected in laboratory thermal gradient ( $T_{set\ low}$ ). Shaded polygons represent the range of environmental temperatures ( $T_e$ ) in three habitat types.



**Figure 4** Relationship between body temperature ( $T_b$ ) and habitat temperature ( $T_e$ ) in open field (a) and under scrubs (b) for *Zamenis longissimus* (in black) and *Hierophis viridiflavus* (in grey). Lines represent linear interpolation of the data. Dashed line of slope 1 indicates perfect thermoconformity ( $T_b = T_e$ ).



**Figure 5** Mean daily deviation of body temperature ( $d_b$ ) from the range of preferred body temperature selected in laboratory thermal gradient ( $T_{set}$ ) for each month of the active season in *Zamenis longissimus* (filled circles) and *Hierophis viridiflavus* (open circles). Circles represent mean  $d_b$  and bars represent SE.

*Zamenis longissimus* maximized crawling speed ( $36.9 \pm 1.4\%$  of observations above 95% of maximal performance), but not digestion (only  $15.4 \pm 0.5\%$  of observations below 3 days of transit). The mean improvement in crawling performance accrued through habitat selection was  $20.2\%$  in *H. viridiflavus* (from  $59.7 \pm 7.7$  to  $79.8 \pm 3.5\%$  of the maximum speed) and  $18.6\%$  in *Z. longissimus* (from  $64.2 \pm 3.4$  to  $82.8 \pm 3.4\%$  of the maximum speed). Digestion transit time decreased from  $8.9 \pm 0.4$  days to  $5.8 \pm 0.3$  days with thermoregulation in *H. viridiflavus* (gain 34.8%) and from  $9.5 \pm 0.4$  days to  $7.9 \pm 0.3$  days in *Z. longissimus* (gain 16.8%).

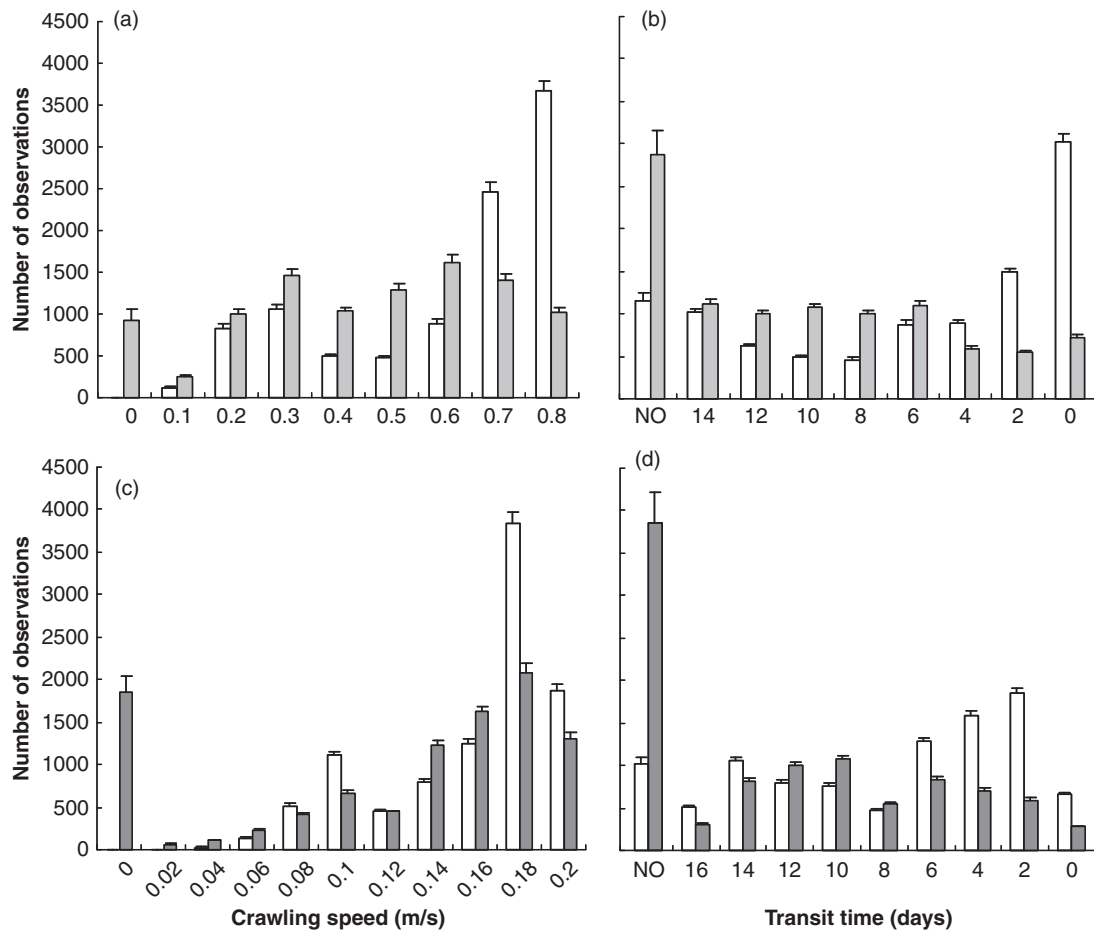
## Discussion

This study provides a clear example of the different relationships linking habitat use and thermal ecology that can occur in

broadly similar coexisting species. Our analyses of habitat use and thermoregulation indicate that reptiles in syntopy exploiting the same trophic resource may profoundly diverge in thermoregulatory strategy, exposure levels and thermal dependence of performance. Importantly, those contrasted ecological strategies may both constitute successful orientations.

As expected, climatic conditions were more constraining for the thermophilic species *H. viridiflavus* than for *Z. longissimus* because of higher thermal requirements. Frequency distribution of  $T_b$  as well as mean daily time above  $T_{set\ low}$  and within  $T_{set}$  showed that *H. viridiflavus* spent 60% less time than *Z. longissimus* at preferred temperatures. During our survey, cold days associated with no thermoregulation opportunities were 10 times more frequent for *H. viridiflavus* than for *Z. longissimus*. Almost 90% of the days allowed *Z. longissimus* to reach its preferred temperature for an extended period (more than 6 h), whereas such possibility was available only on 65% of days for *H. viridiflavus*. Summer months (from June to August) were more favourable to both species. Conversely, in May and September, thermal conditions were more challenging, particularly for *H. viridiflavus*, which had difficulties achieving preferred body temperatures (high  $d_b$ ).

Accordingly, habitat use and level of exposure differed markedly between *H. viridiflavus* and *Z. longissimus*, reflecting their contrasted thermal requirements. In *H. viridiflavus*, thermal specialization for hot temperatures was associated with selection of open areas and more exposure. None of the sampled habitats provided high thermal quality for *H. viridiflavus*. Temperature in open field is highly variable and rarely matches  $T_{set}$  while closed habitats generally remain too cold. This species is thus constrained to active thermoregulation consisting of extended basking in the morning followed by frequent shuttling between sun and shade during the hottest part of the day. On the other hand, in *Z. longissimus*, thermal measurements showed that  $T_b$  precisely matched  $T_e$  under vegetation and that closed habitats were compatible with their lower thermal requirements, notably during summer. Our radio-telemetric surveys confirmed that *Z. longissimus* is a generalist in habitat use that intensively frequents forested areas. Overall, this species displays a secretive lifestyle with limited basking behaviour.



**Figure 6** Impact of thermoregulation on physiological performance in *Zamenis longissimus* and *Hierophis viridiflavus*. Estimated performance was derived by applying the thermal performance equation to the habitat temperature ( $T_h$ , filled bars) and body temperature ( $T_b$ , open bars) distributions. (a) Crawling speed for *H. viridiflavus*, (b) transit time for *H. viridiflavus*, (c) crawling speed for *Z. longissimus* and (d) transit time for *Z. longissimus*. Bars represent standard deviation after bootstrap.

When environmental conditions were favourable, accurate thermoregulation allowed *H. viridiflavus* to maximize crawling speed and digestion capacities. As *H. viridiflavus* is an active, fast-moving forager, prey capture success is presumably strongly determined by locomotor performance. Considering the high level of thermal sensitivity for performance in this species (Lelièvre *et al.*, 2010), optimization of locomotor capacities undoubtedly improves fitness by maximizing foraging and predator avoidance. Moreover, to meet its high-energy requirements, *H. viridiflavus* probably needs to maximize feeding rate by minimizing digestion transit time. In addition, given its lifestyle, rapid digestion is probably necessary to reduce the negative impact of a full digestive tract on locomotion (Garland & Arnold, 1983). We showed that thermoregulation allowed *H. viridiflavus* to decrease significantly transit time, and that *Z. longissimus* is comparatively much slower and its crawling speed is less sensitive to temperature (Lelièvre *et al.*, 2010). We thus expect that locomotor abilities have a weaker impact on

fitness for *Z. longissimus*, which probably relies on crypsis to catch prey and evade predators. Whether our results on performance improvements are typical of other reptiles is difficult to evaluate because despite early pleas to adopt this approach (Hertz *et al.*, 1993), few studies have ensued (Blouin-Demers & Weatherhead, 2008). Our results for *H. viridiflavus* and *Z. longissimus* are comparable with those of Blouin-Demers & Weatherhead (2008), who reported that black ratsnakes in Ontario improved locomotor performance by 18% through habitat-mediated thermoregulation.

*Hierophis viridiflavus* and *Z. longissimus* display highly contrasted thermoregulatory strategies. These strategies undoubtedly arise from differential balance between costs and benefits (Huey & Slatkin, 1976). Trade-off between costs and benefits of thermoregulation is a key aspect of reptile life history (Huey, 1974; Huey & Berrigan, 2001). According to the cost-benefit model of thermoregulation (Huey & Slatkin, 1976), the principal costs associated with basking behaviour are increased predation risk (Martin & Lopez,



1999; Herczeg *et al.*, 2008), time spent which is not available for other activities like foraging or mating (Shine *et al.*, 2000) and greater energy expenditure (Secor & Nagy, 1994). The thermophilic strategy used by *H. viridiflavus* increases energetic requirements (Lelièvre *et al.*, 2010) and exposure to predators, but the benefits in terms of performance gain are likely to offset these potential costs. During hot days when conditions were favourable, *H. viridiflavus* actively selected higher  $T_b$  than *Z. longissimus*. Moreover, *H. viridiflavus* thermoregulated with comparable accuracy and achieved a greater thermal exploitation of the environment. Despite higher requirements, *H. viridiflavus* was able to maintain  $T_b$  above  $T_{set\ low}$  for more than 6 h per day, which was equivalent to *Z. longissimus*.

In our study site, predation mainly arises from raptors: short-toed eagle *Circaetus gallicus* that forage heavily on snakes (Bakaloudis, Vlachos & Holloway, 1998) and common buzzard *Buteo buteo* that is a generalist predator feeding partly on snakes (Selas, 2001). As dense vegetation cover offers effective protection to small reptiles against avian predation (Daly *et al.*, 2008), we may expect a higher risk of predation on *H. viridiflavus* because they use open habitats more frequently and bask more than *Z. longissimus*.

Contrasted thermoregulatory strategies have important impacts on energy budgets and probably drive foraging strategies and feeding rates. Standard metabolic rate measurements in the laboratory (Lelièvre *et al.*, 2010) allowed to estimate that energy expenditure at  $T_{set}$  is 60% higher in *H. viridiflavus* compared with *Z. longissimus*. This estimation is conservative because our calculations do not take activity into account. We expect that the difference in expenditure obtained through field metabolic rate measurements (Secor & Nagy, 1994) would be higher because *H. viridiflavus* showed higher activity than *Z. longissimus* during our study (distance travelled per day and frequency of movement). Thus, higher energy expenditure in *H. viridiflavus* must be counterbalanced by higher foraging efficiency and rate of energy acquisition. Our results suggest that foraging could be maximized by actively selecting higher body temperatures and thereby increasing locomotor and digestive performance.

In conclusion, our results emphasize the importance of complex covariation linking thermal preferences, performance and level of exposure in terrestrial ectotherms that possibly reflect coadaptation patterns (Angilletta *et al.*, 2006). The level of exposure to predation remains a key element to understand thermal adaptation and thermoregulatory strategies of terrestrial ectotherms (Mitchell & Angilletta, 2009). Exploring the consequences of exposure strategies on energetics and performance in reptiles holds promise to unravel the numerous links between habitat selection and fitness. Another research avenue deserving more attention is the possible role of competition in explaining differences in thermal strategies of sympatric species. If the ecophysiological characteristics of sympatric species seem to be of primary importance to explain this difference, behavioural interference for food or basking sites could also play a role (Luiselli, 2008).

## Acknowledgments

We are very grateful to P. Surre, J. Farsy, S. Lecq, P. Ragot, P.A. Raut and J. Tigreat for their assistance in the field. We thank L. Luiselli and one anonymous reviewer for their constructive comments on an earlier version of the paper. We thank the Office National des Forêts for allowing us to work in the reserve. This research was made possible by the financial support of the Conseil Général des Deux-Sèvres, the Région Poitou-Charentes and the ANR (ECTOCLIM project).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Structural variables used in the analysis of habitat use by *Z. longissimus* and *H. viridiflavus* in western France with associated abbreviations and sampling radii.

**Table S2.** Summary statistics for the two principal components (PC) and their pooled within groups correlations (*r*) with the discriminating variables used in the analysis of habitat use by *Z. longissimus* (*Zl*) and *H. viridiflavus* (*Hv*).

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