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Using GAMM to examine inter-individual heterogeneity in thermal performance curves for *Natrix natrix* indicates bet hedging strategy by mothers

Mathew J. Vickers[a], Fabien Aubret[a], Aurélie Coulon[b,c,*]

[a] Station d'Ecologie Théorique et Expérimentale, UMR 5321, CNRS 2 route du CNRS, 09200 Moulis, France
[b] CESCO UMR 7204, Bases écologiques de la Conservation, Muséum national d'Histoire naturelle, 4 Avenue du Petit Château, 91800 Brunoy, France
[c] CEFE UMR 5175, CNRS – Université de Montpellier – Université Paul-Valéry Montpellier – EPHE, Laboratoire Biogéographie et Ecologie des Vertébrés, 1919 route de Mende, 34293 Montpellier, France

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ABSTRACT

The thermal performance curve (TPC) illustrates the dependence on body- and therefore environmental-temperature of many fitness-related aspects of ectotherm ecology and biology including foraging, growth, predator avoidance, and reproduction. The typical thermal performance curve model is linear in its parameters despite the well-known, strong, non-linearity of the response of performance to temperature. In addition, it is usual to consider a single model based on few individuals as descriptive of a species-level response to temperature. To overcome these issues, we used generalized additive mixed modeling (GAMM) to estimate thermal performance curves for 73 individual hatchling *Natrix natrix* grass snakes from seven clutches, taking advantage of the structure of GAMM to demonstrate that almost 16% of the deviance in thermal performance curves is attributed to inter-individual variation, while only 1.3% is attributable to variation amongst clutches. GAMM allows precise estimation of curve characteristics, which we used to test hypotheses on tradeoffs thought to constrain the thermal performance curve: hotter is better, the specialist-generalist trade off, and resource allocation/acquisition. We observed a negative relationship between maximum performance and performance breadth, indicating a specialist-generalist tradeoff, and a positive relationship between thermal optimum and maximum performance, suggesting “hotter is better”. There was a significant difference among matrilines in the relationship between Area Under the Curve and maximum performance - relationship that is an indicator of evenness in acquisition or allocation of resources. As we used unfed hatchlings, the observed matriline effect indicates divergent breeding strategies among mothers, with some mothers provisioning eggs unequally resulting in some offspring being better than others, while other mothers provisioned the eggs more evenly, resulting in even performance throughout the clutch. This observation is reminiscent of bet-hedging strategies, and implies the possibility for intra-clutch variability in the TPCs to buffer *N. natrix* against unpredictable environmental variability.

1. Introduction

Thermal performance curves (TPCs) describe the dependency of biological processes (e.g., metabolism, or sprint speed, (Huey and Kingsolver, 1989)) on body temperature. This dependency is particularly pronounced in ectotherms given the wide potential range of operative body temperature and because body temperature is innately reliant on environmental temperature. TPCs have been used to understand the thermal dependence of processes from cellular (Somero, 1995), to metabolic (Careau et al., 2014b), to whole-organism (e.g. locomotor performance (Artacho et al., 2013)), with broad applications in studying thermoregulation strategies, acclimation and climate-driven range shifts (Bozinovic et al., 2013; Colwell et al., 2008; Herczeg et al., 2003). Most recently, TPCs have been widely used to predict species response to climate change at multiple levels (Kearney and Porter, 2004, 2009; Sunday et al., 2011; Tewksbury et al., 2008). That is, TPCs are used to study behavioral thermoregulation (Lillywhite, 1980; Seebacher and Shine, 2004; Slip and Shine, 1988), which allows the organism to optimize performance through body temperature control; acclimation, the plasticity in the TPC or behavior associated with exposure to different temperatures (Glanville and Seebacher, 2006; Leal and Gunderson, 2012; Seebacher and

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* Corresponding author at: CESCO UMR 7204, Bases écologiques de la Conservation, Muséum national d'Histoire naturelle, 4 Avenue du Petit Château, 91800 Brunoy, France.
E-mail address: acoulon@mnhn.fr (A. Coulon).

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Franklin, 2005); adaptation, permanent changes in the TPC due to climate temperature change, or the lack of change due to the strength of buffering by the Bogert effect (Munoz et al., 2013); and evolution and the cost of TPC maintenance, for example due to tradeoffs (e.g. resource allocation, (Angilletta et al., 2003)) or thermodynamics (e.g. constraint). Despite the utility of the TPC in ecology, there is innate reliance on the assumption of linearity and normality of the residuals of modeled TPC, and little attention paid to the variability (within species, or populations) in TPC shape and location, two issues we focus on here.

To construct a TPC, individual performance is typically measured at approximately 5 ecologically relevant temperatures for $N \approx 5$ individuals (Bulté and Blouin-Demers, 2006), and a linear (Bozinovic et al., 2013), or in some cases a non-linear (Bulté and Blouin-Demers, 2006; Nussey et al., 2007) model is fitted, which becomes the Thermal Performance Curve for the species. Linear models are usually an inappropriate fit for the TPC, as it is commonly acknowledged that the TPC is strongly non-linear ((Huey and Kingsolver, 1989); but see (Angilletta, 2006) for an attempt at linear fitting). Variation in the TPC within populations is rarely considered, and most commonly non-varying TPCs are used in species survival predictions (Sinervo et al., 2010; Sunday et al., 2014; Tewksbury et al., 2008). Individual variation in a phenotype is the substrate on which natural selection acts (Careau et al.,2014a), yet we understand that a variable, heritable phenotype is linked to fitness, and therefore has the capacity to be involved in tradeoffs leading to adaptation and evolution (Angilletta et al., 2002). Further, plasticity, or phenotypic variability, provides resilience for individuals and species to contemporary, or possibly to future, variability in climate and habitat (Gvoždík, 2002). Accordingly, quantifying population, family, and inter-individual variability of the TPC will further our understanding of the evolution and evolutionary constraints of the TPC. Using modern statistical programs, it is relatively easy to fit the TPC using Generalised Additive Mixed Modeling (GAMM), abandoning assumptions of linearity of the relationship and normality of the residuals (e.g. Bozinovic et al. (2013)). With a mixed model approach, intra- and inter-clutch variance can be clearly partitioned to address questions of heritability that predicate literary discussion of adaptation and persistence or TPC traits in a rapidly changing environment.

Probably one of the most interpretable (but also most ecologically relevant) performances to apply TPCs to is locomotor performance. Locomotor performance has obvious implications for fitness. Locomotion is, for instance, involved in predator-prey interactions, dispersal, and mating success (Irschick and Garland, 2001; Miles, 2004). Locomotor performance, as a trait, was even used as an indication of whole-organism fitness for lizards by Bennett (Bennett, 1980), and Miles (Miles, 2004). It is unsurprising then that the thermal performance of locomotion, sprint speed or endurance, is highly studied in ectotherms. Here we introduce the use of Generalized Additive Mixed Models (GAMM, (Wood, 2011)) to fit individual swimming speed TPCs to hatchings of the colubrid snake *Natrix natrix*, and use properties of the TPC to study constraints, tradeoffs and individual and within clutch variability of the TPC.

The constraints on, and evolution of TPCs have been of interest in the literature (Huey and Kingsolver, 1989; Leal and Gunderson, 2012), and will continue to be so as biologists attempt to understand and predict the fate of ectotherms whose temperature-dependent performance is innately linked to environmental temperature. There are three commonly tested constraints on the TPC. First, the specialist-generalist trade off (Huey and Kingsolver, 1989; Latimer et al., 2011) is commonly invoked in discussion of the evolution of the TPCs. This tradeoff invokes the principle of allocation (Levins, 1968) whereby an individual has a pool of resources and can “decide” how to invest them; for example, a jack of all trades or master of one strategy ((Huey and Kingsolver, 1989), but see (Goodman, 2007)), i.e. either low performance over a wide range of environments, or a high performance over a narrow-range of environments respectively. The specialist-generalist tradeoff is generally tested by regressing maximum performance (Max_{perf}) and performance breadth (PB_{80}, the temperature range over which performance exceeds 80% of Max_{perf}). A negative relationship indicates that maximum performance is traded for performance over a wider temperature range. This tradeoff similarly explains observed negative correlations between speed and endurance in some lizards, fish, and mammals (Vanjooydonck et al., 2001). The tradeoff is not ubiquitous though (reviewed in (Angilletta et al., 2003)), and its absence suggests compensation by biological or physiological adaptations (Frazier et al., 2006). The second hypothesis is that hotter-is-better. In this case, it is assumed that the TPC is thermodynamically constrained so that individuals with a low thermal optimum (T_{opt}, the temperature of Max_{perf}) achieve lower Max_{perf} than individuals with high T_{opt}. In other words, if the TPC is thermodynamically constrained, we expect to observe a positive relationship between Max_{perf} and T_{opt}. The last hypothesis was coined the acquisition tradeoff, where the individual sacrifices survivorship, e.g. risk of predation or parasitism, for acquiring resources that can be allocated to performance (Angilletta et al., 2003). This class of tradeoff influences the area under the curve (AUC) of the TPC. The AUC is the integral of the thermal performance, is indicative of total performance, and varies with the shape and height of the TPC. If AUC varies with Max_{perf}, it may indicate that individuals make a sacrifice to acquire more resources and improve total performance.

In this study we demonstrate fitting individual TPCs for swimming speed in 73 new-born semi-aquatic grass snakes (*Natrix natrix*) from 7 clutches. We use GAMM to fit the TPCs and attribute the deviance, or effect size, to the species, matriline, or individual. Properties of individual TPCs were used to test for the existence of the specialist-generalist trade-off and the hotter-is-better hypothesis. We examine AUC, which can be used to examine acquisition by the offspring, however, since we used snakes hatched in the laboratory there was no opportunity for foraging before the experiment. In this case, variability in AUC represents either the baseline inter-individual variation in total performance or maternal allocation of resources among offspring. We used random regression to detect variability in AUC and variability in strategies among matrilines, which suggests maternal allocation strategies.

2. Methods

2.1. Study species

The colubrid snake, *Natrix natrix* (Linnaeus, 1758), is widely distributed across Europe: from Sweden and Norway, through Britain, France, Portugal, east to Turkey, Poland and parts of Russia, from sea level to ~2000 m. The species is a generalist, living in forests, grasslands and river edges. The species is communally nesting, bears clutches of ~8–30 eggs, and has instances of multiple paternity per clutch at a frequency of 27–90% of clutches (Meister et al., 2012).

2.2. Animals used

Seven gravid females were captured in May and June 2014 along a twenty kilometre stretch of river between the localities of Moulis, France (42° 57′ 43″ N; 1° 05′ 30″ E; approximate elevation 500 m) and Le Pont (42° 52′ 32″; N 0° 57′ 19″ E; approximate elevation 800 m). They were then kept in a controlled temperature room at 18 °C wthin the Station d'Ecologie Théorique et Expérimentale. Females were weighed in body mass within a few hours from capture (± 0.1 g). They were housed individually in 60 cm x 40 cm x 50 cm (LBH) plastic boxes, each containing a 5 cm layer of peat, one 40 W heat lamp, and one black 15 cm x 10 cm x 5 cm laying box lined with 2 cm moist vermiculite. After oviposition, females were weighed a second time, measured in snout-vent length (± 0.1 cm) (Supplementary Table 1), and returned to their exact site of capture.

Oviposition dates spanned from 7 July to 13 August 2014. A total of

95 eggs were obtained (N =13.6 ± 6.6 eggs per female). Eggs were collected within 12 h and transferred into an Aqualytic® incubation chamber (Dortmund, North Rhine-Westphalia, Germany) set at a constant 28 °C. Eggs were placed on a 2 cm layer of moist vermiculite for the entire incubation period.

Hatching occurred between 13 August and 20 September 2014. Neonates fully emerged from their eggs within two days of splitting their egg shells. A total of 73 snakes were born, 34 males and 39 females. All neonates were marked by scale-clipping and measured in body mass (± 0.01 g) and snout-vent length (± 0.1 cm) within 12 h of hatching (Supplementary Table 1). Sex was determined by eversion of the hemipenes. Siblings were housed together in plastic boxes (15 cm x 10 cm x 5 cm), with a water dish, shelter, and paper towel as substrate.

2.3. *Swimming performance*

All snakes started the swimming performance tests at 3 weeks old to make sure any undigested yolk absorbed in the stomach had been assimilated (i.e. stomach content may impair locomotion in snakes; Garland, 1983). Swimming performance was tested using a procedure adopted from previous studies (Shine and Shetty, 2001; Aubret, 2004; Aubret et al., 2015). A linear 300 cm x 40 cm x 50 cm swimming track was built (wooden frame painted white with black markers drawn every 10 cm, clear coated with Epoxy resin). A high definition wide angle digital camera (30 fps) was fitted above the track and used to record trials (recording section of 100 cm). The tank was filled with 10 cm of water and fitted with a reverse-cycle water chiller (TECO® TC15, Ravenna, Italy) and water pump (Aquavie® 1264, Connaux, France) that allowed for quick adjustment of water temperature. All snakes were subjected to swimming tests at 5 different water temperatures between 10 and 38 °C, selected from 10, 12, 17, 24, 30, 31, 34, 35, 38 °C. Temperature was continuous in the model so inter-individual variation in temperature tested should be unimportant. Snakes were subjected to one test per day in a balanced temperature order. The video was analysed using the software Tracker 4.87, and we calculated straight-line swimming speed for each trial. After all tests were completed, snakes were given their first meal (small dead minnows ranging from 0.5 to 1 g; supplied by the fish farm Armorvif®, Kerpert, France) and returned to their mother's capture site.

2.4. *TPC curve fitting using GAM*

The fastest three linear speeds for each snake at each temperature were used as the response variable (speed) for the following series of GAM(M)s:

a. species level
b. with matriline as a fixed factor
c. with matriline as a fixed factor, allowing for random intercept of individuals
d. with matriline as a fixed factor, allowing for random intercept and slope of individuals
e. with matriline as fixed factor, and random smooth per individual
f. with random smooth per individual

This design allowed us to compare models using log-likelihood/F tests as models b:f are nested within model a. In this way, we can see whether AIC changes amongst models, and so find the model with the most explanatory power given degrees of freedom, but also whether a given better or worse model explains significantly different amounts of the deviance in the data.

GAM is a piece-wise model estimation, and so can make imperfect predictions outside the range of recorded data. Accordingly, individuals whose predicted critical thermal maximum (the highest temperature where the TPC crosses 0; Ct_{max}) exceeded 48 °C (maximum for metazoans is ca. 45–47 °C (Pörtner, 2001; Chown et al., 2010)) had an additional dummy data point at 44.6 °C, speed=0, which was the mean CT_{max} predicted for all individuals whose CT_{max} was rooted below 48 °C. Similarly, individuals whose predicted critical thermal minimum (the lowest temperature where the TPC crosses 0; Ct_{max}) <0 °C had an additional dummy point added at 5.8 °C, the mean CT_{min} for all others. While not empirically determined critical temperatures, these values are realistic, and appear to be reasonable estimates (P Gordon *pers comm*, T Madsen, *pers comm*). In the end, seven TPCs still did not root (Fig. 3, e.g. 2nd plot in the 1st line or 2nd last plot in the 5th line). The GAMM method estimates the function locally for groups of data, which may have resulted in inflections near the temperature extremes, or otherwise there may have been some measurement error. We have included their GAMM to illustrate this point (Fig. 3). In reality, individuals whose TPC did not converge to 0 within realistic thermal bounds may have curves that are very steep toward the extremes.

For the winning models, we extracted maximum performance, thermal optimum, performance breadth (temperature range of all performance >80% of maximum), and area under the curve for the temperature range 10–38 °C, the range over which we had data.

2.5. *Data analysis*

Statistical tests were performed using the software R version 3.1.2 (R Development Core Team). We tested for a potential effect of sex, mass and SVL on maximum swimming speed with one-way ANOVAs. GAMM models were fitted in R using a cubic regression spline smoother, with the package mgcv (Wood, 2011). Mixed model selection, fitting and validation followed Zuur et al. (2009). TPC properties (Max_{perf}, PB_{80}, T_{opt}, AUC) were used in mixed linear models for hypothesis testing: specialist-generalist tradeoff ($Maxperf{\sim}PB_{80}$); hotter-is-better ($Max_{perf}{\sim}T_{opt}$); and maternal allocation ($Max_{perf}{\sim}AUC$). In the case of no effect of matriline, the random effect was removed, following Zuur et al. (2009), and the final model included no maternal effect.

3. Results

There was no effect of sex, mass or SVL on swimming speed (Supplementary Tables 2 and 3).

Among the candidate GAMs, the winning model was model f, with one unique swimming speed ~ temperature (TPC) curve fit for each individual, without accounting for matriline (Table 1). This model explained 89.44% of the total deviance. The next competing model, which included matriline as a fixed factor with random smooths per individual (e), explained 84.40% of the deviance.

Fitting one common curve (model a, Fig. 1) for the species explained 68.51% of the deviance in the data. Including matriline

Table 1
Characteristics of the six compared models of thermal performance.

Model	Resid. Df	Resid. Dev	AIC	dAIC	Weight	Dev. Expl (%)
a	1223.5	3,371,987.4	13,266.9	634.6	1.60E-138	68.51
b	1192.7	3,236,961.5	13,285.4	653.1	1.50E-142	69.77
c	1131.9	2,389,367.2	13,031.5	399.3	2.00E-87	77.68
d	1116.9	2,295,781.8	13,015.9	383.6	5.00E-84	78.56
e	1033	1,670,783.4	12,825.6	193.3	1.00E-42	84.4
f	899.8	1,130,895	12,632.3	0	1	89.44

Model names as in the text: a is at the species level (data from all individuals pooled without distinction), b includes matriline as a fixed factor, c is similar to b but also allows for random intercept of individuals, d is similar to c but also allows random slopes for individuals, e includes matriline as a fixed factor, and one random smooth per individual, f includes one random smooth per individual.

Resid. DF=residual degrees of freedom, Resid. Dev=residual deviance, Dev. Expl. =deviance explained.

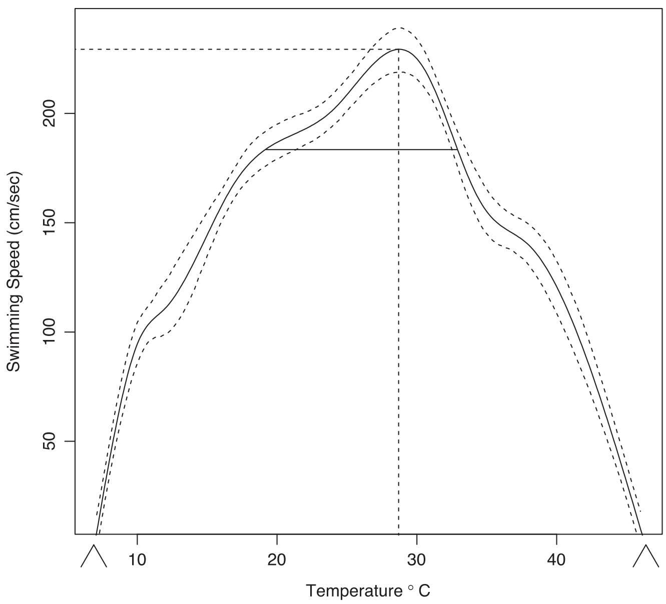

Fig. 1. Thermal performance curve for *N. natrix*, modeled by GAM, with 95% prediction intervals. Model a, based on 73 individuals from 7 matrilines. Vertical/horizontal dashed lines indicate thermal optimum and maximum performance, horizontal solid line 80% performance breadth, critical thermal minimum and maximum occur where the curve crosses zero.

(model b, Fig. 2) increased this by 1.26% (Table 1), indicating that only 1.26% of the variation in *N. natrix* TPC was maternally derived. By degrees, adding more information about individuals increased the deviance explained by the model: allowing a random intercept (model c) added a further 8.21%, random intercept and slope (model d) another 0.58%, unique smooth term per individual (model e) another 5.84%, and finally by removing the constraint of matriline but retaining a unique smoother per individual (model f, Fig. 3), 89.44% of the data in the deviance was explained.

The species summary TPC (model a, Fig. 1) and matriline-level TPC (model b, Fig. 2) bore strong resemblance to the expected hypothetical shape for a TPC, and many published curves. The individual curves (model f, Fig. 3) varied a lot more, including a wide spread in TPC metrics such as height, breadth and area under the curve. These metrics were used to examine tradeoffs in curve dimensions. Twelve of the individual curves were bimodal, which may reflect the terrestrial-aquatic lifestyle of *N. natrix*, with two local thermal optima, one lower for swimming performance, one higher for terrestrial (Fig. 3).

Performance breadth decreased significantly with maximum performance and there was no effect of matriline (Fig. 4), supporting the specialist-generalist trade off hypothesis. There was a weak but significant relationship between $\mathrm{Max}_{\mathrm{perf}}$ and $\mathrm{T}_{\mathrm{opt}}$, with no effect of matriline (Fig. 5), providing weak support for the hotter-is-better hypothesis. There was a significant effect of matriline in the relationship between area under the curve and maximum performance (Fig. 6, Table 2). Six out of seven mothers had a positive relationship between

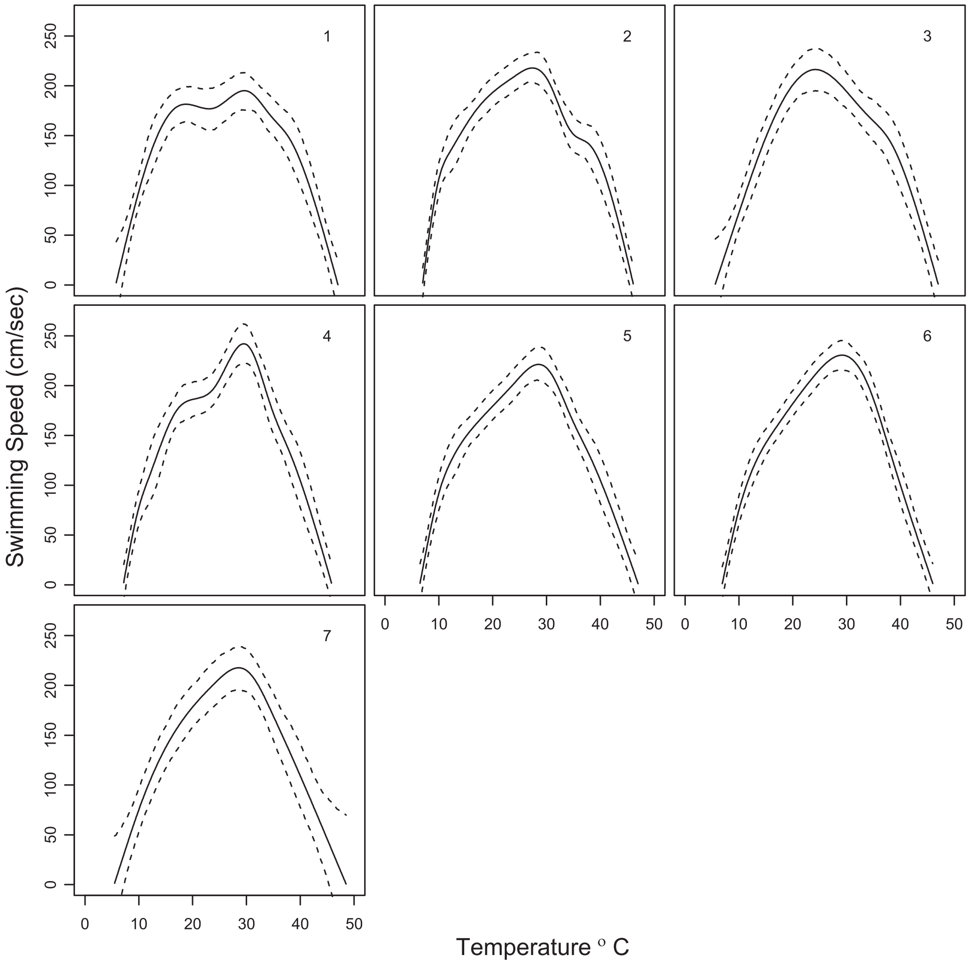

Fig. 2. Predicted TPC GAMs at the matriline level, model b. (Dotted zone is 95% prediction intervals). GAM for species is shown as grey zone (from Fig. 1). Numbers correspond to matrilines.

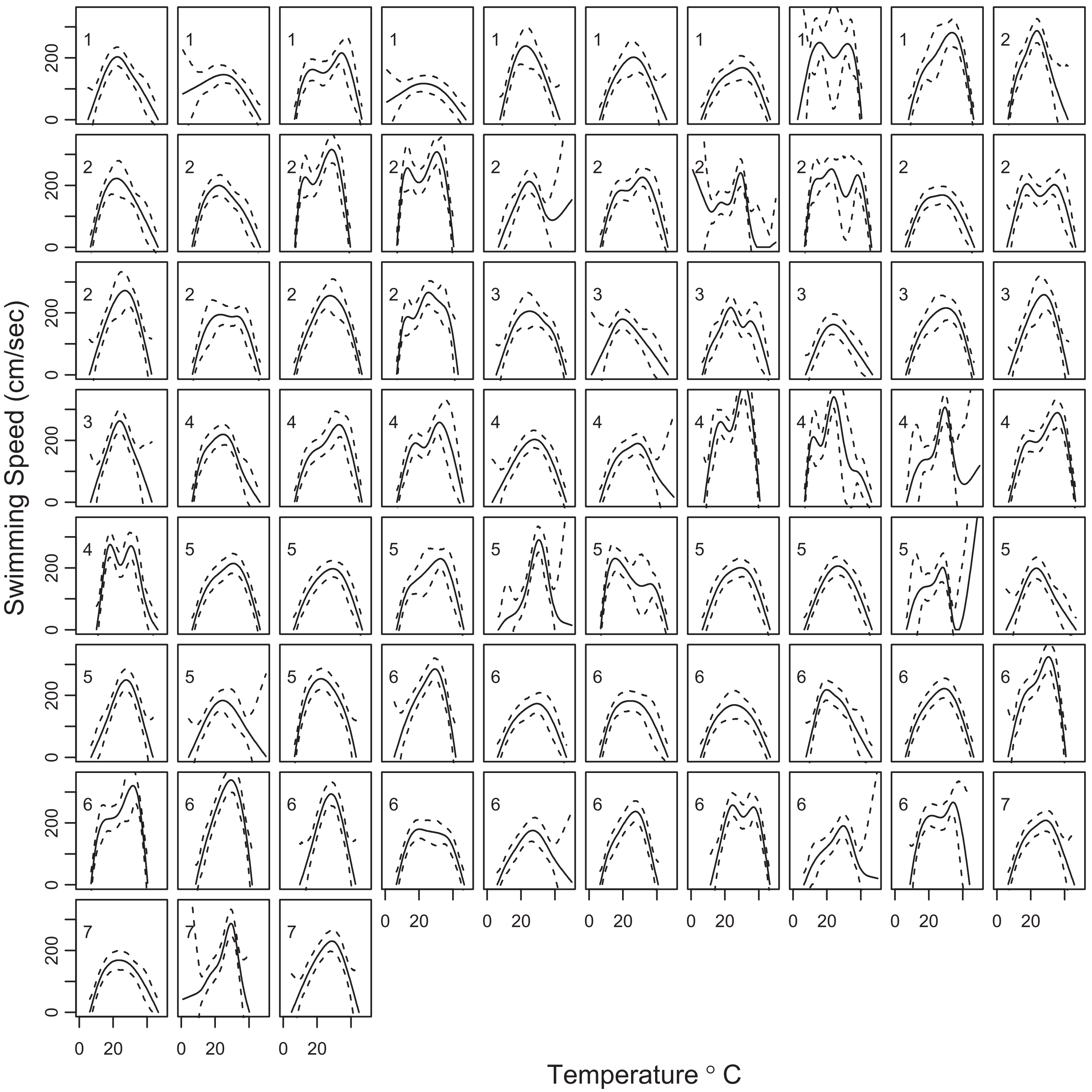

Fig. 3. Predicted TPC GAMMs at the individual level, model f. Dotted zone is individual 95% prediction, grey zone is matriline TPC GAM from Fig. 2, with matriline number indicated.

AUC and Max_{perf} (one significantly weaker than the others, mother #5), indicating better performers had better overall performance. The last mother, mother #7, showed a negative relationship, indicating increased specialization with increased temperature.

4. Discussion

Using GAMM, we demonstrated the allocation of deviance in TPC shape to species, matriline, and individual. Including individual as a random effect in the *N. natrix* TPC model explained 16% more of the deviance compared to the species curve (model f vs model a). This eclipsed variability explained by matriline (1.3%) and significantly improved the model. The high variability in TPC shape among individual *N. natrix* corroborates recent reports of inter-individual heterogeneity in thermal sensitivity of performance (Artacho et al., 2013), and in TPC intercept (Careau et al., 2014a). If such inter-individual variability is general, the implications are broad: on one hand, it means species-curves that do not account for variability have less predictive power than thought (Sunday et al., 2011; Bonebrake and Deutsch, 2012). On the other hand it means the error can be easily calculated and included in predictions drawn by future studies, improving our understanding of biological systems, and the precision of our understanding itself. We found strong support for a specialist-generalist tradeoff between performance maximum and performance breadth and weak support for the hotter-is-better hypothesis that high T_{opt} is accompanied by high performance, and we found mixed support for a relationship between area under the curve and maximum performance, potentially indicating different maternal strategies.

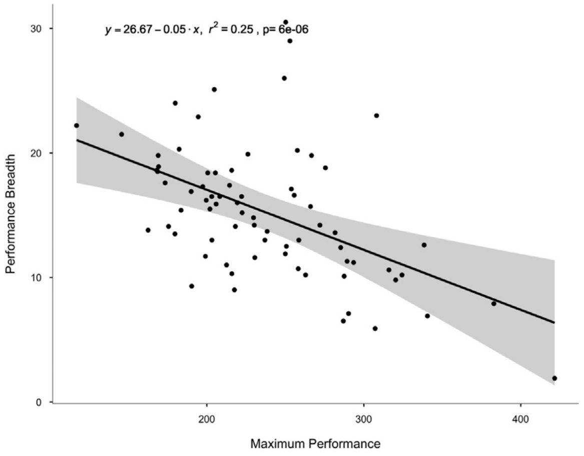

Fig. 4. Negative relationship between Performance Breadth and Maximum Performance, supporting a specialist-generalist trade off: maximum performance is traded with performance over a wide breadth.

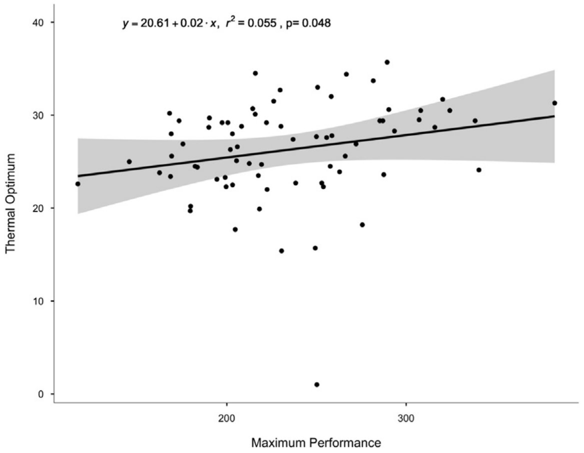

Fig. 5. Positive relationship between Thermal Optimum and Maximum Performance supporting the hotter-is-better hypothesis: thermodynamics constrain maximum performance, and individuals with a higher thermal optimum perform better overall.

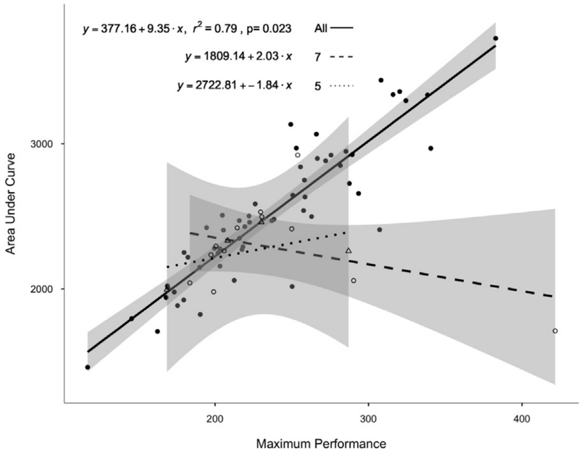

Fig. 6. Relationship between Area under the Curve and Maximum Performance. There was a significant effect of matriline, indicative of varying reproductive strategies.

Inter-individual differences in TPC were stronger than any maternal effect, with the implication that variance within each clutch is as large as total variance in the sampled population. The effect of matriline was barely significant. By comparing a model with its nested mixed subset, we were testing on the boundary which inflates type I error (incorrectly rejecting the null, (Bolker, 2008)): the difference between the species and the matriline models must be interpreted with caution. Given the very small increase in deviance explained, we suspect the observed matriline effect on TPC shape was biologically meaningless. By contrast, inter-individual differences accounted for almost 16% of the total deviance of the model, and this was true within and between clutches: there was as much variation in TPC within clutches as between. This may indicate low heritability of the TPC, though to comment on that requires knowledge of the mother's TPC at hatching, as well as the father's (up to N=5 per clutch, (Meister et al., 2012)). Observed TPC variability may also be beneficial and part of a reproductive strategy.

Table 2
TPC metrics (summary, mean (variance)) by matriline.

Mother	Area Under Curve	Thermal Optimum	Maximum Performance	Performance Breadth
1	15.77 (12.41)	25.93 (35.71)	1.4 (0.12)	17.14 (26.05)
2	16.65 (7.7)	26.25 (15.16)	1.51 (0.07)	18.34 (35.4)
3	16.34 (4.88)	24.76 (11.09)	1.55 (0.07)	14.67 (11.01)
4	16.96 (10.79)	28.42 (27.13)	1.7 (0.19)	12.44 (20.59)
5	15.13 (3.99)	28.48 (67.38)	1.59 (0.2)	13.54 (24.35)
6	17.01 (13.62)	27.6 (14.47)	1.61 (0.17)	15.2 (19.11)
7	16.41 (3.91)	27.58 (7.88)	1.66 (0.27)	13.93 (30.25)

In the absence of costs, we might expect the ultimate snake to have the highest and broadest performance (e.g., superfleas, (Reznick et al., 2000)), and that this should be the evolutionary endpoint. Not all snakes we measured were equal in terms of maximum performance, thermal optimum, or performance breadth, and we observed tradeoffs and variation amongst clutches in these tradeoffs. In general, activities that are considered to impact fitness (e.g. locomotor performance, (Warner and Andrews, 2002; Miles, 2004)) are carried out at T_{opt} (Gilchrist, 1995), and to minimize physiological stress, T_{opt} should match the habitat (Huey, 1991). Similarly we expect niche breadth, in this case performance breadth, to match environmental temperature variation (Huey and Stevenson, 1979; Kassen, 2002). Thermal specialization, low PB_{80}, in TPCs is associated with constant environments and environments with relatively strong within-generation variance in environmental temperature, while thermal generalization (high PB_{80}) is associated with strong among-generation variation (Gilchrist, 1995), and experiments have demonstrated a plastic decrease in maximum performance with increased within-generation environmental thermal variation (Bozinovic et al., 2013). We found a negative association between Max_{perf} and PB_{80}, and high variability within clutches in both T_{opt} and PB_{80}. This variability may be the proximate mechanism that increases habitat exploitation, ultimately reducing competition for space amongst kin and reducing the chance they will interact.

In general, there was a positive relationship between the area under the curve and maximum performance, though three out of four of the highest-maximum-performers have less than average area under the curve. If we consider AUC as the integration of performance over the performance breadth, then AUC becomes the total "performance resource" that an individual has, analogous to the total resource acquisition, and which the individual can allocate among traits. A positive relationship between acquisition (AUC) and allocation (e.g. maximum performance) indicates acquisition is more variable than allocation: how much of the resource an individual has varies more than the strategy they implement to use the resource (Reznick et al., 2000; van Noordwijk and de Jong, 1986). For the case of one matriline, the opposite was true: resource acquisition was less variable than the strategy in using it, and indeed, while there was no heterogeneity of variance detected in maximum performance or AUC among matrilines, matriline 7 had the largest and matriline 5 the second largest variance in maximum performance, and they had the smallest and second smallest variance in AUC. The snakes in the experiment had no foraging opportunity and were operating only on the resources from the egg, this can be seen from the mother's perspective as allocation per offspring unit. By allocating unequal resources to each egg in the clutch, the female may influence her offspring in many ways. Increased variability in the clutch may mean large variance in body size, body condition, survival, performance, habitat preference, or any number of traits (Bonnet et al., 2001; Lourdais et al., 2003; Rugiero et al., 2012). Typically egg resources affect offspring size and performance (Sinervo, 1990), though they may also affect other factors, or indeed, size and performance difference may have flow-on effects throughout the life history; for example slower, smaller individuals are hypothesized to employ different survival tactics and habitat use than larger or faster individuals (Miles, 2004). The end result of this may be variability in habitat preference and use, reducing intra-clutch competition for space and habitat.

In conclusion, our study demonstrated that the GAMM method allows to measure individual TPC metrics with very high precision (maximum performance, AUC etc), with which we examined inter-individual and intra-clutch tradeoffs and resources in a wide-ranging snake species. We found support for a specialist-generalist tradeoff, and to a lesser extent for the hotter-is-better hypothesis. We found unequal acquisition of resources (from the offspring perspective) or allocation (from the mothers'), which opposes the principal of allocation, and weakens our support of the specialist generalist hypothesis, but opens an interesting discussion on the cause for unequal maternal allocation of resources to the egg, and the likely effects of such. Experiments on the variation in energetics among eggs in clutches may be warranted to further explore this.

Data accessibility

Data and R scripts are available on figshare (https://dx.doi.org/10.6084/m9.figshare.4232141).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtherbio.2016.11.003.

References

Angilletta, M.J., 2006. Estimating and comparing thermal performance curves. J. Therm. Biol. 31 (7), 541–545. http://dx.doi.org/10.1016/j.jtherbio.2006.06.002.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.N., 2002. The evolution of thermal physiology in endotherms. J. Therm. Biol. 27, 249–268. http://dx.doi.org/10.2741/E148.

Angilletta, M.J., Wilson, R.S., Navas, C.A., James, R.S., 2003. Tradeoffs and the evolution of thermal reaction norms. Trends Ecol. Evol. 18 (5), 234–240. http://dx.doi.org/10.1016/S0169-5347(03)00087-9.

Artacho, P., Jouanneau, I., Le Galliard, J.-F., 2013. Interindividual variation in thermal sensitivity of maximal sprint speed, thermal behavior, and resting metabolic rate in a lizard. Physiol. Biochem. Zool. 86 (4), 458–469. http://dx.doi.org/10.1086/671376.

Aubret, F., 2004. Aquatic locomotion and behaviour in two disjunct populations of Western Australian tiger snakes. *Notechis ater occidentalis*. Aust. J. Zool. 52 (4), 357–368.. http://dx.doi.org/10.1071/ZO03067.

Aubret, F., Blanvillain, G., Kok, P.J.R., 2015. Myth busting? Effects of embryo positioning and egg turning on hatching success in the water snake *Natrix maura*. Sci. Rep. 5 (April), 13385. http://dx.doi.org/10.1038/srep13385.

Bennett, A.F., 1980. The thermal dependence of lizard behaviour. Anim. Behav. 28 (3), 752–762. http://dx.doi.org/10.1016/S0003-3472(80)80135-7.

Bolker, B.M., 2008. Ecological Models and Data in R. Princeton University Press, New Jersey.

Bonebrake, T.C., Deutsch, C.A., 2012. Climate heterogeneity modulates impact of warming on tropical insects. Ecology 93 (3), 449–455. http://dx.doi.org/10.1890/11-1187.1.

Bonnet, X., Naulleau, G., Shine, R., Lourdais, O., 2001. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. Oikos 92 (2), 297–308.. http://dx.doi.org/10.1034/j.1600-0706.2001.920212.x.

Bozinovic, F., Catalan, T.P., Estay, S.A., Sabat, P., 2013. Acclimation to daily thermal variability drives the metabolic performance curve. Evolut. Ecol. Res. 15 (5), 579–587.

Bulté, G., Blouin-Demers, G., 2006. Cautionary notes on the descriptive analysis of performance curves in reptiles. J. Therm. Biol. 31 (4), 287–291. http://dx.doi.org/10.1016/j.jtherbio.2005.11.030.

Careau, V., Biro, P.A., Bonneaud, C., Fokam, E.B., Herrel, A., 2014a. Individual variation in thermal performance curves: swimming burst speed and jumping endurance in wild-caught tropical clawed frogs. Oecologia 175 (2), 471–480. http://dx.doi.org/10.1007/s00442-014-2925-7.

Careau, V., Gifford, M.E., Biro, P.A., 2014b. Individual (co)variation in thermal reaction norms of standard and maximal metabolic rates in wild-caught slimy salamanders. Funct. Ecol. 28 (5), 1175–1186. http://dx.doi.org/10.1111/1365-2435.12259.

Chown, S.L., Hoffmann, A.A., Kristensen, T., Angilletta, M.J., Stenseth, N., Pertoldi, C., 2010. Adapting to climate change: a perspective from evolutionary physiology. Clim. Res. 43, 3–15. http://dx.doi.org/10.3354/cr00879.

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C., Longino, J.T., 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322 (5899), 258–261. http://dx.doi.org/10.1126/science.1162547.

Frazier, M.R., Huey, R.B., Berrigan, D., 2006. Thermodynamics constrains the evolution of insect population growth rates: “warmer is better”. Am. Nat. 168 (4), 512–520. http://dx.doi.org/10.1086/506977.

Gilchrist, G.W., 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. Am. Nat. 146 (2), 252–270. http://dx.doi.org/10.1086/285836.

Glanville, E.J., Seebacher, F., 2006. Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. J. Exp. Biol. 209 (Pt24), 4869–4877. http://dx.doi.org/10.1242/jeb.02585.

Goodman, B.A., 2007. Microhabitat separation and niche overlap among five assemblages of tropical skinks. Aust. J. Zool. 55 (1), 15. http://dx.doi.org/10.1071/ZO06066.

Gvoždík, L., 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. Can. J. Zool. 80 (3), 479–492. http://dx.doi.org/10.1139/z02-015.

Herczeg, G., Kovacs, T., Hettyey, A., Merilä, J., 2003. To thermoconform or thermoregulate? An assessment of thermoregulation opportunities for the lizard *Zootoca vivipara* in the subarctic. Polar Biol. 26, 486–490. http://dx.doi.org/10.1007/s00300-003-0507-y.

Huey, R.B., 1991. Physiological consequences of habitat selection. Am. Nat. 137 (s1), S91 . http://dx.doi.org/10.1086/285141.

Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4 (5), 131–135. http://dx.doi.org/10.1016/0169-5347(89)90211-5.

Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Integr. Comp. Biol. 19 (1), 357–366. http://dx.doi.org/10.1093/icb/19.1.357.

Irschick, D.J., Garland, T., Jr, 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. Annu. Rev. Ecol. Syst. 32 (1), 367–396. http://dx.doi.org/10.1146/annurev.ecolsys.32.081501.114048.

Kassen, R., 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. J. Evolut. Biol. 15 (2), 173–190. http://dx.doi.org/10.1046/j.1420-9101.2002.00377.x.

Kearney, M.R., Porter, W.P., 2004. Mapping the fundamental nice: physiology, climate, and the distribution of a nocuturnal lizard. Ecology 85 (11), 3119–3131. http://dx.doi.org/10.1890/03-0820.

Kearney, M.R., Porter, W.P., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12 (4), 334–350. http://dx.doi.org/10.1111/j.1461-0248.2008.01277.x.

Latimer, C.A., Wilson, R.S., Chenoweth, S.F., 2011. Quantitative genetic variation for thermal performance curves within and among natural populations of *Drosophila serrata*. J. Evolut. Biol. 24 (5), 965–975. http://dx.doi.org/10.1111/j.1420-9101.2011.02227.x.

Leal, M., Gunderson, A.R., 2012. Rapid change in the thermal tolerance of a tropical lizard. Am. Nat. 180 (6), 815–822. http://dx.doi.org/10.1086/668077.

Levins, R., 1968. Evolution in Changing Environments: Some Theoretical Explorations.

Lillywhite, H.B., 1980. Behavioral thermoregulation in Australian elapid snakes. Copeia (3), 452–458.

Lourdais, O., Bonnet, X., Shine, R., Taylor, E.N., 2003. When does a reproducing female viper (*Vipera aspis*) “decide”on her litter size? J. Zool. 259, 123–129. http://dx.doi.org/10.1017/S0952836902003059.

Meister, B., Ursenbacher, S., Baur, B., 2012. Frequency of multiple paternity in the grass snake (*Natrix natrix*). Amphibia-Reptilia 33, 308–312. http://dx.doi.org/10.1163/156853812X634053.

Miles, D.B., 2004. The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. Evolut. Ecol. Res. 6, 63–75.

Munoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A., Losos, J.B., 2013. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proc. R. Soc. B 281, 20132433. http://dx.doi.org/10.1098/rspb.2013.2433.

Nussey, D.H., Wilson, a.J., Brommer, J.E., 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. J. Evolut. Biol. 20 (3), 831–844. http://dx.doi.org/10.1111/j.1420-9101.2007.01300.x.

Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88 (4), 137–146. http://dx.doi.org/10.1007/s001140100216.

Reznick, D., Nunney, L., Tessier, A., 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol. Evol. 15 (10), 421–425. http://dx.doi.org/10.1016/S0169-5347(00)01941-8.

Rugiero, L., Capula, M., Vignoli, L., Luiselli, L., 2012. Offspring condition determines dispersal patterns in western whip snakes, *Hierophis viridiflavus*. Herpetol. J. 22 (4), 259–261.

Seebacher, F., Franklin, C.E., 2005. Physiological mechanisms of thermoregulation in reptiles: a review. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 175 (8), 533–541. http://dx.doi.org/10.1007/s00360-005-0007-1.

Seebacher, F., Shine, R., 2004. Evaluating thermoregulation in reptiles: the fallacy of the inappropriately applied method. Physiol. Biochem. Zool. 77 (4), 688–695. http://dx.doi.org/10.1086/422052.

Shine, R., Shetty, S., 2001. Moving in two worlds: aquatic and terrestrial locomotion in sea snakes (Laticauda colubrina, Laticaudidae). J. Evolut. Biol. 14 (2), 338–346. http://dx.doi.org/10.1046/j.1420-9101.2001.00265.x.

Sinervo, B., 1990. Evolution of thermal physiology and growth rate between populations of the western fence lizard (Sceloporus occidentalis). Oecologia 83 (2), 228–237. http://dx.doi.org/10.1007/BF00317757.

Sinervo, Barry, Mendez de la Cruz, Fausto, Miles, Donald B., Heulin, Benoit, Bastiaans, E., Villagran-Santa Cruz, M., Lara-Resendiz, R., Martinez-Mendez, N., Calderon-Espinosa, M.L., Meza-Lazaro, R.N., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V., Rocha, Carlos F.D., Ibarguengoytia, N., Puntriano, C.A., Massot, Manuel, Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, Aaron M., Branch, W.R., Clobert, Jean, Sites, J.W., 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328 (5980), 894–899. http://dx.doi.org/10.1126/science.1184695.

Slip, D.J., Shine, R., 1988. Thermoregulation of free-ranging diamond pythons, *Morelia spilota* (Serpentes, Boidae). Copeia 1988 (4), 984–995. http://dx.doi.org/10.2307/1445722.

Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc. R. Soc. B: Biol. Sci. 278 (1713), 1823–1830. http://dx.doi.org/10.1098/rspb.2010.1295.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. USA 111 (15), 5610–5615. http://dx.doi.org/10.1073/pnas.1316145111.

Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Putting the heat on tropical animals. Science 320 (24), 1296–1297.

van Noordwijk, A.J., de Jong, G., 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128 (1), 137–142. http://dx.doi.org/10.1086/284547.

Vanjooydonck, B., Van Damme, R., Aerts, P., 2001. Speed and stamina trade-off in lacertid lizards. Evolution 55 (5), 1040–1048.

Warner, D.A., Andrews, R.M., 2002. Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. Biol. J. Linn. Soc. 76 (1), 105–124.

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc.: Ser. B (Stat. Methodol.) 73 (1), 3–36. http://dx.doi.org/10.1111/j.1467-9868.2010.00749.x.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health. Springer, New York. http://dx.doi.org/10.1007/978-0-387-87458-6.

Mathew Vickers is completing his first post-doc focusing on inter-individual heterogeneity in dispersal characteristics of reptiles under the supervision of Aurélie Coulon and Fabien Aubret. His Ph.D. focused on the thermal biology of reptiles, in particular behavioral thermoregulation.

Fabien Aubret is a French evolutionary biologist and herpetologist. He completed his Doctoral and Post-doctoral studies between 2001 and 2008 in Australia (University of Western Australia and University of Sydney). Since 2009, he has been working as a full time researcher for the CNRS (National Centre for Scientific Research) at the Station of Theoretical and Experimental Ecology (SETE, Moulis, France). Fabien's research is mostly empirical, with an experimental backbone, and involves a variety of snake and lizard models. His research is pluri-disciplinary and involves ecology, physiology, phenotypic plasticity, climate change, thermoregulation and reproductive biology.

Aurélie Coulon has been an associate professor at the Muséum national d'Histoire naturelle (France) since 2008. After completing her PhD on roe deer dispersal (movement ecology, landscape genetics) in Toulouse in 2006 she did a post-doc on Florida Scrub Jay lanscape genetics at the Cornell Lab of Ornithology (USA). She works on the movement ecology of a range of animals, including reptiles. She is particularly interested in the effects of landscape composition and structure on dispersal, and their consequences for population functioning. She also investigates inter-individual heterogeneity in movements.