Gestation, Thermoregulation, and Metabolism in a Viviparous Snake, *Vipera aspis*: Evidence for Fecundity-Independent Costs

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Accepted 2/20/03

ABSTRACT

Oxygen consumption of gestating Aspic vipers, *Vipera aspis* (L.), was strongly dependent on body temperature and mass. Temperature-controlled, mass-independent oxygen consumption did not differ between pregnant and nonpregnant females. Maternal metabolism was not influenced during early gestation by the number of embryos carried but was weakly influenced during late gestation. These results differ from previous investigations that show an increase in mass-independent oxygen consumption in reproductive females relative to nonreproductive females and a positive relationship between metabolism and litter size. These data also conflict with published field data on *V. aspis* that show a strong metabolic cost associated with reproduction. We propose that, under controlled conditions (i.e., females exposed to precise ambient temperatures), following the mobilisation of resources to create follicles (i.e., vitellogenesis), early gestation per se may not be an energetically expensive period in reproduction. However, under natural conditions, the metabolic rate of reproductive females is strongly increased by a shift in thermal ecology (higher body temperature and longer basking periods), enabling pregnant females to accelerate the process of gestation. Combining both laboratory and field investigation in a viviparous snake, we suggest that reproduction entails discrete changes in the thermal ecology of females to provide optimal temperatures to the embryos, whatever their number. This results in the counterintuitive notion that metabolism may well be largely independent of fecundity during gestation, at least in an ectothermic reptile.

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Physiological and Biochemical Zoology 76(4):497–510. 2003. 1522-2152/2003/7604-2041$15.00

Introduction

Reproduction represents a major disruption to the typical day-to-day life of any female organism. The decision to reproduce, or to do so successfully, is often strongly resource orientated, mediated by food availability (Nagy et al. 1984, 1995; Boggs 1992; Meijer and Drent 1999), by body condition (Larsen 1980; Anderson and Karasov 1981; Nagy 1987; Brown 1991; Naulleau and Bonnet 1996; Meijer and Drent 1999), or both (Meijer and Drent 1999; Bonnet et al. 2001*b*). For example, endogenous reserve levels can determine the initiation of vitellogenesis (Bonnet et al. 1994) or reproductive effort and associated costs (Madsen and Shine 1993; Erikstad et al. 1997; Festa-Bianchet et al. 1998; Bonnet et al. 2001*a*, 2002, 2003). The amounts of body reserves can also influence the maintenance of brooding (Chastel et al. 1995; Dearbon 2001), gestation (Boyd 1984; Cresswell et al. 1992), and the intensity of parental care (Olsson 1997).

In many vertebrates, reproduction occurs when an animal is in positive energy balance since folliculogenesis maintains a low priority in the ongoing competition for energy allocation (Bronson 1998; Schneider et al. 2000). Any attempt to reproduce during periods of resource deficiency may potentially decrease both current and future reproductive success (Stearns 1992). The association between reproduction and resources is very intimate because reproduction is an energetically demanding process. An analysis of the available literature suggests that there is a clear difference between the metabolic rate of reproductive and nonreproductive individuals (Birchard et al. 1984; Speakman and McQueenie 1996; Mauget et al. 1997; Angilletta 2000). This conclusion is based on comparisons of rates of metabolism measured during discrete periods of the reproductive cycle (see Guillette 1982). However, to generalise such a broad divergence in metabolism across the whole reproductive process could be slightly hazardous given that this process is clearly not static. For example, the physiological and behavioural mechanisms that underlie folliculogenesis, gestation, and lactation are extremely different (Thibault and Levasseur 1991; Speakman and McQueenie 1996). Even among

males, where the energetic contribution to gametic production is almost negligible in most species, metabolic rate varies with pubertal maturity (Brown et al. 1996) or with the alternation of mating and nonmating periods (Olsson et al. 1997). Moreover, the ecological implications of changes in metabolism over the reproductive period are important considering the fact that food resource and other environmental conditions (temperature, rainfall, etc.) are often limited and/or often fluctuate.

Whatever the taxon or reproductive mode (i.e., viviparity vs. oviparity), the total metabolic effort expended over time is generally higher in reproductive females than nonreproductive females during the reproductive period. Limiting the focus to gestation, and hence considering viviparous species only, the extra metabolic demands associated with the developing foetus are higher than the normal metabolic demands for growth and repair (Hahn and Tinkle 1965; Mauget et al. 1997). Such a difference is detectable even in the late luteal phase of the menstrual cycle (Curtis et al. 1996). In mammals, this greater metabolic expenditure is often driven by a change in the hormonal balance of the reproducing individual that, in turn, provokes an increase in their basal metabolic rate through increased levels of cellular work (protein synthesis, mitosis, ion pumping, etc.) over the reproductive period (Thibault and Levasseur 1991; Howe et al. 1993; Butte et al. 1999).

Physiological changes also occur in viviparous ectotherms to cope with the demand of developing embryos. Birchard et al. (1984) and other authors describe an increase in mass-independent oxygen consumption in reproductive females, suggesting a modification of metabolic regulation set point, and this may also be associated with hormonal changes (Highfill and Mead 1975; Fergusson and Bradshaw 1991; Callard et al. 1992). However, ectothermic vertebrates are limited in the extent to which they can elevate the rate of cellular work and therefore their metabolic rate through purely physiological mechanisms (Harlow and Grigg 1984; Shine et al. 1997; Cadenas et al. 2000; Wang et al. 2001). Instead, ectotherms may depend on the environment to compensate for such a physiological constraint (Bradshaw 1997), using available thermal energy (heat) to increase metabolic reaction rate and to increase development of embryos. It is a common observation that in viviparous reptiles living in temperate areas, gravid females bask for longer periods than nongravid females (Gregory et al. 1987; Seigel and Ford 1987; Bonnet and Naulleau 1996; Shine 1998), with the accelerated development of embryos occurring as a result (Naulleau 1986). Change in body temperature of pregnant female ectotherms is the most effective way to increase the rates of cellular activity involved with production of young, and we may expect that this increase is proportional to the load of active tissues represented by the embryos. Such changes will involve a change in thermoregulatory behaviour. In addition, if we admit that variation in female metabolic rate is an integrative effect of the cellular work of the tissues of the mother plus those of developing embryos, we may expect a positive relationship between the size (and mass) of the litter and maternal mass-independent oxygen consumption. Such a relationship has been previously documented in viviparous reptiles, but the number of studies has been limited (Birchard et al. 1984; Demarco and Guillette 1992).

Importantly, the form of the relationship between fecundity (litter size), reproductive effort (mass-independent oxygen consumption, materials invested into follicles, loss of feeding opportunities, etc.), and the potential costs (decreased reproductive value, here simply viewed as a combination of lower survival and depletion of energy stores) has immense consequences on the evolution of life-history traits and on the underlying physiological regulations (Shine and Schwarzkopf 1992; Stearns 1992; Niewiarowski and Dunham 1994; Sinervo and Svensson 1998). Clearly, both empirical and experimental data are required in this field. For instance, increasing our knowledge in the costs/benefits relationship of pregnancy is a major prerequisite to better understanding the oviparity versus viviparity transition that has evolved independently several hundreds of times in many vertebrate taxa (fishes, amphibians, squamate reptiles; Shine 1985).

Snakes are suitable models for investigations such as this because there are oviparous and viviparous species in the same family group (Shine 1985). Because they are ectothermic, we can also experimentally control body temperature and assess the impact of this factor on metabolism in both reproductive and nonreproductive individuals (Beaupré and Duvall 1998). In addition, the broad range of fecundity among conspecific females allows us to identify any potential fecundity/reproductive effort/cost relationships. Finally, many snake species have a less than annual breeding frequency (Saint Girons 1957; Seigel and Ford 1987). Therefore, within any given year, both reproductive and nonreproductive individuals are influenced by the same variation of climate and resource availability, making the two groups more comparable.

In this investigation, we examined the influence of the reproductive status and fecundity on the metabolic rate of viviparous female snakes. Notably, under the same thermal conditions, are pregnant females more metabolically active than nonreproductive females over the gestative period? As an aside, we demonstrate that broad generalisations on the effects of reproductive status on metabolism, based on laboratory experiments over discrete periods, may potentially limit the value of conclusions in field conditions.

Material and Methods

Origin and Description of Study Animals

In this study, we used wild-caught gravid female Aspic vipers (*Vipera aspis*). We used this species because this snake is abundant in our study area and tolerant to captivity and manipulation, and previous work provides a useful baseline on the reproductive ecology and physiology of this species (Saint Gi-

rons 1957; Saint Girons and Duguy 1992; Bonnet et al. 1994, 1999, 2000*b*, 2001*a*, 2001*b*, 2002, 2003; Aubret et al. 2002; Lourdais et al. 2002*a*, 2002*b* and references therein). The Aspic viper is a stocky, venomous snake species widely distributed throughout western Europe (Naulleau 1997). Average snout-vent length (SVL) is 48.5 cm, and average body mass (BM) is 85.5 g. Females mature at approximately 3 yr (Bonnet et al. 1999), mating occurs in spring (March–April; Saint Girons 1957; Naulleau et al. 1999), and parturition occurs in autumn (September; Saint Girons 1957). Clutch size varies from one to 13 individuals ($\text{mean}_{\text{SVL}} = 17.9 \pm 1.2$ cm, $\text{mean}_{\text{BM}} = 6.3 \pm 1.1$ g; Bonnet et al. 2000*b*). Females in western France do not breed every year (Saint Girons 1957; Bonnet and Naulleau 1996) and delay reproduction until they exceed a minimum body condition threshold through the accumulation of large body reserves such as abdominal fat bodies (Naulleau and Bonnet 1996; Aubret et al. 2002).

Individuals used in this investigation were collected by hand from Les Moutiers-en-Retz and Les Sables d'Olonne, central western France (47°03′N, 02°00′W and 46°30′N, 01°44′W, respectively). Both study sites lie within 60 km of each other. Habitats were consistent between the two study sites, and the climate for the region is a temperate oceanic one (see Bonnet and Naulleau 1993 for average temperatures).

Specimens used for the metabolic tests were collected during early spring, after the mating period and during vitellogenesis (Saint Girons 1957). At this time, reproductive females had already committed to the development of a number of follicles. Individuals were housed in outdoor terraria (8–16 m^2) in our laboratory (46°08′N, 00°25′W) from June to late September and were exposed to a similar climate to the field site from where they were collected. Within each enclosure, snakes were provided with a mosaic of microhabitats, including many shelters and well-exposed sites to facilitate optimal thermoregulation. A unique code of ventral scale clipping identified individuals, and these marks are permanent because the regenerated tissues exhibit a different colour.

Resting Oxygen Consumption

The oxygen consumption of 50 reproductive females and 19 nonreproductive females was measured in the laboratory under controlled environmental conditions. Subjects were tested for rates of oxygen consumption over two phases during the survey period: early gestation and late gestation. A total of 59 snakes were tested during early gestation (40 reproductive and 19 nonreproductive), and a subset of 17 reproductive snakes (among the 40 reproductive females) were used for repeated-measures analysis in late gestation. An additional 12 reproductive snakes were measured in late gestation at 32°C to increase the statistical power of the data set used to derive the relationship with fecundity. Table 1 summarises the number of females sampled and resampled at different temperature regimes and/or at the two periods of gestation.

Table 1: Details on the number of female Aspic vipers assayed for O_2 consumption

Reproductive Status	Gestation Period	17.5°C	25°C	32.5°C
Reproductive	Early	14	13	13
Nonreproductive	Early	6	5	8
Reproductive	Late	7 (7)	None	22 (10)

Note. The first number provides the total number of females used in each treatment group. The number in parentheses refers to females used in repeated-measures experiment over early and late gestation.

Rates of oxygen consumption ($\dot{V}o_2$; mL O_2 h^{-1}) were measured using a flow-through respirometry system. Dry incurrent air was drawn through a small, clear Perspex metabolic chamber at a rate of 204 ± 4 mL min^{-1} by a Byoblock Scientific 6-L air pump, and flow was controlled using a Platon mass flow controller. The chamber, specifically built to accommodate Aspic vipers, was large enough (internal diameter: 15 × 15 × 5 cm) to accommodate snakes up to 200 g without preventing voluntary activity. Oxygen concentration was maintained at approximately 20.1%. The metabolic chamber was located within a sealed Cryosystem temperature-controlled chamber and positioned such that snakes could be observed during the trial through a small viewing port. During early gestation, the body temperature of each individual was checked before commencement of the test using a quick-registering thermometer (Novo) inserted 2 cm into the cloaca until stabilisation of the measure.

Excurrent air was passed through two column desiccators containing drierite, then through a paramagnetic O_2 transducer (Servomex Series 1100). The differential output of the oxygen analyser (ambient air minus excurrent air) was recorded, adjusted to standard temperature and pressure conditions, and plotted on a standard desktop PC. The metabolic chamber was calibrated to the outside atmosphere (Pressure Indicator Druck DPI 260) and set at zero oxygen consumption by running an empty chamber for 1 h before each snake being tested. Differential output was presented graphically as it was acquired, and snakes were run for as long as necessary to obtain stable oxygen consumption for a period greater than 30 min. Monitoring of snakes ensured that fluctuations in $\dot{V}o_2$ were attributed to activity and not to technical perturbations. Oxygen consumption was calculated following the equation of Depocas and Hart (1957).

Reproductive and nonreproductive snakes were run at 17.5°, 25°, and 32.5°C during early gestation, and reproductive snakes only were run at 17.5° and 32.5°C during late gestation. This corresponds to the range of temperatures we have recorded, using radio telemetry in the field in females (reproductive and nonreproductive) during the pregnancy period (Naulleau et al. 1996; G. Naulleau, X. Bonnet, and O. Lourdais, unpublished

data). Before measurements of oxygen consumption, animals were fasted for a minimum of 4 d and introduced to the system on the night before testing. Because the Aspic viper is diurnal, all experiments were conducted during the normal light phase appropriate for the time of year and location (approximately 0600 to 1900 hours). Snakes of both reproductive statuses were tested randomly throughout the day to remove any possible influence of diel cycle. Body mass of snakes was recorded on a top-loading electronic scale (±0.1 g) before each run, and the measurements were used to derive mass-independent volume of oxygen consumed (mass-independent $\dot{V}O_2$).

Measurements of Fecundity

Fecundity was determined by palpation in early gestation and, in this species, follicles as small as 2.0 g can be detected. Fecundity was confirmed at parturition. Live and stillborn neonates were included in the analysis as metabolically active tissues during pregnancy. Unfertile and/or undeveloped eggs (i.e., where only yolk was identifiable) that are frequent in Aspic viper's litters (Bonnet et al. 2001*b*) were not considered as metabolically active tissues during pregnancy because these undeveloped eggs are formed during vitellogenesis but not pregnancy.

Field Body Temperatures

We collected field data to compare the temperature regimes imposed on snakes in our laboratory experiment with the far more complex situation experienced by wild vipers. Using internal temperature radio transmitters, we determined the body temperature of reproductive and nonreproductive females in the field during most of the period of reproduction (April 15, 1996–July 15, 1996), encompassing the last 45 d of vitellogenesis and the first 45 d of gestation. The methodology has been described elsewhere (Naulleau et al. 1996) and has also been used successfully in a closely related species, the adder (*Vipera berus*; Madsen and Shine 1992, 1993).

Radio tracking enabled the collection of over 1,896 temperature records from 21 female Aspic vipers (11 reproductive and 10 nonreproductive). The temperatures were collected without disturbing the snake during the day. Each animal was sampled one to three times per day: in the morning, at midday, and in the afternoon (mean number of records per snake per day was 2.33 ± 0.84, range 1–6 with 99.9% of the cases comprised between 1 and 4 and 97% below 4). Because we sampled each snake randomly irrespective of the reproductive status, the exact time elapsed between two consecutive records was also random. However, it was always greater than 3 h and often greater than 12 h. The duration between samples ensures that consecutive records will reflect the thermal behaviour adopted by each snake rather than the thermal inertia of the body of the snake. Since body temperature is affected by ambient temperature, and ambient temperature increased over our sampling period, we took into account the effect of date in most analyses involving field body temperatures.

Statistical Analysis

Body mass (g) and oxygen consumption ($\dot{V}O_2$; mL O_2 h^{-1}) were $\log_{10}$ transformed to meet the normality assumption (Shapiro-Wilk, $W = 0.986$, $P = 0.731$ for log BM; Shapiro-Wilk, $W = 0.972$, $P = 0.158$ for log $\dot{V}O_2$; Zar 1984). Oxygen consumption data were adjusted for body mass by regressing log $\dot{V}O_2$ on log BM. The residuals from this regression yielded mass-independent oxygen consumption (mass-independent $\dot{V}O_2$), which was used for several of the analyses (see "Results"). We did not use ratios to scale oxygen consumption (Atchley et al. 1976; Packard and Boardman 1988). Instead we performed ANCOVAs, for example, to compare reproductive versus nonreproductive females using BM as a covariate (Garcia-Berthou 2001). Statistical analysis of $\dot{V}O_2$ was performed on the mean rate of oxygen consumption measured over a stable 30-min period for each individual. Analyses were performed using Statistica 5.1 and 6.0 (Statsoft 1995, 2001).

Results

Reproductive Status, Body Size, and Body Condition in Early Gestation

At the beginning of gestation, there was no difference in SVL between reproductive and nonreproductive females (one-factor ANOVA with SVL as the dependent variable and reproductive status as the factor: $F_{1,56} = 2.74$, $P = 0.10$; Table 2). However, reproductive females were significantly heavier than nonreproductive females (same-design ANOVA with BM as the depen-

Table 2: Morphometrics of 40 reproductive and 19 nonreproductive female Aspic vipers used during early gestation

Reproductive Status	Snout-Vent Length (cm)	Body Mass (g)	Adjusted Body Mass (g)
Reproductive	48.80 ± 6.14	99.84 ± 38.01	93.59 ± 3.25
Nonreproductive	46.17 ± 4.11	60.73 ± 18.16	66.98 ± 4.80

Note. Means are expressed ±SD. Comparing reproductive versus nonreproductive females, adjusted body mass (scaled by size using snout-vent length as a covariate) was greater in pregnant females; however, this was not necessarily due to the amounts of body reserves.

dent variable: $F_{1,56} = 17.16$, $P < 0.001$; Table 2) and logically were in better body condition than nonreproductive snakes (ANCOVA with log BM as the dependent variable and log SVL as the covariate: $F_{1,55} = 34.12$, $P < 0.001$; Table 2). Importantly, at the beginning of gestation, the greater body condition of reproductive females relative to nonreproductive females does not mean that they possess larger body reserves. In fact, most of the extra mass of reproductive females is represented by the litter, and despite their external appearance, pregnant females are relatively emaciated during early gestation (Bonnet et al. 2003).

Effect of Body Mass, Ambient Temperature, and Reproductive Status on Oxygen Consumption in Early Gestation

Body mass and body temperature are the two most important determinates of any ectotherm's metabolic rate; therefore, these variables must be controlled before looking for an effect of reproductive status. We performed an ANCOVA with log $\dot{V}O_2$ ($mL^{-1}\ h^{-1}$) as the dependent variable, reproductive status as the factor, and log BM and body temperature as the covariates. The whole model (test of the sum of squares [SS] of the whole model vs. SS residuals) explained a large proportion of the variance in oxygen consumption ($r^2 = 0.60$; $F_{3,54} = 26.59$, $P < 0.0001$; Levene's test for homogeneity of the variance, df = 1,56 for all F's; for log $\dot{V}O_2$: $F = 1.14$, $P = 0.29$; for log BM: $F = 0.47$, $P = 0.50$; for body temperature: $F = 1.46$, $P = 0.23$), reinforcing the notion that body mass and body temperature play a major role in the oxygen consumption of ectotherms (Saint Girons et al. 1985; Beaupré and Zaidan III 2001). Although both covariates strongly and positively influenced oxygen consumption (respectively, log BM: $F_{1,54} = 12.29$, $P < 0.001$; and body temperature $F_{1,54} = 51.76$, $P < 0.0001$), we did not find any significant effect caused by the reproductive status ($F_{1,54} = 2.82$, $P = 0.10$; Fig. 1). Using ambient temperature (17.5°, 25° and 32.5°C) as a second factor (due to its discontinuous nature in the experiment) instead of body temperature as a covariate did not change the result (whole model: $r^2 = 0.61$; specific effect of ambient temperature: $F_{2,51} = 25.07$, $P < 0.0001$) or reveal any significant influence of reproductive status (specific effect of reproductive status: $F_{1,51} = 1.46$, $P = 0.23$). Moreover, there was no interaction between the factors of reproductive status and ambient temperature ($F_{2,51} = 0.50$, $P = 0.61$). When the effects of body mass and body temperature were taken into account, the adjusted mean oxygen consumptions ($\pm$SE) were 0.33 ± 0.05 O_2 $mL^{-1}\ h^{-1}$ and 0.23 ± 0.03 O_2 $mL^{-1}\ h^{-1}$ for nonreproductive and reproductive females, respectively. The increase in ambient temperature from 17.5° to 32.5°C provoked a 2.8-fold increase in oxygen consumption (see Table 3) that represents a Q_{10} of 2.05 for all snakes tested during early gestation. The Q_{10} for the rate of change of oxygen consumption from cold (17.5°C) to medium (25°C) was 2.19 and from medium (25°C) to hot (32.5°C) was 1.91, showing the expected decrease in Q_{10} as temperatures increase (Withers 1992).

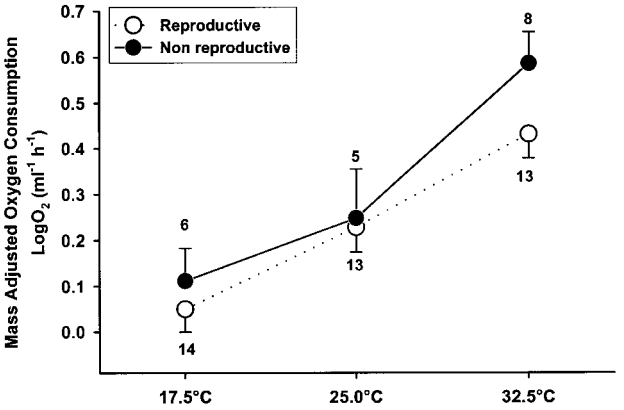

Figure 1. Effect of reproductive status and temperature on oxygen consumption of female Aspic vipers during early gestation. Each point represents the adjusted mean (least squares using log $\dot{V}O_2$ [$mL^{-1}\ h^{-1}$] as the dependent variable, reproductive status and ambient temperature as the factor, and log BM as the covariate; $\pm$SE and sample size) for each group of snakes in each category.

Effect of Gestation Period on Oxygen Consumption

In pregnant females, there was no change between the mean mass-independent $\dot{V}O_2$ (residuals) for the two periods, early and late gestation. To control for possible interindividual differences in reproductive stage or body mass, we used the same females on the two occasions under the same temperature conditions (seven females at 17.5°C and 10 females at 32.5°C). The 17 females were sampled during early gestation and again, 2 mo later, during late gestation. Despite being sensitive enough to detect any potential difference in mass-independent $\dot{V}O_2$ between gestation periods, the t-test for dependent samples provided a nonsignificant result ($t = 1.56$, df = 16, $P = 0.14$; Fig. 2).

Effect of Fecundity on Oxygen Consumption

For the data collected in early gestation, a partial correlation analysis was performed to take into account the effect of body temperature, body mass, and fecundity on oxygen consumption ($r^2 = 0.51$, $F_{3,34} = 11.97$, $P < 0.0001$; specific effect of body temperature, $F_{3,34} = 4.62$; $P < 0.001$; log BM, $F_{3,34} = 2.68$, $P = 0.011$; fecundity, $F_{3,34} = -0.31$, $P = 0.758$). Overall, in early gestation, fecundity per se did not influence significantly oxygen consumption of reproductive individuals (Fig. 3, *top*). For this analysis, the sample size was reduced, and the three ambient temperatures used (17.5°, 25°, and 32.5°C) created discontinuous data between groups of females, making the use of body temperature as an independent variable suspect. For this reason,

Table 3: Comparison of the current mass-specific $\dot{V}O_2$ (mL g^1 h^{-1}) data against data previously collected in ecologically similar species and in ecologically different species tested at three imposed temperature regimes (Secor and Nagy 1994)

		Temperature Category		
Species	Body Mass (g)	Cold (17.5°C)	Medium (25.0°C)	Hot (32.5°C)
Vipera aspis	85	.018	.031	.05
Masticophis flagellum	125	.012	.028	.06
Crotalus cerastes	124	.009	.020	.04

Note. *Vipera aspis* and *Crotalus cerastes* are similar sit-and-wait predators (capital breeders), while *Masticophis flagellum* is a typical active forager (income breeder).

we adjusted the oxygen consumption to the mean value obtained at 32.5°C by multiplying the values obtained under low and medium temperature by 2.8 and 1.6, the factor by which oxygen consumption increased as temperature increased, respectively. Even after adjusting oxygen consumption, the result remained unchanged ($r^2 = 0.18$, $F_{2,35} = 3.90$, $P < 0.03$; specific effect of fecundity, $F_{2,35} = 1.32$, $P = 0.195$).

However, in late gestation, the data show a positive and significant relationship between oxygen consumption and fecundity, measured as the number of offspring produced at parturition (Fig. 3, *bottom*). A partial correlation analysis was performed to take into account the effect of body mass on oxygen consumption. For this analysis the sample size was further reduced ($N = 19$ females), and only two ambient temperatures were used (17.5° and 32.5°C); thus, we used the temperature-adjusted oxygen consumption ($r^2 = 0.38$, $F_{2,16} = 4.96$, $P = 0.02$; specific of fecundity, $F_{2,16} = 2.97$, $P = 0.008$), and despite a small sample size, the power ($1 - \beta$) of this analysis was relatively high (0.83). A similar relationship was observed regressing the mass of live young produced against temperature adjusted $\dot{V}O_2$ ($r^2 = 0.38$, $P = 0.029$, $N = 19$, power = 0.83). Interestingly, we found that postparturient body mass of the females had no influence on the oxygen consumption measured before parturition ($r^2 = 0.004$, $N = 19$, $P = 0.80$), suggesting that the contribution of the fatigued maternal organisms in the total variance was minor in comparison to the contribution of the offspring they carry.

Field Body Temperatures

We found a strong effect of the sampling date on the mean body temperature selected by snakes in the field (ANOVA, $F_{89,1806} = 7.44$, $P < 0.0001$). As expected, the mean body temperature of the snakes increased from midspring to midsummer (correlation between mean body temperature of the females and date: $r^2 = 0.16$, $F_{1,88} = 17.23$, $P < 0.0001$), with important daily fluctuations as expected under temperate-oceanic climate (see Fig. 4).

Field data showed that in the course of the reproductive period, reproductive females maintain higher body temperature (25.01 ± 0.26°C, $N = 989$) than nonreproductive females (22.03 ± 0.27°C, $N = 907$; ANCOVA with reproductive status as the factor, temperature records as the dependent variable, and date as the covariate: reproductive status, $F_{1,1893} = 44.05$, $P < 0.0001$; date, $F_{1,1893} = 59.86$, $P < 0.0001$; Levene's test for homogeneity of the variance: $F_{1,851} = 1.01$, $P = 0.31$ and $F_{1,851} = 0.93$, $P = 0.34$ for body temperature and date, respectively). Such a difference was more pronounced during gestation where the body temperature of reproductive snakes was approximately 3.79°C higher (3.27°C for date-adjusted means), with the mean body temperature of reproductive and nonreproductive females being 26.39 ± 0.29°C (± SD, $N = 605$) and 22.60 ± 0.37°C (± SD, $N = 438$), respectively (same design ANCOVA: reproductive status, $F_{1,1040} = 47.10$, $P < 0.0001$; date, $F_{1,1040} = 15.75$, $P < 0.0001$, Fig. 4). Despite the time elapsed between two consecutive temperature records, each individual was represented more than once, and this may lead to spurious pseudoreplication effects. We checked for the possibility that pseudoreplication generated the observed difference in body temperature between the two classes. We used the mean body

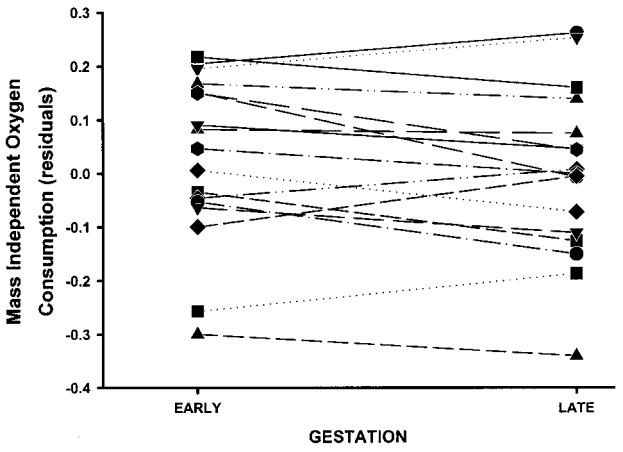

Figure 2. Effect of early (shortly after ovulation) versus late (shortly before parturition) gestation on oxygen consumption in 17 pregnant female vipers. Each female was plotted two times (early and late gestation) with a line connecting the two values.

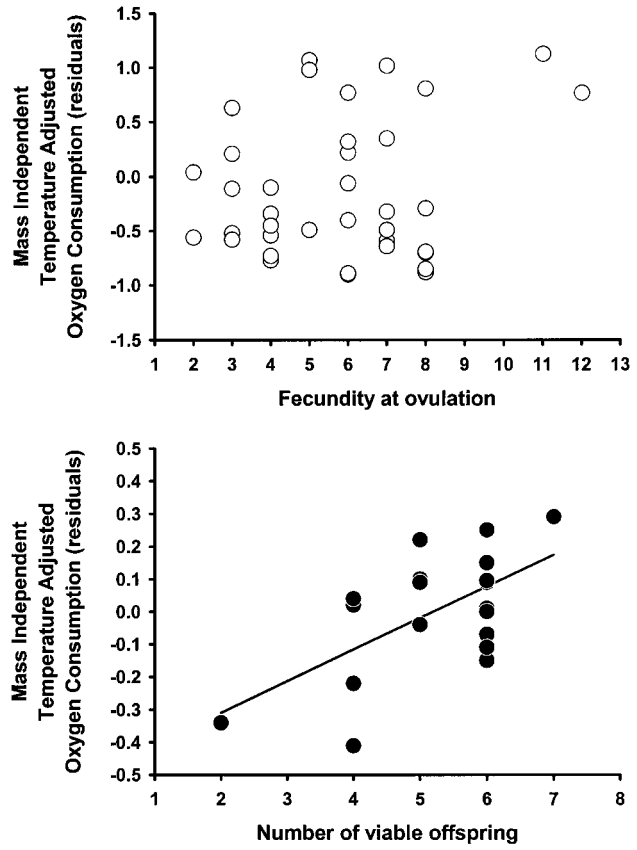

Figure 3. Relationship between oxygen consumption (residuals from the regression of log BM against log $\dot{V}O_2$, adjusted to an ambient temperature of 32.5°C) and fecundity of reproductive female vipers during early gestation (*top*; $N = 38$, $Y = 0.02x - 0.413$, $P = 0.227$), and the number of viable neonates during late gestation (*bottom*; $N = 19$, $Y = 0.610x - 0.501$, $P < 0.006$). Fecundity at ovulation was determined by palpation and was measured as the number of viable neonates or fully developed stillborn young at parturition. A number of ovulated follicles do not develop and lead to unfertile eggs (where only yolk is identifiable), explaining the difference between the ranges of values between the two *X*-axes (Bonnet et al. 2000).

temperature calculated over the reproductive period for each female (generating independent data but weakening the sensitivity of the analysis) and performed an ANOVA with reproductive status as the factor and mean body temperature as the dependent variable. The above results were unchanged (ANOVA, $F_{1,19} = 7.06$, $P = 0.015$), with mean body temperature of reproductive and nonreproductive females being 24.54 ± 1.23°C (± SD, $N = 11$) and 19.80 ± 1.29°C (± SD, $N = 10$), respectively.

A regression of mean body temperature against fecundity also shows that the mean body temperature of reproductive females is not influenced by fecundity ($r^2 = 0.0002$, $P = 0.97$, $N = 10$ [the litter size of one reproductive female was not estimated]; Fig. 5). Since the sample size for this analysis was very small and the analysis crude, great caution is required to interpret this pattern. However, the required sample size ($N > 100{,}000$) to attain conventional significance with low α and β errors (<0.05 and 0.10) suggests that the influence of fecundity on selected body temperature, if any, was probably weak.

Discussion

The data from this investigation suggest that, throughout the reproductive period, metabolism in both reproductive and nonreproductive snakes was strongly affected by both temperature and body mass. An important body of published data has already reported these effects in ectotherms for temperature (Bennett and Dawson 1976; Naulleau et al. 1984; Andrews and Pough 1985; Loumbourdis and Hailey 1985; Bradshaw et al. 1991; Zari 1991; Thompson and Withers 1992) and body mass (Bennett and Dawson 1976; Dmi'el 1986; Zari 1991; Thompson and Withers 1992; Beaupré and Zaidan III 2001). The expected difference in the metabolic rate between reproductive and nonreproductive females, however, was not apparent in our data when the two groups of snakes were placed under the same temperature regime. Furthermore, in reproductive females, metabolism was largely independent of fecundity. With regard to these latter outcomes, our results differ from previous investigations in this field (Guillette 1982; Birchard et al. 1984; Beaupré and Duvall 1998; Kunkele 2000).

Why do our data conflict with other investigations and fail to support some of our initial hypotheses? First, it is necessary to consider the methodology. For example, the period over which oxygen consumption was measured was different between the current and previous studies. In the live-bearing lizard *Sceloporus aeneus* and the garter snake *Thamnophis sirtalis sirtalis*, comparisons were made with reproductive females less than 2 wk before parturition (Guillette 1982; Birchard et al. 1984). Beaupré and Duvall (1998) focused on vitellogenesis, and they showed that reproductive female western diamondback rattlesnakes *Crotalus atrox* consume 1.4 times the amount of oxygen as nonreproductive females; however, it is not made clear exactly when during vitellogenesis (a prolonged period in snakes; 2–4 mo, or more in viperids) the data were collected. Because the metabolic process from the beginning of vitellogenesis to the end of pregnancy would be very dynamic, it is difficult to compare directly these data and previous investigations. In addition, the few snake species studied belong to very different lineages (colubrids vs. viperids, for instance). It may also be that our methodology lacked the capacity to detect subtle differences that may affect the outcome. Important interindividual differences—such as sensitivity to manipulation, hormonal levels related to stress, and state of metabolically active tissue such as the intestine epithelium—would certainly increase the variation in our data set.

Nevertheless, the consistency between published and current

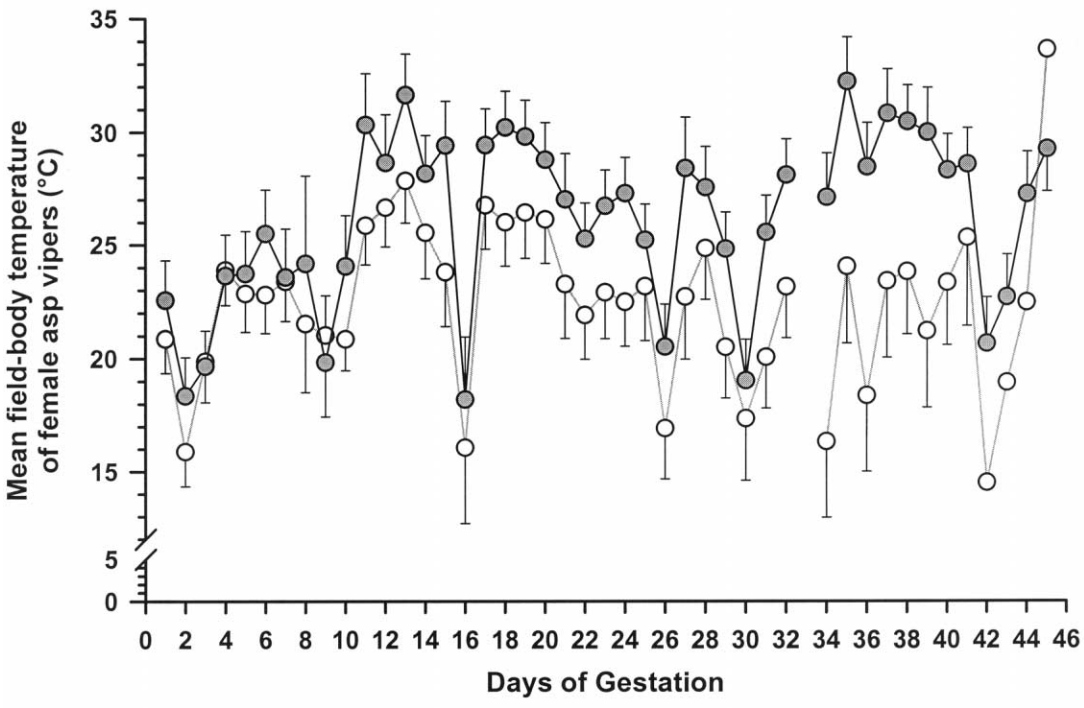

Figure 4. During gestation, reproductive females (*filled circles*) maintain higher body temperatures than nonreproductive females (*open circles*). Each circle represents the mean body temperature (±SE) calculated for several females ($N = 11.85 \pm 5.29$ records on average, range 2–25). Despite marked daily fluctuations most likely due to environmental variations (i.e., passage of very cold fronts in late June, days 26 and 30), the mean body temperature of reproductive females was often several degrees higher compared with nonreproductive females. Body temperature was taken at the point of capture irrespective of behavioural status.

data suggests that the system employed during this investigation leads to reasonable and interpretable results (Table 3), and important effects such as those linked to body mass or body temperature were clearly visible in our data. For instance, our Q_{10} of 2.05 is similar to the typical Q_{10} of between 2 and 3 reported for most reptiles (Bennett and Dawson 1976; Andrews and Pough 1985; Thompson and Withers 1992). The experimental process undertaken, augmented by long-term monitoring of a large number of female Aspic vipers in the field, enables us to propose a number of falsifiable hypotheses to explain our unexpected results. A simple approach is to follow the chronology of reproduction in female viviparous snakes from vitellogenesis to the end of gestation.

Vitellogenesis

Clear differences in the metabolic effort between reproductive and nonreproductive females during vitellogenesis may be expected in capital breeders, such as female rattlesnakes or vipers. In capital breeders, nonreproductive females accumulate energy, sometimes over very prolonged periods (years in vertebrates; Bull and Shine 1979), until a sufficient store (the capital) has been constituted, following which vitellogenesis can be engaged (Drent and Daan 1980; Stearns 1992; Naulleau and Bonnet 1996; Bonnet et al. 1998). During vitellogenesis, there is an extensive mobilisation of maternal body reserves to produce a large number of offspring (Bull and Shine 1979). In snakes, the vitellogenic process is a very intensive physiological event (Bonnet et al. 1994) where the energetic expenditure of reproductive females increases substantially over that of nonreproductive females, as was demonstrated by Beaupré and Duvall (1998) in the viperid *C. atrox*. Although we have no data on the metabolic rate of Aspic vipers during vitellogenesis, indirect information supports the notion that this period corresponds to an increase of oxygen consumption for reproductive females over nonreproductive females. First, large amounts of body resources are mobilised to develop follicles, while nonvitellogenic females do not exhibit any sign of such a mobilisation at that time (Bonnet et al. 1994). Second, vitellogenic females move and forage intensively at that time, mostly to supplement the energy available for follicular development, which in turn improves their reproductive success; at the same time, nonvitellogenic females are more sedentary, probably to save energy and to minimise predation risk (Naulleau et al. 1996; Bonnet et al. 2001*a*, 2002). Third, vitellogenic females engage in energetically demanding acts of sexual behaviour (courtship, mating), while nonvitellogenic females do not (Naulleau et al. 1999;

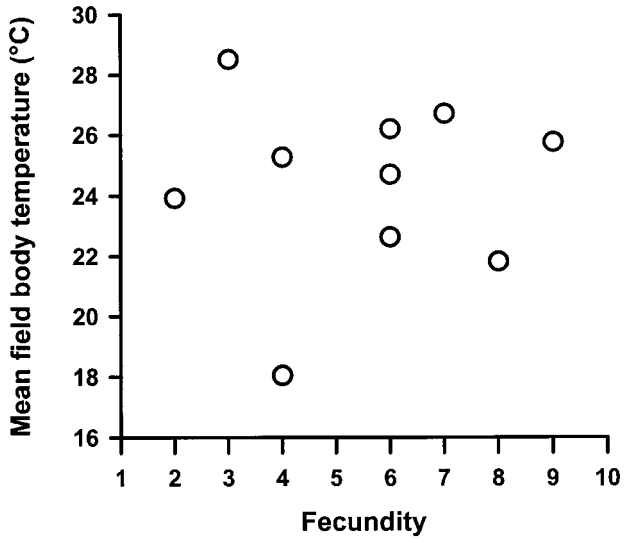

Figure 5. Relationship between the average body temperatures recorded for the reproductive females captured in the field and the fecundity of those females determined by palpation ($N = 10$).

Aubret et al. 2002). Fourth, vitellogenic females bask in the sun much more frequently than nonvitellogenic females (Bonnet and Naulleau 1996), with a concomitant increase in metabolism due to the fundamental effects of increased temperature on reaction rates (see "Results"). Overall, development of follicles during vitellogenesis is dependent on the increased activity of metabolically active tissues such as the liver, the ovaries, the intestine epithelium, and the locomotor muscles, while these tissues are relatively less active in nonvitellogenic females (Secor et al. 1994; Singh and Ramachandran 2000; O'Sullivan et al. 2001). The completion of vitellogenesis occurs in early June (Naulleau and Bidaut 1981), and this period corresponds to the beginning of summer in our study area.

Pregnancy

Pregnancy immediately follows vitellogenesis. At this time, follicular development and the mobilisation of maternal reserves are complete (Bonnet et al. 1994, 2001*a*), and the extra metabolic cost of being pregnant in Aspic vipers may only be a small effort associated with the maintenance of follicles. Our data support this notion, with the decrease in reproductive effort apparent in the lack of difference between the oxygen consumption of reproductive and nonreproductive females. However, during the early stages of pregnancy, a precise comparison of oxygen consumption between the two classes of female snakes is difficult due to the potentially confounding effect of the mass of the yolk follicles. Yolk is comprised mainly of fat (50%) and water, components likely to contribute little to organismal metabolic effort (Darken et al. 1998). In early pregnancy, developing embryos are very small (less than 5 mm in total length; X. Bonnet, unpublished data), and the yolk component is relatively large. This is a methodological difficulty that can only be resolved using techniques such as ultrasound (Beaupré and Duvall 1998) and nuclear magnetic imaging and proton spectroscopy (X. Bonnet, S. Akoka, and S. Villevieille, unpublished data) to measure the exact volume and chemical composition (i.e., water versus lipids using NMRI) of the follicles, from which mass and energy can be calculated in a nondestructive manner.

Field data further support the idea that early gestation is not metabolically demanding. Gravid females in early gestation are often already emaciated at this stage (i.e., fat bodies represent less than 3% of the mass of the postovulatory females; Bonnet et al. 2003). In this situation, any substantial increases in metabolism would lead to a rapid depletion of these reserves before parturition and the possible failure to successfully produce viable offspring. Pregnant females could forage at this time to compensate for their low energy stores. However, field data show that this is not often the case. Instead, they sharply reduce both locomotor and hunting activities during the 2–3 mo of pregnancy, seldom capturing prey (Naulleau et al. 1996; Lourdais et al. 2002*a*). The limited body reserves of pregnant females, sometimes supplemented by energy from prey, provide sufficient energy to sustain the metabolism of the females until parturition (Lourdais et al. 2002*a*). These experimental, field, and physiological (comparative metabolic activity of muscles, liver, or intestinal epithelium between vitellogenesis and gestation; Bonnet et al. 2003) data suggest that early pregnancy is not a costly metabolic phenomenon per se. As a consequence, the absence of a difference in mass-independent $\dot{V}O_2$ between pregnant and nonreproductive female vipers in early gestation is not surprising when temperature is controlled by the experimenters.

In late gestation, embryonic development was mostly complete, and each foetus could potentially contribute to the overall metabolism of the reproductive female. However, the current investigation suggests that reproductive females maintain a similar rate of mass-independent $\dot{V}O_2$ throughout pregnancy. Such a result is partly logical because $\dot{V}O_2$ was systematically scaled by maternal mass, which includes the mass of the embryo and extra embryonic fluids (the total water content of embryo and fluids being close to 80%; X. Bonnet and J. P. Robin, unpublished data) in late gestation. But this is not entirely satisfactory since we may expect a slight increase in the mass-independent oxygen consumption of the females in the course of pregnancy: first, because the overall body composition (mother and embryos) now includes a larger proportion of active tissues (muscles of the embryos, etc., vs. yolk) at the end of gestation. Second, the oxygen consumption by developing embryos is not the only factor of pregnancy that will affect metabolism. Other energetic components, such as supplying oxygen to the foetuses and handling foetal nitrogenous waste, are also paid by the

mother (Clark and Sisken 1956). Birchard et al. (1984) suggest that the contribution of foetal oxygen consumption to the overall oxygen consumption of the mother is the factor responsible for the observed difference in metabolic rate of pregnant versus nonpregnant garter snakes. Perhaps interindividual variance rendered our data too noisy to detect such a difference that is likely to be subtle anyway. In late gestation, there was a significant positive relationship between fecundity and oxygen consumption, though the relationship was weak, indicating that our measurements enabled us to detect the fact that the intrauterine embryos were metabolically active. An alternative explanation may be that any detectable difference generated by the embryos may have been masked by a decrease in maternal metabolism at the end of gestation due to the fatigued body of the reproductive females in the later stages of pregnancy (Bonnet et al. 2003). In fact, there was a cross phenomenon: embryos increased in size while the mother lost body reserves (muscles, digestive tracts, etc. [based on dissection]; Bonnet and Naulleau 1995; Bonnet et al. 2003). Because the relative contributions of the embryos increase in an overall constant viper mass, it is not surprising to detect a positive relationship between fecundity and O_2 consumption shortly before parturition, revealing a state-dependent effect.

Field versus Laboratory Data

Overall, when ambient temperature was imposed in the laboratory, reproductive status and fecundity did not influence, or only weakly influenced, maternal metabolism. However, field body temperatures show that a commitment to reproduction means a dramatic shift in behavioural thermoregulation, with pregnant female vipers maintaining, on average, a 4°C higher body temperature than nonreproductive females. This result is consistent with the observation that, in the field, pregnant females bask more often than nonreproductive females (Bonnet and Naulleau 1996). Given the fact that reproduction (at least vitellogenesis) is an energetically expensive process and considering the difficulties of reproductive females to sustain metabolism at a higher rate than nonreproductive females independently from a shift in thermoregulatory behaviour (since they are ectothermic), it seems intuitive that the maintenance of higher preferred body temperature over prolonged time periods accelerates metabolism to the rate where development of young is completed over an appropriate time scale. Embryos are sensitive to temperature, both in terms of extremes and variations (Burger 1998; Rhen and Lang 1999), and development is usually optimised by precise temperatures (Shine and Harlow 1996; Downes and Shine 1999; Elphick and Shine 1999; Shine 1999; Shine and Downes 1999; Andrews et al. 2000). It is very likely that the optimal temperatures for follicular growth and for embryonic development are the same whatever the number of offspring. This may explain the absence of any relationship in the current investigation between mean field body temperature measured in pregnant females and fecundity.

Conclusion

These results suggest that, in female Aspic vipers, the change of reproductive status corresponds to an all-or-nothing system in terms of energetic metabolism. Vitellogenesis and pregnancy impose a discrete modification in the day-to-day life of the females rather than progressive adjustments to the size of the litter (from one to 13 offspring in our study zone). Capture-recapture data have shown that survival is independent of fecundity in our population (Bonnet et al. 2000*a*). We hypothesise that increased thermoregulatory behaviour linked to vitellogenesis and gestation substantially increase maternal metabolism, provoke a strong emaciation, and result in increased exposure of the females to avian predation. As a result, the majority of the females will not survive a single reproductive event whatever their current fecundity (Bonnet et al. 2002, 2003). Our field and laboratory data support the notion proposed by Bull and Shine (1979) that, in systems where the costs of reproduction independent of fecundity are significant, the emergence of reproductive strategies based on a low breeding frequency should be favoured (which is the case for the many viperid snakes; Saint Girons 1957; Brown 1991; Martin 1993; Bonnet and Naulleau 1996). The notion that costs of reproduction may be partly disconnected from fecundity provides immense potential for better understanding reproductive strategies such as capital breeding and/or semelparity (Bull and Shine 1979; Bonnet et al. 1998; Olsson et al. 2000). Although incomplete, our data clearly show that the true value of understanding the relationships between fecundity, the energetics of reproduction, and reproductive effort lies not in a simple comparison with nonreproductive conspecifics tested under laboratory conditions but in the synthesis of laboratory data and field observation.

Acknowledgments

Thanks to the Centre National de la Recherche Scientifique, the French Embassy in Canberra (A. Moulet, A. Littardi, and J. Mordeck), and the faculty of science and the Zoology department (University of Western Australia) for the financial support that made this project possible. Laurence Pastout taught the use of the metabolic chamber, Guy Merlet solved several technical problems, and Christian Thiburce cared for the animals between measurements. Special thanks for the rigorous review of the manuscript provided by two anonymous referees.

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