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Relative reproductive effort drives metabolic changes and maternal emaciation during pregnancy in a viviparous snake

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Abstract

Most viviparous squamates are lecithotrophic, and maternal effort during pregnancy mainly involves behavioural and thermoregulatory shifts to optimize developmental conditions. Still, pregnancy also imposes specific metabolic demands on the female, known as the metabolic cost of pregnancy (MCP). Contrary to the thermoregulatory shift, these energy constraints should be directly fecundity dependent and their evaluation is important to assess the 'costs' of viviparity. We measured the metabolic rate of aspic vipers *Vipera aspis* at three stages (early pregnancy, late pregnancy and post parturition), and we examined the determinants of maternal metabolic changes over time. We found a 55% metabolic increase over the course of pregnancy that was better explained by maternal relative reproductive effort (relative litter mass) when compared with absolute estimates (litter mass, litter size). After parturition, female metabolism dropped below values recorded at early pregnancy and this decrease was closely related to maternal relative reproductive effort. Our estimates for MCP ranged from 13.9 to 14.7% of maternal metabolic rate, suggesting that specific energetic demands of pregnancy are significant. It appears crucial to consider both direct (MCP) and indirect (thermoregulatory shift) components to evaluate overall maternal metabolic demand during pregnancy. Because females are already emaciated at the onset of pregnancy, these combined constraints are likely costly by inducing structural protein mobilization and altered performances after parturition.

Introduction

Reproduction imposes multiple demands on females that have to allocate a substantial amount of energy and time towards offspring production. Because resources are often limited, optimality models predict that females will adjust their allocation in current reproduction to optimize lifetime reproductive success (i.e. life-history traits; reviewed in Congdon, Dunham & Tinkle, 1982; Charnov, Warne & Moses, 2007). One major aspect in addressing reproductive constraints is the influence of fecundity. For example, mothers will have to allocate nutrients to support offspring development, and a fecundity increase will directly affect maternal effort. Alternatively, reproduction can involve substantial behavioural shifts in activities that are fecundity independent. This is typically the case for accessory activities such as egg guarding, breeding migration or maternal thermoregulation (Adamczewska & Morris, 2001; Steinhart *et al*., 2005; Shine, 2012; Lorioux, Lisse & Lourdais, 2013*a*; Lorioux *et al*., 2013*b*). The fecundity dependence of maternal allocation and associated costs is likely a major determinant of optimal reproductive effort and, more generally, reproductive strategies (Bull & Shine, 1979; Bleu *et al*., 2011).

Viviparity has emerged on repeated occasions among vertebrate lineages including fishes (Dulvy & Reynolds, 1997), amphibians (Blackburn, 1999), reptiles (Tinkle & Gibbons, 1977; Blackburn, 1985; Shine, 1985) and mammals (Zeller, 1999). Within squamates, transition to viviparity appeared more than 100 independent times (Blackburn, 2000). Most squamates are lecithotrophic, and embryonic energy demands are supported by important yolk reserves (Stewart & Thompson, 2000). Maternal effort during pregnancy is therefore primarily linked to thermoregulation (Tinkle & Gibbons, 1977; Shine & Harlow, 1993; Shine, 1995; Bernardo, 1996). That is, pregnant females typically display prolonged basking behaviours, modified thermal preference and more stable body temperatures (Shine, 1980, 2006, 2012; Charland & Gregory, 1990; Lorioux *et al*., 2013*a*). Maternal thermoregulation is beneficial by buffering the impact of environmental fluctuations (Shine, 1985; Stewart & Thompson, 1993; Qualls & Andrews, 1999; Crespi & Semeniuk, 2004; Lorioux *et al*., 2013*b*) on developmental rate (Tinkle & Gibbons, 1977; Shine

& Bull, 1979; Shine, 1983) and offspring quality (Shine, 1995, 2004; Lorioux *et al*., 2013*b*).

Viviparity also imposes substantial constraints on the female. For example, the physical burden imposed by developing embryos will alter female locomotor performance and will increase vulnerability to predators (Shine, 1988; Le Galliard, Le Bris & Clobert, 2003; Schwarzkopf & Andrews, 2012). Moreover, basking behaviour will profoundly affect maternal activity budget and will sometimes translate in reduced or absent food intake and compromised energy balance (Lourdais *et al*., 2004; Lourdais, Lorioux & DeNardo, 2013). Modified thermal preferences will affect female metabolic rate especially in species that shift to higher thermal regimes (Ladyman *et al*., 2003; Lorioux *et al*., 2013*a*). Pregnancy also imposes specific metabolic demand on the female, called metabolic cost of pregnancy (MCP; Birchard *et al*., 1984). Indeed, even in lecithotrophic species, pregnant females have to cope with embryonic oxygen demand and also the processing of embryonic waste (Van Dyke & Beaupre, 2011). For example, pregnant garter snakes display increased heart rate and modified blood parameters to facilitate oxygen delivery to the embryos (Birchard *et al*., 1984). Although embryonic metabolic requirements are minimal at ovulation, they increase progressively along with exponential somatic growth and are likely to affect maternal demand. MCP has already been documented in various viviparous squamates species (Birchard *et al*., 1984; Beuchat & Vleck, 1990; DeMarco & Guillette, 1992; Robert & Thompson, 2000; Schultz, Webb & Christian, 2008; Van Dyke & Beaupre, 2011; Yue *et al*., 2012).

Clarifying the relative costs of pregnancy and the proximate determinants of metabolic changes is important to better understand the energetic implication of viviparity (Bleu *et al*., 2011). Metabolic rate at late pregnancy will result from both maternal contribution (maintenance requirements and MCP) and embryonic metabolism that dramatically increases during somatic growth and foetal life (Robert & Thompson, 2000; Andrews, 2004). Importantly, substantial variations in reproductive output (i.e. litter size) exist within snakes (Bonnet *et al*., 2003), which can reflect either variation in maternal body size or variation in maternal reproductive allocation (relative to maternal size or mass) (Bonnet *et al*., 2003). Maternal relative reproductive effort is likely a relevant parameter that should help us to understand metabolic demand and the specific costs of pregnancy. If MCP is significant, we predict that maternal relative reproductive effort should be a better descriptor of metabolic changes when compared with absolute reproductive output (embryonic contribution). Yet, this parameter remains largely understudied.

In this study, we measured metabolic rate over pregnancy in a viperid snake, the aspic viper *Vipera aspis*. We considered three relevant periods: early pregnancy, late pregnancy and post parturition. We compared different measures of reproductive effort on (1) metabolic increase during pregnancy; (2) metabolic decrease associated with parturition; (3) maternal emaciation. We predicted that maternal relative reproductive effort would be a better predictor of metabolic changes and of female emaciation than absolute output (litter mass, litter size). Finally, we estimated MCP using a method previously described (Birchard *et al*., 1984; Schultz *et al*., 2008).

Materials and methods

Study species

We studied the aspic viper *V. aspis*, a medium-sized venomous snake of the Western Palearctic region (Naulleau, 1997). The aspic viper is known as a typical capital breeder, which means that females store important fat reserve before reproduction and often cease to feed during pregnancy (Bonnet, Bradshaw & Shine, 1998; Bonnet *et al*., 1999; Bonnet, Naulleau & Lourdais, 2002*a*; Bonnet *et al*., 2002*b*; Lourdais, Bonnet & Doughty, 2002*a*). Pregnancy begins after ovulation in early June (Naulleau, 1981) and parturition occurs 2–3 months later from late August to early September (Lourdais *et al*., 2002*a*). Because of high energetic demand of reproduction, females are extremely emaciated after parturition (Bonnet *et al*., 1999, 2002*b*).

Capture and maintenance

In late May to early June 2010, we caught 16 reproductive females [mean ± standard error (SE); body mass (BM): = 84.7 ± 4.4 g; snout-vent length (SVL): = 44.9 ± 0.9 cm] from several neighbouring population in Western-Central France (Vendée, Loire-Atlantique and Maine et Loire District). Reproductive status was first determined with manual palpation of the abdomen and then checked in the laboratory with high-resolution ultrasonography (SonoSite microMaxx, Inc., Bothell, WA, USA). All snakes were weighed (±1 g), measured (±0.5 cm) and individually housed in plastic boxes (30 × 20 × 10 cm). In this species, a skin-shedding episode occurs at the onset of pregnancy (early June) close to ovulation time (see Lorioux *et al*., 2013*b*). For each female, we recorded skin-shedding date as an index of the onset of pregnancy. Females were maintained in climatic chambers (Vötsch, Industrietechnik, VP 600, Balinger, Germany), which allowed us to recreate natural daily temperature cycle (16–33°C). Water was provided *ad libitum*. Because females often cease feeding during pregnancy, individuals were fasted during the experimentation (Lourdais *et al*., 2002*a*) to avoid possible bias caused by food intake (Andrade, Cruz-Neto & Abe, 1997).

At the end of pregnancy, cages were inspected daily to detect parturition. The different components of the litters (neonates, stillborns and undeveloped ova) were counted and weighed (±0.1 g). Offspring and stillborns were also measured (±0.1 cm) and sexed. These measurements allowed us to consider different estimates of reproductive output: litter size (i.e. number of all litter components), fit litter size (i.e. number of viable offspring), litter mass (i.e. mass of all litter components) and fit litter mass (i.e. mass of viable offspring); see details on methods in Lourdais *et al*. (2002*a*). Offspring were housed individually in small plastic containers (10.5 × 30.0 × 16.5 cm)

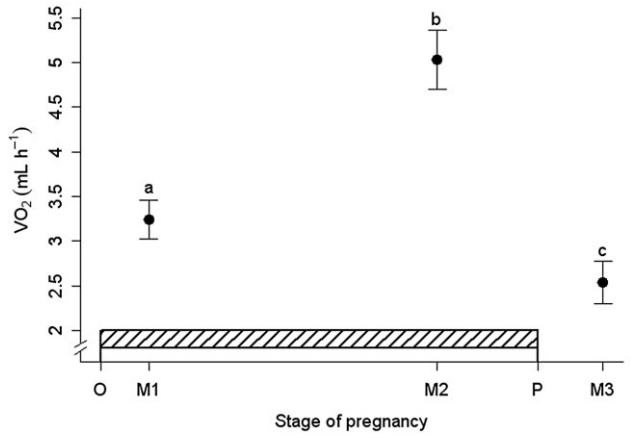

Figure 1 Oxygen consumption (VO_2) of female *Vipera aspis* ($n = 16$) measured at different stages of pregnancy (M1: early stage, 11% of pregnancy duration; M2: late stage, 77% of pregnancy duration; M3: post parturition, 115% of pregnancy duration). O and P designate Ovulation and Parturition (i.e. 0 and 100%, respectively, of pregnancy duration). Each point represents the mean (± standard error) of VO_2 (mL h^{-1}). Different letters above symbols represent significantly different values.

containing a polyvinyl chloride shelter and water *ad libitum* for 1 month before being release with their mother at the exact site of capture.

Experimental design

Metabolic rate was assessed at three stages of pregnancy (early, late pregnancy and post parturition; see Fig. 1). Using shedding (i.e. ovulation) and parturition dates it was possible to estimate accurately at what stages measures were carried. First metabolic rate was measured shortly after ovulation (mean ± SE: 6.9 ± 0.9 days since ovulation) while 11% of pregnancy duration occurred. Then a second measure was carried in late pregnancy (mean ± SE: 51.2 ± 1.2 days since ovulation) when >90% of vitellus was absorbed by embryos (see details on the method in Lorioux *et al*., 2013*b*), while 77% of pregnancy duration occurred. A final measure was carried few days after parturition (mean ± SE days since parturition: 9.2 ± 1.0 days).

Oxygen consumption

We measured standard metabolic rate (SMR) of females using closed-system respirometry. All trials were carried at 25°C as this temperature has previously been used for metabolic measure on aspic viper (Ladyman *et al*., 2003). Snakes were placed into test chambers (2170 mL) in a climatic chamber (±1°C) (Mastercella Cryosystem, SAS ERCO, Niort, France). Snakes acclimatized for 3 h before each run to reach thermal equilibrium and reduce activity. During acclimation, air was continuously flush with a 55 L min^{-1} pump (Bioblock Scientific, AVANTEC, Illkirch, France). A baseline air sample was collected for each individual at the onset of the trial, and the test chamber was then carefully sealed. Average trial duration (mean ± SE) was 131.6 ± 2.7 min. A final sample of air was collected with two 140-mL syringes connected to a three-way stopcock. The oxygen (O_2) proportion was then determined via an O_2 analyser (FoxBox; Sable Systems, Las Vegas, NV, USA). Before entering the analyser, air was dried by passing through a column of self-indicating Drierite and sent with a constant flow (36 mL min^{-1}) controlled with an infusion pump (KDS210; KD Scientific, Inc., Holliston, MA, USA). The O_2 analyser was calibrated before each trial using outdoor air. Finally, O_2 consumption (VO_2 in mL h^{-1}) was obtained by subtracting final from baseline values considering the volume of test chamber (mL) and run duration (h).

Reproductive effort and maternal emaciation

Multiple methods have been described to assess reproductive effort in snakes (see Bonnet *et al*., 2003). Absolute measures of reproductive output (litter mass, litter size, or fit litter mass and fit litter size) are routinely used (Lourdais *et al*., 2002*a*). However, it is crucial to control for allometric influences (Bonnet *et al*., 2003). Therefore, we also considered the relative litter mass (RLM) derived from the ratio between litter mass and post parturition female BM (i.e. RLM, in %). We also used the residuals of the linear relationship between litter mass and maternal SVL (i.e. RLM, residuals; Bonnet *et al*., 2003) as this relation was significant ($F_{1,14} = 5.2$, $P = 0.039$).

We considered the body condition (BC) after parturition (i.e. post-partum BC) as an estimate of maternal emaciation (Bleu *et al*., 2011). Post-partum BC was obtained by extracting residuals from the relation between female BM after parturition against SVL (Jayne & Bennett, 1990).

MCP estimates

We used a previously described method to estimate MCP (Birchard *et al*., 1984). This method consists of plotting the difference between the VO_2 of pre-parturient females and the VO_2 of post-parturient females (i.e. ΔVO_2) against the litter size. The intercept of the regression that represents the VO_2 for a hypothetical pregnant female with no offspring should indicate the MCP. Here, we also estimated MCP by plotting ΔVO_2 against the RLM (%). The RLM (residuals) could not be used as the intercept does not represent a hypothetical absence of embryos.

Statistical analyses

All statistical analyses were performed with R software (R Development Core Team, 2011). First, we used linear mixed model (package nlme) to analyse metabolic change over pregnancy with pregnancy stage (i.e. early, late and post) as a factor and female identity as a random factor to control for repeated contributions. VO_2 was $\log_{10}$ transformed so the distribution of the residuals of the model conformed to the

Table 1 Model selection comparing the relation between different estimates of reproductive effort (litter size, fit litter size, litter mass, fit litter mass or relative litter mass) and metabolic increase (late–early pregnancy) or ΔVO_2 (late pregnancy–post parturition)

	Metabolic increase		ΔVO_2		MCP	
	AIC	*P*	AIC	*P*	Value	*P*
Litter size	60.0	0.947	62.5	0.827	2.24 (44.6%)	0.062
Fit litter size	50.1	0.004	55.9	0.023	0.67 (13.3%)	0.109
Litter mass (g)	52.8	0.013	53.8	0.007	0.74 (14.7%)	0.044
Fit litter mass (g)	49.6	0.003	48.4	0.001	0.70 (13.9%)	0.016
RLM (%)	**40.3**	<0.001	48.9	0.001	0.71 (14.1%)	0.016
RLM (residuals)	43.6	<0.001	**45.4**	<0.001	–	–

Metabolic cost of pregnancy (MCP; mL h^{-1}) was estimated from the intercept of the relation of ΔVO_2 against fecundity or relative litter mass (RLM) and its statistical difference from zero. Boldfaced numbers correspond to the smallest values of AIC. Values between parentheses correspond to the MCP expressed as a proportion of late pregnancy metabolic rate. AIC, Akaike's information criterion. See text for details.

assumptions of normality (Shapiro–Wilk test, $P = 0.342$). We did not adjust VO_2 with BM of females because pregnancy is associated with dramatic variation in BM ($F_{2,30} = 247.8$, $P < 0.0001$). VO_2 was not adjusted to female body size as the relation was not significant at the different stages considered ($F_{1,30} = 2.6$, $P = 0.128$). We used pairwise *post hoc* tests of Tukey (package multcomp) to compare the three stages of pregnancy.

Second, we compared the influence of different measures of reproductive effort (i.e. litter size, fit litter size, litter mass, fit litter mass and the two estimates of RLM) on metabolic changes. We considered separately metabolic increase over pregnancy (late–early pregnancy) and metabolic drop associated with parturition (late–post; ΔVO_2). We considered the model with the smallest Akaike's information criterion (AIC) to determine the best determinant of maternal metabolic changes. When two models differed by less than two AIC, they were considered equivalent.

We used non-linear models to compare the influence of the different determinants of reproductive effort on the ΔVO_2 and to assess the associated values of MCP. A previous investigation on the dataset revealed that the best fitting curve between ΔVO_2 and the different determinants of reproductive effort followed an exponential relation. We used linear models to test the influence of the metabolic increase and RLM (residuals) on female post-partum BC.

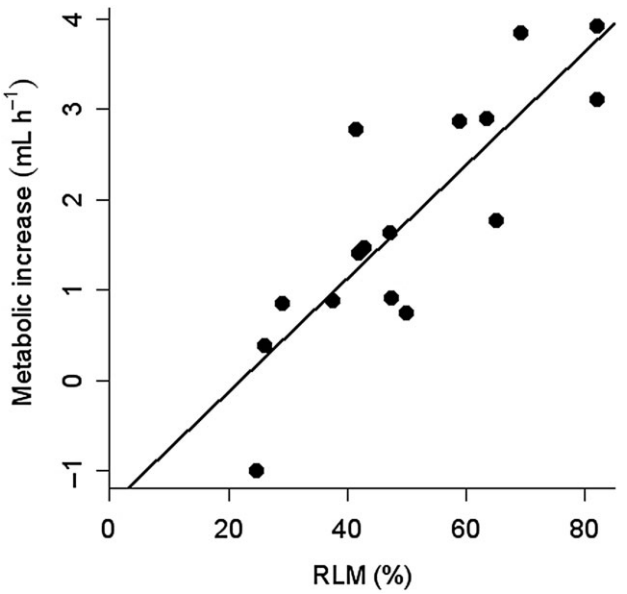

Figure 2 Influence of relative litter mass (RLM; %) on the metabolic increase during pregnancy (mL h^{-1}). Metabolic increase was derived for each individuals ($n = 16$) from the difference in VO_2 between early and late stages. The line represents the fitted linear regression ($P < 0.001$, $r^2 = 0.71$).

Results

Metabolic changes over pregnancy

Oxygen consumption (VO_2) was not constant throughout pregnancy ($F_{2,30} = 28.7$, $P < 0.0001$; Fig. 1). First, a 55% increase in SMR was observed between early and late pregnancy measures (Tukey's test, $P < 0.001$; Fig. 1). Then, metabolic rate significantly dropped after parturition and differed from the two other stages (Tukey's test, all $P < 0.01$; Fig. 1).

Determinants of metabolic changes and maternal emaciation

The metabolic increase between early and late pregnancy was significantly explained by the different estimates of reproductive effort except litter size (Table 1; Fig. 2). Yet, model selection indicated that RLM (%) was the best descriptor (Table 1). Similarly, the drop in metabolic rate after parturition was significantly influenced by each estimate of reproductive effort except litter size (Table 1). Model selection indicated that RLM (residuals) was the best descriptor (Table 1).

Finally, female post-partum BC was significantly influenced by the RLM (residuals) ($F_{1,14} = 6.0$, $P = 0.028$) and by the metabolic increase over pregnancy ($F_{1,14} = 5.8$, $P = 0.030$; Fig. 3).

MCP estimate

MCP estimates were derived from the intercept and were significant when using litter mass, RLM (% and residuals), but

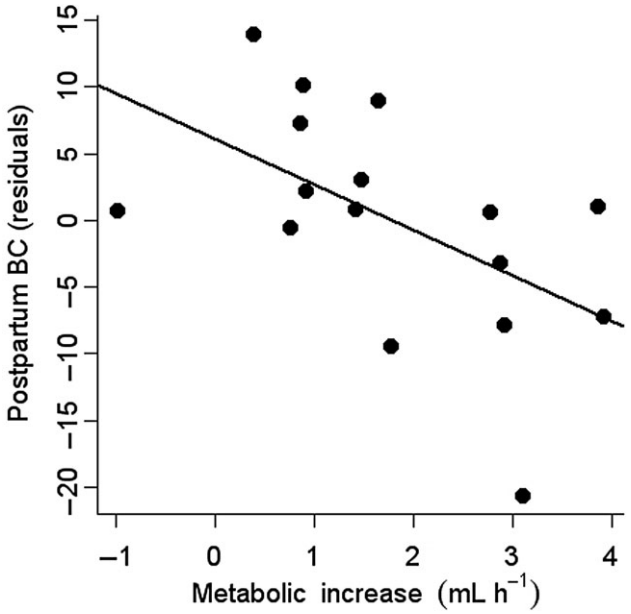

Figure 3 Relation between the metabolic increase during pregnancy and female post-partum body condition (BC; residuals). The line represents the fitted linear regression ($P = 0.030$, $r^2 = 0.29$).

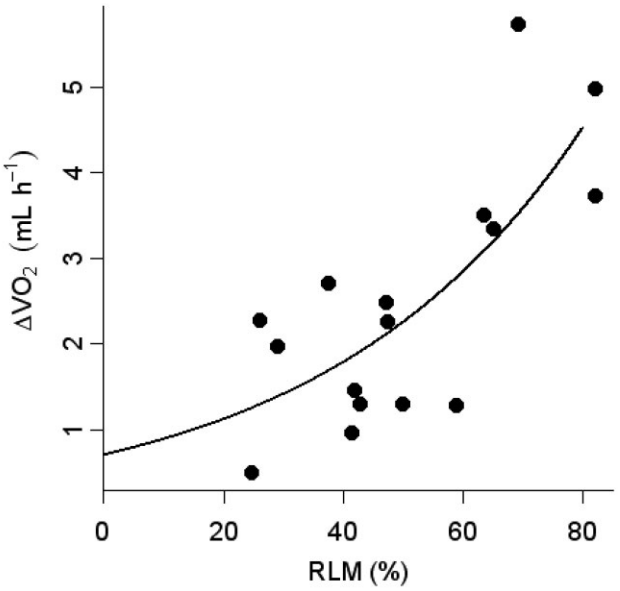

Figure 4 Relation between the relative litter mass (RLM; %) and metabolic drop (ΔVO_2) after parturition. The line represents the fitted curve from exponential regression ($y = 0.705\ e^{0.0232x}$). The metabolic cost of pregnancy corresponds to the intercept (0.705 ± 0.259 mL h^{-1}) of the regression.

not litter size as descriptive variables (Table 1; Fig. 4). Considering only significant contributions, we found close MCP values ranging from 0.70 to 0.74 mL h^{-1}, which represent 13.9 and 14.7%, respectively, of the maternal metabolic rate in late pregnancy (Table 1).

Discussion

Viviparity has emerged independently on a multitude occasions (>110 times) among squamates, and addressing associated 'costs' is critical to better understand this evolutionary transition. Our study, in a lecithotrophic species, highlights that litter mass influences maternal metabolic changes over pregnancy but relative reproductive effort is a better determinant than absolute measures of reproductive output. This component of gestational constraints may contribute to post reproductive costs (maternal emaciation) and thereby favour optimal allocation strategy at earlier stages (i.e. vitellogenesis).

We observed a 55% rise in the metabolic rate between early and late pregnancy. This increase was closely related to the different estimates of reproductive effort (except litter size), illustrating a proximate link between embryo development and maternal metabolic rate. Previous investigations on the same species did not reveal any metabolic change over pregnancy (Ladyman *et al*., 2003). This is possibly due to methodological bias and the fact that different individuals were compared over time. In this study, we used closed-system respirometry with repeated measures, which revealed a clear metabolic increase consistent with other studies (Birchard *et al*., 1984; Beuchat & Vleck, 1990; DeMarco & Guillette, 1992; Robert & Thompson, 2000; Schultz *et al*., 2008; Van Dyke & Beaupre, 2011; Yue *et al*., 2012). This pattern mainly reflects embryonic metabolism that will increase over time due to exponential somatic growth (Robert & Thompson, 2000; Andrews, 2004). Besides, specific maternal physiological adjustments occur such as an increased heart rate (Birchard *et al*., 1984) combined with modified red cell oxygen affinity (Holland *et al*., 1990; Ingermann, Berner & Ragsdale, 1991; Ragsdale & Ingermann, 1991). Such physiological adjustments likely aim at optimizing the transfer of oxygen and water to the embryos along with the excretion of waste (Van Dyke & Beaupre, 2011). Parturition was associated with a major metabolic drop, and the lower metabolic rate observed in post-partum females compared with ovulated ones could reflect female emaciation due to protein catabolism. Females may also already face an increased metabolism early in pregnancy although embryonic metabolic requirements shortly after ovulation are likely negligible.

Body size has a proximate influence on reproductive output (either litter size or mass) in snakes (Bonnet *et al*., 2003), but substantial variation also exists independently of body size reflecting different levels of reproductive allocation. In this context, we expect that a female with a high output relative to its size or mass (i.e. high relative reproductive effort) should face higher costs of supporting pregnancy when compared with a female that allocates in a lower relative reproductive effort. If so, metabolic changes over pregnancy should be more closely related to relative reproductive effort than absolute reproductive output. In turn, if MCP is small or

negligible, metabolic changes should predominantly reflect the embryonic demand, and measures of reproductive output (litter size or litter mass) should then provide better descriptors. Here, we specifically compared the influence of absolute and relative reproductive effort on metabolic changes. We found that metabolic increase over pregnancy and metabolic drop after parturition were closely related to the different estimates considered (see Table 1). Interestingly, the influence of litter size (including non-viable small-sized elements such as undeveloped ova) was not significant, whereas fit litter size and mass were closely related to metabolic changes suggesting that the number or mass of developing embryos (i.e. metabolically active tissues) is more relevant. Still, metabolic changes were best explained respectively by RLM (%) and size-adjusted litter mass (residuals) (Table 1) underlying that female relative reproductive effort will profoundly affect metabolic changes. Maternal emaciation after parturition is an important life-history parameter in viviparous squamates (Bonnet *et al*., 1999; Bleu *et al*., 2011; Baron *et al*., 2012) that influences female survival after reproduction and the time to recoup energy between breeding episodes (Bonnet *et al*., 1999; Lourdais *et al*., 2002*b*). We found that post-partum BC was closely related to metabolic increase over pregnancy as well as maternal relative reproductive effort. Together, these results suggest that relative reproductive effort drives maternal metabolic changes over pregnancy and affects maternal physiological state after reproduction.

We detected significant and close MCP estimates (13.9–14.7% of late pregnancy maternal metabolic rate) with the different measures of reproductive effort. MCP has already been measured in different viviparous squamate species (Robert & Thompson, 2000; Schultz *et al*., 2008; Yue *et al*., 2012), but values derived from other studies were non-significant or negative (Birchard *et al*., 1984; Beuchat & Vleck, 1990; DeMarco & Guillette, 1992; Van Dyke & Beaupre, 2011). It is not clear whether these discrepancies result from biological differences or inherent methodological biases (Van Dyke & Beaupre, 2011). Here again, model selection suggests that maternal relative reproductive effort provides a valuable descriptor. It is worth noting that direct metabolic demands (MCP) are not sufficient to assess the overall energetic impact of pregnancy. Modified thermoregulation also contributes to increase metabolic rate (Bennett & Dawson, 1976; Ladyman *et al*., 2003) notably in species that select higher body temperature during pregnancy such as the aspic viper (Ladyman *et al*., 2003; Lorioux *et al*., 2013*b*). It has been recently demonstrated that thermoregulatory effort is independent of fecundity, and all developing embryos benefit from maternal thermal regimes (Lorioux *et al*., 2013*a*). That is, females will maintain a temperature set point that is driven by the narrow embryonic thermal tolerance. In turn, direct metabolic demand of pregnancy will depend on fecundity and, as demonstrated here, on maternal relative reproductive effort.

Clarifying the metabolic impacts of pregnancy is a key component in improving our understanding of the 'cost' of viviparity (Bleu *et al*., 2011). Van Dyke & Beaupre (2011) suggested that MCP is relatively low when compared with metabolic costs of vitellogenesis. However, although metabolic measures during vitellogenesis and pregnancy estimate energetic requirements, they are poor currencies for actual 'costs' to the mother. Assessing actual costs of reproduction requires quantifying the impact on survival and/or future reproduction (Bonnet *et al*., 2000; Bleu *et al*., 2011). In this context, the metabolic demands of pregnancy (direct and indirect) occur while females are already extensively emaciated with only residual fat reserves and already mobilized muscle protein (Bonnet *et al*., 2002*b*). Pregnant females will have to catabolize critical muscle proteins to provide amino acid for gluconeogenesis (Lourdais *et al*., 2004, 2013). Besides, direct amino acid transfer to the embryos can also occur during pregnancy in lecithotrophic species (Van Dyke & Beaupre, 2012). Compromising structural proteins will translate in altered musculature and performance after parturition (Lourdais *et al*., 2004, 2013). The actual 'costs' induced by pregnancy are likely significant, as suggested by the significant relationship between metabolic changes and maternal emaciation. A recent study on the common lizard *Zootoca vivipara* demonstrated that an experimental litter size reduction during pregnancy was beneficial to the mother resulting in higher BC after parturition (Bleu *et al*., 2011) and such results provide support for high costs induced by pregnancy.

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