

Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*

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SUMMARY

Reproduction is energetically expensive for males as well as for females, but evolutionary biologists have typically regarded the energy costs of sperm production as trivial compared to the energy costs of overt reproductive behaviours, such as mate-searching, courtship, copulation and male–male combat. Adders (*Vipera berus*) offer an ideal model system in which to quantify the relative costs of spermatogenesis (and associated physiological preparations for reproductive activity) versus the costs of overt reproductive behaviours, because (i) these two activities occur sequentially rather than simultaneously, and are separated by a clear indicator (sloughing of the skin); and (ii) males do not feed during either of these phases. Hence, the rate of mass loss by males can be used as an index of energy costs. Surprisingly, the rate of mass loss is at least as high during the phase when males are immobile, building up sperm supplies, as when they move about and engage in reproductive behaviour. Rates of mass loss are not significantly correlated with male size or measures of the male's participation in reproductive activities. Our data suggest that sperm production may be a major energy cost to reproducing male adders, and that this species offers a useful system in which to further investigate this possibility.

1. INTRODUCTION

One of the basic tenets of evolutionary ecology is that 'sperm is cheap'; that is, the physiological costs of actually producing gametes are a trivially small component of the total costs (in energy and risk) of the reproductive activities of males. In contrast, gamete (ovum) production is a major component of total reproductive costs for females, and many differences in sexually selected traits between males and females have been attributed to this initial divergence in the magnitude of costs of gamete production (e.g. Bateman 1948; Trivers 1972; Charnov 1982). Increasingly, however, this simple maxim has been challenged by, for example, evidence that testes' size is only hypertrophied in species with intense sperm competition (e.g. Møller & Briskie 1995) and in populations with high food availability (Rose 1982). Thus, the physiological costs of producing sperm may be relatively high, especially in those species in which males must produce very large quantities (e.g. Birkhead & Møller 1992; Olsson & Madsen 1996). This possibility has strong implications for life history theory, because mathematical models depicting maximization of individual reproductive success predict that the optimal reproductive tactics are very sensitive to the form and magnitude of reproductive costs. For example, high 'fixed costs' (such as gamete production), uncorrelated with realized reproductive success, can favour the evolution of low reproductive frequencies (e.g. Bull & Shine 1979).

For most kinds of organisms, it will be difficult to quantify 'costs' of sperm production because of the

temporal overlap between sperm production and two other processes that affect the animal's energy stores: energy intake (feeding) and energy expenditure on other reproductive activities (e.g. mate-searching, mating, male–male combat). Because of this temporal confounding, overall energy budgets of reproducing males are complex, with variable rates of reproductive expenditure (on sperm and reproductive behaviours) coinciding with variable rates of energy replenishment through feeding. Ideally, then, we need a system in which reproductive activity is divided into two parts: where actual reproductive expenditure through behaviour occurs only after the completion of an initial phase during which the male does not move about or feed, but instead simply enlarges his testes and produces sperm. In such a system, the temporal separation of sperm production versus mating activities allows comparison of mass loss (energy cost) due to each of these activities. In this paper, we present data on a study organism that fulfils these conditions, and thus allows us to quantify different components of overall allocation of energy reserves to reproduction.

2. MATERIALS AND METHODS

Adders (*Vipera berus*) are small (to 65 cm snout–vent length in females, 55 cm in males) venomous snakes that are widely distributed through Europe and Asia (Arnold & Burton 1978). Their ecology and mating systems have been studied by several authors (e.g. Viitanen 1967; Nilson 1980, 1981; Nilson & Andrén 1982; Andrén 1982, 1985, 1986; Andrén & Nilson 1983; Luiselli 1992, 1993*a, b*, 1995), including detailed analysis of a small isolated population near

Smygehuk in extreme southern Sweden (Madsen 1988; Madsen & Stille 1988; Madsen & Shine 1992*a-d*, 1993, 1994, 1995; Madsen *et al.* 1992, 1993, 1996). After emerging from their overwinter dormancy in spring (late March–early April), adult male adders remain virtually sedentary for the next month. They spend most of this time basking whenever the weather is suitable, and do not feed during this period (Prestit 1971). The testes, which are small at emergence, rapidly increase in size and begin producing and maturing spermatozoa (Volsøe 1944; Nilson 1980). The end to this ‘basking’ phase (Viitanen 1967; Prestt 1971) is signalled by sloughing of the skin, at which time the males adopt much brighter colours (Prestit 1971). Immediately following this slough, males begin to move about actively in search of reproductive females, and engage in vigorous courtship and male–male combat. This second phase lasts for approximately three weeks, and males do not feed during this period (Viitanen 1967; Prestt 1971; Nilson 1980).

We have previously analysed costs of reproduction in male as well as female adders at Smygehuk (Madsen & Shine 1993, 1995). However, in that analysis we did not attempt to differentiate mass loss by males into components occurring before versus after the springtime slough. Instead, we made the tacit assumption that all mass loss by males was due to the metabolic costs of mate-searching, courtship and combat activities. More recently, in the course of reviewing sperm competition in reptiles, Olsson & Madsen (1996) concluded that costs of sperm production may not be a trivial component of overall male costs. We then realized that patterns of loss in body mass by the male adders of Smygehuk offer an ideal system in which to test this idea. Hence, the present paper looks in more detail at this issue, and separates out change in body mass in each of the two phases of the male cycle (pre- versus post-slough). We are able to do this because we have data for a subset of males on their body mass at the time they emerged from winter inactivity, at the time of sloughing, and at end of the mating period. Our criteria for these time periods were as follows:

(i) *Immediately post-emergence.* Each year, we visited Smygehuk on every day in early spring when the weather was warm enough for adders to emerge. Because the snakes move so little in the first few weeks after emergence, and individuals use the same specific basking sites year after year, we can be confident that we found males on the first day or two after their emergence from hibernation. Most adders at this time of year were still covered in mud, as further evidence of their recent emergence from underground.

(ii) *At the time of sloughing.* We use only data from adders that we caught and weighed less than 24 h after sloughing. Thus, all these snakes were recorded as being in an unshed condition either the same day as, or the day before, we weighed them post-sloughing.

(iii) *At the end of the mating season.* Mating activity is highly synchronized in this small population, and we monitored this activity intensively. We recaptured and reweighed all adult snakes as soon as mating activity came to an end.

Each male was represented only once in the data set and as there were no significant differences between years for any traits we pooled the data for all six years (ANOVA, year as class variable, $p > 0.45$ for all traits in table 1 and pre- and post-sloughing mass loss). We analyse these data below.

3. RESULTS

Figure 1 shows patterns in the rate of loss in body mass in the male adders of Smygehuk. The clear result is that mass loss occurs in both phases of the male reproductive cycle (i.e. before as well as after slough-

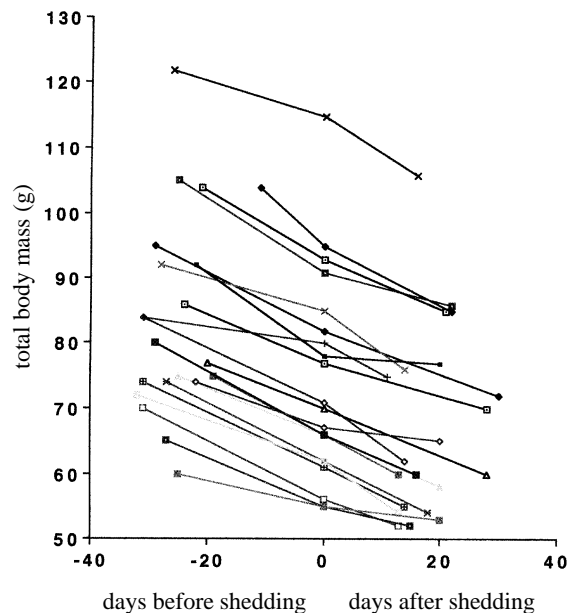


Figure 1. Reduction in body mass of adult male adders, *Vipera berus*, over two periods in springtime: from the time of their emergence from hibernation until they slough their skins; and then until the end of mating activity. Each line shows data for one male, and all shedding dates are standardized to time zero to facilitate comparison. The estimates of mass loss per day are similar in the two periods.

Table 1. Correlations between the rate at which a male adder lost body mass (mass loss per day, as a percentage of his initial [post-emergence] mass) and his body size and reproductive activities

(The table gives values for the Spearman rank correlation coefficient, with mass loss calculated over two periods: the basking phase prior to sloughing, and the subsequent mating period. See text for explanation. $n = 22$ males for all analyses; all p values > 0.05 .)

trait	Spearman rank correlation coefficient	
	pre-slough period	mating season
snout–vent length (cm)	−0.14	−0.16
number of matings	0.33	−0.34
number of combat bouts	0.20	−0.26
number of females visited	−0.06	−0.42
total distance moved	0.01	−0.22
mean distance moved per day	−0.13	−0.20

ing). There was in general little difference between the two periods in the rate of mass loss per day both in absolute terms (g day^{-1}) and in per cent of body weight (paired two-tailed t -test, $t_{21} = 0.25$, $p = 0.80$; $t_{21} = 0.74$, $p = 0.47$). However, contrary to our expectation that all males should lose mass more rapidly subsequent to the onset of mating activities, 13 of 22 males in fact decreased their rate of mass loss subsequent to skin shedding (figure 1). This result suggests that energy investment into reproduction was at least as high

during the pre-slough period (while males were sedentary) as in the post-slough period (when males were moving about and engaging in overt reproductive behaviours).

The rate of mass loss in each of these two phases was not significantly correlated with any of our measures of reproductive activity by the males in question (table 1). Our *a priori* expectation was to find positive correlations between post-slough mass loss and reproductive behaviours, e.g. total distance moved during mate search. However, from the pre- to the post-sloughing phase, there was a shift from no correlative trends towards overall negative correlations between reproductive activities and mass loss (table 1, for pre-sloughing mass loss three of six coefficients < 0 ; for post-sloughing males all six Spearman rank correlation coefficients < 0).

The rates of a male's loss in body mass before versus after sloughing were not significantly correlated (Spearman rank ρ with 22 d.f. = -0.16 , $p = 0.46$). That is, males that lost mass more rapidly than other males in the pre-sloughing period did not consistently lose mass more or less rapidly than other males during the post-sloughing (i.e. mating) period.

4. DISCUSSION

We find these results surprising, in particular the magnitude of the pre-slough costs. Our initial analyses of costs of reproduction in male adders (Madsen & Shine 1993, 1995) quantified mass loss from spring emergence until the end of the mating period, but attributed this loss to the energy costs of reproductive behaviours (such as mate-searching, courtship and combat). This interpretation is clearly falsified by the more detailed information in figure 1, which shows that more than half of this loss in body mass occurs before the males even begin to engage in any of these behaviours. If the two time periods differed in duration so that the pre-slough period was longer, then this can explain a higher cumulative mass loss for this period of time. However, it cannot explain why the rate of mass loss is at least as high during the pre-slough phase when males are sedentary, as during the post-slough phase when they engage in reproductive activities, e.g. mate search and combats (Volsøe 1944; Prestt 1971). Our reanalysis was prompted by the hypothesis that testis enlargement and, hence, sperm production may be a more expensive component of reproduction than has heretofore been assumed, and the pattern revealed by our data is exactly that predicted from this idea. Nonetheless, the magnitude of the mass loss over this one-month period is surprising in view of the relatively small size of the testes in adders ($< 5\%$ of body mass; Volsøe 1944; Nilson 1980). Indeed, even though our data fit the prediction from the 'sperm is costly' hypothesis, there are alternative explanations, most of which we believe we can refute:

(i) The pre-sloughing mass loss may be due to material other than fat reserves (i.e. energy stores). For example, snakes may lose mass because they eliminate waste material (faeces or urine), or because they desiccate over this period. We cannot rule out these

possibilities, but consider them unlikely. Adders typically enter hibernation with empty alimentary tracts (T. Madsen, personal observation). The spring habitat is very moist, making desiccation unlikely. The mass reduction due to mass of the slough is likely to be trivial, because dried sloughs weigh less than 2 g.

(ii) Metabolic rate is temperature dependent (Q_{10} approximately equals 2–3) and, hence, if males maintained a higher body temperature prior to that after sloughing, this could explain the relatively high pre-sloughing mass loss. However, during the early Swedish spring the ambient temperature increases sharply (the key trigger of adder reproduction) and the opposite trends in mass loss would be expected from a relationship based purely on temperatures – metabolic rates. Furthermore, male adders do not engage in mating activities unless they are near preferred body temperatures (*ca.* 30 °C, T. Madsen, personal observation) and, hence, there are *a priori* reasons to believe that mass loss due to a higher mean body temperature should be at least as high, more likely higher, in the post- rather than in the pre-sloughing phase.

(iii) Even if the mass loss is due to metabolic expenditure, it may involve more than simply the production of sperm. As well as the direct energy cost of spermatogenesis, males may incur substantial additional costs associated with increased plasma titres of steroids, such as testosterone and its metabolites. Plasma testosterone levels are known to peak at around the time of sperm production in a number of reptile species (e.g. Licht 1984; Crews & Moore 1986; Moore & Lindzey 1992; Olsson & Silverin 1996), and are believed to be high throughout both the pre-slough and post-slough periods in male adders (Nilson 1980). Elevated testosterone levels may directly increase metabolic rates (Chandola *et al.* 1974; Thapliyal *et al.* 1974; Gupta & Thapliyal 1985), with corresponding mass loss and increased mortality (Marler & Moore 1988*a, b*, 1991).

Even if the third of these hypotheses is valid, we are still left with the conclusion that a significant fraction (at least half) of the energy costs of reproduction in male adders are related to physiological factors (sperm production, metabolic preparation, etc.) rather than to straightforward energetic costs of reproductive activities. Interestingly, neither the pre- nor the post-sloughing components of mass loss are strongly tied to our measures of male activity or success rates during the mating season (table 1). This conclusion mirrors that from our previous analyses of reproductive costs in male adders (Madsen & Shine 1993, 1995).

The existence of strong energy costs of reproduction in males, independent of overt reproductive activities, may have strong implications for life history models. Nonlinearities in the relationship between reproductive 'costs' (as manifested by loss in body mass) versus associated 'benefits' (i.e. reproductive success) can substantially change predictions about optimal life history 'tactics', and especially optimal levels of reproductive effort (Schaffer 1974; Bull & Shine 1979). Thus, further research on this topic would be of value. Our preliminary analysis suggests that it would be

worthwhile taking a closer look at factors such as the energy content of testes, the metabolic rates of males, and the nature and extent of morphological changes occurring in male adders during their period of pre-sloughing inactivity. Perhaps some of the significant costs of reproduction in males relate to these more subtle factors, rather than to the overt and frenzied activity of males during the mating season.

Lastly, we note that reptiles may offer ideal model systems for investigating questions of this type. The temporal separation between gamete production and mating activity (the 'dissociated pattern' of Crews & Moore 1986; see also Smyth 1968; Saint Girons 1982) in many reptiles, plus the frequent occurrence of anorexia during reproduction (e.g. Shine 1980), provides an opportunity to tease apart the costs associated with different components of the reproductive cycle in both males and females.

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