

REPRODUCTION IN A TYPICAL CAPITAL BREEDER: COSTS, CURRENCIES, AND COMPLICATIONS IN THE ASPIC VIPER

XAVIER BONNET,^{1,2,3} OLIVIER LOURDAIS,¹ RICHARD SHINE,² AND GUY NAULLEAU¹

¹Conseil Général des Deux Sèvres, Centre d'Études Biologiques de Chizé, Centre National de la Recherche Scientifique (CEBC, CNRS), 79360, Villiers en Bois, France

²Biological Sciences A08, University of Sydney, New South Wales 2006, Australia

Abstract. Female aspic vipers (*Vipera aspis*) are "capital breeders," and delay reproduction until they have amassed large energy reserves. Data from an eight-year mark-recapture study on free-ranging vipers suggest that potential costs of reproduction were high for these animals, in terms of survival as well as growth and energy storage. Females that reproduced experienced higher mortality rates than nonreproductive females and, hence, exhibited a tendency toward semelparity, grew less, and devoted most of their energy stores to reproduction. Both the depletion of body reserves and the low survival of reproductive females translated into significant costs (decrements of lifetime reproductive success [LRS]). However, the cessation of growth during pregnancy had no detectable effect on LRS. Most females produced only a single litter during their lifetimes. A female's "costs" in energy terms were not negatively correlated with her future reproductive output, probably because female vipers vary considerably in the rate at which they can accumulate energy. This notion is supported by the observations that (1) females with higher initial body reserves expended more energy during reproduction, and (2) females that accumulated energy more rapidly after parturition were more likely to survive and to breed again. This kind of variation among females masks any underlying trade-off between current reproductive effort and probable future reproductive success. Despite this complication, a strong link between rates of survival and postreproductive mass recovery suggests that changes in body reserves govern reproductive effort in this species.

Key words: breeding frequency; capital breeders; cost of reproduction; energy storage; lifetime reproductive success; relative clutch mass; reproductive effort; semelparity; snakes; *Vipera aspis*.

INTRODUCTION

The extensive scientific literature on "costs of reproduction" falls into two main categories, with relatively little overlap. This dichotomy involves theory-based (primarily mathematical) explorations on the one hand, and empirical studies of living animals on the other. Many mathematical models in life history theory incorporate some causal link between an animal's current reproductive expenditure and its probable future reproductive output. These models suggest that the exact nature of that link has profound implications for the kinds of life history strategies that will maximize lifetime reproductive success and, hence, are expected to evolve under the conditions posited in the model (e.g., Williams 1966a, b, Schaffer 1974, Winkler and Wallin 1987, Shine and Schwarzkopf 1992).

Unfortunately, it is difficult to translate these apparently simple notions into practicable measures of reproductive costs (Jönsson and Tuomi 1994, Reznick 1992, Reznick et al. 2000). Thus, much of the empirical literature on costs of reproduction relies on measuring variables that are linked only indirectly to the potential costs experienced by reproducing

organisms (Stearns 1992). Such variables include measures of reproductive output (i.e., clutch sizes), reproductive output relative to maternal size (e.g., relative clutch mass; Cuellar 1984, Seigel and Fitch 1984), or effects of reproduction on maternal traits (e.g., metabolism, locomotor speeds, postparturition maternal body condition; Shine 1980; Birchard et al. 1984, Seigel et al. 1987, Lee et al. 1996). However, it is not easy to determine whether or not these measures correlate with the degree to which current expenditure decreases future probable reproductive success (i.e., decrements in lifetime reproductive success [LRS]; Williams 1966a, b). First, LRS is extraordinarily difficult to measure in mobile or long-lived animals (Clutton-Brock 1988). Second, effects of reproductive output on LRS can be mediated via several different processes. The most obvious dichotomy is between survival costs and energy costs (e.g., Callow 1979), but there are many subtleties even within these two broad categories. For example, higher energy expenditure on current reproduction may reduce LRS via decreased energy stores and/or by decreasing subsequent growth rates (and hence fecundity if body size enhances reproductive success). Third, these currencies are not independent (Bauwens and Thoen 1981, Brodie 1989). Fourth, the magnitude of various "costs" is likely to shift among habitats and years (Festa-Bianchet

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³ E-mail: bonnet@cebc.cnrs.fr

et al. 1998). Fifth, variation in levels of resource availability among individuals may generate a positive rather than negative correlation between current reproductive output and future reproductive success, thereby masking a trade-off between these two traits (Bell and Koufopanou 1986, Van Noordwijk and de Jong 1986). In order to overcome some of these difficulties, such studies should focus on various species that offer logistical advantages for measuring the relevant traits of reproductive expenditure and its consequences (Reznick 1992, Shine and Bonnet 2000). In the current paper, we describe an eight-year study on such a system, the aspic viper (*Vipera aspis*). Aspic vipers are abundant, sedentary (hence, easily recaptured), and live in a relatively cool climate (so that thermoregulatory needs during vitellogenesis and gestation substantially modify patterns of movement and feeding). Perhaps more importantly, females reproduce on a less-than-annual basis, so that we can readily compare females that are in the reproductive vs. nonreproductive years of their cycles (Bonnet et al. 2000a). This species is a typical capital breeder (sensu Stearns 1992). Large body reserves must be accumulated during long periods (years) before reproduction, both for the induction of vitellogenesis and to fuel most of the reproductive effort (Saint Girons 1957b, Bonnet et al. 1994, 2001b, Naulleau and Bonnet 1996). The massive depletion of body reserves in the course of reproduction results in a low breeding frequency and hence can be considered as a typical energy cost (Naulleau and Bonnet 1996, Bonnet et al. 2001b). Like other ectotherms, female aspic vipers are pre-adapted to capital breeding (Pough 1980, Bonnet et al. 1998). In contrast, endotherms are less well suited to long-term storage of body reserves (Jönsson 1997), and these animals tend to rely on "income" rather than "capital" to fuel reproduction (Else and Hulbert 1981, Bonnet et al. 1998, Bronson 1998, Schneider et al. 2000). As a result, changes in body mass during reproduction may be a poor indicator of energy costs of reproduction in mammals and birds. First, such changes may reflect fluctuations in food availability independently of reproductive effort; Second, the high basal metabolic rate of these animals means that body reserves can be depleted rapidly during short periods (days) of starvation in nonreproductive individuals (Nagy 1987). This situation seriously complicates direct comparisons between reproductive and nonreproductive individuals. By contrast, most ectotherms can survive for long periods of time (months to years) during total starvation with minor changes in body mass (Pough 1980). Hence, any massive decrease in body mass that is temporally and physiologically associated with reproductive effort is likely to be a direct consequence of reproduction. The comparison between reproductive and nonreproductive females is thus straightforward: both survival rates and changes in body mass are likely useful candidates for measuring potential costs of reproduction in this snake species.

Body reserves can influence LRS through major components such as breeding frequency (Naulleau and Bonnet 1996), current fecundity (Bonnet et al. 2001b), and survival (Bonnet et al. 2000b). We set out to answer three main questions:

1) What form do "costs of reproduction" take in aspic vipers? We explore three potential currencies in this respect: probabilities of survival, decreases in energy reserves, and rates of growth in body length (because fecundity is generally associated with size in snakes).

2) Because we have long-term data, we can assess whether or not these kinds of "cost" indices (such as reductions in growth and body condition), or the magnitude of expenditure on current reproduction, actually translate into lower reproductive success in the future. This must be true for survival costs, although even here it is possible that the effect is trivial (e.g., if subsequent postreproductive survival rates are so low that few females live long enough to reproduce again anyway). For energy storage costs, is it true that an unusually emaciated female will delay the production of her next litter? For growth costs, will females forfeit fecundity increments in later litters if they grow less after their first reproduction? Are females with high reproductive output in their first litter less likely to reproduce again, or are they likely to produce a small litter if they do?

3) What attributes of a female in the year after she reproduces (e.g., her rate of growth in body length, or her rate of replenishment of body condition) offer the best predictors of her subsequent reproductive output (i.e., determine the time she reproduces, how many offspring she produces, and their size)? Data on this issue can help us to identify which of these traits may offer the best currency in which to measure costs of reproduction in a typical ectothermic capital breeder.

MATERIALS AND METHODS

Animals and study site

The aspic viper (*Vipera aspis*) is a stocky, medium-sized venomous snake species widely distributed through western Europe (Naulleau 1997). Adults in our population average the following size: snout-vent length (SVL), 47.7 ± 3.4 cm; total length (TL), 54.3 ± 3.8 cm (means ± 1 SD). Female vipers mature at an age of approximately three years (Bonnet et al. 1999). Mating occurs in spring (March-April: Saint Girons 1952, 1957a, b, Vacher-Vallas 1997, Naulleau et al. 1999). In our population, females are gravid over summer and give birth to a litter of 1-13 large (20.7 ± 1.2 cm TL, 6.1 ± 1 g) offspring in autumn (late August-September). Most female vipers do not reproduce every year (Bonnet and Naulleau 1996). The exact frequency of reproduction depends upon thermal conditions (especially, length of the activity season) and, food supply, so that reproductive frequencies differ among areas and among years (Saint Girons 1952, 1957a, b, 1996).

This less-than-annual frequency of reproduction results from the time taken to replenish energy stores for the next litter: females delay reproduction until they exceed a minimum body condition threshold (Naulleau and Bonnet 1996).

We studied the aspic viper in a closed population in western central France (Les Moutiers en Retz, 47°03' N; 02°00' W; Bonnet and Naulleau 1996). The study site is 33 ha in extent. It is bordered to the north and east by roads, to the south by the Atlantic Ocean, and to the west by a camping site (Vacher-Vallas et al. 1999). It is a typical parkland habitat that has not been intensively managed for 15 yr. Thus, the hedges form a dense network, and bushes (especially brambles) have invaded the meadows to varying degrees. In some meadows, oak and pine plantations have recently (1993-1995) been established. The climate is a temperate oceanic one (see Bonnet and Naulleau [1993] for mean temperatures).

Procedure

One to three people checked the area almost every sunny day from the time the snakes emerged (late February for the females) to the end of their reproductive period (September), and less frequently in late-September-October. Searching effort averaged 95.3 d per annual activity season (1 SD, 32.2 d; range, 51-124 d) and 523.7 h per annual activity season (1 SD, 198.4 h; range, 232-614 h). Over the period 1992-1999, we hand-captured 469 different adult female vipers. Classification of these animals as adults is based on the minimum size we have recorded for parturition in this population (SVL, 41.5 cm; TL, 47 cm). Each female was individually marked for future identification by scale clipping in 1992, and fitted with a Passive Integrated Transponder (PIT) tag since 1993 (Sterile Transponder TX1400L; Destron/IDi, Lyon, France), measured (to the nearest 0.5 cm, SVL and TL), masses measured (to nearest one gram), palpated for prey, eggs, or embryos, and released at her exact place of capture. Reproductive status was determined by palpation of eggs or embryos, by records of parturition or by obviously postparturient body condition. Immediately after giving birth, females are very emaciated, with a flaccid abdomen and extensive skin folds. Our analyses exclude body mass data taken from individuals containing prey items or oviductal embryos. Recapture probabilities were high (see Bonnet and Naulleau 1996), but few females ($N = 5$) were recaptured in eight consecutive years, due to the low survival rate of reproductive females.

Survival

We scored a female as having died if we failed to locate her after searching >250 d over a period of more than two years. Given the very low vagility (mean value, 5 m/d; Naulleau et al. 1996) and high recapture rates within this closed population, we can be confident

that such animals had died rather than moved away. Only one female escaped capture during three consecutive years (marked in 1993 and not recaptured until 1997), and only eight animals were "missed" during two consecutive years.

Changes in body mass and body size

We measured changes in body mass and body length from the onset of vitellogenesis to the postparturition period. This covers the entire activity season (6-8 mo/yr). Our criteria for inclusion of data in the analyses were as follows: "early vitellogenesis" was defined as the period March-April (Bonnet et al. 1994), and data from snakes captured after this period were not included for analyses of change in body mass or length. However, we did include these later captures for analyses of rates of survival and future reproduction. Changes in body mass and rates of growth in body length were calculated from March-April to August-November within a given year, and (ignoring hibernation) from March-April to the next March-April between years. Our extensive data indicate that vipers did not show any significant change in either body mass or body length over the hibernation period.

Reproductive output

As soon as we recorded the first parturition of the year (generally in the second half of August), we collected all of the gravid females that we could locate, and held them in captivity in individual cages (for periods of up to one month) so that we could count, measure, weigh, sex, and mark the offspring. Captive females had masses measured every two days, and immediately after parturition. We defined relative clutch mass (RCM) as the total mass of the litter (including stillborn offspring, etc.) divided by the postparturient mass of the mother. Data were obtained on 158 litters from 137 different females. Several females were captured shortly before parturition in two different years, so that we were able to quantify reproductive output on each occasion. Data on these animals allowed us to explore the relationship between initial reproductive output and subsequent changes in body size, body condition, and reproductive output. However, for most analyses, data were not available from all of the females (e.g., data for mass change during the reproductive period were available on 301 females).

Activities associated with reproduction likely to be costly in aspic vipers

In combination with extensive studies in other parts of France (e.g., Saint Girons 1952, 1957a, b, 1996), our studies reveal that reproduction imposes marked changes on several aspects of the biology of female vipers. The major modifications are as follows:

1) Relative to males and nonreproductive females, reproductive female vipers become more sedentary in

the course of gestation: mean home ranges decrease sharply from 3000 m² to 300 m² (Naulleau et al. 1996).

2) Gravid vipers spend more of their time in behavioral thermoregulation than do other animals within the population (Bonnet and Naulleau 1996), and hence could be more exposed to predation (mainly by birds; Naulleau et al. 1997).

3) Pregnant females progressively reduce their rate of feeding, and may cease feeding in the latter stages of gestation (Saint Girons 1952, 1957a, b, 1996; G. Naulleau, unpublished data).

4) Female vipers show a consistent pattern of change in body mass over the course of the reproductive cycle. Body condition (mass relative to length) increases during the nonreproductive years, until it exceeds the threshold level required to initiate vitellogenesis (Naulleau and Bonnet 1996). The female's mass drops dramatically at parturition. The magnitude of this decrease in maternal mass (i.e., from the beginning to the end of the reproductive bout) offers a measure of her net energy expenditure over that period: the sum of body reserves invested into the litter and metabolic expenditure, minus food intake.

Analyses

Body condition was calculated as residual values from the logarithmic regression of body mass against body size (Jayne and Bennett 1990). We randomly selected a single record per female to avoid pseudoreplication bias in this analysis. However, ignoring such bias, and including in the analyses 753 females where reproductive status is known, did not change any results significantly. (Note that a total of 853 "female-year" data were collected, and reproductive status was unknown on 100 occasions.) Importantly, the mean body sizes of reproductive females and nonreproductive females were similar (ANOVA with reproductive status as the factor and SVL as the dependent variable; $F_{1,267} = 0.90$, $P = 0.34$), allowing us to compare these two categories of females without having to take into account possible effects of body size on rates of survival or growth (Bonnet et al. 2000a).

The snakes' tendency toward semelparity greatly reduced our sample sizes for tests comparing successive reproductive events by the same female. Conclusions from such tests are problematic because of their low power to reject the null hypothesis. Statistical conventions are much more rigid with respect to α (the decision to reject null hypotheses with an error $\alpha < 0.05$) than with respect to β (the type II error). Statistical textbooks generally recommend that the power of a test ($1 - \beta$) should be ≥ 0.80 . Because statistical tests (especially correlation analyses) have very low power when sample size is small, we performed power analysis to estimate the ability of our statistical tests to detect "significant" effects. In all our ANOVAs, power was close to 1.0 and has not been reported. Correlation analyses are more sensitive; a low correlation between

two variables inevitably (due to the structural trade-off between α and β error rates) leads to a low power of the analysis, even with a large sample size. Such a low power does not invalidate the analysis, but means that caution is needed in interpretation. In such cases, we calculated the sample sizes that would be required to detect a significant result at low α and β error rates (0.05 and 0.10, respectively). The presence of power analyses and required sample size estimates are not a panacea to low sample sizes, but no conflict arose among our different tests. We used Statistica Versions 5.1 and 6.0 (Statsoft, Tulsa, Oklahoma, USA) to perform the statistical analyses.

RESULTS

How high are the potential costs of reproduction for female aspik vipers?

We can estimate these potential costs by comparing females in reproductive years vs. nonreproductive years of their cycles. Our data provide four separate indices: (1) whether or not a female survived to produce her litter, (2) how much her energy stores (as measured by changes in body mass) decreased over the reproductive period (vitellogenesis plus gestation), (3) how much she grew in body length over this period, and (4) her body condition (mass relative to length) after giving birth. The first three variables are self-explanatory; the fourth is relevant because previous studies on two species of viviparous snakes sympatric with *V. aspis* have suggested that this trait (maternal postparturition condition) may be a significant predictor of maternal survival rates (Madsen and Shine 1993, Luiselli et al. 1996). Comparisons between reproductive and nonreproductive females suggest that potential costs are high in each of these four currencies.

Survival rates.—We have data for 381 randomly sampled females caught during 1992-1997 (females caught in 1998 and 1999 are ignored), for which reproductive status and survival are known. Adult female vipers experienced much higher survival during nonreproductive years (123 of 145 records, 85%) than during reproductive years (106 of 236 records, 45%; $\chi^2 = 59.7$, $df = 1$, $P < 0.0001$; see Fig. 1). The total potential cost of reproduction in terms of survival is actually higher and more complex than this, because a female viper's food-gathering activities during her nonreproductive years also constitute a component of reproduction (Bonnet et al. 2000b). However, the clear result is that activities directly associated with the production of the litter cause a substantial decline in annual survival. The major component of the low survival of reproductive females seems to occur mainly before parturition (i.e., during vitellogenesis and gestation), with 88 of 142 females dying before parturition vs. 31 of 78 females dying between parturition and the following spring ($\chi^2 = 10.0$, $df = 1$, $P = 0.0016$; the sample size

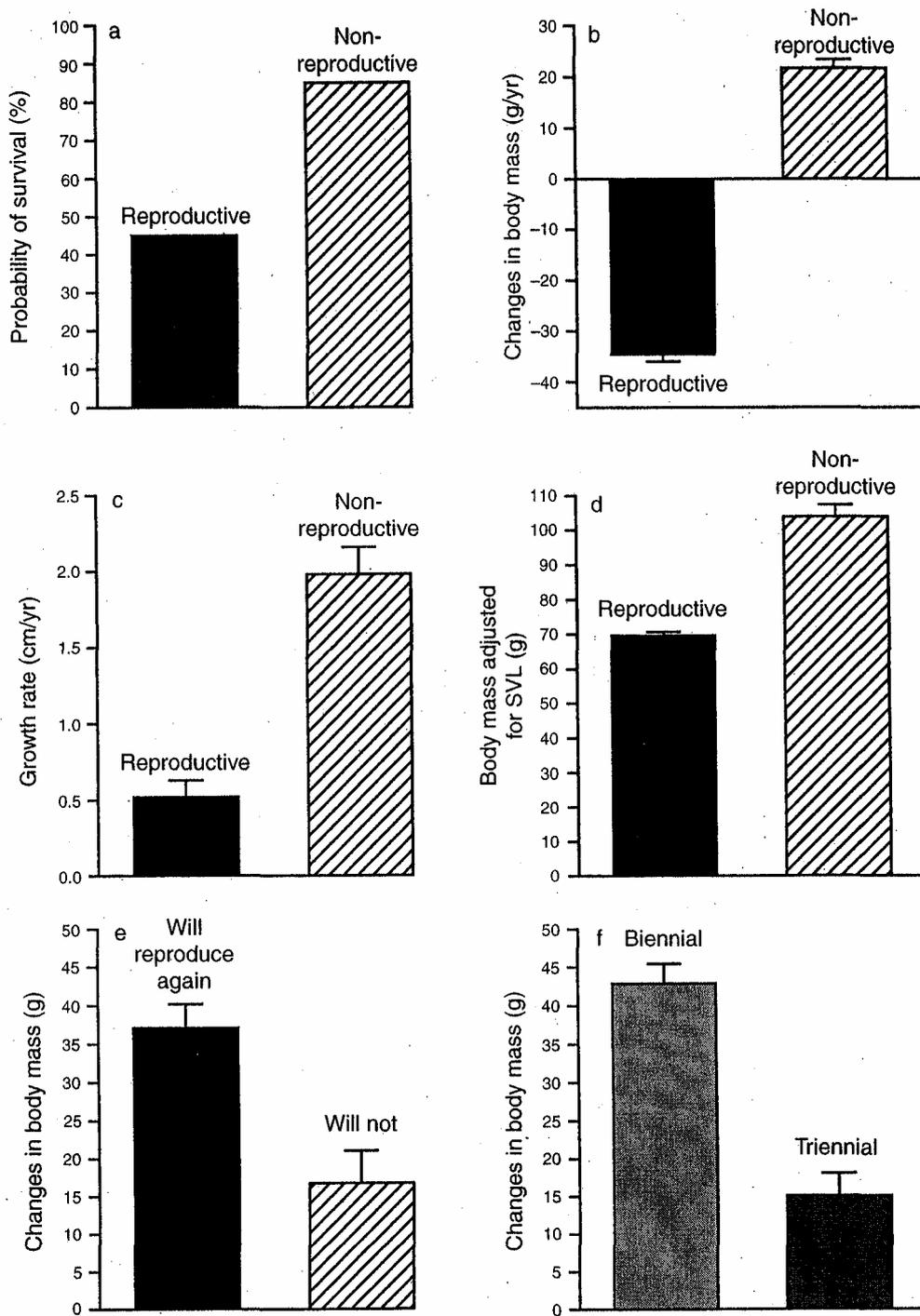


FIG. 1. Comparisons of (a) survival rates, (b) change in body mass, and (c) rate of growth in body length. (d) Maternal body condition (residual scores from linear regression of \ln -transformed mass vs. snout-vent length [SVL]) immediately after the time of parturition in reproductive vs. nonreproductive female vipers (for ease of interpretation, data are shown in terms of body mass adjusted for maternal SVL, rather than the body condition index). (e) Female vipers that regained mass more rapidly after reproduction were more likely to reproduce again and did so after a briefer delay. (f) Mean mass changes of females that had an interlitter interval of two (biennial) or three (triennial) years.

was reduced for this test because we selected females caught several times during a given year).

Considering the 236 reproductive females, the studied population exhibited a tendency toward semelparity: 182 females became vitellogenic only once, 43 twice, and 11 were vitellogenic on three different occasions; leading to a mean number of 1.28 reproductions per female during their life. Importantly, many

(~50%) of these females died before giving birth, and the mean total number of litters per "reproductive female" was actually less than one. For reliability, we excluded from this analysis the few females that survived over long periods, but that we failed to recapture in some years (for example, caught in 1992 and in 1995 but missed in 1993 and 1994), because of uncertainty about their number of reproductive episodes.

TABLE 1. Mean values (± 1 SD) of mass loss, growth rate, relative litter mass, and postpartum body condition recorded in the course of reproduction in wild female aspik vipers.

Trait	Will reproduce again	Will not reproduce again	N
Mass loss (g)	-37.2 \pm 17	-35.1 \pm 16	71
Growth rate (cm/yr)	0.32 \pm 1.46	0.87 \pm 1.46	73
Relative litter mass (%)	-0.55 \pm 0.22	-0.51 \pm 0.24	137
Postpartum body condition	0.005 \pm 0.1	-0.002 \pm 0.1	119

Notes: The changes in mass and growth rate were calculated from the onset of vitellogenesis to parturition. All of the females used in this analysis survived to produce their first litter and were classified based upon whether or not they also reproduced again in the future. We found no significant differences between the two groups of females. Growth rate statistics: ANCOVA with snout-vent length (SVL) as the covariable and growth rate the dependent variable, $F_{1,70} = 2.53$, $P = 0.12$. See Results for remaining statistics.

Changes in maternal body mass.—All reproductive females (regardless of whether we used the full data set or only one randomly selected data point per female) lost body mass over the period from spring (vitellogenesis) to autumn (postparturition). In contrast, non-reproductive females generally gained substantially in mass (ANOVA with reproductive status as the factor: $F_{1,299} = 616.8$, $P < 0.0001$; see Fig. 1). This decrease corresponds to the depletion of body reserves that are transferred in the embryos plus the metabolic costs of vitellogenesis and gestation (six months). Factoring out the potential effect of size through ANCOVAs (reproductive status as the factor, changes in body mass as the dependent variable, and body size as the covariate) leads to similar results ($F_{1,298} = 606$, $P < 0.0001$).

Growth rates in body length.—Most (57 of 68, 84%) reproductive females showed no detectable growth in body length during the reproductive period, whereas nonreproductive females showed significant growth (ANOVA with reproductive status as the factor: $F_{1,375} = 37.2$, $P < 0.0001$; see Fig. 1).

Body condition postparturition.—Females that reproduced were in much lower body condition after giving birth than were nonreproductive females at the same time of year (ANOVA with reproductive status as the factor: $F_{1,255} = 229.7$, $P < 0.0001$; see Fig. 1). In fact, almost all postparturient females were in poor body condition, as indicated by their abundant skin folds, typical of snakes with minimal body reserves (Bonnet 1996).

Do these "costs" mean lower future reproductive output?

Although it seems plausible that future reproductive output will be compromised by high levels of current expenditure (an index of energetic reproductive effort), this assumption requires empirical verification. To do this, we can examine the relationship between our measures of potential cost (survival rate, mass loss, growth rate, and maternal body condition postpartum) and reproductive output (relative clutch mass) on the one hand, and future reproductive output on the other. That is, do high levels of potential cost or reproductive out-

put correlate with lower levels of future output, as predicted by the "costs" hypothesis? In other words, do our measures of potential costs reveal real costs of reproduction?

Survival rates.—Obviously, females who die during reproduction are less likely to breed again than are surviving females. Our data clearly show that reproductive females have a low probability of survival. However, the degree to which this is a significant cost to lifetime reproductive success (LRS) depends not only upon survival probabilities in the absence of reproduction, but also upon a postpartum female's probability of reproducing again even if she survives until the following active season. If most "surviving" postparturient females in this population actually die before a second reproductive opportunity (regardless of their reproductive output in the first litter), then there may be little difference in the incidence of second litters between animals that survive to their first parturition vs. those that die during their first reproductive year. If so, the high mortality associated with reproduction will no longer be a real cost. This is not the case in our population. Almost 50% (59 of 123) of the reproductive females that survived initiated reproduction a second time. Although a significant proportion of these animals undoubtedly died during their second pregnancy and thus did not actually produce two litters, females that survive to reproduction have significant opportunity to reproduce again. Overall, the low survival rate of reproductive females, regardless of the period during which mortality peaks (i.e., before or after parturition), entails significant costs by strongly decreasing future probabilities of reproduction.

Changes in maternal body mass.—Were females that lost less body mass over the reproductive period more likely to breed again? Our data show that this was not the case (logistic regression, $\chi^2 = 2.16$, $df = 1$, $N = 71$, $P = 0.14$; Table 1). Similarly, we might expect females that lost more mass when producing their first litter to delay subsequent reproduction for a longer period in order to recoup their energy stores. No such effect was apparent in our data ($\chi^2 = 0.77$, $df = 1$, $N = 64$, $P = 0.38$). Lastly, we might also predict that

females who lost more mass would produce smaller than average litters and/or smaller than average neonates at their next reproductive episode. Neither of these patterns appeared in our data set (litter size $r = 0.24$, $N = 20$, $P = 0.29$; offspring size, $r = 0.18$, $N = 10$, $P = 0.64$). However, we note that the sample sizes were small for these later tests, and hence that the power of these analyses was low (0.27 and 0.13, respectively), perhaps reflecting the nonsignificance of the results. Nonetheless, the correlations were positive rather than negative, and the sample sizes that would be required to obtain a significant effect were high (178 and 320 respectively). Thus, any "undetected" effects were probably weak or negligible.

Growth rates in body length.—The notion that growth costs decrease future reproductive output depends upon the assumptions that (1) a decrease in growth rate during reproduction will influence body size at the next reproduction, and (2) a larger body size will allow a larger reproductive output. Neither of these assumptions is well supported by our data. In our population, body size influences reproductive output only slightly (see Bonnet et al. [2000a] for a detailed discussion of this issue). In addition, the extent of a female's growth during reproduction did not correlate with her body size at the next reproduction ($r = 0.24$, $N=15$, $P=0.39$). The power (0.22) of this analysis was low, and we may have failed to detect a slight effect. Nonetheless, the weak influence of maternal size on reproductive output (Bonnet et al. 2000a) suggests that growth effects of reproduction probably have little effect on future fecundity. Thus, the almost total inhibition of growth by reproducing female vipers did not translate into a significant cost of reproduction.

Relative clutch mass (RCM).—Female vipers that produced small litters relative to their own body size were no more likely to reproduce again than were conspecifics producing larger litters ($\chi^2 = 0.78$, $df = 1$, $N = 137$, $P < 0.37$; Table 1. Female that produced large first litters (high RCM) did not exhibit low rather than high RCMs in their second litters ($r = 0.45$, $N = 14$, $P = 0.11$). The power of this analysis was only 0.51. Regardless, the positive rather than negative correlation strongly suggests, counterintuitively, that high initial RCM does not translate into a reduced subsequent RCM. Females with high RCMs in their first litter did not produce smaller offspring (relative to other females) in their second litters ($r = -0.20$, $N = 12$, $P = 0.54$; but the power was low = 0.15) and did not delay their subsequent breeding attempts relative to other females ($\chi^2 = 0.04$, $df = 1$, $N = 63$, $P = 0.82$). A ratio measure such as RCM facilitates intuitive understanding of reproductive output relative to maternal size, but may introduce statistical artifacts into analyses (e.g., Seigel and Ford 1987). To overcome this problem, we repeated all of these analyses using alternative measures of reproductive output: either absolute mass of the litter, or residual scores from the general linear regression of litter

mass to body mass. We obtained similar results in each case.

Maternal body condition postpartum.—Female vipers are emaciated immediately after parturition, and have relatively low energy reserves at this time. Nonetheless, the degree of maternal emaciation did not correlate with a female's probability of survival to the next season (logistic regression, $\chi^2 = 1.88$, 1 df , $N = 119$, $P = 0.17$), or with her probability of breeding again (analysis restricted to the females that survived; logistic regression, $\chi^2 = 1.15$, $df = 1$, $N = 71$, $P = 0.28$; Table 1). Similarly, her litter size at the next reproduction ($r = -0.25$, $N = 14$, $P = 0.36$), or offspring size at the next reproduction ($r = 0.18$, $N = 12$, $P = 0.58$), was not strongly influenced by a postparturition female's body reserves. The low power of these two later analyses (0.22 and 0.14, respectively) require caution in interpretation; but the required sample sizes to obtain a level of $\alpha < 0.05$ were relatively high (164 and 320, respectively), suggesting that any "missed" effect was weak. Postpartum maternal body condition tended to affect the number of years' delay until her next reproduction, but this trend did not attain the conventional level of statistical significance ($\chi^2 = 3.39$, $df = 1$, $N = 63$, $P = 0.065$).

What currencies of potential costs influence future reproductive output?

Another way to identify the appropriate currency in which to assess "costs" is to ignore reproductive output per se, and focus instead on the long-term consequences of rates of change in the attributes that we know to be affected by reproductive expenditure. Body reserves and growth rates can readily be examined in this way.

Maternal change in body mass after the first litter.—If body reserves are important, we expect that females that recoup their energy (body mass) reserves rapidly will be more likely to breed again (and will breed sooner) than females that regain mass only slowly. Analysis supports this proposition for the female's probability of reproducing again ($\chi^2 = 10.53$, $df = 1$, $N = 55$, $P < 0.001$) and for the duration of the delay to her next reproduction ($\chi^2 = 27.70$, $df = 1$, $N = 42$, $P < 0.0001$; see Fig. 2). The female's litter size and neonate size at her second reproduction showed no significant relationship with her rates of mass increase after the first litter (litter size, $r = 0.45$, $N = 14$, $P = 0.10$; neonate size, $r = 0.29$, $N = 11$, $P = 0.38$). Although our sample sizes were small and associated statistical power low (0.51 and 0.22, respectively), these correlations were positive rather than negative.

Rate of growth in body length after the first litter.—Faster growing females were more likely to breed again ($\chi^2 = 6.10$, $df = 1$, $N = 89$, $P = 0.01$), and bred sooner than slower growing animals ($\chi^2 = 4.1$, $df = 1$, $N = 58$, $P = 0.04$). Taking into account the effect of a female's mean body size on her growth rate (by using

residuals of the regression between absolute growth rate and initial snout-vent length [SVL]; Bonnet et al. 2000a), does not alter this conclusion (breed again, $\chi^2 = 8.20$, $df = 1$, $N = 67$, $P = 0.004$; and breed sooner, $\chi^2 = 4.90$, $df = 1$, $N = 47$, $P = 0.003$). Reproductive output in the second litter was not associated with rates of body growth in the period following production of the first litter (litter size, $r = 0.05$, $N = 18$, $P = 0.84$; offspring size, $r = 0.12$, $N = 15$, $P = 0.65$). Despite a low power of these analyses (0.05 and 0.07, respectively), the very weak correlations would require sample sizes of 4198 and 725 to attain statistical "significance," and hence suggest an absence of effect.

DISCUSSION

Our analyses of costs of reproduction differ from most previous studies in this field by incorporating two steps into our analyses. First, we have measured the "potential costs of reproduction" for female asp viper in terms of energy and survival. Second, we have examined the consequences of these potential costs in terms of lifetime reproductive success (LRS). That is, we have tried to determine whether or not potential costs actually translate into a significant decrease in future reproductive success. By adopting this method, we can better tease apart two components of the life history: reproductive effort per se, and costs of reproduction (Niewiarowski and Dunham 1994). Our data support four main conclusions:

1) Reproducing female vipers commit themselves to a considerable effort in vitellogenesis and gestation, to the degree that most females produce only a single litter during their lifetime. Such reproductive effort is reflected in high rates of mass loss, a virtual cessation of growth, and a decrease in the probability of survival.

2) The different components of reproductive effort do not systematically, and equally, translate into real costs of reproduction. The cessation of growth rate during reproductive years had no measurable influence on future reproduction. By contrast, the strong mobilization of maternal reserves necessary to fuel reproductive effort had a considerable impact on breeding frequency and lifetime reproductive success through distinct, but interconnected mechanisms. First, vitellogenesis and gestation entail a strong increase in basking frequency and expose females to predation (Bonnet and Naulleau 1996, Naulleau et al. 1997). Second, many postparturient females die from starvation; and, even if a female survives, she delays reproduction for a long period of time (one to three years) until she has restored her body reserves (Naulleau and Bonnet 1996, Bonnet et al. 2000b). The lower survival caused by such thermal and energetic requirements of reproduction, along with the long period between reproductive bouts (that automatically increase mortality from other causes; Bonnet et al. 2000b) constitute a high cost.

Reproductive females that die, thereby lose significant opportunities to reproduce again and to increase the total number of offspring they could produce during their life.

3) The magnitude of reproductive effort in a given reproductive bout does not affect future reproductive success. Levels of reproductive output and our measures of reproductive effort (such as lowered survival, lower maternal body condition, loss in mass, or decrements in growth) were not negatively correlated with future reproductive success.

4) Changes in maternal body reserves over the complex alternation of nonreproductive and reproductive phases play a key role in the reproductive biology of female asp vipers. Notably, the emaciation of postparturient females determines the low breeding frequency and a high proportion of the mortality experienced by reproducing females. Despite the difficulty of detecting trade-off between reproductive investment and future reproductive success, the strong link between rate of mass recovery in postparturient females vs. the duration of delay to production of the next litter (and probability of survival to this time) offers strong evidence that energy stores are a crucial currency. Thus, the rate that a female viper can replenish her energy stores after reproduction may strongly affect her LRS.

These conclusions have several implications for studies on costs of reproduction. For example, they offer a challenge to simplistic attempts to use measures of reproductive output, such as relative clutch mass (RCM), increased metabolism, or cessation of growth, as a shorthand index of costs of reproduction (Shine 1980, Vitt and Price 1982, Birchard et al. 1984, Seigel et al. 1987). The reality is far more complex: even if reproductive effort is high, its magnitude may not correlate with future reproductive output in simple phenotypic comparisons (Bauwens and Thoen 1981, Brodie 1989, Niewiarowski and Dunham 1994, Olsson et al. 2000). Nonetheless, our study is encouraging in that a relatively easily measured trait (changes in maternal mass) may offer a reasonable currency in which to estimate several of the major costs, at least in ectothermic animals. Because they entail significant costs, the physiological mechanisms that control the allocation of body reserves during reproduction should be under strong selection (Sinervo and Svensson 1998, Bonnet et al. 2001a). Such a situation enables us to identify more precisely the ecological context that can favor the emergence of capital breeding instead of income breeding as an alternative reproductive strategy (Stearns 1992, Jönsson 1997, Bonnet et al. 1998).

Although a comparative approach will be needed to examine the evolution of such traits, at present we cannot compare the absolute magnitude or form of reproductive costs in *V. aspis* with that in other reptiles, because most previous studies have relied on indirect measures of cost. Indeed, costs of reproduction may be manifested differently. For example, there is apparently no significant survival cost of reproduction

in Orsini's viper, whereas such costs are high in both adders and aspik vipers (Capula et al. 1992, Madsen and Shine 1992, 1993, Baron et al. 1996, Luiselli et al. 1996). Nonetheless, it may often be true that female viviparous snakes living in cool climates experience such high mortality during reproduction that many females produce only a single litter in their lifetimes, and hence exhibit a strong tendency toward semelparity (Brown 1991). In the case of *V. aspis*, the mortality comes not only from starvation per se (because some females maintain sufficient reserves to avoid this threat), but also from vulnerability to predation during pregnancy (probably due to increased basking) and associated dangers, such as occlusion of the oviducts by inviable embryos (Naulleau 1997). Thus, although energy stores are a crucial currency that limits a female's reproductive output, energy limitation is not the only proximate determinant of reproduction-associated mortality in female vipers. When they engage in reproduction, females shift from a very secretive to a conspicuous way of life (Bonnet and Naulleau 1996). The thermal requirements of vitellogenesis and gestation result in high rates of basking in reproductive females (Shine and Harlow 1993), regardless the number of eggs/embryos they carry (X. Bonnet and G. Naulleau, *unpublished manuscript*). Hence, reproductive female vipers are disproportionately exposed to avian predation. Because such costs of reproduction can be independent of fecundity (Bull and Shine 1979), high energy and survival costs are often associated with the extreme reproductive effort in capital breeders such as viperid snakes. The low survival rates of reproductive female aspik vipers are not affected by fecundity in natural conditions (Bonnet et al. 2001a), and females may optimize their reproductive effort by producing the greatest number of offspring per litter in order to minimize the cost paid per neonate. Capitalizing large amount of body reserves prior to reproduction is an elegant way to produce a massive reproductive effort when the probability of experiencing more than a single reproductive bout is low.

Our study revealed another classical complication in studies of costs of reproduction. The expected underlying trade-off between current vs. future reproduction (as evidenced by negative correlations between energy costs and future reproduction) was masked. The comparisons between reproductive and nonreproductive females, and the link between rates of mass recovery and future reproduction, provide strong evidence for the existence of costs. Why, then, are they not manifested in negative correlations between output and costs on the one hand, and future reproduction on the other?

The answer almost certainly lies in substantial differences among females within our population in their ability (opportunities) to gather resources (Glazier 2000). For example, females with large initial body reserves produce larger litters and have a greater output relative to their own body sizes (Bonnet et al. 2001b),

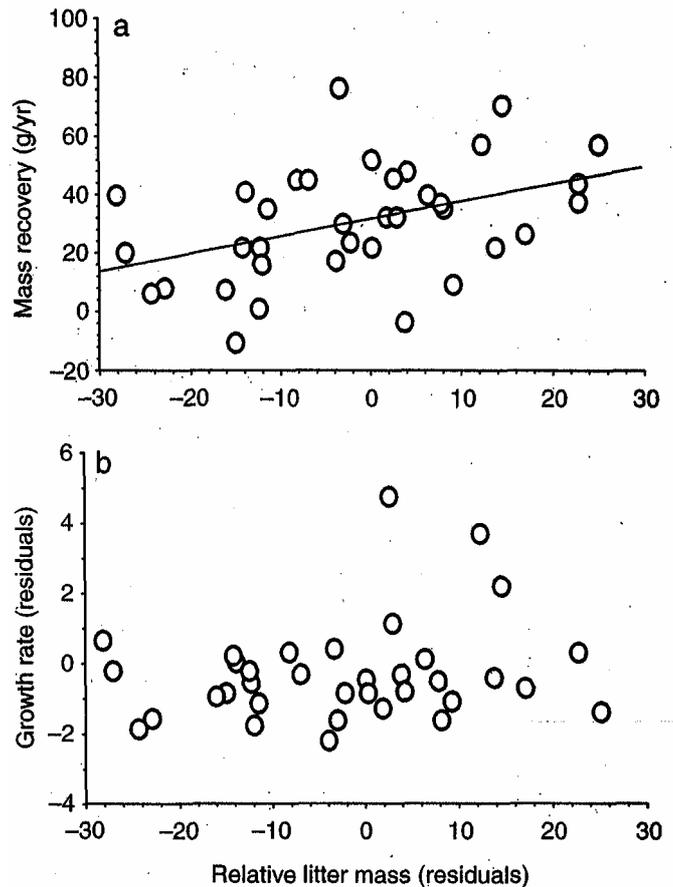


FIG. 2. Relationships between a female viper's reproductive output in her first litter and (a) her subsequent rate of recovery of body reserves and (b) growth rate in body length. Female vipers that invested more in reproduction exhibited higher rather than lower rates of mass recovery or growth, in contrast to predictions from the "costs" hypothesis. Females with higher relative clutch mass (RCM, residuals of litter mass on post-parturient female's mass) recovered their body reserves more rapidly during the following year ($r = 0.43$, $N = 36$, $P = 0.01$; the power of this analysis was 0.85), without trading this body reserve replenishment against growth rate the following year ($r = 0.20$, $N = 36$, $P = 0.20$; power = 0.32). To control for the effect of size on growth rate, growth rate was calculated as the residual values of the regression of absolute gain in size (cm/year) on initial body size (SVL, in cm). We used absolute values for panel (a) because mass recovery was not affected by body size.

but, as we have demonstrated, they are nonetheless no less likely to breed again and they do not produce a relatively smaller litter at their second bout. Despite their high reproductive expenditure, they do not recoup energy reserves more slowly after parturition, and thus they eventually reproduce again without additional delay. That is, females that invest more (higher RCM) in their first litter were not less likely to survive (logistic regression with survival as the dependent variable and RCM [residuals] as the independent variable, $\chi^2 = 1.03$, $df = 1$, $N = 141$, $P = 0.31$), and even showed a tendency to regain mass more rapidly (Fig. 2) than the "less lucky" females who exhibited lower reproductive output and who thus superficially appear to have paid lower potential costs. This situation may re-

fleet strong differences in female "quality" (as manifested in traits such as energy reserves prior to reproduction), as has been documented in other species (e.g., Van Noordwijk and de Jong 1986, Doughty and Shine 1997, Reznick et al. 2000). It may often be true that any given level of reproductive output is a greater cost (e.g., to survival) for a female in poor body condition (Cichon et al. 1998), and for variation in maternal quality to generate positive rather than negative correlations between reproductive output and survival (Bell and Koufopanou 1986, Winkel and Winkel 1995). We have no data on the determinants of female quality, but some correlates suggest that females with the highest reproductive effort (i.e., RCM) also exhibited the highest abilities to recover after parturition (Fig. 2) and hence to reproduce again. This variation might reflect under-lying genetic factors, or processes acting during ontogeny (e.g., developmental temperatures, feeding opportunities early in life, or low parasite numbers). Alternatively, these interindividual variations may simply reflect the fact that some females have been luckier than others during foraging prior to, during, and after reproduction. Food intake can affect reproductive body reserves, reproductive success, and recovery at each of these phases, and these effects can interact strongly (Bonnet et al. 2001b).

Despite the masking of phenotypic trade-off by variations among females, our data nonetheless provide support for the use of changes in maternal body mass as a realistic currency in which to estimate costs of reproduction in female vipers. We base this conclusion on several facts. First, reproduction is expensive energetically: a female viper's reproductive output is tightly linked to her energy stores prior to vitellogenesis (Bonnet et al. 2001b). Second, the rate at which a female can recoup her energy stores (expended during the previous reproduction) is a significant predictor of her future reproductive output, as our analysis has shown. The high growth in body size observed among females that recouped their body reserves rapidly reflects the fact that females with high food availability invested both in body reserve recovery and body growth. Changes observed in body mass integrate these two effects. Third, female vipers postpone reproduction until they have achieved a critical threshold level of body condition (Naulleau and Bonnet 1996). Thus, the substantial variation among postparturient females in their rates of recovery of body condition is convincing evidence that rates of gain in body mass offer a biologically meaningful currency in which to gauge a female's ability to survive and reproduce. Moreover, the absolute mass that postparturient females must regain to reach the threshold for reproduction fits well with the absolute mass loss during reproduction (Fig. 1b, e). The other currencies that we examined appear to be less useful for measuring costs of reproduction. Survival is obviously important and this parameter must be included in analyses, but it is difficult to integrate

with energetic measures in any single currency. Also, the real survival cost of producing a litter involves survival rates over the entire reproductive cycle (not just the year in which the litter is produced), whereas energy allocation can be calculated from the reproductive year only. Litter sizes are predictable from energy stores, as manifested in body condition.

One major advantage of using changes in maternal body mass as a currency for reproductive effort and potential costs, is that such changes are easy to measure under field conditions. Also, reproductive output can be quantified in the same currency, so that the two measures of investment (litter mass plus maternal loss in body mass) can simply be added together to calculate a female's total expenditure on reproduction. This cannot be done if investment in aspects other than the litter is measured in other currencies. Thus, our study is encouraging in that a logistically feasible currency can be used to quantify a female's investment into current reproduction, in terms that can be directly translated into effects on future reproductive success. This currency may well prove to be useful for other species as well especially those in which maternal energy reserves fuel most of the reproductive expenditure, as is the case for capital breeders (Drent and Daan 1980).

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