

Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake

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Summary

1. The ways that fluctuations in prey abundance and weather conditions can affect reproductive output in a 'capital breeding' ectotherm, the aspic viper (*Vipera aspis*) were examined.
2. Our longitudinal study confirms that female aspic vipers adjust reproductive investment by integrating allocations of energy from stores ('capital') and facultative feeding ('income'). Thus, long-term energy storage enabled females to reproduce successfully even in years when prey were scarce.
3. Not surprisingly, temporal changes in body reserves of female vipers preparing for reproduction depended upon current feeding rates. However, the mean environmental temperature during the active season also affected mass gain.
4. Allometric patterns suggest that reproductive output was limited by energy availability in 8 out of the 9 years of our study. In the other year, high prey availability in the preceding season meant that reproductive output was maximized within the constraints set by maternal body size (and thus, abdominal volume).
5. High summer temperatures increased basking opportunities of gravid vipers and thus accelerated gestation. However, maternal metabolic costs also increased in such situations, resulting in low postpartum body condition.

Key-words: capital breeding, ectothermy, environmental fluctuations, food availability, temperature.

Journal of Animal Ecology (2002) **71**, 470–479

Introduction

Variation in reproductive success is a central theme in evolutionary biology. Theoretical models predict that variation in quality among individuals provides the basic substrate for natural selection, resulting in a differential contribution to the number of descendants produced. Intrinsic sources of variation (including genetic effects), however, are only one source of phenotypic variation. Proximate (environmental) factors also affect reproductive performance. In many biological systems, year-to-year variation in environmental characteristics such as food supply or weather conditions can have dramatic repercussions on reproductive traits such as clutch size or reproductive frequency.

Such influences have been documented in a variety of taxa (Lack 1954; Ballinger 1977, 1983; Brand & Keith 1979; Todd, Keith & Fisher 1981; Seigel & Fitch 1985).

To cope with limitations and/or fluctuations of food resources, organisms have evolved a wide range of strategies for energy acquisition and allocation to reproduction. One fundamental dichotomy is between species in which reproduction is fuelled by recently acquired energy ('income breeders') and species where storage constitutes the primary energy source for reproduction ('capital breeders', Drent & Daan 1980). For income breeders, reproductive output should be closely linked to current resource availability, while in capital breeders a temporal separation should exist between the phase of energy acquisition and investment in reproduction. Capital breeding may be especially advantageous to buffer resource fluctuations among years, or if annual food levels are stable but insufficient to permit successful reproduction (Calow

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1979). However, the acquisition and storage of large amounts of energy requires time and is also potentially costly (Jönsson 1997). Therefore, the costs and benefits of alternative tactics of resource use (i.e. capital vs. income breeding) will vary among species. For instance, many features associated with ectothermy preadapt organisms to store large reserves and to use them for reproduction (Bonnet, Bradshaw & Shine 1998). The duration of energy gathering may sometimes cover long periods (years) and therefore often results in a low frequency of reproduction (Bull & Shine 1979).

In vertebrates, capital breeding systems coupled with infrequent (less-than-annual) reproduction have been observed in many reptiles (Saint Girons & Naulleau 1981; Brown 1991; Brana, Gonzales & Barahona 1992; Doughty & Shine 1997; Madsen & Shine 1999). Some of the best examples are among viperid snakes (Madsen & Shine 1992; Brown 1993; Martin 1993), with some species showing very low reproductive rates. For example, female aspik vipers (*Vipera aspis* Linné) in western France do not initiate vitellogenesis until they have accumulated sufficient energy stores to exceed a high body condition threshold (Naulleau & Bonnet 1996). The time necessary to accumulate body reserves entails a delayed maturity (2.5–3.5 years of age with an average lifespan of 5 years; Bonnet *et al.* 1999; X. Bonnet & O. Lourdais, unpublished data) and a low breeding frequency (once every 2–3 years). Owing to high costs of reproduction, most females will produce only one or two litters within their lifetimes (Bonnet *et al.* 2000, 2002), and the same may be true for many temperate-zone viperid species (Madsen & Shine 1992; Brown 1993).

In west-central France, the habitat of the aspik viper is characterized by strong annual fluctuations in availability of rodents that are the snakes' main prey (Naulleau 1997). Thermal conditions also vary significantly among years in this area. For an ectothermic species, variations in the thermal environment may affect the rate of processes such as digestion, metabolism and reproduction (Huey 1982; Naulleau 1983a,b). In the present paper, we use data from a longitudinal study of a population of vipers to examine how annual variation in both food availability and temperature affect reproductive output in a capital breeding ectotherm species. Specifically, we predicted that:

1. The long duration of energy gathering prior to reproduction combined with the female's ability to store large amounts of energy within her body should result in a high investment per reproductive bout. Nonetheless, because 'income' is also allocated to reproduction (Bonnet *et al.* 2001), we expect that litter mass will be influenced by prey abundance in the year preceding reproduction as well as the current year.
2. Capital breeding coupled with a fixed body condition threshold (Naulleau & Bonnet 1996) means that all females initiating reproduction do so with substantial energy reserves and, hence, the success of the litter should not be compromised by an unanticipated

energy shortage. The proportion of healthy offspring vs. nonviable components in a litter should be high, and independent of resource fluctuations.

3. Among non-reproducing females (i.e. individuals preparing for reproduction), body reserves accumulated at the end of the activity period should depend upon current food levels and also be influenced by thermal conditions that determine digestion rate.

4. Thermal conditions should directly affect the rate of embryogenesis and thereby gestation length.

Materials and methods

STUDY ANIMALS

The aspik viper (*Vipera aspis* Linné) is a small viviparous snake of the western-Palearctic region and is locally abundant at the northern limit of its distribution in France. Females mature at 40 cm snout–vent length (SVL), which is attained in 2.5–3.5 years (Bonnet *et al.* 1999). In this area, females typically reproduce at a less-than-annual frequency (Saint Girons 1957a,b; Bonnet & Naulleau 1996; Naulleau & Bonnet 1996; Naulleau *et al.* 1999). Ovulation typically occurs during the first 2 weeks of June with limited geographical or altitudinal variations (Saint Girons 1957b, 1973; Naulleau 1981). Parturition occurs 2–3 months later, from late August until late September (Bonnet *et al.* 2001).

STUDY SITE AND METHODS

The study site is near the village of Les Moutiers en Retz in west-central France (47°03'N; 02°00'W). It is a 33-hectare grove with a mosaic of meadows and regenerating scrubland. The site is characterized by a temperate oceanic climate. From 1992 to 2000, one to four people patrolled the site using a standardized searching method on almost every favourable day during the vipers' annual activity period (late February to late October). The total searching effort exceeded 4000 h. Snakes were caught by hand, sexed by eversion of the hemipenes, weighed to the nearest gram with an electronic scale, and measured (SVL and total length) to the nearest 5 mm. Over 400 adult and subadult female vipers were marked using passive integrated transponder (PIT) tags (Sterile transponder TX1400L, Rhône Mérieux, Lyon, product of Destron/IDI Inc.). Each snake was then released at its exact place of capture. Because the study site is surrounded by habitat unsuitable for vipers (Vacher-Vallas, Bonnet & Naulleau 1999) and this species is highly philopatric (Naulleau, Bonnet & Duret 1996), any snake not captured over a long period (>2 years) had almost certainly died rather than emigrated or avoided capture.

Initial total body length and body mass were measured in March–April (before any significant food intake). Changes in body mass and body length were calculated from March–April to August–November within a given year and from March–April to the next

March–April between years. Since our data indicate that vipers did not show any significant change in either body mass or body length over hibernation, we excluded that time period from our calculations. To provide an index of body condition (mass relative to length), we calculated residual values from the regression of log-transformed body mass against log-transformed body length (Jayne & Bennett 1990). Initial body condition (calculated in March–April) provides an accurate indicator of the level of fat stores in female vipers (Bonnet 1996).

Reproductive status was determined using two methods. First, any female whose initial body condition value was greater than the threshold at this time (March–April) was considered reproductive (see Bonnet, Naulleau & Mauget 1994; Naulleau & Bonnet 1996). Second, from mid-vitellogenesis (May) to the end of gestation (late August) reproductive status was easily determined either by palpation of ova and/or embryos or by records of parturition (Fitch 1987; Naulleau & Bonnet 1996). Gravid females were captured and maintained in captivity after the first parturition of the year was recorded (generally in late August). For each year, mean annual changes in maternal mass prior to parturition were calculated as prepartum body mass minus initial body mass. Date of capture of pregnant females had no significant effect on this parameter for two main reasons. First, all pregnant females were re-captured over a short period (1–2 weeks) at the end of gestation, by which time there was little further mass change before parturition. Second, during the active season, food intake occurs mainly during vitellogenesis in spring and almost ceases after ovulation (Saint Girons & Naulleau 1981; Bonnet *et al.* 2000; Lourdais, Bonnet & Doughty 2002). Overall, then, prepartum mass change provides a robust indicator of food intake during reproduction.

Pregnant females were maintained in separate cages in the laboratory until they gave birth 1–21 days later. Mass was recorded every 2 days and immediately after parturition. For each female, we calculated prepartum and postpartum body condition. Body condition of prepartum females includes both the litter mass and female carcass mass, whereas postpartum body condition offers an indicator of the degree of female emaciation (Bonnet *et al.* 2000).

Reproductive data were obtained on 173 litters from 149 different females. For most individuals (129) only a single litter was obtained, but 16 females produced two litters and 4 individuals produced three litters. Treating these successive litters by the same female as independent data may introduce problems with pseudoreplication. However, none of our conclusions from statistical tests differed depending on whether these ‘repeat’ litters were included or excluded. Thus, the following analysis presents calculations based on the total data set.

The components of the litter were characterized (yolked eggs, dead offspring, healthy offspring), counted and weighed (± 0.1 g). Additionally, young and stillborn were measured (± 0.5 cm) and sexed. For one individual, total litter mass was not available.

We could not distinguish unfertilized ova from ova that had been fertilized but had died early in embryogenesis. Hence, eggs where only yolk was visible (fertilized or not), underdeveloped embryos and stillborn were all grouped in the same ‘nonviable’ category. Litter mass and litter size included all of these elements along with healthy offspring, whereas ‘fit litter mass’ and ‘fit litter size’ included healthy offspring only (Gregory, Larsen & Farr 1992). Relative litter mass (RLM) was defined as the residual score from the general linear regression of litter mass against postparturient mass of the mother. Gestation periods were calculated from parturition dates, assuming a fixed ovulation date in mid June (Naulleau 1981).

ENVIRONMENTAL FACTORS

Food levels

Vipers feed mainly on voles (*Microtus arvalis* Pallas) whose populations typically fluctuate in a 3–4 year cycle (Delattre *et al.* 1992). Variations in vole abundance directly influence rates of feeding by the snakes, as revealed by the proportion of snakes captured with a prey item in the stomach (Bonnet *et al.* 2000, 2001). Data on the annual proportions of adult snakes containing prey at the time of capture were available from 1993 to 2000. In the following analysis this parameter was used as an index of food abundance. This estimator of food levels provides results that are consistent with those from line trapping; for example, the same annual peaks in vole density are detected by both methods (Bonnet *et al.* 2000; Salamolard *et al.* 2000). However, feeding rates of the snakes provide a more sensitive measure of prey abundance, because trapping can fail to detect voles at low population densities. We acknowledge that feeding rates may also be sensitive to environmental temperature (due to thermal effects on snake activity and foraging success) and hence, our measures of annual variation in temperatures and in food supply would not be independent. However, no significant correlation was found between mean annual temperature and the proportion of snakes captured with a prey item in the stomach (see below). Because the asp viper is a very sedentary animal occurring at a high density in our population, feeding rates are likely to be tightly linked to prey abundance.

Temperatures

The study site is near the northern limit of the species’ range and therefore climatic conditions may constrain the animals’ reproductive biology. This inference is supported by the fact that parturition in southern populations occurs in mid-July, 2 months earlier than in West-central France (Zuffi, Giudici & Iolae 1999). Throughout the nine years of our study, daily thermal maxima ($^{\circ}\text{C}$) under shelter were recorded. Two biological periods were considered: the active season

(March to October) and the gestation period (mid-June to August).

Results

FLUCTUATIONS IN ENVIRONMENTAL FACTORS

Food levels

Feeding rates varied markedly during the course of our study ($\chi^2 = 25.53$, d.f. = 7, $P < 0.0006$). Two years were distinguishable, with one year of very low (1994) and one of particularly high (1996) food levels (see Fig. 1).

Temperature

During the course of the study, mean daily temperature during the snakes' active season (and during the gestation period) varied significantly among years (ANOVA, $F_{8,2196} = 3.88$; $P < 0.0001$; $F_{8,819} = 2.89$; $P < 0.0035$, respectively, see Fig. 2). We found no significant relationship between feeding rates and mean temperature during the active season ($r = 0.27$, $n = 8$, $F_{1,6} = 0.5$, $P = 0.50$).

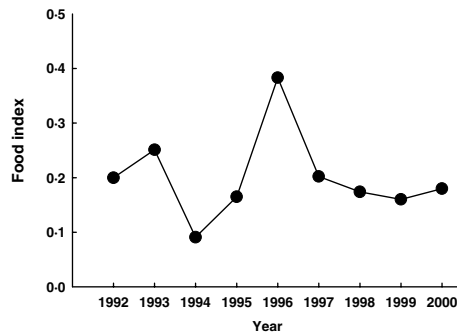


Fig. 1. Annual variation in the proportion of asp vipers containing prey items in the stomach at the time of capture. Analyses in this paper use this proportion as an index of availability of prey for the snakes. Most of these prey were voles (*Microtus arvalis*). See text for details on the method.

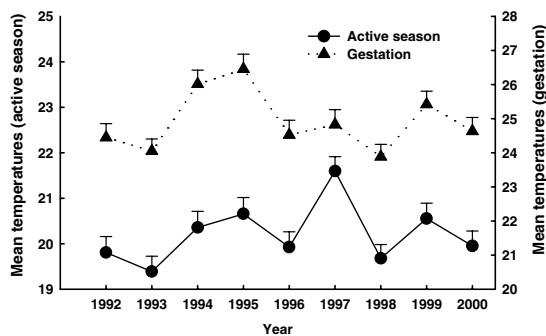


Fig. 2. Annual variation in thermal conditions over the course of our study. Mean ambient temperatures (\pm SE) under cover items were calculated separately for the active season (March to October; black circles, continuous line) and for the gestation period (mid-June to August; black triangles, dashed line).

INFLUENCES ON VIPER REPRODUCTION

1 Annual fluctuations in food levels and reproductive output

(a) *Variation in litter mass and reproductive effort*
Reproductive investment was generally high (mean litter mass = 34 ± 15 g), representing on average 52% of female postpartum body mass. Mean litter mass showed significant annual fluctuations (ANCOVA, $F_{8,160} = 2.26$, $P < 0.02$; using litter mass as the dependent variable, female body length as a cofactor). Among the 173 litters obtained, 15 individuals produced entirely nonviable litters (e.g. only undeveloped ova and still-born offspring) and thereby had very low litter mass values. Even when these nonviable litters were excluded from analyses, mean litter mass displayed significant annual variation (ANCOVA, $F_{7,142} = 3.76$, $P < 0.0009$, Fig. 3). The variation was mainly due to two consecutive years of especially high litter mass values: 1996 (a high food year) and 1997 (an intermediate food year).

Substantial year-to-year variations in maternal characteristics were also detected (see Fig. 4). Mean initial body condition of reproducing females varied

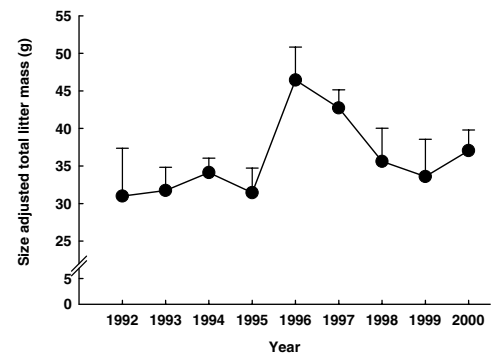


Fig. 3. Annual fluctuations in mean total litter mass (\pm SE) of female asp vipers. Values have been scaled with female body length. Fifteen litters that were entirely nonviable were excluded from this analysis (see text for statistics).

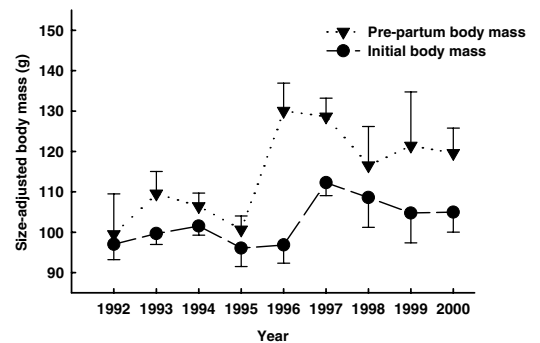


Fig. 4. Annual fluctuations in size-adjusted body mass of reproducing female asp vipers. The black circles and dashed line represent values for body condition of adult female vipers in spring (initial condition), while the black triangles and dotted line represent values for prepartum female body condition. Error bars represent standard errors.

Table 1. Effects of food level in the year of (n) and the year prior to ($n - 1$) reproduction on absolute litter mass of aspic vipers in south-western France. Multiple regression: $r = 0.95$; $r^2 = 0.91$; $n = 7$; $F_{2,4} = 19.745$; $P = 0.008$

	β	Partial correlation	Semi-partial	P -value
Food year n	0.83	0.9	0.82	0.005
Food year $n - 1$	0.57	0.88	0.57	0.019

significantly (ANOVA, $F_{8,257} = 3.53$, $P < 0.0007$) with the highest values observed in 1997 (after a year of high food abundance, see Fig. 2). Prepartum body condition also showed significant variation (ANOVA, $F_{8,160} = 6.27$, $P < 0.00001$) with the highest value observed in 1996 (the high food year). Annual maternal mass change prior to parturition (i.e. mean prepartum mass minus mean initial mass calculated for each year) was significantly correlated with current feeding rates ($r = 0.84$, $n = 8$, $F_{1,6} = 14.62$, $P < 0.008$), prepartum body condition ($r = 0.87$, $n = 9$, $F_{1,7} = 21.89$, $P < 0.002$), and total litter mass ($r = 0.87$, $n = 9$, $F_{1,7} = 21.50$, $P < 0.002$). As food abundance in a given year was independent of food abundance in the preceding year ($r = 0.12$, $n = 8$, $F_{1,5} = 0.074$, $P < 0.79$), we combined those two parameters in a multiple regression analysis which explained 91% of the variance in mean annual litter mass ($r = 0.95$, $n = 8$, $F_{2,4} = 19.74$, $P < 0.009$; Table 1).

(b) *Influence of maternal body size* Because maternal body size is highly correlated with litter size or litter mass in many snakes (Seigel & Ford 1987), we looked for correlations between maternal length and reproductive output (litter size and litter mass) in our population. Combining data for all years, a significant but weak influence of maternal body size on reproductive output was detected ($r = 0.20$, $n = 173$, $F_{1,171} = 7.1$, $P < 0.008$ and $r = 0.24$, $n = 172$, $F_{1,170} = 10.9$, $P <$

0.001, respectively, for litter size and litter mass). This analysis was also conducted separately for the nine years of the study. Because 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. For one year only (1997, i.e. the year following the high food availability year), a significant correlation between maternal size reproductive output was detected with both low α and β error rates (see Table 2).

(c) *Reproductive 'efficiency'* The 'efficiency' of reproduction is defined here as the proportion of viable vs. nonviable offspring (stillborn neonates and undeveloped ova) in a litter. It provides an index of the efficiency in converting yolk (i.e. energy allocated into reproduction) into viable offspring. The production of at least some nonviable items was frequent in this population (93 of 173 litters, 54%). Total reproductive failure (i.e. a litter consisting exclusively of all nonviable components) was, however, rare (8%). The 'efficiency' of reproduction (based on the ratio of fit litter mass to total litter mass) was high during the study. Fit litter mass averaged 86% of total litter mass and, not surprisingly, these two values were strongly correlated ($r = 0.95$, $n = 171$, $F_{1,169} = 1747.0$, $P < 0.0001$).

To avoid statistical problems associated with ratios (Seigel & Ford 1987), we calculated residual values from the regression of fit litter mass against litter mass to provide an index of reproductive 'efficiency'. This adjusted measure of fit litter mass peaked in 1994 (ANOVA, $F_{8,160} = 3.21$, $P < 0.0021$, see Fig. 5; considering only years where data on more than 10 litters were obtained, $F_{3,118} = 5.94$; $P < 0.0008$). Paradoxically, reproductive 'efficiency' was highest in 1994 (i.e. during a year of food scarcity) and lowest in 1996 (high food year). Considering fit litter size rather than fit litter mass did not change those results. This annual variation mostly reflected changes in the proportions of

Table 2. Correlation between maternal body length and reproductive output (litter size and litter mass) for each year of the study. The power ($1 - \beta$) of the analysis and the sample size required for $\alpha < 0.05$ and $\beta < 0.09$ were calculated. A significant correlation with both low α and β error rates was detected in 1997 only (bold). For some years only reduced sample sizes were available (1992, 1995, 1998 and 1999), power test values ($1 - \beta$) were consequently very low preventing any conclusions from being drawn. For other years (1993, 1994, 1996 and 2000), however, power test values were low but the large sample size required to detect a significant effect ($\alpha < 0.05$) suggests a very weak, if any, influence of maternal body size on reproductive output

Year	Litter size					Litter mass				
	r	n	α	($1 - \beta$)	Required n	r	n	α	($1 - \beta$)	Required n
1992	0.62	5	0.26	0.23	23	0.84	5	0.07	0.52	10
1993	0.20	26	0.31	0.17	258	0.21	26	0.35	0.18	234
1994	0.17	40	0.27	0.18	359	0.23	40	0.11	0.30	194
1995	0.34	11	0.30	0.18	86	0.36	11	0.27	0.20	76
1996	0.05	24	0.79	0.05	4199	0.25	23	0.24	0.19	165
1997	0.38	34	0.027	0.62	72	0.49	34	0.003	0.84	40
1998	0.56	8	0.14	0.33	29	0.36	8	0.36	0.15	76
1999	0.51	10	0.12	0.35	36	0.57	10	0.11	0.44	28
2000	0.17	15	0.54	0.1	359	0.1	15	0.73	0.06	1046

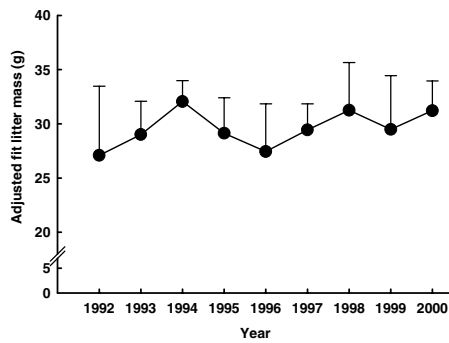


Fig. 5. Annual fluctuations in our measure of reproductive 'efficiency' based on the regression of fit litter mass against litter mass for aspik vipers. For simplicity, the graph shows fit litter mass values adjusted for total litter mass (\pm SE).

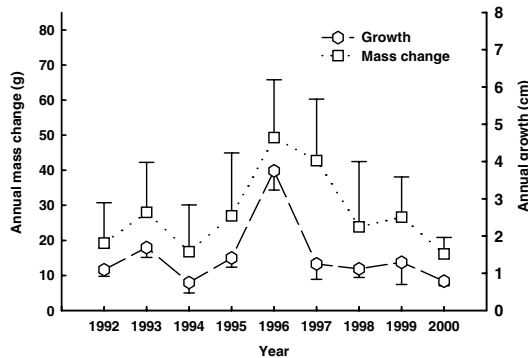


Fig. 6. Fluctuations in annual growth rates (open hexagons, dashed line) and annual mass changes (open square, dotted line) for non-reproducing female *V. aspis*. Values were scaled with female initial (spring) body size. Error bars represent standard errors.

litters with at least one nonviable component ($\chi^2 = 9.92$; d.f. = 3; $P = 0.02$) rather than annual variation in proportional viability among litters with at least one nonviable component (ANOVA, $F_{4,67} = 0.64$, $P = 0.64$).

Females with low reproductive success (i.e. $>60\%$ of the litter nonviable) did not have decreased survival ($\chi^2 = 0.13$, d.f. = 1, $n = 154$, $P = 0.71$), but they did have longer gestation periods (ANOVA, $F_{1,170} = 14.83$, $P < 0.0002$) and a lower probability of reproducing again ($\chi^2 = 6.49$, d.f. = 1, $n = 90$, $P = 0.01$).

2 Influence of food levels on females preparing for reproduction

(a) *Annual variations in mass gain and growth* Strong annual differences were detected in mass change of non-reproducing females (i.e. individuals accumulating energy stores for reproduction the following year, ANCOVA, $F_{8,152} = 5.10$, $P < 0.00001$; using change in body mass as the dependent variable, year as the factor, and initial body size as the co-factor) with the highest values observed in 1996 and 1997 (see Fig. 6). Mean annual mass gain was higher in years when prey were more abundant ($r = 0.79$, $n = 8$, $F_{1,6} = 10.4$, $P < 0.02$).

Table 3. Combined influences of mean ambient temperature during the active season (Mean temp) and food levels on adjusted annual mass change of non-reproducing female vipers. Multiple regression: $r = 0.96$; $r^2 = 0.92$; $n = 8$; $F_{2,5} = 30.9$; $P < 0.0015$

	β	Partial correlation	Semi-partial	P-value
Mean temp.	0.56	0.89	0.54	0.007
Food level	0.95	0.96	0.92	0.0007

Similar annual variation was observed in the growth rates of non-reproducing females (ANCOVA, $F_{8,229} = 9.19$, $P < 0.00001$; using annual growth rate as the dependent variable and female initial body length as a cofactor, Fig. 6). Annual growth rate was closely related to food levels ($r = 0.93$, $n = 8$, $F_{1,6} = 41.2$, $P < 0.0006$). Significant year-to-year variation in average body size was detected. Adult females (including both reproducing and non-reproducing individuals) were significantly longer in 1997 than in 1996 (ANOVA, $F_{1,265} = 5.29$, $P < 0.02$; using initial body size as the dependent variable and year as the factor).

(b) Interaction between food levels and thermal conditions

A multiple regression analysis revealed a significant combined influence of food levels and mean temperature during the active season on annual mass gain of non-reproducing females. These two factors explained 96% of the variance in mean annual mass gain (see Table 3). Interestingly, we were not able to detect any significant interaction between food levels and thermal conditions in the determination of annual rates of growth in body length (partial correlation: $r = 0.14$, $n = 8$, $F_{1,6} = 0.5$, $P = 0.76$).

3 Influence of thermal conditions during the gestation period

(a) *Reproductive success* Mean daily temperature during pregnancy did not affect the proportion of viable neonates ($r = 0.08$, $n = 173$, $F_{1,171} = 1.12$, $P < 0.29$).

(b) *Gestation length* Mean daily temperature during pregnancy strongly affected the length of gestation ($r = -0.47$, $n = 173$, $F_{1,171} = 49.36$, $P < 0.00001$, Fig. 7). Because low reproductive success (i.e. $>60\%$ of litter nonviable) also affected gestation length, we re-analysed the data excluding these litters, thus enabling us to focus on the effect of temperature ($r = -0.61$, $n = 138$, $F_{1,135} = 83.01$, $P < 0.00001$). Mean temperatures during pregnancy explained 40% of the variance in gestation length.

(c) *Maternal condition postpartum* We found a negative correlation between mean summer temperature and mean postpartum body condition ($r = 0.24$,

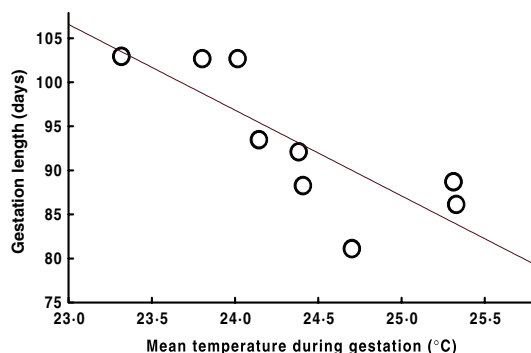


Fig. 7. Influence of mean environmental temperature during the gestation period (summer) on the duration of gestation in free-ranging aspik vipers. For simplicity, the graph shows mean annual gestation length. See text for detailed statistical analyses of these data.

Table 4. Combined influences of temperature during gestation (Ges. temp.) and food intake during the year of reproduction (indirectly measured using prepartum body condition value) on the postpartum body condition of female aspik vipers. Multiple regression: $r = 0.45$; $r^2 = 0.21$; $n = 169$; $F_{2,166} = 22.05$; $P < 0.000001$

	β	Partial correlation	Semi-partial	P-value
Ges. temp.	-0.20	-0.22	-0.20	0.0045
Food intake	0.38	0.38	0.37	0.000001

$n = 172$, $F_{1,170} = 10.7$, $P < 0.00125$). Postpartum body condition in this species is also positively influenced by energy acquired during the year of reproduction (Bonnet *et al.* 2001). A positive correlation was found between mean mass changes prior to parturition and postpartum body condition ($r = 0.84$, $n = 8$, $F_{1,7} = 17.56$, $P < 0.004$). The data were reanalysed holding prepartum body condition constant (i.e. an indirect measure of energy acquired during the year of reproduction). The partial correlation analysis supported our initial finding (Table 4).

Discussion

Previous studies on this population have shown that the reproductive ‘decisions’ of female aspik vipers largely rely on stored energy reserves (‘capital breeders’: Naulleau & Bonnet 1996; Bonnet *et al.* 2001). Demographic data clearly identify prey-driven variations in the proportion of reproducing females in this population, with food levels in a given year influencing the number of reproductive females the following year (Bonnet *et al.* 2001; O. Lourdais, unpublished data). In the present work, we have focused on annual variation in reproductive output to further understand the system of energy acquisition and allocation in this species. As predicted, capital breeding allowed for a high reproductive investment regardless of food availability in the current year. However, reproductive investment varied

among years, with mean litter mass higher in 1996 and 1997 than in other years (Fig. 3). Reproducing females gained substantially in body condition during the year of highest prey availability (1996), reflecting high energy intake during that year. High feeding rates at this time also resulted in high initial body mass of reproducing females early in the following season (1997). That is, the high prey availability of a single year (1996) increased litter masses not only that year but also in the following year as well (by increasing energy storage of non-reproducing females).

This complex system of energy allocation involving both capital breeding and facultative income breeding is well illustrated in the multiple regression analysis combining food levels both in the year of reproduction and in the preceding year (Table 1). A female viper’s reproductive strategy combines both ‘rigid’ and ‘flexible’ components. Firstly, female vipers have to reach a fixed body condition threshold to engage in reproduction (Naulleau & Bonnet 1996). A certain level of flexibility is evident however, with some prereproductive females eating rapidly enough to ‘overshoot’ the body condition threshold, and hence accumulating body reserves above the fixed threshold (Naulleau & Bonnet 1996). These ‘extra’ reserves are invested into reproduction and they positively influence litter size (Bonnet *et al.* 2001). After this initial phase that determines both the female’s reproductive decision and the number of follicles she recruits, facultative income breeding enables her to adjust her reproductive effort to current food levels during vitellogenesis. This composite system of energy allocation is advantageous for at least two reasons. First, reliance upon stored reserves secures a high reproductive ‘efficiency’ independently of current prey availability. The proportion of undeveloped ova or stillborn offspring produced was low and was not related to food levels. Second, instead of a completely ‘rigid’ capital breeding system, facultative income breeding enabled individuals to adjust reproductive investment to local resource levels reproductive and notably to take advantage of occasional periods when prey are abundant. A similar flexibility may occur in the closely related adder, *Vipera berus* Linné (Andren & Nilson 1983).

The effects of marked fluctuations in food levels were also evident in non-reproducing females (i.e. individuals preparing for reproduction). Among these females, change in body mass and growth in body length were both highly dependent on food levels. However, variance in mass gain was linked not only to feeding rates, but also to thermal conditions during the active season. This result may explain the high rates of mass gain observed in 1997, a year when climatic conditions were particularly favourable but prey abundance was only intermediate (confirmed by trapping, Salamolard *et al.* 2000). Thermal conditions may influence average mass change in several ways. Favourable thermal conditions may influence average mass change by accelerating digestion rates whereas low temperatures prolong

digestion and may even stimulate regurgitation of the meal (Naulleau 1983a, 1983b). While our data show no correlation between feeding rates and thermal conditions, we cannot exclude the possibility of undetected complex interactions between concurrent fluctuations in thermal factors and prey availability, and therefore we encourage further study of this relationship.

Several results from our study support an energy limitation model for reproduction in this population. First, elevated rates of body growth and mass gain were observed during a year of high food levels (1996). Second, maternal body size correlated only weakly and inconsistently with reproductive output (litter size and litter mass) in our aspic vipers. This correlation is high in most other snakes (Seigel & Ford 1987), perhaps because maternal abdominal volume (rather than energy supply) generally constrains reproductive output (Shine 1988). In a capital breeder, however, body stores may provide a greater constraint on reproductive output, and may often be below the level at which the litter mass would be constrained by (and, hence, correlated with) maternal body size. Under this scenario, we would expect a positive influence of female body size on fecundity to be more easily detected when energy intake is sufficient to allow maximization of body reserves. This prediction is consistent with our results: the correlation between maternal body size and reproductive output was significant only in 1997, the 'best' year in terms of energy availability.

While the system of energy allocation allows for adjustment to fluctuations in food availability, some aspects of reproduction were directly dependent upon variations in thermal conditions. High midsummer temperature accelerated gestation, as has been reported previously in captive snakes (Blanchard & Blanchard 1941). Our data also suggest that summer temperatures influence 'costs of reproduction' for females. The maintenance of a higher body temperature and thus higher metabolic rate (Saint Girons, Naulleau & Célérier 1985) during pregnancy translated into negative effects on female postpartum body condition. During the course of gestation, female aspic vipers are virtually anorexic and feed only opportunistically. Embryo maintenance generates substantial fecundity-independent mass loss in females and constitutes an important component of energy expenditure (Lourdais *et al.* 2002). Our data suggest that the magnitude of this metabolic cost varied from year to year, depending upon thermal conditions.

This result demonstrates difficulties associated with accurate estimation of reproductive effort in viviparous ectotherms. Classically, 'reproductive effort' has been quantified using simple measures of reproductive output, such as the ratio of absolute litter mass to postpartum female body mass (Seigel & Fitch 1984). However, our results, in combination with related work (Bonnet *et al.* 2001) suggest that the postpartum body condition of a female aspic viper is affected in complex

ways by several factors including her direct investment in the litter (absolute litter mass), her food intake during the year of reproduction, and her metabolic expenditure during gestation. Although all of these factors affect female emaciation (and thus both current and future reproductive effort), they are controlled by very different variables: (1) investment in the litter depends on energy stores combined with current food intake; (2) independent of reproductive investment, food intake during the year of reproduction enhances female postpartum body condition; and (3) female emaciation is also influenced by climatic conditions. Complex interactions between the two varying environmental factors (prey abundance and thermal conditions) are thus likely to affect the degree of female emaciation. Integrative approaches will be needed to identify the different means by which energy is expended during reproduction in ectotherms.

In conclusion, our long-term study clarifies the influence of two major environmental factors (food availability and thermal conditions) on the reproductive biology of female vipers. The snakes' responses to annual fluctuations in food availability support the hypothesis that reproductive output in *V. aspis* is determined by a combination of capital breeding (i.e. the use of energy stores) and income breeding (i.e. the use of energy from current food intake). This mixed (capital plus income) system masks but does not eliminate a trend for correlated fluctuations in prey abundance and reproductive output. The reliance on stored energy also enables a high and relatively invariant 'efficiency' of reproduction (proportion of viable embryos).

Annual fluctuations in thermal conditions also entailed both direct and indirect repercussions on viper reproduction. Among females preparing for reproduction, thermal conditions, in combination with food levels, significantly influenced the acquisition of energy stores. Additionally, thermal conditions have at least two direct effects on reproducing females during gestation period. First, gestation length is influenced by ambient temperatures that determine the thermal regimes experienced by pregnant females (Naulleau 1986). Second, high midsummer temperatures not only shorten gestation, but they also increase maternal metabolism and thus decrease the female's postpartum body condition. Such a decrease may well translate into a lower probability of survival, or into a delay in production of the eventual next litter (Bonnet *et al.* 2002).

Acknowledgements

We thank Gwenaél Beauplet for comments on the manuscript. Financial support was provided by the Conseil Régional de Poitou-Charentes, Conseil Général des Deux Sèvres, the Centre National de la Recherche Scientifique (France). Special thanks to Melle, notably for being her.

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Received 12 September 2001; revision received 8 January 2002