

BENEFITS OF COMPLEMENTARY TECHNIQUES: USING CAPTURE-RECAPTURE AND PHYSIOLOGICAL APPROACHES TO UNDERSTAND COSTS OF REPRODUCTION IN THE ASPIC VIPER (*VIPERA ASPIS*)

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ABSTRACT: The growing sophistication of field techniques during recent decades has profoundly modified the way in which we study free-living animals. For example, energy budget, patterns of movements, food intake, and body temperature has been widely measured in a variety of species. Physiological investigations, nonetheless, have rarely been used in combination with other disciplines to examine demographic parameters such as reproductive decision, reproductive effort, and associated costs (i.e., fecundity or source of mortality in natural populations). This perceived neglect inspired our research group to examine reproductive physiology from a classical approach. We have combined two methods we believe can yield new insights into issues such as, for example, reproductive decision. We have studied the reproductive physiology of the Aspik Viper (*Vipera aspis*) under natural conditions in west-central France since 1985. In a parallel study, we monitored a natural population of *V. aspis* from 1992 to 2000, using classical capture-recapture methods. The combination of both techniques has revealed more on the biology of *V. aspis* than if either was used in isolation. Plasma analyses, for example, indicate the time when females initiate reproduction, the type of body reserves mobilized during vitellogenesis, and precisely when such mobilization occurs. Hence, we have a greater understanding of the reproductive decision, reproductive effort and costs of reproduction in female *V. aspis*. Similarly, analyses of plasma hormones enable us to understand the activity of males in the field. Physiological information can clarify factors that determine catchability and pattern of movements (and hence vulnerability to predators), fecundity, and the tendency toward a semelparous-like condition in the population. Physiological studies of reproduction framed within a demographic perspective provide a strong basis to devise experimental tests of life history concepts, such as the cost of reproduction. In turn, the capture-recapture analysis can highlight the physiological mechanisms that are likely to be under selection in natural populations, thereby enabling us to focus on more relevant issues/aspects.

INTRODUCTION

Variations in reproductive success among individuals constitute the basis of natural selection (Darwin 1859). Variations in reproductive traits, however, are physiologically and genetically linked, and the existence of adaptive trade-offs is a postulate of most theories in evolutionary ecology (Williams, 1966a, b; Stearns, 1989, 1992; MacNamara and Houston, 1996). Resources at the disposal of a given individual (such as time and energy) are limited, and models predict that differential adjustments among competitive ways (i.e., between number and size of offspring) can optimize their respective adaptive value. For example, the effort dedicated to reproduction at any one time can influence future reproductive success by reducing growth and survival. This latter notion has been formalized into the allied concepts of "reproductive effort" and "cost of reproduction" (Fisher, 1930; Williams, 1966b; Roff, 1992; Stearns, 1992). Both reproductive effort and costs of reproduction may be estimated using phenotypic measurements (for a controversial discussion on this topic, see Reznick, 1985, 1992, and Rose et al., 1996 vs Sinervo and Svensson, 1998). Nevertheless, it is widely accepted that physiological mechanisms underlie adaptive trade-offs,

thereby regulating reproductive effort that may determine the final reproductive cost (Sinervo and Svensson, 1998; Reznick et al., 2000). In turn, physiological mechanisms are the expression of the genetic mechanisms that are the ultimate targets of selection (Sinervo et al., 1992; Sinervo, 1994; Sinervo and Svensson, 1998).

The premise that there is a physiological basis to the pattern of resource allocation to reproduction has conceptual and methodological implications. To understand reproductive effort and its potential consequences in terms of costs, it is essential to identify the types of resources invested into reproduction, the precise timing of this investment, and the manner in which such resources are invested. Such a necessity (and its associated complications) is best illustrated with examples. The exact timing at which an individual decides to reproduce (e.g., nest construction) may well be detected long after the actual reproductive decision (e.g., gametogenesis) has been initiated, if the observer ignores the physiological basis of the system (e.g., changes in hormonal levels). This type of error may well falsify correlative analyses between phenotypic characteristics (body size and body condition) and reproductive traits (reproductive decision, duration of reproductive effort, number and size of offspring). Such complications also apply to the potential cost that follows reproductive investment. Reproduction

can reduce the chances of survival through degradation of body condition, and/or an increased risk of predation (as mate searching or pregnancy can expose individuals to predators; Shine, 1980; Madsen, 1987; Moore, 1987; Lima and Dill, 1990; Schwarzkopf and Shine, 1992; Bonnet et al., 1999b). Unfortunately, dead individuals are very difficult to find in the field and are even more rarely dissected. Survival rates in natural populations are often estimated indirectly using capture-recapture data, thereby ignoring the underlying mechanisms. Similarly, future fecundity can be altered by current reproductive effort in the same manner on an absolute scale (same reduction of the number of offspring produced per year), but through different mechanisms.

For example, such a decrease can be caused by a reduction of maternal growth rate if fecundity is related to maternal size, as in many species with indeterminate growth. However, such decrease in growth rate may be due to the depletion of maternal reserves (mobilization of maternal reserves during reproduction), or alternatively due to a decrease in feeding rate if reproduction entails modification of behaviors (e.g., if predator escape tactics move away reproductive individuals from their feeding source). Nevertheless, in both cases the potential costs will be the same. In the first situation current reproductive effort is traded against maternal body reserves (the mechanisms that regulate the mobilization of maternal reserves are involved), while in the latter, current reproductive effort is traded against feeding behaviors (e.g., the neuroendocrine system that regulates feeding behaviors). At the level of the organism all these processes are connected, but decorticating them will be helpful to better understand the resulting costs of reproduction. The challenge then is to distinguish cause from consequence: if the primary trade-off involves feeding behavior, then the decrease in body condition will be a consequence. It is evident that we cannot rely entirely on phenotypic measures (such as changes in body size, body mass or body condition, survival rate) to unravel the different components of reproductive effort and associated reproductive costs. Indeed, these measures taken in isolation often ignore the diversity of the physiological basements through which selection operates.

In snakes, even basic knowledge of the nature and timing of resource investment into reproduction is fragmentary. This situation is somewhat paradoxical given the rapid evolution of field techniques during recent decades. For example, measurements of

energy budgets, patterns of movements, food intake, or body temperatures is now possible in a wide variety of snake species. Nevertheless, studies that combine the "classical" capture-recapture methods with ecophysiological investigations of reproduction are still rare. Herein, we report data that demonstrates the benefits of using complementary techniques, capture-recapture and physiological approaches, to understand the connections between reproductive effort and cost of reproduction in the Aspice Viper (*Vipera aspis*). This paper combines both original and published data and our goal is to show that the combination of the two approaches can tell us much more than either in isolation. This has been clearly shown in other taxa (for an overview, see Sinervo and Svensson, 1998).

MATERIAL AND METHODS

Animals

Vipera aspis is a medium-sized viviparous viper, relatively abundant in west-central France. In adults, it has a mean snout-vent length (SVL) of 48.5 cm, and a mean mass of 85.5 g. In the northern parts of its distribution, females typically reproduce with a lower than annual frequency (Saint Girons, 1957a, b; Bonnet and Naulleau, 1996; Naulleau and Bonnet, 1996). Ovulation typically occurs during the first two weeks of June (Naulleau and Bidaut, 1981), and parturition 2-3 months later from late August until late September. Females can mature at 2.5 years of age, but need to exceed 40.0 cm in SVL (Bonnet et al., 1999a). Males can reproduce annually, although they often skip mating seasons (Vacher-Vallas et al., 1999). The smallest male observed copulating (with sperm transmission) was 36.5 cm SVL and larger individuals were considered to be adults.

Capture-Recapture Study

We studied *V. aspis* in a closed, free-living population in order to examine the three classical aspects of potential cost of reproduction that can result from a given reproductive effort (i.e., investment of time, energy and material into current reproduction): decrease in survival, growth rate, and body condition. The study site (33 ha) was situated close to the village of Les Moutiers en Retz (district 44; 47°03' N, 02°00' W). It is a typical grove with a mosaic of meadows and regenerating shrubland. The climate is temperate oceanic (for average temperatures, see Bonnet and Naulleau, 1993).

Since 1992, one to four people patrolled the site on almost every sunny day during the vipers'

annual activity period (from late February to late October). The total searching effort represents more than 4,000 hours. Snakes were captured by hand, sexed by eversion of the hemipenes, mass determined to the nearest 1 g (electronic scale), measured for total length (TL) and SVL to the nearest 0.5 cm, and the presence of prey items or embryos was determined via palpation of the abdominal region (Fitch, 1987). These measurements were made regularly, depending on individual recapture rates. Reproductive status was determined by palpation of eggs or embryos, and by records of parturition (Naulleau and Bonnet, 1996). More than 1,000 adult or sub-adults vipers have been marked with Passive Integrated Transponders (PIT-tags). Every snake was released at the exact place of capture. The study site is surrounded by habitat unsuitable for vipers (Vacher-Vallas et al., 1999), and this species is highly philopatric (Naulleau et al., 1996). Thus, we are confident that any snake not captured over a long period (> 2 years) almost certainly died rather than emigrated or avoided capture. In support of this, only one female escaped capture during three consecutive years, and only eight animals were "missed" during two consecutive years. Direct (radiotracking) and indirect information (i.e. animals found dead on surrounding roads) indicate that emigration (or immigration) occur only rarely in our study population (Naulleau et al., 1996; Bonnet et al., 1999b).

Pregnant female vipers (N = 154) were captured 1-21 days prior to parturition (i.e., after the first parturition was recorded in the field each year, generally in late August). These females were maintained in separate enclosures in the laboratory (1-30 days, under natural lighting conditions, ambient temperatures close to 20°C) until they gave birth. Mass of females was recorded every second day during this period, and mass of both the female and her offspring were taken immediately after parturition. For every courtship and mating observed in the field we captured the snakes to identify individuals, particularly to obtain the reproductive status of males which is otherwise difficult to determine (Vacher-Vallas, 1997; Vacher-Vallas et al., 1999).

We also monitored more than 80 adults (60 females, 20 males) over a long period (six months per year from 1993 to 1997) by radiotracking to quantify daily patterns of movements and home range (for detailed procedures see Naulleau et al., 1996).

Physiological Investigations

Blood is the principal conjunctive tissue in vertebrates and the vehicle of intermediate forms of resources (energy, materials). As such, it should be helpful in investigating the basis of reproductive decision, effort and costs. Thus, we concentrated most of our effort in plasma analyses. Although some of the physiological measurements (blood samples) were taken in the field, most of this study has been conducted on captive animals, placed either in indoor or outdoor enclosures. Animals originated from neighboring populations of the main capture-recapture study site.

Conditions of captivity.—Snakes kept in outdoor enclosures (Forêt de Chizé, Deux-Sèvres, 46°07' N, 00°25' W) were exposed to natural climatic conditions similar to those experienced by free-living snakes, notably a long (four-month) hibernation. The terraria had a surface area of 8 m² and included three hibernating dens (50 cm underground). Three to 10 vipers were placed in each terrarium. Subjects were fed laboratory mice and water was available ad libitum. Mass of snakes was recorded at least once per month. These snakes reproduced normally and served as models to monitor physiological changes in the course of the complex alternation of reproductive and non-reproductive years.

Indoor snakes were kept in artificial conditions in the laboratory (12L:12D cycle, constant ambient temperature 20°C) in individual cages (L40 x W40 x H40 cm) with a 100 W incandescent light bulb to provide a thermal gradient 12 h/day. Food (laboratory mice: 15-40 g) was provided weekly and water was continually available. These snakes did not engage in reproduction, and provided us with the opportunity to test the influence of exogenous hormones on reproductive behaviors, and/or reproductive effort such as mobilization of body reserves.

Plasma assays.—Blood samples (100 to 500 µl into 1 ml heparinized syringes, needle 27G x 3/4", 0.4 x 20 mm) were taken by cardiac puncture (Naulleau and Fleury, 1990). This technique does not cause any detectable problems for the animals. Blood samples were immediately centrifuged and plasma was collected and stored at - 25°C until assayed. Plasma estradiol, progesterone, testosterone, and thyroxin radioimmunoassays were performed as described by Naulleau et al. (1987), Fleury and Naulleau (1990), Naulleau and Fleury (1990), and Bonnet et al. (1994). Plasma levels of metabolites were determined by an automatic spectrophotometer (Abbott ABA 100) using

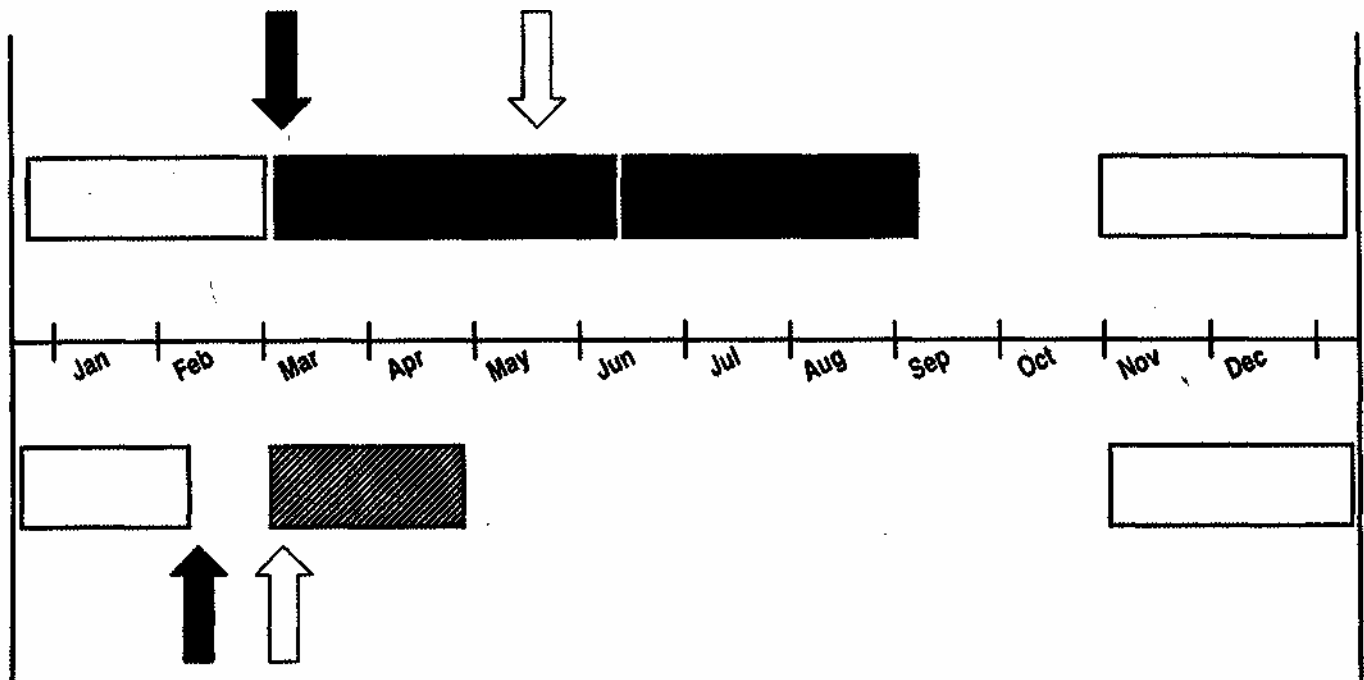


Fig. 1. Schematic of reproductive cycles of female (above) and male (bottom) *Vipera aspis* in west-central France. White boxes = hibernation; black box = vitellogenesis; gray box = gestation; hatched box = mating period. The black arrows indicate the exact timing when vipers "decide" to invest resources into reproduction. Physiological investigations (blood sampling and plasma analyses) have been indispensable to obtain such precise information on "reproductive decision." The white arrows indicate the time when reproductive status become detectable by "classical" capture-recapture approach, notably, palpation of large follicles in females and observation of sexual behaviors in males.

bioMérieux reagent (enzyme kits and references therein; see Bonnet et al., 1994).

Body reserves.— We also gathered information on dead snakes. Some of them were found dead in the field, but most were killed by people (particularly gardeners), predators (cats and birds), and vehicles. Others had died in our enclosures from natural causes such as post-parturition emaciation or from anorexia, which sometimes occurs in captive animals. The snakes were dissected to obtain their body composition (for additional details on dissection procedures, see Bonnet, 1996; Bonnet et al., 1998b). These data provided information about the causes of death, and were used to perform a correlation between morphological characteristics (body size, body condition) and body reserves at different stages of reproduction.

RESULTS

Reproductive success is determined in different ways in females and males and it is therefore possible to examine sexes separately.

When Do Aspic Vipers "Decide" to Invest Resources into Reproduction?

We were able to detect vitellogenic follicles in females by palpation during May. At that time it was

possible to classify females either as reproductive or non-reproductive. However, plasma assays indicate that the decision to reproduce is made much earlier, in late February to early March (Bonnet et al., 1994). The mobilization of maternal resources (mostly maternal reserves) that is necessary for the growing follicles occurs precisely at the time of emergence from hibernation. Thus, in terms of reproductive effort (mobilization of maternal reserves), females decide to reproduce at that time and not one to two months later when a large part of vitellogenesis is already complete (Fig. 1). Some females undergo follicular atresia (X. Bonnet et al., unpublished), hence a second decision, that is to achieve vs to cancel vitellogenesis, may occur after the initial reproductive decision. This certainly further complicates the picture, but this issue is out of the topic of this paper.

For adult males, mate searching, courtship, and matings are the only criteria usable in the field (Vacher-Vallas et al., 1999). This behavior is observed immediately after the emergence of reproductive females, generally in early March (Fig. 1). However, most males emerge from hibernation one to three weeks before the females (as in many other reptiles, depending on climatic conditions), perhaps to prepare their body to the coming mating

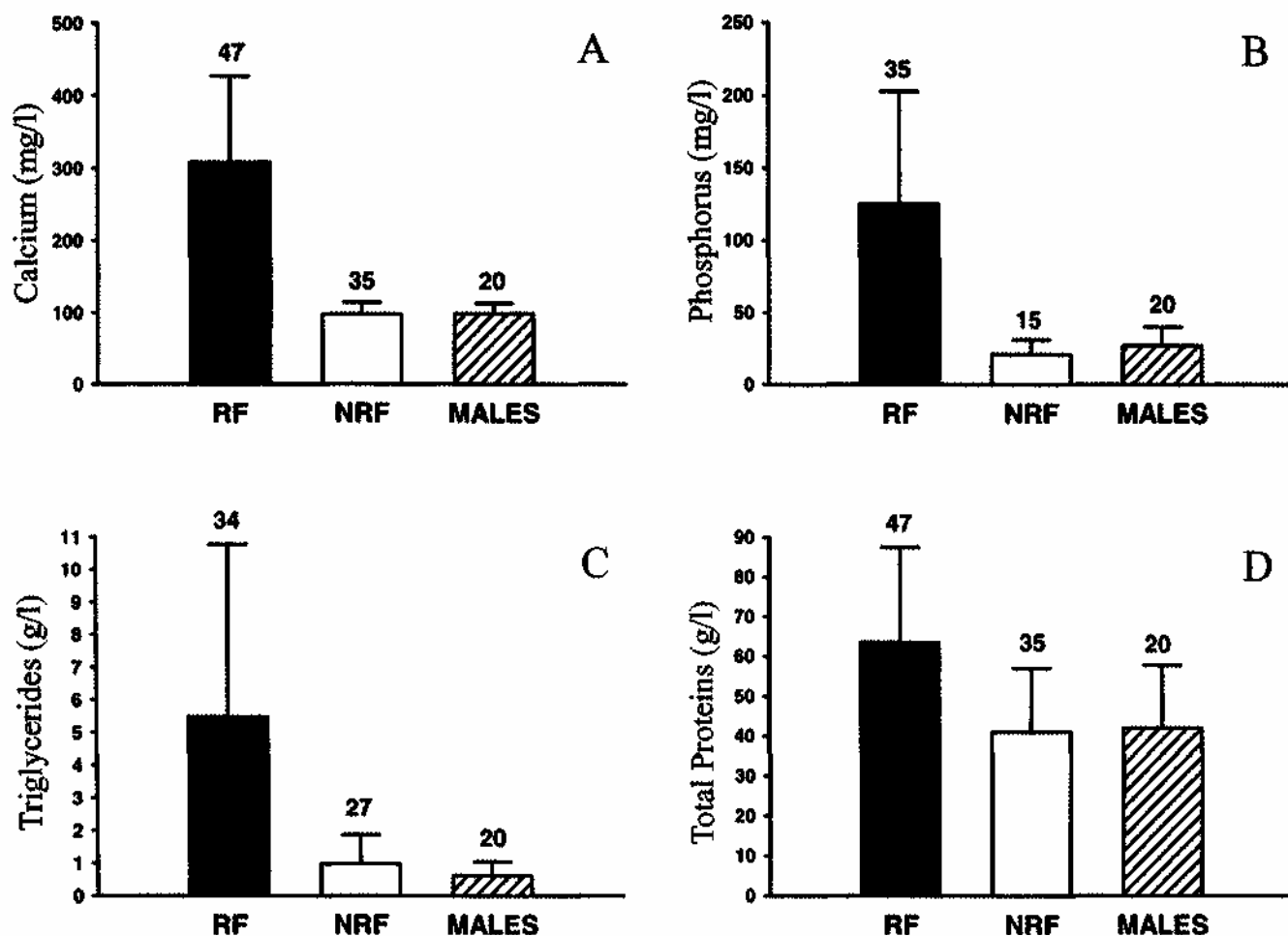


Fig. 2. Plasma levels of several metabolites in reproductive females (RF), non-reproductive females (NRF), and male *Vipera aspis* during the period of vitellogenesis (March until late May). (A, B) Vertebral bones, (C) lipidic reserves (fat bodies and liver), and (D) proteic reserves (mainly muscles) are massively mobilized in reproductive females (Alcobendas 1992; Bonnet et al. 1994; Bonnet 1996). In non-reproductive females, plasma levels of these metabolites remain relatively low, close to those observed in males. Plasma values of reproductive females are significantly greater (ANOVA, all $P < 0.01$) relative to non-reproductive females or males. Numbers indicate samples size.

period following long months of inactivity due to hibernation. Plasma assays support this suggestion: thyroxinemia and glycemia reach their highest levels immediately after hibernation, suggesting that metabolism of males peaks at that time (Fleury and Naulleau, 1987; Naulleau et al., 1987; Bonnet and Naulleau, 1993; Fig. 1). Similarly, testosterone levels also peak during this preparation phase (Naulleau et al., 1987). A delay may thus exist between physiological reproductive decision and the phenotypic expression of such a decision. This may be true also for females, since they may be prepared to engage into vitellogenesis in late fall, long before winter emergence. If so, females make decisions to reproduce prior to the onset of energy mobilization. However, such plausible preparation phase, if it exists in females, is certainly discrete in terms of energy requirements.

What Kinds of Resources are Invested into Reproduction? Over which Period?

Plasma assays show that reproductive females mobilize a wide variety of maternal reserves during vitellogenesis (Fig. 2). Females mobilize fat reserves, muscles, liver, and also skeletal elements (Bonnet and Naulleau, 1995). Such mobilization occurs for three months, is extremely intensive, and enables females to produce vast amounts of vitellogenin that are necessary for the development of the follicles (Bonnet et al., 1994). From the onset of vitellogenesis to ovulation, females increase their body mass ($\bar{x} = 13.0$ g; paired t -test = -5.20 , $df = 44$, $P < 0.0001$, $N = 45$ females monitored in the field from emergence to ovulation). Such increase is mainly due to food consumption that complements the mobilization of maternal body reserves during vitellogenesis

Table 1. Phenotypic characteristics vs body reserves in reproductive female *Vipera aspis* at different stages of reproduction. This table includes data obtained from females monitored in the field in the main capture-recapture site (N = 45, left columns), and from females "accidentally" killed at the same reproductive stages and subsequently dissected (right column, N indicated in brackets). The two data sets are not perfectly comparable because different individuals are involved, but they reveal a complication with biometrical measurements. Although body condition (mass adjusted by size) increases from early vitellogenesis until ovulation, maternal body reserves (here scored as fat stores) decreased at the same time; maternal reserves are massively invested to the growing follicles that are $34.9 \pm 23.7\text{g}$ (N = 17) at ovulation. As a result, females that recently ovulated are heavy but emaciated.

Reproductive stage	Body mass (g)	Body condition	Fat bodies (g)
Early vitellogenesis	101.44 ± 19.07	0.12 ± 0.12	15.5 ± 6.1 (14)
Ovulation	114.09 ± 22.62	0.23 ± 0.16	2.9 ± 1.8 (20)
Post-ovulation	64.00 ± 11.51	-0.35 ± 0.13	1.8 ± 1.5 (31)

(Bonnet et al., 2001). However, at the completion of vitellogenesis in early June, pregnant females are already emaciated, despite the apparent high body condition (mass scaled by size) (Table 1). Following gestation, post-parturient females are in poorer body condition, with minimal body reserves left (Table 1). Overall physiological investigations indicate that most of the reproductive effort, in terms of investment of materials and energy, occurs during vitellogenesis.

Males did not engage in similar body reserves mobilization (Fig. 2). However, during the entire mating season (pre-mating period plus mating period) a strong decrease in body mass occurs (comparing individual body masses recorded during that period, but with more than 15 days elapsed between two consecutive measures: paired t -test = 19.18, $df = 203$, $P < 0.0001$). On average, males lost 10g (12% of their body mass) during the mating season (-0.17 ± 0.13 g/day, N = 204). Dissections reveal that such a decrease is primarily explained by the utilization of fat reserves. A large proportion (44%) of the variance in body condition of males is explained by the mass of the fat bodies (Bonnet, 1996), suggesting that these reserves are mainly used to fuel muscular activity during mate searching, male-male combat, courtship, mating, and to sustain the presumed high metabolic rate of the preparation phase.

Do Changes in Hormone Levels Control Reproductive Effort?

Estradiol is the primary hormone to stimulate vitellogenesis in vertebrate females (Shuk-Mei, 1991). We find high levels of this steroid exclusively in reproductive females, and exclusively during the phase of mobilization of maternal reserves (Fig. 3). Experimental evidence indicates that increases in estradiol levels provoke such mobilization (Bonnet et al. 1994). Thus, in natural populations there are two discrete categories of females: reproductive indivi-

duals with high plasma levels of estradiol that invest a major portion of their body reserves into vitellogenesis, and, non-reproductive females.

In males, high levels of testosterone are linked to mating behavior, although a delay occurs between the peak of testosterone and the appearance of sexual behavior (Naulleau et al., 1987; Saint Girons et al., 1993). We experimentally demonstrated that increases in plasma levels of this steroid initiate male sexual behavior, and furthermore provokes a profound anorexia after a delay of 15 days (Bonnet, 1996). Under natural conditions, we found a correlation between the initial body condition of males (calculated at the beginning of the mating period) and plasma levels of testosterone (Fig. 3). This suggests that males will adjust their reproductive effort depending on their ability to sustain such effort. This allows males with a higher initial body condition index to search for females more intensively. Radiotracking and capture-recapture data indicate that males with larger fat stores covered a greater distance per day during the mating period and obtained more matings (Vacher-Vallas, 1997). In addition, Vacher-Vallas (1997) found that males with higher plasma levels of testosterone covered longer distances. These data suggest that males with higher body condition secrete more testosterone plasma levels, tend to show a higher reproductive effort and have a greater mating success.

The combination of physiological and capture-recapture approaches provides a greater understanding of the reproductive effort in *V. aspis*. The next step is to examine the extent to which such effort translates into reproductive cost.

What Type of Costs will Follow Reproduction?

Broadly speaking, two types of reproductive costs are distinguished: survival costs and energy costs (Shine, 1980). Although survival and energy costs

are physiology connected (low body reserves may reduce survival) it is helpful to analyze them separately.

Reproductive females pay a high survival cost: 54.9 % (N = 237) of the reproductive females die during reproduction, while only 31.4 % (N = 207) of the non-reproductive females died ($\chi^2 = 24.67$, $df = 1$, $P < 0.00001$). Following parturition, reproductive females that survived the six months reproductive effort are extremely emaciated (Table 1). A further survival reproductive cost is paid over a longer time scale (> 1 year) because many emaciated females will not recover and not survive (Bonnet et al., 1999c). This strong depletion of body reserves also affects breeding frequency and subsequent growth rate (Bonnet et al., 2002). Overall, female *V. aspis* in this population exhibit a strong tendency toward a semelparous-like condition.

In males, the depletion of body reserves means that less energy will be available for growth. Because mating success is dependent on body size, and to a lesser extent on body reserves (Vacher-Vallas et al., 1999), such a decrease can be assimilated as an indirect cost. Rate of body reserves utilized in the course of mating did not correlate to survival (logistic regression with daily loss of body mass as the independent variable and survival as the dependent variable: $\chi^2 = 0.96$, $P = 0.33$, N = 224). Thus in males, reproductive effort (as measured by depletion of body reserves) has no direct effect on survival, but may represent an indirect cost through decrease of growth rate. During the mating period, the catchability of males is high in comparison to other periods of the annual cycle (Saint Girons, 1996). Such an increase is particularly marked for those males that exhibit the greater reproductive activity (Vacher-Vallas et al., 1999), suggesting that sexual behavior exposes males to high risks of predation.

DISCUSSION

Broadly speaking, our results on the Aspic Viper (*Vipera aspis*) show that employing a variety of methods to study reproduction will enrich our knowledge on this particular species. This is hardly surprising and, more importantly, the combination of different approaches (physiology and capture-recapture in the present case) has revealed important complications that may eventually lead to further answers. We list and discuss some of these complications in the following section.

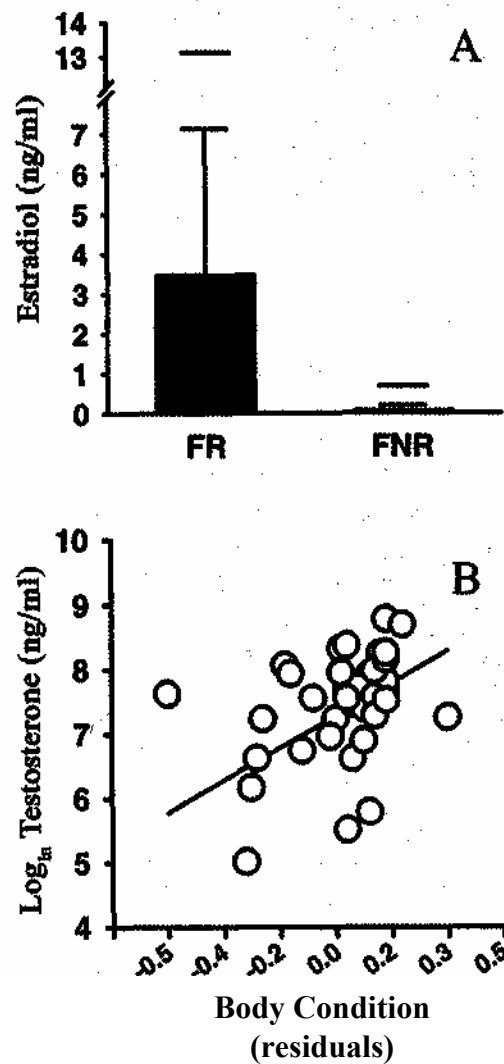


Fig. 3. Steroid values in female and male *Vipera aspis*, (A) Estradiol mean values (\pm SD, the horizontal bar represent highest values) recorded in females during vitellogenesis. In reproductive females (vitellogenic females, black bar, N = 44) levels of 17- β estradiol were high, and non-reproductive females had very low or non-detectable plasma values (the "flat" white bar, N = 35; ANOVA with reproductive status as the factor and plasma estradiol levels as the dependent variable, $F_{1,77} = 34.88$, $P < 0.0001$). (B) Testosterone levels vs body condition. Early body condition (residuals from the regression between log body mass against log SVL) correlated positively to plasma levels of testosterone ($r^2 = 0.21$, N = 36, $P < 0.01$) in males sampled in the field soon after emergence from hibernation.

Benefits of Physiological Approach to Capture-Recapture

We suggest that several aspects of the life history of *V. aspis* may not be revealed without collecting blood samples, and that a number of important questions are better addressed using physiological investigations.

When do females decide to reproduce? What kinds of resources are invested in reproduction?—Plasma analyses reveal two major issues. First, reproductive decision occurs immediately after emergence from hibernation (possibly earlier), precisely when the mating period begins (a decision which is completely undetectable by capture-recapture). Second, such a decision means that females will intensively mobilize their body reserves (lipid, protein, phosphocalcic reserves) for a period of three months.

From a purely methodological perspective, in order to correlate morphological characteristics to effective reproductive output (a central issue in evolutionary biology; see Stearns, 1992), it is essential to capture females at the precise time when the decision to reproduce is made, and not two or three months later. Taking advantage of this physiological information during capture-recapture sessions, we have described for the first time a body condition threshold necessary for the induction of reproduction in a natural population (Naulleau and Bonnet, 1996). The existence of such a threshold was theoretically predicted (see Stearns, 1992:75), but no empirical data were previously available (review in Naulleau and Bonnet, 1996; Bronson 1998). A rapid survey of the snake literature of this specific question indicates that almost, if not all, published studies ignored such a significant methodological complication (King, 1993; Monney, 1994; Baron et al., 1996; Madsen and Shine, 1996; Gregory and Skebo, 1998). Indeed, females may feed a lot, or starve during the first week of vitellogenesis; the resulting contrasted changes in body mass, in combination to those due to uptake of water by growing follicles (gain) and to the metabolism associated to vitellogenesis (loss), make the use of maternal body mass (or body condition) a no more reliable measure of initial body reserves if recorded too late. Many studies have used body condition calculated at ovulation, or even later, in their analyses (see references above). This often led to the conclusion that females with more fat reserves (but see Table 1) were more fecund. Such conclusions may well be accurate, but the analyses are probably questionable. We simply suggest that physiological investigations of the timing of vitellogenesis should be considered more carefully.

Vitellogenesis plus gestation takes approximately six months, but the period with favorable ambient temperatures is relatively short in west-central France. This time constraint on reproduction dictates that vitellogenesis must be terminated in early June. Maintenance of the maternal metabolism at a high

level requires a high body temperature. Thus, it is not surprising to observe that reproductive females are far more catchable than non-reproductive females, as they need to bask in the sun (Bonnet and Naulleau, 1996). This will not surprise many herpetologists, but such a differential catchability has been rarely considered in estimates of breeding frequency, particularly during the vitellogenic phase which extends over periods as long as pregnancy itself (Bonnet and Naulleau, 1996). In addition, we better understand some of the connections between reproductive status and reproductive behaviors. In the field, only vitellogenic females are reproductive and involved in courtship behaviors and readily accept to copulate (Naulleau et al., 1999). This is probably due to the high levels of plasma estradiol that stimulate such behavior. Overall, there are several reasons for determining the timing of vitellogenesis, and identifying which females are vitellogenic is necessary in order to correctly interpret several field data. Physiological exploration is thus highly useful and a relatively easy method to develop.

When do males decide to reproduce?—In comparison to females, the delay between the physiological decision to reproduce and observable sexual behaviors is relatively short in males (three weeks to one month on average). More importantly, the changes in body mass during such a delay can not lead to erroneous results. For example, a male in high body condition is never emaciated, while a heavy female with ovulated follicles may be. Hence, there are fewer complications with males in comparison to females. However, a recent study on a closely related species of adder (*V. berus*) suggests that the preparation phase entails a significant cost (Olsson et al., 1997). Their study found that the changes in body mass during the preparation phase are equivalent to those observed during the mating period, and the authors concluded that the decrease in mass during this preparation-phase is due to the production of sperm (hence sperm may not be as cheap to make as previously viewed). We observed a similar decrease in the body mass of male *V. aspis* during the preparation-phase, but our interpretation is slightly different. We suspect that the hormonal ambience (in particular, the peak of thyroxin) combined with high body temperatures results in an increase in the general metabolism (including testis activity) that in turn promotes the diminution of fat stores. We base this assumption on the fact that the mass of the testis represents only 1.2 % of the total body mass, and hence cannot utilize large amounts of energy. By contrast the liver (5.8 % of total body

mass) and the muscles (55.4 % of total body mass) are very active tissues and are more likely to be better candidates. Certainly more studies are required to tease apart these different hypotheses. Nevertheless, physiological data already indicate that the preparation-phase of mating should be considered carefully in male snakes.

Physiological information is helpful in assessing some ecological questions such as ones about reproductive decision, but this assertion is perfectly reversible. In the following section we provide examples that suggest that conducting physiological explorations of reproduction without the capture-recapture approach will be limited, in an evolutionary perspective.

Benefits of Capture-Recapture to Physiological Investigations

Collecting physiological information on reproduction is often necessary to interpret correctly how "costs" will eventually be manifested. However, only capture-recapture data can tell us the magnitude of these costs, when they will be paid, and ultimately allow us to identify the likely targets of selection. For example, only field observations can tell us if depletion of body reserves during reproduction entails a cost. Hence, we may expect to understand why some physiological mechanisms of reproduction have been selected in natural populations.

Why have female Aspik Vipers evolved physiological mechanisms that produce a brutal, massive, and infrequent reproductive effort?—Capture-recapture data indicate that reproductive females bask more often than non-reproductive females, probably to meet the temperature requirements of vitellogenesis and gestation (Bonnet and Naulleau, 1996). Although not yet precisely measured, such temperature-metabolism needs should be relatively constant regardless of the number of growing follicles or developing embryos (i.e., the optimal temperature for development should be identical for single or multiple embryos). Hence, all females should be exposed to the same degree of risk (as basking exposes snakes to predation, particularly from birds; Naulleau et al., 1997) during the six months of reproduction, regardless of the number of offspring carried. A logistic regression with litter size as the independent variable, and survival as the dependent variable supports this notion ($\chi^2 = 0.13$, $N=181$, $P = 0.72$). Such reproductive costs, which are independent of fecundity (Bull and Shine, 1979; Olsson et al., 2000), together with the strong tendency toward

semelparity, should favor capital breeding where the accumulation of reserves prior to reproduction, eventually over long time periods, is necessary to fuel reproductive effort (Jönsson, 1997; Bonnet et al., 1998a). The cost paid per neonate will be proportionally less in larger, compared with smaller litters. In other words, given the low probability of survival to reproduction, females should maximize their likely unique reproductive bout by producing the larger litter size as possible (increasing litter size and offspring size as well; Bonnet et al., 2001). The physiological data support this idea. High concentrations of plasma estradiol are only found in females that have reached a high body condition index, and that can sustain an important reproductive effort. In such situations, selection for capital breeding may well be favored, especially when several components of the costs of reproduction are independent of the current fecundity (survival independent of fecundity). Capital breeders sometimes need to accumulate body reserves over long periods, and hence often have a low frequency of reproduction (Bull and Shine, 1979).

In males, plasma testosterone levels increased gradually with body condition. This suggests that physiological adjustments exist between body reserves and reproductive effort of males, and that this adjustment is mediated by the endocrine system. Indeed, in contrast to females, males can produce a moderate reproductive effort from which they may withdraw if survival is jeopardized. Our hypothesis is that males that reach a lower body condition threshold will cancel reproductive effort (Cherel and Groscolas, 1999), and males with higher fat reserves will invest more. Both radiotracking and survival data support this notion. Males with large body reserves produce more testosterone, search for mates more intensively, obtain more matings, and invest in more body reserves. Their survival, however, is not markedly less than those males that are already in poor condition and cannot invest a lot of energy to reproduce. By contrast, in order to cancel reproduction, a female must terminate vitellogenesis and/or gestation. Overall, we observe the coexistence of two contrasting reproductive strategies within a single species: an "all-or-nothing" response in females vs a gradual one in males. Focusing exclusively on physiological data will not enable us to interpret this coexistence. Clearly, in capture-recapture programs, physiological data provide important insights and at the very least enables us to construct a number of testable hypotheses regarding costs of reproduction.

Future Directions

In this paper, for reasons of space, we deliberately ignored many phenomena that may influence reproductive costs in natural populations. Notably, fluctuations in food availability, inter-annual variations in climatic conditions, or inter-individual variations in quality can strongly modify the expression, form, and magnitude of reproductive effort and associated costs (Niewiarowski and Dunham, 1994, 1998; Shine et al., 1996; Beaupre, this volume). The relation between reproductive costs and reproductive efforts is rarely constant or linear and depends largely on the ecological context (Congdon et al., 1982; Tuomi et al., 1983). A clear example is provided by the study carried out in Italy on *V. aspis*. Marco Zuffi and his collaborator observed several females that reproduced annually in a natural population monitored close to Pisa (Zuffi, 1999; Zuffi et al., 1999; see Luiselli and Zuffi, this volume). This suggests that the high survival cost we observed in our main study site may not apply in this case. Similarly, in captivity and under controlled climatic conditions, *V. aspis* in France can reproduce with a high frequency (Naulleau, 1970, 1973). It would be interesting to examine the influence of genetic variation and phenotypic plasticity to explain why female *V. aspis* have a tendency to reproduce semelparous-like under certain environmental conditions and iteroparous under others. Such a situation is particularly favorable to investigate to what extent, and under which circumstances, a semelparous-like condition is the optimal strategy (Lessels, 1991; Clutton Brock, 1998). Another point of interest would be to examine whether the body condition threshold for the induction of vitellogenesis is rigid in response to inter-annual and inter-population variations or alter-natively, if such a threshold is much more plastic (Madsen and Shine, 1999). The physiological basis of body condition threshold can readily constitute a target of selection.

The next step would be to measure more precisely the different components of reproductive effort and associated costs of reproduction, and, in particular, to what extent reproductive status and fecundity influence maternal metabolism. Notably, we need to test more accurately the hypothesis that some major components of costs of reproduction (i.e. energy costs) can be independent of fecundity (Bull and Shine, 1979). Assuming that capital breeding is a reproductive strategy that allows females to recoup such costs of reproduction independent of fecundity by producing large litters, we may expect that

physiological mechanisms will underlie the body condition threshold for vitellogenesis. Large changes in plasma estradiol provide important links, but we still need to identify the nature of the signal that indicates to females that they can engage into reproduction: how can the central nervous system, which in turn controls the hypothalamo-hypophysogonadic axis, estimate the magnitude of the capital of body reserves stored under various biochemical forms (lipids, proteins). Understanding proximal mechanisms that regulate reproductive effort is thus a major goal in this field, and leptins are among the most targeted hormonal candidates for future studies (Schneider et al., 2000). Phenotypic engineering, which intergrates physiology and ecology, would be one appropriate method, for example, by injecting sexual hormones to increase RF or offspring size and observing the consequences in the field (Sinervo and Licht, 1991; Sinervo et al., 1992).

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LITERATURE CITED

- ALCOBENDAS, M. 1989. Recherche sur le métabolisme phosphocalcique au cours du cycle annuel et du cycle de la reproduction chez un reptile, *Vipera aspis*. Unpublished thesis, Université Paris VII.
- BARON J. P., R. FERRIÈRE, J. CLOBERT, AND H. SAINT GIRONS. 1996. Stratégie démographique de *Vipera ursinii ursinii* au Mont-Ventoux (France). C. R. Acad. Sci. 319:57-69.
- BONNET, X. 1996. Gestion des réserves corporelles et stratégie de reproduction chez *Vipera aspis*. Unpublished thesis. Université de Lyon I, France.
- , S. D. BRADSHAW, AND R. SHINE. 1998a. Capital *versus* income breeding: an ectothermic perspective. *Oikos* 83:333-342
- , O. LOURDAIS, R. SHINE, AND G. NAULLEAU. 2002. Reproduction in snakes (*Vipera aspis*): costs, currencies and complications. *Ecol.* 83:2124-2135.

- , AND G. NAULLEAU. 1993. Relations entre la glycémie et l'activité saisonnière chez *Vipera aspis* L. *Amphibia-Reptilia* 14:295-306.
- , AND —. 1995. Estimation of body reserves in living snakes using a Body Condition Index (BCI). Pp. 237-240 *In* G. A. Llorente, A. Montori, X. Santos and M. A. Carreto (Eds.), *Scientia Herpetologica*. Barcelona Spain.
- , AND —. 1996. Catchability in snakes: consequences on breeding frequency estimates. *Can. J. Zool.* 74:233-239.
- , —, O. LOURDAIS, AND M. VACHER-VALLAS. 1999a. Growth in the asp viper (*Vipera aspis*): insights from a long term field study. Pp. 63-69 *In* C. Miaud and R. Guyétant (Eds.), *Proceedings of the 9th Ordinary General Meeting, Societas Europea Herpetologica*. Le Bourget du Lac, France.
- , —, AND R. MAUGET. 1994. The influence of body condition on 17- β estradiol levels in relation to vitellogenesis in female *Vipera aspis* (Reptilia, Viperidae). *Gen. Comp. Endocrinol.* 93:424-437.
- , —, AND R. SHINE. 1999b. The dangers of leaving home: dispersal and mortality in snakes. *Biol. Conserv.* 89:39-50.
- , —, —, AND O. LOURDAIS. 1999c. What is the appropriate timescale for measuring costs of reproduction in a typical capital breeder: the asp viper. *Evol. Ecol.* 13:485-497.
- , —, —, AND —. 2000. Reproductive versus ecological advantages to larger body size in female *Vipera aspis*. *Oikos* 89:509-518.
- , —, —, AND —. 2001. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos*, 92:297-308.
- , R. SHINE, G. NAULLEAU, AND M. VACHER-VALLAS. 1998b. Sexual dimorphism in snakes: different reproductive roles favour different body plans. *Proc. Royal. Soc. London, Series B* 265:1-5.
- BRONSON, F. H. 1998. Energy balance and ovulation: small cages versus natural habitats. *Reprod. Fertil. Develop.* 10:127-137.
- BULL, J. J., AND R. SHINE. 1979. Iteroparous animals that skip opportunities for reproduction. *Am. Nat.* 114:296-316.
- CHEREL, Y., AND R. GROSCOLAS. 1999. Relationship between nutrient storage and nutrient utilisation in long-term fasting birds and mammals. Pp. 17-34 *In* N. J. Adams and R. H. Slotow (Eds.), *Proceedings of the 22nd International Ornithological Congress*. Durban, Johannesburg.
- CLUTTON-BROCK, T. H. 1998. Studying reproductive costs. *Oikos* 83:421-423.
- CONGDON, J. D., A. E. DUNHAM, AND D. W. TINKLE. 1982. Energy budgets and life histories of reptiles. Pp. 233-271 *In* C. Gans and F. H. Poug (Eds.), *Biology of the Reptilia*, Vol. 13. Academic Press, New York.
- DARWIN, C. 1859. *The Origin of Species*. John Murray, London.
- FISHER, R. A. 1930. *The General Theory of Natural Selection*. Dover, New York.
- FITCH, H. S. 1987. Collecting and life history techniques. Pp. 143-164 *In* R. A. Seigel, J. T. Collins, and S. S. Novak (Eds.), *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.
- FLEURY, F., AND G. NAULLEAU. 1987. Relations entre l'hivernage et la reprise des activités endocrines, testiculaires et thyroïdienne, chez *Vipera aspis* L. (Reptilia: Viperidae). *Gen. Comp. Endocrinol.* 68:271-277.
- , AND —. 1990. Évolution de la progestérone plasmatique chez la femelle *Vipera berus* L. (Reptilia: Viperidae) au cours du cycle sexuel. *Amphibia-Reptilia* 111:61-66.
- GREGORY, P. T., AND K. M. SKEBO. 1998. Trade-offs between reproductive traits and the influence of food intake during pregnancy in the garter snake, (*Thamnophis elegans*). *Am. Nat.* 151:477-486.
- KING, R. B. 1993. Determinants of offspring number and size in the brown snake, *Storeria dekayi*. *J. Herpetol.* 27:175-185.
- LESSELLS, C. 1991. The evolution of life histories. Pp. 32-68 *In* R. Krebs and N. D. Davies (Eds.), *Behavioural Ecology*, 2nd ed. Blackwell, Oxford.
- LIMA, S. L., AND L. M. DILL. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68:619-640.
- MACNAMARA, J. M., AND A. I. HOUSTON. 1996. State dependent life histories. *Nature* 380:215-221.
- MADSEN, T. 1987. Cost of reproduction and female life-history tactics in a population of grass snakes, *Natrix natrix*, in southern Sweden. *Oikos* 49:129-132.

- , AND R. SHINE. 1996. Determinants of reproductive output in female water pythons (*Liasis fuscus*: Pythonidae). *Herpetologica* 52:146-159.
- , AND —. 1999. The adjustment of reproductive threshold to prey abundance in a capital breeder. *J. Anim. Ecol.* 68:571-580.
- MONNEY, J. C. 1994. Note sur la reproduction et la taille des nouveau-nés chez la vipère aspic (*Vipera aspis*) et la vipère péliade (*Vipera berus*) dans l'Oberland bernois. *Bull. Soc. Frib. Sc. Nat.* 83:61-74.
- MOORE, S. D. 1987. Male-biased mortality in the butterfly *Euphydryas editha*: a novel cost of mate acquisition. *Am. Nat.* 130:306-309.
- NAULLEAU, G. 1970. La reproduction de *Vipera aspis* en captivité dans des conditions artificielles. *J. Herpetol.* 4:113-121.
- , 1973. Reproduction twice in one year in a captive viper (*Vipera aspis*). *British J. Herpetol.* 5:353-357.
- , AND C. BIDAUT. 1981. Intervalle entre l'accouplement, l'ovulation et la parturition chez *Vipera aspis* L. (Reptiles, Ophidiens, Vipéridés), dans différentes conditions expérimentales, étudié par radiographie. *Bull. Soc. Zool. Fr.* 106:137-143.
- , AND X. BONNET. 1996. Body condition threshold for breeding in a viviparous snake. *Oecologia* 107:301-306.
- , —, AND S. DURET. 1996. Déplacements et domaines vitaux des femelles reproductrices de vipères aspic *Vipera aspis* (Reptilia, Viperidae) dans le centre ouest de la France. *Bull. Soc. Herpetol. Fr.* 78:5-18.
- , —, M. VACHER-VALLAS, R. SHINE, AND O. LOURDAIS. 1999. Does less than annual production of offspring by female vipers (*Vipera aspis*) mean less than annual mating? *J. Herpetol.* 33:688-691.
- , AND F. FLEURY. 1990. Changes in plasma progesterone in females *Vipera aspis* L. (Reptilia, Viperidae) during the sexual cycle in pregnant and non pregnant females. *Gen. Comp. Endocrinol.* 78:433-443.
- , —, AND J. BOISSIN. 1987. Annual cycles in plasma testosterone and thyroxine for the male aspic viper, *Vipera aspis* L. (Reptilia, Viperidae), in relation to the sexual cycle and hibernation. *Gen. Comp. Endocrinol.* 65:254-263.
- , C. VERHEYDEN, AND X. BONNET. 1997. Prédation spécialisées sur la Vipère aspic *Vipera aspis* par un couple de buses variables *Buteo buteo*. *Alauda* 65:155-160.
- NIEWIAROWSKI, P. H., AND A. E. DUNHAM. 1994. The evolution of reproductive effort in squamate reptiles: costs, trade-offs, and assumptions reconsidered. *Evolution* 48:137-145.
- , AND —. 1998. Effect of mortality risk and growth on a model of reproductive effort: why the Shine and Schwarzkopf model is not general. *Evolution* 52:1236-1241.
- OLSSON, M., T. MADSEN, AND R. SHINE. 1997. Is sperm really so cheap? Cost of reproduction in male adders, *Vipera berus*. *Proc. Royal Soc. London, Series B* 264:455-459.
- , R. SHINE, AND E. BAK-OLSSON. 2000. Locomotor impairment of gravid lizards: is the burden physical or physiological? *J. Evol. Biol.* 13: 263-268.
- REZNICK, D. N. 1985. Cost of reproduction: an evaluation of empirical evidence. *Oikos* 44:257-267.
- , 1992. Measuring costs of reproduction. *Trends Ecol. Evol.* 7:42-45.
- , L. NUNNEY, AND A. TESSIER. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol. Evol.* 15:421-425.
- ROFF, D. A. 1992. *The Evolution of Life Histories*. Chapman & Hall, New York.
- ROSE, M. R., T. J. NUSBAUM, AND A. K. CHIPPINDALE. 1996. Laboratory evolution: the experimental wonderland and the Cheshire Catsyndrome. Pp. 221-241 *In* M. R. Rose and G. V. Lauder (Eds.), *Adaptation*. Academic Press, San Diego.
- SAINT GIRONS, H. 1957a. Le cycle sexuel chez *Vipera aspis* (L). dans l'ouest de la France. *Bull. Biol. Fr. Belg.* 91:284-350.
- , 1957b. Croissance et fécondité de *Vipera aspis* (L). *Vie et Milieu* 8:265-286.
- , 1996. Structure et évolution d'une petite population de *Vipera aspis* (L) dans une région de Bocage de l'ouest de la France. *Terre et Vie* 51:223-241.
- , S. D. BRADSHAW, AND F. J. BRADSHAW. 1993. Sexual activity and plasma levels of sex steroids in the Aspic viper *Vipera aspis* L. (Reptilia, Viperidae). *Gen. Comp. Endocrinol.* 91:287-297.
- SCHNEIDER J. E., D. ZHOU, AND R. B. BLUM 2000. Leptin and metabolic control of reproduction. *Horm. Behav.* 37:306-326.
- SCHWARZKOPF, L., AND R. SHINE. 1992. Costs of reproduction in lizards: escape tactics and susceptibility to predation. *Behav. Ecol. Sociobiol.* 31:17-25.

- SHUK-MEI, H. 1991. Vitellogenesis. Pp. 91-126 *In* P. K. T. Pang and M. P. Schreibman (Eds.), *Vertebrate Endocrinology: Fundamentals and Biomedical Implications*, Vol. 4, Part A. Academic Press, San Diego.
- SHINE, R. 1980. "Costs" of reproduction in reptiles. *Oecologia* 46:92-100.
- , L. SCHWARZKOPF, AND M. J. CALEY. 1996. Energy, risk and reptilian reproductive effort: a reply to Niewiarowski and Dunham. *Evolution* 50:211-2114.
- SINERVO, B. 1994. Experimental tests of reproductive allocation paradigms. Pp. 73-90 *In* L. J. Vitt and E. R. Pianka (Eds.), *Lizard Ecology: Historical and Experimental Perspectives*. Princeton University Press, Princeton, New Jersey.
- , AND R. DOUGHTY, R. B. HUEY, AND K. ZAMUDIO. 1992. Allometric engineering: a causal analysis of natural selection on offspring size. *Science* 258:1927-1930.
- , AND P. LICHT. 1991. Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *J. Exp. Zool.* 257:252-264.
- , AND E. SVENSSON. 1998. Mechanistic and selective causes of life-history trade-offs and plasticity. *Oikos* 83:432-442.
- STEARNS, S. C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.* 3:259-268.
- . 1992. *The Evolution of Life Histories*. Oxford University Press, New York.
- TUOMI, J., T. HAKALA, AND E. HAUKIOJA. 1983. Alternative concepts of reproductive effort, costs of reproduction and selection in life-history evolution. *Amer. Zool.* 23:25-34.
- VACHER-VALLAS, M. 1997. Comportement sexuel, condition corporelle et stratégie de reproduction chez *Vipera aspis*: étude en milieu naturel. Unpublished thesis, Université François Rabelais, Tours.
- , X. BONNET, AND G. NAULLEAU. 1999. Relations entre les comportements sexuels et les accouplements chez *Vipera aspis*: étude en milieu naturel. *Revue d'Ecologie, Terre et Vie* 54:375-391.
- WILLIAMS, G. C. 1966a. *Adaptation and Natural Selection*. Princeton University Press, Princeton, New Jersey.
- . 1966b. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am. Nat.* 100:687-690.
- ZUFFI, M. 1999. Activity patterns in a viviparous snake, *Vipera aspis* (L.), from Mediterranean central Italy. *Amphibia-Reptilia* 20:313-318.
- , F. GIUDICI, AND P. IOALÉ. 1999. Frequency and effort of reproduction in female *Vipera aspis* from a southern population. *Acta Oecol.* 20:633-638.