

The Evolution of Semelparity

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17.1 OVERVIEW

Mathematical modeling has been the major source of progress in the understanding of the evolution of two contrasting reproductive strategies: semelparity (death following a single reproduction) versus iteroparity (iterative reproduction). However, current models do not allow us to understand why some animal groups (e.g., insects and fish) are more oriented towards semelparity compared to others (e.g., birds and mammals), in which this strategy is under-represented. In addition, the putative links between allelic combinations and their associated respective reproductive strategies (semelparity versus iteroparity) rely on the personal choice and convenience of the modeler, and hence are subject to speculation. Based on field and laboratory research on the reproductive traits of the viviparous Aspik Viper (*Vipera aspis*), this chapter proposes a different approach and a novel scenario for the tendency toward semelparity observed in a snake population monitored in the field. The main purpose of this scenario is to provide rational links between physiological requirements for reproduction, lifetime reproductive success (a proxy of Darwinian fitness) and demographic consequences. This scenario is testable both in the field and in the laboratory and consequently it also opens a door for modeling, criticisms and generalization.

17.2 WHAT DOES SEMELPARITY MEAN?

17.2.1 From the Myth

Sémélé, the daughter of the king of Thebes named Cadmos, was strikingly beautiful (Hesiod 700BC). She was also a mistress of Zeus who commanded every mortal and the Gods. Hera (Zeus' wife) became aware of the love affair and she hatched a cruel revenge. Using a subterfuge,

Era instilled a fatal doubt in the heart of Sémélé. Tormented, the princess asked Zeus to take an oath to fulfill her most desired vow. Because nothing was supposed to be impossible to him, Zeus imprudently swore the oath. Sémélé then asked Zeus to present himself in his full glory to prove he was Zeus and not a usurper. Despite desperate attempts to get free from the oath in order to not kill his most beloved Sémélé, Zeus complied. As no one, divine or mortal, could sustain the manifestation of Zeus in his full power, Sémélé died under raging thunderbolts. At the time Sémélé was pregnant and Zeus rescued the unborn child from Sémélé by sewing it into his thigh (and thereby was consequently a successful precursor to Rick Shine in manipulating viviparity). A few months later, Dionysus was born, and became reputed for his own reproductive career (Ovidius Naso 8).

17.2.2 To the Concept

Likely, the term semelparity derived from this myth (in Latin “pario” means giving birth). Death following reproduction has been documented in various species from a wide diversity of taxa, across bacteria, plants, and almost all animal classes (Cole 1954; Wodinsky 1977; Finch 1990; Young and Augspurger 1991; Stearns 1992; Rodhouse 1998; Hautekète *et al.* 2001; Karsten *et al.* 2008; Mayor *et al.* 2009). Several authors even employed unbridled terminology such as “suicidal reproduction” to describe this strategy (e.g., Smith and Charnov 2001). However, all researchers agree that death is a consequence of an intense reproductive effort, and not a consequence of a voluntary sacrificial behavior; therefore research has focused on the costs versus benefits of various reproductive investments per reproductive bout (Stearns 1992; Hautekète *et al.* 2001; Crespi and Teo 2002). In fact, semelparity represents one extremity of a reproductive strategy gradient, with long life species that can reproduce many times representing the other extreme. Although Cole (1954) was probably not the first to coin the term, he published a seminal article comparing population consequences, notably lifetime reproductive success, associated with the respective life history traits (such as maturity and fecundity), of semelparous and iteroparous organisms. Cole (1954) produced the first significant mathematical model that provided a solid background to further developments. From this pivotal publication, major improvements and multiple refinements permitted the resolution of early paradoxical conclusions, notably via incorporating various key factors such as survival rates of different age classes, competition, stability of population dynamics, density dependence and resource availability (Bryant 1971; Charnov and Schaffer 1973; Young 1981; Bulmer 1985; Ranta *et al.* 2000a, b; 2002; Davydova *et al.* 2005; Zeineddine and Jansen 2009). One of the main outcomes of the numerous developments is a shift away from a simple dichotomized selection scheme (e.g., the r-K selection paradigm) towards more realistic multi-polar systems (Stearns 1992; Benton and Grant 1999; Reznick *et al.* 2002). There is no doubt that this theoretical progress

represents the major contribution to our understanding of the evolution of semelparity as an extreme reproductive strategy.

However, whatever the level of sophistication of theoretical developments, modeling relies heavily on demographic parameters (e.g., maturity schedule and density) in relation to environmental factors (e.g., predation and resource availability). Therefore, an important step from genetic make-up to population make-up was bypassed: the genetic and physiological regulatory mechanisms that underlie phenotypes (Franklin 1989; Stern 1998; Zera and Harshman 2001; Nachman *et al.* 2003; Abzhanov *et al.* 2004; Burggren and Warburton 2005; Carroll *et al.* 2005; Breuker *et al.* 2006; Davidson and Erwin 2006). Various studies clearly reveal that endocrine systems effectively play a central role in the negative consequences of reproduction for parental survival, thereby establishing functional links between the physiology of reproduction and semelparity (Wodinsky 1977; Bradley *et al.* 1980; Carruth *et al.* 2002; Barry *et al.* 2010). Other physiological parameters such as metabolic mode and hormonal regulation of follicle recruitment, although probably largely involved, have not yet been integrated.

17.2.3 Sex and Metabolic Modes

Strictly speaking, semelparity refers to species in which individuals die after a unique reproductive bout (Cole 1954; Zeineddine and Jansen 2009). Implicitly, this notion applies to species that reproduce sexually; and thus excludes vegetative reproduction where individuals cannot die from reproduction. For simplicity, I also discard social species (e.g., social ants) where the distinction between the individual's and the colony's life history traits, notably the reproductive strategy, is somewhat tricky (i.e., is it the queen or the colony that can be semelparous? If so what is the appropriate time scale to consider?). From this starting point, an important distinction in terminology should be raised when sex and lineage are taken into account. In plants, hermaphroditic or dioecious species classified as semelparous effectively die shortly after reproduction (e.g., annuals) and the terminology is strictly respected. In the animal kingdom, whatever the phylum, researchers classify females as typically semelparous when they die after their first and thus unique reproductive episode; this implies a single reproductive event before death (Morse and Stephens 1996; Bilde and Lubin 2001; Crespi and Teo 2002; Morse 2009; but see Futami and Akimoto 2005 for an interesting complication). In mammals however, males are considered as semelparous if they die after a single reproductive season, irrespective of the number of copulations or the number of different partners; and in fact multiple copulations with different females seems common (Bradley *et al.* 1980; Oakwood *et al.* 2001; Smith and Charnov 2001; Holleley *et al.* 2006; Martins *et al.* 2006). Such a flexible definition does not apply equally for other lineages where semelparity is generally considered under its absolute definition. For instance, male spiders are

usually classified as semelparous if they die after a unique copulation (generally killed by the female), with extreme cases where the transfer of sperm necessitates the perforation of the male by the fangs of the female to secure the mating (Andrade 1996); but usually not if they die after a reproductive season with multiple mating events. Apparently trivial, this neglected distinction has an immediate broad consequence. Strict (or absolute) semelparity has been observed in ectotherms solely. Indeed, semelparity remains undocumented for endotherm females, and the application of the definition remains unclear for males. I therefore suspect that metabolic mode is a key factor for the expression of semelparity.

In the following paragraphs, I report a case study that identifies the proximal physiological and environmental factors, along with their interactions, that favor semelparity in a free ranging ectothermic vertebrate, a viviparous snake. Long-term studies of *Vipera aspis* in France suggest that under certain environmental conditions this species exhibits a marked trend toward semelparity (Bonnet *et al.* 2002). As males are clearly iteroparous, the focus is on females. Most of the results discussed below have been published in previous articles. Importantly, the results presented have never been assembled to form a broader picture and the scenario currently proposed was only slightly evoked in a paper published a decade ago (Bonnet *et al.* 1998). The synthetic approach is therefore the main contribution of this chapter. I fully realize that proposing a novel scenario for the evolution of a reproductive strategy is somewhat perilous, especially using a single species, and I therefore emphasize that my goal is essentially to attract criticisms and to stimulate further discussion.

17.3 A CASE STUDY FROM REPRODUCTIVE PHYSIOLOGY TO POPULATION DYNAMICS

17.3.1 Overview

In western central France, reproductive females (more than 500 individuals monitored in the field as well as laboratory studies) exhibit a low survival rate and consequently tended to breed only once during their lifespan. Prior to reproduction females store large amounts of body reserves in well-developed fat bodies, the liver, locomotor muscles and a dense vertebral skeleton. Immediately after emergence from hibernation in spring, a sharp elevation of plasma levels of oestradiol (oestradiol-17 β) provokes the mobilization of maternal body reserves (e.g., lipids, amino-acids, minerals etc.) required for yolk formation. Such a physiological shift is associated with a marked exposure to predators because reproductive females must often bask in the sun to meet the high temperature (metabolic) requirements for vitellogenesis. Similarly, gestation imposes strong metabolic and predatory costs notably through important sun basking behaviors required to elevate body temperature (hence oxygen consumption of the entire organism). Importantly, a substantial proportion of the costs of reproduction are

independent of fecundity. Indeed, the optimal body temperatures (close to 30°C) for vitellogenesis and embryogenesis are not influenced by offspring number; hence maternal metabolism and the risk of being killed by a predator are determined by reproductive status rather than fecundity. To minimize such costs, selection favors a maximal increase of fecundity per reproductive bout instead of iteroparity. Capital breeding offers a solution to increase offspring number. Under constraining climatic or environmental conditions this strategy can evolve towards semelparity.

17.3.2 Reproductive Phenology of Females in Western Central France

Vipera aspis is a medium sized polymorphic viviparous snake (~50 cm snout-vent length for adult females) distributed in France, Switzerland and Italy. Current taxonomy is still imprecise and subject to discussion. The species “Aspic Viper” probably includes a complex of species and/or sub-species (Golay *et al.* 2008; Barbanera *et al.* 2009). This snake has been the subject of numerous ecological and physiological studies (Saint Girons 1957a,b; Naulleau 1970, 1973; Naulleau and Bidaut 1981; Naulleau and Fleury 1990; Saint Girons *et al.* 1993; Bonnet *et al.* 1994, 2001; Naulleau *et al.* 1999; Zuffi *et al.* 1999; 2009). Consequently, the reproductive biology and ecology of *V. aspis* is relatively well studied.

The phenology of reproduction is variable within and among populations; with climatic factors playing a major role and generating strong geographic and inter-annual fluctuations. In western central France, females usually emerge from hibernation in March after a succession of sunny and windless days. Mating is observed in March and April. Roughly, one third of the adult females undergo vitellogenesis immediately after emergence, and the two other thirds skip reproduction for one year or more. Vitellogenesis is a physiologically demanding process that broadly lasts three months with ovulation occurring in early June, followed by fertilization. Gestation takes place in summer, and parturition occurs from late August to late September. With the cooling of the ambient temperatures in October, the snakes progressively return to their winter shelter for a four-month hibernation period on average.

17.3.2.1 Methods

The results used in the current manuscript were obtained in the field and in captivity, several individuals being transported from one field site to the laboratory for various (usually short: days or weeks) periods of time, and often released at their initial place of capture. The main field site, Les Moutiers-en-Retz (Loire Atlantique, 47°03'N, 02°00'W), is situated at the western-northern limit of the distribution area of the species. Captive studies were conducted in the CEBC-CNRS also situated in western central France. The field and laboratory sites are separated by 120 km and are characterized by similar climatic oceanic conditions. Several females that were involved in the laboratory observations were captured in other

locations. These additional field sites were located in a radius of 50 km around the CEBC-CNRS. Overall, all the snakes involved originated from previously interconnected populations living in a relatively homogenous oceanic climate characterized by a low occurrence of prolonged hot sunny periods. This means that the snakes must often bask in the sun to reach elevated body temperatures, especially in spring and in autumn.

The main population of *Vipera aspis aspis*, monitored in the field between 1992 and 2008 in Les Moutiers-en-Retz, was large and well isolated by roads and a village. Guy Naulleau initiated this population study with XB, and over time more than 500 females have been marked individually and regularly recaptured. Broadly 150 gravid females were captured shortly before parturition, brought to the lab and maintained in individual cages until they gave birth. This technique allowed us to collect precise data on litters and post-parturient females. Newborns, stillborns and undeveloped eggs were counted and measured. Soon after the birth, females were released with their progeny at the last place of capture. Each year, field sessions were organized from hibernation emergence until the return to the winter hibernacula in October. The snakes were sexed, measured (± 1 cm) weighed (± 1 g), individually marked with PIT tags and precisely (± 2 m) localized on a map. During successive recaptures, females were palpated to detect the presence of prey, follicles and embryos. Each year, ten to thirty females were fitted with temperature sensitive radio-transmitters (forced ingurgitation) and radio-located at least once a day for one to five consecutive months. Since 2002, the population declined sharply following pine tree plantation that entailed a rapid closing of the habitat, and the results presented here are limited to the field research undertaken between 1992 and 2002.

In parallel studies, we investigated different aspects of the reproductive physiology of captive females maintained under natural climatic conditions in outdoor enclosures. Females were weighed regularly, at least once a week, and fed regularly with dead mice. Reproductive individuals were placed in individual cages before parturition in order to obtain data on their litter. Palpation determined reproductive status around mid-vitellogenesis (follicles larger than 2 cm) and estimated litter size with almost no error. Another set of females was maintained in individual cages fitted with artificial grass substratum, a water dish, a shelter and a source of heat (halogen lamp). Several females were examined using ultra-sonography and nuclear magnetic imaging (Bonnet *et al.* 2008). These two techniques were useful to monitor developing follicles during vitellogenesis and embryos over the entire pregnancy. Blood of both reproductive and non-reproductive females (along with males) was sampled via intra-cardiac punctures. Several steroid hormones (oestradiol-17 β , progesterone and testosterone) and plasma metabolites (total calcium, phosphorus, triglycerides, glucose, total protein, and albumin) were assayed over the complete annual cycle, thereby encompassing the reproductive cycle and hibernation (see Bonnet *et al.* 1994 for description of the methods).

We also studied individuals kept under controlled laboratory conditions. This methodology was essential to identify and better describe the key phase of the mobilization of maternal resources during vitellogenesis. Using indirect calorimetry (oxygen consumption), we assessed the metabolic rate of adult reproductive and non-reproductive females placed under different ambient temperatures. Individuals found dead in the field (killed by predators and road kills) or in the lab (emaciated and/or anorexic snakes) were dissected in order to determine their body composition. More details on the techniques and methods briefly presented above are available in previous publications (Bonnet *et al.* 1994, 1999, 2000, 2001a,b, 2003a,b, 2008; Bonnet and Naulleau 1996; Naulleau and Bonnet 1996; Naulleau *et al.* 1999; Vacher-Vallas *et al.* 1999; Aubret *et al.* 2002; Lourdais *et al.* 2002a,b,c, 2003; Ladyman *et al.* 2003).

17.3.2.2 Maternal reproductive physiology: Plasma metabolites and hormones

Vitellogenesis involves a succession of physiological stages from the initial recruitment of the follicles that will then undergo full development to ovulation. Although all the successive phases are essential, I will focus on the aspects that are the most demanding in terms of resource investment following early recruitment steps. Broadly speaking, the results presented concern follicles larger than 0.5 cm in diameter.

The analyses below combine data collected directly in the field, in captive snakes maintained in outdoor enclosures and in laboratory-maintained individuals. Our empirical and experimental data clearly show that during the spring phase of vitellogenesis, immediately after emergence from hibernation until ovulation in June, the maternal organism is heavily oriented to fuel the demands of the rapidly developing follicles. Over an average of three months, reproductive females display distinctive physiology and ecology compared to non-reproductive females. Such divergence is so pronounced that for many plasma parameters non-reproductive females are more similar to males rather than to reproductive females (Fig. 17.1). The main maternal body reserve components are involved (Fig. 17.1). Importantly, the plasma concentration values of the metabolites presented below were recorded in non-digesting snakes because of the confounding effects of postprandial physiology. The plasma metabolite concentration values result from the mobilization of maternal reserves from different storage tissues that are directed to the follicles, with a fundamental detour for most of them via the liver that synthesizes yolk precursors; consequently they mix various types of elements. For example the total plasma calcium incorporates the portion bound to the vitellogenin. Below, the main types of maternal reserves are described in a concise manner.

- 1) Mineral reserves (Fig. 17.1A): the maternal skeleton is heavily mobilized during vitellogenesis; the vertebrae are subject to a deep demineralization (osteoporosis) that results in extremely high plasma

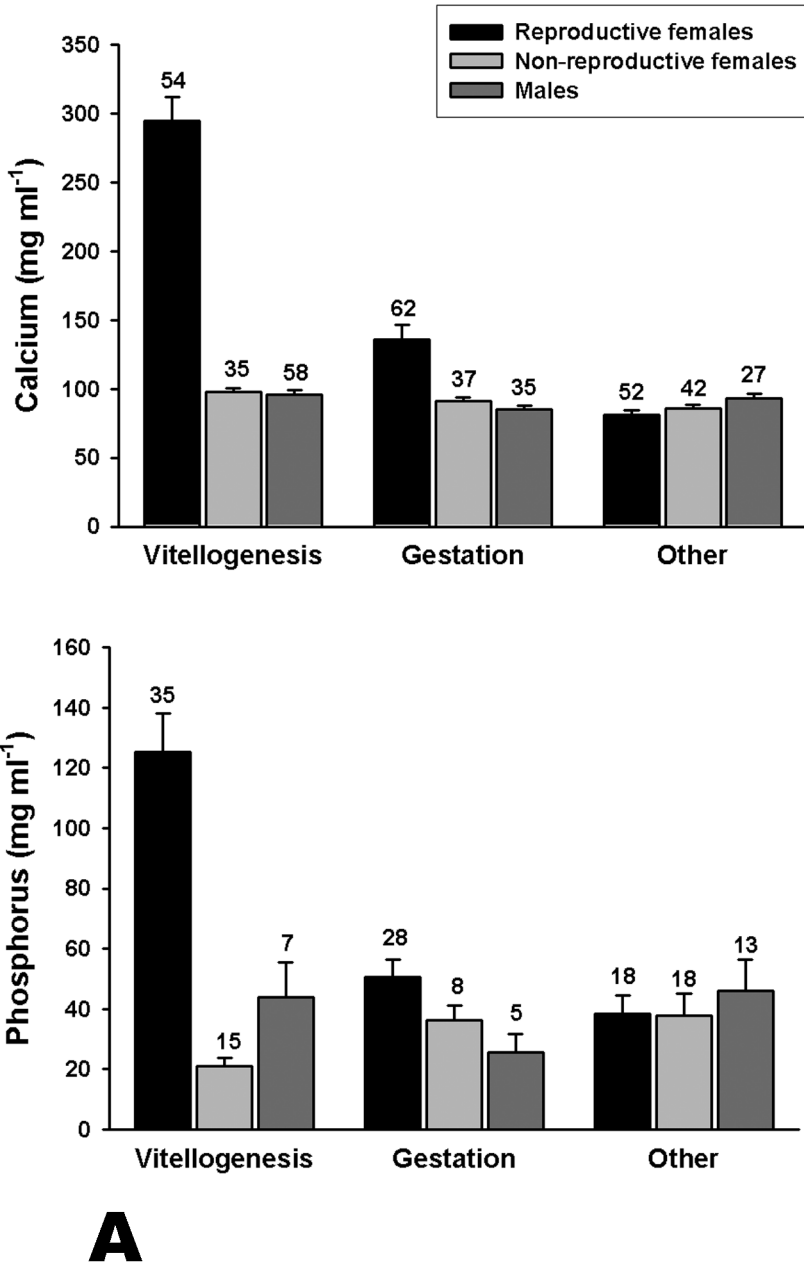
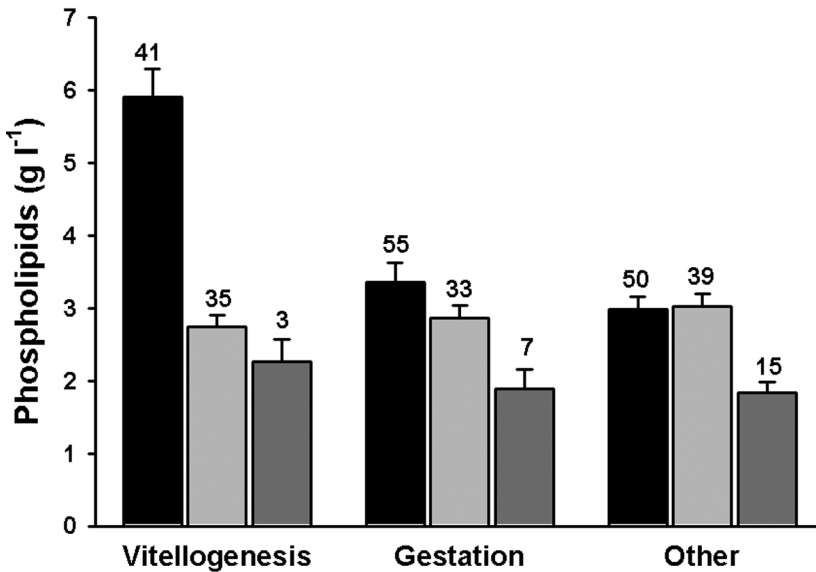
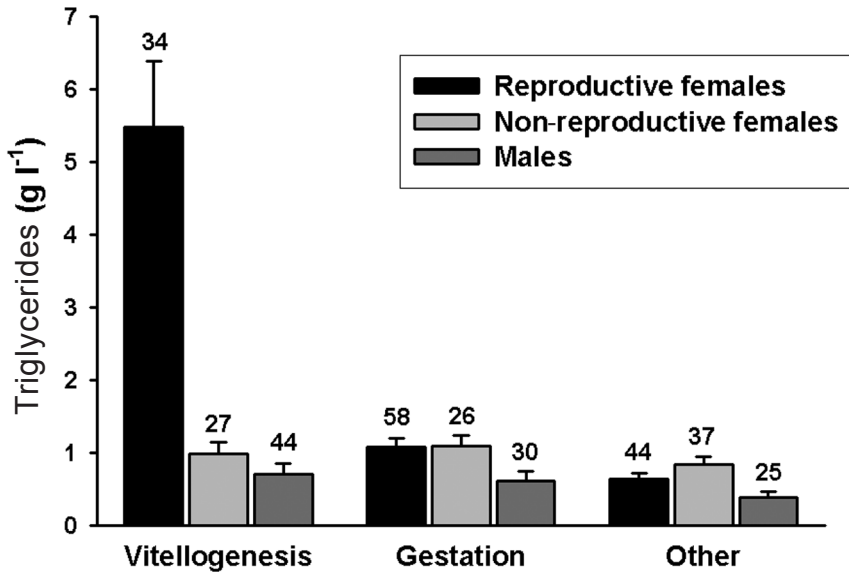


Fig. 17.1 Comparison of plasma metabolite levels of reproductive female *Vipera aspis* (black bars) relative to non-reproductive females (light grey bars) and males (dark grey bars) during vitellogenesis (~3 months), gestation (~3 months), and after parturitions (other, ~2 months before hibernation). **A.** The very high calcium and phosphorus plasma values observed in

Figure 17.1 Contd. ...

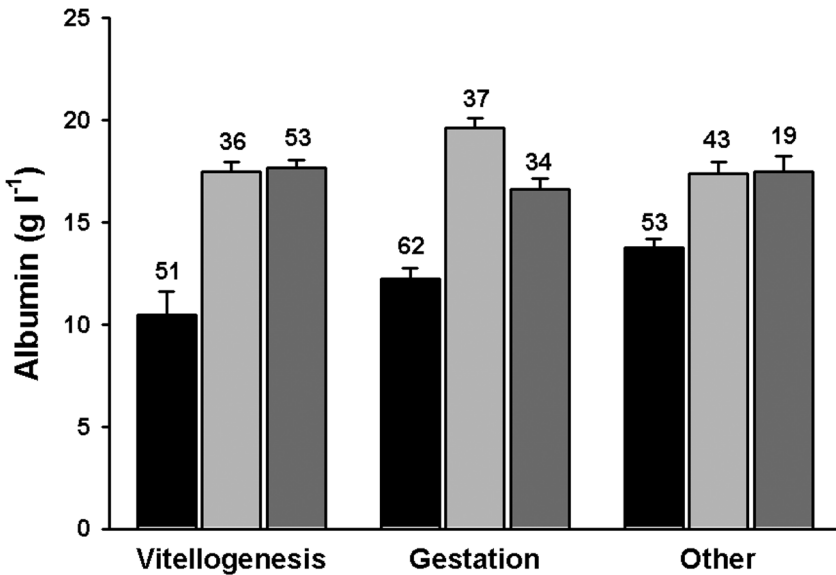
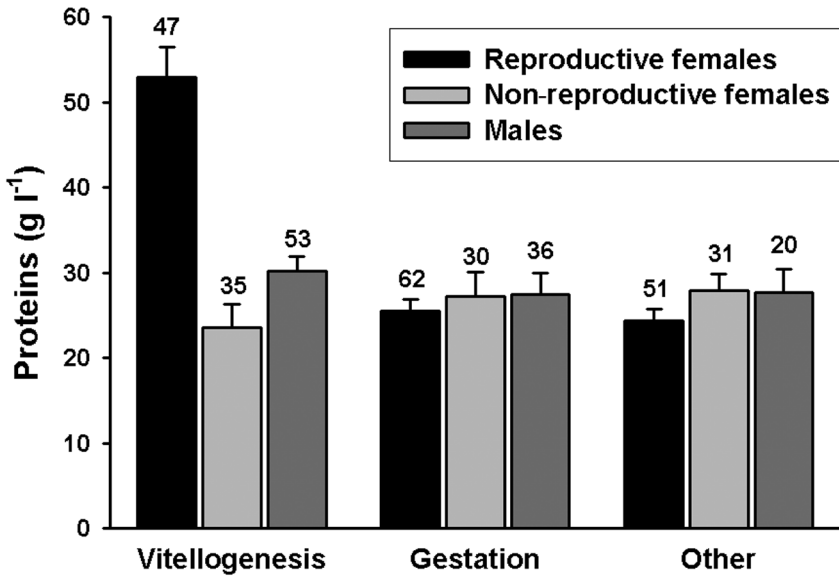


B

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vitellogenic females are caused by the strong mobilization of maternal skeleton. **B.** Similar patterns are observed for the metabolites involved in the mobilization of maternal lipid body

Figure 17.1 Contd. ...



C

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reserves (tryglycerides, phospholipids). **C.** The massive synthesis of vitellogenin (yolk precursor) by the liver entails both an elevation of plasma protein concentration and a decrease of albumin levels due to a trade-off between vitellogenin *versus* albumin syntheses by the hepatocytes. Means \pm SE, sample size above each bar.

values of total calcium and phosphorus (Alcobendas 1989; Alcobendas *et al.* 1991; Bonnet *et al.* 1994). Both elements are essential for the formation of the yolk. The mean plasma value calculated over three months, close to 300 mg per ml (maximal value >500 mg/ml), is amongst the highest ever recorded in vertebrates (including pathologies). After ovulation, the plasma values return to normal concentrations as observed in non-reproductive females and males.

- 2) Lipid reserves (Fig. 17.1B): similarly, the rapid mobilization of the lipids stored in the form of fat bodies and in the liver provokes an extreme elevation of the plasma values. In fact, the plasma becomes very thick, with values culminating above 20 g/l for triglycerides and above 10 g/l for the phospholipids.
- 3) Vitellogenin synthesis: The liver produces and releases this macromolecule into the general circulation, this entails a strong elevation of plasma proteins (indeed vitellogenin is a large phospho-lipo-protein that binds calcium; Fig. 17.1C). Such production requires the mobilization of large amounts of amino acids, and consequently the locomotor muscles are subjected to a marked catabolism (also observed in other snakes species; Lourdais *et al.* 2004). Interestingly, the production of albumin drops during vitellogenesis (this plasma protein is not incorporated into vitellogenin), suggesting that the liver cannot simultaneously increase drastically the production of vitellogenin and maintain the production of albumin. Such a trade-off between competing functions has been observed at the cellular level: the experimental hormonal stimulation of vitellogenesis by the hepatocytes massively recruits the intra-cellular machinery (e.g., mRNA synthesis), and other protein biosyntheses are markedly slowed (Ho *et al.* 1982; Callard *et al.* 1990).

Overall, over three consecutive months, the massive changes in plasma metabolite concentrations observed in reproductive females reveal a pronounced and prolonged physiological shift. In terms of mobilization of maternal resources, vitellogenesis is a more extreme process than gestation and there are major metabolic and behavioral consequences associated with the development of the follicles. Surprisingly, almost all field ecologists ignore this crucial phase whilst gestation has received considerable attention. I do not dispute the importance of gestation, but I suggest that neglecting vitellogenesis may lead to erroneous, or at least incomplete, understanding of the reproductive biology and ecology of many animal species that produce large and/or numerous follicles. For the purpose of the current manuscript, the drastic shift imposed by vitellogenesis over three months on the maternal organism in terms of investment in body reserves, metabolism and behaviors (see below) is a major element that favors semelparity as explained below. Information on the mechanisms that control vitellogenesis is therefore interesting.

Our data clearly established that oestradiol is the key hormone that initiates the massive mobilization of all the maternal reserves during the

spring phase of vitellogenesis (Fig. 17.2). An artificial increase in plasma oestradiol provokes identical changes in the plasma composition compared to naturally vitellogenic females, including the marked decline in the plasma levels of albumin (Bonnet *et al.* 1994). In all vertebrate species investigated, oestradiol has been identified as the primary stimulus for vitellogenesis in the hepatocytes (Ho *et al.* 1982). However, in snakes this role has been questioned (Saint Girons *et al.* 1993). I suspect that this disagreement was simply the result of the misclassification of the females (reproductive versus not), especially as our experimental data are particularly clear regarding the influence of elevated circulating levels of oestradiol on the mobilization of body reserves. The other important steroid hormone involved in the regulation of female reproduction, progesterone, is not (or perhaps indirectly through complex mechanisms?) involved in the mobilization of maternal reserves; an elevation of plasma values was observed in pregnant females only, hence after the completion of vitellogenesis. In addition, experimental elevation of the plasma concentration of progesterone was not accompanied by the characteristic oestradiol induced changes in the plasma metabolite composition (Bonnet *et al.* 2001a). Overall, oestradiol is the key hormone for the drastic mobilization of the maternal body reserves required to fuel vitellogenesis, which induces (directly or indirectly) a rapid catabolism of the vertebrae, fat stores, locomotor muscles, and liver, and that also stimulates the synthesis of vitellogenin by the liver. Because the concentrations we observed are particularly elevated, I suspect that the variations in plasma levels of this steroid are an important determinant of reproductive effort (although the complex but not-investigated systems that involve the receptors and numerous intra-cellular regulations also play

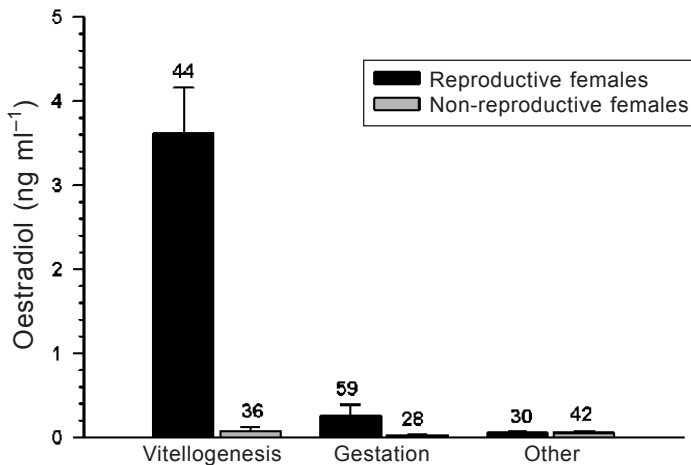


Fig. 17.2 Oestradiol (E2) is the key hormone in the extremely intense mobilization of maternal reserves during vitellogenesis in *Vipera aspis*. Three main periods of female cycle are considered: vitellogenesis (~3 months), gestation (~3 months), and post-parturition (~2 months before hibernation). Means \pm SE, sample size above each bar.

central roles). The other major hormones that likely control the number of follicles at an early stage of vitellogenesis (gonadotrophins and leptins; Gobbetti *et al.* 1994; Schneider *et al.* 2000) remain virtually unexplored in snakes in general.

17.3.2.3 Changes in body condition over time

Considering the prolonged and intensive phase of spring vitellogenesis, we may expect significant consequences for maternal condition with an increasing depletion of reserves during the course of reproduction. Using different techniques (e.g., dissection of accidentally killed females and NMI, Nuclear Magnetic resonance Imaging) we observed that at the end of vitellogenesis females are very emaciated, despite an apparent external high body condition, in fact slightly higher than at the onset of vitellogenesis (Bonnet *et al.* 2003b). Comparison of the main body reserves of adult females dissected (death was always accidental, hence sample sizes follow availability of dead snakes) shows that at the onset of vitellogenesis, reproductive females possess large body reserves (fat bodies, liver and muscles), but that almost all the reserves have been transferred to the follicles by ovulation, and that post-parturient females are even more emaciated (Table 17.1). Other (non-reproductive) adult females are in intermediate position. In the course of vitellogenesis, almost all the fat bodies have been degraded, two thirds of the liver also, and a large proportion of the body (essentially represented by locomotor muscles) as well. Gestation generates further degradation of maternal reserves, and after birth, the females have almost no body reserves. Non-reproductive females are not emaciated, but their body reserves are significantly reduced compared to reproductive females at the onset of vitellogenesis; they can be considered as being in an intermediate stage.

Table 17.1 Dissections of adult female *Vipera aspis* provided the mass of the main body reserve tissues at different stages (1-4). 1: "Vitello" means at the onset of vitellogenesis (N=14), 2: "Ovulation" means shortly after ovulation (N=5), 3: "Parturition" means after parturition and before hibernation (N=31), and 4: "Other" means females not reproductive during the year they died (N=27). The last columns refer to comparisons between the stages, e.g., 1/2 is the comparison (%) between the masses of the body reserves observed in ovulated versus early-vitellogenic females (Bonnet *et al.* 2003b). The mean body size (SVL) was not significantly different among four categories.

Tissue (g)	1 Vitello	2 Ovulation	3 Parturition	4 Other	1/2	2/3	1/3	1/4
Fat	15.5±6.1	2.6±1.8	1.7±1.5	7.8±4.5	83%	34%	89%	50%
Liver	10.8±6.7	3.2±1.5	3.1±1.4	5.2±2.1	70%	2%	71%	52%
Carcass	77.5±23.9	46.1±8.9	41.6±10.2	58.2±12.3	41%	10%	46%	25%

These results show that at ovulation reproductive females are already in poor body condition, although large ovulated eggs distend their abdomen. During the course of gestation they use the small amounts of reserves left, and their extremely poor condition is clearly revealed after parturition:

large abdominal skin folds and very poor stamina characterize this category of females.

Given the marked physiological differences between reproductive and non-reproductive females, we may expect parallel divergences in terms of their behavioral ecology in the field.

17.3.2.4 Field behavior

Reproductive females are considerably more often exposed, basking in the sun, compared to non-reproductive females (Bonnet and Naulleau 1996). On average the annual capture frequency of individual reproductive females was 2.64 ± 1.74 (mean \pm SD, range 1-8 per year) captures per year, and 1.76 ± 1.02 (range 1-5 per year) captures per year in non-reproductive females (Bonnet and Naulleau 1996). As a result, most of the females captured in the field are reproductive, although non-reproductive females represent an average two thirds of the adult female population. Part of the greater visibility of reproductive females is explained by mating behavior in March and April, but the trend for a higher capture rate of reproductive females increases over time and is not associated with copulation: the proportion of reproductive females shifts from 45% to more than 70% between March and late August (Bonnet and Naulleau 1996). This is largely due to the progressive increase of ambient temperatures. In early spring when air temperatures often remain below 15°C, non-reproductive females are forced to bask in the sun for digestion, sloughing and to forage; in summer they can reach a satisfactory body temperature (25-30°C) with limited sun basking, indeed air temperature is often above 25°C. Consequently, non-reproductive females become progressively more and more secretive. By contrast, reproductive females are relatively easily observed in the field while the air temperature is below 25°C whilst they still need to bask in the sun basking to warm up their body to meet their specific reproductive metabolic requirements. This is typically the case after rainfall or during the first half-hour after sunrise, even in summer. These results show that during vitellogenesis and gestation reproductive females are under strong pressure to maintain their body temperature close to the maximal plateau body temperatures recorded in the field, 30-31°C, with brief peaks at 32-35°C. The difference in visibility is associated with a divergence in the risk of predation: at our field site, raptors, the main predators for *Vipera aspis* (Naulleau *et al.* 1997), patrol the area intensively during the entire activity period of snakes.

Body temperatures (N>2500) recorded from April to November in the field using internal temperature-sensitive transmitters revealed that reproductive females (N=18) maintain their body temperature an average of 3 to 4 degrees above the body temperature recorded in non-reproductive females (N=9). Interestingly, this difference is significant at any time of the day, even at night, suggesting that reproductive females select warmer night refuges than non-reproductive females. In summer, we also observed that non-reproductive females select cool (below 20°C) refuges when they

are not involved in physiologically constraining processes such as skin sloughing or digestion. This means that not only do reproductive females select elevated and stable body temperatures in order to process and rapidly metabolize resources required for vitellogenesis and for precise thermoregulation to provide optimal temperatures to the developing embryos during gestation, but also that non-reproductive females select low body temperatures likely to minimize their metabolic costs when possible. Interestingly, in reproductive females, the mean distance traveled per day and home range decreased rapidly after ovulation (from 5.1 m/day to 2.7 m/day and from 3,187 m² to 670 m², during vitellogenesis [3 months] and gestation [3 months] respectively; Naulleau *et al.* 1996). This behavioral shift corresponds to two contrasting phases in terms of physiological priorities: during vitellogenesis reproductive females are foraging actively in search of prey (voles) to be invested into the yolk (Bonnet *et al.* 2001b), but during gestation they are essentially involved in careful thermoregulation to minimize thermal instability of the developing embryo, an activity somewhat antagonistic to foraging.

Overall, capture rate and body temperature data are complementary; they both indicate that reproductive females spend considerably greater amounts of time basking in the sun to reach elevated temperatures and/or to maintain precise thermoregulation profiles, whilst non-reproductive females minimize their exposure to avian predation and their energy expenditure.

Considering the physiological and behavioral information presented above, it is obvious that reproduction generates cumulative survival risks: indeed, the metabolic requirements of vitellogenesis and gestation result in an almost complete depletion of body reserves, but they also expose reproductive females to an increased predation risk. Therefore, population dynamics of adult females should be largely influenced by reproductive status.

17.3.2.5 Population consequences

In our study population, each year an average of two thirds of the adult females skip reproduction. The very emaciated post-parturient females (from the previous year) represent part of the non-reproductive females, but apparently healthy females that are in an intermediate body condition are the most abundant snakes in this non-reproductive category. These proportions have been calculated using the capture-recapture technique and individual monitoring over several years: adult female *V. aspis* cannot reproduce every year in our study site, and they require an average of two years to restore their body reserves after parturition (Naulleau and Bonnet 1996; Bonnet *et al.* 1999, 2002). This extended recovery process means that most of the females cannot reproduce twice in their life. Indeed, roughly fifty percent of reproductive females do not survive the long, exhausting and perilous period of vitellogenesis + gestation + parturition (see above). The surviving post-parturient females that can safely hibernate are in very

poor body condition most of the time (Table 17.1); about half of them die from extreme emaciation the next active season following spring emergence (Bonnet *et al.* 1999; 2002). The remaining females that are able to capture prey rapidly after hibernation (and hence escape lethal emaciation) must nonetheless survive for two years on average to restore their body condition in order to be able to reproduce again. For that, they must also evade predation during their second period of vitellogenesis and gestation.

From a simplistic perspective, the chances for a female *V. aspis* to breed more than once are limited to 13% in our study area. This value results from the combined survival rates calculated during the first reproductive year (survival of reproductive females is ≈ 0.50), during the recovery year after birth (survival of the very lean post-parturient females is ≈ 0.50), during the following body year of restoring body reserves (survival of intermediate non-reproductive females is ≈ 0.80), and during the second reproduction (survival rate is ≈ 0.65 to parturition; the immediate post-parturition low survival is ignored here). We marked and monitored more than 500 females in the field. Of these, we captured 148 shortly before parturition in order to keep them briefly in captivity to collect precise data on their litters. Some of these females were captured more than once, indicating that they were iteroparous. Interestingly, this number of iteroparous females ($N = 19$) matches almost perfectly the expected number based on our calculated survival rate: 19.2 (148×0.13). Therefore, the average survival rate estimated for the different categories of adult females using the CMR technique (capture-mark-recapture) is somewhat validated by the average respective proportions of semelparous (90%) and iteroparous (10%) females that we have been able to capture and bring to the laboratory for precise examination of their reproductive output.

On average, female *Vipera aspis* exhibits a strong trend toward semelparity, with roughly 90% of the individuals reproducing only once, and the breeding frequency of the few remaining iteroparous females is very low. In fact, these proportions are derived from averaging long-term monitoring data, and significant annual fluctuations have been observed (this important aspect will not be discussed in the current ms; but see Bonnet *et al.* 2000). In terms of the strict definition, *V. aspis* is not semelparous, but our study population is clearly oriented toward this extreme strategy. Strictly speaking, the *V. aspis* is not semelparous, complicating any generalization about this extreme strategy. However, the flexibility of post-reproduction survival that is apparent from our field data provides an opportunity to compare iteroparous versus semelparous individuals, otherwise impossible in species where post-reproduction death is obligate.

17.3.2.6 Lifetime reproductive success

Although poorly represented in our data set, the few iteroparous females that we monitored provide an opportunity to assess important questions. Do semelparous and iteroparous females partition their reproductive

effort differently? Does the total number of offspring produced (Lifetime Reproductive Success, LRS, a proxy of Darwinian fitness) also differ? Are there morphological differences between semelparous and iteroparous females that will impart a survival advantage to iteroparous females?

We found no evidence of partitioning of reproductive effort between reproductive events ($P > 0.5$). On average semelparous females produced 6.0 ± 0.2 (mean litter size adjusted by maternal SVL \pm SE) offspring ($N = 127$ litters), females that reproduced twice produced first 6.1 ± 0.5 and then 7.1 ± 0.5 ($N = 17$ litters) offspring; finally, females that reproduced three times produced successively 5.8 ± 1.0 , 5.9 ± 1.0 , and 6.3 ± 1.0 ($N = 4$ litters). The cumulated mean number of offspring increased linearly ($N = 3$) with the number of successful reproduction events. Clearly, the LRS of iteroparous females increases with reproductive events.

We found no difference between the morphological characteristics of semelparous ($N = 19$) and iteroparous ($N = 129$) females. Both groups were indistinguishable for body size (SVL) and early body condition (recorded at the onset of vitellogenesis) (unpublished). We extended the analyses by incorporating females for which the reproductive strategy could be determined but for which detailed information on reproductive output was not collected (to ensure that capture rate did not falsify our results, we waited at least two years to classify a given female as dead or not; Bonnet *et al.* 1999, 2002). The results remained unchanged. Consequently we found no difference between the two categories of females at the onset of reproduction: body size and/or body reserves did not influence the probability of reproducing more than once.

Perhaps divergent reproductive investment between the two categories of females was involved. Our data do not support this hypothesis either; females that lost less body mass over the reproductive period were not more likely to breed again (Bonnet *et al.* 2002). Similarly, iteroparous females that lost more mass when producing their first litter did not delay subsequent reproduction for a longer period compared to females that invested relatively less resources (Bonnet *et al.* 2002).

However, iteroparous females produced significantly heavier offspring during their first reproduction compared to semelparous ones. Taking into account two important factors that influence reproductive output, maternal SVL and initial body condition (Bonnet *et al.* 2001b) the mean offspring mass of the females that bred only once was 6.2 ± 0.1 g (SE, $N = 52$ litters) versus 6.9 ± 0.3 g (SE, $N = 14$ litters) for the first litter of females that bred more than once. This difference was essentially due to a differential food intake between the two categories of females (Bonnet *et al.* 2001b); iteroparous females consumed more prey (Lourdais *et al.* 2000b, 2003). Food availability varied greatly between years in our study area (Bonnet *et al.* 2000). Pregnant females that were able to capture three or more voles during gestation were also in better condition after parturition (Lourdais *et al.* 2003) and were more likely to survive the following year (Bonnet *et al.* 1999).

Overall, semelparous and iteroparous females were indistinguishable in terms of body size and body reserves at the onset of vitellogenesis and in the number of offspring produced per reproductive event. We nonetheless observed a significant effect of food intake on the mean mass of the offspring and on the post-parturition body condition of the mother. Costs of reproduction represent the second fundamental aspect that must be examined.

17.3.2.7 Fecundity independent costs of reproduction (FIC) and optimal reproductive effort

The notion that low frequencies of reproduction are most likely to evolve in taxa that display a high fecundity-independent cost of reproduction was proposed 30 years ago (Bull and Shine 1979). Our data provide some evidence in support of this idea.

In the field, we found no relationship between fecundity and survival rate. The number of follicles palpated (an accurate estimate of fecundity in this species) varied from one to thirteen, and did not influence the probability of recapturing a female during the following three years ($P > 0.6$; Bonnet *et al.* 1999, 2002). In the laboratory, using three different ambient temperatures (17.5°C, 25.0°C and 32.5°C), we found no (or a very weak) correlation between fecundity and metabolism measured as oxygen consumption (VO_2 calculated in ml/g/h; Ladyman *et al.* 2003). By contrast, ambient temperature had a strong effect on oxygen consumption (Ladyman *et al.* 2003). Therefore, at least two types of potentially important costs of reproduction, survival during reproduction and temperature dependent energy consumption during pregnancy, were independent of fecundity.

These counterintuitive results have a strong consequence in terms of optimal reproductive effort. Female *Vipera aspis* are under strong selection to amortize the fecundity independent costs of reproduction: the greater number of offspring they produce per reproduction, the better the level of amortization. However, a significant amount of reproductive effort is also represented by the considerable resources invested in yolk synthesis. The balance between the two types of costs, fecundity dependent versus fecundity independent, is likely a strong determinant of optimal reproductive effort. Because relative litter mass (the ratio of total litter mass to maternal post-partum mass; often been used as a measure of relative reproductive investment) is high in *V. aspis*, close to 50% on average with extreme values above 100% (Bonnet *et al.* 2003a), and because breeding frequency is particularly low, I suggest that FIC are elevated. Further results support this notion.

17.3.2.8 Thermoregulation: A key factor

Regardless of the number of eggs (fecundity), the optimal body temperature required for a female to rapidly complete vitellogenesis (i.e., in early June to leave enough time for gestation after ovulation and before winter) is likely to be the same. Indeed, metabolic rate (the sum of the catabolism for reserve mobilization, blood transport, anabolism in the liver and

ovaries) is highly dependent on maternal body temperature but not on fecundity, and high body temperatures accelerate this physiological process. During vitellogenesis, it is expected that reproductive females would bask with the same intensity irrespective of the number of follicles they carry. Unpublished data gathered by Olivier Lourdais (CEBC-CNRS) on females fitted with temperature data loggers suggested that, during gestation, embryos are sensitive to thermal perturbations: all the gravid females maintained an identical high and stable body temperature (31°C) in order to optimize offspring phenotypes. During the entire reproductive period (6 months on average), all reproductive females tend to have identical thermoregulatory profiles (depending upon ambient conditions) irrespective of their offspring number. Consequently they take identical sun exposure risks with regard to avian predation, and the elevation of their body temperature generates an equal overall energy expenditure of the whole organism. Improving the reproductive process (vitellogenesis, gestation) via selection of high body temperatures automatically results in “parasitic” energy expenditure from the rest of the body not directly involved in reproduction (e.g., skin and muscles during gestation). For instance, an elevation of maternal body temperature from 25°C to 30°C generates a significant increase in oxygen consumption (>40%).

Thus high predation risk and significant parasitic energy expenditure represent typical FIC. Such FIC automatically decrease per offspring with increasing litter size. Our calculations (unpublished) suggest that a female should produce at least four to five offspring per reproduction. Importantly, females undergo vitellogenesis (at least the resource demanding part) immediately after winter emergence, and huge amounts of resources are required for the growth of more than four follicles. This means that the resources for the development of a large litter must be available very early during reproduction. A physiological solution is to store large body reserves prior to reproduction, and to not utilize them unless they are sufficient for the production of a large litter. In other words, this is a definition of the capital breeding strategy in which a body condition threshold is expected to dictate reproduction. This is also exactly what we observed in the field: female *Vipera aspis* do not undergo vitellogenesis unless they reach a high and precise body condition threshold (Naulleau and Bonnet 1996).

A corollary of these assumptions and observations is that we should not observe small litter sizes. However, on a few occasions, we recorded litter sizes of one to three offspring only (Bonnet *et al.* 2001b, 2003a). Repeated magnetic nuclear imaging sessions on the same individuals showed cases of “egg disappearance” during gestation. Combined with the more classical follicle atresia, these physiological processes offer an alternative explanation (Bonnet *et al.* 2008). Unexpected small litter sizes (<4) result from a reduction of fecundity after an initial “normal” recruitment (≥ 4 follicles). Repeated palpations of females maintained in captivity suggest that this process can be common in stressed females (e.g., involved in experiments that require frequent manipulations).

17.4 A NOVEL SCENARIO

By assembling the above conclusions in a single scenario I propose the following chain of causes and effects (Fig. 17.3):

- A) If the costs independent of fecundity (FIC) represent a major proportion of the total costs of reproduction compared to the costs that increase with offspring number, then it is profitable to maximize offspring number (fecundity) per reproductive event. Indeed, the invariable total FIC would be divided per clutch size, and thus the total cost per offspring equally divided. Offspring production would be automatically more efficient. Therefore, selection should favor physiological processes that enable individuals to maximize offspring number. Among others, key endocrine systems such as those involving FSH and or LH in vertebrates should represent potential targets of selection (Gobbetti *et al.* 1994).
- B) However, increasing offspring number is not an easy task because large amounts of resources are required along with space to accommodate the growing follicles. In addition, egg production and embryo development

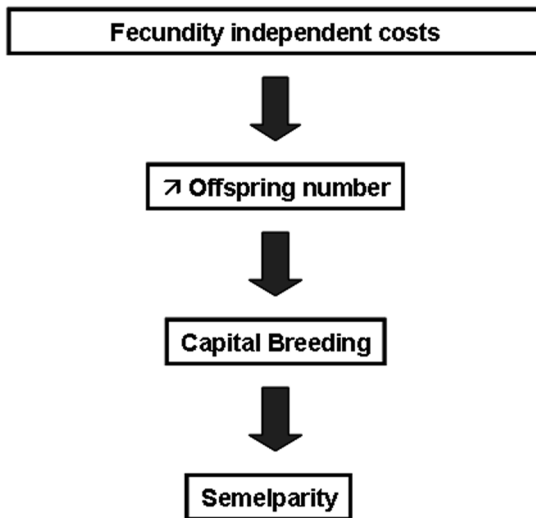


Fig. 17.3 Cascade of causalities from costs of reproduction to extreme physiological exhaustion in the course of a reproductive event. To amortize heavy fecundity independent costs of reproduction (FIC, see text) it is profitable to maximize offspring number per reproductive event. One of the best options to secure a large clutch size is to rely on a capital breeding strategy, and to make a massive reproductive effort. As a consequence, the probability of reproducing again is degraded (e.g., owing to the long time needed to restore body reserves). If FIC become predominant, selection should favor an extreme mobilization of the resources to maximize offspring number. The death of individuals after a single reproduction (semelparity) would be merely a consequence of such intense reproductive investment, not necessarily linked with other demographic traits (e.g., maturity schedule, growth rates), but strongly related to environmental factors (e.g., food availability).

might be strongly constrained by time and climatic factors. Therefore, in many cases the best option to secure a large clutch size is to rely on the accumulation of large maternal body reserves prior to reproduction. Body reserve storage necessitates both time and space, and potentially can handicap individuals (to escape predators for instance). The advantage of body reserve storage in terms of reproductive efficiency is therefore balanced by inevitable costs. A physiological system locking reproduction until sufficient body reserves have been accumulated is theoretically required to prevent inefficient investments. Storage of large body reserves prior to reproduction along with the existence of a body condition threshold to meet optimal reproductive investment requirements corresponds to a definition of a capital breeding reproductive strategy. Therefore, proportionally high FIC favors capital breeding.

- C) For species with low breeding frequency, delayed maturity, or where reproduction entails a large shift in term of survival risk (e.g., long migration observed in European eels or in different species of salmonid fishes; Crespi and Teo 2002), the probability of reproducing twice is low. In fact, most of these traits can be interpreted with respect to the FIC concept (Bull and Shine 1979). In other words, when FIC become especially survival costs over energy costs, selection should favor an extreme mobilization of the resources for reproduction in order to maximize offspring number. The death of individuals would be merely a consequence of an intense reproductive effort.

This scenario allows us to answer one of the questions raised earlier: why are some ectotherms more oriented towards semelparity compared to endotherms?

In an eco-physiological perspective, capital breeding occupies a central position for the evolution of semelparity, but this strategy is more likely to evolve in ectotherms than in endotherms (see Bonnet *et al.* 1998 for further discussion of this issue). In fact, there is as yet no endotherm species in which reproduction is entirely fueled by capital whilst abundant examples have been documented among ectotherms. If we consider that semelparity is an extreme example of capital breeding, then the virtual absence of strict semelparity observed among endotherms is expected.

Secondly, in this scenario, evolution toward semelparity does not rely on the sudden occurrence of a mutation in an iteroparous population. However, one of the basic assumptions of most of the mathematic models in the literature is that the change between semelparity versus iteroparity rests on a single mutation that provokes a rigid all-or-nothing shift between two extremes (McNamara 1997; Ranta, *et al.* 2000). The eco-physiological (bottom up?) approach developed above seriously challenges this theoretical view. It is indeed possible to envisage an uncoupling of the various traits that are usually invoked to differentiate these two strategies (Baird *et al.* 1986). For example, a species simultaneously exhibiting late maturity (Saint Girons

1957b), relatively low fecundity and semelparity would be considered an impossible chimera by currently available demographic models as mixing typical and incompatible iteroparous and semelparous traits. But this is just what *Vipera aspis* does. Such annoying examples have been simply ranked as paradoxes (Stearns 1992). In the scenario above, I propose that the targets for selection are both genetic and hormonal mechanisms that control follicle recruitment, offspring size and the physiological investment underpinning reproduction. The physiological and behavioral systems involved can be gradually modified. The case of *V. aspis* shows that the transformation from iteroparity to semelparity does not involve a rearrangement of alleles with the creation of a genetic barrier between these two extremes, but rather differential environmental constraints. Other studies have reached similar conclusions for both ectotherms and endotherms (Schmidt *et al.* 2006; Mayor *et al.* 2009). For instance different capelin populations exhibit absolute semelparity versus iteroparity strategies; such facultative semelparity results from interactions between spawning habitat, physical forcing, and predatory pressure (Christiansen *et al.* 2008). In a copepod, the probability of death following a massive reproductive investment depends upon food availability (Mayor *et al.* 2009).

17.5 FUTURE DIRECTIONS

17.5.1 Field and Experimental Studies

The notion that FIC can represent a substantial proportion of the total costs of reproduction is not familiar to ecologists, and such costs have been neglected. I suggest devoting more effort in characterizing and estimating FIC versus costs that increase with fecundity in various systems. This can be done both in the field and in the laboratory. For instance, in many ectotherms, survival and metabolic consequences of the shift from non-reproductive to reproductive status could be measured independently from fecundity, for example by estimating the higher metabolic expenditure related to the specific thermoregulatory requirements of reproduction.

Comparative studies with other species represent another important future direction. For instance, although similar data to those presented above are lacking on other snake species, there is strong evidence that the more northerly distributed Adder (*Vipera berus*) exhibits even more semelparous traits than the aspic viper (Madsen and Shine 1993; pers. obs.). Post parturient females of *V. berus* are almost systematically extremely emaciated, and many of them cannot survive whatever environmental conditions (unpublished). Comparative studies would offer an opportunity to better take into account the impact of environmental constraints. As advocated in the current paper, because *V. berus* faces cold climatic constraints, we would expect its reproductive frequency of to be even lower and closer to semelparity than in *V. aspis*. Similarly, intra-specific comparisons of populations distributed over a wide range of

climatic situations (altitude, latitude...) would be particularly useful (e.g., comparing aspik vipers from northern distribution areas with those living in relatively warm areas in several places in Italy).

17.5.2 Mathematical Modelling

Mathematical modeling could incorporate physiological constraints, for instance by estimating the fitness consequences of various combinations of FIC and fecundity dependent costs, both in terms of energy and survival. The potential links between capital breeding and semelparity could help to propose novel scenario for evolution toward this extreme strategy, a possibility clearly outlined in a salmon: "Adaptation-by-time may play an important role in life history evolution within some species, particularly those with breeding systems characterized by semelparity, capital breeding, and heritable breeding times" (Hendry *et al.* 1999). More generally, incorporating physiological constraints in relation to the environment rather than focusing too heavily on demographic parameters might be a fruitful approach (e.g., page 23 in Willson 1997). Finally, considering that some flexibility between iteroparous and semelparous individuals can occur would help to better understand genetic correlates between semelparous versus iteroparous phenotypes, for instance to better quantify the respective importance of gradual versus discrete selection, and therefore the extent of the limitation of gene flow to allow divergence (Mueller 1987; Hendry *et al.* 2003).

17.5.3 Conservation Issues

The low number of offspring produced during lifetime by European vipers in northern or alpine areas suggests that these populations are particularly sensitive to perturbations (climatic changes, habitat loss...). Therefore, it is essential to devote important conservation efforts (monitoring, habitat management...) to the species that exhibit a marked trend toward semelparity, and that simultaneously display reproductive trait typical of long live species, notably low fecundity, low breeding frequency, late maturity. Indeed, this particular combination of traits has been neglected in a conservation perspective, possibly because it was previously considered as improbable (or not easily integrated into current demographic models).

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