Reproductive and Fat Body Cycles of the Lizard, *Lacerta lepida,* in Centra!Spain

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ABSTRACT. -We studied the annual reproductive and fat body cycles of the oviparous lizard *Lacerta Jepida* in central Spain. Testicular recrudescence started in late summer and testes attained their maximal sizes during the following spring. Females exhibited ovarian growth during spring and most oviposited in July.Fat bodies of males were smallest during the mating period. Female fat body volumes declined during vitellogenesis. No significant decline of lipid stores was detected during the hibemation period (October-March).

Reproductive activities of temperate zone lizards are typically restricted to a well-defined annual period, reflecting seasonal changes of gonadal activity and size (reviews by Fitch, 1970; Duvall et al., 1982; Licht, 1984). Lipid stores, with the bulk occurring in the paired abdominal fat bodies, tail, and liver, also cycle annually, and may be used as an energy source for winter maintenance and/or reproductive processes (e.g.,Hahn and Tinkle, 1965;Telford, 1970; Avery, 1974; Derickson, 1976; Etheridge et al., 1986; Ortega, 1986). Although reproductive cyclicity has been studied in numerous temperate zone lizards,the literature on species of the palearctic genera *Lacerta* and *Podarcis* is surprisingly sparse and restricted to a few species (see Saint Girons and Duguy, 1970;Avery, 1974; Xavier, 1982; Brafia, 1984; Bauwens and Verheyen, 1985; Hraoui-Blocquet, 1987; Kwiat and Gist, 1987;Hraoui-Blocquet and Blocquet, 1988).

Lacerta lepida, the largest European lacertid lizard (adult snout-vent length 140-230 mm), is found in a variety of mediterranean habitats throughout most of Spain and Portugal, in southern France, and extreme north-west Italy. This diurnal, heliothermic, terrestrial lizard forages actively on a large variety of prey composed mainly of arthropods. Despite its conspicuousness, information on its reproductive habits is largely fragmentary and/or based on observations in captivity (review by Bischoff et al., 1984).

The present study records seasonal changes in gross gonadal morphology and fat body sizes of *L. lepida* collected in centra!Spain. Our aim is to provide quantitative information on: (1) seasonal changes of gonadal size in males and females; (2) annual cyclicity of fat body volumes;and (3) roles of fat bodies as energy stores for reproduction and hibernation.

MA.TERIALS AND METHODS

We examined 124 male and 103 female *L. lepida* from the collections of the Unidad de Zoologia Aplicada (Madrid, Spain), which had been collected at different localities in the centra! Spanish provinces of Madrid, Ciudad Real, Cáceres and Toledo between 1971 and 1986. Each specimen had the following measurements taken: (1) snout-vent length (SVL); (2) longest and shortest axes of the right testis; (3) maximum diameter of the right epithelio-epididymis; (4) diameter of the largest ovarian follicle; (5) maximum width of the oviduct; (6) length, width and height of the right abdominal fat body; (7) stage of follicular development (nonvitellogenic, vitellogenic, luteal); and (8) presence of oviducal eggs.

An estimate of testicular volume was obtained using the formula for the volume of an ellipsoid ($V = 4/37ra^2b$, where $a = 1h$ shortest diameter and $b = 1h$ largest diameter). Although fat bodies vary in shape, their general form was approximated as the longitudinal half of a flat ellipsoid with unequal lengths for the three axes. Accordingly, we calculated an estimate of fat body volume by the formula $V = 4/67rabc$, where $a = 1h$ length, $b = 1h$ width and $c = 1h$ height.

Testicular volume, maximum diameter of the epididymis,maximum width of the oviduct, and fat body volume each increased significantly with SVL. An index of SVL-adjusted dimensions for each organ was calculated using residuals (observed minus predicted value) from the least-squares regression line between organ size and SVL (both variables ln-transformed). We preferred this method of adjusting organ size to the use of ratios of organ/body size, since the latter procedure may yield biased estimates for small and large individuals when organ size
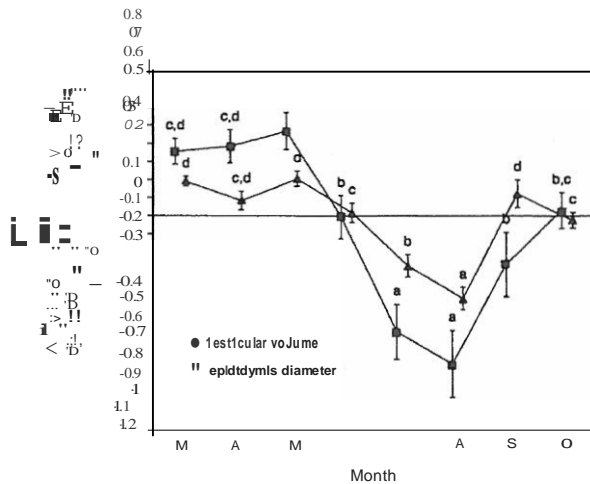
FIG. 1. Monthly changes (mean ± 1 SE) in SVL-adjusted testicular volume (squares) and maximum diameter of the epididymis (triangles) of *Lacerta lepida* throughout its activity period. For each organ size, values with common superscripts are not significantly different (Tukey's a posteriori test, $P > 0.05$).

TABLE 1. Percent of female *Lacerta lepida* in distinct reproductive states during different months. N = sample size; NVF = non-vitellogenic follicles; EVF = enlarged vitellogenic follicles; OE = oviducal eggs; PP = post-parturient.

Month	N	Percent occurrence			
		NVP	EVP	OE	PP
March	10	100	0	0	0
April	13	92	8	0	0
May	24	54	46	0	0
June	19	11	31	26	32
July	13	0	8	31	62
August	15	0	0	0	100
September	9	0	0	0	100

does not change proportionally with body size (Clutton-Brock and Harvey, 1984; Packard and Boardman, 1987).

In a study area near Navas del Rey (40°23'N, 4°15'W, province of Madrid, Spain), observations were made from April 1984 to September 1985 on the timing of emergence and onset of hibernation, courtship, mating, presence of observable oviducal eggs, oviposition, and appearance of hatchlings. Thirteen females hearing oviducal eggs were removed from the study area during June-July 1986 and were housed in outside vivaria in Madrid (40°25'N, 3°40'W). After oviposition, eggs were dug up and buried in soil-filled containers. To mimic natural conditions, the containers were placed outdoors in a shaded place until hatching. The timing of oviposition and hatching was assessed through daily inspections.

To assess differences between means a one-way analysis of variance (ANOVA) was used followed by Tukey's a posteriori test. Analysis of covariance (ANCOVA) tested comparisons between regression equations. Correlations between monthly mean values were examined by Spearman rank correlations (r,).

REsULTS

Field Observations.-Lizards emerged from hibernation during March. Courtship and mating behavior was seen from April to June (extreme dates were 8 April and 24 June). Aggressive encounters between territorial males were frequently observed during this period as well. Females with observable oviducal eggs were encountered from 11 June-19 July. We observed females with abdominal skin folds, which attested to recent oviposition, from 28 June-7 August. Lizards maintained in captivity oviposited between 1 July and 10 August. At the field study site, the first yearlings were recorded during the second half of September. In the vivaria, eggs hatched between 10 and 29 October; the incubation period ranged 71-102 days $(i = 85, SD = 9, N = 13)$. Lizards entered hibernation from the second half of October onwards.

Reproductive Cycles.-Both testicular volume and the maxima! diameter of the epididymis increased with body size (testis: ln Y = -12.814 + 3.515 ln SVL, N = 102, F = 73.709, $P < 0.001$; epididymis: ln Y = -7.140 + 1.602 ln SVL, N = 102, F = 76.027, $P < 0.001$). Significant seasonal variation was evident in both SVL-adjusted testicular volume and diameter of the epididymis (testis: F = 13.978, df = 7, 94, $P < 0.001$; epididymis: F = 16.726, df = 7, 94, $P < 0.001$; Fig. 1). Testicular recrudescence began in September, with peak volumes occurring from March to May. The testes decreased in volume during June and reached their minimum sizes during July and August. A similar pattern was observed for the maximum diameter of the epididymis (Fig. 1).

The first females with enlarged, vitellogenic follicles were collected in April; their relative frequency increased during May and June. Females containing oviducal eggs were encountered in June and July. Post-parturient females were present from June onwards (Table 1).

Differences between females in the timing of reproductive activities were associated with differences in body size. During April-May, individuals that exhibited vitellogenic follicles had larger mean SVL (i = 170.1 mm, SD = 14.7, N = 15) than did females with previtellogenic follicles $(i = 157.3$ mm, SD = 15.6, N = 22; t = 2.54, $P < 0.02$). Likewise, in June-July gravid and post-parturient females were larger $(i =$
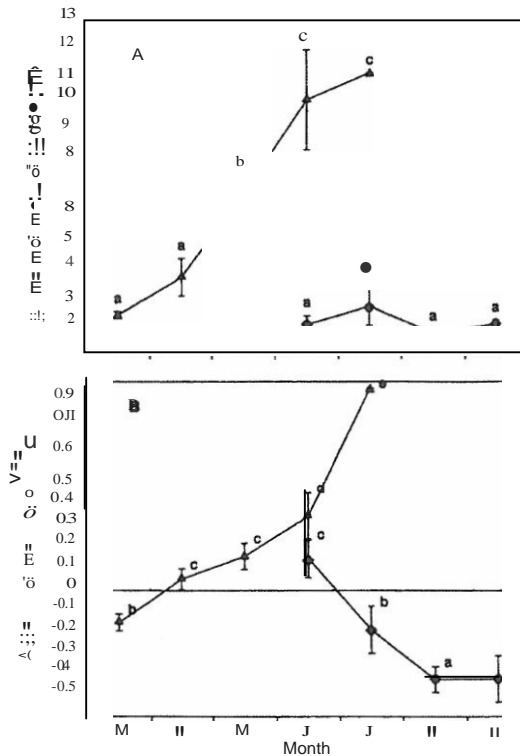
FIG. 2. Monthly changes (mean ± 1SE) in maximum diameter of follicle (A), and SVL-adjusted diameter of the oviduct (B), of *L. lepida* throughout its activity period. Triangles = pre-ovulatory females; squares = gravid and post-parturient individuals. For each organ size, values with common superscripts are not significantly different (Tukey's a posteriori test, *p* > 0.05).

FIG. 3. Monthly changes (mean ± 1 SE) in SVL-adjusted fat body volume in male and female *L. lepida* (squares= pre-ovulatory; triangles = gravid and post-parturient females) throughout its activity period. Within a sex, values with common superscripts are not significantly different (Tukey's a posteriori test, *p* > 0.05).

167.2 mm, SD = 8.9, N = 23) than pre-ovulatory individuals *(i* = 159.4 mm, SD = 9.7, N = 9; *t* = 2.19, *p* < 0.05).

The ovarian follicles from females collected in March were translucent and 3 mm or less in diameter (Fig. 2). Follicular growth started during the second half of April. Most females collected in May and June possessed vitellogenic follicles whose size varied between 5-19 mm in diameter. All post-parturient females collected from June onwards had regressed ovaries containing follicles less than 3 mm in diameter. None of the females exhibited the simultaneous presence of yolked follicles and oviducal eggs or corpora lutea. A total of three atretic follicles was encountered in the ovaries of two females.

Females with oviducal eggs had dilatated oviducts and were excluded from the subsequent analysis. Maximum diameter of the oviduct increased with body size (ln Y = -13.669 + 2.992 ln SVL; N = 77, F = 47.861, *P* < 0.001). Maximum diameter of the oviduct, adjusted for SVL, exhibited seasonal changes (F = 12.211, df = 8, 68, *P* < 0.001; Fig. 2). The width of the oviduct
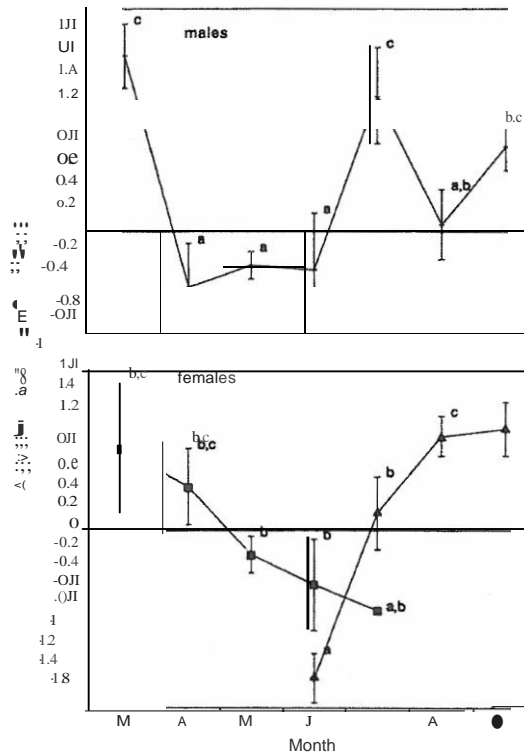
increased gradually in pre-ovulatory females and showed a progressive decrease after oviposition.

Fat Body Cycles.-Fat body volumes were related to SVL in both males (ln Y = -20.444 + 4.985 ln SVL; N = 71, F = 20.297, *P* < 0.001) and females (ln Y = -26.627 + 6.681 ln SVL; N = 68, F = 19.025, *P* < 0.001). Estimates of SVL-adjusted fat body volume varied seasonally in both sexes (males: F = 3.257, df = 6, 64, *P* < 0.01; females: F = 5.849, df = 8, 60, *P* < 0.001; Fig. 3). Fat bodies of males were significantly smaller during April-June than in the other months of the year. In pre-ovulatory females, adjusted fat body volume decreased gradually as vitellogenesis proceeded. Fat bodies reached their lowest volumes in gravid and post-parturient females collected during June. Lipid stores of post-parturient females increased until the onset of hibernation. It should be noted that neither sex showed a significant decrease in fat body volumes over the hibernation period.

The monthly rate of change in lipid stores was estimated by the difference in mean fat

body volume between succeeding months. In females, this index was inversely correlated with the between-month changes in mean follicle diameter (r, = -0.762, P < 0.05). In males, the between-month changes in fat body and testicular volumes were not correlated (r, = 0.107, P > 0.70).

We examined sexual differences in the amount of lipid stores by comparing regression lines of estimated fat body volumes versus SVL. Separate comparisons were carried out for lizards collected during months with high (males: March, July, September; females: March, April, August, September) and low quantities of lipid stores (males: April, May, June; females: June, July). No significant differences in slopes or intercepts of regression equations were detected in either data set (ANCOVA: P > 0.30 in all cases). Hence, fat bodies of males and females have similar SVL-adjusted volumes.

DISCUSSION

The male and female reproductive cycles of L. *lepida* are similar to those of many other temperate zone lizards (Fitch, 1970; James and Shine, 1985), and particularly to those of other species of the genera *Lacerta* and *Podarcis* (Saint Girons and Duguy, 1970; Xavier, 1982; Brana, 1984; Hraoui-Blocquet, 1987; Kwiat and Gist, 1987; Hraoui-Blocquet and Blocquet, 1988). Testicular recrudescence started in late summer and proceeded until the onset of hibernation; testes attained their maximum volumes from spring emergence through late May. The latter interval coincided with the period during which courtship and mating was observed in the field. This pattern, in which a single spermatogenic cycle is completed in two succeeding activity seasons, corresponds with the "mixed type" spermatogenic cycle of Saint Girons (1982).

Vitellogenesis began in early spring, with ovulation occurring in June-July and oviposition mainly during July. Reproduction was rather synchronized: differences among females in the timing of reproductive activities were restricted to a 2 month period, the interval during which we found most females to be in a given reproductive condition.

Several factors may contribute to the observed variation in reproductive timing. First, as our samples carne from different locations and years, we cannot dismiss the possible effect of variation in proximate factors such as ambient temperature (Duvall et al., 1982), that in-.fluence follicular growth. Second, smaller females exhibit a delay in reproduction relative to the larger individuals. A similar phenomenon has been documented in *Lacerta vivipara* (Bauwens and Verheyen, 1985) and other lizards (Van Loben Sels and Vitt, 1984). Apparent

differences in reproductive timing could be induced by the production of multiple clutches by individual females. However, we found no indications for the production of more than one clutch per season in our lizards from central Spain, although it is known that this species may lay two clutches per year in southeastern Spain (Castilla and Bauwens, 1989), and in captivity (Peters, 1961; Hofmann, 1963; Howard, 1985).

In estimating fat body volumes, we unrealisticially assumed that these have a uniform and fixed shape. Therefore, our estimates are subject to error that would increase variance within the sampling periods. Even so, a fat body cycle emerges which is consistent with those of other temperate zone lizards (e.g., Ballinger and Schrank, 1972; Avery, 1974; Derickson, 1976; Etheridge et al., 1986). Fat body lipids represent only a fraction of a lizard's total fat stores, which also include those in the tai!, liver and carcass. In the congeneric *Lacerta vivipara* (Avery, 1974) and other lizards (Vitt and Cooper, 1985; Etheridge et al., 1986) tail and/or liver lipids cycle in a manner similar to that of the fat bodies. These results justify the use of fat body volumes to estimate total fat cycles.

Fat body cycling in male and female L. *lepida* is closely tied with the timing of reproductive activities. In females, vitellogenesis is associated with a decline in fat body volume, suggesting that stored lipids contribute energy to follicular maturation and yolking. Hahn and Tinkle (1965) provided experimental evidence of the mobilization of fat body lipids for vitellogenesis in the lizard *Uta stansburiana;* numerous other studies have shown an inverse relation between fat body volume and follicular growth in a variety of lizards, mainly from temperate climate zones (e.g., Derickson, 1976; Van Loben Sels and Vitt, 1984; Guillette and Sullivan, 1985; Loumbourdis and Kattoulas, 1985; Ortega, 1986).

Fat body volumes in males showed a precipitous drop during early spring and remained at a low level throughout the mating period (April-June). It could be hypothesized that fat body lipids decline due to the intense spermatogenic activity during this period. However, this hypothesis is falsified by our observation of an increase of body fat volume coinciding with testicular recrudescence in late summer, and by the fact that energy requirements for sperm production are low (Krebs and Davies, 1981). We alternatively suggest that males invest considerable amounts of energy, or at least expend more than they can assimilate, in behavioral activities associated with reproduction (see Vitt et al., 1978; Ortega and Barbault, 1986). These include acquisition and defense of territories,

searching for females, mate-guarding, courtship, and mating. During both periods before and after reproductive activities, no sexual difference in fat body volumes was found, indicating that the amount of fat stores mobilized by males during the reproductive period approximated that invested by females.

Fat body volumes of lizards collected . immediately following spring emergence were not significantly smaller than those of pre-hibernating lizards. Therefore, the energetic requirements during hibernation appear to be only a rather small fraction of the fall lipid stores. This pattern of fat body cycling is similar to that of most temperate lizards, where reproductive demands on lipid stores seem greater than maintenance needs during winter dormancy (Derickson, 1976).

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