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The Annual Reproductive Cycle of the Male and Female Desert Tortoise: Physiology and Endocrinology

VALENTINE A. LANCE[1] AND DAVID C. ROSTAL[2]

[1]*Center for Reproduction of Endangered Species, P.O. Box 120551, San Diego, California 92112 USA [E-mail: lvalenti@sunstroke.sdsu.edu; Fax: 619-557-3959];*
[2]*Department of Biology, Georgia Southern University, Statesboro, Georgia 30460 USA*

ABSTRACT. – **Data from a captive population of 20 male and 30 female desert tortoises (*Gopherus agassizii*) held under semi-natural conditions in the Las Vegas area, and from free-ranging tortoises fitted with radiotransmitters in the same area, are compared during two successive reproductive cycles. In April female tortoises show elevated plasma estradiol, testosterone, corticosterone, and lipid. During April and May ovulation and egg-laying occur and progesterone levels increase, but rapidly return to baseline once eggs are laid. By May and June all hormones and lipids decline to their lowest levels, except thyroxine which is lowest in October. In August and September plasma estradiol, lipid, and calcium increase coincident with vitellogenesis and follicular growth. When males emerge in April testosterone is relatively low, but continues to decline until May. During June and July testosterone increases and reaches its highest levels in August and September when male-male aggression, mating activity, and spermatogenesis are greatest. Corticosterone levels are also highest when testosterone is highest. Plasma corticosterone is significantly higher in males than in females in all months of the year, and plasma lipids are significantly higher in females than in males in all months of the year. Total white cell counts in both sexes were low from April through June, but increased significantly in August through October.**

KEY WORDS. – **Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; reproduction; steroid hormones; corticosterone; testosterone; thyroxine; lipids; cholesterol; triacylglycerol; phospholipids; cholesterol; stress; blood cells; Nevada; USA**

In the northern part of its range the desert tortoise, *Gopherus agassizii,* inhabits an extremely harsh and variable environment where there is considerable annual variation in temperature and unpredictable rainfall. Thus in a "good" year in which ample rainfall occurs and food plant production is adequate, female tortoises may lay up to two clutches of eggs (rarely three), whereas in "bad" years they may produce only one small clutch, or fail to nest altogether (Turner et al., 1986; Mueller et al., 1998; Wallis et al., 1999). The activity cycles of the male and female tortoise are closely tied to this annual variation in temperature and water availability. In the cold winter months they remain dormant in their burrows, and in the extreme heat of mid–summer they also spend most of the day underground, emerging only for short periods in the early morning and late afternoon (Ruby et al., 1994).

The peak in mating activity and in male-male aggression takes place in August through September. In April when animals emerge from their burrows there is a less vigorous bout of mating. There is a good probability that insemination occurs during the fall mating and that the spermatozoa are stored in the female reproductive tract until the following spring when ovulation occurs (Gist et al., 1990; DCR, unpubl. data).

Earlier we presented a review of the reproductive cycle of the male and female desert tortoise that included some preliminary data on circulating hormone levels (Rostal et al., 1994a). We later published some additional hormonal information on the species (Lance et al., 1995). Since that time some additional papers on tortoise reproductive cycles have appeared: Gonzalez-Trapága (1995) on Bolson's tortoise (*Gopherus flavomarginatus*) in Mexico; Schramm et al. (1999) on the Galapagos tortoise (*Geochelone nigra*) on Santa Cruz, Galapágos; and Ott et al. (1999) on the gopher tortoise (*Gopherus polyphemus*) in southwest Georgia.

We have collected a considerable amount of additional hormonal and lipid data on both male and female desert tortoises in captivity, on free ranging tortoises in the Eastern Mojave in the Las Vegas area, and on some blood from tortoises sampled in Arizona. Thyroid hormone (Kohel et al., 2001), corticosteroid hormones (Lance et al., 2001), and seasonal changes in plasma lipids (Lance et al., 2002) of male and female desert tortoises have been analyzed. In this paper we summarize all of the hormonal and physiological data we have collected (published and unpublished) on both captive and free-ranging desert tortoises from 1991–93 and from animals sampled in 2000.

METHODS

Animals. — Fifty adult tortoises collected from various development sites around Las Vegas in 1991 were maintained in ten separate pens of 30 m x 15 m at the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada. Each pen contained two males and three females and each had five artificial burrows. Male mean straight cara-

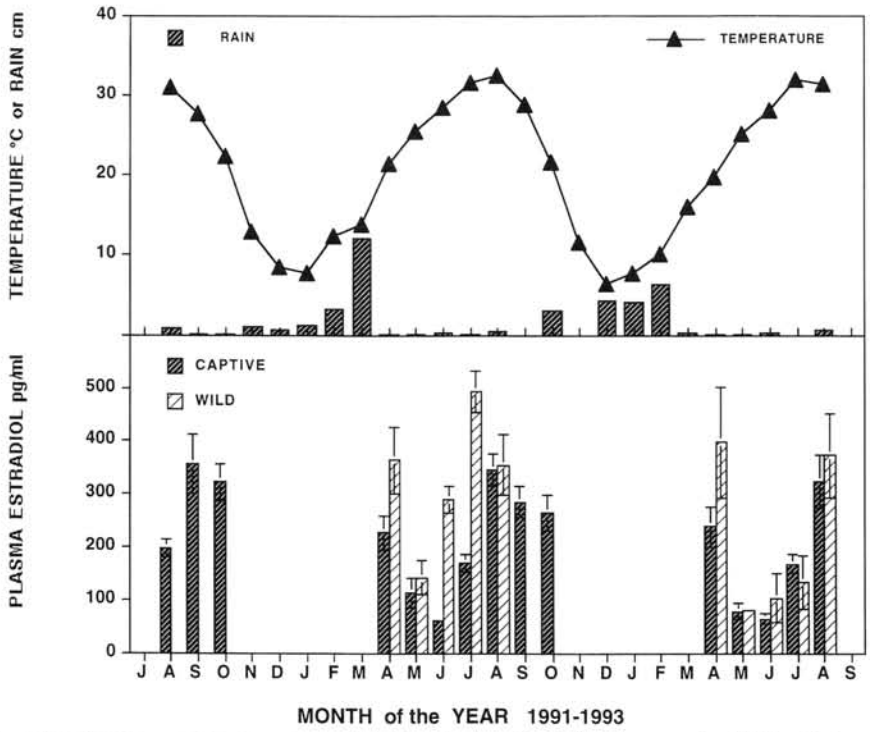

Figure 1. Meteorological data (upper panel) and mean monthly female desert tortoise plasma estradiol levels (lower panel) from August 1991 to August 1993 in Las Vegas, Nevada . The mean monthly temperature (°C) is represented by solid triangles and rainfall amounts in cm by hatched bars. The dark-hatched bars in the lower panel indicate mean monthly plasma estradiol levels in pg/ml in captive tortoises, and the lightly-hatched bars the levels in free-ranging wild tortoises. The lines above the hatched bars represent the standard error of the means (SEM). No samples for estradiol levels were obtained from November through March.

pace length (CL) was 261.3 ± 3.93 mm (SEM; n = 20); female mean straight CL was 241.1 ± 2.32 mm SEM; n = 30). The tortoises were exposed to ambient lighting and weather conditions (i.e., air temperature, humidity, and rainfall). In each pen water, natural vegetation, and a watered grass plot provided browse supplemented with alfalfa hay. In 1992–93, blood samples (3–5 cc) were collected from all 50 tortoises once a month from April to October from the jugular vein (Jacobson et al., 1992). Only a limited number of blood samples were collected in the cooler months from November to April when the tortoises remain dormant in their burrows, but the results from these are not included as this procedure appeared to cause a marked stress response (see Rostal et al., 1994, for details). The blood samples were centrifuged and the plasma stored at -20°C until tested for the presence of antibodies to *Mycoplasma agassizii* (Brown et al., 1994), the causative agent for upper respiratory tract disease (URTD) or assayed for hormones and lipids. Total white cell numbers were determined on blood smears from ten male and ten female desert tortoises at each sampling period. In 2000 we returned to Las Vegas and took blood samples from the same set of captive desert tortoises that were studied in 1992–93. Remarkably 28 of the original 30 females and 16 of the 20 males were still in the research pens at the DTCC. These animals were again sampled at monthly intervals from April to October. This second study was to assess the long-term effect of URTD on reproduction (Rostal and Lance, in prep.).

Ultrasound Examinations. — The reproductive status of the females was monitored using ultrasonography similar to that described by Rostal et al. (1990, 1994a). Females were scanned every second month during the pre- and post-nesting periods to track ovarian follicular growth. During the nesting season, females were scanned every two weeks to monitor oviductal egg development and to track nesting.

Histology. — Male reproductive tissues were provided from animals sacrificed for upper respiratory tract disease (URTD) studies conducted by E. Jacobson, College of Veterinary Medicine, University of Florida. Tissues from diseased and healthy desert tortoises euthanized during URTD research were available for histology. Additional samples from healthy desert tortoises sacrificed during nutritional studies at the DTCC were obtained from Perry Barbosa. In 1996 an additional 23 testis specimens were obtained from tortoises euthanized in Las Vegas. A total of 55 male reproductive tracts were examined. These tissues came from animals sacrificed in January, April, July, August, October, and December. Tissues were fixed in 10% buffered formalin or Bouin's solution. Fixed tissues were trimmed, dehydrated through a series of ethanol, then embedded in Paraplast. Sections of 4 µm were prepared on glass slides and routinely stained with hematoxylin and eosin or Masson's trichrome.

A group of sub-adult male tortoises (the sex verified by laparoscopy, Rostal et al., 1994b), were also bled for over one summer to assess any hormonal changes in this population (n = 9). Sub-adult tortoises are animals with CL between 187–208 mm, the minimum CL at sexual maturity is 210 mm (Turner et al., 1987).

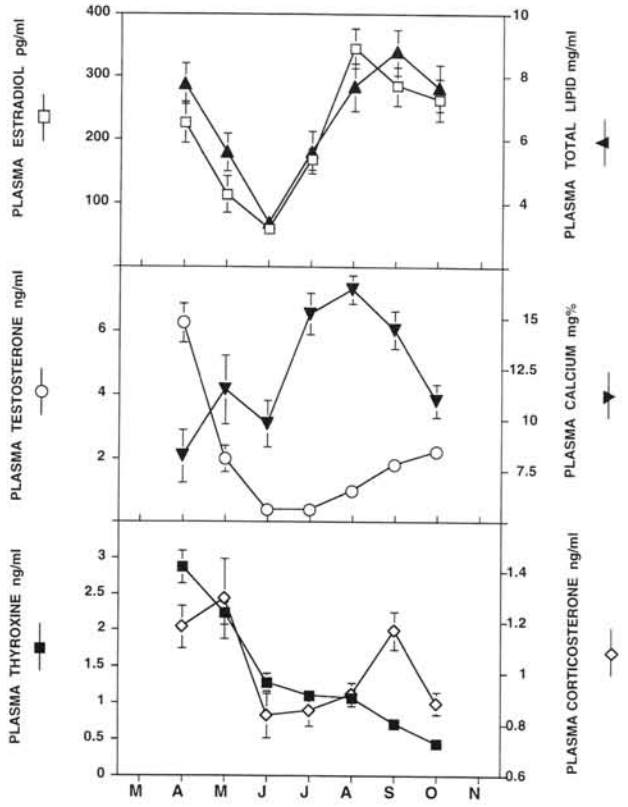

Figure 2. Plasma hormones and lipids in female desert tortoises. The months of the year from March to November are indicated as letters. No samples were collected through the winter months, November to March. In the upper panel are monthly mean plasma estradiol (open squares), and plasma total lipid (solid triangles). The bars above and below the symbols represent the SEM. In the middle panel are shown monthly mean testosterone values (open circles), and mean calcium values (solid inverted triangles). Plasma thyroxine (solid squares), and plasma corticosterone (open diamonds), are shown in the lower panel.

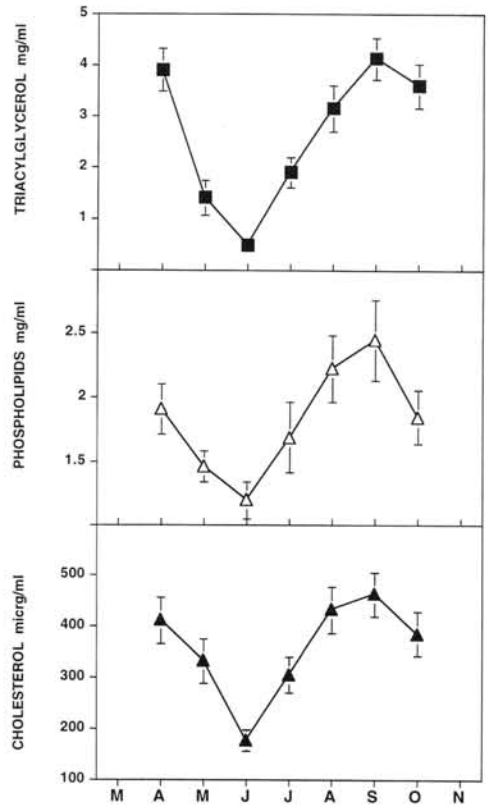

Figure 3. Plasma triacylglycerol (solid squares), phospholipids (open triangles), and cholesterol (solid triangles) in female desert tortoises during the annual reproductive cycle. Months of the year and SEMs as in Fig. 2.

Blood samples from free-ranging adult female tortoises in the Las Vegas area fitted with radiotransmitters (O'Conner et al., 1994), were also assayed for hormones. A small set of blood samples from free-ranging tortoises at several field sites in Arizona was also assayed for reproductive steroids.

Reproduction and Stress Studies. — In addition to the reproductive cycle study, experiments were carried out to assess the effect of stress on reproductive hormones. Blood samples from male tortoises held in boxes for several hours were assayed for corticosterone and testosterone. An additional experiment was carried out to test the effects of adrenocorticotropic hormone (ACTH) on circulating levels of corticosterone and testosterone in adult male tortoises. Sixteen adult male tortoises were divided into three groups: group 1, six animals (mean body mass 3.17 ± 0.2 kg; mean CL 255 ± 7 mm) high dose ACTH; group 2, six animals (mean body mass 2.94 ± 0.27 kg; mean CL 254 ± 7 mm) low dose ACTH; and group 3, four animals (mean body mass 3.04 ± 0.43 kg; mean CL 258 ± 15 mm), saline control. Group 1 received the high dose of synthetic ACTH 1-24 (Sigma), 1 µg/ kg/body wt, and group 2 received 0.1 µg kg/ body wt dissolved in physiological saline. The tortoises in group 3 received saline alone. The drugs were injected directly into the jugular vein after the initial blood sample was drawn. Blood samples were taken at 1, 2, 4, 8, and 24 hrs post-injection and assayed for testosterone and corticosterone. For detailed information on the hormonal and biochemical assays see Rostal et al. (1994a), Kohel et al. (2001), and Lance et al. (2001, 2002). The data were subjected to a repeated measure analysis of variance (ANOVA) followed by Sheffé's multiple range test.

RESULTS

Although data were collected from August 1991 until August 1993, the samples from 1992 were subjected to an extensive analysis for all hormones, lipids, and inorganic ions, whereas samples from 1991 and 1993 were analyzed for hormones only. Therefore, for the purposes of clarity of presentation (other than the data in Fig. 1), data from 1992 will be focused upon to exemplify a typical reproductive cycle.

Females. — In Fig. 1 (upper panel) meteorological data for the Las Vegas, Nevada region from August 1991 to August 1993 are shown, and in the lower panel plasma estradiol values from captive and wild female tortoises in the same area are shown. The year-to-year variation in mean monthly temperature was similar, and rainfall was low in both winters. In 1992 estradiol values for wild tortoises in June and July were significantly higher than in the captive

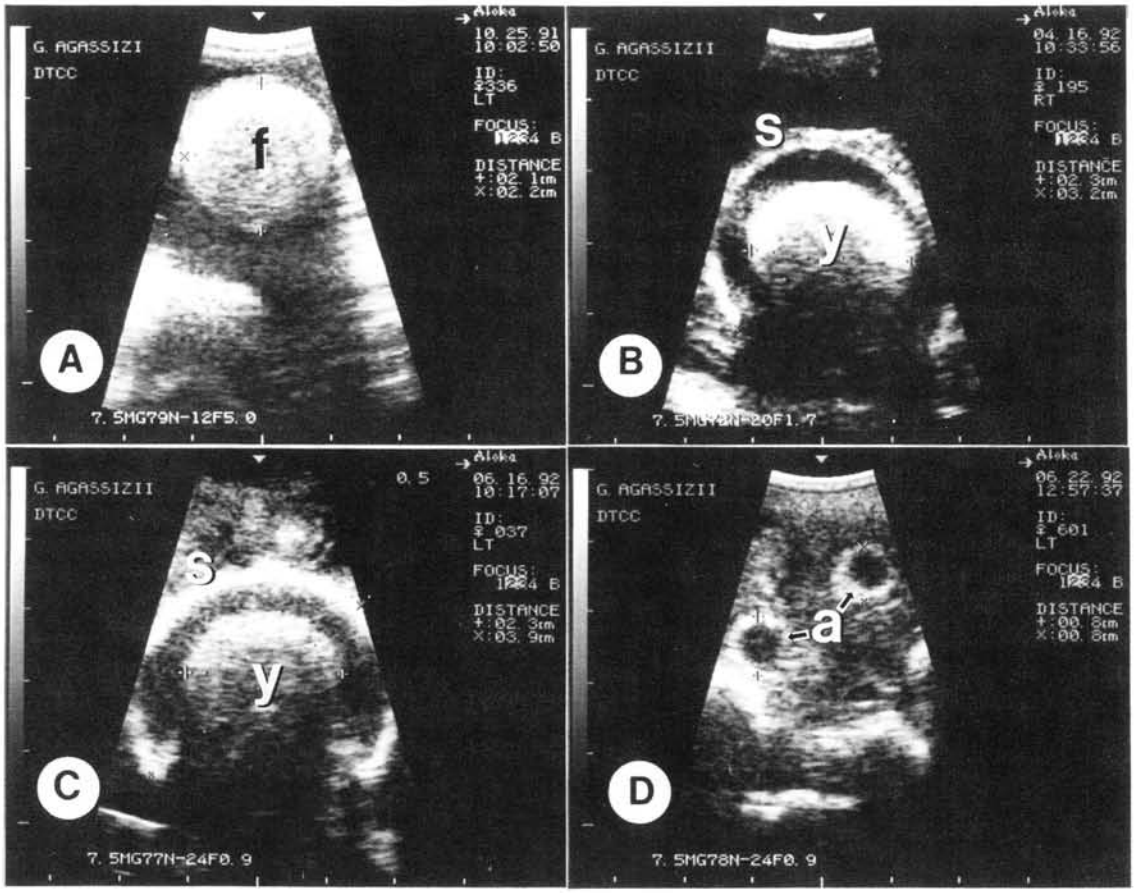

Figure 4. Ultrasound images of desert tortoise ovaries and oviductal eggs. **(A)** Image of a large vitellogenic follicle, (f, 2.1 cm diam.) in October. **(B)** Image of a recently ovulated egg in the oviduct (less than 10 days post-ovulation) showing a well defined yolk (y, 2.3 cm diam.) and a thinly calcified shell (s). **(C)** Image of a fully developed oviductal egg (between 20–30 days post-ovulation) showing a less clearly defined yolk (y, 2.3 cm diam.) and a well calcified shell (s). Resolution is poorer at this stage due to the heavily calcified shell. **(D)** Image of atretic ovarian follicles (a, 0.8 cm diam.) in late June following completion of the nesting cycle. Figure from Rostal et al., 1994a.

tortoises, but in 1993 there were no significant differences between the two groups. Estradiol in both groups was significantly lower in May than in April, August, September, and October ($p < 0.05$).

Estradiol levels in tortoises from Arizona were in the same range as those from Nevada. Results from two sites in Arizona combined showed estradiol levels of 226 ± 65 in April, $n = 9$; 33 ± 11 in June, $n = 6$; 242 ± 38 pg/ml in September, $n = 9$ (compare with mean values in Fig. 1). Sample sizes in other months were too small for statistical analysis.

Figure 2 is a composite of six separate graphs. In the upper panel are shown the plasma estradiol values and the plasma total lipid values for 1992. Estradiol and total lipid are closely linked; both are lowest in June and both increase significantly ($p < 0.001$) during July and August. In the center panel of Fig. 2 are shown the plasma testosterone and plasma calcium values from the same tortoises. Calcium was highest when estradiol was highest in August, but was low in April when estradiol was still elevated. Testosterone values were highest in April when the mean value reached 6.2 ng/ml, declined to almost undetectable in June and July, then rose slightly from August through October. Plasma thyroxine and plasma corticosterone results are shown in the lower panel of Fig. 2. Highest thyroxine values were seen in April when the tortoises emerged from their burrows, and the lowest were found in October just before the tortoise retreat underground for the winter. Mean corticosterone concentrations showed two distinct peaks, one in April–May and a second in September.

Plasma lipid fraction in female tortoises is shown in Fig. 3. In the upper panel is shown the monthly mean triacylglycerol values, in the middle panel the phospholipid and in the lower panel, the cholesterol values. All showed a similar pattern to total lipid and estradiol with significantly lower values in June and elevated levels in all other months.

In Fig. 4 is an ultrasonograph showing preovulatory follicles, shelled eggs in the oviduct, and atretic follicles. Diameter of the largest ovarian follicles in each month as determined by ultrasonography during vitellogenesis is shown in Fig. 5. Superimposed over the bars representing mean monthly diameter is plasma total lipid. The follicular growth cycle closely matches that of estradiol, lipid, and calcium. There are no follicular diameter data for May and June because shelled eggs in the oviducts obscured the ovary.

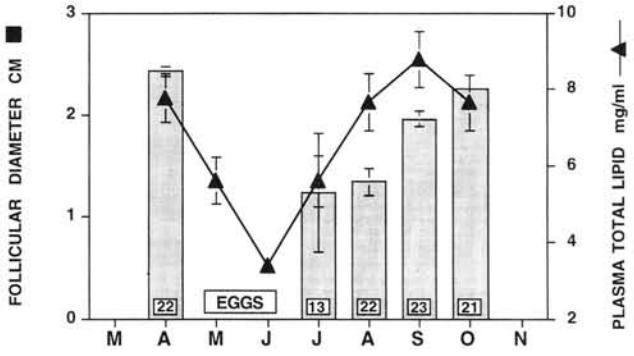

Figure 5. Follicular diameter and plasma total lipid in desert tortoises. No ultrasound data on follicle size are available for the months of May and June because shelled eggs in the oviducts obscured the ovary. Mean diameter of the dominant follicles in each month is represented by the solid bars, and mean monthly plasma lipid by solid triangles. The numbers in the boxes at the base of the bars indicates the number of observations in each month.

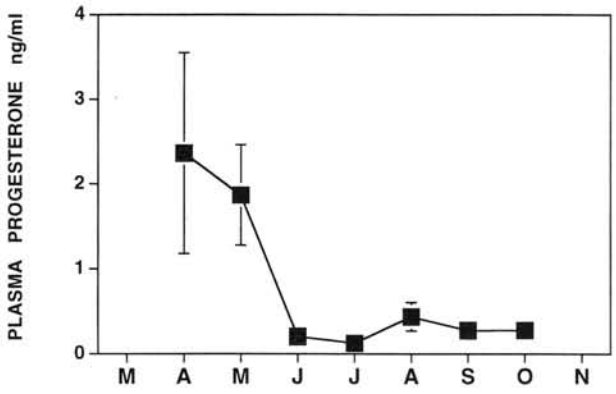

Figure 6. Mean monthly plasma progesterone in female desert tortoises. The April sample has a large standard error because the mean includes samples from tortoises that were preovulatory and had very low progesterone and samples from tortoises that had ovulated and had very high progesterone.

In Fig. 6 are shown the progesterone values for the year. The only significant elevation above baseline occurred in April and May, coincident with ovulation. In April progesterone values ranged from a low of 0.12 ng/ml to a high of 30.70 ng/ml, and in May the range was from 0.20 to 12.70 ng/ml.

Males. — Plasma testosterone in male tortoises showed highly significant seasonal variation with a mean of 18.4 ng/ml in May to mean levels just under 200 ng/ml in August. Individual values of greater than 300 ng/ml were common in the August samples. The highest testosterone values in female tortoises (Fig. 2) were less than 8 ng/ml. Monthly means for male tortoises during 1992 are presented in Fig. 7, upper panel. Plasma corticosterone values in male tortoises showed an almost identical seasonal pattern as the testosterone, and in fact were strongly correlated, $p < 0.001$ (upper panel in Fig. 7). Plasma total lipid in male tortoises is shown in the middle panel of Fig. 7. Total lipid values were lowest in June and July at the beginning of the spermatogenic cycle. Plasma thyroxine levels in male tortoises were highest in April when they emerged from their burrows, declined in May and June then rose again in July and August during the period when male-male aggression was highest (Fig. 7, lower panel). Lowest thyroxine levels were seen in October.

Photomicrographs of testicular histology of tortoises from different phases of the spermatogenic cycle are shown in Fig. 8. Samples from April, upper left panel (A), July, upper right panel (B), October, lower left panel (C), and December, lower right panel (D) representing a fairly complete cycle were available. In April the seminiferous tubules contain the remnants of the previous spermatogenic cycle. By May the testes are fully regressed and the tubules contain only spermatogonia and Sertoli cells. This is also the month during which the lowest testosterone values are seen. In July the seminiferous tubules show active spermatogenesis, but few mature spermatozoa are seen. Circulating testosterone is still however, relatively low. In October tubule diameter is greatest, spermiogenesis is occurring and testosterone levels are high. The December section shows a seminiferous tubule devoid of spermatozoa just after spermiation.

In order to emphasize the significant differences between males and females, a comparison of the corticosterone and total lipid cycles are shown in Fig. 9. Corticosterone values of males are higher than females in all months, and total lipid values of females are higher than males in all months.

The seasonal variation in plasma testosterone in a group of immature male tortoises is shown in Fig. 10. No samples were available in April, but from May to August the pattern

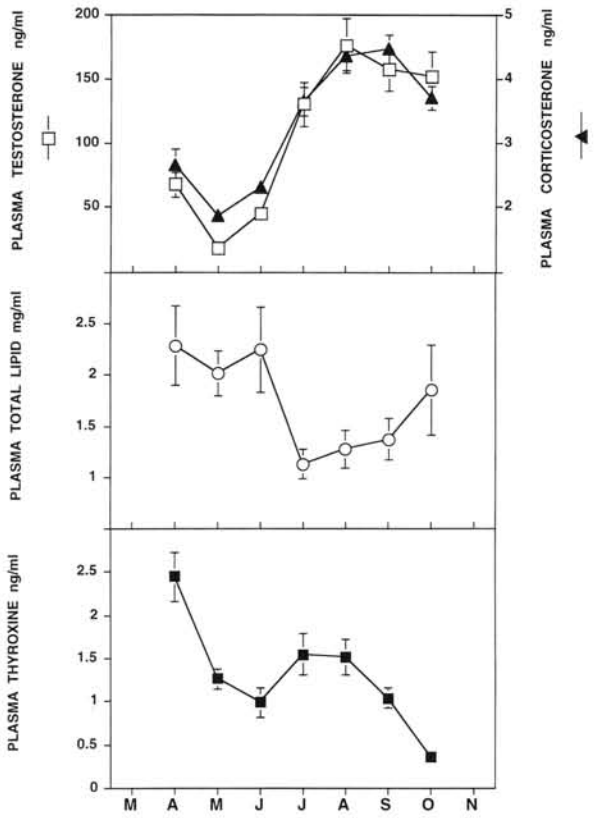

Figure 7. Seasonal changes in plasma hormones and total lipids in male desert tortoises. In the upper panel, monthly mean plasma testosterone (open squares) and corticosterone (solid triangles) are shown. In the middle panel, the monthly mean total plasma lipid is shown, and in the lower panel, monthly mean plasma thyroxine.

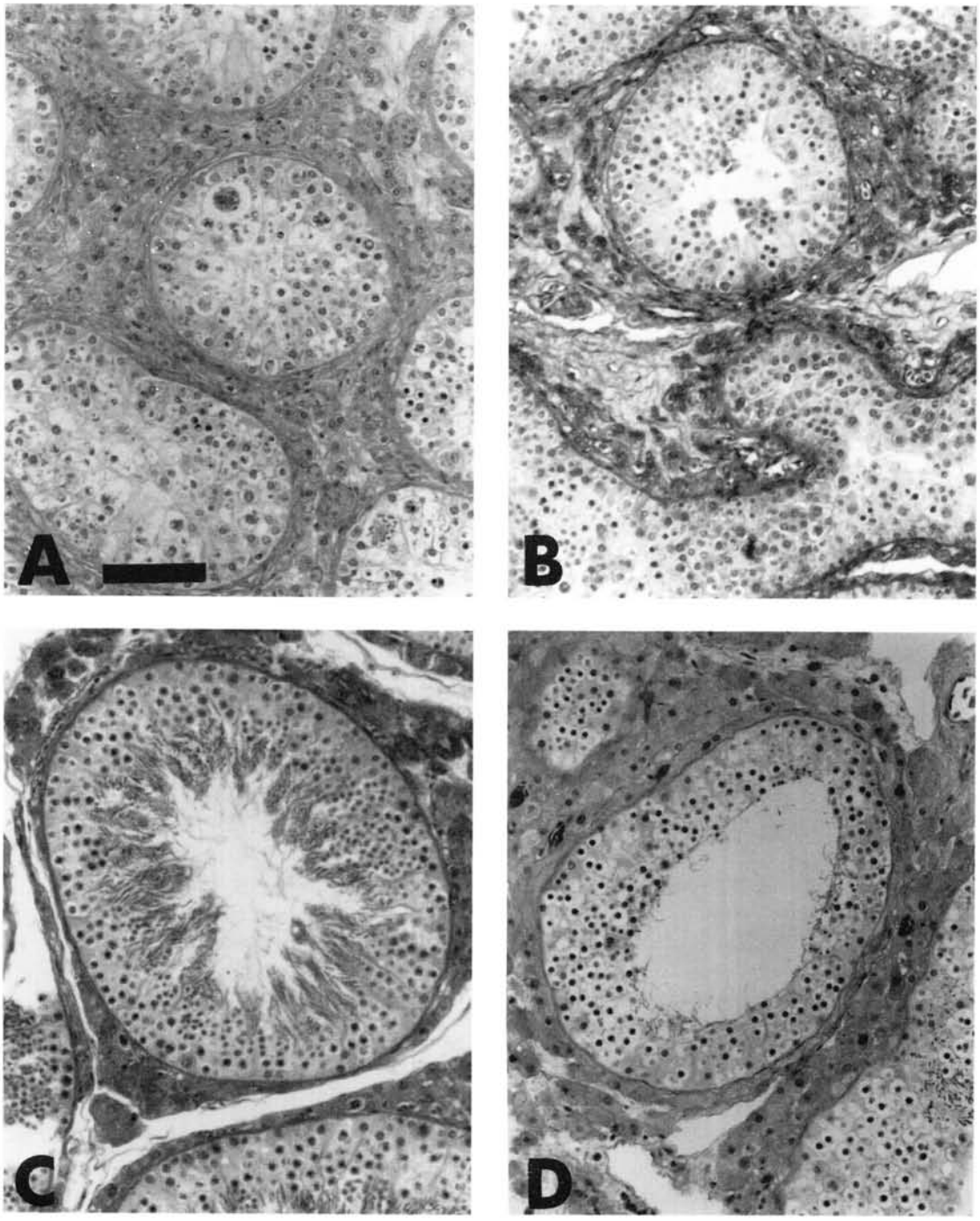

Figure 8. Testicular histology of desert tortoises during the annual spermatogenic cycle. The black bar in the photomicrograph A is equal to 100 μm and all four plates are at the same magnification. **(A)** Testis from an animal sacrificed in April. Seminiferous tubules are completely regressed and contain the remnants of spermatozoa, Sertoli cells, and spermatogonia. The interstitial area is filled with Leydig cells. **(B)** Section of a testis from a tortoise sacrificed in July. The seminiferous tubules have enlarged and show signs of active spermatogenesis. Abundant spermatogonia, spermatocytes, and spermatids are present, but no mature spermatozoa are visible in this particular section. **(C)** Section of a testis from an animal sacrificed in October. Seminferous tubules are at their greatest diameter and spermatogenesis is at a maximum. Note Leydig cells filling the interstitial space as compared with section B from July. **(D)** Section of a testis from an animal collected in December. The seminferous tubule is empty with a few residual spermatozoa visible, but still containing large numbers of spermatogonia and spermatocytes.

is similar to that of adult males. The concentration of testosterone in these tortoises, however, is about half that of adults.

ACTH Experiment. — The results of the ACTH experiment are shown in Fig. 11. In the upper panel is shown change in plasma corticosterone in response to a single injection of ACTH. The high dose of ACTH caused a rapid and significant rise in corticosterone that declined to baseline by 8 hrs. The responses to the low dose of ACTH (results not shown) or an injection of saline were not significant. In the lower panel in Fig. 11 are shown the percent change in

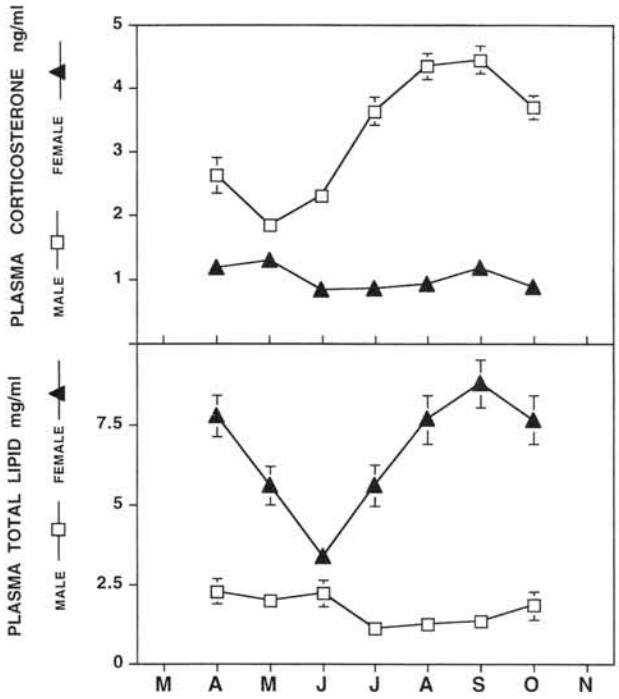

Figure 9. In the upper panel are shown the mean monthly corticosterone values (males, open squares; females, solid triangles), and in the lower panel are shown the mean monthly plasma total lipid values (symbols as in upper panel) for desert tortoises

plasma testosterone in response to the high dose of ACTH and saline. As there was a very large variation in testosterone concentrations (range from 11.9 ng/ml to 91.2 ng/ml) at the initial sample, the results are presented as percentage change from that value over time. The change in testosterone following ACTH showed considerable variation and was not significant.

White Blood Cells. — The seasonal changes in total white cell numbers in both males and females are shown in Fig. 12. In both sexes there are significant increases in total cell numbers in August, September, and October as compared to April through July ($p < 0.005$).

DISCUSSION

Two obvious features of the habitat in the Eastern Mojave Desert are very little annual precipitation and extreme temperatures. The weather patterns were similar during the two years of our study as were the reproductive cycles in both years. There were insufficient samples for a complete cycle for wild male tortoises, but there were sufficient wild female samples in both years to show a seasonal cycle similar to that of the captive animals. The year-to-year reproductive cycles showed some minor differences, but in general the same pattern was apparent. In free-ranging tortoises the peak in estradiol levels in 1992 was seen in August, whereas in 1993 peak estradiol was seen in July. In the captive tortoises that received water and food supplements the year-to-year variation was less noticeable. Mean estradiol levels in the field samples from Arizona were similar to those of the captive Nevada tortoises, with very

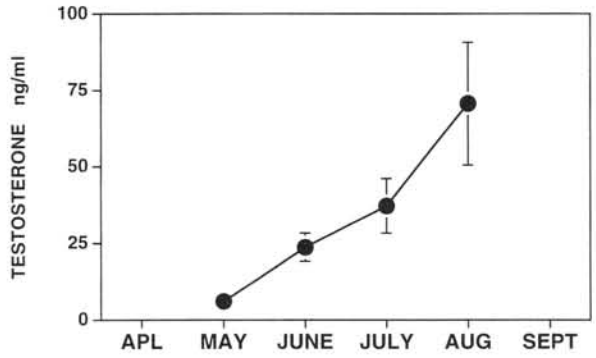

Figure 10. Changes in mean plasma testosterone in sub-adult male desert tortoises from May through September. Compare with adult males (Fig. 7).

low levels in June and high levels in September. Clutch sizes in the captive and wild populations were not significantly different (Rostal et al., 1994), again emphasizing that the reproductive cycles in the two groups are comparable.

The chelonian ovarian cycle is characterized by a long, slow growth phase during which the follicles increase in diameter and accumulate yolk over several months. This prolonged vitellogenic phase is characterized by elevated plasma estradiol, testosterone, lipids, calcium, and phospho-

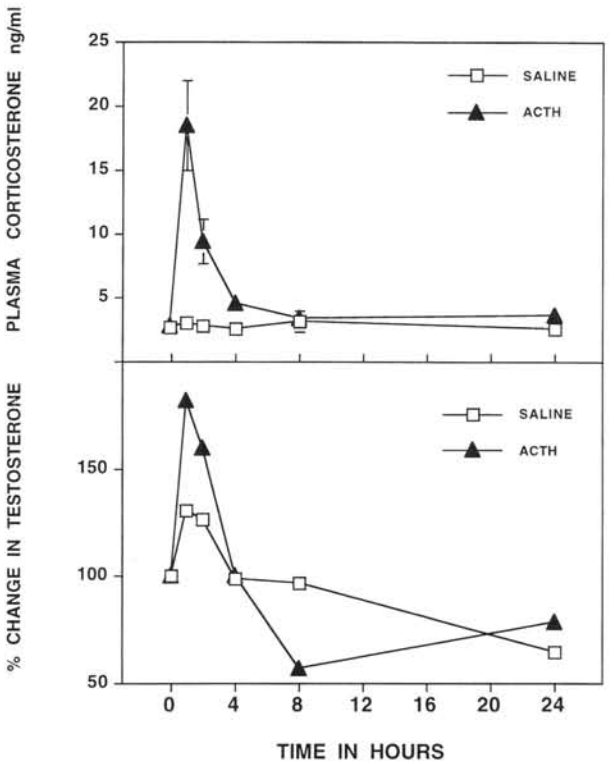

Figure 11. Changes in plasma corticosterone and testosterone in response to a single injection of ACTH or saline in male desert tortoises. Plasma corticosterone (upper panel) increased significantly by 1 hr in response to ACTH (solid triangles) and returned to baseline by 4 hrs. There was no significant change in plasma corticosterone in response to saline injections (open squares). Plasma testosterone increased by 1 hr in response to ACTH (solid triangles) then decreased to below baseline by 8 hrs. The saline injected control tortoises (open squares) also exhibited a slight increase in plasma testosterone by 1 hr before returning to baseline by 8 hrs.

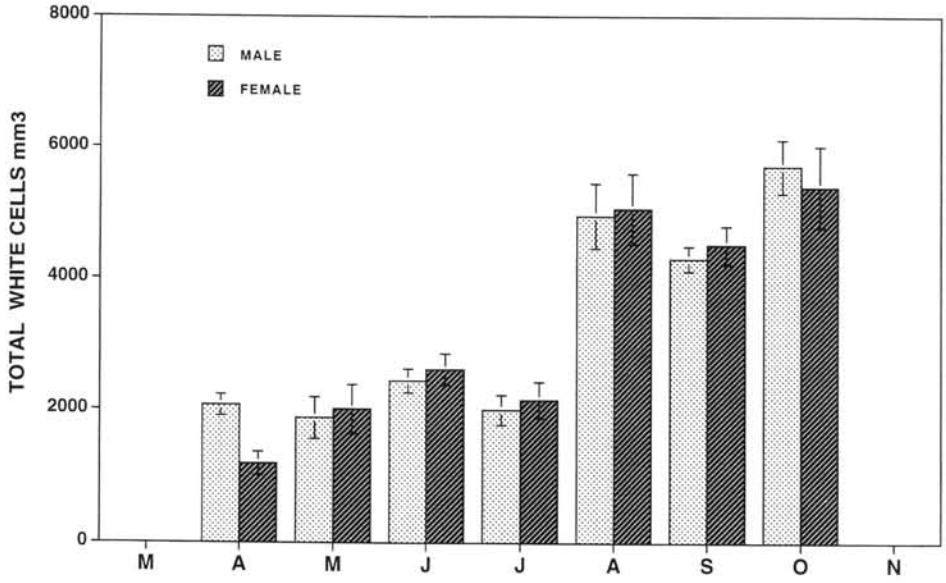

Figure 12. Changes in white blood cell numbers in male and female desert tortoises during one year. Ten samples for each sex from April to October were analyzed. Monthly means of total white cell counts are represented by dark bars (male) and light stippled bars (females). Total numbers were significantly higher in August, September, and October than in the months of April to June. There was no significant difference between males and females.

rus. In female tortoises elevated estradiol and lipids were seen during the months when follicular growth was apparent by ultrasonographic examination (Figs. 4 and 5). Follicular growth and vitellogenesis begin in July and continue through October. During the winter there is little change until ovulation occurs shortly after the females emerge from their burrows in April. Some individuals had shelled eggs in the oviduct in April suggesting that ovulation occurred prior to emergence, and thus fertilization was achieved from spermatozoa stored in the oviduct over winter. Christopher et al. (1999) provided hematologic and blood biochemical values from a large number of free-ranging desert tortoises sampled over a five-year period at multiple sites in the Mojave Desert in California. Their data set was broken down into four separate seasons rather than by months, and reproductive vs. non-reproductive females were not identified, so comparisons are difficult. In general, however, their results showed a pattern similar to ours. Calcium and phosphorus in their data set showed changes that are typical of vitellogenesis in that they were significantly higher in females than in males. They also showed higher levels of cholesterol and triacylglycerol in females than in males. A similar study on wild desert tortoises in Arizona showed an equal difference in male and female lipids (Dickinson et al., 2002).

The lipids, minerals, and protein circulating in the blood are deposited in the yolk of the developing follicle. These physiological changes are driven by estradiol secreted from the granulosa of the developing follicles. Estradiol acts on the liver to induce the synthesis and secretion of the calcium-binding yolk precursor protein, vitellogenin, and the very low-density lipoproteins that help transport the large amounts of triacylglycerol, phospholipid, and cholesterol to the developing follicle (Speake et al., 1998; Lance et al., 2002). Similar changes in plasma lipid, phosphorus, and calcium can be elicited in juvenile and male reptiles by injecting estrogen (Ho et al., 1982; Magliola, 1984). One lipid fraction did not follow this pattern. Plasma cholesterol ester remained low when estradiol was high (Lance et al., 2002). All plasma lipid fractions, however, were higher in females than in males in all months (Fig. 9).

The low calcium levels in female tortoises in April when estradiol was relatively high was probably due to the fact that some of the females had completed vitellogenesis and had already ovulated. In the data of Christopher et al. (1999) calcium levels in female tortoises in spring were significantly higher than in males.

The reptile ovary secretes testosterone during folliculogenesis with the highest levels seen prior to ovulation, but peak concentrations are generally less than 10% of those seen in males. The larger the number of preovulatory follicles the higher the concentration of circulating testosterone (Rostal et al., 1998). The role of testosterone in reproduction in female reptiles is not clear. It may be necessary for oviduct development prior to ovulation as has been proposed for birds (Kawashima et al., 1999), and it may be important for sex behavior, but data are lacking.

Just before ovulation there is a rapid drop in estradiol and testosterone and a rise in progesterone. The granulosa cells of the mature follicle down-regulate the genes that control synthesis and secretion of the two follicular stage hormones and undergo a preovulatory luteinization. Progesterone continues to be secreted from the post-ovulatory follicle (or corpus luteum) during the time eggs are in the oviduct. The estimated interval from ovulation to oviposition in the desert tortoise is about 30 days (DCR, unpubl. obs.). Obtaining an accurate record of this periovulatory surge in progesterone is difficult when blood samples are collected only once a month. The large variation in proges-

terone values in the months of April and May are due to the fact that some of the tortoises had ovulated and some had not. Nevertheless, progesterone levels in the female tortoises in these months when ovulation and egg-laying occurred were significantly higher than in all other months of the year.

Both plasma thyroxine and plasma corticosterone showed significant seasonal variation in female tortoises. Thyroxine was highest in April when tortoises first become active and search for food. Increased thyroid hormone levels have been associated with this active feeding behavior in reptiles (Kohel et al., 2001) and increased plasma corticosterone concentrations have been associated with increased mating activity (Schramm et al., 1999). The peaks in corticosterone in April and September were associated with the spring and late summer mating periods, whereas the peak in thyroxine was associated only with the emergence from winter dormancy and the increased feeding activity in spring. When estradiol secretion is at its highest thyroxine is lowest. Thyroxine may play a role in reproduction in female tortoises, but where and how is not clear.

Although the sample size was extremely small the histology of the male gonadal cycle was clear. The spermatogenic cycle of the desert tortoise is similar to what has been described in other temperate zone chelonians (Lance, 1984; Licht, 1984; Moll, 1979; Kuchling, 1999). When the tortoises emerge from their winter torpor in April the testes are fully regressed and contain only primary spermatogonia, Sertoli cells, and a large amount of cell debris from the previous cycle, whereas the epididymides are packed full of spermatozoa. The tortoises undergo an abbreviated mating period at this time, but most successful copulations are believed to occur in August and September (DCR, unpubl. obs.). In May the seminiferous tublules contain only Sertoli cells and spermatogonia and the cell debris has disappeared. In early July the seminiferous tubules show active cell divsion of the spermatogonia and abundant spermatids. By September and October spermatogenesis is complete; the tubules of the testis are at their greatest diameter and are full of mature spermatozoa. During this period testosterone and corticosterone levels are highest, frequent mating activity is observed, and male-male aggression is at its most intense. Male desert tortoises possess a pair of mental glands on the chin that show a significant increase in development and secretory activity when testosterone levels are highest in late summer (Alberts et al., 1994), but the role of these glands in reproduction remains unclear.

The seasonal pattern in plasma testosterone in male desert tortoises held at the DTCC was remarkably similar in 1992 and 2000. This virtually identical pattern and similar hormone levels in a population sampled at an eight-year interval emphasizes how closely the annual reproductive cycle in this species is tied to the seasonal weather pattern. In both 1992 and 2000 there was little rain and very warm summers.

Plasma testosterone levels in *Gopherus* species are considerably higher than levels reported in other chelonian species. Individual levels of greater than 300 ng/ml were seen in male desert tortoises in August and September. Mean plasma testosterone over 300 ng/ml were reported in *G. polyphemus* (Ott et al., 2000), and levels in excess of 1000 ng/ml in *G. flavomarginatus* (Gonzalez-Trapága, 1995). Peak levels of testosterone in male Galapagos tortoises were less than 50 ng/ml (Schramm et al., 1999). The reason for these differences is not known.

Plasma thyroxine in male tortoises was highest in April, declined in June, then showed a second increase in July and August before dropping to its lowest point in October. This pattern differs somewhat from that of the female where hormone levels continued to decline in late summer. Kohel et al. (2001) suggested that the elevated levels of corticosterone and testosterone in late summer stjeimulate thyroid activity in males, and that elevated thyroid hormone is related to increased energy expenditure. Males probably do expend more energy than females in late summer when intense male-male combat often takes place. Triiodothyronine (T3) in both male and female tortoises was undetectable. Likewise, in an aquatic turtle, *Chrysemys picta*, thyroxine was measurable and increased in response to TSH, but T3 was undetectable (Sawin et al., 1981).

The seasonal pattern in total plasma lipid in male tortoises is remarkably similar to that reported in an aquatic turtle, *Sternotherus odoratus* (McPherson et al., 1982). At the onset of spermatogenesis total lipids decline sharply and remain low until the cycle is complete in late September. The increased metabolism of lipid associated with reproduction is well documented for other reptiles in which abdominal fat bodies decline as testis mass increases (Derickson, 1976). Chelonians lack discrete fat bodies associated with the gonads, thus a relationship between gonadal cycles and fat metabolism are difficult to demonstrate. The decline in circulating lipid during spermatogenesis in the desert tortoise and various aquatic turtles suggest that there is a relationship between reproduction and lipid metabolism in these reptiles.

Our results demonstrate a clear and significant seasonal cycle in male plasma corticosterone, and a clear but less marked seasonal pattern in female plasma corticosterone. What was also apparent was a highly significant difference between the sexes in each month of the cycle. It is not clear why male tortoises have higher glucocorticoid levels than females. There is no information available at present on the relative size of the adrenal tissue or relative blood flow to the adrenal in the two sexes that could help explain this difference. What was even more remarkable in the male was the close association of corticosterone and testosterone ($p < 0.0001$). The cycles for the two hormones are virtually identical. The reason for this link between the secretion of the two hormones is not known. The close anatomical association of the gonad and adrenal gland in the Testudines and the intimately related vascular systems of the two tissues may be important. Increased blood flow to one organ could thus result in increased blood flow to the other and an increase in hormone secretion. It is possible that secretion of both ACTH and gonadotropin are increased in response to

the same stimuli, or that ACTH activates gonadotropin receptors on the Leydig cells.

Another explanation for the sex difference in plasma glucocorticoid concentration is greater activity in male tortoises than in female tortoises. There are a number of studies that document larger home ranges for male tortoises than for females (Berry, 1986; O'Conner et al., 1994). Increased glucocorticoid secretion is associated with increased activity, especially mating. Thus both the intense mating activity and male-male combat in August and September, and the larger home range of males may account for their higher corticosterone levels throughout the year.

Immature (sub-adult) male tortoises show a seasonal change in plasma testosterone similar to that of the adult males. The concentration of testosterone in these tortoises is, however, about half that of the adults. It is not known if these animals are undergoing spermatogenesis, but they lack the full suite of secondary sex characters, and have not been observed to copulate with females. Reptiles do not exhibit puberty as is seen in mammals, but achieve sexual maturity over several seasons as they gradually reach adult body size. The gradual increase in circulating testosterone reflects this developmental pattern.

Stress is known to suppress reproductive hormone secretion in all vertebrate groups. Acute stress (short term) has been shown to shut down gonadal secretion of testosterone in male reptiles within hours (Lance, 1994). In male chelonians, however, acute stress has a paradoxical effect on circulating testosterone. Mahmoud et al. (1989) reported that in male snapping turtles, *Chelydra serpentina*, there is an increase in testosterone during the first several hours post-capture then a gradual decrease, such that by 24 hrs plasma levels are significantly lower than at the initial sample. A similar pattern is seen in the desert tortoise subjected to restraint stress. Tortoises held in boxes and taken from the field before blood is drawn have significantly higher corticosterone and testosterone concentrations than tortoises bled immediately in the field (Classen, 1994). A similar pattern is seen in response to a single intravenous injection of ACTH. There is a rapid rise in corticosterone secretion and an equally rapid increase in plasma testosterone levels (Fig. 11). By 8 hrs post-injection corticosterone had returned to baseline and testosterone had fallen to about 50% of the initial value. The close relationship between plasma testosterone and corticosterone during the annual reproductive cycle of the male (Fig. 7) also suggests that secretion of the two hormones is somehow linked. The mechanism for this relationship, however, remains unknown.

In all temperate-zone reptiles that have been studied there is a large variation in circulating white blood cell (WBC) numbers during the year. In general WBC numbers are low during cooler months and high when ambient temperature increases in summer (see Lance, 1994, for a review). In both male and female desert tortoises in Nevada the number of WBC increased significantly during August and September (Fig. 12). Neither Christopher et al. (1999) nor Dickinson et al. (2002) found any significant seasonal differences in WBC numbers in wild populations of desert tortoises in California and Arizona, respectively. The peak in WBC numbers in the captive desert tortoises occurs when both testosterone and adrenal steroid secretion are greatest in the male, and when estradiol secretion is greatest in the female. Current theory suggests, however, that the immune system and WBC numbers are suppressed when the concentration of circulating steroids is high (Zapata et al., 1992). The results from the desert tortoise run counter to this model: WBC numbers are highest when steroid secretion in highest, and low when steroids are low. If steroids are modulating the immune system in reptiles the mechanism remains obscure.

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