General and Comparative Endocrinology 117, 335–342 (2000)
doi:10.1006/gcen.2000.7426, available online at http://www.idealibrary.com on IDEAL®

Seasonal Patterns of Plasma Steroid Hormones in Males and Females of the Bearded Dragon Lizard, *Pogona barbata*

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Accepted April 27, 1999

Pogona barbata is an Australian lizard that produces several large clutches of eggs between August and December (spring to early summer). Mating takes place around ovulation. The seasonal pattern of reproductive hormones in males and females of *P. barbata* was determined by radioimmunoassay of plasma progesterone (P), estradiol-17**β** (E-17**β**), corticosterone (B), and total androgen (TA). In females, P began to rise in August and was elevated from September to December. Corticosterone and TA were detectable but low and did not vary with time of year or reproductive condition. Estradiol-17**β** was only detectable in a few females and exhibited no elevation with vitellogenic activity. These results suggest that B and TA are not involved in female reproduction. Estrogens may be either so low they could not be detected or they were present in a form other than estradiol-17**β**. The high sensitivity of the estradiol-17**β** radioimmunoassay suggests the latter. In males, TA peaked at the beginning of spring. They then declined to a minimum during November and December. However, concentrations recovered in the postbreeding activity period, January to April (summer and autumn). These patterns are consistent with the observation of maximum spermatogenic activity in spring, followed by the cessation of spermatogenesis directly after the breeding period and testicular recrudescence in February (late summer).

Key Words: Pogona barbata; Agamidae; reproductive endocrinology.

The eastern bearded dragon, *Pogona barbata*, is a large (~250-mm snout–vent length, SVL) semiarboreal lizard found in a wide range of habitats in eastern and southeastern Australia. Mating and egg-laying occur through spring to early summer (late August to December, personal observation, Badham, 1971). The species lays up to three large clutches of up to 35 eggs each in a year and can do so for several years (personal observation, Bustard, 1966; Greer, 1989; Heatwole and Taylor, 1987). This is in contrast to most other large lizards, which tend to lay single, small clutches annually (Schwarzkopf, 1994). Most models of reproductive endocrinology assume a single reproductive event per breeding period and do not discuss species where several reproductive events may occur in each breeding period and may even overlap (Callard *et al.,* 1990; Duvall *et al.,* 1982; Lofts, 1985). To deepen our understanding of reptilian reproductive biology, the endocrine patterns mediating annual reproduction in each sex of *P. barbata* were investigated. We assayed the steroids most likely to be important: progesterone (P), estradiol (E-17β), and androgens (TA). Corticosterone was also investigated, because of the effects of stress on reproduction and the possible influence of corticoste-

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0016-6480/00 $35.00

rone itself on reproduction (Whittier *et al.*, 1997; Wilson and Wingfield, 1994).

METHODS

Sampling Methods

Animals of each sex were captured every month between March 1994 and October 1995, except for the months May and June 1994. Animals were collected monthly by hand from private lands in and around the Shire of Laidley, Queensland (27°35′ S, 152°18′ E), and the grounds of the University of Queensland, St. Lucia campus (27°30′ S, 153°01′ E). They were blood sampled immediately, within 3 min of capture. Blood (0.5–1.0 mL) was collected from the caudal vein, a few centimeters posterior to the cloaca. A heparinized 75-mm capillary tube was filled with blood from this sample for hematocrit determination. The blood was kept on ice until spun. Blood samples and capillary tubes were centrifuged at 3000 rpm for 10 min. Plasma was frozen at −5° until assayed. Sex of the animals was determined as follows. Mature males were large and heavily built (235 ± 4 mm SVL, average ± standard error, 393 ± 17 g body mass, $n = 51$) with wide heads, large black beards, and large femoroanal pores. Eversion of hemipenes was rarely possible but breeding males' inverted hemipenes were probed with a blunt probe. During the breeding season, the cloacae of males usually had a white paste and their snouts were often scarred from aggression with other males. Females tended to be smaller (193 ± 3 mm SVL, 239 ± 10 g body mass, $n = 72$) with longer heads, small beards, and small pores. Females often had bite wounds on the back of the neck from copulation. Reproductive condition could be determined by abdominal palpation and was classed as nonreproductive, vitellogenic, or gravid. Vitellogenic follicles could be felt as a collection of soft, round objects, approximately 4–20 mm in diameter. Oviductal eggs could be felt as firm, ellipsoid objects filling the entire abdominal cavity.

Lizards were weighed to the nearest gram on a Pesola spring scale balance and snout–vent length was measured to the nearest millimeter with a rigid ruler. They were toe clipped before release to ensure individuals were only sampled once.

Radioimmunoassay (RIA)

The assay used was developed for reptilian plasma (Whittier *et al.*, 1997, 1987). Samples (100 µL) were extracted twice with diethyl ether (AnalaR grade, BDH Chemicals). Tritiated hormone (25 µL) was added for each hormone recovery determination (P: 1,2,6,7,21-[^{3}H]P, NET724; TA: 1,2,6,7-[^{3}H]T, NET370; E-17β: 2,4,6,7-[^{3}H]E-17β, NET317; B: 1,2,6,7-[^{3}H]B, NET399; Amersham International). Extracted samples were evaporated to dryness and then resuspended in RIA buffer (500 µL, solution: 8.66 g anhydrous $Na_6(PO_4)_2$, 4.70 g Na_3PO_4, 9.0 g NaCl, 1.0 g NaN_3, and 1.0 g bovine albumen in 1.0 L double-distilled H_2O; all reagents from Sigma-Aldrich).

Duplicate samples (2 × 200 µL from resuspended sample) were assayed with tritiated hormone and specific antisera (P: P11-192, 1:80 dilution; T: T-1500, 1:60 dilution; E-17β: E26-47, 1:60 dilution; B: B3-163, 1:50 dilution; Endocrine Sciences). The testosterone antiserum (T) cross-reacts significantly with dihydrotestosterone (DHT: 44.0%), Δ-1-T (41.0%), and Δ-1-DHT (18.0%). Due to this, we report total androgen (TA). Cross-reactivity of other antisera was insignificant (<1%). Standard solutions were created from crystalline steroid (P: P-1030; T: T-1500; E-17β: E-8875; B: C-2505; Sigma-Aldrich) in ethanol covering the range 1.25 to 1000 pg/100 µL. Dextran-coated charcoal solution was used to separate bound and free fractions. Incubation period was 12 min (P and B), 16 min (TA), and 18 min (E-17β). Bound fraction was counted in an aqueous-based scintillant (NBCS104, Amersham International) on a Beckman LS6000.

Data were analyzed using the Immunofit v 2.00 and RIAPRO programs (Beckman Instruments). Validation assays were performed for each steroid. Water blanks averaged 100 ± 7% of B_{Max} (average ± SE), stripped plasma blanks 98 ± 8%. Recoveries averaged 84 ± 4% and accuracies 120 ± 35%. Individual samples were corrected for individual sample recovery. Sensitivities were 0.25 ng/mL (P and B), 0.075 ng/mL (TA), and 0.062 ng/mL (E-17β). Intra-assay variability averaged 14.8 ± 4.3% and interassay variability 17 ± 2%. Parallel binding was established with three standard concentrations (0.1, 1.0, and 10 ng/mL) in stripped plasma. The slope of actual versus measured concentration averaged 0.882 ± 0.223 and the correlation (r^2) was 0.876 ± 0.105.

Stripped plasma blanks were created from approximately 5 mL of sea turtle (*Caretta caretta*) plasma. Turtle plasma was readily available in large quantities and was not expected to be significantly different from *P. barbata* plasma. High and low samples were created for each hormone by pooling appropriate plasma samples. Samples from mature males during the breeding season taken within 3 min of capture were used for a pool high in TA and low in P, E-17β, and B. Samples from late-vitellogenic females taken within 3 min of capture were used for a pool high in P and E-17β and low in B and TA. Samples from animals which had been kept in a cloth bag for 3 h were used to create a pool of high B.

Data Analysis

All averages are given ± standard error. Hormone values were log transformed before statistical testing as significant kurtosis of untransformed data was observed. The following variables were tested for their relationship with hormone values: SVL, mass, body condition (mass / SVL × 100), hematocrit, date (pooled into two monthly samples), breeding period (August–December vs January–July), hour of sampling, female reproductive condition (nonreproductive, vitellogenic, or gravid) and values of the other hormones. Hematocrit and body condition were transformed using $\sqrt{(x+1)}$. The Minitab 11.11 program was used for all statistics. Analysis of covariance and general linear model ANOVAs were used to define the influence of these variables. Fisher's pairwise comparisons were used as post hoc tests. A probability of $P = 0.05$ was accepted as significant. Only the highest F value and smallest P value of nonsignificant results for each hormone within each sex are reported.

RESULTS

Males

Total androgen varied significantly between months ($df = 49$, $F = 2.90$, $P = 0.032$; Fig. 1). Total androgen showed a large peak of 27.58 ± 9.05 ng / mL ($n = 5$) in July–August. Concentrations declined over the succeeding months to a minimum in November–December of 2.97 ± 0.73 ng / mL ($n = 11$). Total androgen concentra-

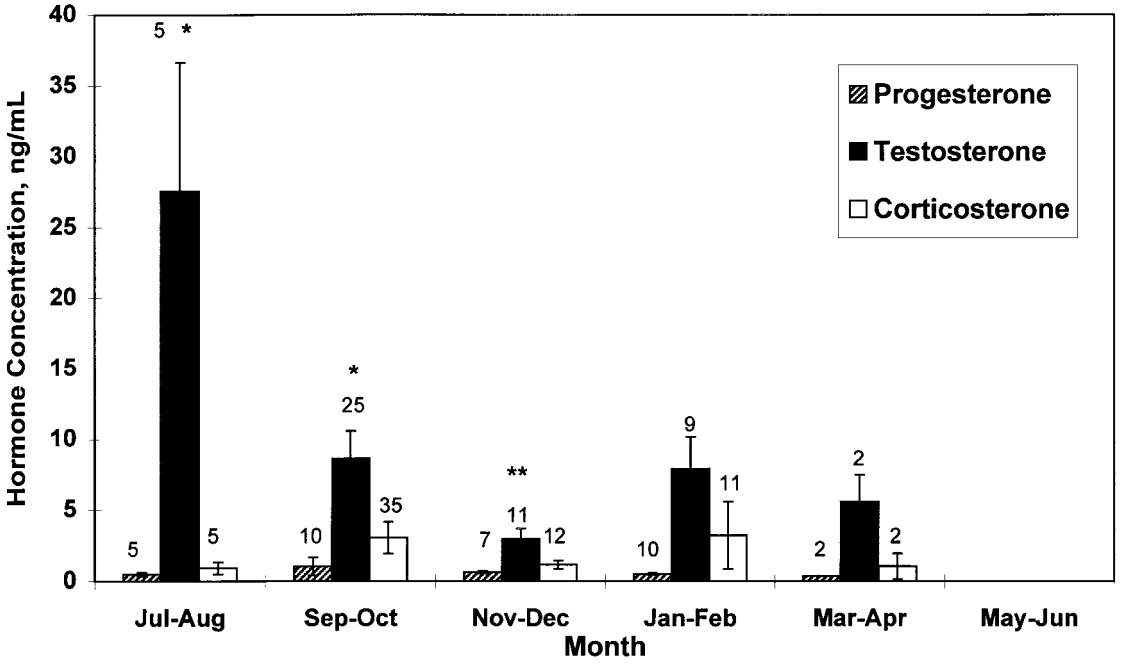

FIG. 1. Plasma hormone levels in males of *P. barbata* throughout the year. Single and double asterisks mark significant groupings according to Fisher's pairwise comparisons test. Sample sizes (*n*) are given above bars.

tions during July to October were significantly different from November to December. There was also a significant relationship between hour and TA ($df = 48$, $F = 2.46$, $P = 0.022$) due to a significant covariance between hour and month (analysis of covariance, $df = 48$, $F = 2.28$, $P = 0.033$). All other factors did not explain significant variation in TA concentrations ($F < 1.23$, $P > 0.278$).

Elevated concentrations of P were detected in the plasma of males (averaging 0.48 ± 0.03 ng / mL, $n = 28$; Fig. 1). However, these concentrations did not vary significantly with any variable ($F < 2.56$, $P > 0.119$). Similarly, B was detected (average 2.58 ± 0.74 ng / mL, $n = 63$; Fig. 1) but did not show relationships with any variable measured ($F < 1.42$, $P > 0.242$).

Females

Progesterone exhibited significant variation among months ($df = 75$, $F = 4.88$, $P = 0.002$; Fig. 2). Concentrations began to rise in August (3.42 ± 3.06 ng / mL, $n = 3$) but reached their peak and remained elevated over September to December (5.27 ± 1.18 ng / mL, $n = 50$). A decline then occurred. Fisher's pairwise comparisons demonstrated that the September to December period was significantly different from the January to April period. Vitellogenic females showed higher concentrations (5.63 ± 1.57 ng / mL, $n = 25$) than the other two reproductive conditions and this difference was statistically significant ($df = 69$, $F = 4.83$, $P = 0.011$; Fig. 3). In female *P. barbata*, neither TA nor E-17β showed any significant correlation with any measured variables (TA: $F < 2.57$, $P > 0.115$; E-17β: $F < 2.30$, $P > 0.137$). Total androgen averaged 0.85 ± 0.29 ng / mL ($n = 50$; Fig. 2). Estradiol-17β was usually undetectable (40 / 48 females) and averaged 0.13 ± 0.09 ng / mL ($n = 48$; Fig. 2). The eight females with detectable E-17β were from all three reproductive conditions. Corticosterone averaged 6.89 ± 2.56 ng / mL ($n = 78$, Fig. 2). There was a significant positive relationship between B and hematocrit ($df = 43$, $F = 6.64$, $P = 0.014$; Fig. 4). There were no other significant correlations between B and other variables in females ($F < 1.53$, $P > 0.202$).

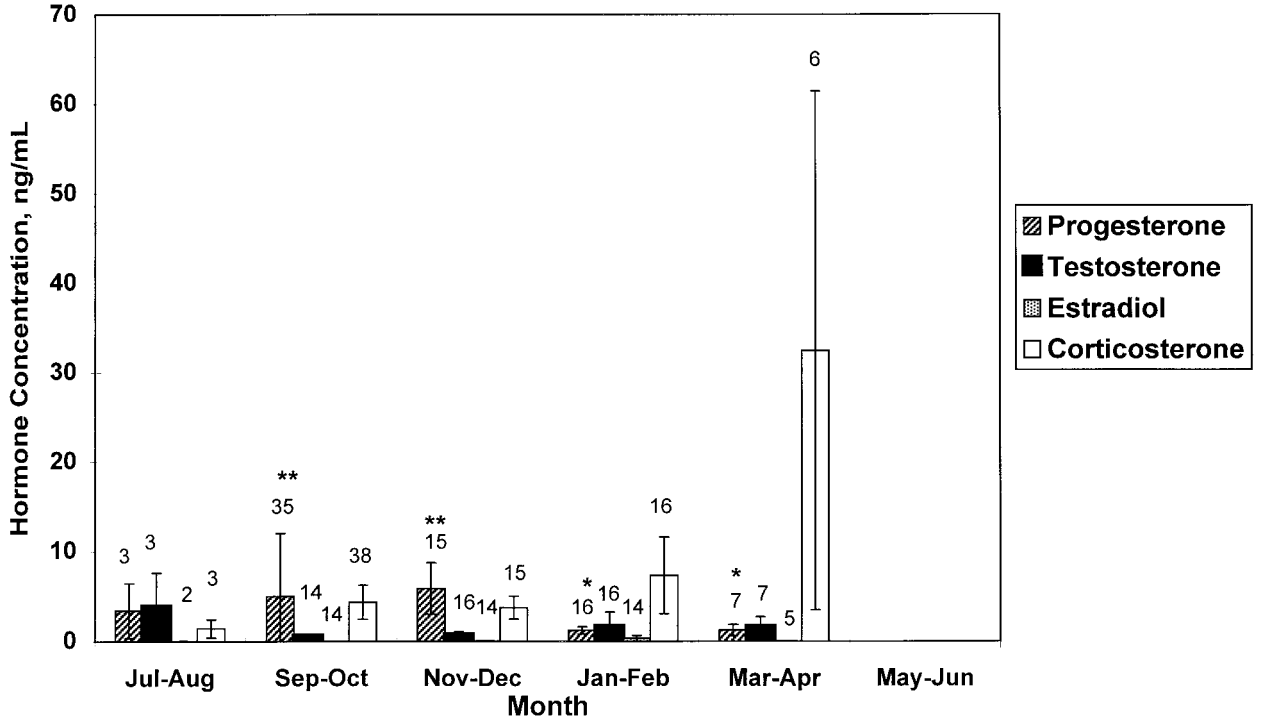

FIG. 2. Plasma hormone levels in females of *P. barbata* throughout the year. Single and double asterisks mark significant groupings according to Fisher's pairwise comparisons test. Sample sizes (n) are given above bars.

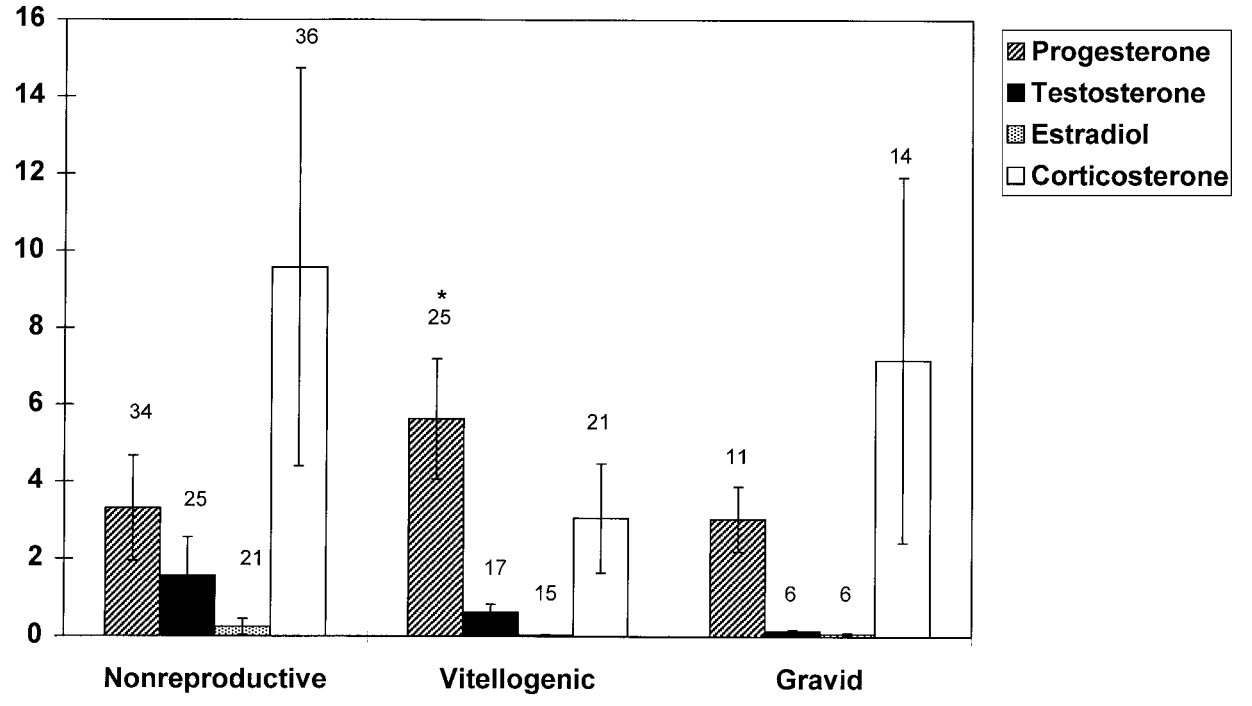

FIG. 3. Plasma hormone concentrations of females in the three reproductive conditions. Asterisk shows significantly different group according to Fisher's pairwise comparisons. Sample sizes (n) are given above bars.

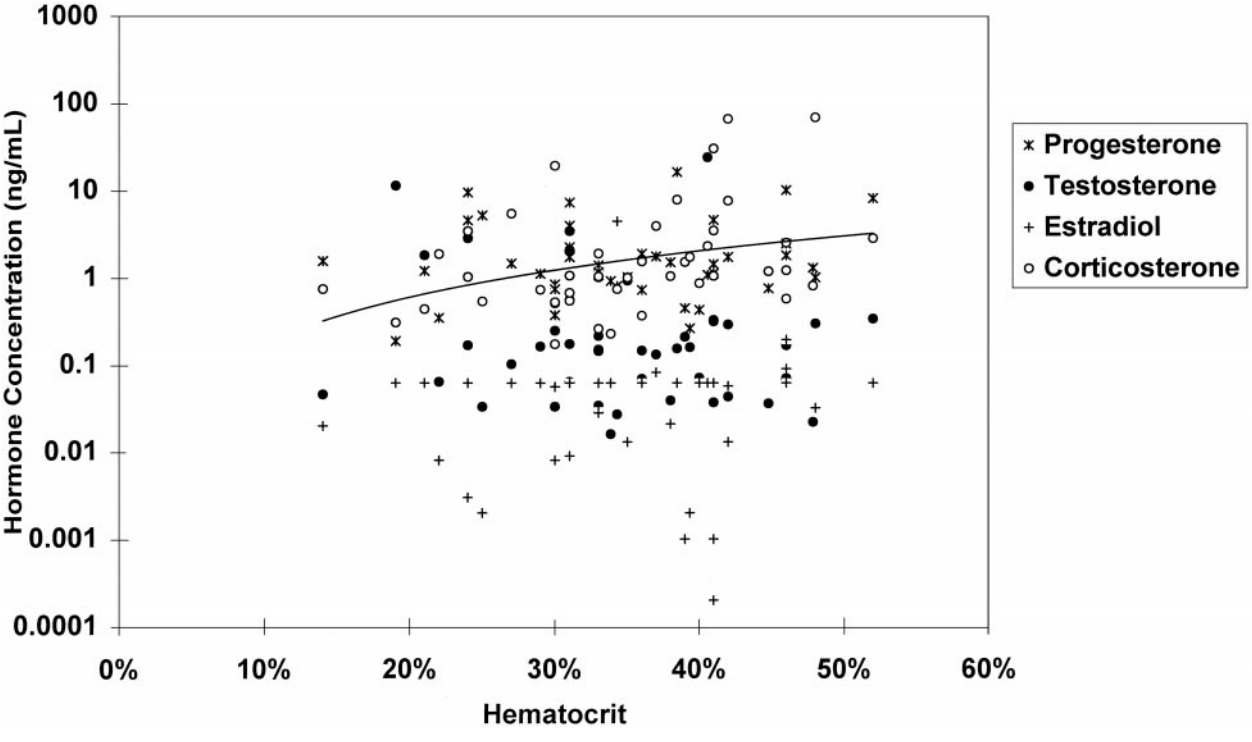

FIG. 4. Plasma hormone concentrations against hematocrit in females of *P. barbata.* Plotted line is regression of corticosterone against hematocrit, the equation of which is $B = 0.0002558 \times \mathrm{Hct}^{2.790}$, $n = 44$, $r = 0.3501$.

DISCUSSION

The spring breeding cycle of *P. barbata* is reflected in the cyclicity of its plasma sex steroid concentrations. This was expected in a species showing the associated reproductive strategy (Crews and Gans, 1992). However, the seasonal hormonal pattern of *P. barbata* also showed several unique features. Females do not appear to use E-17β to stimulate vitellogenesis. Significant levels of androgens were detected in males from all months sampled with minimum concentration only for a short period after reproduction.

Progesterone concentrations in females correlated strongly with the reproductive cycle. The hormone started to increase in July–August, once the lizards had emerged from winter torpor, and reached a peak by September. High concentrations were maintained until breeding finished in December. Variability in plasma P concentrations within the breeding period reflected a significant correlation with reproductive condition and the fact that different females were in different stages of vitellogenesis and gravidity within each month. The fact that P was highest in vitellogenic females can be interpreted as due to P rising during vitellogenesis and then slowly declining during gravidity. This pattern has been observed in a wide range of other oviparous reptile species such as *Sphenodon punctatus* (Cree *et al.*, 1992), *Eumeces obsoletus* and *Sceloporus jarrovi* (Guillette and Jones, 1980), and *Sternotheres odoratus* (McPherson *et al.*, 1982). The similarity to *S. odoratus* is particularly interesting as this turtle also produces multiple clutches within a breeding period. Before ovulation, the main source of P is the developing follicles. The corpora lutea continue this role after ovulation (Duvall *et al.*, 1982; Lance and Callard, 1978). However, in *P. barbata*, we hypothesize that, as in turtles, the corpora lutea are relatively short-lived. Rather than maintaining pregnancy, the main functions of P in oviparous species such as *P. barbata* are the stimulation of follicle maturation and ovulation and preparation of the oviduct for this event (Wibbels *et al.*, 1992).

Estradiol-17β is stated to be the main form of E present in vertebrates (Breuer, 1962; Callard *et al.*, 1990) and it is a potent stimulator of vitellogenesis (Cree *et al.*, 1992; Gavaud, 1986; Ho *et al.*, 1985; Mahmoud *et al.*, 1985). However, only very small amounts were observed in vitellogenic females of *P. barbata*. Most individuals in fact had nondetectable concentrations. The assay used was sensitive to 0.0625 ng / mL. Other reptile species that have been studied have low concentrations of E-17β but they would still have been detected by this assay. For example, vitellogenic *Vipera apsis* had plasma concentrations of 4.83 ± 1.27 ng / mL (Bonnet *et al.*, 1994), *Podarcis sicula* showed a similar peak (Carnevali *et al.*, 1991), *Niveoscincus metallicus* reached 0.714 ± 0.114 ng / mL (Jones and Swain, 1996), and *Lissemys punctata* had plasma concentrations of 0.598 ng / mL at ovulation (Sarkar *et al.*, 1996). Only a small number of females of *P. barbata* had detectable concentrations and these were from all reproductive conditions and all times of the year.

This suggests that E-17β is not of major significance to *P. barbata*. It is possible that another form of estrogen, for example, estrone or estriol, is more important. Estradiol-17β may simply be a short-lived metabolite of this more abundant estrogen. Although this possibility in other reptiles has been raised (Owens and Morris, 1985; Whittier *et al.*, 1997; Wibbels *et al.*, 1992), an alternative estrogen has not yet been demonstrated.

Androgens have been assigned numerous roles in female reptiles' reproduction, such as follicle maturation and ovulation (Wibbels *et al.*, 1992), receptivity, and migration (Wibbels *et al.*, 1990). They may be used in a way similar to B to mobilize lipids, carbohydrates, and proteins during vitellogenesis (Whittier *et al.*, 1997). In *P. barbata*, however, low but detectable concentrations were not found to vary significantly between months or reproductive conditions. We found no evidence for a role of TA in female reproduction in this species.

Similarly, B also did not vary between months and reproductive conditions for either sex. However, the interindividual variation was large with many individuals showing high concentrations. There was a significant relationship between B and hematocrit in females. The classical role of B as a ''stress'' mediator seems the best explanation for this. High hematocrit is an indicator of dehydration and it may be this stressor that stimulated B secretion. Studies on the influence of B on female reproduction in other reptile species have produced a variety of results. Some have shown a positive correlation between B and vitellogenesis (Wilson and Wingfield, 1992, 1994), a negative correlation (Grassman and Crews, 1990), a seasonal change due to

reproductive condition (Whittier *et al.,* 1997), elevation during gravity (Tyrrell and Cree, 1998), or a seasonal change regardless of reproductive condition (Girling and Cree, 1995; Whittier *et al.,* 1987). The role of B in female reptilian reproduction is currently poorly understood but it is obviously complex and variable between species. In *P. barbata,* the lack of correlation with any reproductive condition or month suggests a lack of involvement in the reproductive process.

Androgens were the only hormones to show any correlation with other variables in males. A large peak in plasma androgens was observed at the beginning of spring. Concentrations then declined to their lowest in November–December. This low concentration was still substantially above that observed in females. Once breeding had finished, concentrations rose again. The decrease in November–December and subsequent recovery preceded a brief cessation of spermatogenesis followed by recrudescence in January (personal observation, Badham, 1971). Significant concentrations of androgens outside the breeding period may be required to stimulate spermatogenesis through summer and autumn. The early spring peak in androgens may stimulate spermatogenesis again after winter torpor. It would also stimulate secondary sexual characters, such as the black beard, aggression, and courtship. The lower concentrations after breeding may be sufficient to stimulate spermatogenesis but be insufficient to stimulate secondary features without the primer peak. It is commonly found that a large dose of hormone may be required to stimulate a response while a smaller one is sufficient to maintain it (for example, Carnevali *et al.,* 1991; Paolucci *et al.,* 1992; Wibbels *et al.,* 1992).

In summary, this study has found that plasma P was present in significant amounts only during breeding in female *P. barbata* and showed a long peak from September to December. The positive correlation of high plasma P concentrations with vitellogenesis indicated its major role in follicle maturation and ovulation. Neither TA nor B appears to have important roles in female *P. barbata* reproduction. Males experienced a sharp peak in TA at the beginning of spring, coinciding with the commencement of spermatogenesis, development of secondary sexual characteristics, and aggression and courtship. Androgens remained elevated after breeding and correlated with continued spermatogenesis through summer and autumn. Corticosterone concentrations in both sexes do not indicate elevated stress during the breeding period.

ACKNOWLEDGMENTS

We thank Laidley Golf Club for allowing access to animals on their grounds. This study was partly supported by an Australian Postgraduate Award scholarship to A.P.A. and an Australian Research Council grant to J.M.W. We thank anonymous reviewers for constructive criticism to an earlier draft of the manuscript.

REFERENCES

Badham, J. A. (1971). "A Comparison of Two Variants of the Bearded Dragon, *Amphibolurus barbatus* (Cuvier)." Ph.D. thesis, University of Sydney, Sydney.

Bonnet, X., Naulleau, G., and Mauget, R. (1994). The influence of body condition on 17-b estradiol levels in relation to vitellogenesis in female *Vipera aspis* (Reptilia, Viperidae). *Gen. Comp. Endocrinol.* **93,** 424–437.

Breuer, H. (1962). The metabolism of the natural estrogens. *Vit. Horm.* **20,** 285–335.

Bustard, H. R. (1966). Notes on the eggs, incubation and young of the bearded dragon, *Amphibolurus barbatus barbatus* (Cuvier). *Brit. J. Herp.* **3,** 252–259.

Callard, I. P., Riley, D. L., and Perez, L. (1990). Vertebrate vitellogenesis: Molecular model for multihormonal control of gene regulation. *In* "Progress in Comparative Endocrinology" (A. Epple, C. G. Scanes, and M. H. Stetson, Eds.), pp. 343–348. Wiley–Liss, New York.

Carnevali, O., Mosconi, G., Angelini, F., Limatola, E., Ciarcia, G., and Polzonetti-Magni, A. (1991). Plasma vitellogenin and 17β-estradiol levels during the annual reproductive cycle of *Podarcis s. sicula* Raf. *Gen. Comp. Endocrinol.* **84,** 337–343.

Cree, A., Cockrem, J. F., and Guillette, L. J. (1992). Reproductive cycles of male and female tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. *J. Zool.* **226,** 199–217.

Crews, D., and Gans, C. (1992). The interaction of hormones, brain and behavior: An emerging discipline in herpetology. *In* "Physiology, Hormones, Brain, and Behavior: Biology of the Reptilia" (C. Gans and D. Crews, Eds.), Vol. 18, pp. 1–23. Univ. of Chicago Press, Chicago.

Duvall, D., Guillette, L. J., and Jones, R. E. (1982). Environmental control of reptilian reproductive cycles. *In* "Physiology and Physiological Ecology: Biology of the Reptilia" (C. Gans and F. H. Pough, Eds.), Vol. 14, pp. 201–231. Wiley, New York.

Gavaud, J. (1986). Vitellogenesis in the lizard *Lacerta vivipara* Jacquin 2: Vitellogenin synthesis during reproductive cycle and its control by ovarian steroids. *Gen. Comp. Endocrinol.* **63,** 11–23.

Girling, J. E., and Cree, A. (1995). Plasma corticosterone levels are not significantly related to reproductive stage in female common geckos (*Hoplodactylus maculatus*). *Gen. Comp. Endocrinol.* **100,** 273–281.

Grassman, M., and Crews, D. (1990). Ovarian and adrenal function in the parthenogenetic whiptail lizard *Cnemidophorus uniparens* in the field and laboratory. *Gen. Comp. Endocrinol.* **76,** 444–450.

Greer, A. E. (1989). ''The Biology and Evolution of Australian Lizards.'' Surrey Beatty & Sons, Sydney.

Guillette, L. J., and Jones, R. E. (1980). Arginine vasotocin-induced *in vitro* oviductal contractions in *Anolis carolinensis:* Effects of steroid hormone pretreatment *in vivo. J. Exp. Zool.* **212,** 147–152.

Heatwole, H., and Taylor, J. L. (1987). ''Ecology of Reptiles.'' Surrey, Beatty & Sons, Sydney.

Ho, S. M., Wangh, L. J., and Callard, I. P. (1985). Sexual differences in the *in vitro* induction of vitellogenesis in the turtle (*Chrysemys picta*): Role of the pituitary and growth hormone. *Comp. Biochem. Physiol. B* **81(2),** 467–472.

Jones, S. M., and Swain, R. (1996). Annual reproductive cycle and annual cycles of reproductive hormones in plasma of female *Niveoscincus metallicus* (Scincidae) from Tasmania. *J. Herp.* **30(2),** 140–146.

Lance, V. A., and Callard, I. P. (1978). Hormonal control of ovarian steroidogenesis in nonmammalian vertebrates. *In* ''The Vertebrate Ovary'' (R. E. Jones, Ed.), pp. 361–140. Plenum, New York.

Lofts, B. (1985). Environmental control of reptilian reproduction. *In* ''The Endocrine System and the Environment'' (B. K. Follett, S. Ishii and A. Chandola, Eds.), pp. 93–103. Springer-Verlag, Berlin.

Mahmoud, I. Y., Cyrus, R. V., Woller, M. J., and Bieber, A. (1985). Development of the ovarian follicles in relation to changes in plasma parameters and 5Δ-3β-hydroxysteroid dehydrogenase in snapping turtle, *Chelydra serpentina. Comp. Biochem. Physiol. A* **82(1),** 131–136.

McPherson, R. J., Boots, L. R., MacGregor, R., and Marion, K. R. (1982). Plasma steroids associated with seasonal reproductive changes in a multiclutched freshwater turtle, *Sternotherus odoratus. Gen. Comp. Endocrinol.* **48,** 440–451.

Owens, D. W., and Morris, Y. A. (1985). The comparative endocrinology of sea turtles. *Copeia* **1985(3),** 723–735.

Paolucci, M., di Fiore, M. M., Ciarcia, G., and Botte, V. (1992). Plasma sex steroid binding proteins (SSBP) in the male lizard, *Podarcis s. sicula,* during the reproductive cycle. *Gen. Comp. Endocrinol.* **87,** 232–239.

Sarkar, S., Sarkar, N. K., and Maiti, B. R. (1996). Seasonal pattern of ovarian growth and interrelated changes in plasma steroid levels, vitellogenesis, and oviductal function in the adult female soft-shelled turtle *Lissemys punctata punctata. Can. J. Zool.* **74(2),** 303–311.

Schwarzkopf, L. (1994). Measuring trade-offs: A review of studies of costs of reproduction in lizards. *In* ''Lizard Ecology'' (L. J. Vitt and E. R. Pianka, Eds.), pp. 7–29. Princeton Univ. Press, Princeton, NJ.

Tyrrell, C. L., and Cree, A. (1998). Relationships between corticosterone concentration and season, time of day and confinement in a wild reptile (tuatara, *Sphenodon punctatus*). *Gen. Comp. Endocrinol.* **110,** 97–108.

Whittier, J. M., Corrie, F. K., and Limpus, C. J. (1997). Plasma steroid profiles in nesting loggerhead turtle (*Caretta caretta*) in Queensland, Australia: Relationship to nesting episode and season. *Gen. Comp. Endocrinol.* **106,** 39–47.

Whittier, J. M., Mason, R. T., and Crews, D. (1987). Plasma steroid hormone levels of female red-sided garter snakes, *Thamnophis sirtalis parietalis:* Relationship to mating and gestation. *Gen. Comp. Endocrinol.* **67,** 33–43.

Wibbels, T., Owen, D. T., Limpus, C. J., Reed, P. C., and Amoss, M. S. (1990). Seasonal changes in serum gonadal steroids associated with migration, mating and nesting in the loggerhead sea turtle, *Caretta caretta. Gen. Comp. Endocrinol.* **79,** 154–164.

Wibbels, T., Owens, D. W., Licht, P., Limpus, C. J., Reed, P. C., and Amoss, M. S. (1992). Serum gonadotropins and gonadal steroids associated with ovulation and egg production in sea turtles. *Gen. Comp. Endocrinol.* **87,** 71–78.

Wilson, B. S., and Wingfield, J. C. (1992). Correlation between female reproductive condition and plasma corticosterone in the lizard *Uta stansburiana. Copeia,* **1992(3),** 691–697.

Wilson, B. S., and Wingfield, J. C. (1994). Seasonal and interpopulational variation in plasma levels of corticosterone in the side-blotched lizard (*Uta stansburiana*). *Physiol. Zool.* **67,** 1025–1049.