ed by MIDGLEY[7]. The protocol for pituitary No. 4 was varied slightly to include an extra $^1/_2$ h of pre-incubation during which time the pituitary fragments were exposed to no additives (controls) or 400 ng/ml of estradiol (E_2), testosterone (T), or progesterone (P), respectively. These fragments were then removed to the 1 ml incubation media containing no additives (controls) or 10 ng/ml of GRH. After incubation, the pituitary fragments were weighed to the nearest 0.01 mg on a micro balance.

Results. Results as presented in the Figure are expressed as ng of LER907 present in the medium per mg of pituitary tissue at the various time intervals. Control values, or baseline release, were somewhat variable. However, all the pituitaries showed some response to the varying doses of GRH either at 15 or 60 min. Only 1 pituitary fragment (no. 2 with 25 µg of GRH) was less than or equal to the control. The release of LH and FSH (as measured in no. 3 and 4) were of the same order of magnitude with release of both hormones significantly above the control at 15 min. Pre-incubation for $^1/_2$ h with 400 ng of E_2, P or T appeared to decrease the response to 10 ng of GRH in pituitary No. 4. Despite the responses seen with most fragments, a dose-response effect was not apparent in these experiments. 1 and 10 ng doses of GRH appeared to be as effective as the larger doses.

Discussion. The relative unavailability of normal human pituitary for in vitro study is a limiting factor in this study. Nevertheless, the data presented indicate that synthetic GRH is capable of releasing both LH and FSH from human pituitaries in vitro. As little as 1.0 ng of GRH appears capable of causing this release. This is in agreement with in vitro studies in rats where nanogram amounts of GRH released significant amounts of LH and FSH[8]. In vivo human studies have indicated that i.v. injections of GRH result in maximum elevation of serum LH and FSH within 15 min to 1 h[9]. Our studies indicate that isolated pituitaries are also capable of such release within 1 h, though the response over baseline was not always as marked. In the 2 pituitaries where concomitant LH and FSH release was studied, FSH-RH activity of GRH was equal to, or greater than, the LH-RH activity of this molecule. This is in contrast to most humanin vivo studies where quick i.v. injection of GRH results in proportionally greater serum LH levels than FSH levels[9–11].

Large doses of estradiol, testosterone, and progesterone had significant suppressive effects on both LH and FSH release in the one pituitary in which this was studied. This in vitro phenomenon suggests a direct effect of steroids on human pituitary tissue.

Zusammenfassung. Menschliche Hypophysen wurden mit und ohne LH-RH inkubiert und eine Freisetzung von LH und FSH in vitro gefunden, die sich durch Sexualsteroide hemmen lässt.

F. J. SANDERS, P. B. MAY and R. K. DONABEDIAN[12]

Department of Laboratory Medicine, Yale University School of Medicine, 133 Cedar Street, New Haven (Connecticut 06510, USA), 18 February 1974.

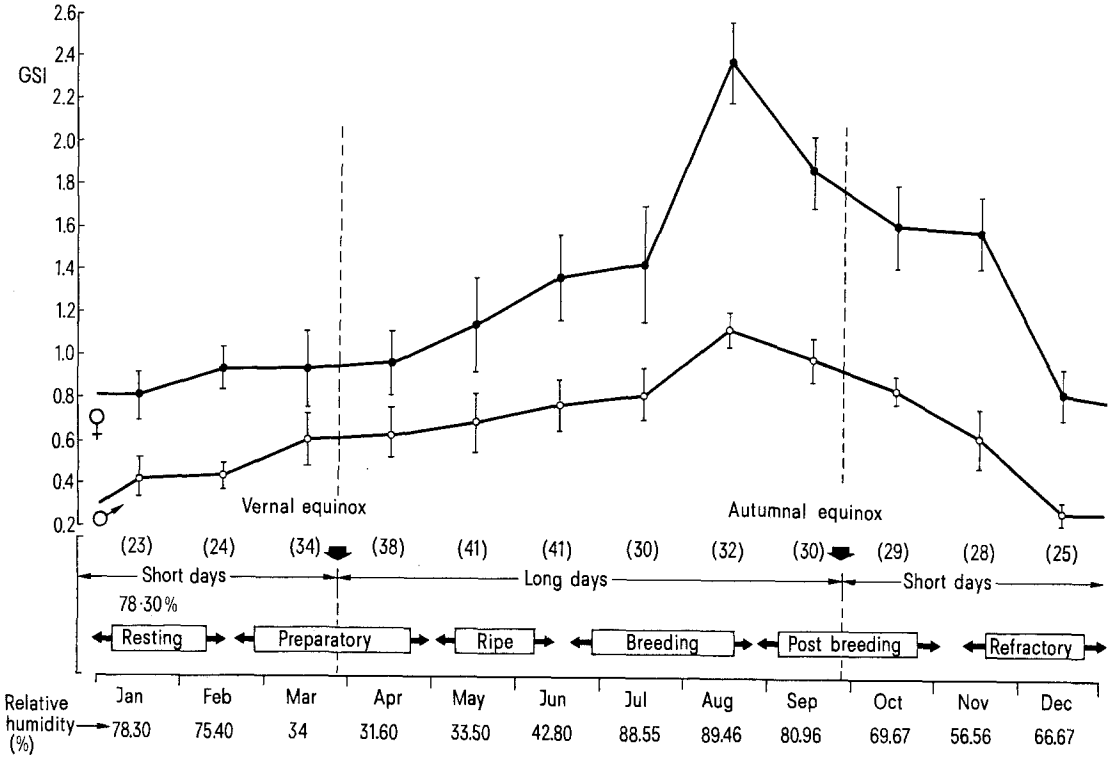
[7] A. R. MIDGLEY JR., Endocrinology *79*, 10 (1966).
[8] A. V. SCHALLY, T. W. REDDING, H. MATSUO, A. ARIMURA, Endocrinology *90*, 1561 (1972).
[9] S. S. C. YEN, G. VANDENBERG, R. REBAR and Y. EHARA, J. clin. Endocrin. Metab. *35*, 931 (1972).
[10] S. S. C. YEN, R. REBAR, G. VANDENBERG, F. NAFTOLIN, Y. EHARA, S. ENGBLOM, K. J. RYAN and K. BENIRSCHKE, J. clin. Endocrin. Metab. *34*, 1108 (1972).
[11] G. MILHAUD, P. RIVAILLE, P. GARNIER, J. L. CHAUSSAIN, E. BINAT and J. C. JOB, C.r. Acad. Sci., Paris *273*, 1858 (1971).
[12] The authors wish to thank Dr. J. VANGILDER of the Department of Neurosurgery who graciously supplied the pituitary specimens for this study. This study was supported by United States Public Health Service Training Grant No. 5T01 GM00696.

Analysis of Environmental Factors Regulating the Gonadal Cycle in a Tropical Pond Turtle, *Lissemys p. granosa* (Schoepff.)

The role of external factors in regulating the sexual cycle of mammals and birds has been extensively studied, but the present literature regarding similar studies in reptiles is meagre[1]. Further, the studies on the gonadal changes in the species of order chelonia appear not to have attracted much attention[2] and hence remain the most unexplored in the whole reptilian group. Some data which are available have been exclusively devoted to temperate species of turtle[3–12] and apparently no literature is at hand on sexual cycle of tropical forms. In this report an attempt has been made to study the seasonal gonadal cycle in a tropical pond turtle *Lissemys p. granosa* in relation to natural environmental changes, particularly photoperiod, temperature, rainfall and humidity.

The gonosomatic index (GSI, weight of gonads divided by body weight and quotient multipled by 100)[13], volume of gonads and histological studies were the parameters used for the evaluation of gonadal cycle. The results obtained from various parameters for the assessment of sexual cycle of *Lissemys p. granosa* were uniform. 6–8 specimens of both sexes were collected in every month for 12 months from the ponds in and around Varanasi and were sacrificed after recording their body weight individually at a preset date similar for each month. The data for environmental changes were collected from the local newspaper office. *Lissemys p. granosa* exhibited marked seasonal cyclical changes in its gonadal activity. In natural habitat, this turtle breeds once a year during July–August and fertilized eggs are laid in September. Its sexual cycle has been divided into six phases (Figure). The cycle in both sexes began from the later half of January

[1] D. P. SINGH, Indian Biol. *4*, 1 (1973).
[2] I. Y. MAMMOUD and J. KLICKA, J. Herpet. *6*, 183 (1972).
[3] I. Y. MAHMOUD, Copeia *2*, 314 (1967).
[4] P. D. ATLAND, J. Morph. *89*, 599 (1951).
[5] F. R. CAGLE, Copeia *3*, 108 (1948).
[6] P. L. RISLEY, J. Morph. *63*, 301 (1938).
[7] C. B. POWELL, Can. Fld Nat. *81*, 134 (1967).
[8] D. W. TINKLE, Ecology *42*, 68 (1961).
[9] J. W. GIBBONS, Ecology *49*, 399 (1968).
[10] J. W. GIBBONS and D. W. TINKLE, Ecology *50*, 340 (1969).
[11] J. M. LEGLER, Univ. Kans. Publs Mus. nat. Hist. *11*, 527 (1960).
[12] C. H. ERNST, Biol. Bull. *140*, 191 (1971).
[13] T. P. SINGH, Endocrinologie *56*, 292 (1970).



Annual reproductive cycle of *Lissemys p. granosa* in relation to the changes in day-length, temperature (given in parentheses) and humidity.

with the beginning of increased day-length. This initial phase of gonadal maturation was slow till the end of February. An accelerated activity was encountered during March–April (after vernal equinox) with the rise of temperature and also when the day-length has increased appreciably (Figure). The gradual increase in gonadal activity continued through the succeeding months, and the peak was registered during July–August with the lengthiest day-length on mark. This peak phase of gonad activity also coincided with the heaviest average rainfall and maximum relative humidity records. The climax in sexual cycle was evident by all the parameters used, as GSI, gonadal volume showing maximum readings (Figure). However, the maximum temperature was recorded during May–June but the threshold of average minimum temperature for 24 h was highest during the months of May through August. A down trend in the gonadal activity became apparent from September (Autumnal equinox) with significant reduction in day-length and temperature. The sexual cycle ebb was noticed in December during short day-length. September through November were the months of post breeding and regression. The refractory and resting phases were short-lived, of about $1^1/_2$ months duration (Figure). The sexual cycle of *Lissemys p. granosa*, however, was not dependent upon rainfall and humidity but the breeding occurred only when these 2 environmental factors were in full effect. It seems that the rainfall and humidity are complementary and additive to the effects produced by long day-length and increased temperature upon sexual cycle. Male gonadal cycle of *Lissemys p. granosa* resembles those of temperate species[2–5,8–12], while the female cycle differs in timing[2–12].

In *Lissemys p. granosa* external environmental factors such as photoperiod and temperature play a profound role and seem to regulate the rhythm of sexual cycle by triggering the maturation and ripening of gonads, and breeding appears to be achieved with the help of rainfall and humidity working in association with the above factors. At present, however, it is not justified to assign the inducive role with certainty to any one of the factors, but it is apparent that photoperiod, temperature, rainfall and humidity are important external environmental factors influencing the gonadal cycle of *Lissemys p. granosa*[14].

Zusammenfassung. Nachweis, dass die Schildkröte *Lissemys p. granosa* in der freien Natur zyklische Veränderungen ihrer Gonadenaktivität bei beiden Geschlechtern zeigt, wobei Umweltbedingungen wie Tageslänge, Temperatur und Luftfeuchtigkeit die Rhythmik beeinflussen und regulieren dürften.

D. P. SINGH

Post-graduate Department of Zoology, Udai Pratap College, Varanasi (India), 1 October 1973.

[14] I am grateful to Dr. G. S. SHUKLA, Department of Zoology, Gorakhpur University, for guidance, to Dr. B. PRASAD, Department of Zoology, Banaras Hindu University, for encouragements and to Dr. T. P. SINGH for his helpful suggestions during the preparation of manuscript.