Seasonal male reproductive cycle of the estuarine snake *Enhydris enhydris* Schneider

R.N. Jadhav[1] and A.S. Padgaonkar[2]
[1]Department of Zoology, ESA College of Science, Vasai Road, Maharashtra 401202, India
[2]E/2-Sagar Vaibhav Society, Dahisar (W), Mumbai 400068, India

Summary

The testis of *Enhydris enhydris* undergoes a definite seasonal reproductive cycle. Breeding takes place during the month of October, following which the testes regress. There is definite arrest of spermatogenesis till the next breeding season. Based on the histological examination of the seminiferous epithelium, the testicular activity is divided into four phases, recrudescent, breeding, degenerate and regressed. Recrudescence begins in the month of June. The testis becomes spermatogenically functional at the beginning of October. The testicular weight and gonado-somatic index show a gradual increase during the recrudescent phase (June –Sep) reaching the peak in the month of October. At the onset of degenerative phase the testicular weight and gonado-somatic index start decreasing. A corresponding seasonal variation is reflected in the ductus epididymidis and ductus deferens as observed from the epithelial cell height and the diameter of the respective ducts. On the other hand the sexual segment of kidney never reaches peak and does not indicate any seasonal cycle.

***Key words** :* Snake, male gonad, reproductive cycle, testis, seasonal cycle

Introduction

The reproductive cycles of the snakes have been investigated by many authors (Aldridge and Metter, 1973; Bauman and Metter, 1977; Aldridge, 1979; Goran 1980; Gorbman et al., 1981; Lemen and Voris, 1981; Weil and Aldridge,1981; Johnson et al., 1982; Saint Girons, 1982; Crew, 1984; Krohmer et al., 1987; Aldridge et al., 1990; Saint Girons et al., 1993; Butler, 1993; Schuett et al., 1997, 2002, 2006; Bertona and Chiaraviglio, 2003; Almeida-Santos et al., 2004; Taylor and DeNardo, 2004, 2005; Graham et al., 2008; Lind et. al., 2010; Taylor, 2010). There are a few reports on the reproductive cycles of the tropical snakes, *Natrix piscator* (Shrivastava and Thapliyal, 1965; Guraya, 1973; Sadhu, 1982; Haldar and Pandey, 1989), *Naja naja* (Lofts et al., 1966; Lofts, 1968), *Natrix tessellate* (Amer, 1976), *Acrochordus granulates* (Samuel, 1991; Wangkulangkul et al., 2005), and *Cerberus rhynchops* (Jadhav and Padgoankar, 2002). It was therefore thought worthwhile to study the male reproductive cycle of the rainbow mud snake, *Enhydris enhydris,* from the Vasai coast, India (19 º 16 ' N and 72 º 4'E). According to Whitaker and Captain (2004) the genus Enhydris is represented in India by four species, *Enhydris dussumierii*, *Enhydris plumbea*, *Enhydris sieboldii* and *Enhydris enhydris*. They are widespread and abundant in some habitats of both lotic and lentic ecosystems (See: Murphy, 2007).

Materials and Methods

For the present study the snakes were collected from Vasai coast every month over a period of one year. They were brought to the laboratory and kept in aquarium tanks for 48 hours. The mature males were sacrificed under an overdose of anesthesia (Sodium pentothal, 2.5 mg / 100g body wt). Both the testes were carefully removed, blotted on a filter paper and weighed to the nearest milligram. The left testis along with the ductus epididymidis, ductus deferens and kidney of that side were fixed in Bouin's fluid for histological analysis. The tissues were processed for routine paraffin embedding. Sections were cut at 5µm to7µm thickness, stained with hematoxyline and eosin and examined in a research microscope. The diameters of the seminiferous tubules, ductus epididymidis and ductus deferens were measured using a calibrated ocular micrometer.

Observations

Changes in the testicular weight

The data on the average testicular weight and gonado-somatic index (GSI) of *Enhydris enhydris* showed a definite cyclical pattern like on other seasonally breeding vertebrates. The maximum combined weight (CTW) of the testes was recorded in the month of October (2342 .50 ± 412.92 mg) (Fig. 1). This period coincided

Correspondence to be addressed to: Dr. Rahul N. Jadhav, Ph.D. E-mail.jadhav2010@rediffmail.com

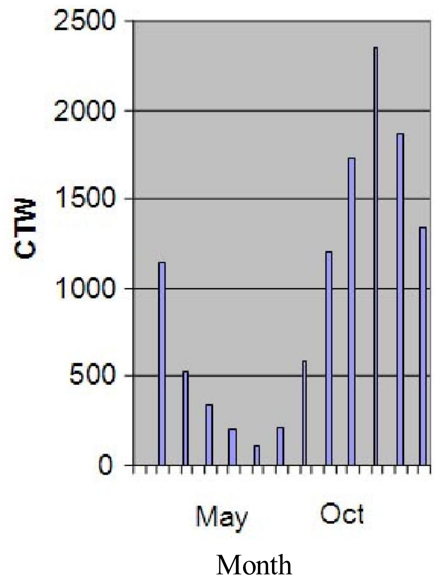

Fig. 1. Combined testicular weight in mg

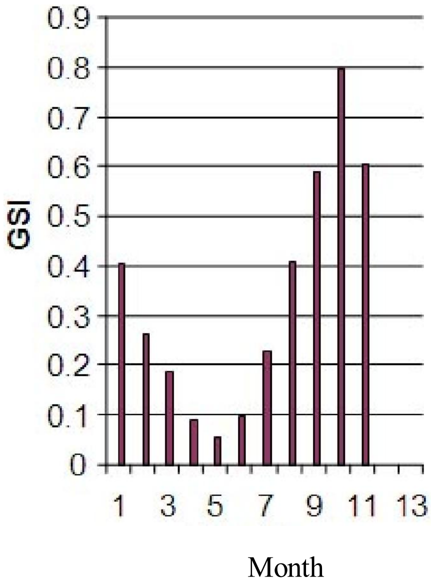

Fig.2. Gonado-somatic Index

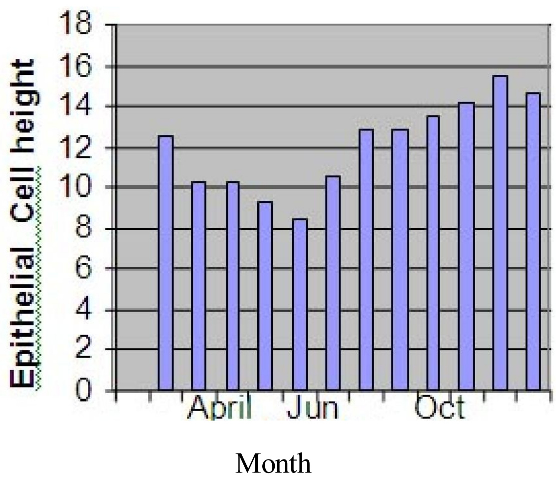

Fig.3. Epithelial cell height of the ductus epididymid is in um

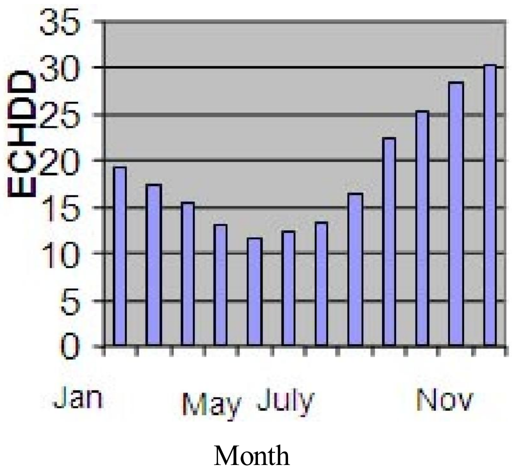

Fig.4. Epithelial cell height of the ductus deferens in um

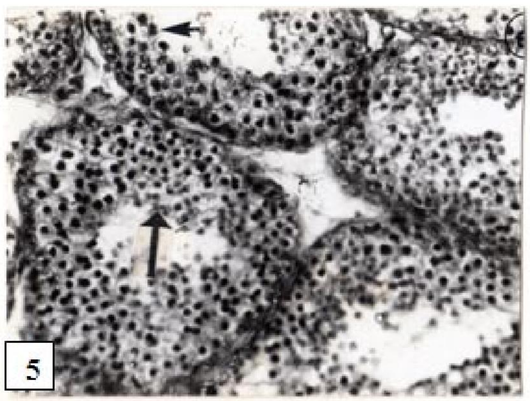

Fig.5. Transverse section of the testis of the snake in the recrudescent phase-x400

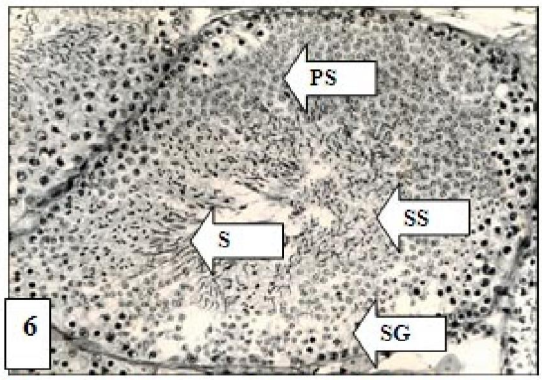

Fig.6. Transverse section of the testis of the snake in the active phase x-400

SG - Spermatogonia S - Sperm
PS - Primary Spermatocytes
SS - Secondary Spermatocytes

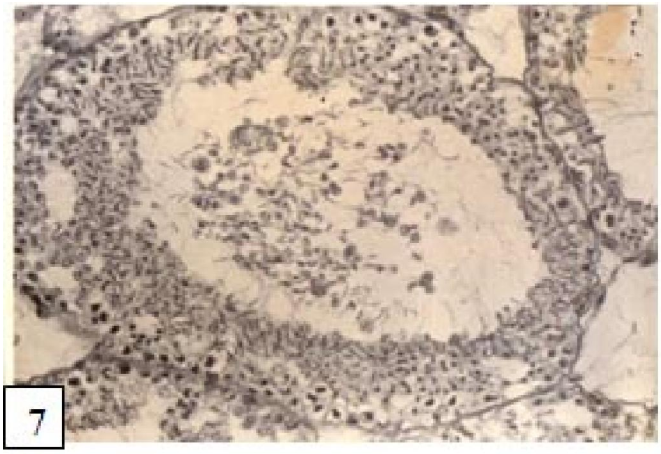

Fig.7. Transverse section of the testis of snake in the degenerative phase. x- 400

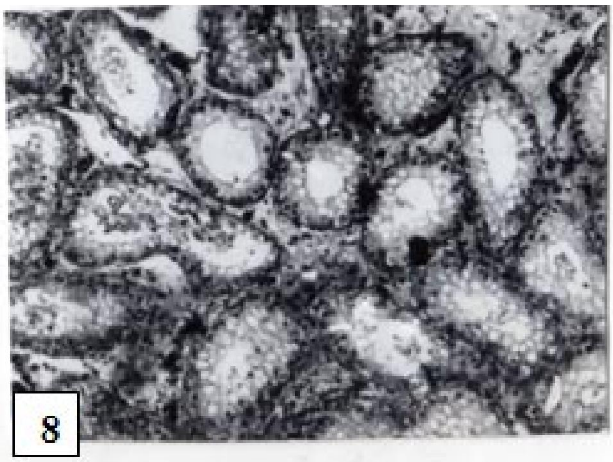

Fig. 8.Transverse section of the testis of the he snake in the regressed phase. x- 400

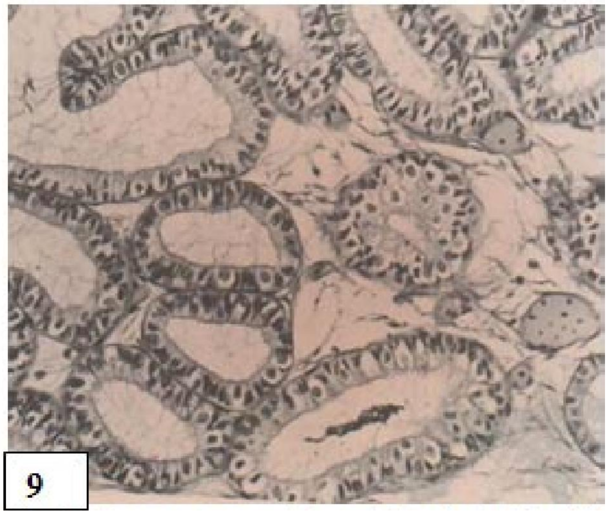

Fig.9. Transverse section of the ductus epididymidis of the snake in the active phase. x - 400

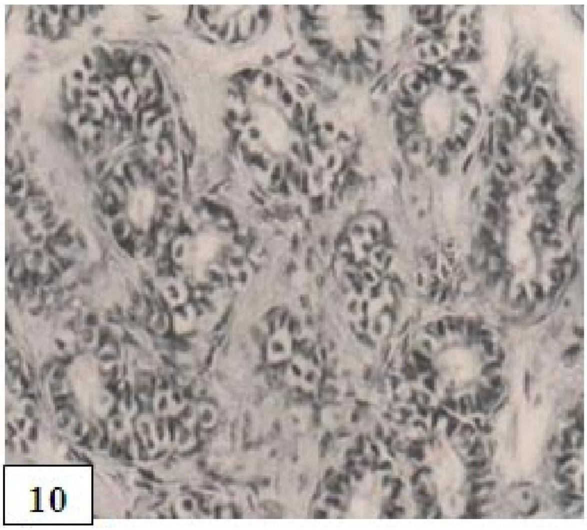

Fig. 10. Transverse section of the ductus epididymidis of the snake in the regressed phase. x- 400

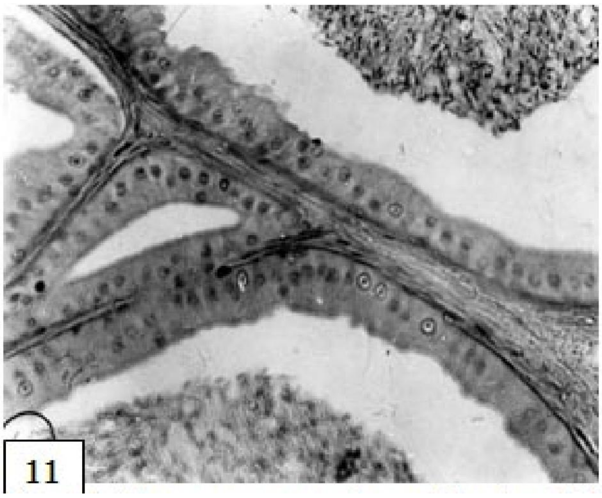

Fig.11. Transverse section of ductus deferens of snake during active phase. x- 400

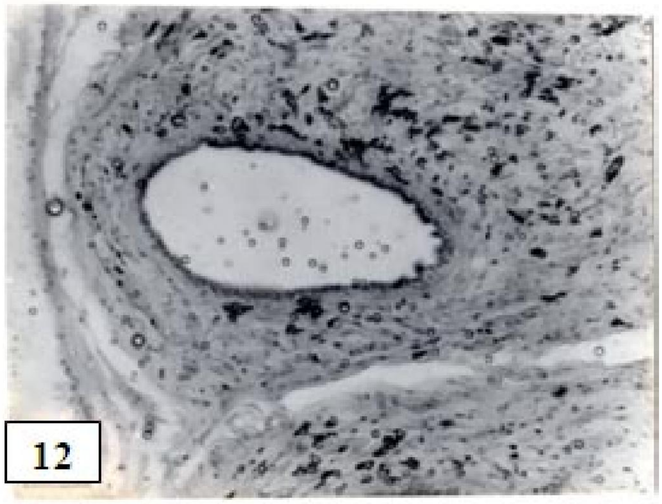

Fig. 12 - Transverse section of the snake ductus deferens during regressed phase. x- 400

with the period of maximum sperm production. The average weight of the testes started decreasing in the month of November (1870.35 ± 341.61 mg). This decrease continued till May when the average testicular weight reached its minimum (1012.64 ± 187.53 mg). The gonado-somatic index (GSI) was at its minimum (0.0584 ± 0.019) in the month of May. It was maximum (0.7984 ± 0.0769) in October (Fig.2).

Seminiferous epithelial cycle

Based on the histological changes in the seminiferous epithelium, the reproductive cycle was divided into four phases.

Recrudescent phase - June to September. Spermatogenic activity begins in the month of June. From July onwards the seminiferous tubules contains mainly spermatogonia, primary spermatocytes and a few secondary spermtocytes. Spermatids appear in the month of September (Fig. 5).

Breeding phase - October. The spermatogenic activity reaches its peak. A large number of spermatozoa are produced, which accumulate in the lumen of the seminiferous tubules (Fig. 6).

Degeneration phase - November to February. Few spermatozoa are found in the lumen of the seminiferous tubules. The tubular lumen is now filled with cell debris formed due to degeneration of germinal elements (Fig. 7).

Regression phase - March to May. Spermatozoa are totally absent. One or two layers of spermatogonia and primary spermatocytes remain in the lumen. The diameter of the seminiferous tubules and height of the seminiferous epithelium greatly decrease, and inter- tubular spaces increase (Fig. 8).

Changes in the accessory ducts

The maximum epithelial cell height of ductus epididymidis was recorded in November, immediately following peak of spermatogenesis (Figs. 3, 9) while minimum was recorded in May (Figs. 3, 10). The ductus deferens had the maximum epithelial cell height during the month of December (Figs. 4,11). The lumen of the ductus deferens showed dense sperm during the period from November to January (Fig. 11) and it was the lowest during May (Fig. 12).

Discussion

Observations made on anatomy, morphology and histology of the testes and accessory ducts of *Enhydris enhydris* throughout the year indicate that this snake is a seasonal breeder. The spermiogenesis occurs in September. Total regression of the testes begins in the month of March and continues till May. Seasonal breeding pattern has been reported in tropical snakes such as *Natrix piscator* (Shrivastava and Thapliyal, 1965; Guraya, 1973; Sadhu, 1982; Haldar and Pandey, 1989), *Naja naja*, (Lofts et al., 1966), *Natrix tessellate* (Amer, 1976), *Enhydrina schistoza* (Voris and Jayne, 1979), *Acrochordus granulatus* (Samuel, 1991; Wangkulangkul et al., 2005), and *Cerberus rhynchops* (Jadhav and Padgoankar, 2002). This type of spermatogenesis, known as pre-nuptial spermatogenesis**,** occurs in pikilothermic vertebrates, viz., fishes, amphibians and reptiles. In this type the seminiferous tubules of the testes contain advanced germinal stages soon after the breeding period. The spermatogenic activity continues till the formation of the spermatozoa, well before the onset of the next breeding season. The testicular cycle of the *Enhydris enhydris* clearly shows that spermatogenesis in the snake is pre-nuptial type.

Prenuptial spermatogenesis has been described in the *Naja naja* (Lofts et al., 1966), *Enhydrina schistoza* (Voris and Jayne, 1979), *Acrochordus granulatus, Cerberus rhynchops, Laticauda colubrine* (Gorbman et al., 1981), *Acrochordus granulates* (Samuel, 1991; Wangkulangkul,2005) and *Cerberus rhynchops* (Jadhav and Padgaonkar, 2002) where the breeding period is immediately succeeded by a testicular regression and a complete suppression of spermatogenesis for a period. Once the spermatogenic process sets in it is very rapid and leads to formation of mature sperm within a short time.

In *Enhydris enhydris* the epithelial cell height of the ductus epididymidis and ductus deferens also undergo seasonal change. Similar observations have also been reported in *Vipera berus* (Nilson, 1980; Fox, 1952), *Natrix piscator* (Shrivastava and Thapliyal, 1965), *Natrix tessellate* (Amer, 1976), *Acrochordus granulatus* (Voris and Glodek, 1979; Samuel, 1991; Wangkulangkul, 2005) and *Cerberus rhynchops* (Gorbman et al., 1981; Jadhav and Padgaonkar, 2002).

Acknowledgment

Rahul N. Jadhav is thankful to University Grants Commission, New Delhi, for financial assistance in a minor research project [F-No.47/201/2008-09 (WRO) Pune].

References

Aldridge RD (1979) Seasonal spermatogenesis in sympatric *Crotalus viridis* and *Arizona elegana* in New Mexico. *J Herpetol* **13:**187-192.

Aldridge RD, Metter DE (1973) The reproductive cycle of the Western worm snake *Carhophis vermis* in Missouri. *Copeia* **1973:**472-477.

Aldridge RD, Greenhaw JJ, Plummer MV (1990) The male reproductive cycle of the rough green snake (*Opheodrys aestivus*). *Amphibia-Reptilia* **11**:165-172.

Almeida-Santos SM, Abdalla FMF, Silveira PF, Breno YN, Salombo MG (2004) Reproductive cycle of the Neotropical *Crotalus durissus terrificus*. I. Seasonal level and interplay between steroid hormones and vasotosinase. *Gen Comp Endocrinol* **139**:143-150.

Amer FI (1976) Seasonal variation in the testis and epididymis of the aquatic snake, *Natrix tessellate*. *Ann Zool* (AGRA) **12:**97-112.

Bertona M, Chiaraviglio M (2003) Reproductive biology, mating aggregation and sexual dimorphism of the Argentine boa constrictor (*Boa constrictor occidentalis*). *J Herpetol* **73**:510-516.

Bauman AM, Metter DE (1977) Reproductive cycle of the Northern water snake, *Natrix s. sipedon* (Reptilia, Serpents, Colubridae). *J Herpetol* **11**:51-59.

Butler JA (1993) Seasonal changes in the African olive grass snake *Psammophis phillipi* (Serpentes: Colubridae). *J Herpetol* **27:**144-148.

Crew D (1984) Gamete production, sex hormone secretion and mating behavior uncoupled. *Horm Behav* **18**:22-28.

Fox W (1952) Seasonal variations in the male reproductive system of Pacific coast Garter snakes. *J Morphol* **90**:481-553.

Goran N (1980) Male reproductive cycle of the European Adder, *Vipera berus* and its relation to annual activity periods. *Copeia* **1980**:729-737.

Gorbman GC, Licht P, McCollum F (1981) Annual reproductive patterns in three species of marine snakes from the Central Phillippines. *J Herpetol* **15**:335-354.

Graham SP, Earley RL, Hoss SK, Schuett GW, Grober MS (2008) The reproductive biology of male cotton moths (*Agkistrodom piscivorus*): Do plasma steroid hormones predict the mating season? *Gen Comp Endocrinol* **159**:226-235.

Guraya S.S.1973.Histochemical observations on the interstitial (Leydig) cells of the snake testis .*Acta Morphol Acad Sci Hung* **21:**1-12.

Halder C, Pandey R (1989) Effect of pinealectomy on annual testicular cycle of Indian chequered snake *Natrix piscator.* *Gen Comp Endocrinol* **76:**214-222.

Jadhav RN, Padgaonkar AS (2002) The male gonadal cycle of the estuarine snake *Cerberus rhynchops* (Schneider). *Trends in Life Science* (India) **17:**47-59.

Johnson LF, Jacob JS, Torrance P (1982) Annual testicular and androgenic cycles of the cotton moth (*Agkistrodom piscivorus*) in Alabama. *Herpetologia* **38**:16-25.

Krohmer RW, Grassman M, Crews D (1987) Annual reproductive cycle in the male red-sided Garter snake *Thamnophis sirtalis parietalis*: field and laboratory studies. *Gen Comp Endocrinol* **68**:64-75.

Lemen CA, Voris HK (1981) A comparison of reproductive strategies among marine snakes. *J Anim Ecol* **50**:89-102.

Lind CM, Husak JF, Eikenaar C, Moore IT, Taylor EN (2010) The relationship between plasma steroid hormone concentrations and the reproductive cycle in the Northern Pacific rattle snake *Crotalus oreganus*. *Gen Comp Endocrinol* **166**:590-599.

Lofts B (1968) In: Barrington EJW, Jorgenson B (Eds) P*erspectives in Endocrinology*. pp 230-340. New York: Academic Press.

Lofts B, Phillips JG, Tam WH (1966) Seasonal changes in the testes of the cobra *Naja naja* (Linn). *Gen Comp Endocrinol* **6**:466-475.

Murphy JC (2007) Homalopsid snakes: Evolution in the mud. p249. Malbar, FL, USA: Krieger Pub Co.

Nilson Goran 1980.Male reproductive cycle of the Europian Adder,*Vipera berus* and its relation to annual activity periods. Copeia.**1980:**729-737.

Sadhu T.K. 1982.On the reproductive cycle of the *Natrix piscator* Schneider (Reptilia, Ophidia) Proc. 69th Cong. Part. III. Abstract pp58.

Saint Girons H (1982) Reproductive cycles in male snakes and their relationship with climate and female reproductive cycles. *Herpetologica* **38:**516.

Saint Girons H, Bradshaw D, Bradshaw FJ (1993) Sexual activity, plasma level of sex steroids in the aspic viper, *Vipera aspis* L (Reptilia, Viperidae). *Gen Comp Endocrinol* **91:**287-297.

Samuel J (1991) Studies on the male reproductive cycle and relation of endocrine glands to reproduction in the snake *Acrochordus granulates* (Schneider). M.Sc. Thesis, Univ of Bombay, India.

Schuett GW, Harlow HJ, Rose JD, Van Kirk EA, Murdoch WJ (1997) Annual cycle of plasma testosterone in male copperhead *Agkistrodon contortrix* (Serpentes, Viperidae): Relationship to timing of spermatogenesis, mating and agonistic behavior. *Gen Comp Endocrinol* **105**:417-427.

Schuett GW, Carlisle SL, Holycross AT, O'Leile JK, Hardy DL Sr, Van Kirk EA, Murdoch WJ (2002) Mating system of male Mojave rattle snakes *(Crotalus scutulatus*): seasonal timing of mating, agonistic behavior, spermatogenesis, sexual segment of the kidney and plasma sex steroids. In: Schuett GW, Hoggren M, Douglas ME, Grecne HW (Eds) *Biology of the Vipers.* pp 515-532. Eagle Mountain, Utah: Eagle Mountain Publishing.

Schuett GW, Repp RA, Taylor EN, DeNardo DF, Early RL, Van Kirk EA, Murdoch WJ (2006). Winter profile of plasma sex steroid level in free-living male western diamond–backed rattle snake *Crotalus atrox* (Serpentes, Viperidae). *Gen Comp Endocrinol* **149**:72-80.

Shrivastava PC, Thapliyal JP (1965) The male sexual cycle of the chequered water snake *Natrix piscator. Copeia* **1965:**410-415.

Taylor EN (2010) Hormones and reproductive cycles in snakes. *Hormones and Reproduction in Vertebrates* **3**:355-372.

Taylor EN, DeNardo DF (2004) Seasonal steroid hormonal level and there relation to reproduction in the Western diamond-backed rattle snake *Crotalus atrox* (Serpentine: Viperidae). *Gen Comp Endocrinol* **136**:328-337.

Taylor EN, DeNardo DF (2005) Reproductive ecology of Western diamond –backed rattle snake (*Crotalus atrox*) in the Sonoran Desert. *Copeia* **2005:**152-158.

Wangkulangkul S, Thirakhupt K, Voris HK (2005) Sexual size dimorphism and reproductive cycle of the little file snake *Acrochordus granulates* in Phangnga Bay, Thailand. *Science Asia* **31**:257-263.

Weil MR, Aldridge RD (1981) Seasonal androgenesis in the male water snake, *Nerodia sipedon. Gen Comp Endocrinol* **44:**44-53.

Whitaker R, Captain A (2007) *Snakes of India- The Field Guide*. p481. Chennai: Draco Books.

Voris HK, Jayne BC (1979) Growth, reproduction and population structure of a marine snake, *Enhydrina schistoza* (Hydrophidae). *Copeia* **1979**:307-317.

Voris HK, Glodek GS (1980) Habitat, diet and reproduction of the file snake, *Acrochordus granulatus*, in the Strait of Malacca. *J Herpetol* **14:**108-111.