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Pattern of Ovarian Activity in the Indian Toad *Bufo melanostictus* (Schn.)

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Oogenesis in the toad is of *continuous* type occurring throughout the year. The prefollicular cells divide mitotically to increase their number, to surround the developing follicles. The vitellogenesis begins from periphery and progresses towards the centre. The theca and granulosa layers in the mature follicles cannot be clearly differentiated. Binuclear oocytes are rarely found.

The ovarian cycle of the toad can be broadly classified into prespawning (April-May), spawning (June-August) and postspawning-cum-preparatory period (September-March). The classification is based on the seasonal changes in the gonadosomatic index, diameter of the largest oocytes and ovarian histology. The breeding activity of the toad coincides with the rainy months of monsoon.

Key Words: Oogenesis, Ovarian cycle, Vitellogenesis, Spawning, Gonadosomatic index

Introduction

The seasonal variation in the ovarian development of lower vertebrates has been recently reviewed (Lofts 1974, Rowlands & Weir 1977, Jones 1978 and Jørgensen et al. 1979). Among amphibians clear cut seasonal changes in the ovary have been recorded mostly in the temperate species and, studies on the tropical species are relatively few (Lofts 1974, Saidapur & Nadkarni 1974 and Jørgensen et al. 1979). The present work on the toad *Bufo melanostictus* was undertaken to study in detail the oogenesis and ovarian cycle, as the toad belongs to tropical regions and is highly terrestrial in its habitat.

Materials and Methods

Adult female toads (*B. melanostictus*) weighing above 60 g were collected from surrounding areas of Dharwad city, Karnataka State, India (15° 17'N 75° 3'E) every month for a period of 13 months. A minimum of ten toads were used in each monthly sample. The toads were weighed and killed by decapitation,

ovaries were weighed and the gonado-somatic index (GSI) was calculated for each month. Small pieces of ovaries were fixed in Bouin's fluid for histology.

The diameter of largest oocytes present in each monthly sample was determined by measuring the diameter of 50 oocytes from the sections of each ovary. The ovaries of five toads were used for determining the monthly mean diameter of largest oocytes.

Meterological data used in this study (figure 1) was obtained from Agricultural College, Dharwad.

The seasonal changes in the ovarian mass were determined by the Analysis of Co-variance (Steel & Torrie 1960) to find out: (i) the effects of months on the ovarian mass, and (ii) influence of body weight on the ovarian mass. This was followed by multiple t test to determine the level of significance. The differences were judged as significant if $P < 0.05$.

Observations

The ovaries of adult toad are paired organs attached to the median surface of the kidneys by mesovarium. Each ovary is a hollow sac-like structure the wall of which is thrown into folds and consists of a narrow cortical region covered by surface or germinal epithelium. The ovarian stroma is scarce.

Oogonia

Oogonia were found in the germinal epithelium throughout the year but they were abundant in the ovaries after spawning. The size of oogonia varied from 9 to 19μm and their nuclei measured 6 to 9 μm (figure 2).

Oocyte Development

The oogonium, in some cases the oogonial nest and young primary oocytes be come surrounded by the prefollicular cells (figure 3) which arise from fibroblast cells of the ovarian stroma. These cells divide mitotically (figures 4 & 5), increase in number and later organise into follicular layers. The smallest oocytes observed in the toad ovary ranged from 20 to 30μm and their nuclei measured 12 to 18μm. The oocytes then enter previttellogenic primary growth phase during which their size increases and the yolk nucleus becomes visible at the juxtanuclear position (figure 6) when the diameter of oocytes is 45–60μm. The yolk nucleus enlarged and broke into irregular masses of various sizes (figure 7).

Table 1 *Analysis of Covariance of Ovary in* B. melanostictus

Source	d.f.	S^2x	S^2y	Sxy	$d.f.$	Adjusted $S\,S$	F value
Total	119	141553.25	8417.83	19966.86	118	5597.83	*2.0855 (H_1)
Lots	11	44493.25	1486.74	2747.55	11		*4.315 (H_2)
Error	108	97069.00	6931.69	17219.31	107	3877.69	

Lot=Month; X=Body wt.; Y=Ovary wt.; H_1=effect of months; H_2=Results are conditioned by body wt.: F=values are significant at 5% level of significance.

The fragments of the yolk nucleus then moved towards the periphery of oocytes. At one stage the yolk nucleus material is uniformly distributed in the cytoplasm in later stages the same is distributed along the inner border of the plasma membrane of previtellogenic oocytes.

The secondary growth phase of the primary oocyte is characterised by the accumulation of yolk. Yolk vesicles were seen at the beginning of the yolk deposition. The onset of yolk deposition occurred when the follicles attained a size of 230 μm to 270μm. The accumulation of yolk started from periphery (figure 8) and progressed towards the centre (figure 9). The oocytes measured 283 to 376μm when vitellogenesis was completed. The nuclear diameter of these oocytes ranged from 135 to 160 μm.

After completion of vitellogenesis the follicle undergoes further growth and attains a size of 1058μm in size. Nuclear diameter of these oocytes ranged from

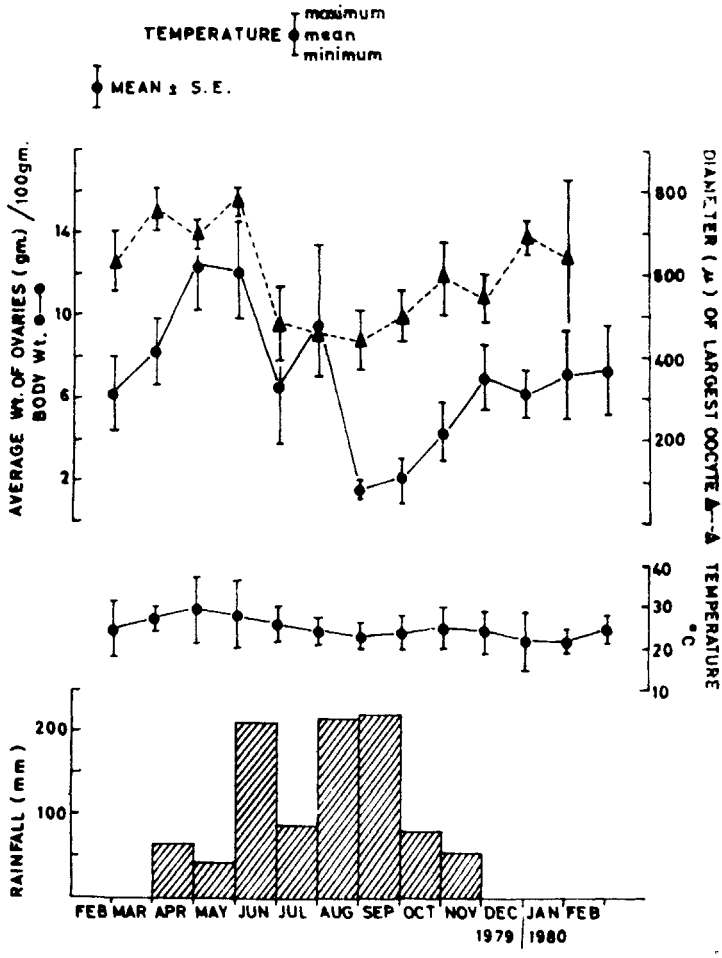
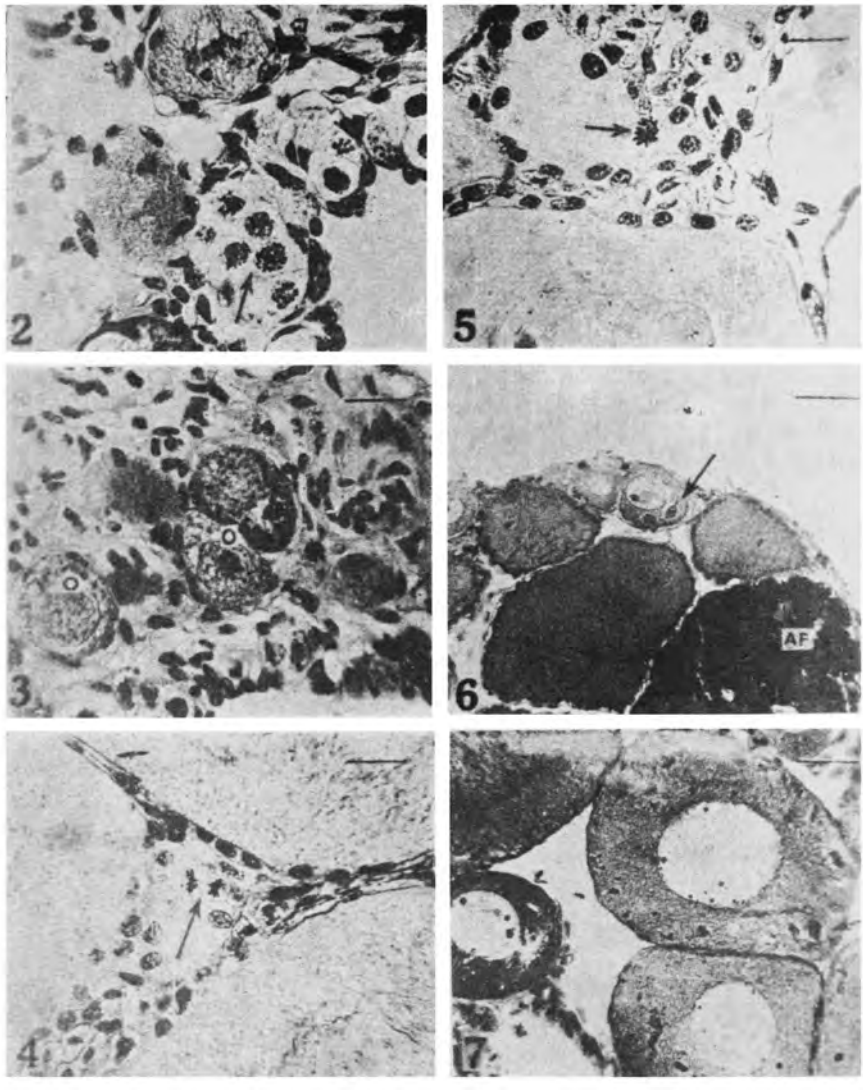
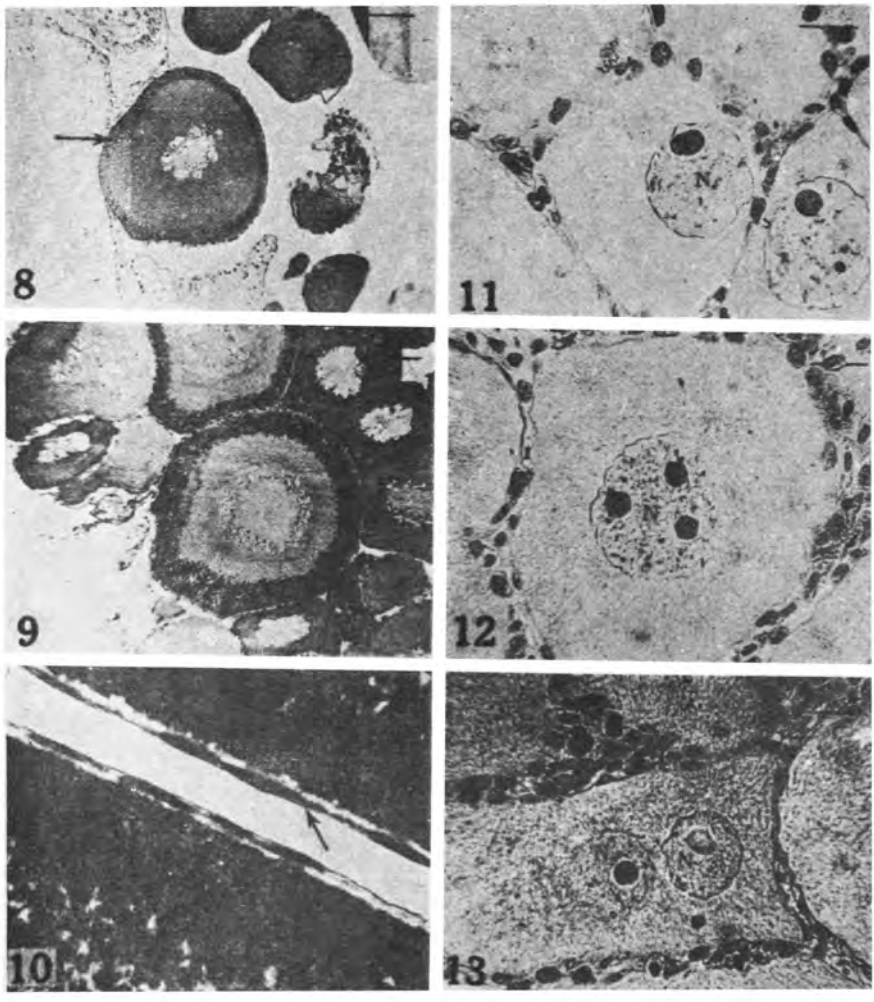


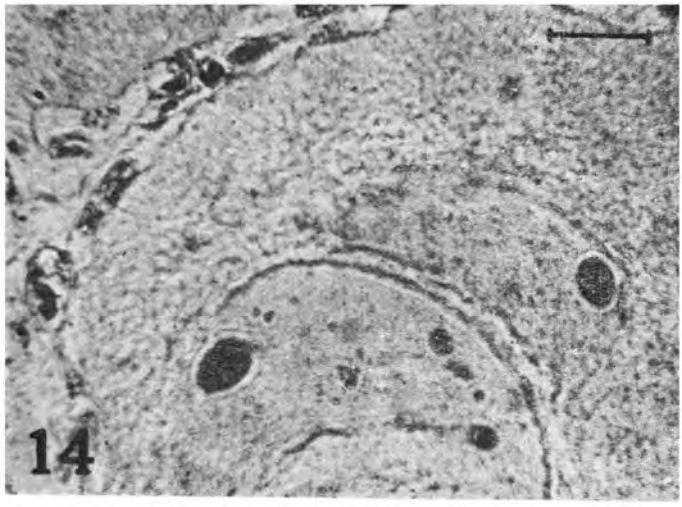
Figure 1 Seasonal changes in the average weight of ovaries (g/100g body wt (GSI), diameter (μm) of largest oocyte, temperature (°C) and rainfall (mm). February 1979 to March 1980. Vertical bars indicate standard error.

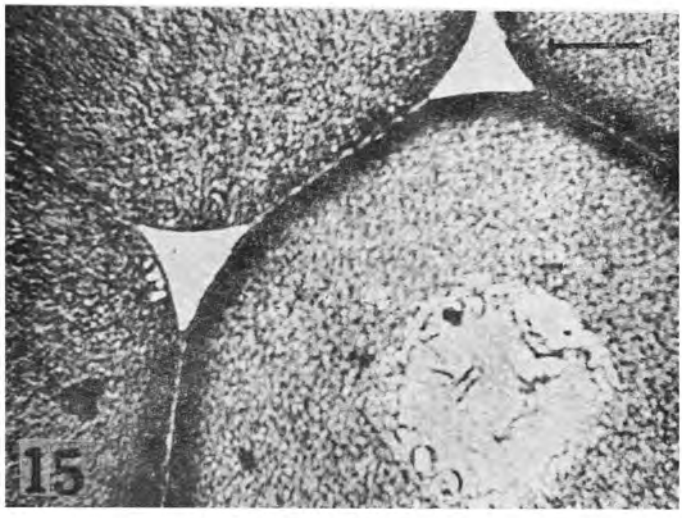


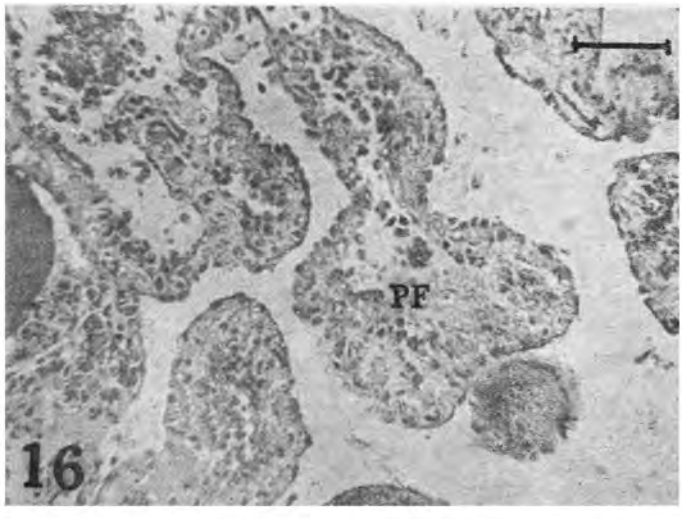
Figures 2–7 *2*, Oogonial cell nest (arrow). The oogonia are in early prophase stage. *3*, Primary oocyte (**O**) surrounded by prefollicular cells; *4-5*, Mitotic division metaphase in figure 5 and anaphase in figure 4; of prefoliicular cells (arrows). *6*, Yolk nucleus (arrows) in the small previtellogenic follicle. **Af**, atretic follicle; **7**, Distribution of yolk material (arrow) in the large previtellogenic follicle.
Scale lines indicate 20, 25, 20, 20, 50 and 50 μm respectively on figures 2, 3, 4, 5, 6 and 7.



Figures 8-13, *8-9,* Early (figure 8) and slightly advanced (figure 9) vitellogenic follicles. The vitellogenesis starts from periphery of the oocyte (arrow) and spreads towards the centre; *10,* Undifferentiated theca and granulosa layer (arrow) in the fully grown vitellogenic follicles; *11,* Presence of one nucleolus in the nucleus (N) of previtellogenic follicle; *12,* Presence of three nucleoli in the nucleus of previtellogenic follicle; *13,* A binucleate oocyte.
Scale lines indicate 100, 100, 20, 20, 20, and 25 μm respectively on figure 8, 9, 10, 11, 12 and 13.







Figures 14-16 *14*, A binucleate oocyte under higher magnification; *15*, May ovary showing mature and ovulatory oocytes (Prespawning period); *16*, June ovary after spawning showing many postovulatory follicles (PF)
Scale lines indicate 20, 100 and 100 μm respectively on figures 14, 15 and 16.

283–307μm. In the toad vitellogenic stages occurred throughout the year. In May, the ovaries contained large preovulatory and small previtellogenic follicles.

The theca and granulosa layers cannot be clearly distinguished from each other at any stage of oocyte development in the toad. In the fully grown oocytes the follicular cells appear flat and elongated (figure 10). At this stage the theca and granulosa layers together measured 1.7 to 2.4 μm.

During the previtellogenic phase the germinal vesicle grows into a spherical structure. The germinal vesicle of young oocyte consisted of one nucleolus (figure 11) and as the development progressed their number increased (figure 12). The nucleoli were arranged adjacent to the nuclear membrane. With further growth the nuclear membrane became lobulated and the lobulations increased with the progress of vitellogenesis. The nucleoli were found inside these lobulations. In the ovary binucleate follicles were occassionally found (figures 13 & 14).

Ovarian Cycle

Based on the seasonal changes in the gonadosomatic index (GSI) diameter of the largest oocytes (figure 1) and gross histological changes in the ovary of *B. melanostictus* the ovarian cycle can be arbitrarily divided into the following phases.

1. *Prebreeding Period (April–May):* The ovary during these months contained large preovulatory follicles (figure 15) and groups of small oocytes which subsequently undergo vitellogenesis after breeding. During this period the values of GSI and the diameter of largest oocytes were highest (figure 1).

2. *Breeding Period (June–August):* This period can be recognised as the breeding period. The ovaries were fully gravid. The onset of monsoon rains in June acts as a trigger for breeding of toads which have completed their ovarian development. Majority of the toads breed only once in the season and almost all the preovulatory follicles are shed at one time and rarely very few are retained. The ovary which weighed 30g before ovulation weighed about 0.5 to 0.8 g after spawning (figure 1). Histological sections commonly showed oogonia, postovulatory luteal bodies (figure 16) young oocytes, a few vitellogenic follicles and some unspent mature follicles. Average ovarian weight and the diameter of largest oocytes declined significantly following the breeding in June–July. The ovary attained minimum weight in the month of August (figure 1).

3. *Postbreeding - cum - Preparatory Period (September–March):* The period between September-March may be recognised as the postbreeding-cum-preparatory period when the oogonia divide and give rise to new batches of oocytes. During this period, the ovary contained: (a) oogonia and very small primordial follicles, (b) previtellogenic follicles, (c) follicles undergoing vitellogenesis, and (d) follicles which have completed vitellogenesis.

In many toads, vitellogenic follicles appeared by September. The weight of the ovary increased from September to November. Between November and March the relative ovarian weights remained almost constant (figure 1). The diameter of largest oocytes slightly increased in October-November and remained constant upto February and showed tendency to increase in March.

Discussion

Oogonia and Oocyte Development

In amphibians with one ovarian cycle a year, oogonia divide and form oogonial cell nests around breeding time (Jørgensen 1973 and Tokarz 1978). In *B. melanostictus* also oogonial population increased after ovulation. Oogonia are initially associated with the flattened prefollicular cells, which are opposed to the oogonial cell membrane. Oogonial nests are formed (figure 2) after repeated mitosis. When oogonia enter into the I meiotic division they are converted into primary oocytes. When the oogonial nest converts into oocytic nest, the oocytes become separated from each other during oogenesis and each developing oocyte gets surrounded by prefollicular cells (figure 3). By the diplotene stage, these cells become organised into follicular epithelium.

In amphibians the wall of growing follicles consists of a zona pellucida, follicular epithelium, basal lamina and theca; a surface epithelium lies outside the thecal layers. The follicular epithelium remains as a single layer of cells throughout the whole period of maturation and this differs from the multicellular granulosa tissue of higher vertebrates. A thecal layer then becomes associated with the developing follicle. In *B. melanostictus* separation of distinct thecal and granulosa layers was not possible even under higher magnification (1000 ×) (figure 10) as in *R. cyanophlyctis* and *R. tigrina* (Saidapur & Nadkarni 1974).

The occurrence of yolk nucleus in early previtellogenic stages is debated in amphibians (Lofts 1974 and Guraya 1979). The absence of yolk nucleus in some species may be attributed to its sparse nature in those amphibian oocytes (Guraya 1965). In *B. melanostictus* the yolk nucleus appears in oocytes of 45 to 60μm whereas in *X. laevis* the yolk nucleus appears when oocytes reach a size of 100–300μm (Follett & Redshaw 1974). The yolk nucleus in *Bufo stomaticus* consists of proteins, RNA and some lipoproteins similar to that reported in reptiles, birds and mammals (Guraya 1979). The function of the yolk nucleus is not properly understood and probably it is not related to yolk formation because yolk synthesis begins in the peripheral cytoplasm before the yolk nucleus reaches the region. Tokarz (1978) suggested that the yolk nucleus seems to serve as a specialized mechanism of transferring nuclear information to the peripheral cytoplasm.

In *B. melanostictus* vitellogenesis begins in the oocytes which measure 230 μm to 270μm. Yolk vesicles were seen peripherally at the beginning of secondary growth phase. In *R. pipiens* vitellogenesis is reported to begin when the oocytes are of 100–150μm in diameter (Follett & Redshaw 1974). Thus, there seems to be a considerable species variation with regard to the onset of vitellogenesis in relation to the size of oocytes. The presence of numerous nucleoli in the nucleoplasm of the toad oocytes is in agreement with the previous reports on other species (Brown & David 1968, Al-Mukhtar & Webb 1971 and Dumont 1972).

Ovarian Cycle

The ovaries of many amphibians exhibit marked changes in activity with season. These changes include (1) a period of quiscence, during which, the ovaries contain only nests of oogonia and primary oocytes, previtellogenic follicles, corpora atretica and in some species, postovulatory corpora lutea of a transient form

(2)—a period of follicular growth, during which some of the previtellogenic follicles undergo vitellogenesis, and, (3) a period of postvitellogenic stasis and ovulation of a batch of oocytes. Seasonal proliferation of oogonia, differentiation of some oogonia into oocytes, and formation of new primordial follicles occurs in adult amphibians. The time of year in which these ovarian stages occur varies among species (Jones 1978). Gallien (1959) considered that seasonal cycle of reproduction in female amphibian generally shows three phases; (i) breeding phase (ii) ovarian regression, and (iii) ovarian growth and vitellogenesis. This is generally true of all the temperate species where clear cut changes are encountered due to the variations in the environmental temperature, whereas in species inhabiting tropical regions where the environmental fluctuations are not marked, the gametogenesis is *continuous or potentially continuous type* (Saidapur & Nadkarni 1974).

In *B. melanostictus*, it is difficult to differentiate the ovarian changes with the season as explained by Gallien (1959) or Jones (1978). This is due to the fact that oogenesis in the toad is a continuous process. Based on the overall consideration of changes in the GSI, diameter of largest oocytes and histological observations it is possible to broadly divide the ovarian cycle in the toad into prebreeding phase (April–May), breeding phase (June-August) and post-breeding-cum-preparatory phase (September-March). During the prebreeding-period homoplastic pituitary extract injection leads to partial spawning (Kanamadi & Saidapur unpublished observations), whereas during June–August pituitary injections induce shedding of almost all mature eggs. In fact, several toads caught after the heavy showers in these months spontaneously spawned in the laboratory without pituitary injection. These toads were in amplexus at the time of field collection. The postovulatory luteal structures were seen in the ovaries of June–August toads only. Therefore, all these observations support the broad classification proposed above for the ovarian cycle of the toad. The present study does not support the views of Alexander (1933 cited in Jørgensen 1973) that in nature *B. melanostictus* can breed at any time of the year. The present authors have collected the toads personally throughout the year without any difficulty. The toads do not seem to undergo hibernation. Therefore, in this species as in *R. cyanophlyctis* (Saidapur & Nadkarni 1974) the oogenesis is of *continuous type* although breeding seems to be an annual event coinciding with the monsoon rains (figure 1).

References

Al-Mukhtar K A K and Webb A C 1971 An ultrastructural study of primordial germ cells oogonia and early oocytes in *Xenopus laevis; J. Embryol. Exp. Morphol.* **26** 195–217

Brown D D and Dawid I B 1968 Specific gene amplication in oocytes. Oocyte contain extrachromosomal replicas of the genes for ribosomal RNA; *Science* **160** 272–280

Dumont J N 1972 Oogenesis in *Xenopus laevis*. I. stages of oocyte development in laboratory maintained animals; *J. Morphol.* **136** 113–180

Follett B K and Redshaw M R 1974 The Physiology of vitellogenesis; in *Physiology of Amphibia* Vol. II pp. 219–309 ed B Lofts (New York; Academic Press)

Gallien L 1959 Endocrine basis for reproductive adaptations in Amphibia; in *Comparative Endocrinology* pp 479–487 ed. A Gorbman, (New York: Wiley)

Guraya S S 1965 A comparative histochemical study of fish *(Channa maruleus)* and Ambhibia *(Bufo stomaticus)* oogenesis; *Zeifschrift fur Zellforschung* **65** 662–700

—— 1979 Recent advances in the morphology, cytochemistry and function of Balbiani's vitelline body in animal oocyte; *Int. Rev. Cytol.* **59** 249–322

Jones R E 1978 Ovarian cycle in Nonmammalian vertebrates; in *The Vertebrate Ovary* ed. R E Jones (New York: Plenum Press)

Jorgensen C B 1973 Mechanisms regulating ovarian function in amphibians (toads); in *The Development and Maturation of the Ovary and its Functions* pp. 133–151 ed. H Peters (Amsterdam: Excerpta Medica)

——, Lissen L S and Lofts B 1979 Annual cycles of fat bodies and gonads in the toad *Bufo bufo bufo* (L). compared with cycles in other temperate-zone Anurans; *Biologiske Skrifter* **22** 1–37

Lofts B 1974 Reproduction; in *Physiology of Amphibia* Vol. II pp. 107–219 ed B Lofts (New York: Academic Press)

Rowlands I W and Weir J B 1977 The Ovarian cycles in vertebrates; in *The Vertebrate Ovary* pp. 217–259 eds. S Zuckerman and Weir J B (New York: Academic Press)

Saidapur S K and Nadkarni V B 1974 A study of female reproducive cycle in two species of frogs, *Rana cyanophlyctis* (Schn.) and *Rana tigrina* (Daud). *Karnatak Univ. J. Sci.* **19** 23–27

Steel R G D and Torrie J N 1960 in *Principles and Procedures of Statistics* (New York: McGraw-Hill)

Tokarz R D 1978 Oogonial proliferation, oogenesis and folliculogenesis in nonmammalian vertebrates; in *The Vertebrate Ovary* pp. 145–173 ed. R E Jones (New York: Plenum Press)