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Annual Changes in the Somatic Weight, Hypophysial Gonadotrophs, Ovary, Oviduct and Abdominal Fat Bodies in the Indian Bull Frog, *Rana tigerina*

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The changes in the pituitary gonadotrophs (B_2 cells), ovary, oviduct, fat body and body weight occurring during the annual reproductive cycle of the female frog *Rana tigerina* are discussed. Soon after breeding in July oogonial proliferation results in the appearance of small primary oocytes in August–September. The first growth phase (FGP) of the previtellogenic oocytes is completed by April, and the vitellogenesis or secondary growth phase (SGP) is completed by the end of May when the ovaries become filled with yolky oocytes. The ovarian mass in May–June accounts for about 12% of the body weight. Following breeding in July the ovarian weight declines to about 0.3% of the body weight. The follicular growth is synchronous and at all times the largest oocytes are also the dominant type in the ovaries. Folliculogenesis and oocyte growth in adults are completed within a year.

The somatic weight (body weight minus ovarian weight) exhibits seasonal changes irrespective of changes in ovarian weights. Body weight decreases in June and tends to increase from September onwards. In adults ovarian weight is related to the body weight/size so that larger frogs have higher fecundity.

The hypophysial gonadotrophs (B_2 cells) show clear-cut seasonal changes in their cytomorphology (nuclear, cytoplasmic and cell areas), staining intensity and cytoplasmic granulation. In the post-breeding period (August–March) B_2 cells are regressed and generally devoid of secretory granules. From April to June the B_2 cell size increases 8–10 fold and the cytoplasm becomes filled with secretory granules. They also show partial degranulation. In July B_2 cells are completely depleted of their secretory products. The B_2 cells then regress and decrease in size (8–10 folds). The seasonal changes in B_2 cell activity correlate with ovarian follicular development. The seasonal changes in the oviduct weight are correlated with the changes in the ovary.

The fluctuations in the weight of the fat bodies are inversely correlated with that of the ovary except in July-August, when both show a decrease.

Key Words: *Rana tigerina*, **Ovarian cycle, Folliculogenesis, Gonadotrophs, Oviduct cycle, Fat body cycle**

Introduction

Even though the spermatogenetic cycle of amphibians is extensively investigated (reviews by Lofts 1974, Saidapur 1983) studies on the ovarian cycle are surprisingly limited (Lofts 1974, Jones 1978, Jørgensen et al. 1978, 1979) especially on the tropical and subtropical species. While correlative seasonal changes in the hypophysial gonadotrophs and testicular activity are documented in several male anurans (van Oordt 1974, Kasinathan et al. 1977, Kurian & Saidapur 1984) such studies are rare for female anurans (Rastogi & Chieffi 1970).

Previous studies on the ovarian cycle of anurans have mostly dealt with the changes in the gonadosomatic index (GSI) and/or gross histological changes (Jones 1978). Detailed investigations on ovarian cycle have been made in the European species, *Bufo bufo* (Jørgensen et al. 1979) and *Rana temporaria* (Jørgensen 1981). The cyclic changes in the ovarian activity in amphibians are generally attributed to the seasonal fluctuations in the climatic factors and nutritional requirements (Salthe & Mecham 1974, Jones 1978, Jørgensen et al. 1979). In recent years body size, body weight, body growth and abdominal fat bodies etc., have been shown to influence the fecundity in female amphibians (Salthe & Mecham 1974, Jørgensen et al. 1979) though relationships between these and the ovarian cycle have not been fully elucidated.

We have studied here the seasonal changes in the somatic weight (body minus ovary weight), GSI, ovarian mass, follicular development, % weights of abdominal fat bodies and oviducts, and cytomorphology of the hypophysial gonadotrophs (B_2 cells) and discussed the factors controlling the ovarian cycle in *R. tigerina*

Materials and Methods

Adult female frogs were collected monthly, around the coastal region of Karwar from September 1979 to December 1980 (except in December 1979). Five frogs were used every month for the histological study of the changes in the ovary. They were weighed before decapitation. The combined weight of the two ovaries was also recorded. Representative pieces of ovaries were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 7μm thickness and stained with haematoxylin-eosin. The oocyte and nuclear diameters were measured from 20 largest oocytes from each monthly sample. Scatter diagrams of body weight, ovary weight and the size of oocytes were plotted to elucidate the relationship, if any, between these factors. Similarly, the oocyte diameter versus the nuclear diameter was plotted on a scatter diagram.

The pituitary glands with adjacent portions of brain from frogs collected from January to December 1980 were fixed in Bouin Hollande sublimate for 24 hr, embedded in paraffin wax, serially sectioned at 4μm and stained with Herlant's Alcian blue periodic acid Schiff orange G technique (van Oordt 1974) using the same stock solutions. The outlines of 20 randomly selected gonadotrophs (Masood Hussain & Saidapur 1983) and their nuclei from each pituitary were drawn using camera lucida at the magnification of 1000×. The cell and the nuclear areas were measured with a planimeter. The cytoplasmic area was calculated by substracting the nuclear area from the cell area (Masood Hussain & Saidapur 1983). Monthly means were then calculated. Seasonal variations in the cytoplasmic granulation and degranulation of B_2 cells were also recorded (table 1).

Five adult females were sacrificed from January to December 1982. The weights of body, ovaries, oviduct and of the abdominal fat bodies were recorded. The monthly means of GSI, the somatic weight, ovary,

Table 1 *Cytoplasmic granulation and degranulation of B_2 cells in the pars distalis.*

Months (Year 1980)	Granulation	Degranulation
January	traces	?
February	++	+
March	++	+
April	++++	+++
May	++++++	++
June	++++++	++
July	++	++++++
August	traces	?
September	,,	,,
October	,,	,,
November	,,	,,
December	,,	,,

The extent of granulation/degranulation was visually quantified from minimum (+) to maximum (++++++); ? = doubtful or not clear

oviduct and of abdominal fat bodies were then calculated.

The meteorological data (figure 1) were obtained from the Government of India, Meteorological Centre, Bangalore and Positional Astronomy Centre, Calcutta.

Influence of season on the body, ovary, oviduct and fat body weights was evaluated by analysis of covariance (ACOVA). The interrelationships between these factors were likewise tested by ACOVA (Steel & Torrie 1960).

Results

Changes in the Body Weight

The data for the year 1980 are based on the frogs collected in Karwar (14°50′N 74°15′E) while in 1982 the frogs were collected around Dharwad city (15°17′N 75°3′E). ACOVA test (table 2) suggests that there is a seasonal variation in the body weight which is directly correlated with the changes in the ovary weights (figure 2). The pattern being identical, especially between May–January, for both the years even though the frogs were collected from different regions. In March–April of 1980 frogs supplied were smaller than those collected in the coressponding months in 1982, a decrease in body weight during these two months in the former may not be real. The body weight began decreasing prior to breeding (figure 2). Later, starting from September the body weight increased gradually up to May.

Changes in the B_2 Cells (Gonadotrophs)

The B_2 cells were columnar in shape and their nuclei were located in the basal region or in

Table 2 *Seasonal variation in body, ovary, oviduct and fat body weights*

	Analysis of Covariance between	'F' value	Remarks
1.	Months *vs* body weight	5.43*	Body, Ovary, Oviduct and Fat body weights undergo significant seasonal changes
2.	Months *vs* ovary weight	68.70*	
3.	Months *vs* oviduct weight	15.11*	
4.	Months *vs* fat body weights	5.18*	
5.	Months *vs* body and ovary weight relation	17.34*	Season has significant effect on the correlative changes in body-ovary, body-fat body, ovary-fat body and ovary-oviduct weights
6.	Months *vs* body and fat body weight relation	6.05*	
7.	Months *vs* ovary and fat body weight relation	4.23*	
8.	Months *vs* ovary and oviduct relation	8.39*	

**F* values are significant at 5% level of significance

the centre with one or two nucleoli. The rostral B_2 cells were larger in size. The cytoplasm contained both cyanophilic and orangeophilic granules. The cyanophilic granules were PAS and AB positive and stained bluish purple with AB-PAS-OG technique. The pituitary gland as well as the B_2 cells in *R. tigerina* were bigger in size in females than in the males.

The cell, cytoplasmic and nuclear areas of gonadotrophs underwent clearcut seasonal changes (figure 1). In the postbreeding period (August to January) a marked decrease in number and size of gonadotrophs was noted, cytoplasmic granules were few and the staining intensity was markedly reduced. A few granulated B_2 cells were, however, seen in the rostral region. In February-

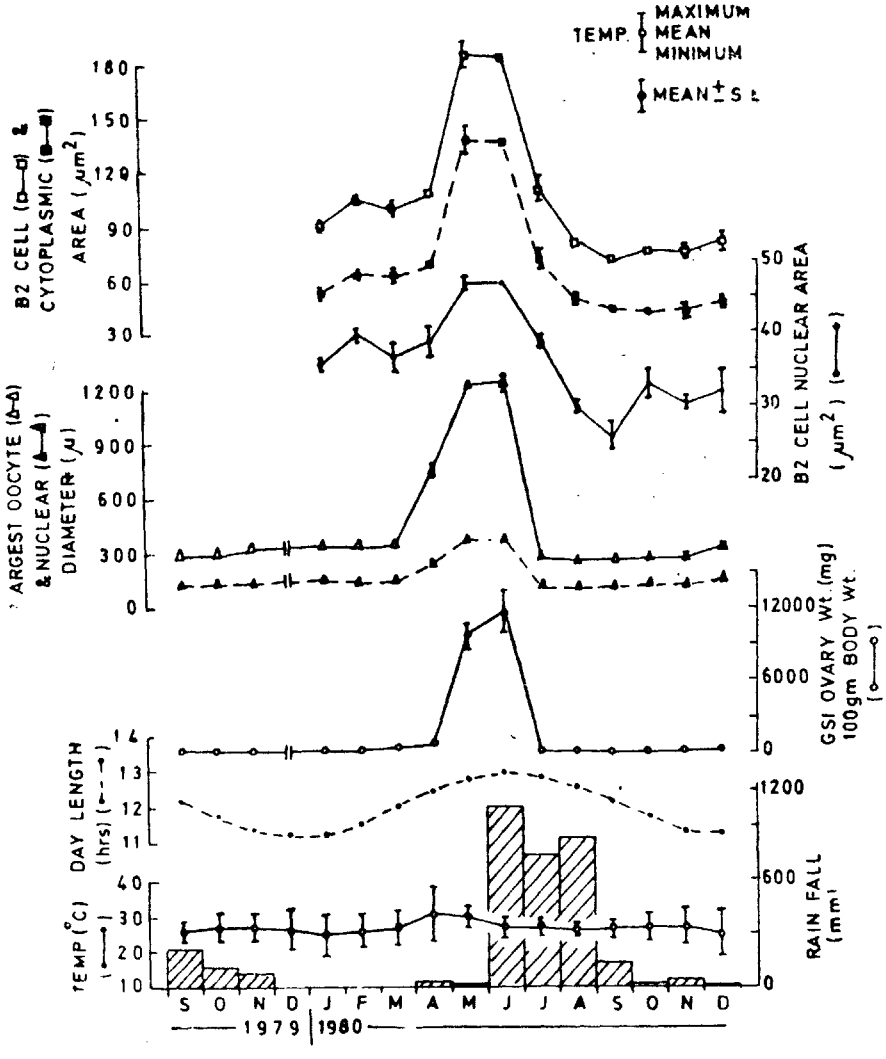


Figure 1 Seasonal changes in GSI (mean weight of ovaries in mg/100 g body weight), mean diameter of largest oocytes and their nuclei (μm), B_2 cell, cytoplasmic and nuclear areas (μm^2) in *R. tigerina* in relation to temperature (°C), rainfall (mm) and day length (hr). September 1979 to December 1980

March, signs of recovery and slow accumulation of secretory granules were seen in B_2 cells (table 1). There was a slight increase in the number and size of B_2 cells. In April B_2 cell size and number increased markedly (figure 1). They were generally filled with secretory granules. However, some B_2 cells showed complete or partial degranulation and vacuolization. Extracellular orangeophilic globules were occasionally seen. During May-June most B_2 cells were densely granulated (figure 3) and stained deep bluish purple. During May-June the B_2 cells were uniformly distributed throughout the pars distalis. In July B_2 cells were markedly reduced in size (figures 1 & 4), degranulated and poorly stained (figure 4).

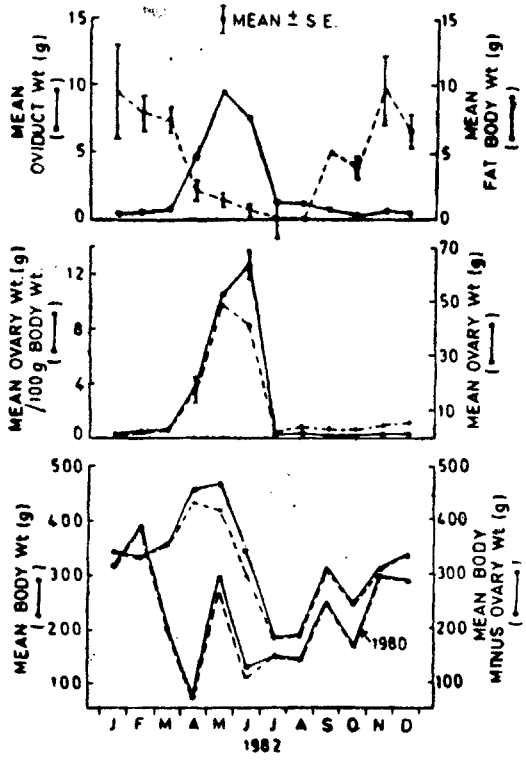


Figure 2 Seasonal changes in mean body weight, body minus ovary weight (somatic weight), GSI, ovary weight, oviduct weight and fat body weight in *R. tigerina*.

Histological Changes in the Ovary

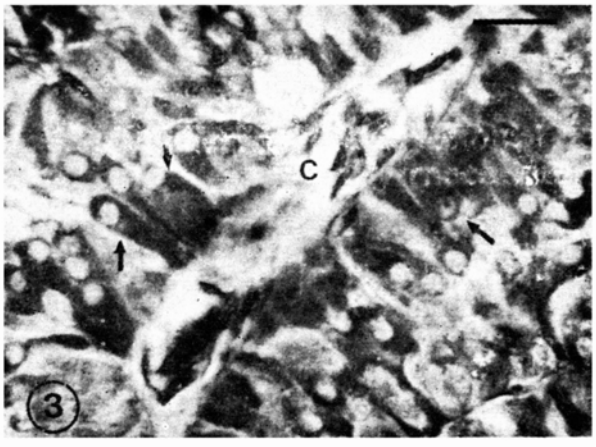
Oogonia

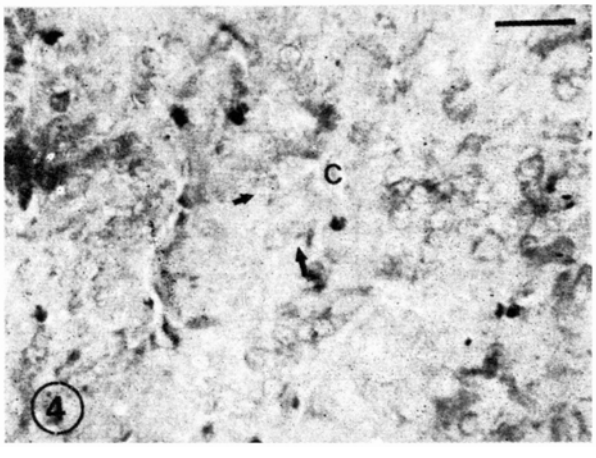
They were small in size (9.3 to 18 μm in diameter) with large nucleus (4.6–9 μm in diameter) and little cytoplasm. The dividing oogonia and the resulting oogonial nests were conspicuous during the early post-breeding period (August–October). Oogonia were seen thghourout the reproductive cycle.

Oocyte

In all adults examined the oocytes were of approximately of the same size (figure 5). The development of oocytes during the annual ovarian cycle is described below:

Post-breeding Regression Phase (August–March): Following the breeding in July, the ovaries became highly regressed in August. Between August–March oocytes were recruited to the first growth phase (FGP) but their growth was very slow (figure 1). With the development of young primary oocytes, the prefollicular cells started surrounding the oocytes, increasing in number by mitosis, and later organised into follicular layers which in turn were covered by the thecal elements. The diameter of the smallest oocyte observed in the ovary of *R. tigerina* ranged from 22 to 37 μm and its nucleus from 11 to 18 μm. In FGP oocytes, the follicle cells remained closely attached to each other and their nuclei showed well-stained chromatin material and smooth nuclear membrane. Many small nucleoli were seen in the periphery of the nucleoplasm. Occasionally, these oocytes also showed 1–3 large nucleoli. In previtellogenic oocytes the yolk nucleus appeared first as a single entity in the ooplasm at juxta nuclear position. At this stage diameter of oocyte was $162\pm7\,\mu$m and its nucleus $68\pm3\,\mu$m. Later, this perinuclear entity appeared in the form of cresent-shaped bits around the nucleus. Gradually as the oocyte increased in size the yolk nuclear substances aggregated to form a ring around the nucleus and





Figures 3 & 4 Sagittal sections of pituitary pars distalis of *R. tigerina*—showing B_2 cells in June (figure 3) and July (figure 4). Note the highly hypertrophied B_2 cells (arrow) with abundant secretory granules, conspicuous nuclei and nucleoli in June. In July (figure 4) these cells become highly regressed and devoid of secretory granules (Herlant's AB-PAS-OG) C, capillary. Scale lines 20μm.

lied in close association with the nuclear membrane. The diameter of the oocyte with fully formed yolk nucleus was 339$\pm$17 μm and that of its nucleus was 150$\pm$1 μm. Such oocytes were seen in January-February. Subsequent to this stage yolk vesicles appeared at the periphery of the ooplasm and the sizes of the oocyte (370$\pm$7 μm) and its nucleus (178$\pm$5 μm) increased further (March).

Preparatory/Prebreeding Phase (April-May): In April, the yolk vesicles appeared throughout the ooplasm except around the nucleus. This marked the beginning of the SGP or the vitellogenic phase. The oocytes entered into

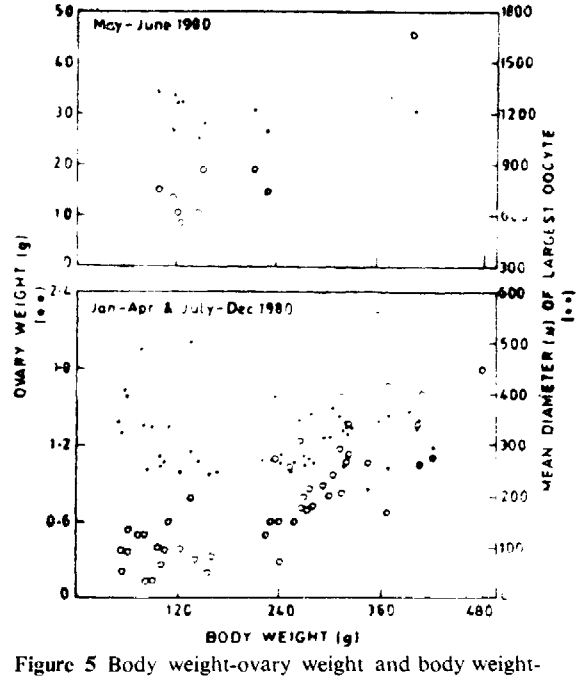


Figure 5 Body weight-ovary weight and body weight-diameter of largest oocyte relationships in *R. tigerina*. It may be noted that ovarian mass increases with body weight. However oocyte sizes are not related to the body weight

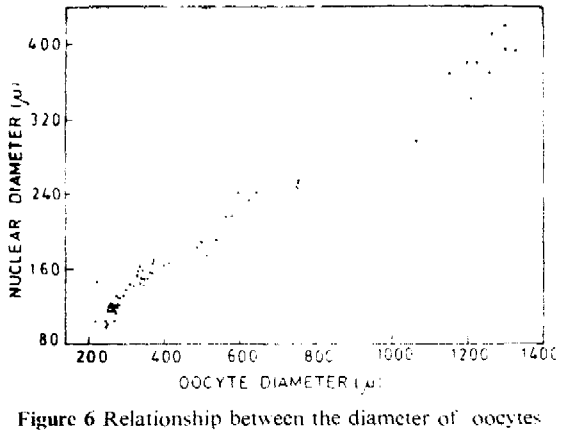


Figure 6 Relationship between the diameter of oocytes and their nuclei in *R. tigerina*

the SGP when they measured 494±17 μm a d their nuclei 195±10 μm. With the onset on SGP there was a breakdown in the yolk nfuclear substance. At this stage the oocytes showed clear theca/granulosa layers and thickened blood vessels in the thecal region. Zona pellucida was seen clearly. Nucleus showed enormous folding of its membrane and many prominent nucleoli.

A small population of previtellogenic oocytes measuring 228±8 μm or smaller in size was also present in the ovaries. Some oocytes ranging from 288 to 483 μm in diameter undergoing degeneration were commonly seen during the preparatory and also after the breeding period.

As the oocyte grew to full size there was unequal distribution of yolk, less in the animal and more in the vegetal pole. Fully grown oocytes were about 1297 μm in diameter. The nucleus of these oocytes was oval-shaped, situated away from the centre, and had a diameter of about 383 μm. The oocyte development was completed in April–May.

Breeding Phase (June-July): The size of the largest oocyte in the ovary dropped to 277±4 μm (figure 1). Immediately after ovulation the ruptured follicles give rise to short-lived postovulatory follicles/corpora lutea. The postovulatory follicles were generally seen in the ovaries of frogs during the first week after the spawning. In some of the thecal and granulosa lutein cells of 3-4 days old postovulatory follicles mitotic divisions were encountered. After breeding the ovary contained small previtellogenic oocytes and atretic follicles.

Atresia: Throughout the year, atretic oocytes were seen in the ovaries. The unspent oocytes undergoing atresia were commonly encountered in the ovaries during August-September.

Changes in the Ovary Weight and GSI

Scatter diagram of the absolute body weight versus ovary weight suggests that the frogs with higher body weight had higher ovarian mass as compared to the adult frogs of lower body weight collected in the same month (figure 5). Changes in the mean ovary weight and GSI during the annual reproductive cycle are directly related except in June when somatic weight decreased causing an elevation of the GSI (figure 2). The ovarian weight was influenced by the body weight (table 2, also see figure 5).

The monthly means of the cell, nuclear and cytoplasmic areas of B_2 cells, GSI, and diameter of largest oocytes (figure 1) reveal that the ovarian cycle is directly related to the B_2 cell activity.

Changes in the Oviduct Weight

In the prebreeding period oviduct weight increased gradually attaining maximum weight (2.2% of the body weight) in May/June (figure 2). The increase or decrease in the oviduct weights during the annual cycle followed the cyclical seasonal changes in the ovarian weights (figure 2). After spawning, the oviduct decreased in weight and showed signs of regression. The oviducts weighed minimum in July (0.0043% of the body weight). The ACOVA test revealed that there was a significant seasonal variation in the oviduct weight which is conditioned by the ovary weight (table 2).

Changes in the Abdominal Fat Body Weight

The abdominal fat bodies were larger in the females compared to the males at all times during the annual cycle. The ACOVA test indicates that there was a significant seasonal variation in the fat body weight which is influenced by the body weight (table 2). There was an inverse relationship between the changes in the ovarian weight and that of the fat body weights except in June-July (figure 2).

Discussion

The majority of the amphibians are seasonal

breeders (Lofts 1974). However, it is now known that diverse patterns in the gametogenetic activity occur among the closely related tropical/subtropical species inhabiting the same area (Saidapur 1983). In the temperate zones a fall in the ambient temperature is the main factor limiting the gametogenetic activity during the winter months (Lofts 1974). Temperature above 20°C is found to be stimulatory for gametogenetic activity in the temperate anurans (van Oordt 1960, Lofts 1974) and such favourable temperature prevails for most part of the day throughout the year in the South Indian regions. Consequently, continuous type of gametogenetic activity is found in both male and female species of *Rana cyanophlyctis* and *Bufo melanostictus* (Lakshman 1965, Saidapur & Nadkarni 1974, Kanamadi & Saidapur 1982, Saidapur 1983). In these species clear-cut seasonal changes do not occur in the histology of gonads. On the other hand, in the ovaries of *R. tigerina* clear-cut seasonal changes occur.

Furthermore cyclical changes also occur in the somatic weight, gonadotrophs, oviduct and fat body weights which are obviously interrelated and bear relationship with the ovarian cycle.

Body weight, Ovary and Fat body Relationships

It is evident that somatic weight in *R. tigerina* undergoes seasonal changes regardless of whether they live in Karwar or Dharwad (figure 2). Further, the changes in the body weight are not due solely to changes in the ovarian mass (see figure 2). Therefore, the somatic weight changes are real and may be related to altered nutritional conditions. Abdominal fat bodies are known to reflect the nutritional status in anurans (Fitzpatrick 1976, Jørgensen et al. 1979).

The decrease in the fat body size in the frog is associated with the vitellogenic growth of oocytes vis-a-vis increase in the ovarian weight (figure 2). Studies on *B. bufo* suggest that fat bodies are not involved in the vitellogenic processes (Jørgensen et al. 1979) whereas earlier authors in other species suggested that fat bodies are essential for the vitellogenic growth of oocytes (Fitzpatrick 1976, Milone et al. 1978, Jørgensen et al. 1979). In *R. tigerina* also, bilateral fat body excision, in the first week of April, markedly reduced the vitellogenic growth of oocytes (Pramoda & Saidapur 1984). However, it is not clear whether the decrease in the fat body size is the result of vitellogenic growth of oocytes or due to utilization of the fat reserves as source of energy or both. Some amphibians are known not to feed around the breeding season (Jørgensen et al. 1979) which may contribute to the depletion of fat stores in the fat body.

In general, changes in the GSI and mean weight of ovaries in the frog run parallel except in June (figure 2). In June due to a decrease in the body weight the GSI value increased even though mean weight of ovaries did not increase (figure 2). In view of the parallel changes taking place in the somatic weight and mean ovarian weight it is suggested that GSI method for assessment of ovarian development is reliable in *R. tigerina* practically for all months of the year. In *R. tigerina* as in few other amphibians (Salthe & Mecham 1974) the body weight influences the ovarian weight (table 2, figure 5) which in turn determines the fecundity of the animal.

Ovary-Oviduct Relationships

In *R. tigerina* growth of oviduct was closely correlated with the growth of the ovaries (figure 2) as reported in *B. bufo* and other temperate anurans (Jørgensen & Vijayakumar 1970, Jørgensen et al. 1979). It is well known that growth of oviducts is stimulated by the ovarian estrogen. The fact that oviducts are well developed only during April–June suggests that ovarian estrogens are elaborated during this period when vitellogenic follicles are present in the ovaries.

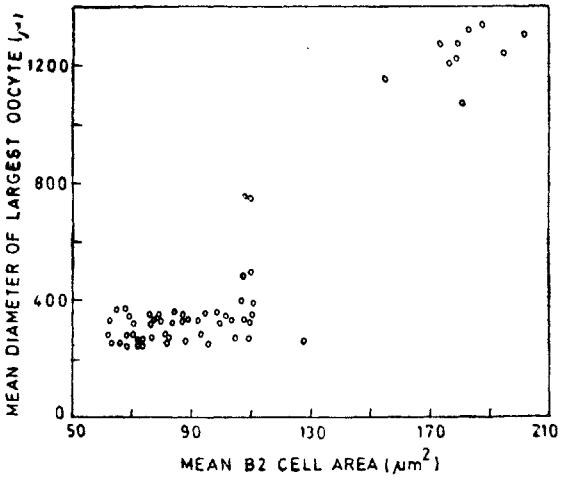


Figure 7 Relationship between the B_2 cell area and the mean diameter of the largest oocytes in *R. tigerina*. Note the rapid increase in oocyte sizes is directly related to an increase in the B_2 cell size

Ovary-B_2 Cell Relationships

The seasonal changes in the folliculogenesis of the frog are very well correlated with the changes in B_2 cells. The fact that the B_2 cells are regressed and inactive during the postbreeding period suggests that oogonial proliferation and early growth of FGP oocytes in the frog may not require gonadotrophins as suggested for certain anurans (Jørgensen 1973, Jones 1978, Tokarz 1978). Nevertheless, these small oocytes respond to exogeneous gonadotrophins (pituitary extracts, HCG and PMSG) and undergo development (Pramoda & Saidapur 1982). The growth of SGP oocytes is associated with the increase in the B_2 cell size. The oocytes between 300–500 μm sizes when not recruited to SGP invariably undergo atresia while oocytes less than 300 μm remain unaffected. The oocyte growth is completed in May and the frogs breed following the onset of heavy monsoon rains in June/July. Apparently, the breeding activity requires high gonadotrophin levels and therefore the B_2 cells empty their products completely in contrast to the situation prevailing in males of the same species (Kurian & Saidapur 1984). In a related species, *R. cyanophlyctis* seasonal changes in the ovarian development are not clearly marked since oocyte recruitment and growth are asynchronous and hence mature eggs are present in the ovary throughout the year (Ramaswami 1962, Lakshman 1965, Sarkar & Rao 1968, Saidapur & Nadkarni 1974). In this species cytomorphological changes in the B_2 cells during the annual reproductive cycle are also less clear (Masood Hussain & Saidapur, unpublished observations).

The environmental factors which influence ovarian activity in *R. tigerina* are not clearly elucidated. It is of interest to note that *R. cyanophlyctis* and *B. melanostictus*. which also inhabit the same area as *R. tigerina* and hence subjected to the same environmental cues show different pattern of ovarian activity. This suggests that genetic factors in addition to environmental factors may also be involved in determining the pattern of reproductive cycle in the tropical anurans.

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