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Dynamics of oogenesis in the tropical anuran *Rana tigrina* (Amphibia: Ranidae) with special reference to vitellogenic cycles in wild-caught and captive flogs

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Abstract. The ovarian cycle of *Rana tigrina* was analysed by quantifying the developing oocytes (classified into stages on the basis of diameter) and atretic ones at monthly intervals. Stages I to IV represent oocytes in the first growth phase and the remaining ones the vitellogenic or second growth phase. Stages I—III occurred year round but exhibited significant variation in their number. The number of stage II oocytes always dominated the other stages. Recruitment of oocytes to stages IV and V in April marked the initiation of vitellogenic growth in all specimens. Of the 30 to 35% second growth phase oocytes, 25 to 28% reached ovulatory sizes by June. After spawning the ovarian mass declined drastically from 15 to 0·2% of body mass in July. Atresia was maximal (5%) in August. In other months, it was less than 1·5% of the total oocytes. Oogenic episodes occurred in March and July yielding new oocytes.

The number of first growth phase oocytes fluctuated from 65 to 95%. The fluctuation was inversely correlated with the second growth phase oocytes indicating a 30 to 35% annual turnover rate of oocytes in the frog. The final egg number/ovarian mass is positively correlated with the snout-vent length as well as body mass of the frogs. *R. tigrina* produces about 4000 eggs/100 *g* body mass. Further, the mean number of yolky eggs/100 g body mass and the total volume (V) of eggs/frog were highly correlated.

Frogs living in captivity produced fewer eggs compared to the wild ones (3594 ± 227 in captive *vs* 4704 ± 317 in wild frogs). Also, these frogs failed to breed though they showed amplexus with breeding males. Injection of desoxycorticosterone acetate however induced spawning in 4 out of 5 frogs. They released about 3000 eggs each. Captivity seems to mainly impair breeding and to a little extent the vitellogenic growth of oocytes in *R. tigrina.*

Keywords. Frog; oogenesis; fat body.

1. Introduction

Among anuran amphibians cyclic ovarian changes have been reported in *Xenopus laevis* (Dumont 1972); *Bufo bufo* (Jørgensen *et al* 1979); *Rana esculenta* (Rastogi *et al* 1983); *Rana cyanophlyctis* (Pancharatna and Saidapur 1985); *Pachymedusa dacnicolor* (*Iela et al* 1986); *Bufo melanostictus* (Kanamadi *et al* 1989); *Rana perezi* (Delgado *et al* 1990); *Polypedates maculatus* (Kanamadi and Jirankali 1991) and *Hyla pulchella andina* (Montero and Pisano 1991). These studies reveal that amphibians have diverse reproductive cycles and, as a result, various reproductive outputs (Saidapur 1989). Temperate zone female anurans typically have annual ovarian cycles that are seasonally correlated (Jorgensen *et al* 1979; Rastogi *et al* 1983). In contrast, tropical anurans have diverse patterns of ovarian follicular

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development even among sympatric species (Saidapur 1989). In species studied to date, three different patterns have been described: (i) continuous annual oogenetic activity in individuals and populations, (ii) continuous at the population level, but discontinuous in individuals, and (iii) discontinuous at both the individual and population levels.

The pattern of folliculogenesis and size frequency distribution of oocytes have been elucidated recently in wild caught and laboratory maintained *R. cyanophlyctis* (Pancharatna and Saidapur 1992) and *B. melanostictus* (Jørgensen *et al* 1986; Kanamadi *et al* 1989), which exhibit the first and second pattern of activity listed above, respectively. *R. tigrina* seems to belong to the third category based on changes in the gonadosomatic index and gross histological study of the ovary (Pramoda and Saidapur 1984a). However, the dynamics of oogenesis, pattern of vitellogenic cycle, extent of follicular atresia, fecundity, relationship between snout-vent length, body and fat body masses, and production of yolky oocytes (number and size) are unknown. The present work was undertaken to elucidate these aspects of the reproductive biology of *R. tigrina* in nature and to study the vitellogenic cycle in captive individuals. Such studies are expected help in developing techniques of amphibian husbandry with special reference to the Indian bull frog which is an economically important species since it is widely used in educational institutions for biology teaching and also in research besides being consumed as food.

2. Materials and methods

Adult female *R. tigrina* (250–650 g) were collected near Dharwad city (15° 17"N, 75° 3"E) monthly from November, 1990 to October, 1991. Six frogs were autopsied soon after their arrival in the laboratory. The weights of the body, ovary and fat body were recorded. The ovaries were then fixed in Bouin's fluid and 10% of the ovarian mass was used for analysis of oocyte growth in each frog. For the size frequency distribution study oocytes were classified into one of six stages as shown in table 1. Concurrent histological study showed that previtellogenic follicles undergoing atresia appear transparent in Bouin's fixed ovaries, whereas yolky atretic follicles appear flabby with black pigment scars. Oocytes less than 646 μm

Table 1. Characteristics of oocytes of different stages.

Stage	Oocyte diameter (μm)	Oocyte characteristics
I	≤ 107	Previtellogenic oocytes in first growth phase (FGP)
II	108–215	
III	216–430	
IV	431–646	Largest previtellogenic and early vitellogenic oocytes with light but visible pigmentation (FGP)
V	647–1077	Uniformly pigmented medium sized vitellogenic/ second growth phase oocytes (MSGP) lacking distinction between animal and vegetal poles
VI	≥ 1078	Large yolky oocytes with clear distinction between animal and vegetal poles (LSGP)

in diameter represent first growth phase (FGP). The medium sized second growth phase (MSGP) and large sized second growth phase (LSGP) oocytes together constitute total second growth phase (SGP) oocytes.

The total volume of SGP oocytes/frog was estimated by multiplying the total number of SGP oocytes with the mean spherical volume (V) assuming that the eggs form hexagonal packing, by using the formula:

$$V = \frac{\pi}{6} \times d^3,$$

where d = diameter of SGP oocytes.

To examine correlations between snout-vent length (SVL) and body mass one hundred frogs covering a wide range of SVL (7·8–20·7 cm) and body weight (55–700 g) were randomly sampled over a period of 12 months (January to December). Similarly, to ascertain correlation between SVL and final ovarian mass, fourteen frogs (weighing 190–700 g) collected during the breeding period covering wide range of SVL (11·8–20·7 cm) and ovarian mass (13·8-96·6 g) were used.

2.1 *Ovarian growth in captive flogs*

Eighty adult female frogs (250–675 g) were collected from a wild population in February, 1992 and placed in an open cement tank (3 × 3 meters) that was exposed to the ambient photoperiod and temperature. They were fed guppies (*Gambusia affinis*) *ad libitum* 6 days a week. Frogs were autopsied from this stock in batches of six in the 2nd week of March, April, May and June. At the corresponding times, frogs obtained from wild populations were used for comparison. Six captive frogs maintained until September were also used for analysis of their ovarian condition.

2.2 *Spawning*

Of the twenty captive frogs 6 were placed individually in glass aquaria and 14 others together in a tank (3 × 3 meters), along with an equal number of adult male frogs (200–400 g) freshly collected from their natural breeding grounds following heavy monsoon rains, in mid June. They were exposed to ambient climatic factors and observed for 5 weeks. None spawned. Hence, five of them were injected (ip) with 2 mg desoxycorticosterone acetate (DOCA) in 0·25 ml distilled water for 1, 2, or 3 days to induce spawning during mid July.

2.3 *Statistical analysis*

Monthly changes in ovarian weight, fat body weight, total number of oocytes, total number of FGP oocytes and also oocytes of stages I to III and, influence of body and ovarian weights on number of oocytes were tested by analysis of variance (ANOVA) and analysis of covariance (ANCOVA) (Steel and Torrie 1980) respectively (table 2). Correlation coefficient r was calculated by Pearson's formula and also

checked for statistical significance at the 5% level of significance. The Mann-Whitney U test (Campbell 1989) was used for comparison of oocytes between wild-caught and captive frogs (table 3).

Table 2. **'F'** values for the various comparisons made by ANOVA or ANCOVA in the frog *R. tigrina.*

Comparisons	Test	$F_{(df: 11,60)}$
Months *vs*		
Fat body mass	ANOVA	6.99*
Ovarian mass	"	88·16*
Stage I oocyte	"	14·54*
Stage II oocyte	"	42·23*
Stage III oocyte	"	13·24*
Total FGP oocyte	"	1·98**
Total number of oocyte	"	0·94
Months *vs*		$F_{(df: 11,59)}$
Body mass and total number of oocytes	ANCOVA	5·23*
Ovary mass and number of oocytes	"	84·25*

Significant at *1% and **5% levels of significance.

Table 3. Showing ovarian and fat body condition in captive* and wild-caught *R, tigrina* during the vitellogenic phase.

	Per 100 g body weight		
	Ovary (g)	SGP oocytes	Fat body (g)
March			
Wild	1·04 ± 0·07	–	3·15 ± 0·45
Captive	0·96 ± 0·10	–	2·01 ± 0·38
April			
Wild	6·91 ± 0·62	3178 ± 168	3·05 ± 0·48
Captive	5·71 ± 1·00	2768 ± 348	0·85 ± 0·18**
May			
Wild	12·72 ± 0·70	4540 ± 211	1·22 ± 0·31
Captive	11·72 ± 0·71	3684 ± 332	0·39 ± 0·16**
June			
Wild	14·80 ± 0·40	4704 ± 317	0·10 ± 0·03
Captive	12·60 ± 1·04	3594 ± 227**	0·09 ± 0·03

Values (per 100 g body weight) are presented as Mean ± SE.
*Captive since 2nd week of February, 1992. Six frogs were used in each group.
**Significant ($P < 0·05$) compared with wild-caught frogs.

3. Results

3.1 *Fat body and ovarian cycles*

A significant annual variation in the abdominal fat body mass (figure 1) was observed (table 2). Between September and April they were 1·16 to 3·0% of body mass. Subsequently their mass declined to 0·1 % of body mass by June. During June and July they remained very small. Size of fat bodies was correlated closely with the body mass (r= 0·91). The ovarian mass fluctuated also (figure 1) significantly (table 2) during the annual cycle. After breeding in July it declined from 15 to 0·2% of body mass.

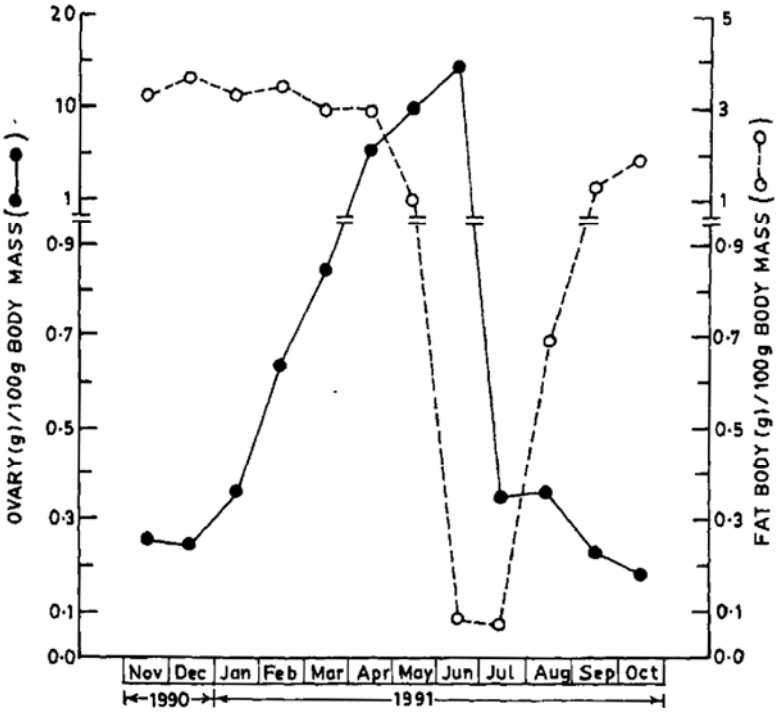

Figure 1. Annual changes in the fat body and ovarian masses in *R. tigrina.*

3.2 *Relation between SVL, body mass and ovary mass*

With the onset of vitellogenesis in April, ovarian mass began to increase in relation to body mass and this trend continued till June. Thus, at completed vitellogenic growth of oocytes in June, the ovarian mass was highly correlated with body mass (r = 0·93). The SVL and body mass also showed a high degree of correlation (r = 0·84). Likewise, SVL and final ovarian mass showed a high positive correlation (r = 0·74).

3.3 *Number and size of eggs*

In April, oocytes greater than 1 mm in diameter were present in the ovaries (figure 2).

In May, both number (figure 2 and 3) and diameter of eggs increased (1·3-1·5 mm). In June, LSGP oocytes were maximal (figure 3). Also, in June, the vitellogenic growth was complete and the largest oocytes represented the ovulatory size (1·4-1·6 mm). The increases in egg numbers and mean volume (V) of eggs/frog are also correlated positively, with *r* values being 0·91 and 0·86 in May and June, respectively. The growth of SGP oocytes was highly synchronous within the individual frogs and also within the population (figure 4).

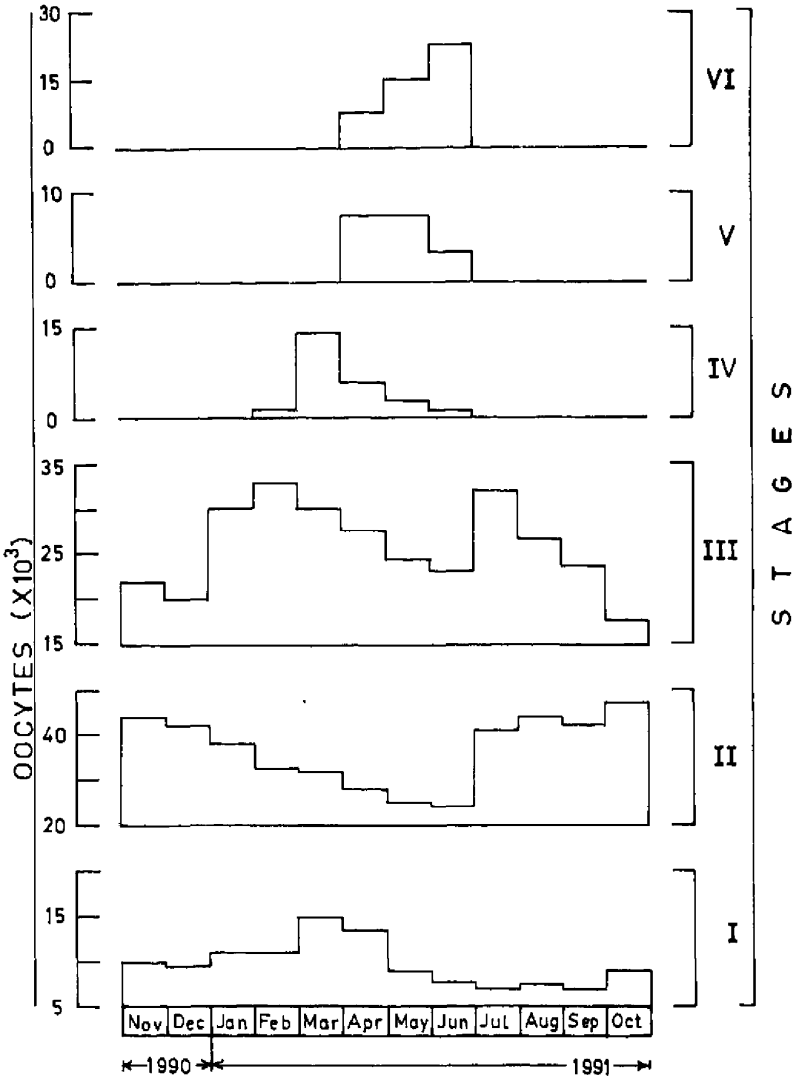

Figure 2. Annual changes in the number of different oocytes (stages I-VI) in *R. tigrina.*

3.4 *Body mass and number of eggs*

The number of eggs produced is closely related to the body mass (r = 0·93 in June). The total volume of SGP oocytes *vis-a-vis* yolk produced is also related

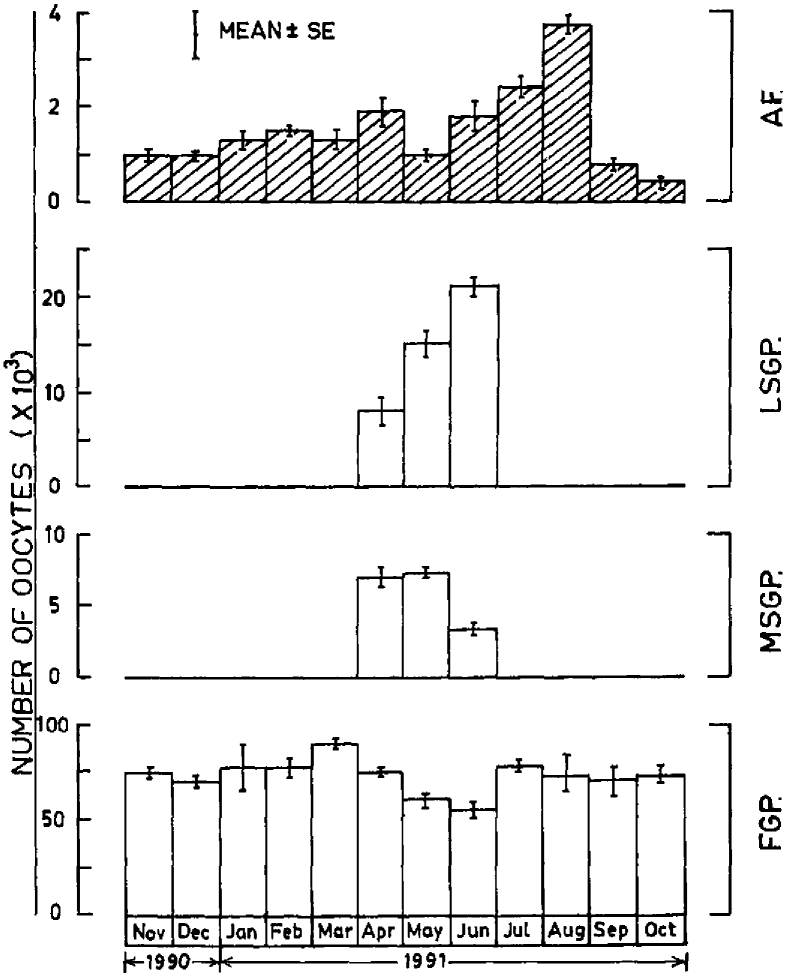

Figure 3. Annual changes in the number of FGP, MSGP, LSGP, and atretic oocytes (AF) in *R. tigrina*.

closely to the body mass (r = 0·52; P < 0·05). In *R. tigrina* on an average 4000 eggs/100 g body mass are produced.

3.5 *Changes and relation between number of small and large oocytes*

Oocytes in the smallest size range (≤ 107 μm) were minimal between June and September (figure 2); their number increased during October–November, and then remained constant until February. In March they increased again. Stage II oocytes were the most common (figure 2), but varied significantly in number during the annual ovarian cycle (table 2). In July, after spawning stage II oocytes increased markedly in size and this was followed by small increments till December (figure 2). From January onwards their number progressively declined reaching lowest level in April with no further change until June. The oocytes in stage III also showed

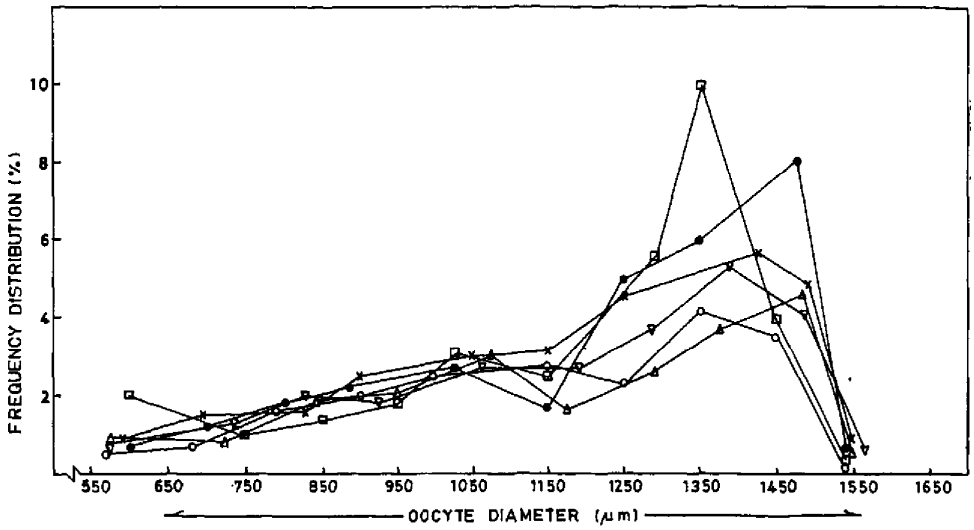

Figure 4. Frequency distribution pattern of oocytes in six frogs autopsied in May. Note that recruitment of oocytes to final growth phase occurs synchronously in all individuals.

significant annual variations (table 2) with peaks in February and July (figure 2). The conversion of stage II oocytes into stage III mainly occurred in January and February (figure 2). Stage III oocytes formed in July gradually degenerated in subsequent months. Stage IV oocytes were produced in March. They grew rapidly to form medium as well as large sized SGP oocytes in subsequent months. Their number declined in a step wise manner in April–May. In June, they were rarely found (figure 2). The SGP oocytes reached final sizes (1·4–1·6 mm) by June. There was a step-wise increase in the number of large eggs between April-June (figures 2 and 3). The frogs spawned in late June or early July following the South–West monsoon rains (figure 5). Each frog laid eggs in a single clutch. Following breeding the ovaries become devoid of oocytes above 0·4 mm until next March (figure 2). The FGP oocyte number underwent significant annual variations with peaks in March and July respectively (figure 5).

The oocyte number shows a positive correlation with body mass (r = 0·42) but it bears no correlation (r = 0·18) with ovary mass. FGP and SGP oocyte numbers exhibit an inverse correlation (r = –0·82) with each other (figure 5) . The SGP oocyte number is highly correlated with ovarian mass (r= 0·92) and also with the total volume of these oocytes (r = 0·91).

3.6 *Atretic oocytes*

Degenerating oocytes were found year round (figure 3). After spawning their number increased to 4·8% of the remaining oocytes in August. Atresia was minimum in October (0·6%). Between September-May it was less than 2% of the total oocytes.

3.7 *Ovarian follicular development/vitellogenesis in captive frogs*

In captive frogs vitellogenesis commenced in April and was completed in June as

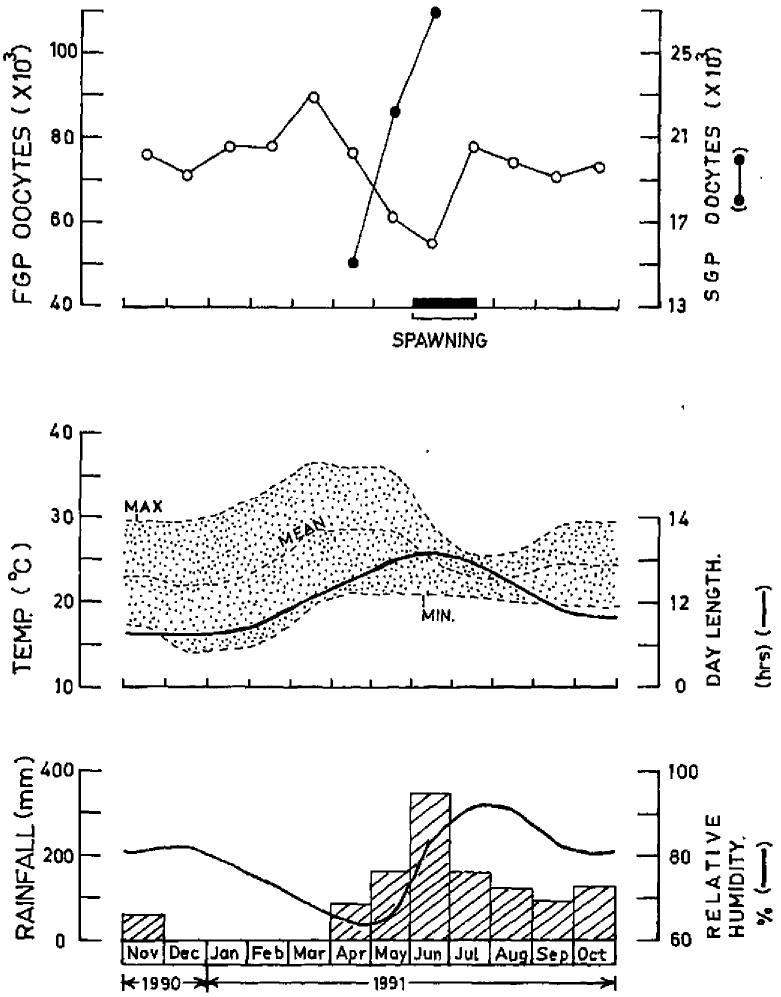

Figure 5. Changes in the FGP and SGP oocytes during the annual ovarian cycle of *R.. tigrina* shown in relation to climatic factors (data obtained from the University of Agricultural Sciences, Dharwad).

in the wild-caught frogs (figure 6). The pattern of changes in the number of different oocytes was also similar between the two groups. However, captive frogs produced fewer eggs than wild-caught ones (table 3). Further, in captive frogs SGP oocytes tended to be more atretic. Secondly their fat bodies became depleted faster than in the wild caught ones (table 3). By September the ovaries of the captive frogs were populated with 24% atretic and 76% FGP oocytes.

3.8 *Courtship and spawning in captive frogs*

The male frogs placed along with the female captive frogs gave breeding calls throughout day and night. For one week there was no amplexus despite breeding calls and rains. Later, 3 frogs in aquaria and one in the cement tank exhibited amplexus with females which lasted for 12–48 h. In forming amplexus the males

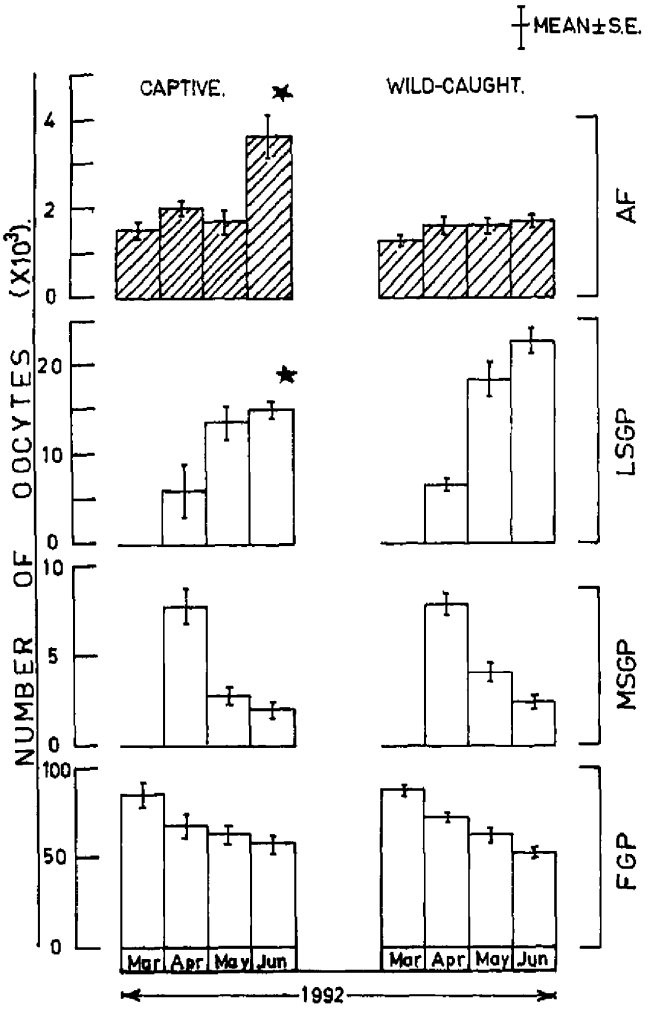

Figure 6. Number of FGP, MSGP, LSGP and atretic oocytes (AF) in captive and wild-caught *R. tigrina.* *$P < 0{\cdot}05$.

did not discriminate between differently sized females. The chorus of males diminished with increase in the duration of the captivity. Once separated, they did not form amplexus a second time. None of the frogs spawned. However, injection of 2 mg DOCA induced spawning in 2 out of 5 frogs after 12 h; they released 2069 and 3000 eggs respectively. Of the remaining 3 frogs two more spawned releasing 3116 and 3829 eggs respectively 12 h after the 2nd injection of DOCA. The fifth frog failed to spawn even after the administration of 3rd dose of DOCA.

4. Discussion

In temperate zones, due to distinct seasonal changes in the temperature, photoperiod, rainfall and abundance of food (insects, meal worms, etc.) amphibians exhibit clear cut and predictable seasonal changes in their ovarian cycles (Jørgensen *et al* 1979).

Whereas, in tropical and subtropical zones such as in Southern India the anurans exhibit diverse reproductive patterns (examples and references in §1). The findings on *R. cyanophlyctis* and the sympatric anuran *B. melanostictus,* besides demonstrating two types of ovarian cycles, signify the value of comparative studies. The present study attempts to analyse changes in nutritional status, dynamics of oogenesis, vitellogenic cycle and atresia in the wild as well as captive *R, tigrina.*

4.1 *Annual changes in nutritional status and ovarian cycle*

The abdominal fat bodies in anurans serve as reliable indicators of nutritional condition in individual specimens (Jørgensen *et al* 1979; Saidapur 1989; Delgado *et al* 1990). Thus, annual changes in the fat body size of *R. tigrina* indicate shifts in the energy balance; the observed inverse correlation between SGP oocytes and fat body mass ($r = -0{\cdot}71$) appears to reflect a negative food balance during vitellogenic phase. Previous studies on *R. tigrina* (Pramoda and Saidapur 1984b) and *R. cyanophlyctis* (Prasadmurthy and Saidapur 1987) have shown that fat bodies play a supporting role in vitellogenic growth of oocytes *vis-a-vis* female reproduction. Following breeding in July the frogs apparently feed actively and their fat bodies become repleted again around September attaining a maximum size by October, *i.e*., well before the winter months. Interestingly, there is no utilization of fat reserves from the fat bodies in winter (November-January) or early summer (February–March) since there appears little change in their size between October–April. *R. tigrina* is not an obligatory hibernator in Southern India and therefore in winter also it appears to feed efficiently to keep a positive food balance. However it exhibits distinct annual ovarian changes as in temperate anurans but quite unlike *R. cyanophlyctis,* a sympatric species living in identical surroundings. In *R. tigrina* the vitellogenic cycle is an annual event occurring 2–3 months prior to breeding (figure 5). On the other hand, in *R. cyanophlyctis* multiple vitellogenic cycles occur concurrently year round (Saidapur 1989).

4.2 *Resting period*

In temperate zones, female anurans are known to exhibit a 2–3 month-long resting phase before initiation of the next ovarian cycle (Jørgensen *et al* 1979. In anurans with multiple vitellogenic cycles within the individuals (*R. cyanophyctics*) it is difficult to identify and determine the duration of the resting period precisely. In *B. melanostictus* the vitellogenic cycle began 4-5 months after breeding (Kanamadi *et al* 1989). The tree frog *P. maculatus* also exhibits a resting phase of about 4–5 months (Kanamadi and Jirankali 1991). In *R. tigrina,* from February onwards ovarian weights begin to increase due to the initiation of ovarian cycle (figure 1). Hence, resting period extends from August to January in the frog. In south Indian anurans (that exhibit a resting period) the quiescent phase is longer than that seen in the temperate anurans. This is evidently due to the fact that the temperate anurans following breeding in spring-summer have little time to complete vitellogenic growth before winter hibernation. On the other hand, in the tropics anurans may or may not hibernate, and, as their breeding is synchronized with precipitation, they can afford a longer resting phase.

4.3 *Dynamics of oogenesis*

4.3a *Egg number and size:* In *B. bufo, Rana temporaria,* and *Bufo viridis* the number and size of eggs are invesely correlated (Jørgensen *et al* 1979; Jørgensen 1981, 1984). This implies that individuals producing a large number of eggs have smaller sized eggs and *vice-versa.* This is believed to be due to the production of a fixed amount of yolk which is distributed over a few large eggs or over many small eggs. In *Rana dalmatina* there was a definite correlation (r= 0·73) between egg diameter and clutch volume *vis-a-vis* total yolk volume (Waringer-Löschenkohl 1991). Similarly, in *R. tigrina* the mean number of eggs produced per 100 g body mass and the volume of eggs (V) are positively correlated. Therefore, both size and number of eggs increase concurrently throughout the vitellogenic growth phase. Also, the fact that final ovarian mass (which is a function of total number of yolky eggs) is positively correlated with SVL as well as body mass suggests that fecundity greatly depends upon the size of the frog.

4.3b *FGP oocyte pool*: As in other anurans, in *R. tigrina,* FGP oocytes always form the major component despite their fluctuation in number (figure 5). The decline in their number is clearly associated with the recruitment of oocytes from this pool to final vitellogenic growth between April-June and hence an inverse correlation between FGP and SGP oocytes is seen (figure 5). In the frog out of the FGP oocyte pool 30–35% oocytes enter SGP but only 25–28% reach the final ovulatory sizes. About 3–4% of these oocytes remain MSGP and eventually degenerate after breeding. After spawning there is little delay, if any in restoring the pool size of FGP oocytes (figure 5). This is apparently due to rapid oogonial proliferation and their differentiation into new oocytes (0·1–0·2 mm) in July (see stage II oocytes in figure 2). In *R. tigrina* the annual turnover rate of oocytes is 30–35%.

4.3c *Atresia:* Quantitative studies on the atresia of oocytes are scarce (Saidapur 1978). During the ovarian cycle of *R. tigrina* 0·5 to 5% oocytes undergo atresia. Atresia is maximal in the breeding period (figure 3), and following spawning nonovulated large oocytes and all SGP oocytes invariably undergo degeneration. Atresia is more easily recognized among the larger oocytes and little is known about artesia of FGP oocytes. In *R. tigrina* about 0·6 to 1·5% atresia was recorded between September-February, when the ovaries lack SGP oocytes. This suggests that atresia during the above period is due to the degeneration of possibly stage IΠ oocytes. Apparently, the increased production of large sized stage III oocytes in July (figure 2) and on going production in subsequent months is in vain as these oocytes may degenerate due to the lack of gonadotrophins (Pramoda and Saidapur 1984a)

4.3d *Correlation of ovarian cycle with climatic factors:* In nature, vitellogenesis in *R. tigrina* is associated with a rise in temperature and daylight (figure 5). Temperature seems to play an important role in the control of ovarian cycle in *R. tigrina* (Pancharatna and Saidapur 1990) as in temperate anurans (Jørgensen *et al*

1978). However, the role of daylight, if present, remains uncertain due to paucity of studies. All frogs and toads studied so far from southern India are known to breed during the rainy months of monsoon irrespective of whether they show continuous or discontinuous type of ovarian cycles. Therefore, rainfall seems to trigger breeding activity in these anurans (Saidapur 1989).

4.4 *Ovarian follicular dynamics and spawning in captive frogs*

Prolonged captivity is known to reduce fat bodies (Saidapur and Prasadmurthy 1988; Kanamadi *et al* 1989; Pancharatna and Saidapur 1992) and increase atresia of oocytes in all anuran species studied so far (*X. laevis,* Shapiro and Shapiro 1934; *R. pipiens,* Smalley and Nace 1983; and *R. cyanophlyctis,* Saidapur and Prasadmurthy 1988; Pancharatna and Saidapur 1992). In comparison however, in *R. tigrina* captivity resulted in a small decrease in the eggs produced (table 3, figure 6).

Interestingly, despite completion of vitellogenesis captive frogs failed to breed even though they formed amplexus with males. It appears that failure of frogs to reach their natural spawning grounds results in nonovulation. Consequently, captivation beyond the breeding season resulted in atresia of all yolky eggs. However, in July, they could be induced to spawn using the corticosteroid hormone DOCA. These findings suggest that the frogs could be held under captivity without severely affecting their ovarian recrudescence and, spawning could be induced in them using hormones.

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