

Effect of photoperiod and temperature on ovarian cycle of the frog *Rana tigrina* (Daud.)

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Abstract. The effect of varying photoperiod regimes (LD: 20,4; 4,20; 6,18; 18,6 and 12,12) on ovarian follicular development was analysed in the frog *Rana tigrina* maintained at ambient and constant $30^{\circ} \pm 1^{\circ}\text{C}$ for 3 months. The experiments were conducted in early recrudescence and quiescent phases. The frogs were fed guppies *ad libitum* on alternate day. None of the photoperiod regimes had any effect on the ovaries or the fat bodies, whereas exposure to constant high temperature (regardless of photoperiod) during recrudescence phase induced production of greater number of eggs (~ 18000 vs 13000 in controls) of ovulatory sizes ($> 1400 \mu\text{m}$) compared to the corresponding controls maintained at ambient temperature. Hence, ovarian mass also increased in these frogs. In the quiescent phase, high temperature merely enhanced growth of previtellogenic oocytes. In both the phases high temperature caused a reduction in the fat bodies over the respective controls, possibly due to increased metabolic activity.

The above findings indicate that temperature plays a key role in the regulation of ovarian cycle of *Rana tigrina* and that the photoperiodic mechanisms may not govern the annual recrudescence of ovaries in the frog. The study also shows that the frog exhibits the phenomenon of "phenotypic plasticity" in its reproductive behaviour by producing significantly greater number of eggs in response to elevated temperature.

Keywords. Photoperiod; ovarian cycle; fat body; follicular development; *Rana tigrina*.

1. Introduction

In some vertebrates, especially birds and mammals photoperiod acts as a chief proximate factor in the regulation of seasonal reproduction (Lofts 1975; Meier 1975; Whitter and Crews 1987; Wingfield and Kenagy 1991). Photoperiod changes accurately and reliably around the year, so that animals can use this cue for prediction of seasonal changes and accordingly programme their gonadal development and breeding activities. Conversely, in ectothermic vertebrates environmental temperature is believed to play a key role in the control of metabolic activity, behaviour and reproduction (Jørgensen *et al* 1978; Rastogi *et al* 1978; Licht 1984; Fraile *et al* 1989).

Among amphibians, the ovarian cycle has been studied in several temperate (Jørgensen *et al* 1979) and tropical (Saidapur 1989) representatives but little is known about the proximate factors affecting or controlling the seasonal ovarian development (Saidapur 1989; Whittier and Crews 1987; Wingfield and Kenagy 1991). The studies on the role of photoperiod in female amphibians are limited to *Bufo fowleri* (Bush 1963) and *Rana catesbeiana* (Horseman *et al* 1978). In *B. fowleri*

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long photoperiods were more stimulatory than short photophases for gonadosomatic index (Bush 1963). In *R. catesbeiana* the photoperiod cycle of LD: 12,12 (25°C) appeared to prevent ovarian regression and atresia in both laboratory reared and wild-caught specimens. Similarly, there is a good evidence that temperature can affect ovarian follicular growth in *Bufo bufo* (Jørgensen *et al* 1978), *Rana esculenta* (Rastogi *et al* 1983) and *Rana tigrina* (Pancharatna and Saidapur 1990). However, in these studies influence of photoperiod, if any, was not studied.

In southern India *R. tigrina* breeds seasonally in June-July (Hoque and Saidapur 1994). It exhibits clear-cut seasonal changes in its ovarian development despite the fact that ambient temperature and photoperiod do not undergo seasonal extremes, and often there are regularly recurring wet and dry periods. The present study covering most part of the ovarian cycle of *R. tigrina*, deals with the long-term effects of short, medium and long photoperiods in relation to ambient and constant high temperature ($30^{\circ} \pm 1^{\circ}\text{C}$). Because abdominal fat bodies are known to have a supportive role in ovarian growth (Pramoda and Saidapur 1984; Prasadmurthy and Saidapur 1987), changes in their mass were also recorded in this study.

2. Materials and methods

Adult female *R. tigrina* (320–450 g) collected around Karwar city (14°50" N, 74°15" E) were used. Two separate experiments, each lasting for 90 days were conducted. The frogs were autopsied on day 91.

Experiment I began in mid February, 1992 (early preparatory period) using freshly collected frogs after 4 day exposure to laboratory conditions. They were divided into six groups, each containing 8 frogs: 1. Initial control. 2. Final control (exposed to natural photoperiod and ambient temperature). 3. LD: 4,20 and ambient temperature. 4. LD: 20,4 and ambient temperature. 5. LD: 12,12 and $30^{\circ} \pm 1^{\circ}\text{C}$. 6. LD: 20,4 and $30^{\circ} \pm 1^{\circ}\text{C}$.

The natural photophase increased gradually from 11 h and 42 min to 13 h and 5 min when the above experiment was conducted. The mean ambient temperature fluctuated from 21°C to 29.5°C.

Experiment II began in mid September, 1992 (post breeding period) using freshly collected frogs. They were divided into the following groups, each containing 6–8 frogs: 1. Initial control. 2. Final control (exposed to natural photoperiod and ambient temperature). 3. LD:6,18 and ambient temperature. 4. LD: 18,6 and ambient temperature. 5. LD: 12,12 and $30^{\circ} \pm 1^{\circ}\text{C}$. 6. LD: 18,6 and $30^{\circ} \pm 1^{\circ}\text{C}$.

Photophase between mid September to mid December when the above experiment was conducted declined gradually from 12 h and 13 min to 11 h and 12 min. As the natural photophase is comparatively shorter in winter than in summer, 18 h photophase (instead of 20 h used in groups 4 and 6 of first experiment) was considered as long photophase in this experiment (groups 4 and 6). Fluctuation in the daily ambient temperature was from 17.7°C to 26.4°C.

Frogs in groups 5 and 6 in both experiments were maintained in an incubator set at $30^{\circ} \pm 1^{\circ}\text{C}$ provided with tube lights and timer to regulate the photoperiod. The intensity of light above the water surface was 800 lux. The aquarium water was renewed daily using water previously equilibrated to the desired temperature. Its level was such that frogs in their sitting position had their heads above water. The frogs were fed guppies (*Gambusia affinis*) *ad libitum* during the photophase on alternate day.

At autopsy, the body, ovary, and fat body weights were recorded. The ovaries were fixed in Bouin's fluid for analysis of ovarian condition. An ovarian sample weighing 10% of the total Bouin's fixed ovary was used for quantification of different types of oocytes. The oocytes were classified into first growth phase (FGP), medium sized second growth phase (MSGP), large sized second growth phase (LSGP), oocytes and atretic follicles (AF) as described earlier (Hoque and Saidapur 1994). A piece of ovary was processed for histology. The diameter of 50 largest oocytes per ovarian sample was measured to determine the maximum size attained by the oocytes.

Data were analysed using a non-parametric Kruskal-Wallis test followed by multiple comparison method (Conover 1980).

3. Results

3.1 Effect of photoperiod and temperature on ovary and fat body in preparatory phase (Experiment 1)

3.1a *Initial and final controls*: The ovaries of initial control frogs weighed 0.57% of body mass (table 1) and contained FGP oocytes which measured $397 \pm 11 \mu\text{m}$ in diameter and about 1.29% AF (table 2). In the final control frogs ovarian mass increased to 10% (table 1) of body mass due to the production of SGP oocytes which had attained the ovulatory sizes ($1418 \pm 19 \mu\text{m}$). In these frogs there was a significant ($P < 0.05$) increase in the number of AF compared to the initial controls.

The initial control frogs possessed well developed fat bodies amounting to 2.86% of body mass but they became significantly ($P < 0.05$) smaller in the final controls (table 1).

3.1b *Frogs exposed to short/long photoperiod and ambient temperature*: The ovaries of frogs exposed to LD: 4,20 or LD: 20,4 and ambient temperature resembled in

Table 1. Effect of photoperiod and temperature on the ovarian and fat body masses in the preparatory period.

Group (n = 8)	Ovarian mass g/100 g body mass (Mean \pm SE)	Fat body (g)/100 g body mass (Mean \pm SE)
1. Initial control	0.57 \pm 0.02	2.86 \pm 0.24
2. Final control	10.16 \pm 0.59 ^a	1.15 \pm 0.20 ^a
3. LD: 4,20 and ambient temp.	9.46 \pm 0.40	1.23 \pm 0.11
4. LD: 20,4 and ambient temp.	9.90 \pm 0.24	1.12 \pm 0.21
5. LD: 12,12 and 30° \pm 1°C	12.95 \pm 0.67 ^{b,c,d}	0.46 \pm 0.10 ^{b,c,d}
6. LD: 20,4 and 30° \pm 1°C	12.42 \pm 0.74 ^{b,c,d}	0.42 \pm 0.12 ^{b,c,d}
Kruskal-Wallis test value	32.01	32.80
	$P < 0.05$	$P < 0.05$
Tabulated $T = 11.07$		

^{a,b,c,d} $P < 0.05$; ^a is a comparison with Group 1; ^b with Group 2; ^c with Group 3 and ^d with Group 4.

Table 2. Effect of photoperiod and temperature on growth and recruitment of oocytes in the preparatory period.

Group (n = 8)	Oocytes ($\times 10^3$)/frog (Mean \pm SE)				Largest oocyte diameter (μm) (Mean \pm SE)
	FGP	MSGP	LSGP	AF	
1. Initial control	77.62 \pm 2.90	—	—	1.00 \pm 0.10	397 \pm 11
2. Final control	61.72 \pm 2.70 ^a	5.44 \pm 0.47	13.60 \pm 1.37	1.45 \pm 0.11 ^a	1418 \pm 19 ^a
3. LD: 4,20 and ambient temp.	64.77 \pm 2.65	4.86 \pm 0.65	11.03 \pm 1.23	1.71 \pm 0.12	1405 \pm 20
4. LD: 20,4 and ambient temp.	59.66 \pm 2.21	5.08 \pm 0.49	12.98 \pm 1.14	1.30 \pm 0.08 ^c	1429 \pm 17
5. LD: 12,12 and 30° \pm 1°C	55.88 \pm 2.03 ^{b,c}	5.80 \pm 0.52	17.73 \pm 1.47 ^{b,c,d}	1.53 \pm 0.12	1445 \pm 17
6. LD: 20,4 and 30° \pm 1°C	51.99 \pm 2.38 ^{b,c,d}	4.91 \pm 0.34	18.19 \pm 1.73 ^{b,c,d}	1.39 \pm 0.11 ^c	1453 \pm 22
Kruskal-Wallis test value	24.98*	2.58	13.73*	14.85*	21.82*
	(K = 6)	(K = 5)	(K = 5)	(K = 6)	(K = 6)

Tabulated $T=11.07$ (when $K=6$) and 9.49 (when $K=5$).

*Significant at 5% level of significance.

^{a,b,c,d} $P < 0.05$; ^ais a comparison with Group 1; ^bwith Group 2; ^cwith Group 3 and ^dwith Group 4.

all respects those of the controls exposed to natural photoperiod and ambient temperature for 3 months (table 2). Moreover, irrespective of the duration of photophase normal complement of oocytes attained the ovulatory sizes (table 2). However, in frogs exposed to longer scotophase follicular atresia was comparatively high (table 2).

The changes in photoperiod had no effect on fat body mass of the frogs as compared to the controls maintained under natural photoperiod and temperature (table 1).

3.1c *Frogs exposed to LD: 12,12 or 20,4 and constant 30° \pm 1°C*: In frogs held at 30° \pm 1°C for 3 months inspite of changes in the photoperiod the ovarian masses increased significantly over the final controls (12% of the body mass) due to increased production of yolky eggs (tables 1 and 2). AF were comparable to those of the controls (table 2). The fat body mass decreased drastically (table 1).

3.2 *Effect of photoperiod and temperature on ovary and fat body in post breeding regression phase (Experiment II)*

3.2a *Initial and final controls*: The ovaries of the initial control frogs contained 99% FGP oocytes which were less than 300 μm in diameter. AF were about 1% of total oocytes. The ovaries weighed 0.28% of body mass. The fat bodies amounted to 1.26% of body mass (table 3).

The ovaries of final control frogs (exposed to natural photoperiod and ambient temperature) were similar to those of the initial controls except that the diameter of the largest FGP oocytes increased significantly (table 4). Also, the fat bodies in these frogs increased to almost 3% of body mass (table 3) as found in nature.

Table 3. Effect of photoperiod and temperature on the ovarian and fat body masses in the quiescent phase.

Group	Ovarian mass	Fat body
	g/100 g body mass (Mean \pm SE)	g/100 g body mass (Mean \pm SE)
1. Initial control (8)	0.28 \pm 0.01	1.26 \pm 0.29
2. Final control (8)	0.31 \pm 0.02	2.84 \pm 0.44 ^a
3. LD: 6,18 and ambient temp. (7)	0.29 \pm 0.02	2.82 \pm 0.31
4. LD: 18,6 and ambient temp. (8)	0.30 \pm 0.02	2.83 \pm 0.38
5. LD: 12,12 and 30° \pm 1°C (6)	0.47 \pm 0.02 ^{b,c,d}	1.72 \pm 0.31 ^{b,c,d}
6. LD: 18,6 and 30° \pm 1°C (7)	0.48 \pm 0.02 ^{b,c,d}	1.77 \pm 0.18 ^{b,c,d}
Kruskal-Wallis test value	27.64	17.25
	$P < 0.05$	$P < 0.05$
Tabulated $T = 11.07$		

Numbers in parentheses indicate number of frogs.

^{a,b,c,d} $P < 0.05$; ^a is a comparison with Group 1; ^b with Group 2; ^c with Group 3 and ^d with Group 4.

Table 4. Effect of photoperiod and temperature on growth and recruitment of oocytes in the quiescent phase.

Group	Oocytes ($\times 10^3$)/frog (Mean \pm SE)		Largest oocyte diameter (μm)
	FGP	AF	
1. Initial control (8)	70.53 \pm 4.95	0.71 \pm 0.11	251 \pm 7
2. Final control (8)	84.25 \pm 5.40	1.00 \pm 0.11	279 \pm 8 ^a
3. LD: 6,18 and ambient temp. (7)	78.87 \pm 4.13	1.15 \pm 0.09	281 \pm 8
4. LD: 18,6 and ambient temp. (8)	89.06 \pm 5.43	1.03 \pm 0.10	274 \pm 9
5. LD: 12,12 and 30° \pm 1°C (6)	72.16 \pm 4.14	1.50 \pm 0.18 ^{b,d}	392 \pm 14 ^{b,c,d}
6. LD: 18,6 and 30° \pm 1°C (7)	80.89 \pm 3.05	1.39 \pm 0.10 ^{b,d}	419 \pm 15 ^{b,c,d}
Kruskal-Wallis test value	10.94	18.09	30.59
	NS	$P < 0.05$	$P < 0.05$
Tabulated $T = 11.07$			

Numbers in parentheses indicate number of frogs. NS, Nonsignificant.

^{a,b,c,d} $P < 0.05$; ^a is a comparison with Group 1; ^b with Group 2; ^c with Group 3 and ^d with Group 4.

3.2b *Frogs exposed to short/long photophase and ambient temperature:* In these frogs exposed to ambient temperature and short or long photoperiod (LD: 6,18 or LD: 18,6) there was no difference in the ovaries or fat body masses compared to those of the controls (table 3).

3.2c *Frogs exposed to LD: 12,12 or LD: 18,6 and constant 30° \pm 1°C:* In these frogs, regardless of the photoperiod regime, there was an increase in the per cent weight of ovaries, size of oocytes and also number of AF (tables 3 and 4). Yet, the oocytes were in the FGP as in the controls. The frogs had significantly smaller fat bodies (table 3).

4. Discussion

In temperate zone anurans which breed in early spring, gametogenesis occurs in summer, and the gonads are quiescent in winter (Rastogi 1976; Jørgensen *et al* 1979). Whereas, most tropical anurans reproduce seasonally in environments that have a seasonal wet/dry periods. For instance, *Phyllomedusa callidryas* and *Phyllomedusa dacnicolor* (Pyburn 1970) in southern Veracruz, Mexico, the Old World anurans *Phrynobatrachus natalensis* and *Bufo regularis* (Inger and Greenberg 1956) in Katanga Province, Congo and *Rana hexadactyla*, *Rana cyanophlyctis*, *Cacopus systoma*, *Rana tigrina*, *Bufo melanostictus* (references in Saidapur 1989) and *Polypedatus maculatus* (Kanamadi and Jirankali 1991) inhabiting peninsular India breed during rainy season after the pools become filled with rain water. Therefore, these species seem to anticipate the onset of the wet season to complete gonadal development, but the cues to which they are responding are not known.

It is generally believed that temperature is the primary cue that regulates reproduction in amphibians and day length has no role. This is evidently based on a few observations that warm temperature at any time of the year stimulates spermatogenesis (e.g. *R. esculenta* and *Notophthalmus viridiscens*) and cool temperature in summer halts spermatogenesis. Similar observations are made in few other anurans and urodeles. It is reported that optimum photoperiod is required for normal spermatogenesis and steroidogenesis in a few amphibians (Paniagua *et al* 1990) while in many others photoperiod had no effect on testicular activity (Wingfield and Kenagy 1991).

Studies on the effect of photoperiod and temperature with well controlled experimental protocols are very scarce in female amphibians and as such the role of proximate factors in the control of their reproduction is far from clear. What is known, however, is that, temperature affects vitellogenesis in *B. bufo* (Jørgensen *et al* 1978), *R. esculenta* (Rastogi *et al* 1983) and *R. tigrina* (Pancharatna and Saidapur 1990). For instance, experiments on *B. bufo* have shown that in toads with ovaries containing oocytes in their final growth stage fail to show any further development when kept at 4°C for 10 weeks, but resume a normal rate of growth when returned to 20°C temperature (Jørgensen *et al* 1978). In *R. esculenta*, 15°±2°C appeared to be optimum for ovarian integrity and vitellogenic growth of oocytes (Rastogi *et al* 1983). Whereas, in *R. tigrina* exposure to LD: 12,12 and 30°C in winter stimulated growth of previtellogenic follicles, while exposure to 22°C in summer retarded vitellogenesis (Pancharatna and Saidapur 1990). These experiments did not elucidate the separate effects of photoperiod and temperature.

In the experiments of early recrudescence phase in *R. tigrina* regardless of the short (LD: 4,20) or long (LD: 20,4) photoperiod the frogs kept at ambient temperature or at constant 30° ± 1°C for 3 months developed normal complement of fully grown oocytes (table 2). It is noteworthy that frogs exposed to constant 30° ± 1°C produced significantly greater number of yolky eggs (table 2) and hence the ovarian mass increased over that of the frogs exposed to ambient temperature (table 1). The increased production of eggs in these frogs was associated with the corresponding decreases in the number of FGP oocytes (table 2). At the same time fat body mass was reduced significantly, evidently due to mobilization of its contents to support vitellogenesis and/or increased metabolism due to high temperature. That fat bodies may play a supporting role in vitellogenesis has been shown in *R. tigrina*

(Pramoda and Saidapur 1984) and *R. cyanophlyctis* (Prasadmurthy and Saidapur 1987). The above findings also suggest that *R. tigrina* exhibits phenotypic plasticity in its reproductive behaviour by producing greater number of eggs in response to higher temperature in recrudescence phase.

In quiescent phase exposure of *R. tigrina* to high temperature (LD: 12,12 or 18,6) elevated mean per cent ovarian weight due to increase in the size of FGP oocytes (table 3). However, no SGP oocytes were produced in them even after 3 month exposure to high temperature, long photophase and adequate food. In these frogs FGP oocyte grew to gonadotrophin dependent sizes (> 400 µm) and eventually became atretic evidently due to an expectant inadequate amount of gonadotrophins in the quiescent phase.

Whether photoperiodic mechanisms play a role in amphibian reproduction is not clear. However, the pineal gland in amphibians is known to serve as a photoreceptor, thermoregulator and neuroendocrine transducer (De Vlaming and Olcese 1981). Further, administration of melatonin is known to retard vitellogenesis in *R. cyanophlyctis* (Kupwade and Saidapur 1986) and also inhibit ovarian compensatory hypertrophy in unilaterally ovariectomized *R. tigrina* (Hoque *et al* 1993) suggesting that pineal/photoperiodic mechanisms (involving melatonin) may be involved in the regulation of their ovarian cycles. In *R. catesbeiana* Horseman *et al* (1978) reported that both short and long photoperiods increase atresia while LD: 12,12 is optimum for ovarian integrity. However, considerably short or long photoperiod used in the present study had no effect on ovarian recrudescence of *R. tigrina*.

The present study on adult *R. tigrina* shows that photoperiod and temperature do not influence oogonial proliferation/production of new oocytes. Further, it demonstrates that the effects of temperature are manifested independent of any photoperiodic mechanisms in the frog.

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