I. GENERAL INTRODUCTION

Seasonality is the initiation-termination-reinitiation of a physiological process and is an adaptation for survival in most of the species. Amphibians are seasonal. Adult amphibians exhibit seasonal cycle in several functions including gain and loss in body weight, body fattening, parasitic load, gonadal growth and development, hormone levels, immune system and hibernation. Most of the seasonal events are centred on reproduction. David Lack (1968) defined "each species of bird has presumably evolved timing of its breeding so that it raises most offspring" and "laying is normally so timed that young are in the nest when there is enough food for them to be raised…".

Seasonality in reproduction and associated events is species specific, and is timed such that when breeding occurs plenty of preferred food is available for both parents and their offspring to make sure that chances of survival of offspring are maximum. Different environmental factors help in timing, which includes seasonal variations in day length, temperature, rainfall, humidity and vegetation (Immelmann, 1971). Baker (1938) for the first time categorised these factors into two types: the proximate and ultimate factors. Proximate factors "gate" a temporal window during the year for any seasonal event, the ultimate factors fine tunes that the actual seasonal events occur during the gated window. In most of vertebrate species to time seasonal reproduction, day length acts as the proximate factor, while

food/temperature acts possibly as an ultimate factor. Although the proximate and ultimate factors act in coordination, and not in isolation. In amphibians being poikilotherms temperature and rainfall may play a crucial role in timing seasonal reproduction as desiccation of pools and ponds will have severe impact on the developing tadpoles.

Rhythmic breeding, timed developmental and other physiological processes are evolved through the development of timing mechanisms that are governed by the oscillatory clock systems. These systems are innate (endogenous), inheritable and genetic in origin (Aschoff, 1981; Kumar, 2001, 2002; Kumar *et al*., 2004). In natural environment, these oscillatory systems are synchronized with daily cycle of Sun, and thus, are represented as daily overt rhythms. When the organisms are exposed to constant environmental conditions (constant dim light, LL_{dim} or constant darkness, DD) under laboratory conditions, they exhibit their endogenous periodicity (Aschoff, 1981) with period (interval between two identical phases) close to 24 hour (24 h), which is referred to as 'circadian' (*circa* – about, *dies* – day; Halberg, 1969), or close to one year, which is referred to as 'circannual' (*circa* – about, *annum*- year). Both circadian and circannual rhythms (clocks) are believed to be involved in regulation of seasonal phenomenon in wide range of organisms.

Two mechanisms have been suggested to be involved in the regulation of seasonality. Photoperiodism, where photoperiodic conditions of the environmental (day light cycle) times the component events of seasonality. Second the circannual rhythm generation, where a self-sustained endogenous rhythm of approximately 1-year times these seasonal processes. However, how both of these mechanisms act, we still don't know. But it appears that they may not be acting mutually exclusive and, might interact closely, albeit as per adaptive needs of the species. However, many workers believe that photoperiodism and circannual rhythm generations are

evolved as separate mechanisms. In general, a commonly held view is that a photoperiodic species lacks a strong circannual component, and a circannual species lacks a strong photoperiodic component. Part of this widely held assumption stems from studies that (1) show maintenance of the post-reproductive photorefractoriness until a long day photoperiodic species is kept under stimulatory long day lengths (Sansum and King, 1976), and (2) show circannual rhythm generation in species that are not considered typically photoperiodic species (Gwinner, 1986).

The most reliable environmental cue is the daily cycle of light dark cycle as in the form of changes in illumination at earth's surface emanating from earth's rotation on its axis and around sun. Length of light phase (day length/photoperiod) changes with season (at least away from the equator) and intensity of any one time of day changes with the time of year and local weather conditions. For example, measured from one midday to the next or one midnight to the next, it is always 24 h. Several other environmental factors vary more or less reliably in parallel with daily changes in illumination, such as temperature and humidity, but these environmental factors are also subjected to change as a result of local weather conditions and therefore none of these are as reliable as day length. Therefore, most of organisms use this daily cycle of light and dark as dominant entraining agent (*zeitgeber* – *zeit* = time*; geber* = giver*;* German word) for their daily rhythms.

William Rowan (1926) for the first time demonstrated the role of photoperiod in avian seasonal events. In the slate-coloured junco (*Junco hyemalis*) long day lengths induces premigratory fattening and gonadal growth. Since then role of day length in control of seasonal premigratory fattening and reproduction has been confirmed in many high-, mid-, and low-latitude species (Murton and Westwood, 1977; Farner *et al*., 1983; Follett, 1984; Wingfield and

Farner, 1993; Kumar, 1997; Hau, 2001; Deviche and Small, 2001). Further, other factors, such as food availability, also influence gonadal growth and development in several species (Wingfield and Farner, 1993; Kumar *et al*., 2001; Bhardwaj and Anushi, 2004). Temperature (Hoffman, 1968), food (Hau and Gwinner, 1996), sound (Menaker and Eskin, 1966), social cues (Viswanathan and Chandrashekaran, 1985; Mrosovsky *et al*., 1989), and endocrine secretions (Turek and Gwinner, 1982) also play role in the synchronization of circadian functions in many vertebrates.

The present thesis

The thesis includes investigations on the population of Terai tree frog (*Polypedates teraiensis*) that lives in and around Mizoram University campus (23ºN and 92º E). In particular, emphasis in this study is placed on studying seasonal responses of tree frogs under natural environment, effect of light duration and temperature on gonadal recrudescence and regression cycle under laboratory conditions and role of photic and non-photic cues on the growth, development and metamorphosis of their tadpoles. Various studies are summarized in following sections.

Section 1. Study of reproductive cycle under natural environmental conditions

This section deals with changes in body mass, gonadal weight and gonadosomatic index in frogs collected every month from their natural habitat.

Section 2. Effect of light on the reproduction under laboratory conditions

This section includes results from the following experiments performed to investigate short and long-term responses under a variety of photoperiodic conditions.

Experiment 1: Role of day length in the regulation of gonadal cycle

This experiment examined if there was a differential effect of photoperiodic conditions on the responsiveness of photoperiodic response system of tree frog to short and long day lengths.

Experiment 2: Role of light intensity in the regulation of gonadal cycle

Here, we investigated whether light intensity plays critical role in photoperiodic responses of tree frog.

Experiment 3: Role of light spectrum in the regulation of gonadal cycle

In this study we studied response of tree frog to different light spectrum to understand the dynamics of their photoperiodic response system.

Section 3. Effect of temperature on the reproduction under laboratory conditions

In this section, we have analysed the role of temperature in the regulation of gonadal growth regression cycle of tree frog.

Section 4. Effect of photoperiod on the growth and development of tadpoles

This section includes experiments, on tadpoles in which we measured growth, development and metamorphosis rate under varying photoperiodic conditions.

Experiment 1: To study the effect of Natural light, continuous light and continuous dark on growth, development and metamorphosis of the tadpoles

In this experiment we examined the role of NDL, LL and DD in growth, development and metamorphosis of tadpoles.

Experiment 2: To study the effect of light duration on the growth, development and metamorphosis of the tadpoles

Here we tested the involvement of varying photoperiod on growth and development of tadpoles.

Experiment 3: To study the effect of light spectrum on growth and development of the tadpoles

In this experiment we addressed the role of light quality in metamorphosis.

Section 5. To study the effect of non-photic cues on the growth and development of tadpoles

Here we examined the role of food and temperature in the regulation of growth, development and metamorphosis of tadpoles.

Experiment 1: To study the effect of timed food availability on the growth and development of tadpoles

This experiment examined the role of food duration and food timing on growth and development of metamorphosis of tadpoles.

Experiment 2: To study the effect of food quality on the growth and development of tadpoles

Here we tested if protein rich food can alter the rate of development.

Experiment 3: To study the effect of temperature on growth and development of tadpoles

This experiment addressed the involvement of temperature in the development of tadpoles.

Section 6: To study the daily expression of clock genes in tree frog

In this objective we studied daily expression of clock genes in brain, liver and testis tissues collected at six time of the day.

II. GENERAL MATERIALS AND METHODS

1. Terai tree frog (*Polypedates teraiensis*): the model species

Experiments were carried out on adult Terai tree frogs (*Polypedates teraiensis*), commonly known as Perching frog or six-lined tree frog. In Mizoram it is locally known as Chang ban. This is a species belonging to the Rhacophoridae. It is distributed in eastern Nepal, eastern, peninsular, and north-eastern India (Assam, Meghalaya, Mizoram, Manipur, West Bengal, Nagaland, Sikkim, Arunachal Pradesh, Gujarat and Madhya Pradesh) and Bangladesh, Myanmar and China (Dutta *et al.,* 2009). There is a sexual dimorphism in this species; a male measure about 40-60 mm in length while female is bigger in size and measures 60-100 mm. In general, they emerge from hibernation in the month of March after heavy rain and can be seen till the month of October/November in their natural habitat. They live in isolation and during breeding period male give mating call and attracts female for mate. Breeding period is reported between March and August. They eat mostly small insects. After mating they lay their egg in the form of nest which may consists of 300-700 eggs. Foam nests are usually laid above the water surface as a protective mechanism from the tadpoles of other species like *Rhacophorus maximus* which shares the same breeding site and breeds earlier than the *Polypedates teraiensis*. No parental care

is reported in this species. Once tadpoles hatch out they enter in water and feed on both vegetarian and non-vegetarian food.

2. **Procurement and maintenance**

Adult frogs of both sexes were procured locally by fish net or hand pick up method within or nearby Mizoram University campus. Mizoram University campus is located in the western side at a distance of about 15 km away from the state capital Aizawl below Tanhril Village. The area of Mizoram University campus lies between 23ºN and 92ºE longitudes. Wild-caught frogs were acclimatized to captive conditions in laboratory before starting experiments. In laboratory frogs were kept pair wise. Each pair of frog was kept in one polypropylene cages (size 28.5 x 22.1 x 14 cm) with water and twigs for perching. Food (larvae of silk moth; *Bombyx mori* and meal worm; *Tenebrio molitor*, cultured in the laboratory) and water were available ad libitum, and replenished once daily during daytime. Cages were cleaned every day. For tadpole's studies freshly laid foam nest was collected from their natural breeding sites (local water bodies within Mizoram University campus or nearby areas) and were kept in cages with water, tadpoles were allowed to hatch out and day one old tadpoles were used for various studies mentioned in the thesis. Tadpoles were fed with freshly chopped and boiled cabbage (*Brassica oleracea*) unless other wised mentioned. Food and water was replenished every day. Frogs and tadpoles maintained good health under captive conditions.

3. Lighting conditions

Experiments on adult frogs were performed within the laboratory. Light was available from compact fluorescent tubes (CFL, Phillips) at desired intensity and desired wavelength as indicated in the respective experiments. No light was available during the night hours. Automatic time switches (Frontier digital timer) controlled the periods of light and dark. In caged condition, frogs were kept pair wise per cage (size 28.5 x 22.1 x 14 cm) for photoperiodic experiments. Similarly, in studies performed on tadpoles, lighting was provided as mentioned in the case of adult frogs.

4. **Experimental design and data collection**

All sections of present thesis comprise several experiments, and each of these experiments had a specific experimental design. We have detailed the experimental design in respective experiments. We had following measurements for determining the effects of an experiment. Data from these measurements were collected at the beginning and at the end of experiment, and at appropriate intervals during the experiment.

4.1. Body mass: We recorded body mass of an individual (frog/tadpole) on a top pan balance with an accuracy of 0.1g. A frog was placed in a polythene bag that was initially weighed and tared. However, tadpole was placed directly on the platform of the balance.

4.2. Gonadal growth and regression and gonadosomatic index: Weight of gonad was considered as an index of gonadal growth and regression. For example, changes in weight of testis in male frog was considered as an effect of treatment over a period of time on gonadotropin secretion (Lofts, 1974). The weight measurements of testes were done under anaesthesia. Briefly, after

anaesthesia, abdominal cavity was cut open and testes were located and dissected out. Weight of both testes were measured on top pan balance. Gonadosomatic index was calculated by taking total weights of the gonads (TWG) divided by weight of the frog (TWF) multiplied by 100.

$$GSI = \frac{\text{Total weight of gonads}}{\text{Weight of frog}} \times 100$$

4.3 Morphological parameters of tadpoles: We measured morphological characteristics of tadpoles as proposed by Altig (2007). Fallowing parameters were measured:

4.3.1 Body length: Body of tadpole included from the tip of head till the beginning of tail.

4.3.2 Tail length: Tail starts at the end of body of tadpole.

4.3.3 Maximum tail height: Tail height was measured at their maximum height place.

4.3.4 Internarial distance: Distance between the two spiracles.

4.3.5 Interorbital distance: Distance between the two eye orbits.

4.4 Histology and imaging:

Histology of testis was done to examine the effects on reproductive maturation stage of testes. Testis were dissected out and immediately fixed in Bouin's fluid for 24 hrs. The gonads were then processed following established protocol for preparation of histological slides. In brief, gonads were washed and dehydrated in different grades of ethyl alcohol, cleared in xylol, embedded in paraffin wax (melting point: 60 ºC- 61ºC, Qualigens Fine Chemicals) and paraffin blocks were prepared. After trimming the blocks, 5 µ thick sections were cut using a rotary microtome (Leica RM 2125 RTS). Then the sections were stretched on glass slides. The slides were incubated overnight at 35 ºC in a hot air oven. After that de-waxed in xylol and rehydrated using different grades of ethyl alcohol. After rehydration, sections were stained with aqueous

haematoxylin. The haematoxylin stained sections were dehydrated using 30%, 50% and 70% ethyl alcohol, and then stained with eosin stain. Then the sections were dehydrated in 90% and absolute alcohol, cleared in xylol and mounted with a cover slip using DPX. The double stained sections were observed under a light microscope. Photographs of histological sections were taken with the help of Olympus CX 41 microscope. The images of the testis were captured at 4X, 10 X and 40 X magnifications, respectively.

5. **Statistical analysis**

Data obtained from different experiments were further analyzed statistically. One-way analysis of variance with repeated measures (1-way RM ANOVA), followed by Newman-Keuls post hoc tests if ANOVA indicated significance of difference, was employed to examine effect of various treatment over a period of time. Similarly, two-way ANOVA was also employed to analyze the effects when two factors (e.g. photoperiod and duration of the treatment) were taken into consideration, and Bonferroni test was used as post hoc test for group comparisons. Significance was always taken at $P < 0.05$. Sokal and Rohlf (1981) was consulted and used for the purpose of statistical analysis. All statistical analysis was done using graph pad prism version 5.

III. SECTION 1: *STUDY OF REPRODUCTIVE CYCLE UNDER NATURAL ENVIRONMENTAL CONDITIONS*

1. ABSTRACT

Seasonality is defined as an initiation-termination-reinitiation of any physiological processes. Most of the seasonal processes are centered around reproduction. Majority of organisms are seasonal breeders and live in seasonally varying environment. Almost all Amphibians studied are seasonal breeders. Most of the Amphibians studies emphasized their breeding ecology. Limited knowledge is available for Amphibians inhabiting in Northeastern region of India. In present section we describe breeding biology of Terai tree frog (*Polypedates teraiensis*) inhabiting Aizawl, Mizoram. Beginning from the month of March till October 2016, we collected adult male and female frogs at the middle of each month. Monthly observations were recorded for body weight and gonadal weight and GSI was calculated. We found the annual change in body mass and gonadal weight and GSI in both male and female with high GSI values during March to June. These results suggest that Terai tree frog is seasonal breeders and their breeding correspond with long days and monsoon season.

2. INTRODUCTION

Almost all animals studied are seasonal and breed in seasonally fluctuating environment. These seasonal breeders reproduce during the time of year when chances for survival of their offspring are maximum. Baker (1938) classified the factors that affect animal reproduction into two groups: a) ultimate factors, such as an abundant food supply, exert a selective pressure and ensures that a species breeds during a precise, optimal period of the year: and b) proximate factors, which are environmental factors directly affecting physiological processes including those involved in reproduction. Photoperiod, temperature, and rainfall are the major proximate factors. Animals inhabiting geographic areas with considerable seasonal changes in environmental conditions adapt to these variations and synchronize their reproductive activity to that time of the year when conditions are optimal for survival of offspring. Thus, reproduction is limited to times with most suitable climatic conditions and food supply (Lofts, 1975).

Reproduction is the fundamental characteristics of all living organisms. An essential attribute of any surviving species or population is the ability to produce offspring. Class Amphibian represents one of the best examples of seasonal vertebrates. Almost all amphibian species that have been studied thus far exhibit seasonality in several functions, such as body mass, gonadal recrudescence and regression, hormonal levels and hibernations etc. Successful reproduction of amphibians depends on the location of potential mates (Wells, 2007), stimulation of mates (Wells, 2007), and site selection for breeding (Kaefer *et al.*, 2007). Most species exhibit in tropical and subtropical regions are capable of reproducing throughout the year. In anurans of tropical and subtropical regions reproductive biology is closely associated with rainfall (Lynch and Wilczynski, 2005). Therefore, rainfall appears extrinsic factor controlling timing of

reproductive activity (Lynch and Wilczynski, 2005). In wild, amphibian-breeding activity is regulated by several ecological factors such as increasing temperature (Saidapur and Hoque, 1995), rainfall (Lynch and Wilczynski, 2005), photoperiod (Saidapur, 1989; Blair, 1960, 1961; Dixon and Heyer, 1968; Whitaker, 1971; Blankenhorn, 1972; Salthe and Mecham, 1974; Obert, 1975; Collins and Wilbur, 1979; Hoogmoed and Gorzula, 1979; Kluge, 1981; Díaz-Paniagua, 1986, 1988; Moreira and Lima, 1991; Donnelly and Guyer, 1994; Bertoluci, 1998), nutrient availability (Girish and Saidapur, 2000) and pond hydrology (Hagman and Shine, 2006). In general, reproductive activity of the temperate region anurans is dependent upon a combination of rainfall and warm temperatures (Blair, 1960; Wiest, 1982; Díaz-Paniagua, 1986; Salvador and Carrascal, 1990; Ritke, *et al.,* 1992). However, in seasonal tropical environments breeding season of most species are typically associated with rainy season (Hoogmoed and Gorzula, 1979; Toft and Duellman, 1979; Aichinger, 1987; Hero, 1990; Gascon, 1991; Duellman, 1995; Bevier, 1997; Bertoluci, 1998).

Temporal regulation of reproduction in anuran is represented by two categories: prolonged breeding and explosive breeding. The spatial and temporal distributions of frogs determine the sex specific (male-male) competition (Arak, 1983). In general, males of explosive breeders category engage in scramble competition in dense aggregations and attempt amplexus with every possible female individuals. These males struggle among themselves for possession of mating female partner. However, males of prolonged breeders usually make mating calls from a stationary position and attract potential mating partner (females) and often maintain some sort of inter male spacing. Selection of mating partner can be achieved by visual cues (Summers *et al*., 1999), olfactory cues (Woodley, 2014), auditory cues (Simmons, 2004), or tactile means (physical contact of the male by the female) or combinations of these (Wells, 2007). Some

anurans, mate with little or no specialized behaviours, whereas others, especially those characterized by complex social interactions, represents diverse kinds of courtship and is an integral part of mate selection and mating. On other hand female selects male by their body size, number of nights of calling per male, location and nature of calling sites (Mitchell, 2001; Wells, 2007). Site selection for eggs deposition can be influenced by the physical features of breeding sites as well as by the presence of predators, competitors and communal nesters (Magnusson and Hero, 1991). Female anurans select oviposition sites based on various physio-chemical characteristics such as water depth, water temperature, water pH, or absence of predators (Hadded and Prado, 2005). In case of arboreal species, breeding sites generally lay above the ground on the trees, hence there is a question of desiccation of water, whereas in terrestrially breeding species, more commonly males choose the nest, defend it from rival males, and call to attract the females for breeding activity near the vicinity of water bodies.

Histological studies demonstrated that in anurans of temperate regions, the sperm cells mature uniformly throughout the testis, but in species of tropical regions which breed throughout the year, testis contains sperm cells in various stages of maturation (Brown and Zippel, 2007). During the breeding season, prominent morphological changes in testis and thumb pad of male takes place (*Rana ridibunda*). In anuran testis is structurally simple and show growth and regression cycle as represented by increase in size and weight. Spermatogenesis and steroidogenesis undergo seasonal variations during the reproductive cycle in amphibians. However, In *Rana perezi*, testicular weight is independent of seasons, and no seasonal variations have been observed (Delgado *et al*., 1989, 1992). Histological evidence suggests that although cell nests of different types are present every month of the year, the most important spermatogenetic activity is initiated in summer (Delgado *et al*., 1989). In case of *Rana*

dalmatine, reproductive activity (spermatogenesis, fat-body weight and thumb pad) showed a markedly discontinuous spermatogenetic cycle (Guarino and Bellini, 1993). Significant seasonal variations were observed in testis and fat-body weight, thumb pad epithelium, and plasma testosterone but not in plasma androstenedione in *Rana dalmatine* (Guarino and Bellini, 1993). Seasonal changes have also been reported in spermatogenesis and fat bodies of testis of *Polypedates maculatus* (Kannamadi and Jirankali, 1993).

In species, which exhibit continuous spermatogenesis (e.g., *Rana cyanophlyctis*, *Rana hexadactyla*, and *Bufo melanostictus*), all stages of spermatogenesis are present in the testis round the year (Huang *et al*., 2004). However, in the species, which exhibit potentially continuous (e.g., *Rana tigerina* and *Bufo marinus*) or discontinuous type (e.g., *R. temporaria*) of spermatogenetic activity, the various stages of spermatogenesis undergo distinct seasonal variations (Huang *et al*., 2004). In bull frog (*Rana catesbeiana*) spermatozoa are retained in the testis in winter and are released in the following breeding season (Sprando and Russell, 1988). Seasonal changes in gonadal morphology, secondary sexual characters and plasma sex steroids have been studied in male *Bombina pachypus* (Guarino *et al*., 1998). The mechanism regulating the ovarian cycle in anurans has been found to be under the control of photoperiod and temperature (Saidapur, 1989; Pancharatna and Saidapur, 1990). Number of studies have been performed on breeding behaviour of Amphibians residing in Western Ghats of India, *Viz.*, *Polypedates maculates* (Mohanty and Dutta, 1988), testis and thumb pad of *Rana tigrina* (Saidapur and Nadkarni, 1975), *Polypedates maculatus* (Dutta *et al*., 2001) and *Rhacophorus lateralis* (Dinesh *et al*., 2010). *Ramanella montana* has been documented to lay eggs in holes of tree trunks (Krishna *et al*., 2004). However, In shrub frog, *Philautus femoralis* (Bahir *et al*., 2005) and in the ground dwelling frog, *Raorchestes resplendens*, development is direct and there

is no tadpole stages (Biju *et al*., 2010). In North Eastern India, breeding behaviour has been studied in *Hyla annectans* (Ao and Bordoloi, 2001), *Chirixalus simus* (Deuti, 2001), *Polypedates leucomystax*, *Rhacophorous bipunctatus* (Iangrai, 2007) and *Hylarana humeralis* (Bortamuli *et al*., 2010). Few studies also report on the histology of testis and ovary of *Rana cyanophlyctis*, *Rana hexadactyla*, *Bufo melanostictus* (Saidapur, 1989), *Rana curtipes* and *Rana cyanophlyctis* (Pancharatna and Saidapur, 1990). Though the North-Eastern India possesses a very rich diversity of amphibians including several endemic and endangered species, there is paucity of information on the breeding behaviour and seasonal changes in the histology of gonads. Little is known about the breeding ecology of the Terai tree frog (*Polypedates teraiensis*). Here we describe the annual breeding cycle of the tree frog studied at the Aizawl, Mizoram at 23^0N 92 ^{0}E.

3. MATERIAL AND METHODS

The study was performed on adult frogs of both sexes procured locally (within the Mizoram University campus) during the middle of each month. We measured the various morphological features of both male and female frogs. Observations on body mass and gonadal weight were made on both male and female frogs (n = 5 - 6) that were procured every month (from March 2016 to October 2016; we did not find any frogs from the months of November to February in their natural habitat). Corresponding change in day-length, temperature and humidity were also recorded during each month. In order to know the seasonal variations in gonadal activity, gonadosomatic index (GSI) of males and females were calculated as mentioned in the general materials and method section. The testes were dissected out and immediately fixed in Bouin's fluid for 24h for histological studies. The gonads were then processed following established protocol for the preparation of histological slides. Histological studies have been performed as mentioned in the general materials and method section. We also observed the mating call and courtship behaviour, oviposition sites, clutch size in their natural habitat.

3.1 **Statistical analysis**

Data are presented as mean ± SE. They were analysed using 1-way analysis of variance (1-way ANOVA) followed by Newman-Keuls post-hoc tests if ANOVA indicated a significance of difference. Significance was taken at $P < 0.05$.

4. RESULTS

Plate one shows adult male and female individuals. There is a sexual dimorphism in the tree frog. Male frogs are lighter in weight and smaller in size (Table1) while the female frogs are much heavier in weight and larger in size. In general, male frogs (Plate1) weighed between 4.8 g to 11.7 g (mean body mass 7.7±0.2 g) and female frogs weighed between 15.4 g to 37.2 (mean body mass; Table 26.2±1.2 g 1). Total length of male frogs ranged between 43.17 mm to 58.24 mm (mean 54.29±0.78 mm) while female frogs ranged between 65.61 to 96.12 mm (mean 79.52 ± 1.79 mm; Table 1). Head length of male frogs ranged between 16.77 mm to 19.57 mm (mean 17.83±1.8 mm; Table 1) while female frogs ranged between 22.09 to 29.26 mm (mean 24.69 ± 0.41 mm; Table 1). Head width of male frogs ranged between 13.8 mm to 16.8 mm (mean 15.57±1.5 mm; Table 1) while female frogs ranged between 19.23 to 26.68 mm (mean 22.49 ± 0.44 mm; Table 1). Eye-Snout length of male frogs ranged between 5.8 mm to 6.6 mm (mean 6.23±0.06 mm; Table 1) while female frogs ranged between 7.38 to 10.16 mm (mean 8.51 ± 0.16 mm; Table 1). Eye diameter of male frogs ranged between 5.01 mm to 6.51 mm (mean 5.82±0.09 mm; Table 1) while female frogs ranged between 6.91 to 9.19 mm (mean 8.0 ± 0.16 mm; Table 1). Inter-orbital space of male frogs ranged between 13.29 mm to 17.75 mm (mean 15.44±0.27 mm; Table 1) while female frogs ranged between 14.97 to 26.0 mm (mean 21.53 ± 0.59 mm; Table 1). Forelimb length of male frogs ranged between 28.88 mm to 40.87 mm (mean 34.96±0.66 mm; Table 1) while female frogs ranged between 42.16 to 64.17 mm (mean 53.20 ± 1.40 mm; Table 1). Hand length of male frogs ranged between 13.8 mm to 19.69 mm (mean 17.54±0.41 mm; Table 1) while female frogs ranged between 21.01 to 32.6 mm (mean 26.27 ± 0.75 mm; Table 1). 1st finger length of male frogs ranged between 6.81 mm to 9.24 mm (mean 7.77±0.15 mm; Table 1) while female frogs ranged between 9.71 to 18.91 mm (mean 12.75 ±

0.50 mm; Table 1). 2nd finger length of male frogs ranged between 7.42 mm to 11.08 mm (mean 9.20±0.20 mm; Table 1) while female frogs ranged between 10.8 to 18.49 mm (mean 13.86 ± 0.51 mm; Table 1). 3rd finger length of male frogs ranged between 11.96 mm to 16.05 mm (mean 13.96±0.25 mm; Table 1) while female frogs ranged between 16.48 to 25.69 mm (mean 20.77 ± 0.65 mm). 4th finger length of male frogs ranged between 9.3 mm to 13.69 mm (mean 12.04±0.25 mm; Table 1) while female frogs ranged between 14.39 to 23.87 mm (mean 17.81 ± 0.49 mm; Table 1). Hind-limb length of male frogs ranged between 75.13 mm to 88.02 mm (mean 82.94±0.89 mm; Table 1) while female frogs ranged between 101.74 to 140.9 mm (mean 120.53 ± 2.7 mm; Table 1). Foot length of male frogs ranged between 19.29 mm to 24.68 mm (mean 22.71±0.31 mm; Table 1) while female frogs ranged between 29.1 to 41.94 mm (mean 34.12 ± 0.95 mm; Table 1). 1st toe length of male frogs ranged between 5.49 mm to 9.11 mm (mean 7.86±0.20 mm) while female frogs ranged between 10.46 to 18.08 mm (mean 13.79 ± 0.44 mm; Table 1). 2nd toe length of male frogs ranged between 6.3 mm to 11.67 mm (mean 9.44±0.26 mm; Table 1) while female frogs ranged between 12.44 to 18.72 mm (mean 15.35 ± 0.38 mm; Table 1). 3rd toe length of male frogs ranged between 11.17 mm to 15.64 mm (mean 13.37±0.32 mm; Table 1) while female frogs ranged between 18.07 to 26.63 mm (mean 22.00 ± 0.65 mm; Table 1). 4th toe length of male frogs ranged between 16.34 mm to 23.34 mm (mean 19.9±0.37 mm; Table 1) while female frogs ranged between 20.17 to 40.94 mm (mean 30.56 ± 1.04 mm; Table 1). 5th toe length of male frogs ranged between 12.83 mm to 17.7 mm (mean 15.53±0.39 mm; Table 1) while female frogs ranged between 20.89 to 30.94 mm (mean 24.89 ± 0.63 mm; Table 1).

Sl. No.	Characters	Males (N=48)		Females (N=38)	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	Body weight	4.8-11.7	7.67±0.2	15.4-37.2	26.2±1.2
2	Total length	43.17-60.6	55.44±0.44	65.61-96.12	77.80±1.28
3	Head length	16.77-21.52	18.43±0.20	22.09-29.26	24.14±0.29
4	Head width	13.8-17.39	15.76±0.11	19.23-26.68	21.97±0.29
5	Eye-Snout length	5.8-6.85	6.31±0.05	7.38-10.16	8.47±0.11
6	Eye diameter	5.01-7.13	6.19±0.08	6.91-9.71	8.02±0.12
7	Inter-orbital space	13.29-17.75	15.78±0.15	14.97-26	21.47±0.38
8	Forelimb length	28.88-42.51	36.34±0.38	42.16-64.77	52.43±0.92
9	Hand length	13.8-20.72	17.50±0.22	21.01-32.6	25.05±0.48
10	1st finger length	6.81-9.24	7.67±0.11	9.71-18.91	12.09±0.30
11	2nd finger length	7.42-11.45	9.16±0.13	10.38-18.49	13.33±0.31
12	3rd finger length	11.96-16.05	14.11±0.16	16.48-25.69	19.84±0.44
13	4th finger length	9.3-13.89	12.12±0.15	14.39-23.87	17.13±0.34
14	Hindlimb length	75.13-92.69	83.69±0.64	101.74-140.9	117.27±1.76
15	Foot length	19.39-28.66	23.12±0.27	29.1-41.94	32.53±0.77
16	1st toe length	5.45-11.18	8.44±0.15	10.46-18.08	13.20±0.29
17	2nd toe length	6.3-11.67	9.99±0.18	12.44-18.72	15.01±0.23
18	3rd toe length	11.17-17.64	14.20±0.22	18.07-26.63	21.26±0.42
19	4th toe length	16.34-24.04	20.74±0.33	20.17-40.94	29.80±0.65
20	5th toe length	12.83-20.51	16.47±0.27	20.89±30.94	24.51±0.46

Table1: Table one shows morphological parameters in male (N=48) and female (N=38) individuals.

When we studied change in body mass, it underwent significant changes over the year ($F_{(7,40)}$=2.326, P = 0.0150; 1-way ANOVA; Figure 2a). Maximum gain in body mass was recorded in the month of April (Fig. 2a). Testes underwent significant growth-regression cycle as indicated by the change in the testes weight ($F_{(7,40)}$=20.48, P<0.0001; 1-way ANOVA; Figure 2b). Maximum testis weight was observed in the month of April (0.28 ± 0.02 gm; Fig. 2b). There was a significant variation in the GSI throughout the year ($F_{(7,40)}$=13.47, P<0.0001; 1-way ANOVA; Figure 2c). Testes were found stimulated in the month of March, April and May (Fig. 2b. By the month of June, testes started regressing and fully regressed testes were observed in the month of July (mean TV=0.06 ± 0.01 gm; Fig. 2b).

In female frogs, body mass underwent significant changes over the year ($F_{(7,40)}$=5.727, P=0.0007; 1-way ANOVA; Figure 2d). In females, there were two peaks in body mass, one in May (mean body mass = 41.6±6.6 g; Fig. 2d) and the other in September (mean body mass = 43.6±7.6 g; Fig. 2d). Ovarian growth and regression also followed seasonal variation ($F_{(11,74)}$=7.409, P=0.0002; 1-way ANOVA; Figure 2e). Bigger ovaries were observed in the month of April and by May, follicles started regressing. Seasonal variations in the size of ovaries were also reflected by GSI values ($F_{(11,74)}$=5.787, P=0.0006; 1-way ANOVA; Figure 2f). Higher GSI values were observed from the month of April till September (Fig. 2f).

We observed that usually in tree frogs amplexus occurs at or near the oviposition site (photo plate 2), in general male approaches the breeding ground and starts the vocalization and mating advertisement call. In response to mating call female approaches to male from their surrounding areas and finally male rides the back of female and forms the amplecting pair. Usually the pairs remain in amplexus for only an hour or two followed by formation of foam nest. The most common and widespread site of oviposition is in free flowing or standing water.

We observed that the tree frogs deposit their eggs in a form of a foam nest usually attached to vegetation above or near water (Photo plate 3, 4). The spawning sites include leafy twigs, hanging over water, grassy bank of ponds, under stones and also on tree trunks. The humidity keeps eggs moist, and then as soon as they hatch, tadpoles fall into water and develop in aquatic environment. The clutch size of foam nest ranged between 250-700.

Histological study of the testis of *Polypedates teraiensis* showed seasonal variations in terms of spermatogenesis (photo plate 5,6). We observed the presence of spermatogenic cells during the different periods of the breeding cycle. During the time of emergence seminiferous tubules were distinct and spermatids were appeared in cluster. As they entered during the breeding phase the size of seminiferous tubes increased in diameter due to the increase in rate of multiplication of spermetogenic cells. During breeding phase seminiferous tubules were having slender and filamentous Spermatozoa. During the post-breeding phase most spermatogonia and only a few spermatids were observed.

5. DISCUSSION

The data are presented in figures 1 and 2. Figure 1 shows annual change of temperature, rainfall and humidity at this latitude. Tree frogs showed clear sexual dimorphism and female frogs are much bigger than males (Photo plate 1, Table 1). Tree frogs spend most of their time outside the water in nearby grass and trees and reached the potential breeding grounds during night. Males approaches first and start vocalization and attracts females. Multiple males were observed nearby breeding sites. Female do get attracted to the mating calls and slowly approach the male and finally, they make amplecting pairs. After one to two hours female laid the foam nest and both male and female get separated. There is no parental care in this species.

Our results show that both the sexes of tree frog show annual variations in body mass and gonadal growth regression cycle. At this latitude (23^0N, 92^0E), annual rainfall is generally predictable, and monsoon increases creates temporary water bodies and abundance of food availability, so it is reasonable to assume that rainfall could act as a primary timer in the control of amphibian reproductive cycle (Lynch and Wilczynski, 2005; Saidapur, 1989; Blair, 1960, 1961; Dixon and Heyer, 1968; Whitaker, 1971; Blankenhorn, 1972; Salthe and Mecham, 1974; Obert, 1975; Collins and Wilbur, 1979; Hoogmoed and Gorzula, 1979; Kluge, 1981; Díaz-Paniagua, 1986, 1988; Moreira and Lima, 1991; Donnelly and Guyer, 1994; Bertoluci, 1998). However, given that internal changes in amphibian reproductive system must start functioning at least 6-8 weeks in advance of actual breeding, rainfall cannot be the primary factor, although it can facilitate breeding by enhancing the chances of pairing between the opposite sex, with increased green vegetation for the tadpoles and attractive sights of ponds and pools (Lynch and Wilczynski, 2005).

We observed that tree frogs emerged from its hibernation in the month of March after only couple of heavy rainfall and spawning took place from the month of April, when enough water was accumulated so that frogs can perform the successful breeding without chances of desiccation of pools/ponds or temporary water bodies. Different seasonal cycles, especially cycle in the gonadal development, remarkably follow changes in day length. There was a seasonal variation in body mass both in male and female tree frogs (Figs. 2). For example, males had gained in body mass in March and attained their peak in the month of April. However, in female two peaks in body mass was observed; first peak in the month of May and second peak in the month of September (Fig. 2). Thus, in females the first peak in body mass was during the reproductive phase, and second peak before the time of hibernation. One speculation can be that frogs gain weight early in the year to meet an expensive physiological activity, i.e. reproduction, and late in the year to meet energy required for phase of hibernation.

Testes began to recrudesce in March and high testicular weight was recorded from the month of April to June when day lengths were increasing, and this is the time of monsoon at this latitude. Testes began regressing well before summer solstice when day lengths were still increasing (Figs. 2). Initially, this type of testicular growth-regression was considered to discount the role of day length as a "driver" in control of annual testicular cycle. However, a series of early investigations on control of annual reproductive cycle of other seasonal vertebrates Viz., birds (Bissonnette, 1931; Burger, 1949; Farner, 1959; Murton and Westwood, 1977) have established that such gonadal regression is due to the development of refractoriness to stimulatory effects of day lengths; this is called photorefractoriness (Farner *et al*., 1983; Nicholls *et al*., 1988). This can also be called by different names depending on the physiological variable involved. For example, the terms "gonadal refractoriness" and "metabolic refractoriness" are

used to describe the phenomenon of photorefractoriness observed with reference to gonads and body fat, respectively (Bissonnette, 1931; Burger, 1947; Farner and Mewaldt, 1955; King *et al*., 1960).

However, in bullfrog *Lithobates catesbeianus* in southern Brazil; male GSI did not vary significantly among seasons (Kaefer *et al.,* 2007), similar phenomenon has also been reported for captive-bred populations of *L. beianus* in southeastern Brazil (Sasso-Cerri *et al.,* 2004). This result is in agreement with previously reported data on other animals that show continuous spermatogenic cycle, (Rastogi *et al.,* 1976, Delgado *et al.,* 1989), including *L. catesbeianus* (Licht *et al.* 1983). Photorefractoriness is as an adaptive phenomenon of annual reproductive cycle, and has been reported in annual cycles of a number of seasonal breeders (Hamner, 1968; Farner, 1962; Farner and Lewis, 1971; Lofts and Murton, 1968; Murton and Westwood, 1977; Kumar, 1997). How exactly photorefractoriness develops and what is the molecular mechanism involve is not known and in absence of an exact etiology of photorefractoriness, a mechanistic explanation can be that after a period of intensive gonadal activity the underlying physiological mechanisms get 'fatigued' and so animals require some sort of physiological rest and the intervening time is utilized for preparations to start afresh the next annual cycle. It is worth recalling that the impracticability of a continuous reproductive season, which is an expensive life process, is the basis for the evolution of seasonality in vertebrates.

Our results are consistent with the previous report on this species (Lalchhanhimi and Lalremsanga, 2017). They showed that vocalization by male during the breeding season was primarily a reproductive function and to attract mating partners (females) to breeding areas. In our studied we observed foam nest of different sizes and clutch size varied according to the foam nest size. Clutch size ranged from 250 to 700. Lalchhanhimi and Lalremsanga also reported 600-

650 clutch size, however the foam nest observed in their study was formed in artificial tanks hence having limited space. We observed that tree frogs made their foam nests on above the water it may be on the side walls of temporary water pools, tree branches, between leaves or any other substratum. It has been observed in several studies that frogs deposit eggs in a wide range of habitats including underground breeding chambers, foam nests (Haddad *et al.,* 1988), and on tree roots or creek banks as well as aquatic habitats (Richard and Alford, 1992). Making of foam nest above the water is a type of protective mechanism. As breeding ground of tree frog is also shared by the giant tree frogs (*Rhacophorus maximus*) which breeds earlier than the tree frogs and hence the tadpoles of the *Rhacophorus* grows earlier than *Polypeadtes*. *Rhacophorus* tadpoles prefer to feed on the foam nest and young tadpoles of *Polypedates*. The spatial abundance, calling activity, spawning of bullfrogs in southern Brazil has shown that the abundance of individuals is more during long photoperiod and high temperature suggesting that the peak timing of reproduction (Medeiros *et al.*, 2016). In our study we observed higher gonadal activity during the month of March to June; the period corresponds with long photoperiod and monsoon activity at this latitude. In bullfrog (*Lithobates catesbeianus*) gonadal analyses suggest that mature males are available throughout the year. However, females produce gametes in advanced developmental stages only in spring and summer (Kaefer *et al.,* 2007). Another most common frog species *Rana tigrina* are also seasonal breeders and breeds between June-July (Saidapur and Hoque, 1995) when the day length is long. It shows definite seasonal variations in ovarian development although the ambient temperature and photoperiod do not undergo seasonal extremes, and usually there exhibit regularly recurring wet and dry periods.

These frogs are available in their natural habitat from the month of March till October/November and rest time they hibernate due to extreme environmental conditions.

Together with this study we suggest that the tree frogs are seasonal breeders and prefer breeding time is between March to June although some late breeders are also observed during this study.

IV. SECTION 2: EFFECT OF LIGHT ON THE REPRODUCTION UNDER LABORATORY CONDITIONS

1. ABSTARCT

Most of the organisms are light sensitive. Photoperiod is most predictable environmental cue which organism use to time their daily physiology and seasonal functions. Most circadian and photoperiodic studies have shown the importance of daylight in controlling daily and seasonal functions in vertebrates. In natural light environmental conditions, day and night components change in terms of duration, intensity and spectrum of light available. In many vertebrate species daytime light intensity and spectrum of light play a critical role in gonadal recrudescence-regression cycle. Less is known how it affects gonadal growth of amphibians. In present section we tried to address the involvement of light duration, light intensity and light spectrum on gonadal growth regression cycle of tree frog. Three experiments were performed on adult male frogs. In experiment one frogs were exposed to four different photoperiods; 8L:16D, 12L:12D, 14L:10D and 16L:8D. in experiment two frogs were exposed to 12L:12D photoperiod but to four different light intensities i.e., 10 lux, 20 lux, 50 lux and 100 lux. In experiment three frogs were exposed to 12L:12D photoperiod and their light phase consists of either red light (wavelength; 640 nm) or blue light (wavelength; 450 nm) or green light (wavelength; 540 nm) or white light. Light intensity was maintained at 0.45W/m^2 at cage level. Body weight and testicular weight was recorded at regular intervals. The results from these experiments were suggest that long

photoperiod promotes gonadal growth regression cycle but light intensity and light spectrum has limited role in photoperiodic induction of gonadal growth regression cycle.

2. INTRODUCTION

Almost all organisms, which have been investigated, are light sensitive. Photoperiod is most dominant external factor that regulates reproduction. In majority of organisms, especially those living away from equator, annual solar cycle plays a role in regulation of various seasonal functions (Murton and Westwood, 1977; Thapliyal, 1981; Hoffman, 1981). At equator where changes in daily length are small, changes in light intensity across seasons influences seasonal responses (Gwinner and Scheuerlein, 1998). These photoperiodic cues are used by many species as a seasonal indicator, as changes in photoperiod are constant throughout the year. Role of day length (= photoperiod) in control of seasonal functions was first demonstrated in birds that the photoperiod (= day length) could act as a temporal information dates back to eighteenth century when Dutch netters realized that the song associated with breeding activity could be induced in males of several species of passerine birds by keeping them first in darkness from May to August and then returning to natural day lengths (cited in Hoos, 1937). However, William Rowan (1925); for the first time experimentally demonstrated the involvement of photoperiod in gonadal development of a passerine bird species, slate-coloured junco (*Junco hyemalis*). Rowan demonstrated that vernal migration and gonadal recrudescence could be induced out of season by exposure of junco in laboratory to increasing day lengths (Rowan, 1926, 1928, 1929, 1932). A relationship between photoperiod and gonadal function has been shown in many higher vertebrate species (Bissonnette, 1931; Burger, 1947; Bullough, 1961; Henderson, 1963; Licht, 1966, 1971; Thapliyal, 1968; Benoit and Assenmacher, 1970; Baggerman, 1972; Elliot *et al.*, 1972; de Vlaming, 1974; Saxena and Saxena, 1975; Bittman *et al.,* 1983; Dawson *et al*., 1986; Karsch *et al.,* 1989; Boswell, 1991; Czykier and Sawicki, 1999).

The seasonal reproduction and associated events are species specific, and is timed such that when breeding occurs the chance of survival of offspring are maximum and plenty of preferred food is available for both the parents and their offspring. Several environmental factors help in this timing, which include seasonal variations in day length, temperature, rainfall, humidity and vegetation (Immelmann, 1971). Baker (1938) classified these factors into two types: the proximate and ultimate factors. Whereas proximate factors "gate" a temporal window during the year for a seasonal event, ultimate factors ensure that the actual seasonal events occur during the gated window. In timing the seasonal reproduction, day length acts as proximate factor, while food/temperature may act as an ultimate factor. It may be noted that the proximate and ultimate factors act in coordination, and not in isolation and may be species-specific.

Poikilothermic vertebrates exhibit a reproductive dependency on photoperiod however, the contribution of photoperiod is species specific (Licht, 1972; Borg, 1982; Fraile *et al.,* 1988, 1989a, b). Among vertebrates, amphibians have pronounced seasonal cycles in various behavioural and physiological processes, and number of them are influenced by annual changes in day length. Breeding activity in amphibian is more unequivocally impacted by abiotic than by biotic components: natural factors, for example, light, rain-fall and temperature (Wells 2007). The influence of seasonal change on the behaviour and physiology of organisms is a wide-spread phenomenon, and photoperiod is the most reliable predictor of environmental changes (Vaze and Sharma, 2013). Furthermore, photoperiod, is a highly predictable abiotic factor at any latitude, influences timing of reproduction in subtropical anuran populations (Both *et al.,* 2008; Canavero and Arim 2009), by regulating gametogenesis and anuran calling (Hatano *et al.,* 2002; Kaefer *et al.,* 2009). In general Amphibian reproductive activity is associated with long photoperiod. Photoperiod is also known to regulate vocalization for anuran assemblages in subtropical regions

(Both *et al.,* 2008, Kaefer *et al.,* 2009, Canavero and Arim, 2009). Photoperiod is also positively related to weight gain and metamorphosis rates of *L. catesbeianus* tadpoles and is responsible for reproductive organ development in this species in captivity (Figueiredo *et al.,* 2001). The effect of photoperiod is one of the most important adaptations to seasonal variation, with direct implications on survival and reproductive success (Vaze and Sharma, 2013). The distinct behavioural patterns of male bullfrogs between spring and summer months may be related to the differences in photoperiod and temperature between these two periods. Thus, longer days in the summer could influence males to start calling and to reach their peak activity later in night than in spring nights. Some of the notable studies done on different amphibians have been summarized below.

Sl. No.	Species name	Work done	Author
1	*Rana cascadae*	Breeding biology	Briggs, 1987
2	*Rana capito*	Breeding biology	Palis, 1998
3	*Rana catesbeiana*	Photoperiod	Horseman *et al., 1987*
4	*Pachymedusa dacnicolor*	Reproduction	Iela *et al.*, 1996
5	*Rana esculenta*	Spermatogesesis	Rastogi *et al.*, 1983a
6	*Lithobates catesbeianus*	Reproductive biology	Kaefer *et al.*, 2007
7	*Eleutherodactylus cooki*	Reproductive biology	Rogowitz *et al.*, 2001
8	*Eleutherodactylus jasperi*	Reproductive biology	Wake, 1978
9	*Rana perezi*	Photoperiod	Delgado *et al.*, 1989
10	*Rana esculenta*	Spermatogenesis	Rastogi *et al.*, 1983b
11	*Polypedates maculatus*	Hematology	Mahapatra *et al.*, 2012
12	*Polypedates maculatus*	Behavioural ecology	Lillywhite *et al.*, 1998
13	*Polypedates maculatus*	Oogenesis	Kanamadi and Jirankali, 1993
14	*Polypedates teraiensis*	Hematology	Das and Mahapatra, 2012
15	*Polypedates maculatus*	Acoustic communication	Kanamadi *et al.*, 1993
16	*Microhyla ornata*	Hematology	Hota *et al.*, 2013
17	*Euphlyctis cyanophlyctis*	Endocrinology	Phuge *and* Gramapurohit, 2013
18	*Euphlyctis cyanophlyctis*	Breeding	Tabassum *et al.*, 2011
19	*Polypedates megacephalus*	Genetics	Zhang *et al.*, 2004
20	*Polypedates megacephalus*	Spermatogenesis	Chen and Liao, 2016
21	*Xenopus laevis*	Genetics	Roe *et al.*, 1985
22	*Kaloula pulchra*	Breeding	Kanamadi *et al.*, 2002
23	*Xenopus laevis*	Cell biology	Gerhart *et al.*, 1984
24	*Xenopus laevis*	Molecular development	Sargent and Dawid, 1983
25	*Rana perezi*	Endocrinology	Isorna *et al.*, 2003
26	*Rana esculenta*	Endocrinology	Dupont *et al.*, 1979
27	*Rhacophorus omeimontis*	Breeding	Liao and Lu, 2010
28	*Nanorana parkeri*	Genome study	Sun *et al.*, 2015
29	*Xenopus tropicalis*	Genetics	Nakayama *et al.*, 2013

30	*Phyllomedusa hypochondrialis*	Breeding	Pyburn and Glidewell, 1971
31	*Pelodytes punctatus*	Reproduction	Toxopeus *et al*., 1993
32	*Hyla arborea*	Conservation biology	Pellet, 2005
33	*Rana pipiens*	Developmental biology	Cross, 1981
34	*Xenopus laevis*	Developmental biology	Stolow *et al.*, 1996
35	*Hyla faber*	Reproductive biology	Martins and Haddad 1988
36	*Xenopus laevis*	Behaviour	Chum *et al.*, 2013
37	*Rana temporaria*	Development	Ryser, 1988
38	*Rana pipiens*	Cell biology	Kelly and Smith, 1964
39	*Rana ridibunda*	breeding	Kyriakopoulou-Sklavounou and Loumbourdis, 1990
40	*Xenopus laevis*	Developmental biology	Nishikawa *et al.*, 1992
41	*Triprion petasatus*	Biology	Duellman, *1995*
42	*Rana palustris*	Reproductive biology	Resetarits and Aldridge, 1987
43	*Discoglossus pictus*	Reproduction	Campanella *et al.*, 1997
44	*Rana lessonae*	Ecology	Gulve, 1994
45	*Hyla versicolor*	Ecology	Kiesecker and Skelly, 2001
46	*Adelotus brevis*	Reproductive biology	Katsikaros and Shine, 1997
47	*Limnomedusa macroglossa*	Breeding	Kaefer *et al*., 2007
48	*Rana pipiens*	Physiology	Jaffe and Schlichter, 1985
49	*Rana tigrina*	Reproductive biology	Saidapur *et al.*, 2001

Seasonally varying environmental conditions appear to modify reproductive performances. Common coqui of Puerto Rico (*Eleutherodactylus coqui*) deposits egg clutches throughout the year, but clutch does not develop during cooler and drier months (Stewart and Pough, 1983; Townsend and Stewart, 1994). A similar reproductive phenomenon has been observed in *Eleutherodactylus johnstonei* from Guyana, where clutches deposition is throughout the year but successful clutches production occurs during the wettest months (Bourne, 1997). Seasonal variation in clutch deposition, with a potential effect of temperature or moisture, is also apparent for the cave coqui of Puerto Rico, *E. cooki* (Joglar *et al.*, 1996; Joglar, 1999).

3. METHODS

The study was done using adult males. Following three experiments were performed using male frogs that were caught locally and acclimatized to captive NDL (natural daylength conditions) conditions for at least 4 days. Observations on changes in body mass, testicular weight and GSI were recorded at the beginning and at the end of experiment, and at appropriate intervals during the experiment.

The other experimental procedures were similar as described in General materials and methods. The data are presented as mean ± SE and were analysed by 1-way ANOVA. 2-way ANOVA was used to analyse the effects of photoperiod, time, and interaction between photoperiod and time. Post-hoc tests were used to make pair-wise comparison if ANOVA indicated the significance of difference. Significance was taken at $P \leq 0.05$.

Three experiments were performed:

3.1 Experiment 1. Role of day length in the regulation of gonadal cycle

The study was started in the month of march 2017 when frogs came out from their hibernation state. Adult male frogs were procured within Mizoram university campus by hand and with the aid of fishing nets and brought to indoor laboratory conditions. Frogs were kept in polypropylene cage (28.5 x 22.1 x 14 cm; two frogs were kept in each cage). Photoperiodic experiments were performed to understand the role of photoperiod in initiation and termination of gonadal growth-regression cycle. Adult male frogs (N= 16/group) were divided into four groups and subjected to artificial light-dark cycles of either short photoperiod (8L:16D; light on 06:00; light off 14:00), equinox photoperiod (12L:12D; light on 06:00; light off 18:00) or long photoperiods (14L:10D; light on 06:00; light off 20:00 and 16L:8D; light on 06:00; light off 22:00) under photoperiodic

boxes (2 x 2 x 2 fit) under laboratory conditions. Food (larvae of silk moth and meal worm) and water was available ad libitum. Light was provided by 14W fluorescent CFL at top of the cage. Light cycle was regulated by automatic timer. Temperature was maintained at 24 ^{0}C. Observations on change in body mass and testicular weight was made on day 0, day 30, day 75 and day100 of respective photoperiod treatment.

3.2 Experiment 2. Role of light intensity in the regulation of gonadal cycle

The study was started in the month of march 2017; immediately after frogs emerged out from their hibernating state. Adult male frogs were procured within Mizoram university campus by hand and with aid of fishing nets and brought to indoor laboratory conditions. Frogs were kept in polypropylene cage (28.5 x 22.1 x 14 cm; two frogs were kept in each cage). Adult male frogs (N= 16/group) were divided into four groups and subjected to artificial light-dark cycles of equinox photoperiod (12L:12D; light on 06:00; light off 18:00) but of different intensities of light. Group one was exposed to 10 lux, group two was exposed to 20 lux, group three was exposed to 50 lux and group four was exposed to 100 lux light intensity under photoperiodic boxes (2 x 2 x 2 fit) under laboratory conditions. Food (larvae of silk moth and meal worm) and water was available ad libitum. Light was provided by 14W fluorescent CFL at the top of cage. Light cycle was regulated by automatic timer. Temperature was maintained at 24 ^{0}C. Observations on change in body mass and testicular weight was made on day 0, day 30 and day 70 of respective treatments.

3.3 Experiment 3: Role of light spectrum in the regulation of gonadal cycle

The study was started in the month of march 2017; immediately the frogs emerged out from their hibernating state. Adult male frogs were procured within Mizoram university campus by hand and with the aid of fishing nets and brought to indoor laboratory conditions. Frogs were kept in polypropylene cage (28.5 x 22.1 x 14 cm; two frogs were kept in each cage). Adult male frogs (N= 16/group) were divided into four groups and subjected to artificial light-dark cycles of equinox photoperiod (12L:12D; light on 06:00; light off 18:00) but of different wavelengths of light. Group one was exposed to red light (wavelength; 640 nm), group two was exposed to blue light (wavelength; 450 nm), group three was exposed to green light (wavelength; 540 nm) and group four was exposed to white light and served as control. Light intensity was maintained at 0.45W/m^2 at cage level. Food (larvae of silk moth and meal worm) and water was available ad libitum. Light was provided by 14W fluorescent CFL at top of cage. Light cycle was regulated by automatic timer. Temperature was maintained at 24 ^{0}C. Observations on change in body mass and testicular weight was made on day 0, day 30 and day 70 of respective treatments.

3.4 Statistical analysis

The data is represented as mean ± SE. One-way analysis of variance (1-way ANOVA), followed by Newman-Keuls post hoc tests if ANOVA indicated significance of difference, was employed to examine effect of various treatment over a period of time. Similarly, two-way ANOVA was also employed to analyze effects when two factors (e.g. photoperiod and duration of the treatment) were taken into consideration, and Bonferroni test was used as post hoc test for group comparisons. Significance was always taken at $P < 0.05$.

4. RESULTS

4.1 Role of day length in the regulation of gonadal cycle

Figure 3 shows change in body weight, testicular weight and GSI over a period of time under different photoperiodic conditions. There was a significant change in body weight under all photoperiodic conditions (8L:16D: $F_{(3,15)}$= 11.08, P=0.0009; 12L:12D: $F_{(3,15)}$= 10.73, P=0.0010; 14L:10D: $F_{(3,15)}$= 14.44, P=0.0003; and 16L:8D: $F_{(3,15)}$= 48.81, P<0.0001; 1-way ANOVA; Figure 3a). Irrespective of photoperiodic duration, there was a significant decrease in body weight as time progressed and by day 100 body weight was significantly reduced (Fig. 3a). Two Way ANOVA revealed that there is only effect of time but not the photoperiodic treatment or interaction of photoperiodic treatment x time on body weight (Treatment: $F_{(3,48)}$= 2.504, P=0.0903; time: $F_{(3,48)}$= 64.46, P<0.0001; interaction of treatment x time: $F_{(9,48)}$= .7100, P=.6969; Two Way ANOVA; Figure 3a).

There was a significantly gain and loss in the gonadal weight as time progressed under each photoperiodic condition (8L:16D: $F_{(3,15)}$= 7.636, P=0.0041; 12L:12D: $F_{(3,15)}$= 10.93, P=0.0010; 14L:10D: $F_{(3,15)}$= 19.46, P<0.0001; and 16L:8D: $F_{(3,15)}$= 11.70, P=0.0007; 1-way ANOVA; Figure 3b). There was a gain in gonadal weight on day 30 under exposure to different photoperiodic treatment followed by the loss in gonadal weight (Fig. 3b) and minimum gonadal weight was recorded on day 100. There was an effect of photoperiodic treatment, time and interaction of photoperiodic treatment x time on gonadal weight (Treatment: $F_{(3,48)}$= 2.955, P=0.0417; time: $F_{(3,48)}$= 41.59, P<0.0001; interaction of treatment x time: $F_{(9,48)}$= 2.218, P=0.0369; Two Way ANOVA; Figure 3b). Body weight was significantly lower on day 30 in 8L:16D group than 12L:12D, 14L:10D and 16L:8D (P<0.05; Bonferroni test).

When we studied GSI there was a significantly gain and loss in GSI as time progressed under long photoperiods (12L:12D: $F_{(3,15)}$= 10.91, P=0.0010; 14L:10D: $F_{(3,15)}$= 36.78, P<0.0001; and 16L:8D: $F_{(3,15)}$= 9.810, P=0.0015; 1-way ANOVA; Figure 3c). However, there was no effect of short photoperiod (8L:16D) on GSI as time progressed (8L:16D: $F_{(3,15)}$= .6656, P=0.589; 1-way ANOVA; Figure 3c). There was a progression of GSI values and on day 30 there was a significantly higher than other days (Fig. 3c) and GSI values had achieved minimum on day 75 of the study. Two way ANOVA revealed effect of photoperiod, time and interaction of photoperiod and time on the GSI values of tree frog (photopeiod: $F_{(3,48)}$= 3.013, P=0.0390; time: $F_{(3,48)}$= 34.09, P<0.0001; interaction of photoperiod x time: $F_{(9,48)}$= 2.855, P=0.0089; Two Way ANOVA; Figure 3c). GSI under short photoperiod (8L:16D) was significantly lower on day 30 in comparison to long photoperiod 12L:12D; 14L:10D and 16L:8D (P<0.05; Bonferroni test; Fig. 3c).

4.2 Role of light intensity in the regulation of gonadal cycle

Figure 4 shows change in body weight, testicular weight and GSI over a period of time under different light intensity treatment. There was a significant change in body weight under all light intensity treatments (10 lux: $F_{(2,32)}$= 32.65, P<0.0001; 20 lux: $F_{(2,34)}$= 18.81, P<0.0001; 50 lux: $F_{(2,37)}$= 16.82, P<0.0001; and 100 lux: $F_{(2,36)}$= 27.64, P<0.0001; 1-way ANOVA; Figure 4a). Irrespective of light intensity treatment, there was a significant decrease in body weight as time progressed and by day 70 body weight reduced significantly (Fig. 4a). Two Way ANOVA revealed that there is only effect of time but not of light intensity or interaction of light intensity and time on body weight (light intensity: $F_{(3,119)}$= 1.017, P=.3877; time: $F_{(2,119)}$= 74.42, P<0.0001; interaction of light intensity x time: $F_{(6,119)}$= 1.161, P=.3319; Two Way ANOVA; Figure 4a).

There was a significantly gain and loss in gonadal weight as time progressed under each light intensity condition (10 lux: $F_{(2,9)}$= 10.11, P=0.0050; 20 lux: $F_{(2,9)}$= 11.33, P=0.0035, 50 lux: $F_{(2,9)}$= 19.38, P=0.0005; and 100 lux: F $F_{(2,9)}$= 24.13, P=0.0002; 1-way ANOVA; Figure 4b). There was a gain in gonadal weight in all groups on day 30 followed by loss of gonadal weight (Fig. 4b) and minimum gonadal weight was recorded on day 70. There was only effect of time but not of light intensity and interaction of light intensity and time on gonadal weight (light intensity: $F_{(3,36)}$= 1.214, P=0.3185; time: $F_{(2,36)}$= 60.85, P<0.0001; interaction of light intensity x time: $F_{(6,36)}$= .6492, P=0.6904; Two Way ANOVA; Figure 4b).

There was a significantly gain and loss in the GSI as time progressed under all light intensity treatments (10 lux: $F_{(2,9)}$= 16.14, P=0.0011; 20 lux: $F_{(2,9)}$= 12.91, P=0.0023, 50 lux: $F_{(2,9)}$= 38.30, P<0.0001; and 100 lux: F $F_{(2,9)}$= 26.63, P=0.0002; 1-way ANOVA; Figure 4c). GSI values were significantly higher on day 30 in comparison to other days (P<0.05; Newman-Keuls Multiple Comparison test); Fig. 4c. Two Way ANOVA revealed that there is only effect of time but not the light intensity treatment or interaction of light intensity treatment and time on GSI (light intensity treatment: $F_{(3,36)}$= 1.109, P=.3581; time: $F_{(2,36)}$= 84.33, P<0.0001; interaction of light intensity treatment x time: $F_{(6,36)}$= .7127, P=.6417; Two Way ANOVA; Figure 4c).

4.3 Role of light spectrum in the regulation of gonadal cycle

Figure 5 shows change in body weight, testicular weight and GSI over a period of time under different light quality conditions. There was a significant change in body weight under all photoperiodic conditions (white light: $F_{(2,27)}$= 12.88, P=0.0001; red light: $F_{(2,36)}$= 37.69, P<0.0001; blue light: $F_{(2,41)}$= 40.06, P<0.0001; and green light: $F_{(2,35)}$= 25.08, P<0.0001; 1-way ANOVA; Figure 5a). Irrespective of different wavelength of light treatment, there was a significant decrease in body weight as time progressed and by day 70 body weight reduced

significantly (Fig. 5a). Two Way ANOVA revealed that there is an effect of light treatment, time and interaction of light treatment x time on body weight (light reatment: $F_{(3,133)}$= 5.398, P=0.0015; time: $F_{(2,133)}$=83.46, P<0.0001; interaction of light treatment x time: F $F_{(6,133)}$=4.863, P=0.0002; Two Way ANOVA; Figure 5a). There was a slight increase in body weight under white light group on day 70 and body weight on day 70 in white light group was significantly higher than red, blue and green light groups (P<0.05; Bonferroni test; Fig. 5b).

There was a significantly gain and loss in the gonadal weight as time progressed under each photoperiodic condition (white light: $F_{(2,9)}$= 5.579, P=0.0256; red light: $F_{(2,9)}$= 12.79, P=0.0023; blue light: $F_{(2,9)}$= 19.01, P=0.0006; and green light: $F_{(2,9)}$= 32.76, P<0.0001; 1-way ANOVA; Figure 5b). There was a gain in gonadal weight in all groups on day 30 followed by the loss of gonadal weight (Fig. 5b) and minimum gonadal weight was recorded on day 70. There was no effect of light quality on gain and loss of gonadal weight but there was an effect of time and interaction of light quality and time on gonadal weight (light treatment: $F_{(3,36)}$= 2.015, P=0.1292; time: $F_{(2,36)}$= 53.46, P<0.0001; interaction of light treatment x time: $F_{(6,36)}$= 2.565, P=0.0358; Two Way ANOVA; Figure 5b).

There was a significantly gain and loss in GSI as time progressed under different spectral light conditions (white light: $F_{(2,9)}$= 21.40, P=0.0004; red light: $F_{(2,9)}$= 11.68, P=0.0032; blue light: $F_{(2,9)}$= 28.33, P=0.0001; and green light: $F_{(2,9)}$= 37.75, P<0.0001; 1-way ANOVA; Figure 5c). GSI values were significantly higher on day 30 in comparison to other days (P<0.05; Newman-Keuls Multiple Comparison test; Figure 5c). Two Way ANOVA revealed that there was effect of time but not the light treatment or interaction of light treatment and time on GSI (light treatment: $F_{(3,36)}$= 2.330, P=.0907; time: $F_{(2,36)}$= 84.16, P<0.0001; interaction of light treatment x time: $F_{(6,36)}$= 1.488, P=.21077; Two Way ANOVA; Figure 5c).

5. DISCUSSION

Our results shows that photoperiod has an effect on reproductive cycle of terai tree frog. Experiment one demonstrates that under captivity as the time progresses there is a reduction in body mass irrespective of the photoperiod, suggesting that long photoperiod is not promoting gain in body mass at-least under laboratory conditions (Fig 3a). However, we clearly observed the differential effect of photoperiod on testicular growth as measured by testicular weight. When frogs were exposed to long photoperiod (12L:12D; 14L;10D and 16L:8D) there was gain in testes weight, however short-day conditions (8L:16D) could not induce the significant change in the testes weight and therefore on day 30 of experiment testicular weight in long photoperiod groups was significantly higher in comparison to the short photoperiod group (8L:16D). Further, within the long photoperiods we did not find difference in gain in testicular weight (Fig. 3b). It seems that once photoperiod has crossed the critical day length it has limited role in the gonadal growth recrudescence cycle. As in case of many amphibians the breeding phase is extended from March till late October where natural day length may vary from 12 h to 16h and hence could be an adoptive mechanism for breeding biology of amphibians. Similar inductive effects of long photoperiod have been reported in ovarian cyclicity of *Bufo fowleri*, where long photoperiods (12L:12D, 16L:8D, 20L:4D) were more stimulatory than short photoperiods (4L:20D, 8L:16D) for gonadosomatic index (Bush, 1963). Similarly, in *Rana catesbeiana***,** long photoperiod (12L:12D) appeared to prevent ovarian regression and atresia in both laboratory reared and wild-caught specimens (Horseman *et al.*, 1987). There are limited studies available in literature which deals with the role of photoperiod on reproduction in amphibians. Werner (1969) exposed salamanders (*Plethodon cinereus*) to various photoperiods and observed that LD 12:12 and 16:8 were more stimulatory than LD 8:16. However, during late fall and early winter, long

photoperiod could not stimulate gonadal growth and hence suggesting that animals entered in the "refractory" phase. Photorefractoriness is considered as an adaptive phase of the annual cycle, and has been reported in the annual cycles of a number of other vertebrates (Hamner, 1968; Farner, 1962; Farner and Lewis, 1971; Lofts and Murton, 1968; Murton and Westwood, 1977; Kumar, 1997). How exactly photorefractoriness develops is not well understood. In the absence of an exact etiology of photorefractoriness, a mechanistic explanation can be that after a period of intensive gonadal activity the underlying physiological mechanisms get 'fatigued' and so animals require some sort of physiological rest and the intervening time is utilized for preparations to start afresh the next annual cycle. It is probably the impracticability of a continuous reproductive season, which is an expensive life process, is the basis for the evolution of seasonality in vertebrates.

Amphibian breeding behaviour is significantly influenced by abiotic factors than by biotic factors: environmental variables such as light, rainfall and temperature (Wells, 2007). Photoperiod affects the timing of reproduction in subtropical anuran assemblages and regulates gametogenesis and calling of anuran (Both *et al.,* 2008; Canavero and Arim, 2009; Hatano *et al.,* 2002; Kaefer *et al.,* 2009). Role of photoperiod in vocalization and calling behaviour for anuran has been suggested (Both *et al.,* 2008; Kaefer *et al.,* 2009; Canavero and Arim, 2009). Bambozzi *et al.* (2004) demonstrated that this variable is positively related to weight gain and metamorphosis rates of *L. catesbeianus* tadpoles. Further, photoperiod is responsible for reproductive organ development in *L. catesbeianus* in captivity (Figueiredo *et al.,* 2001). The effect of change of season on behaviour and physiology of organisms is a widespread phenomenon, and photoperiod is most reliable predictor of these changes (Vaze and Sharma, 2013). The response to photoperiod is considered one of the most important adaptations to

seasonal variation, with direct implications on survival and reproductive success (Vaze and Sharma, 2013).

The differential behavioural patterns of male bullfrogs during spring and summer months may be related to the differences in photoperiod and temperature between these two periods. Thus, longer days in summer could influence males to start calling and to reach their peak activity later in night than in spring nights (Both *et al.,* 2008, Kaefer *et al.,* 2009, Canavero and Arim, 2009). Most of the research on amphibian reproduction has centred on reactions to exogenously administered hormones and have used wild animals maintained in laboratory for only short periods of time (Lofts, 1974). Henderson (1963) studied the involvement of photoperiod and temperature in regulation of reproductive cycle of brook trout (*Salvelinus fontinalis*). They observed that the development of female's first complement of eggs proceeded to maturation on either a long (LD 20:4) or short (LD 4:20) photoperiod. However, the photoperiodic conditions used by Henderson in the above study were extreme (20L:4D or 4L:20D). Laboratory-reared female bullfrogs achieved mature ovary development on either long (14L:10D) or short (8L:16D) photoperiods (Henderson, 1963). However, ovaries begin rapid reabsorption and "asynchrony" developed i.e., the ovaries having both reabsorbing follicles and vitellogenic follicles, but there was no predominant group of vitellogenic oocytes which could be expected to reach maturity at approximately the same time. In another study when leopard frog females subjected to an artificial hibernation period (8L:16D; 4 ^{0}C), injected with pituitary extract injection appeared to increase both the rate of ovulation and rate of normal development of ovulated eggs (Lehman, 1977).

Our results show that there is no additive effect of light intensity and light quality on the gonadal growth of tree frog. As in both the experiments (experiment 2 and experiment 3; Fig. 4 and 5) all groups were exposed to 12L:12D (a stimulatory photoperiod), probably we did not observe any additive effect of light quality other than the effect of light duration. So, it appears that light duration is important but the other properties of light has limited role in regulation of gonadal growth of tree frog. There is a limited literature available for involvement of light spectrum and light intensity in regulation of gonadal growth regression cycle in amphibians. Role of light intensity and wavelength of light has been reported for higher vertebrates. The wavelength of light could be critical for photoperiodic regulation of physiological processes in many avian species (Benoit, 1964; Oishi and Lauber, 1973; Rani *et al.,* 2002, Malik *et al.,* 2014). In birds, the circadian and photoperiodic responses depend on subjective interpretation of illumination during the day and night (Meyer *et al.,* 1988, 1991); blackheaded bunting, *Emberiza melanocephala* (Kumar *et al.,* 1992); Indian weaver bird, *Ploceus philippinus* (Singh *et al.,* 2012). The spectral composition of daylight can affect the circadian and photoperiodic responses in vertebrates, including birds (Rani *et al.,* 2002). The involvement of spectral composition and intensity of light needs to be investigated in more amphibian species for better understanding of its involvement in photoperiodic responses.

V. SECTION 3: EFFECT OF TEMPERATURE ON THE REPRODUCTION UNDER LABORATORY CONDITIONS

1. ABSTRACT

Amphibians are seasonal animals and their annual reproductive cycles dependent on seasonal changes in environmental factors. In tropical conditions many species show a clear relationship between reproduction and seasonal distribution of rainfall. In temperate conditions, reproduction is usually centered during spring and summer seasons when environmental conditions are favourable. Poikilotherms are highly sensitive to change in environmental temperature and their physiology and metabolic activities depends on environmental temperature. Seasonal changes in environmental temperature are consequently important in regulation of reproductive cycles. Both light and temperature have a remarkable influence on annual testicular activity of amphibians. However, temperature, appears to be the most important environmental cue involved in control of reproduction; photoperiod acts only in a permissive way to facilitate the temperature response. Present study demonstrates the effects of high and low temperature on body weight and testicular growth under stimulatory photoperiod. Adult male frogs were divided into two groups and subjected to artificial light-dark cycles of equinox photoperiod (12L:12D). Group one was exposed to high temperature ($34 \pm 2\ ^0C$), while group two was exposed to low temperature ($20 \pm 2\ ^0C$). Observations on change in body mass and testicular weight was made on day 0, day 30 day 60 and on day 90 of respective treatments. Results of the present study suggest that lower temperature promotes the testicular recrudescence of tree frog under laboratory conditions.

2. INTRODUCTION

Almost all amphibian species having annual reproductive cycles dependent on seasonal changes in environmental factors. In general, temperature and photoperiod in temperate zones and rainfall in tropical areas appear to be the most important environmental factors influencing breeding activity (Lofts, 1984; Whittier and Crews, 1987). In tropical and equatorial regions, annual fluctuations in photoperiod and temperature is limited. There is a seasonal rhythm in annual rainfall in tropical zones and, consequently, seasonal fluctuations in availability of food supply. In these areas, many species show a clear relationship between reproduction and seasonal distribution of rainfall (Gwinner, 1981; McArthur, 1981). In temperate conditions, reproduction is usually centred during spring and summer seasons when environmental conditions are favourable. With increase in latitude, the favourable environmental conditions shorten and, hence, the reproductive period (Baker, 1938b; Saboureau and Outourne, 1981; Vivien-Roels and Pévet, 1983). A similar phenomenon can be observed with increasing altitude which reduces the favourable climatic period (Lofts, 1974; Callard and Ho, 1979).

Influence of photoperiod on reproduction is mediated by the pineal gland in fishes and in many mammals (Fiske *et al.*, 1960; Wurtman *et al.*, 1968; Reiter, 1980). In poikilothermic vertebrates, study of photoperiod effects is of particular interest since, in most of these animals, the pineal gland has a photoreceptive structure that can be stimulated by direct light and therefore, its function may be independent of ocular photoreception (Dodt, 1973; Oksche and Ueck, 1974; Fraile *et al.*, 1988, 1989a). However, Poikilothermic vertebrates are highly sensitive to change in environmental temperature as their physiology and metabolic activities depends on body temperature, and this in turn depends on environmental temperature. Seasonal changes in environmental temperature are consequently important in regulation of reproductive cycles. It

appears that temperature is the principal external factor which regulates reproductive cycle in many amphibians and reptiles (Licht, 1972; Rastogi *et al.,* 1978; Moll, 1979; Fraile *et al.,* 1989b). Studies suggest that both light and temperature have a remarkable influence on the annual testicular activity of amphibians. However, temperature, being an ectotherm vertebrate, appears to be the most important environmental cue involved in control of reproduction; photoperiod acts only in a permissive way to facilitate the temperature response (Rastogi *et al.,* 1978). Relative role of photoperiod and temperature has been proposed for *Rana perezi* where annual testicular cycle is regulated by both temperature and photoperiod, and then effects of these two factors can vary seasonally, depending on phase of annual reproductive cycle (Delgado *et al.,* 1992).

Mechanism of action of temperature in regulation of breeding is still not clear. However, it has been proposed to act via hypothalamus (Porter and Eicht, 1986). Further, the direct effect of temperature on gonads has also been proposed (Jorgensen *et al.,* 1978). In ectotherm animals along with photoperiodic conditions, temperature also seems to be important in regulation of lpine activity via a melatonin-mediated system (Underwood, 1990). Role of temperature in regulation of spermatogenesis in amphibians have been studied. The relationship between annual changes in temperature and spermatogenesis is observed in amphibians that inhabit cold/temperate areas (Galgano, 1932,1934,1936; Galgano and Lanza, 1951) and urodele (Ifft, 1942). The development of discontinuous cycles was attributed to the occurrence of a cold season during which most activities, including spermatogenesis, were arrested.

Proposed effect of temperature on reproductive activity is mediated by hypothalamus, which regulates pituitary gonadotropin secretion (Mazzi, 1970; Ball, 1981). However, environmental temperature is also involved in regulation of melatonin synthesized by pineal

gland (de Vlaming and Olcese, 1981; Vivien-Roels and Pévet, 1983). Pinealectomy hinders temperature stimulation of testis in *R. esculenta* (Rastogi *et al.,* 1976). Differential role of temperature has been observed in different species. Temperature below 12 ^{0}C spermatogenesis is arrested in the urodele *Notophthalmus viridiscens,* and the spermatids and spermatocytes that are present degenerate (Ifft, 1942). Similar observation has been observed in *T. cristatus* (Steinborn, 1984). However, in, *Plethodon cinereus,* temperature of 10 ^{0}C permits primary spermatocyte proliferation during the quiescent period and causes only some delay in spermatogenic development during the period of spermatogenesis (Werner, 1969). These results suggest that the optimum temperature range for spermatogenesis varies species to species. Low temperatures (below 10 ^{0}C) enhance the proliferation of primary spermatogonia and hinder the development of secondary spermatogonia and primary spermatocytes in *R. esculenta* (Rastogi *et al.,* 1976). Temperature under 4 ^{0}C arrests proliferation of spermatogonia type in *Bufo spinulosus* (Bustos-Obregón, 1979). However, mild temperatures affect spermatogenesis favourably even when the photoperiods are short. In Newts *(Notophthalmus viridescens)* maintained at 20 ^{0}C with a short photoperiod (8-10 h of light daily) for three months during the phase of testicular quiescence develops spermatogenesis until it reaches the round spermatid stage (Ifft, 1942). Similar results have been obtained with the marbled newt (*T. marmoratus)* (Fraile *et al.,* 1988). In similar photoperiod and temperature conditions, complete spermatogenesis was observed in *T. cristatus* with only 6 weeks of exposure during the quiescent period (Steinborn, 1984).

On the other hand, moderately high temperatures affect different phases of the spermatogenic cycle in *T. marmoratus* differently. Newts maintained at 30 °C for 3 months during the quiescent period with the naturally short photoperiod showed the same spermatogenic development as those maintained at 20 °C (Fraile *et al.,* 1989b). If animals were exposed to 30

°C during the period of germ cell proliferation (March-June) with a naturally long photoperiod (12-14 h of light daily), spermatogenic development was normal, like that of newts maintained in their natural environment or those kept in the laboratory at 20 °C (Fraile *et al.,* 1989b). However, when newts were maintained at 30 °C during the period of spermatogenesis (July-September), spermatogenesis was arrested at round spermatid level (Fraile *et al.,* 1989b). Diminished spermiogenesis was also observed by Ifft (1942) in newts *(N. viridescens)* maintained at 25 ^{0}C during the period of spermiogenesis. Therefore, the effect of moderately high temperature is similar to that of mild temperatures during the phase of germ-cell proliferation and meiosis, and detrimental during the phase of spermiogenesis. In earlier studies dealing with the effects of temperature on the ovarian function of amphibians, exposure to high temperature was found to stimulate vitellogenic growth of oocytes. However, response of the ovary varied with the temperature used, duration of the exposure, phase of the reproductive cycle and pattern of oogenetic activity (Jorgensen *et al.,* 1978; Pancharatna and Saidapur, 1990; Pancharatna and Kulkarni, 1993).

3. MATERIALS AND METHODS

Study was started in the month of March 2017; immediately after frogs emerged out from their hibernating state. Adult male frogs were procured within Mizoram university campus by hand and with the aid of fishing nets and brought to indoor laboratory conditions. Frogs were kept in polypropylene cage (size 28.5 x 22.1 x 14 cm; two frogs were kept in each cage). Adult male frogs (N= 12/group) were divided into two groups and subjected to artificial light-dark cycles of equinox photoperiod (12L:12D; light on 06:00; light off 18:00). Group one was exposed to high temperature 34 ± 2 ^{0}C, while group two was exposed to low temperature 20 ± 2 ^{0}C. Food (larvae of silk moth and meal worm) and water was available ad libitum. Light was provided by 14W fluorescent CFL at top of cage. Light onset and offset was regulated by automatic timer. Observation on changes in body mass and testicular weight was made on day 0, day 30 day 60 and on day 90 of respective treatments.

3.1 Statistical analysis

Data is represented as mean ± SE. One-way analysis of variance (1-way ANOVA), followed by Newman-Keuls post hoc tests if ANOVA indicated the significance of difference, was employed to examine the effect of various treatment over a period of time. Similarly, two-way ANOVA was also employed to analyze effects when two factors (e.g. temperature and duration of the treatment) were taken into consideration, and Bonferroni test was used as post hoc test for group comparisons. Significance was always taken at $P < 0.05$.

4. RESULTS

Figure 6 shows changes in body weight, testicular weight and GSI over a period of time under different temperature conditions. There was a significant change in body weight in both the groups (high temperature: $F_{(3,12)}$= 25.99, P<0.0001; low temperature: $F_{(3,12)}$= 9.710, P=0.0016; 1-way ANOVA; Figure 6a). Frogs, under high temperature treatment lost the body weight continuously and maximum body weight was recorded on day 0 (7.04±0.19 gm; Fig. 6a) mean body weight: and minimum body weight (4.45±0.37 gm; Fig 6a) was recorded on day 90. In contrast to high temperature, frogs under low temperature first gained in body weight and then started losing body weight and maximum body weight was recorded on day 30 (7.9±0.27 gm; Fig. 6a) and minimum body weight (4.76±0.60 gm; Fig. 6a) was recorded on day 90. Two Way ANOVA revealed that there is an effect of temperature, time and interaction of temperature treatment x time on body weight (temperature treatment: $F_{(1,24)}$= 4.724, P=0.0398; time: $F_{(3,24)}$=23.80, P<0.0001; interaction of temperature treatment x time: $F_{(3,24)}$= 3.587, P=0.0284; Two Way ANOVA; Figure 6). On day 30 body weight under low temperature was significantly higher than high temperature group (P<0.05; Bonferroni test).

There was a significantly gain and loss in gonadal weight as time progressed in both the groups (high temperature: $F_{(3,12)}$= 15.51, P=0.0002; low temperature: $F_{(3,12)}$= 11.31, P=0.0008; 1-way ANOVA; Figure 6b). There was a gain in gonadal weight in both groups on day 30 followed by loss of gonadal weight (Fig. 6b) and minimum gonadal weight was recorded on day 90. However, the regression of testes was much faster in high temperature group as compared to low temperature group. Two Way ANOVA revealed that there is an effect of temperature, time and interaction of temperature treatment x time on gonadal weight (temperatue reatment: $F_{(1,24)}$= 12.51, P=0.0017; time: $F_{(3,24)}$=20.64, P<0.0001; interaction of temperature treatment x time:

$F_{(3,24)}$= 3.997, P=0.0193; Two Way ANOVA; Figure 6b). On day 30 testes weight under low temperature was significantly higher than high temperature group (P<0.05; Bonferroni test; Fig. 6b). Gain in testes weight on day 30 in low temperature group was significantly higher than high temperature group.

There was a significantly gain and loss in GSI as time progressed in both the groups (high temperature: $F_{(3,12)}$= 9.539, P=0.0017; low temperature: $F_{(3,12)}$= 131.2, P<0.0001; 1-way ANOVA; Figure 6c). There was a gain in GSI in both the groups on day 30 (Fig. 6c) however, gain in low temperature group is significantly higher than high temperature group. Two Way ANOVA revealed that there is an effect of temperature, time and interaction of temperature treatment x time on gonadal weight (temperature reatment: $F_{(1,24)}$= 35.02, P<0.0001; time: $F_{(3,24)}$=48.08, P<0.0001; interaction of temperature treatment x time: $F_{(3,24)}$= 14.02, P<0.0001; Two Way ANOVA; Figure 6c). On day 30 GSI values are much higher in low temperature group in comparison to high temperature group (P<0.05; Bonferroni test; Fig. 6c). However, by day 90 GSI values were similar in both the groups.

5. DISCUSSION

The present study demonstrates the effect of temperature on photoperiodically controlled phenologies expressed at the seasonal levels in the tree frog (cf. fig. 6). Under poststimulatory photoperiod (12L:12D), there was a significant difference in the body fattening and testicular recrudescence between the two temperature groups (Fig. 6a). Low temperature promoted the body weight gain and testicular recrudescence as reflected by the gain in testes weight and increase in GSI (Fig. 6a). In comparison to low temperature, high temperature leads the decrease in body weight and small gain in testicular weight on day 30 of treatment. There was an increase in GSI on day 30 under both the temperature groups but in comparison to low temperature the gain in GSI under high temperature treatment was significantly less (Fig. 6c). The rate of photoperiodic induction under high temperature was slower, resulting in reduced body fattening and delayed and attenuated testicular maturation in the high temperature group compared with the low temperature group (cf. figures 6). Thus, the effects of temperature appear to be on the rate of photoperiodic induction.

These results (Fig. 6) suggest the response specific effects of temperature in the tree frog exposed to long days (Wingfield, *et al.,* 2003). Temperature appears to have differential effects on the processes underlying the fat deposition and testicular recrudescence, as is evident by the response of the high temperature group to the 12 L photoperiod (Fig 6). There was no gain in body weight of tree frogs in the high temperature group on day 30 (Fig. 6a), but testicular weight was increased (Fig. 6b**)**. It appears that body fattening and testicular recrudescence in tree frog are regulated by separate photoperiodic phenomena which is evident in other vertebrate species (Kumar, 1988; Jain 1993; Misra *et al.,* 2004). Further, these results on tree frogs are indicative of that temperature affects both the timing and duration of a life-history stage in the tree frog

differently. When compared with the low temperature group, high temperature group had a shortened reproductively active phase under the stimulatory photoperiod of the 12L:12D, caused by delayed maturation and advanced regression of the testes (Fig. 6). There was no gain in body weight under the high temperature but they continued to loss body weight even under stimulatory photoperiod (Fig. 6). We can speculate the low temperature and stimulatory photoperiod (12L:12D), acted as a spring season and therefore resulted in gain in body weight and testicular recrudescence while stimulatory photoperiod (12L:12D) combined with high temperature acted like peak summer time and hence could be unfavourable time for breeding hence limited and attenuated gain in the testicular weight.

The involvement of thermal environment in the regulation of reproductive behaviour has been shown in the multiple species across many different habitats (Calder, 1971; Daunt *et al.,* 2006; Doody *et al.,* 2006; Post and Forchhammer, 2008; Singh *et al.,* 2012). Temperature dictate the various parameters such as timing of seasonal migrations, incubation timing and durations, sex ratios, and the length of the breeding, either at the population level or at individual attributes (Sexton *et al.,* 1999; Bowen *et al.,* 2005; Daunt *et al.,* 2006; Doody *et al.,* 2006; Singh *et al.,* 2012). With the increase of urbanization and global climate warming, understanding how endangered species respond to these changes is essential (Williams *et al.,* 2008). It is particularly important for poikilotherms, such as amphibians, whose body temperatures is closely indicator and reflector of their surroundings. The length of the internesting interval, timing of courtship, fecundity, clutch size and egg maturation are regulated by temperature (Sato *et al.,* 1998; Hays *et al.,* 2002; Hamann *et al.,* 2003; Cheng *et al.,* 2009; Weber *et al.,* 2011; Lamont and Fujisaki, 2014). Some recent studies suggest that increase in temperatures have impacted the nesting phenology of marine turtles, including initiation of nesting and duration of the nesting season,

(Weishample *et al.*, 2004, 2010; Hawkes *et al.*, 2007; Pike, 2008; Pike *et al.*, 2006). In Columbia spotted frogs (*Rana luteiventris*), relationships among local climate variables, annual survival and fecundity, and population growth rates suggest that an increase in survival and breeding probability of these frogs as severity of winter decreased. Therefore, it appears that warming climatic conditions with less severe winters is likely to promote population viability in Columbia spotted frog (McCaffery and Maxell, 2010).

Studies on amphibian reproduction have suggested that moderate temperature plays a primary role in stimulating gametogenesis. In some species rainfall regulates the timing of breeding and reproductive behaviour (Lofts, 1984; Whittier and Crews, 1987; Saidapur, 1989). The relatively moderate temperature stimulates vitellogenic growth of oocytes within the ovary. However, the response of the ovary depends on the temperature used, duration of the exposure, phase of the reproductive cycle, and pattern of oogenetic activity (Jorgensen *et al.*, 1978; Pancharatna and Saidapur, 1990; Kanamadi and Jirankali, 1993; Pancharatna and Kulkarni, 1993). In general, most of the studies on the environmental control of amphibian reproduction have focused on temperate species (Jorgensen *et al.*, 1978; Lofts, 1984; Saidapur, 1989), temperature regulation of the ovarian cycle has also been documented in some tropical species: *Rana tigrina* (Pancharatna and Saidapur, 1990), *Polypedates maculatus* (Kanamadi and Jirankali, 1993), and *Rana cyanophlyctis* (Pancharatna and Kulkarni, 1993; Kulkarni and Pancharatna, 1994). These effects of temperature on the ovary are mediated through the pituitary gland in *R. tigrina* (Pancharatna and Saidapur, 1990) and *P. maculatus* (Kanamadi and Jirankali, 1993).

Studies on female *Rana cyanophlyctis* suggest that relatively high temperature stimulates growth of previtellogenic oocytes leading to the formation of SGP oocytes as in the ovary of adult anurans (Pancharatna and Patil, 1997; Jorgensen *et al.*, 1978; Pancharatna and Saidapur,

1990; Kanamadi and Jirankali, 1993; Pancharatna and Kulkarni, 1993). Further, when frogs exposed to constant high temperature in coupled with long photoperiods, the stimulatory effect of high temperature was magnified. In these frogs, LSGP oocyte numbers doubled compared to frogs exposed to high temperature and 12L:12D photoperiod. It suggests that long photoperiod coupled with high temperature favours the growth of SGP oocytes. Our results are in consistent with these findings. In *P. maculatus,* vitellogenesis was unaffected in the ovaries of the frogs exposed to short (6L: 18D) photoperiod (Kanamadi and Jirankali, 1993). Further, light is known to be the proximate factor in regulating reproductive cycles in *Plethodon cinereus, Xenopus laevis, Triturus cristatus,* and *Hymenochirus boettgeri* (Lofts, 1984).

Finally, there could be the adaptive significance of the role of temperature in photoperiodic induction of seasonal phenologies in poikilotherms, e.g. amphibians. Is it advantageous for a photoperiodic seasonal breeders to evolve a second regulatory mechanism involving the environmental temperature. Probably, the photoperiod and temperature act in synergistically to synchronize biological clock-mediated functions that underlie daily and seasonal cycles in amphibian species in order to maximize their survival and reproductive success. Also, temperature influences food availability, which has significant effects on photoperiodic induction of daily and seasonal responses (Kumar *et al.*, 2001). However, still we do not have much understanding about the mechanism involve in the regulation of temperature induced reproductive processes; future studies should focus on investigating how the biological clock regulating daily and seasonal responses adjusts to sudden temperature changes in the environment and the mechanism involved.

VI. SECTION 4: EFFECT OF PHOTOPERIOD ON THE GROWTH AND DEVELOPMENT OF TADPOLES

1. ABSTRACT

Amphibians are seasonal breeders and live in seasonally varying environments having time constraints to achieve their annual life history states and their survival is often strongly dependent on successful timing of certain critical life history decisions. In general, in larval amphibian development, growth and metamorphosis are strongly time-limited due to variations in seasonal factors. Photoperiod is known to influence wide range of physiological processes in seasonal breeders in general and reproduction in particular. Most of studies of rearing conditions on growth, development and metamorphosis of amphibian's tadpoles have been focused on laboratory species such as the genus *Xenopus*. Very less is known about other seasonally breeding amphibians. In present study we addressed the effects of photic conditions (light quantity and light quality) on growth, development and metamorphosis of Terai tree frog (*Polypedates teraiensis*). Three experiments were performed. In experiment one we studied development and metamorphosis of tadpoles under NDL (but captivity) conditions and compared it with the development under continuous light (LL-bright light) and continuous dark (DD). In experiment two we addressed effects of light duration and in experiment three we addressed effects of light quality (spectral light) on growth, development and metamorphosis of Terai tree frog tadpoles. Our results suggest that light conditions influence the growth, development and metamorphosis of tree frog.

2. INTRODUCTION

Seasonally breeding amphibians have time constraints to achieve their annual life history states and their survival is often strongly dependent on successful timing of certain critical life history decisions. The geographical variation in time constraints, *Viz*, altitudinal and latitudinal gradients, seasonal variations in temperature and optimal growth season, often create genetic clines in growth and development rates (Conover and Schultz, 1995; Arendt 1997). In many organisms these traits are phenotypically plastic (Arendt, 1997; Case, 1978; Newman, 1992; Gotthard and Nylin, 1995). This phenotypic plasticity in relation to seasonal cues is often adaptive and assures successful growth and development strategies in seasonal environments. In comparison to other vertebrates, amphibians having a complex life cycle which includes intermediate feeding larval stage, 'tadpole' which undergoes metamorphosis and timing of metamorphosis is important fitness components.

Light conditions during early development may or may not affect development depending on the organism and the behaviour. In various mammals, development is abnormal if the animal is deprived of light or exposed to an abnormal visual field during an early, critical period of development (Blakemore and Cooper, 1970; Grobstein and Chow, 1975; Hubel and Wiesel, 1963). In general, for amphibian behaviours, development proceeds normally without reference to sensory experience or other external events. Depriving frog *Rana pipiens* of light during larval stages, does not alter the initial development of the visual system (Jacobson, 1971; Jacobson and Hirsch, 1973). Similarly, when a tadpole's eye is rotated at an early developmental stage revealed that genetic instructions and not altered afferent input are responsible for guiding retina's developing connections to the tectum (Beazley, 1979; Jacobson and Hirsch, 1973);

consequently, visually directed orientation is permanently misguided in frogs with rotated eyes. In *Xenopus* tadpoles near metamorphosis, occlusion of one eye decreases precision and rotation of one eye alters initial development of connections between that eye and ipsilateral visual tectum (Keating, 1977; Keating and Feldman, 1975) suggesting that light play role in development of this species. Furthermore, binocular visual experience contributes to proper formation of binocular cells in visual tectum of *Xenopus* (Gaze *et al.,* 1970), and visual experience helps maintain normal visual connections in adult *Xenopus* (Chung *et al.,* 1973). Further, early exposure to light is necessary for development of photopositive behaviour in *Xenopus laevis* tadpoles (Copp and McKenzie, 1984). Light-deprivation during the tadpoles' first 10 days of development lead in a long-lasting reduction in tadpoles' light preference. Lengthening tadpoles' exposure to light during first 10 days of development produces increasingly strong light preferences (Copp and McKenzie, 1984).

In general, in larval amphibian development, growth and metamorphosis are strongly time-limited due to variations in the seasonal factors (Berven *et al.,* 1979; Merila *et al.,* 2000) or pond desiccation (Newman, 1992; Denver *et al.,* 1998; Laurila and Kujasalo, 1999). Ecological models of metamorphosis have used growth rate (Wilbur and Collins, 1973), or growth rate mortality trade-off in different habitats (Werner, 1986; Ludwig and Rowe, 1990; Rowe and Ludwig, 1991). Role of photoperiod, wavelength and light intensity is documented in affecting behaviour and physiology of various species (White *et al.,* 1994; Aarseth and Schram, 1999; Phillips and Lomas, 2001; Oishi and Lauber, 1973; Prayitno *et al.,* 1997; Kumar *et al.,* 2000; Rani *et al.,* 2001; Miklosi *et al.,* 2002). In vertebrates most of the photoperiodic studies have been conducted on avian system as birds represent a good model for seasonal breeders. These studies of avian system indicate that their photoperiodic response system can discriminate

between different wavelengths of light and requires a minimum light intensity threshold for photo-stimulation (Malik *et al.,* 2014). There are differential effects of wavelength and intensity of light on the circadian processes mediating photoperiodic regulation of daily and seasonal responses in the birds (Malik *et al.,* 2014) and circadian behaviour on the Indian Weaver bird, *Ploceus philippinus* (Pandey and Bhardwaj, 2011). There are some evidences that photoperiod affect development of amphibian larvae in laboratory (Wright *et al.,* 1988; Crawshaw *et al.,* 1992), but how the different light quality influences the growth and development patterns of larval amphibians is largely unclear.

Most of the studies of rearing conditions on growth, development and metamorphosis of amphibians tadpoles have been focused either on laboratory species such as the genus *Xenopus* (Rose, 1960; Frazer, 1976; Smith and Van Buskirk, 1995; Halliday, 1999; Reed, 2005), or on farmed species such as the American Bullfrog; *Rana catesbeiana* (Hutchison and Hill, 1978; Collins, 1979; Menke and Claussen, 1982; Culley and Sotiaridis, 1984; Lima and Agostinho, 1984, 1992; Benitez-Mandryano and Flores–Nava, 1997) or have generally been limited to certain species (Pough *et al.,* 2001; Gillespi *et al.,* 2007). Previously we have shown the effects of photic and non-photic cues on the growth development and metamorphosis of *Rhacophorus maximus* (Borah *et al.,* 2018). However, no previous studies have been reported concerning the development and metamorphosis of *Polypedates teraiensis*. Here we addressed the effects of photic cues on growth, development and metamorphosis of *Polypedates teraiensis* tadpoles under laboratory conditions. We exposed tadpoles to different photoperiod treatments. Our goal was to determine how light conditions affect development and growth of *Polypedates teraiensis* tadpoles.

3. MATERIALS AND METHODS

Polypedates teraiensis (Terai tree frog) during breeding period lay eggs in foam nests built above pools and ponds. This study was started in the month of March 2017. The newly laid foam nest was collected from natural pond within Mizoram University campus at Aizawl, Mizoram (23^0N, 92^0E) and allowed to hatch in the laboratory. Experiments were performed on day 1 old tadpoles. During experiments tadpoles were kept in a clean and easily manageable polypropylene cage (size 28.5 x 22.1 x 14 cm) and were fed ad libitum. Water was changed every day. Morphological measurement was taken at regular intervals using a Vernier Calliper for each experimental tadpole to study the effects on growth and development of tadpoles. Body mass of an individual tadpole was recorded on a top pan balance with an accuracy of 0.01g. Body length, tail length, interorbital distance (IOD), internarial distance (IND) and maximum tail height (MTH) of an individual tadpole was recorded as per Altig (2007) using a Vernier Calliper with an accuracy of 0.01mm.

Three experiments were performed:

3.1 Experiment 1: To study the effect of natural light, continuous light and continuous dark on growth, development and metamorphosis of tadpoles

Experiment began in month of April 2015. Day one old tadpoles were divided in three groups (N=30 each group). Group one was exposed to NDL (natural day length conditions), group 2 was exposed to continuous light LL (LL-bright light) and group 3 was exposed to continuous dark (DD). Food was available ad libitum and temperature of room was maintained at 24±2 ^{0}C. All the observations were made at the interval of ten days.

3.2 Experiment 2: To study the effect of light duration on growth, development and metamorphosis of the tadpoles

Day one old tadpoles were divided in three groups (N=30 each group). Group one was exposed to SD (8L:16D), group 2 was exposed to long photoperiod LD (13L:11D) and group 3 was exposed to further long photoperiod (16L:8D). All groups were receiving light intensity of 300 ± 10 lux at the level of bottom of cage. Food was available ad libitum and temperature of room was maintained at 24 ± 2 ^{0}C. All the observations were made at the interval of ten days.

3.3 Experiment 3: To study the effect of light spectrum on growth and development of tadpoles

One-day old tadpoles were divided in three groups (N=30 each group). All the groups experienced similar duration of photoperiod (12L:12D; 12h light and 12h dark) and intensity (0.45 W/m^2 at floor level of cage) but different wavelength of light. Desired light intensity of a particular wavelength was achieved using monochromatic filters (Supergel Rosco, Germany) and covering black pastel sheet papers. Group 1 was exposed to short wavelength (blue light; wavelength 450 nm), group 2 was exposed to long wavelength (red light; wavelength 660 nm) and group three was exposed to white light and served as control. Food was available ad libitum and temperature of room was maintained at 24 ± 2 ^{0}C. All the observations were made at the interval of ten days.

3.4 Statistical analysis

Data are represented as mean and standard error (Mean± SE). One-way analysis of variance (one-way ANOVA) followed by the Newman–Keuls test tested significant differences in body weight, body length, tail length, IOD, IND and MTH within the group. Similarly, two-way ANOVA followed by Bonferroni post hoc test compared effects of photoperiodic conditions (factor 1) and time of day (factor 2) on different parameters studied.

4. RESULTS

4.1 Experiment 1: To study the effect of natural light, continuous light and continuous dark on growth, development and metamorphosis of tadpoles

Irrespective of the light treatments, there was a significant growth and development in the tadpoles of each group as the time progressed; NDL (body length: $F_{5,134} = 555.9$, $P < 0.0001$; tail length: $F_{5,134} = 41.11$, $P < 0.0001$; IOD: $F_{5,134} = 202.2$, $P < 0.0001$; IND: $F_{5,134} = 66.59$, $P < 0.0001$; MTH: $F_{5,134} = 111.5$, $P < 0.0001$; 1-way ANOVA; Figure 7a-e; Table 2), LL-bright (body length: $F_{8,158} = 455.7$, $P < 0.0001$; tail length: $F_{8,158} = 60.66$, $P < 0.0001$; IOD: $F_{8,158} = 149.1$, $P < 0.0001$; IND: $F_{8,158} = 36.61$, $P < 0.0001$; MTH: $F_{8,158} = 66.97$, $P < 0.0001$; 1-way ANOVA; Figure 7a-e; Table 3) and DD (body length: $F_{5,143} = 281.2$, $P < 0.0001$; tail length: $F_{5,143} = 75.91$, $P < 0.0001$; IOD: $F_{5,143} = 151.3$, $P < 0.0001$; IND: $F_{5,143} = 50.24$, $P < 0.0001$; MTH: $F_{5,143} = 255.9$, $P < 0.0001$; 1-way ANOVA; Figure 7a-e; Table 4).

There was a significant effect of light conditions on growth of tadpoles; body length (light treatment: $F_{2,387} = 144.1.$, $P < 0.0001$; time: $F_{4,387} = 1255$, $P < 0.0001$ and interaction of light treatment X time: $F_{8,387} = 18.08$, $P < 0.0001$; two way ANOVA; Figure 7a); tail length (light treatment: $F_{2,387} = 8.358.$, $P < 0.0001$; time: $F_{4,387} = 186.6$, $P < 0.0001$ and interaction of light treatment X time: $F_{8,387} = 12.08$, $P < 0.0001$; two way ANOVA; Figure 7b); IOD (light treatment: $F_{2,387} = 134.8.$, $P = 0.0001$; time: $F_{2,387} = 605.3$, $P < 0.0001$ and interaction of light treatment X time: $F_{2,387} = 10.63$, $P < 0.0001$; two way ANOVA; Figure 7c); IND (light treatment: $F_{2,387} = 5.919.$, $P = 0.0030$; time: $F_{2,387} = 142.2$, $P < 0.0001$ and interaction of light treatment X time: $F_{2,387} = 15.69$, $P < 0.0001$; two way ANOVA; Figure 7d) and MTH (light treatment: $F_{2,387} = 170.7.$, $P = 0.0028$; time: $F_{2,387} = 575.0$, $P < 0.0001$ and interaction of light treatment X time: $F_{2,387} = 22.66$, $P = 0.06289$; two way ANOVA; Figure 7e).

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)
1	3.38±.03	6.56±.03			
10	7.58±0.08	16.23±0.16	3.81±0.03	1.63±0.02	3.94±0.05
20	9.95±0.17	21.56±0.23	4.97±0.05	1.88±0.03	5.05±0.10
30	13.15±0.26	28.01±0.49	6.38±0.10	2.70±0.06	6.72±0.11
40	14.39±0.27	21.29±2.60	6.34±0.11	2.39±0.08	6.54±0.16
50	13.49±4.17	18.45±4.97	6.01±0.93	2.18±0.92	5.76±0.90

Table 2: Table 2 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to NDL (natural day length) as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.37±.02	6.56±.03			
10	6.53±0.08	11.71±0.28	3.69±0.04	1.68±0.03	3.74±0.05
20	8.13±0.18	15.64±0.42	4.67±0.09	1.82±0.04	4.81±0.15
30	10.49±0.16	22.59±0.38	5.46±0.07	2.25±0.05	6.81±0.15
40	11.25±0.20	23.20±0.49	5.69±0.09	2.34±0.05	7.28±0.13
50	12.39±0.25	25.08±0.77	6.14±0.11	2.47±0.04	7.53±0.17
60	13.91±0.30	23.52±2.48	6.45±0.10	2.39±0.10	7.44±0.35
70	14.49±0.20	29.55±0.72	7.08±0.06	2.86±0.14	8.45±0.46
80	15.65±0.61	20.13±7.01	6.64±0.37	2.43±0.25	7.11±1.76

Table 3: Table 3 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to LL-bright (continuous light) as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.36±.03	6.56±.03			
10	7.38±0.10	13.91±0.15	4.14±0.06	1.94±0.02	4.49±0.08
20	11.28±0.44	22.19±0.36	5.79±0.09	2.33±0.04	7.19±0.10
30	13.24±0.20	28.07±0.32	6.86±0.09	2.88±0.05	8.39±0.13
40	14.83±0.28	20.91±2.00	6.42±0.10	2.31±0.07	7.44±0.11
50	15±0.30	2.99±2.39	5.73±0.05	1.80±0.04	

Table 4: Table 4 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to DD (continuous dark) as time progressed.

Fastest development was observed in DD group followed by NDL and slowest development was in LL group (Fig. 8). Under DD, 82% tadpoles had forelimb developed by week 6, and by week 8 all tadpoles had forelimb developed (Fig. 8a). In NDL 43% tadpoles had forelimb by week 6 however, by week 8 all tadpoles had forelimb developed. Slowest forelimb development was observed under LL conditions where only 6% tadpoles had forelimb developed by week 8 and it took 12 weeks to have forelimb in all tadpoles (Fig. 8a).

Froglet appearance followed forelimb development pattern (Fig. 8b). Under DD froglet formation also started by the end of week 6 and 11% tadpoles got metamorphosed in froglet, while 81% tadpoles metamorphosed by week 7 and by end of week 9 all tadpoles metamorphosed. In NDL 19% froglets were formed by week 6, 50% were metamorphosed by week 7 and metamorphosis was completed by week 8 (Fig. 8b). Under LL, metamorphosis was delayed and by week 9 only 18% tadpoles metamorphosed and it took 13 weeks to complete metamorphosis (Fig. 8b).

4.2 Experiment 2: To study the effect of light duration on the growth, development and metamorphosis of tadpoles

Tadpoles showed the growth and development under all the photoperiodic treatment; 8L:16D (body length: $F_{6,151} = 272.3$, $P < 0.0001$; tail length: $F_{6,151} = 44.12$, $P < 0.0001$; IOD: $F_{6,151} = 53.74$, $P < 0.0001$; IND: $F_{6,151} = 14.16$, $P < 0.0001$; MTH: $F_{6,151} = 78.55$, $P < 0.0001$; 1-way ANOVA; Figure 9a-e; Table 5), 13L:11D (body length: $F_{8,184} = 179.7$, $P < 0.0001$; tail length: $F_{8,184} = 61.91$, $P < 0.0001$; IOD: $F_{8,184} = 62.63$, $P < 0.0001$; IND: $F_{8,184} = 22.50$, $P < 0.0001$; MTH: $F_{8,184} = 56.51$, $P < 0.0001$; 1-way ANOVA; Figure 9a-e; Table 6),) and 16L:8D (body length: $F_{7,176}= 253.2$, $P < 0.0001$; tail length: $F_{7,176}= 27.68$, $P < 0.0001$; IOD: $F_{7,176}= 60.60$, $P <$

0.0001; IND: $F_{7,176}$= 9.884, P < 0.0001; MTH: $F_{7,176}$= 69.16, P < 0.0001; 1-way ANOVA; Figure 9a-e; Table 7).

There was a significant effect of photoperiod duration on growth of tadpoles; body length (light treatment: $F_{2,481}$ = 28.38., P < 0.0001; time: $F_{5,481}$ = 838.1, P < 0.0001 and interaction of light treatment X time: $F_{10,481}$ = 2.581, P = 0.0047; two way ANOVA; Figure 9a); tail length (light treatment: $F_{2,481}$ = 2.946., P = 0.0061; time: $F_{5,481}$ = 167.0, P < 0.0001 and interaction of light treatment X time: $F_{10,481}$ = 4.576, P < 0.0001; two way ANOVA; Figure 9b); IOD (light treatment: $F_{2,394}$ = 9.020., P = 0.0001; time: $F_{4,394}$ = 227.4, P < 0.0001 and interaction of light treatment X time: $F_{8,394}$ = 227.4, P < 0.0001; two way ANOVA; Figure 9c); IND (light treatment: $F_{2,394}$ = 4.513., P = 0.0115; time: $F_{4,394}$ = 59.42, P < 0.0001 and interaction of light treatment X time: $F_{8,394}$ = 4.321, P < 0.0001; two way ANOVA; Figure 9d) and MTH (light treatment: $F_{8,394}$ = 5.974., P = 0.0028; time: $F_{4,394}$ = 258.4, P < 0.0001 and interaction of light treatment X time: $F_{8,394}$ = 0.7736, P = 0.06289; two way ANOVA; Figure 9e).

Under 8L:16D forelimb developed in 8% tadpoles by week 5, and it took 8 weeks to have forelimb in all the tadpoles. Under 13L:11D 20% population had forelimb by week 6 and all tadpoles had forelimb by week 12. In 16L:8D 21% tadpoles had forelimb by week 6 but it took 13 weeks to have forelimb in all the tadpoles (Fig. 10a). 15% tadpoles metamorphosed into froglet by week 6 under 8L:16D, and by week 9 all tadpoles were converted into froglets. Under 13L:11D only 3% froglets were formed and it took 13 weeks to complete the metamorphosis. Development was much slower under 16L:8D where 21% froglets formed by week 7, and it took 14 weeks to complete the metamorphosis (Fig. 10b).

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.35±.01	6.56±.03			
10	7.4±.15	13.97±.32	4.18±.10	1.85±.03	4.20±.12
20	9.91±.24	21.55±.61	5.45±.11	2.26±.12	6.45±.15
30	12.66±.27	26.08±.58	6.31±.12	2.75±.06	7.39±.16
40	14.08±.36	21.67±2	6.21±.11	2.4±.07	7.64±.20
50	13.99±.31	16.99±3.82	6.11±.17	2.39±.14	7.35±.44
60	16.5±.7	1.84±.05	5.97±.08	1.87±.01	6.95±.42

Table 5: Table 5 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to short photoperiod (8L:16D) as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.36±.03	6.56±.03			
10	6.49±0.15	12.04±0.34	3.72±0.08	1.68±0.05	3.62±0.12
20	9.08±0.30	18.45±0.70	4.97±0.13	2.00±0.06	5.86±0.24
30	11.27±0.31	23.68±0.59	5.86±0.14	2.42±0.07	6.91±0.17
40	11.92±0.19	24.52±0.46	6.02±0.09	2.36±0.04	7.45±0.15
50	12.72±0.23	26.27±0.98	6.28±0.09	2.58±0.06	7.55±0.15
60	13.72±0.30	25.41±2.23	6.59±0.19	2.47±0.13	7.75±0.48
70	14.22±0.29	14.81±5.22	6.32±0.09	2.34±0.18	8.06±0.17
80	15.5±0.68	2.8±1.46	5.8±0.08	1.9±0.01	

Table 6: Table 6 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to long photoperiod (13L:11D) as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.38±.01	6.56±.03			
10	7.40±0.12	14.37±0.23	4.21±0.08	1.96±0.03	4.05±0.10
20	9.89±0.16	20.87±0.31	5.49±0.05	2.26±0.04	6.40±0.09
30	11.60±0.26	24.74±0.49	5.98±0.12	2.43±0.06	7.28±0.19
40	12.93±0.32	23.95±1.51	6.08±0.10	2.47±0.06	7.67±0.17
50	13.29±0.26	21.01±2.47	6.11±0.11	2.33±0.08	7.51±0.19
60	13.55±0.37	22.63±3.36	6.59±0.14	2.43±0.11	7.65±0.41
70	13.95±0.76	14.94±8.09	6.23±0.24	2.30±0.21	8.59±0.37

Table 7: Table 7 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to long photoperiod (16L:8D) as time progressed.

4.3 Experiment 3: To study the effect of light spectrum on growth and development of tadpoles

Irrespective of light quality treatment, there was a significant growth and development in the tadpoles of each group as the time progressed; blue light (body length: $F_{5,153}$ = 574.8, P < 0.0001; tail length: $F_{5,153}$ = 54.58, P < 0.0001; IOD: $F_{5,153}$ = 145.3, P < 0.0001; IND: $F_{5,153}$ = 18.70, P < 0.0001; MTH: $F_{5,153}$ = 115.8, P < 0.0001; 1-way ANOVA; Figure 11a-e; table 8), red light (body length: $F_{6,155}$ = 223.3, P < 0.0001; tail length: $F_{6,155}$ = 34.68, P < 0.0001; IOD: $F_{6,155}$ = 25.38, P < 0.0001; IND: $F_{6,155}$ = 22.41, P < 0.0001; MTH: $F_{6,155}$ = 79.12, P < 0.0001; 1-way ANOVA; Figure 11a-e; table 9) and white light (body length: $F_{6,162}$ = 410.8, P < 0.0001; tail length: $F_{6,162}$= 38.03, P < 0.0001; IOD: $F_{6,162}$= 72.56, P < 0.0001; IND: $F_{6,162}$= 17.38, P < 0.0001; MTH: $F_{6,162}$= 89.05, P < 0.0001; 1-way ANOVA; Figure 11a-e; table 10).

However, there was a significant effect of the light treatment (light wavelength) on the growth of tadpoles; body length (light treatment: $F_{2,463}$ = 10.70, P < 0.0001; time: $F_{5,463}$ = 15733, P < 0.0001 and interaction of light treatment X time: $F_{10,463}$ = 3.143, P = 0.0007; two way ANOVA; Figure 11a); tail length (light treatment: $F_{2,463}$ = 9.492., P < 0.0001; time: $F_{5,463}$ = 132.7, P < 0.0001 and interaction of light treatment X time: $F_{10,463}$ = 5.779, P < 0.0001; two way ANOVA; Figure 11b); IOD (light treatment: $F_{2,376}$ = 3.498., P = 0.0312; time: $F_{4,376}$ = 351.9, P < 0.0001 and interaction of light treatment X time: $F_{8,376}$ = 4.848, P < 0.0001; two way ANOVA; Figure 11c); IND (light treatment: $F_{2,376}$ = 1.322., P = 0.2677; time: $F_{4,376}$ = 58.45, P < 0.0001 and interaction of light treatment X time: $F_{8,376}$ = 3.549, P < 0.0001; two way ANOVA; Figure 11d) and MTH (light treatment: $F_{2,376}$ = .7908., P = 0.4545; time: $F_{5,376}$ = 420.5, P < 0.0001 and interaction of light treatment X time: $F_{10,376}$ = 2.910, P = 0.0089; two way ANOVA; Figure 11e).

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.38±.03	6.56±.03			
10	7.18±0.11	13.71±0.19	4.01±0.05	1.91±0.02	4.24±0.08
20	11.04±0.22	21.47±0.36	5.77±0.10	2.36±0.04	7.12±0.16
30	13.01±0.18	25.93±0.32	6.65±0.08	2.82±0.05	7.93±0.12
40	13.99±0.27	21.15±1.86	6.36±0.08	2.48±0.16	6.90±0.25
50	14.28±0.14	7.57±3.32	5.90±0.20	1.99±0.10	8.39±0.21

Table 8: Table 8 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to blue light in the tadpoles as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.36±.03	6.56±.03			
10	7.37±0.14	13.30±0.25	4.09±0.07	1.96±0.04	4.15±0.10
20	11.40±0.15	21.99±0.36	5.81±0.07	2.40±0.03	6.84±0.13
30	12.96±0.24	25.82±0.53	6.61±0.10	2.77±0.06	7.96±0.14
40	14.72±0.29	18.65±2.26	6.29±0.10	2.46±0.08	7.65±0.22
50	14.56±0.31	23.00±2.88	6.53±0.18	2.34±0.14	7.34±0.33
60	15.81±1.23	27.95±1.39	7.01±0.51	2.19±0.24	6.52±1.24

Table 9: Table 9 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to red light in the tadpoles as time progressed.

Days	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	3.37±.01	6.56±.03			
10	7.52±0.12	14.60±0.23	4.35±0.06	1.95±0.02	4.27±0.09
20	10.92±0.18	22.32±0.33	5.66±0.06	2.39±0.03	6.88±0.15
30	12.29±0.23	26.18±0.41	6.19±0.11	2.64±0.06	7.86±0.12
40	13.20±0.22	23.59±1.63	6.13±0.08	2.39±0.07	7.65±0.18
50	13.96±0.30	19.30±3.10	6.27±0.13	2.28±0.13	7.26±0.48
60	15.63±0.75	10.71±5.97	6.13±0.20	2.06±0.16	8.55±0.56

Table 10: Table 10 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to white light in the tadpoles as time progressed.

Development was fastest in the blue light followed by red light and slowest development was recorded in white light group. Development of forelimb in tadpole was also fastest in blue light group and by sixth week of experiment forelimb developed in 48% population and by end of ninth week forelimb developed in all tadpoles exposed to blue light (Fig.11a). In comparison to blue light growth was delayed under red light and by the end of sixth week of experiment only 42% population had forelimb while it took ten weeks to finally develop the forelimbs in all tadpoles (Fig.12a). Growth was slowest in white light group and 32% tadpoles had forelimb developed in the sixth week of exposure to white light and it took twelve weeks to finally develop forelimb in all tadpoles under white light conditions (Fig.12a). Similarly, froglet formation was fastest in blue light started by sixth week and completed in ninth week followed by red-light group where it started by seventh week and completed by eleventh week (Fig.12b). Metamorphosis was slowest in white light group started by sixth week and completed by twelfth week (Fig.12b).

5. DISCUSSION

In this study, we examined the effects of photoperiodic conditions on growth and development of *P. teraiensis* tadpoles. Here we demonstrate that both light quantity and light quality affects growth and development of *P. teraiensis* tadpoles. In experiment 1, we observed that growth, development and metamorphosis is faster under DD followed by NDL and slowest growth, development and metamorphosis was observed under LL conditions. Delay in the growth under continuous bright light group may be because of light stress. It could also be result of loss of melatonin rhythms. Although we have not done melatonin assay for this particular study but literature is well supported with the fact that continuous bright light conditions leads loss of melatonin production (see review, Trivedi *et al.,* 2017; Trivedi and Kumar, 2014). Our results support our prediction that the growth and development rates are influenced by photoperiodic conditions in *P. teraiensis* tadpoles and under blue light growth and development was fastest followed by red and white light (Fig. 11 and 12). Previous studies have suggested that the photoperiodic conditions may influence growth and development rates of amphibian larvae (Crawshaw *et al.,* 1992; Wright *et al.,* 1988; Delgado *et al.,* 1987). The hormonal mechanisms of metamorphosis, and manipulations of photoperiod have usually been suggested (i.e. continuous light or darkness compared to a more even photoperiod). In the tadpoles of *Rana* increase in day length leads increased growth and development rates. In our study we used long photoperiod (13L:11D) which is correspond to photoperiod that adults experience at time of breeding in nature, therefore all groups showed growth and development at the end of study. However, differential growth under different light quality suggests that some other factor(s) is/are responsible for observed pattern. One reason could be of differential activation of deep brain photoreceptors present in brain regions. Number of opsin molecules has been identified as

extraretinal photoreceptors in Amphibians (Okano *et al.*, 2000; Currie *et al*., 2016). (The opsin-like immunoreactivities were found in anuran amphibians, *Xenopus laevis, Rana catesbeiana, Rana nigromaculata,* and *Bufo japonica* (Okano *et al.,* 2000). In our study we found that exposure to blue light initiates faster development in tadpoles in comparison to red light. These results are consistent with the findings of Currie *et al.,* (2016), where they have suggested the *Xenopus laevis* larvae having short wavelength-sensitive deep brain photoreceptors. They studied the effects of ambient light on spontaneously generated fictive locomotion of prometamorphic *Xenopus laevis* larvae. The response was found to be tuned to short-wavelength (390–410 nm) UV illumination, and immunostaining for OPN5 and cryptochrome 1, a blue-light sensor, reveals cells in this region of the tadpole diencephalon that express proteins with an appropriate spectral sensitivity (Currie *et al.,* 2016). Role of endocrine mechanisms in mediating evolutionary changes in metamorphic life history strategies has been defined (Currie *et al.,* 2016). The difference in light quality may mechanistically results alteration in thyroid axis, primary endocrine regulator of metamorphosis (Buchholz *et al.,* 2011; Page *et al.,* 2009; Safi *et al.,* 2006; Elinso, 2013). The thyroid axis consists of a series of central regulators that mediate the production and release of TH from thyroid gland and peripheral regulators that mediate tissue-specific responses to circulating hormone (Buchholz, 2011). Alterations that affect metamorphic timing potentially occur at any level of thyroid axis, and evolutionary changes at one level will often have effects at other levels.

Study on *Xenopus laevis* tadpoles has shown that low ambient light levels can affect the vertical distribution of tadpoles by influencing their swimming behaviour and these responses to dimming are mediated by the pineal eye (Jamieson and Roberts, 2000). Metamorphosis is the

changes in body form as an amphibian develops from the tadpole into an adult form. Metamorphosis is strictly regulated by a cascade of hormones including thyroid hormones (THs), corticosterone and prolactin. These hormones regulate the rate and timing of metamorphosis via inhibitory and excitatory signals acting through the hypothalamo–pituitary–thyroid axis (Tata, 1999). Corticosterone has a stimulating effect on metamorphosis both centrally through the adrenocorticoid system via corticotropin-releasing factor, which has a dual role in the stimulation of the pituitary to release both thyroid-stimulating hormone and adreno-corticotropic hormone; and locally through stimulating the conversion of T4 to T3, and enhancing the binding of T3 to the thyroid receptor (Tata, 1996). Corticosterone has also been reported to inhibit metamorphosis during early larval development (Hayes, 1997). Timing of metamorphosis is extremely important for offspring survival and there is a trade-off between capitalising on an abundant food supply in the aquatic environment to attain a large size at metamorphosis, and the greater risk of residing in the aquatic habitat, where the animals are more susceptible to predation. Many external factors, such as nutrition, competition, and temperature, can dramatically influence the rate of metamorphosis, and some of these external factors are thought to be mediated by the 'stress' hormone, corticosterone, such as the acceleration of metamorphosis during pond drying (Denver, 2009).

Our study shows that with varying environmental light conditions as happens in nature with varying season; growth and development rate vary hence the timing of metamorphosis *of Polypedates teraiensis* tadpoles. The relatively short photoperiod and shorter wavelength favours the development over long photoperiod and long wavelength respectively.

VII. SECTION 5: TO STUDY THE EFFECT OF NON-PHOTIC CUES ON GROWTH AND DEVELOPMENT OF TADPOLES

1. ABSTRACT

Amphibians are among the most threatened vertebrate taxa experiencing severe population declines. They are highly sensitive to a wide array of environmental factors, such as habitat loss and fragmentation, environmental contamination, direct exploitation, and alien species introduction. Their life history traits are tightly coupled with environmental temperature and food supply. The environmental conditions strongly influence the energy use, activity patterns and the initiation of critical life history states such as hibernation, breeding or development. Most Amphibians species have a complex life cycle that inhabits both aquatic (egg laying, tadpole development) and terrestrial environments (other life history traits). Such reliance on diverse habitats induces a strong sensitivity to terrestrial and aquatic conditions. In this section we addressed the role of food and temperature on the growth, development and metamorphosis of tadpoles. Together three experiments were conducted. In experiment one we addressed the time of food availability on development, in experiment two we addressed the nutritional quality of food on development and in experiment three we addressed the role of temperature on development of tadpoles. Our findings suggest that food restriction delays the development, nutritional food promotes faster metamorphosis and high temperature accelerates faster growth in *P. teraiensis* tadpoles.

2. INTRODUCTION

The anthropogenic activities, for example, loss and fragmentation of habitat, contamination in environmental, direct exploitation, and introduction of alien species (Collins and Storfer, 2003; Beebee and Griffiths, 2005) influence amphibians. These amphibians are among the most threatened vertebrate taxa experiencing severe population declines (Houlahan *et al.,* 2000; Stuart *et al.,* 2004; Beebee and Griffiths, 2005). They are also very sensitive to climate change (Blaustein *et al.,* 2010). Life history traits of amphibians are greatly influenced by environmental temperature and hydric conditions (Jørgensen, 1986; Köhler *et al.,* 2011; Gao *et al.,* 2015a). These environmental conditions (for example moisture and temperature) greatly affect the energy use (Reading and Clarke, 1995; Reading, 2007; Dillon *et al.,* 2010), activity patterns (e.g., mobility of feeding, Jørgensen, 1986; Chan-McLeod, 2003; Köhler *et al.,* 2011) and the initiation of critical life history states such as hibernation, breeding or development (Gao *et al.,* 2015a, b). Most Amphibians species have a complex life cycle that inhabits both aquatic (egg laying, tadpole development) and terrestrial environments (other life history traits). Such reliance on diverse habitats induces a strong sensitivity to terrestrial and aquatic conditions (Collins and Storfer, 2003; Beebee and Griffiths, 2005). Amphibian dispersal is also limited (e.g., up to 11–13 km for anurans, Smith and Green, 2005), therefore reducing amphibians' capacity to escape locally adverse conditions (Araújo *et al.,* 2006; Lawler *et al.,* 2009).

Studies also suggest quick responses of amphibians for changing climatic conditions (Blaustein *et al.,* 2010). Breeding biology of several amphibian species has been influenced by rise in temperatures (Beebee, 1995; Gibbs and Breisch, 2001; Tryjanowski *et al.,* 2003; Carroll *et al.,* 2009; Benard, 2015), and droughts have lead severe population decreases (Daszak *et al.,* 2005; Rittenhouse *et al.,* 2009; Cayuela *et al.,* 2016a). Modifications of the thermal and hydric

conditions are also induce shifts in species distribution (Araújo *et al.,* 2006; Lawler *et al.,* 2009). Climate can synergistically interact with other factors that may impact amphibian populations (Blaustein *et al.,* 2010; Venesky *et al.,* 2014; Davis *et al.,* 2017; Crump and Houlahan, 2017).

In general, rainfall and temperature effects on amphibian physiology and breeding biology can be complex as these climatic factors may interact synergistically or antagonistically (Amburgey *et al.,* 2017). Warmer temperatures prior to breeding season could lead loss in adult survival and fecundity (*Bufo bufo*: Reading, 2007; *Triturus cristatus*: Griffiths *et al.,* 2010; *Bombina variegata*: Cayuela *et al.,* 2017). Further, mild winter conditions could lead enhance of recruitment probability of newly-metamorphosed amphibians (*Triturus cristatus*: Cayuela *et al.,* 2017). Pre-reproductive rainfall increases survival or breeding probability of *Bombina variegata* (Cayuela *et al.,* 2014), and to enhance colonization rates at breeding ponds for several species (Cayuela *et al.,* 2012). Besides important consequences of extreme events (droughts, floods) that can cause high mortalities or reproduction failing in several species (*Triturus cristatus*: Griffiths *et al.,* 2010; *Bombina variegata*: Cayuela *et al.,* 2015a, 2016a), persistence of climate-induced changes in demographic parameters through years can affect population growth rates and induce severe population declines, as in *Bufo bufo* (Reading, 2007) and *Triturus cristatus* (Griffiths *et al.,* 2010; Cayuela *et al.,* 2017). Other factors such as density dependence have also been implemented in strongly influencing population growth rates (*Hyla arborea,* Pellet *et al.,* 2006; *Rana dalmatina*, Băncilă *et al.,* 2016). Effects of climatic conditions on amphibian can be context-dependent, as organismal response may vary according to the species and its life history traits (Cayuela *et al.,* 2017), population studied and may depend on latitudinal, topographical, hydrometric, and climatic context (Cayuela *et al.,* 2016b, 2017; Amburgey *et al.,* 2017), and age of individuals (Cayuela *et al.,* 2016b).

The diet composition of Amphibians may be influenced by size, mobility, availability, and abundance of prey in the environment, and Amphibians can modify their diet according to season, sex, or age (Toft, 1980; Donnely, 1991). Interspecific and intraspecific differences play a role in community structure (Toft, 1980). Selection of food composition and its variations can provide clues about species' foraging behaviour. Active foragers feed more on small, slow prey, especially prey that occur in social aggregations, such as ants or termites, whereas ambush foragers typically feed on big and more mobile prey. In this study, we studied effects of temperature and food availability on growth development and metamorphosis of tree frog tadpoles under laboratory conditions.

3. MATERIALS AND METHODS

Newly laid foam nest was collected from natural pond within Mizoram University campus at Aizawl, Mizoram (23^0N, 92^0E) and allowed to hatch in the laboratory. Experiments were performed on day 1 old tadpoles. During experiments tadpoles were kept in a clean and easily manageable polypropylene cage (size 28.5 x 22.1 x 14 cm) and were fed ad libitum. Water was changed every day. Morphological measurement was taken at regular intervals using a Vernier Calliper for each experimental tadpole to study effects on growth and development of tadpoles. Body mass of an individual tadpole was recorded on a top pan balance with an accuracy of 0.01g. Body length, tail length, interorbital distance (IOD), internarial distance (IND) and maximum tail height (MTH) of an individual tadpole was recorded as per Altig (2007) using a Vernier Calliper with an accuracy of 0.01mm.

Three experiments were performed:

3.1 Experiment 1: To study the effect of timed food availability on the growth and development of tadpoles

One-day old tadpoles were divided into nine different groups (N=20 each group). All groups were exposed to 12L:12D (12h light and 12h dark) photoperiod and were fed with freshly chopped boiled cabbage. Group one was fed ad libitum, group 2 was fed for 12h beginning from light on till light off (ZT 1 to ZT12), group 3 was fed for the first 4h of light phase (ZT 1 to ZT4), group 4 was fed for 4h during the middle of the day (ZT4 to ZT8), group 5 was fed for last 4h of day (ZT8 to ZT12), group 6 was fed for 12h during dark hours (ZT12 to ZT24), group 7 was fed for first 4h of dark phase (ZT12 to ZT16), group 8 was fed for 4h during the middle of dark hours (ZT 16 to ZT20), and group 9 was fed for last 4h of dark phase (ZT 20 to ZT24).

Temperature of the room was maintained at 24 ± 2 ^{0}C. All observations were made at the regular interval of ten days.

3.2 Experiment 2: To study the effect of food quality on the growth and development of tadpoles

One-day old tadpoles were divided into two different groups (N=20 each group). Group one was fed daily with freshly chopped boiled cabbage while group two was fed with protein rich food (Hello pets, Maharashtra aquarium, Mumbai India). Protein rich food consists of 35% crude protein, 3% crude fibre, and 4% of crude fat. In both the groups food were available ad libitum and were exposed under 12L:12D (12h light and 12h dark) photoperiod and temperature of the room was maintained at 24±2 ^{0}C. All observations were made at the interval of ten days.

3.3 Experiment 3: To study the effect of temperature on the growth and development of tadpoles

One-day old tadpoles were divided into two groups (N= 30 each group). The first group was exposed to higher temperature (30 ±2 ℃) while the second group was exposed to lower temperature (20 ±2 ℃). Both the groups were exposed under 12L:12D photoperiod and food was available ad libitum. All observations were made at the interval of ten days.

3.4 Statistical analysis

Data are represented as mean and standard error (Mean± SE). One-way analysis of variance (one-way ANOVA) followed by the Newman–Keuls test tested significant differences in the

body weight, body length, tail length, IOD, IND and MTH within the group. Similarly, two-way ANOVA followed by the Bonferroni post hoc test compared the effects of Photoperiodic conditions (factor 1) and time of day (factor 2) on different parameters studied.

4. RESULTS

4.1 Experiment 1: To study the effect of timed food availability on the growth and development of tadpoles

As time progressed there was growth in the tadpoles under all the food conditions; Food ad libitum (body weight: $F_{4,159} = 89.80$, $P < 0.0001$; body length: $F_{3,51} = 276.5$, $P < 0.0001$; tail length: $F_{3,51} = 57.89$, $P < 0.0001$; IOD: $F_{3,51} = 8.399$, $P < 0.0001$; IND: $F_{3,51} = 152.8$, $P < 0.0001$; MTH: $F_{3,51} = 40.37$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 11), 12h day food group (body weight: $F_{10,114} = 57.24$, $P < 0.0001$; body length: $F_{10,114} = 176.5$, $P < 0.0001$; tail length: $F_{10,114} = 57.97$, $P < 0.0001$; IOD: $F_{10,114} = 2.685$, $P = 0.0070$; IND: $F_{10,114} = 60.38$, $P < 0.0001$; MTH: $F_{10,114} = 53.15$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 12), first 4h day time food group (body weight: $F_{10,76} = 17.39$, $P < 0.0001$; body length: $F_{10,76} = 33.71$, $P < 0.0001$; tail length: $F_{10,76} = 25.57$, $P < 0.0001$; IOD: $F_{10,76} = 2.250$, $P = 0.0276$; IND: $F_{10,76} = 15.01$, $P < 0.0001$; MTH: $F_{10,76} = 10.86$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 14), middle 4h day time food group (body weight: $F_{10,94} = 42.23$, $P < 0.0001$; body length: $F_{10,94} = 33.71$, $P < 0.0001$; tail length: $F_{10,94} = 104.9$, $P < 0.0001$; IOD: $F_{10,94} = 3.139$, $P = 0.0022$; IND: $F_{10,94} = 59.04$, $P < 0.0001$; MTH: $F_{10,94} = 21.42$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 15), last 4h day time food group (body weight: $F_{10,78} = 9.660$, $P < 0.0001$; body length: $F_{10,78} = 28.14$, $P < 0.0001$; tail length: $F_{10,78} = 20.82$, $P < 0.0001$; IOD: $F_{10,78} = 10.80$, $P < 0.0001$; IND: $F_{10,78} = 11.06$, $P < 0.0001$; MTH: $F_{10,78} = 15.20$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 16), 12h night food group (body weight: $F_{10,104} = 78.87$, $P < 0.0001$; body length: $F_{10,104} = 183.0$, $P < 0.0001$; tail length: $F_{10,104} = 49.40$, $P < 0.0001$; IOD: $F_{10,104} = 120.6$, $P = 0.0070$; IND: $F_{10,104} = 65.80$, $P < 0.0001$; MTH: $F_{10,104} = 8.690$, $P < 0.0001$; 1-way ANOVA; Figure 13-18; table 13), first 4h night food group (body weight: $F_{10,90} = 16.70$, $P <$

0.0001; body length: $F_{10,90}$ = 41.31, P < 0.0001; tail length: $F_{10,90}$ = 49.88, P < 0.0001; IOD: $F_{10,90}$ = 8.881, P = 0.0070; IND: $F_{10,90}$ = 20.04, P < 0.0001; MTH: $F_{10,90}$ = 18.60, P < 0.0001; 1-way ANOVA; Figure 13-18; table 17), middle 4h night food group (body weight: $F_{10,38}$ = 64.75, P < 0.0001; body length: $F_{10,38}$ = 227.6, P < 0.0001; tail length: $F_{10,38}$ = 123.2, P < 0.0001; IOD: $F_{10,38}$ = 115.4, P = 0.0070; IND: $F_{10,38}$ = 47.95, P < 0.0001; MTH: $F_{10,38}$ = 33.20, P < 0.0001; 1-way ANOVA; Figure 13-18; table 18), and last 4h night food group (body weight: $F_{10,169}$ = 36.89, P < 0.0001; body length: $F_{10,169}$ = 227.6, P < 0.0001; tail length: $F_{10,169}$ = 83.14, P < 0.0001; IOD: $F_{10,169}$ = 28.26, P = 0.0070; IND: $F_{10,169}$ = 27.07, P < 0.0001; MTH: $F_{10,169}$ = 27.61, P < 0.0001; 1-way ANOVA; Figure 13-18; table 19).

Two-way ANOVA revealed that there is an effect of time of food availability on growth and development of tadpoles. When we compared among food ad libitum group, 12h day and 12h night time food availability group we found there is an effect of food timing availability on growth and development; body weight (food treatment: $F_{2,163}$ = 95.78, P < 0.0001; time: $F_{3,1163}$ = 257.3, P < 0.0001 and interaction of food treatment X time: $F_{6,163}$ = 22.90, P < 0.0001; two way ANOVA; Figure 13); body length (food treatment: $F_{2,163}$ = 205.3, P < 0.0001; time: $F_{3,163}$ = 2008, P < 0.0001 and interaction of food treatment X time: $F_{6,163}$ = 116.7, P < 0.0001; two way ANOVA; Figure 14); tail length (food treatment: $F_{2,163}$ = 98.78, P < 0.0001; time: $F_{3,163}$ = 319.7, P < 0.0001 and interaction of food treatment X time: $F_{6,163}$ = 34.70, P < 0.0001; two way ANOVA; Figure 15); IOD (food treatment: $F_{2,122}$ = 21.69, P < 0.0001; time: $F_{2,122}$ = 29.59, P < 0.0001 and interaction of food treatment X time: $F_{4,122}$ = 1.302, P = 0.2732; two way ANOVA; Figure 16); IND (food treatment: $F_{2,122}$ = 18.77, P = < 0.0001; time: $F_{2,122}$ = 219.3, P < 0.0001 and interaction of food treatment X time: $F_{4,122}$ = 31.58, P < 0.0001; two way ANOVA; Figure 17); MTH (food treatment: $F_{1,122}$ = 11.841, P < 0.0001; time: $F_{3,122}$ = 196.2, P < 0.0001 and

interaction of food treatment X time: $F_{27,122}$ = 12.91, < 0.0001; two way ANOVA; Figure 18). In comparison to 12h food at either time of the day growth was faster in ad libitum group (P<0.05; Bonferroni test; Figure 13-18). However, 12h food at either time of the day had similar response.

When we compared among the groups having food only during the day time we found effect of time of food availability on growth development and metamorphosis; body weight (food treatment: $F_{3,356}$ = 325.4, P < 0.0001; time: $F_{10,1356}$ = 90.733, P < 0.0001 and interaction of food treatment X time: $F_{30,356}$ = 12.71, P < 0.0001; two way ANOVA; Figure13); body length (food treatment: $F_{3,356}$ = 54.56, P < 0.0001; time: $F_{10,1356}$ = 172.9, P < 0.0001 and interaction of food treatment X time: $F_{30,356}$ = 3.048, P < 0.0001; two way ANOVA; Figure 14); tail length (food treatment: $F_{3,356}$ = 214.8, P < 0.0001; time: $F_{10,1356}$ = 142.3, P < 0.0001 and interaction of food treatment X time: $F_{30,356}$ = 8.169, P < 0.0001; two way ANOVA; Figure 15); IOD (food treatment: $F_{3,360}$ = 7.067, P < 0.0001; time: $F_{3,360}$ = 29.59, P < 0.0001 and interaction of food treatment X time: $F_{3,360}$ = .5493, P = 0.9690; two way ANOVA; Figure 16); IND (food treatment: $F_{3,360}$ = 62.80, P = < 0.0001; time: $F_{3,360}$ = 15.98, P < 0.0001 and interaction of food treatment X time: $F_{3,360}$ = 6.859, P < 0.0001; two way ANOVA; Figure 17); MTH (food treatment: $F_{1,360}$ = 169.5, P < 0.0001; time: $F_{3,360}$ = 60.09, P < 0.0001 and interaction of food treatment X time: $F_{27,360}$ = 4.098, P < 0.0001; two way ANOVA; Figure 18). Compared to 12h food, 4h groups had further delayed in the development of tadpoles (Fig. 13-18).

When we compared among the groups having food only during the night time we found effect of time of food availability on growth development and metamorphosis; body weight (food treatment: $F_{3,446}$ = 301.4, P < 0.0001; time: $F_{3,446}$ = 164.9, P < 0.0001 and interaction of food treatment X time: $F_{3,446}$ = 10.58, P < 0.0001; two way ANOVA; Figure 13b); body length (food treatment: $F_{3,446}$ = 184.0, P < 0.0001; time: $F_{3,446}$ = 401.0, P < 0.0001 and interaction of

food treatment X time: $F_{3,446}$ = 6.062, P < 0.0001; two way ANOVA; Figure 14b); tail length (food treatment: $F_{3,446}$ = 135.7, P < 0.0001; time: $F_{3,446}$ = 246.8, P < 0.0001 and interaction of food treatment X time: $F_{3,446}$ = 5.279, P < 0.0001; two way ANOVA; Figure 15b); IOD (food treatment: $F_{3,384}$ = 54.31, P < 0.0001; time: $F_{3,384}$ = 120.5, P < 0.0001 and interaction of food treatment X time: $F_{3,384}$ = 1,693, P = 0.0180; two way ANOVA; Figure 16b); IND (food treatment: $F_{3,384}$ = 63.74, P = < 0.0001; time: $F_{3,384}$ = 134.5, P < 0.0001 and interaction of food treatment X time: $F_{3,384}$ = 2.213, P = 0.0006; two way ANOVA; Figure 17b) and MTH (food treatment: $F_{3,384}$ = 59.98, P < 0.0001; time: $F_{9,384}$ = 107.3, P < 0.0001 and interaction of food treatment X time: $F_{27,384}$ = 3.212, P < 0.0001; two way ANOVA; Figure 18b). Compared to 12h food, 4h groups had further delayed in the development of tadpoles (Fig. 13-18).

When we compared between the first 4h day and first 4h night time food availability groups, we found effect of time of food availability on growth, development and metamorphosis; body weight (food treatment: $F_{1,164}$ = 58.97, P < 0.0001; time: $F_{10,164}$ = 22.35, P < 0.0001 and interaction of food treatment X time: $F_{10,164}$ = 2.759, P = 0.0036; two way ANOVA; Figure 13d); body length (food treatment: $F_{1,164}$ = 85.16, P < 0.0001; time: $F_{10,164}$ = 63.85, P < 0.0001 and interaction of food treatment X time: $F_{10,164}$ = 3,871, P < 0.0001; two way ANOVA; Figure 14d); tail length (food treatment: $F_{1,164}$ = 101.0, P < 0.0001; time: $F_{10,164}$ = 67.62, P < 0.0001 and interaction of food treatment X time: $F_{10,164}$ = 4.834, P < 0.000; two way ANOVA; Figure 15d); IOD (food treatment: $F_{1,122}$ = .01234, P =1.000; time: $F_{9,122}$ = 30.02, P = 1.000 and interaction of food treatment X time: $F_{9,122}$ = 0.0322, P = 1.0000; two way ANOVA; Figure 16d); IND (food treatment: $F_{1,122}$ = .01321, P = 1.000; time: $F_{9,122}$ = 40.07, P = 1.0000 and interaction of food treatment X time: $F_{9,122}$ = .1214, P = 1.0000; two way ANOVA; Figure 17d) and MTH (food treatment: $F_{3,122}$ = 89.31, P < 0.0001; time: $F_{9,122}$ = 22.01, P < 0.0001 and

interaction of food treatment X time: $F_{27,122}$ = 4.101, P = 0.0051; two way ANOVA; Figure 18d). Growth was relatively faster under first 4h night group comparison to first 4h day group ($P < 0.05$; Bonferroni test; Figure 13-18).

Days	Body weight (g)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.36±.03	6.56±.03			
10	0.20±0.01	7.73±0.10	16.60±0.31	4.06±0.04	1.50±0.02	4.63±0.10
20	0.55±0.04	12.70±0.36	31.01±0.87	6.18±0.14	2.57±0.06	7.36±0.19
30	0.48±0.04	15.35±0.73	13.90±4.20	5.76±0.14	1.98±0.06	5.56±0.68

Table 11: Table 11 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food ad libitum conditions in the tadpoles as time progressed.

Days	Body weight (g)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.38±.01	6.56±.03			
10	0.08±0.00	6.36±0.07	13.62±0.22	3.39±0.05	1.35±0.03	3.97±0.09
20	0.20±0.02	9.05±0.22	20.92±0.24	4.70±0.09	1.84±0.05	6.24±0.13
30	10.62±0.19	25.62±0.40	5.39±0.10	2.16±0.05	6.99±0.14	0.29±0.02
40	0.39±0.02	11.44±0.22	28.16±0.72	5.67±0.10	2.37±0.05	7.13±0.19
50	0.48±0.03	12.57±0.31	29.58±0.78	6.09±0.10	2.45±0.05	7.44±0.16
60	0.50±0.04	13.03±0.41	27.76±2.60	6.20±0.12	2.55±0.09	6.45±0.20
70	0.49±0.04	13.26±0.47	27.21±2.88	6.27±0.15	2.68±0.13	6.61±0.24
80	0.61±0.05	13.8±0.47	31.26±0.97	6.86±0.15	2.96±0.09	8.24±0.26
90	0.67±0.08	14.22±0.40	32.82±0.69	6.98±0.11	2.90±0.08	8.08±0.12
100	0.67±0.03	14.12±0.29	32.10±0.83	6.78±0.13	2.89±0.04	8.40±0.27

Table 12: Table 12 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during 12h of light phase in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.35±.03	6.56±.03			
10	0.08±0.01	6.39±0.09	14.13±0.15	3.48±0.04	1.41±0.03	3.94±0.08
20	0.19±0.01	8.92±0.27	21.08±0.41	4.70±0.09	1.86±0.04	5.90±0.14
30	0.34±0.02	10.95±0.25	26.72±0.47	5.46±0.09	2.16±0.06	6.87±0.16
40	0.42±0.02	11.99±0.25	28.04±0.54	5.77±0.07	2.48±0.05	7.45±0.20
50	0.39±0.02	12.69±0.44	22.83±3.29	5.70±0.11	2.26±0.08	7.25±0.26
60	0.44±0.04	12.57±0.31	27.98±0.92	5.98±0.11	2.48±0.06	6.86±0.34
70	0.51±0.05	12.99±0.45	27.59±1.34	6.36±0.13	2.73±0.07	7.29±0.36
80	0.57±0.04	13.67±0.26	28.37±0.99	6.87±0.08	2.88±0.04	8.37±0.24
90	0.62±0.05	14±0.35	28.9±0.98	6.7±0.15	2.86±0.06	7.96±0.16
100	0.69±0.01	13.99±0.20	31.12±0.53	6.66±0.17	2.58±0.11	7.22±0.36

Table 13: Table 13 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during 12h of dark phase in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.39±.03	6.56±.03			
10	0.03±0.00	5.2±0.05	11.41±0.22	3.01±0.02	1.17±0.01	3.18±0.05
20	0.02±0.00	5.27±0.08	11.33±0.36	2.93±0.04	1.24±0.02	2.91±0.06
30	0.05±0.00	5.80±0.15	12.06±0.68	3.19±0.10	1.29±0.04	3.01±0.12
40	0.06±0.00	6.26±0.34	13.38±0.78	3.26±0.15	1.45±0.04	3.35±0.18
50	0.07±0.01	6.76±0.35	14.42±1.07	3.75±0.13	1.51±0.05	3.82±0.33
60	0.10±0.03	7.20±0.34	15.58±1.32	3.85±0.27	1.56±0.10	3.93±0.37
70	0.12±0.04	7.9±0.81	16.48±2.16	4.12±0.45	1.71±0.21	4.53±0.80
80	0.15±0.07	8.67±1.20	16.50±1.75	4.67±0.50	1.93±0.24	5.20±0.91
90	0.23±0.13	9.8±2.40	19.75±5.30	5.25±0.92	2.1±0.57	5.35±0.92
100	0.24±0.13	9.57±3.24	18.79±5.88	4.99±0.91	2.05±0.42	6.05±1.74

Table 14: Table 14 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during first 4h of light phase (ZT1- ZT4) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.38±.03	6.56±.03			
10	0.05±0.00	5.48±0.08	12.46±0.15	3.09±0.02	1.21±0.02	3.27±0.05
20	0.05±0.00	6.23±0.09	13.81±0.19	3.42±0.05	1.41±0.03	3.55±0.09
30	0.14±0.01	7.44±0.22	15.66±0.68	4.00±0.08	1.57±0.04	4.75±0.30
40	0.17±0.02	8.77±0.30	18.73±0.64	4.54±0.16	1.78±0.06	5.29±0.24
50	0.19±0.02	9.28±0.32	20.27±0.75	4.84±0.15	1.87±0.07	5.71±0.22
60	0.24±0.02	9.92±0.26	21.15±0.73	5.24±0.09	2.09±0.06	6.15±0.28
70	0.25±0.03	10.01±0.51	21.95±0.81	3.47±0.68	2.16±0.06	6.05±0.36
80	0.27±0.03	10.67±0.42	22.09±0.92	5.59±0.14	2.32±0.08	6.63±0.36
90	0.29±0.03	11.07±0.47	22.58±1.25	5.72±0.22	2.35±0.12	6.22±0.56
100	0.32±0.05	11.04±0.45	22.48±1.29	5.58±0.18	2.28±0.09	6.01±0.33

Table 15: Table 15 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during middle 4h of light phase (ZT4- ZT8) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.37±.03	6.56±.03			
10	0.05±0.00	5.59±0.07	12.60±0.18	3.11±0.03	1.22±0.02	3.24±0.08
20	0.05±0.00	6.25±0.13	13.97±0.61	3.42±0.07	1.37±0.05	3.47±0.14
30	0.08±0.01	6.99±0.27	15.96±0.76	3.90±0.19	1.59±0.07	4.12±0.27
40	0.11±0.02	7.42±0.34	16.65±0.99	4.14±0.21	1.70±0.07	4.62±0.35
50	0.12±0.02	7.81±0.43	17.26±1.24	4.18±0.22	1.70±0.10	4.88±0.42
60	0.13±0.04	7.76±0.62	17.36±2.00	4.33±0.31	1.76±0.12	4.68±0.70
70	0.15±0.04	8.26±0.81	17.99±2.13	4.60±0.41	1.88±0.15	5.15±0.63
80	0.18±0.05	9.04±0.78	18.32±2.26	4.78±0.42	1.93±0.16	5.38±0.79
90	0.22±0.06	9.36±1.04	19.26±2.60	5.06±0.53	2.10±0.22	6.28±0.83
100	0.25±0.06	10.01±1.01	20.17±1.40	5.48±0.52	2.21±0.23	6.81±0.93

Table 16: Table 16 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during last 4h of light phase (ZT8- ZT12) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.36±.03	6.56±.03			
10	0.04±0.00	5.52±0.09	12.61±0.13	3.53±0.41	1.19±0.03	3.25±0.06
20	0.06±0.01	6.51±0.35	12.18±0.59	3.57±0.17	1.40±0.08	3.40±0.26
30	0.14±0.03	8.02±0.60	17.00±1.05	4.28±0.25	1.69±0.10	4.93±0.44
40	0.18±0.03	8.71±0.47	19.33±1.23	4.73±0.29	1.89±0.13	5.83±0.53
50	0.22±0.04	9.52±0.55	20.83±1.28	5.14±0.24	2.05±0.10	6.31±0.38
60	0.25±0.04	10.25±0.54	22.06±1.39	5.42±0.25	2.23±0.12	6.78±0.48
70	0.26±0.05	10.22±0.69	22.39±1.37	5.51±0.29	2.27±0.15	6.79±0.57
80	0.28±0.05	10.81±0.78	22.81±1.51	5.83±0.36	2.40±0.17	6.83±0.62
90	0.34±0.06	11.6±0.68	24.8±1.53	6.07±0.27	2.52±0.14	7.23±0.64
100	0.40±0.08	11.65±0.89	24.53±1.58	5.91±0.34	2.43±0.17	7.92±0.56

Table 17: Table 17 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during first 4h of dark phase (ZT12- ZT16) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.37±.01	6.56±.03			
10	0.04±0.00	5.61±0.11	12.21±0.17	3.12±0.04	1.23±0.08	3.16±0.08
20	0.07±0.00	6.83±0.13	12.52±0.52	3.76±0.07	1.61±0.03	3.81±0.10
30	0.13±0.01	8.45±0.19	17.37±0.47	4.59±0.06	1.73±0.03	5.03±0.15
40	0.15±0.01	8.85±0.12	18.55±0.40	4.80±0.08	1.86±0.04	5.59±0.14
50	0.16±0.01	9.17±0.11	19.86±0.48	5.20±0.09	2.03±0.04	6.02±0.15
60	0.19±0.01	9.53±0.11	20.64±0.36	5.27±0.06	2.13±0.04	6.29±0.11
70	0.20±0.01	10.28±0.19	20.65±0.61	5.38±0.06	2.17±0.04	6.05±0.15
80	0.23±0.01	9.90±0.13	21.13±0.51	5.51±0.10	2.26±0.06	6.31±0.21
90	0.22±0.02	10.01±0.23	21.20±0.59	5.49±0.11	2.25±0.06	6.48±0.23
100	0.23±0.02	10.13±0.27	21.39±0.81	5.53±0.11	2.25±0.07	6.08±0.20

Table 18: Table 18 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during middle 4h of dark phase (ZT16- ZT20) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.37±.03	6.56±.03			
10	0.04±0.00	5.78±0.10	11.59±0.19	3.24±0.03	1.26±0.02	3.40±0.09
20	0.08±0.01	7.09±0.15	12.88±0.38	4.01±0.09	1.66±0.03	3.92±0.16
30	0.12±0.01	8.36±0.24	17.20±0.59	4.48±0.14	1.73±0.05	4.57±0.18
40	0.14±0.01	8.50±0.25	17.27±0.61	4.47±0.17	1.73±0.06	4.92±0.22
50	0.17±0.02	9.18±0.33	19.24±0.76	5.00±0.22	1.97±0.0	5.59±0.27
60	0.21±0.02	9.52±0.34	20.36±0.70	5.26±0.20	2.12±0.10	5.98±0.36
70	0.24±0.02	9.92±0.38	20.33±0.76	5.39±0.18	2.22±0.11	6.34±0.24
80	0.23±0.03	10.41±0.42	21.53±0.96	5.51±0.20	2.28±0.11	6.34±0.42
90	0.25±0.03	10.56±0.43	22.55±0.98	5.61±0.20	2.36±0.13	6.74±0.33
100	0.29±0.03	11.06±0.46	22.71±1.09	5.88±0.15	2.46±0.09	7.24±0.29

Table 19: Table 19 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to food available during last 4h of dark phase (ZT20- ZT24) in the tadpoles as time progressed.

When we compared between the middle 4h day and middle 4h night time food availability groups, we found effect of time of food availability on growth development and metamorphosis; body weight (food treatment: $F_{1,230}$ = 25.62, P < 0.0001; time: $F_{1,230}$ = 105.7, P < 0.0001 and interaction of food treatment X time: $F_{1,230}$ = 2.823, P = 0.0025; two way ANOVA; Figure 13e); body length (food treatment: $F_{1,230}$ = 14.56, P < 0.0001; time: $F_{1,230}$ = 342.7, P < 0.0001 and interaction of food treatment X time: $F_{1,230}$ = 4.972, P < 0.0001; two way ANOVA; Figure 14e); tail length (food treatment: $F_{1,230}$ = 4.185, P = 0.0292; time: $F_{1,230}$ = 225.2, P < 0.0001 and interaction of food treatment X time: $F_{1,230}$ = 1.427, P = .1694; two way ANOVA; Figure 15e); IOD (food treatment: $F_{1,179}$ = .75.60, P =1.000; time: $F_{9,179}$ = 4.698, P < 0.0001 and interaction of food treatment X time: $F_{9,179}$ = 1.501, P = .1506; two way ANOVA; Figure 16e); IND (food treatment: $F_{1,179}$ = 3.569, P = .0451; time: $F_{9,122}$ = 101.8, P < 0.0001 and interaction of food treatment X time: $F_{9,179}$ = .1.706, P = .0895; two way ANOVA; Figure 17e); MTH (food treatment: $F_{3,179}$ = 1.685, P = 0.1958; time: $F_{9,202}$ = 77.65, P < 0.0001 and interaction of food treatment X time: $F_{9,202}$ = .5540, P = 0.8334; two way ANOVA; Figure 18e).

When we compared between the last 4h day and last 4h night time food availability groups, we found effect of time of food availability on growth development and metamorphosis; body weight (food treatment: $F_{1,192}$ = 19.06, P < 0.0001; time: $F_{10,192}$ = 37.74, P < 0.0001 and interaction of food treatment X time: $F_{10,192}$ = 1.076, P = 0.3826; two way ANOVA; Figure 13f); body length (food treatment: $F_{10,192}$ = 54.57, P < 0.0001; time: $F_{10,192}$ = 98.98, P < 0.0001 and interaction of food treatment X time: $F_{10,192}$ = 1.884, P = 0.0495; two way ANOVA; Figure 14f); tail length (food treatment: $F_{10,192}$ = 15.98, P < 0.0001; time: $F_{10,192}$ = 81.56, P < 0.0001 and interaction of food treatment X time: $F_{10,192}$ = 2.075, P = .0283; two way ANOVA; Figure 15f); IOD (food treatment: $F_{1,163}$ = 41.28, P < 0.0001; time: $F_{9,163}$ = 33.89, P < 0.0001 and interaction

of food treatment X time: $F_{9,163}$ = .9730, P = .4644; two way ANOVA; Figure 16f); IND (food treatment: $F_{1,163}$ = 34.00, P < 0.0001; time: $F_{9,163}$ = 34.15, P < 0.0001 and interaction of food treatment X time: $F_{9,163}$ = 1.166, P = .3204; two way ANOVA; Figure 17f); MTH (food treatment: $F_{1,163}$ = 17.60, P < 0.0001; time: $F_{9,163}$ = 26.13, P < 0.0001 and interaction of food treatment X time: $F_{9,163}$ = .6919, P = 0.7156; two way ANOVA; Figure 18f).

4.2 Experiment 2: Effect of food quality on the growth and development of tadpole

There was differential effect of food quality on growth and development of tadpoles; cabbage group (body weight: $F_{4,68}$ = 272.1, P < 0.0001; body length: $F_{4,68}$ = 681.1, P < 0.0001; tail length: $F_{4,68}$ = 685.4, P < 0.0001; IOD: $F_{4,68}$ = 136.0, P < 0.0001; IND: $F_{4,68}$ = 43.51, P < 0.0001; MTH: $F_{4,68}$ = 77.60, P < 0.0001; 1-way ANOVA; Figure 19; table 20), protein rich food (body weight: $F_{4,80}$ = 162.2, P < 0.0001; body length: $F_{4,80}$ = 441.0, P < 0.0001; tail length: $F_{4,80}$ = 359.9, P < 0.0001; IOD: $F_{4,80}$ = 110.3, P < 0.0001; IND: $F_{4,80}$ = 52.23, P < 0.0001; MTH: $F_{4,80}$ = 136.0, P < 0.0001; 1-way ANOVA; Figure 19; table 5; table 21).

There was a significant effect of the food quality on the growth of the tadpoles as observed in the study; body weight (food treatment: $F_{1,148}$ = 55.84, P < 0.0001; time: $F_{4,148}$ = 331.3, P < 0.0001 and interaction of food treatment X time: $F_{4,148}$ = 9.218, P < 0.0001; two way ANOVA; Figure 19a); body length (food treatment: $F_{1,148}$ = 20.47, P < 0.0001; time: $F_{4,148}$ = 934.7, P < 0.0001 and interaction of food treatment X time: $F_{4,148}$ = 3.362, P = 0.0115; two way ANOVA; Figure 19b); tail length (food treatment: $F_{1,148}$ = 42.48, P < 0.0001; time: $F_{4,148}$ = 782.5, P < 0.0001 and interaction of food treatment X time: $F_{4,148}$ = 6.445, P < 0.0001; two way ANOVA; Figure 19c); IOD (food treatment: $F_{1,113}$ = 1.333, P = .2507; time: $F_{3,113}$ = 216.8, P <

0.0001 and interaction of food treatment X time: $F_{3,113}$ = 1.194, P = 0.3155; two way ANOVA; Figure 19d); IND (food treatment: $F_{1,113}$ = .07684, P = .7820; time: $F_{3,113}$ = 91.44, P < 0.0001 and interaction of food treatment X time: $F_{3,113}$ = 2.685, P = 0.0491; two way ANOVA; Figure 19e); MTH (food treatment: $F_{1,113}$ = 32.24, P < 0.0001; time: $F_{3,113}$ = 190.4, P < 0.0001 and interaction of food treatment X time: $F_{3,113}$ = 4.442, P = 0.0055; two way ANOVA; Figure 19f). in general growth was faster under protein rich food comparison to the cabbage group (P< 0.05; Bonferroni test; Figure 19).

Forelimb appearance was directly influenced by the quality of food supplied (Fig. 20a). In protein rich food 47% tadpoles had forelimb by the fifth week and developed in all tadpoles by seventh week (Fig. 20a). In cabbage group forelimb started to develop by sixth week and took eight week to had forelimbs in all tadpoles (Fig. 20a). Metamorphosis showed the similar trend and in protein rich food froglet were started to be formed by the fifth week and all tadpoles were metamorphosed by the seventh week (Fig. 20b). While in cabbage group metamorphosis started by the sixth week completed by the eight week (Fig. 20b).

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.37±.03	6.56±.03			
10	0.09±0.01	7.80±0.12	17.17±0.36	4.53±0.06	1.77±0.03	4.71±0.13
20	0.28±0.02	11.30±0.22	23.63±0.50	5.90±0.11	2.29±0.05	6.11±0.16
30	0.50±0.03	13.20±0.26	28.63±0.41	6.71±0.09	2.77±0.06	7.65±0.13
40	0.81±0.03	15.16±0.17	34.00±0.53	7.21±0.13	2.79±0.12	7.82±0.23

Table 20: Table 20 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to normal food (cabbage) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.35±.03	6.56±.03			
10	0.11±.01	8.03±.11	18.37±0.39	4.42±0.06	1.83±0.02	4.78±.12
20	0.41±0.03	12.06±0.34	27.34±0.73	5.94±0.15	2.35±0.07	7.18±0.16
30	0.71±0.03	14.51±0.22	33.12±0.84	6.98±0.09	2.92±0.08	8.62±0.16
40	1.10±0.06	16.27±0.25	36.93±1.11	7.44±0.07	2.40±0.14	8.66±0.21

Table 21: Table 21 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to protein rich food in the tadpoles as time progressed.

4.3 Experiment 3: Effect of temperature on the growth and development of tadpoles

Temperature played a significant role on the growth development cycle of tadpoles and differential effects were observed under high and low temperature treatments. In general growth and development took place under both the temperatures, body weight: (high temperature: $F_{3,96}$ = 411.7, P < 0.0001; low temperature: $F_{10,183}$ = 73.36, P < 0.00011-way ANOVA; Figure 21a; table 22, 23), Body length: (high temperature: $F_{3,96}$ = 1317, P < 0.0001; low temperature: $F_{10,183}$ = 125.8, P < 0.00011-way ANOVA; Figure 21b), tail length: (high temperature: $F_{3,96}$ = 1908, P < 0.0001; low temperature: $F_{10,183}$ = 196.4, P < 0.00011-way ANOVA; Figure 21c), IOD: (high temperature: $F_{3,96}$ = 77.67, P < 0.0001; low temperature: $F_{10,183}$ = 80.72, P < 0.00011-way ANOVA; Figure 21d), IND: (high temperature: $F_{3,96}$ = 131.0, P < 0.0001; low temperature: $F_{10,183}$ = 56.75, P < 0.00011-way ANOVA; Figure 21e), MTH: (high temperature: $F_{3,96}$ = 4.505, P = 0.0146; low temperature: $F_{10,183}$ = 34.12, P < 0.00011-way ANOVA; Figure 21f; table 6).

There was a significant effect of temperature conditions on growth and development of tadpoles; body weight (temperature treatment: $F_{1,178}$ = 342.3, P < 0.0001; time: $F_{3,178}$ = 385.2, P < 0.0001 and interaction of temperature treatment X time: $F_{3,178}$ = 43.21, P = 0.0616; two way ANOVA; Figure 21a); body length (temperature: $F_{1,178}$ = 127.09, P < 0.0001; time: $F_{3,178}$ = 637.7, P < 0.0001 and interaction of temperature treatment X time: $F_{3,178}$ = 19.73, P < 0.0001; two way ANOVA; Figure 21b); tail length (temperature: $F_{1,178}$ = 608.6, P < 0.0001; time: $F_{3,178}$ = 1670, P < 0.0001 and interaction of temperature treatment X time: $F_{3,178}$ = 71.67, P = 0.2877; two way ANOVA; Figure 21c); IOD (temperature: $F_{1,127}$ = 150.4, P < 0.0001; time: $F_{1,127}$ = 120.6, P < 0.0001 and interaction of temperature treatment X time: $F_{1,127}$ = 4.882, P = 0.0091; two way ANOVA; Figure 21d); IND (temperature: $F_{1,127}$ = 185.8, P < 0.0001; time: $F_{1,127}$ = 176.3, P < 0.0001 and interaction of temperature treatment X time: $F_{1,127}$ = 5.251, P = 0.0063;

two way ANOVA; Figure 21e) and MTH (temperature: $F_{1,127} = 120.5$, $P < 0.0001$; time: $F_{1,127} = 26.10$, $P < 0.0001$ and interaction of temperature treatment X time: $F_{1,127} = 5.695$, $P = 0.0042$; two way ANOVA; Figure 21f). In general, growth and development were faster under high temperature ($P < 0.05$, Bonferroni test; Figure 21).

Temperature has clear effects on rate of metamorphosis. Forelimb development was much faster in high temperature group. By 4th week of experiment 35% tadpoles had forelimb and by week 5 85% tadpoles had the forelimb developed and reaming tadpoles had forelimb developed by week 6 of study (Fig. 22a). Rate of forelimb development was much slower under low temperature treatment. Only 15% tadpoles had forelimb by week 9 and it took 12 weeks to have forelimb in all the tadpoles (Fig. 22a). Froglet formation was significantly faster in high temperature treatment group and by week 5 39% froglets were formed and by week 6 all the tadpoles metamorphosed into froglets (Fig. 22b). Under low temperature treatment rate of metamorphosis was slowed and by week 10 46% tadpoles metamorphosed into froglets while it took 14 weeks to complete the metamorphosis (Fig. 22b).

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IND (mm)	MTH (mm)
1	0.01	3.48±.03	6.56±.036			
10	0.38±0.01	12.33±0.19	22.99±0.29	5.70±0.09	2.12±0.04	6.77±0.19
20	0.50±0.02	13.56±0.18	26.92±5.09	6.55±1.24	2.79±0.53	7.38±1.39
30	0.71±0.02	14.61±0.15	30.54±0.31	7.13±0.05	3.10±0.04	7.46±0.23

Table 22: Table 22 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to high temperature (30 ±2℃).) in the tadpoles as time progressed.

Days	Body weight (gm)	Body length (mm)	Tail length (mm)	IOD (mm)	IN (mm)	MTH (mm)
1	0.01	3.38±.01	6.56±.03			
10	0.11±.01	7.76±.21	15.21±.21	4.27±.1	1.73±0.04	5.01±0.12
20	0.25±0.02	11.21±0.60	19.78±0.49	5.34±0.12	2.09±0.06	5.66±0.16
30	0.37±0.02	11.93±0.26	22.63±0.55	6.35±0.12	2.58±0.06	6.67±0.14
40	0.47±0.02	13.01±0.19	25.19±0.37	6.59±0.10	2.75±0.06	7.24±0.15
50	0.58±0.04	13.95±0.23	27.55±0.57	6.89±0.09	2.95±0.04	7.37±0.20
60	0.72±0.05	14.84±0.32	29.53±0.78	7.28±0.11	3.11±0.10	7.46±0.21
70	0.65±0.06	14.97±0.54	28.66±1.50	7.23±0.11	3.19±0.11	7.88±0.24
80	0.76±0.09	15.34±0.62	30.17±1.12	7.58±0.28	3.20±0.15	8.01±0.36
90	0.97±0.12	16.93±0.81	32.25±1.23	8.16±0.27	3.47±0.10	9.06±0.31
100	1.07±0.11	17.23±1.06	34.99±1.63	7.96±0.25	3.03±0.17	8.57±0.51

Table 23: Table 23 shows various morphological parameters (Mean ± SE values) of the tadpoles exposed to low temperature (20 ±2℃).) in the tadpoles as time progressed.

5. DISCUSSION

In this study, we examined the effects of food and temperature on the growth and development of *P. teraiensis* tadpoles. Here we demonstrate that timed restricted food availability has great impact on growth and development of tadpoles. Food restricted only to 12h has significant reduction in growth compared to ad libitum group. Similarly, food available only for 4h has further delayed the developmental processes in tadpoles. 4h food restriction during the day time has more severe effect comparison to the night time as tadpoles under 4h food during first phase of night had faster growth compare to the group having food at first 4h during day time. Present study also emphasise that protein rich food promotes the faster growth as shown previously for *Rhacophorus maximus* tadpoles which share same breeding habitat with *P. teraiensis* (Borah *et al.*, 2018).

In our study we clearly observed the effects of nutritional quality of food on growth and development. Tadpoles fed on food rich in nutrition (protein rich food) have much faster growth and development in comparison to tadpoles fed on cabbage (less nutritional food). In protein rich food group 47% of population was metamorphosed by fifth week of experiment and all tadpoles metamorphosed by sixth week of study. However, in cabbage group growth was relatively much slower and metamorphosis begins by sixth week of experiment and all tadpoles metamorphosed by eight week of study. Effect of food availability on growth and development has been documented in few studies. In larvae of yellow spotted mountain newts *Neurergus microspilotus*, interaction of temperature and food levels influenced the growth and development rates. Earlier metamorphosis was shown in the larvae grown at the high food level and high temperature and exhibited greatest snout to vent length (SVL) compared with individuals raised at relatively low food level and low temperature (Vaissi and Sharifi, 2012). Few previous studies have

demonstrated that dietary protein accelerates metamorphosis in tadpoles (Kupferberg, 1997; Álvarez and Nicieza, 2002; Denver, 1997), by influencing the thyroid hormone production. Our results are consistent with these findings, where the protein level in the diet appears to have some effect on growth, development and metamorphosis which is accelerated with diet having supplementary nutrition.

Among ectotherms, lower temperature conditions of winter season are unfavourable for growth and reproduction. Most of these ectotherms must attain a certain developmental stage or a minimum body size before the winter approach, and then enter a dormant period (Tauber *et al.*, 1986; Danks, 1987; Gotthard, 2001, 2008; Bradshaw and Holzapfel, 2007). Our results also suggest that temperature has significant effect and plays crucial role on growth and metamorphosis of *Polypedates teraiensis* tadpoles. Over the experimental period, tadpole growth rate was higher at high temperature. Similar results have also been observed in yellow spotted mountain newts *Neurergus microspilotus* where tadpoles grown at relatively low temperature, metamorphosis time was significantly longer compared with individuals raised at relatively high temperature. High temperature also favours the growth and development in the endangered green and golden bell frog; *Litoria aurea* (Browne and Edwards, 2003). Role of temperature has also been studied in feeding behaviour of tadpoles and high temperature favours high ingestion in *Rana clamitans* (Warkentin, 1992). As the higher temperature is the indicator of the summer season and therefore it appears to favour the faster growth and development in comparison to the lower winter temperature. Effect of temperature on feeding rate of green frog tadpoles (*Rana clamitans*) demonstrated that the tadpoles ingest significantly less under low temperature comparison to high temperature (Warkentin, 1992).

Our study shows that with varying environmental conditions with the timed food availability and type of food availability and temperature as it may vary in the wild with changing environmental conditions may influence the growth and development rate hence the metamorphosis timing of *Polypedates teraiensis* tadpoles.

VIII. SECTION 6: TO STUDY THE DAILY EXPRESSION OF CLOCK GENES IN TREE FROG

1. ABSTRACT

In Amphibians, independent circadian clocks have been documented in the photoreceptor organs; retina. However, less is known of the molecular clockwork, residing in the brain and other peripheral tissues. In the present study we investigated the molecular clock by monitoring the daily expression of four canonical clock genes (*Bmal1, Clock, Per3* and *Cry*2) in Terai tree frog (*Polypedates teraiensis*). We measured their 24-h profiles in brain, liver and testes tissues, collected at six times; ZT1 1 ZT5, ZT9, ZT13, ZT17, and ZT21. All four-clock gene studied showed the daily variation in all the three tissues studied. The results are consistent with the autoregulatory circadian feedback loop as reported for other vertebrates, and indicate a conserved tissue-level circadian time generation in Terai tree frogs. Variable phase relationships between gene forming positive and negative limbs of the feedback loop suggest the tissue-specific contribution of individual core circadian genes in the circadian time generation.

2. INTRODUCTION

Almost all organisms exhibit circadian (circa = about; dies = day) rhythms. These robust circadian rhythms in physiological functions and behaviours are conserved across all organisms, from cyanobacteria to humans. These rhythms are output of an intrinsic endogenous mechanism called the circadian clock, which generates self- sustaining rhythms with a periodicity of about (*circa*) 1 day (*dies*). The circadian rhythms are intrinsic, however, living organisms are also under the influence of cyclic changes of environments, such as day–night cycles and seasonal changes in photoperiods and temperature. Theses environmental cues or "Zeitgebers" help organisms to time their clock and help to maintain periodicity referred as entrainment. For all organisms, light is the strongest Zeitgeber. The circadian clock entrains to a 24-h light–dark (LD) cycle resulting in diurnal, nocturnal, or crepuscular behaviour. Most of our understanding for the circadian clock comes from the studies on mammalian system. The mammalian circadian system comprises a central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus which regulates additional circadian rhythms found in non-SCN brain regions and in other peripheral organs throughout the body (Weaver and Reppert, 2002, Mohawk *et al.,* 2012, Yamazaki *et al.,* 2000). In mammals the light signals are transmitted to the SCN via the retinohypothalamic tract (RHT). The SCN is the only clock that is reset by the light from the environment. Once the SCN pacemaker is light-entrained, it synchronizes the peripheral clocks to the same 24-h cycle through mechanisms that remain largely unknown.

The first known mammalian clock gene, *Clock,* encoding a basic helix-loop-helix (bHLH) Period-Arnt-Sim (PAS)- type transcription factor, was cloned in 1997 (Gekakis *et al.,* 1998), which was followed by the cloning of *Period 1* (*Per1*) (Tie *et al.,* 1997) and *Bmal1* (Ikeda and Nomura, 1997, Honma *et al.,* 1998), encoding a PAS protein and a bHLH-PAS transcription factor, respectively. BMAL1 heterodimerizes with CLOCK and binds to an E-box enhancer element of the *Per* gene and induces transcription of *Per*. The PER protein then translocates to the nucleus with cryptochrome (CRY) and binds to the CLOCK/ BMAL1 heterodimer to inhibit enhancer activity (Kume *et al.,* 1999). One cycle of this negative feedback loop takes about 24h, and generates circadian rhythmicity. Along with the core molecular feedback loop consisting of *Per1, Per2, Cry1, Cry2, Clock*, and *Bmal1*, interlocking feedback loops have also been identified to regulate circadian rhythms. *Bmal1* is regulated by a retinoic acid receptor-related orphan receptor (ROR) enhancer site located upstream of the *Bmal1* gene; *Ror* binding activates gene expression, while *Rev-Erbα* binding inhibits transcription (SatoTK Panda *et al.,* 2004). *Reb-Erbα* itself is regulated by an E-box enhancer located upstream of its transcription start site, resulting in an expression pattern that subsequently puts *Bmal1* completely out of phase with *Per1* and *Per2* (Preitner *et al.,* 2002). *Dec1* and *Dec2* are other two elements of an interlocking loop which negatively affect circadian rhythms (Honma *et al.,* 2002). Both *Dec1* and *Dec2* have E-box enhancers and are positively regulated by *Clock* and *Bmal1*, and negatively by *Per1*, *Per2*, *Cry1*, and *Cry2*. Furthermore, the transcription of *Dec1* and *Dec2* is self-regulated by their encoded proteins. DECs also form a mutually interlocked molecular loop with the *Per* loop. These interlocked multiple molecular loops are thought to be advantageous by providing stability and fine tuning to the circadian periodicity (Ueda *et al.,* 2005).

In non-mammalian vertebrates most of the literature available from studies on birds. In birds, separate circadian oscillators lie in the retina, pineal, and hypothalamus, interacts with each other and with the external periodic environment (e.g., day-night cycle) produce timing at the physiological level (Kumar *et al.,* 2004). Thus, birds are recognized with having a central circadian pacemaker system comprising three independent oscillators, although the degree of their self-sustainment and contribution to the circadian time generation can vary among the bird species (Gwinner and Brandstatter, 2001; Kumar *et al.,* 2004). The avian orthologues of core circadian clock genes have been cloned and characterized in few avian species, namely Japanese quail (*Coturnix c. japonica*; Yasuo *et al.,* 2003; Yoshimura *et al.,* 2000), chicken (*Gallus domesticus*; Bailey *et al.,* 2003, 2004; Karaganis *et al.,* 2008, 2009), house sparrow (*Passer domesticus*; Abraham *et al.,* 2002, 2003; Brandstatter *et al.,* 2001; Helfer *et al.,* 2006), *Emberiza melanocephala* (Singh *et al.,* 2015). However, general mechanism of working of avian circadian clock is similar to the mammals.

However, knowledge about clock genes expression in amphibian tissues is limited to the photoreceptor organs. In present study we examine daily expression of transcripts levels of four clock genes (*Bmal1, Clock, Per3* and *Cry2*) in three tissues (brain, liver and testis) of Terai tree frog.

3. MATERIAL AND METHODS

The experiment was done on male frogs that were procured in the month of April. Animals (N=5 per time) were sacrificed at six times at ZT1, ZT5, ZT9, ZT13, ZT17, and ZT21 (ZT0, zeitgeber time 0, sun rise time). The brain, liver, and testes were immediately removed, kept in RNA Later solution (Thermo Fisher Scientific; AM 7020), and stored at -80 ^{0}C. The experiment was carried out in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

3.1 RNA isolation and measurement of mRNA expression:

The total RNA was extracted using Trizol solution (Ambion AM9738; USA), as per manufacturer's protocol. Trizol is a mixture of guanidine thioacyanate and phenol, which effectively dissolves DNA, RNA and protein on homogenization or lysis of tissue sample. This procedure is very effective for isolating RNA molecules of all types from 0.1 to 15kb.

3.1.1 Reagents required:

Fallowing reagents were required to extract RNA

Trizol

Chloroform

Ethanol absolute

Isopropanol

1.5 And 2ml eppendorf tubes – autoclaved and oven dried.

5ml tubes for homogenization

DNase-RNase free water

3.1.2 RNA preparation:

1. Homogenization:

Tissue samples were homogenized by adding 1 ml of Trizol (Life Technologies; 1556018) reagent with silica beads (1.0 mm dia. Zirconia/Silica; Unigenetics Instruments Pvt; Ltd.;11079110z) in a homogenizer tube using mini bead-beater (Biospec products). Homogenized samples were allowed to stand for 5 minutes at room temperature.

2. Phase separation:

0.3 ml of chloroform was added per 1ml of Trizol used. The samples were capped tightly and vortexed vigorously for 15 seconds. Then allow to stand for 5 minutes at room temperature. The resulting mixture was centrifuged at 12,000rpm for 15min at 4 ^{0}C. Centrifugation separates the mixture into lower red, phenol-chloroform phase, an interphase and upper aqueous phase (containing RNA). The colourless upper aqueous phase was then transferred to a new clean tube, using a 200 µl pipette and avoiding the interphase (containing the DNA).

3. Total RNA precipitation:

0.75ml of isopropanol was added per 1ml of Trizol reagent and mixed gently by inverting the sample for 15 sec and incubated at room temperature for 10 min. The mixture was centrifuged at 13,000 rpm for 15 min at 4 ^{0}C. The RNA precipitates, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube after centrifugation.

4. RNA wash:

The supernatant was removed and the RNA pellet was washed by adding 1ml of 75% ethanol per 1ml of Trizol used in sample preparation. It was mixed gently by inverting the sample a few times. The above washing process was repeated twice

5. RNA drying and resuspension:

The supernatant was removed and air dried for 5-10 minutes by inverting the tube on a clean wipe**.**

6. Resuspension of pellet in DNase-RNase free water:

RNA was dissolved in DNase-RNase free water (Ambion; AM 9938) by passing solution a few times through a pipette tip.

3.1.3 Spectrophotometric analysis

2ul of the suspended RNA and 298 µl of DEPC treated water were mixed. Using 300, OD was taken at 260 nm and 280 nm to determine sample. Concentration and purity using spectrophotometer (Eppendorf; Biophotometer plus). Apply the convention that 1 OD at 260 equals 40 µg /ml RNA.

3.2 cDNA synthesis

1 µg RNA treated with DNase (Promega, M610A) was used for cDNA preparation by Revert Aid First Strand cDNA synthesis kit (Thermo Scientific, K1622).

3.2.1 DNAse Treatment:

DNAse treatment was done using Promega kit (Promega; RNase-free DNase; M610A). The following reagents were added into a sterile, nuclease free tube on ice in the indicated order:

DNA/RNAse free H2O – 6 µl

RNA - 2 µl

RQ Buffer - 1 µl

RQ Enzyme - 1 µl

PCR was performed in a thermal cycler for 30 minutes.

Followed by adding 1 ul of stop solution. – 10 minutes

Buffer - 4 µl

dNTP mix - 2 µl

Random primers – 1 µl

Ribolock - 1 µl

Rivertaid - 1µl - 1 hour

3.3 Quantitative (Real-time) RT-PCR (qPCR)

The quantitative PCR (qPCR) measured the transcripts of *Bmal1*, *Clock*, *Per3* and *Cry2* genes using gene-specific primers (Table 24). Primer 3 plus online program was used to design the qPCR primers. qPCR was performed by Applied Biosystems Quant Studio 5 real-time PCR, using power SYBR green PCR master mix (Applied Biosystems, Life Technology, 4367659). A total reaction volume of 7 µl for each gene comprised 1 µl each of cDNA and forward and reverse primers, 3 µl Power SYNR Green PCR Master mix (ABI 4387669) and 1 µl of nuclease-free water. Each PCR plate included beta-actin as an endogenous control, and the non-template and sample controls that lacked reverse transcriptase in the cDNA synthesis. Each sample was run in duplicate. The fluorescence exceeding the background level gave the cycle threshold (Ct) for every replicate, which was averaged and used to calculate ΔCt for each sample, using the formula (Ct [target gene] – Ct [reference gene]). Thus, we had five ΔCt values (one per sample) for each time point. At ZT1, the highest ΔCt value was identified, which was subtracted individually from other remaining ΔCt values of ZT1 and all other time points; this gave the

ΔΔCt. From this, the fold change in relative mRNA expression levels was calculated using the formula $2^{-(\Delta\Delta Ct)}$ (Livak and Schmittgen, 2001).

3.4 Statistical analysis

To test the significance in variation in transcripts levels among six daily time points we performed one-way analysis of variance (one-way ANOVA) followed by Newman-Keuls post hoc test. Values describing daily Clock gene profiles were analysed by cosinor analyses based on unimodal cosinor regression [$y=A.+ (B. \cos (2\pi (x - C)/24))$], where A, B, and C denote the mean level (mesor), amplitude, and acrophase of the rhythm, respectively (Cuesta et al., 2009). The significance of regression analysis determined at $p < 0.05$ was calculated using the number of samples, R^2 values, and numbers of predictors (mesor, amplitude, and acrophase; Soper, 2013; http://www.danielsoper.com/statcalc3/calc.aspx?id=15).

4. RESULTS

Figure 23 shows daily profile of *Bmal1, Clock, Per3* and *Cry2* expression in brain, liver and testis tissue of tree frog at six times during the month of April. All the four genes showed daily variation in all the three tissues, *Bmal1* (brain: $F_{5,35}$ = 10.75, P <0.0001; liver: $F_{5,35}$ = 11.03, P <0.0001; testis: $F_{5,35}$ = 8.610, P <0.0001; One way ANOVA; Figure 23a,e,i), *Clock* (brain: $F_{5,35}$ = 11.75, P <0.0001; liver: $F_{5,35}$ = 10.03, P <0.0001; testis: $F_{5,35}$ = 3.386, P = 0.0153; One way ANOVA; Figure 23b,f,j), *Cry2* (brain: $F_{5,35}$ = 4.196, P = 0.0052; liver: $F_{5,35}$ = 5.303, P = 0.0013; testis: $F_{5,35}$ = 6.311, P = 0.0004; One way ANOVA; Figure 23c,g,k) and *Per3* (brain: $F_{5,35}$ = 4.273, P = 0.0045; liver: $F_{5,35}$ = 2.731, P = 0.0378; testis: $F_{5,35}$ = 3.464, P = 0.0137; One way ANOVA; Figure 23d,h,l). We observed tissue specific peak expression of clock genes. *Bmal1* transcript peak expression was observed during middle of day in the brain and liver tissue i.e., at ZT 7.3 in brain and at ZT 8.3 in liver (Table 8). However, in testis Bmal1 transcripts peaked at the late day time i.e., at ZT 11(Table 8). Clock transcript was also peaked during day time in brain (ZT 8) and liver tissue (ZT 9.5) while during the late day time in testis (ZT 11.5). Cry2 transcript peaked during the day time in all the tissues (brain: ZT6, liver: ZT 7.3 and testis: ZT9). Per3 transcripts peak was observed during late night hours in brain (ZT 20.5), early morning in liver (ZT 1.2) and during late day *time* in testis (ZT 9.3, Table 8).

We determined the relationship in the peak expression timing of clock genes expression in different tissues with reference to their expression in the brain, as brain is the central clock in all vertebrates. As compared with the peak expression time in the brain, *Bmal1* peak was delayed in the liver and testis by 1 and 4 h respectively (Figure 23; Table 8). Similarly, *Clock* peak was delayed in liver by 1.5h and in testis by 5.5h h. *Cry2* peak expression times in liver was delayed

by 1.3h and in testis by 3h (Table 8). Further, as compared with the time of peak in the brain, *Per3* mRNA peak was delayed by about 4h in liver and by 13h in testis (Figure 23; Table 8).

Gene	F-Primer for qPCR	R- Primer for qPCR
Beta-actin	5’ AGC CAT GCC AAT CTC ATC TC 3’	5’ AAA TGC TTC TAG GCG GAC TG 3’
Bmal1	5’ AGA TGT TCC AAT GGC TGA CC 3’	5’ TGG TAA TCG GTC AAG CTT CC 3’
Clock	5’ TAG GAG CCA TGC AAC ATC TG 3’	5’ TGA CCA TGG ACC ATC TGA AG 3’
Cry2	5’ AGG TGAAAC AGA AGC CTT GG3’	5’ TCTC GGG CCT TTC ATA GTT GG 3’
Per3	5’ AAG GTC ACA AAC GGC AAG AG3’	5’ TGT CAT CAT GAT CGG GGT AG 3’

Table 24: List of forward and reverse primers used for various gens for qPCR analysis.

Gene	Tissue	Mesor	Amplitude	Acrophase
Bmal1	**Brain**	0.7	0.4	7.3
	Liver	0.4	0.4	6.3
	Testis	0.9	1.0	11
Clock	**Brain**	0.8	0.5	9.6
	Liver	0.6	0.4	10.7
	Testis	.0	0.6	11
Per3	**Brain**	1.4	0.6	20.4
	Liver	0.3	0.2	1.2
	Testis	0.6	0.2	1.2
Cry2	**Brain**	0.7	0.5	6
	Liver	0.4	0.5	4.2
	Testis	0.5	0.7	9

Table 25: Table 25 shows various parameters of different genes studied during daily expression of clock genes.

5. DISCUSSION

This is the first comprehensive molecular study of the canonical clock genes in any amphibian species. Present study demonstrates the daily expression of clock genes in the brain, a clock centre, liver a metabolic centre and in testis a reproductive organ and their phase relationships within and between tissues in the central and peripheral tissues. Tree frog clock genes had high sequence homology with those from other species. The 24-h expression profiles (Figure 23) of four clock genes studied also show similarity to those reported in other vertebrates, namely mouse (Gekakis *et al.,* 1998; Jin *et al.,* 1999; Kume *et al.,* 1999) Japanese quail (Yasuo *et al.,* 2003; Yoshimura *et al.,* 2001), chicken (Karaganis *et al.,* 2009), house sparrow (Abraham *et al.,* 2002, 2003; Helfer *et al.,* 2006), Redheaded bunting (Singh *et al.,* 2013; Trivedi *et al.,* 2016), blackheaded bunting (Singh *et al.,* 2015). When we compared the expression timing of *Bmal1* and *Per3* mRNA oscillations, they were anti phased in brain and liver there was peak time expression phasing in the testis tissue (Figures 23). Similar findings have also been reported for other vertebrate species as reported in the rat SCN and peripheral tissues (Oishi *et al.,* 1998), chicken (Okano and Fukada, 2001; Karaganis *et al.,* 2009), and quail SCN, MBH, and pineal (Yasuo *et al.,* 2003), red headed bunting (Singh *et al.,* 2013). Clock mRNA transcripts had variable temporal patterns among tree frog tissues but showed daily oscillation in all the tissues (Figure 23). A daily oscillations of *Clock* transcripts has also been reported in other vertebrates. Clock mRNA is reported acyclic in quail brain (Yoshimura *et al.,* 2000), sparrow hypothalamus (Helfer *et al.,* 2006). On the other hand, *Clock* shows a constant expression level throughout the day in many tissues in mammals, although a low amplitude oscillation with high and low levels around mid-day and mid-night, respectively, is reported in the rat SCN (Abe *et al.,* 1999). In

Drosophila, however, Clock mRNA cycles with two peaks (ZT5 and ZT23; Darlington *et al.*, 1998).

The *Per3* mRNA expressed in all the three tissues and had a significant daily rhythm, with high levels during late night in brain, early daytime in liver and during middle of day in testis (Figure 23). Similar oscillations of period gene have been reported in other vertebrates as in the *Per* gene oscillations in mice (Albrecht *et al.,* 1997), quail (Yoshimura *et al.*, 2000), and chicken (Bailey *et al.,* 2004; Okano *et al.,* 2001), house sparrow (Helfer *et al.,* 2006). *Per3* mRNA expression in brain of house sparrow is reported 2h advance than *Per3* mRNA and it peaked at ZT 22.5 (Helfer *et al.,* 2006). It supports the idea of PER abundance are critical for sustaining the circadian oscillations (Chen *et al.*, 2009; Yamamoto *et al.*, 2005). *Cry* genes which constitute the negative-limb partner genes, had similar transcriptional rhythms, but established a different phase relationship with the light phase (Figure 1). *Cry2* mRNA peaked in all the tissues, between ZT6 and ZT9 (Figures 23), as in the chicken retina (ZT8; Haque *et al.*, 2002), sparrow hypothalamus (ZT6.5; Helfer *et al.,* 2006), and quail central and peripheral tissues (e.g., pineal, hypothalamus, muscle, liver, and skin; Fu *et al.*, 2002; Yasuo *et al.*, 2003), bunting (ZT 8-10; Singh *et al.*, 2013). The phase relationship between *Per* and *Cry* (Figure 23) suggests that *Cry* is necessary for the circadian clock function in tree frogs (Ukai-Tadenuma *et al.*, 2011).

In *Xenopus laevis* circadian clock has been documented in the photoreceptors that controls the synthesis and release of melatonin (Cassone, 1998; Green, 2003). *Per1*, *Per2* and *Cry1* transcripts rhythmically expresses in the retina of *Xenopus leavis* in LD with a phase difference of 4–8 h. *Per1* peaks at light onset and *Per2* peaks during the light phase in response to light (Zhuang *et al.*, 2000; Zhu and Green, 2001). Clock gene oscillation has also been

demonstrated in in cultured melanophores under light–dark cycles. The expression of *Per1*, *Per2* and *Bmal1* showed temporal variation exclusively in LD (Moraes *et al.*, 2014).

In summary, our data confirm the existence of similarities between the amphibian and other vertebrate circadian clockwork. However, difference occur regarding phase relationships of clock genes with the light zeitgeber as well as phase relationships of certain genes to each other. As most of other studies had done on the mammalian and avian system very limited knowledge is available for amphibian clock work. Therefore, the present study on non-mammalian circadian clockworks, might represent a useful tool to clarify aspects of the evolution of molecular timing systems in vertebrates.

REFERENCES

Aarseth KA, Schram TA. (1999). Wavelength-specific behaviour in *Lepeophtheirus salmonis* and *Calanus finmarchicus* to ultraviolet and visible light in laboratory experiments (Crustacea: Copepoda). Mar. Ecol. 186: 211–217.

Abe H, Honma S, Namihira M. (1999). Phase dependent induction by light of rat clock gene expression in the suprachiasmatic nucleus. Mol. Brain. Res. 66: 104–10.

Abraham U, Albrecht U, Gwinner E, Brandsta¨tter R. (2002). Spatial and temporal variation of passer Per2 gene expression in two distinct cell groups of the suprachiasmatic hypothalamus in the house sparrow (*Passer domesticus*). Eur. J. Neurosci. 16: 429–36.

Abraham U, Albrecht U, Brandsta¨tter R. (2003). Hypothalamic circadian organization in birds. II. Clock gene expression. Chronobiol. Int. 20: 657–69.

Aichinger M. (1987). Annual activity patterns of anurans in a seasonal neotropical environment. Oecologia. 71: 583-592.

Albrecht U, Sun ZS, Eichele G, Lee CC. (1997). A differential response of two putative mammalian circadian regulators, mPer1 and mPer2, to light. Cell. 91: 1055–64.

Altig R. (2007). A primer for the morphology of Anuran tadpoles. Herpitol. Conserv. Biol. 2: 71-74.

Álvarez D, Nicieza G. (2002). Effects of temperature and food quality on anuran larval growth and metamorphosis. Funct. Ecol. 16: 640-648.

Ao JM, Bordoloi S. (2000). Annual breeding cycle and spawning behaviour of *Hyla annectans*, Jerdon, 1870 in Nagaland. India. Cur. Sci. 79(7): 943-945.

Amburgey SM, Miller DAW, Campbell GEH, Rittenhouse TAG, Benard MF *et al.*, (2017). Range position and climate sensitivity: the structure of among-population demographic responses to climatic variation. Glob. Chang. Biol. 24: 439-454.

Arak A. (1983). Sexual selection by male–male competition in natterjack toad choruses. Nature. 306: 261–262.

Araújo M, Thuiller W, Pearson R. (2006). Climate warming and the decline of amphibians and reptiles in Europe. J. Biogeogr. 33(10): 1712–1728.

Arendt JD. (1997). Adaptive intrinsic growth rates: an integration across taxa. Q. Rev. Biol. 72: 150–177.

Aschoff J. (1981). A survey on biological rhythms. Handbook of behavioural Neurobiology. 4: 3-10.

Baggerman B. (1972). Photoperiodic responses in the stickleback and their control by a daily rhythm sensitivity. Gen. Comp. Endocrinol. Suppl. 3: 466-476.

Bahir MM, Meegaskumbura M, Manamendra-ArachchiK, Schneider CJ, Pethiyagoda R. (2005). Reproduction and terrestrial direct development in Sri Lankan shrubfrogs (Ranidae: Rhacophorinae: Philautus). The Raffles Bulletin of Zoology. 12: 339–350.

Bailey MJ, Beremand PD, Hammer R. (2003). Transcriptional profiling of the chick pineal gland, a photoreceptive circadian oscillator and Pacemaker. Mol. Endocrinol. 17: 2084–95.

Bailey MJ, Beremand PD, Hammer R. (2004). Transcriptional profiling of circadian patterns of mRNA expression in the chick retina. J. Biol. Chem. 279: 52247–54.

Baker JR. (1938a). The evolution of breeding seasons. Evolution. 161-177.

Baker JR. (1938b). The relation between latitude and breeding seasons in birds. Proc. Zool. Soc. Lond. 108A: 557-582.

Ball JN. (1981). Hypothalamic control of the pars distalis in fishes, amphibians, and reptiles. Gen. Comp. Endocrinol. 44: 135-170.

Bambozzi AC, Filho JTS, Thomaz LA, Oshiro LMY. (2004). Efeito do fotoperíodo sobre o desenvolvimento de girinos de rã-touro (*Rana catesbeiana* Shaw, 1802). R. Bras. Zootec. 33: 1-7.

Băncilă RI, Ozgul A, Hartel T, Sos T, Schmidt BR. (2016). Direct negative density-dependence in a pond-breeding frog population. Ecography (Cop). 39(5): 449–455.

Beazley LD. (1979). Intertectal connections are not modified by visual experience in developing *Hyla moorei*. Exp. Neurol. 63: 411-419.

Beebee TJC. (1995). Amphibian breeding and climate. Nature. 374(6519): 219–220.

Beebee TJC, Griffiths RA. (2005). The amphibian decline crisis: a watershed for conservation biology? Biol. Conserv. 125(3): 271–285.

Benard MF. (2015). Warmer winters reduce frog fecundity and shift breedingphenology, which consequently alters larval development and metamorphic timing. Glob. Chang. Biol. 21(3):1058–1065.

Benitez-Mandryano MA, Flores–Nava A. (1997). Growth and metamorphosis of *Rana catesbeiana* (Shaw) tadpoles fed live supplementary feed, using tilapia, *Oreochromis niloticus* (L) as biofertilizer. Aquac. Res. 28: 481–486.

Benoit J. (1964). The structural components of the hypothalmo-hypophyseal pathway, with particular refrence to photostimulation of the gonads in birds. Ann N Y Acad Sci. 117: 204-216.

Benoit J, Assenmacher 1. (1970). La photorrégulation de la reproduction chez les oiseaux et les marnmifbres. CNRS, Paris.

Bertoluci J. (1998). Annual patterns of breeding activity in Atlantic Rainforest anurans. J. Herpetol. 32: 607-611.

Berven KA, Gill DE, Smith-Gill SJ. (1979). Counter gradient selection in the green frog, *Rana clamitans*. Evolution. 33: 609–623.

Bevier CR. (1997). Breeding activity and chorus tenure of two neotropical hylid frogs. Herpetologica. 53: 297-311.

Bhardwaj SK, Anushi. (2004). The effect of duration and time of food availability on the photoperiodic response in the male house sparrow, *Passer domesticus.* Reprod. Nutr. Dev. 44: 29-35.

Biju SD, Shouche Y, Dubois A, Dutta SK, Bossuyt F. (2010). A ground-dwelling rhacophorid frog from the highest mountain peak of the Western Ghats of India. Curr. Sci. 98: 1119–1125.

Bissonnette TH. (1931). Studies on sexual cycle of birds. IV. Experimental modification of the sexual cycle in males of the European starling *(Sturnus vulgaris)* by changes in daily period of illumination and by muscular work. J. Exp. Zool. 58: 281-320.

Bittman EL, Karsch FJ, Hopkins JW. (1983). Role of the Pineal Gland in Ovine Photoperiodism: Regulation of Seasonal Breeding and Negative Feedback Effects of Estradiol upon Luteinizing Hormone Secretion. Endocrinol. 113: 329–336.

Blair WF. (1960). A breeding population of the mexican toad (*Bufo valliceps*) in relation to its environment. Ecology. 41: 165-174.

Blair WF. (1961). Calling and spawning seasons in a mixed population of anurans. Ecology. 42: 99-110.

Blakemore C, Cooper GF. (1970). Development of the brain depends on the visual environment. Nature. 228: 477-478.

Blankenhorn HJ. (1972). Meteorological variables affecting onset and duration of calling in *Hyla arborea* L. and *Bufo calamita calamita* Laur. Oecologia. 9: 223-234.

Blaustein AR, Walls SC, Bancroft BA, Lawler JJ, Searle CL, Gervasi SS. (2010). Direct and indirect effects of climate change on amphibian populations. Diversity. 2(2): 281–313.

Borah BK, Renthlei Z, Trivedi AK. (2018). Photic and non-photic regulation of growth, development, and metamorphosis in ginat tree frog (Rhacophorus maximus) tadpoles. Bio. Rhyth. Res. 1-13.

Borg B. (1982). Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the three-spined stickleback *Gasterosteus aculeatus* L. Can. J. Zoo. 60(12): 3377-3386.

Bortamuli T, Bordoloi S, Ohler A, Grosjean S. (2010). External morphology, bucco pharyngeal anatomy and development rate of the tadpoles of two Asian Ranidae (Amphibia: Anura), *Hylarana humeralis* (Boulenger, 1887) and *Hylarana leptoglossa* (Cope, 1868). J. Nat. Hist. 44(7&8): 421-445.

Boswell T. (1991). The Physiology of migratory fattening in the European quail*(Coturnix coturnix)*. Ph.D. Thesis. Department of Zoology, University of Bristol, U.K.

Both C, Kaefer IL, Santos TG, Cechin STZ. (2008). An austral anuran assemblage in the Neotropics: seasonal occurrence correlated with photoperiod. J. Nat. Hist. 42: 205-222.

Bourne GR. (1997). Reproductive behavior of terrestrial breeding frogs *Eleutherodactylus johnstonei* in Guyana. J. Herpetol. 31: 221-229.

Bowen KD, Spencer RJ, Janzen F. (2005). A comparative study of environmental factors that affect nesting in Australian and North American freshwater turtles. J. Zool. Lond. 267: 397–404.

Bradshaw WE, Holzapfel CM. (2007). Evolution of animal photoperiodism. Annu. Rev. Ecol. Evol. Syst. 38: 1–25.

Brandstatter R, Abraham U, Albrecht U. (2001). Initial demonstration of rhythmic Per gene expression in the hypothalamus of a non-mammalian vertebrate, the house sparrow. Neuroreport. 12: 1167–70.

Briggs SR. (1987). Breeding Biology of the Cascade Frog, *Rana cascadae*, with Comparisons to R. aurora and R. pretiosa. Copeia. 1: 241-0245.

Brown RK, Zippel K. (2007). Reproduction and larval rearing of Amphibians. J. ILAR. 48(3): 214-234.

Browne RK, Edwards DL. (2003). The effect of temperature on the growth and development of the endangered green and golden bell frog (*Litoria aurea).* J. Therm. Biol. 28: 295–299.

Buchholz DR, Moskalik CL, Kulkarni SS, Hollar AR, Ng A. (2011). Hormone regulation and evolution of frog metamorphic diversity. Mechanisms of Life History Evolution. 87–97.

Bullough WS. (1961). Vertebrate sexual cycles. Methuen. 123.

Burger JW. (1947). On the relation of day length of the spermatogonic cycle of the starling. J. Exp. Zool.105: 259- 267.

Bush FM. (1963). Effects of light and temperature on the gross composition of the toad *Bufo fowleri*. J. Exp. Zool. 153: 1–13.

Bustos-ObregónE. (1979). Efectosde latemperatuay la luzsobre la actividad testicular en vertebrados. Arch. Biol. Med. Exp. 12: 133-142.

Calder WA. (1971). Temperature relationships and nesting of the Calliope hummingbird. Condor. 73: 314–321.

Callard IP, Ho SM. (1979). Seasonal reproductive cycle in reptiles. Prog. Reprod. Biol. 5:5-38.

Campanella C, Carotenuto R, Infante V, Maturi G, Atripaldi U. (1997). Sperm-egg interaction in the painted frog (*Discoglossus pictus*): An ultrastructural study. Mol. Reprod. Dev.:47(3): 323-333.

Canavero A, Arim M. (2009). Clues supporting photoperiod as the main determinant of seasonal variation in amphibian activity. J. Nat. Hist. 43: 2975-2984.

Carroll EA, Sparks TH, Collinson N, Beebee TJC. (2009). Influence of temperature on the spatial distribution of first spawning dates of the common frog (*Rana temporaria*) in the UK. Glob. Chang. Biol.15(2): 467–473.

Case TJ. (1978). On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. Q. Rev. Biol. 53: 243–282.

Cassone VM. (1998). Melatonin's role in vertebrate circadian rhythms. Chronobiol. Int. 15: 457–473.

Cayuela H, Besnard A, Béchet A. (2012). Reproductive dynamics of three amphibian species in Mediterranean wetlands: the role of local precipitation and hydrological regimes. Freshw. Biol. 57(12): 2629–2640.

Cayuela H, Besnard A, Bonnaire E, Perret H, Rivoalen J, Miaud C, Joly P. (2014). To breed or not to breed: past reproductive status and environmental cues drive current breeding decisions in a long-lived amphibian. Oecologia. 176(1): 107–116.

Cayuela H, Arsovski D, Boitaud S, Bonnaire E, Boualit L, Miaud C, Joly P, Besnard A. (2015) Slow life history and rapid extreme flood: demographic mechanisms and their consequences on population viability in a threatened amphibian. Freshw. Biol. 60(11): 2349–2361.

Cayuela H, Arsovski D, Bonnaire E, Duguet R, Joly P, Besnard A. (2016a). The impact of severe drought on survival, fecundity, and population persistence in an endangered amphibian. Ecosphere. 7(2): e01246.

Cayuela H, Arsovski D, Thirion J-M, Bonnaire E, Pichenot J, Boitaud S, Miaud C, Joly P, Besnard A. (2016b). Demographic responses to weather fluctuations are context dependent in a long-lived amphibian. Glob. Chang. Biol. 22(8): 2676–2687.

Cayuela H, Joly P, Schmidt BR, Pichenot J, Bonnaire E, Priol P, Peyronel O, Laville M, Besnard A. (2017). Life history tactics shape amphibians' demographic responses to the North Atlantic Oscillation. Glob. Chang. Biol. 23(11): 1–19.

Chan-McLeod ACA. (2003). Factors affecting the permeability of clearcuts to red-legged frogs. J. Wildl. Manag. 67(4): 663–671.

Chen C, Huang YY, Liao WB. (2016). A comparison of testes size and sperm length between Polypedates megacephalus populations at different altitudes. J Herpetol. 26(3): 249-252

Chen R, Schirmer A, Lee Y. (2009). Rhythmic PER abundance defines a critical nodal point for negative feedback within the circadian clock mechanism. Mol. Cell. 36: 417–30.

Cheng IJ, Huang CT, Hung PY, Ke BZ, Kuo CW, Fong CL. (2009). Ten years of monitoring the nesting ecology of the green turtle, *Chelonia mydas*, on Lanyu (Orchid Island), Taiwan. Zoological Studies. 48: 83–94.

Chum HH, Felt SA, Garner JP, Green SL. (2013). Biology, behavior, and environmental enrichment for the captive African clawed frog (Xenopus spp). Applied Animal Behav. Sci. 143(2-4): 150-156

Chung SH, Gaze RM, Stirling RV. (1973). Abnormal visual function in Xenopus following stroboscopic illumination. Nature. 246: 186-189.

Collins JP. (1979). Intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeiana*. Ecology. 60: 738–749.

Collins JP, Storfer A. (2003). Global amphibian declines: sorting the hypotheses. Divers. Distrib. 9(2): 89–98.

Collins JP, Wilbur HM. (1979). Breeding habits and habitats of the amphibians of the Edwin S. George Reserve, Michigan, with notes on the local distribution of fishes. Occas. Pap. Univ. Michigan Mus. Zool. 686: 1-34.

Conover DO, Schultz ET. (1995). Phenotypic similarity and the evolutionary significance of counter gradient variation. Trends. Ecol. Evol. 10: 248–252.

Copp NH, McKenzie T. (1984). Effects of Light-Deprivation on Development of Photopositive Behavior in *Xenopus laevis* Tadpoles. J Exp. Zool. 230: 219-228.

Crawshaw LI, Rausch RN, Wollmuth LP, Bauer EJ. (1992). Seasonal rhytms of development and temperature selection in larval bullfrogs, *Rana catesbiana.* Shaw. Physiol. Zool. 65: 346–359.

Cross NL. (1981). Initiation of the activation potential by an increase in intracellular calcium in eggs of frog, *Rana pipiens*. Dev. Biol. 85: 380-384.

Crump P, Houlahan J. (2017). The impacts of overwintered green frog larvae on wood frog embryo mortality under a wetter climate. Freshw. Biol. 62(7): 1244–1254.

Culley DD, Sotiaridis PK. (1984). Progress and problems associated with bullfrog tadpole diets and nutrition. 3rdAnnual Dr. Scholl Conference on Nutrition of wild and captive wild animals. l23–148.

Currie SP, Doherty GH, Sillar KT. (2016). Deep-brain photoreception links luminance detection to motor output in Xenopus frog tadpoles. Proc. Nat. Acad. Sci. USA. 113: 6053-6058.

Czykier E, Sawicki B. (1999). Postnatal development of the European bison spermatogenesis. Acta Theriol. 44: 77-90.

Danks HV. (1987). Insect Dormancy: An Ecological Perspective. Biological Survey of Canada Monograph series, No1. Entomological Society of Canada, Canada.

Darlington TK, Wagner-Smith K, Ceriani FM. (1998). Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. Science. 280: 1599–603.

Das M, MahaPatra PK. (2012): Blood cell profiles of the tadoles of the dubois's tree frog, *Polypedates teraiensis* (Dubois), 1986 (anura: rhacophoridae).- The Scientific World Journal.

Das M, Mahapatra PK. (2014). Hematology of wild caught dubois's tree frog *Polypedates teraiensis*, Dubois, 1986 (Anura: Rhacophoridae). The Scientific World Journal.

Daszak P, Scott DE, Kilpatrick AM, Faggioni C, Gibbons JW, Porter D. (2005). Amphibian population declines at Savannah River Site are linked to climate, not chytridiomycosis. Ecology 86(12): 3232–3237.

Daunt F, Afanasyey V, Silk RD, Wanless S. (2006). Extrinsic and intrinsic determinants of winter foraging and breeding phenology in a temperate seabird. Behav.Ecol. Sociobiol. 59: 381–388.

Davis CL, Miller DAW, Walls SC, Barichivich WJ, Riley JW, Brown ME. (2017). Species interactions and the effects of climate variability on a wetland amphibian metacommunity. Ecol. Appl. 27(1): 285–296.

Dawson A, Goldsmith AR, Nicholls TJ. (1986). Seasonal changes in testicular size and in plasma FSH and prolactin concentrations in thyroidectomised male and thyroidectomized castrated starlings *(Sturnus vulgaris)*. Gen. Comp. Endocrinol. 63: 38-44.

Delgado MJ, Gutierrez P, Alonso-Bedate M. (1987). Melatonin and photoperiod alter growth and larval devel opment in *Xenopus laevis* tadpoles. Comp. Biochem. Physiol. A. 86: 417–421.

Delgado MJ, Gutierrez P, Alonso-Bedate M. (1989). Seasonal cycles in testicular activity in the frog, *Rana perezi*. Gen. comp. endocrinol. 73(1): 1-11.

Delgado MJ, Alonso-Gomez, Alonso-Bedate M. (1992). Role of environmental temperature and photoperiod in regulation of seasonal testicular activity in the frog, *Rana perezi*. Can. J. Physiol. Pharmacol. 70: 1348-1352.

Deuti K. (2001) Breeding ecology of Annandale's tree frog *Chirixalus simus* (Anura: Rhacophoridae) near Kolkata, West Bengal. J. Bombay nat. Hist. Soc 98(3): 341-346.

de Vlaming UL. (1974). Environmental and endocrine control of teleost reproduction. In: Control of Sex in Fishes (Schreck ed.) Sea Grant U.P.I. and S.U., Blacksburg, Va. 13-83.

de Vlaming VL, Olcese J. (1981). The pineal gland and reproduction in fish, amphibians and reptiles. In: The Pineal Gland Vol 2. Reiter R.J. (ed.). C.R.C. Press. Boca Raton.1-30.

Denver RJ. (1997). Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. Am. Zool. 37: 172-184.

Denver RJ. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. Gen. Comp. Endocrinol. 164: 20–31.

Denver RJ, Mirahadi N, Phillips M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology. 79: 1859–1872.

Deviche P, Small T. (2001). Photoperiodic control of seasonal reproduction: Neuroendocrine mechanisms and adaptation. Avian Endocrinol. 113-128.

Díaz-Paniagua C. (1986). Reproductive periods of amphibians in the biological reserve of Donaña (Huelva, Spain). Amphibia-Reptilia. 6: 307-322.

Díaz-Paniagua C. (1988). Temporal segregation in larval amphibian communities in temporary ponds at a locality in SW Spain. Amphibia-Reptilia. 9: 15-26.

DillonME, Wang G, Huey RB. (2010). Global metabolic impacts of recent climate warming. Nature. 467(7316): 704–706.

Dinesh KP, Radhakrishnan C, Gururaja KV, Zacariya A. (2010). New locality record of *Rhacophorus lateralis* Boulenger, 1883 (Amphibia: Anura: Rhacophoridae), in Western Ghats, India. J. Threatened. Texa. 2(6): 986-989.

Dixon JR, Heyer WR. (1968). Anuran succession in a temporary pond in Colima, Mexico. Bull. South. California Acad. Sci. 67: 129-137.

Dodt E. (1973). The panetal eye (pineal and parapineal organs of lower vertebrates). Handbook of Sensory Physiology. 7(B):113-140.

Donnelly MA, Guyer C. (1994). Patterns of reproduction and habitat use in an assemblage of neotropical hylid frogs. Oecologia. 98: 291-302.

Doody J, Guarino S, Georges EA, Corey B, Murray G, Ewert M. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. Evolutionary Ecology. 20: 307–330.

Duellman WE, Klaas LT. (1964). The biology of the hylid frog *Triprion petasatus*. Copeia: 308-321.

Duellman WE. (1995). Temporal fluctuation in abundances of anuran amphibians in a seasonal Amazonian rainforest. J. Herpetol. 29: 13-21.

Dupont W, Bourgeois P, Reinberg A, Vailland R. (1979). Circannual and circadian rhythms in the concentration of corticosterone in the plasma of the edible frog (*Rana esculenta* L.). J. Endocrinol. 80: 117-125

Dutta SK, Mishra S, Rath S. (2001). Breeding and development of *Polypedates maculatus* (Anura: Rhacophoridae). Pranikee: J. zoo. Soc. Orissa. 121-134.

Dutta SK, Nair MV, Mohapatra PP, Mahapatra AK. (2009). Amphibians and reptiles of similipal biosphere reserves. Regional. Plant. Resource. Centre. 52–53.

Elinson RP. (2013). Metamorphosis in a frog that does not have a tadpole. Curr. Top. Dev. Biol. 103: 259–276.

Elliot JA, Stetson MH, Menaker M. (1972). Regulation of testis function in golden hamsters: A circadian clock measures photoperiodic time. Science. 178: 771-773.

Farner DS. (1959). Photoperiodic and related control of annual gonadal cycles in birds. In: Photoperiodism and related phenomena in plants and animals, Eds. R.B. Withrow. Am. Assoc. Advance Sci.Washington. D.C. 716-750.

Farner DS. (1962). Hypothalamic neurosecretion and phosphatase activity in relation to photoperiodic control of testicular cycle of *Zonotrichia leucophrys gambelii*. Gen. Comp. Endocrinol. 1: 160-167.

Farner D, Lewis RA. (1971). Photoperiodism and reproductive cycles in birds. Photophysiology. 6: 325-370.

Farner DS, Mewaldt LR. (1955). Is increased activity or wakefulness an essential element in the mechanism of the photoperiodic response of avian gonads? Northwest. Sci. 29: 53-65.

Farner DS, Donham RS, Matt KS, Mattocks PW, Moore MC, Wingfield JC. (1983). The nature of photorefractoriness. Avian Endocrinology, Environmental and Ecological Perspective. 149-166.

Figueiredo MRC, Lima SL, Agostinho CA, Baêta FC. (2001). Efeito da Temperatura e do Fotoperíodo sobre o Desenvolvimento do Aparelho Reprodutor de Rã-touro (*Rana catesbeiana* Shaw, 1802). R. Bras. Zootec. 30: 916-923.

Fiske VM, Bryant K, Putman J. (1960). Effect of light on the weight of the pineal in the rat. Endocrinology. 66: 489-491.

Follett BK. (1984). Birds. Marshall's physiology of reproduction. 283-350.

Fraile B, Paniagua R, Rodríguez MC. (1988). Long day photoperiods and temperature of 20° C induce spermatogenesis in blinded and non-blinded marbled newts during the period of testicular quiescence. Biol. Reprod. 39: 649-655.

Fraile B, Paniagua R, Rodríguez MC, Sáez FJ. (1989a). Effect of photoperiod on testicular function in blinded and non-blinded marbled newts, *Triturus marmoratus* (Caudata, Salamandridae). J. Morphol. 200: 151-162.

Fraile B, Paniagua R, Sáez FJ, Rodriguez MC. (1989b). Effects of moderately high temperature on the testis of the marbled newt. *Triturus marmoratus.* Amphibia-Reptilia. 10:
117-124.

Frazer JFD. (1976). Anura (Frogs and Toads). UFAW Handbook. 5:516–524.

Fu Z, Inaba M, Noguchi T, Kato H. (2002). Molecular cloning and circadian regulation of cryptochrome genes in Japanese quail (*Coturnix coturnix japonica*). J. Biol. Rhythms.17: 14–27.

Galgano M. (1932). Prime richerche intorno all'inflenza della temperatura sui processi spermatogenetici normale ed aberrantidi *Ranaesculenta.*L. Monit. Zool. Ital. 43: 156-160.

Galgano M. (1934). L'influenza della temperatura sui proccesi spermatogenetic in or male edaberrantidi *Ranaesculenta.* L. Monit. Zool. Ital. 45,83.

Galgano M. (1936). lntorno all'influenza del clima sulla espermatogenesi di *Rana esculenta.*Arch. Ital. Anat. Embriol. 35: 511-541.

Galgano M, Lanza B. (1951). L Influenza della temperatura sulla spermatogenesis e sopra I caracteri sessuali Lau. Monit. Zool. Ital. 51: 166-181.

Gao X, Jin C, Camargo A, Li Y. (2015a). Allocation trade-off under climate warming in experimental amphibian populations. PeerJ. 3: e1326.

Gao X, Jin C, Llusia D, Li Y. (2015b). Temperature-induced shifts in hibernation behavior in experimental amphibian populations. Sci. Rep. 5(1):11580.

Gascon C. (1991). Population- and community-level analyses of species occurrences of central amazonian rainforest tadpoles. Ecology. 72: 1731-1746.

Gaze RM, Keating MJ, Szekely G, Beazley L. (1970). Binocular interaction and the formation of specific intertectal neuronal connections. Roc. R. Soc. Lond. Biol. 175: 107-147.

Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. Science. 280: 1564–1569.

Gerhart J, Wu M, Kirschner M. (1984). Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. J. Cell. Biol. 98(4): 1247-1255.

Gibbs JP, Breisch AR. (2001). Climate warming and calling phenology of frogs near Ithaca, New York, 1900-1999. Conserv. Biol. 15(4): 1175–1178.

Gillespie G, Traher R, Banks C. (2007). ARAZPA Amphibian Action Plan. Report to the Australasian Regional Association of Zoological Parks and Aquaria. 245.

Girish S, Saidapur SK. (2000). Interrelationship between food availability, fat body, and ovarian cycles in the frog, *Rana tigrina*, with a discussion on the role of fat body in anuran reproduction. J. Exp. Zool. 286(5): 487-93.

Gotthard K. (2001). Growth strategies of ectothermic animals in temperate environments. Environment and Animal Development. 287–304.

Gotthard K. (2008). Adaptive growth decisions in butterflies. Biosci. 58: 222–230.

Gotthard K, Nylin S. (1995). Adaptive plasticity and plasticity as adaptation: a selective review of plasticity in animal morphology and life history. Oikos. 74: 3–17.

Gotthard K, Nylin S, Wiklund C. (1994). Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. Oecologia. 99: 281–289.

Griffiths RA, Sewell D, McCrea RS. (2010). Dynamics of a declining amphibian metapopulation: survival, dispersal and the impact of climate. Biol. Conserv. 143(2): 485–491.

Green DM. (2003). The ecology of extinction: population fluctuation and decline in amphibians. Biol. Conserv. 111(3): 331–343.

Grobstein P, Chow KL. (1975). Receptive field development and individual experience. Science. 24: 352- 358.

Guarino FM, Bellini L. (1993). Reproductive activity and plasma androgen concentrations in the male of *Rana dalmatina*. Boll. Zool. 60: 281-286.

Guarino FM, Bellini L, Mazzarella G, Angelini K. (1998). Reproductive activity of *Bombina pachypits* from southern Italy. Ital. J. Zool. 65: 335-342.

Gulve PS. (1994). Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. Ecology. 75(5): 1357-1367.

Gwinner E. (1981). Annual rhythms: perspectives, Handbook of Behavioral Neurobiology. Biological rhythms. 4: 381-389.

Gwinner E. (1986). Circannualrhythms: Endogenous annual clocks in the organization of seasonal processes. Zoophysiology series 18. Springer-Verlag, Berlin.

Gwinner E, Scheuerlein A. (1998). Seasonal changes in day-light intensity as a potential zeitgebers of circannual rhythms in equatorial stonechats. J. Ornithol. 139: 407-412.

Gwinner E, Brandstatter R. (2001). Complex bird clocks. Phil. Trans. R. Soc. Lond. B. 356: 1801–10.

Haddad CFB, Prado CPA. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. Bio. Sci. 55: 207–217

Haddad CFB, Andrade GV, Cardoso AJ. (1988). Anfı´bios anuros no Parque Nacional da Serra da Canastra, Estado de Minas Gerais. Brasil. Florestal. 64: 9–20.

Hagman M, Shine R. (2006). Spawning site selection by feral cane toads (*Bufo marinus*) at an invasion front in tropical Australia. Austral. Ecology. 31(5): 551 – 558.

Halberg F. (1969). Chronobiology. Ann. Rev. Physiol. 31: 675-725.

Halliday TR. (1999). Amphibians. UFAW Hand book on the Care and Management of Laboratory Animals. Oxford. 2: 90–102.

Hamner WM. (1968). The photorefractory period of the house finch. Ecology. 49: 211-227.

Hamann M, Limpus CJ, Whittier JM. (2003). Patterns of lipid storage and mobilization in the female Green Sea Turtle (*Cheloniamydas*). J. Comp. Physiol. B. 172: 485–493.

Haque R, Chaurasia SS, Wessel JH, Iuvone PM. (2002). Dual regulation of cryptochrome1 mRNA expression in chicken retina by light and circadian oscillators. Neuroreport. 13: 2247–51.

Hau M. **(**2001). Timing of breeding in variable environments: Tropical birds as model systems. Horm. Behav. 40: 281-290.

Hau M**,** Gwinner E. (1996). Food as a circadian zeitgeber for house sparrows: the effect of different food access durations. J. Biol. Rhythms. 11: 199-210.

Hatano FH, Rocha SFD, Van Sluys M. (2002). Environmental factors affecting calling activity of a tropical diurnal frog (*Hylodes phyllodes*: Leptodactylidae). J. Herpetol. 36: 314-318.

Hawkes LA, Broderick AC, Coyne MS, Godfrey MH, Godley BJ. (2007). Only some like it hot quantifying the environmental niche of the Loggerhead Sea Turtle. Diversity and Distribution. 13: 447–457

Hayes TB. (1997). Steroids as potential modulators of thyroid hormone activity in anuran metamorphosis. Am. Zool. 37: 185–194.

Hays GC, Broderick AC, Glen F, Godley BJ, Houghton JDR, Metcalfe JD. (2002). Water temperature and internesting intervals for Loggerhead (*Caretta caretta*) and Green (*Chelonia mydas)* Sea Turtles. J. Therm. Biol. 27: 429–432.

Helfer G, Fidler AE, Vallone D. (2006). Molecular analysis of clock gene expression in the avian brain. Chronobiol. Int. 23: 113–27.

Henderson NE. (1963). Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchell). J. Fish. Res. Bd. Can. 20: 859-897

Hero JM. (1990). An illustrated key to tadpoles occurring in the Central Amazon forest, Manaus, Amazonas, Brazil. Amazoniana. 11: 201-262.

Hoffman K. (1968). Synchronization der circadianer aktutaetsperio von Eidechsen durch temperatur cyclen verschied ner. Amplitude. Z. Val. Physiol. 58: 225-228.

Hoffman K. (1981). Photoperiodic function of the mammalian Pineal organ. In: The pineal organ: Photobiology-Biochronometery-Endocrinobiology, Eds. A. Oksche and P. Pevet. Elsvier/North-Holland Biomedical Press. 123-138.

Honma S, Ikeda M, Abe H, Tanahashi Y, Namihira M, Honma K, Nomura (1998). Circadian oscillation of BMAL1, a partner of a mammalian clock gene Clock, in rat suprachiasmatic nucleus. Biochem. Biophys. Res. Commun. 250: 83–87.

Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, Kato Y, Honma K. (2002). Dec1 and Dec2 are regulators of the mammalian molecular clock. Nature. 419: 841–844.

Hoogmoed MS, Gorzula SJ. (1979). Checklist of the savanna inhabiting frogs of the El Manteco region with notes on their ecology and the description of a new species of treefrog (Hylidae, Anura). Zool. Meded. 54: 183-216.

Hoos D. (1937). De vinkenbaan. Hoe het er toeging en wat er mee in verband stond. Ardea. 26: 173-202.

Horseman ND, Smith CA, Culley DD Jr. (1987). Effects of age and photoperiod on ovary size and condition in bullfrogs (*Rana catesbeiana* Shaw) (Amphibia, Anura, Ranidae); J. Herpetol. 12: 287–290.

Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. (2000). Quantitative evidence for global amphibian population declines. Nature. 404(6779): 752–755.

Huang WS, Cheng, YS, Tu HY. (2004). Reproductive Patterns of Two Sympatric Ranid Frogs, *Rana latouchii* and *R. sauteri*, with Comments on Anuran Breeding Seasons in Taiwan. Coll. and Res. 17: 1-10

Hubel DH, Wiesel T. (1963). Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J. Neurophysiol. 26: 994-1002.

Hutchison VH, Hill CG. (1978). Thermal selection of bullfrog tadpoles (*Rana catesbiana*) at different stages of development and acclimation temperatures. J. Therm. Biol. 3: 57–60.

Horseman ND, Smith CA, Culley Jr DD. (1978). Effects of Age and Photoperiod on Ovary Size and Condition in Bullfrogs (*Rana catesbeiana* Shaw) (Amphibia, Anura, Ranidae). J. Herpetol. 12(3): 287-290.

Hota J, Das M, Mahapatra PK. (2013). Blood cell profile of the developing tadpoles and adults of the ornate frog, *Microhyla ornata* (Anura: Microhylidae). Int. J. Zool.

Iela LJ, Powell JF, Sherwood N. (1996). Reproduction in the Mexican leaf frog, *Pachymedusa dacnicolor*. VI. Presence and distribution of multiple GnRH forms in the brain. Gen Comp Endocrinol. 103(3): 235-343.

Ifft JD. (1942). The effect of environmental factors on the sperm cycle of *Truiturus viridescens*. Biol. Bull. 83: 111-128.

Ikeda M, Nomura M. (1997). cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix/PAS protein (BMAL1) and identification of alternatively spliced variants with alternative translation initiation site usage. Biochem. Biophys. Res. Commun. 233: 258–264.

Immelmann K. (1971). Ecological aspects of periodic reproduction. Avian Biol.1: 341-389.

Isorna E, Anton AG, Delgado MJ, Bedate MA, Alonso-Gomez AL. (2003). Characterization of melatonin binding sites in the brain and retina of the frog *Rana perezi*. Gen. Comp. Endocrinol. 135(3): 259-67

Isorna E, Delgado M J, Guijarro AI, Lopez-Patino MA, Alonso-Bedate M, Alonso-Gomez AL. (2004). 2-[125I]-Melatonin binding sites in the central nervous system and neural retina of the frog *Rana perezi*: regulation by light and temperature. Gen. comp. endocrinol. 139(2): 95-102.

Iangrai AJ. (2007). Studies on ecology, breeding behaviour and metamorphosis of tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctaus* (Anura: Rhacophoridae) in Meghalaya (North-East India). Ph. D. thesis, North-Eastern Hill University, Shillong- 793022.

Jacobson M. (1971). Absence of adaptive modification in developing retinotectal connections in frogs after visual deprivation or disparate stimulation of the eye. Proc. Natl. Acad. Sci. USA. 68: 528-532.

Jacobson M, Hirsch HVB. (1973). Development and maintenance of connectivity in the visual system of the frog. The effects of eye rotation and visual deprivation. Brain. Res. 49: 47-65

Jaffe LA, Schlichter LC. (1985). Fertilization-induced ionic conductances in eggs of the frog, *Rana pipiens*. J. Physiol. 358(1): 299-319.

Jain N. (1993). Strategies for endogenous programming in the migratory blackheaded bunting, *Emberiza melanocephala Scopoli*. PhD thesis, Meerut University, Meerut, India.

Jamieson D, Roberts A. (2000). Responses of young *Xenopus laevis* tadpoles to light dimming: possible roles for the pineal eye. J Exp Biol. 203: 1857-1867.

Jeffrey L, Briggs, Sr. (1987). Breeding Biology of the Cascade Frog, *Rana cascadae*, with Comparisons to *R. aurora* and *R. pretiosa*. Copeia. 241-245.

Jin X, Shearman LP, Weaver DR. (1999). A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. Cell. 96: 57-68.

Joglar RL. (1999). Los coquies de Puerto Rico: su historia natural y conservaci6n. Univ. of Puerto Rico Press, San Juan.

Joglar PA, Burrowes, Rios N. (1996). Biology of the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*, and some recommendations for conservation. Contributions to West Indian herpetology: a tribute to Albert Schwartz. Contribution to Herpetology. 12: 251-258.

Jørgensen CB. (1986). External and internal control of patterns of feeding, growth and gonadal function in a temperate zone anuran, the toad *Bufo bufo*. J. Zool. London. 210(2): 211–241.

Jφrgensen CB, Hede PE, Larsen LO. (1978). Environmental control of annual ovarian cycle in the toad *Bufo bufo* L. Environ. Endocrinol. 28–36.

Kaefer IL, Boelter RA, Cechin SZ. (2007). Reproductive biology of the invasive bullfrog *Lithobates catesbeianus* in southern Brazil. Ann. Zool. Fenn. 44: 435-444.

Kaefer IL, Both C, Cechin SZ. (2009). Breeding biology of the rapids frog *Limnomedusa macroglossa* (Anura: Cycloramphidae) in southern Brazil. J. Nat. Hist. 43: 1195-1206.

Kanamadi RD, Jirankali CS. (1993). Role of temperature and light in regulation of ovarian cycle of tree frog, *Polypedates maculatus*. Indian J. Exp. Biol. 31: 732-737.

Kanamadi RD, Schneider H, Hiremath CR Jirankali CS. (1993). Vocalization of the tree frog *Polypedates maculatus* (Rhacophoridae). J. Biosci.18: 239-245.

Kanamadi RD, Kadadevaru GG, Schneider H. (2002). Advertisement call and breeding period of the frog, *Kaloula pulchra* (Microhylidae). Herpetol. Review. 33(1):19-21

Karaganis SP, Kumar V, Beremand PD. (2008). Circadian genomics of the chick pineal gland in vitro. BMC Genomics. 9: 206–222.

Karaganis SP, Bartell PA, Shende VR. (2009). Modulation of metabolic and clock gene mRNA rhythms by pineal and retinal circadian oscillators. Gen. Comp. Endocrinol. 161: 179–92.

Karsch FJ, Robinson JE, Woodfill CJ, Brown MB. (1989). Circannual cycles of luteinizing hormone and prolactin secretion in ewes during prolonged exposure to a fixed photoperiod: evidence for an endogenous reproductive rhythm. Biol. Reprod. 41:1034–1046.

Katsikaros K, Shine R. (1997). Sexual dimorphism in the tusked frog, *Adelotus brevis* (Anura: Myobatrachidae): the roles of natural and sexual selection. Biol J. Linnean Socie. 60(1): 39-51.

Keating MJ. (1977). Evidence for plasticity of intertectal neuronal connexions in adult Xenopus. Philos. Trans. R. Soc. Lond. Biol. 278: 377-294.

Keating MJ, Feldman SD. (1975). Visual deprivation and intertectal neuronal connections in Xenopus. Proc. R. Soc. Lond. Biol. 191: 467-474.

Kelly DE, Smith SW. (1964). Fine structure of the pineal organs of the adult frog, *Rana pipiens*. J Cell biol. 22(3): 653-674.

Kiesecker JM, Skelly DK. (2001). Effects of disease and pond drying on gray tree frog growth, development, and survival. Ecology. 82(7): 1956-1963.

King JR, Mewaldt LR, Farner DS. (1960). The duration of post nuptial metabolic refractoriness in the white-crowned sparrow. Auk. 77: 89-92.

Kluge AG. (1981). The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Misc. Publ. Univ. Michigan Mus. Zool. 160: 1-170.

Köhler A, Sadowska J, Olszewska J. (2011). Staying warm or moist? Operative temperature and thermal preferences of common frogs (*Rana temporaria*), and effects on locomotion. J. Herpetol. 21: 17–26.

Krishna SN, Krishna SB, Vijayalaxmi KK. (2004). Breeding ecology of a rare microhylid, *Ramanella Montana,* in the forests of Western Ghats, India. Cur. Sci. 87: 80-82.

Kulkarni JT, Pancharatna K. (1994). Role of temperature in the initiation of vitellogenic cycle in the ovary of immature frog, *Rana cyanophlyctis* (Schn.). J. Advance. Zool. 15: 102-106.

Kumar V. (1988). Investigations of photoperiodically induced fattening in migratory blackheaded bunting (*Emberiza melanocephala*) (Aves). J. Zool. Lond. 216: 253–263.

Kumar V. (1997). Photoperiodism in higher vertebrates: An adaptive strategy in temporal environment. Indian J. Exp. Biol. 35: 427-437.

Kumar V. (2001). Melatonin and circadian rhythmicity in birds. Avian Endocrinology. 93-112.

Kumar V. (2002). Biological Rhythms (Ed.) Narosa Publishing house. New Delhi, Chennai, Mumbai, Kolkata.

Kumar V, Kumar BS, Singh BP. (1992). Photostimulation of blackheaded bunting: subjective interpretation of day and night depends upon both photophase contrast and light intensity. Physiol. Behav. 51: 1213–1217.

Kumar V, Rani S, Malik S. (2000). Wavelength of light mimics the effects of the duration and intensity of a long photoperiod in stimulation of gonadal responses in the male blackheaded bunting (*Emberiza melanocephala*). Cur. Sci. 79: 508–510.

Kumar V, Singh S, Misra M, Malik S. (2001). Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting *Emberiza melanocephala*. J. Exp. Biol. 204: 2843–2848.

Kumar V, Singh BP, Rani S. (2004). The bird clock: A complex, multi-oscillatory and highly diversified system. Biol. Rhythms Res. 35: 121-144.

Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, Maywood ES, Hastings MH, Reppert SM. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell. 98: 193–205.

Kupferberg SJ. (1997). The role of larval diet in anuran metamorphosis. Am. Zool. 37: 146-159.

Kyriakopoulou-Sklavounou P, Loumbourdis N. (1990). Annual Ovarian Cycle in the Frog, Rana ridibunda, in Northern Greece. J. Herpetol. 24(2):185-191.

Lack D. (1968). Bird migration and natural selection. Oikos. 19: 1-9.

Lalchhanhimi J, Lalremsanga HT (2017). Observation on the breeding biology of *Polypedates teraiensis* (Dubois, 1987) (Amphibia: Rhacophoridae). Sci. Vision. 17: 25-32.

Lamont MM, Fujisaki I. (2014). Effects of Ocean Temperature on Nesting Phenology and Fecundity of the Loggerhead Sea Turtle (*Caretta caretta*). J. Herpetol. 48: 98–102.

Laurila A, Kujasalo J. (1999). Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. J. Anim. Ecol. 68: 1123–1132.

Lawler JJ, Shafer SL, Bancroft BA, Blaustein AR. (2009). Projected climate impacts for the amphibians of the Western hemisphere. Conserv. Biol. 24(1): 38–50.

Lehman GC. (1977). Environmental influence on ovulation and embryonic development in *Rana pipiens*. J. Exp. Zool. 199: 51-56.

Liao WB, Lu X. (2010). Breeding behaviour of the Omei tree frog *Rhacophorus omeimontis* (Anura: Rachophoridae) in a subtropical montane region. J. Nat. His. 44: 2929-2940.

Licht P. (1966). Reproduction in lizards: Influence of temperature on photoperiodism in testicular recrudescence. Science. 154: 1668-1670.

Licht P. (1971). Regulation of the annual testis cycle by photoperiod and temperature in the lizard *Anolis carolinensis*. Ecology. 52: 240-252.

Licht P. (1972). Environmental physiology of reptilian breeding cycles: role of temperature. Gen Comp. Endocrinol. (Suppl). 3: 477-488.

Licht P, McCreery BR, Barnes R, Pang R. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in bullfrog, *Rana catebeiana*. Gen. Comp. Endocrinol. 50: 124-145.

Lima SL, Agostinho CA. (1984). Ranicultura: Tecnicas e propostas para alimentção de rãs. Informaçion Téchnica 5, Universidade Federal de Viçosa.

Lima SL, Agostinho CA. (1992). A tecnologia de criação de rãs. Universidade Federal de Viçosa, Viçosa.

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(–ddC(T)) method. Methods. 25: 402–8.

Lofts B. (1974). Reproduction. Physiology of the Amphibia. 2:108-218.

Lofts B. (1984). Amphibia. Reproductive cycles in vertebrates. Marshal's physiology of reproduction. 1: 127-2645.

Lofts B, Murton RK. (1968). Photoperiodic and physiological adaptations regulating avian breeding cycles and their ecological significance. J. Zool. 155: 327-394.

Lillywhite HB, Mittal AK, Garg TK, Agrawal N. (1997). Wiping behavior and its ecophysiological significance in the Indian tree frog *Polypedates maculatus*. Copeia. 88-100

Lillywhite HB, Mittal AK, Garg T, Das I. (1998). Basking Behavior, Sweating and Thermal Ecology of the Indian Tree Frog, *Polypedates maculatus*. J. Herpetol. 32(2): 169-175.

Ludwig D, Rowe L. (1990). Life-history strategies for energy gain and predator avoidance under time constraints. Am. Nat. 135: 686–705.

Lynch SK, Wilczynski W. (2008). Reproductive hormones modify reception of species-typical communication signals in a female anuran. Brain. Behav. Evol. 71(2): 143–150.

Magnusson WE, Hero JM. (1991). Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. Oecologia. 86(3): 310-318.

Mahapatra BB, Das M, Dutta SK, Maahapatra P.(2012). Hematology of Indian rhacophorid tree frog *Polypedates maculatus* Gray, 1833 (Anura: Rhacophoridae). Comp. Clin. Pathol. 21: 453-460.

Malik S, Yadav G, Rani S, Kumar V. (2014). Light wavelength dependent circadian and seasonal responses in black headed bunting. Indian J. Exp. Biol. 52: 448–459.

Martins M. (1993). Observations on nest dynamics and embryonic and larval development in the nest building gladiator frog, *Hyla faber*. Amphibia-Reptilia. 14(4): 411-421.

Martins M, Haddad CFB. (1988). Vocalization and reproductive behavior in the Smith Frog, *Hyla faber* Wied (AmphibiaL Hylidae). Amphibia-Reptilia. 9: 49-60.

Mazzi V. (1970). The hypothalamus as a thermodependent neuroendocrine center in urodeles. The hypothalamus. 663-676.

McArthur RM. (1981). Geographical ecology. In: the pineal organ: Photobiology, Biochronometery and Endocrinology. Elsevier. North Holland. Amsterdam.

McCafferya RM, Maxell BA. (2010). Decreased winter severity increases viability of a montane frog population. PNAS. 107: 8544-8649.

Medeiros C, Both C, Kaefer IL, Cechin SZ. (2016). Reproductive phenology of the American Bullfrog in subtropical Brazil: photoperiod as a main determinant of seasonal activity. An. Bra. Acad. Sci. 88(3):1909-1921.

Menaker M, Eskin A. (1966). Entrainment of circadian rhythms by sound in *Passer domesticus*. Science. 154: 1579-1581.

Menke M, Claussen DL. (1982). Thermal acclimation and hardening of the tadpoles of the bullfrog, *Rana catesbiana*. J. Therm. Biol. **7**: 215–219.

Merila J, Laurila A, Laugen AT, Rasanen K, Pahkala M. (2000). Plasticity in age and size at metamorphosis in *Rana temporaria* comparison of high and low latitude populations. Ecography. 23: 457–465.

Meyer WE, Millam JR, Bradley FA. (1988). Photostimulation of Japanese quail by dimlightdepends upon photophase contrast, not light intensity. Biol. Reprod. 38: 536–543.

Meyer WE, Millam JR. (1991). Plasma melatonin levels in Japanese quail exposed to dimlight are determined by subjective interpretation of day and night, not light intensity.Gen. Comp. Endocrinol. 82: 377–385.

Miklosi A, Gonda ZS, Osorio D, Farzin A. (2002). The effects of the visual environment on responses to colour by domestic chicks, J. Comp. Physiol. A. 188: 135–140.

Misra M, Rani S, Singh S, Kumar V. (2004) Regulation of seasonality in the migratory male blackheaded bunting (*Emberiza melanocephala*). Reprod. Nutr. Dev. 44: 341–352.

Mitchell NJ. (2001). Males call more from wetter nests: effects of substrate water potential on reproductive behaviours of terrestrial toadlets. Proc. Biol. Sci. 268(1462): 87-93.

Mohawk JA, Green CB, Takahashi JS. (2012). Central and peripheral circadian clocks in mammals. Ann. Rev. Neurosci. 35: 445–462.

Mohanty HP, Dutta SK. (1988). Life history of the common Indian tree frog, *Polypedates maculatus* (Gray, 1834) (Anura: Rhacophoridae). J. Bombay nat. Hist. Soc. 85: 512-517.

Moll OE. (1979). Reproductiove cycle and adaptations. Turtles, perspectives and research. 305-331.

Moraes MNCM, Poletini MO, Ramos BCR, Leonardo Henrique Ribeiro Graciani de Lima LHRG, Ana Maria de Lauro Castrucci AML. (2014). Effect of Light on Expression of Clock Genes in *Xenopus laevis* Melanophores. Photochem. Photobiol. 90: 696–701.

Moreira G, Lima AP. (1991). Seasonal patterns of juvenile recruitment and reproduction in four species of leaf litter frogs in central Amazonia. Herpetologica. 47: 295-300.

Mrosovsky M, Salmon PA, Ralph MR. (1989). Non photic phase shifting in hamster clock mutants. J. Biol. Rhythms. 7(1): 41-49.

Murton RK, Westwood NJ. (1977). Avian breeding cycles.

Nakayama T, Fish MB, Fisher M, Oomen-Hajagos J, Thomsen GH, Grainger RM. (2013). Simple and efficient CRISPR/Cas9-mediated targeted mutagenesis in *Xenopus tropicalis*. Genesis. 51(12): 835-43.

Newman RA. (1992). Adaptive plasticity in amphibian metamorphosis. Bio.Science. 42: 671–678.

Nicholls TJ, Goldsmith AR, Dawson A. (1988). Photorefractoriness in birds and comparison with mammals. Physiol. Rev. 68: 133-176.

Nishikawa S, Hirata J, Sasaki F. (1992). Fate of ciliated epidermal cells during early development of *Xenopus laevis* using whole mount iummunostaining with an antibody against chondroitin 6-sulfate proteogland antitubulin: transdifferentiation or metaplasis of amphibian epidermis. Histochem. 98(6): 355-358.

Obert HJ. (1975). The dependence of calling activity in *Rana esculenta* Linne 1758 and *Rana ridibunda* Pallas 1771 upon exogenous factors (Ranidae, Anura). Oecologia 18: 317-328.

Oishi T, Lauber JK. (1973). Photoreception in the photo sexual response of quail. Effects of intensity and wavelengths. Am. J. Physiol. 4: 880–886.

Okano K, Okano T, Yoshikawa, Masuda A, Fukada Y, Oishi T. (2000). Diversity of opsin Immunoreactivities in the extraretinal tissues of four Anuran Amphibians. J. Exp. Zool. 286: 136–142.

Okano T, Fukada Y. (2001). Photoreception and circadian clock system of the chicken pineal gland. Microsc. Res. Tech. 53: 72–80.

Okano T, Yamamoto K, Okano K. (2001). Chicken pineal clock genes: implication of BMAL2 as a bidirectional regulator in circadian clock oscillation. Genes. Cells. 6: 825–36.

Oksche A, Ueck M. (1974). The nervous system. Physiology of the Amphibia. 3:313.

Page RB, Monaghan JR, Walker JA, Voss SR. (2009). A model of transcriptional and morphological changes during thyroid hormone-induced metamorphosis of the axolotl. Gen. Comp. Endocrinol. 162: 219–232.

Palis JG. (1998). Breeding Biology of the Gopher Frog, *Rana capito*, in Western Florida. J. herpetol. 32: 217-223.

Pancharatna K, Saidapur SK. (1990). Role of temperature in regulation of ovarian cycle in bull frog *Rana tigrina*. Indian J. Exp. Biol. 28: 806-811.

Pancharatna K, Kulkarni JT. (1993). Control of ovarian cycle in tropical frog, *Rana cyanophlyctis*: J. Advance Zool. 14: 76-83.

Pancharatna K, Patil MM. (1997). Role of Temperature and Photoperiod in the onset of sexual maturity in female frogs, *Rana cyanophlyctis*. J. Herpetol.31: 111-114.

Pandey RK, Bhardwaj SK. (2011). Circadian and seasonal responses in Indian weaver bird: subjective interpretation of day and night depends upon both light intensity and contrast between illuminations. Chronobiol. Int. 28(9): 758–763.

Pellet J. (2005). Conservation of a threatened European tree frog (*Hyla arborea*) metapopulation. PhD thesis, Laboratory for Conservation Biology, University of Lausanne, Switzerland.

Pellet J, Schmidt BR, Fivaz F, Perrin N, Grossenbacher K. (2006). Density, climate and varying return points: an analysis of long-term population fluctuations in the threatened European tree frog. Oecologia. 149(1): 65–71.

Phillips CJC, Lomas CA. (2001). The perception of color by cattle and its influence on behavior, J. Dairy Sci. 84: 807–813.

Phuge SK, Gramapurohit NP. (2013). Gonadal sex differentiation, development up to sexual maturity and steroidogenesis in the skipper frog, *Euphlyctis cyanophlyctis*. Gen. comp. endocrinol. 181: 65-71.

Pike DA. (2008). Environmental correlates of nesting in loggerhead turtles, *Caretta caretta*. Anim. Behav. 76: 603–610.

Pike DA, Antworth RL, Stiner JC. (2006). Earlier nesting contributes to shorter nesting seasons for the Loggerhead Sea Turtle, *Caretta caretta*. J. Herpetol. 40: 91–94.

Porter DA, Licht P. (1986). Effects of temperature and mode of delivery on responses to gonadotropin-releasing hormone by superfused frog pituitaries. Gen. Comp. Endocrinol. 63: 236 -244.

Post E, Forchhammer MC. (2008). Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. Philos. Trans. R. Soc. 363: 2369–2375.

Pough FH, Andrews RM, Cadle JE, Crump ML, Savitzky AH, Wells KD. (2001). Herpetology. 2

Prayitno DS, Phillips CJC, Stokes DK. (1997). The effects of color and intensity of light on behavior and leg disorders in broiler chickens. Poult. Sci. 76: 1674–1681.

Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U. (2002) The orphan nuclear receptor REVERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 110:251–260.

PyburnWF, Glidewell JR. (1971). Nests and breeding behavior of *Phyllomedusa hypochondrialis* in Colombia. J. Herpetol. 5: 49-52.

Rani S, Singh S, Misra M, Kumar V. (2001). The influence of light wavelength on reproductive photorefractoriness in migratory black headed bunting (*Emberiza melanocephala*), Reprod. Nutr. Dev. 41: 277–284.

Rani S, Singh S, Kumar V. (2002). Light sensitivity of the biological clock. Biological Rhythms. 232–243

Rastogi RK, Iela L, Saxena PK, Chieffi G. (1976). The control of spermatogenesis in the green frog, *Rana esculenta*. J. Exp. Zool. 196: 151-166.

Rastogi RK, Lela L, Delrio G, di Meglio M, Russo A, Chieffi G. (1978). Environmental influence on testicular activity in the green frog, *Rana esculenta*. J. Exp. Zool. 206: 49-63.

Rastogi RK, Iela L, Meglio MD, Matteo LD, Minucci S, Izzo-Vitiello I. (1983a). Initiation and kinetic profiles of spermatogenesis in the frog, *Rana esculenta* (Amphibia). J. Zool. 201(4): 515-525.

Rastogi RK, Izzo-Vitiello I, DiMeglio M, Dimatteo L, Franzese R, Dicostanzo MG, Minnucci S, lela L, Chieffi G. (1983b). Ovarian activity and reproduction in the frog *Rana esculenta*. J. Zool. London. 200: 233–247.

Reading CJ. (2007). Linking global warming to amphibian declines through its effects on female body condition and survivorship. Oecologia. 151(1): 125–131.

Reading CJ, Clarke RT. (1995). The effects of density, rainfall and environmental temperature on body condition and fecundity in the common toad, *Bufo bufo*. Oecologia. 102(4): 453–459.

Reed BT. (2005). Guidance on the housing and care of the African clawed frog *Xenopus laevis*. Royal Society of the Prevention of Cruelty to Animals, Research Animals Department, London.

Reiter RJ. (1980). The pineal glands and its hormones in the control of reproduction in mammals. Endocrinol. Rev. 1: 109-129.

Resetarits Jr WJ, Aldridge RD. (1988). Reproductive biology of a cave-associated population of the frog *Rana palustris*. Can. J. Zool. 66(2): 329-333.

Richards SJ, Alford RA. (1992). Nest construction by an Australian rainforest frog of the Litoria lesueuri complex (Anura: Hylidae). Copeia. 1120–1123.

Ritke ME, Babb JG, Ritke MK. (1992). Temporal patterns of reproductive activity in the gray treefrog (*Hyla chrysoscelis*). J. Herpetol. 26: 107-111.

Rittenhouse TAG, Semlitsch RD, Thompson FR. (2009). Survival costs associated with wood frog breeding migrations: effects of timber harvest and drought. Ecology. 90(6): 1620–1630.

Roe BA, Ma DP, Wilson RK, Wong JF. (1985). The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. J. Biol. Chem. 260: 9759-9774.

Rogowitz GL, Candelaria CL, Denizard LE, Melendez LJ, Price AH. (2001). Seasonal Reproduction of a Neotropical Frog, the Cave Coquí (*Eleutherodactylus cooki*). Copeia. 2: 542-547.

Rose SM. (1960). A feedback mechanism of growth control in tadpoles. Ecology.41: 188–198.

Rowan W. (1926). On photoperiodism, reproductive periodicity and the annual migrations of birds and certain fishes. Proc. Boston Soc. Nature Hist. 38: 147-189.

RowanW. (1928). Reproductive rhythms in birds. Nature. 122: 11-12.

RowanW. (1929). Experiments in bird migration. III. Manipulation of the reproductive cycle: Seasonal histological changes in the gonads. Proc. Boston. Soc. Nature. Hist. 39: 151-208.

Rowan W. (1932). Experiments in bird migration III. The effects of artificial light, castration and certain extracts on the autumn movements of the American crow *(Corvus brachyrhynchos).*Proc. Nat. Acad. Sci. USA.18: 639-654.

Rowe L, Ludwig D. (1991). Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology. 72: 413–427.

Ryser J. (1988). Determination of growth and maturation in the common frog *Rana temporaria*, by skeletochronology. J. Zool. Lon. 216: 673–685.

Saboureau M, Dutourne B. (1981). The reproductive cycle in the male hedgegog (*Erinaceus europeaus L.*): A study of endocrine and exocrine testicular function. Reproed. Nutr. Dev. 21: 109-126.

Safi R, Vlaeminck-Guillem V, Duffraisse M, Seugnet I, Plateroti M, Margotat A, Duterque-Coguillaud M, Crespi EJ, Denver RJ, Demeneix B, Laudet V. (2006). Paedomorphosis revisited: thyroid hormone receptors are functional in Necturus maculosus. Evol. Dev. 8: 284–292.

Saidapur SK. (1989). Reproductive cycles of Indian amphibians. Reproductive cycles of Indian vertebrates. 166–224.

Saidapur SK, Nadkarni VB. (1975). The effect of 17 β-estradiol acetate on the testis and thumb pad of *Rana cyanophlyctis* (Schn.) and *Rana tigrina* (Daud.). Gren. Comp. Endocrinol. 27(3): 350-357.

Saidapur SK, Hoque B. (1995). Effect of photoperiod and temperature on ovarian cycle of the frog *Rana tigrina* (Daud.). J. Biosci. 20(3): 445-452.

Salthe SN, Mecham JS. (1974). Reproductive and courtship patterns. Physiology of the Amphibia. 309- 521.

Salvador A, Carrascal LM. (1990). Reproductive phenology and temporal patterns of mate access in Mediterranean anurans. J. Herpetol. 24: 438-441.

Sansum EL, King JR. (1976). Long term effects of constant photoperiod on testicular cycles of white-crowned sparrows *(Zonotrichia leucophrys* gambelii*)*. Physiol. Zool. 49: 407-416.

Sargent TD and Dawid IB. (1983). Differential gene expression in the gastrula of *Xenopus laevis*. Science. 222(4620): 135-139.

Sasso-Cerri E, Faria FP, de Freymüller E, Miraglia SM. (2004). Testicular morphological changes during the seasonal reproductive cycle in the bullfrog *Rana catesbeiana*. J. Exp. Zool. 301: 249–260.

Sato K, Matsuzawa YH, Tanaka H, Bando T, Minamikawa S, Sakamoto W, Naito Y. (1998). Internesting intervals for loggerhead turtles, *Caretta caretta*, and green turtles, *Chelonia mydas*are affected by temperature. Can. J. Zool. 76: 1651–1662.

SatoTK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB. (2004). A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron. 43:527–537.

Saxena VL, Saxena AK. (1975). Effect of thyroidectomy on body weight and gonadal volume of rain quail, *Coturnix coturnix* coromendelica. Indian J. Zool. 16: 113-116.

Sexton OJ, Phillips C, Bramble JE. (1999). The effects of temperature and precipitation on the breeding migration of the Spotted Salamander. Copeia. 781–787.

Simmons AM. (2004). Call recognition in the bullfrog, *Rana catesbeiana*: generalization along the duration continuum. J. Acoust. Soc. Am. 115(3): 1345-55.

Singh J, Budki P, Rani S, Kumar V. (2012). Temperature alters the photoperiodically controlled phenologies linked with migration and reproduction in a night-migratory songbird. Proc. R. Soc. B. 279: 509-515.

Singh D, Rani S, Kumar V. (2013). Daily expression of six clock genes in central and peripheral tissues of a night-migratory songbird: evidence for tissue-specific circadian timing. Chronobiol. Int. 30(10):1208-17.

Singh D, Trivedi AK, Rani S, Panda S, Kumar V. (2015). Circadian timing in central and peripheral tissues in a migratory songbird: dependence on annual life-history states. FASEB J.29(10): 4248-4255.

Smith MA, Green DM. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations?. Ecography. 28(1): 110–128.

Smith DC, VanBuskirk J. (1995). Phenotypic plasticity and ecological performance in two tadpole species. Am. Nat. 145: 211–233.

Sokal RR, Rohlf FF. (1981). *Biometery*, 2nd ed., Freeman and Co., San Francisco.

Soper DS. (2013). F-Value and p-Value Calculator for Multiple Regression [online software]. Available at: http://www.daniel- soper.com/statcalc.

Sprando RL, Russell LD. (1988). Spermiogenesis in the bullfrog (*Rana catesbeiana*): a study of cytoplasmic events including cell volume changes and cytoplasmic elimination. J. Morphol. 198(3): 303-19.

Steinborn J. (1984). Einflusse von photoperiod und wassertemperatur au die Spermatogenese des Kammolches (*Triturus C. cristatus*) im naturlinchen Jahresgan und im Winter. Bull Soc. Zool. Fr. 100: 653-663.

Stewart MM, Pough. (1983). Population density of tropical forest frogs: relation to retreat sites. Science. 221: 570-572.

Stolow MA, Bauzon DD, Li J, Sedgwick T, Liang VC, Sang QA, Shi YB. (1996). Identification and characterization of a novel collagenase in *Xenopus laevis*: possible roles during frog development. Mol. Biol. Cell.7(10): 1471-83.

Summers K, Symula R, Clough M, Cronin T. (1999). Visual mate choice in poison frogs. Proc. Biol. Sci. 266(1434): 2141-2145.

Sun YB, Xiong ZJ, Xiang XY, liu SP, Zhou WW, Tu XL, Zhu CL. (2015). Whole-genome sequence of the Tibetan frog *Nanorana parkeri* and the comparative evolution of tetrapod genomes**.** Proc. Natl. Acad. Sci. U S A. 112(11): E1257–E1262.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, Waller RW. (2004) Status and trends of amphibian declines and extinctions worldwide. Science. 306(5702): 1783–1786.

TabassumF, Rais M, Anwar M, Mehmood T, Hussain I, Alikhan S. (2011). Abundance and Breeding of the Common Skittering Frog (*Euphlyctis cyanophlyctis*) and Bull Frog (*Hoplobatrachus tigerinus*) at Rawal Lake, Islamabad, Pakistan. Asian Herpetological Research 2(4): 177–250.

Tata JR. (1996). Amphibian metamorphosis: an exquisite model for hormonal regulation of postembryonic development in vertebrates. Dev. Growth. Differ. 38: 223–231.

Tata JR. (1999). Amphibian metamorphosis as a model for studying the developmental actions of thyroid hormone. Biochimie. 81: 359–366.

Tauber MJ, Tauber CA, Masaki S. (1986). Seasonal Adaptations of Insects.

Thapliyal JP. (1981). Endocrinology of avian reproduction. Sectional Presidential address, Indian Science Congress, Varanasi, India.

Thapliyal JP. (1968). Body weight cycle of the spotted-munia, *Uroloncha punctulata*. Bull. Natl. Sci. India. 36: 154-166.

Toft CA, Duellman WE. (1979). Anurans of the lower Rio Llullapichis, Amazonian Peru: a preliminary analysis of community structure. Herpetol. 35: 71-77.

Townsend DS, Stewart MM. (1994). Reproductive ecology of the Puerto Rican frog *Eleutherodactylus coqui*. J. Herpetol. 28: 34-40.

Toxopeus AG, Ohm M, Arntzen AW. (1993). Reproductive biology of the parsley frog, *Pelodytes punctatus*, at the northernmost part of its range. Amphibia-Reptilia.14(2): 131-147.

Trivedi AK, Kumar V. (2014). Melatonin: an internal signal for daily and seasonal timing. Indian J. Exp. Biol. 52(5): 425-37.

Trivedi AK, Malik S, Rani S, Kumar V. (2016). Pinealectomy abolishes circadian behaviour and interferes with circadian clock gene oscillations in brain and libver but not retina in a migratory songbird. Physiol Behav. 156: 156-63.

Trivedi AK, Singh D, Dixit, AS, Kumar V. (2017). Pineal gland, melatonin and time keeping in Nonmammalian vertebrates: Avian perspective. In (Ed. Vinod Kumar): Biological timekeeping, clocks, rhythms and behavior. Sporinger: 521-541.

Tryjanowski P, Rybacki M, Sparks T. (2003). Changes in the first spawning dates of common frogs and common toads in western Poland in 1978-2002. Ann. Zool. Fennici. 40: 459–464.

Turek FW, Gwinner E. (1982). Role of hormones in the circadian organization of vertebrates. Vertebrate circadian systems.

Ueda HR, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, Iino M, Hashimoto S. (2005). System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nat. Genet. 37:187–192.

Ukai-Tadenuma M, Yamada RG, Xu H. (2011). Delay in feedback repressionby cryptochrome 1 is required for circadian clock function. Cell. 144: 268–81.

Underwood H. (1990). The pineal and melatonin: regulators of circadian function in lower vertebrates. Experientia. 46: 120-128.

Vaissi, Sharifi M. (2012). Changes in food availability mediate the effects of temperature on growth, metamorphosis and survival in endangered yellow spotted mountain newt: implications for captive breeding programs. Biologia. 71: 444-451.

Vaze KM, Sharma VK. (2013). On the Adaptive Significance of Circadian Clocks for Their Owners. Chronobiol. Int. 30: 413-433.

Venesky MD, Raffel TR, Mcmahon TA, Rohr JR. (2014). Confronting inconsistencies in the amphibian-chytridiomycosis system: implications for disease management. Biol. Rev. 89(2): 477–483.

Vishwanathan N, Chandrashekaran MK. (1985). Cycles of presence or absence of mother mouse entrain the circadian clock of the pups. Nature. 317: 530-531.

Vivien-Roels B, Pévet P. (1983). The pineal gland and the synchronization of reproductive cycles with variations of the environmental climate conditions, with special reference to temperature. Pin. Res. Rev. 1: 91-143.

Wake MH. (1978). The reproductive biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with comments on the evolution of live-bearing systems. J. Herpetol. 121-133.

Warkentin KM. (1992). Effects of Temperature and Illumination on Feeding Rates of Green Frog Tadpoles (*Rana clamitans*). Copeia. 3: 725-730.

Weaver DR, Reppert SM. (2002). Coordination of circadian timing in mammals. Nature. 418: 935–941.

Wells KD. (2007). The Ecology and Behavior of Amphibians. Chicago University Press, Chicago, 1148 p.

Werner EE. (1986). Amphibian metamorphosis: growth rate, predation risk and the optimal size at transformation. Am. Nat. 128: 319–341.

Werner JK. (1969). Temperature-photoperiod effects on spermatogenesis in the salamander Plethodon cinereus. Copeia. 592-602.

Weber SB, Broderick AC, Groothuis TGG, Ellic J, Godley BJ, Blount JB. (2011). Fine-scale thermal adaptation in a green turtle nesting population. Proc. R. Soc. B. 279: 1077–1084.

Weishampel JF, Bagley DA, Ehrhart LM. (2004). Earlier nesting by Loggerhead Sea Turtles following sea surface warming. Glob. Change. Biol. 10: 1424–1427.

Weishampel JF, Bagley DA, Ehrhart LM. Weishampel AC. (2010). Nesting phonologies of two sympatric sea turtle species related to sea surface temperatures. Endan. Spec. Res. 12: 41–47.

Whitaker JO Jr. (1971). A study of the western chorus frog, *Pseudacris triseriata*, in Vigo County, Indiana. J. Herpetol. 5: 127-150.

Whittier JM, Crews D. (1987). Seasonal reproduction: patterns and control. Hormones and reproduction in fishes, amphibians and reptiles. 385-409.

White RH, Stevenson RD, Bennett RR, Cutler DE, Haber WA. (1994). Wavelength discrimination and the role of ultraviolet vision in the feeding behavior of hawk moths, Biotropica. 26: 427–435.

Wiest JA Jr. (1982). Anuran succession at temporary ponds in a post oak-savanna region of Texas. U.S. Fish and Wildlife Res. Rept. 13: 39-47.

Wilbur HM, Collins JP. (1973) Ecological aspects on amphibian metamorphosis. Science. 182: 1305–1314.

Williams SE, Shoo LP, Isaac JL, Hofmann AA, Langham G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol. 6(12):e325.

Wingfield JC, Farner DS. (1993). Endocrinology of reproduction in wild species. In: Avian biology. Vol. IX. Academic Press. 163-277.

Wingfield, JC, Hahn TP, Maney DL, Schoech SJ, Wada M, Morton ML. (2003). Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys* oriantha. Gen. Comp. Endocrinol. 131: 143–158.

Woodley SK. (2014). Chemical signalling in Amphibians. In: Mucignat-Caretta C, editor. Neurobiology of Chemical Communication. Boca Raton (FL): CRC Press/Taylor & Francis.

Wright ML, Jorey ST, Myers YM, Fieldstad ML, Paquette MC, Clark MB. (1988). Influence of photoperiod, day length, and feeding schedule on tadpole growth and development. Dev. Growth. Differ. 30: 315–323.

Wurtman RJ, Axelord J, Kelly DE. (1968). The pineal. 1-199.

Yamamoto K, Okano T, Fukada Y. (2001). Chicken pineal Cry genes: light-dependent upregulation of cCry1 and cCry2 transcripts. Neurosci. Lett. 313: 13–6.

Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. Science. 288: 682–695.

Yasuo S, Watanabe M, Okabayashi N. (2003). Circadian clock genes and photoperiodism: comprehensive analysis of clock gene expression in the mediobasal hypothalamus, the suprachiasmatic nucleus, and the pineal gland of Japanese quail under various light schedules. Endocrinol. 144: 3742–8.

Yoshimura T, Suzuki Y, Makino E. (2000). Molecular analysis of avian circadian clock genes. Mol. Brain. Res. 78: 207–15.

Yoshimura T, Yasuo S, Suzuki Y. (2001). Identification of the suprachiasmatic nucleus in birds. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280: 1185–9.

Zhang P, Zhou H, Liang D, Liu Y, Chen YQ, Qu L. (2004). The complete mitochondrial genome of a tree frog, Polypedates megacephalus (Amphibia: Anura: Rhacophoridae), and a novel gene organization in living amphibians. Gene. 346: 133-143.

Zhuang M, Wang Y, Steenhard BM, Besharse JC. (2000). Differential regulation of two period genes in the Xenopus eye. Brain. Res. Mol. Brain. Res. 82: 52–64.

Zhu H, Green CB. (2001). Three cryptochromes are rhythmically expressed in Xenopus laevis retinal photoreceptors. Mol. Vis. 7: 210–215.