CHAPTER II

EXTRARETINAL PHOTORECEPTION IN LACERTILIAN TAIL REGENERATION- THE LATERAL EYES DO NOT PARTICIPATE IN PHOTOPERIODICALLY SIGNIFICANT PHOTORECEPTION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*.

Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in the photoperiodic response in *Anolis carolinensis* (Undeerwood, 1975). Accordingly, long stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded Anoles. Similar studies have been conducted on a variety of vertebrate species, such as fishes and birds where testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods (Underwood, 1979). The first demonstration that extraretinal photoreceptors could participate in photoperiodic responses of vertebrates occurred more than 50 years ago when Benoit showed that light could induce testicular growth in blinded ducks (Benoit, 1935). One of the techniques developed to ascertain the potential contribution of eyes and extraretinal receptors in birds involved opaquing the heads of birds while leaving the eyes exposed (McMillan *et al*., 1975). In the house sparrow so treated, no response occurred when the birds were maintained under long stimulatory photoperiods,despite the fact that the eyes were exposed showing that extraretinal receptors were solely involved in mediating photoperiodic photoreception in these birds.

Other studies indicated that the eyes or vision, are not necessary for photoperiodic induction of gonadal growth in the chicken, *Gallus domesticus* (Hurs, 1935; Harvey, 1965;Ookawa, 1970), the common coturnix or Japanese quail, *Coturnix coturnix japonica* (Sayler and Wolfson, 1968; Homma and Sakakibara, 1971), and the house sparrow, *Passer domesticus* (Menaker, 1971). Furthermore, after maturity, blinded and intact birds were equally responsive to changes in enviornmental light as indicated by the testes weight in the common coturnix (Oishi *et al.*, 1966) and by the time of oviposition in the domestic fowl (Harrison and Becker, 1969).

According to Maier and Singer (1977) and Turner and Tipton (1972), long length photoperiod can speed up the rate of forelimb regeneration in the newt and tail regeneration in lizards, respectively. This effect is not mediated by the optic system as blinded newts kept in continuous light regenerated their forelimbs more rapidly than their sighted counterparts kept in total darkness (Maier and Singer, 1977). With the above mentioned literatures in view and owing to the fact that no investigation has yet been carried out on extraretinal photoreception in relation to tail regeneration in lizards in general and Hemidactylus in particular, the present investigation was deemed appropriate and timely. To demonstrate the potential contribution of the lateral eyes, or retinae, on photoperiodic photoreception in *H. flaviviridis*, both the lateral eyes were surgically removed (bilateral orbital enucleation) and the enucleated animals exposed, along with their sighted (unoperated) counterparts, to eight different photoregimes during the process of tail regeneration.

MATERIALS AND METHODS

A total of 640 lizards was used in this investigation and they were divided into two groups and exposed to the eight photoperiodic schedules as described on pages 12 and 13.

Group 1 - Experimental (BL) :

The first group of 320 lizards ~~were~~ WAS blinded (BL) by surgical removal of both the lateral eyes (bilateral orbital enucleation). A circular cut was made with a sharp scissors on the skin that surrounds the eye-ball. The eyeball was then lifted up with a sterilized forceps and the optic nerves were cut with a curved scissors. Bleeding occurred and the blood was wiped off with a piece of clean cotton wool. A small quantity of plaster of Paris was sprinkled inside the eyesocket which helped in stopping the bleeding. Blinding of each animal took about two minutes and BL lizards were allowed 5 days recovery period in order to eliminate any traumatic side effect due to surgery. They were divided into eight batches of 40 lizards each and were acclimated for another 7 days to the eight different photoperiodic conditions prior to autotomy. Mortality in BL lizards was negligible. A paste of cockroaches was prepared and enucleated animals were force-fed with the paste for a period of 10 days after eye surgery. After this period, blinded Hemidactylus could easily locate the live cockroaches and began feeding <u>ad libitum</u>.

<u>Group 2</u> - <u>Controls</u> (NL) :

A second group of 320 lizards had both the lateral eyes intact without any operation performed. Forty lizards each were then exposed to the eight photoregimes. Food and water were provided <u>ad libitum</u>.

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of new growth (regenerate), in mm, was measured with a graduated meter rule and recorded at fixed time intervals of 5,10,20,30,40,50 and 60 days post-caudal autotomy. This investigation was conducted during the post-breeding monsoon months (August - October) and the recorded average monthly ambient, room and cage temperatures are given in table 2. The average daily temperatures at the level of the animals in the lighted and dark chambers did not differ by more than 2°C at any stage. The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and further to Duncan's multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, 1955).

RESULTS

The results are depicted in table 1 and figures 1-3. The blastemic stage appeared in NL as well as BL groups of animals exposed to LL (H), LL (L) and LD 18 : 6 photoperiods by day 5 to day 7 and in LD 6 : 18 and 0 : 24 exposed animals by day 12 to day 14 post-caudal autotomy. In the intermediate photoperiods of NLD;LD 12 : 12 and LD 16 : 8, the regeneration process started by day 8 to day 10 while in LD 8 : 16 exposed animals it occurred by day 10 to day 12. The arbitrary stages of regeneration shown in table 1 are described in chapter 1.

Growth rate and total length regenerated :

A measurable growth occurred in NL and BL groups of animals exposed to LL and LD 18 : 6 lighting regimes by day 5 while in LD 12 : 12, LD 16 : 8 and LD 8 : 16 groups of animals it occurred between days 5 and 10. However, in lizards exposed to LD 6 : 18 and 0 : 24 a measurable growth occurred only between days 10 and 15. The regeneration process was completed in LL (H), LL (L) and LD 18 : 6 groups of animals by the 50th day at which time the total length of the tail regenerated was 41.7 mm (NL) and 42.0 mm (BL), 33.3 mm (NL) and 33.0 mm (BL), and 38.7 mm (NL) and 38.3 mm (BL) respectively. In the other groups of lizards, the regenerative growth ceased by day 60 and the least length regenerated, 28.2 mm (NL), 27.6 mm (BL) and 27.4 mm (NL), 27.0 mm (BL) were in the LD 6 : 18 and LD 0 : 24 groups. The total length of tail regenerated in the remaining groups of animals were nearly similar and were NL (33.6 mm) and BL (33.9 mm) in LD 16 : 8, NL (33.0 mm) and BL (34.0 mm) in LD 12 : 12 and NL (31.0 mm) and BL (31.3mm) in LD 8 : 16. The pattern of growth rate depicted in figure 2 indicates two peaks of growth spurts in both NL and BL groups of animals exposed to LL (H), LL (L) and LD 18 : 6 photoperiods, one during the first ten days and the other between twenty and forty days. In contrast, LD 16 : 8, LD 12 : 12, LD 8 : 16, LD 6 : 18 and LD 0 : 24 produced a sigmoid growth curve with the asymptote occurring between days twenty and forty, corresponding to the second growth peak in the long-day photoperiods. Another observable effect is a delayed temporal shift by five days in the case of LD 6 : 18 and ten days in the case of LD 0 : 24.

Total percentage replacement :

Percentage replacement calculated in terms of total length of tail regenerated and total length of tail autotomized revealed a minimum of 50.5 (NL) and 50.4 (BL) in DD exposed lizards and a maximum of 75.3 (NL) and 75.1 (BL) in LL (H) exposed animals followed by 70.4 (NL) and 70.3 (BL) in LD 18 : 6. LL (L), NLD and LD 16 : 8 recorded nearly similar replacement of 62.5% (NL) and 62.3% (BL), 61.7% (NL) and 61.4% (BL) and 62.7% (NL) and 62.3% (BL), respectively. Lizards exposed to 8 hours of light showed slightly reduced percentage replacement of 57.8% in both (NL) and (BL) while those exposed to 6 hours of light produced a replacement of 52.7% (NL) and 51.5% (BL), more like the DD exposed animals.

All possible comparisons between the eight experimental set ups (Duncan's multiple range test) revealed no statistical significance between normal (NL) and blinded (BL) groups of animals exposed to DD and LD 6 : 18 on the one hand and between NLD, LL (L) and LD 16 : 8 on the other. However, all other comparisons other than these were statistically significant at both 5% and 1% levels.

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN NORMAL AND BLINDED H. FLAVIVIRIDIS.

	WOUND HEALING	BLASTEMA	EARLY DIFFERENTIATION	DIFFERENTIATION	LATE DIFFERENTIATION	GROWTH	FULLY REGENERATED TAIL
LL (H)	3	5 - 7	7 - 9	10	20	30	50
LL (L)	3	5 - 7	7 - 9	10	30	40	50
LD 18 : 6	3	5 - 7	7 - 9	10	25	35	50
LD 16 : 8	5	8 - 10	12 - 14	20	30	40	60
LD 12 : 12	5	8 - 10	12 - 14	20	30	40	60
LD 8 : 16	5	10 - 12	14 - 16	22	30	40	60
LD 6 : 18	8	12 - 14	16 - 18	25	35	45	60
LD 0 : 24	8	12 - 14	16 - 18	25	40	45	60

LL (H) - CONTINUOUS LIGHT (HIGH INTENSITY)
LL (L) - CONTINUOUS LIGHT (LOW INTENSITY)
LD 18:6 - 18 HOURS LIGHT (HIGH INTENSITY) AND 6 HOURS DARKNESS
LD 16:8 - 16 HOURS LIGHT (HIGH INTENSITY) AND 8 HOURS DARKNESS
LD 12:12 - 12 HOURS LIGHT (HIGH INTENSITY) AND 12 HOURS DARKNESS
LD 8:16 8 HOURS LIGHT (HIGH INTENSITY) AND 16 HOURS DARKNESS
LD 6:18 - 6 HOURS LIGHT (HIGH INTENSITY) AND 18 HOURS DARKNESS
LD 0:24 - CONTINUOUS (TOTAL) DARKNESS

TABLE 2. AVERAGE AMBIENT, ROOM AND CAGE TEMPERATURES DURING THE PERIOD OF STUDY.

MONTHS*	TEMPERATURE AMBIENT		MEASUREMENT ROOM		IN °C. CAGE		HUMIDITY	
	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.
AUGUST 1986	29.0	23.9	28.0	21.0	28.0	22.0	99%	42%
SEPTEMBER 1986	35.4	24.6	32.0	22.0	33.0	21.0	94%	40%
OCTOBER 1986	37.7	22.0	34.0	20.0	35.0	20.0	93%	13%

AVERAGE DAILY TEMPERATURES

+-LIGHTED CHAMBER = 27°C

DARK CHAMBER = 25°C

* POST-BREEDING MONSOON SEASON.

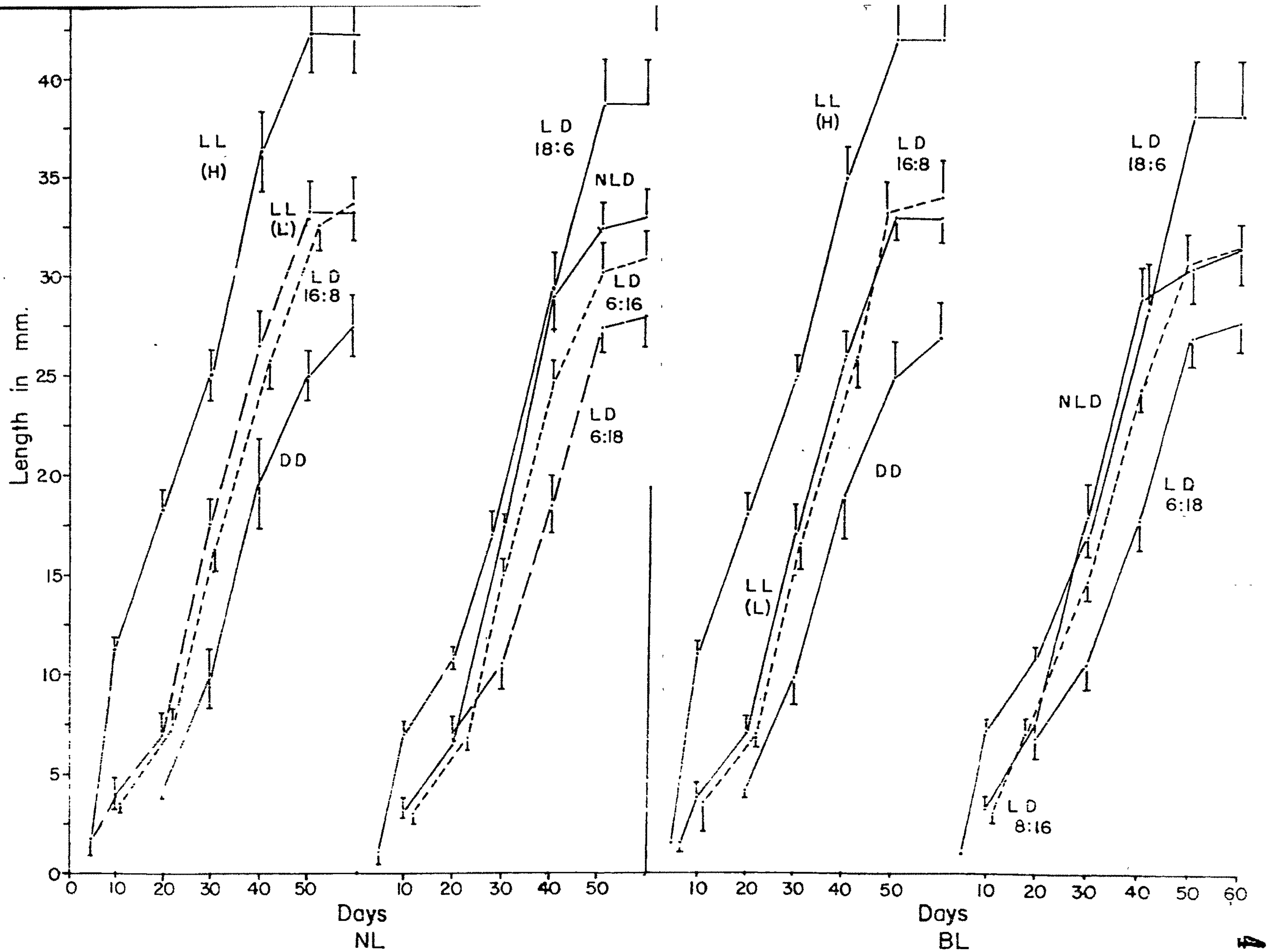

Fig.1. Regenerative tail elongation in sighted and blinded hemidactylus under different photoperiodic regimens.

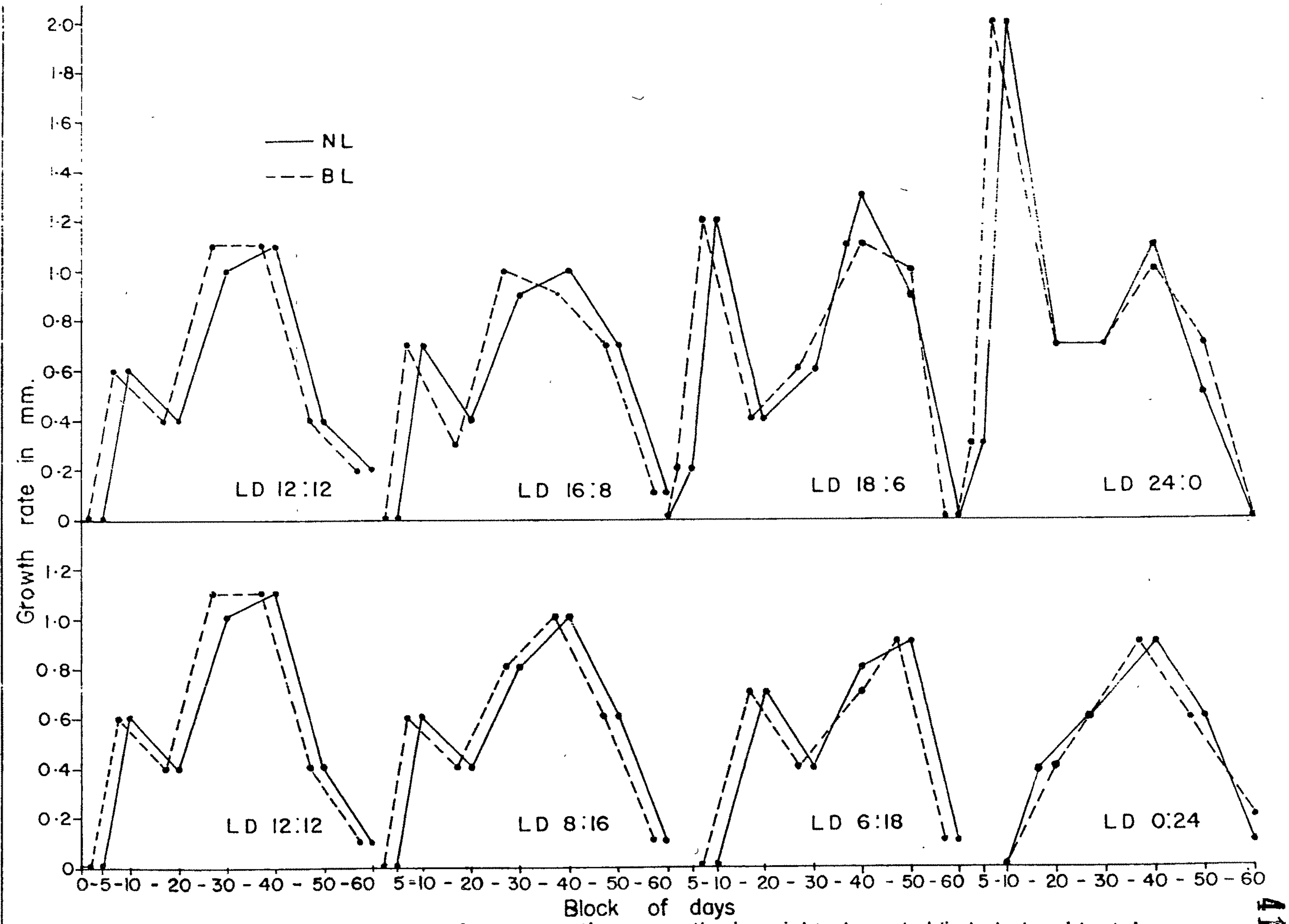

Fig.2. Per day rate of regenerative growth in sighted and blinded hemidactylus under different photoperiodic regimens.

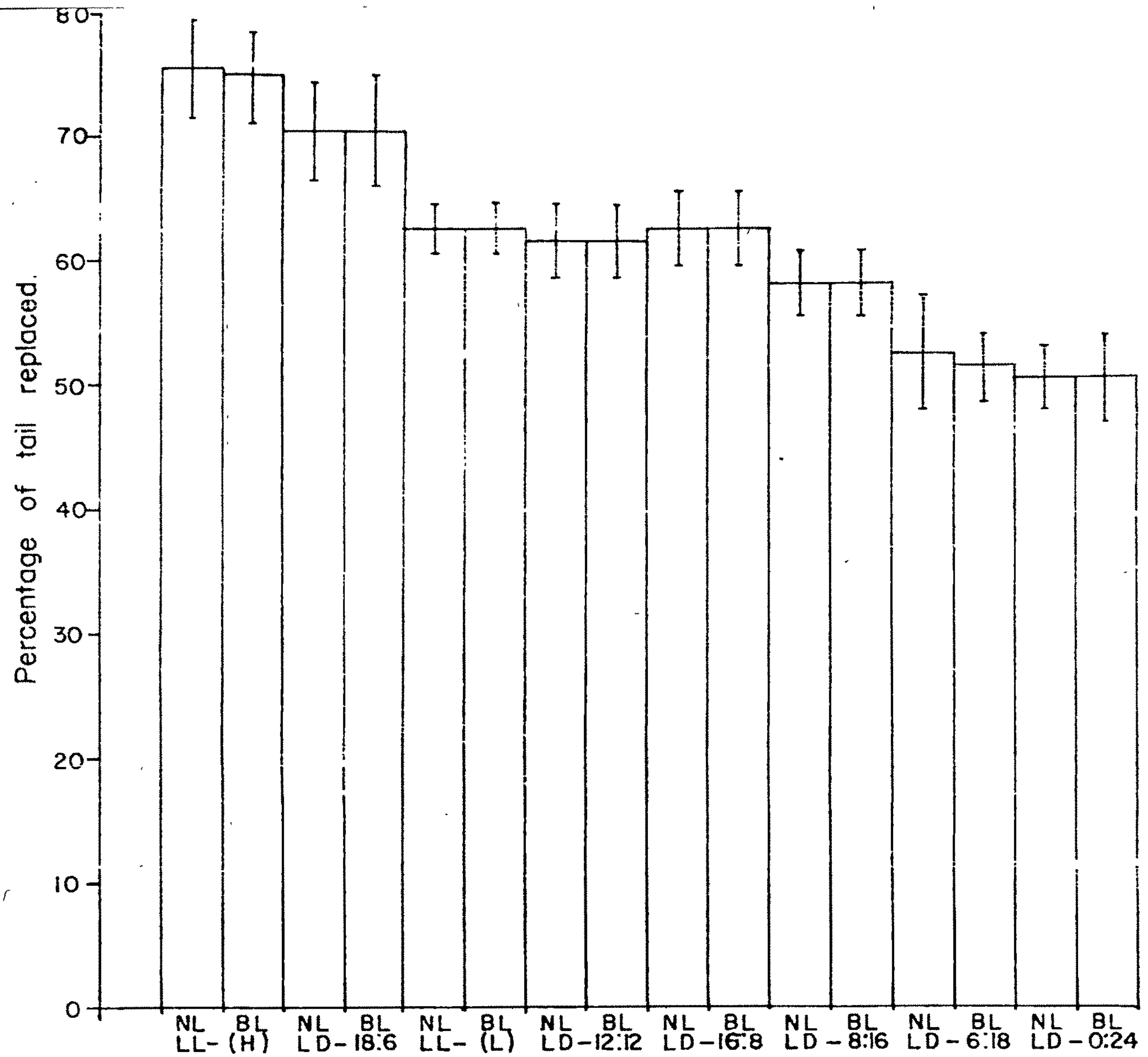

Fig.3 Percentage replacement during tail regeneration in sighted and blinded hemidactylus under different photoperiodic regimens

LL(H) continuous light, high intensity; LL(L)-continuous light, low intensity; DD-continous (total) darkness; NLD- normal light and darkness; 18LL:6DD-18 hours light and 6 hours darkness; 6LL:18DD-6 hours light and 18 hours darkness; 16LL:8DD-16 hours light and 8 hours darkness; 8LL:16DD-8 hours light and 16 hours darkness.

DISCUSSION

Long-day photoperiod stimulates tail regeneration in the Gekkonid lizard *Hemidactylus flaviviridis*, whereas short-day photoperiod has no effect (Ndukuba and Ramachandran, 1989~~c~~ chapter 1). This stimulatory photic effect is not mediated by the lateral eyes, or retinae, as blinded Hemidactylus regenerated their lost (autotomized) tails similar to their sighted (unoperated) counterparts. It is, therefore, presumed that an extra-retinal photoreceptor(s) situated in the brain region of the lizard head mediate in photoperiodic photoreception during the process of tail regeneration.

Most investigations on a potential role for retinal receptors have been conducted with birds. A long series of investigations by Benoit on the domestic duck demonstrated the participation of extraretinal receptors in the photoperiodic response of ducks (Benoit, 1935), Many other different combinations of experiments led Benoit to conclude that both retinal and extraretinal photoreceptors are involved (Benoit, 1970). However, a careful reconsideration of the published work of Benoit by McMillan *et al.* (1975) led them to conclude that a retinal participation in photoperiodism in ducks has not been conclusively demonstrated. The participation of extra-retinal receptors in testicular responses in a second avian species, the house sparrow, *Passer domesticus*, was shown in 1968 (Menaker and Keatts, 1968). A series of experiments with house sparrows, utilizing several different experimental approaches demonstrated that the eyes are not involved in photoperiodic photoreception; extraretinal receptors located in the brain are fully capable of mediating this response (Underwood and Menaker, 1970; Menaker *et al.*, 1970; McMillan *et al.*, 1975). Subsequently, other avian species (Chickens, Japanese quail, and white - crowned and golden crowned sparrows) have been investigated (Gwinner *et al.*, 1971; Homma and Sakakibara, 1971; Harrison, 1972; Oishi and Lauber, 1973; Turek, 1975), and no clear demonstration of a retinal involvement in the stimulation of gonadal recrudescence in birds has been shown, with blinded birds responding as well as intact birds to stimulatory photoperiods.

It is well established that among fishes, amphibians and reptiles the pineal organ, a small structure embedded in the top of the brain, and such associated structures as the parietal "eye" are sensitive to light. The function of the pineal organ in the life of the lower vertebrates remains uncertain, and it has not been shown that the structure acts as a light detector in either birds or mammals. Benoit (1935) reported finding a light sensitive area in the brain of Peking ducks; direct illumination of the duck's head stimulates the growth of the testes. Benoit's work was the first indication that complex image-forming eyes, in animals that possess them, are not necessarily the exclusive mediator of photoperiodism. Although Benoit and his colleagues persevered in their study of extraretinal phtoreception and did uncover many fascinating phenomena, the photo-detecting structure itself was not identified.

In this study of extraretinal photoreception in relation to tail regeneration in the lizard, *H. falviviridis*, a technique of shielding the lateral eyes with a piece of dark cloth while leaving the head region exposed to light was first applied. This technique proved unsuccessful because the lizards frequently removed the coverings by scratching their heads against the walls of the wooden cages. Subsequently, both the lateral eyes were surgically extirpated (Bilateral orbital enucleation) and the observations showed that Hemidactylus accepts enucleation very well and the mortality rate was negligible. From the time of autotomy till the completion of the regeneration growth, there was no significant alteration either in the initiation and onset of regeneration, the daily growth rate, the total new growth (regenerate) produced at the end of regenration or the total percentage replacement of the lost (autotomized) tails in blinded lizards as compared to their sighted (unoperated) counterparts exposed to similar experimental photoperiodic schedules.

Two different light intensities were used in this investigation - a high light intensity of 2500 lux units and a low intensity of 638 lux units. Although the higher light intensity produced a better regenerative performance, the lateral eyes did not play any significant role in photoperiodic photoreception since enucleated animals regenerated their autotomized tails similar to their sighted counterparts. Underwood (1980) demonstrated that the eyes were not involved in testicular recrudescence in <u>Anolis</u> <u>carolinensis</u> exposed to light of 40 lux units intensity and doubted whether they could be involved at higher intensities. Although Underwood's work was not available at the time this investigation was carried out on extraretinal photoreception on tail regeneration in the lizard, Hemidactylus, the observations in this report can satisfactorily erase that doubt since at either 638 or 2500 lux units of light intensity, the eyes did not participate in photoperiodically significant photoreception.

In mammals, intact retinae appear to be required for lighting information to influence most endocrine systems (Brownman, 1937; Hollwich, 1964) and Circadian rhythmicity (Snyder <u>et</u> <u>al</u>., 1965; Reiter et al., 1965). However, Ganong <u>et</u> <u>al</u>. (1963) have found that measurable amounts of light can penetrate the skull to the brains of mammals without the intervention of the eyes. Other workers have obtained evidence suggesting that light can directly affect hypothalamic neurones in the duck (Benoit, 1964) and rat (Lisk and Kannwischer, 1964). The pineal gland of rats is markedly affected by exposure of the animals to varying periods of light and darkness. After maintaining adult rats in continuous lighting, there is significant decrease in pineal weight (Fiske <u>et</u> <u>al</u>., 1960) and in the enzymatic capacity of the pineal gland to synthesize melatonin (Wurtman <u>et</u> <u>al</u>., 1963) and an increase in the activity of the serotonin-synthesizing enzyme, 5-hydroxytryptophan decarboxylase, in the pineal gland (Snyder *et al.*, 1964; Snyder and Axelrod, 1964; Snyder *et al.*, 1965). These effects of light exposure are abolished by bilateral orbital enucleation (Snyder *et al.*, 1964; Snyder and Axelrod, 1964 ; Snyder *et al.*, 1965; Wurtman *et al.*, 1964).

In the present investigation, blinded and sighted Hemidactylus responded similarly to continuous illumination as well as to the other experimental lighting regimes. From figures 1 and 3, it becomes obvious that the total length of tail regenerated and the percentage replacement are maximal under LL (H) and minimal under LD 0 : 24 in both NL and BL groups of animals. Though the values with regards to these two parameters were quite similar in LL (L), LD 16 : 8 and LD 12 : 12 on one hand and LD 6 : 18 and 0 : 24 on the other, a definite linear correlation between the length of tail regenerated and total percentage replacement can be inferred. This fact is confirmed by the observed values under LD 18 : 6 which were significantly more than all the groups except for LL (H). Another striking inference that could be drawn by careful study of figure 2 is the biphasic growth spurt, in both NL and BL groups of animals exposed to LL (H), LL (L) and LD 18 : 6 during the process of tail regeneration, and a delayed temporal shift by five and ten days, respectively, in the LD 6 : 18 and 0 : 24 exposed groups of lizards.

These results demonstrate that the lacertilian lateral eyes, or retinae, do not participate in photoperiodically significant photoreception. On the basis of current knowledge, the most likely photoreceptor is the pineal organ. Over the years, a body of information, based largely upon indirect morphological evidence, has accumulated to the effect that the epiphyseal complex of the lower vertebrates is responsive to light and darkness. A few electrophysiological studies now lend direct evidence of such activity in fishes (Hangri _et_ _al_., 1969) and reptiles (Miller and Wolbarsht, 1962).

How the pineal may respond to affect the rate of tail regeneration can only be speculated upon. Melatonin is produced by the pineal gland and is a mitotic inhibitor (Banerjee and Margulis, 1973). Melatonin levels are lowest during the day and can be suppressed by extended exposure to light (Brownstein, 1975). Litwiller (1940) demonstrated that the mitotic rate of blastemal cells peaks during the light phase of the diurnal cycle. Results from this investigation demonstrate that it is during the pre-blastemic, blastemic and early differentiation stages of regeneration, characterized by high mitotic potential, that the positive influence of increasing photoperiodism on the regenerative performance is essentially exerted. Apparently, photic input is being transduced and translated into hormonal and, or, physiological responses favouring growth potential, though the exact action at the cellular level remains speculative. It may be that the increased mitotic rate during the day light hours and its subsequent decline during the dark phase bears a causal relation to the melatonin cycle. Alternatively, increased or decreased lengths of light may affect the production of prolactin which is a known growth promoter (Crim, 1975). Bourne and Tucker (1975) had, in fact, demonstrated the positive influence of increasing lengths of light on the level of serum prolactin. Serotonin could, in this respect, mediate the light affect since it is enhanced by light (Brownstein, 1975). Moreover, serotonin and its precursors have been shown to elevate serum prolactin levels (Lu and Meites, 1973) and, therefore, could operate as a mitotic stimulator by way of its ability to induce prolactin release.