CHAPTER IV

TAIL REGENERATION IN NORMAL, BLINDED AND PINEALECTOMIZED GEKKONID LIZARDS, *HEMIDACTYLUS FLAVIVIRIDIS*, EXPOSED TO FOUR DIFFERENT LIGHT CONDITIONS DURING THREE SEASONS (TEMPERATURES).

Many vertebrate species living in mid and high latitudes use the annual changes in day length to time such important events as moulting, fattening, migration and reproduction. Among poikilotherms, both photoperiod and temperature are important stimuli regulating annual gonadal cycle as in the iguanid lizard, *Anolis carolinensis*, with the importance of the two stimuli varying with the phase of the cycle (Licht, 1971).

The perception of light provides important information for the organism about its environment. For this purpose most animals possess well developed photoreceptors and neuronal networks in the retinae of their lateral eyes. Interestingly, even in species with highly organised ocular photoreceptors, additional photoreceptive structures - extra-ocular photoreceptors - are utilized in the transmission of photic information about the day-night schedule and seasonal photoperiod changes. Considerable evidence supports the view that the pineal organ is the principal site of extraocular photoreception in lower vertebrates (Cf. Meissl and Dodt, 1981). Recent radioimmunoassay studies have revealed the presence of circadian oscillators in the isolated pineal organs of the lizard, *A. carolinensis* (Menaker and Wisner, 1983). Circadian rhythm are characterized by three major properties; they oscillate under constant conditions (free-run), they can be synchronized by environmental light-dark (LD) cycles (entrainment), and their periods vary only slightly with changes in ambient temperature (temperature-compensation). The pineal organ of *A. carolinensis*, contains one or more temperature-compensated circadian oscillators coupled with photoreceptors on the input side and to melatonin synthetic pathways on the output side. In *A. carolinensis*, some of the photoreceptors are coupled with the circadian oscillators that regulate the synthesis of melatonin since the rhythm in isolated Anolis pineals can be entrained by LD cycles (Menaker and Wisner, 1983).

The newts, Triturus-*Notophthalmus viridescens*, exposed to cold temperature, regenerate their forelimbs more slowly than those at higher temperatures (Schauble and Nentwig, 1974). Schauble (1972) demonstrated that animals kept in identical temperature and photoperiodic conditions regenerated more rapidly in the summer months than in the winter. Turner and Tipton (1972) showed that the lizard *A. carolinensis* regenerates its tail more rapidly when exposed to a long-day photoperiod

(18 hours) than to a short one (6 hours). Maderson and Licht (1968) and Tassava and Goss (1966) demonstrated the influence of temperature on the final form of the regenerated tail in <u>A. carolinensis</u>, a smaller proportion being replaced at a lower temperature (20°C) and a bigger proportion at a higher temperature (32°C).

Several species of lizards have been shown to respond to changes in day length (Clausen and Poris, 1937; Bartholomew, 1950, 1953; Fox and Dessauer, 1958; Mayhew, 1964), but the photoperiodic influence has not yet been clearly related to lacertilian tail regeneration, nor have possible interactions with temperature been adequately examined. The present investigation was designed to elucidate the effect of different photoperiodic schedules and temperature conditions on tail regeneration in normal, blinded and pinealectomized Gekkonid lizards, <u>Hemidactylus flaviviridis</u> during Summer (30°C), monsoon (26°C) and winter (17°C) seasons.

MATERIALS AND METHODS

A total of 480 lizards was used in each of the three seasons, and they were divided into three groups and exposed to the four different photoperiodic schedules (LL(H), LL(L), LD12:2 & LD0:24) as described on pages 12 and 13.

<u>Group 1-Experimental (PX)</u>

The first group of 160 lizards was pinealectomized by surgical removal of the pineal organ (See Chapter III for operation procedure). Pinealectomy in each animal took about three minutes, and PX lizards were allowed 5 days recovery period in order to eliminate traumatic side effects due to surgery. They were then divided into 4 batches of 40 lizards and exposed to the four different photoperiodic conditions for 7 days prior to tail autotomy. Mortality in Px animals during the recovery period was negligible (2%). Microscopic examination of Px lizards showed that the pineal was removed completely and no damage was done to the brain. Food and water were provided ad libitum.

Group 2 - Experimental (BL):

The second group of 160 lizards was blinded (BL) by surgical removal of both the lateral eyes (Bilateral orbital enucleation); (see chapter II for operation procedure). Blinding of each animal took about two minutes, and BL lizards were allowed 5 days recovery period in order to eliminate traumatic side effects due to surgery. They were also divided into 4 batches of 40 lizards each and were acclimated for another 7 days to the four different photoperiodic conditions prior to tail autotomy. Mortality in BL lizards during the recovery period was negligible (1%). 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 were fed and provided with water *ad libitum*, thereafter.

Group 3 - Control (NL) :

A third group of 160 intact lizards, divided into 4 batches of 40 each served as control (NL). They were acclimated for 7 days to the four photoperiods prior to tail autotomy. Food and water were provided *ad libitum*.

Lizards in all the groups were fed with cockroaches *ad libitum* during the entire period of study (60 days). None of the animals in any of the experimental set ups showed any noticeable behavioural change. Moreover, mortality was nil in all the groups of animals from the time of autotomy till the end of the experiment.

Tail autotomy was induced in the three groups of lizards at the level of the third segment by applying mild pressure with the thumb and the forefinger. The animals were exposed to the four different photoperiodic conditions during the entire process of tail regeneration. The length of tails removed from the lizards varied from 50 mm to 60 mm. The length of new growth (regenerate), in mm, was measured every day with a graduated meter rule and the measurements obtained at fixed time intervals of 5,10,20,30,40,50 and 60 days post-caudal autotomy were used for morphometric calculations. 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 shown in tables 1 and 2 and figures 1-3. The pattern of regeneration observed from this investigation showed that the initiation of regeneration, the daily growth rate, the total new growth (regenerate) produced at the end of regeneration and the percentage replacement of the lost (autotomized) tails were all significantly affected by both temperature and photoperiod. There was no weight loss in any of the groups of lizards at any stage during the entire period of experimentation thus over ruling any possibility of the nutritional status influencing the observed differences (see Fig.4).

The arbitrary stages of regeneration shown in table 1 are described in chapter I. The main difference between Px and NL or BL lizards in this investigation was a temporal delay in the stages of regeneration in Px animals and, at the completion of the regeneration process, a retarded regenerate was obtained in all Px lizards.

Summer season :

The influence of various photoperiodic schedules on tail regeneration in Hemidactylus during the summer season is shown in figures 1-3. The average daily temperature at the level of the animals was 30°C. The positive influence of long-day photoperiod was evident in NL and BL lizards exposed to LL schedules, whereas a negative influence of short-day photoperiod was the case in their counterpart exposed to LD 0 : 24 . Pinealactromy, however, abolished the stimulatory effect of continuous illumination and retarded regeneration, as Px animals were not affected by either increased or decreased photoperiodism during the process of tail regeneration . The initiation of regeneration marked by a measurable growth occurred earlier with increasing light schedules and pinealectomy delayed it maximally (Table 1). The regeneration process was completed in NL, BL and PX lizards exposed to LL conditions by the 50th day and in LD 12:12 and 0 : 24 by the 60th day post-caudal autotomy.

The pattern of growth rate shown in figure 2 indicates a positive increase peaking at 30 - 40 days in LD 12 : 12 and LD 0 : 24 schedules . However, LL(H) and LL(L) photoperiodic conditions produced a biphasic growth rate curve in NL, BL and PX lizards by inducing a very significant initial growth spurt. The positive influence of photoperiodism was further revealed by the gradually decreasing peak growth rate from LL to LD 0:24 schedules.

Monsoon season :

The average daily temperature at the level of the animals during the monsoon season was 26°C. The positive influence of long-day photoperiod and the negative influence of short-day photoperiod as well as pinealectomy on lacertilian tail regeneration were also discernible in monsoon months. The time taken to reach the various arbitrary stages of regeneration in the three groups of lizards under the various lighting schedules is shown in table-1. The regeneration process was completed in all the three groups of animals exposed to LL photoperiodic schedules by the 50th day and in LD 12:12 and 0:24 by the 60th day post-caudal autotomy. The pattern of growth rate in the monsoon season was similar to that in summer.

Winter season :

During the winter season, the average daily temperature at the level of the animals was 17°C. The positive influence of long-day photoperiod, observed in summer and monsoon months was abolished during the winter season and there was a delay in the formation of the blastema. The delay in reaching the various stages of regeneration during this season is represented in table - 1.

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES ON REGENERATION IN NORMAL, BLINDED AND PINEALECTOMIZED H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT, NORMAL LIGHT AND DARKNESS AND CONTINUOUS DARKNESS DURING SUMMER, MONSOON AND WINTER SEASONS.

SEASONS AND LIGHTING REGIMES	WOUND HEALING		BLASTEMA		EARLY DIFFERENTIATION		MID DIFFERENTIATION		LATE DIFFERENTIATION		GROWTH		FULLY REGENERATED TAIL	
	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px
SUMMER														
LL (H)	1	3	3–5	5–7	5–7	7–9	8	10	14	18	20	24	50	50*
LL (L)	1	3	3–5	5–7	5–7	7–9	8	10	14	18	20	24	50	50
LD 12:12	3	5	5–7	8–10	10–12	13–15	16	20	23	23	32	36	60	60
LD 0:24	6	8	9–11	12–14	14–16	17–19	20	25	28	33	35	41	60	60
MONSOON														
LL (H)	3	5	5–7	8–10	7–9	10–12	10	15	20	25	30	32	50	50
LL (L)	3	5	5–7	8–10	7–9	10–12	10	15	30	35	40	42	50	50
LD 12:12	5	7	8–10	15–17	12–14	18–20	20	25	30	35	40	42	60	60
LD 0:24	8	10	12–14	18–20	16–18	24–26	25	38	40	42	45	47	60	60
WINTER														
LL (H)	7	12	8–10	16–18	25–27	32–35	30	38	33	40	38	45	50	50
LL (L)	7	12	8–10	16–18	25–27	32–35	30	38	35	40	42	45	50	50
LD 12:12	11	17	22–24	25–27	32–34	38–40	38	42	40	44	42	46	60	60
LD 0:24	14	20	28–30	32–34	36–38	40–42	40	44	43	46	46	48	60	60

LL (H) - CONTINUOUS LIGHT (HIGH INTENSITY)
LL (L) - CONTINUOUS LIGHT (LOW INTENSITY)
LD 12:12 - 12 HOURS LIGHT (HIGH INTENSITY) AND 12 HOURS DARKNESS
LD 0:24 - CONTINUOUS (TOTAL) DARKNESS
NL - NORMAL LIZARDS
BL - BLINDED LIZARDS
Px - PINEALECTOMIZED LIZARDS
* - DAYS POST-CAUDAL AUTOTOMY

TABLE 2. THE PERCENTAGE INCREMENT IN TAIL REPLACEMENT IN THE THREE LIGHTING SCHEDULES UNDER INCREASING TEMPERATURES.

TEMPERATURE VARIATION	% increment in tail replacement					
	LD 0 : 24		LD 12 : 12		LD 24 : 0	
	NL	Px	NL	Px	NL	Px
18°C - 27°C	145.1	281.0	98.71	191.18	30.75	124.96
27°C - 31°C	3.0	2.0	15.0	0.2	17.0	2.0

TABLE 3. AVERAGE MONTHLY CAGE TEMPERATURE DURING SUMMER, MONSOON AND WINTER SEASONS.

MONTHS AND SEASONS	CAGE TEMPERATURE MEASUREMENTS IN °C. MAXIMUM	MINIMUM	HUMIDITY MAXIMUM	MINIMUM
SUMMER				
MARCH, 1987	35.0	21.0	77%	07%
APRIL, 1987	37.0	23.0	88%	03%
MAY , 1987	41.0	27.0	83%	04%
	AVERAGE CAGE TEMPERATURE LIGHTED CHAMBER = 31°C DARK CHAMBER = 29°C = 30°C			
MONSOON				
AUGUST, 1986	28.0	22.0	99%	42%
SEPTEMBER, 1986	23.0	22.0	94%	40%
OCTOBER , 1986	35.0	20.0	93%	13%
	AVERAGE CAGE TEMPERATURE LIGHTED CHAMBER = 27°C DARK CHAMBER = 25°C = 26°C			
WINTER				
NOVEMBER, 1986	21.0	11.0	82%	18%
DECEMBER, 1986	26.0	13.0	84%	16%
JANUARY , 1987	23.0	12.0	83%	22%
	AVERAGE CAGE TEMPERATURE LIGHTED CHAMBER = 18°C DARK CHAMBER = 16°C = 17°C			

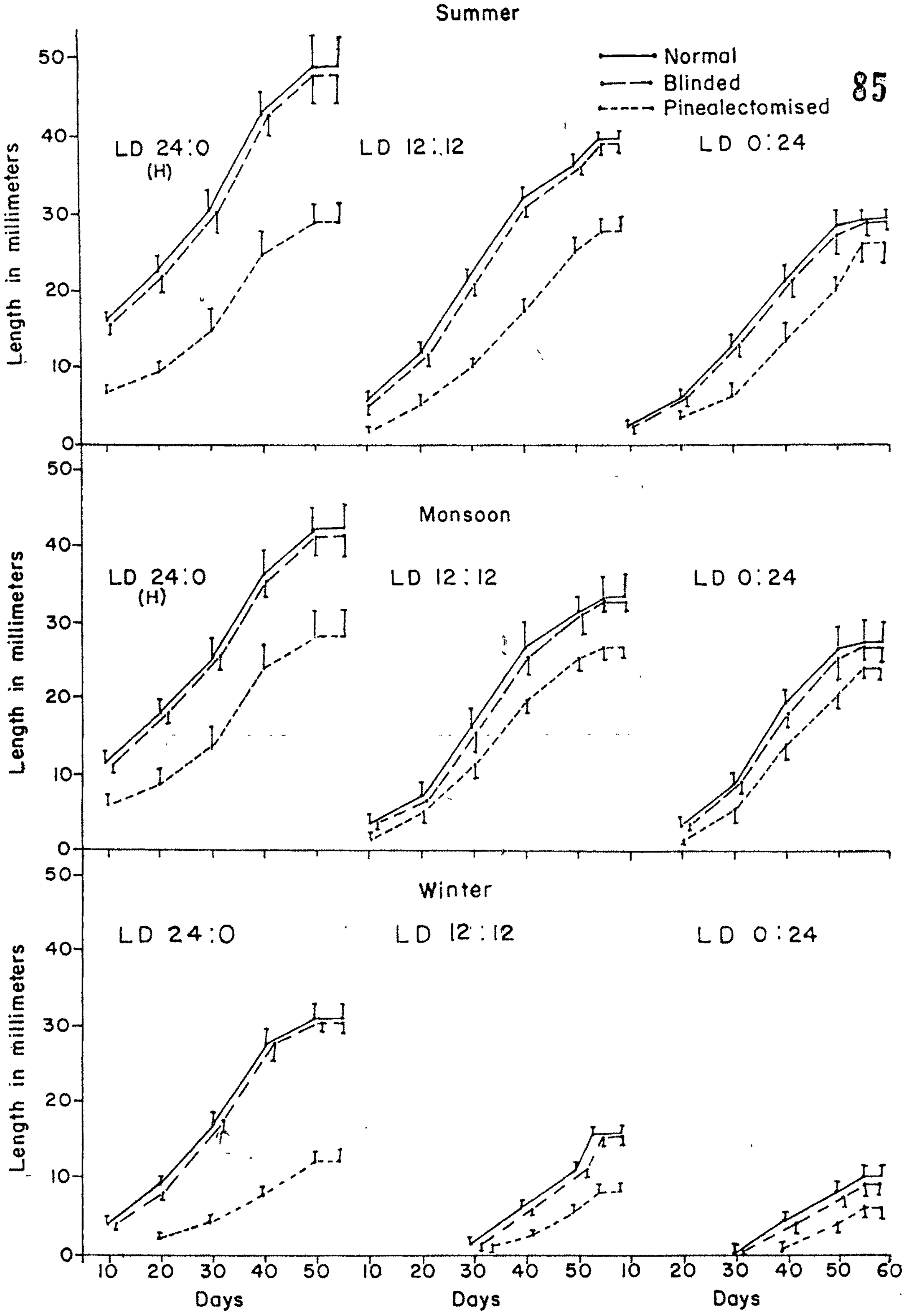

Fig. 1 Seasonal regenerative tail elongation in relation to different photoperiodic schedules in normal, blinded and pinealectomized *Hemidactylus flaviviridis*.

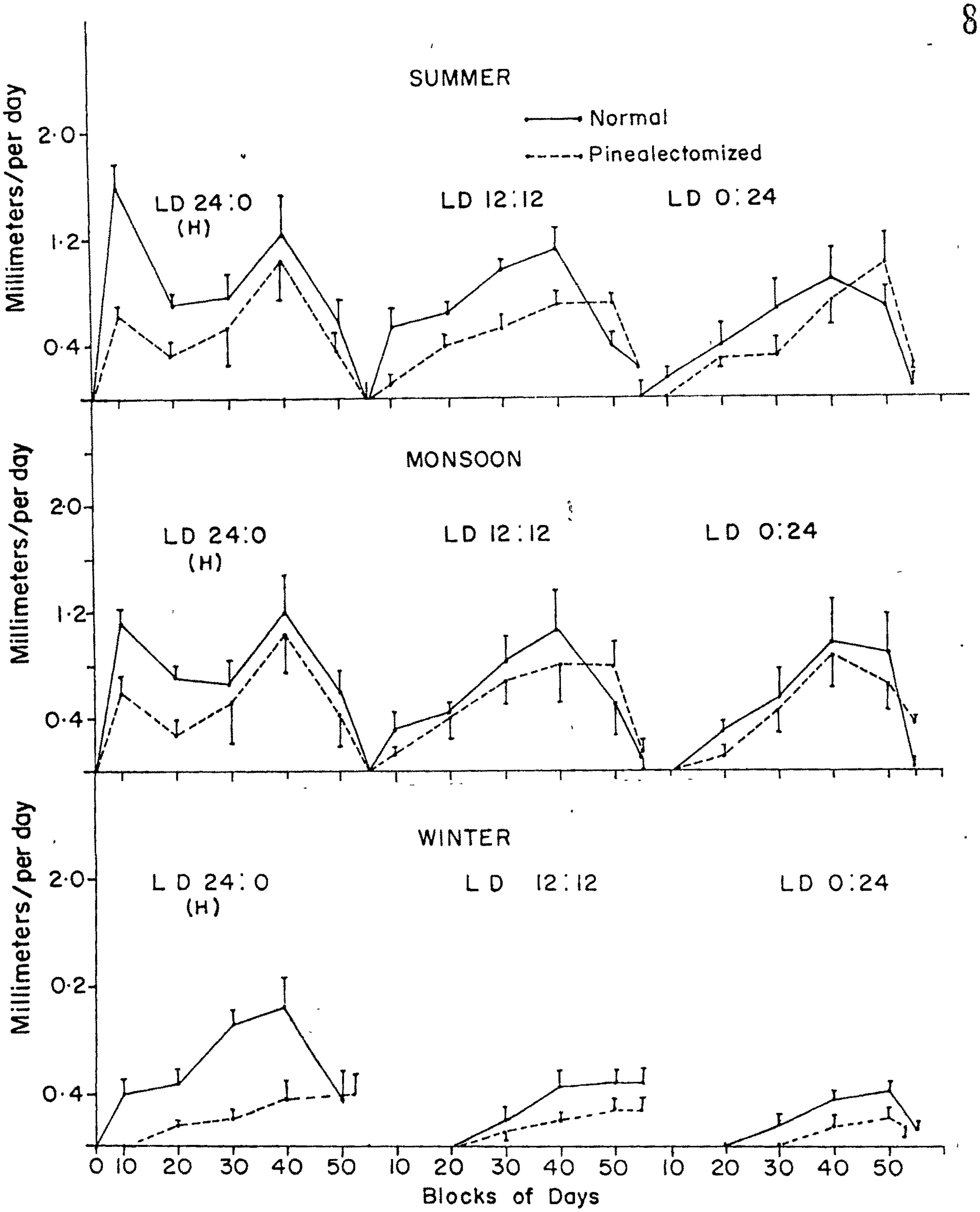

Fig. 2 Seasonal growth rates in relation to different photoperiodic schedules in normal and pinealectomized *Hemidactylus flaviviridis*

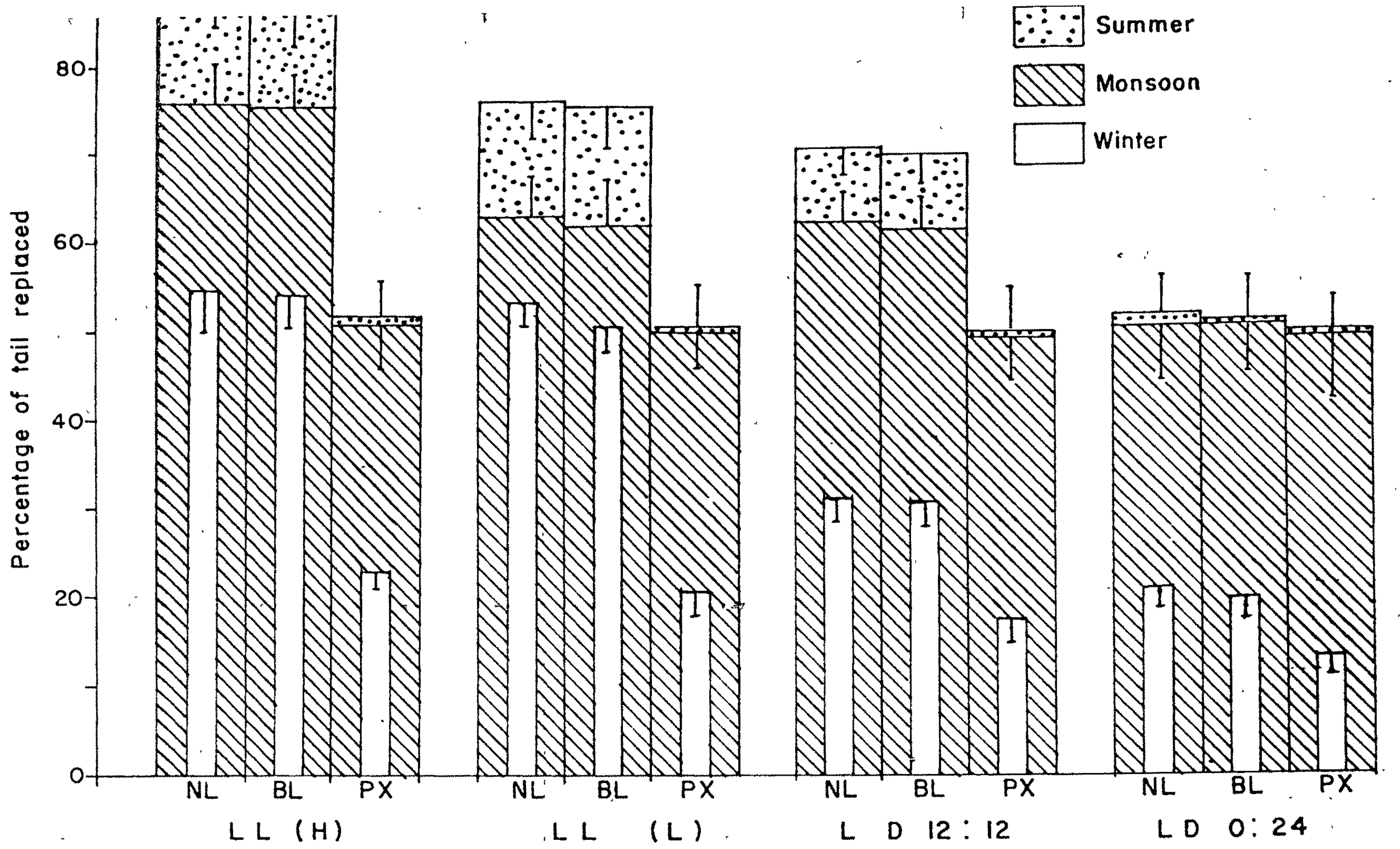

Fig. 3: Seasonal differences in percentage tail replacement under different photoperiodic schedules in normal, blinded and pinealectomized Hemidactylus flaviviridis

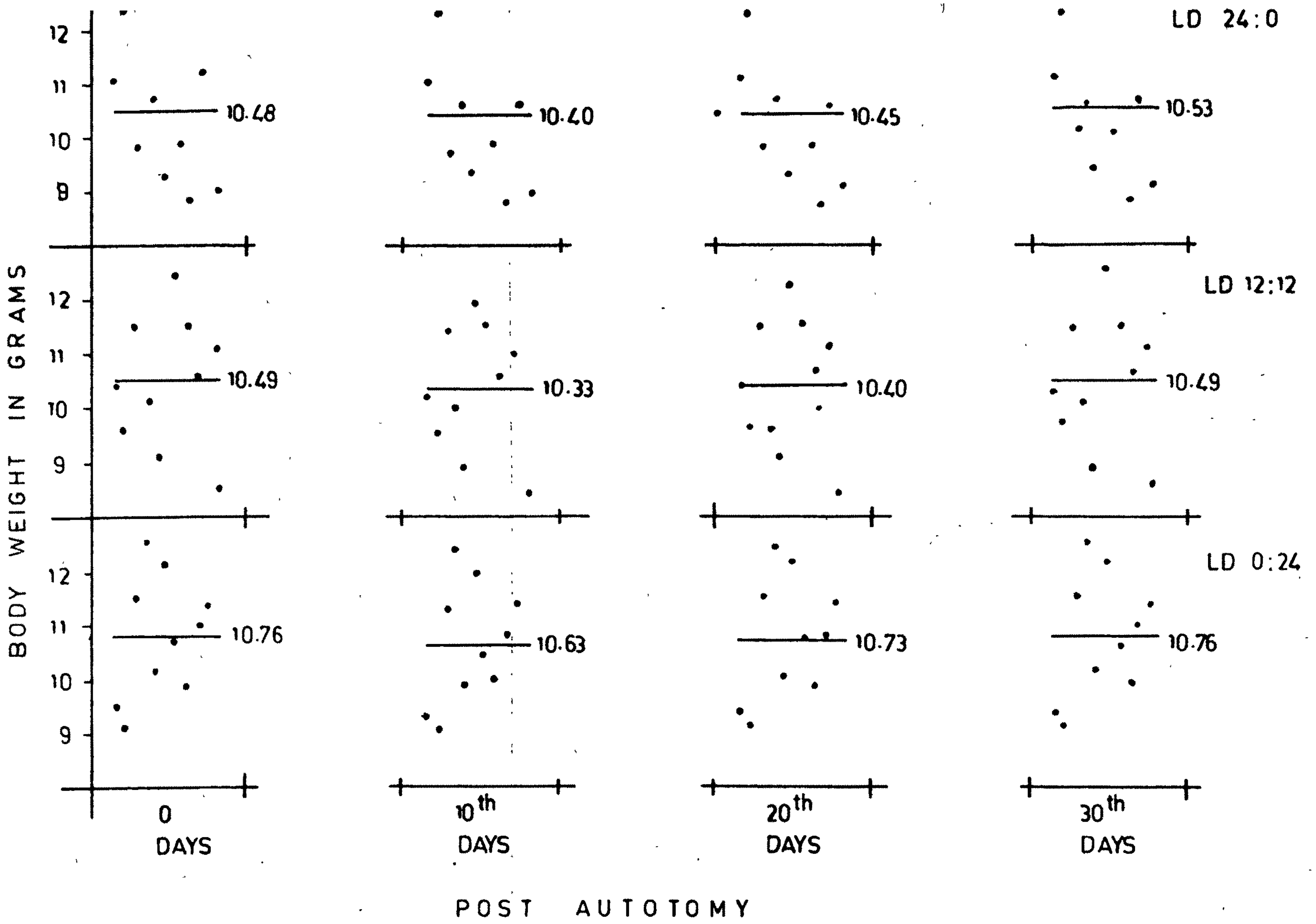

FIG.-4 SHOWING THE BODY WEIGHT (MEAN AND INDIVIDUAL) OF 10 LIZARDS DURING 30 DAYS OF TAIL REGENERATION POST- AUTOTOMY UNDER LD 24:0, LD 12:12 AND LD 0:24

The regeneration process was completed in NL,BL and Px animals exposed to LL schedules by the 50th day and in LD 12:12 and LD 0:24 by the 60th day post-caudal autotomy. The biphasic growth rate curve, observed in summer and monsoon seasons in NL,BL and Px groups of animals exposed to LL schedules was completely obliterated during the winter months.

All possible comparisons between NL,BL and Px in LL(H), LL(L),LD 12:12 and LD 0:24 on one hand and between NL,BL and Px in summer, monsoon and winter seasons on the other (Duncan's multiple range test) showed no statistical significance between NL and BL groups of animals. However, all other comparisons other than these were statistically significant at both 5% and 1% levels (Duncan, 1955).

DISCUSSION

Seasonal differences on lacertilian tail regeneration have received scant attention previously. Day length and temperature are important determinants of seasonality in most ectothermic vertebrates. Turner and Tipton (1972) showed that the lizard, Anolis carolinensis regenerates its tail more rapidly when exposed to a long-day photoperiod (18 hours) than to a short-one (6 hours). Maderson and Licht (1968) and Tassava and Goss (1966) demonstrated the influence of temperature on the final form of the regenerated tail in A. carolinensis, a smaller proportion being replaced at a lower temperature (21°C) and a bigger proportion at a higher temperature (32°C).

Results of this investigation in which normal (NL), blinded (BL) and pinealectomized (Px) Gekkonid lizards, Hemidactylus flaviviridis were exposed to LL(H), LL(L), LD 12:12 and LD 0:24 photoperiodic schedules, demonstrated that season may influence at least 4 different aspects of the regeneration process. A comparative evaluation of results obtained in summer (March-May), monsoon (August-October) and winter (November-January) seasons indicates that the initiation of regeneration, the daily growth rate, the final length of tail replaced at the end of regeneration and the total percentage replacement of the lost (autotomized) tails are all enhanced during the summer months and retarded in the winter months with the regenerative performance during the monsoon season in between. Secondly, there was no statistical difference in the regenerative tail elongation in NL and BL Hemidactylus in any season, however, Px lizards generally showed inhibited regenerative performance when compared to their NL or BL counterparts. Furthermore, the observations demonstrate that low temperature may mask photoperiodic influence during winter (17°C) but as it increases through monsoon (26°C) to summer (30°C) seasons, photoperiodic influence becomes markedly enhanced (Tables 2 and 3) and (figures 1 and 3).

This investigation further demonstrates that in NL and BL lizards during summer and monsoon seasons, the positive influence of continuous illumination on the regenerative performance is essentially exerted in the initial blastemic and early differentiation stages which are characterized by high mitotic potential. 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. The stimulatory effect of light in NL and BL groups of animals is reduced during the winter season and abolished by pinealectomy since the regeneration process in Px lizards is not affected by either continuous light (LD 24:0) or continuous darkness (LD 0:24). The biphasic growth rate curve, characteristic of NL and BL animals exposed to continuous illumination in summer and monsoon seasons but not in the winter months,was also discernible in Px lizards. It is difficult to give a sound interpretative explanation for the observed biphasic growth rate curve in Px groups of animals. However, Rusak and Zucker (1979) had shown that the suprachiasmatic nucleus of the hypothalamus is light sensitive and, therefore, it is presumed that in the absence of the pineal-the principal photoreceptor organ in lacertilians - some amount of light may reach the suprachiasmatic nucleus through an extrapineal pathway to produce the biphasic growth rate curve observed in Px Hemidactylus.

A number of factors other than photothermal influences in lacertilian tail regeneration have been suggested. Tassava and Goss (1966) and Bryant and Bellairs (1967) demonstrated that rates of regeneration (but not the final proportion regenerated) increased in proportion to the amount of tail removed. Hughes and New (1959) and Bryant and Bellaris (1967) showed that repeated autotomies lowered the regeneration rates. It will also be presumed that the size and sex of the experimental animals as well as the individuals' nutritional state may effect the capacity for tail regeneration. Animals of similar size were used and they were composed of equal numbers of males and females in each experimental cage. The animals were fed with cockroaches <u>ad libitum</u> throughout the entire period of the experiment. In the present investigation, each experimental lizard was autotomized at the third segment from the vent, thereby, maintaining a consistency at the plane of autotomy as well as the length of tail removed. Thus none of the extra-photothermal factors mentioned above would presumable give rise to variations beyond those reported here. Comparison of the values obtained in the three different seasons further confirm the conclusion that the final length of tail regenerated as well as the total percentage replacement of the lost (autotomized) tail are photoperiod-temperature dependent. The observed positive influence of continuous illumination on tail regeneration in <u>H</u>. <u>flaviviridis</u> during summer (30°C) and monsoon (26°C) suggests that environmental cues play important roles in the initiation of regeneration, the subsequent progressive elongation of the regenerating tail and the final length of the full regenerate. Both photoperiod and temperature appear to be potential modifiers of the regeneration process. The results show that temperature may possess a dominant role over photoperiod in as much as an optimal temperature has to be maintained for the photoperiodic influence to be exerted. The importance of temperature in the photoperiodic response in NL, BL and Px Hemidactylus during their tail regeneration is further illustrated by the marked reduction in the effect of continuous light and the obliteration of the biphasic growth rate curve during the winter season (17°C).

Calculation in terms of percentage increment or decrement in percentage tail replacement represented in Table 2 shows that an increase in temperature of 9°C from the average winter temperature of 17°C to the monsoon temperature of 26°C improves the regenerative performance of both NL and Px lizards to a great degree under all the three photoperiodic schedules. However, a further increase in temperature by 4°C from that of monsoon to that of summer (30°C) elicites better and increasing regenerative performance in NL lizards with increase in photoperiodic condition, while in Px lizards, there was hardly any improvement. Apparently, any increase in temperature from the winter temperature (17°C) to that of monsoon (26°C) is perceived by the body independent of the pineal. However, at temperatures above that of the monsoon months, the pineal organ is the essential receptor and synchronizer of the photothermal input in lacertilians during their tail regeneration.

From this investigation, 4 important conclusions have emerged : (1) there is a positive correlation between an increase in photothermal input and the total amount of tail regenerated and total percentage of the autotomized tail replaced (2) both temperature and photoperiod affect lacertilian tail regeneration, and the effect of one may complement the influence of the other (3) the influence of temperature at 17°C masked that of photoperiod but even then a closer scrutiny of the data reveals that the involvement of photic input is still noticeable, though in a much attenuated fashion, (4) the pineal organ of Hemidactylus may be involved in thermosensitivity at temperature ranges above the optimum (monsoon temperature - 26°C) just as it has been demonstrated (Ramachandran and Ndukuba, 1989a) to be directly involved in photoreception during the process of lacertilian tail regeneration.