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CHAPTER V

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EFFECTS OF PHOTOPERIODISM, PINEALECTOMY AND SEASONAL VARIATION IN TEMPERATURE ON TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS.

Most living organisms use the annual changes in some environmental variables such as photoperiod. temperature. rainfall, or food supply to synchronize several physiological or endocrine functions such as reproduction, hibernation, or thermoregulation with the seasons (see Vivien-Roels et al., 1988). 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 retinat of their lateral eyes. Interestingly, even in species with highly organized ocular photoreceptors, additional photoreceptive structures - extraocular photoreceptors - are utilized in the transmission of photic information about the day - night schedule and seasonal photoperiodic 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) Chapter 3 and in lizards (Ramachandran and Ndukuba 1989). The pineal system (pineal organ and parietal eye) is light sensitive on the basis of neurophysiological and cytological evidence

(Wurtman <u>et al.</u>, 1968). A feature of every vertebrate pineal is its capacity to synthesize different indoleamines including serotonin (5-hydroxytryptamine) and melatonin (5methoxy-N-actyltryptamine). The daily rhythmic secretion of the pineal indoleamine, melatonin, is able to interpret seasonal and photoperiodic changes (for refereces see, Tamarkin <u>et al.</u>, <u>1985</u>; Skene <u>et al.</u>, 1987). Light and temperature are two important stimuli that can affect pineal melatonin levels in vertebrates, and alterations in the length of light have been shown to alter the levels of nocturnal melatonin secretion in some nonmammalian vertebrates, such as the quail (Underwood and Siopes, 1985), the rainbow trout (Duston and Bromage, 1987), the laying chicken (Lious <u>et al.</u>, 1987), the box turtle (Vi**w**ien-Roels <u>et al.</u>, 1988) and the anole lizard (Underwood, 1985).

Enviornmental cues are utilized by various reptiles to maintain annual cycles in growth, spontaneous activity and reproduction. For example, additional illumination during the normal quiscent phase of reproduction is reported to stimulate testicular growth in a variety of species. In the field, lizards depend upon solar radiation for elevation of body temperature and, thus, the duration of body heating is established by the daily photoperiod (Bartholomew, 1959; Licht, 1967). In the laboratory, it was found that low temperatures retarded development in embryonic primordia (Piatt, 1971) and regeneration in hydra (Corff and Burnett, 1970). Temperature, however is not the sole factor controlling the rate of regeneration in the newt, <u>Notophthalmus virideScens</u> was noted under controlled environmental conditions at 20°C. Linear regenerative

growth was enhanced in the summer compared to winter and reached its maximum rate in late spring-early summer (Schauble, 1972).

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. To our knowledge, there are no reports on the influence of photoperiodism, pinealectomy and seasonality on tail regenera-*Chapter 7* tion in a tropical saurian (Ndukuba and Ramachandran 1989a) *Chapter 3 bf* Ramachandran and Ndukuba 1989a,b). The present study was designed to investigate the influence of normal seasonal variation in temperature in relation to varying lengths of photic exposure on tail regeneration in normal, blinded and pinealectomized Gekkonid lizards, <u>Hemidactylus flayiyiridis</u>.

MATERIALS AND METHODS

A total of 600 lizards was used in each of the three seasons and they were divided into three groups and exposed to photoregimes LD 18 : 6, LD 16 : 8, LD 12:12, LD 8 : 16 and LD 6 : 18 as described on pages 12 and 13.

Group 1 - Experimental (PX) :

The first group of 200 lizards was pinealectomized (PX) by surgical removal of the pineal organ. Pinealectomy was performed as described in chapter.3. Pinealectomy in each animal took about three minutes and PX lizards were allowed 5 days recovery period in order to eliminate any traumatic side effect due to surgery. They were then divided into 5 batches of 40 lizards each and exposed to the five different photoperiodic conditions prior to tail autotomy. Mortality in PX animals was negligible. Microscopic examination of PX lizards showed that the pineal gland was removed completely and no damage was done to the brain. Food and water were provided <u>ad libitum</u>.

Group 2 - Experimental (BL)

The second group of 200 lizards was blinded (BL) by surgical removal of both the lateral eyes (Bilateral orbital enucleation). Blinding was done as described in *chapter*. 2. 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 also divided into 5 batches of 40 lizards each and were acclimated for another 7 days to the five different photoperiodic schedules prior to tail 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 live cockroaches and began feeding <u>ad libitum</u>.



Group 3 - Control (NL)

A third group of 200 lizards, divided into 5 batches of 40 lizards each served as control (NL). NL lizards remained sighted with the pineal organ intact. They were acclimated for 7 days to the five experimental photoperiods prior to tail autotomy. Food and water were provided <u>ad libitum</u>.

Lizards in all the groups were fed with cockroaches ad <u>libitum</u> during the entire period of study (60 days). None of the animals in any of the experimental set ups showed any noticeable behavioural changes. Moreover, no mortality was observed in any of the groups of animals at the end of the experiment.

Tail autotomy was carried out by pinching off the tails at the third segment from the vent, and the animals were exposed to the five different photoperiodic schedules during the entire process of tail regeneration. The length of tails removed from the lizards varied from 50 mm to 60 mm depending on the length of each tail from the third segment to the tip of the tail. 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. This investigation was extended over three seasons : Summer (March - May), Monsoon (August -October) and Winter (November - January). The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and further to Duncan's

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multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, 1955).

RESULTS'

The results are shown in tables 1-4 and figures 1-5. The initiation of regeneration, the daily growth rate, the total length of new growth (regenerate) produced at the end of regeneration and the total percentage replacement of the lost (autotomized) tails were all significantly affected by both season (temperature) and photoperiodism. A comparative evaluation of the regenerative performance in NL, BL and PX Hemidactylus in three different seasons showed enhancement during the summer season and depression during the winter months with the regenerative performance during the monsoon season in between. Furthermore, there was no significant difference between any of the parameters in NL and BL groups of lizards, however, PX animals produced significant retardation in tail elongation when compared with their NL or BL counterparts (Figs 1 and 3). During summer and monsoon seasons, there was a positive influence of increasing lengths of light on tail regeneration in NL and BL groups of lizards exposed LD 16:8 and LD 18:6 from the average photoperiodic schedule of LD 12:12, whereas a negative influence of decreasing photoperiodism was the case in their counterparts exposed to LD 8:16 and LD 6:18 conditions. Pinealectomy abolished the stimulatory influence of increasing photoperiodism and

significantly retarded the regeneration process. Further, the positive influence of increasing lengths of light observed in summer and monsoon seasons, was abolished during the winter season and there was a pronounced delay in the formation of the regeneration blastema (Figs. 1,3 and Table 1).

The pattern of growth during summer and monsoon seasons (Fig. 2) indicates a positive increase peaking at 30-40 days in NL,BL and PX lizards exposed to all the photoperiodic schedules except LD 18:6, LD 18:6 condition 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 LD 18:6 to LD 6:18 schedules (Fig 2). In the winter months, the pattern of growth rate showed a positive increase peaking at 30-40 days in the LD 18:6 condition and 40-50 days in LD 16:8, LD 12:12, LD 8:16 and LD 6:18 photoregimes. The biphasic growth rate curve, observed during summer and monsoon seasons in NL,BL and PX groups of animals exposed to LD 18:6 was completely abolished during the winter season (Fig 2).

All possible comparisons between NL,BL and PX in LD 18:6, LD 16:8, LD 12:12, LD 8:16 and LD 6:18 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 difference between NL and BL groups of animals. However,all

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APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN NORMAL, BLINDED AND PINEALECTOMIZED H.FLAVIVIRIDIS EXPOSED TO FIVE DIFFERENT PHOTO-REGIMES DURING THREE SEASONS (TEMPERATURES). • •---TABLE

	f-	Рх		60	60	60	60	50		60	60	60	60	50
/	STOP OF GROWTH	- NL/BL		60	60	60	60	50		60	. 09	60	60	50
		Ρx		41	39	36	36	24		47	44	42	42	32
	GROWTH	NL /BL		35	34	32	32	20		45	42	40	40	30
	I N	Рх		33	31	29	29	<u>00</u>		42	38	35	35	25
	LATE DIFFEREN- TIATON	NL/BL		29	27	23	23	14		40	32	30	30	20
	EN-	Px		25	23	20	20	10		38	30	25	25	15
	MID DIFFEREN-	NL/BL		20	18	16	16	ω		25	22	20	20	10
	- LN	Ρx		17-19	15-17	13-15	13-15	7-9		24-26	20-22	18-20	18-20	10-12
	EARLY DIFFERE	NL/BL PX		14-16	12-14	10-12	10-12	5-7		16-18	14-16	12-14	12-14	7- 9
	Ł	Px		12-14	10-12	8-10	8-10	5- 7		18-20	15-17	12-14	12-14	8-10
	BLASTEMA	NL/BL Px		9-11	7-9	2 2	5- 7	3- 5		12-14	10-12	8-10	8-10	2-1
	U	×'		ω	9	വ	ß	ო		10	ω	7	7	۱Ω
	WOUND	NL/BL		9	4	ო	ო	~~		8	9	വ	വ	ო
ويوا الما إمار بيني فينا فينا ألفا الما المالية المالية المالية عنه في	SEASONS AND	CONDITIONS	SUMMER	LD 6:18	LD 8:16	LD 12:12	LD 16: 8	LD 18: 6	NOOSNOM	LD 6:18	LD 8:16	LD 12:12	LD 16: 8	LD 18: 6

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	OF H Px	00000 00000	
	STOP GROWTH NL/BL	60 60 60	
	PX	48 46 46	45 50 50 50 50 50 50 50 50 50 5
	GROWTH NÍL/BL	46 42 42 42	
	х d	4 4 4 4 4 4 6 4 4 5 4 6 4 4 5 4 6 4 5 4 6 4 5 4 6 4 6	
-	LATE DIFFERENT- IATION NL/BL	4 4 4 4 4 4 0 4 0 4 0 0 4 0 0 0 0 0 0 0	and
	SENT- I Px	44 42 42 42	
	MID DIFFERENT- IATION NL/BL Px	33 33 40 33 33 40	5 30 (HIGH IN (HIGH (HIGH IN (HIGH IN (HIGH IN (HIGH (HIGH (HIN (HIGH (HIGH (HIGH (H
	- TNI Px	40-42 39-41 38-40 38-40	-27 32–35 30 3 -27 32–35 30 3 URS LIGHT (HIGH INTEN URS LIGHT (HIGH INTEN LECTOMIZED LIZARDS LECTOMIZED LIZARDS POST-CAUDAL AUTOTOMY
	EARLY DIFFERENT- IATION NL/BL F	36-38 34-36 32-34 32-34	
-	ХД	32-34 28-30 25-27	16-18 2 16-18 2 16-18 2 176-18 2 176-18 4 176-18 4 176-18 1 18 1 18 1 18 1 18 1 18 1 19 1 10 10
	BLASTEMA NL/BL	28-30 25-27 22-24	8 - 10 8 - 10 8 - 10 12 - 12 18 - 6 18 - 6
	X A	20 17 17	
	WOUND HEALING NL/BL '	4015	- 12
LOI	SEASONS AND LIGHT CONDITIONS	WINTER LD 16:18 LD 8:16 LD 12:12 LD 12:12	

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

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UNDER INCREASING TEMPERATURES.

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IEMFERALURE VARIATION	NL PX	PX	NL	NL PX	NL PX	PX	NL PX	1	NL PX
WINTER - MONSOON 17°C - 26°C.	94.4	132 . 5	73.5	94.9	59.8 75 . 5	75.5	50.3 65.7	45.1	55.2
MONSOON – WINTER 26°C – 17°C	48.5	57.0	42.5	48.7	37.4 43.1	43.1	33 . 4 39 . 6	28.6	35.5
MONSOON - SUMMER 26°C - 30°C	4 . 5	0•2	12.4	0•3	15.0	0.2	15.6 0.3	16.0	0.5
SUMMER - MONSOON 30°C - 26°C	2°2	0•2	11.0	0.4	12.9	12.9 0.4	13.5 0.4	13.8	3 0 • 6

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TO TO	IMPROVEMENT IN REGENERATIVE PERFORMANCE IN <u>H.FLAVIVIKIUIS</u> FROM LD 6: 18 TO LD 18: 6 DURING SUMMER, MONSOON AND WINTER SEASONS (TEMPERATURES).	DRMANCE IN POON AND WINT	N REGENERALIVE PERFORMANCE IN <u>H.FLAVIVIKIUIS</u> FROM LD 6 : 18 DURING SUMMER, MONSOON AND WINTER SEASONS (TEMPERATURES).
SEASONS - AND TEMPERATURES.		RIMENTAL PHOTOPERIODIC S LD 6 : 18 to LD 18 : 6 NL PX	EXPERIMENTAL PHOTOPERIODIC SCHEDULES LD 6 : 18 to LD 18 : 6 NL PX
WINTER			
1'7°C		78.9	51.6
MONSOON			
26°C	29	29 . 0	1. 2
SUMMER			
30°C	43	43.1	1°5

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α. IMPROVEMENT IN REGENERATIVE DEREORMANCE IN H FLAVIVIRIDIS FROM I.D 6 TABLE 3.

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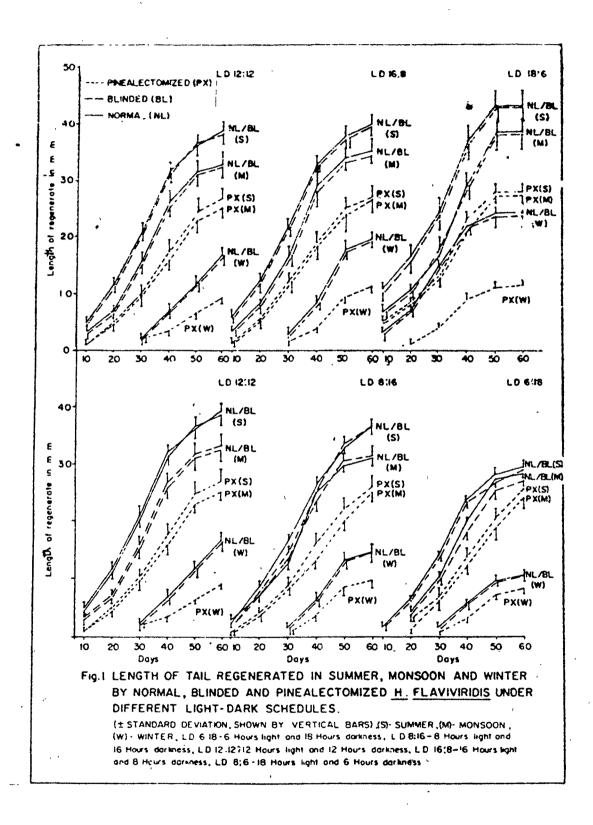
MONTHS AND SEASONS	CAGE TEMPERATURE A HIGHEST MAXIMUM	TEMPERATURE MEASUREMENTS IN °C. HEST LOWEST AUM MINIMUM	HUMIDITY HIGHEST MAXIMUM	LOWEST MINIMUM
SUMMER MARCH, 1987	35 . 0°C°	21 . 0°C	77%	. %20
APRIL, 1987	37.0°C	23 . 0°C	88%	03%
MAY, 1987	41.0°C 27.0°C AVERAGE CAGE TEMPERATURE	27 . 0°C AATURE = 30°C	83%	04%
NONSOON				
AUGUST, 1986	28 . 0°C	22.0°C	%66	42%
SEPTEMBER, 1986	23 . 0°C	22 . 0°C	94%	40%
OCTOBER,1986	35.0°C AVERAGE CAGE TEMPERA	20.0°C Temperature = 26°C:	63%	13%
WINTER				
NOVEMBER,1986	21 • 0°C	11.0°C	82%	18%
DECEMBER,1986	26 . 0°C	13.0°C	84%	16%
JANUARY, 1987 H	23.0°C 12.0°C AVERAGE CAGE TEMPERATURE = 17°C	$12_{0}^{\circ}0^{\circ}C$ ATURE = $17^{\circ}C$	83%	22%

TABLE 4. AVERAGE MONTHLY CAGE TEMPERATURE DURING SUMMER, MONSOON AND WINTER SEASONS.

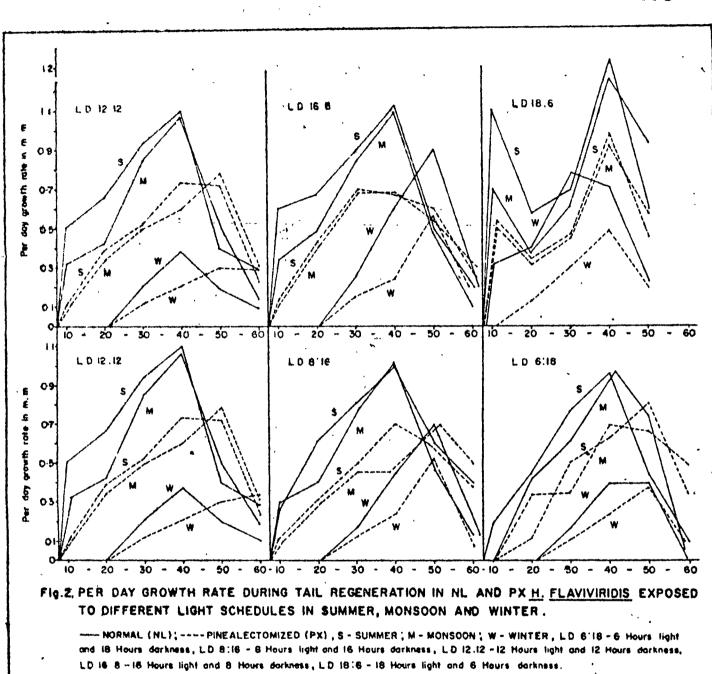
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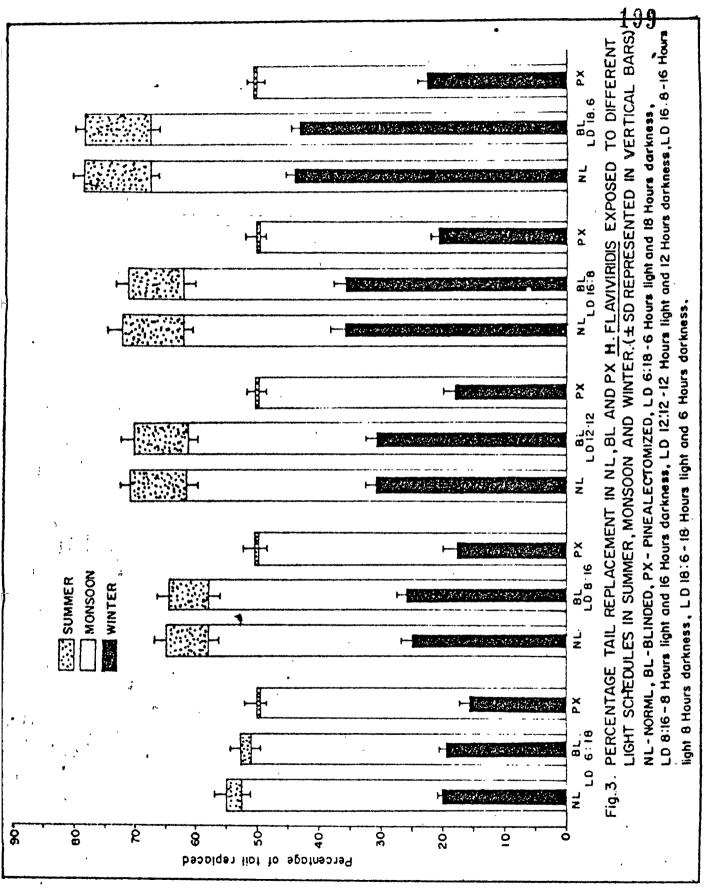
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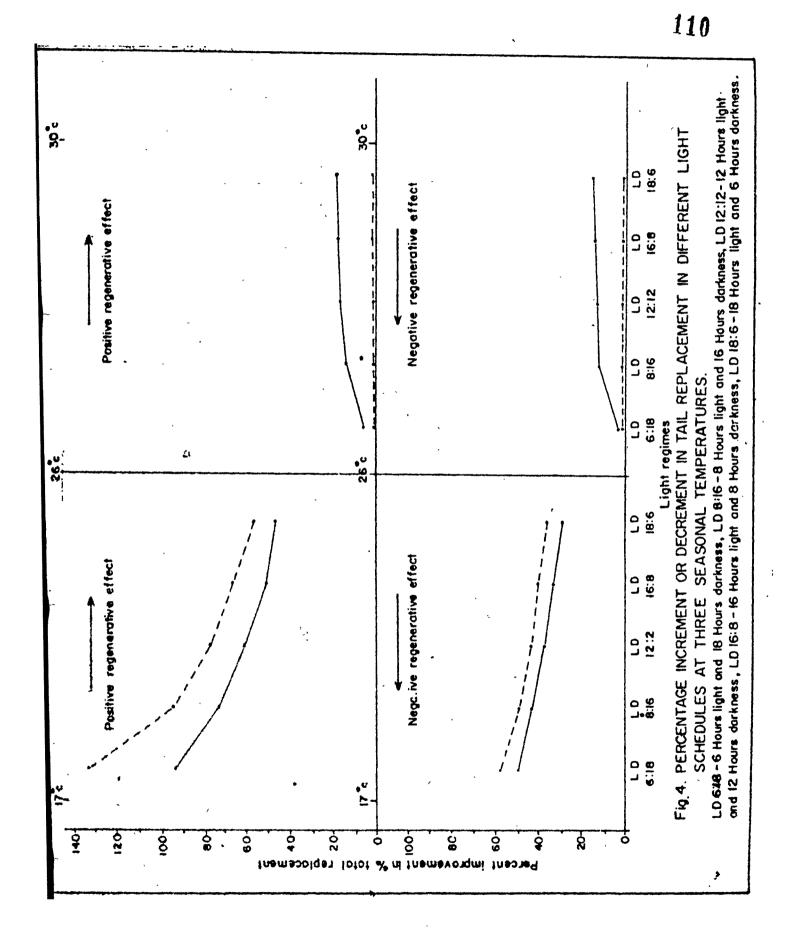


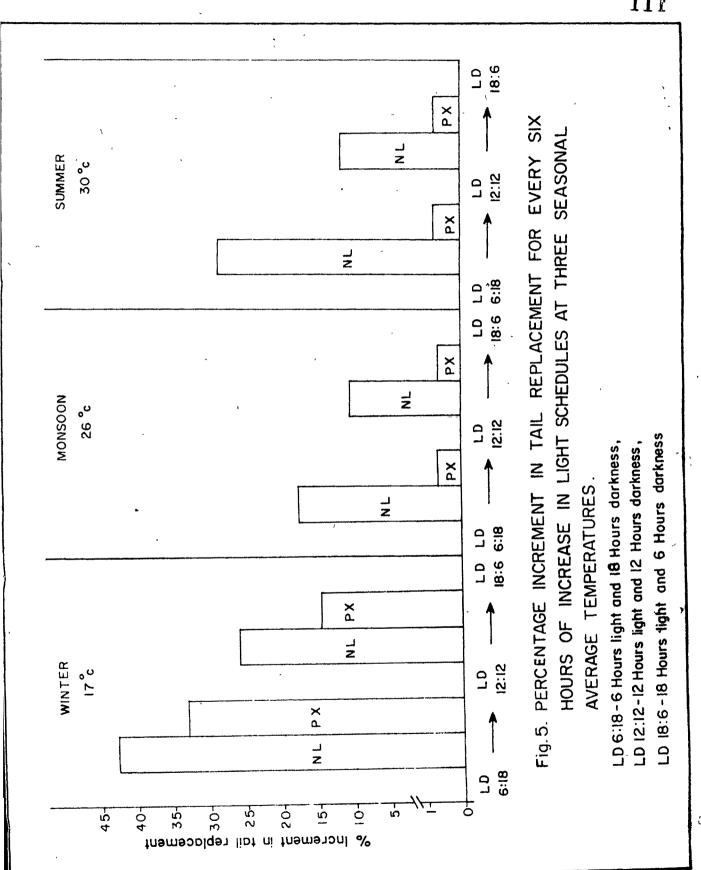
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other comparisons other than these were statistically significant at both 5% and 1% levels (Duncan, 1955).

DISCUSSION

Previous results from our laboratory demonstrated that continuous light stimulates tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis while continuous darkness depresses it (Ndukuba and Ramachandran 1989a) and further, the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception since blinded lizards regenerated their autotomized tails like their sighted counterparts exposed to similar experiment photoperiodic chapter 2 schedules (Ndukuba and Ramachandran 1988). Moreover, the pineal organ is the principal site of extraretinal photoreception in Hemidactylus since both pinealectomy as well as light deprivation to the pineal abolished the stimulatory influence of increasing photoperiodism and significantly retarded the regeneration process (Ramachandran and Ndukuba Chapter 3 198**9**a,). The present investigation was an attempt to ascertain the influence of different seasons (temperatures) on photoperiodic control of tail regeneration in lacertilians. NL. BL and PX Hemidactylus were exposed to increasing and decreasing lengths of light in summer (March - May), monsoon (August - October) and winter (November - January) seasons. Increasing photoperiodism had a positive influence on tail regeneration while decreasing photoperiodism produced a negative influence in NL and BL groups of lizards. PX lizards were not

affected by either increased or decreased photoperiodism. These results, coupled with the observation that the regenerative performance was enhanced during the summer season and depressed in the winter months with the performance during the monsoon season in between, leads to the suggestion that tail regeneration in lizards is influenced by photoperiodism, pinealectomy and seasonal (temperature) variations.

Pineal organs of nonmammalian vertebrates contain photoreceptor cells or their derivatives. An evolutionary sequence in the restructuring of these photoreceptor cells, observed in anamniotes, through transitional cell types - noted in reptiles - to pinealocytes has been well documented (Collin et al., 1986; Gern and Greenhouse 1988). In its most diverse form, the reptilian pineal complex is composed of a superficially situated parietal eye (parapineal) and a deeper pineal . organ on the dorsal aspect of the diencephalon (see Firth et al., 1988). The pineal complex of lizards, the most extensively studied group of reptiles is photosensory Chapter 3 (Ramachandran and Ndukuba 1988a). Many lizard species have as part of the pineal complex a superficial parietal or "third" eye (Gundy and Wurst, 1976). The eye, an excellent wavelength discriminator, is more highly organized than the homologous frontal organ of amphibians (Dodt and Scherer, 1968). In Crotaphytus collaris, the parietal eye and the intracranial pineal organ have a feedback relationship wherein the parietal eve sends afferent impulses to the pineal body and the pineal



body sends efferent signals to the parietal eye (Englebtson and Lent, 1976). A parietal nerve in Lacerta viridis projects into the habenular region and a nerve from the pineal body reaches the subcommisural organ with some fibers traversing the posterior commisure (Kapper, 1967). The reptilian pineal complex transmits photic and perhaps other environmental information to the brain (Oksche, 1984). The complex is involved in numerous functions, including reproduction (De Vlaming and Olcese, 1981), circadian activity rhythms (Menaker and Wisner, 1983; Underwood, 1984), and thermoregulation (Ralph; 1984; Firth et al 1988). It is becoming increasingly apparent that, in addition to their photoreceptive function (Meissl and Dodt, 1981; Ramachandran and Ndukuba 1983a), the pineal photoreceptor cells or their derivatives are the site of an active indoleamine biosynthetic pathway with melatonin being a major secreted product (Juillard et al., 1983; Falcon et al., 1985, 1987; Collin et al., 1986; Gern and Greenhouse,1988).

Calculation on the basis of figure 3, represented in onel figures 4 and 5 tables 2 and 3, showed that an increase in temperature of 9°C from the average winter temperature of 17°C to the monsion temperature of 26°C, decreased the predicted improvement in regenerative performance with increasing photoperiodic schedules in both NL and PX lizards. Further, with an increase in temperature by 4°C from the average monsoon temperature of 26°C to the average summer temperature of 30°C,

the expected improvement in regenerative performance was increased with increasing photoperiodism in NL lizards while there was no noticeable effect in PX groups of animals. These observations indicate a seasonal (temperature)compensated effect by light when the temperature falls from monsoon to winter in both NL and PX lizards. Apparently, the light effect in the PX animals is essentially mediated by the lateral eyes probably to compensate for both the fall in temperature as well as the lack of the photoreceptive pineal organ. This supports the earlier conclusion of light perception by the lateral eyes in the absence of the pineal organ with regard to photoperiodic control of regeneration (Ramachandran and Ndukuba 19888 - Chapter 3). Another interesting revelation that comes to light is that when the temperature fell from summer to monsoon, increasing photoperiodism supplemented the negative seasonal (temperature) effect on regenerative performance in NL animals but not in PX groups of lizards. In other words, an increase in temperature above that of monsoon produced a cumulative photothermal effect in NL lizards. Apparently, temperature range around that of monsoon (26°C) showed minimal variation with various light schedules (Figures 4 and 5 and Tables 1 and 4) and hence, this temperature could be considered the optimum for regenerative performance in <u>H. flaviviridis</u>. Furthermore, it has earlier been demonstrated (Ramachandran and Ndukuba 1989 - chapter 4) that NL groups of animals produced improvement in the

regenerative performance with increase in temperature evel16 when exposed to continuous darkness (0 hours) which was not shown by PX lizards. This improvement in regenerative performance of NL lizards completely deprived of light, coupled with the present finding of the influence of increasing photoperiodism and the pineal organ above the optimum temperature, may indicate a fine thermosensitivity of the pineal organ in Hemidactylus at temperature ranges above the optimal.

This investigation further demonstrates that, in NL and BL lizards, during summer and monsoon seasons, the positive influence of increasing lengths of light 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 increasing photoperiodism in NL and BL groups of animals was reduced during the winter season and abolished by pinealectomy since the regeneration process in PX lizards was not affected by either increased or decreased lengths of exposure to light. The biphasic growth rate curve, characteristic of NL and BL animals exposed to LD 18:6 schedule in summer and monsoon seasons but not in winter months, was also discernible in PX lizards (Fig 2).



It is difficult to give a sound interpretative explanation for the observed biphasic growth rate curve in Px lizards exposed to LD 18:6 photoperiodic condition. However, Rusak and Zucker (1979) have demonstrated that the suprachiasmatic nucleus of the hypothalamus is light sensitive and, thus, it may be presumed that in the absence of the pineal organ, the principal photoreceptor organ in lacertilians (Ramachandran and Ndukuba 198**9**a - chapter 3) and under long length photoperiods, some photic information may reach the suprachiasmatic nucleus through an extrapineal pathway to produce the biphasic growth rate curve observed in Px Hemidactylus.

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> DIAGRAMATIC REPRESENTATION OF SECTION 1.

SECTION 2.

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Neuropharmacological Studies

on Lizard

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