CHAPTER

1

Photothermal Influences on Tail Regeneration: A Seasonal Evaluation

Many vertebrate species use the annual changes in day length to time such important events as moulting, fattening, migration and reproduction. Among poikilotherms, both day length and temperature are important factors regulating the annual gonadal cycle, as in the case of the lizards (Clausen and Poris, 1937; Fox and Dessauer, 1958; Licht, 1966, 1967a, b, 1971a,b; Noeske and Meier, 1983; Underwood and Hall, 1982; Mayhew, 1964; Licht *et al.,* 1969; Marion, 1976; Botte *et al.,* 1978). Though the initiation of regeneration is an innate process, it is however sensitive to modulation by various endogenous and exogenous factors. Amongst the environmental factors, though temperature variations on a seasonal basis do exert an influence, light or photoperiod is also a major factor involved in the regulation of various endogenous rhythms capable of modulating regenerative potential. However, the photothermal influence on vertebrate appendage regeneration has not been fully explored. It was shown by Schauble and Nentwig (1974) that newts, *Notophalmus viridescens*, exposed to cold temperatures regenerated their forelimbs more slowly than those at higher temperatures. Schauble (1972) demonstrated that animals kept in identical temperature and photoperiodic conditions regenerated their limbs more rapidly in summer months than in winter. Turner and Tipton (1972) showed that the lizard. *A. carolinensis*, regenerated its tail more rapidly when exposed to a long photoperiod (18hrs) than a short one (6 hrs). Maderson and Licht (1968) and Tassava and Goss (1966) demonstrated the influence of temperature on the final form of

regenerated tail in *A. carolinensis*; a smaller proportion being replaced at 21°C and a bigger proportion at 32°C. A more detailed study on photoperiodic influences on tail regeneration in *H. flaviviridis*, involving different light schedules and seasons has been carried out in this laboratory (Ramachandran and Ndukuba, 1989a, Ndukuba and Ramachandran, 1991b). It is the importance of light and temperature *per se* on regeneration and, the inadequate literature on lizard tail regeneration on this aspect, that has formed the basis for the present detailed evaluation of photothermal influences on regeneration in *H. flaviviridis*.

MATERIAL AND METHODS

Adults lizards, *H. flaviviridis* of both sexes weighing 10±2 gms and measuring 80±5mm snout-vent length were procured from a local animal supplier. They were maintained on a diet of cockroaches and water *ad libitum* for a period of seven days to acclimatize them to laboratory conditions.

The experiments were carried out over a period of 3 years i.e., 1993-1995 and consisted of three experimental schedules :

1. Influence of different temperature ranges on regenerative growth under LD 12:12 (NLD) and LD 0:24 (DD) conditions.

2. Impact of different intensities of light on regenerative growth at a constant average temperature of 30°C under LD 12:12 and LD 24:0 (LL) conditions.

3. Compensatory effect of light on temperature and that of temperature on light.

Schedule I (Temperature)

These experiments were done in different months during the 3 years period involving different temperature ranges under a constant light schedule of LD 12:12 or continuous darkness (LD 0:24). Two groups of lizards maintained under LD 12:12 and LD 0:24 were studied for their regenerative ability at 17°C, 23°C, 26°C, 29°C, 31°C and 33°C. The experiments were repeated at least twice during the 3 years and atleast 10 lizards were used in each experimental group. The average maximum and minimum temperature ranges and the months are given in table 1.

Schedule II (Light intensity)

These experiments were done during the months when the average temperature was 30°C. Four different light intensities ranging from a basal intensity of 150 lux units and 3 enhanced intensities of 300, 600 and 1200 lux were used. The experimental groups for each intensity of light consisted of 10 lizards.

TABLE - 1

Showing the average maximum, minimum and monthly mean temperatures for the three years of study

Months	1993		1994		1995	
JAN.	22.0 12.40	17.20	23.01 13.00	18.00	24.02 14.00	19.01
FEB.	28.01 15.11	21.56	29.52 17.65	23.58	29.1 19.08	24.09
MAR.	36.42 23.02	29.72	35.00 23.02	29.01	37.12 23.11	30.115
APR.	39.00 27.50	33.25	27.57 25.12	31.34	39.22 23.86	31.54
MAY	40.21 28.96	34.58	39.12 27.00	33.06	40.00 26.08	33.04
JUN.	34.52 27.52	31.02	33.30 26.90	30.1	37.00 25.00	31.00
JUL.	31.00 25.00	28.00	29.51 26.05	27.78	31.98 25.14	28.56
AUG.	28.00 24.00	26.00	27.12 24.62	25.87	29.00 25.02	27.01
SEP.	30.53 27.61	29.07	31.73 23.07	27.4	33.58 26.00	29.79
OCT.	34.12 25.93	30.02	35.96 23.52	29.74	36.60 25.62	31.11
NOV.	28.00 18.00	23.00	29.66 20.50	25.08	29.83 18.52	24.175
DEC.	24.55 14.52	19.5	25.02 16.00	20.51	28.57 15.18	21.87

Schedule III

A total of 70 lizards divided into 7 groups of 10 each were exposed to 7 different photic schedules of LD 0:24, LD 6:18, LD 8:16 LD 12:12, LD 16:8, LD 18:6 and LD 24:0 in the month of January when the average temperature of the month was 17°C. The regenerative responses obtained in these experiments were compared with the regenerative responses obtained under 3 different temperatures i.e., 17°C (winter), 26°C (monsoon) and 30°C (summer) in animals maintained under continuous darkness (LD 0:24). This was done to evaluate the temperature compensation effect of light and light compensation effect of temperature and decipher their relative importance.

Experimental Protocol

The cages housing the animals measured 18"x15"x10" with one side made of transparent glass and ventilated on three sides; each cage housed a total of 10 lizards and they were balanced for size and sex. For the experiments involving temperature, studies were carried out during various months during the three year period. Ultimately, some temperatures were chosen i.e., 17°C, 23°C, 26°C, 29°C, 31°C and 33°C and the months during which these average temperatures were obtained and the average upper and lower range of temperatures for the month are represented in table 1.

For the experiments involving different intensities of light, the cages housing animals were placed, glass surface up, under suspended cool 40 watt fluorescent lamp thereby facing the source of illumination. The inner side of the wooden cages was lined with aluminium foil in the case of animals exposed to 1200 lux units. The distance from the fluorescent lamps to the glass surface of the cages was 15" and to the floor level 25". The light intensities was measured at the floor level using a digital lux meter. The various light intensities were generated by suspending one fluorescent lamp (150 lux), 2 fluorescent lamps (300 lux), 3 flourescent lamps (600 lux) or 3 flourescent lamps with aluminium foil lining of the cages (1200 lux).

The experiments involving various light schedules i.e., continuous light (LD 24:0; LL), 18 hours of light and 6 hours of dark (LD 18:6), 16 hours of light and 8 hours of dark (LD 16:8), 12 hours of light and 12 hours of dark (LD 12:12), 8 hours of light and 16 hours of dark (LD 8:16), 6 hours of light and 18 hours of dark (LD 6:18) and continuous darkness (LD 0:24; DD) were carried out using the light intensities of 1200 lux. The cages housing animals for continuous darkness were placed in a dark chamber completely sheilded from light with a black cloth, except for a period of about 2 minutes exposures to dim red light for taking measurements. These animals were maintained in complete darkness. For the various light schedules, the cages were placed in a lighted chamber at 0700 hrs and were shifted into the dark chamber at the end of respective lengths of exposure.

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of tail removed was from the animal varied between 60 ± 2. The length of new growth in mm was measured with a graduated meter rule and recorded at fixed time intervals 5, 10, 15, 20, 25 and 30 days post-caudal autotomy. The data was subjected to analysis of variance and Duncan's multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, 1955).

RESULTS

Schedule I :

The number of days taken to attain the various arbitrary stages in LD 12:12 and LD 0:24 lizards at different temperatures is shown in table 2. The number of days taken to attain the various arbitrary stages was more under the lower temperatures. This became significantly less at higher temperatures. There was only a difference of a day between LD 12:12 and LD 0:24 lizards. The total length of tail regenerated at the end of 30 days, the total percentage replacement and the per day growth rate are given in figures 1 & 2 and tables 3-5. The percentage difference in regenerative growth and also the increment in regenerative growth for every 1°C increase amongst animals under LD 12:12 or LD 0:24 at different temperature ranges and, the percentage difference between the two groups at different temperatures are represented in figures

TABLE - 2

Showing the number of days taken to attain the various arbitrary stages of regeneration under different temperatures in NLD and DD lizards

TEMP	PHOTIC SCHEDULE	WOUND HEALING	PREBLASTEMA	BLASTEMA	INITIATION OF GROWTH
17 °C	NLD	24	25	26	27
	DD	25	26	27	28
23 °C	NLD	12	13	14	15
	DD	17	18	19	20
26 °C	NLD	6	7	8	9
	DD	12	13	14	15
29 °C	NLD	6	7	8	9
	DD	6	7	8	9
31 °C	NLD	5	6	7	8
	DD	5	6	7	8
33 °C	NLD	4	5	6	7
	DD	4	5	6	7

NLD - normal light and dark, DD - continuous darkness.

TABLE - 3

Total length of tail regenerated under LD 12:12 (NLD) and LD 0:24 (DD) at different temperatures at the end of 30 days.

PHOTIC SCHEDULE	17 °C	23 °C	26 °C	29 °C	31 °C	33 °C
NLD	3.0 ± 0.6	10.4 ± 1.8	15.54 ± 1.90	22.66 ± 2.8	27.96 ± 3.4	29.00 ± 2.99
DD	1.8^{b} ± 0.26	7.25^{b} ± 0.99	9.70^{c} ± 1.06	19.66^{b} ± 2.89	24.00^{a} ± 3.12	29.66 ± 3.0

NLD - normal light and dark, DD - continuous darkness.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

TABLE - 4

Total percentage of tail replaced in NLD and DD lizards at the end of 30 days under different temperatures

PHOTIC SCHEDULE	17 °C	23 °C	26 °C	29 °C	31 °C	33 °C
NLD	4.90	17.04	25.47	37.14	45.83	47.54
DD	2.90^{b}	11.88^{b}	15.90^{c}	32.22^{b}	39.34^{a}	48.62

NLD - normal light and dark, DD - continuous darkness.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

TABLE - 5
Per day growth rate (mm) in NLD and DD lizards at different temperatures.

TEMP	PHOTIC SCHEDULE	5-10	10-15	15-20	20-25	25-30
17 °C	NLD	-	-	-	-	0.6
	DD	-	-	-	-	0.36
23 °C	NLD	-	-	.49	.90	0.68
	DD	-	-	-	.55	.90
26 °C	NLD	.20	.65	1.33	0.4	0.44
	DD	-	-	.50	.68	.76
29 °C	NLD	.25	.94	1.62	.97	.73
	DD	.20	1.53	.74	.81	.64
31 °C	NLD	.43	2.17	1.51	.99	.48
	DD	.28	1.82	.87	1.00	.82
33 °C	NLD	.47	2.21	1.55	1.03	.51
	DD	.50	2.04	1.10	1.22	1.05

Fig.1 The length of tail regenerated at different temperatures in lizards under LD 12:12 & LD 0:24.

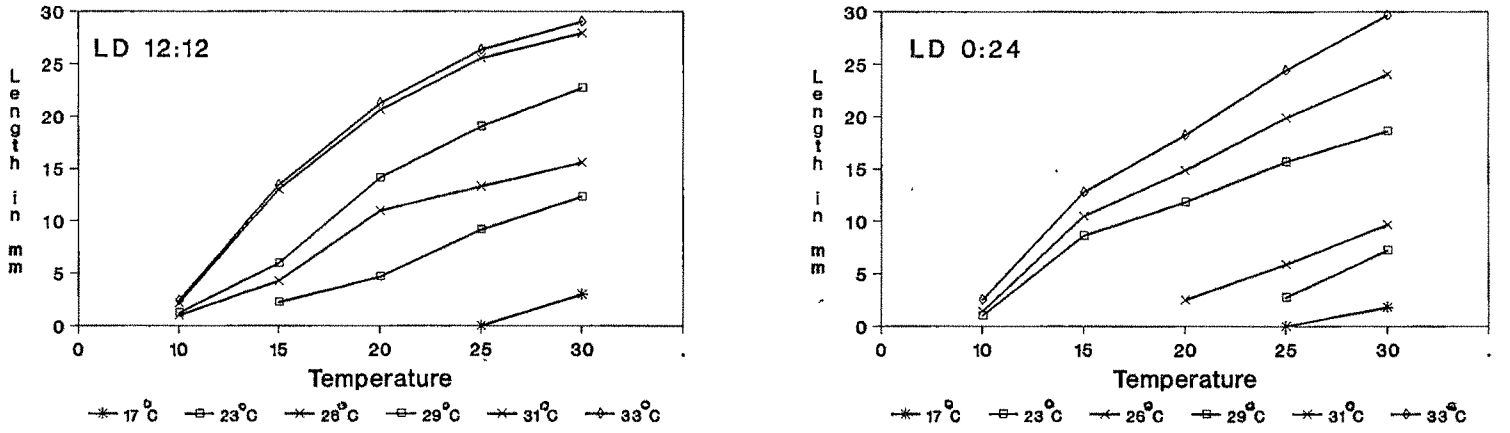
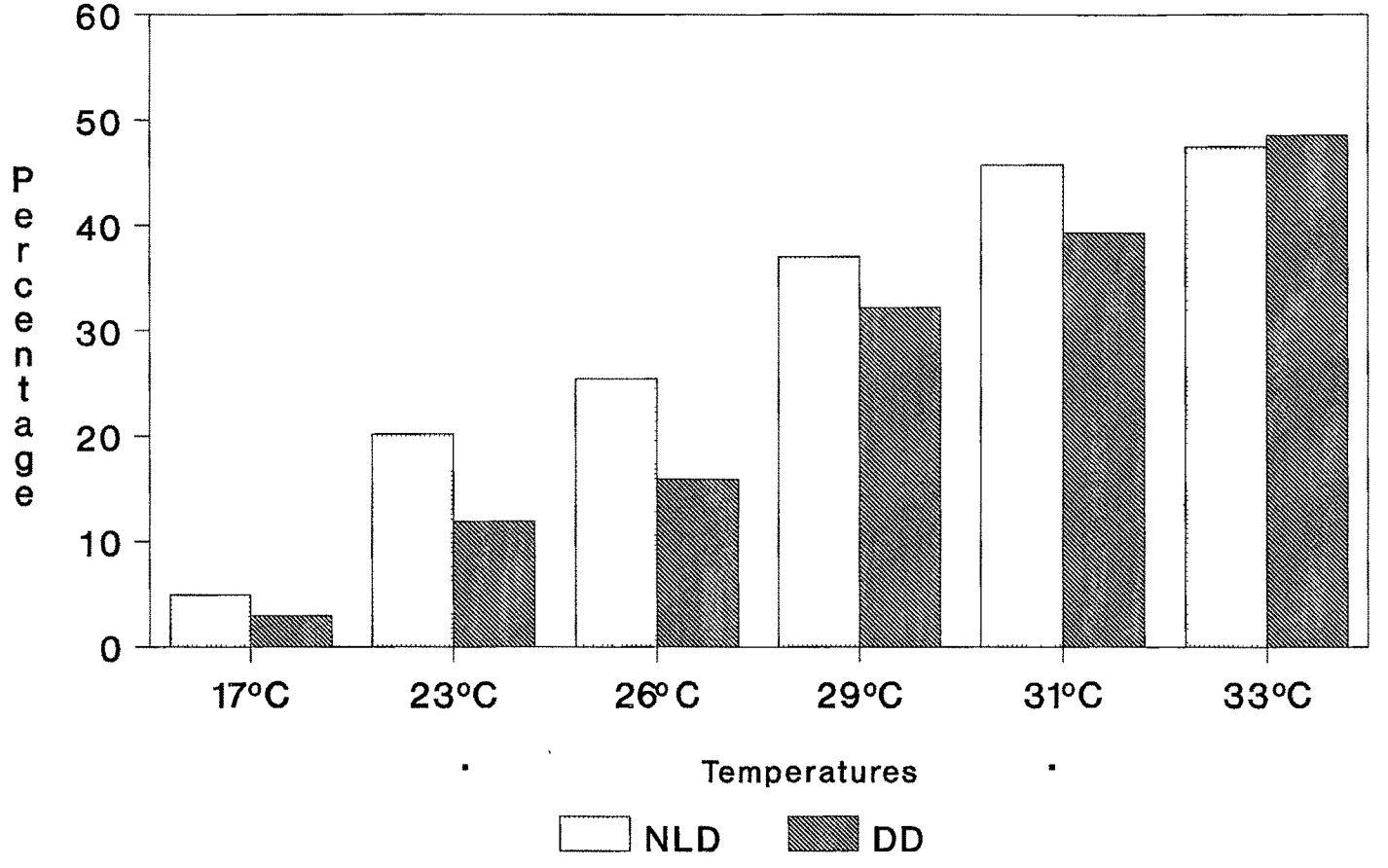


Fig.2 Total percentage replacement at different temperatures in lizards under NLD and DD.



NLD-Normal Light & Dark DD-Continous Darkness

3 & 4 and tables 6 & 7. The regenerative growth in LD 12:12 animals was greater than in DD upto 26°C. Between 26°C and 30°C this difference got narrowed and ultimately at 33°C the difference was totally nullified. These differences in regenerative growth are well reflected in the percentage difference between the two groups as well as in the observed regenerative increment for every 1°C increase in temperature.

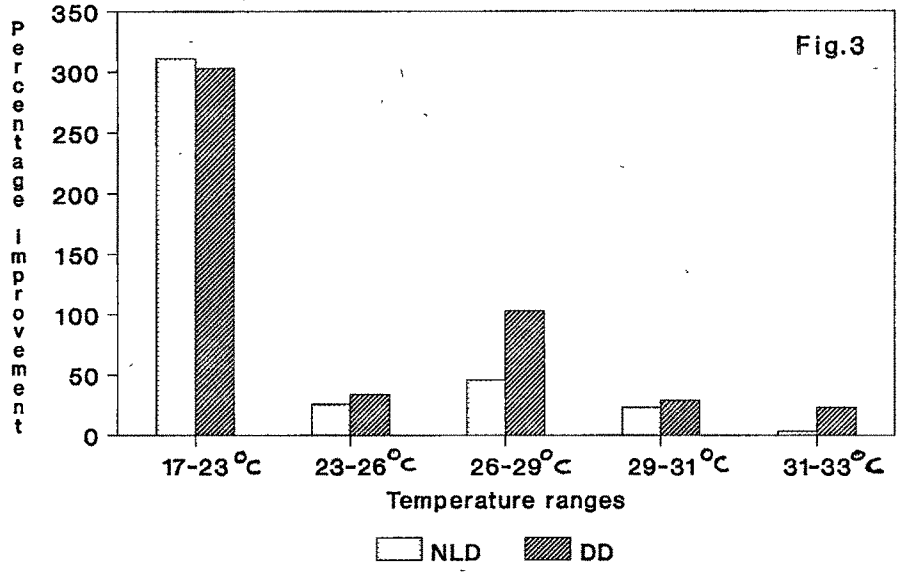
Schedule II

The number of days taken to attain the various arbitrary stages of regeneration did not show much change with intensity of light in lizards housed under LD 12:12. Lizards under LD 24:0 attained the various stages a day earlier with 300 and 600 lux intensities while with 1200 lux, the attainment of stages occurred two days earlier (see table 8). The total length of tail regenerated, the growth rate and the percentage replacement were very much the same in both NLD and LL lizards with 150 or 300 lux light intensities. Significant difference occurred only with 600 and 1200 lux light intensities in both the groups of lizards, more markedly in the LL group (Tables 9-11, Figs. 5 - 9).

Schedule III

The length of tail regenerated at the end of 30 days, the percentage of tail replaced, the percentage increment in regenerative growth and the growth rate per

Figs.3&4 Percentage improvement & growth rate increment in mm per degree centrigrade increase in temp. under different temp. ranges in NLD and DD.



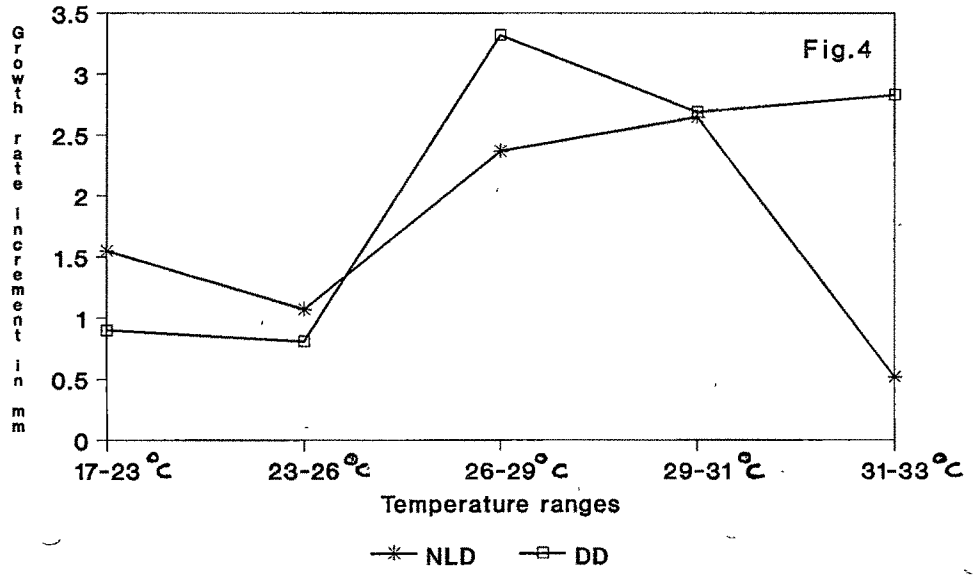


TABLE - 6

Table showing percentage increment and increment in growth rate (mm) per degree centigrade increase in temperature in LD12:12 (NLD) and LD0:24 (DD) Lizards at different temperature ranges.

TEMPER-ATURE RANGES	Percentage increment between different temperature ranges		Increment in regeneration for every 1°C increase in temperature at different temperature ranges		Percentage improvement for every 1°C increase in temperature at different temperature ranges	
	NLD	DD	NLD	DD	NLD	DD
17-23	311	303	1.55	.90	18	89
23-26	26.0	34	1.07	.816	31	30
26-29	45.8	103	2.37	3.32	3	9
29-31	23.38	28.9	2.65	2.69	49	22
31-33	3.71	23.58	.52	2.83	3	23

NLD - normal light and dark, DD - continuous darkness.

TABLE - 7

Percentage difference in terms of tail replaced at the end of 30 days in LD 12:12 (NLD) Lizards in relation to LD 0:24 (DD)

TEMPERATURE RANGE (IN CENTIGRADE)					
17°C	23°C	26°C	29°C	31°C	33°C
+ 66.6	+ 70.06	+ 63.4	+ 15.25	+ 16.5	- 2.2

TABLE - 8

Number of days taken to attain the various arbitrary stages in animals exposed to different light intensities under LD 12:12 (NLD) and LD 24:0 (LL) at 30 °C.

LIGHT INTENSITIES (in LUX)	PHOTIC SCHEDULE	WOUND-HEALING	PREBLA-STEMA	BLAST-EMA	INITIATION OF GROWTH
150	NLD LD 12:12	6.00	7.00	8.00	9.00
	LL LD 24:0	6.00	7.00	8.00	9.00
300	NLD LD 12:12	6.00	7.00	8.00	9.00
	LL LD 24:0	5.00	6.00	7.00	8.00
600	NLD LD 12:12	6.00	7.00	8.00	9.00
	LL LD 24:0	5.00	6.00	7.00	8.00
1200	NLD LD 12:0	5.00	6.00	7.00	8.00
	LL LD 24:0	4.00	5.00	6.00	7.00

TABLE - 9

Total length of tail regenerated in lizards exposed to LD 12:12 (NLD) and LD 24:0 (LL) at different light intensities at the end of 30 days.

PHOTIC SCHEDULE	LIGHT INTENSITIES (IN LUX)			
	150	300	600	1200
NLD	20.16 ± 2.1	20.97 ± 1.55	22.98[a] ± 2.1	26.15[c] ± 2.22
LL	20.88 ± 1.8	21.91 ± 1.91	24.80[b] ± 1.66	31.66[c] ± 3.16

NLD - normal light and dark, DD - continuous darkness.

a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$ compared to corresponding 150 lux.

TABLE - 10
The total percentage replacement in Lizards exposed to LD 12:12 (NLD) and LD 24:0 (LL) at different light intensities.

PHOTIC SCHEDULE	LIGHT INTENSITIES (IN LUX)			
	150	300	600	1200
NLD	33.04	34.37	37.67[a]	42.86[c]
LL	34.22	35.91	40.65[b]	51.90[c]

NLD - normal light and dark, DD - continuous darkness.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$, compared to corresponding to 150 Lux.

TABLE -11
Per day growth rate (mm) in NLD and LL lizards exposed to different light intersities.

LIGHT INTENSITIES	PHOTIC SCHEDULE	5-10	10-15	15-20	20-25	25-30
150 LUX	NLD	.311	.781	1.23	1.097	.613
	LL	.33	.809	1.25	1.12	.641
300 LUX	NLD	.33	.821	1.27	1.13	.653
	LL	.37	.850	1.29	1.16	.682
600 LUX	NLD	.318	.904	1.33	1.19	.854
	LL	.359	.97	1.39	1.25	.923
1200 LUX	NLD	.40	1.04	1.46	1.32	.992
	LL	.62	1.68	1.26	1.54	1.21

NLD - normal light and dark, LL - continuous light.

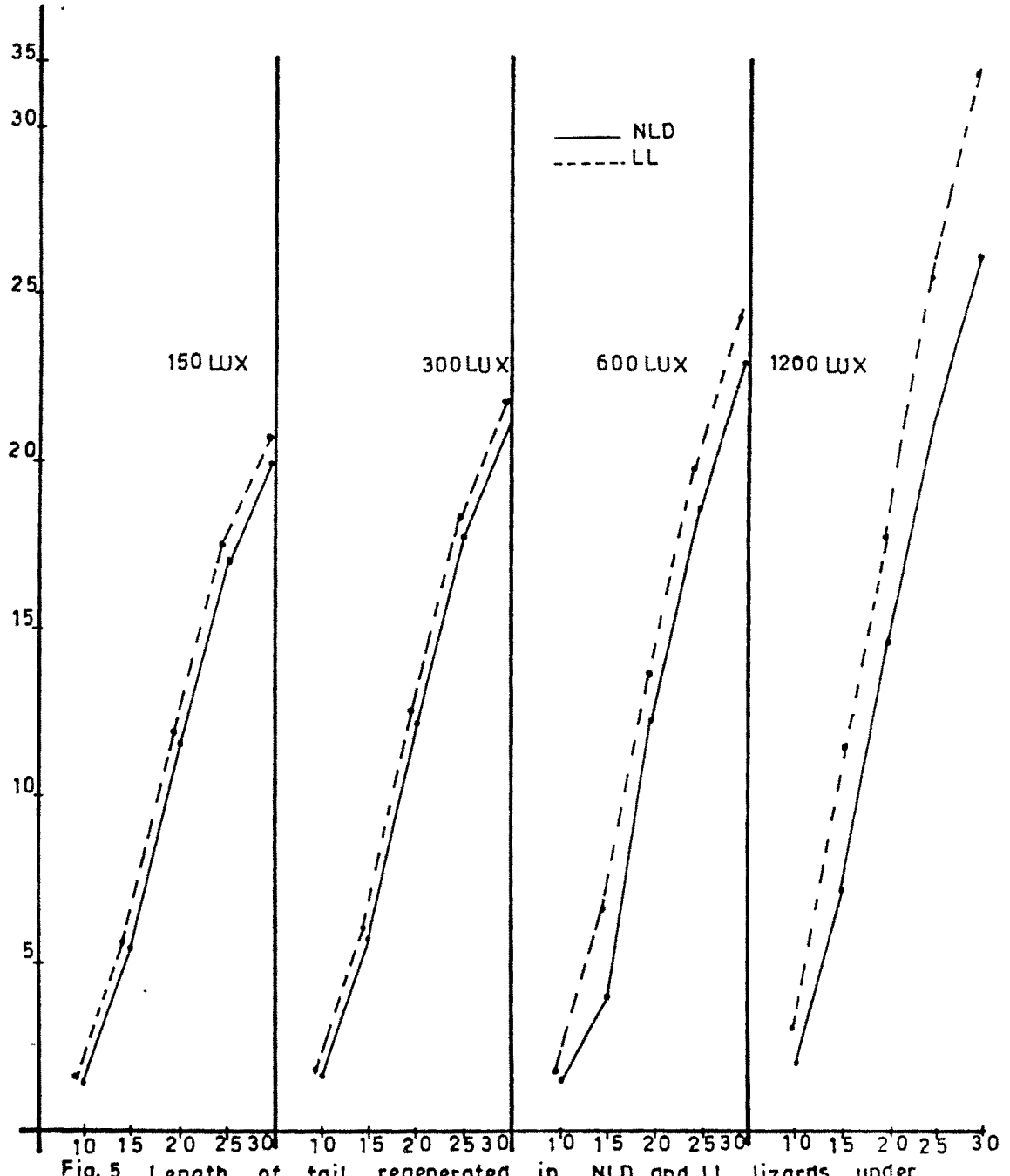
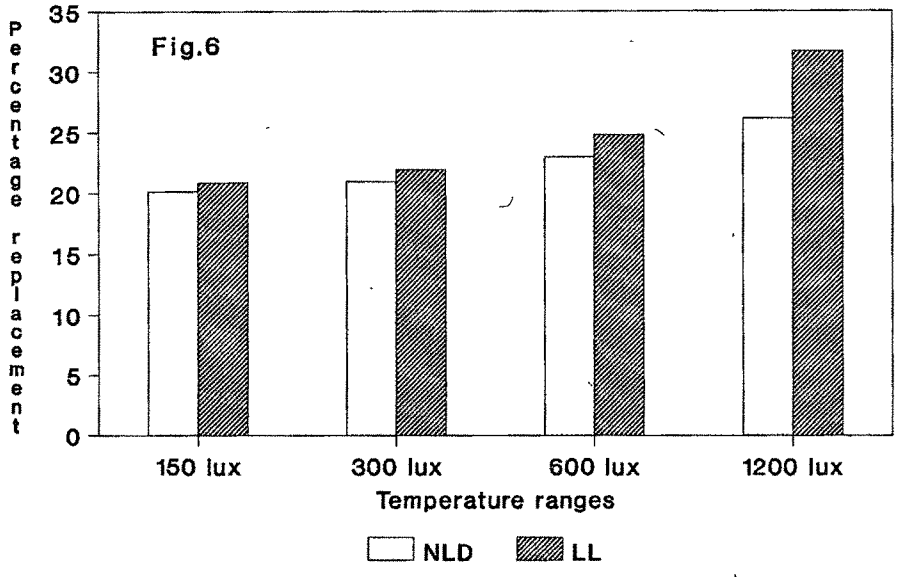
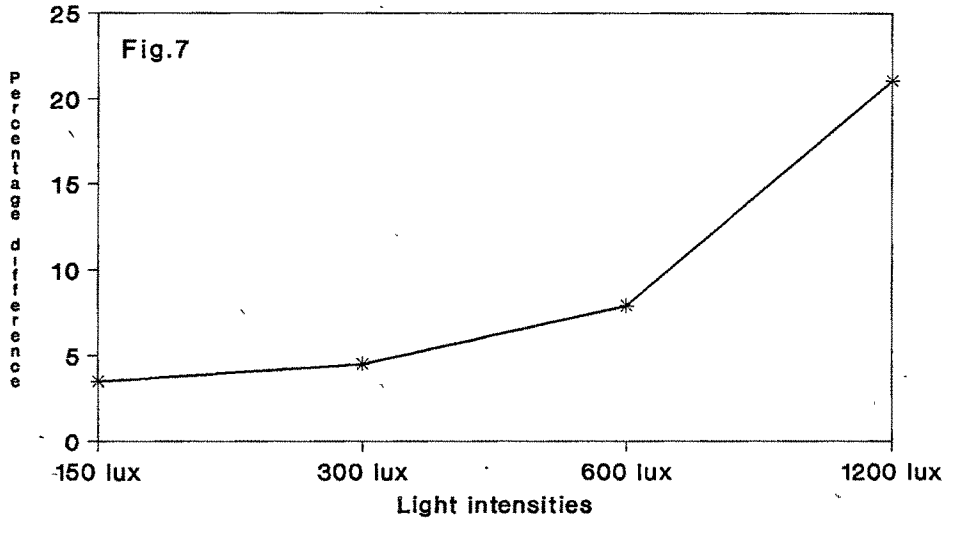


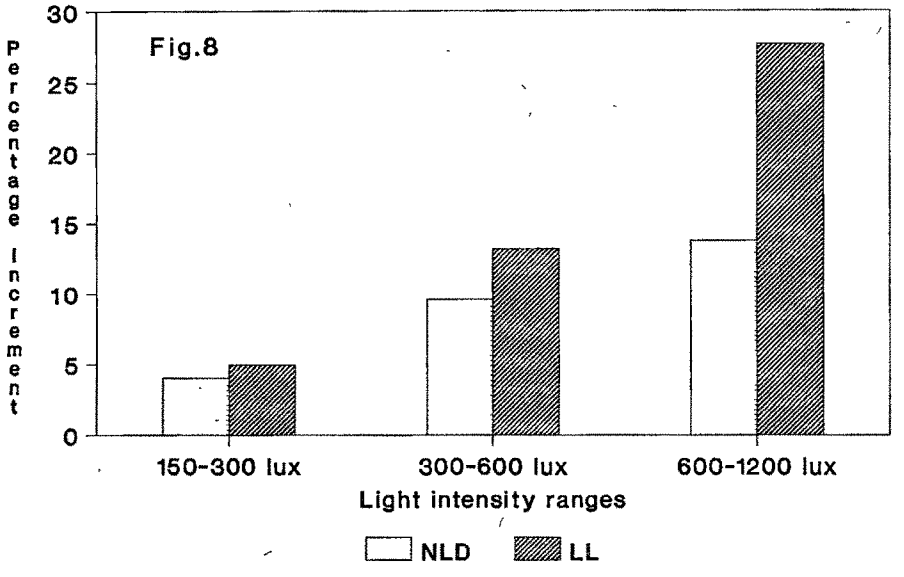
Fig. 5 Length of tail regenerated in NLD and LL lizards under different intensities of light.

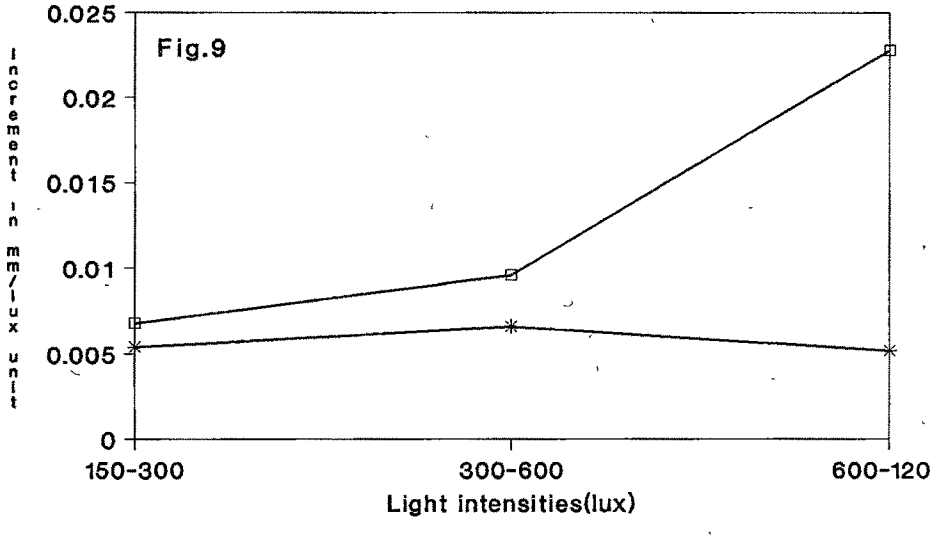
Figs.6&7 Showing percentage tail replaced in NLD and LL lizards and the percentage difference between NLD and LL with diff. intensities of light.





Figs.8&9 Percentage increment in various light intensities and the increment rate in mm for every lux unit increase in light under NLD and LL conditions.





degree centigrade increment in temperature at 17°C, 26°C and 30°C in lizards in DD and NLD are shown in figures 10-13, tables 12 & 13. The increment in regenerative growth from 17°C to 26°C in both DD and NLD groups of lizards was 400% while that between 26°C to 30°C was 45.8% in NLD and 106% in DD. The growth rate for every degree centigrade increase in temperature between 17°C to 36°C was 0.8mm in DD and 1.39mm in NLD. Between 26°C and 30°C, the same was 2.4mm in the former and 1.78mm in the latter. The overall growth improvement from 17°C-30°C was 655% in NLD and 934% in DD.

The length of tail regenerated and the percentage tail replacement at the end of 30 days under increasing photic schedules of LD 0:24, LD 6:18, LD 8:16, LD 12:12, LD 16:8, LD 18:6, and LD 24:0 at 17°C as well as the percentage increment with increasing light schedule and the growth rate per hour increase in light schedule are represented in tables 14 & 15 and figures 14-17. With increasing light schedule there was continuous improvement in tail regeneration from a minimum of 1.8mm in LD 0:24 to a maximum of 16mm at 17°C and, at 30°C, the same ranged from 18mm in LD 0:24 to 28mm in LD 24:0. The overall improvement in regenerative performance at 17°C was 789% while the same at 30°C was 56%. The maximum percentage increment and growth rate were seen between 12 hrs. and 18 hrs. of photic schedule at 17°C, the same occurred between 18 hrs. and 24 hrs. of light at 30°C.

Fig.10 The length of tail regenerated at the end of 30 days in DD and LL lizards at 17, 26 and 30 degree centigrade.

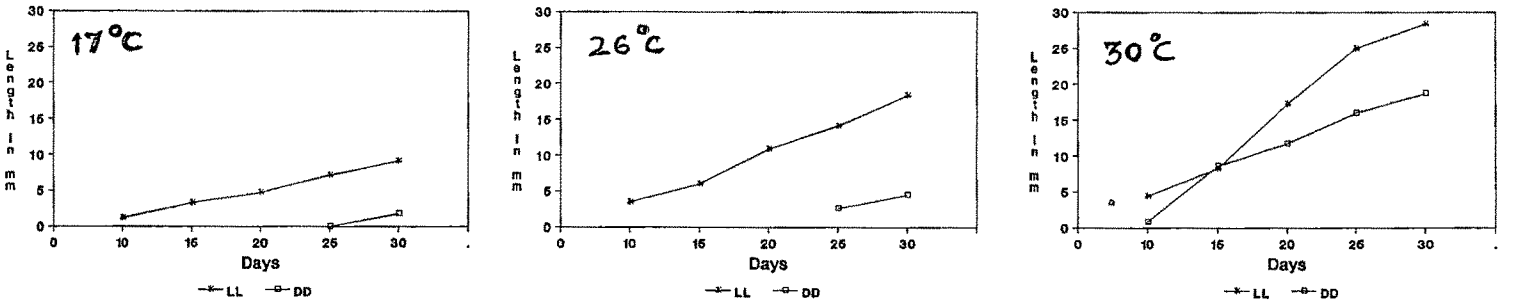
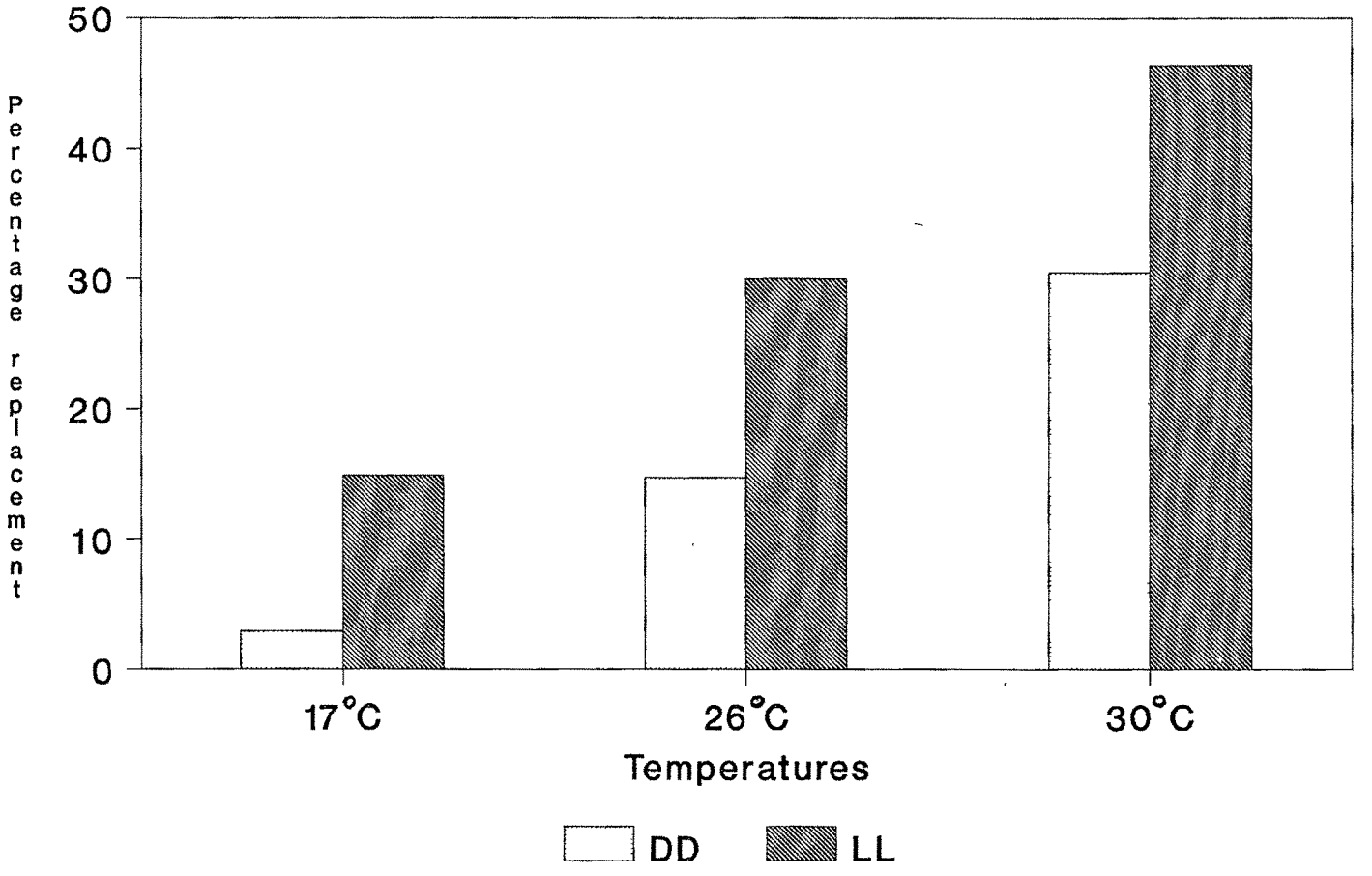
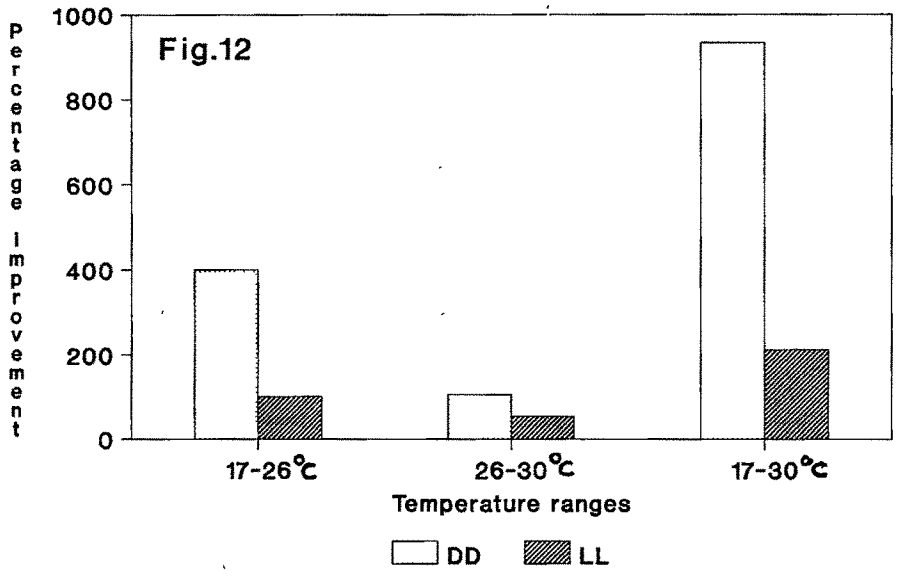
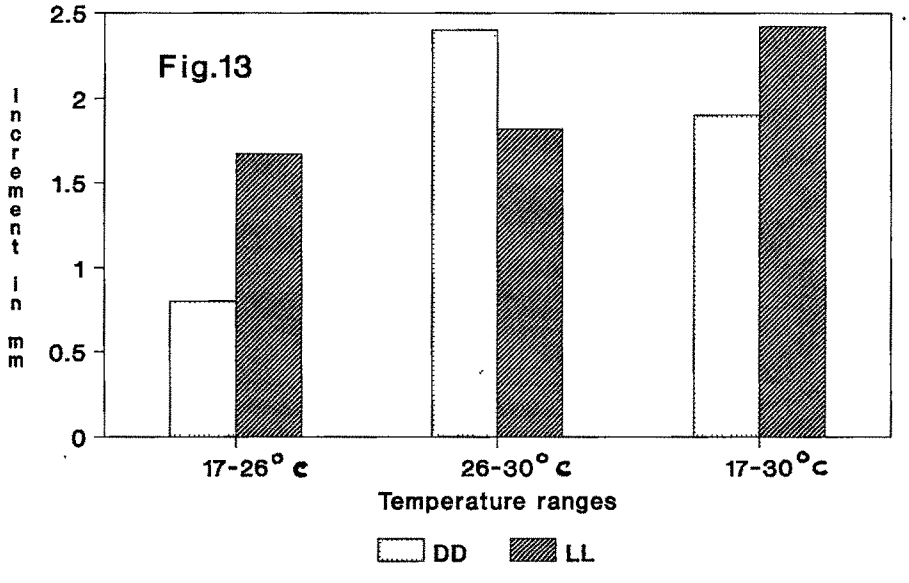


Fig.11 Showing percentage replacement at the end of 30 in DD and LL lizards at 17, 26 and 30 degree centrigrade.



Figs.12&13 Percentage improvement and increment in growth rate in DD and LL lizards under different temperature ranges.





DD-Continuous darkness LL-Continuous light

TABLE - 12

% improvement in regeneration in NLD and LL Lizards under different ranges of light intensity.

Light Intensities	NLD	LL
150-300 Lux	4.06	4.95
300-600 Lux	9.62	13.20
600-1200 Lux	13.74	27.69
150-1200 Lux	29.7	51.6

NLD - normal light and dark, LL - continuous light.

TABLE - 13

Increment in Growth rate (mm) for every Lux increase.

Light intensities	NLD	LL
150-300 Lux	.0054	.0068
300-600 Lux	.0066	.0096
600-1200 Lux	.00528	.0228

NLD - normal light and dark, LL - continuous light.

TABLE - 14
The length of tail regenerated at the end of 30 days in DD, NLD, and LL lizards at 17°C, 26°C and 30°C.

PHOTIC SCHEDULE		TEMPERATURES	
	17°C	26°C	30°C
DD	1.8 ± 0.31[a]	9.00 ± 1.1[c]	18.61 ± 2.42[c]
NLD	3.0 ± 0.91	15.54 ± 1.83	22.66 ± 2.86
LL	14.9 ± 1.46[c]	30.00 ± 2.67[c]	46.40 ± 3.68[c]

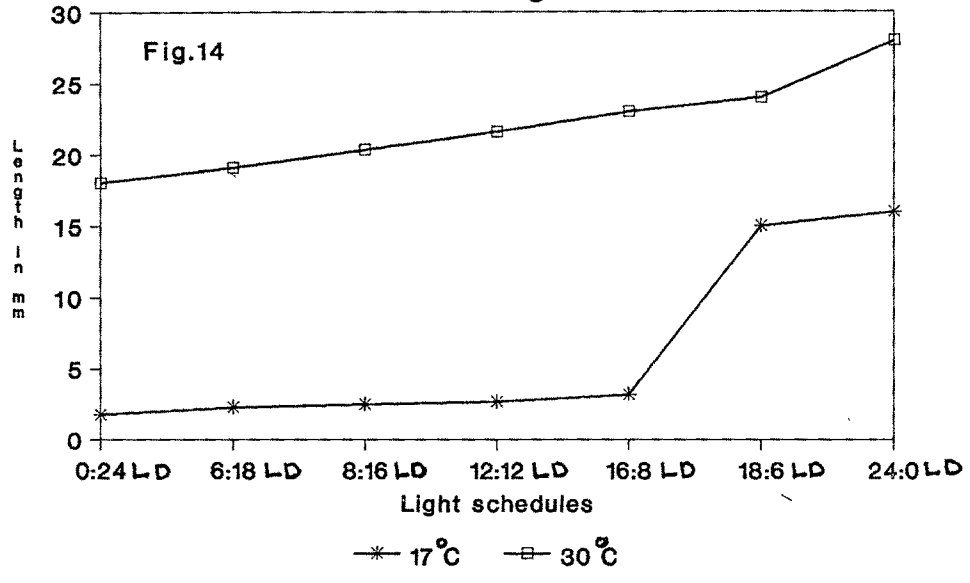
NLD - normal light and dark, LL - continuous light, DD - continuous darkness.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$ compared to NLD.

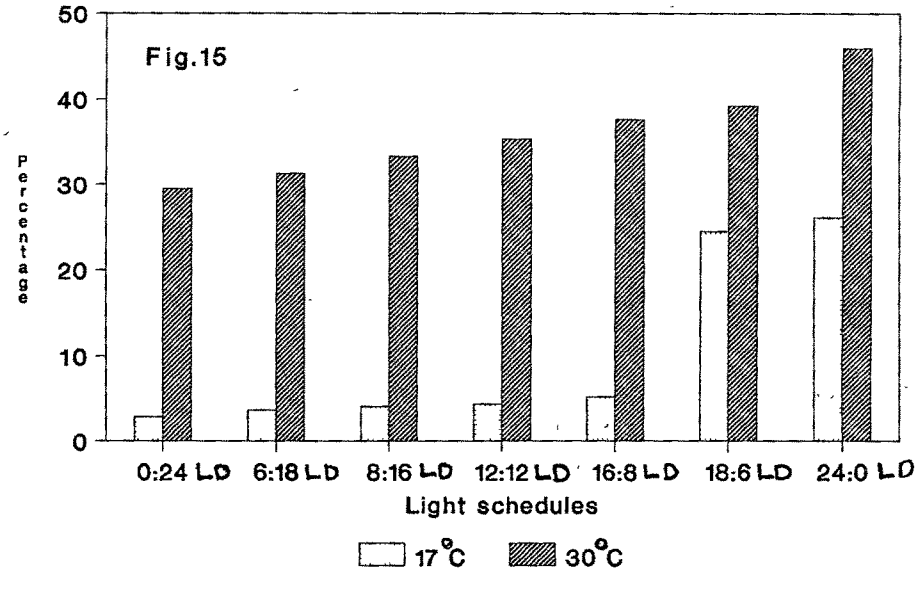
TABLE - 15
The total percentage replacement at the end of 30 days in DD, NLD and LL lizards at 17°C, 26°C and 30°C

PHOTIC SCHEDULES	TEMPERATURES		
	17°C	26°C	30°C
DD	2.9[c] ± 0.32	14.70[c] ± 1.60	30.50[b] ± 3.12
NLD	4.90 ± 0.65	25.54 ± 2.84	37.14 ± 4.11
LL	24.42[c] ± 2.58	49.18[c] ± 4.31	76.06[c] ± 8.52

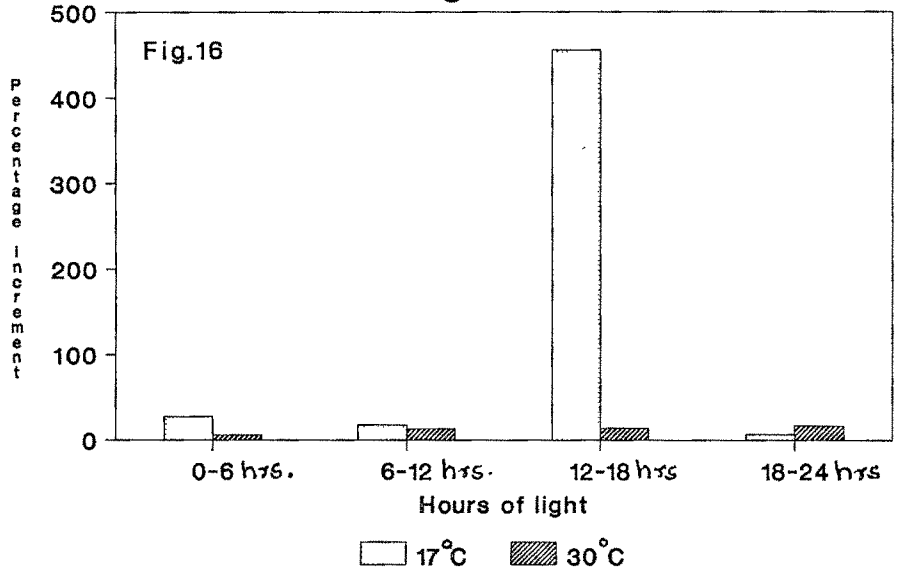
NLD - LD 12 : 12; DD - LD 0 : 24; LL - LD 24 : 0.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$ compared to NLD.

Figs.14&15 Length of regenerate attained at 30 days and percentage increment in lizards at 17°C and 30°C at different light schedules.





Figs.16&17 Percentage increment and growth rate increment in mm per hour increase in light at 17°C and 30°C at different light schedules.



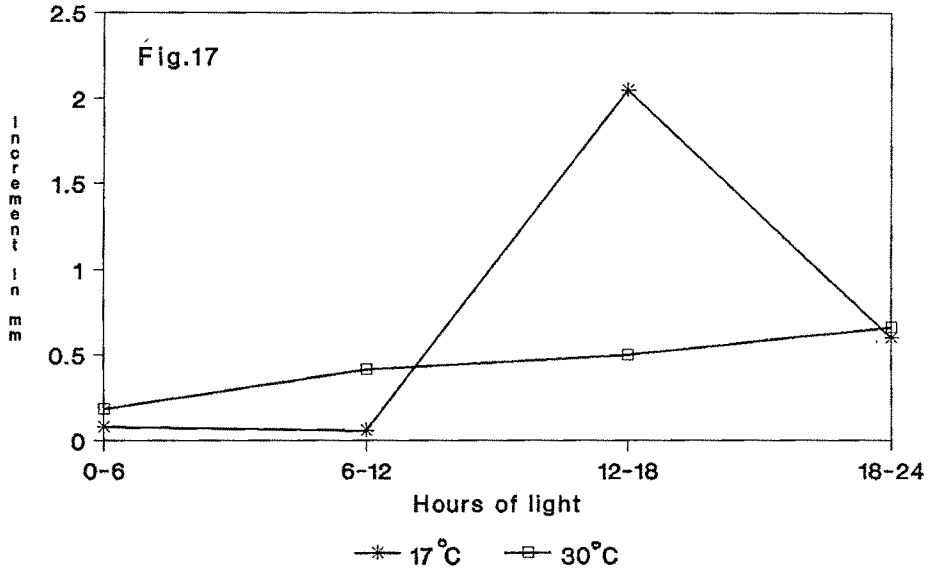


TABLE - 16

Total length regenerated at the end of 30 days under different photic schedules at 17°C and 30°C.

PHOTIC SCHEDULES	TEMPERATURES	
	17°C	30°C
LD 0 : 24	1.8 ± 0.34	18.0 ± 1.4
LD6 : 18	2.3 ± 0.61[a]	19.11 ± 1.68
LD 8 : 16	2.5 ± 0.66[a]	20.34 ± 2.01[a]
LD 12 : 12	2.7 ± 0.58[a]	21.60 ± 1.99[a]
LD 16 : 8	3.2 ± 0.71[b]	23.0 ± 2.46[b]
LD 18 : 6	15 ± 1.8[c]	24.0 ± 2.48[c]
LD 24 : 0	16 ± 1.67[c]	28.0 ± 2.96[c]

a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$ compared to LD 0: 24

TABLE - 17

Total percentage replacement at end of 30 days under different photic schedule under 17°C and 30°C.

PHOTIC SCHEDULE	TEMPERATURES	
	17°C	30°C
LD 0 : 24	2.9 ± 0.61	29.5 ± 3.66
LD6 : 18	3.7 ± 0.73	31.3 ± 3.31
LD 8 : 16	4.09 ± 0.96[a]	33.3 ± 2.98
LD 12 : 12	4.40 ± 0.88[b]	35.4 ± 4.01[b]
LD 16 : 8	5.24 ± 0.89[b]	37.7 ± 3.5[b]
LD 18 : 6	24.5 ± 3.4[c]	39.3 ± 3.9[c]
LD 24 : 0	26 ± 2.8[c]	45.9 ± 4.8[c]

a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$ compared to LD 0:24.

DISCUSSION

Experiments carried out herein at different temperatures indicate a gradient effect of temperature on regeneration as, tail elongation was hastened when temperature increased from 17°C to 33°C. The experiments were not done at fixed temperatures as the animals do not encounter such conditions in nature. The essential purpose was to assess the regenerative performance in relation to naturally occurring seasonal variations in temperature. However, the animals were maintained at a constant photoperiod of LD 12:12 or in total darkness (LD 0:24) in all the seasons so as to nullify the influence due to change in photoperiod. The temperatures mentioned actually represent the average monthly means in a given season and, the range of maximum-minimum temperatures during the select months is shown in table 1. A justification for this is a recent report that lizards obeying a light dark cycle of LD 12:12 showed a less pronounced melatonin rhythm at a constant temperature of 30°C and no rhythmicity at constant 15°C, however lizards in a thermal cycles (fluctuating temperature) of 30°C/15°C showed a very robust melatonin rhythm (Firth and Kennaway, 1987).

The gradient effect is manifested in the form of progressive increase in tail elongation with increase in temperature. This effect is seen in lizards obeying a photoperiodic schedule of LD 12:12 as well as animals in continuous darkness.

Influence of temperature on regeneration has been shown by other workers as well. The regeneration rate of tadpole tails was shown to increase with increase in temperature between 19°C and 28°C and was further shown that regeneration ceases below 14°C and above 28°C (Ellis, 1909). Higher temperatures were shown to accelerate both rates of blastema formation and the subsequent regeneration rates in *Anolis carolinensis* (Maderson and Licht, 1968). In the present experiment, both NLD and DD lizards showed significant increment in regenerative growth with increase in temperature. Animals in DD showed relatively greater percentage increment (1457% vs 866%). Comparison of the length of tail replaced at the end of the 30 days in NLD and DD lizards at different temperature ranges reveals maximum percentage increment at the lowest temperature range of 17°C to 23°C, which amounted to 311% in NLD and 303% in DD. The next highest percentage increment occurred between 26-29°C. The DD lizards showed a higher percentage increment (103%) in relation to NLD lizards (46%). At the other temperature ranges, i.e., 23°C to 26°C, 29°C to 31°C and 31°C to 33°C, the DD lizards showed increment ranging between 24-34%. In the NLD lizards, at temperature ranges between 29°C to 31°C and 23°C to 26°C, the percentage increment was 23.3% and 26% respectively. At the highest temperature range of 31°C to 33°C the NLD lizards depicted a very insignificant increment (3.7%), while the DD lizards showed 24%. A comparison of increment in linear growth with increase in every one degree centigrade showed maximum increment of 2.37mm and

2.65mm between 26°C-29°C and 29°C-31°C respectively by NLD lizards, while DD lizards showed maximum increment of 3.32mm between 26°C to 29°C and slightly lower but significant increments of 2.69mm and 2.83mm between 29 to 31°C and 31°C to 33°C respectively. The NLD lizards however depicted an insignificant increment of 0.52mm between 31°C to 33°C. Probably 31°C represents the upper limit to stimulation of regeneration process in NLD lizards. Similar upper limit to stimulation of regeneration process was also defined by Schauble and Nentwing (1974) in the newt, *N. viridescens*. These authors found regeneration to be stimulated between 15°C and 25°C with no further significant change beyond 25°C.

Evidently, 26°C-29°C appears to be the optimal temperature range for regenerative performance in both NLD and DD lizards. However, temperatures, above 31°C were less productive in NLD lizards while the DD lizards maintained a steady increment rate. Apparently, the linear tail elongation in lizards under constant darkness remains enhanced at higher temperatures. This becomes obvious from the fact that, the regenerative performance in the DD animals which was only 33% of the NLD animals at 17°C, improved considerably with increase in temperature and at 33°C it became 2% more than the NLD lizards.

The experiments with different intensities of light did reveal a stimulatory influence with increasing intensities of light under both NLD and LL conditions.

However, increase in intensities from 150 to 300 lux units was inconsequential and the percentage increment in tail growth was marginal and identical in both NLD and LL lizards. Significant difference in regenerative growth manifested only beyond 300 lux units of light. The increase was progressive with greater increment being registered between 600 and 1200 lux units than between 300 and 600 lux. The difference in regenerative growth was significantly greater in LL lizards than in NLD lizards. The growth rate per lux unit of light at the different ranges of light intensities i.e., 150-300, 300-600 and 600-1200 was constant in NLD while there was progressive augmentation under LL with a very pronounced one between 600-1200 lux. Even the percentage difference in the regenerative output between NLD and LL lizards was less significant between 150 and 600 lux units and, it became greatly amplified at 1200 lux units and was almost double in LL lizards in relation to NLD lizards. It is conceivable that there is a cumulative effect of duration and intensity of light. Influence of light in stimulating regeneration in newts has been documented previously (Maier and Singer, 1977; 1982).

The third set of experiments performed for evaluating the relative effects of light and temperature, have revealed importance of both these factors on regenerative tail elongation in lizards. The various calculuses, project the potent influence of both photic and thermal components on regeneration, though on a relative basis the latter had greater impact than the former. An increase of 934% in regenerative tail

elongation with rise in temperature from 17°C to 30°C, with an average increment of 1.3mm per degree centigrade increase in DD lizards, as against 789% increase in tail length with increase in photoperiod from LD 0:24 to LD 24:0 and an average increment of 0.6mm for every hour of light at 17°C, amply validates the espoused relative importance of temperature. A careful scrutiny and detailed analysis reveal that while the photic influence is principally exerted at the higher photic schedules (LD 12:12 to LD 24:0), the thermal influence is manifested equally well at both the lower and upper temperature ranges though slightly more in the upper temperature range. *Inter alia* comparison reveals that both temperature and light have compensatory influence over each other. Comparison between the effect of increasing photic schedule at the lowest temperature and that of increasing tempeatures under continuous darkness indicate light compensation effect on temperature and temperature compensation effect on light; again the temperature compensation effect on light was more pronounced than the light compensatory effect over temperature. The increase in photic schedule from 0000 hrs to 2400 hrs of light bettered the regenerative performance by 789% in 17°C while the same at 30°C yielded only 56% improvement. Maximum percentage improvement of 17% and an average increase of 0.66mm for every hour of light occurred between LD 18:6 and LD 24:0 at 30°C, while, a maximum percentage replacement of 56% and an average growth increment of 2.05mm for every hour of light occurred between LD 12:12 and LD 18:6 at 17°C.

Apparently, the observed changes at 30°C are a consequence of cumulative photo-thermal influence but, the specific effects seen between 12 hrs. to 18 hrs of light at 17°C is a case of light compensatory effect over temperature and, the optimum duration of light for this is evidently between 12 and 18 hours. In terms of compensatory effect over light, whereas there was better regenerative growth by 934% under LD 0:24, improvement under LD 24:0 was a meager 75%. This temperature compensatory effect over light was maximally expressed at the higher temperature range between 26°C-30°C by an increased growth rate of 2.4mm per degree centigrade. The alluded dominant effect of temperature compared to light is also brought out clearly when the percentage difference in the regenerative growth between DD and LL at 17°C and 30°C and that under LD 0:24 and LD 24:0 is made. Whereas in the latter case the difference was reduced from 900% to 75%, in the former, it was reduced from 788% to 50%.

The entire gamut of observations and analysis thereat, reviewed above, signify the potential ability of both light and temperature to favourably influence the course of regeneration in lizards. Though photoperiod has been considered the principal factor in the entrainment of the pineal meletonin rhythm in mammals (see Vivien-Roels and Pevet, 1983), in poikilotherms like reptiles, even temperature has been implicated as an entrainment cue (Vivien-Roels and Arendt, 1981; 1983; Vivien-Roels, 1985; Underwood, 1985a). Many of the recent studies on the influence of altered

temperature and light cycles in reptiles have provided evidences for temperature cycles to be of primary importance in maintaining the melatonin rhythm though, light cycles could also maintain the same (Vivien-Roels *et al.,* 1979, 1988, Firth and Kennaway 1987; Mendonca *et al.,* 1995). A consensus that emerges from the observations of the above studies is that temperature is responsible for the amplitude of the melatonin signal while photoperiod is responsible for the duration of the signal. An interesting observation in this context was the abolition of melatonin rhythm by both constant light and constant darkness under constant temperature conditions in the lizard *Trachydosaurus rugosus* (Firth *et al.,* 1979). The common house lizard, *H. flaviviridis*, the present experimental model, are generally found indoors remaining hidden in dark crevices or in concealed places. They are rarely active during the day and are active only during the early part of night when the indoor lightings are on. Considering their habits and habitats, it is clear that they are not generally exposed to any regular photoperiodic cycles while, they are exposed to the circadian and circannual variations in temperature cycles. Apparently, the thermal cycles could be considered the most potent cue for entraining a melatonin rhythm in these animals, while photoperiod could play a secondary role and even exert a modifying influence mostly by controlling the duration of melatonin signal as inferred earlier. The present findings on the regenerative performance of animals maintained in constant darkness at different temperatures provides ample justification for the temperature controlled

neuroendocrine rhythms favouring tail regeneration. However, the mechanisms responsible for the transduction of these environmental factors into linear growth need to be elucidated. In view of the previously documented importance of prolactin (PRL) in inducing linear growth of the autotomized tail (Ndukuba and Ramachandran, 1989a), this hormone appears to be the likely agent modulated by both temperature and light. It is conceivable that PRL secretion is upregulated by both light and temperature. It can be speculated that light increases PRL secretion by increasing the hypothalamic serotonergic activity (potent secretogogue of prolactin) while temperature augments PRL release by decreasing dopaminergic activity (potent PRL release inhibitor) as evidences in favour are forthcoming from studies in mammals. (Brown and Forbes, 1980; Munro *et al.,* 1980; Adams *et al.,* 1989, 1992; Maywood *et al.,* 1990; Tucker *et al.*, 1991; Grosse *et al.,* 1993; Asher *et al.,* 1994; Maywood and Hastings 1995; Houghton *et al.,* 1995).

Enough evidences are available in this context which buttress the fact that PRL mediated regenerative growth can be modulated by temperature and light. Whereas, Maier and Singer (1977, 1982) have implicated PRL in the light mediated stimulatory influence on regeneration, Schauble (1972), Schauble and Tyler (1972) and Schauble and Nentwig (1974) have reported the significant influence of temperature on regeneration and the ability of PRL to nullify the inhibitory influence on growth manifested by lower temperatures. Moreover, a previous study from this laboratory

has shown that para-chlorophenylalanine (p-CPA), a 5-HT synthesis inhibitor, could effectively nullify the continuous light induced increment on regenerative growth alluding the role of light in mediating PRL release by increasing 5-HT. A consideration of these purported neural mechanisms controlling PRL secretion and the melatonin rhythms suggest, that the dopaminergic and serotonergic rhythms are not driven either by melatonin or a common oscillator (Ramachandran and Ndukuba, 1989c). They appear to be driven by separate oscillators though a coupling is possible as becomes evident from the other studies in later chapters. Substaintiation for this notion comes from previous observations that pinealectomised lizards showed some degree of increment in regenerative growth both with increase in photoperiod as well as increase in temperature on a seasonal basis (Ndukuba and Ramachandran, 1991b) conveying a staunch impression that though pineal is the principal mediator, there is some extra-pineal photothermal perception.

Overall, based on the present observation it can be surmised that :

1. There is a positive photo-thermal influence on tail regeneration.
2. Increased temperature and light intensity as well as duration of light, all exert a positive influence on regeneration.
3. Both temperature and light have compensatory influence on the another, but the thermal influence is relatively dominant of the two.

SUMMARY

Since both temperature and light exert control over various activities in ectotherms, a detailed analysis of photothermal influences on tail regeneration in lizards was envisaged. To this end, the Gekkonid lizard, *Hemidactylus flaviviridis* has been used and experiments were carried out under three schedules. (1) Influence of different temperature ranges on a seasonal basis on the course of tail regeneration under constant photoperiods of LD 12:12 and LD 0:24, (2) Impact of different intensities of light on tail regeneration in lizards exposed to LD 12:12 and LD 24:0 under a constant average temperature of 30° C, and (3) Comparison of regenerative performance in lizards exposed to different light schedules of LD 0:24, LD 6:18, LD 8:16, LD 12:12, LD 16:8, LD 18:6, and LD 24:0 at an average temperature of 17° C with that of lizards at 17° C, 26°C and 30°C in LD 0:24 to assess light compensatory and temperature compensatory effects. Both the NLD and DD lizards showed increasing regenerative performance with increasing temperature, the percentage increment being more pronounced between 17° C to 23° C. Whereas the DD lizards showed significant growth increment even beyond 31° C, the NLD lizards showed insignificant response. Increasing intensities of light also produced increment in regenerative growth but, only at intensities more than 300 Lux, more pronounced in LL than NLD. The third experimental schedule revealed compensating influence of

both factors on each other, with temperature compensation being more pronounced than the light compensation effect. These results are discussed in the text in terms of the transduction of photo-thermal cues into neuroendocrine responses favouring regenerative growth.