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Temperature is Relatively More Important than Light for Regenerative Tail Growth in Tropical Lizards: Observations from Seasonal and Experimental Studies in *Hemidactylus flaviviridis*

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Abstract: The influence of both temperature and light over various activities in ectotherms necessitated a detailed analysis of photothermal influences on tail regeneration in a tropical lizard. To this end, the Gekkonid lizard, *Hemidactylus flaviviridis* received experimental scrutiny under three different schedules. The three schedules were: 1) different seasonal temperature ranges 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 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, with more pronounced increment 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 compensatory influence of both factors on each other though, temperature compensation effect was dominant to light compensation effect. These results stand discussed in the text in terms of the transduction of photo-thermal cues into neuroendocrine responses favoring regenerative growth.

Key words: Tail regeneration · Temperature · Light · Lizard

INTRODUCTION

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 seasonal functions and behavior of lizards. 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 remains not fully explored. Schauble and Netwig [1] were the first to demonstrate the retardatory influence of cold temperature on forelimb regeneration in the newt, Notopthalmus viridiscens. Schauble [2] demonstrated that animals kept in identical temperature and photoperiodic conditions regenerated their limbs more rapidly in summer months than in winter. Turner and Tipton [3] showed that the lizard, *A. carolinensis*, regenerated its tail more rapidly when exposed to a long photoperiod (18 hrs) than a short one (6 hrs). Maderson and Licht [4] and Tassva and Goss [5] demonstrated the influence of temperature on the final form of regenerated tail in *A. carolinensis*; a smaller proportion being

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replaced at 21°C and a bigger proportion at 32°C. Previously, we had investigated the photoperiodic influence on tail regeneration in *H. flaviviridis* involving different light schedules and seasons [6, 7]. It is the importance of light and temperature *per se* on regeneration and, the inadequate literature on lizard tail regeneration, especially on a tropical species, that gave impetus for the present detailed evaluation of photothermal influences on regeneration in *H. flaviviridis.*

MATERIALS AND METHODS

Adult lizards, *H. flaviviridis* of both sexes weighing 10±2 gm and measuring 80±5 mm snout-vent length were procured from a local animal supplier. They were maintained on a diet of nymphs 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 and consisted of three experimental schedules:

- Influence of different temperature ranges on regenerative growth under LD 12:12 (NLD) and LD 0:24 (DD) conditions.
- Influence 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.
- Light compensatory effect on temperature and temperature compensatory effect on light.

Schedule I (Temperature): These experiments were done in different months during the 3 years period involving different temperature ranges under a constant schedule of LD 12:12 or continues 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 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 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.

Schedule III: 70 lizards divided into 7 groups; 10 of each were exposed to seven different 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 was 17°C. The regenerative responses obtained in this experiment 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 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 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 aluminum foil in the case of animals exposed to 1200-lux units. The distance from fluorescent lamps to the glass surface of the cages was 15” and to the floor level 25”. The light intensities were measured at the floor level using a digital lux meter. The various light intensities were obtained by suspending one fluorescent lamp (150 lux), two fluorescent lamps (300 lux), three fluorescent lamps (600 lux) or four fluorescent lamps with aluminum 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 light intensity of 1200 lux. The cages housing animals for continues darkness were placed in a dark chamber completely shielded 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 hours and were shifted into the dark chamber at the end of respective lengths of exposure.

Autotomy of tail was performed by pinching off the tail at the third segment from the vent. The length of tail removed from the animal varied between 60±2 mm. The length of new growth in mm was measured with a graduated meter rule and recorded at fixed time intervals of 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 1: The number of days taken to attain the various arbitrary stages in LD 12:12 and LD 0:24 lizards at different temperatures are shown in table (1). 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 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 and 2) and table (2). The percentage difference in regenerative growth and also increment in regenerative growth for every 1°C increase

Table 1: Showing the number of days taken to attain the various arbitrary stages of regeneration under different temperatures in NLD and DD lizards

Temperature	Photic schedule	Wound healing	Preblastema	Blastema	Initiation of growth
17°C	NLD	24±1.00	25±0.98	26±0.89	27±1.50
	DD	25±1.21^{ns}	26±1.11^{ns}	27±1.21^{ns}	28±1.42^{ns}
23°C	NLD	12±0.89	13±1.01	14±1.00	15±0.89
	DD	17±1.00**	18±0.89**	19±0.90**	20±0.96**
26°C	NLD	6±0.78	7±0.55	8±0.77	9±0.95
	DD	12±0.98***	13±0.99***	14±1.00***	15±1.00***
29°C	NLD	6±0.55	7±0.70	8±0.75	9±0.80
	DD	6±0.65^{ns}	7±0.56^{ns}	8±0.71^{ns}	9±0.79^{ns}
31°C	NLD	5±0.43	6±0.53	7±0.67	8±0.79
	DD	5±0.45^{ns}	6±0.55^{ns}	7±0.77^{ns}	8±0.80^{ns}
33°C	NLD	4±0.34	5±0.44	6±0.50	7±0.71
	DD	4±0.32^{ns}	5±0.40^{ns}	6±0.60^{ns}	7±0.67^{ns}

NLD- normal light and dark; DD- continuous darkness. Where, **p<0.01, ** p<0.005, ***p<0.001 and ^{ns}Non-significant compared to NLD

Table 2: Per day growth rate (mm) in NLD and DD lizards at different temperatures

Temperature	Photic schedule	5-10	10-15	15-20	20-25	25-30
17°C	NLD	-	-	-	-	0.6
	DD	-	-	-	-	0.36
23°C	NLD	-	-	0.49	0.90	0.68
	DD	-	-	-	0.55	0.90
26°C	NLD	0.20	0.65	1.33	0.40	0.44
	DD	-	-	0.50	0.68	0.76
29°C	NLD	0.25	0.94	1.62	0.97	0.73
	DD	0.20	1.53	0.74	0.81	0.64
31°C	NLD	0.43	2.17	1.51	0.99	0.48
	DD	028	1.82	0.87	1.00	0.82
33°C	NLD	0.47	2.21	1.55	1.03	0.51
	DD	0.50	2.04	1.10	1.22	1.05

NLD- normal light and dark; DD- continuous darkness

Table 3: Number of days taken to attain the various arbitrary stages in animal exposed to different light intensities under LD 12:12 (NLD) and LD 24:0 (LL) at 30°C

Light intensity (LUX)	Photic schedule	Wound healing	Preblastema	Blastema	Initiation of growth
150	NLD DD 12:12	6±0.43	7±0.56	8±0.66	9±0.77
	LL LD 24:0	6±0.45^{ns}	7±0.51^{ns}	8±0.61^{ns}	9±0.71^{ns}
300	NLD DD 12:12	6±0.56	7±0.62	8±0.67	9±0.80
	LL LD 24:0	5±0.45*	6±0.60*	7±0.59*	8±0.67*
600	NLD DD 12:12	6±0.51	7±0.43	8±0.56	9±0.69
	LL LD 24:0	5±0.42*	6±0.46*	7±0.51*	8±0.71*
1200	NLD DD 12:12	5±0.33	6±0.51	7±0.57	8±0.71
	LL LD 24:0	4±0.31*	5±0.41*	6±0.59*	7±0.65*

Where, *p<0.01, ** p<0.005, ***p<0.001 and ^{ns}Non-significant compared to NLD

Table 4: 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

	Light intensity (LUX)			
Photic schedule	150	300	600	1200
NLD	20.16±2.1	20.97±1.55 ^{ns}	22.98±2.1*	26.15±2.22***
LL	20.88±1.8	21.91±1.91^{ns}	24.80±1.66**	31.66±3.16***

NLD- normal light and dark; LL- continuous light. Where, *p<0.01, ** p<0.005, ***p<0.001 and ^{ns} non-significant compared to corresponding 150 LUX.

Table 5: Total percentage replacement in lizards exposed to LD 12:12 (NLD) and LD 24:0 (LL) at different light intensities at the end of 30 days

	Light intensity (LUX)			
Photic schedule	150	300	600	1200
NLD	33.04	34.37	37.67	42.86
LL	34.22	35.91	40.65	51.90

NLD- normal light and dark; DD- continuous darkness

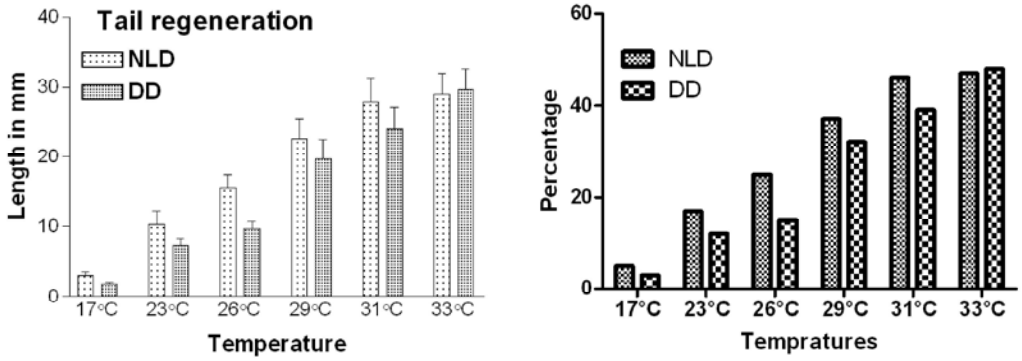

Fig. 1a,b: Length of tail regenerated and percentage replacement respectively at the end of 30 days at different temperatures under LD 12:12 and LD 0:24.

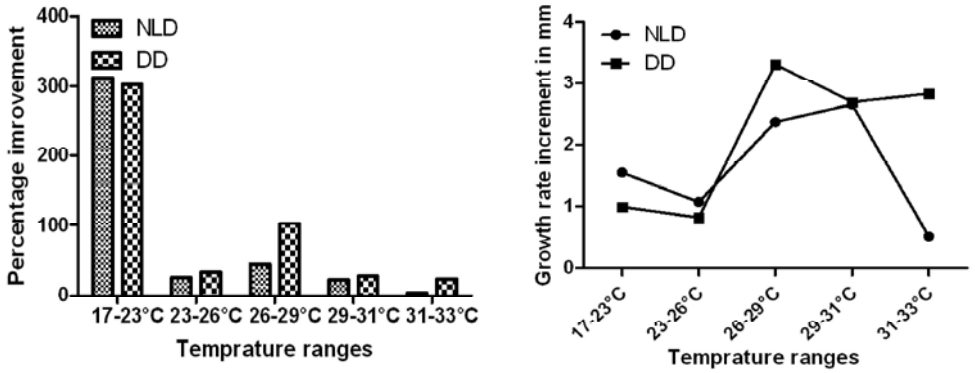

Fig. 2a,b: Percentage improvement and growth rate increment in mm respectively per degree centigrade increase in temperature under different temperature ranges in NLD and DD.

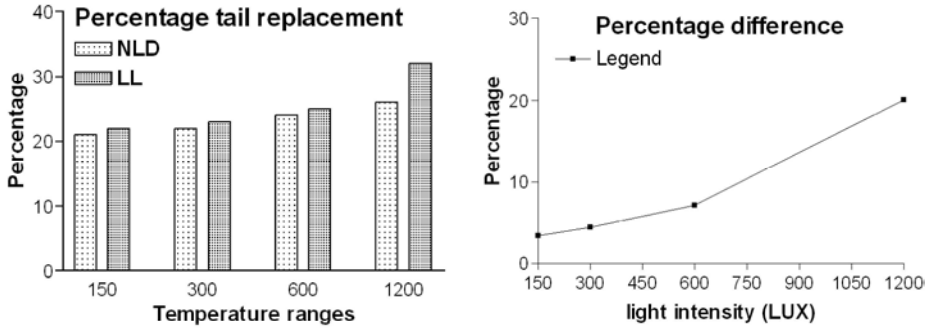

Fig. 3a,b: Showing percentage tail replaced in NLD and LL lizards and the perecentage difference between NLD and LL with different intensities of light.

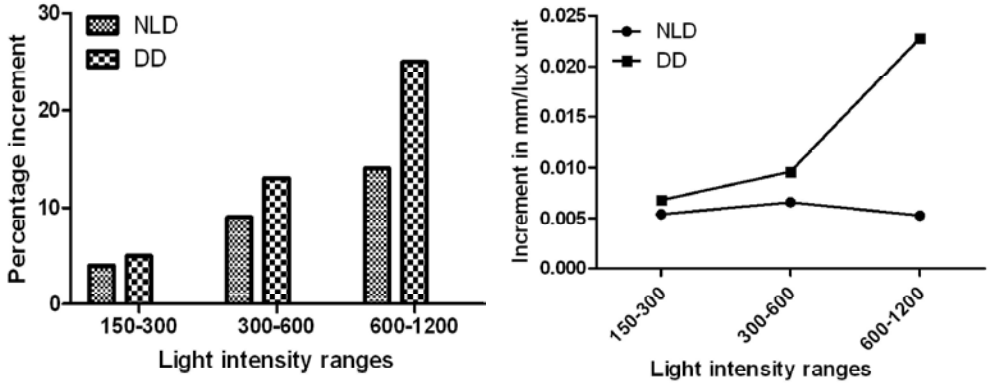

Fig. 4a,b: Percentage increment under various light intensities and, increment rate in mm respectively for every LUX unit increase in light under NLD and LL conditions.

Table 6/7: Percentage improvement in regeneration in NLD and LL lizards under different ranges of light intensity

Light intensity (LUX)	NLD	LL
150-300	4.060	4.950
300-600	9.620	13.200
600-1200	13.740	27.690
150-1200	29.700	51.600

NLD-normal light and dark; DD- continuous darkness

Table 7/8: Increment in growth rate for every LUX increase

Light intensity (LUX)	NLD	LL
150-300	0.0054	0.0068
300-600	0.0066	0.0096
600-1200	0.00528	0.0228

Table 8/9: Total length regenerated at the end of 30 days under different photic schedules at 17°C and 30°C

	Temperature	
Photic schedule (Light:Dark)	17°C	30°C
0:24	1.8±0.34	18.0±1.4
6:18	2.3±0.61*	19.11±1.68
8:16	2.5±0.66*	20.34±2.01*
12:12	2.7±0.58*	21.60±1.99*
16:8	3.2±0.71**	23.0±2.46**
18:6	15.0±1.8***	24.0±2.48***
24:0	16.0±1.67***	28.0±2.96***

Where, *p<0.01, ** p<0.005, ***p<0.001 and ^{ns} Non-significant compared to Light: Dark 0:24.

Table 9/10: The length of the tail regenerated at the end of 30 days in NLD, DD and LL lizards at 17°C, 26°C and 30°C

	Temperature		
Photic schedule	17°C	26°C	30°C
NLD	3.00±0.910	15.54±1.830	22.66±2.860
DD	1.80±0.310*	9.00±1.100***	18.61±2.420***
LL	14.90±1.460***	30.00±2.670***	46.40±3.680***

NLD- normal light and dark; DD- continuous darkness, LL- continuous light, where, *p<0.01, ** p<0.005, ***p<0.001 and ^{ns} non-significant compared to NLD.

Table 10/11: The total percentage replacement at the end of 30 days in NLD, DD and LL lizards at 17°C, 26°C, 30°C

	Temperature		
Photic schedule	17°C	26°C	30°C
NLD	4.90±0.16	25.54±2.84	37.14±4.11
DD	2.9±0.32***	14.70±1.60***	30.50±3.12**
LL	24.42±2.58***	49.18±4.31***	76.06±8.52***

NLD- normal light and dark; DD- continuous darkness, LL- continuous light, where, *p<0.01, ** p<0.005, ***p<0.001 and ^{ns} non-significant compared to NLD

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 (3 and 4) and tables (6 and 7). The regenerative growth in LD 12:12 animals was greater than in DD up to 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 (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 in both the groups of lizards, more markedly in the LL group (Tables 9-11 and figures 5-9).

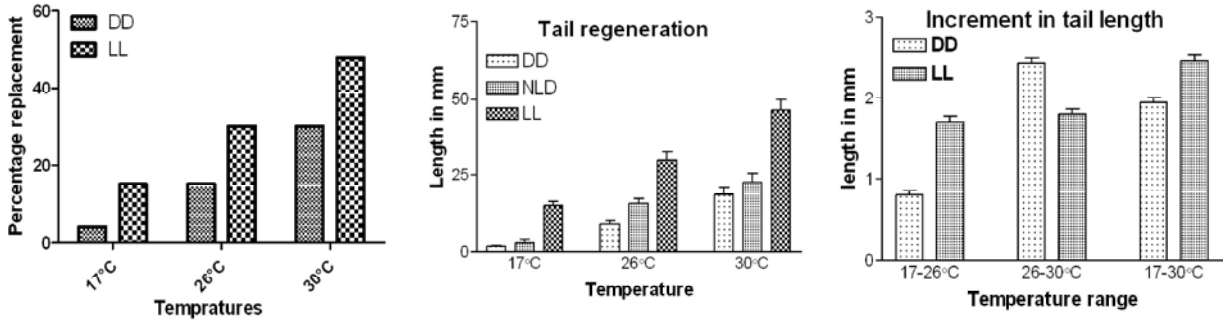

Fig. 5a,b,c: The length of tail regenerated and percentage replaced respectively at the end of 30 days in DD and LL lizards at 17, 26 and 30°C.

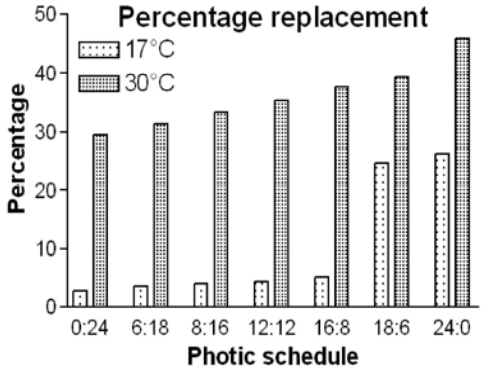

Fig. 7a: Percentage replacement in lizard at 17°C and 30°C at different light schedules

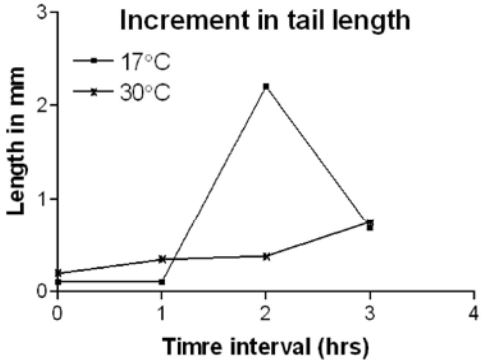

Fig. 7: Growth rate increment in mm per hour increase in light at 17°C at 30°C at different light schedules.

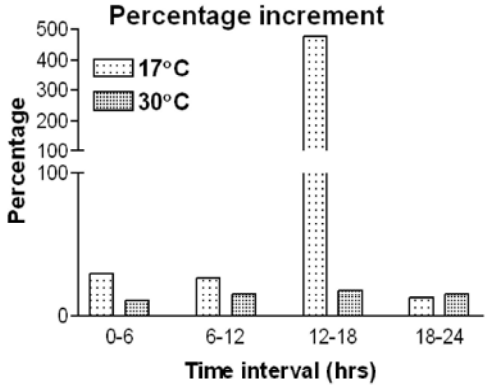

Fig. 7b: Percentage increment in light at 17°C at 30°C at different light schedules

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 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 and 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 1°C increment from 17°C to 36°C was 0.8 mm in DD and 1.39 mm in NLD. Between 26°C and 30°C, the same was 2.4 mm in the former and 1.78 mm 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 and 15) and figures (14-17). With increasing light schedule there was continuous improvement in tail regeneration from a minimum of 1.8 mm in LD 0:24 to a maximum of 16 mm at 17°C and at 30°C, the same ranged from 18 mm in LD 0:24 to 28 mm 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 same 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.

DISCUSSION

Experiments carried out herein at different temperatures indicate a gradient effect of temperature on regeneration as, tail elongation hastened when temperature increased from 17°C to 33°C. Fixed temperatures were not employed for the experiments, as animals do not encounter such conditions in nature. The essential purpose was to assess the regenerative performance in relation to naturally occurring seasonal variation 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 to nullify the influence due to change in photoperiod. The temperatures mentioned actually represent the average monthly means in a given season and, table (1) represents the range of maximum-minimum temperatures during the select months. A justification for this is the 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 thermal cycles (fluctuating temperature) of 30°C/15°C showed a very robust melatonin rhythm [8].

The gradient effect is manifest in the form of progressive increase in tail elongation with increase in temperature as seen in lizards obeying a photoperiodic schedule of LD 12:12 as well as DD. Influence of temperature on regeneration has been shown by other workers as well. The regenerative rate of tadpole tail increases with increase in temperature between 19°C and 28°C and regenerative growth ceases below 14°C and above 28°C [9]. Higher temperatures accelerate both rates of blastema formation and the subsequent regeneration rates in *Anolis carolinensis* [4]. 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 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 2.37 mm and 2.65 mm between 26°C-29°C and 29°C-31°C respectively in NLD lizards. The DD lizards showed maximum increment of 3.32 mm between 26°C to 29°C and slightly lower but significant increments of 2.69 mm and 2.83 mm between 29to 31°C and 31°C to 33°C respectively. The NLD lizards however depicted an insignificant increment of 0.52 mm between 32°C to 33°C. Probably 31°C represents the upper limit to stimulation of regeneration process in NLD lizards. Schauble and Nentwing [1] also defined similar upper limit to stimulation of regeneration process in the newt, *N. viridiscens*. 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 form 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 occurring 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 but 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 the newt stands documented previously [10,11]

The third set of experiments performed for evaluating the relative effects of light and temperature, has revealed importance of both these factors on regenerative tail elongation in lizards. The various calculations project the potent influence of both photic and thermal components on regeneration, though on a relative basis the latter has greater impact than the former. The DD lizards registered 934% increase in tail elongation and an average increment of 1.3 mm per degree centigrade with rise in temperature from 17°C to 30°C. On the other hand, lizards at 17°C registered 789% increase in tail length and an average increment of 0.6 mm for every hour of light with increase in photoperiod from LD 0:24 to LD 24:0. A comparison of the two validates the espoused relative importance of temperature over light. 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 temperature under continuous darkness indicate light compensation effect on temperature and temperature compensation effect on light; again, the temperature compensation effect on light being 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% at 17°C while, the same at 30°C yielded only 56% improvement. Maximum percentage improvement of 17% and an average increase of 0.66 mm 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 2.05 mm 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 photothermal 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 24:0 was a meager 75%. This temperature compensatory effect over light, manifests maximally at the higher temperature range between 26°C–30°C as an increased growth rate of 2.4 mm per degree centigrade. The alluded dominant effect of temperature compared to light stands out clearly when compared with the percentage difference in regenerative growth between DD and LL at 17°C and 30°C and that under LD 0:24 and LD 24:0. Whereas in the latter case the difference reduces form 900% to 75%, in the former, it reduces from 788% to 50%.

The entire gamut of observations and analysis reviewed above, suggest the potent favorable influence of both light and temperature on the course of regeneration in lizards. Though photoperiod has been considered the principal factor in the entrainment of pineal melatonin rhythm in mammals [12], in poikilotherms like reptiles, even temperature has been implicated as an entrainment cue [13-15]. 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 cycle could also maintain the same [16-18] 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* [17] 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 seem 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, provide ample justification for the temperature controlled neuroendocrine rhythms favoring tail regeneration. However, the mechanisms responsible for the transduction of these environmental factors into linear growth need elucidation. In view of the

previously documented importance of prolactin (PRL) in inducing linear growth of the autotomized tail [19] this hormone appears to be the likely agent modulated by both temperature and light. Up regulation of PRL secretion by both light and temperature is conceivable. Present observations clearly suggest a light augmented PRL secretion by increased hypothalamic serotonergic activity (potent secretogogue of PRL) and a temperature augmented PRL release by decreased dopaminergic activity (potent PRL inhibitor). This contention finds support from the results of many studies in mammals [20-26] Enough evidences are available in this context, which buttress the fact that, both temperature and light can modulate PRL mediated regenerative growth. Whereas, [27, 28] have implicated PRL in the light mediated stimulatory influence on regeneration, Schauble [2], Schauble and Tyler [29] and Schauble and Netwig [1] have reported 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-cholrophenylalanine (p-CPA), a 5-HT synthesis inhibitor, could effectively nullify the continuous light induced increment on regenerative growth, alluding to 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 rhythm seems to suggest neither melatonin nor a common oscillator as responsible for driving the dopaminergic and serotonergic rhythms [7]. Separate oscillators seem to drive them though a coupling is possible as evident from our other studies. Substantiation for this notion comes from previous observations that, pinealectomised lizards show some degree of increment in regenerative growth both with increase in photoperiod as well as increase in temperature on a seasonal basis [7], conveying a staunch impression that though pineal is the principle mediator, there is some extra-pineal photothermal perception.

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

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

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