CHAPTER I

EFFECT OF DIFFERENT PHOTOPERIODIC LENGTHS ON TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS.

Many animals 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). For example, the length of the day can serve as a reliable cue for the regulation and timing of reproductive cycles and other physiological processes that must be correlated with different times of the year. This response to seasonal changes in the length of the day is somewhat loosely called "photoperiodism". Day length may have an important influence on reproductive activity in some reptiles. While not all reptiles examined showed distinct photo-sexual responses (Mellish, 1936; Tinkle and Irwin, 1965), artificially increased photoperiods have been demonstrated to accelerate testicular recrudescence and egg laying in several species of lizards (Bartholomew, 1950, 1953; Fox and Dessauer, 1958, Mayhew, 1961, 1964; Licht, 1966, 1967a, 1967b). Changes in day length may also have other effects in reptiles, such as on appetite and growth (Fox and Dessauer, 1957; Mayhew, 1965) and thermal acclimation (Hutchinson and Kosh, 1965; Licht, 1968).

The problem of regeneration in reptiles has attracted attention of several workers in the past who studied extensively histomorphological aspects of normal and regenerating tails of lizards. Amongst those who have contributed to such studies are Woodland (1920) on Hemidactylus flaviviridis, Slotopolsky (1922) on Lacerta, Barber (1944) and Kamrin and Singer (1955) on Anolis carolinensis, Hughes and New (1959) on the Gekkonid lizard, Sphaerodactylus, Simpson (1964) on Lygosoma laterale, Moffat and Bellairs (1964) on Lacerta vivipara Jacquin, Bryant and Bellairs (1967) on Anguis fragilis and Lacerta dugessi, Shah and Chakko (1968) on Hemidactylus flaviviridis and Cox (1969) on Anolis carolinensis.

Reports on photoperiodic influence on lacertilian tail regeneration is scanty. Turner and Tipton (1972) showed that the lizard A. carolinensis regenerated its tail more rapidly when exposed to a long-day photoperiod (18 hours) than to a short one (6 hours). To date, no investigation has yet been carried out on the influence of photoperiodim on tail regeneration in a tropical saurian. In fact, Bellairs and Bryant (1985) in their recent review of literature on reptilian tail regeneration have proposed the necessity of more work on photoperiodism to integrate the couple of observations available.

The present investigation, in this context, evaluates the effect of different photoperiodic lengths on the rate of tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis.

MATERIALS AND METHODS

Eight different photoperiodic lengths were investigated as detailed on pages 12 and 13, and each schedule consists of 40 lizards.

This investigation was conducted during the post-breeding monsoon months (August - October) and the recorded average monthly ambient, room and cage temperatures are given in table 3. All groups of lizards in the present experiment were kept under similar conditions of temperature and humidity. The average daily temperature at the level of the animals in the lighted and dark chambers did not vary by more than 2°C at any stage (Table 3). The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and further to Duncan's Multiple range test with an alpha level of both 0.05 and0.01 (Duncan, 1955).

RESULTS

The results are depicted in tables 1 and 2 and figures 1-3. The blastemic stage appeared in LL and LD 18 : 16 exposed animals by day 5 to 7 and in LD 6 : 18 and 0 : 24 exposed animals by day 12 to day 14 post-caudal autotomy. In the intermediate photoperiods of LD 12 : 12 and LD 16 : 8, the regeneration process started by day 8 to day 10 (Table 1). This temporal difference in regenerative outgrowth persisted until the early differentiation phase after which it became minimized during the late differentiation and growth phases. The arbitrary stages of regeneration shown in table 1 can be described as:(1) Wound healing, when the cut surface of the tail stump is completely covered by epithelium ;(2) blastema, a dome shaped outgrowth from the tail stump which contains the primordial cells of the regenerate ;(3) early differentiation, ~~can be~~ visualized as a small elongation from the blastema ; (4) mid differentiation, at this stage the regenerate tapers slightly from the blastema cone; (5) late differentiation, at this stage the tapering of the regenerate becomes pronounced ;(6) growth, the regenerate is up to 10 mm at this stage and will begin to take the form of a functional organ, resembling the lost tail; (7) fully regenerated tail, when daily measurement of the regenerate showed no additional growth. After a period of twenty days : we considered it as a fully regenerated tail (Shah and Chakko, 1968; Radhakrishnan and Shah, 1986).

Growth rate and total length regenerated.

A measurable growth occurred in animals exposed to LL (H), LL (L) and LD 18 : 6 photoregimes by day 5 while in the LD 12 : 12,LD 16 : 8 and LD 8 : 16 groups of animals it occurred between days 5 and 10, However, in lizards exposed to LD 6 : 18 and LD 0 : 24 , a measurable growth occurred only between days 10 and 15. The regeneration process was completed in LL(H), LL(L) and LD 18 : 6 groups of animals by the 50th day at which time the total length of the tail regenerated was 41.7 mm, 33.3 mm and 38.7 mm, respectively. In the other groups of lizards, the regenerative growth ceased by day 60 and the least regeneration (28.2 mm and 27.4 mm) was found in the LD 6 : 18 and LD 0 : 24 groups. The total length of tail regenerated in the remaining groups of animals was nearly similar and were 33.6 mm in LD 16 : 8, 33.0 mm in LD 12 : 12 and 31.0 mm in LD 8 : 16.

The pattern of growth rate (figure 2) indicates a linear increase peaking at 30-40 days in all photoperiodic regimes from LD 0 : 24 to LD 16 : 8. However, LD 18 : 6, LL (L) and LL (H) regimes induced a very significant initial growth spurt which rendered the growth rate curve a biphasic one with increasing photoperiodism beyond 16 hours having a definite stimulatory influence on this initial spurt. The stimulatory influence of photoperiodism on lacertilian tail regeneration was further revealed by the gradually decreasing peak growth rate from LD 16 : 8 to LD 0 : 24.

Total percentage replacement :

Percentage replacement calculated in terms of total length of tail regenerated and total length of tail autotomized revealed a minimum of 50.5 in LD 0 : 24 exposed lizards and a maximum of 75.3 in LL (H) exposed animals followed by 70.4 in LD 18 : 6.LL (L),LD 12 : 12 and LD 16 : 8 recorded nearly similar replacement of 62.5%, 61.7% and 62.7%, respectively. Lizards exposed to eight hours of light showed slightly reduced percentage replacement of 57.8 while those exposed to six hours of light produced a replacement of 52.7%,more like the LD 0 : 24 exposed animals. In the present experiment, none of the groups of lizards fully replaced the length of the original tail and the maximum replacement obtained was only 75.3% under LL (H).

All possible comparisons between the eight experimental set ups (Duncan's multiple range test) revealed no statistical significance between LD 6 : 18 and LD 0 : 24 exposed groups of animals on one hand and between LL (L), LD 12 : 12 and LD 16 : 8 groups on the other. However, all other comparisons other than these were statistically significant at both 5% and 1% levels.

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN <u>H</u>. <u>FLAVIVIRIDIS</u>.

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

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

TABLE 2. GROWTH RATE DURING DIFFERENT PERIODS OF TAIL REGENERATION IN _H_. _FLAVIVIRIDIS_.

PHOTOREGIMES	DAYS 0 - 10	DAYS 10 - 20	DAYS 20 - 30	DAYS 30 - 40	DAYS 40 - 50
LL (H)	1.12 ± 0.11*	0.71 ± 0.09	0.67 ± 0.16	1.20 ± 0.30	0.59 ± 0.15
LL (L)	0.40 ± 0.16*	0.30 ± 0.10	0.87 ± 0.15	1.04 ± 0.09	0.70 ± 0.09
LD 18 : 6	0.70 ± 0.10*	0.37 ± 0.07	0.61 ± 0.14	1.25 ± 0.28	0.92 ± 0.30
LD 16 : 8	0.34 ± 0.10	0.48 ± 0.02	0.95 ± 0.20	1.15 ± 0.27	0.48 ± 0.19
LD 12 : 12	0.32 ± 0.11	0.42 ± 0.07	0.85 ± 0.15	1.07 ± 0.26	0.30 ± 0.19
LD 8 : 16	0.30 ± 0.10	0.40 ± 0.10	0.79 ± 0.08	1.02 ± 0.16	0.56 ± 0.13
LD 6 : 18	- -	0.43 ± 0.11	0.61 ± 0.11	0.97 ± 0.06	0.57 ± 0.15
LD 0 : 24	- -	0.31 ± 0.05	0.57 ± 0.26	0.95 ± 0.31	0.86 ± 0.34

* Significantly high compared to the corresponding next period.

TABLE 3. AVERAGE AMBIENT, ROOM AND CAGE TEMPERATURES DURING THE PERIOD OF STUDY.

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

AVERAGE DAILY TEMPERATURES.
LIGHTED CHAMBER = 27°C
DARK CHAMBER = 25°C

* POST-BREEDING MONSOON SEASON.

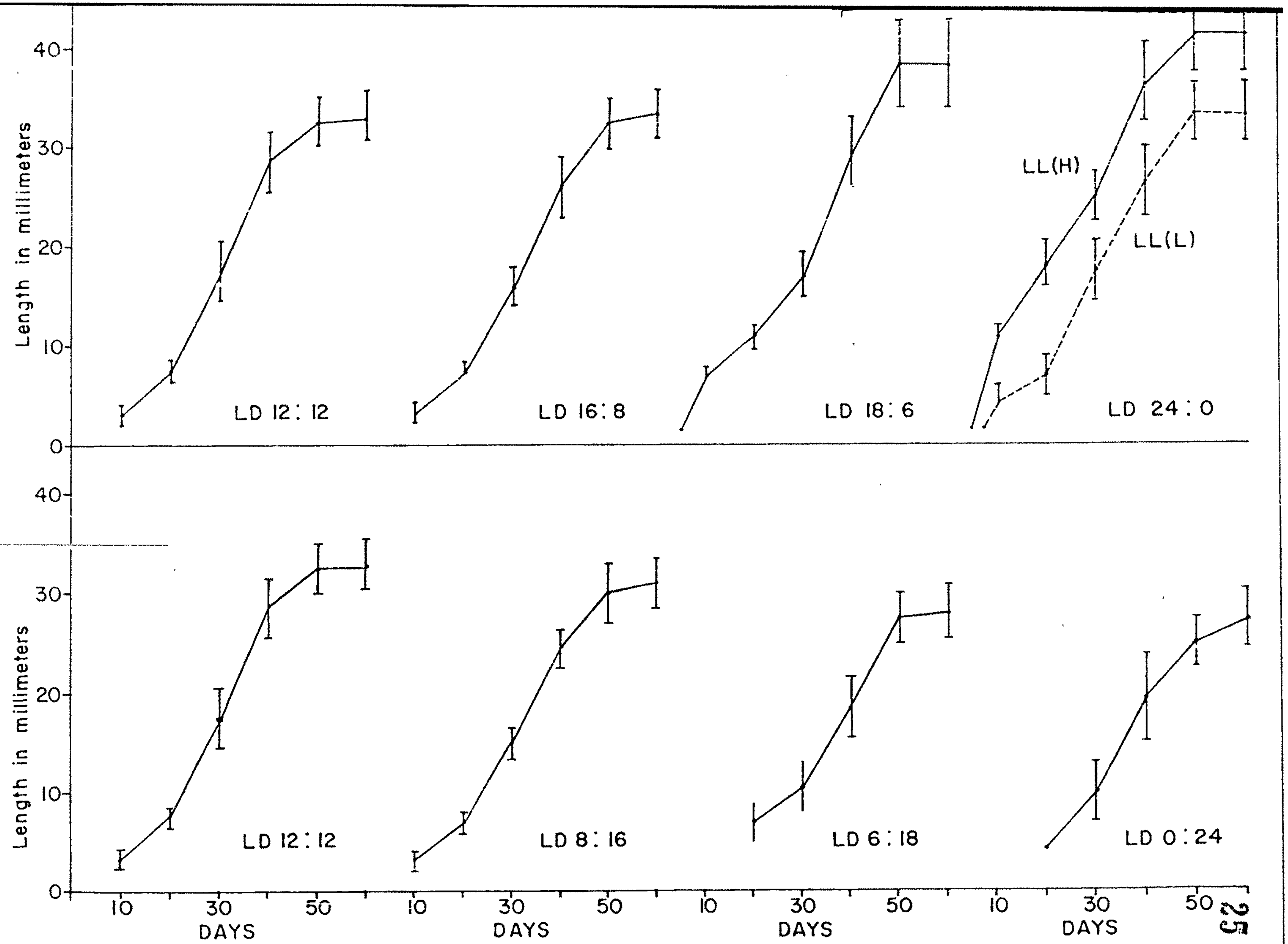

Fig. I THE TOTAL LENGTH OF THE TAIL REGENERATE AT VARIOUS TIME PERIODS IN *H. FLAVIVIRIDIS* (± SD)

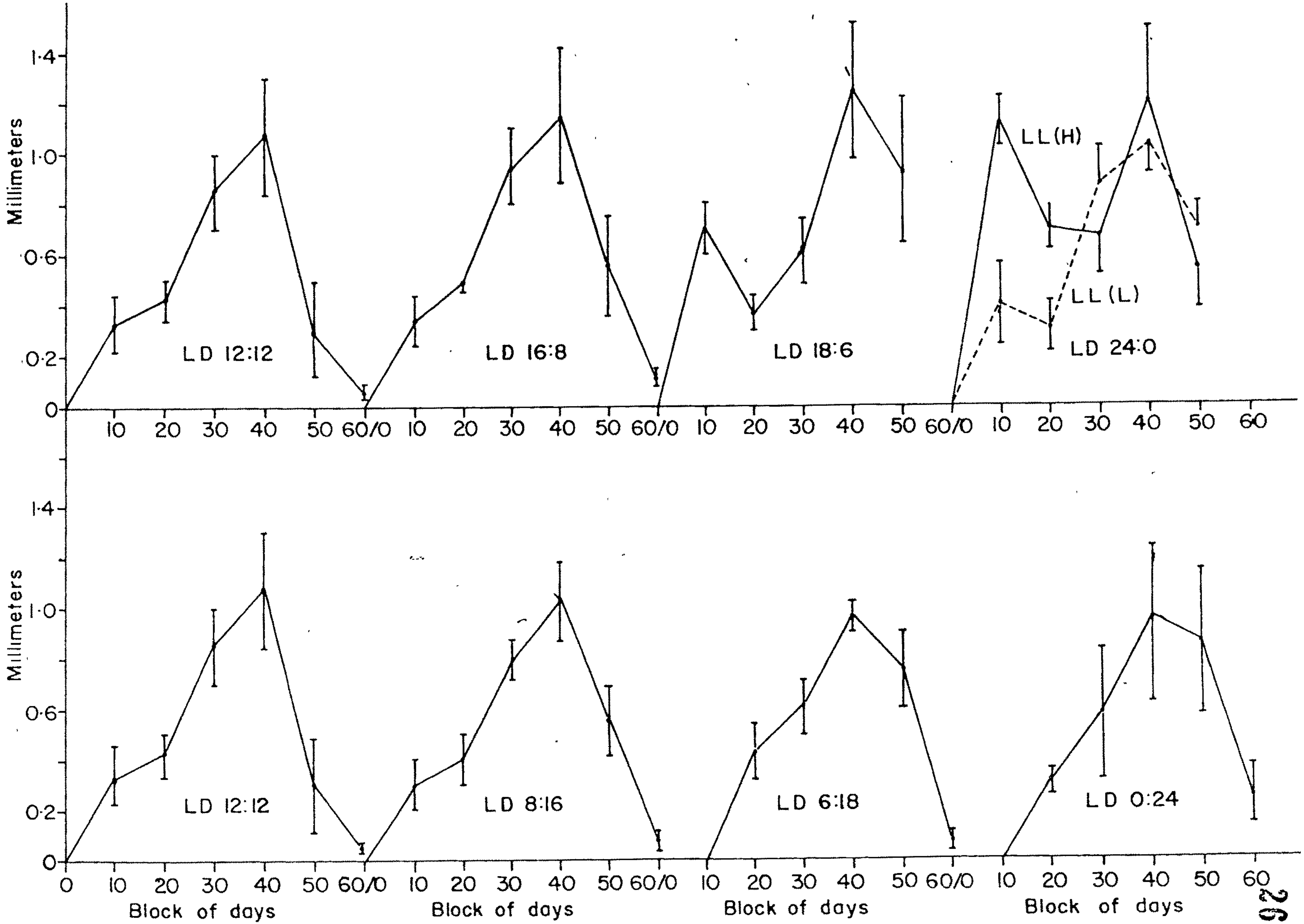

Fig.2. THE PER DAY RATE OF GROWTH IN BLOCKS OF DAYS DURING TAIL REGENERATION IN *H. FLAVIVIRIDIS* (± SD)

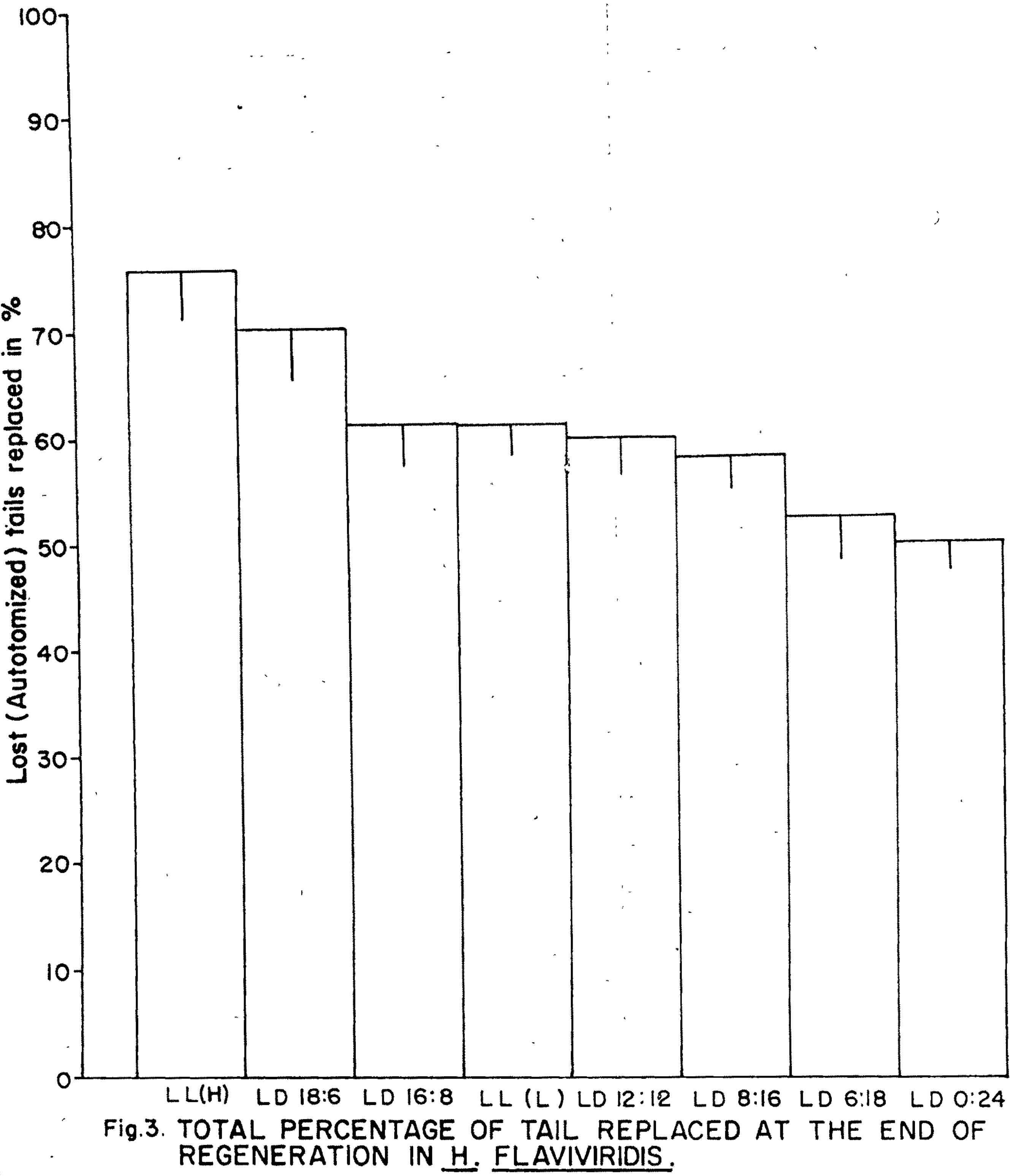

Fig.3. TOTAL PERCENTAGE OF TAIL REPLACED AT THE END OF REGENERATION IN H. FLAVIVIRIDIS.

LL(H) continuous light, high intensity; LL(L)-continuous light, low intensity; LD 18:6-18 hours light and 6 hours darkness; LD 16:8-16 hours light 8 hours darkness; LD 12:12-12 hours light and 12 hours darkness; LD 8:16-8 hours light and 16 hours darkness; LD 6:18-6 hours light and 18 hours darkness; LD 0:24-continuous (Total) darkness.

DISCUSSION

This study evaluated the effect of different photoperiodic lengths on tail regeneration in the Gekkonid lizard Hemidactylus flaviviridis. The regeneration process is stimulated by continuous light and depressed by continuous darkness. Furthermore, the stimulatory effect of light has also been shown to be dependent on both the duration and intensity. A comparative assessment of the new growth (regenerate) reveals that the onset of regeneration,the daily growth rate, the final length of tail replaced at the end of regeneration and percentage replacement of the autotomized tail are all significantly affected by the length of exposure to light as well as its intensity. The investigation also demonstrates that a short-day photoperiod of LD 6:18 has no stimulatory effect on the regenerating lizard tail, whereas the opposite long day photoperiod of LD 18:6 has a definite stimulatory influence.

From figures 1 and 3, it becomes obvious that both the total length of tail regenerated and the percentage replacement are maximal under LL (H) and minimal under LD 0:24. Though the values with regards to these two parameters were quite similar in LL (L), LD 12 : 12 and LD 16 : 8 on one hand and LD 6:18 and 0:24 on the other, a definite linear correlation between the length of photoillumination and the ultimate length of tail regenerated and total percentage replacement can be inferred. This fact is confirmed by the observed values under LD 18:6 which were significantly more than all the groups except for LL (H). 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. The present work employing greater number of photoperiodic regimes and larger sample size as compared to the above two works have definitely underscored the stimulatory influence of increasing lengths of light on regenerative performance and, in this context, the results obtained by Maier and Singer (1977) in the newt and Turner and Tipton (1972) in lizards are consistent with the present findings in as much as a greater proportion of the lost appendage is replaced at long-day photoperiods and a smaller proportion at short-day photoperiods. It may be added here that higher light intensities enhance the stimulatory influence of long length photoperiods on lacertilian tail regeneration.

Another striking inference that could be drawn by careful study of figure 2 is the biphasic growth spurt in LL (H), LL (L) and LD 18:6 photoregimes and the significant linear positive influence of increasing light exposure on the peak growth spurt. The observable effect of decreasing light schedules on the initial regenerative growth is a delayed temporal shift by five and ten days, respectively, in the LD 6:18 and LD 0:24 groups of animals while there is no remarkable effect of increasing photoperiodism on the maximal regenerative spurt. It is, however, difficult at this juncture to give any interpretive explanation on the observed initial growth spurt in LD 18:6 and LL regimes and the resultant biphasic growth rate curve. Nevertheless, it is very clear that the stimulatory influence of incfeasing lengths of light on the regenerative performance is essentially exerted in the initial blastemic and 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 observations of Litwiller (1940) of increased mitotic rate of blastemal cells of amphibia during the light phase of the diurnal cycle coupled with the demonstration of Bourne and Tucker (1959) of increased serum prolactin level with longer lengths of light in mammals and the report of Crim (1975) of prolactin as a growth promoter in amphibia are relevant and may provide a causal explanation to our observations.