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Influence of photoperiod on number of young ones laid down (fecundity) of freshwater snail *Bellamya dissimilis*

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Abstract
Light is the synchronizing factor as in many animals and photoperiod play an important role in reproduction. This experiment was designed to investigate the influence of photoperiod on the reproduction/fecundity (no. of young ones) of the viviparidae snail *Bellamya dissimilis*. Reproduction/Fecundity of the snail *Bellamya* were examined at four different groups i.e. long day (16L: 8D), short day (8L: 16D), medium day (12L: 12D) and control group kept in an artificial photoperiod experiment for 21 days. This result indicates that artificial photoperiod affects the frequency of young ones. Snails kept in long day laid maximum number of young ones (153 young ones/25snails/21days) and those kept in short day laid minimum number of young ones (43 young ones/25snails/21days) and snails kept in medium day and in control group laid 45 young ones and 33 young ones respectively. There was a significant difference ($p<0.01$) between the long day photoperiod 16L: 8D and in control group. Mortality rate was less in long day (16%) than in short day (40%) though long day has maximum number of young ones. This study conclusively shows that long day regimes have stimulatory effects on sexual maturation. Further investigations will be put on the difference in quality between young ones laid in different seasons.

Keywords: snail, *Bellamya dissimilis*, fecundity, mortality, photoperiod

1. Introduction

Reproductive behavior or the occurrence of reproductive activity in mollusk is influenced by the environmental factors like temperature, salinity, photoperiod, O_2, CO_2 content and availability of food. In Gastropod molluscs reproductive behavior or the reproductive activity is controlled by environmental parameters, such as temperature, salinity, photoperiod, food availability, water quality etc. [30, 18]. The behavioral pattern and the metabolic processes in snails are due to the light regimes to which the snails are affected [39, 15]. The driving force in life cycle of a snail is reproduction. Because reproduction is the only way that an individual contributes its genotype to future generations; natural selection should favor organisms with generations determined traits that increase their likelihood of surviving to sexual maturity [33]. Reproductive cycles of animals like Pulmonate gastropods which are living in the temperate regions mostly are influenced by the effects of photoperiod and temperature [20].

Light is the synchronizing factor as in many animals [16] and photoperiod play an important role in reproduction in many vertebrates [5]. Many authors have been worked on the effects of photoperiod on reproduction mostly in basommatophorans species of lymnaeids and planorbids [25], in *Melampus* [41], in *Lymnaea stagnalis* [7, 12, 32] and in *Bulinus truncates* [4]. Again in snails, the species *Helix aspersa* shows much more importance of photoperiod on the control of reproduction by [51, 3, 35, 22]. In *Cepaea nemoralis*, the effect of photoperiod seems less pronounced than in *Helix aspersa* because [24] obtained egg laying in animals raised in short days just as well as those raised in long days.

Trails in a controlled environment showed that in long days egg laying can be more achieved than in natural conditions [22]. We have therefore undertaken systematic study of the artificial photoperiod on reproduction in snail *Bellamya dissimilis*.

The freshwater prosobranch snail *Bellamya dissimilis* Muller 1774, belongs to viviparidae family which gives directly birth to the young ones, instead of laying egg masses, they brood their young ones. This snail gives birth to a large number (120-130) of fully developed young ones [40]. Viviparidae are generally hermaphrodite where both sperm and eggs produced either simultaneously or sequentially in the same individual. Although they are hermaphroditic, they generally copulate for exchange of sperms.

This species seems to be highly adaptable to varying environmental conditions in the field, there are few laboratory studies which are been examined that how they get affected by the environmental factors like photoperiod, temperature, food preference etc.

External factors such as photoperiod, light intensity, and temperature were found to be directly involved in the induction of egg laying [42]. Photoperiodism appears to influence all aspects of somatic physiology in pulmonates, addition to having obvious effects on reproductive maturation and activity [56]. Studied the effect of photoperiod and temperature on egg-laying behaviour in the marine mollusk, *Aplysia californica*. They found that reproductive system of an *A. Californica* is responsive to photoperiod for egg laying.

Functional adaptations to withstand a particular environmental parameter are necessary for the survival of the organism. The photoperiod is one of the factors that determine the induction of breeding in freshwater snail [1, 41].

Several molluscs studies have been carried on effect of environmental parameters on reproduction wherein light and temperature have been considered as test conditions [45, 38]. The effects of photoperiod, temperature and population density on adult fecundity and juvenile growth in *Marisa cornuarietis* was described by [29]. Numerous studies on the pond snail *L. stagnalis* have shown that photoperiod [13] light intensity, food availability, temperature and atmospheric pressure all have long term effects on reproductive activities [53, 54, 52, 32]. The influence of environmental factors on the reproduction of *Lymnaea stagnalis* have been described by [50]. Photoperiod influences snail behavior in conjunction with the seasons of the year, therefore in relation to reproductive processes it is an important factor that also need to be controlled [19]. In natural photoperiodic condition of 12 hour or shorter occur in autumn, winter, and early spring, when little food is available [46]. The environmental regulation of seasonal reproduction had focused on describing the effects of various environmental factors on reproduction, the endocrine system involved, the role of circadian system in photoperiodic responses. The photoperiod changes the animal's reproductive activity after many weeks of exposure [55].

Effect of photoperiod on female reproductive activity of *L. stagnalis* kept under laboratory conditions shows production of eggs and egg masses is much higher at a long day photoperiod (L:D 16:8) than at shorter photoperiods [7]. Several authors have been studied the reproduction in Molluscs is effected by various environmental factors such as photoperiod, temperature etc. [8, 17, 9, 10, 43, 48, 32, 49, 56, 27, 19, 55]. [49] Reported that maturation of slug *Limax maximus* reproductive system is dependent on photoperiodic signals. Slugs exposed to long day-lengths stimulate the growth and development of gonad, penis, albumen gland, various female accessory sex organs, and maturation of sperms.

Mating behavior shows that are only sensitive to photoperiod, which are numerous in long days and rare in short days [20]. Further investigations will be put forth on the difference in quality between eggs laid in different seasons.

2. Material and methods

2.1 Animals

The freshwater snail *Bellamya dissimilis* (length 3.3-4.3cm, width 4.5-5.9cm, weight 2.38-4.9g) were collected from the collection site (Godavari river near Kaygaon Toka).the geospatial location of collection spot is at Latitude: 19˚37'57.44" N and Longitude 75˚14'30.41"E.The animals were brought safely to the laboratory, snails were washed with tap water and were maintained in large plastic trough with continuous water refreshment and aeration.

2.2 Experiment

100 animals of *Bellamya dissimilis* of equal size and weight were selected for experiment. The snails were divided into four groups to following different photoperiodic experiment, viz., Group I Long day (16L:8D), Group II Short day (8L:16D), Group III Medium day (12L:12D) and Group IV as Control. The trough was covered with wire netting to prevent the animals from escaping. In this experiment there were total three replicate troughs for each above the photoperiodic treatments, each containing 25 healthy, mature snails of *Bellamya dissimilis* and were placed in large plastic trough with 6 liters of water. Each trough was illuminated 1 ft. above with separate table lamps with bulbs of Anchor Company (60WATT and 260 VOLTS). The lighting was provided with an intensity of 1479 lux. A carefully routinized schedule of feeding and water change every second day was followed throughout the experiment period, snails were fed with algae, mulberry leaves and spinach. Room temperature was 38ºC to 40ºC at the time of experiment; Sunrise and Sunset were ranged between 5:49am to 7:06am with the help of mobile phone which was recorded daily. During the experiment when groups were supposed to keep in darkness the groups were covered totally with the help of bed sheet to see the effect of photoperiod on young ones laid down during darkness. The experiment was done for 21 days from 19th May to 8th June 2017.

2.4 Statistical analysis

The significance difference between the groups was calculated by using Students t – test to compare the experimental groups with control. A value of $p<0.01$ was taken as statistically significant and the results were as mean with standard deviation (±SD) values for the experimental data.

2.3 Observations

Snails were checked daily to see the young ones laid down from each group. Mortality rate was also evaluated by the percentage of snails dying during the experiment in each group.

3. Results

Fig 1: Showing new young ones laid by mature snails.

Fig 2: Showing experimental setup of photoperiod.

3.1 Comparisons of total number of young ones laid down in the four groups

Comparisons of the rates of reproduction obtained in the different groups (Table 1) shows clearly that the best reproduction and the greatest number of young ones were obtained with long days (16L:8D) group while snails kept at short days (8L:16D) group laid very less young ones.

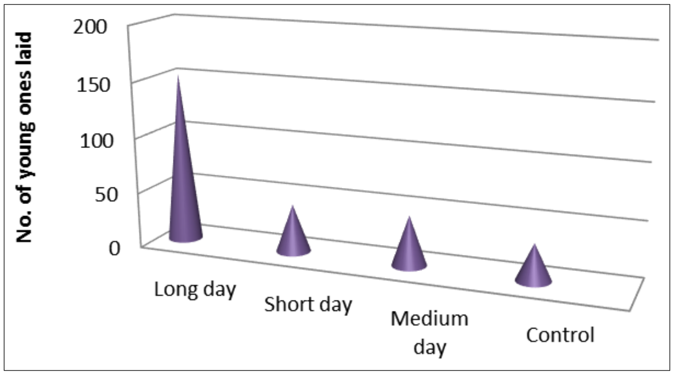

Fig 3: Variations in number of young ones laid down by different treatments of photoperiod.

Table 1: Effect of photoperiod on number of young ones laid down (Fecundity) of snail *Bellamya dissimilis*.

Environmental parameter/ Conditions	Type of treatment	Number of young ones laid/25snails /21days	Mean± SD	Mortality
Photoperiod	Long day (16L:8D)	153	7.28±6.82	4 (16%)
	Short day (8L:16D)	43	2.04±2.33	10 (40%)
	Medium day (12L:12D)	45	2.14±3.59	5 (20%)
	Control	33	1.57±2.58	5 (20%)

Number of young ones observed during long day group was 7.28±6.82 that of in short day group was 2.04±2.33 and number of young ones observed during medium day and in control group was 2.14±3.59 and 1.57±2.58 respectively.

The effect of photoperiod on reproductive activity in *Bellamya dissimilis* has been depicted in fig. 3 photoperiod affect the frequency of laying young ones in *B. dissimilis.* Snails kept in group A i.e. Long day (16L: 8D) photoperiod laid maximum number of young ones during the experimental period while snails kept in group B i.e. Short day (8L:16D) photoperiod laid minimum number of young ones as compared to long day and medium day.

Snails maintained in group A i.e. long day regimes laid 153 young ones/25snails/21days and those kept in group B short day (8L:16D) laid 43 young ones/25snails/21days. In group C i.e. medium day (12L: 12D) laid 45 young ones/25snails/21days and those kept in group D i.e. control laid 33 young ones/25snails/21days.

Although there were no considerable differences in number of young ones laid down for long day, short day, medium day and control photoperiodic regimes. Major difference was observed in the number of young ones laid down in each group. The short day animals laid very less number of young ones i.e. 43 opposed to 153 by long day animals. This shows that long day lengths have stimulatory effects.

Snails acclimated to long day photoperiod was higher than those acclimated to control group. Statistical analysis show that number of young ones during short day photoperiod and medium day were more than in control, however these difference were not significant ($p<0.01$).There was a significant difference ($p<0.01$) between the long day photoperiod 16L:8D and control in terms of number of young ones laid down.

3.2 Comparisons of the rate of mortality

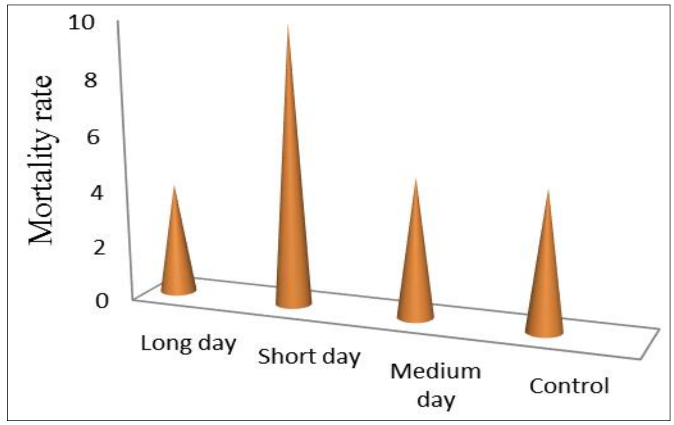

Fig 4: Mortality rate of different groups.

In group A i.e. long days (16L:8D) mortality was less as compared with group B i.e. short days (8L:16D) in which long days had a higher rate of young ones. During the experimental period which lasts for 21 days the mortality in group A i.e. long day was 16% (4 of 25 animals) and those in group B i.e. short day which laid minimum number of young ones had mortality of 40% (10 of 25 animals), that in group C medium day and group D control shows equal number of mortality rate i.e. 20% (5 of 25 animals).Long days was compatible with a good survival rate and with more number of young ones laid down by the snails (Fig.4).

4. Discussion

Observations on the influence of photoperiod for egg laying by [20] showed that number of eggs laid was 45-50/snails and the frequency of egg laying (90-130%) were greater in long days than in short days i.e. 16-35/snails and 27-77%. In their experiment long day photoperiods brought about cyclic reproduction over a period which plays an important role on the neuroendocrine control of egg laying in snail *Helix pomatia.*

The present experiment shows that only the long day photoperiod was able to induced a prolonged egg laying behavior for 21days (Fig.1).In short day photoperiod, egg laying was less as compared with long day photoperiod. It shows the importance of factor of daily lighting in the determination of reproduction.

Under medium day (L: D 12:12) photoperiodic conditions starvation stop oviposition, where as egg laying continues under long day conditions, ending in premature death of the snails [31, 14]. [28] Studied the effect of temperature and artificial photoperiod on growth and subsequent fecundity of *H. aspersa* var. *maxima*, under laboratory conditions.

Studies on the pond snail *L. stagnalis* in past have shown that five major external signals, viz., photoperiod, temperature, food consumption, water quality and the parasites, regulate reproduction and associated behaviors [32]. The studies carried out by [7] indicates that long day photoperiodic regimes stimulate sexual maturation and egg laying in *L. stagnalis* as compared to animals kept either in short days or neutral day's regimes. In addition, the snails kept in long day lengths are slightly more sensitive to hormonal induction of egg laying than those animals kept in medium days [14]. The most prominent effect of photoperiod is on the number of eggs laid [32]. When *Helix aspera* was submitted to a decreased light regime in a 24 hr. light/dark cycle or a fixed short day (8L:16D), it presented a partial or complete reduction in reproductive activities such as mating, egg laying, and ovulation [3, 21].

Snail *I. exustus* exposed to different treatments of photoperiods had no significant effect on somatic growth but it affects on ovipositon in long day photoperiod, snails lay more number of egg masses and number of eggs per egg masses as compare to short day and medium day photoperiod [37]. [24] Observed during a study of *Cepaea nemoralis* in which they concluded that gametogenesis and functional development of the reproductive system were neither prevented nor retarded by exposing the animals to short-day photoperiods. They also concluded that photoperiod was involved in lesser effects on reproduction; similar observations were recorded for *H.pomatia* [20]. These results indicate a photoperiod is dependent control of the neuroendocrine centers responsible for ovulation.

[56] Showed that animals maintained in warm temperature, short day lengths (8L: 16D) enhanced the frequency of egg laying in mature *Aplysia* compared with those animals kept on long day (16L: 8D). Effect of photoperiod on egg laying behavior, this response is not robust compared with the effect of temperature. The Pulmonate snail *Heliosoma duryi* [34] showed increased growth in darkness compared to 12L: 12D and continuous light [57]. [29] Observed that there is no effect of photoperiod on adult fecundity or egg hatching and on juvenile growth and development. Increase in egg laying with increased photoperiod in *Lymnaea stagnalis* has been reported by [6].

Long day lengths (L16:8D) have stimulatory effects on sexual maturation, long day photoperiod showed maximum egg masses. Snails maintained on long days slightly more sensitive to hormonal induction of egg laying than those kept on medium days. The most prominent effect of photoperiod is on the number of eggs laid. Snails reared in the laboratory under artificial photoperiodic conditions laid significantly greater number of eggs and laid eggs more frequently under long days than in medium or short days [37]. Studies on the mating behavior of *H. aspera var. aspera* indicated more significant role of photoperiod and the observation that snails of this species receiving 9h of light did not lay eggs [51] and this studies further confirmed by [3, 35,]. Long day photoperiodic factor is a crucial factor in egg laying in *Lymnaea* [7, 52].

In the photoperiodic experiment of [23] the snails exposed in light for 6h in 24 h dark period shows higher egg laying as compared to snails kept in dark for 24h. They observed that the egg laying was higher in control group with respect to eggs kept for 24h in the dark. There is no significant change in the egg laying behavior of *L. acuminata* which was kept in the dark for 24h with respect to control snails reported by [47].

Results obtained from the studies of [2] shows that the effect of two photoperiodic on the production of eggs are three times more egg laying in 12L: 12D treatment than 0L: 24D treatment for the species studied i.e. *Achatina achatina* (Linne, 1758) *Achatina fulica* (Bowdich, 1820) and *Archachatina ventricosa* (Gould, 1850). According to them the fertility of snails in breeding is influenced by the photoperiod. Whatever the species may be, the 12 hours daylight/12 hours dark treatment shows a fertility superior to that for the 0 hours daylight/24 hours dark treatment.

In [36] work reported that photoperiod influences snail behavior in conjuction with the seasons of the year. It was found that in *Melania scabra* during monsoon season young ones laid were more in number than in winter and summer and young ones laid during long day photoperiod were more than short day photoperiod.In the studies of [28] they observed the effect of photoperiod on the weights of the snails *Helix aspera var. Maxima* and mortality the weights of the snails reared at 16-h darkness exceeded those of snails reared in total darkness by 11% and mortality did not exceed over 10% and no effects were observed on snail oviposition.

However, in present study we have observed that number of young ones laid down was higher in long day (16L: 8D) group as compared to short day (8L: 16D) or control group and mortality rate shows snails reared in long day by 16% and those reared in short day shows 40% of mortality. It indicates that light stimulation plays an important role in the fecundity of snails. [44] Reported that in long day photoperiod snails *Helix asperta* had significantly higher body weights as compared to short day photoperiod with respect to the seasonal groups in spring snails and autumn snails and mortality was low of only 3.3-8% of snails from each group. Further investigations are necessary to determine the role of photoperiod on the neuroendocrine system of snail *Bellamya dissimilis*.

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