

Research & Reviews in



Regular Paper

RRBS, 9(6), 2014 [223-230]

Advancement in the spawning time of goldfish *Carassius auratus* under various temperature and photoperiod shifts

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ABSTRACT

Our aim of the present study is to manipulate the spawning time of goldfish Carassius auratus by using different Photoperiod and Temperature regimes so that there fry will be available all the year around. 4 photoregimes were used representing 3 treatments and control. The first photoregime (control) were subjected to the natural light at room temperature. The second aquaria treatment 1 were kept under 17L/7D at 24°C. The third aquaria representing treatment 2 were subjected to 13L/11D Photoperiod at 24°C. Photoperiod of last aquaria was adjusted 15L/9D at 24ºC. The first sample was taken after 30 days from the beginning of the experiment. The second one was after 50 days from the beginning of the experiment. The third was carried out after 70 days from the start of the experiment, and last one was after 90 days respectively. After 30 days from the start of the experiment, 17L/7D at 24°C reached the ripe stage earlier than the other ones (40 days earlier than the control group). After 50 days from the beginning of the experiment 15L/9D at 24°C followed treatment 1. The spawning time of 15L/9D at 24°C started 20 days earlier than the control. After, 70 days control was the third to reached the ripe stage. The last treatment that reached egg-ripening stage was the 13L/11D at 24°C after 90 days from the beginning from the experiment. The spawning time of second treatment also extend to overlap with that of the control later 20 days. Accordingly, there was an artificial spawning time (additional 40 days) that prolonged the natural spawning season and extended the availability of *Carassius* fry for a longer period Weight gain, Feed conversion ratio and Gonadosomatic index, were also discussed. © 2014 Trade Science Inc. - INDIA

INTRODUCTION

Environmental control of teleost reproductive cycles has been reviewed by^[19,7,18]. Photoperiod and temperature, among the factors concerned, have been considered to be much important in the regulation of teleost reproductive cycles.

Manipulation of photoperiod can accelerate, maintain or delay sexual maturation and spawning of broodstock so that spawning may occur out of season^[14,15]. Developmental and maturational events are dominated and coordinated by seasonal changes in photoperiod, temperature, rainfall etc^[21]. In this concern, some authors studied the role of photoperiod and temperature in freshwater fish^[4]. studied the effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout. He concluded that early spawning was achieved when accelerated photoperiod regimes were applied^[17], studied photosensitive development of the ovary in the Mosquitofish, *Gambusia qfflnis qffinis*. In his experiment, stimulation of ovaries was induced in fish exposed to a 16L-32D or a 8L-28Dlight cycle, though it did not occur in the fish maintained under a short photoperiod of 8L-16D.

Shaikh and Hafeez^[22] on cyprinid fish, Cyprinion

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watsoni assessed different photoperiod-temperature regimes using gonadosomatic index and histological criteria in different seasons, prespawning, spawning and post spawning. Their results revealed that long photoperiod-warm temperature accelerated gametogenic progress in the prespawning season. Short photoperiod-warm temperature had inhibitory or regression effect in most seasons. In the spawning season, warm temperature stimulated spawning and low temperature inhibited it regardless of photoperiod.

Marshall and Bielic^[16] investigated the Periodicity of reproductive behaviour by the blue tilapia, *Oreochromis aureus*. Their observations assured that shifting the light cycle by seven hours resulted in a corresponding shifting in time of spawning^[8,9]. combined effect of temperature and photoperiod on the induction of reproduction and growth of freshwater amphipod *Hyalella azteca*. He considered the photoperiod as the main cue in the induction and termination of reproduction, however active reproduction did not take place unless temperature was within certain range. Optimum reproduction and growth rates occurred in this range.

Additionally, several researchers clearly noted that photoperiod and light intensity manipulation have been successfully used to improve the growth of larval and juvenile stages of a number of fish species. Also, improved fish growth in relation to light regime has been attributed to a number of factors including higher food conversion efficiency and growth parameters and also lower activity and lower oxygen consumption^[1,20].

The aim of this work was to test different temperature-photoperiod regimes on the reproductive behavior and growth of the Goldfish *Carassius auratus* to improve the production of fry.

MATERIAL AND METHODS

The experiment started on 1/4/2012 and lasted for 90 days. As shown in TABLE 1, 20 fish per aquarium (*Carassius auratus* female) with an average body weight of 50.12 ± 2.18 g were collected from commercial dealer and randomly divided into 4 groups representing three treatments and a control. Glass aquaria 4 x 4 x 2 feet each, were used. 5 males were taken randomly from a group of males with an average body weight of 50.26 ± 3.24 g and placed with females of each aquarium. This experiment was carried out in the Fisheries research Laboratory in the Department of Zoology, Agra college Agra, India.

Experimental design

4 photoregimes were used representing 3 treatments and control. The first photoregime (control) was subjected to the natural light and at room temperature. The second group (treatment 1) was kept under 17L/7D photoperiod water temperature was increased and maintained at (24°C). The third aquaria representing treatment 2 was subjected to photoperiod (I3L/11D) and their water temperature was kept as (24°C). Photoperiod of the last aquaria (treatment 3) was adjusted 15L/9D and water temperature maintained as (24°C). The first sample was taken after 30 days from the beginning of the experiment. The second one was after 50 days from the beginning of the experiment The third was carried out after 70 days from the start of the experiment, and last one was after 90 days respectively. The fish samples were sacrificed and ovaries were extruded carefully and weighed to calculate the gonadosomatic index.

On 1 April 2012 four experimental treatments were created within the aquriums as follows:

- Control: Natural light and at room temperature
- Treatment-1 : Long Photoperiod 17L: 7D at temperature (24 °C)
- Treatment-2: Photoperiod 13L:11D at tempera-

TABLE 1 : Number and average initial weight of female goldfish under different experiment regimes at the start of the experiment. Data are presented as Mean \pm Sem. Average initial weight of goldfish under different experimental regimes in the same column are statistically not significantly different (p>0.05)

| Experimental Regimes | No. of Fish per aquarium | Average Initial body weight Female (gm) |
|--------------------------------|--------------------------|---|
| Control | 20 | 50.42 ± 0.082 |
| 1.17L:7D at 24 ⁰ C | 20 | 49.78 ± 0.24 |
| 2.13L:11D at 24 ^o C | 20 | 49.8 ± 0.014 |
| 3.15L:9D at 24 ⁰ C | 20 | 0.032 |



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ture (24 °C).

 Treatment-3: Long Photoperiod 15L:9D at temperature (24 °C)

Environmental conditions

Aerated and dechlorinated water with flow rate of 1.5 L min⁻¹ 9 ppm dissolved oxygen, 7.8 pH and 102 mg as CaCO₃ total water hardness was used. Experiment lasted for 90 days. The fishes were fed twice daily with commercial fish meal at 4 % of total biomass at 10:00 am and 14:00 pm. Each aquarium was constantly aerated by electric aerators. After 2 hour after feeding uneaten feed were removed by the sandpipe at the bottom of the tank, dried and weighed to calculate net feed utilized by the fish. Stocking density was maintained at 25 fish (20F+5M).

40 l-¹ water to avoid overcrowding and the fish were treated with tetracycline bath (0.012g.l-¹) to prevent the outbreak of bacterial infection. Each tank with an artificial photoperiod regime was enclosed within a box made from carton and black plastic sheeting to prevent the escape of light to the surrounding tanks and enable complete isolation from natural light. Illumination was supplied with daylight fluorescent tubes (100 W) suspended 30 cm above the water surface and automatically controlled by a timer. Water temperature with a commercial aquarium thermostat and heater.

Gonadosomatic index (GSI %)

Weight of Gonad is expressed as percentage of the total body weight

GSI (%) = gonad weight (g) x100 /total body weight (g).

Weighing

To calculate and monitor growth parameters and predict a daily food ratio, fish were individually weighed and zoometric measurements were taken before the start of the trial and then at the last. Fish were placed on laminated graph paper.

The total length (TL) of the fish was measured from the tip of the anterior or part of the mouth to the caudal fin using meter rule calibrated in centimeters. Fish were measured to the nearest centimeter. Fish weight was measured after blot drying with a piece of clean hand towel. Weighing was done with a table top weighing balance, to the nearest gram. Length, from the mouth to caudal peduncle, and depth, from the deepest point of the body to base of dorsal fin, were measured (mm). Both fish and food weight data were used to calculate the food conversion ratio, using the equations below.

Feed conversion ratio (FCR)%=Feed in take (g) /body weight gain (g)

Weight gain

Weight Gain (WTG) = Wi – Wo

where Wi = final mean weight (g), Wo = Initial mean weight (g)

Percentage Weight Gain (%) = $[(Wi - Wo)/Wo] \ge 100$ where Wi = final mean body weight (g), Wo = Initial mean body weight (g)

Four samples were taken during the whole experiment. The first sample was taken after 30 days from the beginning of the experiment. The second one was after 50 days from the beginning of the experiment. The third was carried out after 70 days. The fourth were after 90 days from the beginning of the experiment respectively.

Statistical analysis

Data were analyzed by students t'test. Data are presented as mean \pm Sem. The values of p<0.05 were considered significantly different.

OBSERVATIONS AND RESULTS

4 photoregimes were used representing 3 treatments and control. Under first photoregime (control) fish were subjected to the natural light at room temperature. Under second photoregime (treatment 1), fish were kept under 17L:7D photoperiod, temperature of water was maintained at (24°C). Fish, under treatment 2 were subjected to photoperiod (13L:11D) and their water temperature was kept as (24°C). Photoperiod given under treatment 3 was adjusted to 15L:9D and water temperature was maintained at (24°C). After 30 days from the beginning of the experiment, treatment 1 (17L:7D at 24^oC) reached the ripe stage earlier than the other ones (40 days earlier than the control group) as shown in (TABLE 2). After 50 days from the beginning of the experiment treatment 3 (15L:9D & 24°C) followed Treatment 1. The spawning time of treatment 3 started 20 days earlier than the control (TABLE 3).

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TABLE 2 : Showing GSI % and average body weight of female goldfish after 30 days of exposure to different Photoperiod and temperature shifts. Data are presented as Mean ± Sem. (Exp.regime 1-17L:7D at 24 °C, Exp.regime 2-13L:11D at 24 °C, Exp.regime 3- 15L:9D at 24 °C, ^R-Ripe).

| Photoperiodic regimes | GSI % (gm) | Average body weight (gm) |
|---------------------------|-----------------------|--------------------------|
| Control | 4.02 ± 0.022 | 51.13 ± 0.02 |
| Exp.regime 1 ^R | 10.35 ± 0.058 *** | 60.12 ± 0.09 *** |
| Exp.regime 2 | 3.72 ± 0.014** | 51.22 ± 0.22 |
| Exp.regime 3 | 6.81 ± 0.025 *** | 55.43 ± 0.217*** |

** Sig.different than the control (P<0.01); *** Sig. different than the control (p<0.001)

After 70 days, Control group was the third one to reach the ripe-egg stage (TABLE 4). The last treatment that reached egg-ripening stage was the 13L:11D at 24°C after 90 days from the beginning of the experiment (TABLE 5). The spawning time of the second treatment also extended to overlap with that of the control later 20 days. Accordingly, there was an artificial spawning time (additional 40 days) that prolonged the natural spawning season and extended the availability of *Carassius* fry for, a longer period Initial body weight of Fish in different experimental regimes were not significantly different (p>0.05) as shown in (TABLE 1).

At First sampling the gonadal development of

TABLE 3 : Showing GSI % and average body weight of female goldfish after 50 days of exposure to different Photoperiod and temperature shifts. Data are presented as Mean \pm Sem. (Exp.regime 1-17L:7D at 24 °C, Exp.regime 2-13L:11D at 24 °C, Exp.regime 3- 15L:9D at 24 °C, ^R – Ripe).

| Photoperiodic regimes | G.S.I % (gm) | Average body weight (gm) | | |
|---------------------------|----------------------|-----------------------------|--|--|
| Control | 7.27 ± 0.29 | 57.6 ± 0.16 | | |
| Exp.regime 2 | 4.84 ± 1.28 *** | 53.3 ± 0.032 *** | | |
| Exp.regime 3 ^R | 10.72 ± 0.49 *** | 60.2 ± 0.048 *** | | |

** Sig.different than the control (P<0.01); *** Sig. different than the control (p<0.001)

TABLE 4 : Showing GSI % and average body weight of female goldfish after 70 days of exposure to different Photoperiod and temperature shifts. Data are presented as Mean ± Sem. (Exp.regime 1-17L:7D at 24 °C, Exp.regime 2-13L:11D at 24 °C, Exp.regime 3- 15L:9D at 24 °C, ^R – Ripe).

| Photoperiodic regimes | GSI % (gm) | Average body weight (gm) | | |
|--------------------------|----------------------|-----------------------------|--|--|
| Control ^R | 10.08 ± 0.046 | 58.85 ± 0.09 | | |
| Exp.regime 2 | 7.92 ± 0.055 *** | 55.4 ± 0.042 *** | | |

** Sig.different than the control (P<0.01); *** Sig. different than the control (p<0.001)

TABLE 5 : Showing GSI % and average body weight of female goldfish after 90 days of exposure to different photoperiod and temperature shifts. Data are presented as Mean \pm Sem. (Exp.regime 1-17L:7D at 24 °C, Exp.regime 2-13L:11D at 24 °C, Exp.regime 3- 15L:9D at 24 °C, ^R-Ripe).

| Photoperiodic regimes | GSI % (gm) | Average body weight (gm) | | |
|---------------------------|-----------------|-----------------------------|--|--|
| Exp.regime 2 ^R | 9.94 ± 0.18 | 58.16 0.065 | | |

females in Experimental regime 1 (17L:7D at 24°C) was at peak earlier than the other experimental regimes and the control (40 days earlier than the control) females of Experimental regime 1 (17L:7D at 24°C) were fully ripe and were ready to spawn and the G.S.I reached at 10.35±0.058 % (TABLE 2) which was significantly different than the control (p<0.001) and second sampling was done after 50 days and it was observed that at this time females of experimental regime 3 (15L:9D at 24°C) showed the signs of egg ripening (20 days earlier than the control).G.S.I % averaged 10.72 ± 0.49 which was significantly different than the control (p<0.001) represented in (TABLE 3). After 70 days third sampling was done and at this time females of control group were ready to spawn G.S.I % reached at 10.08 \pm 0.046 (TABLE 4) and last sampling was done after 90 days from the beginning of the experiment and females of Experimental regime 2 ((13L:11D at 24°C) were the last to reached at the ripe stage (20 days later than the control) G.S.I reached at 9.94 ± 0.18 represented in (TABLE 5).

According to the results of the Experimental regime 1(17L:7D at 24°C). Weight gain and feed conversion ratio were also at the peak in comparison with control and the Experimental regimes.(TABLE 6).

Experimental regime 3 (15L:9D at 24°C) followed the experimental regime 1 after 20 days from the beginning of the experiment average body weight was 60.2 ± 0.048 (TABLE 4), significantly different than the control (p<0.001) The control group females were the third to reached the ripe stage with average body weight of 58.85 ± 0.09 as represented in (TABLE 4), weight gain and F.C.R were $8.43 \pm$ 0.09 and 2.68 ± 0.042 (TABLE 6)

Females of experimental regime 2 (13L:11D at 24°C) reached the ripe stage after 90 days from the beginning of the experiment with average body weight of 58.16 ± 0.065 TABLE 6, weight gain and F.C.R were 8.36 ± 0.049 and 2.42 ± 0.019 which were statistically not different in comparison with control p>0.05 (TABLE 5)

As indicated in (TABLE 7) the average body weight of fish in the control group in sample 1,2 and 3 were 51.13 ± 0.02 (p<0.01), 57.6 ± 0.16 and 58.85 ± 0.09 (p<0.001) were significantly higher than the initial body weight.

Average body weight of Experimental regime 1 was 60.12 ± 0.09 which was significantly different than the 0 sample p<0.001.(TABLE 7;Figure 1)

In treatment 2 average body weight of fish at 1,2,3 and 4 sampling time were 51.22 ± 0.22 (p<0.01),

TABLE 6 : Showing weight gain and feed conversion ratio of female goldfish exposed to different photoperiod and temperature shifts. Data are presented as Mean \pm Sem. (Exp.regime 1-17L:7D at 24 °C, Exp.regime 2-13L:11D at 24 °C, Exp.regime 3- 15L:9D at 24 °C, ^R – Ripe).

| Photoperiodic regimes | Weight gain (gm) | FCR |
|-----------------------|-----------------------|------------------------|
| Control | 8.43 ± 0.09 | 2.68 ± 0.042 |
| Exp.regime 1 | 10.35 ± 0.041 *** | $3.62 \pm 0.028^{***}$ |
| Exp.regime 2 | 8.36 ± 0.049 | 2.42 ± 0.019 |
| Exp.regime 3 | 9.98 ± 0.035 *** | $3.2 \pm 0.058 **$ |

** Sig. different than the control (p<0.01); *** Significantly different than the Control (p<0.001).

 53.3 ± 0.032 , 55.4 ± 0.042 , and 58.16 ± 0.065 (p<0.001) which was significantly different than the initial reading (TABLE 7; Figure 1).

In all groups slight increase in G.S.I % was also recorded at different sampling time as represented in (TABLE 7).

In experimental regime 3 average body weight at 1 and 2 sampling time were 55.43 ± 0.217 and 60.2 ± 0.048 significantly different than the Initial body weight (p<0.001).

The weight gain in control group, Experimental regime 1, Experimental regime 2 and Experimental regime 3 were 8.43 ± 0.09 , 10.35 ± 0.041 , 8.36 ± 0.049 and 9.98 ± 0.035 respectively, weight gain of Experimental regime 1 and 3 differed significantly than the control (p<0.001) but weight gain of Experimental regime 2 did not differ significantly to the control (p<0.05). (TABLE 6)

The Feed conversion ratio of control of Experimental regime 1, 2 and 3 were 2.68 ± 0.042 , 3.62 ± 0.028 , 2.42 ± 0.019 and 3.2 ± 0.058 respectively F.C.R of Experimental regime 1 differed significantly than the control (p<0.001) but F.C.R of Experimental regime 2 was not significantly different to the control (p>0.05) (TABLE 6).

Results show that exposure to 17L/7D at 24°C resulted in 40 days advancement than control group under 15L/9D at 24°C, fish attained reproductive maturity and riped 20 days earlier than control group and 20 days later than 17L/7D photoregime. Weight gain and feed conversion ratio were also high in 17L/7D photoregime.

DISCUSSION

Our aim of the present study is to manipulate the spawning time of the Goldfish *Carassius auratus*

 TABLE 7 : Average body weight (gm) and G.S.I % of goldfish under different experimental regimes at different time intervals. Data are presented as Mean ± Sem

| Days of Exposure | Control | | 17L:7D at 24 ⁰ C | | 13L:11D at 24 ⁰ C | | 15L:9D at 24 ⁰ C | |
|---------------------|--------------------|----------------------|-----------------------------|----------------------|------------------------------|---------------------|-----------------------------|------------------------|
| | G.S.I % | Avg. body wt. | G.S.I % | Avg.body wt. | G.S.I % | Avg. body wt. | G.S.I % | Avg.body wt. |
| Initial | - | 50.42 ± 0.082 | - | 49.78 ± 0.24 | - | 49.8 ± 0.044 | - | 50.22 ± 0.032 |
| 30 | $4.02\pm\!\!0.022$ | $51.13 \pm 0.02 **$ | 10.35 ± 0.058 | $60.12 \pm 0.09 ***$ | 3.72±0.014 | 51.22 ± 0.22 ** | 6.81 ± 0.025 | 55.43±0.217*** |
| 50 | 7.27 ± 0.29 | 57.6±0.16*** | - | - | 4.84 ± 0.28 | 53.3±0.032*** | 10.72±0.49 | $60.2 \pm 0.048^{***}$ |
| 70 | 10.08 ± 0.046 | $58.85 \pm 0.09 ***$ | - | - | 7.92 ± 0.055 | 55.4±0.042*** | - | - |
| 90 | - | - | - | - | 9.94 ± 0.18 | 58.16±0.065*** | - | - |

** Significantly different than the 0 sample (p<0.01); *** Significantly different than the 0 sample (p<0.001)



Figure 1 : Showing body weight of goldfish in experimental regime and control after 30, 50, 70 and 90 days of exposure. Data are presented as mean \pm Sem. 0 days means initial.

by using different photoperiod and temperature regimes so that there fry will available all the year round.

Temperature, and especially photoperiod, manipulation is widely practiced commercially by the aquaculture industry. What we currently know concerning photoperiodic influences on reproduction is commonly used to modify the phase of reproductive rhythms, advancing or delaying the spawning season, Investigations on seasonal variation in the reproductive activity of fish and the factors responsible for such variations have revealed that the seasonal changes in the day-length and water temperature are the major factors controlling the reproductive cycle^[12,2].

The Spawning time of Goldfish *Carassius auratus* in the nature is during July and August. In this study Fish were kept in 3 Experimental regimes 17L:7D at 24°C, 13L:11D at 24°C, 15L:9D at 24°C and 1 in control group at natural conditions and our results revealed that Long photoperiod with warm temperature (17L:7D at 24°C) accelerated advanced shifts in the spawning time of Goldfish *Carassius auratus*, in this experimental regime fish attained maturity with 40 days advancement control group. Weight gain and Feed conversion ratio is also significantly higher than the control Group. The long photoperiods are required for the final stages of gonadal maturation in *Phoxinus*^[5].

The long photoperiods with warm temperature during winter and spring brought about sexual maturity in female goldfish^[13]. The fish of experimental regime 3 (15L:9D at 24^oC) reached maturity stage after 50 days (20 days later than experimental regime 1 and 20 days earlier than the control) and control was the third one to reached the maturity stage and Experiment 2 was the last one. Therefore, these results revealed that photoregime 15L:9D is also effective because in this regime fish were ripe 20 days earlier than the control^[10]. exposed goldfish *Carassius auratus* to various photoperiods at several different times of the year, long photoperiods stimulated gonadal maturation in *Carassius* during spring. A long photoperiod warm temperature regime also promotes sexual maturation in other fishes *Carassius auratus*^[13] and *Notropis bifrenatus*^[11,8]. also considered that the photoperiod is the main cue in the induction of gonadal development.

In this experiment long Photoperiod with warm temperature (17L:7D at 24°C) play expected role when fish were exposed to this regime. The fish of this regime were preceding to maturation earlier than other groups and control. For most temperate water or higher latitude fish, spawning is an annual event controlled by endogenous (including endocrine) and exogenous (including photoperiod and temperature) cues^[24]. Feed conversion ratio (FCR) was significantly (p<0.05) affected by photoperiod treatments, and the best FCR was recorded in the 17 hrs light regime with 24°C. The feed conversion ratio (FCR) were increased with extended duration of light regime and significant differences (P<0.05) were found in comparison with control group. The feed conversion increased when fish were exposed to long photoperiod regime 17L/7D at 24° C (3.62 ± 0.078) in comparison with 13L/ 11D at 24°C (2.42 \pm (0.019), 15L/9D at $24^{\circ}C$ (3.2 ± 0.058) and Control (2.68 ± 0.042) . Weight gain was also recorded at peak in 17L/7D at 24 °C (10.35±0.041). Although the best values were obtained with the long photoperiod; this same photoperiod also gave the best results in growth, short light periods do not appear to be the most suitable for obtaining a favourable conditions for better development in Goldfish.

It is clear through our results that the positive effects of long-term photoperiod on fish growth rates are achieved through increased specific growth rate and food conversion ratio rather than stimulation of feeding. In our experiment the highest feed conversion ratio and weight gain was observed in 17L:7D at 24° C.

The use of artificial photoperiods to manipulate the timing of maturation is now a well-recognized tool within the aquaculture industry. By photoperiod applications, the period of gonadal development was brought to a half at spawning periods causing an increase in the live weights of salmons^[18]. A similar situation was found in the sea bass, *Dicentrarchus labrax*, with the rate of maturation being increased in fish exposed to compressed photoperiod cycles^[6]. Blythe *et al.*, (1994a) suggested that temperature may regulate the process of oocyte development by controlling metabolism.

Our study suggests that the annual cycle of reproduction is controlled by an endogenous rhythm or clock whose periodcity is circannual, i.e. approximately a year in length. Under ambient conditions the periodicity of this clock is closely entrained by the seasonally changing pattern of day length so that its period length is exactly one year.

ACKNOWLEDGEMENT

The authors are thankful to the Authorities and Head of the department, Department of Zoology Agra college Agra for providing the facilities.

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