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Determination of optimum temperature and photoperiod for mass production of *Oxya hyla hyla* (Serville)

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Abstract: Insects are natural food for many vertebrates. Being nutritionally rich, acridids can be used to produce high quality feed for livestock industries. For a sustainable supply for the feed manufacturing companies, a huge acridid biomass must be obtained on a regular basis. Therefore, for successful acridid farming, a laboratory rearing system in semi-controlled conditions is proposed to produce a huge acridid biomass. Experiments were conducted to determine a favorable temperature and photoperiod for rearing the chosen multivoltine species, i.e. *Oxya hyla hyla*. For this purpose nymphal mortality, growth rate, fecundity, fertility, and adult dry weight were determined. The results revealed that, at 35 ± 2 °C with a L:D photoperiod of 12:12, fecundity and fertility were maximum, while nymphal mortality, egg incubation period, nymphal duration, adult life span, and adult dry weight were favorable. It was concluded that 35 ± 2 °C with a photoperiod of 12:12 is suitable for mass production of *Oxya hyla hyla* in acridid farms.

Key words: Acridid farming, *Oxya hyla hyla*, photoperiod, temperature

Introduction

Most underdeveloped and developing countries are facing difficulties of acute shortage of animal protein that adversely affects the protein intake level of the country's population and results in malnutrition. Therefore, enhancement of these livestock industries is becoming increasingly essential. However, livestock industries are facing difficulty to obtain a sufficient amount of feed due to a sharp rise in their price. Generally, feed accounts for more than 60% of the total cost of raising farm animals (Onimisi and Omage, 2006). Shortage of the conventional animal feedstuffs like fish, and plant feedstuff like maize and soybean, is occasioned by the competition between man and livestock for these feed sources (Vander, 1997). Hence, there is a need to look for an alternative, nonconventional, economic, protein rich, natural food source that is not competed for by man. Such replacement may include insects that can ultimately serve as a protein source for humans. In this regard Ramos-Elorduy (1984) reported that protein content of short-horned grasshoppers (acridids) varies from 52.1% to 77.1%. Recently, Wang et al. (2007) formulated high protein diets with the Chinese acridid, *Acrida cineria* and proved the insect to be an acceptable feed for broilers without any adverse effect on weight gain, feed intake, or gain:feed ratio. However, there should be a strategy for a continuous and sustainable supply of acridids to the livestock feed developing companies. In this context, Haldar et al. (1999) proposed the idea of acridid farm establishment by their mass rearing with suitable

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food plant at optimum environmental conditions (i.e. temperature and photoperiod). If a huge biomass of acridids could be produced at those farms, more and more protein-rich raw materials could be supplied to the feed producing companies to manufacture low cost feed for the livestock that might ultimately enhance livestock production worldwide.

Despite farming potentiality, information on any effort to use acridids as a food source for a wide variety of livestock animals is scanty. Thus, the aim of the present study was to find out the optimum temperature and photoperiod for mass rearing and high production of a common Indian multivoltine acridid *Oxya hyla hyla* (Serville). In this study, wide ranges of temperature and photoperiodic regimes were selected and some biological traits of the chosen acridid species were observed.

Materials and methods

Collection of *Oxya hyla hyla*

The acridid of interest, i.e. *O. hyla hyla*, was collected from nearby agricultural fields and grasslands of Santiniketan (23°39′N, 87°42′E), India, by the sweeping method, using 30-cm diameter insect net. They were reared in the insectariums of the Entomology Research Unit, Dept. of Zoology, Visva-Bharati University, adopting the strategies proposed by Ewen and Hinks (1886) until they laid egg pods. When nymphs emerged from the egg pods they were used for the following experiment.

Nymphal mortality

For the study of nymphal mortality, a total of 300 newly hatched nymphs were collected and separated into 4 sets with 5 replicates, in transparent plastic jars of 3 L capacity ($10 \times 10 \times 25$ cm^3), containing 15 nymphs per jar. The floor of each jar was covered with fine, sterilized sand of about 3-4 cm thickness and few drops of water were sprinkled regularly to keep it moist. The mouths of the jars were covered with fine nylon net. In order to feed the insects, a sufficient amount of *Brachiaria reptans* (L.) (one of their natural food plants) was offered to the nymphs ad libitum in a 100 mL conical flask. The 1st, 2nd, 3rd, and 4th sets were kept at 25 ± 2 °C, 30 ± 2 °C, 35 ± 2 °C, and 40 ± 2 °C, respectively, in an environmental chamber. For the experiment, relative humidity, light intensity, and photoperiod were selected as 60%-90%, 500 ± 25 lux, and 12:12, respectively. A similar procedure was followed with 375 nymphs for preparing another 5 sets of jars for 5 different L:D photoperiods of 0:24, 6:18, 12:12, 18:6, and 24:0. For this experiment, the temperature was fixed at 35 ± 2 °C as nymphal mortality was moderate at this temperature regime.

Day to day observation was done to observe molting, if any. Since all of the nymphs in a jar were of the same age (0 days old, 1st instar), it was expected that molting would occur on the same day, but in practice it was found that there were 1-3 days of deviation in molting from the 1st instar to the 2nd instar, from the 2nd to the 3rd, and so on, up to the 6th. To avoid errors, the newly emerged 2nd instar nymphs (0 days old, 2nd instar) were carefully collected from all 5 replicates with soft cotton balls and transferred to a separate jar for rearing. The same procedure was followed for the latter instars. In each jar of each set, dead specimens were counted accordingly. The nymphal mortality percentage (NM%) of respective instar stages were calculated.

Growth rate

For the study of growth rate, 4 sets with 4 different prefixed temperature regimes, and 5 sets with 5 different photoperiodic regimes were prepared with 0-day-old and 1st instar nymphs in an environmental chamber following the above mentioned procedure. Nymphs were observed daily to observe molting, if any. Life spans of all of the intermediate instars were recorded. The instar wise growth rate (GR) was calculated by the following formula:

GR = (weight gained by the insect) × [duration of feeding period (days) × (mean weight of insect during feeding period)]$^{-1}$ (Ananthakrishnan, 1986).

Egg pods per female

For the experiment with newly emerged 0-day-old adults (fledgling), 1 pair (male and female) were taken from each of the 20 jars of the 4 different temperature regimes, and 4 new sets each with 5 replications were prepared. The sets were again placed in an environmental chamber with 4 different regimes of temperature. Photoperiod, light intensity, and relative humidity were restricted to 12:12, 500 ± 25 lux, and 65%-75%, respectively. Similarly, 5 sets were prepared for different photoperiodic regimes

with fledglings by following the above mentioned method, where the temperature was restricted to 35 ± 2 °C, as their maximum fecundity was found at this temperature. The sand was examined regularly, every morning (1000 hours) by sieving with a fine mesh to count the egg pods, if any.

Eggs per pod

To count the number of eggs per pod, the egg pods were kept in moist tissue paper on petri dishes for a period of 48 h and were then placed in an environmental chamber at 32 ± 2 °C for 5 days to allow the eggs to swell. Then the frothy material was removed from the egg pods by nylon haired brushes and forceps, and the swollen eggs were separated out easily and their numbers per pod were counted accordingly (Das et al., 2001).

Fecundity and fertility

The egg pods laid per pair were divided into 2 groups with an equal number of individuals. Fecundity was calculated from the 1st group by multiplying the mean number of egg pods laid per female to the mean number of eggs per pod. Fertility was calculated from the 2nd group by multiplying the mean number of egg pods laid per female to the mean number of fertile eggs per pod. To calculate the fertile eggs per pod, the eggs hatched per pod was calculated by the following experiment.

Eggs hatched per pod

Egg pods were placed inside the 1-cm thick sand of a 150 mL beaker and the mouths of the beakers were covered with nylon net. Thus 4 sets were prepared, each with 5 beakers and incubated at the 4 above mentioned temperature regimes. Then 5 other sets were prepared, each with 5 beakers and kept at 5 different photoperiod regimes and other parameters were restricted, as mentioned earlier. On emergence of the hatchlings (0 days old, 1st instar), they were counted. For these hatchlings, continuous day to day observation was done, as all of the viable eggs did not hatch on the same day.

Adult dry weight

To calculate adult dry weight, adult males, virgin females, and mated females were freeze killed and then the specimens were kept in thermoresistant glass vials and placed in a microwave oven at 45 °C for 72 h. The dried specimens were then weighed to calculate the dry weight.

Statistical analysis

Data are presented as means ± SD. For each set, 5 replicates were carried out. For nymphal mortality percentage, the instar developmental period and dry adult body weight at different temperatures and photoperiods were compared via two-way analysis of variance (ANOVA). One-way ANOVA was carried out on growth rate, reproductive ability, and total nymphal developmental period at different temperatures or photoperiodic regimes. For adult life span at different temperatures, two-way ANOVA was done taking temperature and sexes as factors, but the two-way ANOVA did not show a significant difference for the adult life span at different photoperiodic regimes. Hence, one-way ANOVA was carried out for males, virgin females, and mated females individually. One-way ANOVA was carried out to observe if there was any significant effect of temperature and photoperiod on the reproductive ability of *O. hyla hyla*. Duncan's multiple range tests (DMRT) were carried out for each case followed by ANOVA in order to separate the mean values according to significance. For the effect of photoperiod on the total nymphal duration, DMRT did not show any significant difference between the mean values, though values for 24:0 seemed to be higher than the same of 12:12 and 6:18. Hence, a t-test was carried out to ensure the significance of these variations. All of the analyses were carried out using Microsoft Excel 2000 Software.

Results

When the jars were kept at different temperature regimes, the results showed that the nymphal mortality gradually increased with the rise of temperature from 25 ± 2 °C to 40 ± 2 °C (Table 1). Although for the 1st instar this increase was significant ($df = 3, 15$; $F = 24.63$; $P < 0.001$) for all of the temperature regimes, for the 2nd instar the results were insignificant between 30 ± 2 °C and 35 ± 2 °C (DMRT), whereas for the 3rd instar onwards, the nymphal mortality did not show any significant variation between the regimes of 25 ± 2 °C, 30 ± 2 °C, and 35 ± 2 °C. On the other hand, when the mortality percentages were

Table 1. Nymphal mortality percentage of *O. hyla hyla* at different temperature regimes. Different letters within a row indicate significant differences between mean values (two-way ANOVA, DMRT, $P < 0.001$).

Instar	Temperature			
	25 ± 2 °C	30 ± 2 °C	35 ± 2 °C	40 ± 2 °C
1	21.8 ± 1.3a	24.4 ± 0.55b	26.8 ± 0.84c	57.8 ± 1.48d
2	19.4 ± 0.55a	21.2 ± 0.84b	22 ± 1b	51.2 ± 0.84c
3	17.6 ± 0.55a	18.2 ± 1.1a	18.6 ± 0.55a	47.6 ± 0.55b
4	15.2 ± 1.64a	16.6 ± 1.95a	17.6 ± 1.52a	40.4 ± 1.14b
5	13.4 ± 0.89a	14.2 ± 1.3a	14.6 ± 1.14a	35.6 ± 0.55b
6	10 ± 1.58a	10.4 ± 1.52a	10.6 ± 1.34a	32.4 ± 1.14b

observed at different L:D photoperiodic regimes, it was recorded that the results gradually decreased up to 12:12 and then again gradually increased (Table 2). Maximum mortality ($df = 4, 20$; $F = 21.76$; $P < 0.001$) was noted in the sets kept in total darkness, followed by the sets kept in total light. For the 1st instar, less nymphal mortality was observed at 12:12 and 6:18; however, from the 2nd instar onwards, less mortality was at 18:6, 12:12, and 6:18.

When growth was compared within instars between the different temperature regimes, it was observed that all of the instars, except the 2nd one, had a similar trend, i.e. growth rate increased with the rise of temperature from 25 ± 2 °C to 35 ± 2 °C (Figure 1). In contrast, in the case of the 2nd instar, the growth rate increased from 25 ± 2 °C to 30 ± 2 °C and then gradually decreased at 35 ± 2 °C and 40 ± 2 °C. Another notable observation was that at 40 ± 2 °C, the growth rate was significantly lower ($df = 15, 119$; $F = 200.42$; $P < 0.001$) for all of the instars. At different photoperiodic regimes, each instar, except for the 3rd, showed a similar trend; growth rate increased as the duration of light increased from 0 h to 12 h (i.e. 24:0 to 12:12), followed by a gradual decrease of growth for further increase of light duration (Figure 2). In the case of the 3rd instar, the growth rate initially increased from 24:0 to 18:6 and then a gradual decrease was observed.

Table 2. Nymphal mortality percentage of *O. hyla hyla* at different L:D photoperiodic regimes. Different letters within a row indicate significant differences between mean values (two-way ANOVA, DMRT, $P < 0.001$).

Instar	Photoperiod				
	24:0	18:6	12:12	6:18	0:24
1	60 ± 1.58d	29.4 ± 1.14b	24.6 ± 1.14a	26.6 ± 0.89a	52.6 ± 0.55c
2	55 ± 0.71c	26 ± 0.71a	22 ± 1.41a	23.2 ± 1.48a	46 ± 0.71b
3	50.2 ± 0.84c	22.2 ± 0.84a	19.4 ± 0.89a	20 ± 1.58a	38.6 ± 1.95b
4	44.2 ± 0.84c	19.4 ± 1.14a	17.4 ± 1.14a	17.6 ± 1.34a	35.6 ± 1.52b
5	38.4 ± 0.89c	17.4 ± 1.34a	15.4 ± 0.89a	15.6 ± 1.14a	32 ± 1b
6	34 ± 1c	11.4 ± 1.14a	10.6 ± 1.14a	11 ± 0.71a	29 ± 1b

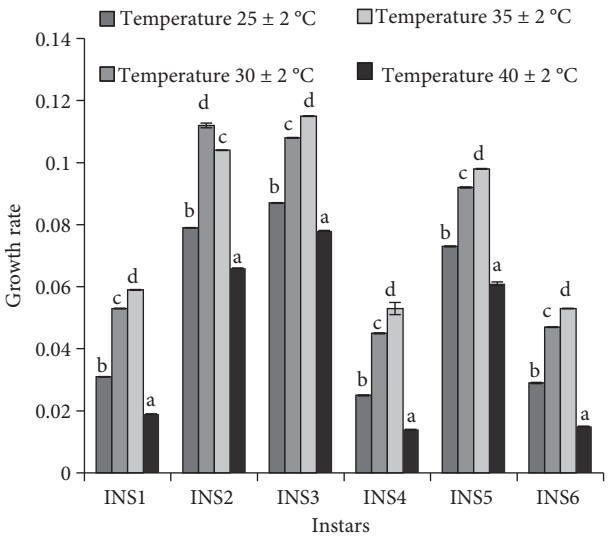

Figure 1. Growth rate of *O. hyla hyla* at different temperature regimes. Bars containing different letters varied significantly (one-way ANOVA, DMRT, $P < 0.001$).

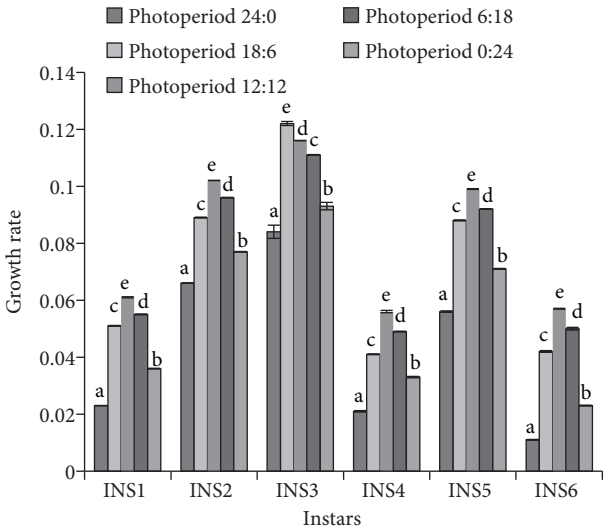

Figure 2. Growth rate of *O. hyla hyla* at different L:D photoperiodic regimes. Bars containing different letters varied significantly (one-way ANOVA, DMRT, $P < 0.001$).

For egg pods laid per female, fecundity and fertility, the temperature regime 30 ± 2 °C to 35 ± 2 °C was recorded as the optimum range because the regimes below and above this range gave significantly lower values (df = 19, F = 11.96, $P < 0.001$; df = 17.8, F = 75, $P < 0.05$; df = 19, F = 233.37, $P < 0.001$, respectively) (Table 3). Number of eggs per pod (df = 19, F = 14.15, $P < 0.001$) and fertile eggs per pod (df = 19, F = 233.37, $P < 0.001$) were significantly lower at the highest temperature regime, i.e. 40 ± 2 °C. No significant variation was observed among the rest of the regimes. Egg incubation period decreased with increased temperature (df = 3, 16; F = 50.02; $P < 0.001$). However, no significant variation was observed between the 2 higher (i.e. 35 ± 2 °C and 40 ± 2 °C) and the 2 lower (i.e. 25 ± 2 °C and 30 ± 2 °C) temperatures. All of the chosen reproductive parameters, except for egg incubation period, arbitrarily gave better results along with increased light duration up to 12:12 and a further increase of light duration gave lower values (Table 4). However, egg pods per female showed no significant variation among the 5 different photoperiodic regimes. The other 4 parameters showed significantly higher values at 12:12 (eggs per pod: df = 4, 20; F = 61.4; $P < 0.001$, fertile eggs per pod: df = 4, 20; F = 6.43; $P < 0.01$, fecundity: df = 4, 20; F = 292.08; $P < 0.001$, and fertility: df = 4, 20; F = 433.55; $P < 0.001$). The mean values of egg incubation period gradually decreased with the increased light duration, though the results of 12:12, 6:18, and 0:24 showed insignificant variation.

Table 3. Reproductive ability of *O. hyla hyla* at different temperature regimes. Different letters within a column indicate significant differences between mean values (one-way ANOVA, DMRT, $P < 0.001$).

Temperature	Egg pods laid per female	Eggs per pod	Fertile eggs per pod	Fecundity	Fertility	Eggs incubation period
25 ± 2 °C	8.6 ± 0.89a	13 ± 1b	11.8 ± 0.84b	111.2 ± 5.93b	101 ± 6b	32.6 ± 1.14b
30 ± 2 °C	11.4 ± 1.14b	12.4 ± 1.52b	11.4 ± 1.34b	140 ± 5.79c	128.8 ± 5.02c	32.2 ± 1.3b
35 ± 2 °C	11.4 ± 1.14b	13.2 ± 0.84b	12.2 ± 0 .84b	149.8 ± 7.7c	138.4 ± 6.8c	27.2 ± 0.84a
40 ± 2 °C	8.4 ± 1.14a	7.6 ± 10.14a	6.6 ± 0.89a	62.8 ± 1.64a	54.8 ± 4.38a	25.2 ± 1.3a

Table 4. Reproductive ability of *O. hyla hyla* at different L:D photoperiodic regimes. Different letters within a column indicate significant differences between mean values (one-way ANOVA, DMRT, P < 0.001).

Photoperiod	Egg pods laid per female	Eggs per pod	Fertile eggs per pod	Fecundity	Fertility	Eggs incubation period
24:0	8.4 ± 1.14a	7.4 ± 0.89a	5.6 ± 0.55a	61.4 ± 3.44a	46.6 ± 3.13a	35.2 ± 0.45c
18:6	9.4 ± 0.55a	11.4 ± 0.55b	10 ± 1b	107 ± 4.58c	93.6 ± 4.93c	32.4 ± 0.89b
12:12	10.6 ± 0.55a	13.4 ± 0.55c	12.4 ± 0.55c	141.8 ± 1.64d	131.2 ± 1.1d	27.2 ± 0.84a
6:18	9.6 ± 0.55a	10.6 ± 0.55b	9.6 ± 0.55b	101.6 ± 4.72c	92 ± 4.47c	26.6 ± 1.67a
0:24	8.6 ± 0.89a	9.6 ± 0.55b	8.4 ± 0.89b	82.2 ± 0.89b	71.6 ± 0.89b	26.4 ± 1.14a

When the instar-wise variations of nymphal developmental period at different temperature regimes (Table 5) were considered, it was observed that the mean values of nymphal developmental periods were always high at 25 ± 2 °C. However, from the 3rd instar onwards, no significant variation was observed within the instars when the results were statistically compared between different temperature regimes, whereas the shortest (df = 4, 20; F = 3.88; P < 0.001) duration for the 2nd instar was observed at 40 ± 2 °C. Total nymphal duration was also calculated; however, no significant variation was observed (P > 0.05) between the selected temperature regimes (Figure 3). When the effect of different photoperiodic regimes on the instar wise nymphal developmental periods (Table 5) was observed, higher durations were recorded at the 2 extreme photoperiodic regimes (i.e. 24:0 and 0:24). Lower mean values were observed at the 18:6 to 6:18 photoperiodic range. When the data were statistically compared between 18:6, 12:12, and 6:18, significantly lower values (df = 4, 20, F = 6.43, P < 0.05) were observed at 12:12 for 5th instar only. Here also, the effect of photoperiod on the total nymphal duration was calculated (Figure 4). Though DMRT result did not show any significant variation between the results of different photoperiodic regimes, the t-test revealed that 24:0 has higher value than 12:12 and 6:18 (P < 0.05, t-test).

Table 5. Instar-wise nymphal developmental period (days) of *O. hyla hyla* at different temperature and L:D photoperiodic regimes. Different letters within a column indicate significant differences between mean values (two-way ANOVA, DMRT, P < 0.05 for temperature; two-way ANOVA, DMRT, t-test P < 0.05 for photoperiod).

Temperature	1	2	3	4	5	6
25 ± 2 °C	10 ± 1.41b	12.8 ± 1.3b	6 ± 0.71a	9 ± 1a	7 ± 0.71a	10 ± 1.58a
30 ± 2 °C	7.4 ± 5.5a	12.4 ± 0.89b	5.4 ± 0.55a	8 ± 1.22a	6.6 ± 1.52a	8.6 ± 1.34a
35 ± 2 °C	7 ± 0.71a	12 ± 1.58b	5 ± 1a	8 ± 1.22a	6.6 ± 1.52a	8 ± 1a
40 ± 2 °C	7 ± 0.71a	9 ± 1.22a	5 ± 1a	7.6 ± 1.34a	6 ± 1.22a	8 ± 1a
Photoperiod						
24:0	10.6 ± 0.89b	13.6 ± 0.55a	7 ± 1a	9.6 ± 0.55a	7.6 ± 0.55b	10 ± 1a
18:6	8 ± 1a	12.6 ± 0.55a	6 ± 0.71a	9 ± 1a	6.6 ± 0.55b	9 ± 0.71a
12:12	7 ± 0.71a	12 ± 1.58a	5 ± 1a	8 ± 1.71a	5.6 ± 0.55a	8 ± 1a
6:18	7 ± 0.71a	12.4 ± 0.55a	5.4 ± 5.5a	8.4 ± 0.89a	6.6 ± 0.55b	8.4 ± 0.55a
0:24	10 ± 1.41b	13 ± 1a	6.6 ± 1.34a	9 ± 1a	7 ± 0.71b	9.6 ± 0.55a

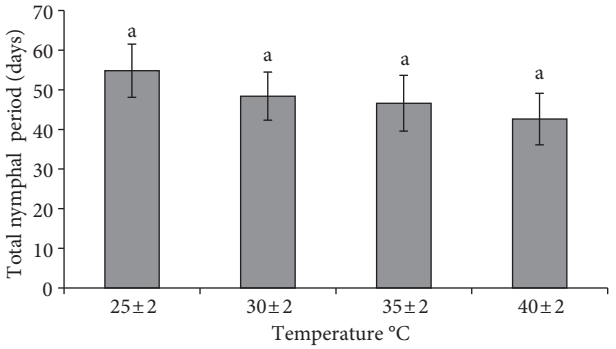

Figure 3. Total nymphal developmental period (days) of *O. hyla hyla* at different temperature regimes. Bars containing same letters are not significant (one-way ANOVA, DMRT, P > 0.05).

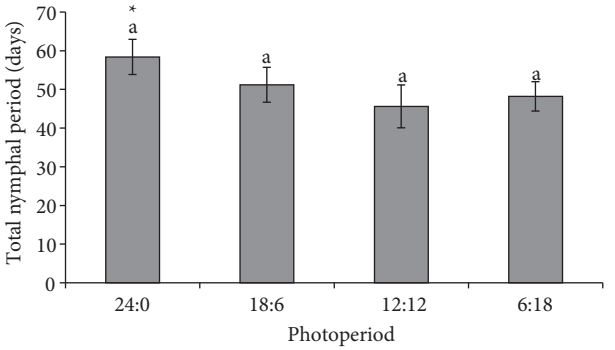

Figure 4. Total nymphal developmental period (days) of *O. hyla hyla* at different L:D photoperiodic regimes. Bars containing same letters are not significant (one-way ANOVA, DMRT, P > 0.05). * denotes the value is significantly higher than the sets kept under the 18:6 and 12:12 photoperiodic regimes (t-test, P < 0.05).

Among the 4 different temperature regimes, adult life span of males, virgin females, and mated females gradually increased with increased temperature from 25 ± 2 °C to 35 ± 2 °C, whereas the 40 ± 2 °C temperature regime showed the lowest values for all (df = 2, 6; F = 3.08; P < 0.05) (Table 6). Adult life span was longest (df = 6, 59; F = 3.08; P < 0.05) in virgin females and shortest in males at the first 3 temperature regimes. Variations in photoperiodic regimes in the adult life span (Table 6) showed that mean values increased up to 12:12, and then shortened gradually. It varied between 7 to 12 weeks; however, they did

Table 6. Adult lifespan (week) of *O. hyla hyla* at different temperature and L:D photoperiodic regimes. Different letters within a column indicate significant differences between mean values (two-way ANOVA, DMRT, for temperature P < 0.05, for photoperiod P = 0.799).

Temperature	Adult (week)		
	Male	Virgin female	Mated female
25 ± 2 °C	10.2 ± 0.45a	11.4 ± 0.55b	9.2 ± 0.84b
30 ± 2 °C	10.6 ± 0.55a	11.6 ± 0.55b	9.2 ± 0.84b
35 ± 2 °C	11.4 ± 0.89a	12.4 ± 0.55b	9.6 ± 0.89b
40 ± 2 °C	9.2 ± 0.84a	8.4 ± 0.55a	7.6 ± 0.55a
Photoperiod			
24:0	9.2 ± 0.84a	9.6 ± 0.89a	7.4 ± 0.55a
18:6	10.2 ± 0.45a	10.6 ± 0.55a	8.6 ± 0.55a
12:12	11.4 ± 0.89a	12.4 ± 0.55a	9.6 ± 0.89a
6:18	10.6 ± 0.55a	11.4 ± 0.89a	9.2 ± 0.84a
0:24	9.6 ± 0.89a	10 ± 0.45a	7.6 ± 0.55a

not show any significant variation. Adult life span also did not vary significantly within photoperiods between males, virgin females, and mated females (P = 0.799, two-way ANOVA).

Effect of temperature on adult dry body weight (Table 7) showed that both males and virgin females attained maximum weight at 30 ± 2 °C and 35 ± 2 °C, whereas the same of mated female was maximum at the first 3 temperature regimes, but the data did not show any significant variation. Adult male and virgin female dry body weights were minimum at the 2 extreme temperature regimes, but the dry weight of mated female was significantly lower at 40 ± 2 °C (df = 3, 6; F = 3.81; P < 0.01). Mated female dry weight was significantly higher than virgin female and male dry weight at each temperature (df = 6, 59; F = 3.81; P < 0.01) and photoperiodic regime (df = 8, 74; F = 2.76; P < 0.05). Between different photoperiodic regimes, virgin females and mated females attained maximum (df = 4, 8; F = 2.76; P < 0.05) weight (Table 7) at 12:12, and adult male dry weight was maximum at 18:6, 12:12, and 6:18, where data did not show any significant variation. Lower dry body weight was observed at the 24:0 and 0:24 photoperiodic regimes for males, virgin females, and mated females.

Discussion

Temperature and photoperiod affect different biological traits of insects (Piesik, 2006; Nabeta et al., 2005; Malina and Praslicka, 2008). Hinks and Erlandson (1994) opined that temperature is a very important and determining factor for grasshopper rearing. Moreover, it has also been reported that reproductive potential in grasshoppers is highly temperature dependent (Whitman, 1998; Blanford and Thomas, 2000). Post-embryonic development of grasshoppers and locusts is also dependent on temperature (Ali, 1982). In the present study, during the development from 1st instar to 6th instar, it was observed that the highest nymphal growth rate was at the 3rd instar stage, in the case of both the temperature and photoperiodic regimes. Kohler et al. (1987) observed the highest growth rate at the 3rd instar stage of 3 European acridids, namely *Chorthippus parallelus*, *C. biguttulus*, and *Gomphocerus* sp. According to Nath and Haldar (1992a), *Acrida exaltata* also showed a higher growth rate at the 3rd instar stage. Nymphal mortality has a great role on population size, structure, and dynamics. The lower the nymphal mortality is, the higher the population output is and the higher biomass production is.

Table 7. Dry adult body weight (g) of *O. hyla hyla* of acridid at different temperature and L:D photoperiodic regimes. Different letters within a column indicate significant differences between mean values (two-way ANOVA, DMRT, P < 0.01 for temperature; two-way ANOVA, DMRT, P < 0.05 for photoperiod).

Temperature	Male	Virgin female	Mated female
25 ± 2 °C	0.065 ± 0.0025a	0.154 ± 0.0032a	0.177 ± 0.0017b
30 ± 2 °C	0.076 ± 0.0026b	0.166 ± 0.0027b	0.179 ± 0.0022b
35 ± 2 °C	0.079 ± 0.0042b	0.171 ± 0.0054b	0.184 ± 0.0013b
40 ± 2 °C	0.063 ± 0.0028a	0.152 ± 0.0019a	0.17 ± 0.0032a
Photoperiod			
24:0	0.059 ± 0.0021a	0.148 ± 0.0019a	0.166 ± 0.0024a
18:6	0.071 ± 0.0017b	0.161 ± 0.0025b	0.173 ± 0.0025b
12:12	0.078 ± 0.0019b	0.17 ± 0.0023c	0.182 ± 0.0026c
6:18	0.075 ± 0.0033b	0.163 ± 0.0021b	0.176 ± 0.0024b
0:24	0.061 ± 0.0027a	0.15 ± 0.0029a	0.168 ± 0.0031a

Nymphal mortality was maximum at the extreme temperatures of 4 different selected temperature regimes, possibly because *O. hyla hyla* could not tolerate the high temperature (i.e. 40 ± 2 °C). Therefore, it could be assumed that extreme high, and maybe the extreme low temperatures were not favorable for the survival of the selected species. The temperature regime from 25 ± 2 °C to 35 ± 2 °C has been found to be the optimum zone for the survival of *O. hyla hyla*. According to Ward and Stanford (1982) and Robinson et al. (1983), metabolic rate was directly influenced by temperature. An increase of temperature up to 35 ± 2 °C favored the growth rate, which might be due to enhanced metabolic activity with increased temperature. Giberson and Rosenberg (1992) found a similar trend of results for ephemeropteran insects. At the extreme high temperature (i.e. 40 ± 2 °C), decreased growth rate might have been observed due to normal metabolic activity hampered in *O. hyla hyla*. For the 1st and 2nd instars, though nymphal mortality was moderate at 35 ± 2 °C, at this regime, it was low for other instars, whereas the growth rate was high for all of the instars. Thus, it could be stated safely that the 35 ± 2 °C temperature regime was ideal for growth and survival. On the studies of *Locusta* sp. and *Schistocerca gregaria*, Uvarov (1966) reported similar observations. Information about influence of photoperiod on the biological traits of *O. hyla hyla* is very scarce. Improved knowledge of the relationship between photoperiod and selected biological parameters of *O. hyla hyla* might be useful in developing acridid farms and determining the best time of year for *O. hyla hyla* rearing. Musolin and Saulich (1997) reported that the nymphal growth rate of Orthoptera was accelerated by long days and retarded by short days. The present study also revealed a similar trend of observations, where the growth rate increased up to 12:12. From studies on *Locusta* sp. and *Schistocerca gregaria*, Hamilton (1950) found that the fastest development and highest survival was at the photoperiod of equal span of day and night. In the case of nymphal survival, the present study supports this observation. Therefore, the 12:12 photoperiodic regime was identified as optimum for the maximal growth and survival of *Oxya hyla hyla*.

Various reproductive traits gave the most favorable results at the 2 moderate temperatures of the 4 different temperature regimes. On the other hand, egg pod laying frequency increased with the increased temperature. Ali (1982) also reported that with the increase in temperature from 20 °C to 37 °C the egg pod laying capability increased in *Acrida exaltata*. Egg mortality increases with the increased temperature from 35 ± 2 °C onwards. Giberson and Rosenberg (1992) observed that the fecundity of mayfly was increased with the rise in temperature. In the present study, similar results for fecundity and fertility were found when the temperature was increased up to a certain level and then declined when the optimum range was exceeded. The egg incubation period varied from 25 to 33 days at different temperatures, which was shortest at 35 ± 2 °C. A short egg incubation period might lead to early adult stage. Therefore, in a year, more life cycles might be completed and more acridid biomass could be obtained. Thus, it could be stated that the 35 ± 2 °C temperature regime is ideal for reproduction. "Photoperiod is an ecological factor that controls oogenesis through the neuroendocrine system" (Malaquias et al., 2009). According to Tauber et al. (1986) and Lenteren (1999), "when exposed to long photoperiod of light, the physiology of many adult insect species is directed to reproduction, whereas short-day conditions can deactivate the corpora allata, thereby reducing juvenile hormone secretion and consequently induce diapause". This explains the fact of increased number of eggs per pod, fertile eggs per pod, and fecundity and fertility with increased photoperiod from 24:0 to 12:12. However, the egg incubation period gave reverse results and was lowest at 12:12, which favors acridid rearing.

For nymphal duration, 35 ± 2 °C and 12:12 was considered optimum, as short duration is very important for the fitness and survival of the insects (Price et al., 1980). Adult life span was shortest at the highest temperature regime for males, virgin females, and mated females, which might be due to metabolic hazards. Different photoperiodic regimes did not show any variation in the adult life span for males, virgin females, and mated females. Adult males and mated females, which lived longer, had a better chance to copulate and laid a higher number of eggs. A shorter nymphal duration and a longer adult life span will produce a high amount of biomass (Ganguly et al., 2010). Adult dry body weight was higher at the 2 moderate temperatures and at equal span of light

and dark. Mated female individuals attained higher dry weight than virgin females, possibly due to high consumption (Abdel-Rahman, 2001) and hormonal effect in mated acridid females. The dry weights of virgin females were greater than those of the males. Nath and Haldar (1992b) observed similar results for *Acrida exaltata*. Nymphal duration was shortest and adult life duration was longest at 35 ± 2 °C and 12:12, and other biological traits, i.e. growth rate, fecundity, fertility, and adult dry body weight, showed the most preferable results for *O. hyla hyla* rearing.

O. hyla hyla is a multivoltine acridid. Anand et al. (2008a) reported that a huge biomass of acridids could be obtained within 1 year from only a single pair taken as a starting point. Hence, by using sufficient amounts of its most suitable food plant and maintaining optimum temperature and photoperiod, an exponential biomass of *O. hyla hyla* might be obtained, which may provide a low cost protein supplement for formulating high protein feed for livestock.

Acridids are superior to the conventional protein supplements such as soybean meal (48% crude protein) and fish meal (50%-55% crude protein) because they have a higher protein content (60%-66%) and a good amount of calories (4.5 to 7 kcal g^{-1}); total fat (approximately 6%-7.5%), total carbohydrate (approximately 3.6%-7.5%), and mineral contents (Anand et al., 2008b). The temperature regime 35 ± 2 °C gave maximum values for growth rate, egg pod laying frequency, fecundity, fertility, adult dry body weight, and adult male life span, whereas the same regime gave minimum results for egg incubation period, nymphal mortality from the 3rd instar onwards and nymphal duration for the 2nd instar onwards. Nymphal mortality of the 1st and 2nd instars were minimum at 25 ± 2 °C and nymphal duration of the 2nd instar was minimum at 40 ± 2 °C, but most other parameters showed favorable results at 35 ± 2 °C, and remarkably worse at either 25 ± 2 °C or 40 ± 2 °C. All of the chosen biological traits showed better results at 12:12 except the growth rate of the 3rd instar. Therefore, when all of the parameters were taken under consideration, 35 ± 2 °C and a photoperiod of 12:12 were identified as the optimum regimes for mass culture of *O. hyla hyla* under laboratory conditions. These results strongly support the idea of establishment of acridid farms where huge biomass of this acridid species could be yielded to provide raw materials for the poultry, fish, and shrimp industry, and make them viable and attractive. Ultimately, sufficient animal protein will be easily available to the populace.

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