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Temperature and Relative Humidity-Mediated Immature Development and Adult Emergence in the Mulberry Silkworm *Bombyx mori* L.

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ABSTRACT

Ranges of two vital environmental factors like temperature and relative humidity (RH) were utilized to assess their impacts on the life-history parameters such as immature development (hrs), adult emergence (%) and sex-ratio (male: female) in the mulberry silkworm *Bombyx mori* L. under laboratory conditions. Disease-free layings (DFLs) from M_2P_2 variety of the silkworm were reared in a digitalized environmental growth chamber (EGC) at 25°-38° C and corresponding 60-95% RH to record the incubation, larval and pupal developmental periods (in hrs), and subsequent adult emergence and sex-ratio. Results revealed that the rise in temperature and RH significantly ($P<0.001$) shortened the immature developmental periods in the experimental insects, but the adult emergence and sex-ratio were not affected ($P>0.05$), even though the number of males outnumbered the females. Negative coefficient of correlation (r) values existed between the environmental factors and all the life-history parameters of *B. mori*, suggesting further that elevated temperature and RH had adverse impacts on the reproductive biology of this commercially important species. Relevance of the present findings to the climate change issues on the silkworm productivity has been discussed.

Introduction

The changing global climate seems to have profound effect on lepidopteran insects including moths and butterflies and other insects which have started to receive concern in recent years[1-5]. Global climate is changing with the evidence that increase in global mean surface air temperature over the last century range between 0.3ºC-0.6ºC, the recent years being particularly warm[3]. Current scenario of changing global climate appears to be one of the major hindrances in the growth and development of silkworm *Bombyx mori*. Various climatic factors such as temperature, humidity, precipitation, light and air bears influence on the developmental process in this insect, and any change in the ratio of these climatic factors may lead to pessimistic result in terms of commercial productivity[5-6].

Being poikilothermic organisms, silkworms are subjected to temperature- and humidity-mediated embryonic and postembryonic development. This is because these environmental factors influence the protein and carbohydrate profile of the insects, in which fluctuations in temperature with low humidity cause delay and decrease in egg hatching whereas higher temperature with low humidity result in death of embryos during early stage[7]. Several previous reports revealed that increased humidity was conductive to the larval growth and better silk yield[8], spinning rate and quality of cocoons were determined by temperature and humidity[9], and these two environmental factors had profound impacts on egg hatchability, larval mortality, pupation and performance of the silkworm lines[10-11]. Moreover, such reproductive potentials as the number of eggs/g and percentages of laying recovery, unfertilized eggs and adult emergence[12], cocoon weight, shell weight and cocoon-shell ratio[13] and the growth, development, productivity and quality of silk in *B. mori*[4] were affected by temperature and humidity. Apart from the mulberry silkworms, however, effects of these two environmental factors have also been assessed on the Muga silkworm *Antheraea assamensis* Helfer[3,14] and the Eri silkworm *Philosamia ricini* L.[15].

The effects of different temperature and humidity ranges on larval mortality, growth and weights of cocoons and pupae[6], growth and development of the 5th instar larvae and their silk glands[16], cocoon quality[17] and commercial cocoon production[18-19] have recently been evaluated. Taking the aforesaid review of literature in consideration, the present work was designed at analyzing the effects of temperature and relative humidity fluctuations on the immature development of the mulberry silkworms, coupled with the impacts of the two vital environmental factors on the adult emergence and sex-ratio in the experimental *B. mori*. The present findings may help understand the effects of climate change on the commercial sericulture productivity in the tropical countries like Bangladesh and India.

Materials and Methods

The experiments were carried out during July 2016 and June 2017 mostly in the Ecology, Biodiversity & Conservation Laboratory and partly in the Genetics & Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh. Brief protocol and the experimental design are described in the following paragraphs.

Experimental insects

Healthy eggs of M_2P_2 variety of the mulberry silkworm *B. mori* collected from the Germplasm Bank, Bangladesh Sericulture Research and Training Institute (BSRTI),

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Rajshahi, were used in the present investigation. Subsequently, adults, their eggs and hatched out larvae from the disease-free layings (DFLs) from the stock culture were used throughout the experiments.

Rearing techniques

The silkworm rearing techniques followed in the present investigation were those described previously[17,20] with slight modifications. The experimental insects were reared in both circular (140cm diameter × 7.5cm deep) and rectangular (120cm × 180cm) flat bamboo trays. In addition, wooden rearing trays with hardboard bottoms (30cm × 45cm) were used for feeding purposes of the silkworm larvae. The experimental eggs were placed in the wooden trays in the environmental growth chamber (EGC, see below) and were examined twice a day, once in the morning at 08:30 hrs and then in the afternoon at 16:30 hrs to record the hatching time. The larvae were fed four times daily at 04:00, 10:00, 16:00, and 22:00 hrs on fresh and tender mulberry leaves (*Morus* sp.) except during moulting. The unconsumed leaves were cleaned two times daily during the 1st and 2nd instars and once every day after the 2nd moult, and the larvae were transferred into separate pre-disinfected rearing trays. The larvae under moult, however, were not disturbed. To prevent diseases and to maintain good sanitation, the rearing room and other rearing appliances were disinfected with a 2% formaldehyde solution and periodically with bleaching powder (1% chlorine) solution following the procedure suggested earlier[21]. At the end of the 5th instar, larvae showing the symptoms of spinning were collected from the rearing bed and were placed on the plastic collapsible mountages (called *Chandraki* in Bangla). Cocoons were then harvested after 4-5 days of mounting**.**

Environmental growth chamber (EGC)

For estimating the speed and fate of development, silkworms were reared under adjustable temperature (°C) and relative humidity (RH %) in an environmental growth chamber EGC (Gallenkamp, UK). The EGC was an incubator-like culture medium where temperature could be maintained from 4° to 50° C and the RH and light: dark photo regime could be adjusted. The controller of the EGC adopts the up-to-date digital circuit technology, and controls such environmental parameters as temperature, RH, light intensity, and their durations by means of a programmable central processing unit (CPU).

Temperature and RH conditions

Six temperature regimes, *viz*. 25°, 28°, 30°, 32°, 35° and 38^{o} C and a corresponding range of RH from 60% to 95% were used in the EGC to estimate the immature development of *B. mori* under study The L (light): D (dark) photo regime maintained during the experiments was 12 hrs: 12 hrs.

Parameters studies

The durations of the incubation, larval, pupal, and total egg-to-adult development of the experimental insects were recorded in hrs. In addition, environmental treatment effects on adult emergence (%) and male-female sex-ratio in *B. mori*, as well as the regression lines and bivariate correlations between the environmental factors and developmental periods were also estimated. Each experiment was replicated 10 times.

Statistical procedures

Statistical analyses were performed using a statistical package SPSS version 16.0 for Windows. The effects of temperature and RH on the incubation, larval, pupal and egg-to-adult total developmental periods of the insects were analyzed with one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) tests, where P-values of ≤0.05 were regarded as statistically significant[22]. The influence of abiotic factors on these mean life-history traits were estimated using the Karl Pearson's two-tailed bivariate correlation as coefficient of correlation (*r*) values and regression lines between temperature and RH ranges and the developmental periods were estimated[23].

Results

Effects of temperature on immature development

Silkworms completed their life-cycles between 25°-32° C. At 35° C, however, development could proceed only up to the 2nd instar larvae; whereas between 35°-38° C, embryogenesis halted and the eggs did not hatch (data not shown). Fig. 1 shows the effects of temperature range of 25°-32° C on the developmental periods in *B. mori*, where the incubation period decreased from 199.0±4.06 to 146.0±3.97 hrs, the larval period reduced from 698.0±9.96 to 392.2±8.80 hrs, and the pupal period shortened from 264.5±9.09 to 208.5±5.32 hrs, respectively. So, the total developmental period of the silkworm declined from 1161.5±10.82 at 25° C to 746.7±13.47 hrs at 32° C. These results indicated that the rearing temperature had highly significant inverse effects (all F-values at 3, 39 df) on all the immature developmental periods in the experimental insects.

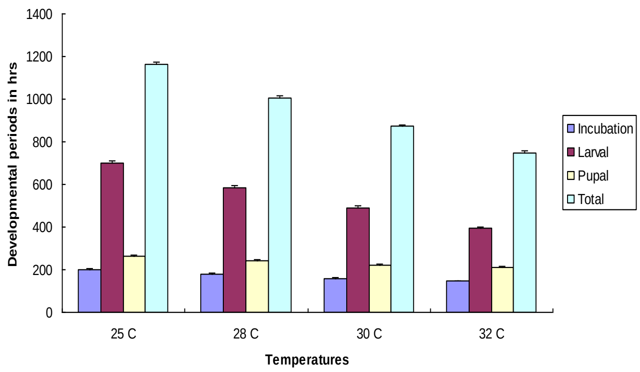


Fig 1. Effects of rearing temperature on developmental periods in the silkworm *B. mori.*

Effects of relative humidity on immature development

Effects of RH on immature development in *B. mori* have been presented in Table 1. Data demonstrate that a range from 66.0±3.16% to 89.7±4.35% RH decreased significantly the incubation((F=306.63;P<0.001)), larval((F= 2021.33; P<0.001)), pupal ((F= 292.84; P<0.001)) and the total ((F= 2383.90; P<0.001)) developmental periods in *B. mori*.

Table 1. Developmental periods influenced by the changes in relative humidity (RH %) in *B. mori* reared in environmental chambers.

RH (%)	Incubation period (hrs)	Larval period (hrs)	Pupal Period (hrs)	Total period (hrs)
66.00±3.16	199.0±4.06a	698.0±9.96a	264.5±2.59a	1161.5±10.82a
64.60±3.20	177.7±4.88b	586.4±9.08b	241.48±4.30b	1005.5±12.60b
84.50±3.03	159.9±3.76c	488.4±9.02c	223.4±5.10c	871.7±8.68c
89.70±4.35	146.0±3.77d	392.2±8.80d	208.5±5.32d	746.7±13.47d
F-values	306.63***	2021.33***	292.84***	2383.90***

All values are mean ±SD; dissimilar letters in each column differ significantly by LSD at P<0.05; F-values are at 3, 39 df; ***= P<0.001; ns= not significant.

The above results suggest that RH had significantly negative impacts on the immature development in the experimental insects under study.

Effects of temperature and RH on adult emergence and sex-ratio

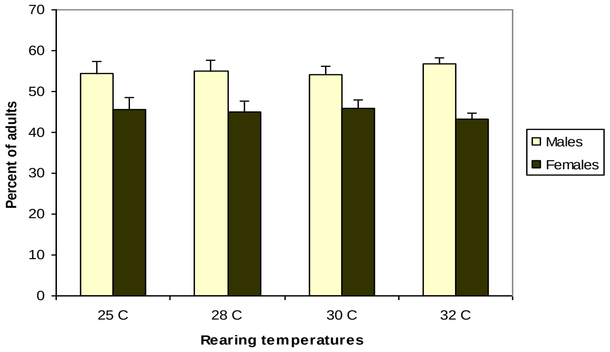


Fig 2. Percentages of *B. mori* adults emerged from broods reared at 25°-32 °C and 60-95% RH in environmental chambers.

Unlike immature developmental periods, neither adult emergence nor sex-ratio was influenced by the rearing temperature and RH in *B. mori* (Fig. 2 and Table 2). A slight increase in the number of males was noticed though, but differences in the sex-ratios did not reach statistical significance level in either case (F= 2.63; P=0.065).

Table 2. Adult emergence and sex-ratio influenced by the changes in temperature and RH in *B. mori* reared in environmental chambers.

Temperature (°C)	RH (%)	Males (%)	Females (%)
25.0±0.12	66.0±3.16	54.3±2.95a	45.7±2.95a
28.0±0.11	64.6±3.20	55.0±2.79a	45.0±2.79a
30.0±0.15	84.5±3.03	54.1±2.13a	45.9±2.13a
32.0±0.16	89.7±4.35	56.8±1.40a	43.2±1.40a
F-values		2.63ns	2.63ns

All values are mean ±SD; dissimilar letters in each column differ significantly by LSD at P<0.05; F-values are at 3, 39 df; ns= not significant.

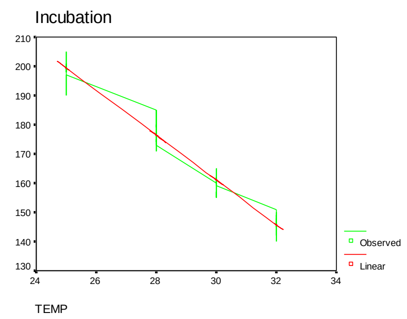
Table 3. Bivariate correlations between the environmental factors and the developmental periods in *B. mori*.

Bivariate correlations between	Karl Pearson's correlation coefficient (*r*)*	Degrees of freedom (df)**	Probabilities
Temperature-Incubation period	-0.980	18	<0.001
Temperature-Larval period	-0.995	18	<0.001
Temperature-Pupal period	-0.980	18	<0.001
Temperature-Total dev. period	-0.996	18	<0.001
RH-Incubation period	-0.864	18	<0.001
RH-Larval period	-0.860	18	<0.001
RH-Pupal period	-0.820	18	<0.001
RH-Total dev. period	-0.860	18	<0.001

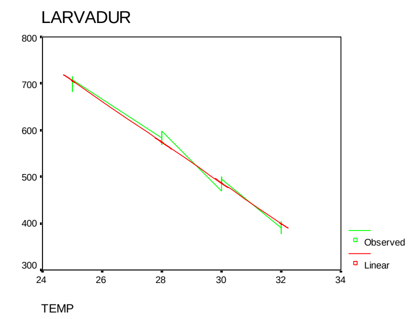
*Two-tailed estimates; **df= n_1+n_2-2

Regression lines and bivariate correlations between environmental factors and developmental periods

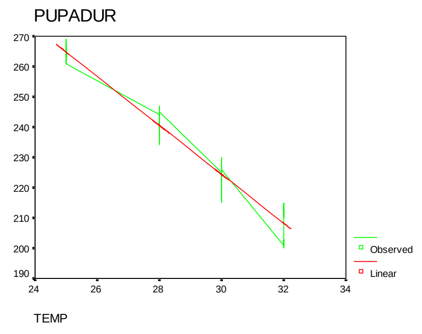
Further analyses of the experimental data confirmed the negative impacts of rising temperature (Fig. 3) and increasing RH % (Fig. 4) on the developmental periods in *B. mori*. Moreover, bivariate correlations between the environmental factors and the egg-to-adult developmental periods also indicated by highly significant negative coefficient of correlation (*r*) values (Table 3).



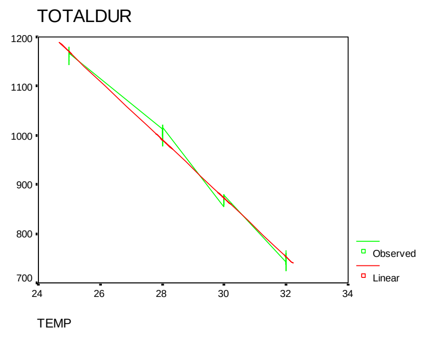
(a) Incubation period



(b) Larval period

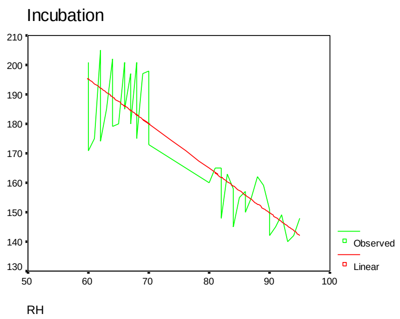


(c) Pupal period

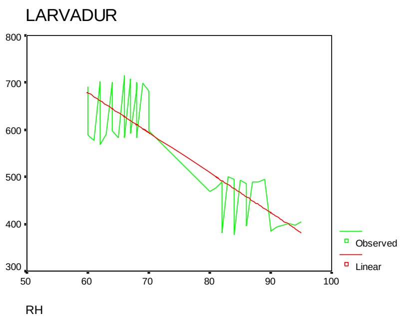


(d) Total developmental period

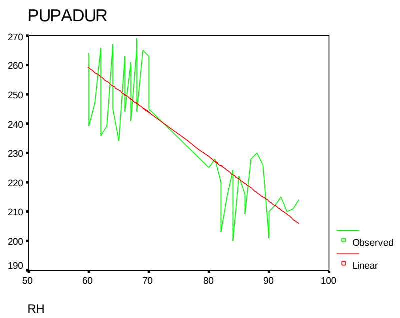
Fig 3. Regression lines showing the negative impacts of rising temperature on various developmental periods in *B. mori*.



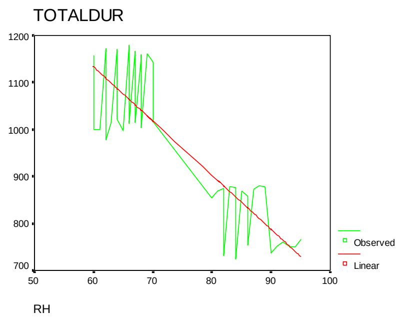
(a) Incubation period



(b) Larval period



(c) Pupal period



(d) Total developmental period

Fig 4. Regression lines showing the negative impacts of RH on various developmental periods in *B. mori.*

Discussion

In the present report a detailed analysis has been carried out to evaluate the impacts of two vital environmental factors on the life-history traits in *B. mori*. Results clearly demonstrated that the temperature and RH had profound effects on incubation, larval and pupal periods as well as on adult emergence and sex-ratio in the experimental insects.

Previously published reports show that the rate of spinning in *B. mori* at 22° C was slow but fast at 38° C; cocoon formation longest at 98±2% RH, but shortest at 40±2% RH; and good quality cocoon was yielded at 22° C and 65±5% RH[9]. Lower RH of 55 and 65% even at 25°C lowered the hatchability and pupation of the silkworm lines and contributed significantly in higher larval mortality, suggesting that temperature and RH in the ranges of 25-26° C and 70-80%, respectively were excellent for egg hatchability, low larval mortality and higher pupation[10]. Further, rearing of larvae under humidity stress conditions resulted in poor performance of the silkworm lines[11]. In addition, sustainable phenotypic output of silkworm crop such as cocoon weight, shell weight, and cocoon-shell ratio[13], and the growth, development, productivity and quality of silk in *B. mori*[14] were found to be influenced by temperature and humidity. Low temperature (25 °C) and high RH (70%) were favourable for higher silk gland, and larval, shell and cocoon weights in this species[16]. These findings corroborate to the present findings in that lower temperatures (25°-28° C) and lower RH (60-80%) were conducive to the immature development and adult emergence in the present silkworm varieties.

In agreement with the present results, recent studies demonstrated that the appropriate temperature and RH for silkworm rearing was 25°C±1 and 80%, respectively[17]; larval mortality was least and the weights of cocoons and pupae reared at temperature 22-26º C and 80-85% RH were more compared to the lower or higher ranges[6] and the best environmental conditions for commercial silkworm rearing was 25º C and 75-80% RH, because the immature mortality rate was minimum at 25º C[19]. As regards the sex-ratio, however, the present data lend support to those reported earlier[24], where the numbers of males generally outnumbered the females, but the differences were insignificant, for examples, 104 males: 91 females; 132 males: 108 females; 116 males: 103 females and 71 males: 65 females, suggesting the sex-ratios very close to 50:50.

Conclusion

It is obvious from the present assessment that the lower temperatures (25°-28° C) and moderate RH (60-75%) are appropriate for the immature development and adult emergence in *B. mori* whereas higher temperatures (28°-32° C) elevated RH (80-95%) shortened immature developmental periods, leading to poor performance of the silkworm lines. These findings correlate to the climate change issue, which has been recognized as a major threat to the survival of species and integrity of ecosystems worldwide. The exact effect of climate change on soil health and sericulture industry is based on prediction and not yet proven, however, several explanations have been proposed in this regard[5]. For example, it may change practices and economy of sericulture drastically in tropical countries like Bangladesh and India

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