# INFLUENCE OF TEMPERATURE ON CERTAIN BIOLOGICAL ATTRIBUTES OF A LADYBEETLE COCCINELLA SEPTEMPUNCTATA LINNAEUS

Shefali Srivastava and Omkar [*]

*Ladybird Research Laboratory , Department Of Zoology , University of Lucknow , Lucknow-226007 , India*

**Abstract**        Influence of temperature on certain biological attributes of an aphidophagous ladybeetle , *Coccinella septempunctata* Linnaeus , feeding on mustard aphid , *Lipaphis erysimi* (Kaltenbach) , at five different temperatures , *viz*. 20 , 25 , 27 , 30 and 35   was investigated. Its developmental period was shortest (11. 7 ± 0. 09 days) at 35   and longest (20. 6 ± 0. 35 days) at 20   . Developmental rate increased with increase in temperature. Hatching percent , larval survival , adult emergence and growth index were maximum at 30   and minimum at 20   . Oviposition period and fecundity were highest at 30   and lowest at 20   . A positive linear relationship exists between temperature and developmental rate and negative correlation between the duration of immature life stages and temperature. The proportion of developmental period allocated to each immature stage was found to be similar at each temperature regime. Thus , 30   was found as the most suitable for *C. septempunctata* amongst the five temperatures tested.

**Key words**        Coccinellidae , ladybeetle , aphids , temperature , biocontrol

## 1   INTRODUCTION

Abiotic factors *viz*. , temperature , humidity and photoperiod are the most important factors , which influence the growth and development of the ladybeetles (Ferran and Larraque 1980). Reproduction in ladybeetles is a crucial factor because slight deviation in temperature affects the reproductive period and performance of the ladybeetles (Ponsonby and Copland 1998). It also governs the ability of ladybeetles to successfully invade new habitats (Phoofolo and Obrycki 2000).

Ladybeetles (Coccinellidae : Coleoptera) are important biocontrol agents , since majority of them are predaceous on several groups of insect pests , such as , aphids , coccids , adelgids and aleyrodids. For the biocontrol of aphid pests , it is desirable to maintain the population of ladybeetles at a level , which may keep the pest population below the eco-

nomic injury level. Amongst coccinellids , *Coccinella septempunctata* Linnaeus , is one the most common , locally abundant and potent predator (Omkar and Srivastava 2001). In developing a reasonable management system for the aphid pests , attention must be paid to the factors responsible for the seasonal variation of *C. septempunctata* populations. Studies on the influence of temperature , relative humidity and photoperiodicity on the rate of development of *C. septempunctata* were made by Hodek (1958) and on the reproductive rate and longevity by Ruzicka *et al*. (1981). Few other studies on the temperature dependent feeding and development of *C. septempunctata* were performed by Ives (1981) , Butler (1982) and Baumgartner *et al*. (1987). Experiments were performed to examine the temperature dependent pre adult development , immature survival and reproduction of *C. septempunctata* at five constant temperatures. The temper-

[*] Address for Correspondence : Dr. Omkar , Reader , Ladybird Research Laboratory , Department of Zoology , University of Lucknow , Lucknow-226007 , India , Ph. No. 0091- 0522-2320080 , E-mail : omkaar55 @hotmail. com

ature relationships will be helpful in identifying the optimal temperature for its propagation in the laboratory and will also be helpful in predicting the development of *C. septempunctata* in the field. The response of *C. septempunctata* to temperature may also provide some evidence to aid in the evaluation of relative competitiveness and adaptability to local climates.

## 2   MATERIALS AND METHODS

### 2. 1   Laboratory maintenance

Adults of *C. septempunctata* were collected from the mustard crop fields ( *Brassica campestris* ) adjoining the city of Lucknow , India , infested with mustard aphid , *Lipaphis erysimi* and brought to the laboratory where the stock culture was maintained at ( 25 ± 20 )   temperature and ( 60 ± 5 ) % R. H. Mating pairs were kept in glass beakers ( 11. 0 cm height and 7. 00 cm diameter ) covered with fine muslin cloths fastened with rubber bands. The ladybeetles were fed on *L. erysimi* on mustard twigs. The left over aphids and dried twigs were replaced daily with fresh ones to avoid contamination. The eggs were collected daily.

### 2. 2   Experimental design

**2. 2. 1   Pre-adult development and immature survival**      Fifty eggs were selected from the laboratory maintained stock and kept in Environmental Test Chamber at 20   temperature in a Petri dish ( 2. 0 cm height and 9. 0 cm diameter ) . The incubation period was recorded after hatching of grubs from the eggs. First instars were transferred from Petri dishes to glass beakers using soft camel hairbrush and provided with aphids. The leftover aphids along with dried host plant twigs were replaced with fresh ones daily. The cast exuviae were noted and removed daily to record the number of moults and number of larvae surviving at each stage. When fourth instar changed into prepupa i. e. it stopped feeding , adhered to leaf surface , and its black colour changed to yellow. This period was recorded as prepupal period. Thereafter , the yellow coloured pre-pupa started developing black. The time duration from this event till the adult emergence was

recorded as pupal period. Number of adults emerged from the pupae were also recorded.

The percent immature survival , adult emergence , development rate and Howe's growth index was calculated.

Howe's Growth Index ( HGI ) $= \log e$ N/AV , where N $=$ Percent adult emergence , AV $=$ Average developmental period which included larval and pupal periods.

To obtain the desired information at each temperature regime , aphid species along with host plant twigs were supplied during the entire life span and humidity was kept constant ( *i. e.* 60 % ± 5 %) . The experiment was repeated at temperatures , 25 , 27 , 30 and 35   and the observations were made in ten replicates.

**2. 2. 2   Reproduction**      Reproductive response of the ladybeetle in terms of oviposition period , fecundity and hatching percentage was recorded at different temperatures. Newly emerged mating pairs along with host plant twigs were kept in the Environmental Test Chamber at 20   and relative humidity , 60 % ± 5 %. Open ends of beakers were covered with the help of fine muslin cloth and rubber bands.

The oviposition period ( the period from first to last day of oviposition ) , fecundity ( egg laying during the entire life span ) and hatching percent was recorded. Similar experiments were designed at temperatures , 25 , 27 , 30 and 35   in a new set up and the observations were made in ten replicates.

### 2. 3   Data Analysis

The data of the above experiments were subjected to one-way ANOVA and Bonferroni test for the comparison of means. Linear regression analysis was applied to determine the relation between : ( 1 ) duration of different immature stages of *C. septempunctata* and temperatures ( 2 ) temperature and fecundity , and ( 3 ) temperature and development rate following the statistical package , Statistix 4. 1 ( 1994 ) on PC.

## 3   RESULTS

### 3. 1   Pre- adult development and immature survival

Table 1 revealed that the incubation period was lowest (2. 1 ± 0. 01 days) at 35℃ and highest (4. 2 ± 0. 14 days) at 20℃ ( $F = 97. 33$ ; $P < 0. 001$ ; df = 4 , 45). The duration of first ( $F = 96. 16$ ; $P < 0. 001$ ; df = 4 , 45) , second ( $F = 30. 91$ ; $P < 0. 001$ ; df = 4 , 45) , third ( $F = 46.$ 45 ; $P < 0. 001$ ; df = 4 , 45) and fourth instars ( $F = 84. 72$ ; $P < 0. 001$ ; df = 4 , 45) differed significantly at different temperatures. Total larval duration was shortest (6. 7 ± 0. 04 days) at 35℃ and longest (11. 9 ± 0. 24 days) at 20℃ ( $F = 180. 25$ ; $P < 0. 001$ ; df = 4 , 45 ).

**Table 1**  Duration (in days) of different life stages of *C. septempunctata* at different temperatures.

| Temp. (in ℃) | 20 | 25 | 27 | 30 | 35 | F-value |
|---|---|---|---|---|---|---|
| Incubation period | 4. 2 ± 0. 14[a] | 3. 1 ± 0. 06[b] | 2. 7 ± 0. 07[c] | 2. 4 ± 0. 07[cd] | 2. 1 ± 0. 01[de] | 97. 33[*] |
| First instar | 3. 1 ± 0. 10[a] | 2. 8 ± 0. 06[b] | 2. 4 ± 0. 04[c] | 1. 9 ± 0. 07[d] | 1. 5 ± 0. 04[e] | 96. 16[*] |
| Second instar | 2. 0 ± 0. 06[a] | 1. 9 ± 0. 06[ab] | 1. 6 ± 0. 03[c] | 1. 5 ± 0. 07[cd] | 1. 3 ± 0. 02[d] | 30. 91[*] |
| Third instar | 2. 8 ± 0. 10[a] | 2. 5 ± 0. 09[b] | 2. 2 ± 0. 02[bc] | 2. 0 ± 0. 05[cd] | 1. 6 ± 0. 03[e] | 46. 45[*] |
| Fourth instar | 4. 0 ± 0. 09[a] | 3. 5 ± 0. 07[b] | 3. 0 ± 0. 04[c] | 2. 9 ± 0. 08[cd] | 2. 3 ± 0. 04[e] | 84. 72[*] |
| Total larval period | 11. 9 ± 0. 24[a] | 10. 6 ± 0. 18[b] | 9. 2 ± 0. 04[c] | 8. 3 ± 0. 13[d] | 6. 7 ± 0. 04[e] | 180. 25[*] |
| Prepupal period | 1. 0 ± 0. 04[a] | 0. 8 ± 0. 04[b] | 0. 8 ± 0. 02[bc] | 0. 6 ± 0. 03[cd] | 0. 6 ± 0. 02[de] | 23. 21[*] |
| Pupal period | 3. 5 ± 0. 10[a] | 2. 9 ± 0. 05[b] | 2. 6 ± 0. 03[c] | 2. 4 ± 0. 04[cd] | 2. 4 ± 0. 04[de] | 57. 70[*] |
| Total pupal period | 4. 5 ± 0. 12[a] | 3. 7 ± 0. 08[b] | 3. 4 ± 0. 04[c] | 3. 0 ± 0. 06[d] | 2. 9 ± 0. 06[e] | 66. 23[*] |
| Complete Development period | 20. 6 ± 0. 35[a] | 17. 4 ± 0. 17[b] | 15. 3 ± 0. 06[c] | 13. 7 ± 0. 15[d] | 11. 7 ± 0. 09[e] | 313. 96[*] |

Values are Mean ± S. E.

Means followed by the same alphabetical letters in a column are not significantly different.

[*] Significant at $P < 0. 001$ ; df = 4 , 45.

The prepupal ( $F = 23. 21$ ; $P < 0. 001$ ; df = 4 , 45) , pupal ( $F = 57. 70$ ; $P < 0. 001$ ; df = 4 , 45) and total pupal periods ( $F = 66. 23$ ; $P < 0. 001$ ; df = 4 , 45) of *C. septempunctata* were significantly different at different temperatures. The complete developmental period was shortest (11. 7 ± 0. 09 days) at 35℃ and longest (20. 6 ± 0. 35 days) at 20℃ ( $F = 313. 96$ ; $P < 0. 001$ ; df = 4 , 45). The proportion of developmental period allocated to each immature stage was found approximately similar at each temperature regime. Table 2 reveals that the ratio of time spent in each instar in

**Table 2**  Regression equations at different durations (days) of immature stages predicting development of various life stages of *C. septempunctata* at different temperatures.

| Duration of developmental stages | Regression equations | $r^2$ |
|---|---|---|
| Incubation period | $Y = 6. 6809 - 0. 1393 X$ | 0. 8129 ; $P < 0. 001$ |
| First instar | $Y = 5. 3662 - 0. 1109 X$ | 0. 8622 ; $P < 0. 001$ |
| Second instar | $Y = 3. 0187 - 0. 0500 X$ | 0. 6933 ; $P < 0. 001$ |
| Third instar | $Y = 4. 4909 - 0. 8230 X$ | 0. 8026 ; $P < 0. 001$ |
| Fourth instar | $Y = 6. 1941 - 0. 1115 X$ | 0. 8521 ; $P < 0. 001$ |
| Total larval period | $Y = 19. 0700 - 0. 3547 X$ | 0. 9271 ; $P < 0. 001$ |
| Prepupal period | $Y = 1. 5823 - 0. 0299 X$ | 0. 6781 ; $P < 0. 001$ |
| Pupal period | $Y = 4. 8374 - 0. 0763 X$ | 0. 7349 ; $P < 0. 001$ |
| Total pupal period | $Y = 6. 4198 - 0. 1061 X$ | 0. 7652 ; $P < 0. 001$ |
| Complete development | $Y = 32. 1710 - 0. 6002 X$ | 0. 9403 ; $P < 0. 001$ |
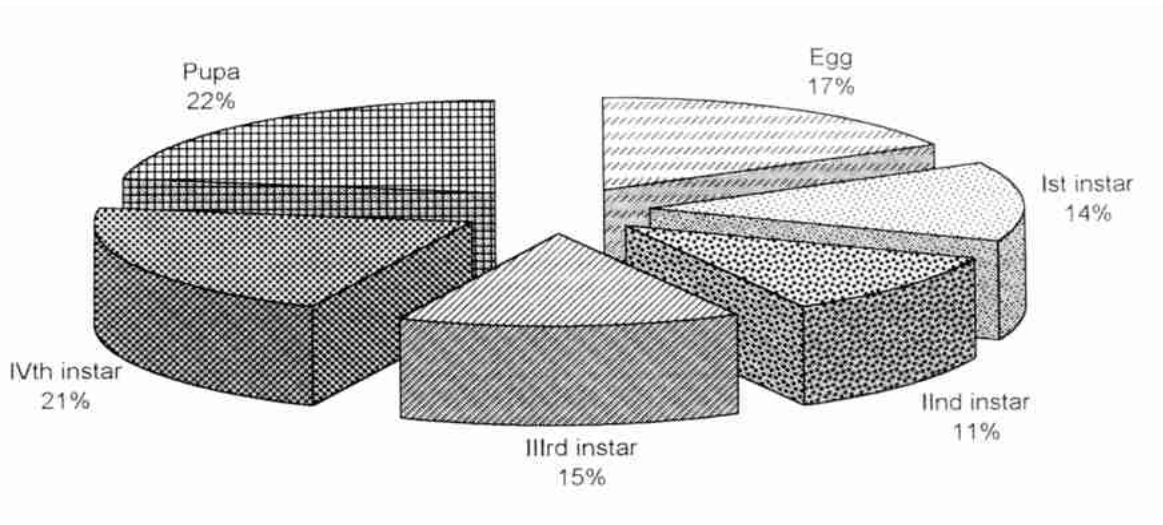
**Fig. 1**   Mean developmental period (days) as a percentage of total development of the various immature stages of
*C. septempunctata* at 30     temperature ( *n* = 10) .

relation to total larval period was nearly same at each temperature regime. Figure1 exhibits the developmental period of each stage at 30  . Fourth instar had comparatively longer developmental period among the instars ; second instar had shortest developmental period in comparison to other instars.

Developmental time was inversely correlated with temperature in the range of 20 —35     (Table-3) . Developmental rate of *C. septempunctata* increased from 0. 05 to 0. 09 as temperature increased from 20 —35   . Thus , increase in temperature increased the rate of development of *C. septempunctata* and a positive linear relationship $Y = 0.1104 + 6.98 E - 03 X$ exists between temperature and developmental rate (Fig. 2) .
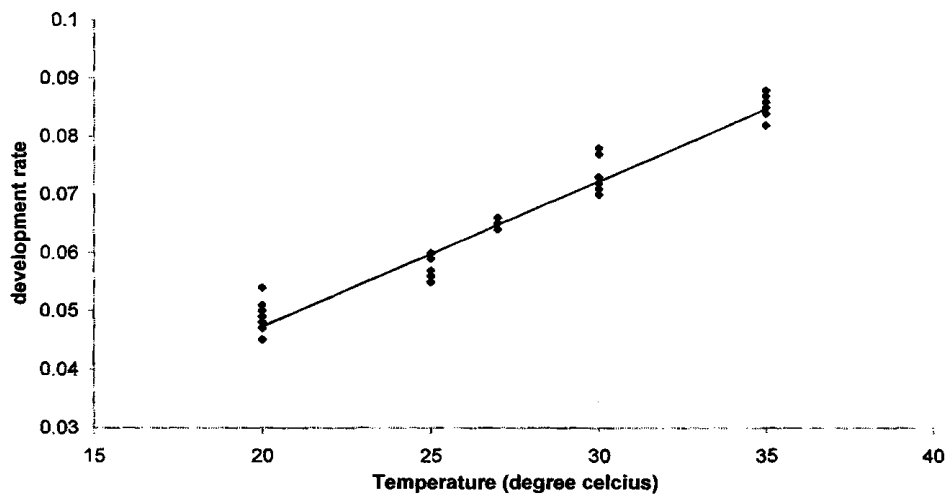


**Fig. 2**   Best fit line for the effect of temperature on the development rate of *Coccinella septempunctata* ,
when the immature predatory stages were fed on apphid ,*L. erysimi*

**Table 3** Ratio of time spent in each instar in relation to total larval period at different temperatures.

| Temperature | Ratio of time spent in first, second, third and fourth instar to the total larval period | | | | |
|---|---|---|---|---|---|
| 20 | 0.25 | 0.17 | 0.24 | 0.34 | 1 |
| 25 | 0.23 | 0.18 | 0.23 | 0.33 | 1 |
| 27 | 0.26 | 0.17 | 0.24 | 0.32 | 1 |
| 30 | 0.23 | 0.18 | 0.24 | 0.35 | 1 |
| 35 | 0.22 | 0.19 | 0.25 | 0.35 | 1 |

Table 4 shows the effect of temperature on percent immature survival and percent emergence of *C. septempunctata*. Percent larval survival increased from 47. 9 ± 0. 94 to 68. 9 ± 0. 57 when temperature increased from 20 to 30 and further decreased (61. 5 ± 0. 54) at 35. Percent adult emergence from the pupae increased from 37. 5 ± 1. 51 to 77. 3 ± 2. 22 when temperature increased from 20—30 and further decreased (66. 9 ± 2. 89) at 35. Growth index increased from 4. 90 ± 0. 29 to 15. 34 ± 0. 49 when temperature increased from 20 to 30 and again decreased to 14.

71 ± 0. 19 at temperature 35.

## 3. 2  Reproduction

The oviposition period of *C. septempunctata* increased from 24. 3 ± 1. 30 to 48. 6 ± 1. 28 days with an increase from 20 to 30, which decreased (34. 2 ± 1. 35 days) when temperature increased upto 35 ($F = 53. 79$; $P < 001$; df = 4, 45). The fecundity increased from 449. 6 ± 7. 23 to 1312. 4 ± 19. 70 eggs when temperature increased from 20 to 30 but decreased (763. 4 ± 23. 04 eggs) at 35. Figure 3 exhibits the best-fit line for predicting effect of temperature on the fecundity of *C. septempunctata*. ANOVA revealed that the fecundity of *C. septempunctata* varied significantly ($F = 334. 00$; $P < 0. 001$; df = 4, 45) at different temperatures. The single polynomial regression analysis of fecundity of ladybeetle at different temperatures yielded the regression equation $Y = -83. 418 + 32. 706 X$. The hatching percent of eggs was highest (93. 7) at 30 and lowest (64. 5) at 20.

**Table 4** Growth and development of *C. septempunctata* at different temperatures.

| Temperature regimes | Percent Hatching | Percent larval survival | Percent emergence | Growth index | Oviposition period (days) | Fecundity (eggs) |
|---|---|---|---|---|---|---|
| 20 | 64. 5 | 47. 9 ± 0. 94 | 37. 5 ± 1. 51 | 4. 90 ± 0. 29 | 24. 3 ± 1. 30 | 449. 6 ± 7. 23 |
| 25 | 76. 5 | 53. 6 ± 1. 28 | 47. 8 ± 0. 64 | 7. 47 ± 0. 12 | 28. 7 ± 0. 89 | 588. 5 ± 13. 20 |
| 27 | 87. 0 | 54. 8 ± 1. 42 | 75. 4 ± 2. 59 | 13. 38 ± 0. 48 | 38. 4 ± 1. 57 | 949. 7 ± 23. 45 |
| 30 | 93. 7 | 68. 9 ± 0. 57 | 77. 3 ± 2. 22 | 15. 34 ± 0. 49 | 48. 6 ± 1. 28 | 1312. 4 ± 19. 70 |
| 35 | 83. 5 | 61. 5 ± 0. 54 | 66. 9 ± 2. 89 | 14. 71 ± 0. 19 | 34. 2 ± 1. 35 | 763. 4 ± 23. 04 |
| F-value | | 64. 65 [*] | 71. 05 [*] | | 53. 79 [*] | 334. 00 [*] |

Values are Mean ± S. E,

[*] Significant at the level of $P < 0. 001$; df = 4, 45.

## 4  DISCUSSION

### 4. 1  Pre- adult development and immature survival

The results reveal that exposure to higher temperature increased the development rate. Consequently, developmental period of life stages decreased. Development of immature stages of ladybeetle, *C. septempunctata* was fastest at 35 and slowest at 20.

Shortest incubation period of eggs of *C. septempunctata* appeared to be due to elevated developmental rate of the embryos. Temperature also influenced the larval development of *C. septempunctata*; possibly by enhancing the metabolic rate of larvae at higher temperatures. Increased larval voracity, on account of high metabolic rate leads to an increased supply of nutrients ultimately resulting in rapid development (Ponsonby and Copland 1996, Omkar and Pervez, unpubl. data). Developmental
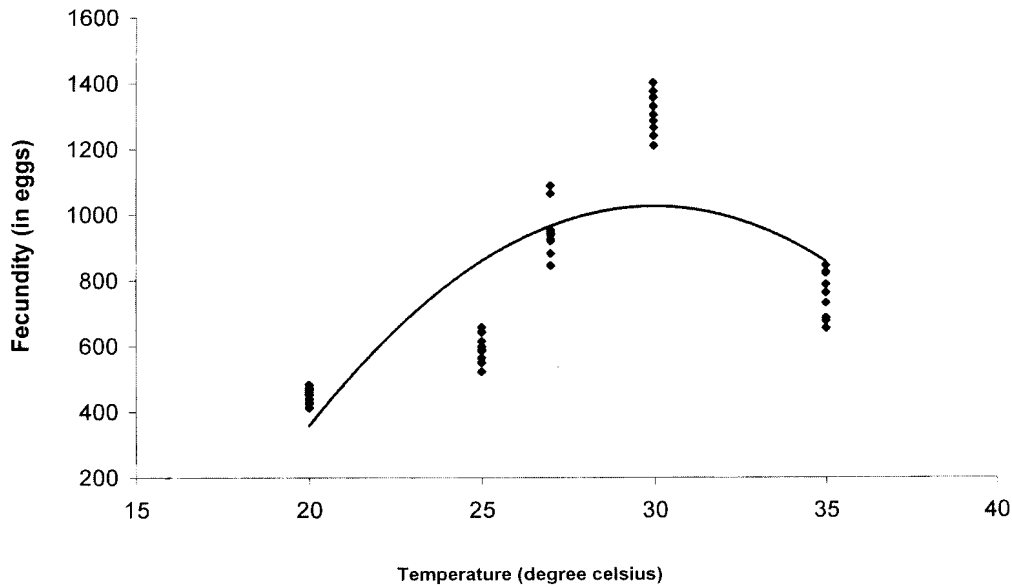
**Fig. 3**   Best fit line for predicting effect of temperature on the fecundity of *C. septempunctata* when fed on
aphid , *L . erysimi*.

rate of ladybeetle is dependent on ambient temperature. The increased temperature resulted in increase in developmental rate. There is a linear relationship between the temperature and the rate of development of the ladybeetle.

Although development rate increased with increase in temperature but the ratio of time spent by the different immature stages did not alter with increase in temperature. The finding suggests the presence of an innate ratio between successive developmental periods of immature life stages. Temperature merely increases the metabolic rate and shortens the period of development , but no significant effect was visible in terms of the relative maturation time of different immature life stages. Similar findings were also recorded in *Chilocorus nigritus* (Fabricius) (Ponsonby and Copland 1996).

The first instar had a developmental period shorter only to the fourth instar ; while second instar had shortest developmental period in comparison to other instars. An inverse relationship exists between temperature and larval period. Similar observations were also reported on other ladybeetles , *viz. Hippodamia convergens* Guerin ( Obrycki and Tauber

1982) , *Hippodamia parenthesis* ( Say) ( Orr and Obrycki 1990) and *Cheilomenes sexmaculata* ( Fabricius) ( Ali Khan and Yousuf 1986). The pupal period also decreased with the increase in temperature , as also recorded by Butler (1982) , Baumgartner *et al.* (1987) , Sakurai *et al.* , (1991) and Singh *et al.* (1994).

Egg viability increased with temperature upto 30  beyond which it decreased. At high temperature ( 35  ) the unhatched eggs , turned flaccid , wrinkled and gave a burnt look ; possibly some of the egg contents get denatured at 35  . Egg viability was significantly low at 20  as compared to 30  and may possibly be attributed to ( i ) increased sperm mortality in the spermathecae , or ( ii ) inhibition of spermatogenesis by low temperature ( Ponsonby and Copland 1998). The egg viabilities at cyclic (14 —30  ) and constant (20  ) temperatures are known to be almost similar ( Ponsonby and Copland 1998) , implying that exposure to low temperature may be the cause of lowered fertility. The significant increase in the egg viability at 30  , increased development rate and increased survival indicates that this temperature is the opti-

mum for progeny survival and development.

Temperatures 20 and below are not deemed suitable for larval survival , as larval survival was 45 % at 20 . High larval mortality at low temperatures is also supported by earlier work on *Scymnus frontalis* (Naranjo *et al.* , 1990) and *H. convergens* (Miller 1992) . The increase in larval mortality at 35 suggests that extremely high temperature is deleterious for the larvae. The internal body temperature may possibly approach lethal point at 35 . The first instar suffered the highest mortality amongst the immature stages and the pupae had the highest survival followed by the fourth instar. The higher mortality at lower instars can be attributed to their thinner and softer cuticle , while in later instars the cuticle is thick due to which mortality reduced. Thus , thicker cuticle and thick pupal case provide protection from unfavourable abiotic conditions. Similar findings were reported in case of *C. nigritus* (Ponsonby and Copland 1996) .

The decrease in larval survival at 35 suggested that larvae were struggling to withstand the imposed stress. At 35 food also became a limiting factor as most of the aphids became alate and a few of them even died. The alate aphids appeared to be distasteful to the coccinellid larvae. The percent adult emergence improved with the increase in temperature from 20 to 30 ; but decreased at 35 . Thus , the optimum temperature for the eclosion of adult *C. septempunctata* was recorded as 30 . Similar findings that increase in larval survival with the increase in temperature were also recorded in *C. sexmaculata* (Alikhan and Yousuf 1986) , *H. convergens* ( Rodriguez and Miller 1995) and *Cryptolaemus montrouzieri* (Jalali *et al.* 1999) . The growth index of the ladybeetle was shortest at 20 and highest at 30 suggests that the slow rate of metabolic activity at low temperature is responsible for the prolonged period of development of *C. septempunctata* at 20 .

## 4. 2    Reproduction

The oviposition period increased with temperature upto 30 but later decreased at 35 . The rapid maturation of the gonads due to increased metabolic activities at higher temperature is probably responsible for the expedited oviposition upto 30 . The lower extreme of temperature reduced the reproductive output and rate to some extent by slowing down the metabolic activities (Rhamhalinghan 1986) . The fecundity was highest at 30 and lowest at 20 . The fecundity followed the same pathway as other parameters. The increased feeding activity at 30 may also be responsible for early ovariole ripening. Reduction in the feeding rate at low temperature might be a cause as reproductive numerical response is directly proportional to prey consumption ( Ofuya and Akingbohungbe 1988) . The increased fecundity with increase in temperature in ladybeetles was also reported in *C. septempunctata* , *Adalia bipunctata* (Linnaeus) , *H. convergens* , *Coccinella californica* Mannerheim , *Coccinella undecimpunctata* Linnaeus , *Cycloneda polita* Casey and *Coccinella trifasciata* Linnaeus ( Frazer and Mcgregor 1992) , *C. sexmaculata* (Ali Khan and Yousuf 1986) , *C. montrouzieri* (Jalali *et al.* 1999) and *Micraspis discolor* (Fabricius) (Omkar and Pervez 2002) .

Thus , it can be inferred that low temperature limits reduce the ability of life to persist for a variety of reasons among which are the inability to support the energy transformation at the minimal level and cessation of some vital reactions. At high temperature (35 ) although the faster preimaginal development of *C. septempunctata* took place but it proved lethal for immature survival etc. and percent viability , larval survival , adult emergence , oviposition period and fecundity was maximum at 30 . Thus , the present study identifies 30 , to be the optimum for effective mass rearing of the predator.

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Shefali Srivastava and Omkar

Ladybird Research Laboratory , Department of Zoology , University of Luckow Lucknnw   226007 , India

*Coccinell septempunctata* Linnaeus    20 ,25 ,27 ,30    35                                  *Lipaphis erysimi*
( Kaltenbach)                                    35    (11. 7 ± 0. 09)    ,                  20
   (20. 6 ± 30. 35)                                                      30

      ,    20                            30               ,    20

  ,                        5              ,30