Effects of Temperature on Survival Rate and Larval Development of Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Under Laboratory Conditions

Efrizal
Departement of Biology, Faculty of Math and Science, Andalas University,
Kampus Unand Limau Manis, Padang, 25163, West Sumatera, Indonesia
Telephone no: 0751-71671
Fax no: 0751-73118
Email: efrizal.unand [AT] gmail.com

ABSTRACT--- An experiment was conducted to determine the effects of temperature on survival rate and larval development of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) under cultured conditions. Five different constant temperatures (26, 28, 30, 32 and 34 ^{o}C) were compared in triplicate. The larval were fed a live diet and examined and daily and death or molts recorded. The result showed that survival rate and larval development were significantly different among treatments due to temperature ($P< 0.05$).). The highest and lowest larval survival rates until C1 stages were promoted by 30 ^{o}C (36.67 %) and 34 ^{o}C (12.22 %). The second best survival rate of C1 (31.11 %) was obtained at 28 ^{o}C. The lowest larval development (3.67 -4.00 days) occurred at 30-34 ^{o}C ($P> 0.05$). At 26^{o}C and 28^{o}C larval development of C1 took around 5.67 days ($P>0.05$).

Keywords--- Portunus pelagicus; larval, temperature, survival rate, development

1. INTRODUCTION

Temperature is one of the most important abiotic factors affecting the growth and survival of aquatic organisms (Kumlu *et al.*, 2000). Larval stages of most decapod species occur in full strength seawater and stable water temperatures. Hence, it is generally accepted that the decapod species are not equipped with capabilities of withstanding major environmental changes during their larval development.

Changes in the environmental conditions of the estuarine and coastal nursery areas of juvenile fish and crustaceans can affect the size of the subsequent yield of adults (Gracia and LeReste, 1981). Studies of the response of juveniles to different environmental factors can therefore lead to a better understanding of stock fluctuations and be used to develop predictive models. They can also provide information on the optimum environmental factors to maximize the reproduction of animals in culture, which is of obvious practical importance for aquaculturists.

Blue swimming crab, *P. pelagicus* (Linnaeus, 1758) is an Indo-Pacific species distributed along in nearshore marine and estuarine waters (Stephenson, 1962 and Kailola *at al*., 1993) and is one of the most important commercial species in this part of the world. In tropical region berried females, *P. pelagicus* spawn throughout the year (Batoy *et al.*, 1987). Whereas, in temperate reproduction regions is restricted to the warmer months (Meagher, 1971; Smith, 1982). The eggs and the larvae of blue swimming crabs are planktonic. The eggs hatch after about 15 days at 24^{o}C. The larval phase consists of five stages. During the larval phases, crabs may drift as far as 80 km out to sea before returning to settle in shallow inshore waters (William, 1982). Bryars (1997) predicted the larval duration of *P. pelagicus* in South Australia to be about 26 to 45 days under average environmental (temperature) conditions. For this, numerous studies have been conducted on the effects of temperature on larval survival and development in many brachyuran species (Anger, 1983; Chen and Cheng, 1985; Dawirs, 1985; Minagawa, 1990; Choy, 1991; Zeng and Li, 1992; Okamoto, 1993; Hamasaki, 1996; 2003), but a few studies have examined the effects of environmental conditions on development and survival of *P. pelagicus* larvae under controlled laboratory conditions.

A better understanding of the effects of temperature on the larval culture of this crab is important in order to provide adequate conditions for an optimal production. The objective of the present study was to determine the development and survival of *P. pelagicus* (Linnaeus, 1758) at five different temperature levels during the larval development from the Z_1 to the first crab stage under laboratory conditions.

2. MATERIALS AND METHODS

Berried blue swimming crab females, with mean body weight (BW) of 149.6 gm, and carapace width (CW) of 114.73 mm were caught from their natural habitat in nearshore marine waters of Port Dickson. They were maintained in

300L fiberglass tanks for disinfection at 100 ppm formaldehyde for 30 minutes prior to stocking in incubation and hatching tanks.

The water quality in the incubation tanks is maintained using a flow-through system (2L/min) and gentle aearation. Water temperature and salinity (measured each morning at 0900) in the tanks varied from 27 - 28^{o}C and 32 ppt, respectively. When hatching appeared imminent, as indicated dark ocular pigmentation and movement of the embryo inside the egg membrane, the water incoming and outcoming were stopped. Hatching occurred during both day and nighttime hours. Larvae are transferred to a separate aquarium for observation and rinsing before being placed into the experimental rearing containers.

Upon hatching, only the healthy Z_1 which swam actively near to water surface were stocked for experiment. The larvae were reared into 3 L cylindrical plastic containers at density of 30 larvae per containers. They were acclimatized to five temperature levels (26, 28, 30, 32 and 34^{o}C) controlled by thermostatically regulated electric heater and air conditioning unit. The water quality parameters were relatively constant throughout the duration of study. Water salinity varied from 28 to 30 ppt, dissolved oxygen was maintained above 6.0 ppm and pH was 7.8 to 8.0.

The larvae were fed *Artemia* nauplii at densities of 5 and 10 ind. ml^{-1} on the first zoea and second zoea to first crab stages respectively. Rotifers and micro-alge *Nannochloropsis oculata* that contained essential fatty acids for larval crabs (Suprayudi *et. al*., 2002) were also given on the first zoea stage at 5 ind. ml^{-1} and 5x10^{3} cell.ml^{-1} , respectively. Gentle aeration provided oxygen saturation and larval suspension and sufficient turbulence to prevent settling of food organism. Larvae were transferred to newly prepared containers with new sea water and prey using a large-mouthed pipette each morning. Larva were observed and counted for survival and larval stages daily. Different stages of zoea were easily distinguished by their body size. Sunken eggs and larval stages of dead larvae were also removed daily from the bottom of the tanks by a large-mouthed pipette.

The data of survival rate (premetamorphic survival and successful metamorphosis) and duration of larval stages (days) were tested using one way ANOVA and Duncan's Multiple Range test was used to compare the mean differences among treatments (Steel and Torrie, 1980). Arcsin transformation was done in the analysis of the data in percentage.

3. RESULTS

Premetamorphic survival and duration of each larval stage of blue swimming crab reared under different temperature conditions are summarized in Table 1. Changes in the number of respective stages of larvae with time after hatching are shown in Figs. 1 to 5. The first zoeal stage (Z_1) and second zoeal stage (Z_2) reared at 26^{o}C, 28^{o}C, 30^{o}C, 32^{o}C, and 34^{o}C displayed higher survival rates in the range of 90.00 to 95.56% and 77.78 to 87.78% respectively, but did not differ statistically (P>0.05). Regression analysis showed a linear relationship between temperature and premetamorphic Z1 and Z2 survival ($PZ_1SR = -0.8332T + 117.8800$; $R^2 = 0.3158$; P>0.05; Fig. 6, and $PZ_2SR = -1.2220T + 119.1000$; $R^2 = 0.3757$; P<0.05; Fig. 7, respectively). The development duration of Z1 (6.67 days) was longer at 26^{o}C compared to other temperature levels (P<0.05). On the other hand, at Z_2, temperature of 34^{o}C gave longer development duration (5.66 days) than the other treatments, this effect was not statistically significant (P>0.05). The relationship between temperature and development duration for Z_1 and Z_2 were quadratic ($DuZ_1 = 0.1071T^2 - 6.6619T + 107.3700$; $R^2 = 0.8688$; P<0.05; Figure 57, and cubic ($DuZ_2 = 0.0174T^3 - 1.5268T^2 + 44.4960T - 424.6100$; $R^2 = 0.3842$; P>0.05; Fig. 9, respectively).

The percentage of premetamorphic survival from Z_1 to Z_3 stage increased with increasing temperature up to 30^{o}C and then decreased at 32^{o}C. Analysis of variance showed a significant effect of temperature on premetamorphic survival rate of Z_3 at 5% level of significance. A further test of significance of the differences in premetamorphic Z_3 survival means showed significant differences between treatment 34^{o}C and treatments 28^{o}C and 30^{o}C. While from the mean development duration of Z_3, low temperature (26-28^{o}C) was found to be prolonged (P<0.05) compare to high temperature (30-34^{o}C). The relationship between temperature and premetamorphic survival and development duration of Z_3 were quadratic ($PZ_3SR = -0.3971T^2 + 22.4920T - 241.3000$; $R^2 = 0.5155$; P<0.05; Fig. 10, and $DuZ_3 = 0.0357T^2 - 2.3095T + 41.1240$; $R^2 = 0.6044$; P<0.05; Fig. 11, respectively).

The premetamorphic survival from Z_1 to Z_4 stages were generally high (62.22 to 66.67%) at all the temperature levels tested, with no significant differences among treatments (P>0.05). The relationship between temperature and premetamorphic survival was cubic with an equation of $PZ_4SR = 0.1967T^3 - 17.7820T^2 + 533.0600T - 5228.5000$ ($R^2 = 0.2450$; P>0.05; Fig. 12). The duration of larval development showed a relatively similar pattern with the previous premetamorphic survival of Z_1, Which the low temperature (26^{o}C) tended to be prolonged development duration (P<0.05) compared to high temperature (28-34^{o}C). Regression analysis showed the relationship to be quadratic ($DuZ_4 = 0.0714T^2 - 4.4524T + 75.9810$; $R^2 = 0.7569$; P<0.05; Fig. 13). During the megalopa stage, the larvae grew best at 26^{o}C to 32^{o}C (42.22 -50.00%). High temperature of 34^{o}C consistently gave the lowest (P<0.05) premetamorphic survival rate of megalopa stage (30%) compared to other treatments. The relationship between temperature and premetamorphic

survival for megalopa was quadratic (PMSR = $0.4366T^2 + 24.0850T – 283.0000$; $R^2 = 0.6286$; $P<0.05$; Fig. 14). The longest ($P<0.05$) development duration was found in premetamorphic M survival held at 26°C to 28°C, which required an average of 8.33-8.67 days, followed by those held at 30°C to 34°C which required an average of 3.67 to 4.00 days. Regression analysis found the relationship to be quadratic (DuM = $0.0655T^2 – 4.1786T + 73.3050$; $R^2 = 0.6654$; $P<0.05$; Fig. 15).

The influence of temperature was more evident on the successful metamorphosis and larval development duration during the first crab stages (C_1). The highest and lowest successful metamorphosis of C_1 stage were obtained at 30°C (36.67%) and 34°C (12.22 %) respectively. The second best successful metamorphosis of C_1 (31.11%) was obtained at 28°C. Statistical analysis using ANOVA with DMRT test demonstrated that successful metamorphosis of C_1 reared at 34% was significantly lower ($P<0.05$) than those at other temperature levels. The shortest larval development duration (3.67-4.00 days) occurred at 30-34°C at all the temperature levels ($P<0.05$). At 26°C and 28°C larval development of C_1 took around 5.67 days. The relationship between temperature and successful metamorphosis and development duration of C_1 were quadratic ($SuMpC_1 = -0.9326T^2 + 54.2340T - 754.4400$; $R^2 = 0.7630$; $P<0.05$; Fig. 16, and $DuC_1 = 0.0476T^2 - 3.1238T + 55.0100$; $R^2 = 0.6392$; $P<0.05$; Fig. 17, respectively).

4. DISCUSSION

Based on the result of correlation coefficient R (Fig. 6, 7, 10, 12, 14 and 16) analysis in temperature and survival relationship; it could be explained that their relationship tended to increase from the stage Z_1 (56.19%), Z_2 (61.29%), Z_3 (71.52%), M (79.28%) and C_1 (87.35%), except for Z_4 (49.49%). For the R^2, survival could be explained by temperature as much as R^2 (%), whereas the remainder could be explained by other factors which were not considered in this research. Starting from stage Z1 to C1 the R^2 value were increased, except for the Z4 as follow 31.58% (Z_1), 37.57% (Z_2) , 51.55% (Z_3), 24.50% (Z_4), 62,86% (M) and 76.30% (C_1).

As can be seen from coefficient of correlation R (Figure 8, 9, 11, 13, 15 and 17) on duration of the development of the larvae, the relationship was varied as follows 93.21% (Z1), 61.98% (Z2), 77.74% (Z3), 87.00% (Z4), 81.57% (M), and 79.95% (C1). In addition, the relationship between larvae survival and the increasing of temperature were varied in every stage of development with value of R^2 as follow 86.88% (Z_1), 38.42% (Z_2), 60.44% (Z_3), 75.69% (Z_4), 66.54% (M) and 63.92% (C_1).

Temperature regulates many processes such as activity, feed consumption and growth in poikilotherms (Winberg, 1959). Effects of temperature on larval survival have been reported for the mud crab species *S. serrata* (Chen and Cheng, 1985; Zeng and Li, 1992). In this study, the survival rate and larval development of blue swimming crab were also shown to be influenced by the levels of temperature. Relatively low temperature (26°C and 28°C) slowed larval development but did not cause high mortality. The larvae subjected to the relatively lower temperature (26 °C and 28°C) displayed higher survival at each premetamorphic survival in comparison to those of higher temperature (32°C and 34°C). This finding agreed with those of Hamasaki (2003) who studied the effects of temperature on survival and larval development of the mud crab *S. serrata* reared in the laboratory. Temperature between 26 to 29°C consistently give a better survival rate for *S. serrata* than temperature between 32 to 35°C. The time taken for metamorphosis into subsequent larval stages decreased when temperature increased to 32°C. Similar results also have been reported for the larvae of various brachyuran species (Anger, 1983; Dawirs, 1985; Minagawa, 1990; Choy, 1991; Okamoto, 1993; Hamasaki, 1996).

The best ($P<0.05$) survival rate to the first crab stages (C_1) was obtained at 30°C (Table 1 and Figs. 1-5). Therefore, the optimum rearing temperature for *P. pelagicus* larvae was at 30°C. Hamasaki (2003) found that the optimum temperature for survival *S. serrata* was 29°C. In the same species, Chen and Chen (1985) kept their culture at three temperatures levels of 22°C, 26°C and 30°C. They reported that the optimum range temperature for survival was between 26 to 30°C. Zeng and Li (1991) also concluded that the optimum temperature for the zoea was between 25-30°C. Anger *et al.* (1981) too demonstrated similars trends among another six species of brachyuran crabs (*Rhithropanopeus harrisii*, *Panopeus herbstii*, *Neopanope sayi*, *Menippe mercenaria*, *Sesarma cinereum*, and *Libinia emarginata*) and noted that the optimal temperatures for these larvae ranged between 25 to 30°C.

In this experiment, the first crab stages reared at 28°C displayed the second highest survival but showed longer larval development period. This fact confirms the suggestion that high temperatures to a certain optimal point increase the molting frequency but reduces the larval survival rate of penaeid shrimp (Parado-Estepa, 1998; Kumlu, 2000). This is possibly because of less protein that is being incorporated into the body tissue (Staples and Heales, 1991; O'Brien, 1994). Hill (1974) found that survival of *S. serrata* zoeae over the first 24 hours from hatching is higher (> 90%) at low temperature of 12-25°C than at high temperature of 25°C to 35°C (50-90%). It is likely that the reduced survival rate of unfed larvae at high temperatures is due to the increased metabolic rate and rapid depletion of limited metabolic reserves rather than a consequence of heat stress.

The thermal preferences of blue swimming crab, *P. pelagicus*, may not be uniform throughout life, and results of this study may not be entirely applicable to all developmental stages of *P. pelagicus*. Blue swimming crab post larvae typically recruit into nearshore areas, where water is shallowest and temperatures highest (Kangas, 2000). As the

animals develop and reach first sexual maturity, they gradually migrate to offshore where water is deeper and temperature is cooler. As such, adult blue swimming crabs may be more suited to lower temperatures than postlarvae, and the thermal requirements of later stage growing animals may need to be investigated separately. The present experiment indicated that *P. pelagicus* larvae would sustain maximum premetamorphic survival and successful metamorphosis rates at 28^{o}C to 30^{o}C. Larvae reared at lower temperatures (26^{o}C) experienced both extended intermolt periods and depressed molting increments, whereas larvae held at higher temperature (32-34^{o}C) seemed to maintain normal moltings, but survival rates of premetamorphic and successful metamorphosis were reduced. These findings are in general agreement with those of Whitam (1973), Lellis and Russell (1990) for *Panulirus argus*, Serfing and Ford (1975) for *Panulirus interruptus* and Hamasaki (2002) for *S. serrata*. Whereas temperature affected both intermolt period and molting increment in the present experiment, Serfling and Ford (1975) reported that accelerated growth at elevated temperatures was attributed solely to reduced intermolt period and not to any change in molting size increment.

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CAPTION OF TABLES AND FIGURES

Table 1: Survival rate (% premetamorphic survival* and successful metamorphosis**) and development duration (days) of different stages of zoea (Z1-Z4*), megalopa (M*), and first crab (C1**) of blue swimming crab, P. pelagicus, reared under different temperature. Value within a given column with different superscripts are significantly different (P<0.05). Values are means ± standard errors from three replicate groups of larvae of the P. pelagicus (means ± SE, n = 3), Du, development duration of larval stages (days); initial number: 90 zoea.

Fig. 1: Percentage survivorship and stage of larvae P. pelagicus reared at 26°C.
Fig. 2: Percentage survivorship and stage of larvae P. pelagicus reared at 28°C.
Fig. 3: Percentage survivorship and stage of larvae P. pelagicus reared at 30°C.
Fig. 4: Percentage survivorship and stage of larvae P. pelagicus reared at 32°C.
Fig. 5: Percentage survivorship and stage of larvae P. pelagicus reared at 34°C.
Fig. 6: Relationship between temperature (T) and premetamorphic Z_1 survival (PZ_1SR) of P. pelagicus larvae.
Fig. 7: Relationship between temperature (T) and premetamorphic Z_2 survival (PZ_2SR) of P. pelagicus larvae.
Fig. 8: Relationship between temperature (T) and development duration of P. pelagicus Z_1 larvae (DuZ_1).
Fig. 9: Relationship between temperature (T) and development duration of P. pelagicus Z_2 larvae (DuZ_2).
Fig. 10: Relationship between temperature (T) and premetamorphic Z_3 survival (PZ_3SR) of P. pelagicus Z_3 larvae.
Fig. 11: Relationship between temperature (T) and development duration of P. pelagicus Z_3 larvae (DuZ_3).
Fig. 12: Relationship between temperature (T) and premetamorphic Z_4 survival (PZ_4SR) of P. pelagicus larvae.
Fig. 13: Relationship between temperature (T) and development duration of P. pelagicus Z_4 larvae (DuZ_4).
Fig. 14: Relationship between temperature (T) and premetamorphic M survival (PMSR) of P. pelagicus larvae.
Fig. 15: Relationship between temperature (T) and development duration of P. pelagicus M larvae (DuM).
Fig. 16: Relationship between temperature (T) and successful metamorphosis of P. pelagicus C_1 larvae ($SuMpC_1$).
Fig. 17: Relationship between temperature (T) and development duration of P. pelagicus C_1 larvae (DuC_1).

Table 1.

Temperature	Variable	Z_1	Z_2	Z_3	Z_4	M	C_1
26°C	% ± SE	95.56 ± 2.72^{a}	87.78 ± 2.72^{a}	74.44 ± 3.60^{ab}	62.22 ± 2.72^{a}	50.00 ± 2.36^{a}	25.56 ± 3.60^{b}
	Du ± SE	6.67 ± 0.41^{A}	5.33 ± 0.41^{A}	5.00 ± 0.00^{A}	8.67 ± 0.41^{A}	8.67 ± 0.41^{A}	5.67 ± 0.41^{A}
28°C	% ± SE	95.56 ± 1.36^{a}	83.33 ± 4.08^{a}	77.78 ± 1.36^{a}	65.56 ± 2.72^{a}	44.44 ± 4.91^{a}	31.11 ± 3.60^{ba}
	Du ± SE	4.67 ± 0.41^{B}	5.33 ± 0.41^{A}	5.00 ± 0.00^{A}	7.00 ± 0.00^{B}	8.33 ± 0.41^{A}	5.67 ± 0.41^{A}
30°C	% ± SE	93.33 ± 2.36^{a}	84.44 ± 5.44^{a}	77.78 ± 5.93^{a}	66.67 ± 6.24^{a}	48.89 ± 3.60^{a}	36.67 ± 2.36^{a}
	Du ± SE	4.00 ± 0.00^{B}	5.00 ± 0.00^{A}	3.67 ± 0.41^{B}	6.67 ± 0.41^{B}	6.33 ± 0.41^{B}	3.67 ± 0.41^{B}
32°C	% ± SE	90.00 ± 2.36^{a}	78.89 ± 3.60^{a}	68.89 ± 2.72^{ab}	56.67 ± 2.36^{a}	42.22 ± 3.60^{a}	23.33 ± 4.71^{b}
	Du ± SE	4.00 ± 0.00^{B}	4.67 ± 0.41^{A}	3.67 ± 0.41^{B}	7.00 ± 0.00^{B}	6.67 ± 0.41^{B}	3.67 ± 0.41^{B}
34°C	% ± SE	90.00 ± 4.71^{a}	77.78 ± 1.36^{a}	65.56 ± 2.72^{b}	63.33 ± 4.71^{a}	30.00 ± 4.08^{b}	12.22 ± 1.36^{c}
	Du ± SE	4.67 ± 0.41^{B}	5.67 ± 0.41^{A}	4.00 ± 0.00^{B}	7.00 ± 0.00^{B}	7.00 ± 0.00^{B}	4.00 ± 0.00^{B}

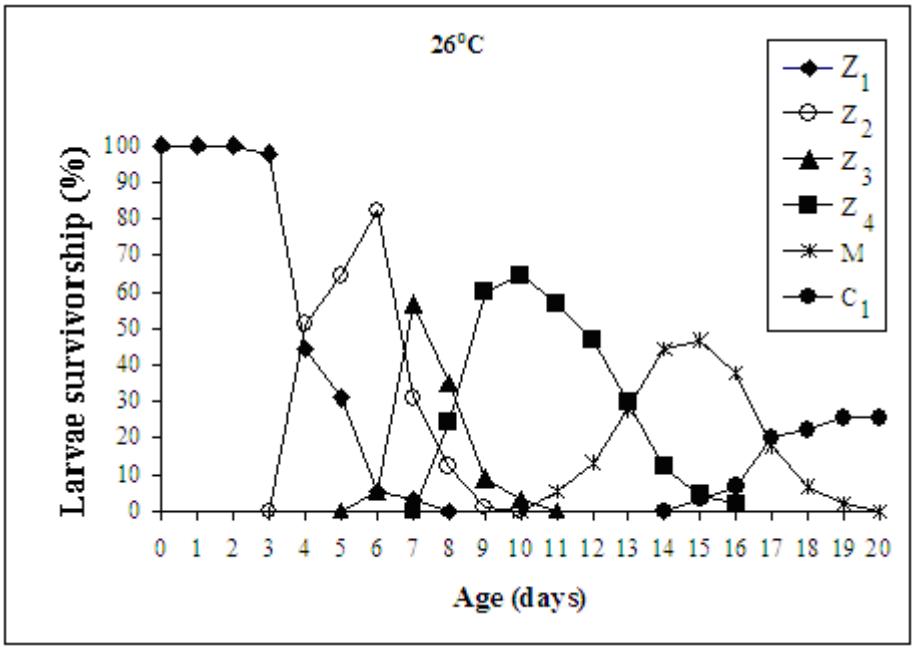
26°C
Z1
Z2
Z3
Z4
M
C1
Larvae survivorship (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Age (days)

Fig. 1

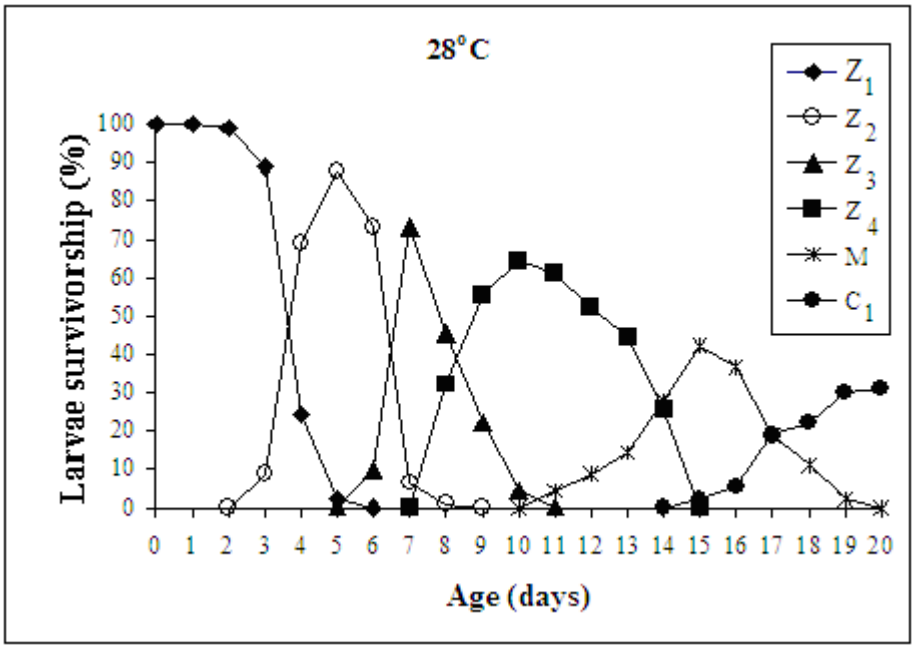
28°C
Z1
Z2
Z3
Z4
M
C1
Larvae survivorship (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Age (days)

Fig. 2

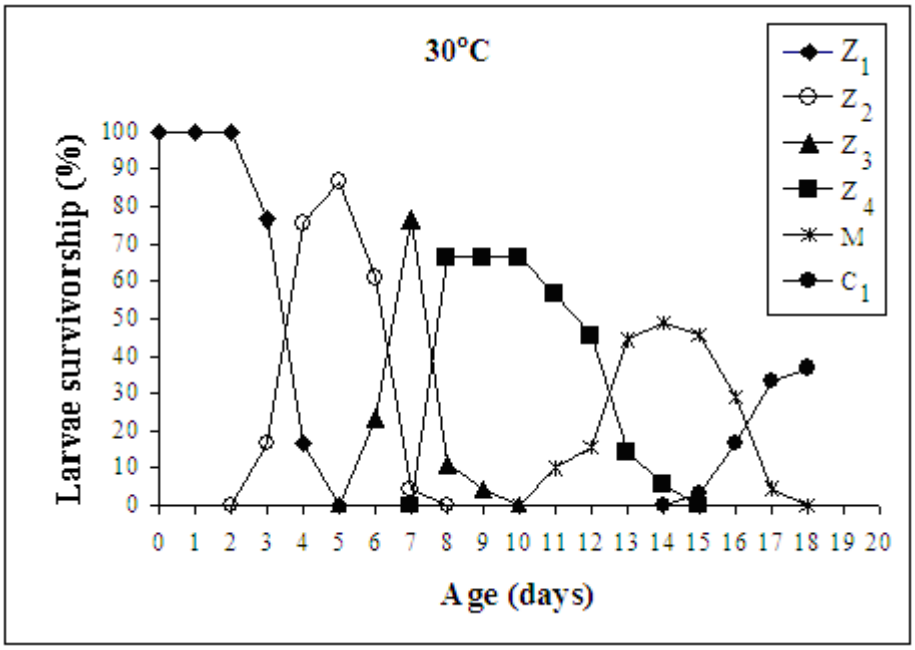
30°C
Larvae survivorship (%)
Age (days)
Z1
Z2
Z3
Z4
M
C1

Fig. 3

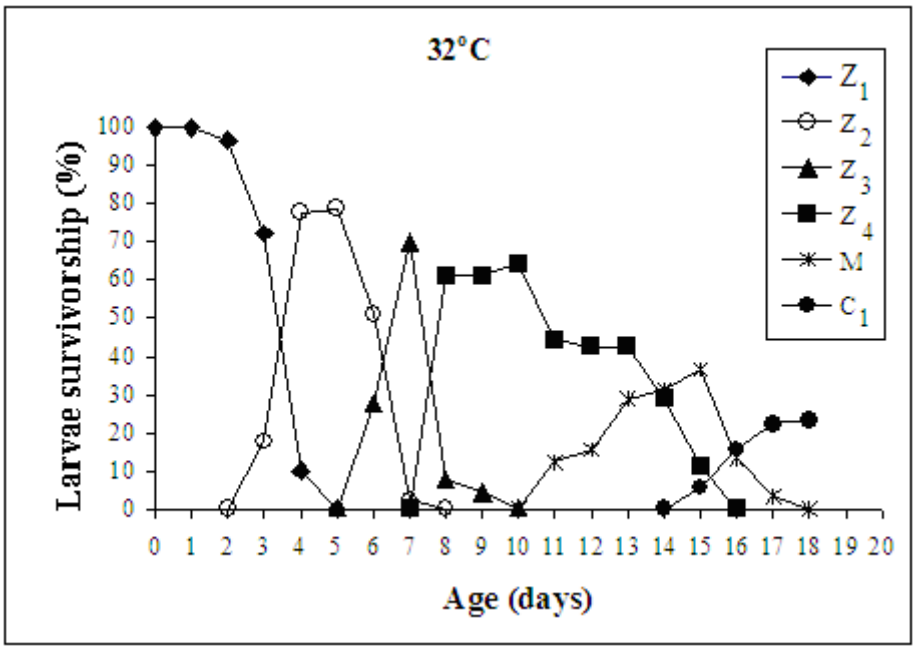
32°C
Larvae survivorship (%)
Age (days)
Z1
Z2
Z3
Z4
M
C1

Fig. 4

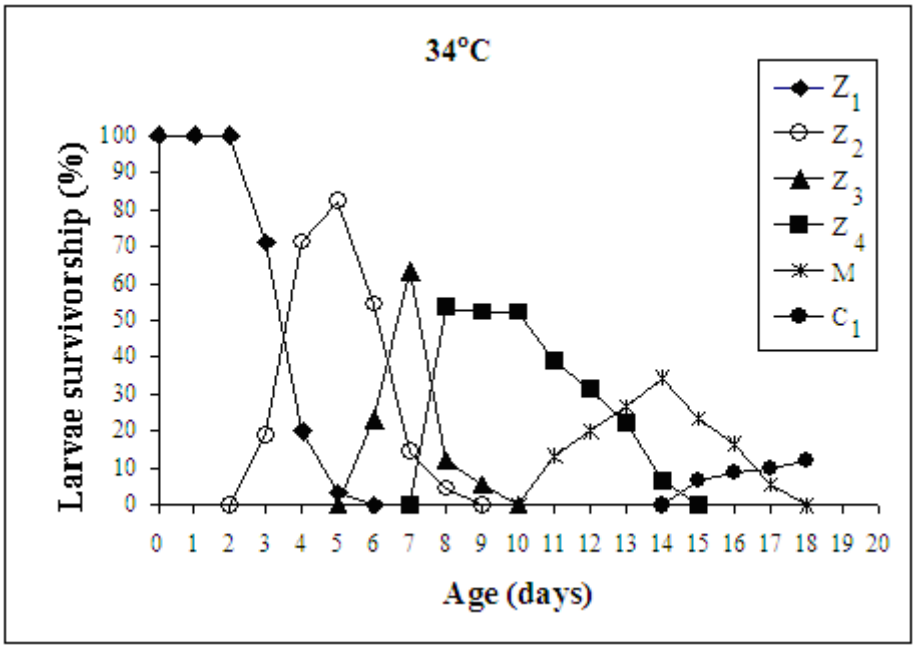
34°C
Larvae survivorship (%)
Age (days)
Z1
Z2
Z3
Z4
M
C1

Fig. 5

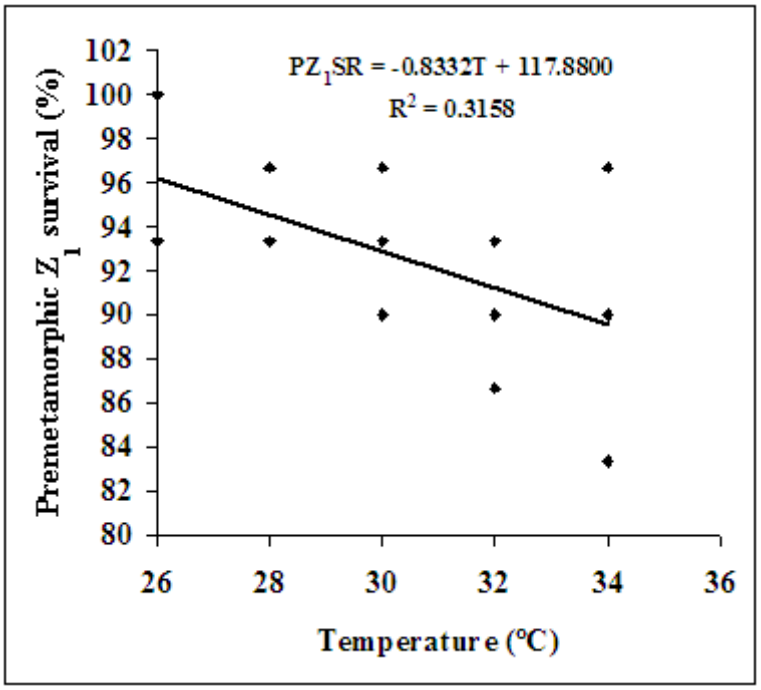
PZ1SR = -0.8332T + 117.8800
R2 = 0.3158
Premetamorphic Z1 survival (%)
Temperature (°C)

Fig. 6

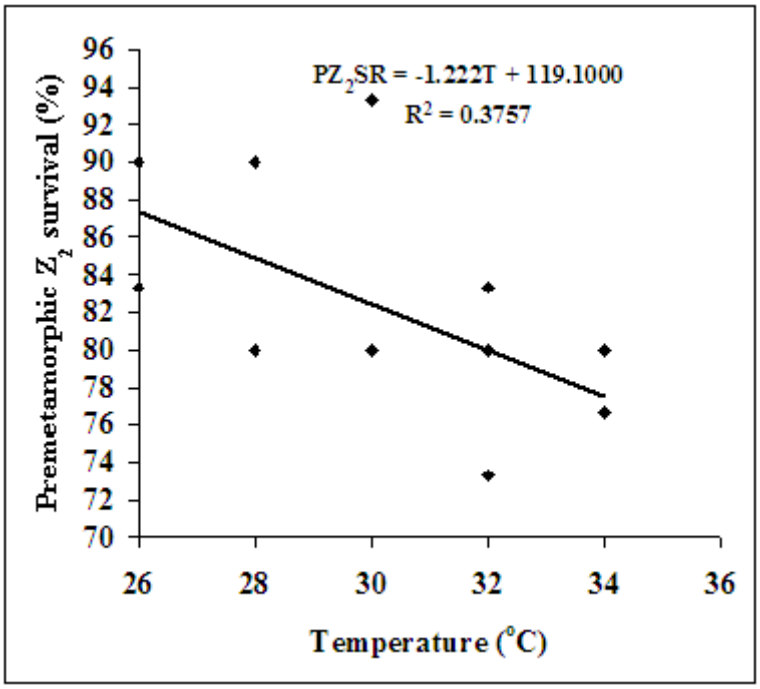
PZ2SR = -1.222T + 119.1000
R2 = 0.3757
Premetamorphic Z2 survival (%)
96
94
92
90
88
86
84
82
80
78
76
74
72
70
26
28
30
32
34
36
Temperature (°C)

Fig. 7

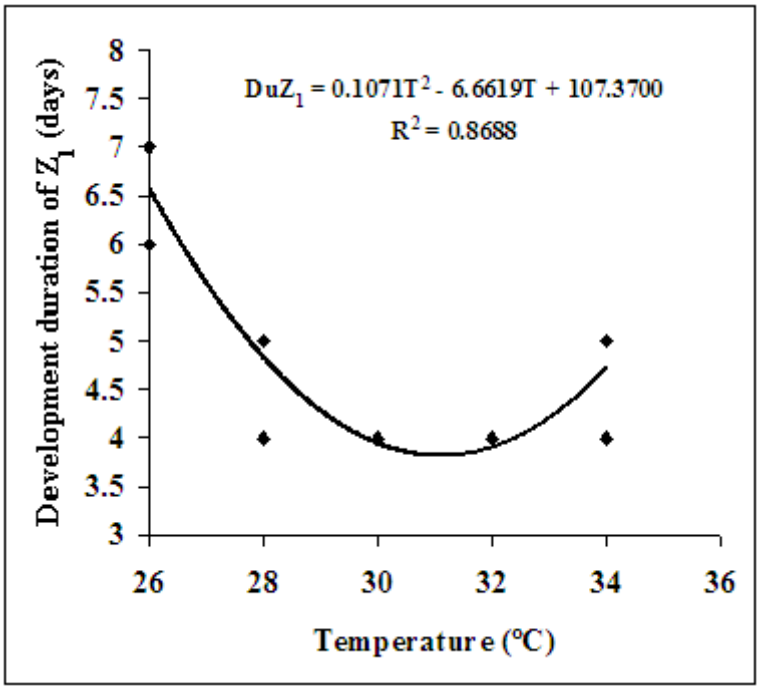
DuZ1 = 0.1071T2 - 6.6619T + 107.3700
R2 = 0.8688
Development duration of Z1 (days)
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
26
28
30
32
34
36
Temperature (°C)

Fig. 8

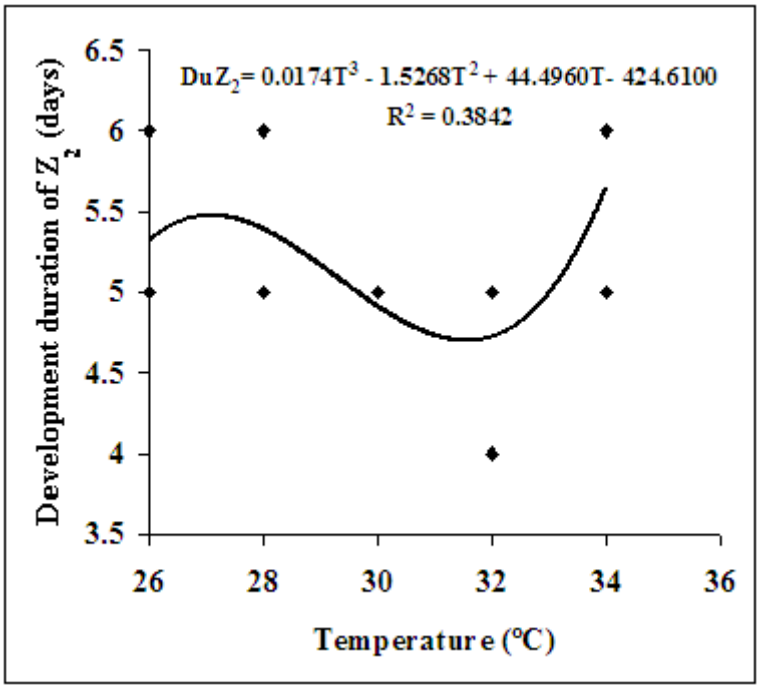
DuZ2= 0.0174T3 - 1.5268T2 + 44.4960T- 424.6100
R2 = 0.3842
Development duration of Z2 (days)
6.5
6
5.5
5
4.5
4
3.5
26
28
30
32
34
36
Temperature (°C)

Fig. 9

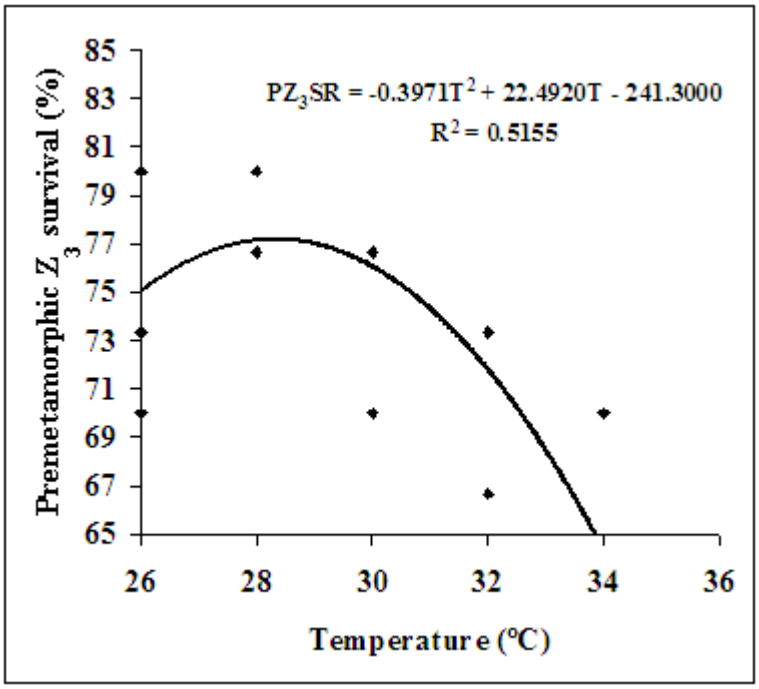
PZ3SR = -0.3971T2 + 22.4920T - 241.3000
R2 = 0.5155
Premetamorphic Z3 survival (%)
85
83
81
79
77
75
73
71
69
67
65
26
28
30
32
34
36
Temperature (°C)

Fig. 10

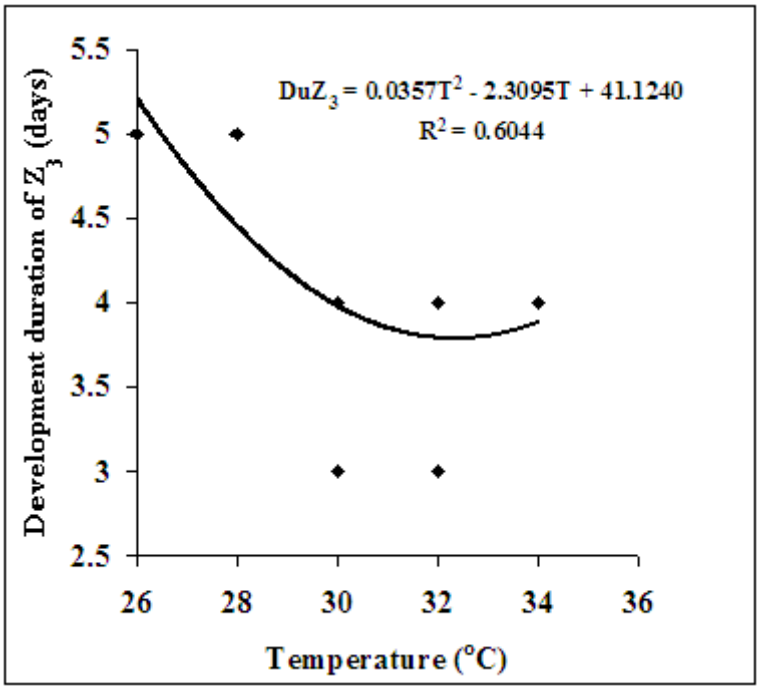
$DuZ_3 = 0.0357T^2 - 2.3095T + 41.1240$
$R^2 = 0.6044$
Development duration of Z_3 (days)
5.5
5
4.5
4
3.5
3
2.5
26
28
30
32
34
36
Temperature (°C)

Fig. 11

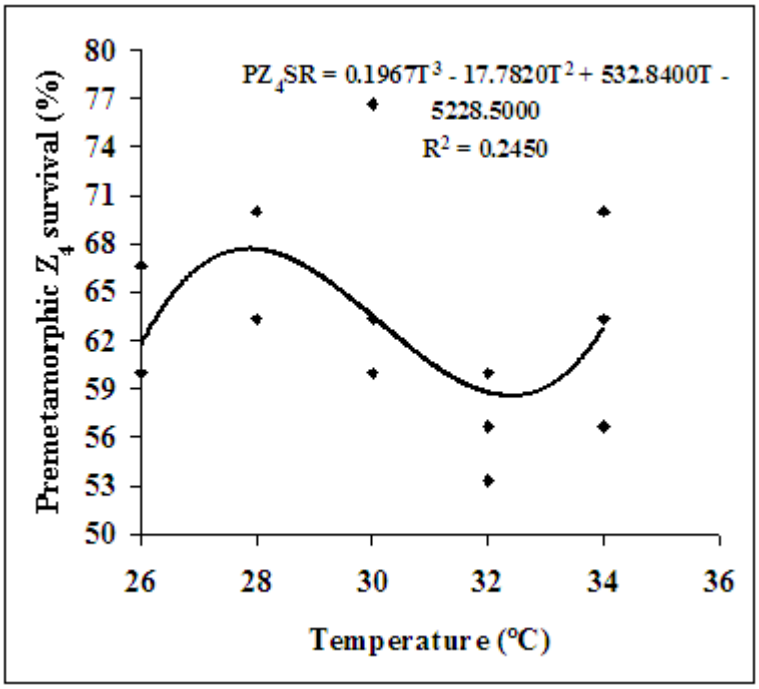
$PZ_4SR = 0.1967T^3 - 17.7820T^2 + 532.8400T - 5228.5000$
$R^2 = 0.2450$
Premetamorphic Z_4 survival (%)
80
77
74
71
68
65
62
59
56
53
50
26
28
30
32
34
36
Temperature (°C)

Fig. 12

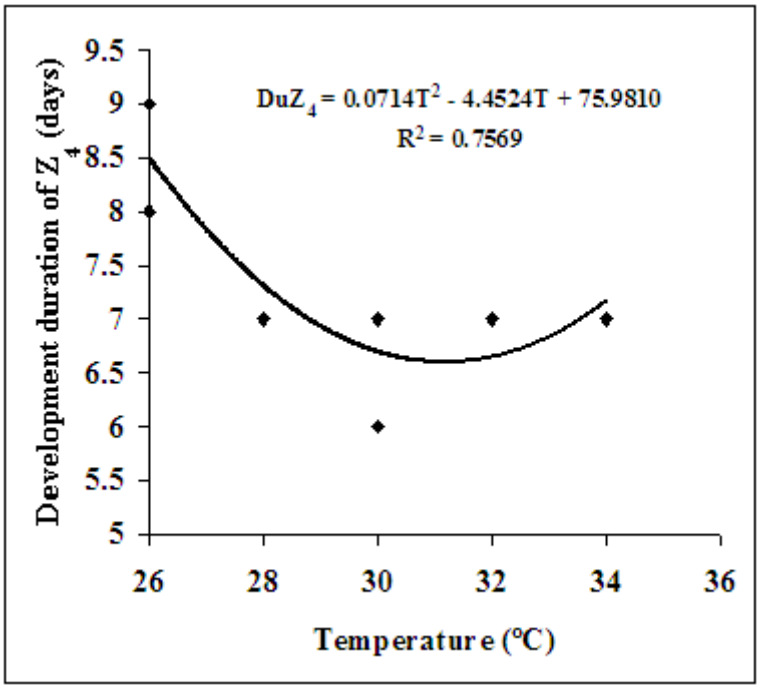
DuZ4 = 0.0714T2 - 4.4524T + 75.9810
R2 = 0.7569
Development duration of Z4 (days)
Temperature (°C)
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
26
28
30
32
34
36

Fig. 13

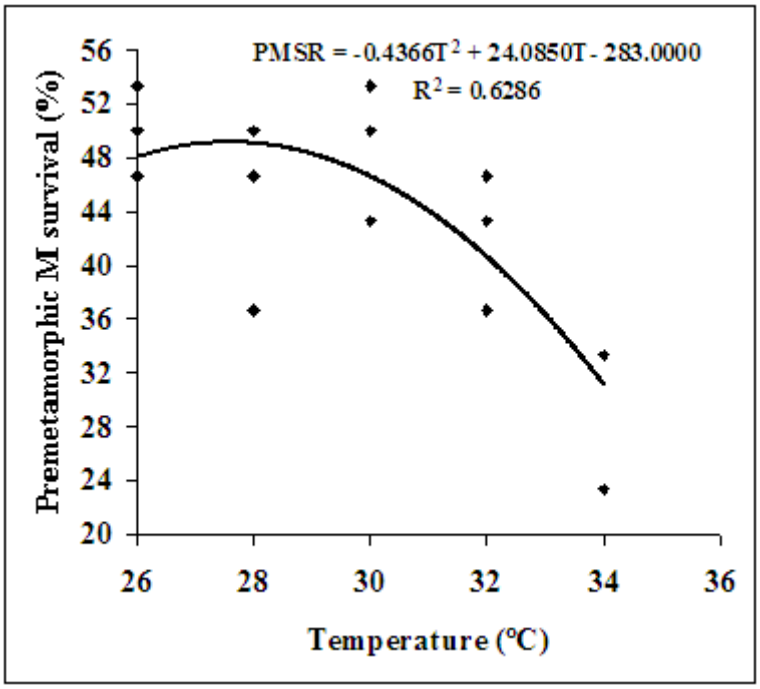
PMSR = -0.4366T2 + 24.0850T - 283.0000
R2 = 0.6286
Premetamorphic M survival (%)
Temperature (°C)
56
52
48
44
40
36
32
28
24
20
26
28
30
32
34
36

Fig. 14

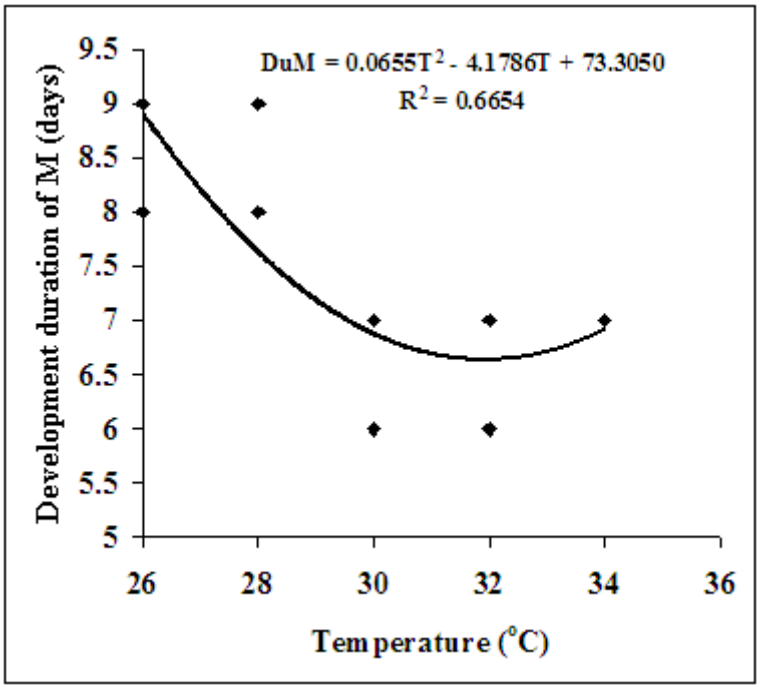
DuM = 0.0655T² - 4.1786T + 73.3050
R² = 0.6654
Development duration of M (days)
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
26
28
30
32
34
36
Temperature (°C)

Fig. 15

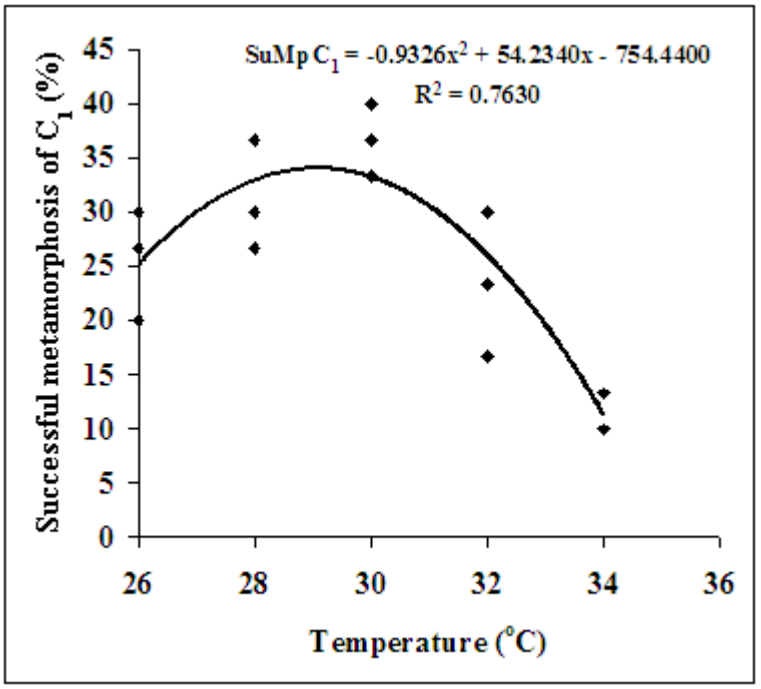
SuMp C1 = -0.9326x² + 54.2340x - 754.4400
R² = 0.7630
Successful metamorphosis of C1 (%)
45
40
35
30
25
20
15
10
5
0
26
28
30
32
34
36
Temperature (°C)

Fig. 16

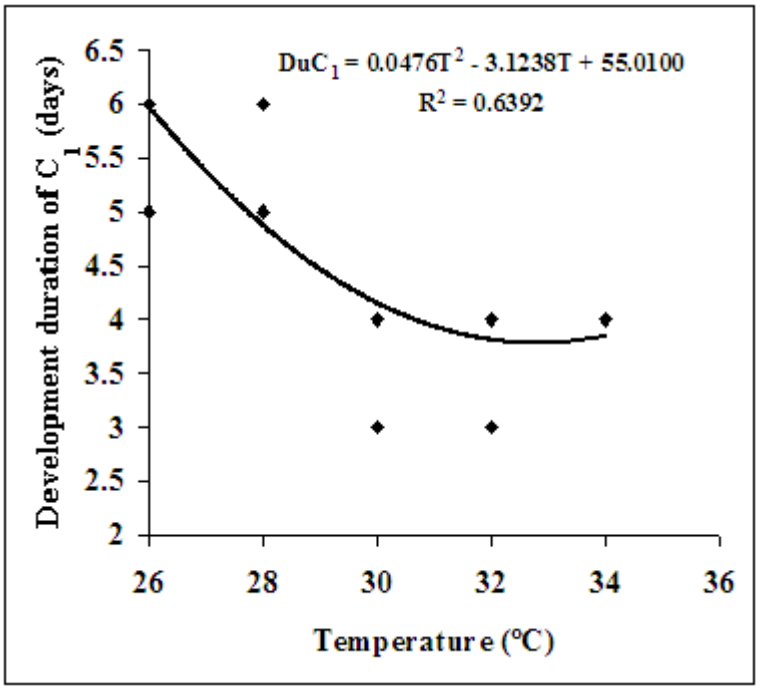
DuC1 = 0.0476T2 - 3.1238T + 55.0100
R2 = 0.6392
Development duration of C1 (days)
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
26
28
30
32
34
36
Temperature (°C)

Fig. 17