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Studies on the effect of low temperature on the duration of embryonic life, hatching percentage, wet weight, water and glucose levels and acid phosphatase activity during embryogenesis in eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)

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ABSTRACT

Laboratory experiments were performed to study the effects of low temperature on the duration of embryonic life, hatching percentage, wet weight, water content, glucose levels and acid phosphatase activity during embryonic development in the rice moth, *Corcyra cephalonica*. A significant increase occurred in the duration of embryonic life with decreasing temperature. Lowering the temperature from 28°C to 24°C did not affect the hatching percentage of eggs. However, when incubated at 28°C, wet weight and water content of eggs significantly decreased with advancing egg age up to 40-48 h and 32-40 h respectively. Moreover, the rate of decrease in wet weight and water content lowered with decreasing incubation temperature. A significant decrease in glucose levels (µg/egg and µg/mg wet weight of egg sample) occurred during the initial stages followed by an increase, during the later stages of embryogenesis in eggs incubated at all three temperatures, although the levels of variation decreased with decreasing temperature. Acid phosphatase activity showed a more or less, sigmoid pattern of change, with a lowering rate of increase, corresponding to decreasing incubation temperature.

Keywords: Acid phosphatase, *Corcyra*, duration of embryonic life, glucose, hatching percentage, temperature, water content, wet weight.

INTRODUCTION

The rice moth *Corcyra cephalonica* (Stainton, 1866) is a notorious pest of stored commodities, especially cereal products and found in Asia, North America, Europe and other tropical and subtropical regions of the world. Its larval stages cause substantial damage to rice, gram, maize, nuts, cotton seeds, peanuts, linseeds, raisins, nutmeg, currants, chocolate, army biscuits and milled products (Atwal, 1976; Piltz, 1977). This pest is adapted for terrestrial mode of life and exists throughout the year. The effects of temperature on various aspects of *Shijimiaeoides divinus barine* have been studied by Nakamura *et al.* (2008) and Koda & Nakamura (2009). Komeyama & Hoshikawa (2007) studied the effect of temperature on larval growth of *Celastrina augitanii*. Lu *et al*. (2009) performed a comparative study of the temperature-dependent life histories of three economically important *Adelphocoris* species. Biochemical investigations of various aspects of the embryonic biochemistry of *C. cephalonica* have also been performed (Chaubey & Bhatt, 1988; Chaubey *et al*., 2002; Chaubey & Misra, 2004; Chaubey, 2007; Chaubey *et al*., 2008).

Insect embryogenesis is characterized by adaptations to the terrestrial mode of life. Terrestrial propagation requires protection of the eggs against desiccation (Sander *et*

al., 1985). Considerable changes in water content (Agrell & Lundquist, 1973), usually loss (Ludwig & Ramazzotto, 1965; Kinsella & Smyth, 1966), may occur during the development of insect eggs. The rate of water loss under various conditions has been studied previously in the eggs of *Rhodnius* (Beament, 1946), *Locusta* (Matthee, 1951) and grasshopper (Leese, 1976). The eggs of *Rhodnius* and probably of many other heteropteran and lepidopteran insects, which are laid in dry, exposed situations, develop without any uptake of water. The moisture content of orthopteran eggs, however, increases significantly (Furneaux & Mc Farlane, 1965; Grellet, 1967, Moloo, 1971). The eggs of many other insects also absorb, water from the environment, which results in a considerable increase in their volume and weight (Chapman, 2000).

Carbohydrates along with proteins and lipids form the principal classes of organic compounds that are found in insects and other organisms. During the embryonic development of insects, yolk carbohydrates and lipids provide the main substrates for energy production. In developing eggs, glycogen is mobilized as glucose and trehalose during embryonic development (Hill, 1945; Chino, 1958; Agrell & Lundquist, 1973; Chippendale, 1978; Bhatt & Krishna, 1982).

Research using both histochemical and biochemical methods to map enzyme patterns in developing has been carried out previously. The wide occurrence of phosphatases in animal tissues is thought to be associated with the (a) transport of metabolites, (b) metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates and (c) synthesis of proteins. The importance of these enzymes in embryonic tissues has also been pointed out by Moog (1946) and Boell (1955). Using histochemical techniques, Yao (1950) reported that acid phosphatase is present in all developmental stages of *Drosophila*, but they found no apparent changes in this enzyme throughout embryogenesis. Biochemical studies on the activities of acid and alkaline phosphatases have been carried out on the eggs of silkworm (Fitzgerald, 1949), grasshopper (Chino, 1961; Sridhara & Bhat, 1963), termite *Odontotermes* (Banerjee, 1964), rice moth (Bhatt & Krishna, 1980; Chaubey & Bhatt, 1988 and Chaubey *et al.* (2002) and house fly (*Musca domestia*) (Ribolla *et al.*, 1992), during oogenesis in cockroach (*Periplaneta americana*) (Oliveira & Machado, 2006), and in the developing eggs of *Spodoptera littoralis* (Mostafa *et al*., 2009).

However, till date, no studies have been conducted on the effect of temperature on water and glucose (reducing sugar) levels and acid and alkaline phosphatase activities, during embryogenesis in the eggs of *C. cephalonica*. The present investigation was, therefore, undertaken with the aim to elucidate the mechanism by which this pest survives periods of low temperature.

MATERIAL AND METHODS

Rice moths, *Corcyra cephalonica* (Stainton, 1866), were reared in the laboratory as described by Chaubey & Bhatt (1988). To prepare egg samples of different age groups, freshly emerged male and female moths were collected and transferred to oviposition chambers. Eggs were collected at regular intervals of 8 h. Collected eggs were counted and weighed to prepare egg samples, with each sample consisting of 500 eggs. To investigate the effect of low temperature on duration of embryonic life the eggs were kept at 26 ± 0.5°C and 24 ± 0.5°C in a BOD incubator. Control eggs were kept at 28 ± 0.5°C. Since control eggs hatched within 90-96 h, biochemical studies were limited to 96 h for control eggs. Lowering in temperature caused increase in duration of embryonic life so experimental egg samples were consisted of 0-8 h, 8-16 h, 16-24 h up to 136-144 h for 26

± 0.5°C and up to 184-192 h for 24 ± 0.5°C. The temperature was maintained almost constant except for a few interruptions due to occasional break in the electric supply.

The wet weight of eggs of different age groups, (2000 eggs/age group) was obtained by weighing them on a single-pan electronic balance. To obtain the water content, egg samples were dried in an electric oven at 60°C for 24 h and then weighed in the same manner. To confirm the dry weights of eggs, all samples were kept at 60°C for additional 24 h and weighed in the same manner. The water content of the eggs (µg/egg) was calculated on the basis of the wet and dry weight measurements.

The method of Folin & Wu (1920) was used to estimate glucose (reducing sugar). Egg homogenates (125 eggs/ml) were made in ice cold double-distilled water and deproteinized by adding 4 ml of tungstic acid (prepared by addition of 0.5 ml of 0.66 N H_2SO_4 to 0.5 ml of sodium tungstate solution). After thorough mixing, the total homogenates were added for deproteinization. After mixing well, the total contents were centrifuged at 5000 g for 20 min at 0°C and supernatants were saved for glucose estimation. For colour development, an equal volume of alkaline copper was added to each Folin and Wu tube containing 2 ml of supernatant. Alkaline copper was prepared by dissolving 40 g of pure anhydrous sodium carbonate in 400 ml of double-distilled water in a 1 L flask and mixing thoroughly. Then, 7.5 g of tartaric acid was added and after its dissolution, 4.5 g of crystallized copper sulphate was added. After thorough mixing the volume was made to 1 L by addition of double-distilled water. After thorough mixing, the contents were heated in a boiling water bath for exactly 8 min and cooled under tap water. To each tube, 2 ml of phosphomolybdic acid reagent was added.. Phosphomolybdic acid reagent was prepared by mixing 35 g molybdic acid, 5 g of sodium tungstate, 200 ml of 10% (w/v) NaOH and 200 ml of double-distilled water. After thorough mixing, the mixture was boiled for 40 min to remove the ammonia present in the molybdic acid, cooled and diluted to 135 ml with double-distilled water and 125 ml of concentrated. (85%, w/v) phosphoric acid was added. The volume was made up to 500 ml with double-distilled water and the mixture was allowed to stand for 5 min for development of a blue colour. Following this the volume in each tube was made up to 25 ml by adding double-distilled water and mixing thoroughly. The absorbance of each sample was measured at 420 nm against a blank, which was prepared simultaneously using 2 ml of double-distilled water instead of egg supernatant. The absorbance was compared with that of known concentrations of glucose (0.125-0.5 mg/ml). The glucose values were calculated as µg glucose/egg and, µg glucose/mg wet weight of the eggs.

Acid phosphatase activity was measured by the method of Bergmeyer (1967). Egg homogenates (125 eggs/ml) were prepared in ice-cold 0.9% NaCl and centrifuged at 5000 g for 15 min at 4°C. Supernatants of 0.2 ml were incubated with 1 ml acid buffer substrate solution (0.05 M citrate buffer, 5.5×10^{-3} M, sodium salt of p-nitrophenyl phosphate, pH 4.8) for 30 min at 37°C to estimate acid phosphatase activity. The reaction was stopped by the addition of an excess of NaOH solution. A yellow colour developed, and its absorbance was measured at 420 nm. Standard curves were derived from known solutions of p-nitrophenol of different concentrations in 0.02 N NaOH. We calculated the amount of p-nitrophenol liberated in each tube by comparing the absorbance of a known solution with that of the unknown solution. The protein content of the enzyme source was measured according to the method of Lowry *et al.* (1951). Results were expressed as µ moles of p-nitrophenol liberated/30 min/mg protein.

STATISTICAL ANALYSIS

Each experiment was repeated six times and the values were expressed as mean ± S.D. Student's t-test was used to compare two sample means.

RESULTS

Relationship between duration of embryonic life and temperature

The effect of temperature on the duration of embryonic life of *C. cephalonica* is shown in Table (1). The minimum duration was 96 h at 28°C. As the temperature decreased to 26°C and 24°C, there was a significant increase in the duration of embryonic life.

Table 1: Effect of temperature on duration of embryonic development and hatching percentage of eggs in *Corcyra cephalonica* (Mean ± S.D.).

Temperature of incubation (°C)	Duration of embryonic life in hours (h)	Hatching percentage
28 ± 0.5	96 ± 07	75 ± 11
26 ± 0.5	144 [a] ± 08	74 [*] ± 09
24 ± 0.5	192 [a] ± 10	76 [*] ± 12

Significance level [a]0.001 and [*]not statistically significant when compared with adjacent means.

Relationship between hatching percentage and temperature

As is evident from Table (1), a decrease in temperature from 28°C to 24°C did not affect the hatching percentage of the eggs.

Relationship between temperature and egg wet weight

A comparison of the wet weight of eggs from different age groups incubated at 28°C clearly indicated that, eggs, in the 0-8 h age group were the heaviest, and there was a significant reduction in wet weight as the age of eggs advanced to 8-16, 16-24, 24-32 and 32-40 h. However, this reduction was not significant in the age groups 40-48, 48-56, 56-64, 64-72, 72-80, 80-88 and 88-96 h, as well as in eggs incubated at 24°C. Lowering the temperature caused a gradual decrease in the wet weight of eggs incubated at 24°C, following a more or less similar pattern of variation (Table 2).

Table 2: Changes in the wet weight of the eggs (µg/egg) at different temperatures during embryonic development of *C. cephalonica* (Mean ± S.D.).

Age of egg samples (h after oviposition)	Wet weight of the eggs at 28°C	Wet weight of the eggs at 26°C	Wet weight of the eggs at 24°C
0-8	36.17 ± 0.46	36.21 ± 0.48	36.23 ± 0.43
8-16	35.05[c] ± 0.43	35.76[*] ± 0.41	36.07[*] ± 0.41
16-24	34.23[c] ± 0.41	35.65[*] ± 0.42	35.89[*] ± 0.42
24-32	33.19[c] ± 0.47	34.93[c] ± 0.45	35.58[*] ± 0.46
32-40	32.18[c] ± 0.45	34.82[*] ± 0.41	35.47[*] ± 0.43
40-48	31.18[c] ± 0.43	34.76[*] ± 0.35	35.31[*] ± 0.47
48-56	31.16[*] ± 0.46	33.89[c] ± 0.47	35.08[*] ± 0.42
56-64	30.38[d] ± 0.42	33.57[*] ± 0.45	34.97[*] ± 0.39
64-72	30.26[*] ± 0.45	32.85[c] ± 0.42	34.78[*] ± 0.42
72-80	30.19[*] ± 0.47	32.59[*] ± 0.41	34.59[*] ± 0.38
80-88	30.17[*] ± 0.46	31.35[*] ± 0.39	34.47[*] ± 0.37
88-96	30.16[*] ± 0.43	31.27[*] ± 0.42	34.38[*] ± 0.41
96-104		31.19[*] ± 0.46	33.89[d] ± 0.35
104-112		31.05[*] ± 0.42	33.67[*] ± 0.43
112-120		30.67[*] ± 0.43	33.51[*] ± 0.42
120-128		30.51[*] ± 0.38	33.43[*] ± 0.41
128-136		30.29[*] ± 0.47	33.24[*] ± 0.39
136-144		30.19[*] ± 0.42	33.03[*] ± 0.41
144-152			32.86[*] ± 0.38
152-160			32.57[*] ± 0.42
160-168			32.42[*] ± 0.39
168-176			32.05[*] ± 0.42
176-184			31.75[*] ± 0.34
184-192			31.63[*] ± 0.41

Significance level [c]0.05, [d]0.1 and [*]not statistically significant when compared with adjacent means.

Relationship between temperature and water content

The amount of water present in the eggs (µg/egg) reduced significantly, with advancing age, from 0-8 to 32-40 h, after which the level became more or less constant.

The level increased significantly in eggs aged 88-96 h at 28°C. The pattern of change in water content, in eggs incubated at lower temperatures was similar to that seen at 28°C, but with a slower rate (Table 3).

Table 3: Effect of temperature on water content (µg/egg) of eggs during embryonic development in *C. cephalonica* (Mean ± S.D.).

Age of egg samples (h after oviposition)	Water content (µg/egg) at 28°C	Water content (µg/egg) at 26°C	Water content (µg/egg) at 24°C
0-8	27.29 ± 0.29	27.31 ± 0.29	27.29 ± 0.23
8-16	26.25 [b] ± 0.31	26.85[d] ± 0.27	27.03 [*] ± 0.31
16-24	25.71 [c] ± 0.27	26.41[c] ± 0.31	26.81 [*] ± 0.21
24-32	23.03 [a] ± 0.32	26.01[c] ± 0.28	26.41 [c] ± 0.25
32-40	22.37 [c] ± 0.28	25.51[d] ± 0.31	26.07 [d] ± 0.29
40-48	22.39 [*] ± 0.31	24.72[c] ± 0.33	25.65 [d] ± 0.21
48-56	22.38 [*] ± 0.27	24.11[c] ± 0.34	25.26 [d] ± 0.31
56-64	22.36 [*] ± 0.28	23.67[d] ± 0.31	24.84 [d] ± 0.27
64-72	22.35 [*] ± 0.27	22.43[b] ± 0.29	24.36 [d] ± 0.32
72-80	22.41 [*] ± 0.25	22.41[*] ± 0.30	23.95 [c] ± 0.29
80-88	22.73 [*] ± 0.26	22.39[*] ± 0.28	23.24 [d] ± 0.31
88-96	23.19 [c] ± 0.21	22.38[*] ± 0.26	22.87 [d] ± 0.32
96-104		22.41[*] ± 0.29	22.45 [*] ± 0.31
104-112		22.39[*] ± 0.32	22.43 [*] ± 0.28
112-120		22.37[*] ± 0.30	22.42 [*] ± 0.33
120-128		22.38[*] ± 0.28	22.43 [*] ± 0.24
128-136		22.56[*] ± 0.24	22.45 [*] ± 0.27
136-144		22.85[*] ± 0.27	22.43 [*] ± 0.37
144-152		23.19[d] ± 0.23	22.41 [*] ± 0.29
152-160			22.47 [*] ± 0.31
160-168			22.59 [*] ± 0.28
168-176			22.73 [*] ± 0.21
176-184			22.91 [*] ± 0.25
184-192			23.18 [*] ± 0.24

Significance level [a]0.001, [b]0.01, [c]0.05, [d]0.1 and [*]not statistically significant when compared with adjacent means.

Relationship between temperature and glucose levels

As is evident from Table (4), the levels of glucose (µg/egg and µg/mg wet weight of egg sample) decreased significantly with advancing age up to 24-32 h at 28°C and up to 56-64 h at 26°C and 24°C. The level of glucose increased continuously with each advancing age group from 32-40 h at 28°C and 64-72 h at 26°C and 24°C.

Relationship between temperature and acid phosphatase activity

At all three temperatures (28°C, 26°C and 24°C) acid phosphatase activity (µ moles of p-nitrophenol liberated/ 30 min/mg protein) was the lowest in newly laid egg samples (0-8 h), and increased significantly with each advancing age group up to the stage of hatching. The level of activity followed a sigmoid pattern at all temperatures. Lowering the temperature caused a reduction in the rate of increase in enzyme activity but did not affect its pattern of variation (Table 5 & Fig. 1).

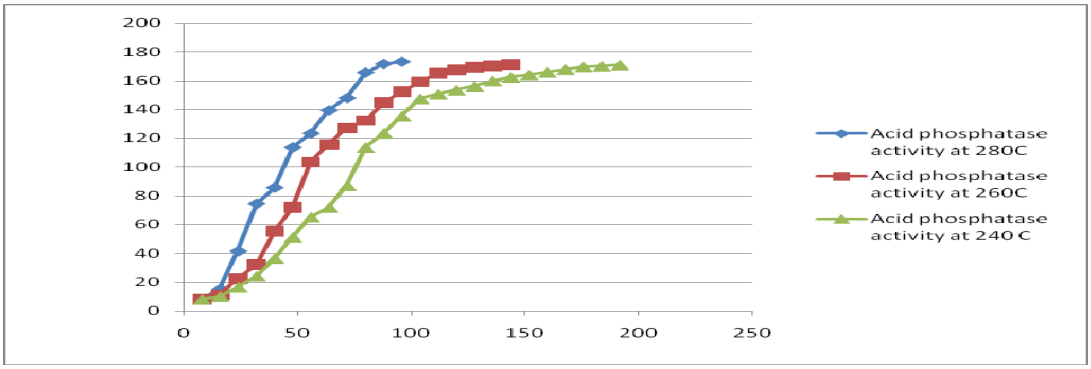
Table 4: Changes in the levels of glucose (µg/egg and µg/mg wet weight of the eggs) at different temperatures in the eggs during embryonic development of *C. cephalonica* (Mean ± S.D.)

Age of egg samples (h after oviposition	Glucose levels at 28°C		Glucose levels at 26°C		Glucose levels at 24°C	
	(µg/egg)	(µg/mg wet weight of egg)	(µg/egg)	(µg/mg wet weight of egg)	(µg/egg)	(µg/mg wet weight of egg)
0-8	0.321 ±0.0038	8.87 ± 0.181	0.319 ± 0.0035	8.81 ± 0.192	0.323 ± 0.0032	8.92 ± 0.195
8-16	0.113[a] ± 0.0035	3.26[a] ± 0.192	0.207[a] ± 0.0038	5.79[a] ± 0.190	0.279[a] ± 0.0037	7.73[a] ± 0.185
16-24	0.087[a] ± 0.0040	2.52[b] ± 0.172	0.165[a] ± 0.0034	4.63[a] ± 0.186	0.216[a] ± 0.0029	6.02[a] ± 0.187
24-32	0.107[a] ± 0.0034	3.22[b] ± 0.181	0.121[a] ± 0.0041	3.46[a] ± 0.185	0.164[a] ± 0.0034	4.61[a] ± 0.168
32-40	0.110[*] ± 0.0037	3.42[*] ± 0.165	0.113[c] ± 0.0036	3.25[*] ± 0.187	0.141[a] ±0.0035	3.98[b] ± 0.176
40-48	0.113[*] ±0.0036	3.62[*] ± 0.167	0.121[c] ± 0.0037	3.48[*] ± 0.188	0.121[a] ± 0.0039	3.43[b] ± 0.184
48-56	0.116[*] ± 0.0032	3.72[*] ± 0.164	0.127[d] ± 0.0038	3.75[d] ± 0.192	0.097[a] ±0.0037	2.77[b] ± 0.190
56-64	0.125[b] ± 0.0038	4.11[c] ± 0.168	0.132[d] ± 0.00335	3.93[*] ± 0.176	0.105[c] ± 0.0041	3.00[d] ± 0.167
64-72	0.131[d] ±0.0041	4.33[d] ± 0.158	0.135[*] ± 0.0037	4.11[*] ± 0.187	0.107[*] ± 0.0038	3.08[*] ± 0.183
72-80	0.137[d] ±0.0039	4.54[d] ± 0.163	0.137[*] ± 0.0042	4.20[*] ± 0.184	0.112[d] ± 0.0037	3.24[*] ± 0.179
80-88	0.141[*] ±0.0037	4.67[*] ± 0.164	0.139[*] ± 0.0040	4.43[*] ± 0.179	0.115[*] ± 0.0035	3.34[*] ± 0.181
88-96	0.149[c] ± 0.0038	4.94[c] ± 0.158	0.142[*] ± 0.0041	4.54[*] ± 0.175	0.119[*] ± 0.0040	3.46[*] ± 0.187
96-104			0.145[*] ± 0.0038	4.65[*] ± 0.186	0.124[*] ± 0.0042	3.66[*] ± 0.190
104-112			0.147[*] ± 0.0036	4.73[*] ± 0.169	0.127[*] ± 0.0039	3.77[*] ± 0.195
112-120			0.147[*] ± 0.0039	4.79[*] ± 0.173	0.131[*] ± 0.0041	3.91[*] ± 0.186
120-128			0.149[*] ± 0.0034	4.88[*] ± 0.168	0.135[*] ± 0.0042	4.04[*] ± 0.178
128-136			0.148[*] ± 0.0038	4.89[*] ± 0.172	0.138[*] ± 0.0041	4.15[*] ± 0.159
136-144			0.151[*] ± 0.0034	5.00[*] ± 0.176	0.141[*] ± 0.0038	4.27[*] ± 0.168
144-152					0.144[*] ± 0.0036	4.38[*] ± 0.174
152-160					0.145[*] ± 0.0037	4.45[*] ± 0.182
160-168					0.147[*] ± 0.0040	4.53[*] ± 0.179
168-176					0.149[*] ± 0.0042	4.65[*] ± 0.168
176-184					0.149[*] ± 0.0041	4.69[*] ± 0.187
184-192					0.152[*] ± 0.0040	4.81[*] ± 0.190

Significance level [a]0.001, [b]0.01, [c]0.05, [d]0.1 and [*]not statistically significant when compared with adjacent means.

Table 5: Changes in the activity of acid phosphatase enzyme (µ moles of p-nitrophenol liberated /30 min /mg protein) at different temperatures in the eggs during embryonic development of *C. cephalonica* (Mean ± S.D.).

Age of egg samples (h after oviposition)	Enzyme activity at 28°C	Enzyme activity at 26°C	Enzyme activity at 24°C
0-8	8.17 ± 1.91	8.03 ±1.05	8.13 ± 1.57
8-16	15.34^{b} ± 2.41	11.25^{c} ± 2.58	10.17^{*} ± 3.05
16-24	41.68^{a} ± 2.53	22.35^{b} ± 3.15	16.58^{c} ± 3.21
24-32	74.51^{a} ± 3.01	32.18^{b} ± 3.21	24.23^{c} ± 4.12
32-40	85.78^{b} ± 2.15	55.28^{a} ± 3.18	36.54^{b} ± 4.25
40-48	113.65^{a} ± 3.75	71.78^{a} ± 3.87	51.37^{b} ± 3.91
48-56	123.37^{b} ± 2.68	103.36^{a} ± 4.65	65.26^{b} ± 4.27
56-64	139.34^{a} ± 3.84	115.67^{b} ± 3.81	72.06^{d} ± 5.03
64-72	147.85^{c} ± 3.93	127.05^{b} ± 4.26	87.21^{b} ± 4.16
72-80	165.58^{a} ± 4.12	132.16^{d} ± 3.54	113.75^{a} ± 3.98
80-88	171.56^{d} ± 4.51	144.78^{b} ± 4.15	123.56^{c} ± 4.51
88-96	173.08^{*} ± 5.07	152.12^{c} ± 3.67	135.43^{b} ± 4.46
96-104		159.05^{c} ± 3.28	147.51^{b} ± 4.19
104-112		165.18^{d} ± 4.61	150.68^{*} ± 4.53
112-120		167.24^{*} ± 4.19	153.57^{*} ± 4.29
120-128		169.17^{*} ± 4.47	156.25^{*} ± 5.06
128-136		170.21^{*} ± 4.08	159.98^{*} ± 4.85
136-144		171.24^{*} ± 5.26	162.48^{*} ± 4.76
144-152			164.16^{*} ± 4.67
152-160			165.97^{*} ± 3.85
160-168			167.89^{*} ± 3.28
168-176			169.71^{*} ± 3.16
176-184			170.27^{*} ± 5.13
184-192			171.08^{*} ± 4.68



Age of egg samples (hours after oviposition)

Fig. 1: Effect of temperature on acid phosphatase activity (µ moles of p-nitrophenol liberated /30m/mg protein) the eggs during embryogenesis of the rice moth (*Corcyra cephalonica*).

DISCUSSION

These results indicate that the wet weight and water content of the eggs of *C. cephalonica* decreased significantly from 0 to 36 h of embryonic development. Thus, it may be concluded that the loss in wet weight, is mainly due to the loss of water from the eggs. Similar water loss has also been reported from the eggs of *Rhodnius* (Beament, 1946), *Locusta* (Matthee, 1951), *Tenebrio* (Ludwig & Ramazzotto, 1965), and grasshopper (Leese, 1976). On the contrary, increases in egg volume and weight during embryonic development in *Ocypus*, *Phyllopertha* and *Dysticus* (Coleoptera), *Notostria*, *Nepa* (Heteroptera), *Culex* (Diptera) and various orthopterans due to absorption of water from the environment have been reported (Chapman, 2000). The wet weight of the eggs

of *C. cephalonica* of 24 to 72 h of age remained fairly constant between 24 and 72 h, while, the egg water content was stable from 24 to 60 h indicating that during later stages of embryonic development in *C. cephalonica,* dehydration is completely checked, which might be responsible of the lack of change in wet weight. A possible protective mechanism involving the utilization of metabolic water produced by lipid oxidation may be responsible for cessation of water loss. The significant increase in water content in egg aged 60-72 h may be due to reduced consumption of water, possibly on account of a low metabolic rate prior to hatching of the larva and increased production of metabolic water from lipid oxidation (Gilbert, 1967). The eggs of *C. cephalonica* probably develop without water uptake from the environment, like those of *Rhodnius* and many other heteropterans and lepidopterans (Chapman, 2000). The loss in wet weight of eggs incubated at low temperatures (26°C and 24°C) was slow in comparison with those incubated at 28^0 C, indicating that temperature plays an important role in the process of water loss from the developing eggs of *C. cephalonica*. Literature regarding the relation between temperature and water loss from the eggs of insects is not available for discussion.

Our data on the effect of temperature on the hatching percentage of eggs clearly indicated that the decrease in temperature from 28°C to 26°C and 24°C did not alter the hatching percentage. Contrary to this result, Koda and Nakamura (2009) reported the highest hatching percentage (88%) at 20°C and a significant decrease in the hatching percentage with decreasing temperature in *Shijimiaeoides divinus barine*.

In the present study, we observed a significant decrease in the glucose content in eggs of early age groups (0-8 h to 16-24 h) incubated at 28°C and a decrease in eggs of older age groups in *C. cephalonica*. A similar pattern of variation in the level of carbohydrate has been reported by Ludwig & Ramazzotto (1965) in the developing eggs of the meal worm, *Tenebrio molitor*. A steady depletion of carbohydrate has been reported in the eggs of *Laccotrephes griseus* during its embryonic development by Premkumar *et al.* (1991). Data on the effect of temperature on carbohydrate metabolism in the developing eggs of insects are not available. In our study, lowering in the temperature caused only a decrease in the rate of variation in glucose levels and did not affect the pattern of variation.

In the present investigation, acid phosphatase activity was the lowest in newly laid eggs (0-8) which increased significantly and continuously with the progression of embryonic development and declined prior to hatching at all temperatures studied. The increase in the activity of this enzyme with advancing embryogenesis, as observed in the present investigation, is consistent with the observations on the eggs of the grasshopper by Fitzgerald (1949), in the eggs of the silk worm by Chino (1961) and Sridhara & Bhat (1963), in the eggs of *Chilomenes sexmaculata by* Sidhu *et al*. (1984) and in the eggs of *Rhodnius prolixu*s by Fialho *et al*. (2002). Using a histochemical technique, Yao (1950) reported that in *Drosophila*, acid phosphatase was present in all stages but no apparent changes occurred in its activity throughout embryogenesis. Literature on temperature related changes in the activity of this enzyme in the eggs of insects during embryogenesis is not available for discussion.

From the findings of the present investigation it can be concluded that the delay in embryonic development with decreasing temperature may be due to retardation in the activity of acid phosphatase, because the wide occurrence of phosphatases in animal tissues is thought to be associated with (a) the transport of metabolites (b) the metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates and (c) the synthesis of proteins. The findings also support the importance of these enzymes in embryonic tissues.

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