4. RESULTS

4.1. Comparative Phenology of Larvae, Pupae and Adults of both Non-diapausing and Diapausing Generations:

4.1.1 Span of the two generations and duration of stadia and moulting :

Bimodal generations of *A. mylitta* was observed during the period of investigation. Rearing of the larvae of ND-generation was started on mid-July after the hatching of larvae from eggs and that of D-generation was started on 1st October of the year. Total life span starting from 0-day of 1st instar larva till the death of moth was recorded to be about 70 and 283 days for ND-and D-generations respectively. However, an instarwise longer larval duration was recorded from the 4th instar onwards in diapause-bound generation. No differences was observed between the two generations in the duration of 1st and 2nd larval moults. But, for the 3rd and 4th moults the diapause generation took about 0.5 day more than those of the ND-larvae. The data on the life span for larvae, pupae and adults are given in Table 1. and Fig. 7.

4.1.2 Larval assessment

Compared to the larvae of ND-generation a higher larval body weight was initially recorded from the late 2nd instar larvae of D-generation. This higher weight was significantly different ($P<0.001$) from the corresponding weight of ND-generation. The incremental weight difference is also reflected in the higher weight of initial 3rd instar larvae of D-generation. In the remaining developmental stages the weight differences increased steadily. The relative growth rate (RGR) decreased gradually from first to fifth larval instars in both the generations. But, the diapause-destined larvae exhibited comparatively a lower RGR than that of the ND-generation. (Table 2;

Table 1 . Life span (days) of different developmental stages of A. mylitta in both non-diapause(ND) and diapause(D) generations.

Developmental stages	Sex	Duration of ND-Generation			Duration of D-Generation		
		Feeding	Moulting	Total	Feeding	Moulting	Total
1L		3.00	1.00	4.00	4.00	1.00	5.00
2L		3.50	1.00	4.50	4.00	1.00	5.00
3L		4.00	1.50	5.50	4.50	2.00	6.50
4L		6.00	2.00	8.00	8.00	2.50	10.50
5L	M	12.00	-	12.00	20.00	-	20.00
	F	13.00	-	13.00	22.00	-	22.00
Total larval	M	28.50	5.50	34.00	40.50	6.50	47.00
duration.	F	29.50	5.50	35.00	42.50	6.50	49.00
Spining	M	-	-	3.00	-	-	8.00
duration.	F	-	-	4.00	-	-	9.00
Pre-pupal	M	-	-	4.00	-	-	9.00
life span	F	-	-	4.00	-	-	9.00
Pupal life span	M	-	-	20.00	-	-	208.00
	F	-	-	21.00	-	-	209.00
Moth life	M	-	-	7.00	-	-	9.00
span.	F	-	-	8.00	-	-	10.00
Total duration from 0-day 1st instar larva to the death of adult.	M	-	-	68.00	-	-	281.00
	F	-	-	72.00	-	-	285.00

L = Larval instar , M = Male , F = Female.

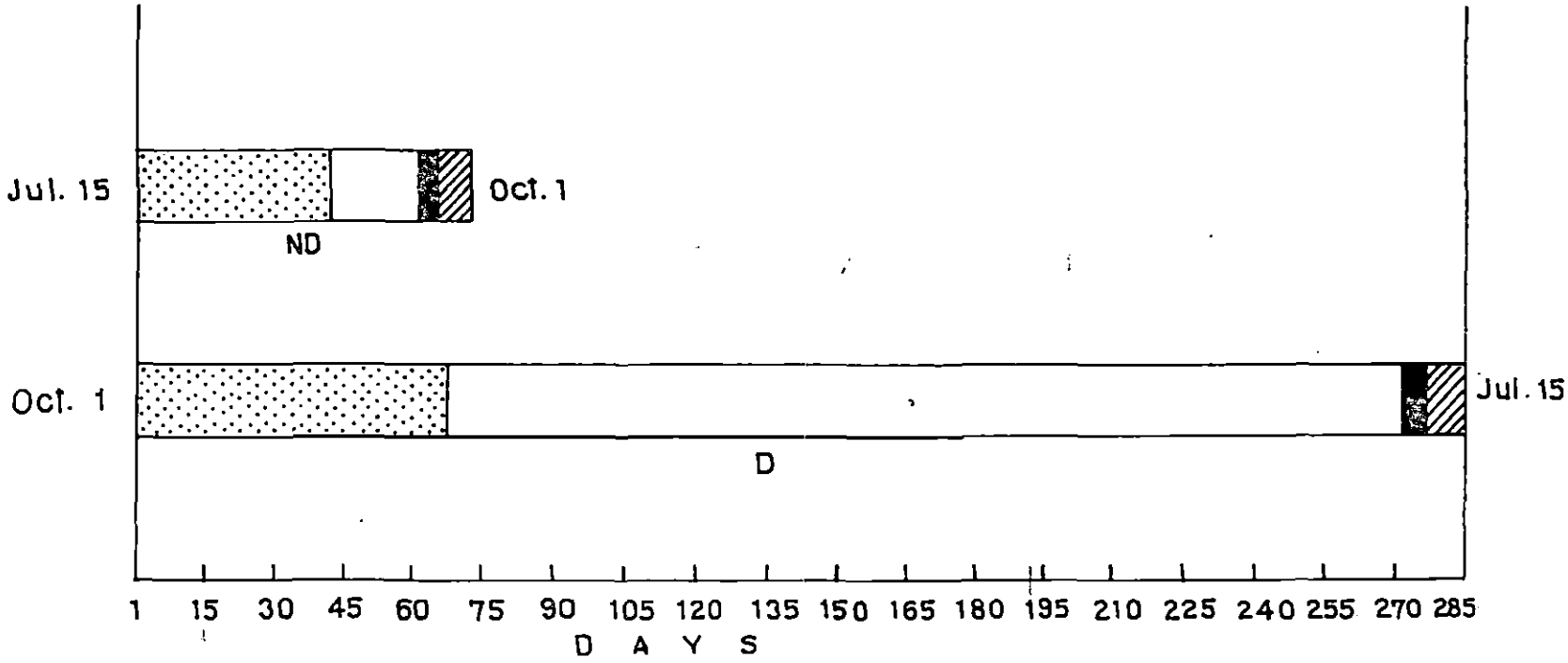


Fig. 7. Life span of A. mylitta in non-diapause (ND) and diapause(D) generation.

Larva (feeding, moulting, spinning and pre-pupal duration)

Pupa.

Moth.

Egg incubation period.

Table 2. Larval weight (mean ± SE) and relative growth rate of *A.mylitta* of non-diapause (ND) and diapause(D) generations.

Larval instars	Sex	Larval weight (g)				Relative growth rate (RGR)	
		ND generation		D-generation		ND generation	D-generation
		0-day	last day	0-day	last day		
1st		0.00842 ±0.00017	0.08474 ±0.00152	0.00881 ±0.00015 NS	0.08699 ±0.00097 NS	0.546	0.408
2nd		0.07854 ±0.00095	0.35170 ±0.00879	0.07885 ±0.00130 NS	0.527 ±0.009 a'	0.423	0.369
3rd		0.31560 ±0.01019	2.12 ±0.07	0.487 ±0.003 a	2.802 ±0.06 a	0.370	0.313
4th		1.90 ±0.03	10.58 ±0.20	2.362 ±0.05 a	11.79 ±0.22 a	0.232	0.166
5th (feeding)	Male	8.98 ±0.16	32.57 ±0.36	9.97 ±0.16 a	35.78 ±0.40 a	0.095	0.056
	Female	11.66 ±0.22	40.83 ±0.40	12.74 ±0.20 a	45.46 ±0.36 a	0.085	0.051
5th (non-feeding)	Male	*20.22 ±0.26	**10.23 ± 0.10	*23.18 ± 0.24 a	**14.03 ± 0.12 a		
	Female	*25.78 ±0.48	**13.56 ±0.12	*28.05 ±0.20 a	**17.34 ± 0.17 a		

* Just after gut purging.
** Pre-pupa.

't' - test probability differences between the two generations.
a = P<0.001.
NS=Non significant.

Fig 8). Thus, the larval developmental rate was faster in the ND-over the D-generation.

4.1.2. Pupal assessment

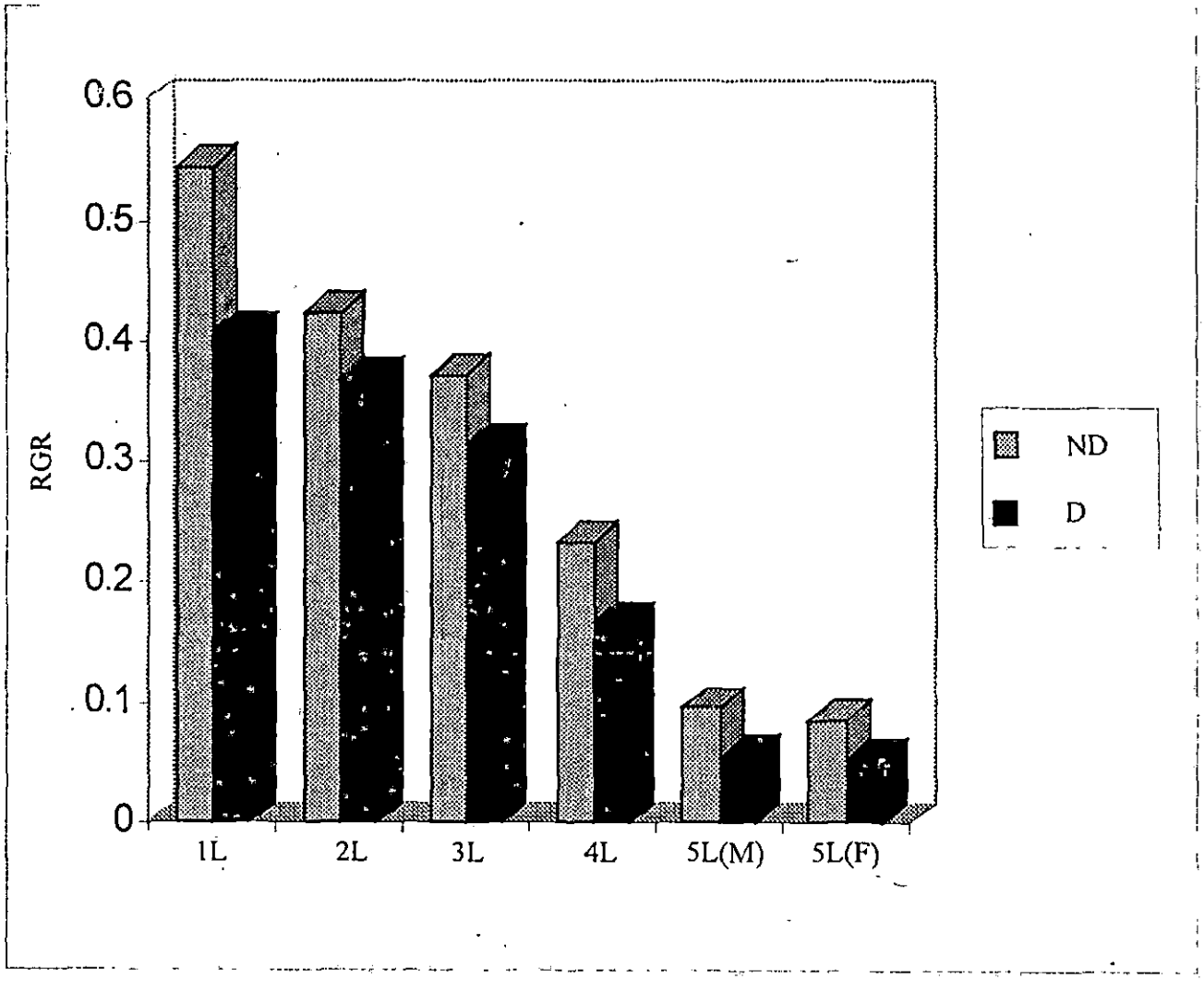
Pupal life span was found to be significantly longer and pupal weight as well as cocoon weight and shell weight in both the sexes of D-generation were higher over these values of the ND-generatio(Table 3). However, a gradual reduction in pupal weight from 0-day onwards till moth eclosion was observed in both the sexes of the two generations. In both the generations pupal weight, cocoon weight and shell weight remained higher in females than these measurements in males except the S.R% which was found to be reverse (Table 3).

4.1.3. Adult weight and egg production

Male and female moth weights as well as fecundity and total egg production (laid + unlaid) per female were found to be significantly lower ($P<0.001$) in the D-generation than those in the ND-generation (Table 3). However, no significant difference was observed in egg incubation period and hatching percentage between the two generations (Table 3).

4.1.4. Influence of environmental factors on the two generations :

Prevailing ambient daylength, temperature, r.h. and rainfall were quite different during the life span of the two generations. The fluctuations in the different environmental parameters in field condition as well as in the insectary are presented monthwise (Table 4) covering the entire period of the observation, together with the ambient conditions experienced by each developmental stage of life history (Table 5, Fig 9). Short day length of less than 12 hrs. (11.65 hrs) in combination with low (minimum)) temperature of less than 20°C (17.68°C) was availed from third instar onwards in case of diapause-destined generation. The minimum average temperature availed during 3rd instar larvae of diapausing -destined generation remained significantly lower ($P<0.001$) than the temperature experienced by the 1st and 2nd larval instar while no significant difference was observed in between



3. 8. Relative growth rate (RGR) of *A.mylitta* in non-diapause (ND) and diapause (D) generation. L=Larval instar, M=Male, F=Female .

Table 3 . Pupal and moth weights, cocoon characters and reproductive performance of *A. mylitta.* The data are mean ± SE.

Pupal age (day)	Weights (g)			
	Non-diapause		Diapause	
	Male	Female	Male	Female
0	9.12 ±0.15	12.89 ±0.10	12.12 ±0.13	15.20 ±0.16
7	8.77 ±0.06	12.03 ±0.06	-	-
14	8.31 ±0.04	11.23 ±0.11	-	-
30	-	-	11.75 ±0.15	14.71 ±0.11
60	-	-	11.60 ±0.11	14.61 ±0.08
90	-	-	11.41 ±0.09	14.51 ±0.07
120	-	-	11.29 ±0.08	14.32 ±0.07
150	-	-	11.00 ±0.05	14.10 ±0.06
165	-	-	10.70 ±0.05	13.63 ±0.06
180	-	-	10.30 ±0.06	12.92 ±0.07
Day before emergence	7.91 ±0.03	9.98 ±0.05	10.12 ±0.05	12.79 ±0.04
Moth	2.97 ±0.06	6.89 ±0.12	2.55 ±0.07	6.32 ±0.16
Cocoon	10.36 ±0.20	13.59 ±0.27	13.90 ±0.19	17.31 ±0.15
Shell	1.37 ±0.02	1.53 ±0.03	2.17 ±0.05	2.45 ±0.06
S.R.(%)	13.34 ±0.30	11.32 ±0.30	15.61 ±0.43	14.15 ±0.34
Egg (laid)	-	257 ±11	-	179 ±5
Production(unlaid)	-	23 ±3	-	36 ±4
(No.) (total)		280 ±10	-	215 ±6
Egg incubation period (days)	-	9.36 ±0.17	-	9.44 ± 0.16
Hatching (%)	-	80.42 ±1.39	-	77.63 ±2.14

Table 4. Environmental conditions experienced by *A.mylitta* during the study period. Each value represents the monthly average ± SE except the rainfall which is total of a month.

Month	Field					Insectary		
	Light(L) : Dark(D) (hrs.)	Max.Temp. (°C)	Min.Temp. (°C)	r.h.(%)	Total rainfall(mm)	Max.Temp. (°C)	Min.Temp. (°C)	r.h.(%)
July	13.33 : 10.67	29.11	22.01	87.95	215.25	27.87	23.81	84.74
	±0.02 ±0.02	±0.39	±0.11	±0.74		±0.25	± 0.13	± 1.06
August	12.86 : 11.14	27.88	21.72	90.74	355.25	26.09	23.55	90.82
	±0.03 ±0.03	±0.24	±0.09	±0.46		±0.21	±0.16	±0.47
September	12.24 : 11.76	28.05	20.35	89.78	245.50	25.87	23.67	81.15
	±0.03 ±0.03	±0.26	±0.22	±0.43		±0.16	±0.18	±1.28
October	11.60 : 12.40	27.05	16.07	84.08	34.50	26.26	20.29	73.92
	±0.04 ±0.04	±0.25	±0.62	±0.78		±0.10	±0.30	±1.17
November	11.05 : 12.95	25.14	9.04	77.47	0.75	20.70	14.07	60.95
	±0.03 ±0.03	±0.28	±0.51	±0.94		±0.19	±0.48	±1.24
December	10.77 : 13.23	21.56	7.41	78.38	45.00	20.13	11.97	61.23
	±0.009 ±0.009	±0.44	±0.47	±5.57		±0.15	±0.32	±1.47
January	10.92 : 13.07	22.00	6.81	73.56	-	15.74	13.68	66.29
	±0.02 ±0.02	±0.62	±0.47	±0.78		±0.39	±0.46	±1.52
February	11.43 : 12.57	24.24	7.93	74.26	17.50	18.43	16.57	63.84
	±0.04 ±0.04	±0.41	±0.39	±0.95		±0.22	±0.23	±1.34
March	12.04 : 11.96	31.98	15.91	61.32	4.50	26.26	23.56	48.68
	±0.04 ±0.04	±0.47	±0.58	±1.16		±0.62	±0.51	±1.45
April	12.71 : 11.29	32.10	19.98	62.87	18.50	30.04	27.63	40.65
	±0.04 ±0.04	±0.77	±0.34	±1.69		±0.37	±0.36	±1.36
May	13.21 : 10.79	32.12	20.75	70.65	67.00	30.00	26.73	53.17
	±0.02 ±0.02	±0.89	±0.39	±1.90		±0.60	±0.62	±3.11
June	13.49 : 10.51	29.04	21.85	81.07	138.75	30.17	27.20	66.03
	±0.005 ±0.005	±0.47	±0.28	±1.09		±0.41	±0.41	±2.71

Table-5. Environmental factors experienced by different developmental stages of *A.mylitta* in both outdoor and the insectary. Values are mean ± SE (except rainfall)

Developmental stages	Daylength (hrs.) (L : D)		Max. Temp.(°C)		Min. Temp .(°C)		r.h.(%)		Total rainfall(mm)	
	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause
IL	13.33 : 10.67 ±0.005 ±0.005	11.89 : 12.11 ±0.02 ±0 .02	27.48 ±0.59	27.44 ±0.60	21.53 ±0.23	19.12 ±0.34	91.13 ±1.19	85.60 ±1.99	56.25	11.50
2L	13.27 : 10.73 ±0.009 ±0.009	11.85 : 12.15 ±0.02 ±0.02	26.80 ±1.19	27.33 ±0.62	21.52 ±0.23	19.57 ±0.41	92.37 ±1.63	89.17 ±0.85	73.00	12.50
3L	13.22 : 10.78 ±0.013 ±0.013	11.65 : 12.35 ±0.02 ±0.02	28.50 ±0.51	27.20 ±0.59	21,78 ±0.08	17.68 0.18	90.67 ±1.28	86.11 ±0.45	38.50	10.50
4L	13.12 : 10.88 ±0.014 ±0.014	11.46 : 12.54 ±0.03 ±0.03	28.07 ±0.25	27.30 ±0.34	21.67 ±0.16	13.02 ±0.58	89.64 ±0.38	81.09 ±0.93	21.25	-
5L(feeding period)	12.93 : 11.07 ±0.019 ±0.019	11.18 : 12.82 ±0.02 ±0.02	28.22 ±0.28	25.97 ±0.31	22.01 ±0.09	11.10 ±0.52	91.00 ±0.69	79.68 ±1.06	139.00	0.75
5L (non-feeding period i.e., spinning to pupation).	12.72 : 11.28 ±0.015 ±0.015	10.91 : 13.09 ±0.02 ±0.02	27.36 ±0.66	23.84 ±0.18	21.61 ±0.12	6.44 ±0.45	90.75 ±0.97	75.21 ±0.91	158.25	-
Pupa in insectary	12.43 : 12.57 ±0.224 ±0.224	12.08 : 11.92 ±0.38 ±0.38	25.70 ±0.16	24.39 ±2.16	23.65 ±0.23	21.05 ±2.37	85.65 ±1.73	57.13 ±3.45	187.25	291.25
Moth in insectary	12.15 : 11.85 ±0.013 ±0.013	13.45 : 11.55 ±0.01 ±0.01	26.29 ±0.26	29.10 ±0.29	24.28 ±0.17	24.20 ±0.24	73.57 ±3.94	79.05 ±1.32	85.75	47.50

L = Larval instar.

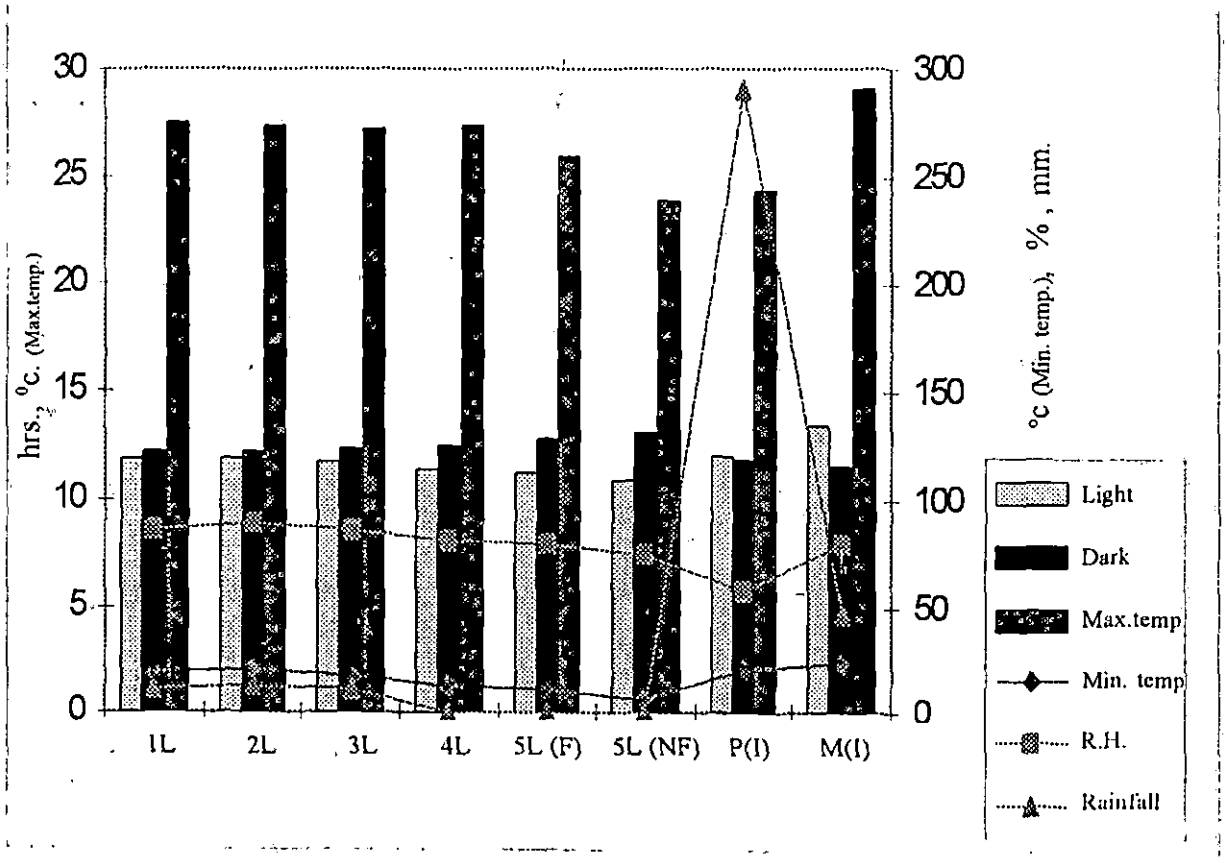


Fig. 9. Environmental factors experienced by different developmental stages of *A. mylitta* in diapause generation in both outdoor and the insectary. L=Larval instar, F=Feeding period, NF=Non-feeding period (spinning to pupation), P(I)=Pupa in insectary, M(I)=Moth in insectary.

first and 2nd instar. However, average maximum temperature was found to be significantly different among the different instars of the same generation and among the corresponding instars of the two generations with the single exception in feeding period of 5th larval instar (table 5).It is to be noted that r.h. % and total rainfall was recorded to be lower in diapause-destined generation than that of the non-diapause one. From the photoperiodic record it was observed that from April upto onwards upto about 150 day of pupal age the daylength suddenly increased above 12 hrs. The average minimum and maximum temperature recorded for this month inside the insectary were found to be a little above 27°C and 30°C respectively. This sudden change in daylength and temperature may influence the initiation of the termination of pupal diapause. The moth emergence and egg laying occurred under long daylength and high temperature in both the generations during or after monsoon.

4.1.5. Grainage performance

Moth Emergence : In the first generation normal moth emergence commenced on the first week of June and continued upto the second week of August covering a period of about 69 days. But in case of the second brood emergence period extended over 42 days from the first week of September upto the third week of October (Table 6 & 7). The time of adult eclosion as recorded from daily observation was mostly between the periods of late photophase and late scotophase in case of both the broods.

In case of first brood, most of the moth emergence took place during the period from fourth week of June to fourth week of July showing a peak on the third week of July, while in the second brood it occurred from second to fourth week of September showing a peak during the third week. Subsequent to the peak a very sharp decline occurred in the daily emergence rhythm. This was consistent in both the generations. However, in ND-generation a total of 85.91% emergence took place in a peak span of 21 days. Whereas in case of D-generation the value was 72.20% spanning a peak period of 30 days (Table 6 & 7). The overall percentage of moth emergence was, however, higher in the second brood than in the first. Moreover, male moths emerged in higher numbers than in females of both

Table 6. Environmental (insectary) conditions and grainage performance of the first brood of *A.mylitta*

Observations at every 3 day from June 1 to August 8.	Temperature(°C) Max.	Min.	Mean ±S.E	R.h% (Mean±S.E)	Emergence (%) Male	Female	Total	Spontaneous mating (%)	Facundity (nos.) (Mean±S.E)	Egg incubation period in days (Mean±S.E)	Hatching (Mean±S.
June 3	31.66	26.33	28.33±0.31	74.33±1.02	0.028	0.019	0.047	50.00	120±5	8.00±0.50	40.50±3.9
6	30.66	24.66	28.91±0.41	66.99±5.03	0.084	0.065	0.149	14.29	161±8	8.00±0.35	47.11±4.6
9	32.00	25.66	29.08±0.49	61.99±3.93	0.084	0.056	0.140	33.33	146±4	9.00±0.35	47.87±3.3
12	31.50	25.50	29.91±0.27	55.50±2.49	0.112	0.103	0.215	15.00	206±8	8.62±0.35	63.58±4.3
15	32.33	24.33	27.04±0.21	78.58±1.19	0.065	0.084	0.149	21.35	209±8	9.33±0.27	73.22±2.7
18	26.00	23.66	24.79±0.16	89.33±0.94	0.261	0.233	0.494	14.00	222±5	9.00±0.21	71.59±2.5
21	27.00	24.33	26.49±0.24	88.16±1.38	1.195	1.008	2.203	20.37	230±5	9.58±0.15	74.60±2.9
24	26.33	24.33	25.00±0.12	89.83±1.08	1.185	0.971	2.156	17.31	234±3	10.08±0.23	76.88±2.4
27	26.33	23.33	25.62±0.21	89.08±1.01	0.355	0.299	0.654	73.75	247±4	10.42±0.19	78.02±2.0
30	26.00	23.66	25.08±0.24	85.33±1.17	2.632	1.596	4.228	43.27	236±4	10.50±0.26	82.61±1.2
July 3	27.00	23.66	24.91±0.17	91.08±0.60	3.669	2.091	5.760	41.52	239±5	10.25±0.46	81.02±2.6
6	25.33	22.66	24.62±0.28	91.74±0.07	2.931	1.727	4.658	48.11	225±6	10.00±0.43	80.29±2.3
9	25.33	22.66	24.54±0.26	91.74±0.07	2.931	2.567	5.498	34.54	235±7	10.33±0.48	78.97±2.8
12	25.00	23.33	24.54±0.16	91.58±0.07	4.574	2.016	6.590	50.00	242±7	10.33±0.33	75.74±2.8
15	25.00	23.00	24.58±0.18	90.58±0.90	6.768	2.688	9.456	60.42	243±6	10.42±0.51	78.06±2.9
18	26.00	23.00	25.12±0.45	87.75±1.88	6.820	4.250	11.070	67.91	237±5	10.33±0.34	82.06±2.0
21	25.00	22.50	24.25±0.37	91.62±0.21	5.320	6.530	11.850	56.57	244±7	10.50±0.40	84.71±3.2
24	25.66	23.00	24.83±0.25	91.91±0.07	2.690	5.250	7.940	23.49	231±5	10.44±0.28	75.50±2.3
27	25.66	23.33	24.95±0.31	91.24±0.30	1.940	3.210	5.150	27.03	233±4	10.35±0.42	76.07±3.3
30	25.50	22.50	24.64±0.14	90.06±0.46	0.887	0.840	1.727	37.78	220±6	10.25±0.29	70.50±2.5
Aug. 2	26.66	23.50	25.12±0.31	84.89±2.69	0.150	0.140	0.290	33.33	202±5	9.70±0.30	61.22±3.4
5	26.50	23.50	25.45±0.28	85.08±1.99	0.080	0.120	0.200	23.08	199±4	9.15±0.35	53.00±3.4
8	27.66	24.50	26.12±0.45	85.99±1.91	0.050	0.030	0.080	33.33	172±6	9.25±0.35	48.50±4.1
AVERAGE :	27.22 ±0.52	23.78 ±0.22	25.87 ±0.37	84.54 ±2.14	44.82	35.89	80.71	43.02	214 ±7	9.73 ±0.16	69.50 ±2.81

Table 7. Environmental (insectary) conditions and grainage performance of the second brood of *A.mylitta*

Observations at every 3 day from Sept.4 to Oct. 15.	Temperature(°C) Max.	Min.	Mean ±S.E	R.h% (Mean±S.E)	Emergence (%) Male	Female	Total	Spontaneous mating (%)	Facundity (nos.) (Mean±S.E)	Egg incubation period in days (Mean±S.E)	Hatching (%) (Mean±S.E)
Sept. 6	26.67	24.67	26.16±0.27	86.92±1.17	0.022	0.00	0.022	0.00	0	0	0
9	27.00	25.00	26.67±0.31	85.67±1.08	0.584	0.007	0.591	0.00	0	0	0
12	27.67	25.33	26.75±0.19	82.16±1.88	6.815	4.540	11.355	53.33	245±6	9.50±0.35	70.57±2.55
15	27.33	25.00	26.42±0.43	83.91±2.07	8.439	5.350	13.789	38.97	252±8	9.50±0.35	86.96±2.42
18	28.67	25.67	27.37±0.32	78.00±2.02	8.826	6.478	15.304	54.51	264±6	9.55±0.46	85.44±4.36
21	27.67	25.33	26.75±0.28	82.25±0.17	8.102	6.225	14.327	40.06	256±3	9.50±0.42	78.73±3.19
24	27.00	25.00	26.62±0.14	83.83±0.04	7.868	5.222	13.090	40.95	241±7	10.00±0.20	82.80±3.20
27	27.67	25.00	27.04±0.26	79.66±1.01	7.600	5.185	12.785	17.62	228±6	10.35±0.25	67.45±2.33
30	27.33	24.67	26.50±0.37	83.42±0.56	2.585	2.677	5.262	15.96	235±7	11.70±0.29	64.22±3.77
Oct 3	26.83	24.66	25.30±0.22	87.08±1.71	0.346	0.418	0.764	10.34	230±5	12.00±0.30	62.50±5.39
6	25.16	23.33	24.62±0.18	89.75±2.39	0.130	0.072	0.202	0.00	0	0	0
9	26.00	23.50	25.06±0.12	83.75±0.30	0.043	0.050	0.093	42.86	225±4	12.00±0.27	64.50±6.33
12	25.83	24.00	24.43±0.27	84.16±1.88	0.043	0.036	0.079	20.00	227±6	12.50±0.27	60.10±5.00
15	25.00	23.00	24.08±0.31	8216±1.36	0.007	0.00	0.007	0.00	0	0	0
Average :	26.84 ±0.28	24.58 ±0.23	25.99±0.28	83.76±0.80	51.41	36.26	87.67	41.51	240±4	10.66±0.39	72.33±0.30

the broods (Table 6 & 7). Emergence rhythm was very slow with the advancement of night, peak was around midnight, than the rate declined gradually till dawn. Daytime emergence is sporadic.

Mating : Spontaneous mating took place at night , particularly around midnight in most of the cases and continued also to the early morning. Mating of most of the moths took place during the period from fourth week of June to third week of July and second to fourth week of September in case of first and second brood respectively after which mating efficacy declined almost to the negligible number. Average spontaneous mating percentage was slightly higher in the first brood than in the second brood though it was only a little over 40% in both the cases (Table 6 & 7).

Fecundity : A lower fecundity was recorded in the first generation during several early (first to third week of June) and late (fourth week of July to second week of August) days of moth emergence, while maximum oviposition was noted in the third week of June to fourth week of July.

In the second brood fecundity remained higher only during the first half of moth emergence period (second and fourth week of September). Thereafter, the fecundity gradually declined.

Relative to the second brood the total fecundity was lower in the first brood (Table 6 & 7).

Egg incubation period : In general, the egg incubation period was not uniform in a population of *A. mylitta.* The daily data revealed a gradual increase in the egg incubation period in each brood. However, in the first brood, egg incubation time decreased at the last phase of the study season (August) and the average egg incubation period was shorter than that of the second brood (Table 6 & 7).

Hatching Performance : Hatching percentage of the eggs of both the broods was almost the same and showed a similar pattern. The eggs obtained from the females emerged during the early and late days of the total emergence span suffered from relatively poor hatchability (Table 6 & 7).

4.2. Determination of Critical Weight of Fifth Instar Larvae and Timings of PTTH Release for Larval-Pupal Transformation

4.2.1 Growth index for control 5th instar larvae

Growth rate was faster and RGR was higher in ND-generation than in the D-generation. Males showed faster RGR than the females of both the generations (Table 10). Overall gain in larval weight during 5th instar was 264.99% in male and 251.22% in female in case of ND-generation and 281.64% and 271.19% respectively in males and females of D-generation (Table 8 & 9) .

4.2.2 Behavioural changes and morphological markers for pupation

Under ambient field conditions the duration of fifth larval instar was 21 days and 39.50 days (irrespective of sex) in ND and D broods respectively. Invariably the phagoperiod including the latent feeding duration, and the non-phagoperiod were longer in diapause-destined generation. Sex specific variation was observed in respect of only latent feeding period irrespective of generations, female took more time than its male counterpart. It is interesting to note that there was no sex-specific differences in non-phagoperiod. After attainment of final (maximum) weight the larvae started moving aimlessly and egested very frequently first solid faeces and later on semisolid ones for 1-1.5 days. Simultaneously the larvae consumed very frequently little amount of leaves. Because of much higher rate of egestion than ingestion and elimination of water through semisolid faeces the body weight declined sharply. Withing about another 6 hrs the gut purging was complete after final elimination of liquid excreta. The maximum body weights after complete gut purging was reduced by about 36% in both the sexes in case of ND-brood and by 33.94% in male and 37.16% in female in case of D-generation.

Spinning continued for 3.25 days and 7.75 days in ND and D-destined broods respectively. A shrinkage of body occurred gradually with the advancement of spinning. Further, during last phase of silk spinning,

Table 8. Daily body weight (g) of non-diapausing brood of *A.mylitta* during the feeding period of 5th larval instar. Values are mean ±S.E. (n=100).

Day / Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
M	8.94 ±0.25	9.87 ±0.34	11.69 ±0.48	15.53 ±0.58	16.03 ±0.60	18.59 ±0.60	20.18 ±0.65	22.40 ±0.87	24.06 ±0.65	26.90 ±0.90 (Lcw)	27.52 ±0.77	30.18 ±0.79	32.63 ±0.61	-	-
F	11.82 ±0.22	14.22 ±0.48	17.14 ±0.74	21.74 ±0.85	25.84 ±0.85	28.37 ±0.85	30.39 ±0.95	31.84 ±0.77	32.05 ±0.36	34.37 ±0.64 (Lcw)	36.58 ±0.93	39.14 ±0.36	40.68 ±0.34	41.55 ±0.40	-

	Percentage increase in weight													Overall increase in wt.(%)
M	10.40	18.44	32.85	3.22	15.97	8.54	11.00	7.41	11.80	2.30	9.67	8.12		264.99
F	20.30	20.53	26.84	18.86	9.79	7.12	4.77	0.66	7.24	6.43	6.99	3.93	2.14	251.52

M = Male ; F = Female.

Table 9. **Daily body weight (g) of diapause-destined brood of *A.mylitta* during the feeding period of 5th larval instar. Values are mean ± S.E.. (n=100).**

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Sex																								
Male	9.97 ±0.23	10.88 ±0.21	12.98 0.19	14.45 ±0.34	15.94 ±0.49	16.36 ±0.36	17.02 ±0.50	19.36 ±0.49	21.48 ±0.48	22.41 ±0.56	23.64 ±0.41	24.33 ±0.43	24.68 ±0.43	25.33 ±0.40	26.18 ±0.54	27.88 ±0.64 (Lcw)	30.44 ±0.49	32.87 ±0.57	34.66 ±0.54	36.6 ±0.29	38.05 ±0.65	-	-	
	12.74 ±0.26	13.97 ±0.26	14.86 ±0.31	16.16 ±0.26	18.77 ±0.26	21.48 ±0.31	23.95 ±0.32	25.13 ±0.34	26.81 ±0.35	28.01 ±0.48	29.87 ±0.51	31.00 ±0.44	32.05 ±0.40	32.75 ±0.44	33.15 ±0.51	35.29 ±0.54 (Lcw)	36.26 ±0.50	38.03 ±0.58	40.31 ±0.55	42.4 ±0.53	44.66 ±0.40	45.97 ±0.33	47.29 ±0.42	
									% increase in weight															overall increase in wt (%)
		9.13	19.3	11.32	10.31	2.63	4.03	13.75	10.95	4.33	5.49	2.92	1.44	2.63	3.36	6.49	9.18	7.98	5.44	5.6	3.96			281.64
		9.65	6.37	8.75	16.15	14.44	11.5	5.05	6.56	4.51	6.6	3.78	3.39	2.21	1.22	6.45	2.75	4.88	3.36	5.18	5.33	2.93	2.87	271.19

Table 10. Relative growth rate (RGR) during the fifth larval instar of *A. mylitta*.

Brood / Sex	Non-diapause(ND)	Diapause(D)	Percentage decrease in D-brood with comparison to ND - brood.
Male	0.095	0.058	38.95
Female	0.085	0.052	38.82

anal prolegs ceased their mobility and finally all the abdominal prolegs became immobile with the attainment of pre-pupal stage. The anal prolegs closed completely after the end of spinning. Irrespective of sexes the duration of pre-pupa was about 4 and 9 days in ND and D generations respectively. (Table 11).

4.2.3 Larval starvation and subsequent biological performances

Starvation stress applied to the larvae of ND-generation upto 6 day age imposed failure to spin and pupate and the larvae eventually died. For the diapause generation this was true upto the age of 12-day. The result was consistent in both the sexes. However, depending on the age of the larvae subjected to starvation and the nature of the two generations the larval survival span varied from 10 to 25 days. Starvation from the age of 9 day onwards and 15 day onwards in ND and D-generation respectively, more than 50% of the starved larvae could ecdyse into pupae showing all the prodorms of pupation and finally could able to emerge as functional adults. Below that age, the functional adults could develop only in negligible numbers (Tables 12 & 13, Figs. 10, 11 and 12). Thus, the larval critical weight(L_{cw}) was attained on the 9th and 15th day of 5th stage larval life for ND and D generation respectively.

In case of ND generation the latent feeding period was 3 and 4 days for males and females respectively. In case of D-generation this period was 5 and 7 days for males and females respectively.

The quality indices such as L_{cw}, larval maximum weight (Lmw), cocoon characters, relative silk conversion efficiency, pupal critical weight (Pcw), pupal maximum weight (Pmw), adult critical weight (Acw) and adult maximum weight (Amw) with relation to the weights of the gut-purged larvae were all related to the length of latent feeding period in both the generations (Table 14 & 15). The indices other than Amw and fecundity of D generation were superior to those of ND-generation. Irrespective of the day of starvation the time required after 4th moult till the onset of spinning did not noticeably differ from the control larvae. However, the overall duration of the 5th stage

Table 11. Span of different functional components during 5th instar larval life of *A. mylitta*

Functional components of 5th stage larval life	Non-diapause(Day)		Diapause (Day)	
	Male	Female	Male	Female
Time required to attain Lcw.	9.00	9.00	15.00	15.00
Latent feeding period	3.00	4.00	5.00	7.00
Total Phagoperiod	12.00	13.00	20.00	22.00
Wandering and gut purging.	1.25	1.25	1.75	1.75
Spinning	3.25	3.25	7.75	7.75
Prepupa	4.00	4.00	9.00	9.00
Total non-phagoperiod	8.50	8.50	18.50	18.50
Total 5th larval life	20.50	21.50	38.50	40.50

Table 12. Effect of starvation on spinning, pupation and moth eclosion performances in non-diapause generation of *A.mylitta*. Control larvae were starved 8-10 hrs. before gut purge. Percentage in each case was calculated out of total population (n=60).

Day of Starvation	Sex	Larval body weight(g)	% of larvae capable to spin	% of larvae capable to pupate	% of larvae capable to emerge as moth
7	M	22.40 ±0.87	8.33	6.67	3.33
	F	31.84 ±0.77	10.00	8.33	5.00
8	M	24.06 ±0.65	15.00	15.00	11.67
	F	32.05 ±0.36	13.33	11.67	10.00
9*	M	26.90 ±0.90	90.00	81.67	68.33
	F	34.37 ±0.64	85.00	75.00	56.66
10	M	27.52 ±0.77	93.33	86.67	75.00
	F	36.58 ±0.93	88.33	80.00	61.67
11	M	30.18 ±0.79	96.67	93.33	78.33
	F	39.14 ±0.36	91.67	90.00	75.00
12	M	32.63 ±0.61	100.00	98.33	90.00
	F	40.68 ±0.34	100.00	93.33	81.67
13	F	41.55 ±0.40	100.00	98.33	90.00
12 (control)	M	26.25 ±0.85	100.00	100.00	93.33
13 (control)	F	31.64 ±0.68	100.00	96.67	91.67

M = Male ; F = Female ; * - Day of critical weight.

Table 13. Effect of starvation on spinning, pupation and moth eclosion performances in Diapause-destined generatio of *A.mylitta*. Control larvae were starved 8-10 hrs. before gut purge. Percentage in each case was calculate out of total population (n=60).

Day of Starvation	Sex	Larval body weight(g)	% of larvae capable to spin	% of larvae capable to pupate	% of larvae capable to emerge as moth
13	M	25.33 ±0.40	6.67	5.00	0.00
	F	32.75 ±0.44	8.33	6.67	0.00
14	M	26.18 ±0.54	13.33	11.67	3.33
	F	33.15 ±0.51	11.66	8.33	3.33
15*	M	27.88 ±0.64	78.33	75.00	51.67
	F	35.29 ±0.54	73.33	70.00	56.67
16	M	30.44 ±0.49	80.00	76.67	55.00
	F	36.26 ±0.50	76.67	71.67	53.33
17	M	32.87 ±0.57	88.33	85.00	55.00
	F	38.03 ±0.58	81.67	75.00	50.00
18	M	34.66 ±0.54	95.00	93.33	63.33
	F	40.31 ±0.55	86.67	81.67	53.33
19	M	36.60 ±0.29	96.67	96.67	68.33
	F	42.40 ±0.53	90.00	86.67	66.67
20	M	38.05 ±0.65	98.33	98.33	73.33
	F	44.66 ±0.40	96.67	91.67	70.00
21	F	45.97 ±0.33	96.67	95.00	68.33
22	F	47.29 ±0.42	100.00	98.33	75.00
20(Control)	M	29.17 ±0.40	100.00	96.67	75.00
22(Control)	F	33.90 ±0.51	100	93.33	73.33

M = Male , F = Female , * - Day of critical weight.

Fig.10 :Photograph showing the results of starvation from different days of non-diapause 5th stage larvae of *A.mylitta*. a.Dead larvae fed upto 8th day. b. Larvae fed upto 9th day, attained Lcw and showing pupal syndrome (prepupa). c. Pupae resulted from the larvae that attained Lcw (as in b).

Fig.11: Photograph of cocoon shells (silken part of cocoon) of *A.mylitta* produced by the 5th stage larvae of non-diapause generation allowed to feed upto different days. a. upto 9th day feeding (age for Lcw), b. upto 11th day feeding, c. upto 13th day (normal feeding period).

Fig.10

Fig.11

Fig. 12 : Photograph of female moths of non-diapause generation of A.*mylitta*. resulted from the larvae fed for normal duration of 13 day. (a) and from the larva fed upto 9 day, the day for attaining Lcw(b).

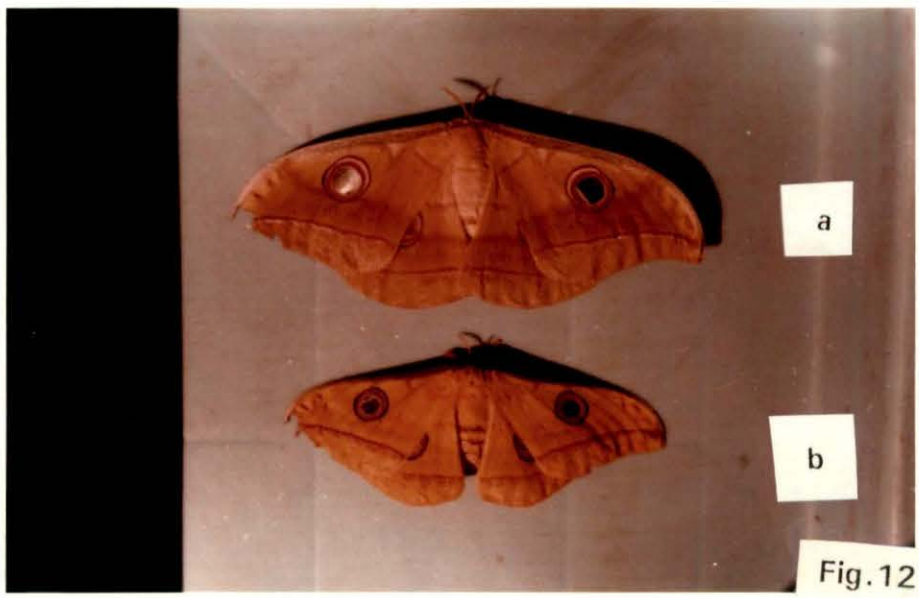


Fig. 12

Table 14. Effect of starvation on biological peformmances and quality indices of non-diapause generation of *A.mylitta*. Control larvae were starved 8-10 hours before comple tion of gut purging. All weights are in (g) and duration in (Day). The values are mean ± SE.

Day of starvation.	Sex	Body wt. on day of starvation.	Body wt.after GPR	Time took for onset of spinning	Spinning duration	Pre-pupal duration	Total 5th larval duration	Relative silk conversion efficiency(%)	Cocoon Characters				Pupal duration	Moth wt.	Egg Production (No.)		
									Coccon wt.	Pupal wt.	Shell wt.	S.R. (%)			Laid	Unlaid	Total
	M	27.10 ±0.61	17.10 ±0.24	3.75 ±0.10	2.75 ±0.15	3.25 ±0.11	18.75 ±0.21	4.561	7.97 ±0.15	7.19 ±0.18	0.78 ±0.03	9.78 ±0.17	17.50 ±1.20	2.39 ±0.07	-	-	-
9	F	34.70 ±0.44	22.65 ±0.21	4.75 ±0.15	2.75 ±0.10	3.25 ±0.14	19.25 ±0.31	3.797	10.71 ±0.19	9.85 ±0.16	0.86 ±0.04	8.03 ±0.10	17.25 ±1.18	5.50 ±0.12	23 ±4	37 ±3	60 ±5
	M	28.09 ±0.53	17.74 ±0.20	3.25 ±0.07	2.75 ±0.14	3.25 ±0.10	19.25 ±0.33	5.411	8.74 ±0.11	7.73 ±0.06	0.96 ±0.06	10.98 ±0.15	18.25 ±0.90	2.55 ±0.08	-	-	-
10	F	36.84 ±0.48	23.24 ±0.21	4.25 ±0.20	2.75 ±0.17	3.25 ±0.16	20.25 ±0.21	3.915	11.39 ±0.10	10.48 ±0.07	0.91 ±0.03	7.99 ±0.07	18.00 ±1.02	5.81 ±0.09	62 ±6	40 ±4	102 ±7
	M	30.53 ±0.36	18.95 ±0.19	2.00 ±0.05	3.00 ±0.14	3.75 ±0.12	19.75 ±0.20	6.227	9.68 ±0.07	8.50 ±0.10	1.18 ±0.04	12.19 ±0.15	19.25 ±0.98	2.74 0.05	-	-	-
11	F	38.98 ±0.51	24.01 ±0.13	2.75 ±0.15	3.00 ±0.09	3.75 ±0.15	20.50 ±0.32	5.206	12.06 ±0.09	10.81 ±0.12	1.25 ±0.05	10.36 ±0.18	18.75 ±1.01	6.26 ±0.10	102 ± 9	70 ±7	172 ±11
	M	32.80 ±0.74	20.39 ±0.24	0.75 ±0.03	3.25 ±0.06	4.00 ±0.11	20.00 ±0.24	6.327	10.26 ±0.08	8.97 ±0.16	1.29 ±0.02	12.57 ±0.22	20.75 ±1.07	2.93 ±0.05	-	-	-
12	F	40.72 ±0.56	24.58 ±0.20	2.00 ±0.04	3.00 ±0.17	3.75 ±0.10	20.75 ±0.31	5.574	12.98 ±0.09	11.61 ±0.15	1.37 ±0.04	10.55 ±0.16	20.00 ±0.96	6.54 ±0.05	152 ±7	58 ±6	210 ±5
13	F	41.77 ±0.38	25.12 ±0.15	0.75 ±0.02	3.25 ±0.12	4.00 ±0.16	21.00 ±0.26	5.732	13.41 ±0.12	11.97 ±0.20	1.44 ±0.06	10.74 ±0.20	21.25 ±1.05	6.98 ±0.06	232 ±8	35 ±6	267 ± 9
12 (Control)	M	26.44 ±0.73	20.46 ±0.21	0.51 ±0.04	3.25 ±0.10	4.00 ±0.14	20.76 ±0.28	6.598	10.34 ±0.20	8.99 ±0.15	1.35 ±0.04	13.05 ±0.30	21.00 ±0.84	3.01 ±0.06	-	-	-
13 (Control)	F	31.86 ±0.61	25.29 ±0.10	0.53 ±0.03	3.25 ±0.16	4.00 ±0.15	21.78 ±0.22	5.783	13.64 ±0.08	12.16 ±0.12	1.48 ±0.03	10.85 ±0.26	22.00 ±1.18	7.24 ±0.10	249 ±10	24 ±4	273 ±11

M = male ; F= female.

Table 15. Effect of starvation on biological pcformmances and quality indices of diapause destined generation of *A.mylitta*. Control larvae were starved 8-10 hours before comple tion of gut purging. All weights are in (g) and duration in (Day). The values are mean ± SE.

Day of starva-tion.	Sex	Body wt. on day of star-vation.	Body wt.after GPR	Time took for onset of spinning	Spinning duration	Pre-pupal duration	Total 5th larval duration	Relative silk conversion efficiency(%)	Cocoon Characters				Pupal duration	Moth wt.		Egg Production (No.)		
									Coccon	Pupal	Shell	S.R.		wt.	wt.	Laid (%)	Unlaid	Total
	M	28.22 ±0.55	18.25 ±0.31	5.75 ±0.15	4.75 ±0.25	5.00 ±0.24	30.50 ±0.41	4.493	8.13 ±0.18	7.31 ±0.28	0.82 ±0.06	10.08 ±0.30	56.80 ±3.21	2.07 ±0.09		-	-	-
15	F	35.44 ±0.73	23.43 ±0.21	8.00 ±0.19	4.75 ±0.27	5.00 ±0.16	32.75 ±0.64	3.841	10.88 ±0.20	9.98 ±0.30	0.90 ±0.04	8.27 ±0.20	62.50 ±3.15	4.86 ±0.12		12 ±2	32 ±4	44 ±6
	M	30.11 ±0.58	19.77 ±0.32	5.25 ±0.20	5.25 ±0.20	5.25 ±0.20	31.75 ±0.52	5.817	9.13 ±0.14	7.98 ±0.22	1.15 ±0.04	12.59 ±0.16	78.20 ±3.86	2.31 ±0.08		-	-	-
16	F	37.02 ±0.72	23.87 ±0.25	7.75 ±0.21	5.25 ±0.19	5.75 ±0.14	34.75 ±0.61	5.027	11.43 ±0.12	10.23 ±0.19	1.20 ±0.05	10.49 ±0.15	84.50 ±2.70	5.09 ±0.10		25 ±3	37 ±4	62 ±6
	M	32.69 ±0.54	20.65 ±0.20	3.75 ±0.15	6.00 ±0.19	5.75 ±0.16	32.50 ±0.73	6.634	10.04 ±0.15	8.67 ±0.25	1.37 ±0.06	13.64 ±0.20	115.00 ±8.65	2.39 ±0.05		-	-	-
17	F	38.33 ±0.71	24.16 ±0.20	6.00 ±0.16	6.00 ±0.16	6.75 ±0.15	35.75 ±0.44	5.877	11.84 ±0.18	10.42 ±0.18	1.42 ±0.08	11.99 ±0.30	132.14 ±4.55	5.21 ±0.08		41 ±2	40 ±3	81 ±5
	M	34.20 ±0.44	21.67 ±0.28	3.00 ±0.10	6.25 ±0.23	6.75 ±0.24	34.00 ±0.38	6.691	10.89 ±0.15	9.44 ±0.20	1.45 ±0.04	13.31 ±0.22	167.20 ±5.32	2.42 ±0.05		-	-	-
18	F	40.07 ±0.58	24.84 ±0.32	4.75 ±0.07	6.25 ±0.24	7.00 ±0.16	36.00 ±0.55	6.583	12.72 ±0.22	11.06 ±0.28	1.66 ±0.08	13.05 ±0.27	157.08 ±5.40	5.85 ±0.14		52 ±4	80 ±6	132 ±8
	M	36.18 ±0.36	22.24 ±0.27	2.00 ±0.07	7.00 ±0.30	8.00 ±0.32	36.00 ±0.36	8.183	11.78 ±0.12	9.96 ±0.15	1.82 ±0.07	15.45 ±0.24	185.33 ±4.95	2.51 ±0.08		-	-	-
19	F	42.04 ±0.66	25.39 ±0.22	3.75 ±0.15	6.75 ±0.27	7.25 ±0.19	36.75 ±0.54	7.286	13.83 ±0.14	11.98 ±0.22	1.85 ±0.04	13.37 ±0.30	162.81 ±6.20	6.08 ±0.12		50 ±3	102 ±8	152 ±9
	M	37.86 ±0.49	22.45 ±0.20	1.50 ±0.06	7.75 ±0.19	9.00 ±0.35	38.25 ±0.72	8.775	12.07 ±0.12	10.10 ±0.15	1.97 ±0.06	16.32 ±0.18	198.00 ±5.90	2.53 ±0.07		-	-	-
20	F	44.49 ±0.53	26.36 ±0.30	3.00 ±0.14	7.75 ±0.21	7.75 ±0.22	38.50 ±0.58	7.777	14.84 ±0.12	12.79 ±0.20	2.05 ±0.13	13.81 ±0.20	170.72 ±4.59	6.13 ±0.08		68 ±5	82 ±4	150 ±8
21	F	45.88 ±0.58	27.29 ±0.42	2.25 ±0.06	7.75 ±0.15	8.25 ±0.30	39.25 ±0.67	8.354	15.38 ±0.15	13.10 ±0.24	2.28 ±0.10	14.82 ±0.21	190.33 ±4.00	6.29 ±0.10		159 ±4	26 ±7	185 ±5
22	F	47.21 ±0.44	27.63 ±0.29	1.50 ±0.05	7.25 ±0.30	9.00 ±0.15	39.75 ±0.44	8.469	15.83 ±0.14	13.49 ±0.21	2.34 ±0.09	14.78 ±0.24	208.66 ±2.70	6.47 ±0.20		168 ±5	36 ±4	204 ±8
20 (Control)	M	29.17 ±0.40	22.83 ±0.28	0.53 ±0.03	7.75 ±0.26	9.00 ±0.24	38.28 ±0.58	9.111	12.33 ±0.12	1025 ±0.15	2.08 ±0.06	16.87 ±0.30	205.12 ±4.02	2.74 ±0.06		-	-	-
22 (Control)	F	33.90 ±0.51	27.78 ±0.30	0.58 ±0.04	7.75 ±0.24	9.00 ±0.36	40.33 ±0.66	8.603	15.91 ±0.10	13.52 ±0.16	2.39 ±0.07	15.02 ±0.28	211.83 ±2.07	6.56 ±0.15		178 ±7	34 ±6	212 ±9

M = Male, F = Female.

larvae slowly increased with the advanced age of larvae subjected to starvation. The starved larvae of early ages took lesser time for completion of spinning. Simultaneously the prepupal duration was also shortened slightly. The observed and predicted values of critical and maximum weights of pupae and adults were very close signifying the accuracy of the experimental results. This was true for both the broods (Table 16).

4.2.4. Ligation

The effect of neck ligation experiment on the gut purging and pupal moult was studied on 'O'- day old 5th stage larvae to the pre-pupae 10-12 hrs before pupation. The results observed were consistent in both the sexes and the broods. Ligation upto the age of 8 day in ND and 14 day in the D-generation debarred the larvae to show any prodormal sign of pupation (except in negligible number of individuals) and such larvae died eventually.(Figs. 13 &14). In case of ligation from the age of 9th or 15th day of the respective broods onwards upto the day of normal gut purge in almost all the ligated larvae gut purging occurred, first surge within 2-24 hrs after ligation followed by intermittent surges which ended within 24-72 hrs after ligation depending on the age of the larvae ligated. The body of the silk-worms had undergone shrinkage together with immobility of anal prolegs when ligated during this period (Table 17, Fig.15). Silkworms when ligatured from the age of 5 hrs after gut purge and upto 3-4 day before pupation, had undergone larval-pupal intermoult in few cases though exhibited all other prodormal signs of pupation such as body shrinkage and loss of mobility of anal prolegs Anteriorily these developed pre-pupal and posteriority (specially abdomen) pupal morphology (Fig. 16). Ultimately these silkworms in both the generations were able to ecdyse into apparently headless pupae (Fig. 17). Moreover, most of the larvae ligated at relatively late-age i.e. more approaching to pupation day survived upto 2-3 months irrespective of sex and generation and ultimately died. Further, the headless pupae could not grow and emerge out as moths and died within 10-60 days.

Table 16. **Quality indices of different stages of *A.mylitta* during two generations. All weights are in grams. Values are means ± S.E. where applicable. Predicted values are in parentheses**

Stages	Non - diapause		Diapause	
	Male	Female	Male	Female
Lcw	26.90 ±0.90	34.37 ±0.64	27.88 ±0.64	35.29 ±0.54
Lmw	32.63 ±0.61	41.55 ±0.40	38.05 ±0.65	47.29 ±0.42
D_p (Decrease of wt. in pupa).	0.724	0.707	0.731	0.714
D_a (Decrease of wt. in adult)	0.908	0.826	0.928	0.861
Pmw	(9.00) 8.99 ±0.15	(12.17) 12.16 ±0.12	(10.26) 10.25 ±0.15	(13.53) 13.52 ±0.16
Pcw	(7.42) 7.19 ±0.18	(10.07) 9.85 ±0.16	(7.50) 7.31 ±0.28	(10.09) 9.98 ±0.30
Amw	(3.00) 3.01 ±0.06	(7.23) 7.24 ±0.10	(2.74) 2.74 ±0.06	(6.57) 6.56 ±0.15
Acw	(2.47) 2.39 ±0.07	(5.98) 5.50 ±0.12	(2.01) 2.07 ±0.09	(4.90) 4.86 ±0.12

Fig.13: Neck-ligated 5th stage larvae of non-diapause gen eration of *A.mylitta*. Ligated larva rolled up the body compared with that of normal one above.

Fig. 14: Neck-ligated 5th stage larvae of non-diapause gen eration of *A.mylitta*. Larvae ligated upto the age of 8th day showing no prodormal sign of pupation and died eventually.

Fig.13

Fig.14

Table 17. **Effect of neck ligation of 5th instar larvae on the expression of prodormal signs for pupation in the two gen erations of *A.mylitta*. Positive responses are expressed by (+) and the negative ones by (-).**

Ligation time	GPR and wandering	Body shrinkage	Immobility of anal prolegs.	Immobility of abdominal prolegs.	Pupal cuticle formation	Larval-pupal intermediates	Pupation
0-day to the day of Lcw.	-	-	-	-	-	-	-
0-hr. of Lcw to 0-hr. of GPR.	+	+	+	-	-	-	-
5-hr. after GPR to 3-4 day before pupation.	+	+	+	+	+ (partial)	+	-
2-3 day before pupation to the day of pupation (before 10-12 hrs.)	+	+	+	+	+	-	+

Fig.15 : Neck-ligation of 5th stage larvae on different ages. a-c. 5-hr after GPR to 3-4 day before pupation. d and e. 3-2 day before pupation.

Fig.16: Larval-pupal intermediates resulted from ligation of larvae 5-hr after GPR to 3-4 day before pupation, corresponding to a-c in figure 15.

Fig.17: Head less pupae resulted from ligation of larvae 3-2 day before pupation, corresponding to d and e in figure 15.

Fig.15

Fig.16

Fig.17

4.2.5 Critical period for gated release of PTTH

On the basis of above findings a sequential model for three step gated release of PTTH is presented in Figures 18 & 19 during the fifth larval life span and upto the larval-pupal moult. The first release appeared to be on the day after attaining the Lcw, second 5 hrs after complete gut purge (GPR) and third step within 2-3 days before pupation.

4.2.6 Climatological conditions encountered by the larvae during starvation and neck ligation experiment

With respect to the max. temp., min. temp., r.h.% , L:D hrs and total rainfall there were considerable difference during the life of the two broods. Rainfall was almost nil during D-generation. L:D hrs were reversed in the two broods. The temperature and r.h.% values were lower in the D-brood (Table 18).

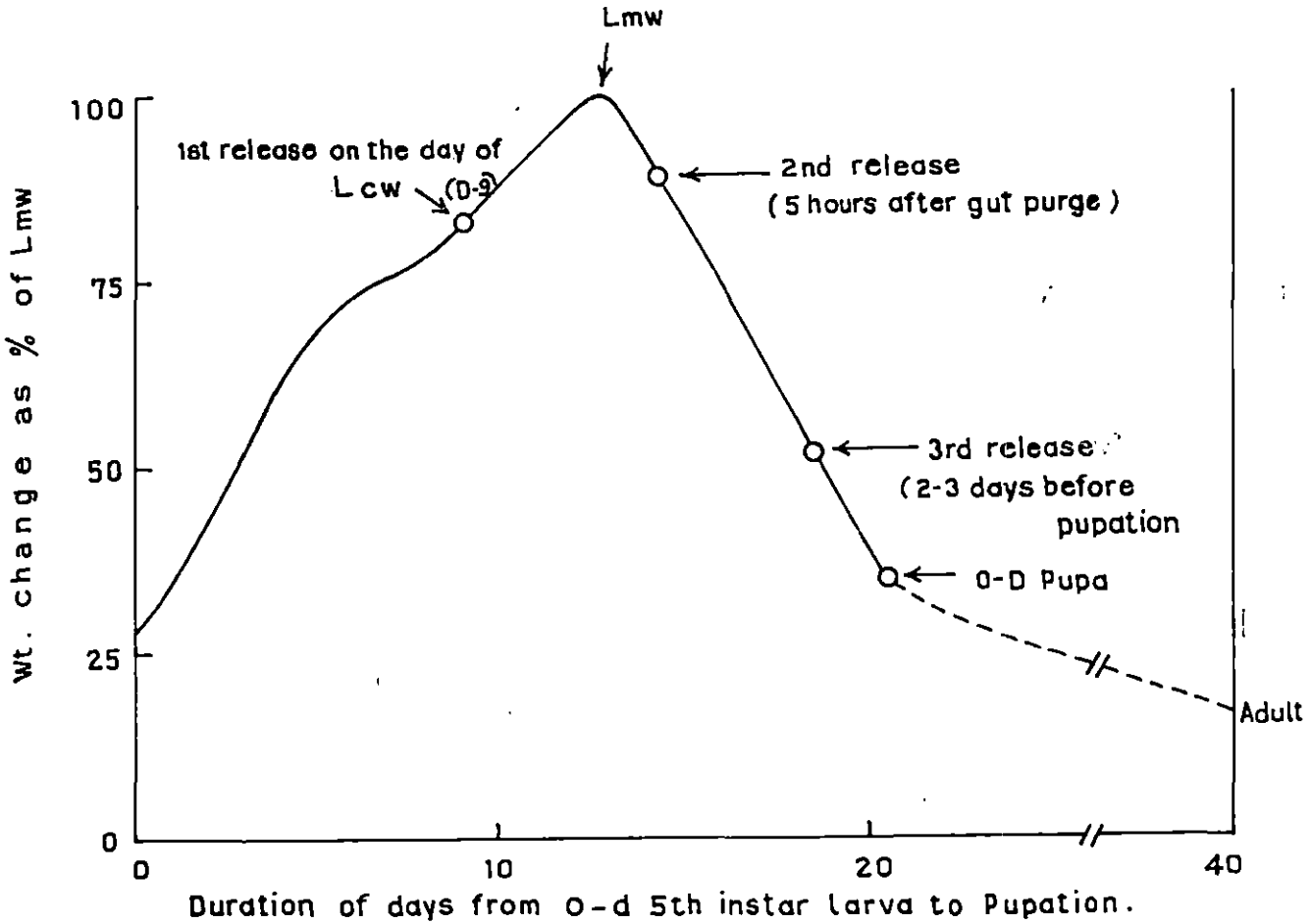


Fig. 18. Model of sequential gated release of PTTH in non-diapause generation of A. mylitta. Lcw = larval critical wt., Lmw = larval maximum wt.

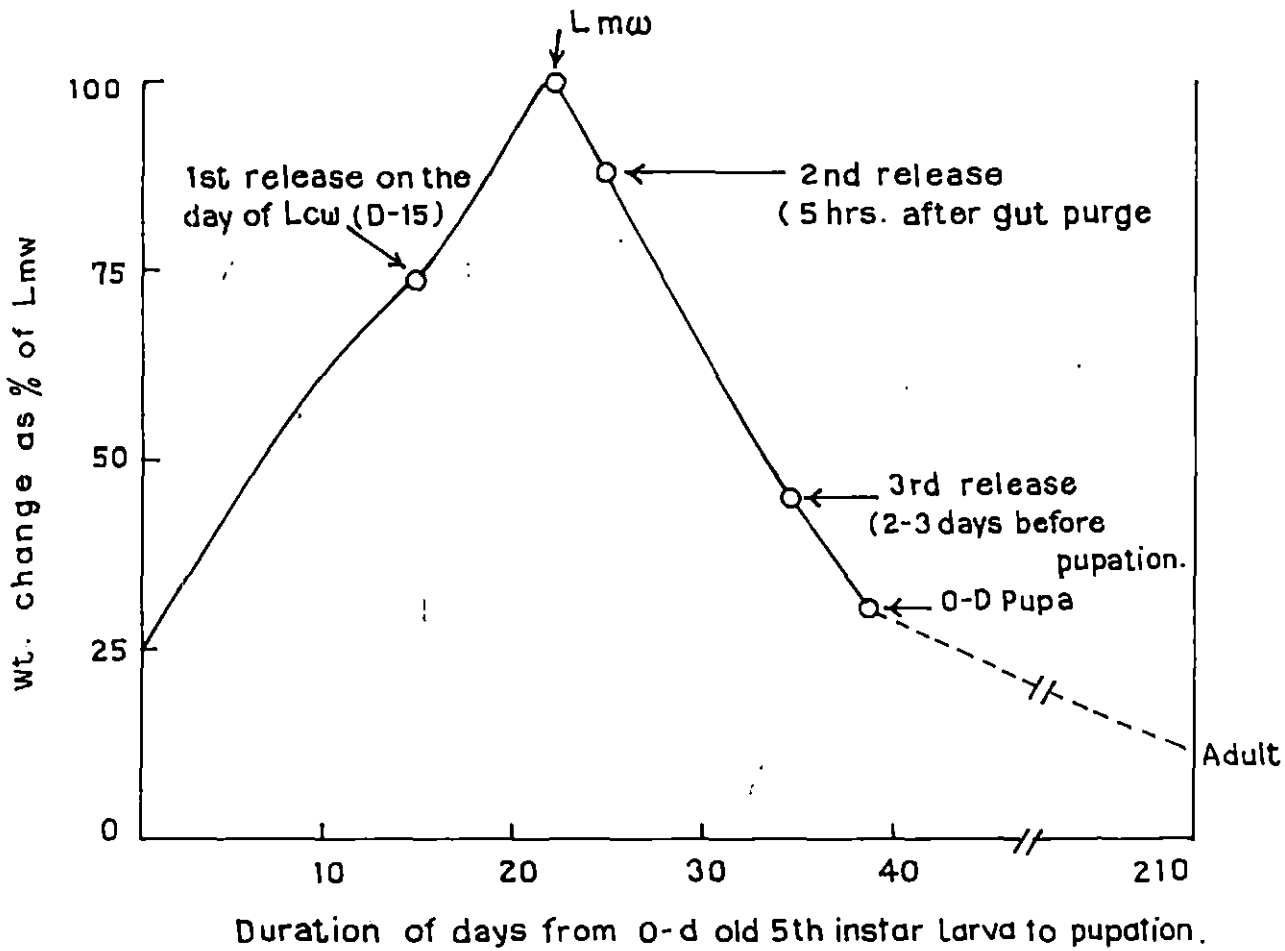


Fig. 19. Model of Sequential gated release of PTTH in diapause-destined generation of A. mylitta. Lcω = larval critical wt, Lmw = larval maximum wt.

Table 18. Climatological conditions availed by the 5th stage larvae during starvation and ligation experiments in the two broods of *A.mylitta*. Values are mean ± S.E.

Brood	Period of 5th larval life	Max . temp. (°C)	Min. temp. (°C)	R.H (%)	L : D (hrs.)	Total rainfall (mm)
ND	Feeding	28.22 ±0.28	22.01 ±0.09	91.00 ±0.69	12.93 : 11.07 ±0.019 ±0.019	139.0
	Non-feeding (upto pupation)	27.36 ±0.66	21.61 ±0.12	90.75 ±0.97	12.72 : 11.28 ±0.015 ±0.015	158.7
D	Feeding	26.65 ±0.31	12.34 ±0.50	80.57 ±0.93	11.33 : 12.67 ±0.03 ±0.03	-
	Non-feeding (upto pupation)	24.90 ±0.37	8.80 ±0.51	77.62 ±1.14	11.02 : 12.98 ±0.02 ±0.02	0.75

ND - Non - diapause; D - Diapause.

4.3. Profile of Cholesterol, Protein, DNA and RNA Contents in Some Tissues of Pre-pupae, Pupae and Adults of Non-Diapause and Diapause Generations

4.3.1. Cholesterol content in haemolymph, fat body and gonad

4.3.1.1. Haemolymph cholesterol :

The cholesterol content of haemolymph plasma of male and female *A.mylitta* of non-diapause generation increased significantly ($P<0.001$) from pre-pupal stage to pupa reaching the peak on day 14 of pupal development. Thereafter, it declined sharply ($P<0.001$) just before pupal - adult moult and in freshly emerged adults ($P<0.01$). Likewise , cholesterol titre in diapause generation increased from pre-pupal age to 15 day old pupa showing the peak level after which gradual decrease in cholesterol level occurred till adult emergence on or about 210 day indicating a slower rate of cholesterol deposition in haemolymph from fat body or other tissue sources and later subsequent utilization and/or transportation for growth and adult development. Compared with the values of males, the degree of rise and fall of cholesterol content in female was higher in most of the cases. However, female haemolymph contained significantly more cholesterol than male counterparts upto 14 day and 150 day of pupal development in non-diapause and diapause generation respectively. Thereafter, cholesterol content was found at lower level upto adult emergence in both the generations (Table 19 & 20, Figs. 20 & 21).

4.3.1.2. Fat body cholesterol :

Cholesterol concentration in non-diapause and diapause generation sharply declined ($P<0.001$) from pre-pupa to 0-day pupa (particularly in female) i.e. during larval-pupal transformation indicating the utilization of the

said biomolecules for pupal organogenesis. Thereafter, gradual increase in cholesterol content was found in non-diapause generation reaching the peak level on 14-day old pupa. On the contrary, the elevation in cholesterol level is very sharp in diapause generation from 0-day pupa to 40-day old pupa and ultimately touched the peak on 150 day of pupal development. However, a reduction in cholesterol titre was recorded in fat body cells on 14-day and 170-day onwards upto adult emergence in non-diapause and diapause generation respectively. The decrease in cholesterol concentration during the later phase of pupal development in both the generations again confirmed the transportation and/or utilization of the cholesterol for pupal - adult transformation. Further, female fat body contained significantly higher cholesterol (µg/100 mg tissue) from pre-pupa to 14-day old pupa in non-diapause generation but only from pre-pupa to 0-day pupa in diapause generation thereafter it was found to be low. Thus, it is evident that the transportation and/or utilization of cholesterol in female fat body started more earlier in diapause generation and continued for a longer period i.e. upto adult emergence than that of non-diapausing ones. However, the reason is unknown (Tables 19 & 20, Figs. 20 & 21).

4.3.1.3. Gonad cholesterol

In gonad (testis and ovary), the cholesterol content showed a specific pattern of variation in both the generations where a gradual increase of the biomolecules upto adult emergence was recorded. Ovary showed always a higher cholesterol titre than testis. It is interesting to point out that in the initial stages i.e.from pre-pupa to 0-day pupa cholesterol level in both the tissues of testis and ovary remained significantly higher ($P<0.02$ - $P<0.001$) in diapausing generation compared to that of non-diapausing ones. But, afterwards it remained low in both the sexes of diapausing generation upto adult emergence (with few exception). This again established the slower rate of cholesterol metabolism (particularly deposition of this biomolecule in gonad) in diapausing generation while the rate was very faster in the non-diapausing animals (Tables. 19 & 20, Figs. 20 & 21).

Table 19. Cholesterol content in different tissues of non-diapausing *A.mylitta* during pre-pupal pupal and adult stages of both the sexes. Each value represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Haemolymph (µg/ml)		Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F	M	F
Pre-pupa	295.49 ±6.27	365.33 ±9.44 c	392.79 ±9.60	901.26 ±7.38 c	128.42 ±4.06	375.00 ±9.11 c
0-day pupa	482.87 ±6.55	914.31 ±9.13 c	275.70 ±4.15	525.32 ±11.15 c	185.75 ±4.96	425.48 ±8.14 c
7-day pupa	685.71 ±12.68	1015.72 ±18.87 c	457.01 ±25.59	684.14 ±28.55 c	457.63 ±9.10	528.33 ±20.30 b
14-day pupa	1110.20 ±55.34	1806.87 ±44.66 c	1492.60 ±32.87	1613.15 ±35.70 a	875.08 ±9.33	1045.40 ±25.31 c
21-day pupa	622.74 ±18.32	543.66 ±22.50 a	1165.06 ±17.39	652.70 ±8.62 c	925.78 ±12.34	1686.80 ±11.49 c
Freshly emerged adult (22 day)	543.90 ±15.42	472.81 ±19.75 a	766.03 ±12.11	418.64 ± 9.31 c	982.61 ±10.29	1875.18 ±35.81 c

't' - test probability differences (male vs. female) : M = Male ; F = Female.
a = P<0.02 ,
b = P<0.01,
c = P<0.001.

Table 20. Cholesterol content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represent Mean±S.E (n=10).

Tissue / Stage of insect	Haemolymph (µg/ml)		Fat body (µg/100mg)		Gonads (µg/100mg)	
	M	F	M	F	M	F
Pre-pupa	338.48 ±5.57	404.15 ±4.94 c	431.06 ±3.37	1023.57 ±6.72 c	186.77 ±3.86	439.98 ±14.21 c
0-day pupa	409.12 ±5.75	494.26 ±11.35 c	310.96 ±7.95	683.84 ±12.39 c	207.24 ±9.68	450.38 ±18.44 c
40-day pupa	740.46 ±16.19	850.86 ±28.15 b	2095.19 ±68.92	1752.88 ±36.09 c	262.89 ±8.95	692.86 ±11.53 c
105-day pupa	778.94 ±10.86	1094.63 ±42.78 c	2567.85 ±86.30	1944.67 ±56.95 c	508.12 ±19.36	1206.18 ±35.97 c
150-day pupa	2172.46 ±52.39	2350.84 ±69.40 a	3765.49 ±68.33	2525.30 ±65.56 c	720.83 ±20.59	1325.60 ±24.35 c
170-day pupa	1922.64 ±42.11	1882.00 ±55.10 NS	2850.94 ±62.44	1878.86 ±51.90 c	815.16 ±15.30	1414.49 ±29.05 c
200-day pupa	1725.72 ±33.43	1573.50 ±48.79 a	1469.08 ±16.44	713.25 ±15.25 c	913.88 ±31.30	1576.77 ±39.40 c
Freshly emerged adult (210-day)	1619.41 ±25.33	922.37 ±38.09 c	965.12 ±31.79	515.58 ±23.43 c	924.35 ±26.30	1751.77 ±56.32 c

't'-test probability differences (Male vs Female) :

a = P<0.05, M = Male ; F = Female.
b = P<0.01,
c = P<0.001,
NS = Not significant.

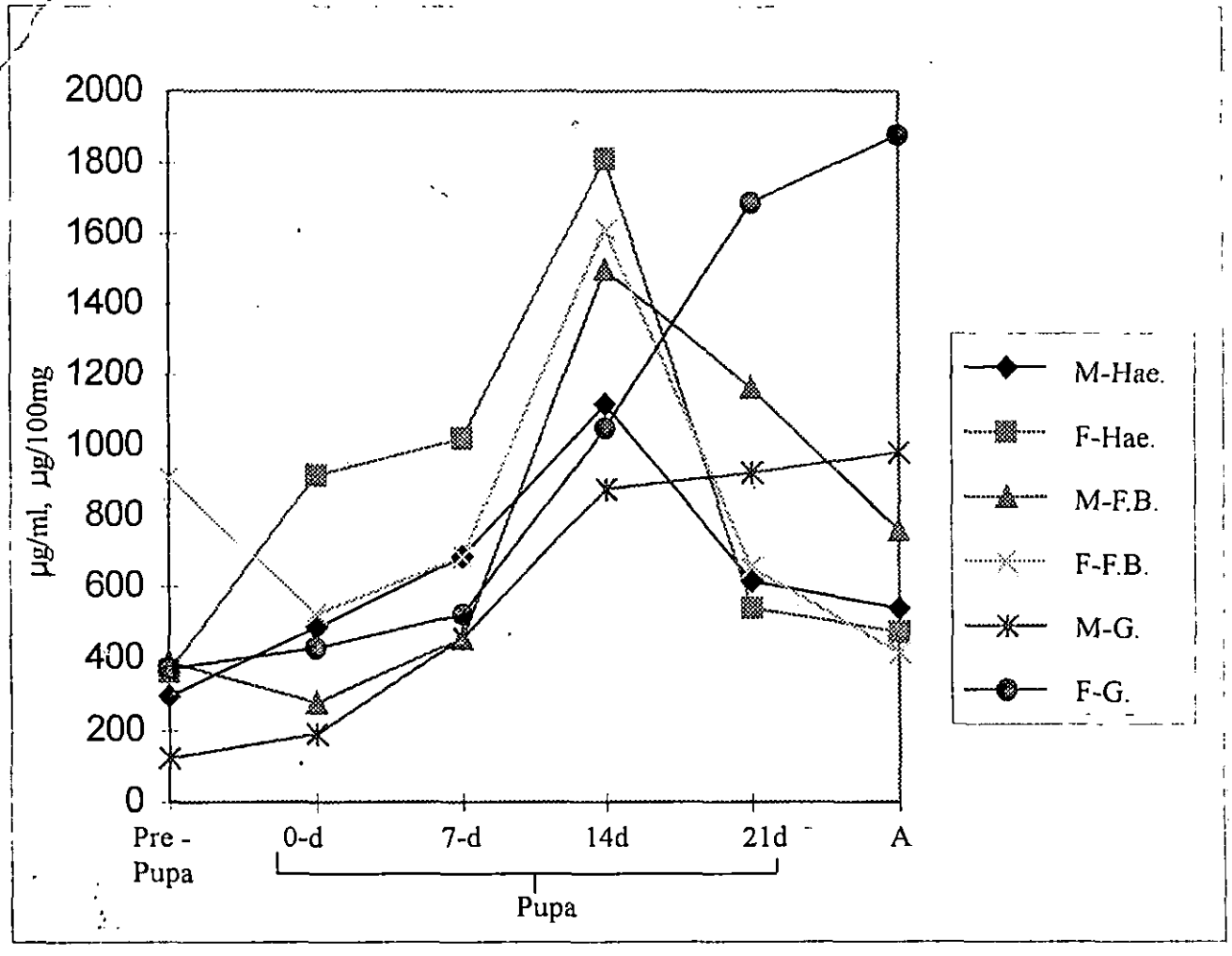


Fig.20. Cholesterol content in different tissues of non-diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes.M= Male, F= Female, A = Adult, Hae.= Haemolymph (µg/ml) F.B.= Fat Body (µg/100mg) , G= Gonad (µg/100mg) .

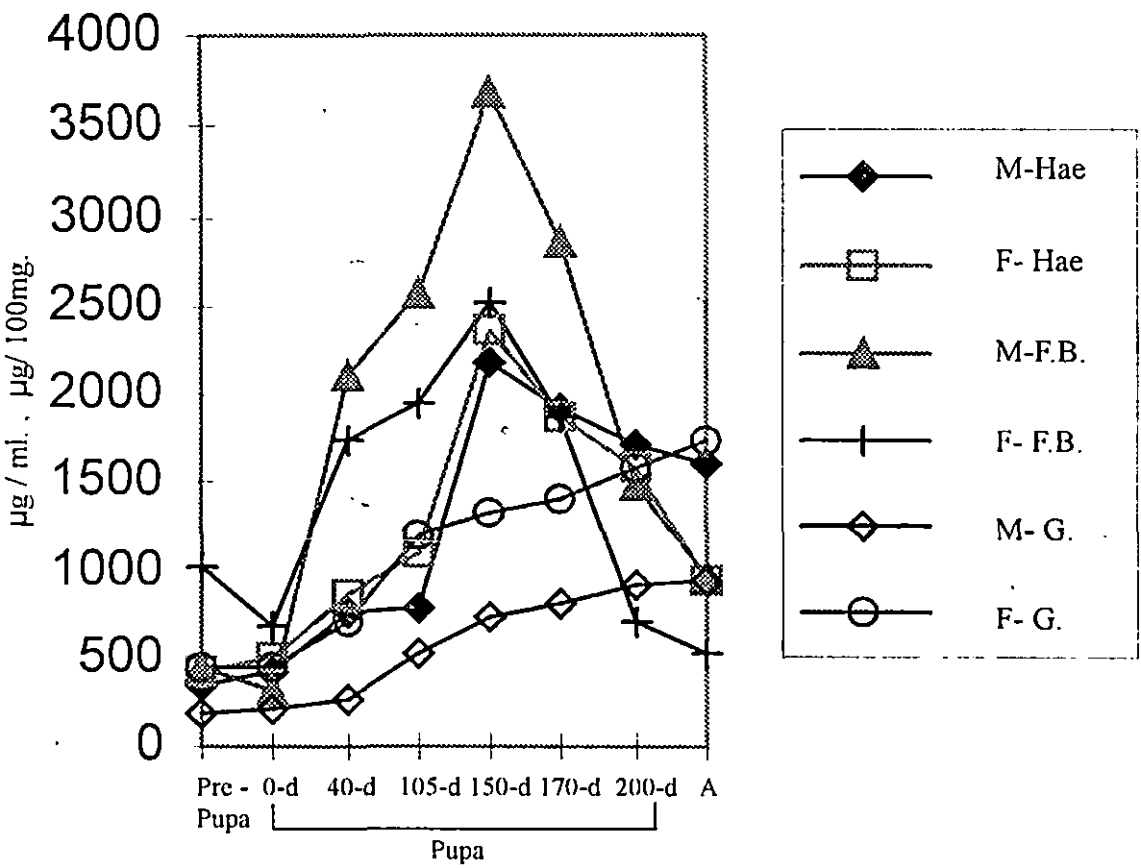


Fig.21. Cholesterol content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, Hae=Haemolymph (µg / ml), F.B.=Fat Body (µg/100mg) , G=Gonad (µg / 100mg), A=Adult.

4.3.2. Total protein content in haemolymph , fat body and gonads

4.3.2.1. Haemolymph protein

Age-dependent changes in haemolymph protein concentration in non-diapause and diapause generations were found to be quite different. In the non-diapause brood animals showed gradual and significant decrease after pre-pupal stage i.e. starting from 0-day pupa to adult emergence. But, in diapause-destined insects peak level of total protein concentration was attained in the pre-pupae and then the protein level decreased slowly but significantly ($P<0.001$) in the 0-day pupa. Thereafter, a sharp decrease in protein titre was recorded in 40 day of pupal age followed by a steep rise in 105 day old pupa. However, this level was maintained upto 150 day during diapause development. Interestingly, after 150 day the total protein content again declined in the subsequent developmental steps gradually and significantly ($P<0.01$ - $P<0.001$) upto the day of adult emergence reaching there at minimum level. During different developmental steps (pre-pupa to adult emergence) the fall and rise in protein level strongly support that the deposition, transportation and utilization of this biomolecule helps to maintain and later on to terminate the diapause state. Thus, 150 day of pupal age may be considered for critical phase for diapause termination in *A.mylitta*. Further, irrespective of sex diapausing taser insects contained higher protein in haemolymph than the non-diapausing ones and females contained significantly more amount of protein than that of males in both the generations (Tables 21 & 22, Figs. 22 & 23).

4.3.2.2 Fat body protein

In the non-diapausing brood total protein content of male and female fat body gradually elevated significantly ($P<0.05$ - $P<0.01$) from pre-pupal stage to 7-day pupa attaining the peak level. Thereafter, protein concentration steadily declined upto adult emergence. In the diapausing generation the fat body protein level increased very slowly upto 150 day of pupal age during diapause development preceded by a significant fall ($P<0.001$) on 105 day old pupa. Then, from 170 day onwards steady decrease

in fat body protein concentration was recorded upto adult emergence. Male and female insects showed age and stage specific pattern of variation in this biomolecule in both the generations which was found to be quite different from each other. However, diapausing generation showed higher level of fat body protein compared to non-diapausing one and females contained more fat body protein then male counterparts in all the cases. The rise and fall in fat body protein concentration in both sexes revealed the synthesis of protein in fat body, then its transportation to the haemolymph and subsequent re-sequestration to the fat body (Tables21 & 22, Figs. 22 & 23).

4.3.2.3. Gonad protein

In general, gonadal protein concentration of male and female insects were found to increase significantly ($P<0.001$) from pre-pupa to adult emergence covering the entire gamut of pupal development in non-diapause and diapause-destined generations. In both the generations the rate of increase of protein level in testis was recorded very low. While in ovary the rise in protein level was very slow only upto 0-day and 40-day old pupa of non-diapause and diapause generation respectively. Again a quantum increase in ovarian protein level was revealed on 7th day of pupal age in non-diapause and 105 day in case of diapausing pupae. Thereafter, the rate of increase of this biomolecule was found to be slow and gradual upto adult stage which showed ultimately the peak level in both the generations. Female gonad contained higher amount of protein throughout the life span with exception in prepupa to 0-day old pupa in case of non-diapause and from pre-pupa to 40-day old pupa for the diapausing generation. It reveals that ovarian maturation took place steadily from 7th day and 105 day onwards in non-diapause and diapause generations respectively and corresponding fluctuations in protein and cholesterol concentration in fat body and haemolymph (stated earlier) also support this observation. Further, male and female gonads in diapausing generation contained higher amount of protein compared to that of non-diapausing ones in most of the cases. (Tables 21 & 22, Figs. 22 & 23).

Table21. Total protein content in different tissues of non-diapausing *A. mylitta* during pre-pupal pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Haemolymph (mg/ml)		Fat body (mg/100mg)		Gonad (mg/100mg)	
	M	F	M	F	M	F
Pre-pupa	22.29 ±0.30	30.20 ±0.12 a	4.12 ±0.09	5.66 ±0.18 a	2.20 ±0.10	0.95 ±0.04 a
0-day pupa	20.56 ±0.10	26.21 ±0.19 a	4.63 ±0.15	6.82 ±0.21 a	3.16 ±0.21	1.55 ±0.20 a
7-day pupa	13.98 ±0.22	21.43 ±0.38 a	5.08 ±0.12	7.60 ±0.25 a	3.85 ±0.16	6.74 ±0.42 a
14-day pupa	12.24 ±0.31	17.61 ±0.44 a	4.11 ±0.21	6.75 ±0.14 a	4.39 ±0.25	10.15 ±0.38 a
21-day pupa	11.10 ±0.11	15.82 ±0.15 a	3.16 ±0.22	4.33 ±0.20 a	5.26 ±0.12	13.03 ±0.39 a
Freshly emerged adult (22-day)	9.62 ±0.18	12.10 ±0.31 a	2.34 ±0.10	3.69 ±0.11 a	5.98 ±0.14	14.14 ±0.50 a

't'-test probability differences (Male vs. Female) M = Male ; F = Female.

a = $P < 0.001$

Table 22. Total protein content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represent Mean±S.E (n=10).

Tissue / Stage of insect	Haemolymph (mg/ml)		Fat body (mg/100mg)		Gonads (mg/100mg)	
	M	F	M	F	M	F
Pre-pupa	28.45 ±0.29	34.19 ±0.32 a	4.46 ±0.13	6.85 ±0.22 a	2.58 ±0.04	1.15 ±0.07 a
0-Day pupa	25.52 ±0.26	30.43 ±0.35 a	5.10 ±0.12	7.90 ±0.21 a	3.97 ±0.06	1.83 ±0.10 a
40-day pupa	4.86 ±0.08	6.74 ±0.33 a	5.65 ±0.17	8.94 ±0.25 a	4.08 ±0.20	2.96 ±0.15 a
105-day pupa	14.28 ±0.17	22.07 ±0.34 a	4.05 ±0.11	6.20 ±0.15 a	4.24 ±0.17	8.52 ±0.20 a
150-day pupa	14.65 ±0.15	22.52 ±0.42 a	6.08 ±0.10	11.85 ±0.12 a	4.97 ±0.16	10.41 ±0.22 a
170-day pupa	12.17 ±0.18	16.42 ±0.34 a	5.40 ±0.14	8.77 ±0.11 a	5.32 ±0.14	11.30 ±0.25 a
200-day pupa	11.45 ±0.10	15.08 ±0.15 a	4.02 ±0.13	7.48 ±0.20 a	5.84 ±0.10	13.54 ±0.35 a
Freshly emerged adult (210 day)	10.14 ±0.22	13.29 ±0.36 a	2.97 ±0.10	4.76 ±0.14 a	6.70 ±0.17	15.68 ±0.40 a

't'-test probability differences (Male vs Female) : M = Male, F = Female

a = P<0.001

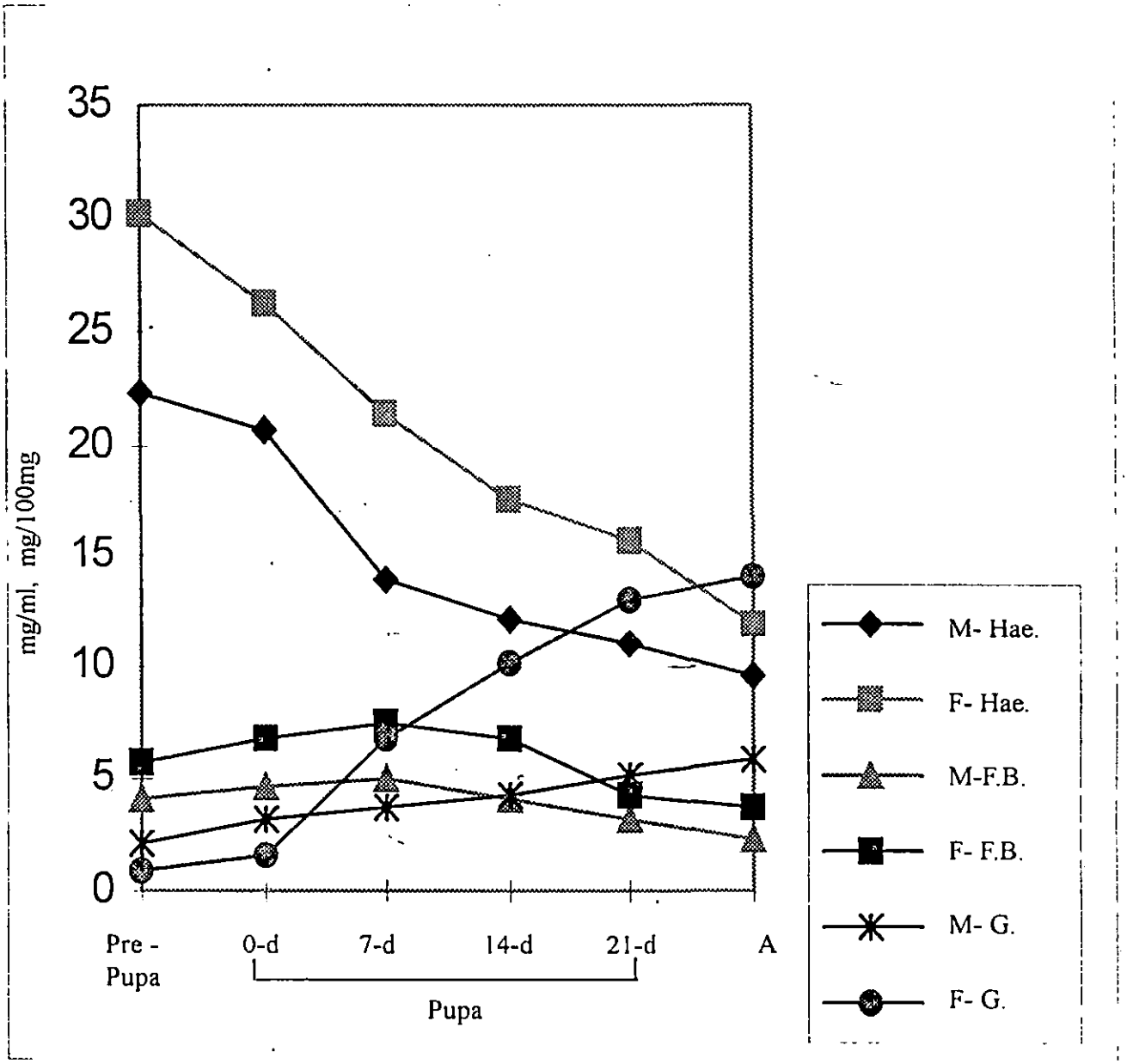


Fig .22. Total protein content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, Hae.= Haemolymph (mg/ml) F.B.= Fat Body(mg/100mg) , G= Gonad (mg/100mg).

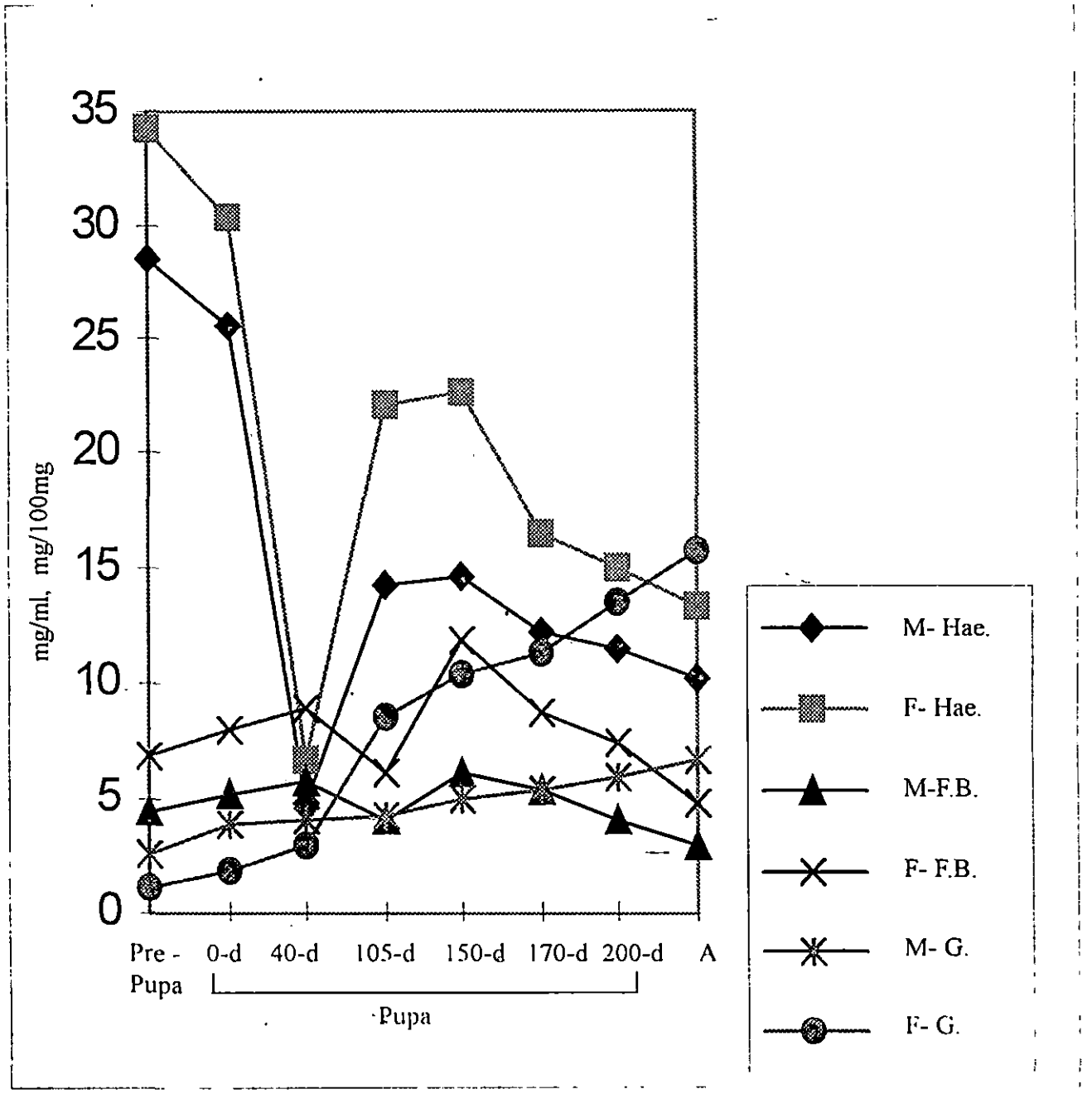


Fig .23. Total protein content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, Hae.= Haemolymph (mg/ml) F.B.= Fat Body(mg/100mg) , G= Gonad (mg/100mg).

4.3.3. DNA content in fat body and gonad

4.3.3.1. Fat body DNA

Pattern of age-dependent variation in DNA content in fat body is not similar between the two generations. In case of non-diapause brood DNA concentration in male and female fat body suddenly increased from pre-pupa to 0-day pupa i.e. after larval-pupal transformation, then gradually declined upto 21 day old pupa and a same level was maintained till adult emergence. Female fat body showed significantly higher value ($P<0.05$ to $P<0.001$) in DNA concentration throughout the pupal phase, but exhibited lower level in DNA titre ($P<0.001$) in pre-pupal stage compared to that of male. Further, there was no significant difference of this biomolecule in freshly emerged adults.

In diapausing generation variation in DNA content in fat body between the two sexes is different in pre-pupa, pupa and adult stages. In male, DNA content in fat body cells showed peak level twice throughout the life span studied from pre-pupa to adult emergence. First, peak of DNA level was observed on 0-day pupa followed by a gradual fall upto 105-day while second peak was recorded on 150 day of pupal age following gradual and significant fall ($P<0.001$) upto 200 day of pupal age and same level was maintained till adult emergence. In female gradual increment of DNA concentration was found upto 150 day pupa exhibiting first peak while second peak was noted on 200-days of pupal development preceeded by a drastic fall on 170-day old pupa. However, no significant difference in DNA concentration of female fat body was observed between 200 day old pupa and adult moths. Unlike in non-diapause brood, female fat body contained less amount of DNA from pre-pupa to 170-day old pupa but it was recorded to be higher only at late phase of life span i.e. 200 day old pupa and freshly emerged adults. It is pertinent to mention here that frequent rise and fall in fat body DNA concentration particularly in diapausing brood irrespective of sexes actually reflects the alteration of metabolic state of fat body tissue particularly in relation to protein and RNA synthesis for maintaining and terminating the diapause state of this insect (Tables23 & 24, Figs. 24 & 25).

4.3.3.2 Gonad DNA

In gonad also the pattern of variation in DNA concentration was different in between the two generations like fat body cells as found earlier. In the non-diapause brood DNA concentration in testis was found to increase sharply from pre-pupal stage onwards to 7th day old pupa and then drastically reduced to a minimum level on day of adult emergence (22 day). The female gonad also maintained the same trend like testis with the difference that ovary always contained high level of DNA. The DNA concentration in diapausing testis was increased day by day from pre-pupal stage and reached maximum level on the day of adult emergence (210 day). A steep rise in DNA level was recorded between 40 to 105 day of pupal age. But in case of female, the DNA titre in ovary increased day by day and touched the peak level on 150 day pupa; thereafter gradual reduction in DNA content was recorded upto the day of adult eclosion. It should be mentioned here that ovary of pre; pupa, 0-day old pupa, 200 day old pupa and freshly emerged adult showed low amount of DNA than their male counterparts which is very unlike from that of non-diapause generation. However, the cause of such variation in DNA concentration is not known. But, it can be presumed the this pattern of variation in DNA level only reflects the RNA and protein synthesis according to the stage and/or age-specific requirement for development and growth during diapause (Table 23 & 24, Figs. 24 & 25).

4.3.4. RNA content in fat body and gonad

4.3.4.1. Fat body RNA

The pattern of variation in RNA content of fat body is similar tin the male and female pre-pupae, pupae and freshly emerged adults of non-diapause generation. In both sexes RNA content in fat body increased significantly ($P<0.001$) from pre-pupa to 0-day old pupa followed by gradual reduction in RNA level upto 7 day pupa. Thereafter, a very sharp decrease in RNA concentration was recorded till adult emergence and reached the minimum level. Females always contained significantly higher amount of RNA in fat body than the males.

In both sexes of diapause generation the pattern of variation in RNA titre of fat body during the different developmental stages is different from that of non-diapausing insects. However, in this brood male and female fat body followed similar pattern of variation in RNA content in every developmental stages studied. The RNA concentration in fat body of two sexes first significantly decreased ($P<0.01$ to $P<0.001$) from peak level in pre-pupa to 40-day old pupa. Thereafter, the concentration of this biomolecule was found to be increased significantly ($P<0.001$) in both the sexes and again touched the peak on 150 day old pupa after which a significant drastic fall ($P<0.001$) in RNA level was recorded in the two sexes from 150 day onwards upto the day of adult emergence and was found lowest amount in freshly emerged male and female moths. Unlike non-diapause generation female fat body contained more amount of RNA than in the male fat body from pre-pupa to 40-day old pupa and then from 200 day of pupal age to freshly emerged adults (210 day). However, RNA level in female fat body was found to be significantly lower in 105-day, 150-day and 170-day old diapausing pupae. (Tables 25 & 26, Figs. 26 & 27).

4.3.4.2. Gonad RNA

In both non-diapause and diapause generations male and female gonads followed the same pattern of variation in RNA concentration from pre-pupal stage to the day of adult emergence. RNA level in both testis and ovary increased gradually and significantly ($P<0.02$ to $P<0.01$) from pre-pupal age to 7-day pupa and 105-day pupa of non-diapause and diapause generation respectively. Thereafter a gradual and significant fall in RNA content ($P<0.001$) was recorded and reached the lowest level in freshly emerged male and female adults of the two generations. Ovary always contained significantly higher ($P<0.01$ to $P<0.001$) amount of RNA than testis from pre-pupa to the day of adult eclosion in both the generations. Thus, it is evident that in case of non-diapause generation day 7 pupa and for diapausing generation 105-day old pupa is the critical phase for the initiation of gonadal development and maturation which is evident from the variations of DNA and protein concentrations of the same tissues (Tables 25 & 26, Figs 26 & 27).

Table 23. DNA content in different tissues of non-diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F
Pre-pupa	235.43 ±6.68	170.31 ±7.10 d	42.15 ±0.20	65.06 ±0.45 d
0-day pupa	265.39 ±6.10	334.10 ±6.19 d	108.20 ±2.91	141.60 ± 2.88 d
7-day pupa	241.60 ±3.39	272.05 ±5.10 d	281.78 ±3.66	392.52 ±8.04 d
14-day pupa	208.51 ±4.64	224.18 ±4.10 a	202.26 ±2.75	254.06 ±5.61 d
21-day pupa	197.70 ±5.69	218.51 ±5.14 b	192.99 ±3.71	210.14 ±2.82 c
Freshly emerged adult (22-day)	200.92 ±6.49	208.15 ±7.08 NS	180.64 ±2.17	201.39 ±4.16 d

't'-test probability differences (Male vs Female) :

a = P<0.05,
b = P<0.02,
c = P<0.01,
d = P<0.001,
NS = Not significant.

M = Male ; F = Female.

Table 24. DNA content in different tissues of diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each value represents Mean± S.E. (n=10)

Tissue / Stage of insect	Fat body (µg/100mg) M	Fat body (µg/100mg) F	Gonads (µg/100mg) M	Gonads (µg/100mg) F
Pre-pupa	246.87 ±7.34	105.72 ±6.86 c	28.67 ±1.09	25.25 ±0.94 a
0-day pupa	271.06 ±6.74	98.07 ±4.26 c	62.51 ±1.78	49.41 ±1.50 c
40-day pupa	239.50 ±4.10	131.15 ±5.24 c	86.24 ±3.42	182.53 ±6.28 c
105-day pupa	182.98 ±7.45	155.34 ±7.10 b	232.90 ±2.67	227.12 ±4.50 NS
150-day pupa	365.84 ±8.60	280.49 ±6.11 c	250.82 ±3.09	304.30 ±5.40 c
170-day pupa	206.35 ±11.69	178.74 ±3.81 a	262.40 ±2.33	271.61 ±7.94 NS
200-day pupa	175.33 ±6.28	266.13 ±9.34 c	285.94 ±3.12	187.63 ±6.45 c
Freshly emerged adult (210 day)	168.34 ±6.83	277.55 ±14.86 c	306.63 ±8.18	180.59 ±5.52 c

't'-test probability differences (Male vs Female) :

a = P<0.05, M = Male ; F = Female.
b = P< 0.02,
c = P<0.001,
NS = Not significant.

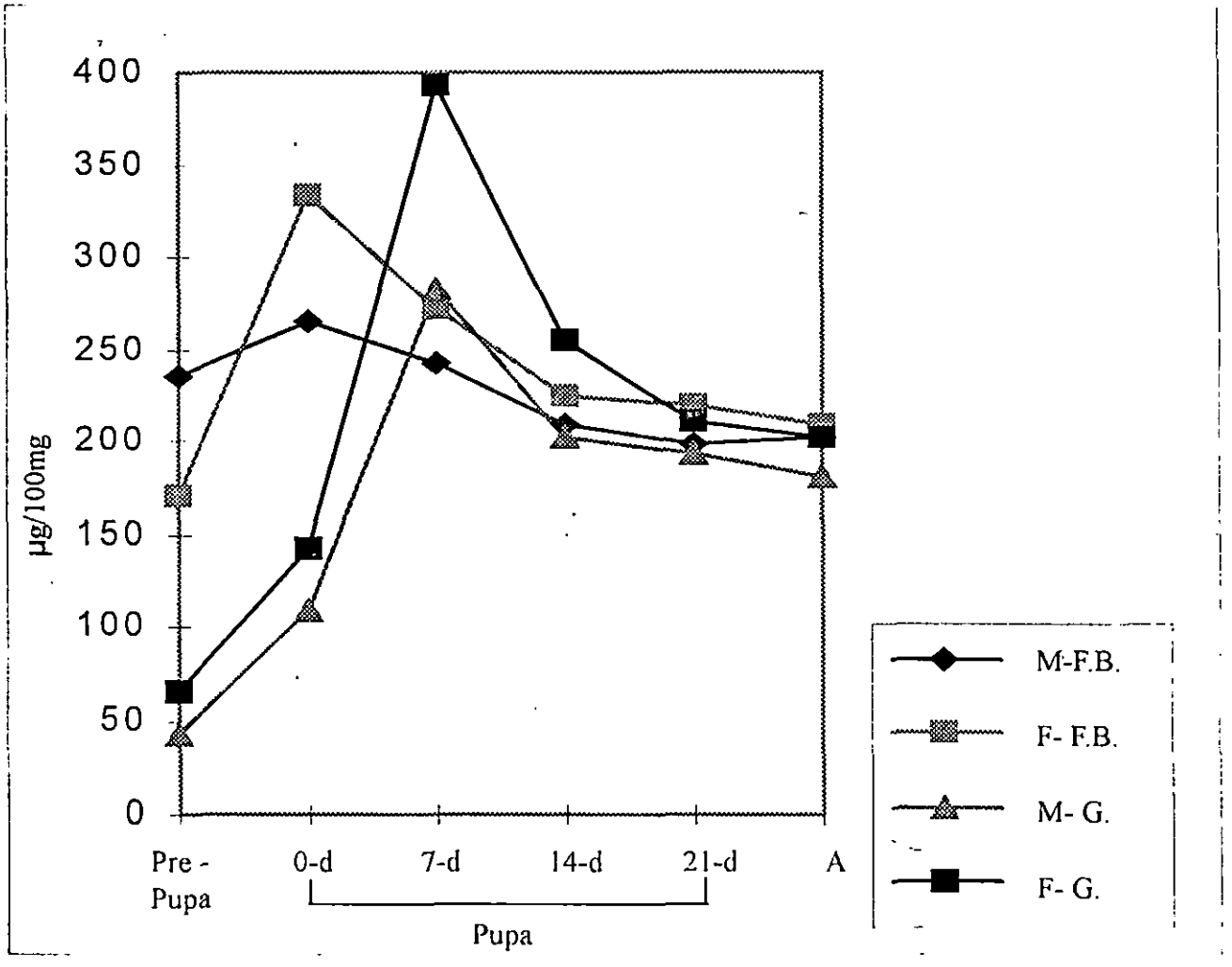


Fig. 24. DNA content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, F.B.= Fat Body (µg/100mg), G = Gonad (µg/100mg).

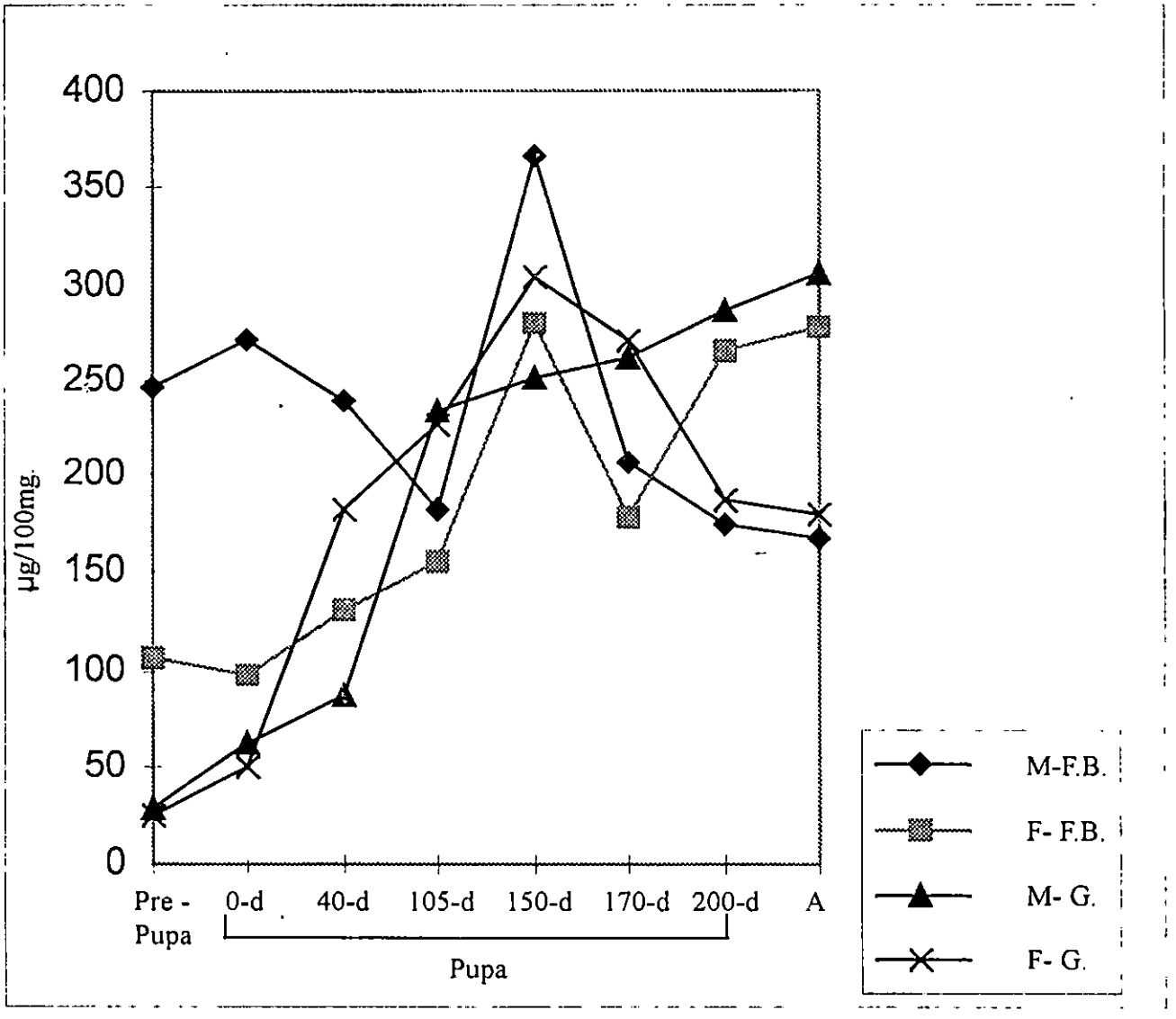


Fig. 25. DNA content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

Tabl 25. RNA content in different tissues of non-diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F
Pre-pupa	3003.50 ±50.66	3215.00 ±32.49 a	278.86 ±8.22	365.71 ±7.70 b
0-day pupa	3285.49 ±45.05	3418.80 ±40.50 a	312.10 ±8.40	472.63 ±8.15 b
7-day pupa	2807.14 ±35.40	3022.25 ±38.52 b	565.66 ±7.14	1092.88 ±15.31 b
14-day pupa	1815.86 ±39.10	2038.55 ±42.26 a	418.00 ±8.49	610.40 ±9.42 b
21-day pupa	618.30 ±20.90	724.02 ±18.21 a	202.70 ±6.15	408.12 ±12.44 b
Freshly emerged adult (22-day)	385.49 ±13.07	478.72 ±12.14 b	152.60 ±4.10	346.80 ±8.40 b

't'-test probability differences (Male vs Female) : M = Male , F = Female.

a = P< 0.01,
b = P <0.001.

Table 26. RNA content in different tissues of diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each value represents Mean± S.E. (n=10)

Tissue / Stage of insect	Fat body (µg/100mg) M	Fat body (µg/100mg) F	Gonads (µg/100mg) M	Gonads (µg/100mg) F
Pre-pupa	3116.87 ±44.65	3385.80 ±50.58 b	305.35 ±9.15	412.27 ±6.60 c
0-day pupa	2824.41 ±46.96	3174.23 ±28.54 c	355.06 ±9.31	510.07 ±9.35 c
40-day pupa	1768.23 ±62.85	1950.20 ±50.70 a	547.64 ±25.20	879.20 ±32.65 c
105-day pupa	2739.06 ±48.31	2318.34 ±87.40 c	718.46 ±16.63	1279.52 ±23.85 c
150-day pupa	3820.45 ±38.62	2635.80 ±42.45 c	595.70 ±25.59	855.83 ±21.52 c
170-day pupa	1510.50 ±43.74	1254.66 ±31.20 c	274.68 ±7.44	653.10 ±14.62 c
200-day pupa	614.26 ±30.30	743.05 ±32.39 a	187.30 ±8.62	325.01 ±15.08 c
Freshly emerged adult (210-day)	406.27 ±17.56	502.46 ±20.52 b	121.81 ±6.93	308.65 ±11.46 c

't'-test probability differences (Male vs Female) : M=Male , F=Female.

a = P<0.02,
b = P<0.01,
c = P<0.001.

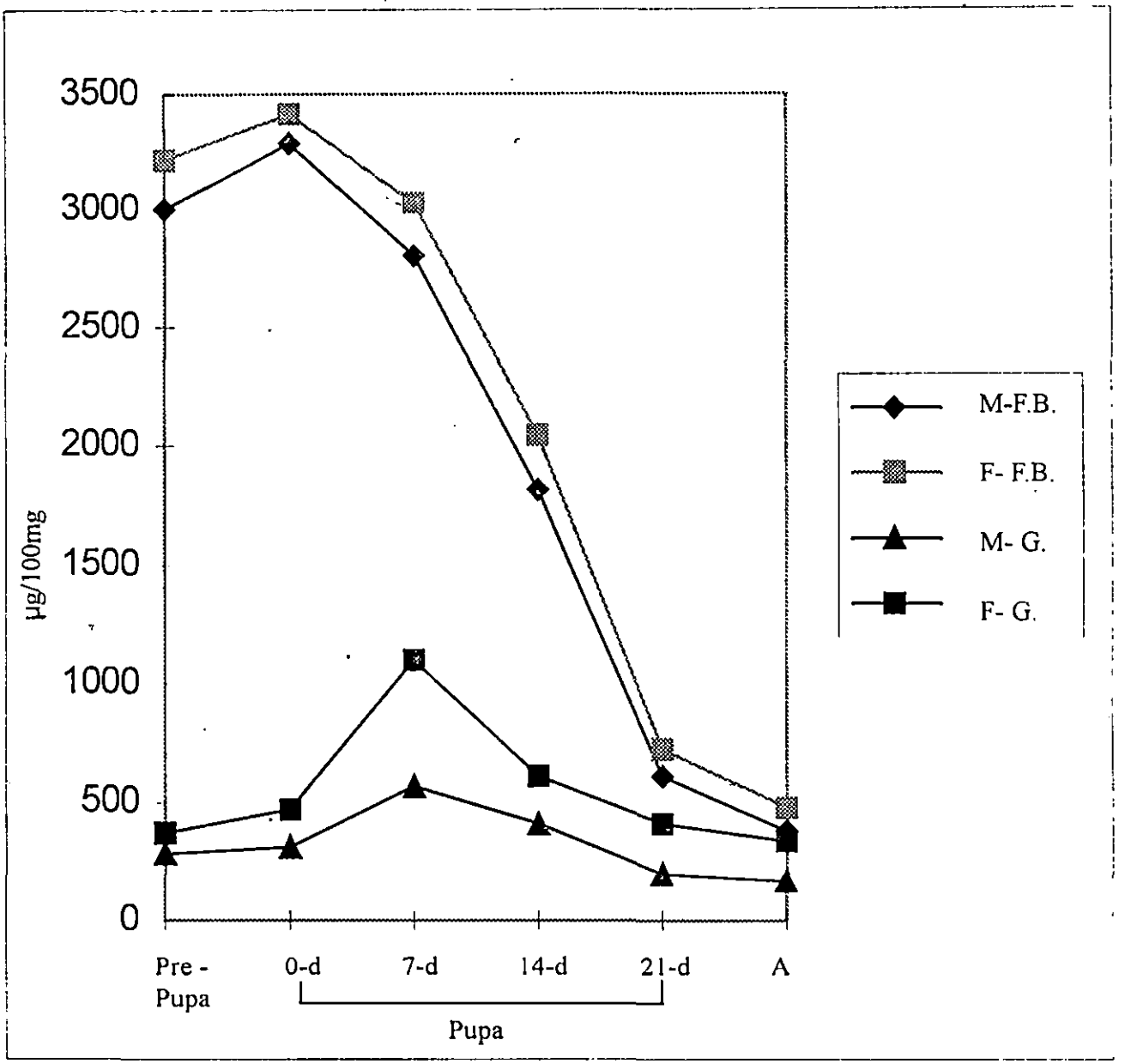


Fig. *26*. RNA content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

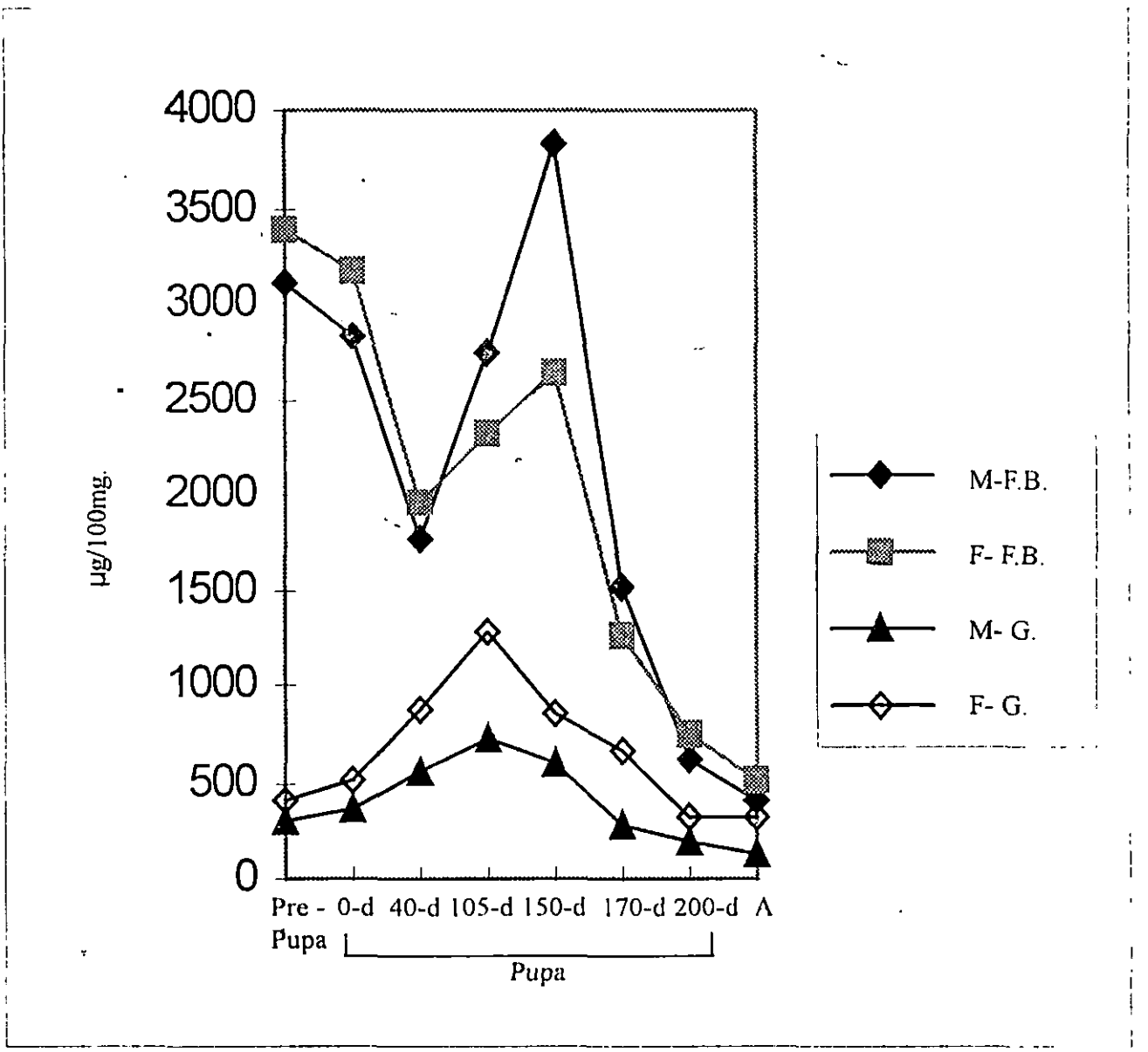


Fig. 27. RNA content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

4.3.5. Weight of gonads of pre-pupae, pupae and adults on non-diapause and diapause generations

Weight of gonad in male and female insects of both non-diapause and diapause generations gradually increased from pre-pupa to adult stage and reached the peak level in the adult moths. It was interesting to note that ovarian weight suddenly increased at about 23.50 times from 0-day to day 7 pupa in non-diapause generation. But, curiously enough the increase in ovarian weight on 200 day old diapausing pupae was found to be 470 times heavier over that of 170 day old pupal ovary. so, it revealed that ovarian growth rate is faster in non-diapause than that of in diapausing moths (Tables 27 & 28, Figs. 28 & 29).

Table 27. Weight of gonads of non-diapausing *A.mylitta* during pre-pupal , pupal and adult stages. Each value represents Mean ±S.E. (n=10).

Tissue / Stage of insect	Weight of gonads (mg) Testis	Ovary
Pre-pupa	18.10 ±0.15	4.14 ±0.20 a
0-day pupa	22.21 ±0.12	12.78 ±0.16 a
7-day pupa	24.92 ±0.14	300.34 ± 4.12 a
14-day pupa	28.15 ±0.17	3968.30 ± 32.24 a
21-day pupa	36.10 ±0.50	5310.26 ±72.43 a
Freshly emerged adult (22-day)	38.20 ±0.61	5419.95 ±88.54 a

't'-test probability differences (Male vs Female) :
a = P<0.001

M = Male ; F = Female.

Table 28. Weight of gonads of diapausing *A. mylitta* during pre-pupal, pupal and adult stages. Each mean value represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Weight of gonads (mg)	
	Testis	Ovary
Pre-pupa	15.19 ±0.21	3.15 ±0.14 a
0-day pupa	15.57 ±0.23	3.32 ±0.18 a
40-day pupa	17.20 ±0.44	4.85 ±0.22 a
105-day pupa	18.62 ±0.80	5.14 ±0.17 a
150-day pupa	20.28 ±0.32	5.75 ±0.11 a
170-day pupa	22.44 ±0.50	6.58 ±0.26 a
200-day pupa	27.86 ±0.41	3094.77 55.93 a
Freshly emerged adult (210 day)	28.58 ±0.59	3311.80 ±97.39 a

't'- test probability differences (Male vs Female) : M = Male, F = Female.

a = P<0.001.

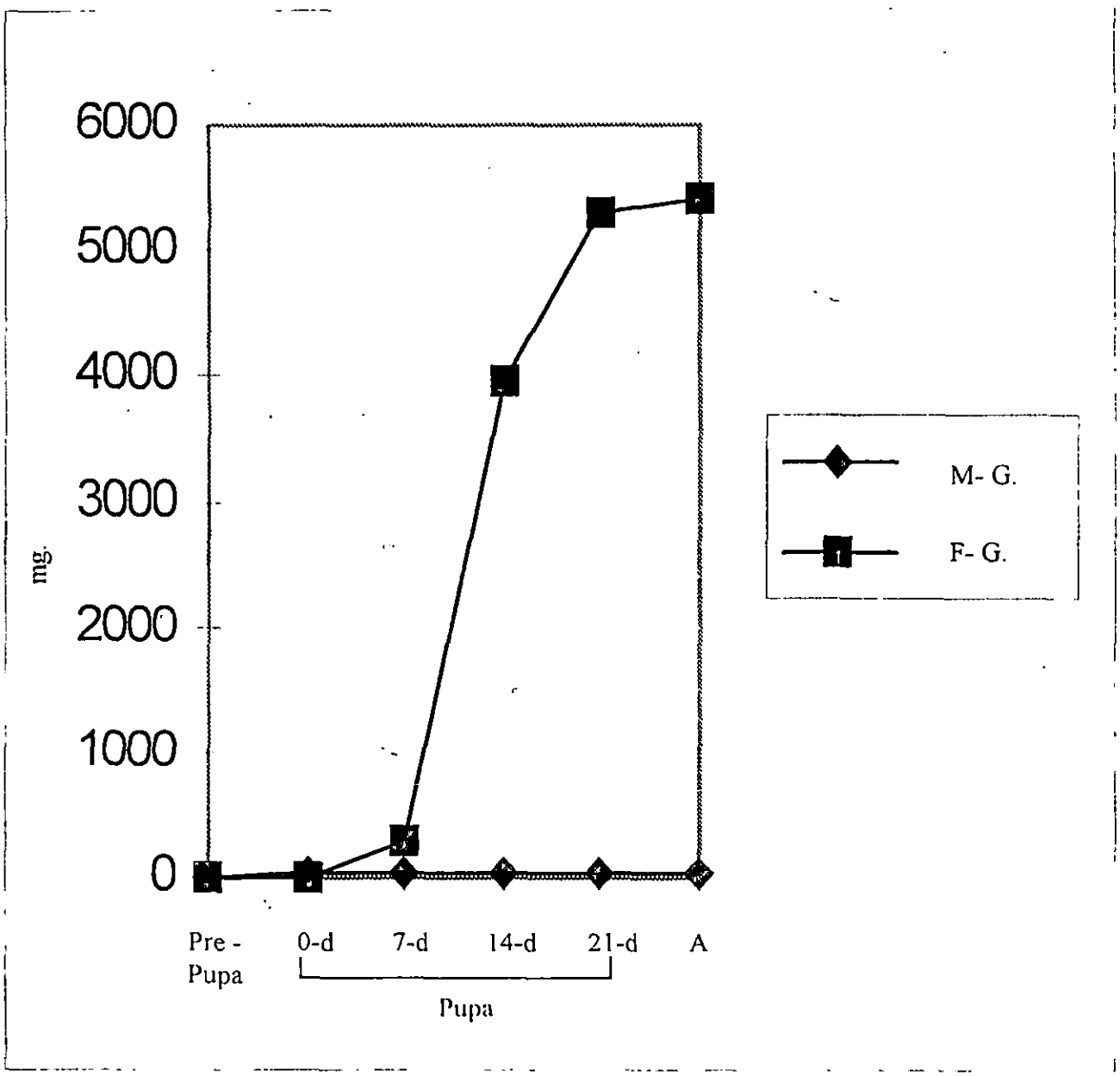


Fig.28. Weight of gonads of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A = Adult, G = Gonad. (mg.)

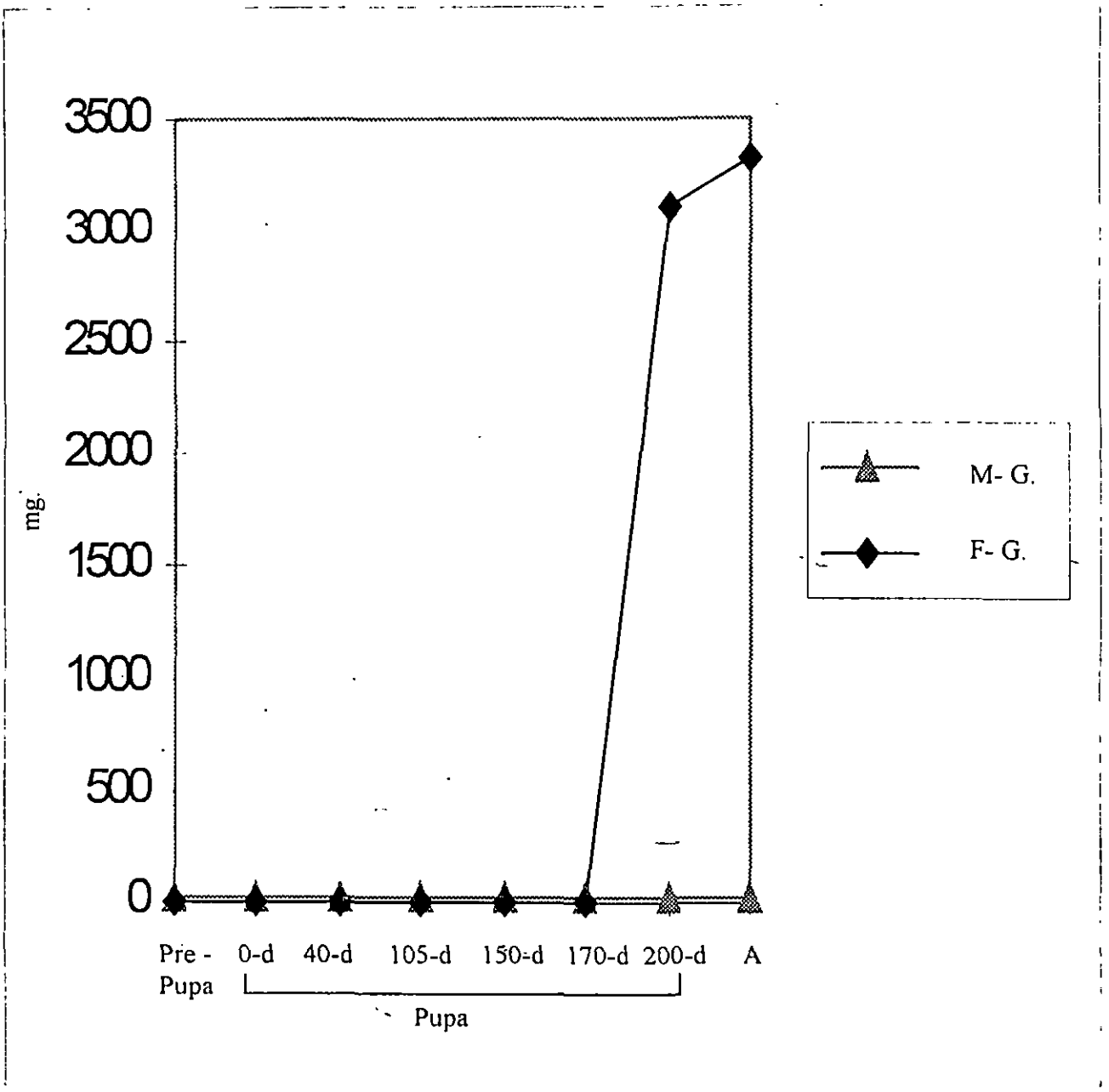


Fig.29- Weight of gonads of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A = Adult, G = Gonad. (mg.)

4.4. Effect of Insulin on Diapause Generation of *A.mylitta*.

4.4.1 Treatment of insulin with diapausing pre-pupae of *A.mylitta*.

Pupal duration : Treatment with Insulin at the doses of 5 µl and 10 µl/pre-pupa significantly shortened the female pupal life span by more than 12 days. However, pupal period in male was significantly reduced by about 11 days only at the dose of 10 µl/pre-pupa (Table 29 & 32)

Moth weight : Moth weight in both the sexes was not significantly enhanced in any of the doses of the applied hormone in comparison to that of control. However, there is a trend in increasing male and female moth weights with 5 µl and 10 µl of insulin although the data were not found to be significant (Table 29).

Egg production and hatching : Eggs laid by a single mother moth (fecundity) as well as the total number of egg production (laid + unlaid) were not significantly influenced by the insulin but an increasing trend was observed in higher doses (5 and 10 µl) although the data were not significant. However, the hatching percentage was also not influenced in any case by insulin in comparison to their control counterparts (Table 29).

4.4.2. Treatment of 40 day old diapausing pupae with insulin

Pupal life span : Pupal life span was significantly shortened in both the sexes after application of insulin at the doses of 5 and 10 µl/pupa in comparison to that of control (Table 30 & 33).

Moth weight : Compared to control all the doses of insulin significantly enhanced both male and female moth weights (Table 30).

Egg production : All the doses of insulin significantly increased the number of eggs laid by a mother moth i.e. fecundity as well as the total egg production (laid + unlaid) when compared to that of control (Table 30).

Hatching performance : No significant effect was observed on hatching percentage due to application of any of the doses of the hormone when compared with the same values of control individuals (Table 30).

4.4.2. Treatment of 150 days old diapausing pupae with insulin

Pupal duration : In case of control the female pupae took more time for emergence than the males. Both the doses of the hormone were able to reduce the average duration of pupal life. (Table 31 & 34).

Moth weight : Female moth weight was significantly increased only with 5 µl dose of insulin while the male moth weight was in no way affected by either of the doses of insulin. (Table 31).

Egg production : Insulin at the dose of 5 µl/pupa significantly increased the total number of egg production (laid + unlaid) as well as fecundity in this insect. But, the dose of 10 µl/pupa significantly decreased the fecundity as well as total egg production. (Table 31).

Hatching performance :

No significant change in hatching percentage was observed in either of the doses of insulin after treatment in comparison with that of control (Table 31).

4.2.3 Effect of insulin on cholesterol content in haemolymph, fat body and gonad of diapausing *A.myllita* during pupal and adult stages of development

In haemolymph : Irrespective of sexes the cholesterol content of haemolymph increased significantly in case of both 5 µl and 10 µl doses of insulin on 150 and 170 day pupa but in adult stage, it decreased significantly compared to that of control. It is interesting to note that 10 µl dose of insulin showed more decreased cholesterol titre ($P<0.02$) during adult phase in both the sexes

Table 29. Effect of single injection of different doses of insulin on diapausing pre-pupae (3 days before pupation) of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unlaid	Total	
Control	208.14 ±3.74	215.17 ±3.28	2.53 ±0.13	6.46 ±0.30	147 ±11	31 ±6	178 ±13	78.58 ±5.36
1 µl/pre-pupa.	203.86 ±1.77 NS	209.68 ±2.89 NS	2.50 ±0.12 NS	6.69 ±0.26 NS	138 ±9 NS	48 ±9 NS	186 ±8 NS	83.60 ±2.88 NS
5 µl/pre-pupa.	202.44 ±3.56 NS	201.93 ±3.01 b	2.63 ±0.07 NS	6.94 ±0.29 NS	191 ±19 NS	32 ±6	223 ±20 NS	83.25 ±3.12 NS
10µl/pre-pupa.	197.30 ±2.84 a	203.12 ±2.12 b	2.67 ±0.16 NS	7.08 ±0.20 NS	195 ±22 NS	16 ±3	211 ±17 NS	84.68 ±2.42 NS

't'-test probability differences (Control vs Treatments) :

a = P<0.05,
b = P<0.01,
NS= Not significant. M=Male . F=Female.

Table 30. Effect of single injection of different doses of insulin on 40-day old diapausing pupae of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unhid	Total	
Control	212.87 ±2.04	215.22 ±1.50	2.37 ±0.10	5.57 ±0.26	153 ±16	13 ±2	166 ±13	80.76 ±4.56
5µl/pupa	203.56 ±1.65 c	201.30 ±2.11 c	2.81 ±0.17 a	6.58 ±0.22 b	239 ±13 c	23 ±3	262 ±18 c	87.92 ±2.14 NS
10 µl/ pupa.	201.84 ±2.42 c	204.60 ±1.67 c	2.79 ±0.16 a	6.71 ±0.12 c	265 ±9 c	43 ±16	308 ±16 c	86.93 ±3.19 NS

't'-test probability differences (Control vs Treatments) :

a = P<0.05 ,
b = P<0.01,
c = P<0.001,
NS= Not significant.

M = Mule F = Female.

Table 31. Effect of single injection of different doses of insulin on 150-day old diapausing pupae of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unlaid	Total	
Control	53.87 ±1.06	58.57 ±2.90	2.38 ±0.08	4.43 ±0.24	154 ±24	29 ±7	183 ±20	78.45 ±3.24
5 µl/pupa	50.37 ±1.02 a	50.95 ±1.19 a	2.54 ±0.08 NS	5.71 ±0.30 b	218 ±17 a	31 ±6	249 ±19 a	84.41 ±2.23 NS
10 µl/pupa	49.27 ±1.80 a	51.78 ±1.07 a	2.37 ±0.12 NS	5.01 ±0.30 NS	73 ±9 b	45 ±10	118 ±12 b	77.20 ±2.93 NS

T-test probability differences (Control vs Treatments)

a = P<0.05, M= Male ; F = Female.
b = P<0.01,
NS= Not significant

Table 32. Rhythm of moth emergence in *A. mylitta* following insulin application to the diapause-destined pre-pupae (n=160).

Observation at every 3 day from May. 25 to July,29.	Control		1 µl/pre-pupa		5 µl/pre-pupa		10 µl/pre-pupa	
	No.	%	No.	%	No	%	No.	%
May 27	0	0	0	0	3	1.875	4	2.50
30	0	0	0	0	0	0	1	0.625
June 2	1	0.625	4	2.50	1	0.625	3	1.875
5	1	0.625	2	1.25	3	1.875	9	5.625
8	4	2.50	4	2.50	7	4.375	11	6.875
11	4	2.50	5	3.125	11	6.875	13	8.125
14	6	3.750	10	6.25	14	8.75	18	11.25
17	5	3.125	19	11.875	13	8.125	24	15.00
20	9	5.625	19	11.875	18	11.25	18	11.25
23	14	8.75	17	10.625	16	10.00	9	5.625
26	12	7.50	15	9.375	14	8.75	8	5.00
29	12	7.50	11	6.875	8	5.00	4	2.50
July 2	11	6.875	7	4.375	4	2.50	1	0.625
5	10	6.25	5	3.125	1	0.625	0	0
8	12	7.50	3	1.875	0	0	1	0.625
11	8	5.00	1	0.625	1	0.625	4	2.50
14	5	3.125	0	0	1	0.625	1	0.625
17	2	1.25	1	0.625	0	0	0	0
20	1	0.625	2	1.25	1	0.625	0	0
23	0	0	1	0.625	1	0.625	0	0
26	1	0.625	0	0	0	0	0	0
29	1	0.625	0	0	0	0	0	0

Table 33. Rhythm of moth emergence in *A.mylitta* following insulin application to the 40-day old diapausing pupae(n=200) .

Observation at every 3 day from May, 26to July,30.		Control		5 µl/ pupa		10 µl/pupa	
		No.	%	No	%	No.	%
May	28	1	0.50	1	0.50	2	1.00
	31	0	0	2	1.00	4	2.00
June	3	0	0	2	1.00	1	0.50
	6	2	1.00	2	1.00	5	2.50
	9	0	0	4	2.00	6	3.00
	12	2	1.00	8	4.00	10	5.00
	15	5	2.50	15	7.50	19	9.50
	18	8	4.00	28	14.00	33	16.50
	21	16	8.00	31	15.50	34	17.00
	24	13	6.50	29	14.50	22	11.00
	27	19	9.50	11	5.50	12	6.00
	30	12	6.00	4	2.00	4	2.00
July	3	14	7.00	4	2.00	2	1.00
	6	16	8.00	0	0	0	0
	9	11	5.50	0	0	1	0.50
	12	10	5.00	1	0.50	1	0.50
	15	9	4.50	2	1.00	1	0.50
	18	3	1.50	3	1.50	1	0.50
	21	1	0.50	0	0	0	0
	24	0	0	0	0	0	0
	27	1	0.50	0	0	0	0
	30	2	1.00	0	0	0	0

Table 34. Rhythm of moth emergence in *A.mylitta* following insulin application to the 150-day old diapausing pupae(n=100) .

Observation at every 3 day from May, 26to July,30.		Control		5 µl/ pupa		10 µl/pupa	
		No.	%	No	%	No.	%
May	28	0	0	0	0	0	0
	31	0	0	0	0	0	0
June	3	0	0	2	2.00	2	2.00
	6	0	0	1	1.00	2	2.00
	9	1	1.00	1	1.00	2	2.00
	12	1	1.00	4	4.00	5	5.00
	15	2	2.00	3	3.00	7	7.00
	18	5	5.00	13	13.00	15	15.00
	21	9	9.00	18	18.00	19	19.00
	24	13	13.00	26	26.00	14	14.00
	27	14	14.00	15	15.00	12	12.00
	30	17	17.00	3	3.00	9	9.00
July	3	11	11.00	2	2.00	3	3.00
	6	6	6.00	3	3.00	0	0
	9	5	5.00	1	1.00	2	2.00
	12	1	1.00	0	0	1	1.00
	15	3	3.00	2	2.00	1	1.00
	18	1	1.00	1	1.00	0	0
	21	1	1.00	0	0	1	1.00
	24	2	2.00	0	0	0	0
	27	1	1.00	0	0	0	0
	30	0	0	0	0	0	0

However, there was uniformity in the pattern of variation of cholesterol in 150 day pupa, 170 day pupa and adult stages. The cholesterol content first elevated significantly ($P<0.001$) on 150 day and then declined significantly ($P<0.001$)) on 170 day pupa and adults ($P<0.001$) in both the sexes of control and treated individuals showing a specific pattern of variation (Table 35).

In Fat Body : In male and female fat body significant enhancement in cholesterol level occurred in both 5 µl and 10 µl doses of insulin on 150 day and 170 day of pupal age while, it was decreased significantly only in the resultant female adults not in the males when compared with that of control. The magnitude of the effect of the insulin remained almost the same in both the doses. Further, the pattern of variation in cholesterol content of fat body in both the sexes was the same as in haemolymph in case of both control and treated groups (Table 35).

In gonad : In case of both control and treatments the cholesterol contents of male and female gonads increased significantly after 40 day of pupal age and reached a peak on adult stage. Compared to control treatment with both the doses (5 and 10 µl) of insulin resulted significantly in higher amount of cholesterol in testis and ovary during the subsequent pupal life and adult stage of development (Table 35).

4.4.4. Effect of insulin on the total protein content in haemolymph, fat body and gonal of diapausing *A.mylitta* during pupal and adult development

In haemolymph : In control and treatments total protein content of haemolymph of each sex first increased on 150 day of pupal age and then declined on 170 day pupa and finally in the adult. However, both 5 and 10 µl doses of insulin significantly enhanced protein level in the two sexes on 150 day and 170 day pupae while the hromone lowered the level of protein in each sex at the adult stage in comparison to those of control individuals. No dose response effect was observed in this case. (Table 36).

In Fat Body : A gradual increase in protein concentration was observed in control and both doses of insulin treated lots, the increase was at the peak on 150 day and then gradually declined upto adult stage establishing a specific pattern of variation of this bimolecule. Insulin (5 and 10 µl/pupa) induced a significant enhancement ($P<0.01$ to $P<0.001$) in protein titre particularly on 150 day and 170 day pupae compared to that of control while the lowest level of the biomolecule was recorded in adult stage. Moreover female fat body contained more amount of protein that male fat body throughout the pupal and adult life span (Table 36)

In Gonad : A specific pattern of variation was observed in gonad protein level in control insects where same was gradually increased upto adult stage with the peak in concentration. Exogenous insulin significantly enhanced the protein titre in testis and ovary throughout the period under study (pupal and adult stages). The specific pattern of variation in protein concentration remained unaltered after insulin treatment. In general, female gonads showed higher amount of protein than male gonads of pupal and adult stages (except in 40 - day old pupa) (Table 36).

4.4.5. Effect of insulin on the DNA content in fat body and gonad of diapausing *A.mylitta* during pupal and adult development

In Fat Body : DNA content of male fat body initially enhanced significantly ($P<0.001$) on 150-day pupa followed by a significant reduction ($P<0.001$) on 170-day old diapausing pupa and reached minimum level at adult stage; while in female, DNA content first increased significantly ($P<0.001$) on 150-day and then declined ($P<0.001$) on 170-day of pupal development but again significantly enchanced ($P<0.001$) in adult stage in both control and treated lots. However, compared to control, the two doses of insulin induced and increase in DNA concentration significantly in fat body of both the sexes of 150 day and 170 day pupae but not in the adults. No dose dependent effect of insulin was observed in DNA titre irrespective of the sex (Table 37).

In Gonad : In control lots DNA content of testis gradually increased significantly from 150 day pupa onwards and touched the peak on the day of

adult emergence; while in ovary it increased on 150 day pupa showing the peak followed by a significant reduction ($P<0.001$) on 170 day old pupa and adults. Both 5 µl and 10 µl doses of insulin expressed significantly higher DNA content in both the sexes over controls in each stage. Further, the age-dependent pattern of DNA in gonad remained unaltered by insulin treatment at any of the doses used. The insulin treated groups also followed the same pattern of variation in DNA concentration like control insects.(Table 37).

4.4.6. Effect of insulin on RNA content in fat body and gonad of diapausing *A.mylitta* during pupal and adult development

In Fat Body : A sharp rise in fat body RNA concentration was recorded in both sexes on 150 day pupa followed by a drastic fall on 170 day and more so during adult stage. Insulin at the doses of 5 and 10 µl/pupa was able to enhance the RNA content only during the pupal stage (150 and 170 day) while no effect of the hormone was recorded in the adult stage. Age-dependent changes in RNA titre remained unaltered after insulin treatment (Table. 38)

In Gonad : RNA levels in control animals was found to be same from 40 to 150 day old diapausing pupae in testis and ovary, then declined from 150 day onwards and reached a minimum level on the day of adult eclosion. Ovary showed higher RNA concentration than testis during pupal and adult stages. Insulin (5 and 10 µl/pupa) significantly elevated the RNA titre from 40-day to 150-day and then from 150 day onwards declined upto the adult stage when compared with RNA content of control lots. It should be mentioned here that insulin was able to change slightly the specific pattern of variation where peak level in RNA concentration was recorded on 150 day and then declined to adult stage (Table 38).

4.4.7. Effect of insulin on gonad weight of diapausing *A.mylitta* during pupal and adult development

Weight of both male and female gonads increased significantly ($P<0.01$ - $P<0.001$) due to the two doses of insulin than that of gonads of

the control individuals at pupal and adult stages. In both control and treatments the weights of testis and ovary gradually increased significantly from 150 day pupa onwards to adult emergence. The rise in the ovarian weight was very sharp approaching the adult stage. Further, during pupal stage (40 to 170 day) ovarian weight remained very low in comparison to testis weight reflecting the commencement of ovarian maturation during very late phase of pupal age. In this case also, no dose response relationship was observed like all other biochemical parameters in this experiment (Table 39).

Table 35. **Cholesterol content in haemolymph , fat body and gonads of pupae and adults of diapausing generation of *A.mylitta* following administration of insulin to 40- day old pupae. Each value represents mean ± S.E. of 10-15 observations.**

Treatment	Sex	Haemolymph(µg/ml)				Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	740.56 ±16.19	2172.46 ±52.39	1922.64 ±42.11	1619.41 ±25.33	2095.19 ±68.92	3765.49 ±68.33	2850.94 ±62.44	965.12 ±31.79	262.89 ±8.95	720.83 ±20.59	815.16 ±15.30	924.35 ±26.30
	F	850.86 ±28.15	2350.84 ±69.40	1882.00 ±55.10	922.37 ±38.09	1752.88 ±36.09	2525.30 ±65.56	1878.86 ±51.90	515.58 ±23.43	692.86 ±11.53	1325.60 ±24.35	1414.49 ±29.05	1751.77 ±56.32
5 µl /pupa	M	698.37 ±24.35 NS	2385.00 ±62.83 a	2079.86 ±45.74 a	1438.50 ±35.36 c	1986.55 ±73.40 NS	3993.15 ±41.26 b	3081.64 ±45.10 b	1020.52 ±32.26 NS	271.35 ±6.91 NS	855.60 ±22.24 c	965.18 ±15.94 c	1056.23 ±25.51 b
	F	902.41 ±39.70 NS	2592.44 ±42.70 b	2151.23 ±55.88 b	718.35 ±25.19 c	1810.97 ±44.15 NS	2875.39 ±49.67 c	2220.58 ±62.63 c	310.20 ±16.74 c	673.49 ±16.32 NS	1468.35 ±26.47 c	1720.87 ±40.69 c	1962.30 ±45.27 b
10 µl /pupa	M	719.50 ±34.23 NS	2422.91 ±51.15 b	2110.87 ±55.49 b	1292.80 ±48.72 c	2148.36 ±57.40 NS	3981.50 ±38.14 b	3150.44 ±55.36 b	983.70 ±46.50 NS	254.90 ±9.05 NS	872.40 ±22.36 c	951.83 ±20.59 c	1082.25 ±28.90 c
	F	837.60 ±19.22 NS	2588.23 ±60.95 a	2227.69 ±41.34 c	632.48 ±20.77 c	1697.10 ±33.64 NS	2930.49 ±52.61 c	2375.10 ±45.22 c	350.40 ±25.81 c	711.36 ±19.20 NS	1446.15 ±25.42 b	1761.44 ±50.39 c	2009.67 ±58.94 b

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001, NS = Not significant

M = Male ; F = Female.

Table 36. **Total protein content in haemolymph , fat body and gonads of pupae and adults of diapausing generation of *A.mylitta* after injection of insulin to 40-day old pupae. Each value represents mean ± S.E. of 10-15 observations.**

Treatment	Sex	Haemolymph(mg/ml)				Fat body (mg/100mg)				Gonad (mg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	4.86 ±0.08	13.56 ±0.15	12.17 ±0.18	10.14 ±0.22	5.35 ±0.17	6.08 ±0.10	5.40 ±0.14	2.97 ±0.10	4.08 ±0.20	4.97 ±0.10	5.32 ±0.14	6.70 ±0.17
	F	6.74 ±0.33	19.20 ±0.42	16.42 ±0.34	13.29 ±0.36	8.94 ±0.25	11.85 ±0.12	8.77 ±0.11	4.76 ±0.14	2.96 ±0.15	10.41 ±0.22	11.30 ±0.25	15.68 ±0.40
5µl/ pupa	M	4.93 ±0.10 NS	14.68 ±0.21 c	13.22 ±0.15 c	9.18 ±0.25 b	5.60 ±0.19 NS	7.10 ±0.15 c	4.82 ±0.11 b	2.23 ±0.12 c	3.97 ±0.18 NS	5.36 ±0.14 a	6.08 ±0.10 c	7.55 ±0.12 c
	F	6.59 ±0.28 NS	24.42 ±0.30 c	18.19 ±0.22 c	11.10 ±0.30 c	9.25 ±0.16 NS	13.25 ±0.18 c	6.47 ±0.20 c	3.10 ±0.19 c	3.04 ±0.11 NS	12.15 ±0.32 c	13.29 ±0.42 c	17.50 ±0.25 c
10µl/ pupa	M	5.01 ±0.07 NS	14.02 ±0.25 c	13.39 ±0.23 c	9.09 ±0.18 c	5.49 ±0.15 NS	7.22 ±0.20 c	4.75 ±0.16 b	2.11 ±0.10 c	4.21 ±0.14 NS	5.40 ±0.15 a	6.28 ±0.15 c	7.59 ±0.14 c
	F	7.43 ±0.30 NS	25.05 ±0.35 c	18.77 ±0.32 c	10.85 ±0.37 c	9.18 ±0.27 NS	13.26 ±0.24 c	6.39 ±0.25 c	3.27 ±0.22 c	3.10 ±0.12 NS	12.06 ±0.38 c	13.46 ±0.30 c	17.68 ±0.36 c

't'-test probability differences (Compared to Control) :
a = P<0.05 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male ; F = Female.

Table 37. **DNA content in fat body and goads of pupae and adults of diapausing generation of A.mylitta following insulin injection to 40 day old pupae. Each value represents mean ± SE of 10-15 observations.**

Treatment	Sex	Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	239.50 ±4.10	365.84 ±8.60	206.35 ±11.69	168.34 ±6.83	86.24 ±6.42	250.82 ±8.09	262.40 ±5.33	306.63 ±8.18
	F	131.15 ±5.24	280.49 ±6.11	178.74 ±3.81	277.55 ±14.86	182.53 ±6.28	304.30 ±5.40	271.61 ±7.94	180.59 ±5.42
5µl/ pupa	M	228.41 ±5.38 NS	462.50 ±8.19 b	280.95 ±6.42 c	155.90 ±8.32 NS	78.06 ±4.23 NS	298.34 ±4.57 b	289.07 ±3.24 b	361.25 ±6.87 b
	F	122.53 ±7.11 NS	351.88 ±9.41 b	197.80 ±4.54 a	274.65 ±7.50 NS	185.33 ±7.60 NS	363.01 ±6.44 b	326.54 ±8.92 b	224.46 ±7.30 b
10µl/ pupa	M	245.26 ±6.90 NS	475.64 ±10.18 b	285.41 ±8.59 c	164.42 ±7.21 NS	91.65 ±5.39 NS	294.28 ±7.95 b	302.49 ±10.05 b	375.41 ±8.20 b
	F	135.60 ±8.14 NS	368.71 ±7.70 b	201.50 ±3.56 c	266.10 ±8.48 NS	176.40 ±9.21 NS	358.20 ±6.82 b	315.18 ±7.22 b	236.19 ±6.89 b

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male : F = Female.

Table 38. **RNA content in fat body and goads of pupae and adults of diapausing generation of A.mylitta following insulin injection to 40 day old pupae. Each value represents mean ± SE of 10-15 observations.**

Treatment	Sex	Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	1768.23 ±62.85	3820.45 ±38.62	1510.50 ±43.74	406.27 ±17.56	547.64 ±25.20	595.70 ±25.59	274.68 ±7.44	121.81 ±6.93
	F	1950.20 ±110.70	2635.80 ±42.45	1154.56 ±31.20	502.46 ±20.52	879.20 ±32.65	855.83 ±21.52	653.10 ±14.62	308.65 ±11.46
5µl/ pupa	M	1747.81 ±55.26 NS	3943.20 ±27.60 a	1715.34 ±33.70 b	417.92 ±11.50 NS	590.15 ±24.48 NS	716.42 ±17.60 c	322.90 ±8.64 c	178.59 ±6.20 c
	F	2069.40 ±59.23 NS	2866.30 ±62.80 b	1297.41 ±34.90 b	556.40 ±41.35 NS	866.84 ±25.36 b	943.61 ±20.42 b	712.90 ±15.18 b	375.44 ±8.10 b
10µl/ pupa	M	1820.16 ±67.30 NS	4010.23 ±39.54 b	1764.29 ±41.80 c	412.75 ±18.42 NS	610.20 ±22.40 NS	736.78 ±15.32 c	331.84 ±9.40 c	190.08 ±7.50 c
	F	1988.71 ±120.45 NS	2920.66 ±51.32 c	1328.73 ±43.20 b	497.41 ±19.25 NS	872.33 ±28.00 NS	950.74 ±18.46 b	722.66 ±15.71 b	362.80 ±7.95 c

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male ; F = Female.

Table 39. Gonadal weights of pupae and adults of diapausing generation of *A.mylitta* after the treatment of 40-day old pupae with insulin. Each value represent mean ± S.E. of 10-15 individuals.

Treatment	Sex	Weight of Gonad (mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult
Control	Male	17.20 ±0.44	20.28 ±0.32	22.44 ±0.50	28.58 ±0.59
	Female	4.85 ±0.22	5.75 ±0.11	6.58 ±0.26	3311.80 ±97.39
5µl /pupa	Male	16.81 ±0.56 NS	21.64 ±0.40 a	24.08 ±0.33 a	31.10 ±0.44 a
	Female	4.94 ±0.31 NS	6.99 ±0.20 b	8.41 ±0.45 b	4140.49 ±102.85 b
10 µl/pupa	Male	17.58 ±0.39 NS	22.04 ±0.30 b	24.22 ±0.18 a	31.64 ±0.35 b
	Female	5.06 ±0.25 NS	7.46 ±0.35 b	9.50 ±0.60 b	4390.15 ±115.16 b

't'-test probability differences (Control vs Treatment)

a = P<0.01, b = P<0.001, NS = Not significant.

4.5. Effect of Exogeneous 20-HE on the Diapause-destined Generation when Applied to the Pre-pupae

4.5.1. On larval - pupal transformation

All the doses of 20-HE caused pupation earlier than in the control, pupation time was shortened progressively with the higher dosages (Table-40). Irrespective of the doses and sex, the pupal mortality did not differ from that of control. The doses other than 1 µg/pre-pupae led to prothetelic development in the pupae such as exposed antennae of pupal-imaginal intermediates and the short crumpled wings . Such developmental derangement was of higher frequency in higher dosages of 20-HE.

4.5.2. Diapause duration and adult development

Irrespective of the sexes 20-HE shortened significantly the duration of pupal diapause with the rise of dosages.Defective pupae were produced due to 20-HE. All the defective pupae resulted from a 2 µg/prepupa dose could develop into normal adults. But, in case of other two higher doses of the hormone only a few of the defective pupae could grow into normal moths, mostly emerged as abnormal adults with crumpled wings, the frequency was highest in case of 10 µg dosage. These moths of two higher dosages were weak in their mating performance than their counterparts of control set. The weak female moths were unable to lay eggs at all although they had fully developed reproductive system. An average number of 193 unlaid eggs per female were counted after dissection whereas in case of control the average number of total (laid unlaid) eggs per female was 217 (Table -41). Further, there was no significant effect of this hormone on the moth weight (Table-40)

4.5.3 Gonad weight and size

Weights of testes and ovaries along with their lengths and widths were significantly increased over those of control pupae on 150 day with 2,5 and 10 µg doses of 20-HE whereas 1 µg dosage was ineffective. 10 µg dosage of the hormone had the greatest effect. Effect of the hormone was reflected in the morphology of average and testis. Ovarian maturation was promoted progressively with the higher dosages of the hormone . This was evident from the impression of the ovarian follicul inside the ovariole, the follicles quickly became distinct when treated with a higher dose (10 µg) (Table 42 and 43).

4.5.4 Reproductive performance

Fecundity and total egg production by a female were recorded to be significantly higher in 5 and 10 µg doses except. In case of 2 µg dose; the fecundity was higher with comparison to that of control. However, hatching percentage was increased over the control only in case of 5 and 10 µg of the hormone. (Table 41).

4.5.5 Protein concentration in the gonads

An increased concentration of protein was recorded in the individuals who received 2, 5 and 10 µg of 20-HE, but 1 µg of the hormone was ineffective and 10 µg was found to be the most effective dose. Further, female gonads contained more protein than the male gonads (Table 43).

Table 40. Effects of exogenous 20-HE on the diapause development after application to the pre-pupae of *A. mylitta*.

Treatment	No-treated	No died	Time took for pupatioin (day) Mean±SE	No. of pupae which terminated diapause in			Duration of diapause (days) Mean±SE	Moth emergence (%)	Moth weight (g)*		Abnormality out of pupae survived (No)	
				<60 days	<200 days	>200 days			Male Mean±SE	Female Mean±SE	Pupa	Adult
Control	50	13	8.36±0.76	0	7	30	217.5±3.5	74.34	2.80±0.46	7.12±0.29	0	0
1µg/prepupa	50	12	5.88±0.63 a	0	14	24	205±3.0 a	75.65	3.25±0.37	7.39±0.29	0	0
2µg/prepupa	50	12	5.96±0.67 a	0	19	19	198.5±4.0 a	76.66	3.36±0.50	7.22±0.33	1	0
5µg/prepupa	50	11	5.48±0.52 a	0	24	15	194.0±5.0 a	78.20	3.00±0.25	7.36±0.30	3	1
10µg/prepupa	45	11	5.20±0.70 a	2	27	5	172.5±7.0 b	75.43	2.91±0.18	7.81±0.31	7	6

't' - test probability differences (Control vs each of the treatments) :

a = P<0.01, b = P<0.001, *Non-significant.

Table 41. Effect of exogenous 20-HE on the fecundity, total egg production and hatching performances when applied to the diapausing pre-pupae of *A. mylitta* Values represent mean ± SE.

Treatment	Egg production (No.)			Hatching (%)
	Fecundity(laid)	Unlaid	Total	
Control	175 ±14	42 ±13	217 ±17	79.59 ±2.50
1 µg/pre-pupa	197 NS ±25	24 ±4	221 NS ±19	79.88 NS ±2.18
2 µg/pre-pupa	256 b ±25	18 ±2	274 NS ±24	83.49 NS ±1.84
5 µg/pre-pupa	248 b ±20	25 ±10	273 a ±21	87.88 a ±2.23
10 mg/pre-pupa	273 c ±17	23 ±4	296 b ±17	86.87 a ±1.81

't' - test probability differences (Control vs each of the treatments) :

a = P<0.05 , b = P<0.01 , c= P<0.001, NS = Non-significant.

Table 42. Effect of exogenous 20-HE on the measurements of the gonads of the diapausing 150 - day - old pupae following application of the hormone to pre-pupae of *A. mylitta*. Values represent mean ± SE.

Treatment	Testis (mm)		Ovary (mm)	
	Length	Width	Length of ovariole	Width of single ovariole
Control	3.525 ±0.023	2.683 ±0.038	5.816 ±0.083	0.177 ±0.007
1 µg/pre-pupa	3.550 NS ±0.045	2.772 NS ±0.051	5.983 NS ±0.095	0.183 NS ±0.008
2 µg/pre-pupa	3.717 b ±0.028	2.877 a ±0.035	9.767 b ±0.601	0.219 b ±0.006
5 µg/pre-pupa	4.167 b ±0.051	3.455 b ±0.045	19.850 b ±0.126	0.211 b ±0.009
10 µg/pre-pupa	4.900 b ± 0.041	3.789 b ±0.042	22.083 b ±0.206	0.360 b ±0.009

't'- test probability differences (control vs each of the treatments) :

a = P<0.05 ; b = P<0.001 NS = Non-significant.

Table 43. Effect of exogenous 20-HE on the weights and protein contents of gonods of diapausing 150-day-old pupae following administration of the hormone to the pre-pupae of *A. mylitta*. Values represents mean ±SE.

Treatment	Testis		Ovary	
	Weight(mg) (2-testes)	Protein content (mg/100mg)	Weight(mg) (2-Ovaries)	Protein content (mg/100 mg)
Control	19.37 ±0.39	4.56 ±0.07	6.10 ±0.54	10.10 ±0.13
1 µg/pre-pupa	20.02 NS ± 0.46	4.49 NS ±0.08	6.25 NS ±0.21	10.32 NS ±0.24
2 µg/pre-pupa	22.49 a ±0.56	5.11 b ±0.06	35.40 c ±1.34	11.35 b ±0.23
5 µg/pre-pupa	24.55 b ±0.87	5.67 c ±0.07	123.80 c ±2.42	12.58 c ±0.25
10 µg/pre-pupa	25.84 b ±1.01	5.98 c ±0.16	145.69 c ±3.67	12.96 c ±0.32

't' - test probability differences (control vs each of the treatments) :

a = P<0.05, b = P<0.01, c = P<0.001, NS = Non-significant.