

CHAPTER 3



RESULT

Objective 1 : Early development of oyster larvae (*C. lugubris*)

The experiment was done at 28.0°C and 28.0 ppt salinity. The early development of the oyster were shown in figure 20 - 34.

The newly released unfertilized egg (fig.20) has a pear shape and compress. Within few minutes of exposing to sea water , the egg becomes globular in shape and with high density (fig.21). About 35 minutes after fertilization , the first polar body (fig.22) appears on animal pole. The second polar body (fig.23) is seen 20 - 25 minutes after the formation of the first polar body. The first cleavage (fig.24) is formed within 75 - 100 minutes after fertilization by the meridional division of blastomere. At the beginning of this stage , the zygote appears to consist of three cells as in the American oyster (*C. virginica*) and Galtsoff called it "trefoil". At the end of this stage , the zygote is divided into two unequal cells. The blastomeres again are divided meridionally into 4 quadrants and the biggest one becomes posterior portion. This stage is called the second cleavage stage (fig.26) which required 120 minutes after fertilization. The third cleavage stage (fig.27) is formed by the equatorial division of the zygote. Since this stage is caused by the first quartet , the micromeres are on the animal pole and the macromeres are on the vegetal

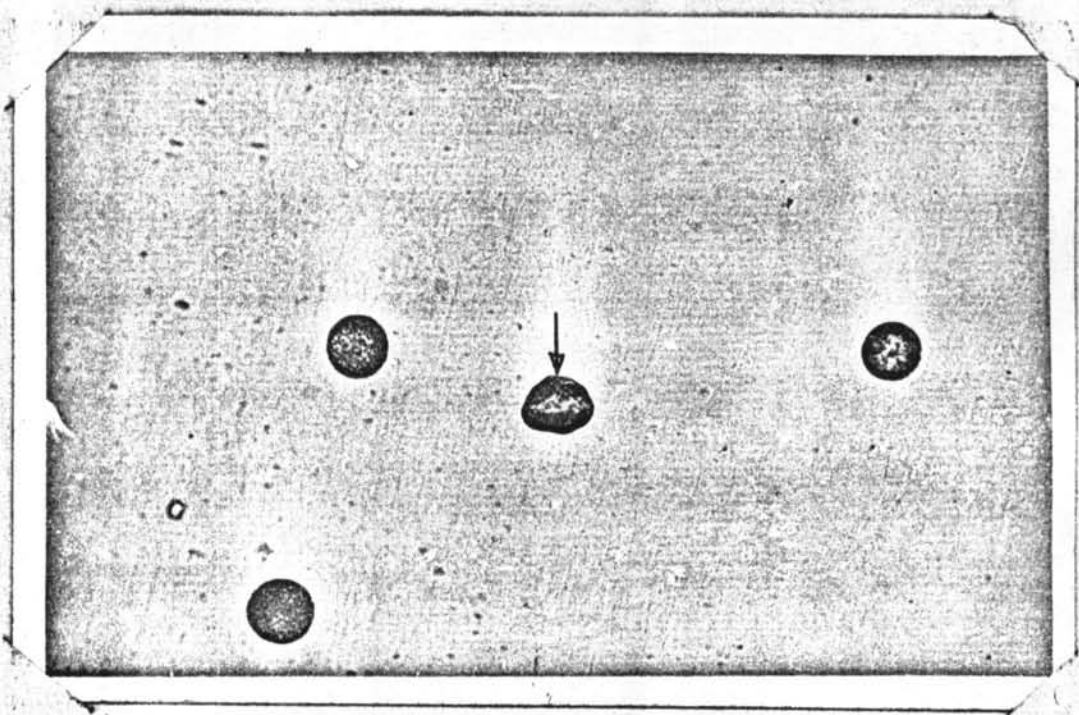


Figure 20: The newly released unfertilized egg of Crassostrea lugubris.

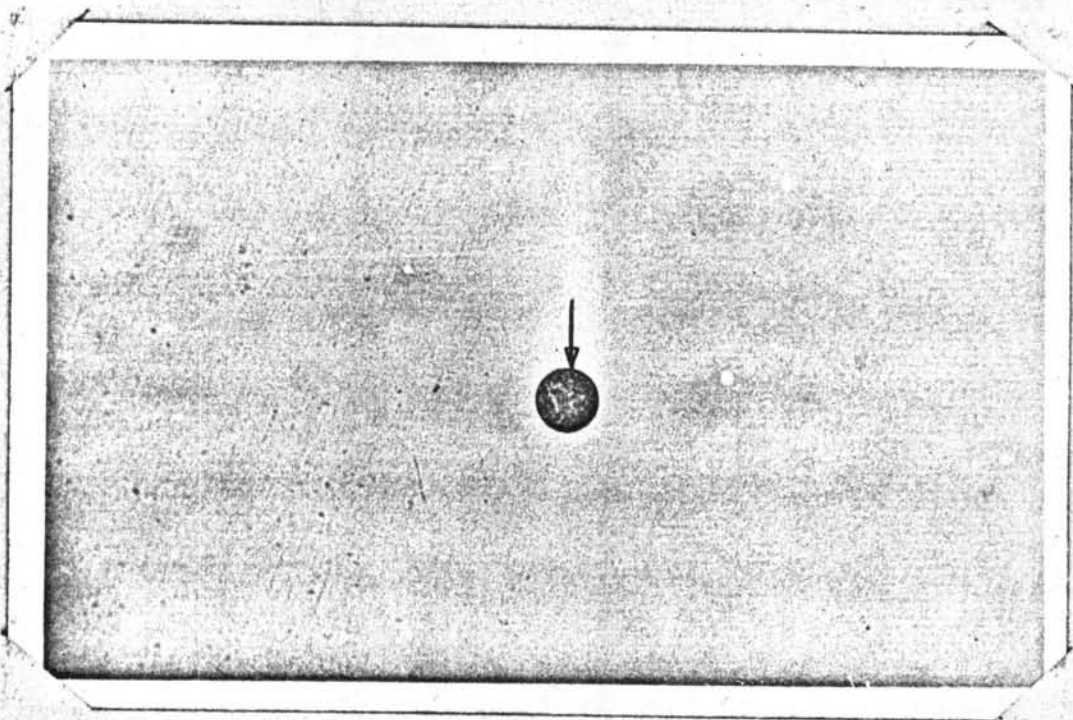


Figure 21 : Within few minutes ,the egg becomes globular in shape and with high density.

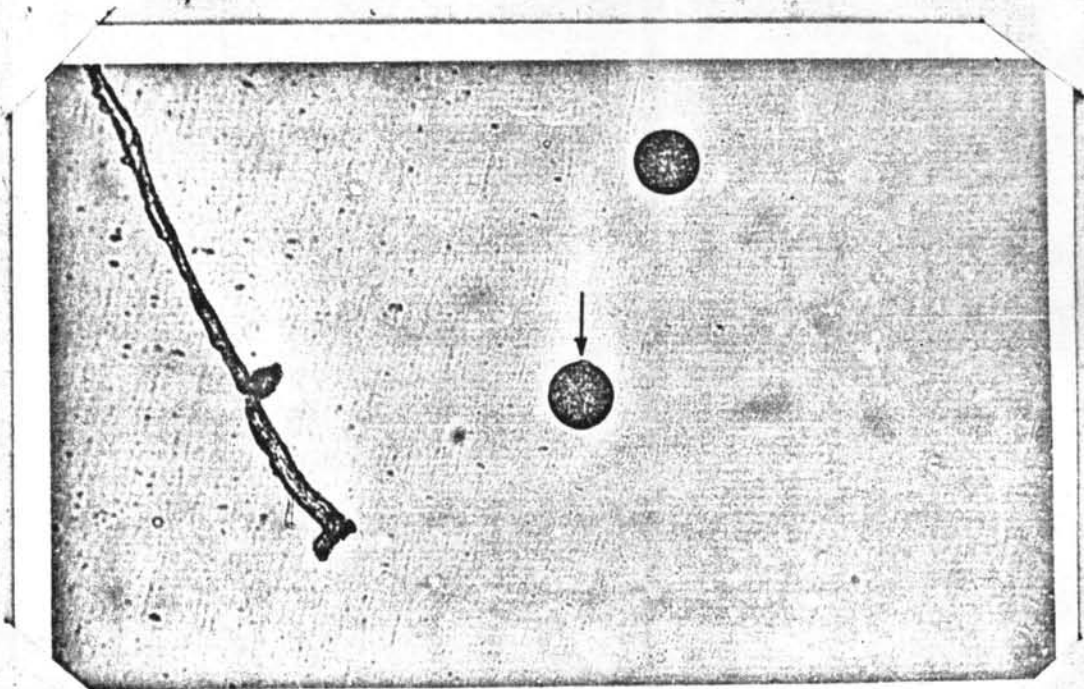


Figure 22 : About 35 minutes after fertilization, the first polar body appears on animal pole.

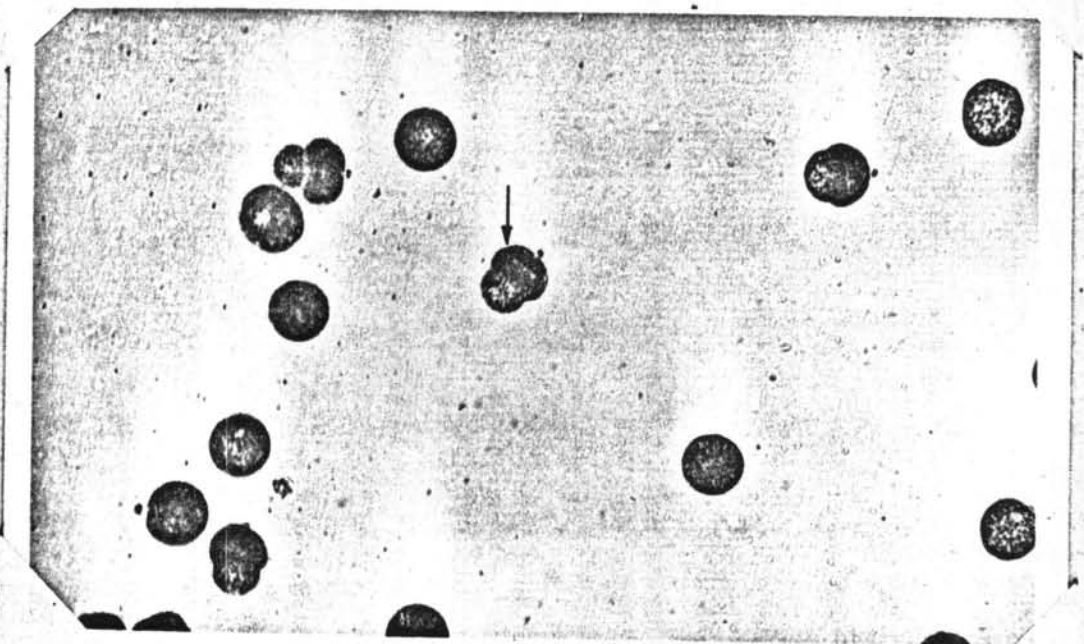


Figure 23 : The second polar body is seen 55-60 minutes after the fertilization.

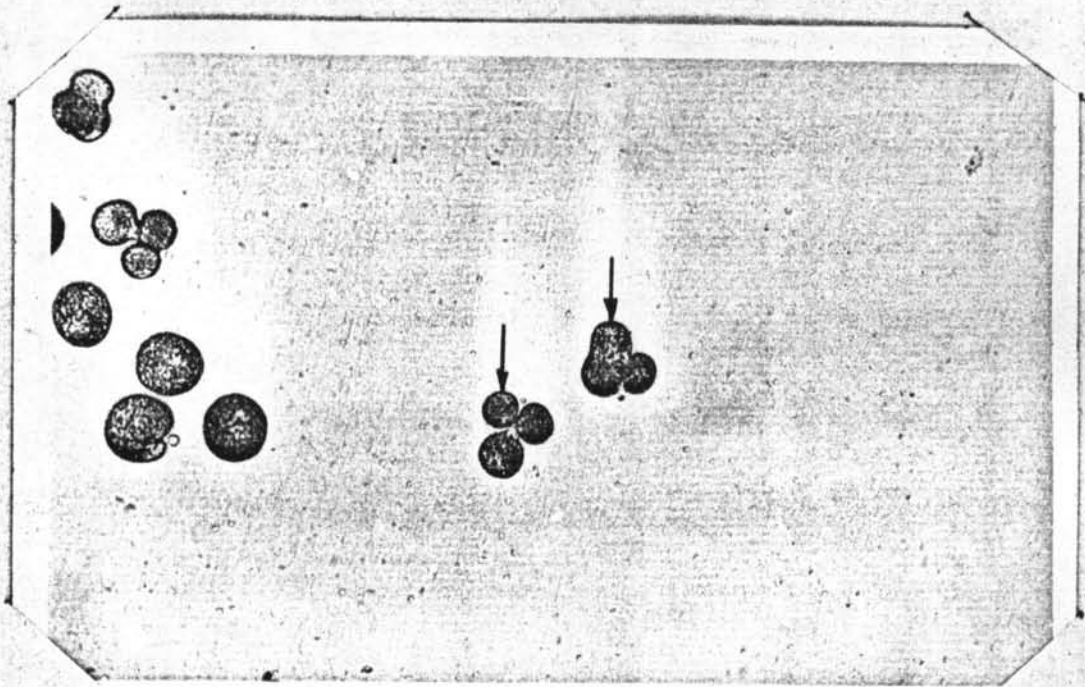


Figure 24 : The first cleavage is formed within 75-100 minutes after fertilization. (trefoil)

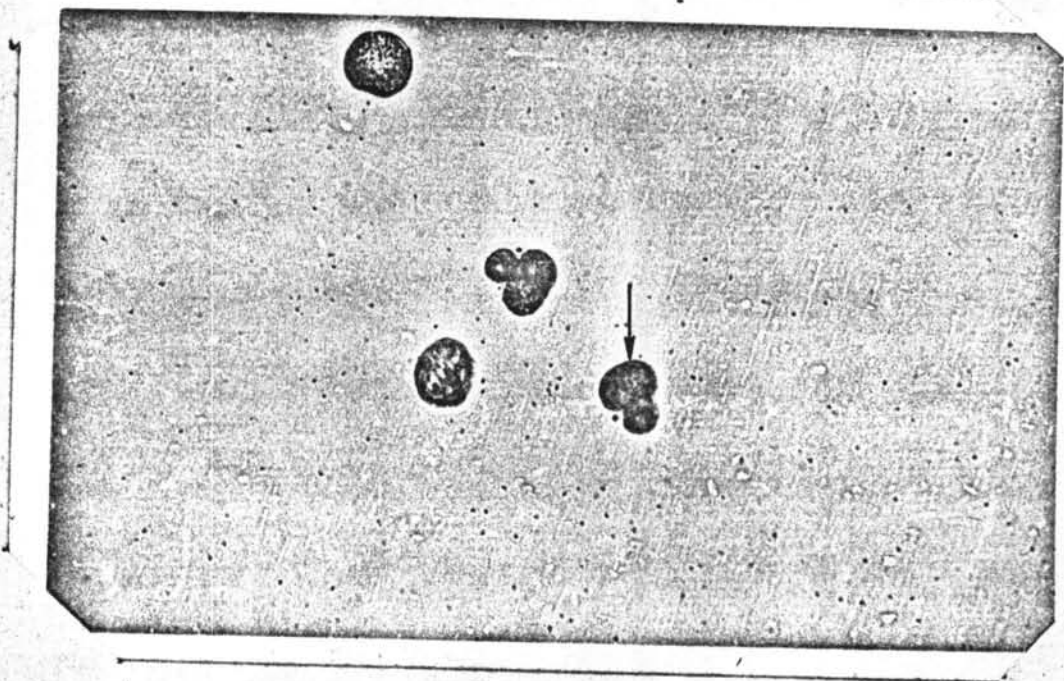


Figure 25 : At the end of the first cleavage stage, the zygote is divided into two unequal cells.

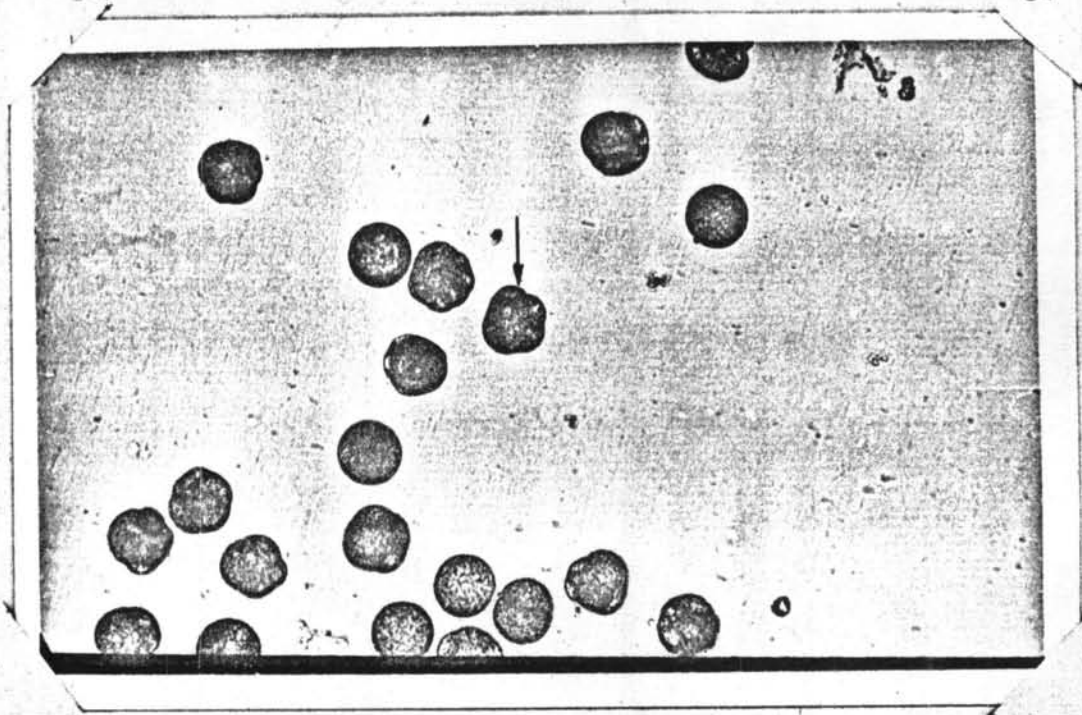


Figure 26 : The second cleavage stage is formed 120 minutes after fertilization.

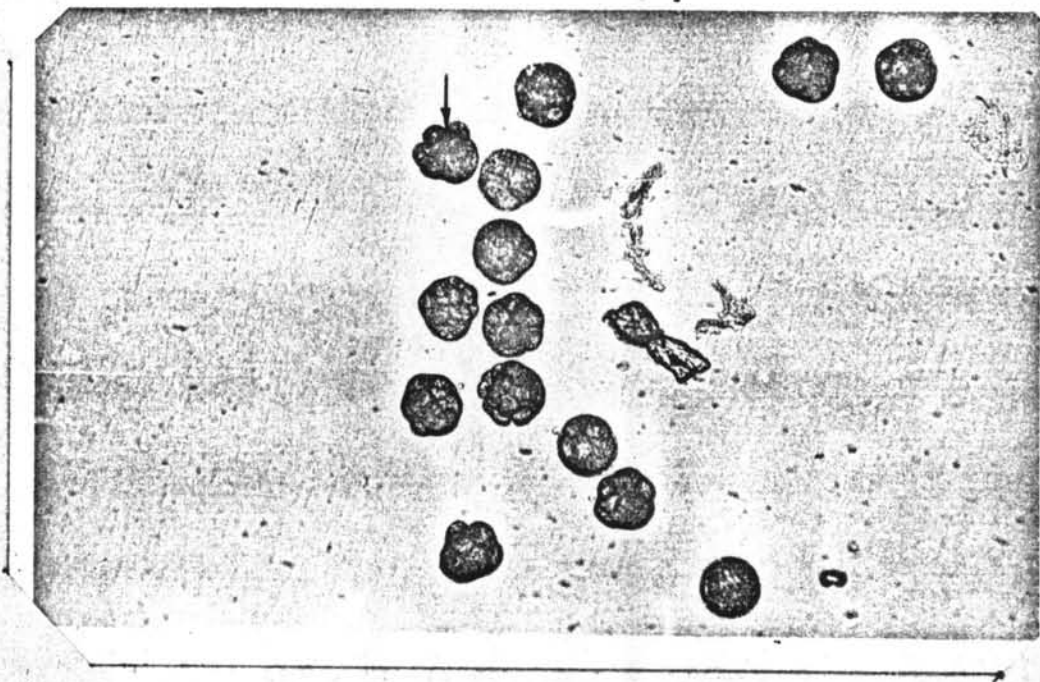


Figure 27 : The third cleavage stage is formed by the equatorial division of the zygote.

pole. The fourth and fifth cleavage is reached by the formation of the second and the third quartet respectively (fig.28 and 29). The multicellular stage or the sixth cleavage stage is reached within 3 hours after fertilization (fig.30). By the epibolic gastrulation of sterroblastula, the moving blastula or the swimming blastula is reached (fig.31) within $4\frac{1}{2}$ hours. The larvae of this stage have developed the strong positive phototaxis character. Brusca (1975) stated in the general patterns of invertebrates development that during the trochophore stage of mollusks, the gut is complete and cilia become visible also shell secretion begins. The trochophore stage of *C. lugubris* took about 20 hours after fertilization. Yonge defined the veliger stage of oyster by the appearance of velum and this can be used for the veliger stage definition of *C. lugubris*. The straight hinge stage or D-shaped larvae (fig.34) is formed within 48 hours after fertilization.

Objective 2 : The effect of temperature on early development of oyster from fertilization to blastula swimming stage.

Observation on the effect of temperatures on early development of oyster were made in two ways ; fresh sample observation and preserved sample counting.

For the fresh sample observation , the time required for the early development of *C. lugubris* from first polar body to D-shaped larvae at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C were shown in table 5. The time required for the development to swimming blastula stage at 23.5°C , 28.0°C and 32.5°C were 360 , 270 and 210 minutes respectively. The data showed that the time required for every

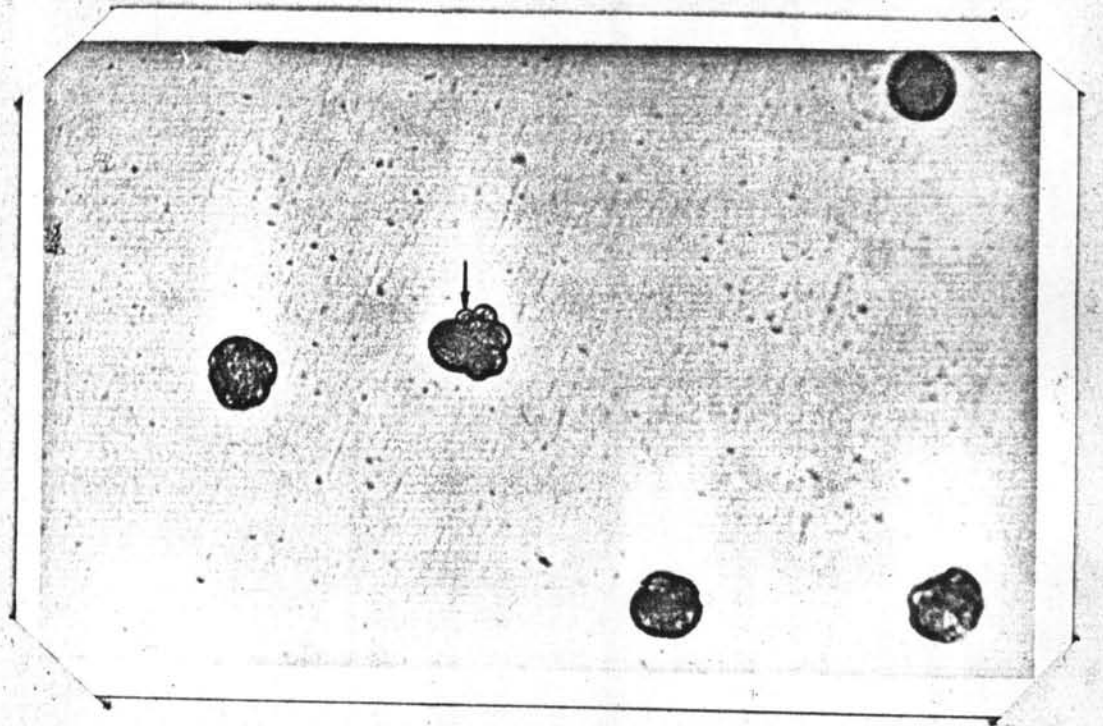


Figure 28 : The fourth cleavage stage is reached by the formation of the second quartet.

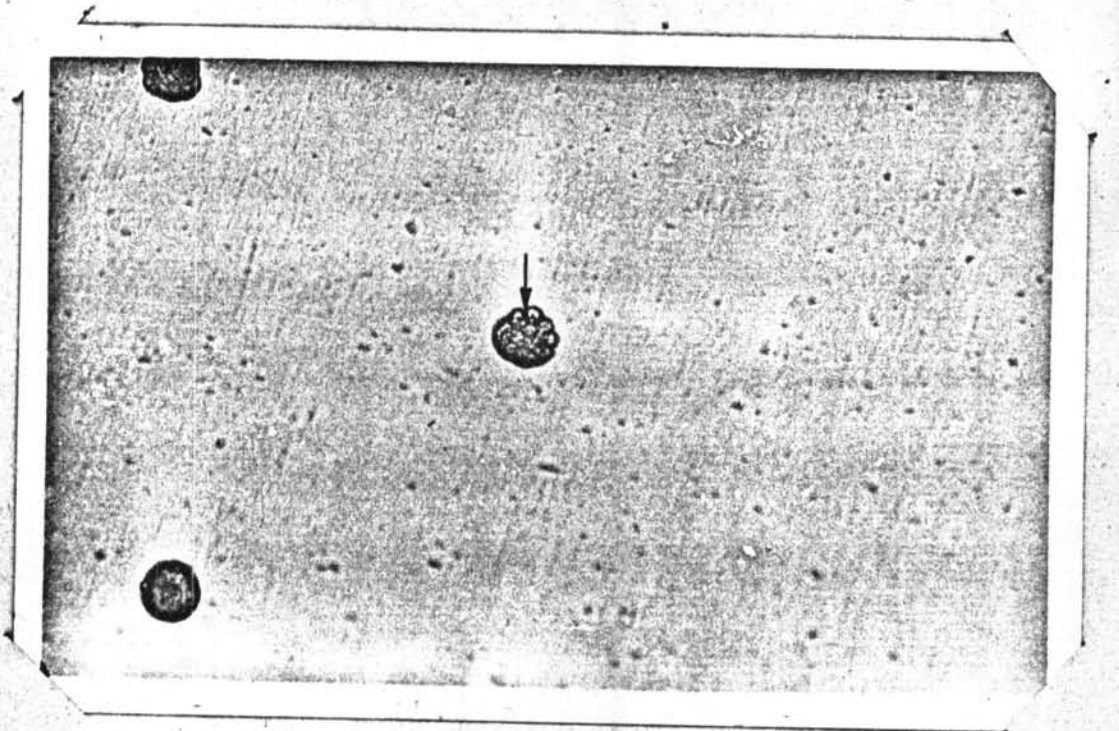


Figure 29 : The fifth cleavage is reached by the formation of the third quartet.

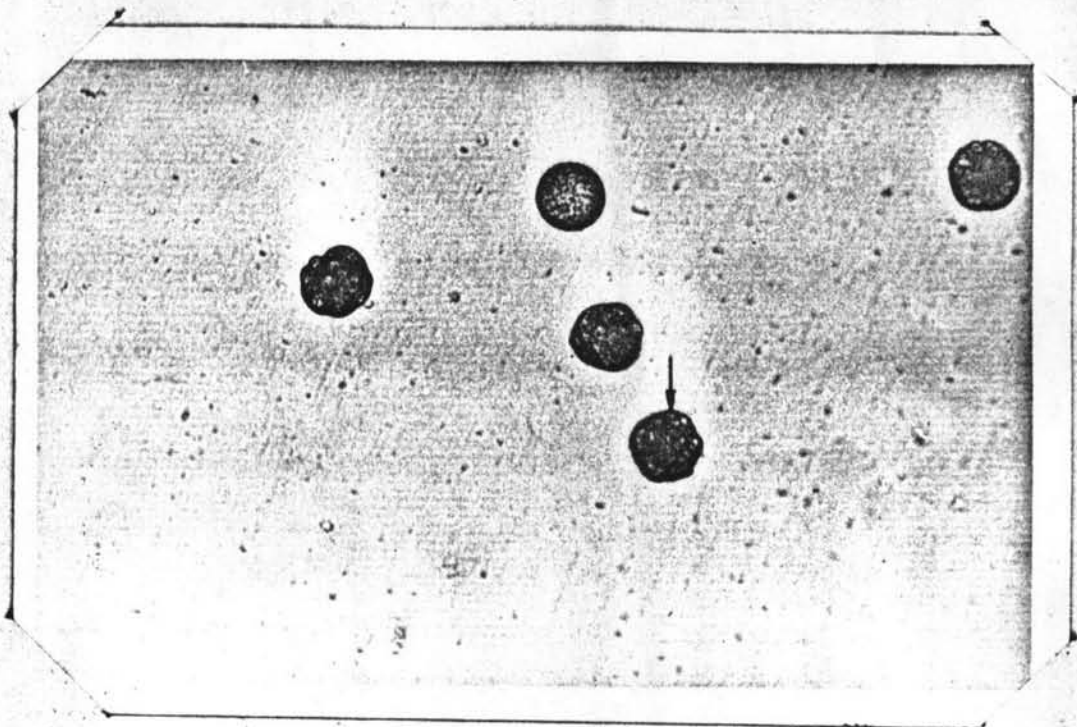


Figure 30 : The multicellular stage or the sixth cleavage stage is formed within 3 hours after fertilization.

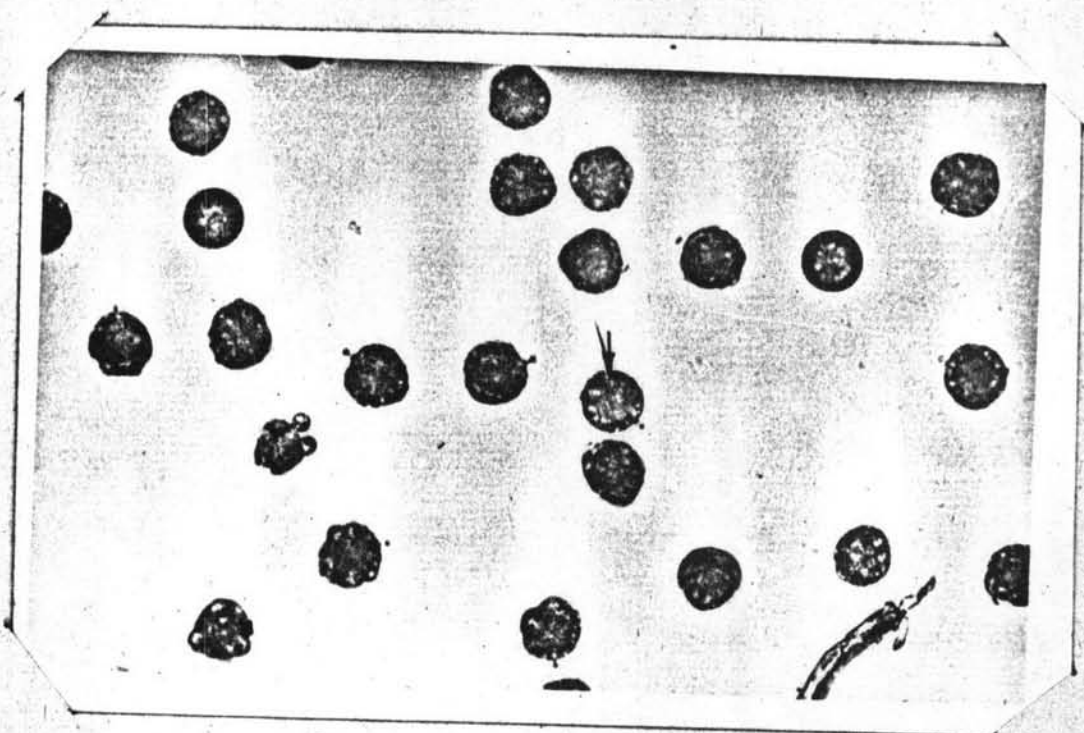


Figure 3I : The moving blastula is reached within $4\frac{1}{2}$ hours after fertilization.

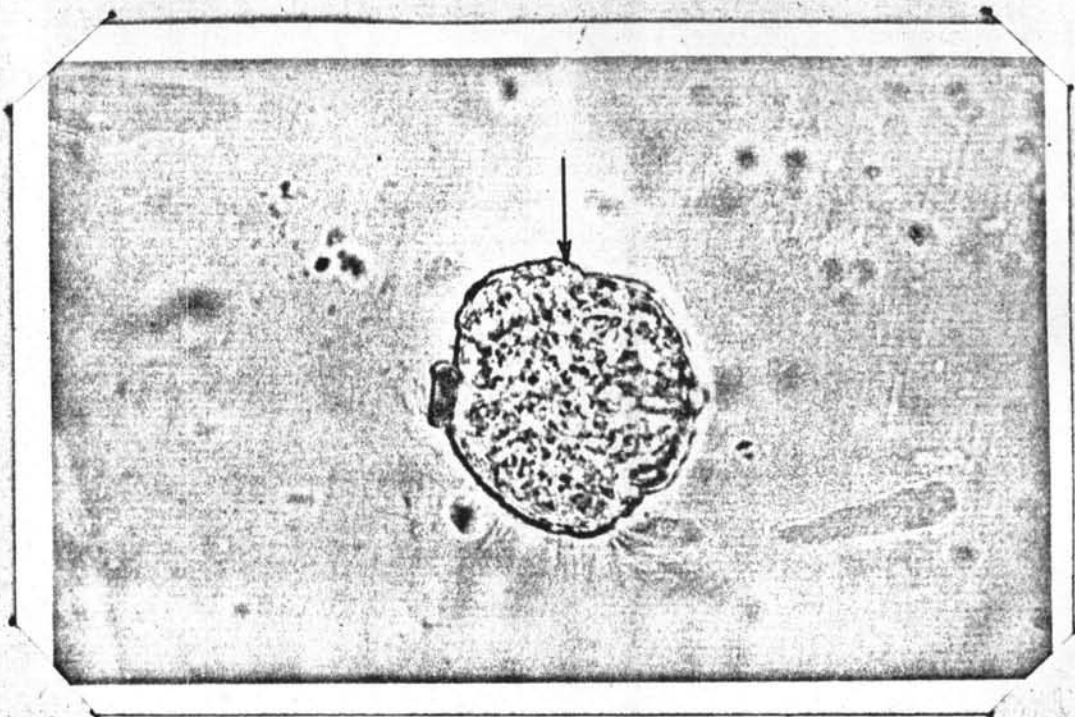


Figure 32 : The trochophore larvae of Crassostrea -
lugubris. (20 hours after fertilization)

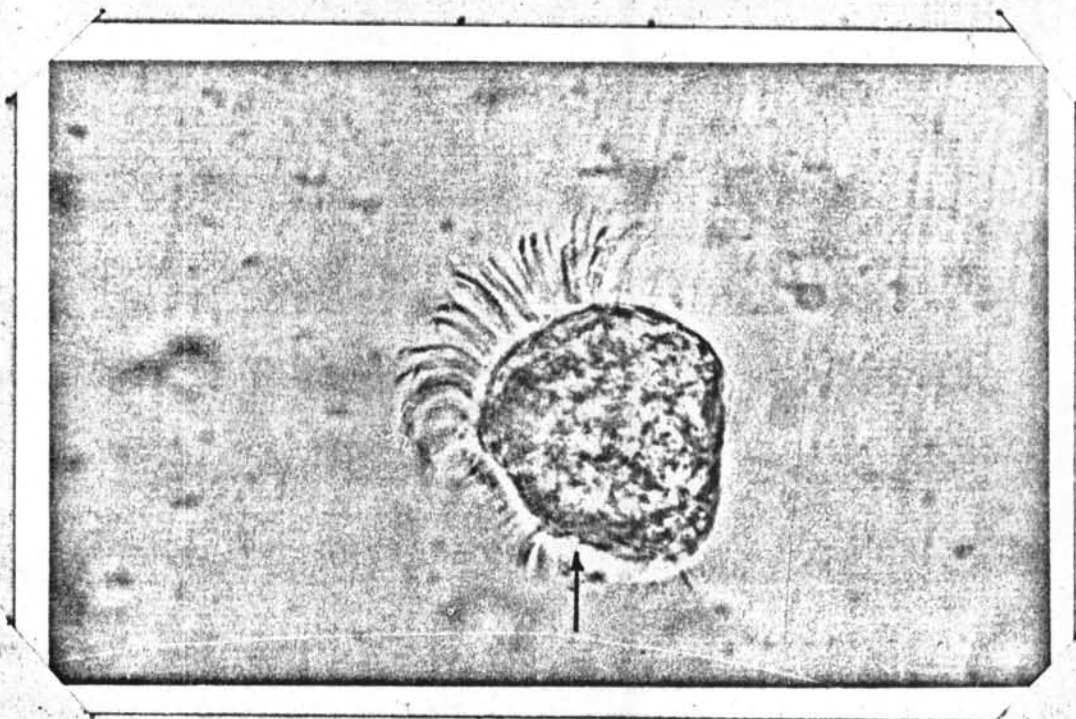


Figure 33 : The veliger larvae of Crassostrea lugubris.

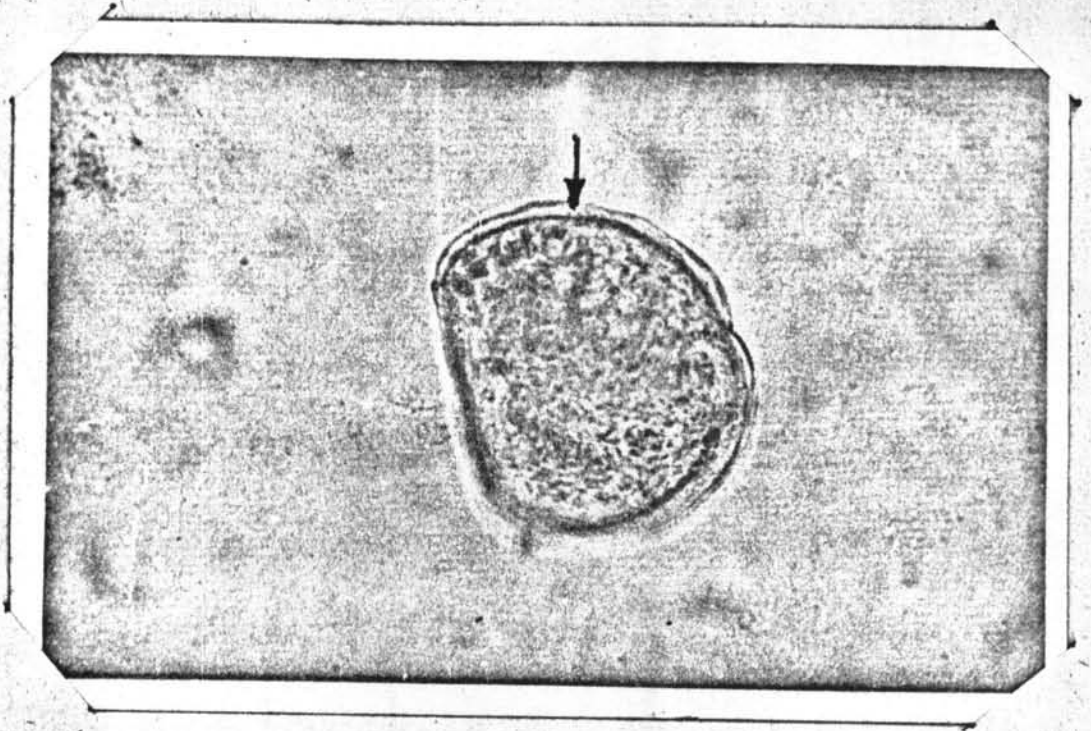


Figure 34 : The straight hinge stage or D-shaped larvae is formed within 48 hours after fertilization.

stage of the development were decreased with the increasing of the incubation temperature.

By counting the preserved sample , the percentage of each stage of the development at every one hour interval from 1 to 6 hours were shown in table 6 , 7 and 8.

At the first hour , the development of the oyster eggs at 23.5°C of incubation temperature reached the first cleavage stage ($8.80 \pm 1.24\%$) but most of them ($56.43 \pm 2.20\%$) were at the first polar body stage. Also the development at 28.0°C of incubation temperature , the percentage of the first polar body stage and the first cleavage stage were 78.66 ± 2.05 and 2.32 ± 1.31 . At the first hour of the development at 32.5°C of incubation temperature , the third cleavage stage were reached and the percentage of the first polar body stage , the second polar body stage, the first cleavage stage , the second cleavage stage and the third cleavage stage were 34.13 ± 2.51 , 14.88 ± 2.56 , 25.27 ± 2.42 , 11.49 ± 2.12 and 1.97 ± 1.19 .

At the second hour period , the development at 23.5°C of incubation temperature reached the fifth cleavage stage ($5.03 \pm 1.43\%$) but most of them ($60.12 \pm 1.27\%$) were still at the first cleavage stage. For 28.0°C of incubation temperature , the development reached the blastula stage or the sixth cleavage stage but the percentage of this stage were only 0.11 ± 0.19 . Most of them were at the first polar body stage ($41.97 \pm 1.43\%$) The second hour of the development at 32.5°C , the development reached the blastula stage with the percentage of 48.91 ± 1.38 . This showed that the blastula stage were reached within the second hour period at 32.5°C

Stage of development	Time required for early development(min.)		
	Acclimation temperature(°C)		
	23.5	28.0	32.5
First polar body	45	35	25
Second polar body	65	55-60	40
First cleavage	90-120	75-100	45-55
Second cleavage	150	120	90
Sixth cleavage	270	180	150
Swimming blastula	360	270	210
Trochophore larvae	1,440	1,200	720
D-shaped larvae	2,880-3,600	2,880	2,160

Table 5 : Time required for early development of fertilized eggs of *C. lugubris* at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C.(From fresh sample observation)

Stage of development	Percentage of each stage of development					
	Period(hour)					
	1	2	3	4	5	6
Undeveloped egg	28.44 (±2.04)	5.05 (±1.21)	2.98 (±1.85)	27.47 (±2.41)	31.42 (±1.63)	35.96 (±3.44)
First polar body	56.43 (±2.20)	60.12 (±1.27)	34.72 (±2.46)	35.00 (±4.69)	11.67 (±2.28)	0.44 (±0.51)
Second polar body	4.18 (±0.85)	2.31 (±1.01)	2.75 (±1.48)	1.54 (±1.66)	0.11 (±0.19)	0.11 (±0.19)
First cleavage	8.80 (±1.24)	8.32 (±1.40)	4.19 (±0.82)	1.31 (±1.18)	0.11 (±0.19)	0
Second cleavage	0	4.71 (±1.30)	4.52 (±0.37)	0.55 (±0.19)	0	0
Third cleavage	0	5.92 (±1.38)	2.75 (±1.15)	0.55 (±0.95)	0.55 (±0.51)	0
Fourth cleavage	0	3.07 (±0.40)	4.30 (±0.86)	1.64 (±1.50)	0	0
Fifth cleavage	0	5.03 (±1.43)	14.12 (±3.80)	2.19 (±1.16)	2.76 (±1.36)	0.22 (±0.39)
Blastula	0	0	20.62 (±2.00)	20.68 (±2.37)	50.31 (±2.17)	57.18* (±2.44)
Abnormal development	2.14 (±0.51)	5.48 (±1.41)	9.05 (±2.52)	9.08 (±0.91)	3.08 (±0.35)	6.09 (±1.94)

* : moving blastula.

Table 6 : The percentage of each stage of early development of *C. lugubris* within six hours period at 23.5°C of incubation temperature.

Stage of development	Percentage of each stage of development					
	Period(hour)					
	1	2	3	4	5	6
Undeveloped egg	2.56 (±1.40)	6.09 (±1.16)	6.53 (±2.17)	5.11 (±2.17)	4.52 (±1.49)	0.11 (±0.19)
First polar body	78.66 (±2.05)	41.97 (±1.43)	8.53 (±2.02)	0.11 (±0.19)	0	0
Second polar body	12.22 (±0.80)	8.31 (±1.51)	1.77 (±1.17)	0	0	0
First cleavage	2.32 (±1.31)	10.19 (±1.97)	4.21 (±1.58)	0.11 (±0.19)	0	0
Second cleavage	0	8.75 (±1.69)	1.55 (±1.26)	0	0	0
Third cleavage	0	3.21 (±1.17)	0.33 (±0.34)	0.11 (±0.19)	0	0
Fourth cleavage	0	8.97 (±1.67)	2.54 (±1.00)	0.22 (±0.39)	0	0
Fifth cleavage	0	4.21 (±1.35)	4.87 (±1.70)	1.00 (±0.58)	0.11 (±0.19)	0.11 (±0.19)
Blastula	0	0.11 (±0.19)	65.45 (±2.34)	85.78 (±3.03)	86.97* (±1.10)	87.25* (±1.78)
Abnormal development	4.23 (±2.02)	8.19 (±1.86)	4.21 (±1.84)	7.67 (±1.20)	8.39 (±0.90)	12.54 (±1.76)

* : moving blastula

Table 7 : The percentage of each stage of early development of *C. lugubris* within six hours period at 28.0°C of incubation temperature.

Stage of development	Percentage of each stage of development					
	Period(hour)					
	1	2	3	4	5	6
Undeveloped egg	1.09 (±1.36)	0.22 (±0.38)	0	0	0	0
First polar body	34.13 (±2.51)	0.22 (±0.19)	1.08 (±1.00)	0.22 (±0.38)	0.11 (±0.19)	0
Second polar body	14.88 (±2.56)	0	0	0	0	0
First cleavage	25.27 (±2.42)	3.04 (±1.01)	0	0	0	0
Second cleavage	11.49 (±2.12)	2.39 (±1.33)	0	0	0	0
Third cleavage	1.97 (±1.19)	3.47 (±1.46)	0	0	0	0
Fourth cleavage	0	0.11 (±0.19)	0	0	0	0
Fifth cleavage	0	26.79 (±2.27)	2.73 (±1.15)	0.22 (±0.38)	0	0
Blastula	0	48.91 (±1.38)	85.84 (±1.48)	96.01 (±1.68)	96.01 (±0.89)	94.22 (±1.02)
Abnormal development	11.16 (±2.05)	14.86 (±2.03)	10.35 (±0.82)	3.55 (±1.72)	3.88 (±1.03)	5.78 (±1.02)

: moving blastula

Table 8 : The percentage of each stage of early development of *C. lugubris* within six hours period at 32.5°C of incubation temperature.

of incubation temperature while most of the development at 23.5°C and 28.0°C of incubation temperature were at the first polar body stage.

At the third hour period , the development at 23.5°C of incubation temperature reached the blastula stage ($20.62 \pm 2.00\%$). For 28.0°C of incubation temperature , the development reached the blastula stage with the percentage of 65.45 ± 2.34 . At the third of the development at 32.5°C of incubation temperature , the percentage of the blastula stage increased to 85.84 ± 1.48 and most of the zygotes completely developed into this stage.

At the fourth hour period , the percentage of each stage of the development at 23.5°C of incubation temperature was slightly different from the development at the third hour within the same incubation temperature. For the percentage of the development at 28.0°C , the percentage of the sixth cleavage stage or the blastula stage increased to 85.78 ± 3.03 and showed clearly difference from the percentage of 23.5°C of incubation temperature within the same fourth period. For 32.5°C of incubation temperature , the percentage of the blastula stage increased to 96.01 ± 1.68 and all of them hatch into larvae or moving blastula. This clearly showed the difference from the development of the other two incubation temperatures.

At the fifth hour period , the percentage of the blastula stage at 23.5°C increased to 50.31 ± 2.17 and there were no moving blastula while the percentage of the blastula stage at 28.0°C of incubation temperature increased to 86.97 ± 1.10 and there were moving blastula. This showed the difference between the development of the zygote at 23.5°C of incubation

temperature and 28.0°C of incubation temperature within the same fifth hour period. For 32.5°C of incubation temperature, the percentage of each stage of the development were almost the same as the percentage at the fourth hour period.

At the sixth hour period, the zygote of 23.5°C of incubation temperature hatched into moving blastula with the percentage of 57.18 ± 2.44 . The percentage of the moving blastula were 87.25 ± 1.78 and 94.22 ± 1.02 for the incubation temperatures of 28.0°C and 32.5°C respectively.

The point in circle showed percent hatchability of the eggs at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C (Table 11, fig.35). The average percentage of hatchability of oyster larvae were increased from 57.18 ± 2.44 to 87.11 ± 0.19 and to 95.41 ± 1.03 with the increasing of incubation temperatures from 23.5°C to 28.0°C and to 32.5°C (Table 11).

The cumulative percentage of abnormal development of the embryonic development at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C were shown in table 9 and fig. 36. The average percentage of abnormal development were increased from 5.82 ± 2.91 to 7.66 ± 3.11 and to 8.26 ± 4.56 with the increasing of incubation temperatures from 23.5°C to 28.0°C and to 32.5°C respectively (Table 11).

The cumulative percentage of undeveloped eggs at three levels of incubation temperatures i.e., 23.5°C , 28.0°C and 32.5°C were shown in table 10 and fig. 37. The average percentage of undeveloped eggs were decreased from 21.89 ± 14.17 to 4.15 ± 2.42 and to 0.22 ± 0.44 with the increasing of temperatures from 23.5°C to 28.0°C and to 32.5°C (Table 11).

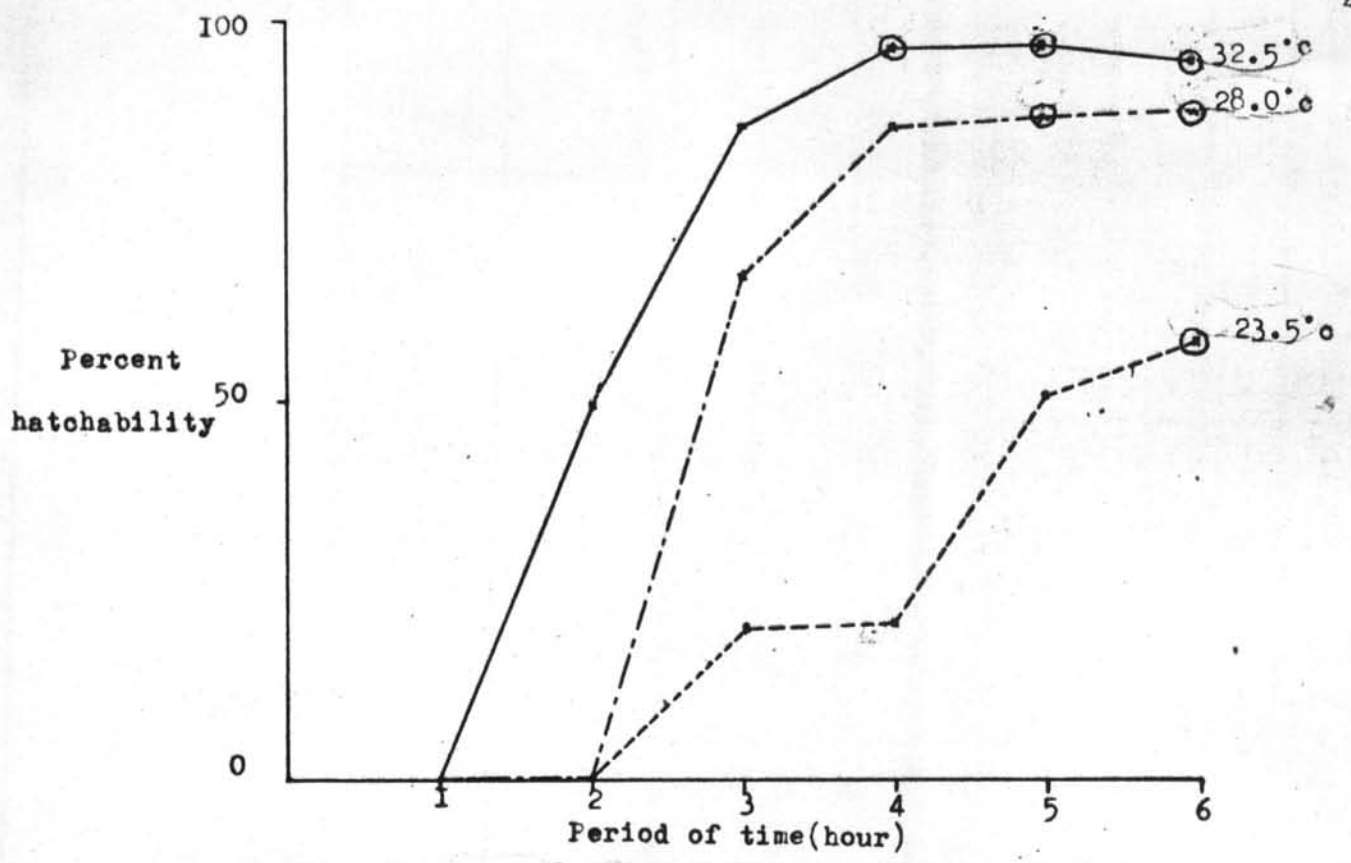


Figure 35 : Points in circles show % hatchability of the eggs at three levels of incubation temperature; 23.5°C, 28.0°C and 32.5°C.

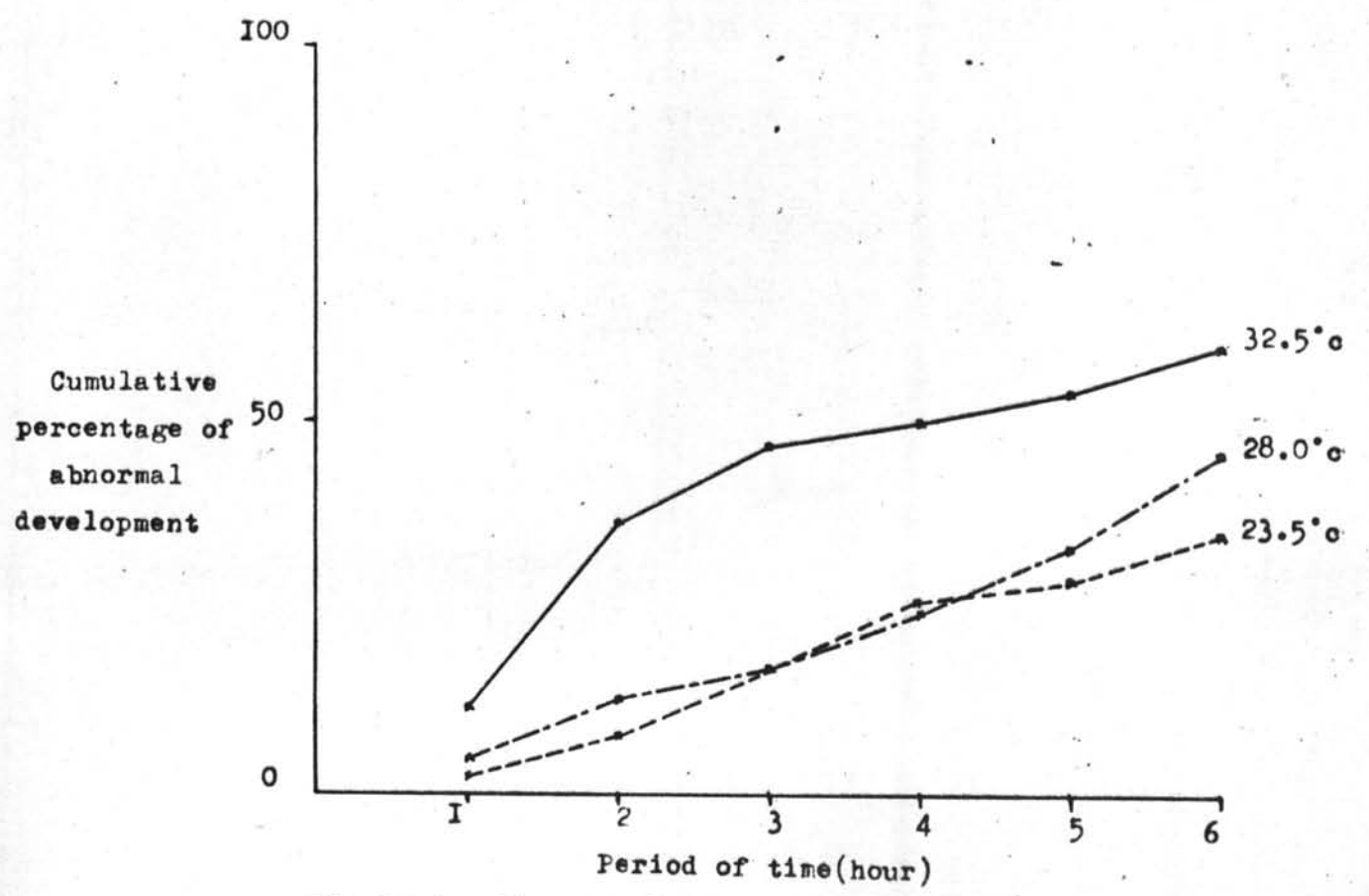


Figure 36 : Show cumulative percentage of abnormal development at three levels of incubation temperature; 23.5°C, 28.0°C and 32.5°C.

Incubation temperature (°C)		Period(hour)					
		1	2	3	4	5	6
23.5	Percent of abnormal development	2.14	5.48	9.05	9.08	3.08	6.09
	Cumulative percentage	2.14	7.62	16.67	25.75	28.83	34.92
28.0	Percent of abnormal development	4.23	8.19	4.12	7.67	8.39	12.54
	Cumulative percentage	4.23	12.42	16.63	24.30	32.69	45.23
32.5	Percent of abnormal development	11.16	14.86	10.35	3.55	3.88	5.78
	Cumulative percentage	11.16	26.02	36.37	39.92	43.80	49.58

Table 9 : Cumulative percentage of abnormal development at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

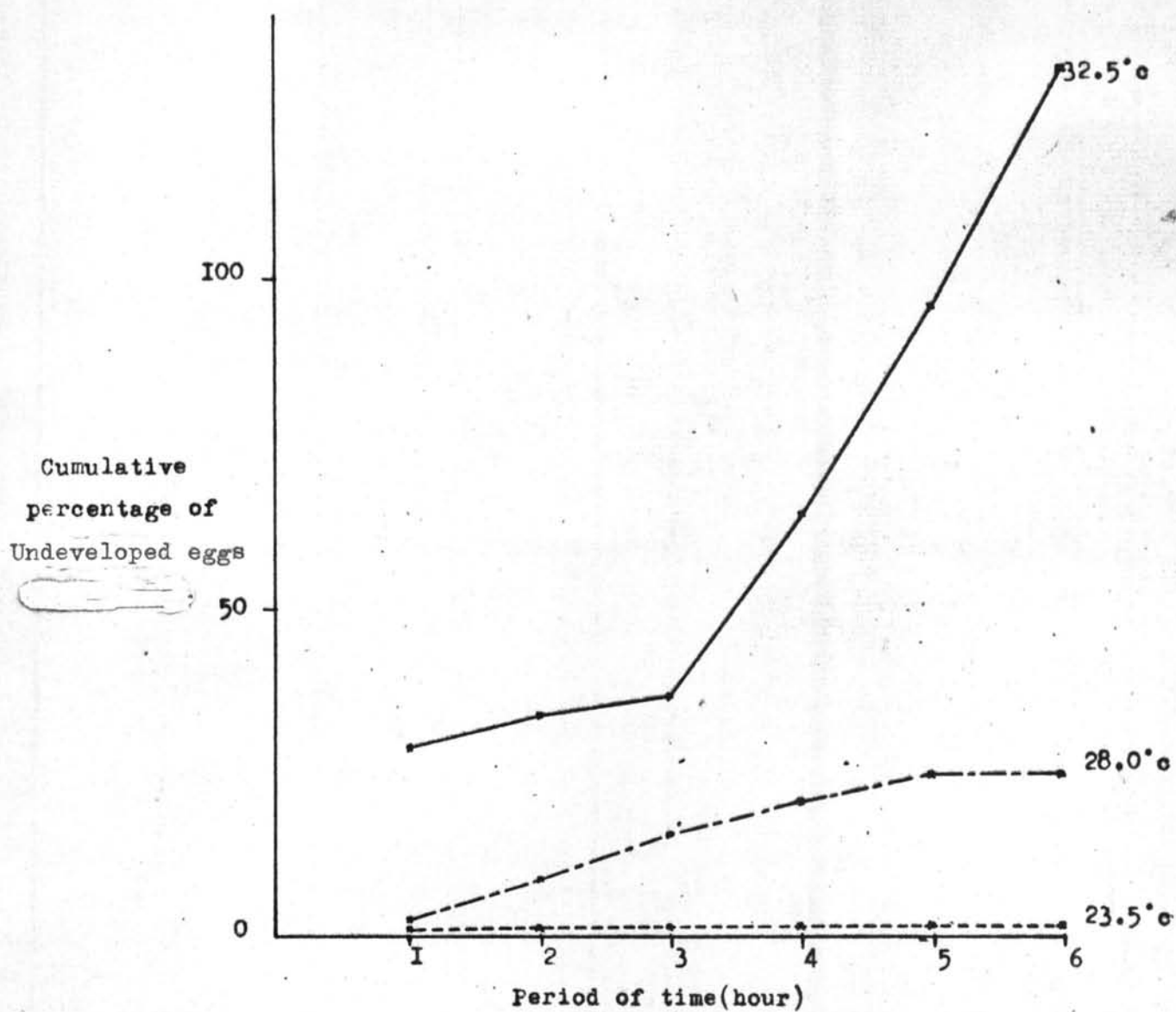


Figure 37: Show cumulative percentage of undeveloped eggs at three levels of incubation temperature; 23.5°C, 28.0°C and 32.5°C.

Incubation temperature (C)		Period(hour)					
		1	2	3	4	5	6
23.5	Percent of undeveloped egg	28.44	5.05	2.89	27.47	31.42	35.96
	Cumulative percentage	28.44	33.49	36.47	63.94	95.36	131.32
28.0	Percent of undeveloped egg	2.56	6.09	6.53	5.11	4.52	0.11
	Cumulative percentage	2.56	8.65	15.18	20.29	24.81	24.92
32.5	Percent of undeveloped egg	1.09	0.22	0	0	0	0
	Cumulative percentage	1.09	1.31	1.31	1.31	1.31	1.31

Table 10 : Cumulative percentage of undeveloped eggs at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

	Incubation temperature(°C)		
	23.5	28.0	32.5
Average percentage of undeveloped eggs	21.89±14.17	4.15±2.42	0.22±0.44
Average percentage of abnormal development	5.82±2.91	7.66±3.11	8.26±4.56
Average percentage of hatchability (4 th hour-6 th hour)	57.18±2.44 (6 th hour)	87.11±0.19 (5 th -6 th hour)	95.41±1.03 (4 th -6 th hour)

Table 11 : The average percentage of undeveloped eggs , abnormal development and hatchability at three levels of incubation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

Objective 3 : Determination of the maximum temperature that prevent hatchability.

The tested temperatures were 34.0°C , 34.5°C , 35.0°C , 35.5°C , 36.0°C , 36.5°C and 37.0°C . After six hours , the samples were collected and examined under a compound microscope. The maximum temperature that prevent hatchability was 35.5°C .

Objective 4 : Determination of the critical thermal maximum (CTM) of blastula swimming stage acclimated in three levels of temperatures i.e., 23.5°C , 28.0°C and 32.5°C .

The critical thermal maximum of blastula swimming stage at three levels of acclimation temperature were all the same at 48.5°C (Table 12). The obtained data showed that there was no relationship between CTM of the larvae and the levels of acclimation temperature.

The behavior of the larvae was observed and noted during the increasing of temperature. From 23.5°C to 32.5°C the larvae moved around by the helping of their cilia. With the increasing of temperature , the larvae moved **faster** and increased the activity of their cilia. The larvae could not moved around at 47.0°C but rotated. Only slightly movement of their cilia could be seen from the samples at 47.5°C and 48.0°C . The larvae died and their cilia were relaxed at 48.5°C .

Objective 5 : Determination of critical thermal maximum (CTM) and lethal temperature of D-shaped larvae at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C .

The critical thermal maximum of D-shaped larvae acclimated at 23.5°C and 28.0°C were 48.17 ± 0.29 C. At 32.5°C of acclimation , the critical

thermal maximum was 48.5°C . The obtained data showed only a slight relationship between the critical thermal maximum and the acclimation temperature (Table 13).

In determining the lethal temperatures, the five levels of test temperature i.e., 45.5°C , 42.5°C , 39.5°C , 36.5°C and 33.5°C were used. The number of dead larvae for each treatment and period of time were shown in table 14. The temperature at 28.0°C was used as a control.

At 45.5°C the larvae of 23.5°C and 28.0°C acclimation were all died within the first hour. Ninety two percent of the larvae acclimated at 32.5°C died within the first hour.

At 42.5°C and 39.5°C , the larvae of three treatments were 100% died within the third hour.

At 36.5°C , the larvae of 23.5°C could not survive over 12 hours but the larvae of 28.0°C and 32.5°C could survive more than 50% after 24 hours.

At 33.5°C , the larvae of three treatments could survive more than 50% after 24 hours.

From Table 14, 12 hr-Lt_{50} and 24 hr-Lt_{50} were determined and showed in fig.38,39 and Table 15.

The 12 hr-Lt_{50} were 34.95°C at 23.5°C of acclimation temperature and 37.95°C at 28.0°C and 32.5°C of acclimation temperature.

The 24 hr-Lt_{50} were 34.5°C at 23.5°C of acclimation temperature, 37.45°C at 28.0°C of acclimation temperature and 37.6°C at 32.5°C of acclimation temperature.

Acclimation temperature(°C)	Critical Thermal Maximum(°C)			
	First treatment	Second treatment	Third treatment	Average
23.5	48.5	48.5	48.5	48.5
28.0	48.5	48.5	48.5	48.5
32.5	48.5	48.5	48.5	48.5

Table 12 : The critical thermal maximum of blastula swimming stage at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

Acclimation temperature(°C)	Critical Thermal Maximum(°C)			
	First treatment	Second treatment	Third treatment	Average
23.5	48.0	48.0	48.5	48.17±0.29
28.0	48.0	48.5	48.0	48.17±0.29
32.5	48.5	48.5	48.5	48.5

Table 13 : The critical thermal maximum of D-shaped larvae at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

TESTED TEMPERATURE (°C)	CONDITION	NUMBER OF DEAD LARVAE AT VARIOUS OF TIME INTERVALS (total count = 100 cells)														
		1 st hour			3 rd hour			6 th hour			12 th hour			24 th hour		
		23.5°C	28.0°C	32.5°C	23.5°C	28.0°C	32.5°C	23.5°C	28.0°C	32.5°C	23.5°C	28.0°C	32.5°C	23.5°C	28.0°C	32.5°C
45.5	TREATMENT	100	100	92	-	-	100	-	-	-	-	-	-	-	-	-
	CONTROL	0	0	6	0	3	0	5	0	1	7	6	3	19	10	4
42.5	TREATMENT	52	41	26	100	100	100	-	-	-	-	-	-	-	-	-
	CONTROL	0	0	6	0	3	0	5	0	1	7	6	3	19	10	4
39.5	TREATMENT	46	18	11	100	100	100	-	-	-	-	-	-	-	-	-
	CONTROL	0	0	6	0	3	0	5	0	1	7	6	3	19	10	4
36.5	TREATMENT	0	0	0	0	0	0	19	5	4	100	10	7	-	37	25
	CONTROL	0	0	6	0	3	0	5	0	1	7	6	3	19	10	4
33.5	TREATMENT	0	0	0	0	0	0	7	4	2	10	1	3	43	10	6
	CONTROL	0	0	6	0	3	0	5	0	1	7	6	3	19	10	4

Table 14 : The number of dead larvae at various of time intervals at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C (control at 28.0°C)

The obtained data showed that both 12 hr-Lt₅₀ and 24 hr-Lt₅₀ were increased with the increasing of the acclimation temperature or the rearing temperature. (Fig.40)

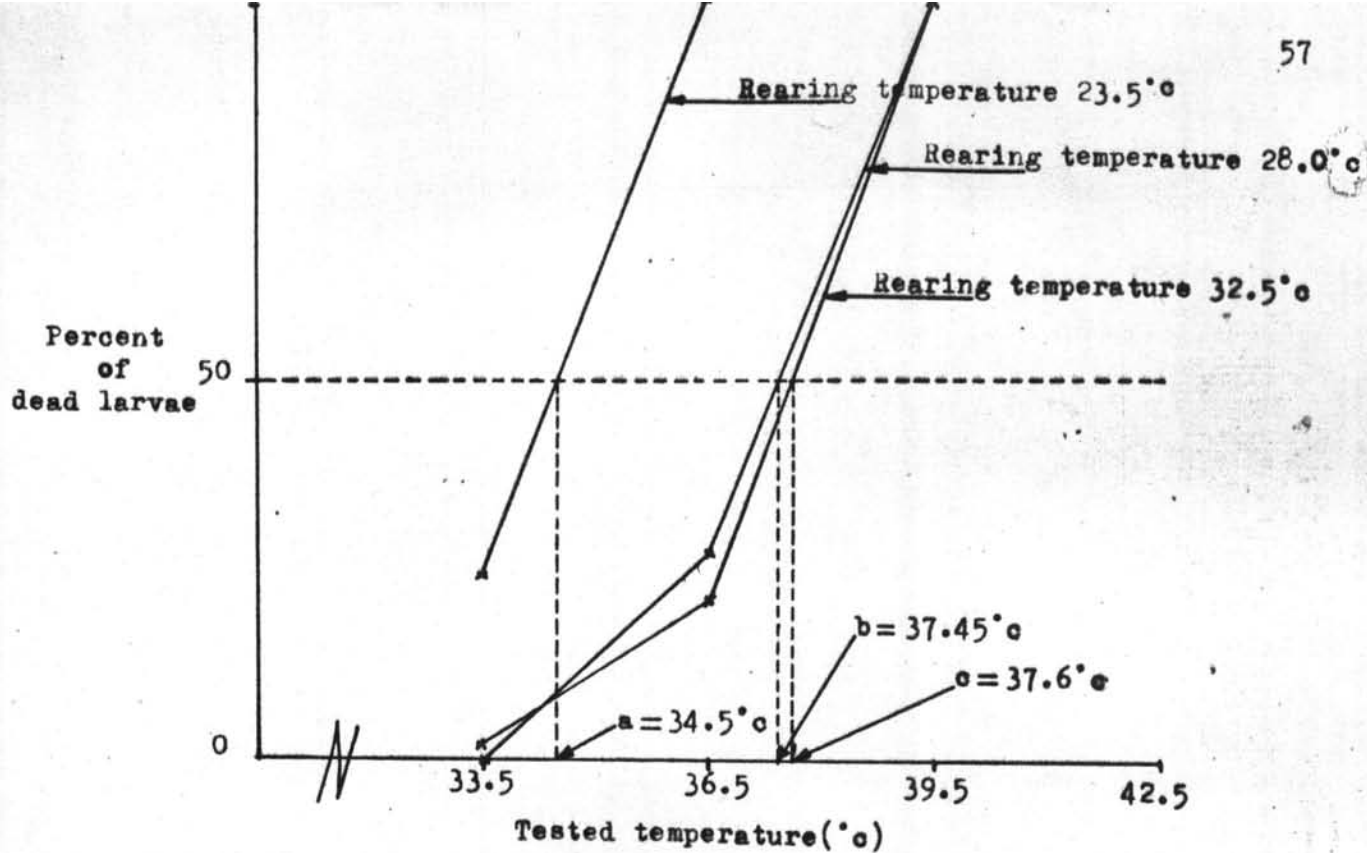


Figure 38 : Show 24 hr. Lt_{50} of D-shaped larvae at three levels of rearing temperature; 23.5°C, 28.0°C and 32.5°C .

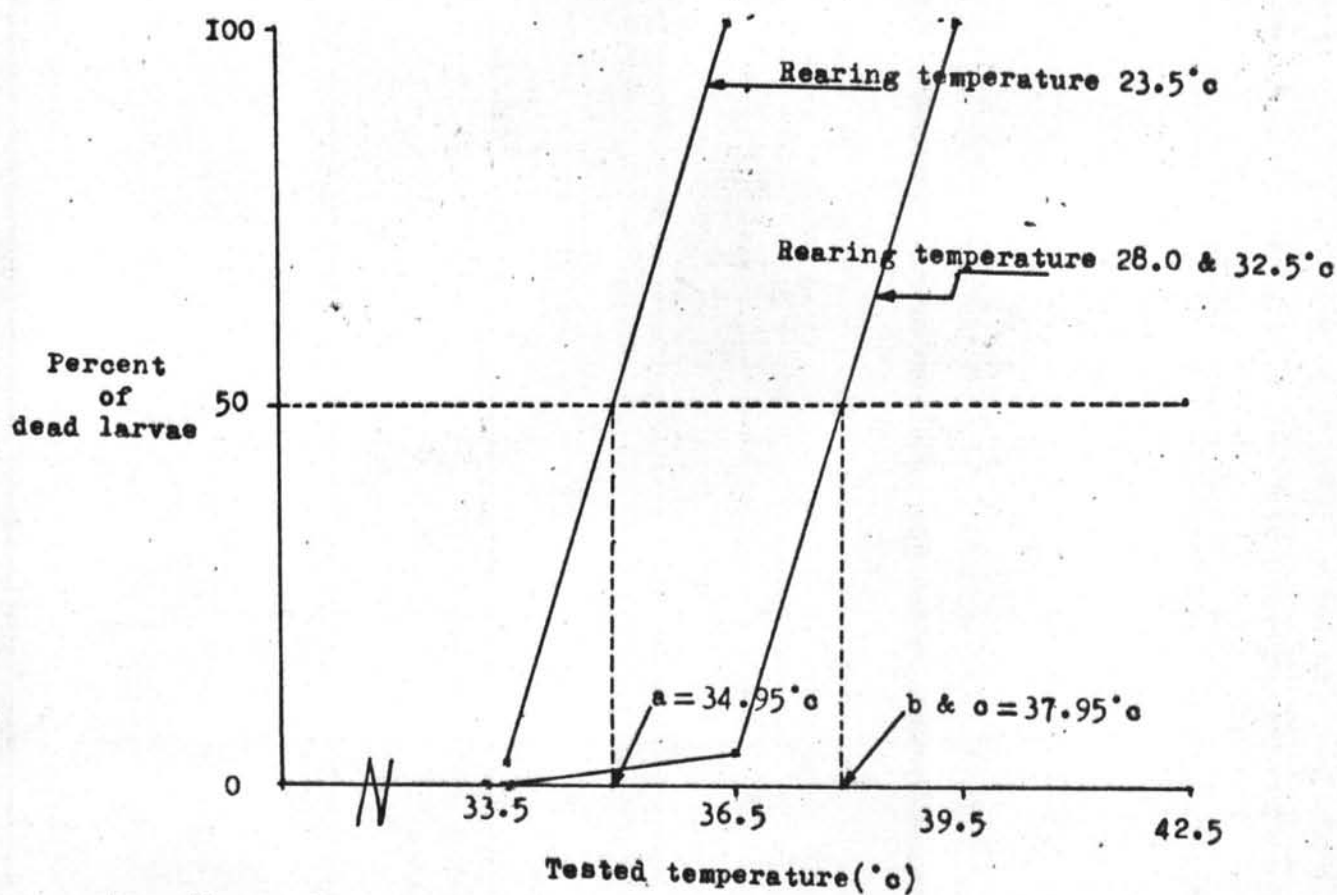


Figure 39 : Show 12 hr. Lt_{50} of D-shaped larvae at three levels of rearing temperature; 23.5°C, 28.0°C and 32.5°C .

acclimation temperature (°C)	12 hr-Lt ₅₀ (°C)	24 hr-Lt ₅₀ (°C)
23.50	34.95	34.50
28.00	37.95	37.45
32.50	37.95	37.60

Table 15: The 12 and 24 hr-Lt₅₀ of D-shaped larvae of Tropical oyster (Crassostrea lugubris) at three levels of acclimation temperature i.e., 23.5°C, 28.0°C and 32.5°C.

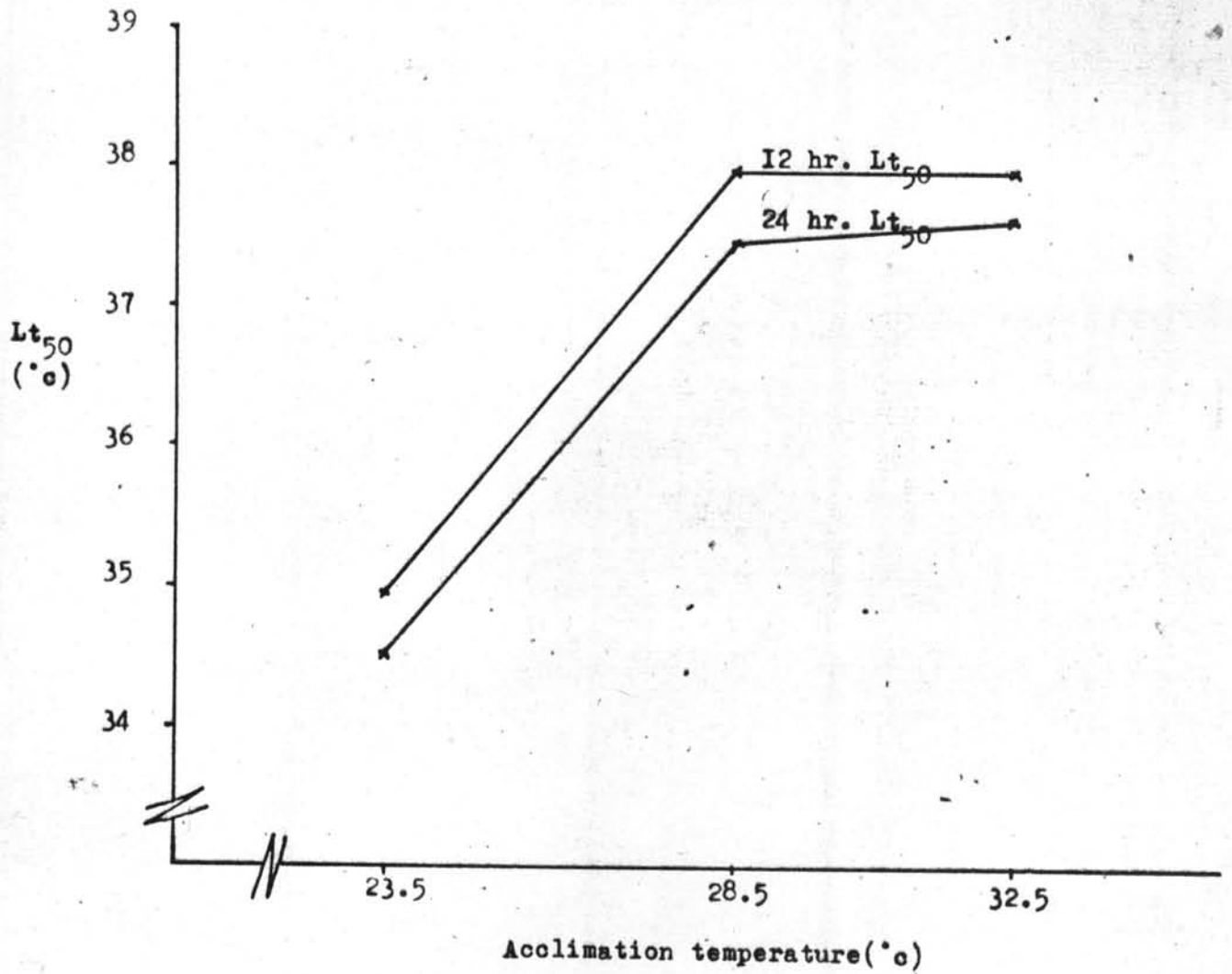


Figure 40 : Show the relation between 12 hr. Lt_{50} , 24 hr. Lt_{50} and acclimation temperature.