CHAPTER 1

INTRODUCTION

Some problems may arise from nuclear power plants which are planned for several countries in Southeast Asia. Thermal pollution is one of those problems. There is a very limited information regarding the thermal pollution in this region. Thailand has a plan to use the nuclear power as a new source of energy. A nuclear power station is expected tobe located on the east coast of the Gulf of Thailand. The coastal water in this area will be used as cooling water for this plant. The yearly ambient sea water temperature usually ranges from 25° to 30° C. It is anticipated that cooling water discharged from the plant will have the temperature about 10°C higher than the ambient sea water temperature. This temperature increment may alter the well being of marine communities within the plant's vicinity. A great deal of information on the effect of temperature on the temperate marine organisms are available , but it is limited for the tropical region. It was concluded that the temperate species could tolerate a wide range of temperature change than the tropical species(Menasveta,1976).

Thermal effluent may affect both aquatic biota and their associated environments in several ways. Temperature is the most important single factor governing the occurrence and behavior of life(Gunther,1957). Van't Hoff Law stated that metabolic activity might increase two-fold

or three-fold with a 10° C rise in temperature. But Q_{10} can be used only within certain limits(Blaxter,1956). These limitation are specific for each species , sex , age and stage in life cycle. Geographic area where organisms live is also important. Below the species specific limitation activities are either non existence or reduced to very low levels. Above this temperature activities increase with the increasing temperature until a point is reached where activities are maximum. Beyond this temperature activities drop off until death occur.

Crassostrea lugubris , the tropical oyster , is chosen as a test organism in this investigation because it is considered as one of the economically important invertebrates in Thailand. They are easily obtained and are easily induced to released sperm and eggs in laboratory. They could be kept in laboratory for a certain period of time for the developmental study. This experiment is designed to work with the early stages of larval development. In Thailand larval development of this oyster has not yet been investigated. Therefore , the information gained from this study can be used for the improvement of oyster culture in Thailand. Furthermore , the information on the temperature effect on oyster can be used in parts for establishing the water quality criteria for heat waste discharge.

This experiment was undertaken to determine the following :

1. Early development of oyster larvae(C. lugubris) from fertilization to D-shaped larvae.

2. Effect of temperature on early development of oyster from fertilization to blastula swimming stage.

3. The maximum temperature that prevent hatchability. This is determined when hatchability percentage is equal to zero.

4. Critical thermal maximum (CTM) of blastula swimming stage at three levels of acclimation temperature i.e., 23.5°C , 28.0°C and 32.5°C.

5. 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.

LITERATURE REVIEW

General Anatomy :

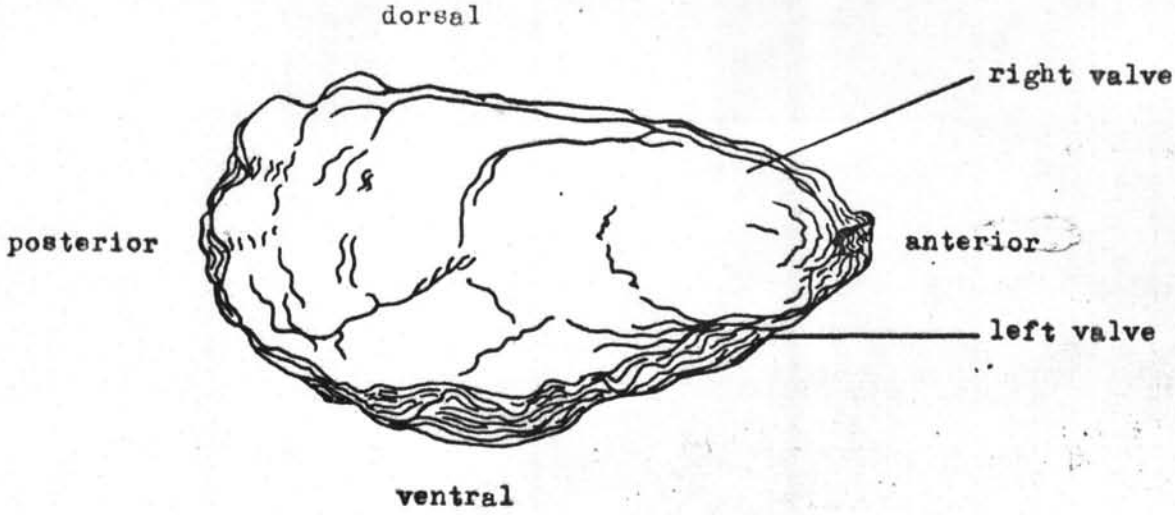
The anatomy of oyster was described in several papers. General account of the anatomy of Crassostrea virginica were given by Moore (1895) , Brooks (1905) , Churchill (1920) and Galtsoff (1958). The structure of the European oyster , Ostrea edulis was described by Orton (1937) , Yong (1960) and Ranson (1965). A brief and partial description on the anatomy of C. angulata was given by Leenhardt (1926) which included the history of the species. The anatomy of the tropical species were also studied. Roughley (1925) had given a short anatomical sketch on the Australian oyster O. commercialis. Awati and Rai in 1931 described the structure of the Bombay oyster O. cucullata.

The general anatomy of C. lugubris is shown in figure 1.

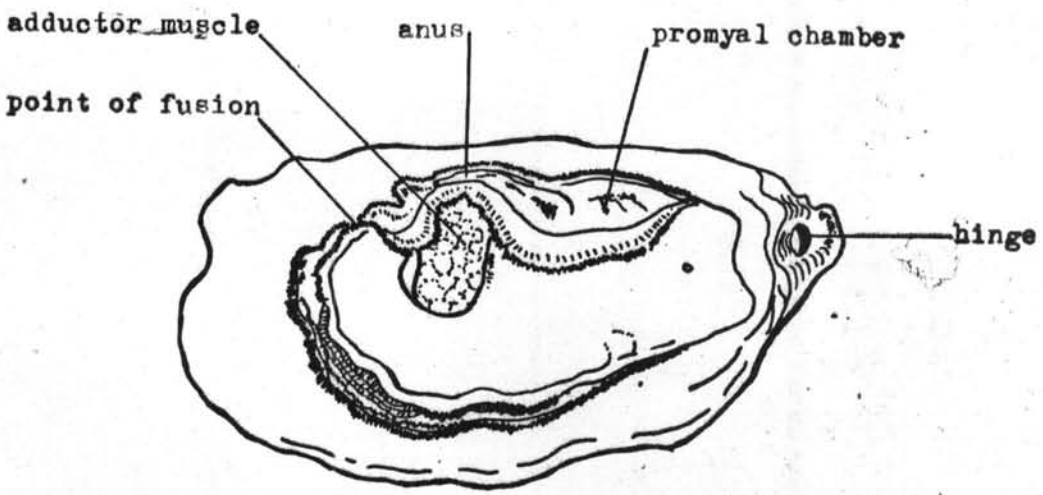
Development of oyster :

Several works had been done on the development of oyster including the embryological development and the larval development.

Brooks1(1880) reported the development of the American oyster (O. virginica). Horst (1882 , 1883) , Huxley (1883) , Danton (1917) and Erdmann (1935) studied the development of the European oyster (O. edulis). The anatomical structure of an oyster larva was also described. Hori (1926) , Fujita (1929) and Anemiya (1929) studied the embryological development of the common oyster of Japan. In 1929 Fujita reported the early development of the common Japanese oyster and in 1934 he also noted on the Japanese oyster larvae (C. gigas). Seno (1929) reported



A.



B

Figure I : The general anatomy of tropical oyster (Crassostrea lugubris).

A: The oyster with both right and left valves.

B: The oyster with right valve removed.

(drawn from fresh specimen)

the most important contribution to the knowledge of the developmemt of O. denselamellosa (larviparous species) which was summarized in figure 2. the publication of Stafford (1913) gave some information on the development of O. lurida and Hori (1933) also studied the development of the Olympia oyster (O. lurida). Icho and Oshima (1938) gave a report on the development of O. gigas which was oviparous species (fig.3). Anemiya (1931) showed that at a water temperature of 25° C the fertilized eggs of the Japanese oyster (C. gigas) could develop into blastula stage within 6 to 8 hours and the trochophore stage was reached within 24 hours after spawning (Table 1). Loosanoff and Davis (1963a , 1963b) reported the technique of artificial rearing of oyster larvae from fertilized eggs. This stimulated the future studies of larval physiology and the understanding of the development of oyster. Galtsoff (1964) reported clearly on the development of the American oyster (C. virginica) from fertilized egg until spat (fig.4-13). He also noted his observations on the time required for artificially fertilized eggs of the American oyster (C. virginica) to reach rotating blastula stage and trochophore stage. The time required were 6½ hours for reaching the rotating blastula stage and were 8 to 9 hours for reaching the trochophore stage at water temperature varying from 23° to 25° C after spawning (Table 2).

Environmental factors and the oyster :

1. Environmental factors and sexuality.

Anemiya (1963) reported that the removal of the gill tissue of Japanese oyster (C. gigas) may have indirectly influenced the development of male sex by reducing the rate of feeding and growth. Sex is ambiguous

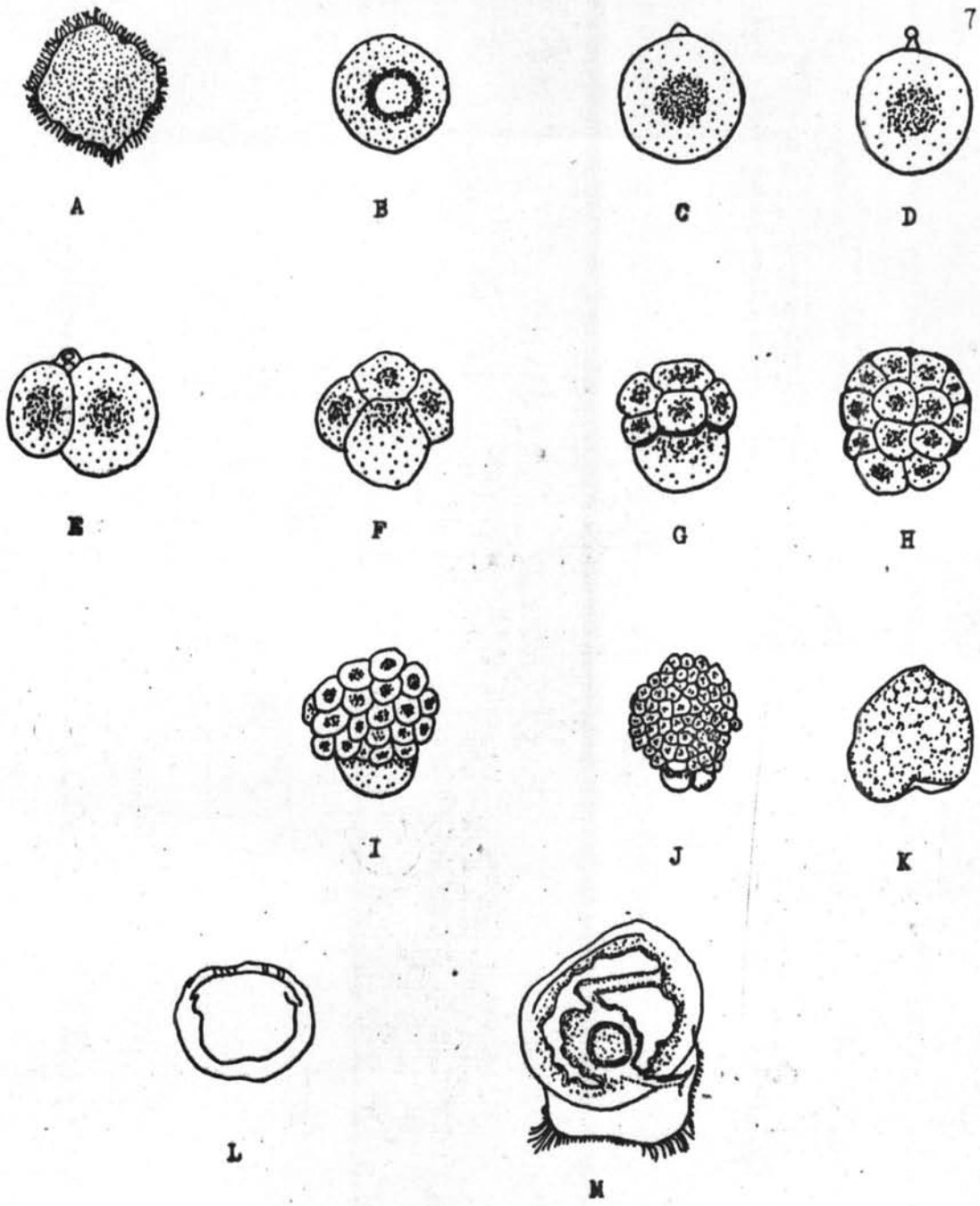


Figure 2 : Development of Ostrea denselamellosa (larviparous species). A. sperm ball, B. unsegmented egg, C. first polar body, D. second polar body, E. first cleavage, F. second cleavage, G. third cleavage, H. fourth cleavage, I. fifth cleavage, J. sixth cleavage, K & L. typical gastrula, M. straight hinge stage. (from Cahn, 1950).

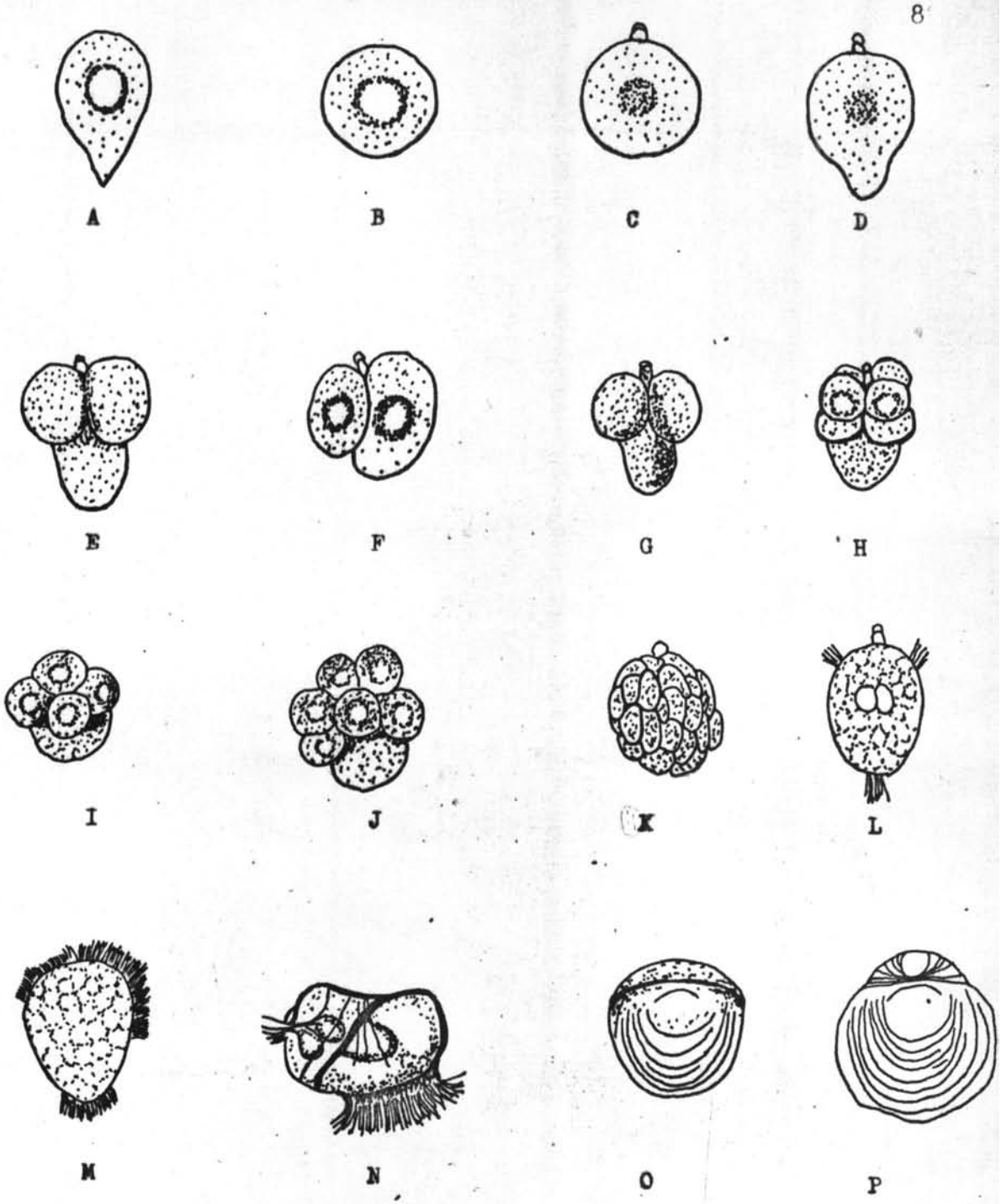


Figure 3 : Development of Ostrea gigas (oviparous species).
A. newly discharged egg,B. spherical,C. first polar body,D. first polar lobe,E. first cleavage,F. resorption of the first polar lobe,G. second polar lobe,H. second cleavage,I. resorption of the second polar lobe, J. third cleavage,K. morula stage,L. gastrula,M&N. trochophore stage, O. umbonem stage,P. shelled larvae. (redrawn from Cahn,1950).

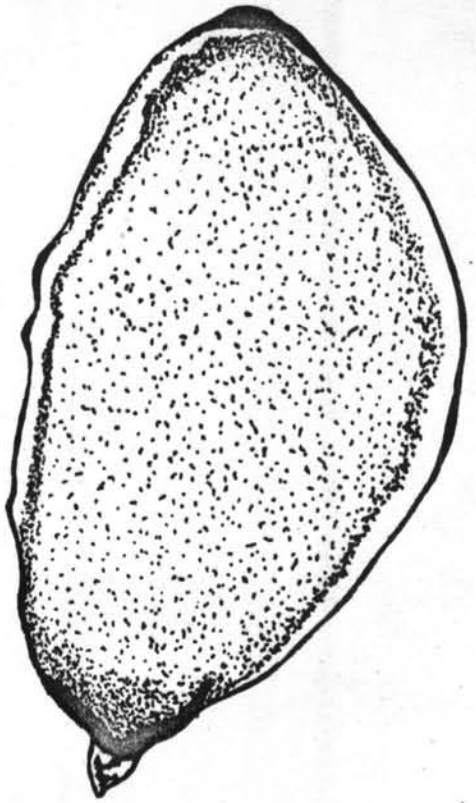


Figure 4 : Unfertilized egg of Crassostrea virginica.(redrawn from Galtsoff,1964).

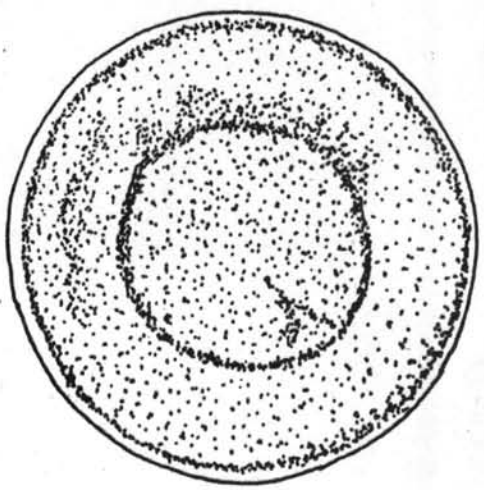


Figure 5 : Unfertilized egg of Crassostrea virginica in spherical form.(redrawn from Galtsoff,1964).

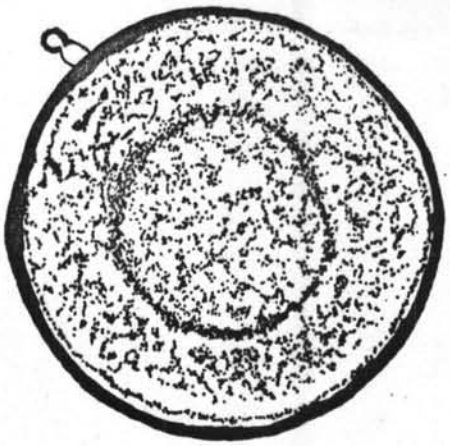


Figure 6 : Fertilized egg of Crassostrea virginica , a few minutes after the formation of the fertilization membrane.(redrawn from Galtsoff,1964).

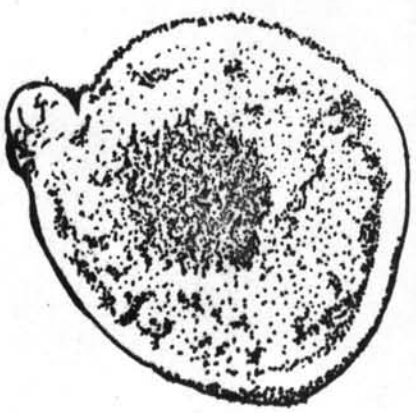


Figure 7 : Fertilized egg of Crassostrea virginica after the formation of two polar bodies.(redrawn from Galtsoff,1964).

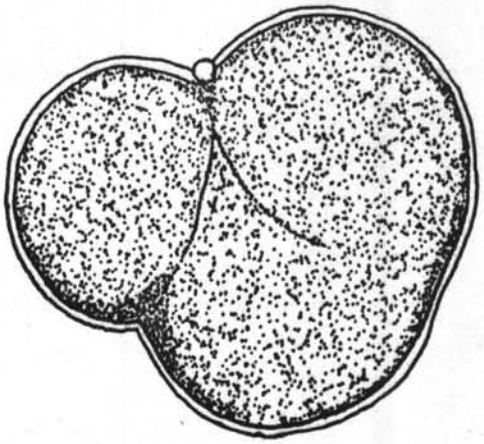


Figure 8 : First cleavage division of the egg of Crassostrea virginica 70 minutes after fertilization called"trefoil".(redrawn from - Galtsoff,1964).

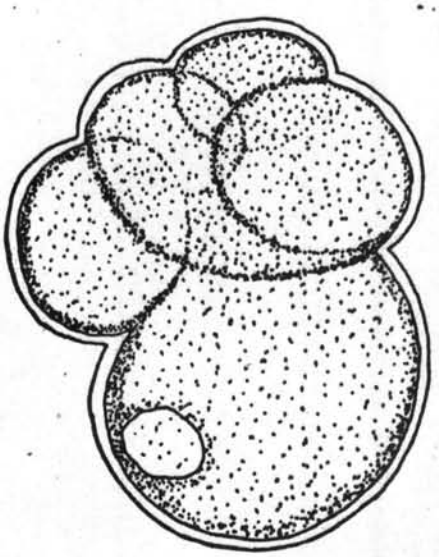


Figure 9 : Third division of fertilized egg of Crassostrea virginica. (redrawn from Galtsoff,1964).

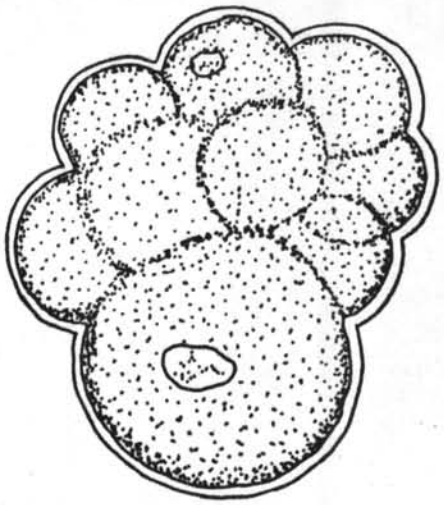


Figure IO : Fourth cleavage of egg of Crassostrea virginica and the formation of 8 micromeres(second quartet).(redrawn from Galtsoff I964).

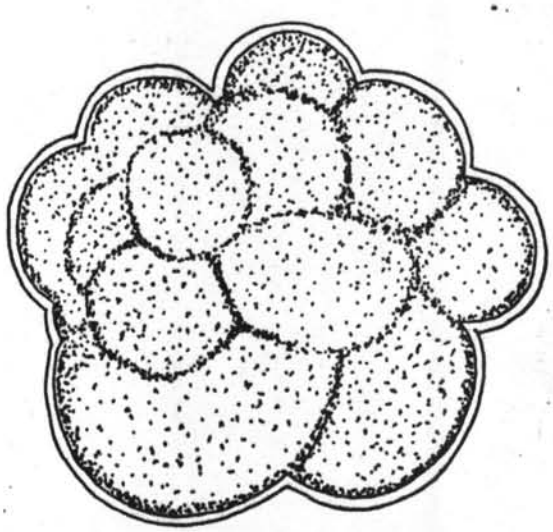


Figure II : Fifth cleavage of egg of Crassostrea virginica and the formation of the third quartet of micromeres(32 cell stage).(redrawn-Galtsoff,I964).

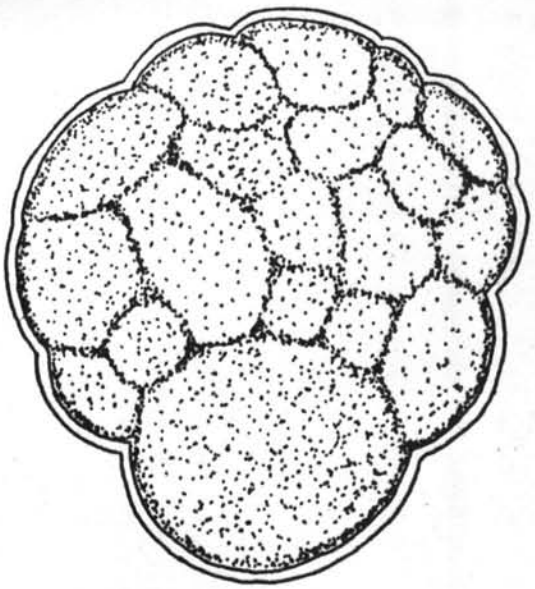


Figure I2 : Formation of sterroblastula in Crassostrea virginica egg(the sixth cleavage stage).(redrawn from Galtsoff,1964).

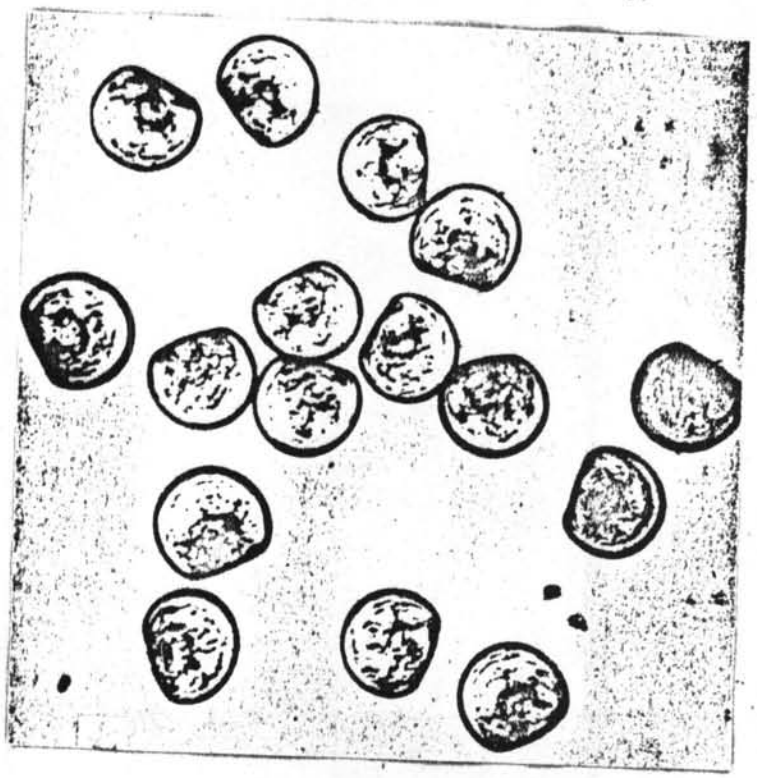


Figure I3 : The early straight-hinge stage larvae of Crassostrea-virginica.(from Galtsoff,1964).

in many bivalves species where hermaphroditism and alternation of sex are common (Galtsoff , 1969). The sex of Pacific oyster may change from year to year and the change take place during the winter (Quayle , 1969). There is general belief that environmental conditions have influence on the determination of sex in oyster. Quayle (1969) stated that whenever the food supply was low , there was a tendency for the female to change into the male. On the other hand , the male would become the female when the food supply was sufficient.

2. Environmental factors and gonad development.

Loosanoff and Engle (1942) noted that the most significant environmental factors for developmental of gonad were temperature , depth , salinity , available food and pollution of oyster bottom. Temperature seemed to be the most important environmental factors controlling the gonad development in oysters. Loosanoif and Davis (1963) showed that the ripe male and female oyster could be obtain e at all season of the year by controlling temperature. Galtsoff (1964) reported that in all species of Crassostrea , the volume of gonads depend on the geography situation. The greatest gonadal development was found in the population of oysters from the northern latitudes north of the Chesapeake Bay rather than in the south Atlantic and Gulf water. This involved the fact that reproductive season in the northern latitudes was rather short approximately 4 to 6 weeks while in the warmer water of the south , the reproductive season lasted for several months. Quayle (1969) reported that the developmental of gonad in Pacific oyster was conducted by the conversion of the winter store of

oysters spawn at rising temperature. This led to the concepte of "critical temperature" (Nelson , 1928a). He also noted that the critical temperature of 20°C was required for the spawning. But later observations by Nelson in 1931 and Loosanoff in 1939 failed to support this concept. Nelson (1931) showed that C. virginica could be induced to spawn at 19.1°C and Loosanoff (1939) reported some of oyster of Long Island sound spawned at 16.4°C. These evidences showed that spawning of the oysters was stimulated by a rapid rise of temperature , not by a specific "critical temperature". Physiological researches at Wood Hole laboratory indicated that temperature or chemical stimulation or both induced spawning in sexually gravid oyster.

In the ripe females of American oyster (C. virginica) , the spawning might be induced by increasing the water temperature from 18°C to 20°C , 22°C or 23°C. A more effective method could be done by rapidly raising the water temperature from 20°C to about 33°C or 34°C (Galtsoff , 1964). He also reported that thermal stimulation methods were more effective for spawning of the males American oyster than the females.

Loosanoff and Davis (1963) used thermal shock to induce ripe oyster to spawn and sometime the suspension of gonadal materials must be added to the water. In this manner the ripe male oyster spawned first and later the eggs were released.

Quayle (1969) indicated that the actual initiation of spawning might be brought out either by thermal shock or chemical stimulation or by combination of both. He stated that the salinity range for breeding

Stage of development	Time after spawning
First polar body extrudes.	50-60 minutes.
Second polar body extrudes.	1 hour , 10 minutes.
First cleavage of zygote occurs.	1 hour , 40 minutes.
Second cleavage of zygote occurs.	2 hours.
Blastula begins to move.	6 hours.
Blastula begins to swim.	8 hours.
Shell appears(trochophore stage).	24 hours.

Table 1 : The time required for development of fertilized eggs of Ostrea gigas to reach trochophore stage at water temperature of 25°C.(Adapted from Anemiya,1931)

Stage of development	Time after spawning
Fertilization membrane.	10 to 25 minutes.
First polar body.	25 to 52 minutes.
Second polar body.	40 to 65 minutes.
First cleavage.	45 minutes.
Second cleavage.	52 to 120 minutes.
Third cleavage.	55 to 195 minutes.
Morula stage.	135 minutes.
Rotating blastula.	6 hours , 30 minutes.
Trochophore stage.	8 to 9 hours.

Table 2 : The time required for development of fertilized eggs of C. virginica to reach trochophore stage at water temperature varying from 23°C to 25°C.(Adapted from Galtsoff,1964)

of Pacific oyster was in the range of 11-32 ppt. From his observation , the Pacific oyster could spawn at temperature as low as 60°F but chemical stimulation should be included.

4. Environmental factors and larval development.

The optimum temperature for the development of O. gigas was in the range of 23° - 25°C (Table 3). The percentage of successful development from egg to straight-hinge stage showed a tendency to fall sharply at temperatures above the optimum and fall slowly at temperature below the optimum (Seno et al , 1926). He also reported that the optimum salinity for the development of O. gigas was in the range of 23 - 28 ppt. The abnormal development occured at extremed salinities. The relationship between salinity and the development was shown in table 4.

Anemiya (1928) reported that the optimum salinity for the development of oyster larvae were not the same at different temperatures even for the same species. The salinity tolerance range was greater at lower temperatures than at higher temperatures.

Embody (1934) reported that at low temperatures , the development of fertilized eggs was slow , on the other hand , at high temperatures, the rate of the development increased.

Cahn (1950) indicated that water temperature had greatly influenced on the development of the egg of O. gigas.

Davis (1967) reported that the developmental rate did not directly proportional to the increasing temperature. He also believed that below or above the optimum developmental temperatures resulted in a greater probability of abnormal fry.

Factor	1	2	3	4	5	6
Mean temperature(°C)	27.7	25.6	23.2 *	20.8	18.6	16.3
Hours between fertilization and shell larvae.	25	23	25	34	53	83
Total number of shelled larvae	3,552	3,971	4,350	3,794	2,295	1,418
Number of abnormal shelled larvae	276	0	209	1,391	1,504	1,418
Percent eggs developed to shelled larvae	81.0	90.0	99.0	86.0	52.0	32.0
Percent abnormal individuals	7.8	0.0	4.8	35.6	65.8	100.0

* Optimum conditions of water temperature.

Table 3 : Effect of water temperature on development of young O. gigas. (Adapted from Seno , Hori and Kusakabe,1926)

Factor	1	2	3	4	5	6
Specific gravity(*)	1.0119	1.0239	1.0155	1.0175	1.0184	1.0208
Salinity(ppt)	16.60	19.22	21.33	23.93	25.10	28.24
Hours between fertilization and shelled larvae	34	28	28	25	22	22
Total number of shelled larvae	444	563	505	627	620	617
Number of abnormal shelled larvae	444	54	90	58	19	5
Percent eggs developed to shelled larvae	68	78	78	96	95	95
Percent abnormal individuals	100.0	9.6	17.8	9.3	3.7	0.3

* Compared at 15°C according to standard procedure.

Table 4 : Effect of salinity on development of O. gigas at water temperature of 25°C.(Adapted from Seno , Hori and Kusakabe,1926)

Tanaka (1975) stated that at temperatures below 15 C and above 30 C , normal development of the fertilized eggs of C. gigas could not occured.