CHAPTER 2

MATERIALS AND METHODS

Materials :

1. Acclimation tank (fig.14) is a 150 litres aquarium containing 35 ppt sea water. Filtration is carried out by sub-sand filter technique. Temperature is controlled at 25°C by air-conditioning.

2. Spawning tank (fig.15) is a 35 litres all glass aquarium containing 20 ppt sea water. Filtration is also carried out by sub-sand filter technique. Temperature is controlled at 30°C by a heater rod.

3. Fertilization tank (fig.16) is a 50 litres all glass aquarium used as water bath. This aquarium contains 3-litres beakers. Each beaker is contained with fertilized eggs. Salinity is maintained at 28 ppt. Temperature in water bath is controlled by using immersion circulator.

4. Plankton netting of two different mesh sizes i.e. , 70 μ and 37 μ (fig.17). The 70 μ is used to remove particles larger than the eggs. The 37 μ mesh is used to remove particles smaller than the eggs. This is done to prevent bacteria and protozoa from damaging the eggs.

5. Incubation tank (fig.18) is similar to the fertilization tank . But 8 of 1-litre flasks containing filtrated and steriled sea water are used instead of the three-litres beakers. They are used for incubation the fertilized eggs to D-shaped stage. Density of fertilized

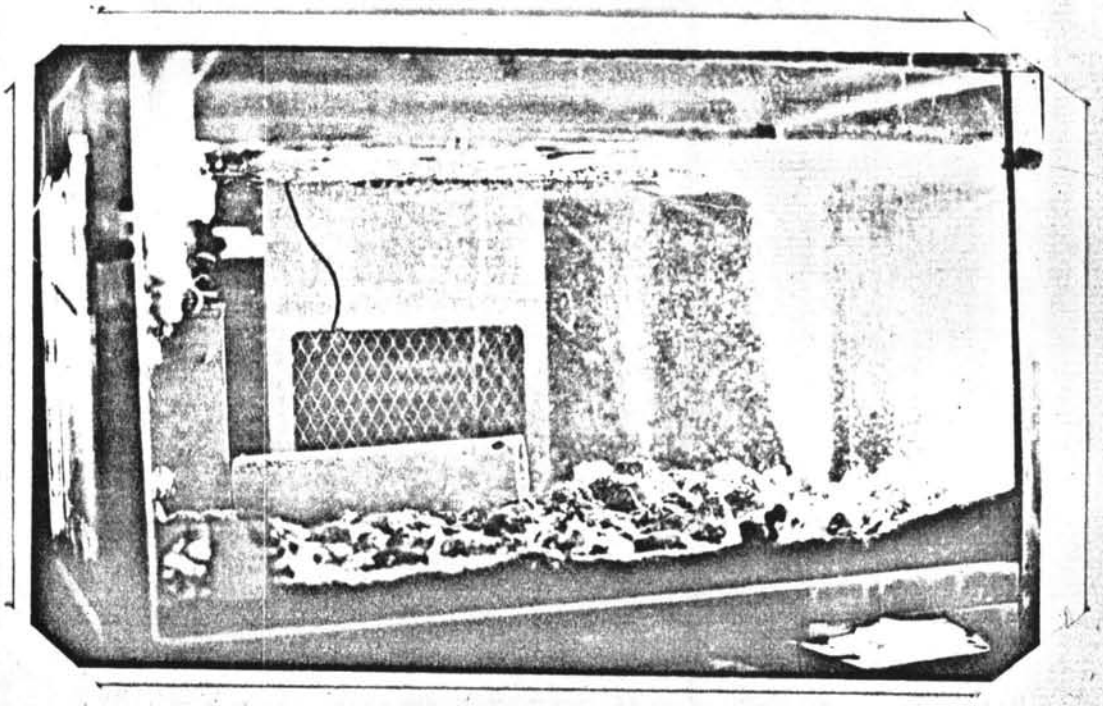

Figure I4 : Acclimation tank for acclimation of adult oyster .

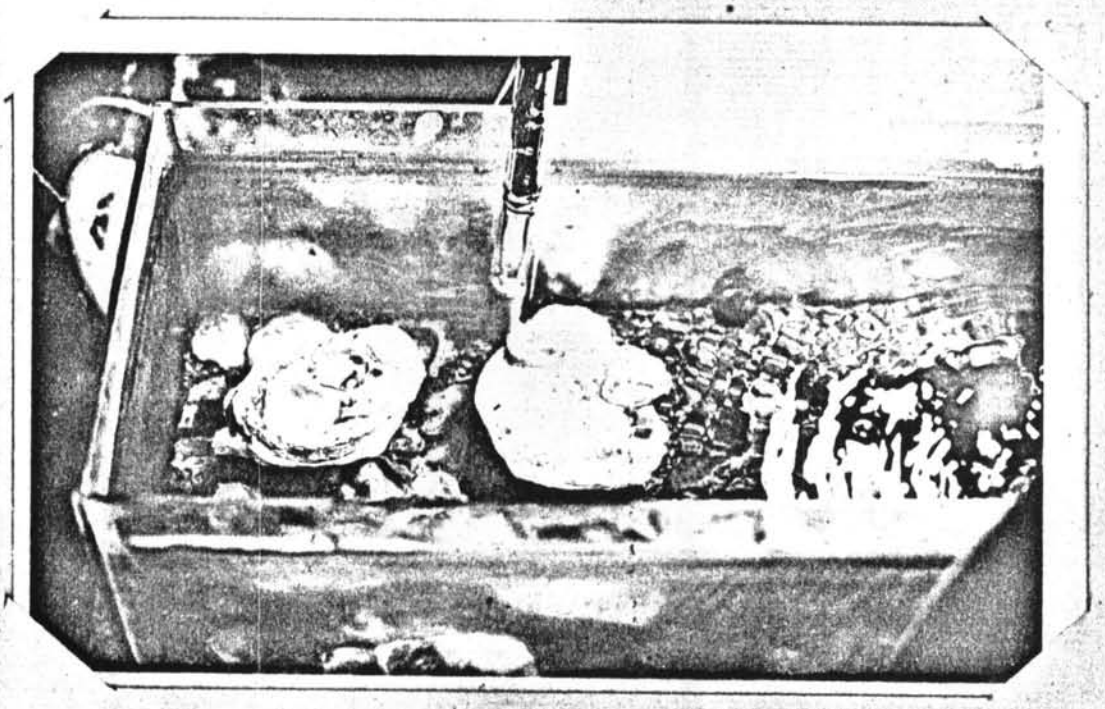

Figure I5 : Spawning tank

eggs per each flask is 20-30 individuals per ml.

6. Experimental tank (fig.19) is a 50 litres all glass aquarium used as water bath. Immersion circulator is used to control temperature. Larvae are placed in test tubes for critical thermal maximum (CTM) and lethal temperature 50 % (Lt_{50}) determination. One litre flasks are used for the study of larval development and the effect of temperature on larval development.

Methods :

This study is divided into four parts (fig.43).

1. Acclimation of adult oyster.

Adult oyster were collected from Ban-Dorn bay , Suradthanee province. The specimen were conditioned to the temperature of 25 °C and salinity at 35 ppt in the acclimation tank (fig.14) until they could be readily spawned in the laboratory. This acclimation period lasted four to six weeks. Usually after this acclimation period , the oysters became gravid males and females. During this period , sampling of oysters to determine various stages of sexual maturity were carried out once every three days by sacrification method (Galtsoff,1964).

2. Induced spawning by increased temperature and decreased salinity.

The gravid specimens were selected from an acclimation tank and transferred into a spawning tank (fig.15) at temperature of 30°C and salinity of 20 ppt. The gravid males would released the sperm first within 2-8 hours after acclimation. The combination of released sperm and increased temperature would then induced one or more females to

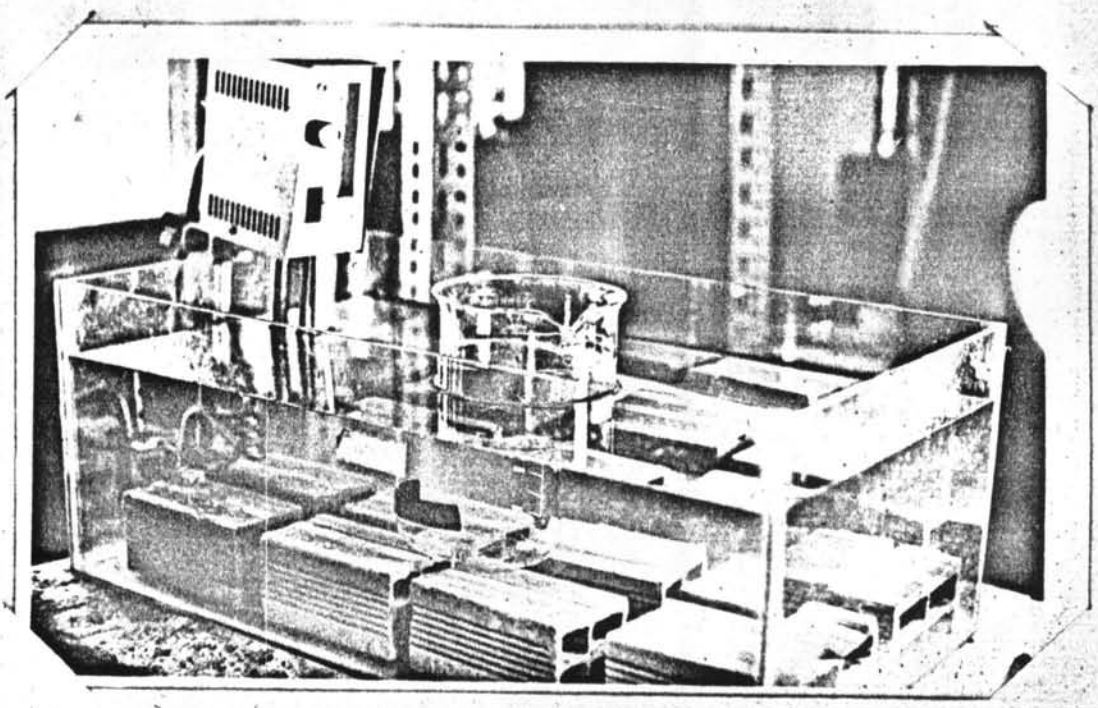

Figure I6 : Fertilization tank

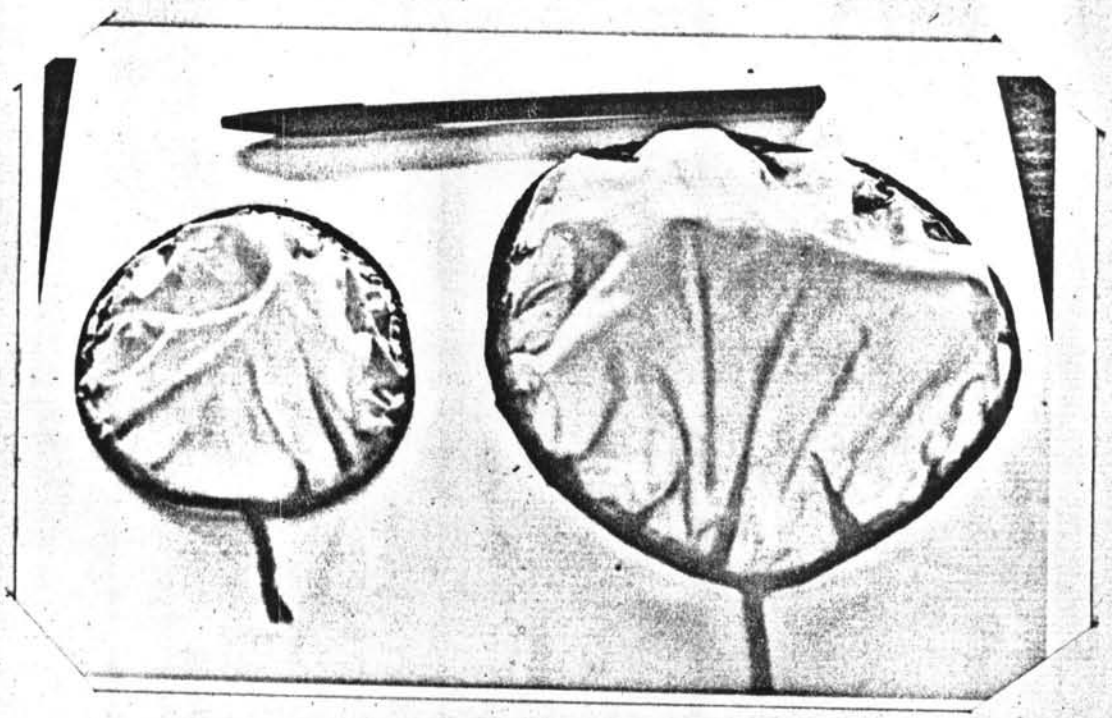

Figure I7 : Plankton netting of two different mesh sizes; 70 u and 37 u .

spawn. The spawn males and females were immediately transferred into the fertilization tank (fig.16). The spawning process will not be interrupted by the transfer process to the new condition in fertilization tank. Eggs and sperm were mixed in this tank at a controlled condition in accord with the objectives of the future experiment.

3. Cleaning of fertilized eggs.

Within 15 minutes after fertilization occurred , eggs were cleaned by egg washing and care process as indicated by Tanaka,1975,for preventing any outbreak of other unwanted organisms such as flagellates. The solution was passed through plankton net of two different mesh sizes. The large particles were first removed by the mesh of 70 μ. The small particles were then removed by the small mesh of 37 μ. Tissues and detritus are considered large particles while immature eggs , excess sperm and blood corpuscles are considered small particles. Fertilized eggs were then seperated and transferred into incubation tanks at density of 20,000-30,000 individuals per litre in steriled 28 ppt sea water.

4. Experimental procedure.

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

Fertilized eggs were transferred into an incubation tank containing steriled sea water at 28 ppt salinity. The developing eggs were sampling from incubation tank every 15 minutes for the first six hours and later at every six hours for two days. After this period , the fertilized eggs would hatched and developed into D-shaped larvae or

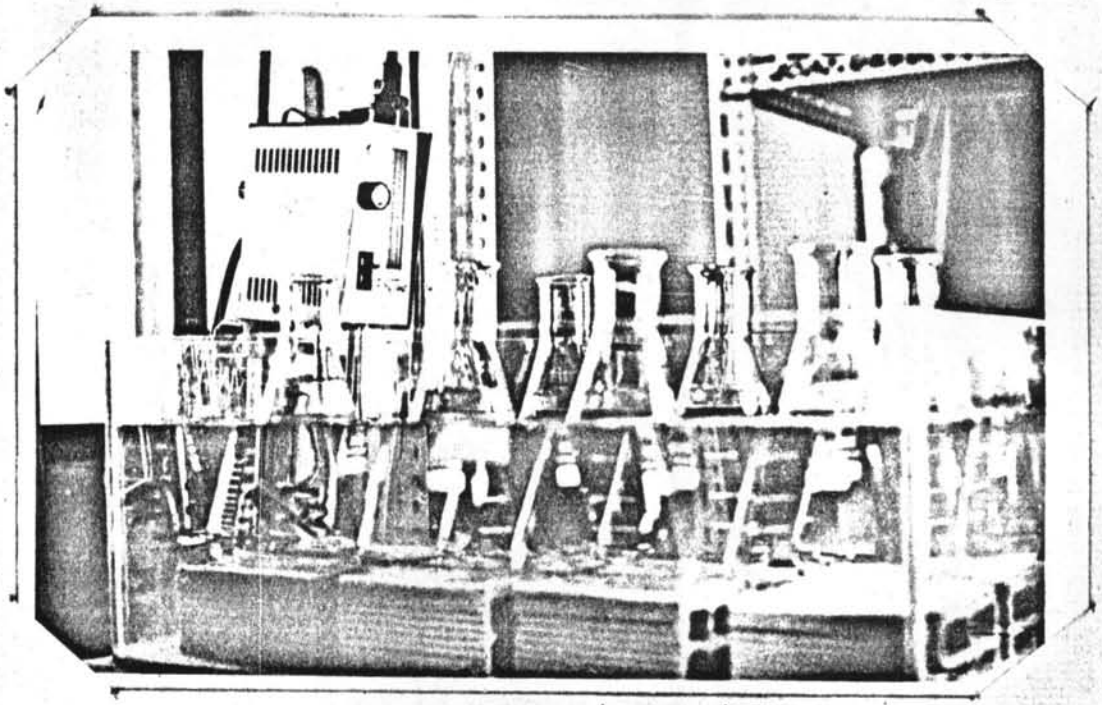

Figure I8 ; Incubation tank.

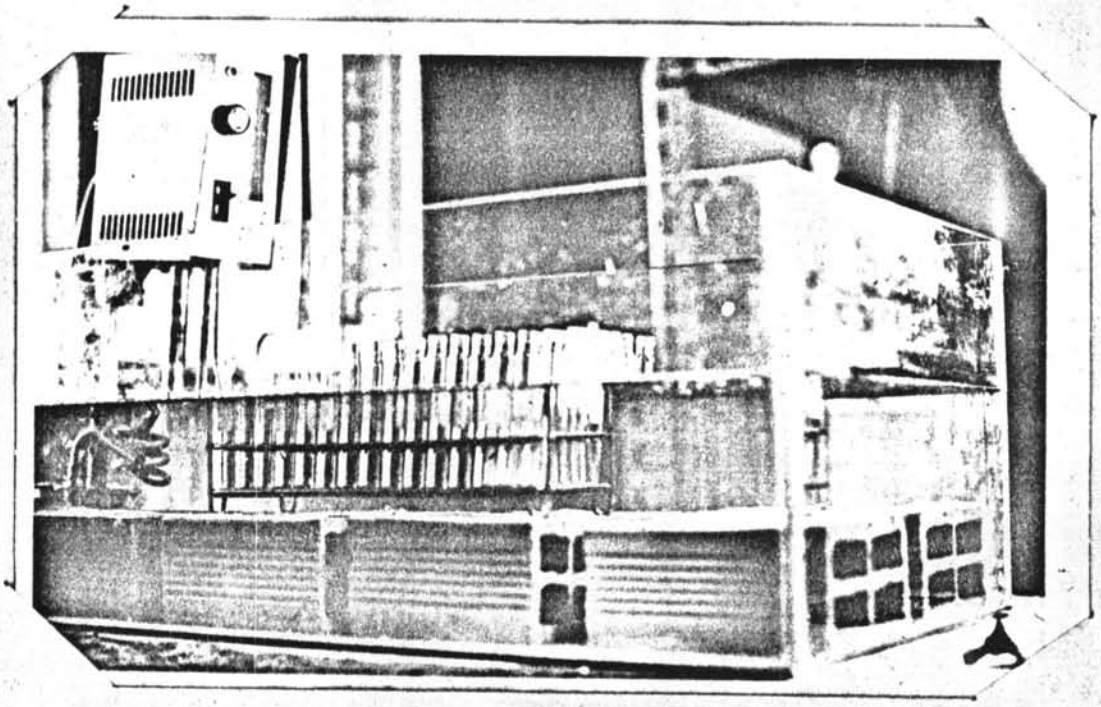

Figure I9 : Experimental tank.

straight hinge stage. Both fresh and preserved in 5% formalin samples were examined under a compound microscope. Various stages of development were photographed.

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

Fertilized eggs were transferred into three incubation tanks containing sea water 28 ppt salinity at the temperatures of 23.5°C , 28.0°C and 32.5°C. The fertilized egg samples were collected from each flask at one hour interval by 37 μ mesh size plankton net and preserved in 5% formalin. This was done until the eggs developed to blastula swimming stage. The number of each embryonic developmental stage in each sample were counted. Three replication were counted and the counted sample were averaged. The total eggs counted for each samples were 300 cells. The results were expressed as the percentage of embryonic developmental stage , percentage of abnormal development , percentage of undeveloped eggs and percentage of hatchability.

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

The cleaned and fertilized eggs were transferred into 6 of 1 litre flasks at a density of 20-30 individuals per ml. for each incubation tank. The temperatures of incubation tanks were set at 32°C , 34°C , 36°C , 38°C and 40°C. Temperature at 28°C was used as the control. After six hours period , fresh samples were collected at each tested temperature by using 37 μ mesh size plankton net and examined under a compound microscope to determine the temperature in which hatchability percentage

was equal to zero.

The experiment were repeated using tested temperatures ranging from 34°C to 37°C at 0.5°C intervals.

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

Fertilized eggs were reared in incubation tanks at three levels of temperatures i.e., 23.5°C , 28.0°C and 32.5°C. The density of eggs was about 20-30 cells per ml. After six hours , the eggs would completely hatch into blastula swimming stage. The surviving larvae in each cultures were collected by using 37 μ net and placed in 10 ml. test tubes in the experimental tank (fig.19). The temperature in the tank was gradually increased to higher degrees at the rate of 0.25°C per minute. Each test tube was sampling at 2 minutes interval or at 0.5°C increased temperature to determine the critical thermal maximum. The temperatures that ciliary movement ceased were recorded.

Three replications for each acclimation temperature were carried out.

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

Fertilized eggs were reared in incubation tanks at three levels of temperatures as follow i.e., 23.5°C , 28.0°C and 32.5°C. The density of eggs was 20-30 cells per ml. Within 48 hours the eggs would completely developed into D-shaped larval stage. The D-shaped larvae were put into

a number of 10 ml. test tubes and placed in each experimental tank. The critical thermal maximum of D-shaped larvae for each acclimation temperature was determined using the method indicated in objective 4. Lethal temperature of D-shaped larvae for each acclimation temperature was determined using results from CTM determination. In each experimental tank , the temperatures were lowered at 3°C interval. The last three temperatures used at which the larvae survived for twenty-four hours were noted. For every acclimation temperature , the number of dead larvae were sampling and counted at the first , the third and the sixth hours period. The larvae were sampling again at twelve hours and twenty four hours periods. From These datas , Lethal temperature 50% (Lt_{50}) could be determined.