CHAPTER 4

DISSCUSSION

Egg and sperm :

Egg ; The newly discharged egg of Crassostrea lugubris have pear shape and compress. The long axis of the eggs varies from 55 μ to 70 μ depending on thier shape ; the width varies from 30 μ to 45 μ.The average diameter of the spherical eggs vary from 50 μ to 55 μ.The eggs are surrounded by vitelline membrane.The cytoplasmic inclusion of the eggs is consisted of protein yolk,lipid yolk,mitochondria and submicroscopic granule (Cleland,1947,1951) . The size of mature eggs for each species were shown in Table 16.

Sperm ; Frazen 1956,Lenhossèk 1898 and Retzius 1904 noted that the spermatozoa of bivalve mollusks appeared to consist of an oval or round head with a pointed front,a middle piece at the lower end of the head and a long tail with a narrow "end piece". By the examining under light microscope with phase contrast oil immersion lens,the structure of live spermatozoan of C. virginica can be seen (fig. 41). The head varies from 1.9 μ to 3.6 μ in length and between 1.0 μ to 2.0 μ in width. The tail is from 27 μ to 39 μ long. The tails are usually slightly curved;specimens with straight tails are rarely found (Galtsoff, 1964). The spermatozoan of Ostrea gigas is 75 μ in total length;the round headhas a diameter of 7.3 μ (Cahn,1950).Tanaka(1975) reported

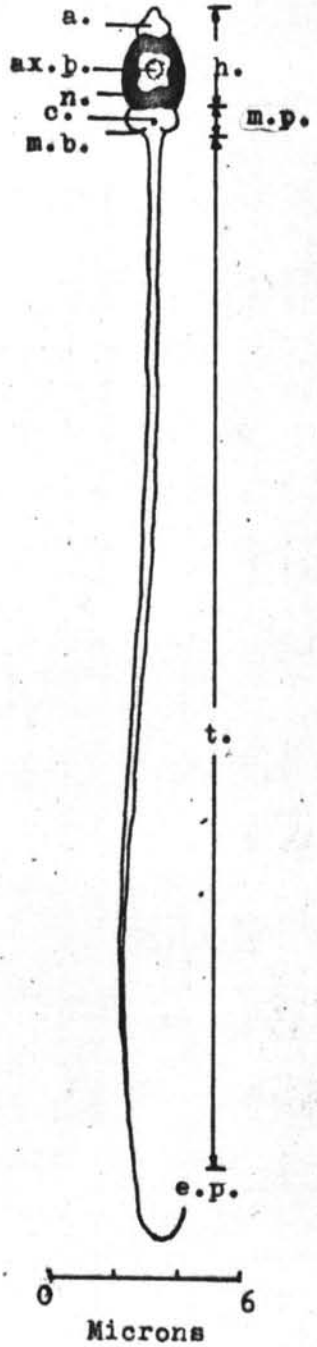

Figure 41 : Live spermatozoon of Crassostrea virginica redrawn from Galtsoff, 1964. a.-acrosome; ax.b.-axial body; c.-centriole; e.p.-end piece of tail; h.-head; m.p.-middle piece; mt.-mitochondrial bodies; n.-nucleus; t.-tail.

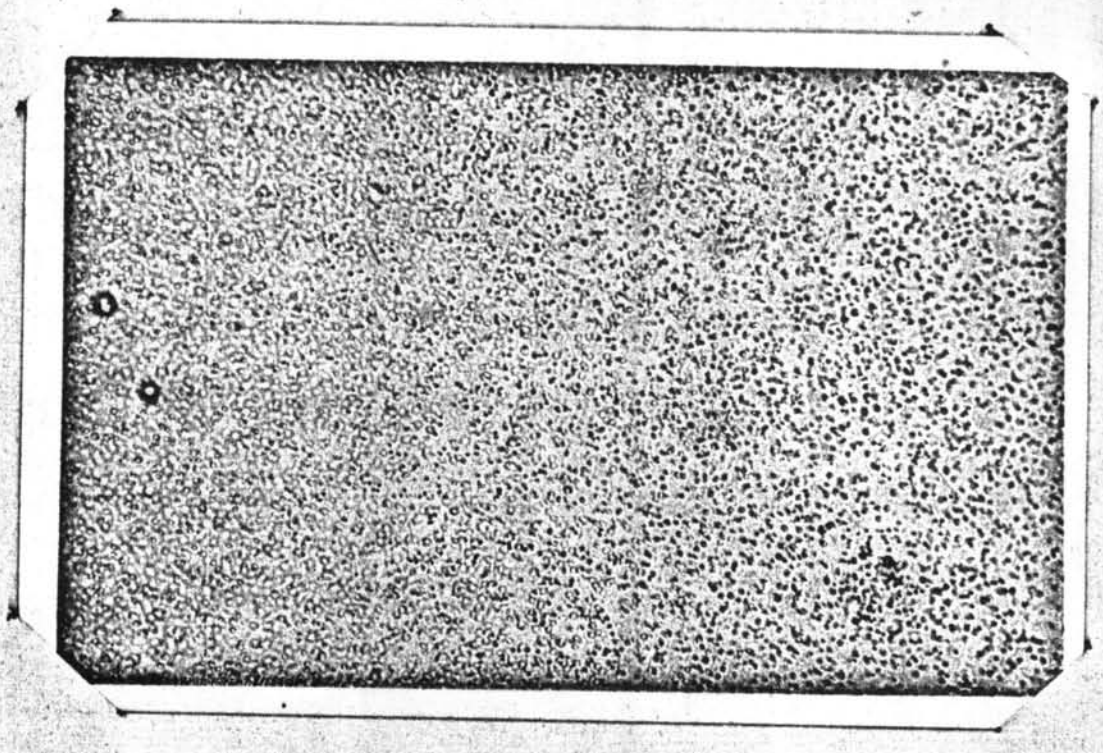

Figure 42 : The lived sperm of Crassostrea lugubris .

that the spermatozoan of C. gigas was 78 μ in total length and the head being 7.8 μ . The spermatozoan of C. lugubris is not clearly studied (fig. 42).

Aging of eggs and sperm :

The longevity of oyster eggs (C. lugubris) were tested in the Bureau's shellfish laboratory at Woodhole. This was determined by their ability to form fertilization membrane and undergo cleavage. The tests were made in water of 31 to 32 ppt salinity and 20.8°C to 21.4°C. During the first 4 hours of aging the percentage of cleaved eggs declined from 90 to 70. After 5 to 6 hours the percentage dropped to 60. The fertilizability decreased to about 20% in 10 hours, and only a few eggs cleaved normally after 12 and 24 hours of aging.

Cahn (1950) stated that the eggs can live and have fertilizability power in sea water for a brief peroid, the viability depending upon the water temperature and salinity. Fujita (1929) found the viability of such eggs of C. gigas to be greatest in salinity from 1.014 to 1.016 (specific gravity) at a temperature of 27°C. Under these conditions, he reported that the eggs still alived at least 15 hours after discharged.

From this investigation, during the first hour of age the ability of eggs to form fertilization membrane and undergo cleavage were not interferred and the eggs could be kept for 3 hours at 23°C and 28 ppt salinity. The eggs have lost their fertilizability at once if the distilled water is used in the eggs washing process. This may be due to the chemical substances covering on the vitelline membrane were washed

Species	Locality	Diameter(mm.)	Authority
O. gigas	Japan	0.051--0.058	Fujita 1929
O. gigas	Japan	0.046--0.053	Anemiya 1928
O. rivularis	Japan	0.049--0.050	Anemiya 1928
O. echinata	Japan	0.048-0.055	Anemiya 1928
O. nippona	Japan	0.047-0.055	Anemiya 1928
O. circumpicta*	Japan	0.102--0.130	Seki 1934
O. densellamellosa*	Japan	0.090-0.110	Anemiya 1928
		0.105	Seno 1929
O. virginica	United States	0.050	Fujita 1933
O. lurida*	United States	0.105	Hori 1933

Table 16: Sizes of mature egg of various oyster species (from Cahn,1950).

* Larviparous species

away by the distilled water.

Embryologists belived that the fertilizing power of spermatozoan is not decreased if the sperm are kept at low temperature about 10°C to 12°C. This is also true for the sperm of C. lugubris .

The fertilizing power is affected by dilution and increased temperature (Galtsoff,1964). At room temperature in a dilute Suspension, the spermatozoan of the American oyster lose their fertilizing ability within 4 to 5 hours. In a concentrated suspension,protected from evaporation and stored in a refrigerator at about 10°C the sperm remains its full fertilizing power for 24 hours and possibly longer. C. gigas in water density of 1.012 to 1.016 gm per cc and at a temperature of 29.0°C,the sperm can live as long as 15 hours union with an egg (Cahn, 1950).

From this investigation,the concentrated sperm suspension of C. lugubris is kept in a small vial with screw cap and stored in a refrigerator at 5°C. The sperm could live and still have their fertilizing power for three days and may be longer. The effect of cold storage on the fertilizability of eggs and sperm of C. lugubris is not clearly known.

Spawning mechanism :

In female oyster,the eggs are released through the normal exhalent channel and are discharged into suprabranchial chambers and again they are forced through the gill ostia into mantle chamber,from which they are ejacculated in a small cloud. The discharged of eggs is intermittent,with a rate of 5-10 times per minute by the adjustment of

the mantle edges and by virgorous action of the aductor muscle.

The male oyster,on the other hand,discharged its sperm in a thin steady stream. The sperm are also released into the suprabranchial chamber,but instead of passing through the gill oâtia against the current aâ do the eggs,they are carried out in the bormal exhalent stream of water.

The spawning mechanism of female and male oyster explained above was found with C. virginica (Galtsoff,1964),C.gigas (Quayle,1969) and was found with C. lugubris in this investigation.

Fertilization of egg :

In fertilization,eggs and sperm secrete substance called gamones which play an important role in fertilization. The secretion from an unfertilized eggs has significant effect on spermtozoa. These substances are also called "egg water" which cause the agglutination reaction in sperm suspension.

The result of this experiment confirm the important role of gamones in fertilization. When the unfertilized eggs were washed distilled water,the fertilization was inhibited. The inhibition may be due to the fact that gamones were washed away and the eggs would lost their ability to stimulate the action of sperm.

Several spermatozoa may attach themselves to an egg but the only one penetrate the cytoplasm. The others called supernumeraries which are cast off when cleavage begins (Galtsoff,1964). The polyspermy case can be occured if the sperm suspension is too concentrated and many spermatozoa enter one egg.These also occured in this experiment and

the normal developement of fertilized egg is interferred. In a normal condition,after a few second of the fertilization,the cytoplasm becomes so dense that the germinal vesicle is no longer visible and a thin transparent fertilization membrane is elevatrd above the vitelline membrane (fig. 22).

Cleavage :

The observation in this experiment showed that the cleavage process of C. lugubris is closely similar to yhe cleavage of C. gigas as reported by Anemiya (1929),Fujita (1929),Hori (1926) and Tanaka (1975) and C. virginica as reported by Galtsoff(1964). Brusca (1975) described the general patterns of invertebrate developement.

The first polar body stage is formed by the appearance of a small spot on the animal pole and the formation of the fertilization membrane (fig. 22).

The second polar body stage is formed by the protrution of the posterior portion or vegetal pole(fig.23).

During the first cleavage,the egg is meridianally divided into two unequal cells (fig.24,25). This stage is also called "trefoil" becaude at the beginning, the egg appears to consist of three cells. Galtsoff (1964) stated that the inequality of the blastomeres at this stage is due to yhe occurrance of the polar lobe.

At the second cleavage the egg is also meridianally divided and both unequal blastomeres are seperated into four cells. The biggest cell becomes the posterior portion (fig.26).

At the third division each cells is equatorial cut and seperated by the formation of the first quartet in dextrotropic direction(to the right) (fig.27). The micromeres are on the animal pole and the macromeres are on the vegetal pole.

At the 16-cells stage or the fourth cleavage stage the zygote is divided by the formation of the second quartet in levotropic direction (to the left). The micromeres overgrow the macromeres (fig.28).

At the fifth cleavage is being result in 32-cells stage by the formation of the third quartet in dextrotropic direction (fig.29).

The multicellular stage or sixth cleavage is reached by the formation of the fourth quartet in levotropic direction and resulting in 64-cells stage or sterroblastula (fig.30).

By the epibolic gastrulation of sterroblastula,the swimming blastula or gastrula stage is reached (fig.31). The larvae of this stage have developed the strong positive phototaxis character.

The cleavage patterns of C. lugubris described above are similar to yhe typical spiral cleavage as described by Brusca (1975).

Larval developement :

At the trochoohore stage the blastopore is closed, the mouth is formed above it and the gut is completed. Some cilia become visible, shellsecretion begins and the larvae begin to eat by the helping of their cilia (fig.32). At this stage, the beating of their cilia is disorganized.

The next stage is known as veliger.The veliger stage is defined by the apperance of "velum" (Yonge,1960). The velum of veliger is used

as the organ of locomotion (fig.33).

At the straight hinge stage or D-shaped larvae,the larvae are completely closed by their valves (fig.34). The size of the larvae varies from 65 μ to 75 μ in length. The larvae can close and open their valves by the helping of the adductor muscle. At this stage, the organs within the shell can be clearly seen through their thin and transparent shell.

Effect of temperatures on early developement of oyster larvae :

The result of this experiment showed that the time required for each stage of developement is shorter at the increase of acclimation temperature from 23.5°C to 32.5°C . This means that cell division and developement activities are directly depended on temperature. The temperature may control every steps of developement from fertilization of egg and sperm,the formation of the fertilization membrane,cleavage until reachinh of spat and so on (Table 5).

The average percentage of undeveloped eggs (Table 11) decreased from 21.89 ± 14.17 to 0.22 ± 0.44 at the increase of acclimation temperatures from 23.5°C to 32.5°C . This shows that at low temperature the activities of eggs and sperm are in low level and result in the high number of undeveloped eggs,on the other hand,at higher temperature the activities of eggs and sperm increase to higher level and result in the low number of undeveloped eggs.

The average percentage of hatchability (Table 11) increased from 57.18 ± 2.44 to 95.41 ± 1.03 at the increase of accclimation temperature from 23.5°C to 32.5°C.This shows that the Higher temperature,the better

results in percentage of hatchability are.

Abnormal developement may be due to three factors i.e.,high density,intrinsic factor (genetics) and polyspermy case. In every experiment the density was controlled at 20-30 cells per ml. Therefore every treatment would have the same condition in density. Because of each treatment,the only one couple of gravid male and female was used, the intrinsic or the biological error could be neglected. Then became the last factor. At high temperature,the eggs and sperm are more active. The probability of an egg to be successfully penetrated by more than one sperm could occur.Therefore the polyspermy case might be the important factor for causing the abnormal developement at higher temperature.

The highest temperature that prevent hatchability :

The highest temperature for thezygote of C. gigas to develope to the shell larval stage was 30°C (Cahn,1950 and Tanaka,1975). For C. lugubris,the highest temperature that completely prevented hatchability was 35.5°C. From this experiment,the percentage of hatchability increased with the increase of tested temperatures. At 35.0°C the zygote could normally develope to straight hinge stage within 24 hours. But at 35.5°C,the fertilization and fertilization membrane were inhibited and the eggs failed to have a successful developement.

From this investigation,no fertilization could occur at 35.5°C. Therefore,the temperature at 35.5°C would lower the activity of sperm to penetrate the eggs or the stimulant abilities of the eggs are inhibited.

The reason why the eggs could not have a successful developement could not explain.

The critical thermal maximum v.s. acclimation temperature :

Since the critical thermal maximum (CTM) of C. lugubris has not been studied before,various methods for finding CTM were designed and the suitable one was explained in the methods and materials part of this thesis. The CTM was recorded when the larvae stopped moving their cilia.There are two objectives for this experiment.First the difference of CTM between blastula swimming stage and D-shaped larvae,and second the relationship between CTM and acclimation temperature of both blastula swimming stage and D-shaped larvae.

In the first objective,The obtained data showed that there were some differences of the CTM between blastula swimming stage and D-shaped larvae.The CTM of D-shaped larvae seem to be less than the CTM of blastula swimming.This circumstance might be due to the age factor. It is generally accepeted among the ecologists that larval stage is more tolerable to the environmental conditions than the advanced form. The result of this experiment was not cleared enough. If it is possible, the CTM of the difference stages of the developement should be studied further.

For the second objective,There are three levels of acclimation temperatures i.e.,23.5°C,28.0°Cand 32.5°C. The acclimation temperatures of blastula swimming stage had no influence on the CTM. This showed that the blastula swimmimg stage had only one CTM at 48.5°C and this value was maximum for this stage. The D-shaped larvae show a sligthly

relationship between CTM and the acclimated temperatures.

In fact, the acclimation temperatures usually shows the positive relation ship with CTM in other organisms (Jones,1969). Nevertheless, the non-relationship between the CTM amd acclimation temperature of the blastula swimming stage can be explained. The blastula swimming stage is found to exist only 6 hours,therefore,the time is too short for a complete acclimation to each temperature. According to this,the relationship between the acclimation temperatures and CTM can not be observed in this stage of developement.

The median tolerance limit (Lt_{50}) of D-shaped larvae and the rearing temperature or acclimation temperature :

In fish,when the acclimation temperatures incteased,the upper and lower lethal temperatures also increased,Brett(1944,1952) , Fry et al.(1942,1946). This temperature relation is also in accord with the finding of this experiment. When the acclimatiom temperature was increased the 12 hr-Lt_{50} and 24 hr-Lt_{50} of the D-shaped larvae also increased. The 12 hr-Lt_{50} of the d-shaped larvae acclimated at 23.5°C, 28.0°C and 32.5°C were 34.95°C,37.95°C and 37.95°C respectively. The 24 hr-Lt_{50} of the D-shaped larvae acclimated at 23.5°C,28.0°C and 32.5°C were 34.5°C,37.45°C and 37.6°C repectively. The obtained data showed that both 12 hr-Lt_{50} and 24 hr-Lt_{50} of the D-shaped larvae increased with the increase of the acclimation temperature.