Environmental Factors Affecting the Early Development of the Sea Urchin (*Tripneustes gratilla*)

Wilfred J. Wilson[1] and Kwo-Yih Yeh

INTRODUCTION

The development of the sea urchin has been the subject of numerous investigations by developmental biologists (Endo, 1952; Runnström, 1952, 1966; Afzelius, 1956; Mitchison, 1956; Rothschild, 1956; Wolpert and Mercer, 1961; Gustafson and Wolpert, 1967; Hinegardner, 1967; Austin,1968).The ease with which the gametes can be collected and utilized as teaching and research tools has also attracted the interest of investigators in other fields of biology, such as cellular physiology and genetics. The present study was thus undertaken in order to present a simple procedure for handling and rearing sea urchin embryos as well as some experimental data on environmental factors affecting the early development of the sea urchin *Tripneustes gratilla* in Taiwan.

Although recent studies have indicated that sea urchins can be raised from fertilizaion through sexually mature adulthood in the laboratory (Hinegardner, 1969; Strathmann, 1971), it is impossible to grow individuals beyond the pluteus stage without resorting to special procedures. Consequently the scope of our study is limited to the induction of gamete shedding, fertilization, cleavage, gastrulation, and development through the pluteus stage. The effects of temperature were found to be particularly critical not only in limiting developmental capacity, but also in affecting the duration of each developmental stage.

1 Visiting Professor, National Taiwan University, 1970-1971; Professor of Zoology, San Diego State College, California, U. S. A.

MATERIALS AND METHODS

Tripneustes gratilla adults were collected at keelung during the month of October, 1970, and were maintained in the laboratory in aerated sea water at room temperature. The following procedure is a standard technique used to collect gametes.

Since the sexes usually occur in a ratio of 50:50 and are indistinguishable externally, a sufficient number of individuals must be available. The animals are induced to shed eggs or sperm by injecting them with a 5% KCl solution by means of a hypodermic needle through the membrane which surrounds the mouth region. Approximately 2-4 ml. is sufficient for a 10cm.×6cm. adult animal. After one or two minutes, eggs or sperm flow through the small genital pores on the aboral surface. Once animals are injected they cannot be used again for several weeks. One female and one male sea urchin provide several ml. of gametes which are sufficient for many students interested in observing fertilization and early developmental larval stages.

The gametes are collected by placing the aboral surface of each injected urchin over a small beaker containing sea water. The eggs are identified by their orange to yellow color; the sperm are white in color. Sperm can retain their abilities to fertilize for at least one hour if stored at about 10° C. Eggs stored under similar conditions can be fertilized several hours after ovulation.

A few drops of sea water with eggs are removed and placed in a large dish free of chemical contaminants and containing clean sea water. One or two small drops of sperm are then added to the dish. Care must be taken to avoid overinseminating the eggs because of the danger of polyspermy. If larval stages are to be grown, it is good technique to remove excess sperm to prevent bacterial buildup after fertilization has been completed.

In order to test the effects of temperature on early development, three incubators were set at 15° C., 22° C., and 29° C., respectively. Containers of sea water were placed in each incubator to allow the sea water to attain the proper temperature before the experiment was begun. After the eggs were fertilized at room temperature, clean pipettes were used to transfer a quantity of zygotes to each of the containers in the three constant-temperature incubators. Samples were periodically taken from each container and examined under a phase contrast microscope.

RESULTS

General Observations. The unfertilized egg shows a small, poorly defined nucleus but a well developed vitelline membrane just peripheral to the plasma membrane. The cytoplasm is yolky, with a cortical region which contains fine granules (Fig. 1). A ferilization membrane forms within a few minutes after the sperm are added to the eggs, Water enters the hyaline space between the fertilization and plasma membranes, although this space remains relatively narrow in *Tripneustes gratilla* (Fig. 2).

The early mitotic divisions proceed at regular intervals to produce 2, 4, 8, and 16-cell stages, and eventually result in a ciliated, hollow blastula (Figs. 3 through 7). Figure 7 shows the ciliated blastula hatching from the fertilization membrane. Shorly after hatching a group of mesenchyme cells in the vegetal end of the blastula begin invagination of the embryo. This invagination will become the primitive gut (archenteron). The blastopore is the opening into this primitive gut and will eventually become the anus of the feeding pluteus larva.

The gastrulation process transforms the blastula into a gastrula (Figs. 8, 9). The shape of the embryo then becomes pyramidal, and the embryo is called a prism larva (Fig. 10). At this time coelomic cavities are formed by budding from the primitive gut. Calcium carbonate spicules from the mesenchyme cells lengthen iuto projections which grow from the base of the prism larva. At this time a mouth forms in the embryo and the digestive tract becomes complete. The larva with feeding ability is now called a pluteus larva (Figs. 11, 12).

Temperature Effects When the eggs are fertilized at room temperature and allowed to develop at 22° C., development is normal and proceeds at a regular rate. The approximate time at which each stage occurs is listed for this temperature in Table 1. These times are roughly similar to those observed at room temperature (25° C). However, development of the 2-cell, 4-cell, and 8-cell stages at room temperature is slightly accelerated over that at 22° C., although more variation is seen at room temperature.

At 29°C. development is normal, but accelerated (Table 1). The first division occurs as early as 55 minutes after fertilization, and early gastrulae can be seen 18 hours after fertilization. The prism stage occurs at approximately 26 hours, and early pluteus larvae are seen at 43 hours.

Development is severely retarded and eventually becomes abnormal at the low temperature of 15°C. (Table 1). The first division does not occur until nearly five hours after fertilization. Well formed blastulae capable of hatching are found in cultures 43 hours after fertilization. At this low temperature, development is arrested at the late blastula stage, and gastrulation does not occur.

TABLE 1

Developmental Rates of *Tripneustes gratilla* Embryos

Developmental Stage	Hours after Fertilization 15°C.	22°C.	29°C.
2-cell	5:00	1:10	0:55
4-cell	7:00	2:25	1:35
8-cell	9:00	3:30	2:15
16-cell	12:00	4:30	3:00
Early blastula	43:00	10:00	8:00
Early gastrula	–	25:00	18:00
Prism	–	44:00	26:00
Pluteus	–	60:00	43:00

DISCUSSION

The results indicate that temperature is one of the most critical parameters with respect to environmental effects on early developmental stages of the sea urchin. At a high temperature (29°C.) the duration of these stages is decreased, suggesting a relatively high metabolic rate. At a low temperature (15°C.) the duration of each stage is prolonged. Of particular interest is the failure to obtain development beyond the early blastula stage at the low temperature. There is evidence that the late blastula and early gastrula stages involve the switching on of the embryonic genome (Ebert, 1970). Low temperature thus appears to prevent this crucial event. However, the embryo does undergo "hatching" although invagination and spicule formation do not occur.

Since temperature shows drastic effects on the developing embryos, care must be taken to avoid temperature changes if the cultures are being maintained for long periods of time. Further studies are also needed to determine the upper and lower limits of temperature at which normal development of *Tripneustes gratilla* can occur.

Although the early stages of sea urchin embryos are relatively easy to rear in the laboratory, certain additional precautions must be taken. Should the embryos fail to develop, various factors may be responsible. One factor is the condition of the culture containers. They must not have been contaminated (even in previous use) with such substances as fixatives, stains, heavy metals, etc. During long term experiments, the dishes should be covered to reduce salinity changes. All glassware should be rinsed with sea water followed by two distilled water rinses. Detergents can inhibit fertilization and develpoment. Volatile toxic substances should be kept out of laboratories and incubators, since many chemicals can saturate a confined air space.

One of the most common hazards in raising these embryos is that of overcrowding. The culture density should be thin, covering no more than 1/3 to 1/4 of the bottom area of a large flattened, culture container. The embryos should be covered with as deep a layer of sea water as is practical. This enables O_2 to diffuse to the developing embryos, and contaminants also will build up more slowly.

Overinsemination is another source of mortality, since the excess sperm usually die and thus encourage bacterial growth. If the sperm density is too great, polyspermy may occur and result in embryo mortality. Culture water can be changed by using a hand centrifuge, should there be any signs of bacterial or protozoal contamination.

SUMMARY

Tripneustes gratilla adults were collected from intertidal areas of Taiwan, and embryos were raised in the laboratory from fertilization through the pluteus larval stage. The influence of various environmental factors was determined, particularly the effects of temperature. Results indicate that at a high temperature of 29°C. development is normal but accelerated, whereas at a low temperature of 15°C. development is retarded and arrested at the blastula stage. Simple procedures are presented for handling embryos, as well as for their use as teaching materials.

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中 文 摘 要

環境因素對海胆 (*Tripneustes gratilla*) 早期發育的影響

魏 彬 爾　　葉 國 義

台灣沿海採得的海胆 (*Tripneustes gratilla*) 用簡單的方法 誘導其排 出卵和精子，觀察其受精作用及由受精卵到幼虫期 (Pluteus stage) 的發生情形。溫度對發生過程具有相當大的影響，在攝氏二十九度之高溫下，加速了發生而且其早期發生相當正常，但在攝氏十五度下，發生緩慢而且停止於囊胚期 (Blastula stage)，由於本實驗材料之取得和操作相當容易，故為教學上之良好材料。

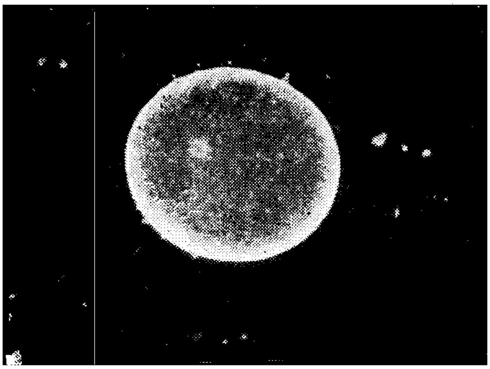

Fig. 1. Unfertilized egg

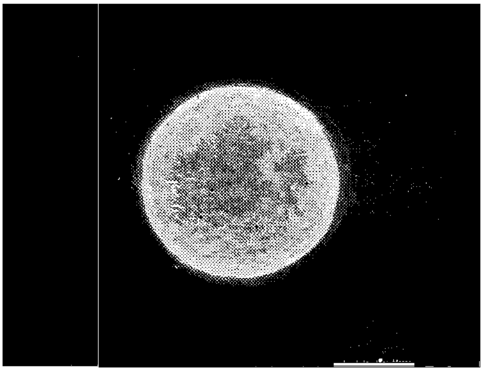

Fig. 2. Fertilized egg

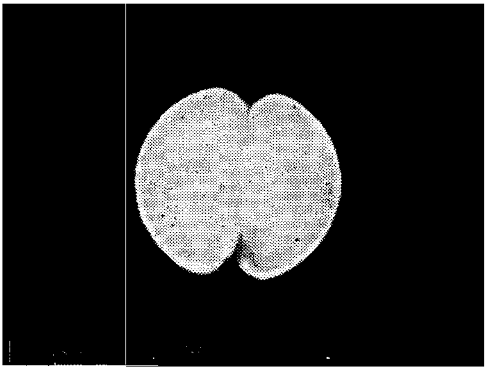

Fig. 3. 2-cell stage

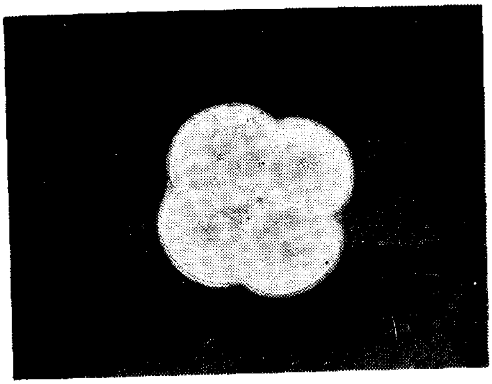

Fig. 4. 4-cell stage

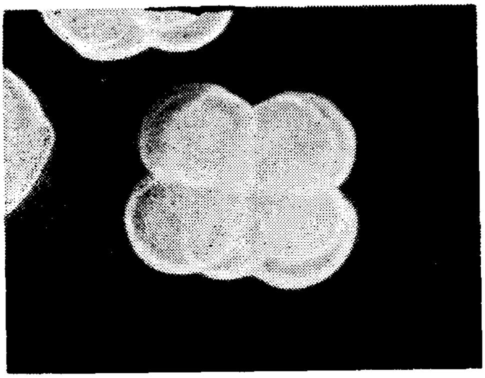

Fig. 5. 8-cell stage

Fig. 6. 16-cell stage

Photomicrographs of stages in the development of the sea urchin, *Tripneustes gratilla.*

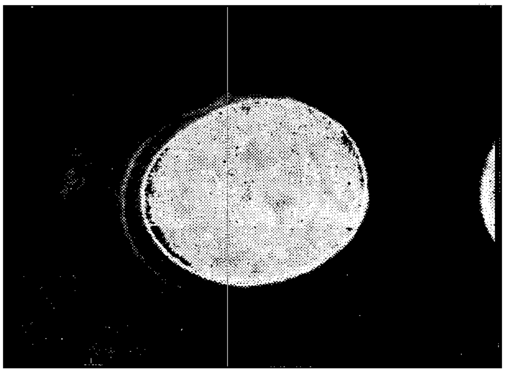

Fig. 7. Hatching blastula

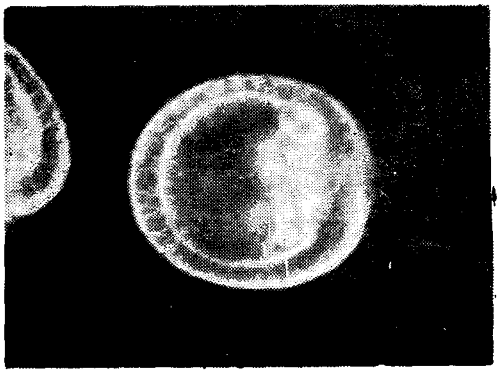

Fig. 8. Early gastrula

Fig. 9. Late gastrula

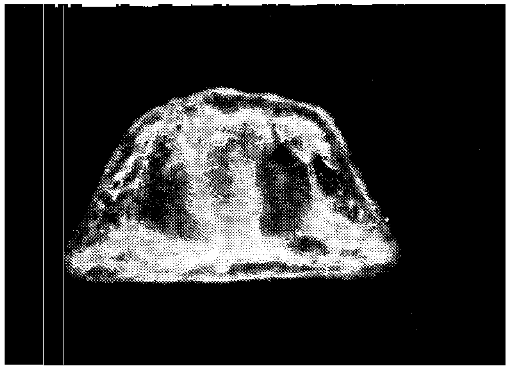

Fig. 10. Prism larva

Fig. 11. Early pluteus larva

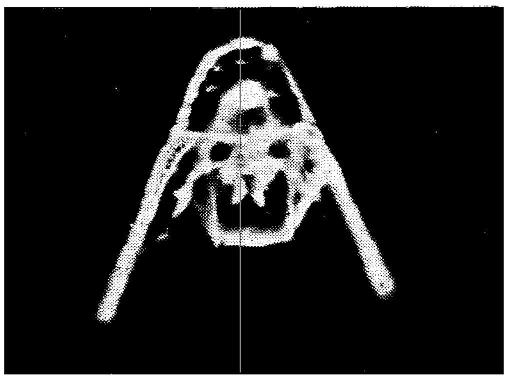

Fig. 12. Late pluteus larva

Photomicrographs of stages in the development of the sea urchin, *Tripneustes gratilla*.