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THE CHANGES IN SEA WATER AND THEIR INFLUENCES UPON MARINE ANIMALS

I. Fertilization and Development of Sea-Urchin Eggs in Artificial Sea Water

BY

TEIJIRO HAYASHI

海水の變化と其の海産動物に及ぼす影響に就て

一、人工海水中に於ける「ウニ」の受精及び發生

林　禎二郎

Apart from the artificial factors, for instance the changes of osmotic pressure, the changes of hydrogenion concentration etc. which check the fertilization and development of the eggs of water inhabitants, there are some natural factors yet unknown, which affect the eggs unfavorably. The fact that in nature even the ripened egg sometimes never starts development by ordinary semination, has long attracted the attention of investigators. The internal factors, for example, the unripened or pathological germ cells which conditions the development of an egg, are not considered in this paper.

In 1922 OSHIMA affirmed that by torturing the Arbacia for a long period it is possible to get the animal to secrete some substances (called dermal secretion by him) which hinder the fertilization of the eggs. The result is of importance inasmuch as it deals with the general phenomenon of the problems. As a matter of fact after a heavy storm it is very difficult to do any experimental work in artificial fertilization until the sea water regains its normal condition.

In order to determine whether any changes, either physical or chemical,

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arising naturally in sea water, affect the animals, the writer has carried out the following experiments in fertilization and development of the sea-urchin in artificial sea water.

The problems might become simple if the artificial solution could be proved to act on the organisms normally as in natural sea water.

The eggs employed as material were from *Strongylocentrotus intermedius* and *S. nudus* which are abundant in the water by the Oshoro Marine Biological Station. In Hokkaido the spawning of both species occurs from the end of August and lasts about a month.

The eggs were obtained by removing the oral parts of the animals and were stripped off into natural sea water in finger bowls. Then one drop of the water containing the germcells was placed in a hard glass vessel which contained 20 cc. of artificial water.

The artificial sea water used in the experiment was prepared after KANDA (1918), which is a modification of VAN'T HOFF's solution; namely

m/2 NaCl	100.0 cc.
m/2 KCl	2.2 cc.
m/2 $CaCl_2$	1.5 cc.
m/2 $MgCl_2$	7.8 cc.
m/2 $MgSo_4$	3.8 cc.

The alkalinity of the solution was regulated by adding m. $NaHCO_3$. All the observations were carried out under a room temperature of 19°C–20°C.

Experiment I

In this case the materials, *S. intermedius* and *S. nudus*, were collected from the sea where the fertilization and the development were going on normally.

Two vessels, one of artificial sea water and the other of natural sea water, containing the ripened eggs were inseminated with the normal germ cells. The process of fertilization and development was observed and the results were arranged separately according to each species in Table I and II. The figures given in the tables show the total number of eggs observed in five different fields under the microscope.

Table I

Time		Unfertilized Eggs	Fertilized Egg									
			1 c.	2 c.	4 c.	8 c.	16 c.	32 c.	Moru.	Blast.	Gast.	T.N.
20 min.	Arti.	1	47	—	—	—	—	—	—	—	—	47
	Cont.	1	51	—	—	—	—	—	—	—	—	51
2 hrs.	Arti.	0	3	42	—	—	—	—	—	—	—	45
	Cont.	1	2	50	—	—	—	—	—	—	—	52
4 hrs.	Arti.	2	—	1	18	25	20	1	—	—	—	65
	Cont.	2	—	—	—	—	73	2	—	—	—	75
10 hrs.	Arti.	6	—	—	—	—	6	20	—	15	1	42
	Cont.	7	—	—	—	—	—	—	—	16	5	21
14 hrs.	Arti.	2	—	—	—	—	—	1	—	15	15	31
	Cont.	0	—	—	—	—	—	—	—	2	23	25

As is seen in the table, in the first twenty minutes after insemination, 47 out of 48 eggs were normally fertilized in the compound sea water, while in the natural control sea water 51 out of 52 eggs were fertilized. After two hours 42 eggs in the artificial sea water and 50 eggs in the control were observed, divided into two cells. In the next two hours, in the prepared sea water 18 eggs reached the four-cell stage, 25 eggs the eight-cell stage and 20 eggs the sixteen-cell stage and in the natural sea water 73 eggs showed the sixteen-cell stage. After ten hours most of the eggs observed in the artificial series were in morula stage while in the control most of them were already in the blastula stage. In the final observation taken 14 hours after the insemination, 15 blastulæ and 15 gastrulæ were observed in the experimental series. By this time in the control there were only 2 blastulæ and all other 23 were gastrulæ.

The numbers of unfertilized eggs given in the first column of the first table, have no significance. They may be simply the unripened eggs as they commonly occur in any case of artificial fertilization; nevertheless the smallness of the numbers as compared to those of the fertilized eggs indicates the favorable condition of the germ cells.

After all, in this experiment the affect of the artifical sea water is not very noticeable in the fertilization and development of the eggs. It is to be generally understood, that, even in normal circumstances, the artificial fertilization and development of *S. nudus* usually takes place with more difficulty than in *S. intermedius*.

Table II

Time		Unfertilized Fggs	Fertilized Eggs									
			1 c.	2 c.	4 c.	8 c.	16 c.	32 c.	Moru.	Blast.	Gast.	T.N.
3 hrs.	Arti.	3	—	2	6	7	—	—	—	—	—	15
	Cont.	1	—	3	3	9	—	—	—	—	—	15
8 hrs.	Arti.	2	—	—	—	—	—	6	8	2	—	16
	Cont.	2	—	—	—	—	—	—	29	4	—	33
24 hrs.	Arti.	3	—	—	—	—	—	—	1	6	20	27
	Cont.	2	—	—	—	—	—	—	—	8	16	24

A glance at the table shows that in this species there is no noticeable difference with regard to the fertilization and development in artificial sea water and in natural sea water.

In the meantime, the processes of development were going along in parallel in both series.

Experiment II

In this case the eggs were fertilized in sea water as usual and then after twenty minutes submitted to the experiment, hence the influences of the artificial sea water upon the germcells and upon the fertilization is negligible.

Both species of the seaurchin were used as material. The results thus obtained are given in Table III for *S. intermedius* and in Table IV for *S. nudus*.

Table III

Time		Unfertilized Eggs	Fertilized Eggs									
			1 c.	2 c.	4 c.	8 c.	16 c.	32 c.	Moru.	Blast.	Gast.	T.N.
20 min.	Arti.	8	48	—	—	—	—	—	—	—	—	48
	Cont.	20	55	—	—	—	—	—	—	—	—	55
2 hrs.	Arti.	9	5	34	1	—	—	—	—	—	—	40
	Cont.	8	—	21	6	—	—	—	—	—	—	27
4 hrs.	Arti.	9	—	—	4	15	25	2	—	—	—	46
	Cont.	18	—	—	—	9	12	36	—	—	—	57
8 hrs.	Arti.	8	—	—	—	1	1	7	28	—	—	37
	Cont.	12	—	—	—	—	—	1	2	43	—	46
12 hrs.	Arti.	8	—	—	—	—	—	1	20	15	—	36
	Cont.	7	—	—	—	—	—	—	—	43	1	44

Table IV

Time		Unfertilized Eggs	Fertilized Eggs									
			1 c.	2 c.	4 c.	8 c.	16 c.	32 c.	Moru.	Blast.	Gast.	T.N.
3 hrs.	Arti.	4	—	—	2	8	3	—	—	—	—	13
	Cont.	0	—	—	2	1	1	—	—	—	—	4
8 hrs.	Arti.	5	—	—	—	1	8	7	5	—	—	21
	Cont.	3	—	—	—	—	3	6	15	—	—	24
24 hrs.	Arti.	3	—	—	—	—	—	—	5	13	2	20
	Cont.	2	—	—	—	—	—	—	—	18	2	20

From the above tables it is to be seen that the artificial sea water again has little affect on the development of the eggs. The results were almost the same as in Table I and II and the relations were just repeated. However, it is worthy of note that many free swimming plutei were later obtained from these experimental series of the artificial sea water.

Experiment III

One day it happened that a heavy storm passed over the sea by the station and the writer had the chance to try the following experiments with the eggs of *S. intermedius* which were collected out of the stormy water.

At first, the process of fertilization in the sea water was observed a day after the storm. Secondly the eggs were inseminated in the sea water and then after twenty minutes were transferred into the artificial sea water, and thirdly, the eggs were inseminated in the artificial sea water. The percentage values of the fertilization were calculated. The results thus obtained are given in the following table.

Table V

	Unfertilized Eggs	Fertilized Eggs	%
I.	22	4	16
II.	9	9	50
III.	3	29	90

From the table it is evident that in the water from the stormy sea the fertilization took place with great difficult, showing only 16 % in the value. In the second series 50 % of the eggs were normally fertilized, while in the third series the value is approximately equal to that in the normal sea water as given in Table I and III.

Therefore, it is to be concluded that the causes of poor percentage in this case did not rise from the interior of the eggs, but was introduced from the exterior by the surrounding waters, the affect of which can be improved by artificial sea water.

Experiment IV

This experiment was undertaken two days after the storm. The material and the method were as in former experiments.

Table VI

	Unfertilized Eggs	Fertilized Eggs	%
I.	12	22	66
II.	3	22	88
III.	7	46	87

In this case the fertilization power in the sea water was not much improved, giving 66 in the percentage value; neverthless it is much higher than in the former experiment. Accordingly the tendency to recover the normal condition in regard to fertilization is evident.

Judging from the percentage values given in the table, in the second or in the third series the solution is normal in this respect as in the other experiments.

Summary

1. The fertilization and the development in sea water were carried out in VAN'T HOFF's artificial sea water modified by KANDA.

2. In the artificial sea water the eggs of *Strongylocentrotus* were fertilized normally as in the natural sea water.

3. In the artificial sea water the fertilized eggs were developed into free swimming plutei.

4. In a storm sea water is changed from its normal condition and affects the fertilization of sea urchin unfavorably.

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摘　　要

海水の變化と其の海産動物に及ぼす影響に就て

(一) 人工海水中に於けるウニの受精及び發生。

自然海水中に偶然生じたる、豫期せざる變化の爲め、海産動物が著しき影響を蒙り、各種の實驗に不慮の障害を齎らしむる事は、屢々經驗せらるる所なれども、其の原因に關しては、未だ明ならざるもの多し。嘗て大島博士はウニに激しき刺激を加ふる事に依り、所謂、皮膚分泌を起さしめ、かゝる分泌物が、其の受精を妨ぐる事を確め得たり。かゝる動物の体内に生ずべき變化も亦考慮せざるべからず。玆に於て、人工海水の生物に及ぼす影響を檢し、若し是が自然海水と同樣の作用をなす事を知り得れば、此種問題の解決に光明を得たるものなりと信ず。依つて其の反應の顯著なる「ウニ」の受精及初期發生に就きて實驗を行ひたり、

本實驗に用ひたる材料は忍路灣に普通なる「ムラサキウニ」 (*Strongylocentrotus intermedius*) 及び「バフンウニ」 (*S. nudus*) にして、此の種「ウニ」の人工受精に於ては普通 85% 乃至 95% の受精率を示すものなるが、こゝに使用せし人工海水中に於ても同等の受精率を示せるのみならず、受精せし卵は其の發生の經過も亦良好にして、此の人工海水中に於て多數の「プルテウス」時期のものを得たり。余の用ひたる「ヴァントホツフ」氏の人工海水は之等海産動物の卵に對し、自然海水と毫も異なる作用を示さざる事を知りたり。余は、又、時化の直后に於ては「ウニ」の受精率極めて悪しく、僅に 16% を示すに過ぎざる事を知りたるが、此の時同じ「ウニ」より得る卵を人工海水にて受精せしめたるに、普通の受精率を示す事實を認めたり。又其の海水中にて、精蟲を加へて後 20 分にして、此の人工海水に移したるものに於ては、其の受精率は兩者の約中間にあり。是は未だ生活力を失はざりし卵及精蟲が人工海水に移されたるため再び活動力を得て受精作用を行ひたるものなり。

風波靜溫になりし后に於ては、自然海水中にても受精は漸次回復せらるるものなり。故に時化に依り海水中に或種の變化の生ずる事は明かなり。