Natural Science Report, Ochanomizu University, Vol. 9, No. 2, 1958
お茶の水女子大学 自然科学報告 第9巻 第2号

Rearing Experiments of *Urechis* larvae

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Introduction

Present report concerning the relation between the diet and the larval developments of *Urechis unicinctus* is a result of experiments designed to find a suitable diet for rearing the larvae of this species and constitutes a part of the projects to acquire a more coherent knowledge about the post-embryonal development of the Echiuroids.

The writer wishes to express her hearty thanks to Professor Matazo KUME of Ochanomizu University, for his continuous directions throughout the work, to Professor Yasuo OHSHIMA and Mr. Reijiro HIRANO of Department of Fisheries, Faculty of Agriculture, University of Tokyo, who kindly supplied diatom stocks and instructed the method of their culture, and to Professor Masumi OHKAWA of Yokohama Municipal University, for his help for securing the materials.

Materials and Method

Materials used in this experiment were all obtained at Kanazawa-Hakkei, Yokohama. The breeding season of this species is comparatively long, as it lasts from the middle of October to the end of March next year, but they are the most prosperous in December.

For fertilization, a necessary quantity of eggs and sperms was obtained by inserting a micropipette into the opening of nephridium. This is a nice method for obtaining the germ-cells without injuring the bodies of the worms (NEWBY, '32). Usually, the inseminations were done within two days after collection, in order to make the developmental conditions as similar as possible.

About 48 hrs after the insemination, most of the eggs develop into the typical trochophora larvae, which then swim about near the surface of the water.

Glass containers 10.2 cm. in diameter and 8.6 cm. in depth were provided and filled with 500 c.c of sea water, in which the developing larvae, 300 individuals in each container, here reared. The larvae in the same container were reared by keeping the same kind of diet through-

out the experiment.

As diet, two species of unicellular marine algae, *Skeletonema costatum* and *Thalassiosira* sp., were used and the results obtained were compared. Besides, in some preliminary experiments a kind of Flagellata was also tried as a sample of zooplankton without getting any positive result, however. The stocks of the algae in this experiment were first supplied from Department of Fisheries, Faculty of Agriculture, University of Tokyo, and were maintained in our laboratory by making unialgal culture.

The trochophore larvae did not feed, even if the diet was offered, until about 6 days after the insemination, when they began to take food, and the food that was taken into the gut of the larvae could actually be observed as green or brownish contents. Therefore, feeding was usually begun 6 days after insemination.

The water and the diet in the containers were changed with fresh ones every ten days, and the inspection of the larvae was made microscopically, and their developmental stages were recorded on the same occasion. Rearings were made in an usual laboratory room, and temperature was about 18.5°C in the daytime. It was strictly avoided to place the containers in the direct sunlights.

Results and Discussion

Developmental stages of the larvae: The developmental stages of *Urechis* larvae can be roughly classified into four according to their morphological features, especially of the external and the behavior.

Until the third week after insemination, the larvae in the same container usually develop uniformly and reach the stage of trochophore designated as the T. stage in this paper. After three weeks, black pigment bands begin to appear in the region of hyposphere which will become afterwards the trunk of the worm. This stage is called the Pre-segmentation stage (Pr.), as the segmentation of the body does not still appear. In the latter half part of this stage, a pair of setae is noticed to appear at the most anterior part of the hyposphere that will later become the first of the body segments.

5 weeks after insemination, there appear in the hyposphere of the trochophore larvae the succesive foldings as the hyposphere elongates, and each one of the black pigment bands comes to lie just on the summit of each of the foldings. These foldings are counted 11 in all. These larvae can be said to have reached the Segmentation stage, but as their behavior of swimming is still maintained, the developmental stage of these larvae is named the first Segmentation stage (S–I). In 5 to 6 weeks after insemination, some of the individuals among the S–I

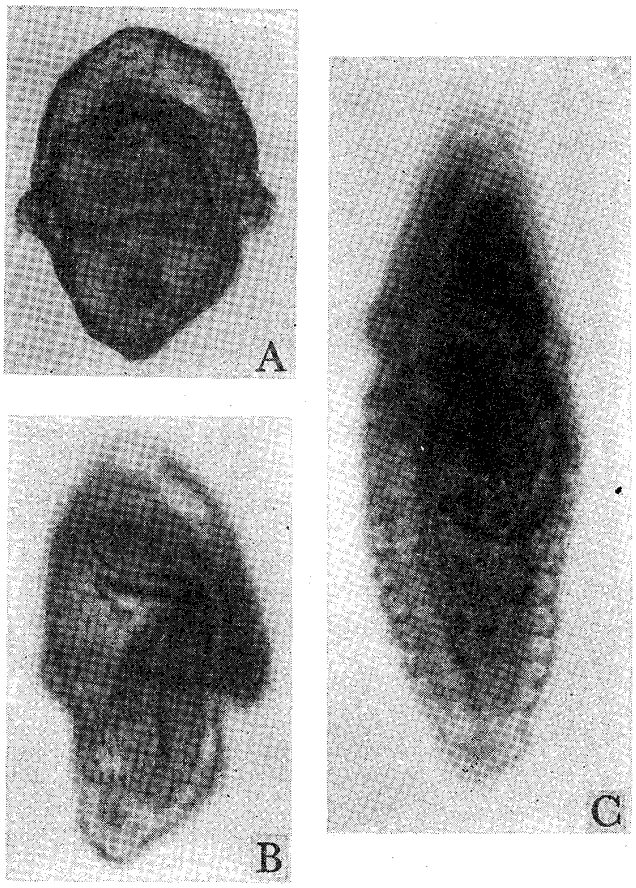

Fig. I. Developmental Stages of *Urechis unicinctus*. (×200)
A. Trochophore stage
B. Pre-segmentation stage
C. 1st segmentation stage

stage begin to sink down to the bottom of the containers, where they move about. The ciliary movement of the larvae does not become strong enough to support their increasing weight of body. Afterwards, the degeneration of ciliary structures begins, and the larval behaviour changes completely into the creeping type. At this time, the segmental structures of the body become very clearly formed, and thus the larvae enter the second Segmentation stage (S-II).

About 7 weeks after insemination, the peristaltic movement of the body of the larvae becomes distinct, but the segmental structures gradually disappear. The remains of the black pigment bands, however, are observed as black dotted lines when the larvae extend their bodies. As this is clearly a sign of the beginning of metamorphosis, the stage of the larvae is named the Metamorphosing stage (M) in this paper.

Feeding Experiments: Rearing of the larvae was repeated several times during the breeding season. However, the experience and the statistical results show that the highest percentage of normal development was obtained from the eggs inseminated during December, and that the mortality was found to be rather high particularly among the larvae developed from the eggs obtained during and after January. Therefore, the writer wishes to describe somewhat in detail the results obtained from one of the several trials performed during December.

In this experiment there were three diet categories; *Skeletonema costatum, Thalassiosira* sp. and no food for comparison.

Table I shows the results obtained from the materials inseminated on December 23rd, 1958.

In this table, a total number of larvae survived and the number of the larvae which attained each of the developmental stages as rear-

ing proceeded, were shown after each diet category.

The same materials were rearranged in Table II (1–5) according to the developmental stages larvae had attained. From these tables, one can observe how each of the developmental stages was distributed as the rearing of the larvae proceeded. Table II–1 concerns with the T. stage.

As said before, the number of individuals in each diet category was 300 when the rearing began, and the same number was maintained until the 4th day when all of the larvae developed uniformly into the T. stage. In the *Skeletonema* group, however, this was decreased to 281 on the 28th day, but, how many of them had proceeded to the next Pre-segmentation stage was unknown since no exact counting was performed at the time of inspection. But, it can be safely inferred that about half of them had already proceeded to the next stage at that time. As seen from table III–A in which correlation between the various developmental stages and the duration of rearing in the *Skeletonema* group is shown, there are found on the 38th day, 64 individuals developed into the S–I stage and 45 into S–II stage. These larvae, a total of 109, might at least be presumed to have been in the Pr. stage at the time of previous inspection.

In the *Thalassiosira* group, however, most of the larvae on the 28th day seemed to have been at the T. stage. This is because 147 out of 243 individuals still remained at the T. stage, and a comparatively few members developed into higher stages on the 38th day.

On the 38th day, 77 out of 280 individuals were at the T. stage in the *Skeletonema* group, while the rest of them, 203 in number, had already been in the more advanced stages as seen from Table I′ or III. In the *Thalassiosira* group, however, more than half of the total individuals, 243 in number, were still at the T. stage.

But on the 48th day the number of trochophore larvae diminished rather suddenly to only 7 in the *Skeletonema* group, while there were still 82 individuals in the *Thalassiosira* group. After the 58th to the 102nd day, there occured in the *Skeletonema* group little numeral changes in the T. stage, where a very few individuals remained constantly. This means that these individuals have no power to develop further in spite of maintaining their life. Such tendency was observed more often in the *Thalassiosira* group than in the *Skeletonema* group, and some of the larvae in the *Thalassiosira* group could continue to live for 102 days without changing their forms as trochophore larvae.

Even in the group of no food, although most of the individuals died out early, some were observed to develop into the T. stage on the 28th day. But these larvae could not proceed to the next stage in spite of maintaining their life over 38 days.

Table I. Relation between diet and developmental stages of larvae
Materials were fertilized on December 23rd, 1957. Exact number of developmental stages on the 28th day was not recorded.

Days after fertilization	Developmental stages	Number of survivals in each group of					
		Skeletonema costatum		*Thalassiosira* sp.		No food	
			Total		Total		Total
4 days	T	300	300	300	300	300	300
28			281		244		56
38	T	77	280	147	243	0	0
	Pr	94		54		0	
	S-I	64		9		0	
	S-II	45		33		0	
48	T	7	263	82	219		
	Pr	21		61			
	S-I	19		18			
	S-II	196		57			
	M	20		1			
58	T	5	250	62	204		
	Pr	14		38			
	S-I	25		29			
	S-II	170		74			
	M	36		1			
70	T	2	63	35	166		
	Pr	11		51			
	S-I	15		24			
	S-II	6		26			
	M	29		30			
80	T	2	8	20	99		
	Pr	0		20			
	S-I	0		19			
	S-II	4		21			
	M	2		19			
91	T	0	0	8	40		
	Pr	0		7			
	S-I	0		11			
	S-II	0		11			
	M	0		3			
102	T			2	15		
	Pr			3			
	S-I			8			
	S-II			2			

T; Trochophore stage Pr; Pre-segmentation stage S-I; First segmentation stage S-II; Second segmentation stage M; Metamorphosingstage

Table II. Tables classified according to the various developmental stages

Table II-1. Trochophore stage

Days after the fertilization	Number of individuals in T. stage/a total number survived		
	Skeletonema costatum	*Thalassiosira* sp.	No food
4 days	300/300	300/300	300/300
28	—/281	—/244	—/ 56
38	77/280	147/243	0/ 0
48	7/263	82/219	
58	5/250	62/204	
70	2/ 63	35/166	
80	2/ 8	20/ 99	
91	0/ 0	8/ 40	
102		2/ 15	

Table II-2 Pre-segmentation stage

Days after the fertilization	Number of individuals in Pr. stage/a total number survived		
	Skeletonema costatum	*Thalassiosira* sp.	No food
4 days	0/300	0/300	0/300
28	—/281	—/244	—/ 56
38	94/280	54/243	0/ 0
48	21/263	61/219	
58	14/250	38/204	
70	11/ 63	51/166	
80	0/ 8	20/ 99	
91	0/ 0	7/ 40	
102		3/ 15	

Table II-3. First segmentation stage

Days after the fertilization	Number of individuals in S-I stage/a total number survived		
	Skeletonema costatum	*Thalassiosira* sp.	No food
4 days	0/300	0/300	0/300
28	—/281	—/244	—/ 56
38	64/280	9/243	0/ 0
48	19/263	18/219	
58	25/250	29/204	
70	15/ 63	24/166	
80	0/ 8	19/ 99	
91	0/ 0	11/ 40	
102		8/ 15	

Table II-4. Second segmentation stage

Days after the fertilization	Number of individuals in S-II stage/a total number survived		
	Skeletonema costatum	*Thalassiosira* sp.	No food
4 days	0/300	0/300	0/300
28	—/281	—/244	—/ 56
38	45/280	33/243	0/ 0
48	196/263	57/219	
58	170/250	74/204	
70	6/ 63	26/166	
80	4/ 8	21/ 99	
91	0/ 0	11/ 40	
102		2/ 15	

Table II-5. Metamorphosing stage

Days after the fertilization	Number of individuals in M. stage/a total number survived		
	Skeletonema costatum	*Thalassiosira* sp.	No food
4 days	0/300	0/300	0/300
28	0/281	0/244	0/ 56
38	0/280	0/243	0/ 0
48	20/263	1/219	
58	36/250	1/204	
70	29/ 63	30/166	
80	2/ 8	19/ 99	
91	0/ 0	3/ 40	
102		0/ 15	

If the above inference is correct, at least about half of the larvae (109 individuals) had already been at the Pre-segmentation stage in the *Skeletonema* group at the time of inspection on the 28th day, while in the *Thalassiosira* group most of the larvae still remained in the T. stage, and then there is found a quite interesting tendency manifested in the developmental patterns of the larvae when the data in Table II (1-5) are compared one another. Namely the number of the larvae at the T. stage is maximum on the 4th day in both the *Thalassiosira* and *Skeletonema* groups (Table I-1). But, the number of those in the Pr. stage reaches a peak (109 individuals) on the 28th day in the *Skeletonema* group, while in the *Thalassiosira* group the same peak (61 individuals) is on the 48th day, if the above inference is correct (Table II-2).

As to the S-I stage, a peak is on the 38th day in the *Skeletonema* group (64 individuals), while it is around the 58th day in the *Thalas-*

Table III. Correlation between developmental stages and the duration of rearing in each group of diet. Maximum numbers were indicated by Gothic letters.

Table III-A. *Skeletonema* group

Stages \ Days after the fertilization	4	28	38	48	58	70	80	91	102 days
T	**300**	281	77	7	5	2	2	0	0
Pr			94	21	14	11	0	0	
S-I			**64**	19	25	15	0	0	
S-II			45	**196**	170	6	4	0	
M				20	**36**	29	2	0	

Table III-B. *Thalassiosira* group

Stages \ Days after the fertilization	4	28	38	48	58	70	80	91	102 days
T	**300**	244	147	82	62	35	20	8	2
Pr			**54**	61	38	51	20	7	3
S-I			9	18	**29**	24	19	11	8
S-II			33	57	**74**	26	21	11	2
M				1	1	**30**	19	3	0

siosira group (29 individuals) (Table II-3).

In the S-II stage, a maximum number exists in the *Skeletonema* group on the 48th day and on the 58th day in the *Thalassiosira* group (Table II-4).

In the M. stage, as shown in Table II-5, there is a maximum in the *Skeletonema* group on the 58th day and on the 70th day in the *Thalassiosira* group. These facts indicate clealy, 1) that although there occur among the larvae many variations in the development as rearing proceeds, the majority of them continues to develop quite regularly and pass successively each of the developmental stages as their development progresses; and 2) that although both algae are used as food for the larvae, their development is faster in the *Skeletonema* group than in the *Thalassiosira* group where considerably many individuals remained as retarded.

Mortality: Mortality was calculated on the basis of the number of survivals found at each inspection, and the results are shown in Table IV.

As indicated in this table, there appears a peak of high mortality on the 28th day after fertilization, which occurs commonly in all three diet categories inspected. The peak is higher in the *Thalassosira* group

than in the *Skeletonema* group and is exceedingly remarkable in the group of no food. This last fact shows that around the 28th day after the insemination there is a limit to maintain their life without taking foods. And a cause of high mortality equally found in other two groups may be ascribed to this, since there must have been some individuals among them which could not adapt themselves to the diet and were under the similar conditions with the individuals in no food group. When the mortality in the *Skeletonema* and *Thalassiosira* groups is compared, that of the latter seems to be somewhat higher than that of the former until the 58th day. This result is in full accord with the microscopical observations on the larvae of the *Thalassiosira* group, whose stomach was not so sufficiently filled with food during the younger periods.

After the 70th day, the mortality rises rather abruptly in both groups. Particularly in the *Skeletonema* group, 187 individuals died until the 70th day, out of 250 individuals which existed on the 58th day. As can be seen from Table II-4, 5 or Table III, there are present a total of 250 individuals on the 58th day, of which 170 individuals belong to the S-II stage. But, on the 70th day the number of the larvae at the S-II stage decreases to only 6 individuals. The cause of this decrease seems not due to the proceeding of the larvae at the S-II stage on the 58th day to the M. stage on the 70th day, that is because the M. stage larvae on the 70th day are only 29 and are smaller in number than those on the 58th day. It is concluded from these facts, that the high mortality appeared on the 70th day is due to the same phenomenon in the larvae of the developmental stages in S-II and in M.

In contrast to this, the mortality in the *Thalassiosira* group from

Table IV. Mortalitiy

Mortality was calculated by whole number.

days after the fertilization	*Skeletonema costatum*			*Thalassiosira* sp.			No food		
	Number of survivals	Number of deaths	Mortality	Number of survivals	Number of deaths	Mortality	Number of survivals	Number of deaths	Mortality
4 days	300			300			300		
28	281	19	6.3%	244	56	18.7%	56	244	81.3%
38	280	1	0.3	243	1	0.3	0	56	18.6
48	263	17	5.3	219	24	8.0			
58	250	13	4.3	204	15	5.0			
70	63	187	62.3	166	38	12.6			
80	8	55	18.3	99	67	22.3			
91	0	8	2.6	40	59	19.6			
102				15	25	8.3			

the 58th to the 70th day is somewhat different from that in the *Skeletonema* group. This number of death in these days is only 38 in contrast to 187 in the correspooding days in the *Skeletonema* group. This is due to the reason that only a small number of individuals are present in the S-II and M. stages on the 58th day, as found in Tables II-4, 5.

The high mortality in the S-II and M. stage is probably caused by a change of the feeding habit accompanied by the larval process of metamorphoses when the creeping behavior on the sea-bed begins. When individuals of these stages are observed under a low power microscop, it is noticed that the diatoms which once filled their gut and colored it greenly or brownish, gradually decrease in number and come to disappear to make the gut completely empty.

Summary

1) In this paper, the results of rearing the larvae of *Urechis unicinctus*, in the laboratory were described.
2) *Skeletonema costatum* and *Thalassiosira* sp. were proved to be effective as diets for the rearing.
3) *Skeletonema costatum* appears more effective as diet than *Thalassiosira* sp., when results on the developmental stages attained by the larvae, and the number of individuals which survived were considered.
4) But, both diets were not suited for the larvae in which the segment had already disappeared and their behavior transformed from that of the free swimmer to the bottom creeper. This was considered as due to the change of their feeding habit during and after the metamorphosis.

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(*Received September 1, 1958*)