THE PHILIPPINE
JOURNAL OF FISHERIES

VOL. 4 JANUARY–JUNE, 1956 NO. 1

THE EMBRYOLOGY OF DIADEMA SETOSUM (LESKE)

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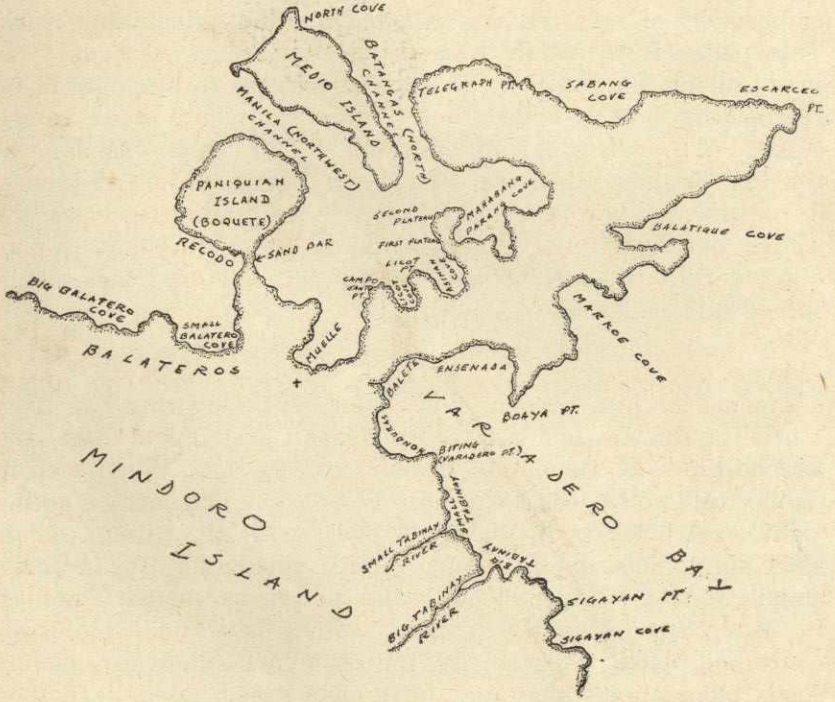
TWO PLATES AND THREE TEXT FIGURES

In the study of the life histories of lower organisms, development of ways and means of rearing them successfully in the laboratory is of great importance. Experimental work on the embryology of invertebrates is a tedious and painstaking work. This paper is primarily a description of some methods tried in culturing an echinoid in the laboratory through artificial fertilization. It is hoped that it may contribute some knowledge to the embryology of echinoids. This work was done at the U.P. Marine Biological Station in Puerto Galera, Mindoro, during the summer session of 1948. With limited facilities, the writer performed the experiments on the embryology of an echinoderm, using *Diadema setosum* (Leske), a black, long-spined and poisonous sea urchin.

MATERIALS

The sea urchins used in this experiment were collected from Muelle of Puerto Galera Bay, Honduras Cove, Balete Cove, and Recodo Shore of Paniquian Island (text fig. 1). When seen in its natural habitat, the sea urchin, *D. setosum*, presents a striking sight. Its black body glows all over, with the orange-yellow anus surrounded by five purple dots, glistening like a jewel among the very long, slender and poisonous primary spines. In young specimens the primary spines have wide bands of white and black. Abactinally, the secondary spines are usually black, but actinally they may have olive green bases, until they become entirely white (or whitish purple) around the peristome

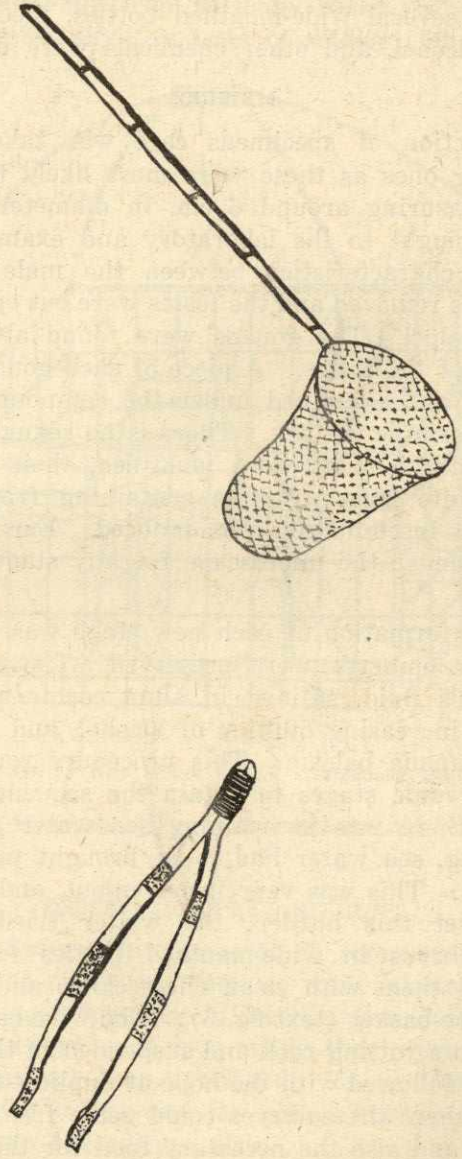
(Roxas 1928). Miliaries, the smallest spines, are black abactinally and white actinally. In life these gregarious creatures are found on rocky and exposed sandy bottoms and coral reefs, traveling by the action of their long spines and tube feet. The species is so abundant all over the shallow waters of Puerto Galera that it has become a menace to fishermen inasmuch as the spines, when stepped upon, may cause intense pain and sometimes fever. This is especially harmful to collectors of specimens who have to walk among the rocks. Next to *Echinotrix calamaris*, this species is the commonest poisonous sea urchin in the Philippines. In spite of its poisonous reputation, however, people of the northern regions from the Ilocos down to the Hundred Islands and as far as Zamboanga in the south, value the sea urchin as a delicacy. The urchins are roasted whole and their testes are opened to get the gonads and the liquid inside. These are eaten with relish. This may become a lucrative business for an enterprising food technologist if he could develop something like the famous caviar of Russia.



TEXT FIG. 1. Map of Puerto Galera Bay and vicinity.

Then, it will no longer be a problem of extermination but one of production.

Materials for collecting consist of a scoop net with a long bamboo handle and a pair of improvised bamboo forceps (text fig. 2). When the tide is high, a banca is used to reach the



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TEXT FIG. 2. Materials used for collecting *D. setosum* (Leske).

collecting grounds, and the specimens are scooped up by means of the net. When the tide is low, specimens are merely picked up with the bamboo forceps.

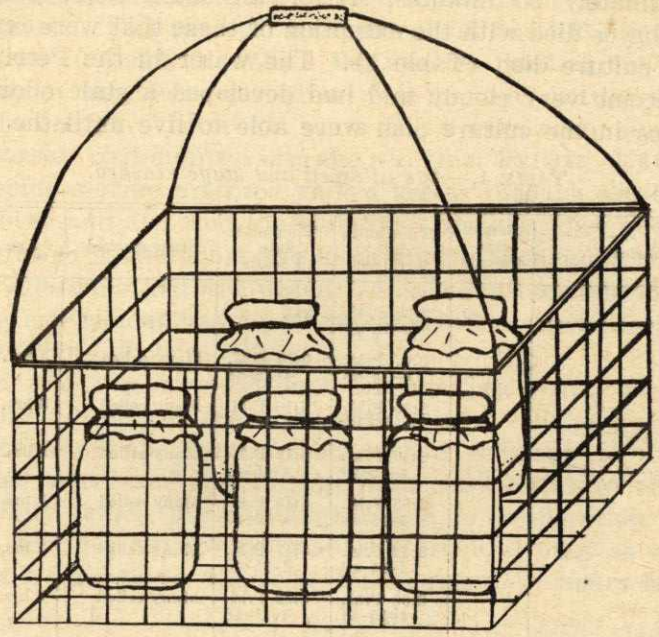
In the experiment itself, a number of Petri dishes, culture dishes, a compound microscope equipped with camera lucida, a dissecting set, several wide-mouthed bottles, pieces of cheesecloth, slides, alcohol, and other chemicals were used.

METHOD

In the collection of specimens care was taken to gather only the bigger ones as these were most likely to be mature. Only those measuring around 6 cm. in diameter were taken. These were brought to the laboratory and examined for any distinguishing characteristics between the male and female. The spines were removed and the testes were cut open in half by means of a scalpel. The gonads were found attached to the abactinal wall of the testes. A piece of each gonad was teased out on a slide and examined under the compound microscope in order to determine the sex. There is no sexual dimorphism. When males and females were identified, their gonads were squeezed out into culture dishes containing fresh sea water where artificial insemination was induced. Each culture was then watched under the microscope for any stages of development.

The time of formation of each new stage was recorded, and samples of the embryos were preserved. The embryos were fixed in Bouin's fluid, stained in alum cochineal, dehydrated by a series of increasing dilution of alcohol, and then mounted on slides in Canada balsam. This procedure was repeated in all other embryonic stages to obtain the sequence of development. Since there was no running sea water supply at the station building, sea water had to be brought up to the laboratory in pails. This was very inconvenient, and it took much time. To offset this burden, the writer tried putting the developing embryos in wide-mouthed bottles filled with sea water, covered them with clean cheesecloth, and placed these bottles in a wire-basket (text fig. 3). The wire-basket was then securely tied to a jutting rock and suspended in the port. This procedure was followed with the hope of duplicating the natural environment where the embryos could get a free circulation of the sea water and also the necessary food for the maintenance of their life.

This experiment was repeated five times on different days with a few variations. When the embryos attained the echinopluteus stage, they were examined once a day until they died. Unfortunately no slides of the echinopluteus larva were prepared as they were destroyed during preparation. It is quite difficult to preserve these larvæ as in many cases the skeleton is dissolved by even the slightest trace of acidity (Mortensen, 1921).



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TEXT FIG. 3. Wire basket with jars containing zygotes.

Illustrations of some cell stages were drawn with the aid of a camera lucida.

OBSERVATIONS AND RESULTS

First trial.—Several specimens of *D. setosum* (Leske) were gathered, and the males and females were selected. Eggs and sperms were placed together in Petri dishes containing fresh sea water, and every once in a while they were examined to see if the eggs had been fertilized. There were eight sets fertilized in Petri dishes, and the remaining reproductive cells were dumped into one culture dish.

The eggs were very small and transparent but heavy, as these were observed to settle down at the bottom of the dishes.

Due to their transparency, the occurrence of the fertilization membrane escaped notice, and fertilization was known to have occurred only when cleavage began. Hence, no record of the time of fertilization was taken. Cleavage was equal and holoblastic. The zygote took almost an hour to develop from the time the egg and sperm were put together until cleavage occurred. From the two-cell stage to the four-cell stage, it took approximately 25 minutes. After the many-celled stage, all the embryos died with the exception of those that were fertilized in the culture dish (Table 1). The water in the Petri dishes had become very cloudy and had developed a stale odor. The embryos in the culture dish were able to live until the following day and developed into ciliated blastulae after about 18 hours. In the afternoon all the ciliated blastulae were dead. These embryos lived for almost 25 hours only. In this experiment the water in the dishes was not changed at all, so that there must have been a lack of oxygen and a probable change in the salinity.

TABLE 1.—*Age attained and stage reached.*

Series	Sets of experiments	Time and date fertilized	Time and date of death	Stage reached	Age attained
A	9	2:35 P.M. 4-15-48	xxx		
		2:47 P.M. 4-15-48	6:15 P.M. 4-15-48	Many celled	3 hrs. 28 min.
		2:50 P.M 4-15-48	6:16 P.M. 4-15-48	Many celled	3 hrs. 16 min.
		2:53 P.M. 4-15-48	6:19 P.M. 4-15-48	Many celled	3 hrs. 26 min.
		2:55 P.M. 4-15-48	6:45 P.M. 4-15-43	Many celled	3 hrs. 50 min.
		3:02 P.M. 4-15-48	6:47-P.M. 4-15-48	Many celled	3 hrs. 45 min.
		3:05 P.M. 4-15-48	6:52 P.M. 4-45-48	Many celled	3 hrs. 47 min.
		3:08 P.M. 4-15-48	5:17 P.M. 4-15-48	16-cell	2 hrs. 9 min.
		3:12 P.M. 4-15-48	4:05 P.M. 4-16-48	Ciliated blastula	27 hrs. 31 min.
B	5	10:20 A.M. 4-16-48	10:00 A.M. 4-18-48	gastrula	47 hrs. 40 min.
		10:24 A.M. 4-16-48	10:00 A.M. 4-18-48	gastrula	47 hrs. 36 min.
		10:27 A.M. 4-16-48	10:00 A.M. 4-18-48	gastrula	47 hrs. 33 min.
		10:40 A.M. 4-16-48	10:00 A.M. 4-18-48	gastrula	47 hrs. 20 min.
		11:20 A.M. 4-16-48	10:00 A.M. 4-18-48	gastrula	46 hrs. 40 min.
C	1	9:30 A.M. 4-20-48	9:30 A.M. 4-21-48	Blastula	24 hrs.
D	1	9:40 A.M. 4-21-48	3:30 P.M. 4-21-48	Many celled	5 hrs. 50 min.
E	1	10:00 A.M. 4-22-48	8:00 A.M. 4-22-48	Echinopluteus	133 hrs.

Second trial.—Another set of eight *D. setosum* was cut open and artificially fertilized with a view to observing their further development. The time of development of the different stages are shown in Table 2. When the zygotes reached the many-celled stage, they were placed in wide-mouthed bottles covered with cheesecloth. After 24 hours the bottles were placed in a wire-basket and dumped into the sea (text fig. 3). Unfortunately, some marine protozoa gained access through the cheesecloth cover and attacked the embryos. Besides, dirt and some algal growth anchored at the cloth covers thus impairing the free circulation of aerated water. As a result the embryos died, and the experiment had to be repeated. The embryos in this trial lived for about 48 hours.

Third trial.—A new set was fertilized following the same procedure. However, this time all the sperm cells and egg cells were reared in one culture dish with a constant addition of fresh sea water and a little tap water, the latter to regulate the salinity which becomes higher due to evaporation. This culture was not any better than the previous ones as all the embryos were dead by the following morning, 24 hours later.

TABLE 2.—*Age in minutes of embryo up to early pluteus stage.*

Series	Two-celled	Four-celled	Eight-celled	Sixteen-celled	32-celled	Many-celled	Blastula	Gastrula	Early pluteus
A	48	77	88	123	158	208			
	55	77	90	116	146	196	(a)	-------	-------
	57	76	102	127	157	206	(a)	-------	-------
	58	75	103	135	164	230	(a)	-------	-------
	55	80	102	129	158	225	(a)	-------	-------
	53	80	100	130	158	227	(a)	-------	-------
	51	79	105	129	(a)	-------	-------	-------	-------
	48	77	95	126	158	-------	1,651	(a)	-------
B	65	100	135	170	191	245	420	1,420	(a)
	63	101	136	167	188	236	421	1,416	(a)
	63	-------	138	153	-------	243	423	1,413	(a)
	-------	-------	135	-------	-------	-------	-------	1,400	(a)
	-------	90	130	-------	-------	-------	375	1,360	(a)
C	60	90	120	150	-------	-------	-------	-------	-------
D	35	-------	-------	140	-------	-------	350	(a)	-------
E	-------	-------	-------	-------	-------	-------	1,320	1,440	2,700
Total	711	1,002	1,579	1,795	1,478	2,016	4,960	8,149	2,700
Average	5.7	87	112.8	138	164.2	224	708.6	1,358	2,700

Fourth trial.—The procedure followed in the third trial was done again to find out why the embryos would not continue to live. In this case the eggs developed only up to the blastula stage and then died. The water in the culture dish also became cloudy as had always happened in the previous trials.

Fifth trial.—This time only the eggs and sperms from one male and one female *D. setosum* were fertilized in a culture dish. Care was taken so that the eggs and sperms placed in the dish are not overcrowded. Instead of placing all the contents of the gonads into this culture dish as was done in previous trials, only a tiny portion of each was taken and mixed together. This met with success as the eggs developed into echinopluteus larvæ which lived four days. The water was constantly changed by decanting the surface liquid and adding fresh sea water with a little tap water. The early echinopluteus larvæ appeared the second day, approximately 45 hours after the eggs were fertilized (Plate 2, fig. 1). The skeleton had started to form and the echinopluteus shape was assumed, but the arms were still very short. The third day they had developed longer processes which appeared to be fenestrated. They were approximately 70 hours old. The fourth day they were bigger in size with mouth, stomach, and anus very distinct. They were then 95 hours old (Plate 2, fig. 2). The fifth day the two arms were very much longer and the larvæ were seen to be moving around. They had been alive for 109 hours. The sixth day the larvæ were dead upon examination. These must have decomposed as only the skeletons could be identified under the microscope, and the water had become milky white in color. From the time of insemination to their death was approximately 133 hours.

Several attempts at artificial fertilization were again made after this successful one, but they proved unsuccessful as the specimens caught all contained immature ova, indicating the fact that the breeding season of *D. setosum* was over. Ripe ova were found only during the middle part of April.

SUMMARY

1. *Diadema setosum* (Leske) breeds during the middle part of April, since the specimens examined after this period were all immature.

2. In artificial fertilization care must be given to the amount of eggs and sperms placed together to prevent overcrowding.

3. Change of sea water for aeration and addition of fresh water for maintenance of salinity must be constant.

4. Cleavage is holoblastic and equal. It occurs approximately every 26.35 minutes.

5. The blastula is ciliated and appears after about 18 hours.

6. The echinopluteus larva appeared after two days, approximately 45 hours after insemination. The skeleton had started to form and the echinopluteus shape was assumed, but the arms were still short.

7. After 70 hours and 15 minutes, the arms were longer and appeared fenestrated.

8. After 95 hours, the larvæ were bigger in size with the mouth, stomach, and anus very distinct.

9. At 109 hours old, the two arms were very much longer and the larvæ were seen to move around.

10. After 133 hours all the larvæ died.

ACKNOWLEDGMENT

The writer wishes to thank Dr. Amado T. Feliciano and Dr. Lucia Manikis, both of the Zoology Department, University of the Philippines, for their unselfish guidance in the performance of the experiments; Mr. Jose Domantay, for the use of his private library; Mr. A. Joson and Mr. B. Escuadro, for putting the illustrations in final order; and Messrs. Claro Martin, Guillermo Blanco, and Inocencio Ronquillo, for going over the paper and making constructive criticisms thereon.

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ILLUSTRATIONS

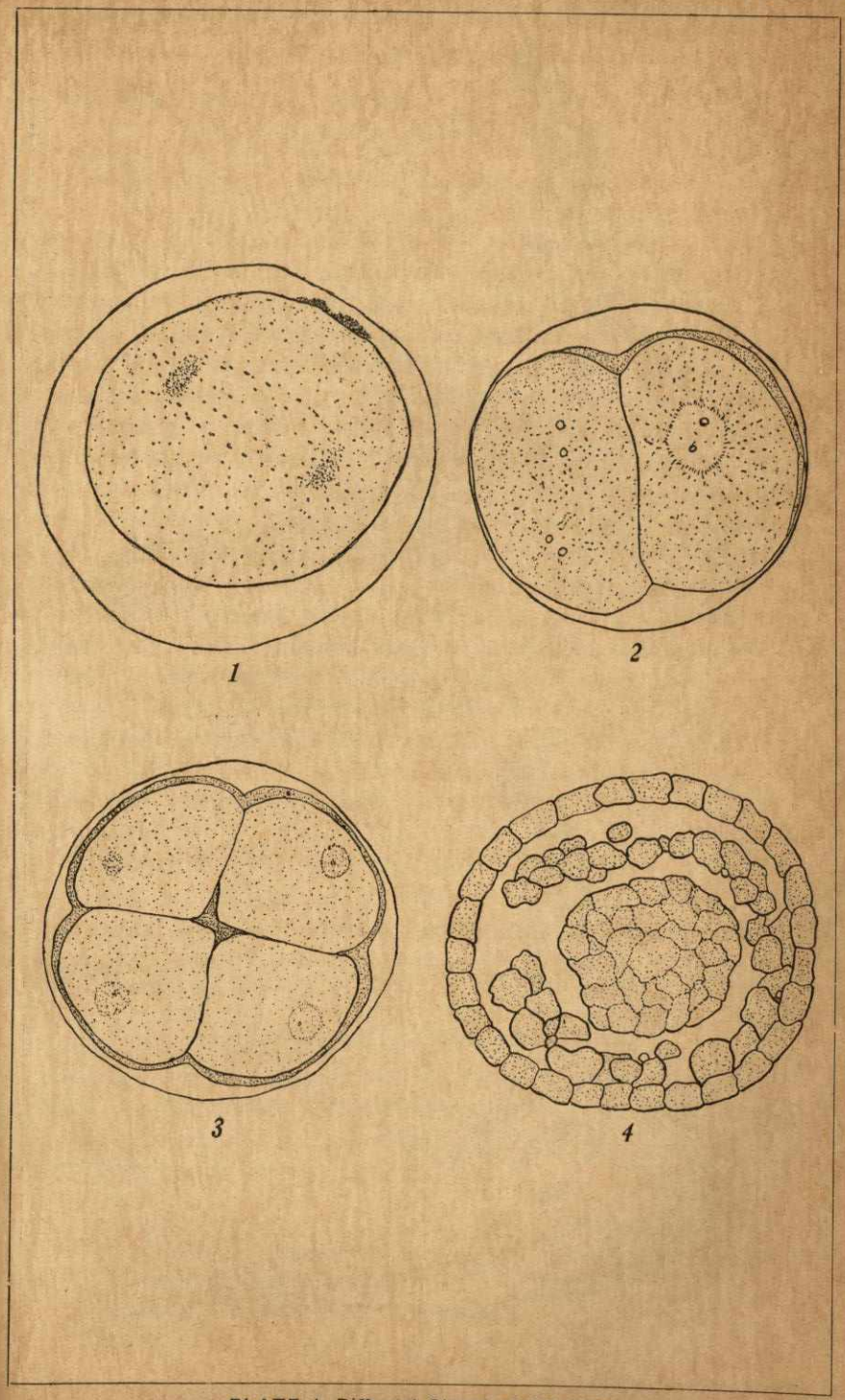

PLATE 1. Different Stages of Cleavage

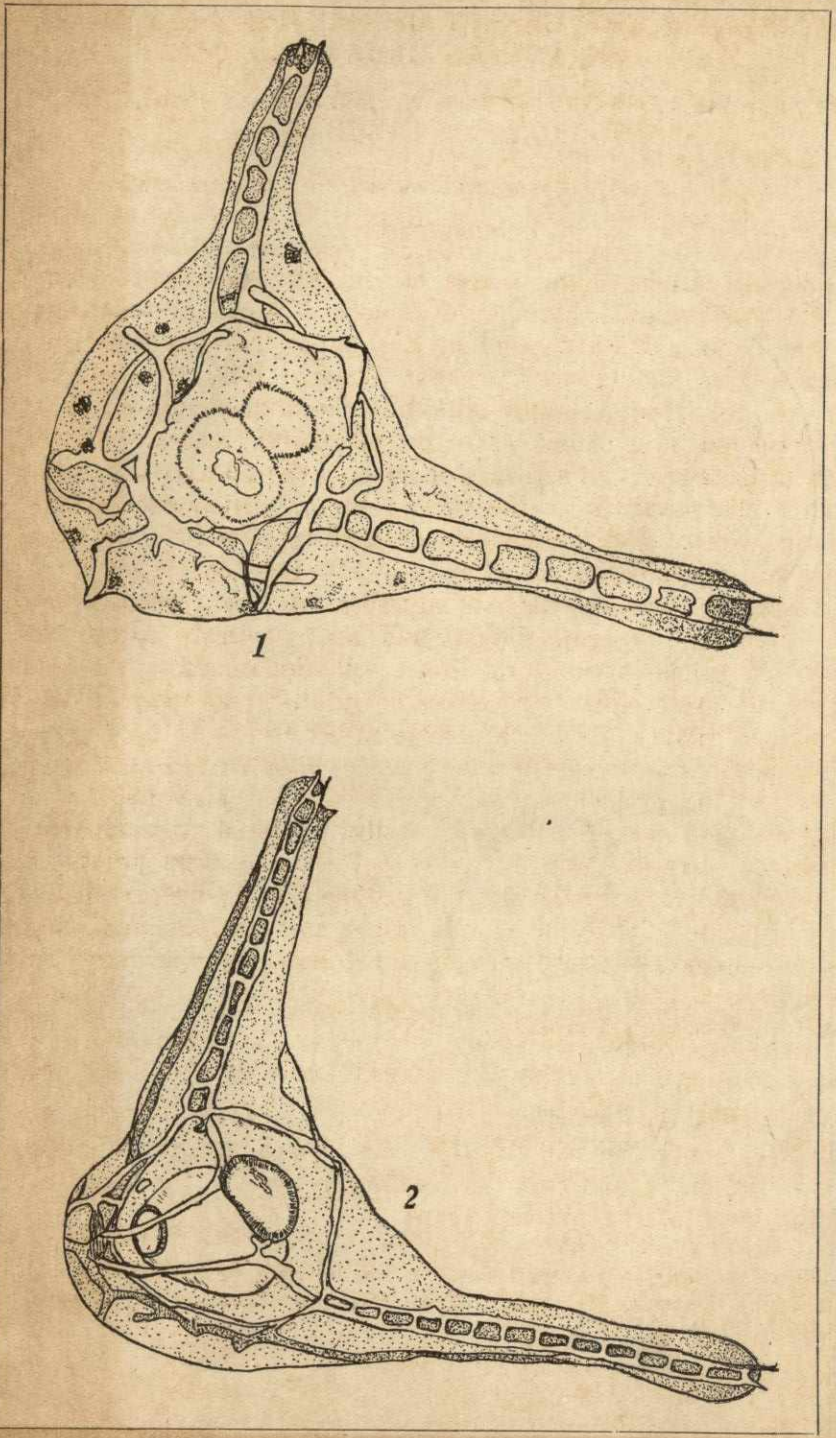

PLATE 2. Two Stages of the Echinopluteus Larva