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ON REARING SEXUALLY MATURE PLATYNEREIS MEGALOPS FROM EGGS

I

THE literature affords so few cases of marine animals reared under laboratory conditions that the writer ventures to communicate his successful attempts to carry through to sexual maturity the nereid, *Platynereis megalops,* from eggs laid in the laboratory. This work had its origin in a suggestion made by Dr. F. R. Lillie in 1911 that the capacity for cross fertilization between *Nereis limbata* and *Platynereis megalops* be tested. At that time, however, since we knew so little of the life history of these forms, we felt that it was necessary to get all data possible on each life history in order to have a standard of comparison for the life history of the hybrids. So far all efforts to cross these nereids have failed. The difference in the breeding habits of *Nereis* and *Platynereis* is so striking that this alone might account for the failure of cross fertilization. *Nereis* sheds eggs into the sea-water where fertilization takes place; *Platynereis* lays inseminated eggs soon after copulation. However, this very difference is calculated to enhance the interest attaching to the cross fertilization. It might be possible to study the inheritance of the egg-laying reactions. In addition, early observations revealed that the young *Platynereis megalops* closely resemble *Nereis dumerilii.* Since, as is well known, *Nereis dumerilii* has a complex life history, we felt that the life history of *Platynereis* might well repay study for its own sake.

II

PLATYNEREIS MEGALOPS REARED UNDER LABORATORY CONDITIONS TO SEXUAL MATURITY

The writer has found that it is possible to rear *Platynereis megalops* to sexual maturity under laboratory conditions. This was first accomplished in 1913–1914, repeated in 1920–1921, and again in 1921–1922. The results may be briefly recounted.

Methods

Males and females caught with a hand net in the evenings of the July and the August full moon are kept in separate dishes. In the laboratory as shortly after capture as possible a male and

a female are placed in a finger bowl of clean sea-water. After copulation and egg-laying the animals are removed from the finger bowl. After the jelly has been extruded by the eggs, the supernatant sea-water is poured off, leaving the eggs in the mat of jelly stuck to bottom of the bowl. At first cleavage, which is invariably one hundred per cent., the jelly-mass is gently broken up and the eggs equally distributed among seven to ten finger bowls of clean sea-water. Early the next morning the sea-water is changed. At this time all eggs that possess fewer or more than four oil drops, one in each macromere, are discarded. Only those larvæ that possess four oil drops evenly distributed among the four macromeres give rise to normal swimming larvæ. As the trochophores rise to the surface in each dish they are pipetted off. Trochophores that fail to swim at the surface in twenty-four hours lack the viability of those that rise earlier.

The young larvæ are kept in subdued light a few in each dish because they tend to aggregate in such dense masses that many die off. This tendency to collect in one spot makes it easy to change the water and thus avoid too great rise in temperature, which is fatal to the animals. The larvæ will reach the stage of three swimming segments without the addition of food.

When the segments appear, the larvæ must now be watched very carefully in order that food may be given at the proper time. *The criterion for the initial feeding is the complete disappearance of the oil drops from the entoderm cells.*

In the eggs of both *Nereis* and *Platynereis* there is at the time of fertilization a girdle of some eighteen to twenty-two oil drops in the equatorial zone. These oil drops in the maturation stages following insemination move to the vegetative pole. During cleavage the number of oil drops is reduced to four large globules which normally are distributed to the cells of the gut. Beginning with the third or fourth day after laying, the oil in the gut cells of the larvæ begins to form an emulsion of smaller and smaller drops. It is thus possible to follow the history of the oil drops very fully in these creatures that make veritable living test tubes in a fat-digestion experiment. If food is given the worms before the oil has been completely used, they are killed in large numbers. On the other hand, food must not be withheld too long after the disappearance of the oil. The first feeding consists of ten c.c. of a diatom culture known by previous examination under the microscope to be free of metazoa or larvæ,

strained through three thicknesses of bolting silk of very fine mesh. As the larvæ add segments more food is given. When the larvæ build their tubes both food and mud are added until the bottom of the dish is well covered. The method of preparing the diatom culture may now be considered.

In 1911 I procured food according to the method described by Hempelmann in his study of the life history of *Nereis dumerilii* at Naples. This method consists principally in scraping the growths from the live tables. The bottom and sides of aquaria under running sea-water frequently show a felt-like growth of diatoms and protozoa. Scrapings from such aquaria suspended in sea-water will give food sufficient to keep a few young worms of both *Nereis limbata* and *Platynereis*. The method does not allow the rearing of any large number of worms. Similarly, attempts at a pure culture of diatoms gave poor results.

In 1913 through the kindness of Dr. Caswell Grave I procured a remarkably fine culture of diatoms from Beaufort, N. C. With this, the first sexually mature worms were obtained. But obviously, *Platynereis* at Woods Hole must live on food got in Woods Hole waters. I, therefore, made various attempts to get an adequate diatom culture from the immediate vicinity. The successful method follows.

At the beginning of the season mud is taken from Eel Pond, near-by flats, or scraped from eel grass, together with animal and plant life. This is placed in jars with the addition of an equal volume of sea-water. The jars are then covered with glass plates and set aside in subdued light. In a day or so all metazoa—worms, crustacea, ascidians, etc.—are dead. After a period of putrefaction, the culture purifies itself and a rich growth of diatoms begins.

For young worms a suspension of diatoms strained through several thicknesses of bolting silk is used. The diatoms for this purpose are previously examined under the microscope, one c.c. at a time; usually no metazoa are found. The suspension is then made up in filtered sea-water. As the larvæ increase in size and vigor food is added in greater quantities.

A brief summary of the three cultures of *Platynereis megalops* reared to sexual maturity may now be given.

The Larval Cultures

The 1911 and 1912 larvæ were not kept after September first.

The 1913 Culture.—During August, 1913, from eggs laid in

the laboratory by twenty females over 100,000 larvæ were reared. On the eve of my departure from Woods Hole the larvæ were all carefully removed from their tubes and placed in half-gallon Mason jars. Each jar contained 500 c.c. of the rich Beaufort diatom culture and sea-water to within ten centimeters of the top. The jars were then tightly covered and set aside. After the worms had had time to build new tubes, the jars were shipped to Washington, D. C. The worms at this time averaged about 10 mm. in length. Early in June, 1914, these worms were shipped from Washington to Woods Hole. Relatively few survived the journey. The largest worms (females), brought carefully in a hand bag, died before the journey was half made. Since during the winter hundreds of these worms had been killed periodically for future study, the number left from the 1913 culture had been greatly reduced. Some animals of this culture were carried through the summer of 1914. They never reached sexual maturity. They were taken back to Washington at the end of 1914. In 1915 they were brought back to Woods Hole and returned to Washington that fall. During this period they still showed no change.

In Washington the animals were kept in the clamped jars without any change of water or additions of distilled water. One culture was kept in a battery jar covered with a glass plate. Nor was any addition ever made to this culture. The jars were kept at room temperature in subdued light. To avoid contamination worms removed for study were from the culture in the battery jar only. After observing the worm I never replaced it, but killed it for *in toto* mount or sectioning.

In 1917 several very fine cultures of worms were started, but they died in transit to Washington. In 1918 and 1919 no worms were reared.

The 1920 Culture.—In August, 1920, very beautiful cultures of about 50,000 larvæ were started: unfortunately, the majority of these died very suddenly late in August. About a thousand worms survived. These were distributed among twelve dishes with food and left over winter in the heated laboratory at Woods Hole. The dishes were left covered with glass plates exposed to north light. No change was made in the water or any additional food given during the period September 1, 1920, to June 1, 1921. On May 17, 1921, about 200 worms of different sizes were found in the dishes. Of these some were preserved from time to time.

The history of the others shows that the first female with ripe eggs appeared June 5. She was discovered slowly crawling around the dish near the surface of the water. In color and in form this animal resembled the females collected during the breeding season. In size she was rather below the average and somewhat more sluggish. The eggs in size, color and form were identical with the eggs got from animals captured during the breeding season. Subsequently, mature females were found at intervals through the summer. No males appeared until late in June. Eventually, thirty-two females and twelve males fully mature were got from this 1920 culture.

Males got from the sea copulate with females reared in the laboratory; such females lay normal eggs that give rise to larvæ of a high degree of viability. Males reared in the laboratory copulate with females taken from the sea. The eggs are perfectly normal. On only one occasion did I find a male and female from this 1920 culture sexually mature at the same time. They copulated in normal fashion. The eggs laid were normal in every respect and gave rise to larvæ that I kept for two weeks before discarding. These larvæ could not be distinguished from larvæ resulting from eggs laid by animals taken from the sea.

The 1921 Culture.—At this writing only two mature animals (females) have appeared—one May 1 and one May 6, 1922.[1]

The Rate of Growth

Some idea of the rate of growth in these worms may be obtained from data collected from the 1913 culture. This was the only culture on which I had the opportunity to make continuous observations.

Life History

Observations so far made on cultures of *Platynereis megalops* reared in the laboratory from eggs laid by animals taken from the sea do not reveal any indication of a sexually mature intermediate form. So far, all eggs obtained appear to be identical with those got from animals in nature. This would seem to suggest that the life history of *Platynereis* is simple—without the complexity of form and sexual condition found in *Nereis dumerilii,* which *Platynereis* so closely resembles. It must be clearly

[1] Since the above was written, 23 animals have reached maturity—17 females and 5 males. One reason for this sex ratio is that the males have difficulty in getting out of their tubes; their mortality is therefore high.

stated, however, that on this point the observations so far made are not conclusive. In order to determine fully that the eggs laid by worms in the cultures in the laboratory are from the same worms started in the culture and not from an intermediate form and are the only eggs laid, it would be necessary to make continuous observations on isolated worms. So far it has not been feasible to do this, since it would mean practically continuous residence at Woods Hole through the winter.

TABLE I

RATE OF GROWTH OF PLATYNEREIS MEGALOPS FROM EGGS LAID ON THE EVENING OF JULY 21, 1913

Date	No. of Segments with Parapodia	Length in Mm.
July 28, 1913	3	
July 29	4 to 5	
July 30	5	
July 31	6	
August 2	10	
August 6	16	2
August 8	22	4
August 12	26	5
August 26		7
Sept. 15		14
Oct. 1		18
Nov. 1		20
Dec. 1		25
Jan. 10, 1914		30
Feb. 2		33
March 1		40–45
April 3		40–50
May 6		50–60
May 28		40–50

On the other hand, it is just barely possible that in a state of nature the life history is more complex than in the laboratory cultures. Under operation of changes in such factors as density of the sea-water, food, and temperature, the life history of the worms may be modified. That this possibility deserves some consideration we may conclude from the sex ratio, if such meagre data will allow. In the laboratory cultures females appeared first in all three years and they outnumber the males. In nature just the reverse is true.

Whatever our conclusions as to the interpretation of these observations, it seems to the writer that the life history of this interesting nereid is worthy of further study.

A Comparison with Other Forms

The method used for rearing sexually mature *Platynereis* from the fertilized egg has been used to rear other worms through to the adult stage: namely, *Pectinaria gouldii, Diopatra, Nereis limbata,* and *Chætopterus.* In all cases the worms were reared from eggs cut out of the females and inseminated in sea-water. In no case were the worms kept beyond September 15 (from one to three months). Though it is usually stated that artificial insemination of *Diopatra* eggs is not possible, every attempt made by the writer in 1911, 1912, 1913, 1914 and 1915 was successful. There is one danger to avoid with these eggs—initiation of development by mechanical shock. The worms reared from *Diopatra* eggs are if anything more hardy than those of *Platynereis.* In 1913 I reared *Diopatra* in a watch glass to a length of four centimeters.

Pectinaria gouldii are likewise readily reared from eggs inseminated in the laboratory. These eggs are extremely beautiful, small, and almost wholly transparent. They are easy to handle. I have found them the best eggs in my experience for study under high power (oil immersion lenses).

The specimens used were from the Eel Pond and are normally smaller than *Pectinaria* found outside of Eel Pond. They are infested with a distome and an interesting ciliate; the latter I did not find in the larger specimens (1911). This, if it be generally true, together with the size of the Eel Pond specimens makes an interesting case from the point of view of ecology.

Among the shed spermatozoa of *Pectinaria* are many in bundles that break up after a short time in the sea-water. In addition to these one can always get bundles of spermatocytes, immature sperm, etc., by puncturing the body wall. It is a very excellent form to use for the study of cytoplasmic inclusions: it is possible to get the whole history of the sperm on one slide.

My object in studying these ova was to try to learn if size, opacity, and yolk influence the ease with which the animals can be reared under laboratory conditions. I found no correlation. Thus, the egg of *Platynereis* is almost transparent; it measures 180–200 μ. *Nereis* egg has more color and measures about 100 μ. The *Nereis* egg is the hardest of all to carry through. The egg of *Pectinaria* is small and almost wholly transparent. It is readily reared. The *Chætopterus* egg has more color than that of *Nereis* and is smaller. It is easier to rear than the egg of *Pectinaria.* The *Diopatra* egg is wholly opaque; it is the largest