10-1-1914

Breeding Habits Of The Heteronereis Form Of Platynereis Megalops At Woods Hole, Mass.

E. E. Just

[Reprinted from Biological Bulletin, Vol. XXVII., No 4, Oct., 1914.]

BREEDING HABITS OF THE HETERONEREIS FORM OF PLATYNEREIS MEGALOPS AT WOODS HOLE, MASS.

E. E. JUST.

Verrill ('73) first described *Platynereis megalops* figuring in his "Report" a male of the heteronereid phase. Later ('79) he figured the nereis-form and the female of the heteronereis-form changing the name he first gave, *Nectonereis megalops*, to *Nereis megalops*. Andrews, who ('91) had discovered the egg *Nereis limbata*, in a paper on the eyes of annelids speaks of the worm as *Nereis alacris*. I am indebted to Dr. J. Percy Moore who identified the animal as *Platynereis megalops*, Verrill. The belief seems to prevail that in the study of the cell lineage of *Nereis* ('92) Wilson indiscriminately used the males and females of *Nereis* and *Platynereis*. But this belief is by no means founded on any statement in Wilson's paper. Bonnevie ('08) has perhaps strengthened popular misconception through her descriptions of the "two varieties" of *Nereis limbata* at Woods Hole.

I. Swarming Habits.

The swarming of *Platynereis* is closely similar to that of *Nereis* (cf. Lillie and Just). There seems to be some variations as noted below. The behavior shows as that of many other forms a definite lunar periodicity: the sea-urchins (Tennent), the Japanese palolo (Izuka), the Pacific palolo (Woodworth and others), the Atlantic palolo (Mayer), *Amphitrite* (Scott), *Nereis dumerilii* (Hempelmann), etc.

Observations were made during the seasons of 1911, 1912 and 1913 at the Marine Biological Laboratory, Woods Hole, Mass. The swarming habits of *Platynereis* have not been worked out as fully as have been those of *Nereis limbata* (Lillie and Just, '13). In the first place during 1911 and 1912 attention was focused mainly on the swarming habits of *Nereis;* moreover, at all times the primary object in the collecting of *Platynereis* was for experimental study. Strict watch, however, was kept on these worms throughout the summers named. This is especially true for the summer of 1913; during June, July, and August I went out every night, giving attention wholly to *Platynereis* swarming.

The animals on swarming nights swim near the surface of the sea, the males invariably appearing first, the females later. The females rarely exceed fifteen, and indeed on some nights no females swim, while the number of males may be very large. Verrill ('73) says that the worms swim at noon. I have never noted this.[1] Hempelmann ('11) might lead one to think that *Nereis dumerilii* swarms early in the morning. I looked for this during August, 1913, but did not find *Platynereis* swarming before or at sunrise. The evening swarm may last two hours.

The small reddish males swim with great rapidity in an ever more narrowing circle within the patch of light thrown by the observer's lantern until the swarm is at its height. Here and there often at a greater depth than the males swims with slow and even laborious movements, the larger female, pale yellow in color with a thin dorsal line of green—the remnant of the empty gut. One cannot but suspect that the sex ratio in some way depends on the rate of movement: the females are easy prey for fish, the males must easily escape their enemies. The sex ratio of the captured animals must be also influenced by the fact that the females tend to keep further below the surface than the males. This is true of *Autolytus* to a marked degree as I have repeatedly observed. (So too, Andrews, '92, and Mensch.) Verrill, however, says of *Nereis limbata* that in their burrows "there are few males in proportion to the females"—as in the case of *Platynereis*, the reverse is true of these worms during swarming.

As the male comes in the vicinity of female he swims very rapidly in spirals tangential to the surface. They swim together and after copulation and egg-laying, the female slowly sinks from view.

The swarming occurs nightly throughout the months of July and August during the dark of the moon. From new moon to full moon, whether there be moonlight or not the animals do not swarm. Only mature animals swarm.

[1] In July, 1914, I found spent males swimming during the day.

I have never taken this Heteronereid at Woods Hole earlier than June 29. In 1911 I remained at Woods Hole until September 18; I took no worms after August 24. For 1913, August 19 is the date of last capture.

The following tables selected from data of 1911, 1912 and 1913 give some idea of the lunar periodicity of the swarming:

TABLE I. 1911.

(Date of first capture, July 20.)

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	August 8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
	16	1	6
Third Quarter	17	0	0
	18	0	0
	19	0	0
	20	0	0
	21	4	5
	22	6	6
New Moon	23	8	8
	24	10	10
	25	0	0
	26	0	0
	27	0	0
	28	0	0

Comparison with *Nereis* shows in the first place that the number of worms swarming is not so great. It was found, for instance, in collecting *Nereis* to be practically impossible to make an accurate estimate of the number of males; for that reason a record was kept of the females only. On two or three nights only did I find it impossible to estimate the number of *Platynereis* males swarming; on other evenings it was easily possible to count them. The swarm of males on the evening of August 11, 1912, was wonderful. For a few minutes the sea was alive with thousands of the rapidly swimming Heteronereids. In 1913 there was a similar swarm of females, but in no such numbers. As in the case of *Nereis* the collections were made in one place during the three years.

The season, moreover, appears to be shorter than that of

Table II. 1912.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 4 to July 2	None	None
	July 3	0	1
	4	0	0
	5	0	0
	6	0	0
Third Quarter	July 7	0	0
	8	0	0
	9	0	0
	10	1	3
	11	3	3
	12	3	3
	13	0	2
New Moon	14	2	2
	15	0	2
	16	0	1
	17	0	0
	18	0	0
	19	0	0
	20	0	0
First Quarter	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	2	1
	26	1	0
	27	0	1
Full moon	28	0	0
	29	0	0
	30	15	30
	31	2	30
	August 1	4	30
	2	3	30
	3	0	20
	4	0	0
Third Quarter	5	8	20
	6	14	20
	7	0	0
	8	12	10
	9	10	50
	10	12	100's
	11	15	1000's
New Moon	12	18	30
	13	0	0
	14	0	0
	15	3	6
	16	0	0
	17	0	0
	18	0	0
First Quarter	19	0	0
	20	0	0
	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	0	0
	26	0	0

TABLE II. 1912.—*Continued.*

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	27	0	1
	28	0	1
	29	0	0
	30	0	0
	31	0	0

TABLE III. 1913.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 13–28	None	None
	29	0	1
	30	0	0
	July 2	0	1
		None	None
	17	0	1
Full moon	18	0	10
	19	0	30
	20	0	20
	21	1	25
	22	0	10
	23	0	8
	24	2	4
	25	50	30
Third Quarter	26	2	4
	27	5	3
	28	16	20
	29	5	8
	30	20	16
	31	21	8
	August 1	6	6
New Moon	2	1	20
	3	4	25
	4	0	16
	5	0	0
	6	0	1
	7	0	0
First Quarter	8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
Full Moon	16	0	2
	17	1	0
	18	0	1
	19	0	2
	20	0	0
	21	0	0

Nereis. In this my observations approximate those of Verrill. Also, the yearly swarming shows more variations than that of

Nereis. This is strikingly brought out by a study of the tables—especially when one recalls that I gave attention wholly to *Platynereis* for the year 1913. Curves of the runs of *Platynereis* would show that the heights tend to fall in with those of *Nereis*. The lunar periodicity is therefore more like that of *Nereis limbata* than that of *N. dumerilii* which in some respects *Platynereis* resembles.

II. Egg-Laying.

Males and females caught with a hand-net in the evening at the surface of the water and kept in separate dishes may be studied in the laboratory. If a male be transferred with a female to a dish of clean sea-water, the phenomena observed in the sea may be readily followed. The female packed eggs discernible through her pale thin body wall swims slowly in a straight line; or, with head bent at right angles to the body describes a circle of which the head is the center. The male swims in spirals tangential to the surface of the water. Soon his spirals are along the course of the female, her body finally becoming the long axis of his helical body. He entwines the female through this performance and straightens out, thus clutching her in the twist of his body. If this embrace be in the posterior region of the female's body, the male loosens slightly and pulls himself along the female's body. The task appears to be exacting. Often I have observed a rather small male that had worked himself forward after having grasped an unusually large female near the anal segment fall apparently too exhausted to complete the courtship. As the male slips along forward over the female, he lashes his tail back and forth. The female bends her head as if seeking the tail. If the female keep her body in a straight line, the male must move anteriorly until he entwines her body in the pharyngeal region. He now forms a coil around her head of which his tail is the apex. He thrusts his tail down into the coil of his own body and so into the waiting jaws of the female. The female is quiescent throughout. About six seconds after the female has received the anal segment of the male, the animals separate and eggs stream from the posterior segments of the female. The male may be held for a time by the female; if so he swims around, dragging her. I believe that the eggs escape, not through gonopores or the like, but through lesions of the body wall (cf. Scott.) Eggs escape from three or more posterior segments, occasionally from anterior segments. If escape by way of the posterior segments be experimentally inhibited, or if the female be slightly disturbed, the eggs seem to burst through the body wall at segments more anterior than otherwise. Females killed at the moment of oviposition show tears in the body wall.

After oviposition—and the whole process just described is in general the event of ten seconds—the female sinks to the bottom of the dish, a mere shred. In the laboratory placed in a little water it remains an irritable sticky mass for a time—incapable of exciting fresh males and finally dies, greatly shrivelled and blackened. Often, however, if flooded with fresh sea water it revives, expands to previous size, and swims around actively, almost perfectly transparent. I have kept these spent females alive for several hours. Since there are no sexual segments as in some annelids, but the whole body is little more than a locomotor ovary, it seems safe to assume that this egg-laying marks the end of the worm's existence.

Both animals must be in healthy condition for this behavior. Active males sometimes grasp females which because of rough handling in capturing are doubtless weak and fail to respond. The active males on the other hand are not very hardy: in the laboratory they rarely live twenty-four hours; one experiment made in 1913, failed to show any difference in the vitality of spent and unspent males. Normal females when placed in dishes with males fail to complete the courtship if the vitality of the male as by rough handling be impaired. Males and females may be kept in the same dish until death; if there be no courtship, there is no oviposition. Female *Platyneris* and male *Nereis* show no excitement when in the same dish, so male *Platynereis* and female *Nereis*. The male *Platynereis* ordinarily will embrace only an unspent female *Platynereis*. But on one occasion (July 23, 1913) all (8) males captured in turn and repeatedly embraced a *Nereis virens* eight inches long whose posterior segments had been lost. Once only I saw a male clutch a female which had extruded part of her eggs after a previous courtship.

The animals will go through this courtship when placed in a very dense suspension of India ink in sea-water; or total darkness. The reaction, therefore, cannot be due to sight. It is more likely due to some chemical emanation from the gravid female only since the spent female is not attractive to the male (Cf. F. R. Lillie on *Nereis*, '12, '13.)

A male *Platynereis* will embrace at least four females. On the evening of August 24, 1911, for instance, I put a male and a female in a dish. They swam around for a time, then the male wrapped himself about the female just back of the head, he let go, uncoiled himself, his tail remaining in the female's mouth. Immediately after release, he was placed with a second female; a minute later he induced oviposition. After intervals of five minutes he embraced a third and fourth female. In all cases the worms shed eggs. The male placed in fresh sea-water with an active female after an hour (11 P.M., about two hours in the laboratory after capture) failed to make a fifth clutch. Other males embraced two females. During 1912 and 1913 these observations were verified.

If after this egg laying behavior, both animals be removed from the dish or if the eggs be pipetted off as laid the eggs develop and normal swimming larvæ much like those of *Nereis limbata* result. If at the moment of her release by the male the female be put in a dish of clean fresh sea-water, eggs will stream out and subsequently develop.

In all these cases sperm are attached to the vitelline membrane within a hull of jelly which has been secreted through the breakdown of the cortical protoplasm of the egg. As in *Nereis* this jelly formation begins at the moment that the sperm touches the membrane. In *Platynereis* it is easily demonstrated that the inseminated eggs have this jelly when laid. Mechanical pressure either by the male, experimentally, or otherwise, as has been repeatedly demonstrated, will not induce oviposition. Mere clutching however recurrent—even by more than one male is not sufficient stimulus for oviposition. The head of the worm may be crushed—eggs will not escape; if she be cut in two, a few eggs escape. Only after thorough drying on filter paper or on sheer dry linen will the eggs burst through the body wall. If the female be finely minced in sea-water practically all the eggs may be procured. But eggs got in this way do not develop after insemination; they will not fertilize in sea-water. I have sections of uninseminated eggs killed after having remained upwards of two hours in sea-water; the cortical layer and the germinal vesicle are intact. (So *Nereis*.)

Eggs removed from worms after clutching only have the appearance of eggs from unembraced females—no sperm attached, subsequently no trace of development. Sperm are not found on the female's body (*e. g.*, hypodermic impregnation: cf. Whitman, Gardiner, etc.) or near the anus at the time of egg extrusion.

It appears, therefore, that mechanical stimulus is not sufficient to excite oviposition or sperm shedding. The eggs are not laid during or after the embrace nor are sperm shed unless the male's tail has been in the female's jaws. This, then, is a case of copulation followed by internal insemination. And indeed, the very elaborate and precise behavior indicates this. The sperm swallowed by the female inseminate the eggs in the body cavity, oviposition following immediately.

In 1911 gravid females before and after copulation were killed in Meves fluid but proved too refractory for cutting; in 1912, special precautions were taken. The following fixatives were used: Bouin, Gilson, 10 per cent. formalin, and Hennings mixture. With these mixtures the yolk and oil of the eggs are dissolved out, but the chitin of the jaws still makes the procuring of good sections difficult. In 1912 I thought that I had solved the difficulty when after experiments with various agents I procured with KCl, and KCN in sea-water eversion of the pharynx. But in 1913, these methods gave very indifferent results. Dissection of the jaws gave almost negative results. My best sections are those of July, 1912, killed in Gilson, Series A; those of August, 1912, killed in formalin, Series B, and those of 1913 kept in formalin for five months.

Sections of gravid females killed before courtship show no sperm in the body cavity. Sections of gravid females just after copulation show sperm among the antennæ, in the mouth, in the pharynx, and in the body cavity. The sperm may be traced, therefore, entering the mouth, passing down the pharynx whence they escape through lesions in the pharyngeal wall to the cœlom.

They may be found also attached to the vitelline membrane of the eggs. If one minces a male, one procures not only sperm but large numbers of corpuscles. Apparently, these are not injected into the female's body (cf. Scott on *Amphitrite*).

Since the mechanical pressure of the male, though often repeated, is not sufficient stimulus for egg-laying, it may be assumed that either the sperm or some secretion with, or of them stimulates in the female movements which bring about oviposition. In some cases males after having induced oviposition in two or three females cause egg-laying in a third or fourth as noted above. A slight amount of this substance, therefore if such there be in addition to the sperm themselves, is sufficient to initiate egg-laying. The injected substance, on the same ground could scarcely exert sufficient pressure to stimulate oviposition.

I had projected for 1913 various experiments to determine this point. The first experiment on the list, however, was clear enough to warrant abandoning the others. I put a female in a dish with no water. If a drop of sea-water be put on her head there is no response. Only complete drying causes breakdown of body wall. If instead of pure sea-water the minced female be added there is no response. But if a drop of minced male be added oviposition follows. This observation was made several times.

The following protocol from notes of the night of July 25, 1913, is typical:

Experiment.—Six males cut up in water adherent to their bodies (*i. e.*, not dried). Dried female put in this sperm suspension. No oviposition. A second female placed in the sperm suspension; and a third. No oviposition.

2. Three males cut up in three drops of sea-water. Two successive females used. No oviposition.

3. Six dried males cut up. Two dried females placed with heads in the sperm suspension. Eggs laid. Next day: trochophores.

4. Three males cut up in two drops of sea-water. Two dried females placed with heads in the sperm suspension. (Both females later copulated with males and laid eggs.) No eggs laid.

Oviposition, then, is clearly brought on through the ingesting of sperm with very little sea-water.

Nereis diversicolor O. F. Muller gives birth to living young. *Autolytus* (Agassiz) carries its larvæ in a brood pouch. In both of these forms there is probably internal insemination. Eisig has described copulation in an annelid, *Capitella*. *Platynereis* is of interest in that oviposition so quickly follows copulation.

III. The Nereis Form of Platynereis Megalops.

As in the case of *Nereis* an attempt has been made to rear the larvæ of *Platynereis*. Best results were obtained during 1913. A table was kept of the development of the young worms and their characteristics noted. They closely resemble the larvæ of *Nereis dumerilii* described by Hempelmann which he obtained from his cultures. Some of my worms aged six months measured four centimeters. It is hoped that a study of these forms will give a clue to the swarming habit.

LITERATURE REFERRED TO.

Agassiz, A.

'62 Alternate Generation in Annelids and the Embryology of the *Autolytus cornutus*. Boston Journal Nat. Hist., Vol. 7.

Andrews, A. E.

'91 Report on the Annelida polychæta of Beaufort, N. C. Proc. U. S. Nat. Mus., Vol. 14.

'92 Eyes of Polychaetous Annelida. Jour. Morph., Vol. 7.

Bonnevie, K.

'08 Chromosomenstudien. II. Heterotypische Mitose als Reifungscharakter. Arch. für Zellforsch., Bd. 2.

Eisig, H.

'87 Fauna und Flora des Golfes von Neapel. Monographie 16: Capitelliden.

Gardiner, Ed. G.

'98 The Growth of the Ovum, Formation of the Polar Bodies, and the Fertilization in *Polychoerus caudatus*. Jour. Morph., Vol. 15.

Hempelman, Fr.

'11 Zur Naturgeschichte von *Nereis dumerilii* Aud. et Edw. Zoologica, Bd. 25, Lief 1 (Heft 62).

Izuka, Akira.

'03 Observations on the Japanese Palola, *Ceratocephale osawai*, n. p. n. sp. Jour. Coll. Sci. Imp. U. of Tokyo, Vol. 17.

Lillie, F. R.

'12 The production of Sperm Iso-agglutinins by Ova. Science, N. S., Vol. 36, No. 929.

'12 Studies of Fertilization, V. The behavior of Spermatozoa of *Nereis* and Arbacia the special reference to egg-extractives. Jour. Ex. Zool., Vol. 14.

Lillie, F. R., and Just, E. E.

'13 Breeding Habits of the Heteronereis Form of *Nereis limbata* at Woods Hole, Mass. Biol. Bull., Vol. 24.

Mayer, A. G.

'08 The Annual swarming of the Atlantic Palolo. Publication 102, Carnegie Institution of Washington.

Mensch, P. Calvin.

'00 Stolonization in *Autolytus varians*. Jour. Morph., Vol. 16.

Scott, J. W.

'09 Some Egg-laying Habits of *Amphitrite ornata*, Verrill. Biol. Bull., Vol. 17.

Tennent, D. H.

'10 Variation in *Echinoid Plutei*. A study of variation under laboratory conditions. Jour. Ex. Zoöl., Vol. 9., No. 4.

Verrill, A. E.

'73 Report upon the Invertebrate Animals of Vineyard Sound and the Adjacent Waters, with an Account of the Physical Characters of the Region. U. S. Com. of Fish and Fisheries, Part I. Washington.

'79 New England Annelida, Part I. Historical Sketch with Annotated Lists of the Species hitherto recorded. Trans. Conn. Acad. Arts & Sci., Vol. 4.

Whitman, C. O.

'91 Spermatophores as a Means of Hypodermic Impregnation. Jour. Morph., Vol. 4.

Wilson, E. B.

'92 Cell Lineage of *Nereis*. Jour. Morph., Vol. 6.

Woodworth, W. Mc.

'07 The Palolo Worm, *Eunice viridis* (Gray). Bull. Mus. Comp. Zool., Vol. 51.