Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part II. On the Gonad of Egg-Spawning Individuals.

By

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With 12 Figures in the Text.

In an earlier publication (**1**, 1927) it has been shown that female-functioning oysters (*O. edulis*) normally begin to develop spermatozoa with great rapidity immediately after extruding a batch of eggs. Thus, while the extruded eggs are developing in the mantle cavity of the oyster, spermatozoa are developing at the same time within the body of the oyster. In these circumstances sperm develop so quickly that a few hours after the extrusion of eggs small clumps of characteristic spermatocytes can be detected in the gonad either in the fresh material or in microscopic sections. Within about 14 hours from the extrusion of ova, relatively large clumps of spermatocytes are easily recognisable in preparations of the fresh gonad ; while within about 45 hours the gonadial tubes become filled with maturing sperm, and in some cases sperm-morulæ may already occur. After $3\frac{1}{2}$ days from egg-spawning the percentage of gonads containing ripe sperm rapidly increases, until at about $8\frac{1}{2}$ days after the extrusion of ova—when the larvæ which the oyster is carrying have become fully developed veligers ready to be extruded—sperm development may have proceeded so far that the gonadial ducts themselves become filled with ripe sperm-morulæ as shown in Figure 1, p. 316. The rates of development quoted above are averages for those occurring in English oyster beds ; but are with little doubt mainly dependent on the prevailing temperature.

After an oyster has extruded its larvæ sperm-production continues for a few weeks, but begins to wane about one month after egg-spawning (see **1**, Table XII, p. 1035, 1927). Two months after the last egg-spawning (if this has occurred in early- or mid-summer) sperm-production has usually ceased ; the gonad passes into a quiescent condition (in British waters and

under normal conditions) and a period of "fattening," or the accumulation of reserves, usually follows. If egg-spawning occurs late in the summer, the sperm subsequently developed may be retained over the winter period. A full discussion of such cases will, however, be given later when data can be presented.

In this paper figures are given to illustrate successive stages of the gonad after egg-spawning, and also the state of the ripe female gonad in the spawning condition. As the condition of the gonad can usually be easily and quickly ascertained by an examination of a fresh preparation of the gonad, figures of the microscopic fields obtained from fresh preparations

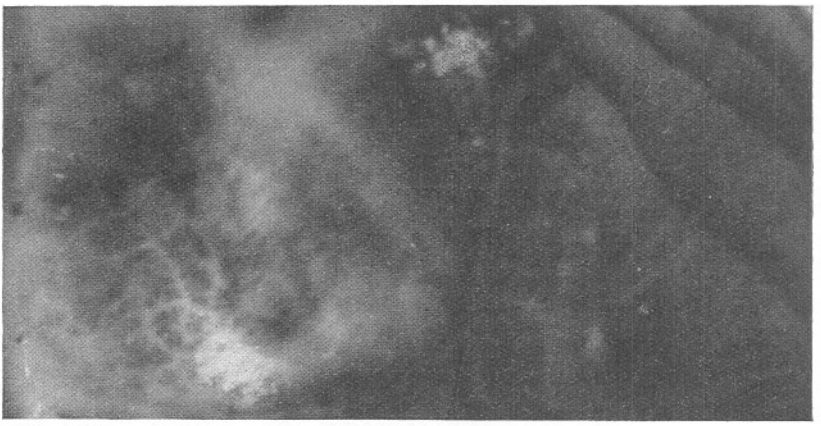

Photo. A.J.S.

FIG. 1.—View of the visceral mass of a recently black sick oyster with gonoducts already filled with ripe sperm-morulæ.

(Photo of the living animal by Mr. A. J. Smith.)

are given as well as those of microscopic sections. In order to obtain a fresh preparation of the gonad of the oyster a cut may be made in the visceral mass near the pericardium or dorsally near the loop of the intestine which encircles the stomach (see **2**, Plate XII). In this way is avoided a possible incision of the main gonoducts which lie mostly superficially. When it is obvious that the gonoducts are empty, the visceral mass may be cut across entirely. When the cut is made a microscope slide is pressed gently against the cut in order to collect a little of the fluid which is exuded. This drop of fluid is examined at once under the microscope without a cover-slip, and without adding sea-water; it is advisable to try several samples until familiarity with the method is obtained. To test the ripeness of the sperm, sea-water may afterwards be added, when—if the preparation has not been allowed to dry—it will be found that ripe sperm-morulæ break up into separate active sperm on the slide.

In Table I, p. 318, are given full details of the series of figures shown herein with the correlated states of development of both the embryos (or larvæ) carried by an oyster, and the sperm-morulæ within the body of the same individual.

The arbitrary periods, A, B, C–F, are defined in **1**, p. 983, and the arbitrary categories I, III–X, are shown in **1**, Table II, p. 981 ; arbitrary periods G to J are defined *loc. cit.* on p. 1022. The significance of the correlation between the arbitrary period and the arbitrary categories associated in Table I herein can be seen at a glance in **1**, Table XII, p. 1035.

ACKNOWLEDGMENTS.

One of us (C. A.) takes this opportunity to thank the Council of the Marine Biological Association for the kind hospitality extended to him to work in its laboratory ; and to express his gratitude to Dr. E. J. Allen and Professor D. M. S. Watson for their encouragement and help.

Our thanks are due to Mr. A. J. Smith for the photograph for Fig. 1.

SUMMARY.

Illustrations are given of successive stages of the gonadial products of the European oyster (*O. edulis*) after the act of egg-spawning. Both microscopic and fresh preparations are figured.

REFERENCES.

1. ORTON, J. H. 1927. Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part I. The Change from Female to Male. Journ. Mar. Biol. Assoc., N.S., Vol. XIV, No. 4, pp. 967–1045.
2. ORTON, J. H. 1924. An Account of Investigations into the Cause or Causes of the Unusual Mortality among Oysters in English Oyster Beds during 1920 and 1921. Part I. Fish. Invest., London, Ser. II, Vol. VI, No. 3, 1923 (1924).

TABLE I.

CORRELATED STATES OF DEVELOPMENT OF SPERM AND EMBRYO IN INDIVIDUALS OF *O. EDULIS*, ILLUSTRATED HEREIN.

	Condition of eggs, embryos or larvæ.	Approx. age of embryos or larvæ.	Date and locality.	Arbitrary period.	Sex-cells in gonad. sperm-morulæ ripe.	sperm-morulæ unripe.	ripe ova.	Arbitrary category.	Description of illustration.
Fig. 2	Ripe eggs; from female in act of spawning.	0 hours	22 June, 1926 Truro beds	A	none	none	∞	I	Section of gonad.
Fig. 3	1 and 2-celled embryos	2 ,,	31 May, 1927 West Mersea	A	none	fair no. very young	few spots	III	Section of gonad microscopic field.
Fig. 4	1 and 2-celled with 2 to 5 nuclei	2½ ,,	30 Aug., 1929 R. Yealm	A	none	few up to 40μ	∞ in patches	III	From living gonad microscopic field.
Fig. 5	10% in 32-celled stages	20 ,,	14 Oct., 1927 Truro beds	B	none	fair no. up to 60μ	f. ∞ in spots	IV	From living gonad.
Fig. 6	about 64-celled stages	46 ,,	16 Sept., 1929 R. Yealm	C	none	∞ ca. 60 μ	none	IV	Microscopic field from living gonad.
Fig. 7	Ciliated embryos with trace of shell	3 days	1 June, 1927 West Mersea	D	none	∞ large nearly ripe	rare	IV	Section of gonad.
Fig. 8	White-sick; larval shell about 100μ	4½ ,,	31 July, 1930 R. Yealm	E	few to fair no.	∞	rare	V	Microscopic field from living gonad.
Fig. 9	Black-sick; fully shelled larvæ	6–8 days	8 Oct., 1926 Truro beds	F	∞	∞	none	VI	Section of gonad.
Fig. 10	Black-sick; larval shell 200μ	,,	25 July, 1929 R. Yealm	F	∞	∞	none	VI	Microscopic field from living gonad.
Fig. 11	Post-sick oyster	estimated 1–2 months	16 Sept., 1929 R. Yealm	H or I	∞	none	none	VIII	Microscopic field from living gonad.
Fig. 12	Post-sick oyster	ca. 50 days	10 Sept., 1930 R. Yealm Spawned 31.7.30	H	few or fair no.	none	none	IX	Microscopic field from living gonad.

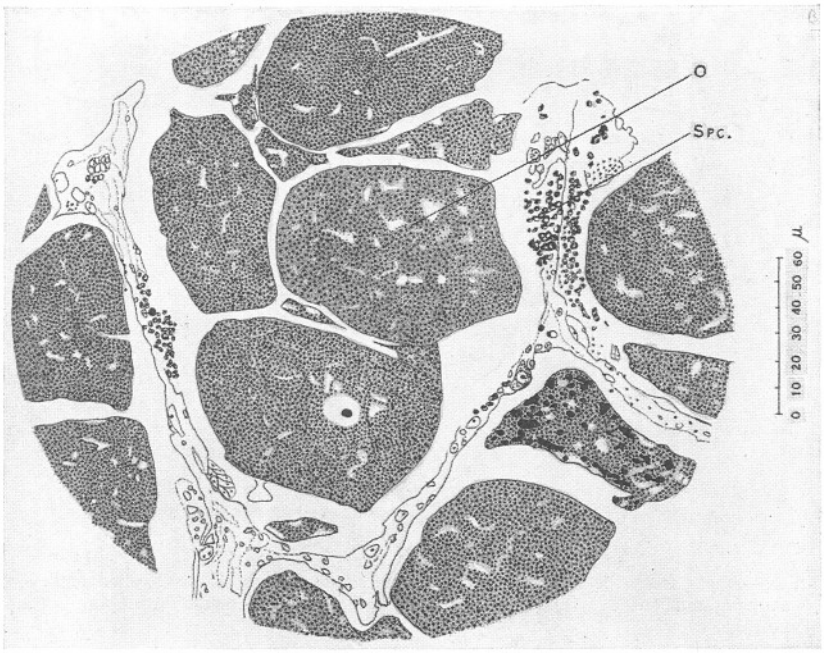

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Fig. 2.—Microscopic section of the gonad of an individual of *O. edulis* caught in the act of spawning, 22 June, 1926. O. mature ova filling the gonadial tubules. Spc. gametogonia destined to become spermatocytes.

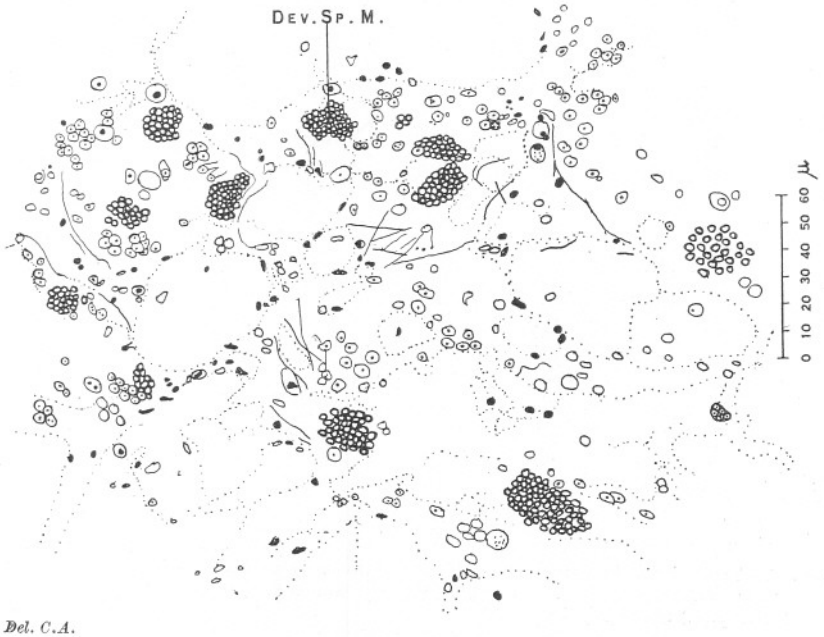

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Fig. 3.—Microscopic section of gonadial tubules of *O. edulis* carrying 1 to 2-celled embryos showing very young developing sperm-morulæ. (Dev. Sp. M.)

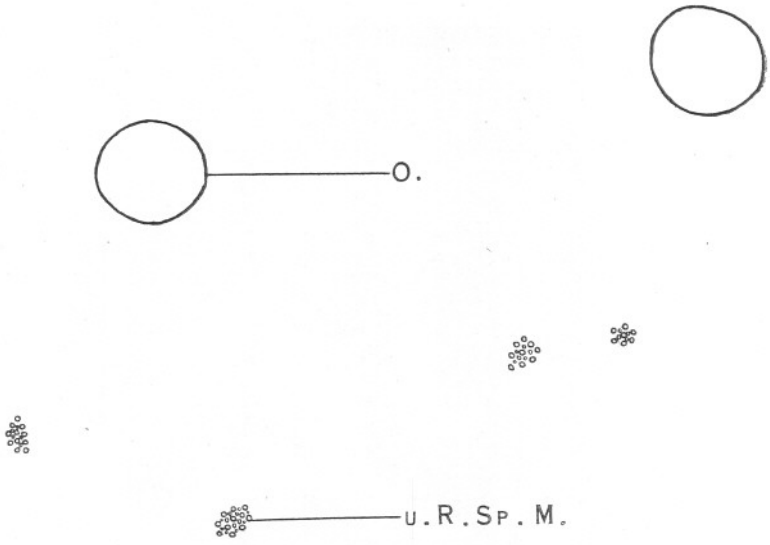

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FIG. 4.—Smear from the living gonad of an *O. edulis* carrying 1 to 2-celled embryos, showing unspent ova, O (about 150μ in diameter), and small developing sperm-morulæ.

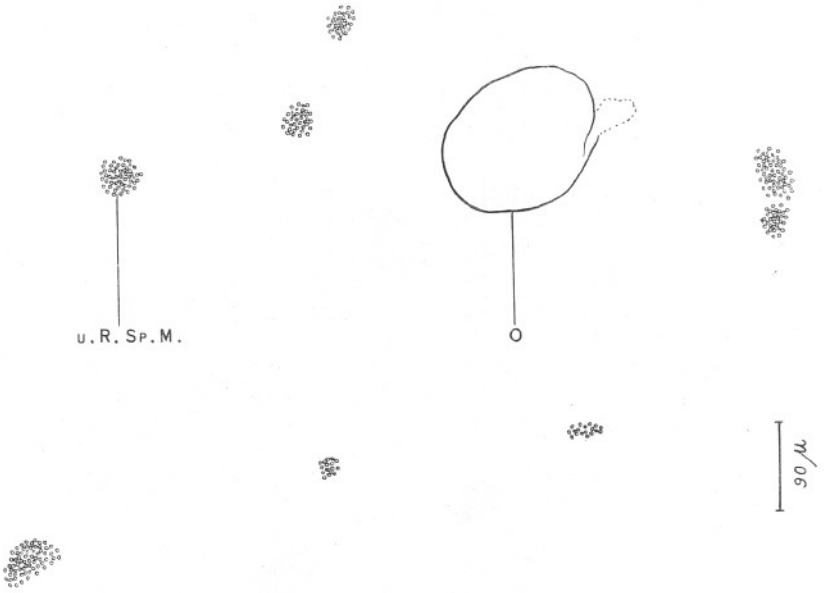

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FIG. 5.—Smear from the living gonad of an *O. edulis* carrying embryos in about 32-celled stages, showing unspent ova, O, and fairly numerous developing sperm-morulæ (u.R.Sp.M.) about 60μ long.

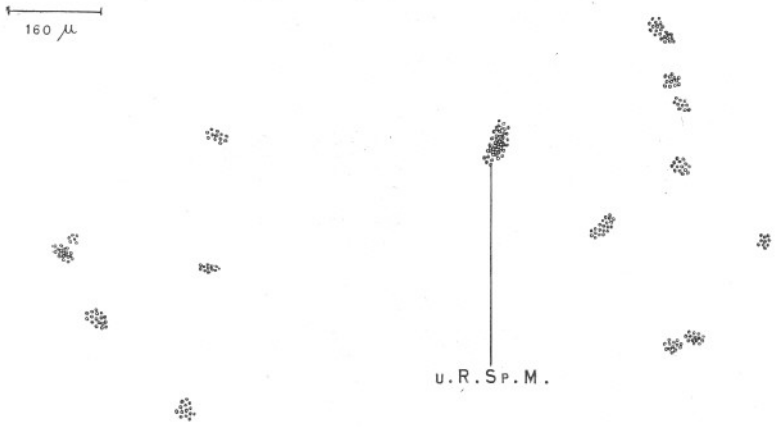

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FIG. 6.—Smear from the living gonad of an *O. edulis* carrying 64-celled embryos, showing numerous large developing sperm-morulæ (u.R.Sp.M.) about 60μ long.

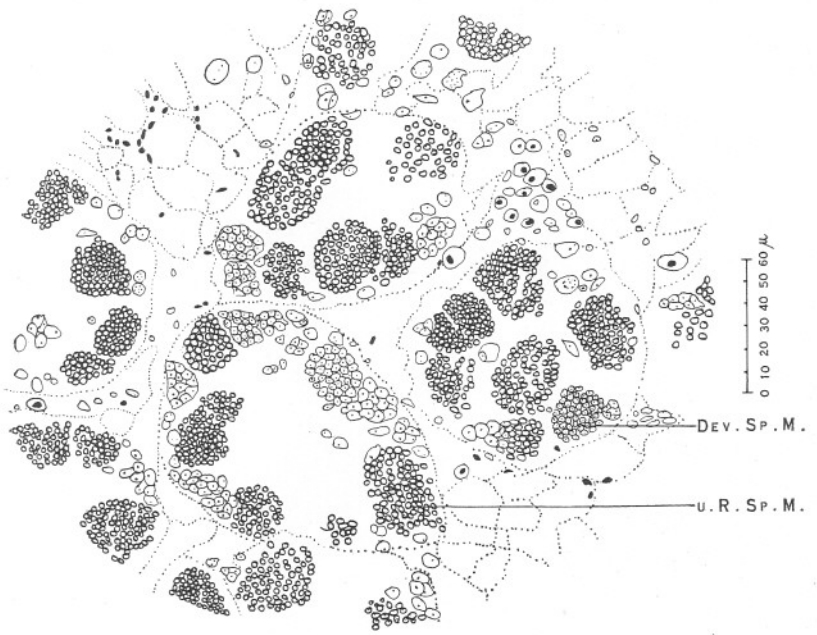

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FIG. 7.—Microscopic section of gonad of *O. edulis* carrying embryos which are ciliated and show a trace of the developing shell, showing numerous large and almost ripe sperm-morulæ (u.R.Sp.M. and Dev.Sp.M.).

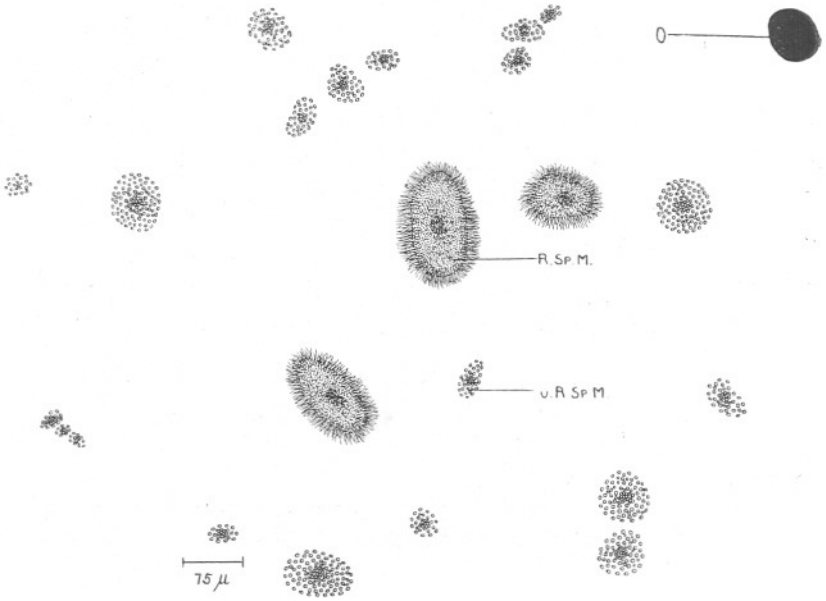

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FIG. 8.—Smear from the living gonad of a grey-sick *O. edulis*, i.e. carrying embryos with half-developed shells and no pigment in the digestive organ, showing numerous large developing sperm-morulæ (u.R.Sp.M.), a fair number of ripe sperm-morulæ (R.Sp.M.), and a degenerating ovum, O.

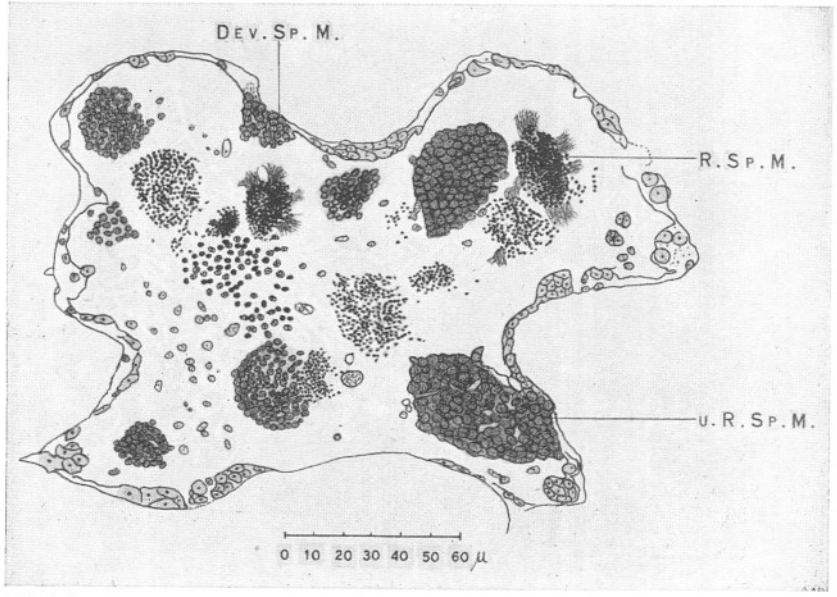

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FIG. 9.—Microscopic section of gonadial tubule of a black-sick oyster carrying fully-developed larvæ (i.e. shells 200μ long) and showing ripe (R.Sp.M.) and large developing sperm-morulæ (u.R.Sp.M.), and also younger masses (Dev.Sp.M.) *in situ*.

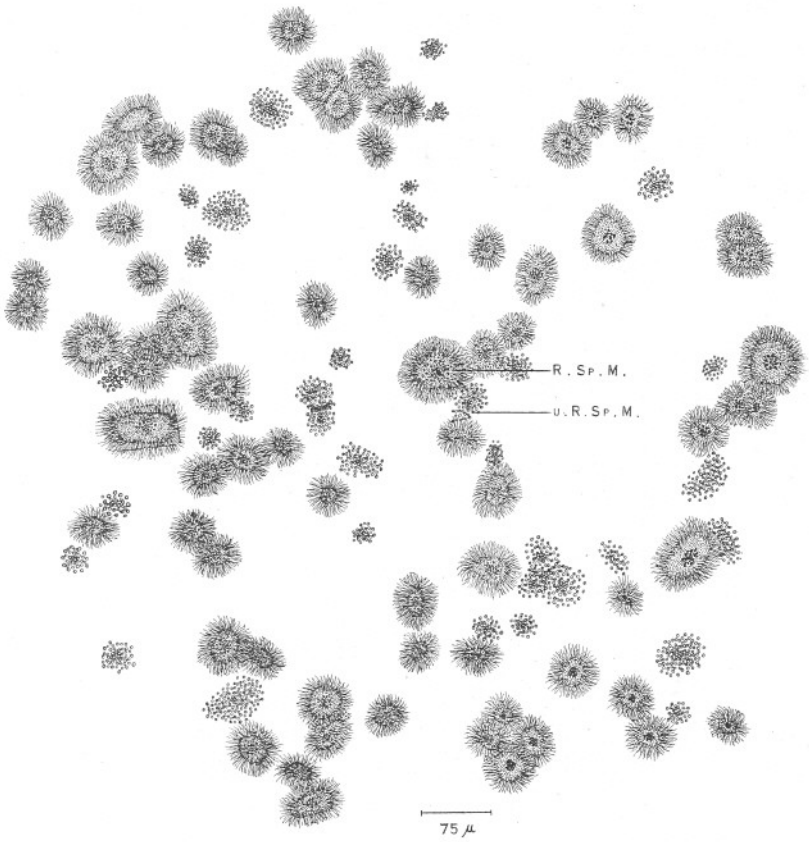

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FIG. 10.—Smear from the living gonad of a black-sick oyster, showing numerous ripe (R.Sp.M.) and numerous large unripe sperm-morulæ (u.R.Sp.M.) in a small portion of the microscopic field.

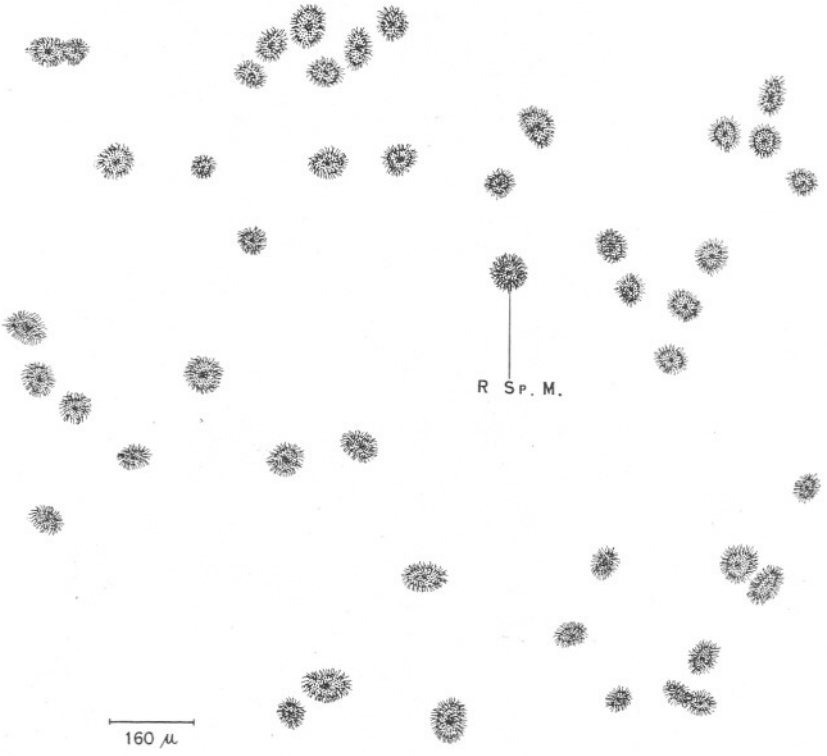

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FIG. 11.—Smear from the living gonad of a post-sick *O. edulis* (identified from seasonal studies in sex-change) showing numerous ripe sperm-morulæ only.

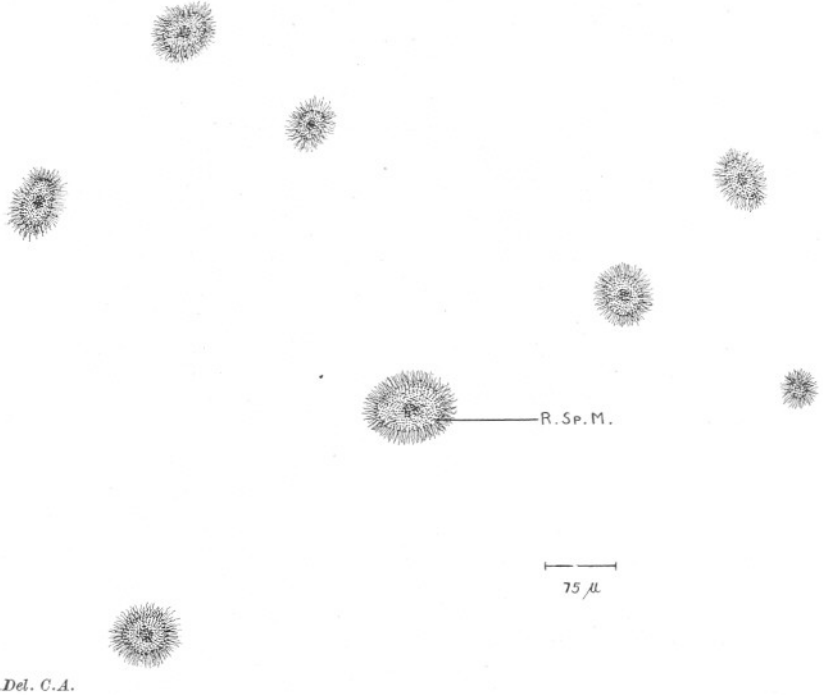

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FIG. 12.—Smear from the living gonad of a post-sick *O. edulis*, showing a fair number of ripe sperm-morulæ only. (White-sick 31 July, 1930 ; kept in tanks afterwards at Plymouth until 10 September, 1930.)