

THE PHYSIOLOGY OF MATURATION AND
FERTILIZATION IN *POMATOCEROS*
TRIQUETER (L.) I. THE NATURE
OF THE MATERIAL

By J. B. Cragg, M.Sc.

Assistant Lecturer in Zoology, University College of North Wales, Bangor

(Text-figs. 1-5)

While investigating the physiology of maturation and fertilization in the marine worm, *Pomatoceros triqueter*, it soon became evident that there was great variability in the reactions of the material, not only among the egg batches from different worms but even in the eggs liberated from one worm. For accurate interpretation of the results it was important that some knowledge of this variability should be obtained. This paper is therefore devoted to the individual and seasonal variations shown by the material.

MATERIAL

The animals were collected from the north side of Cullercoats Bay. The principal sources were the "Gut" and the hollow close to the north breakwater.

During the course of the work over 3000 worms were examined, the sex ratio being about five females to one male. The distribution of the sexes appeared to be haphazard, but the material was selected to give large numbers of eggs, large specimens forming the greater number of those examined. A statistical study of different size classes might show a reversal of sex in the animal or a size difference between the sexes.

METHODS

The material for the studies on seasonal variation, carried out between September 1934 and September 1935, was collected the day prior to or on the morning of the experiment. For other work, apart from certain special cases to be referred to later, the material was never kept in the aquarium for more than three days before use.

To collect the germinal products the stone on which the animal had formed its tube was first washed with tap water, the tube chipped away, and the worm removed. If a female, it was quickly rinsed in tap water, then in Berkefeld filtered sea water, and finally placed in the dish of sea water or experimental solution in which the eggs were required. Since liberation proceeds very rapidly once the tube is broken, the above steps must be carried out quickly.

Practically complete liberation occurs within 10 min. of opening the tube. Uniform crystallizing dishes about 5 cm. in diameter and in depth were used for the experiments. No special precautions were taken to control the temperature, but records were kept throughout each experiment. The pH of the experimental solution was determined colorimetrically before and after each experiment.

Previous workers have usually paid little attention to the accuracy of results obtained from egg counts, although Fuchs (1915) working on *Ciona* drew attention to the possible errors associated with egg counts and the desirability of making check counts. The chief source of error in the present work was the difficulty of obtaining a uniform mixture of eggs for distribution to the various solutions. The best method was to shake the eggs thoroughly in 25 c.c. of solution and then by a rotary motion collect them in the centre of the dish. A fine pipette was then used to transfer the eggs. In filling the pipette eggs were collected from different regions of the egg mass and approximately the same number of eggs was present in each transfer. Counts were made in the dish, not by transfer to a slide which in itself introduces a source of error. Unfertilized eggs were fixed by the addition of formalin after a lapse of $4\frac{1}{2}$ hr., for then practically all eggs capable of maturing had done so. Results had sometimes to be based on counts of only 300-400 eggs. The degree of accuracy of the method as tested by repeated countings was $\pm 3\%$ for mature eggs and fertilization stages and $\pm 10\%$ for immature oocytes.

Males were transferred immediately to about 5 c.c. of sea water instead of being washed in tap water. After liberation of the sperm the worm was removed and the mixture shaken. When more than five batches of eggs had to be fertilized the method advised by Fuchs for making a sperm suspension proved more satisfactory; that is, to dilute the original suspension and ensure thorough mixing by pouring the liquid from one vessel to another about twenty times. Fertilizations were made by adding one or more drops of sperm suspension and fertilized batches were fixed 5 hr. after the addition of sperm.

THE FORM OF THE EGG

When shed from the worm the eggs are primary oocytes (Fig. 1) and they are usually light red in colour. Each egg possesses a large, clear germinal vesicle, the diameter of which is half that of the egg. A large asymmetrically placed nucleolus is present in each nucleus. The cytoplasm is finely granular, with the granules evenly distributed. The egg is surrounded by a vitelline membrane 2μ in thickness, the surface of which is covered by a system of fine furrows or indentations. By cytolysing eggs in distilled water the membrane was shown to be very tough. When liberated the eggs are usually irregular or flattened. The mean diameter of flattened eggs, based on measurements of 50-100 eggs, varies with the batch. In the majority it is $65-70\mu$, but eggs giving a mean diameter of over 80μ have been recorded.

A varying percentage of the eggs liberated, depending on the ripeness of the batch, mature. This change is characterized by the breakdown of the germinal vesicle, the egg becoming purple-red in colour and approximately spherical. The mean diameter of such eggs was 63–68 μ . A comparison of the measurements of flattened primary oocytes and mature eggs indicates the marked variation in the form of the primary oocyte (Fig. 2). The change from primary oocyte to mature egg took place mainly in the 4 hr. following liberation; longer intervals in sea water without fertilization resulted in cytolysis. The common cytolytic form superficially resembled the blastula stage of a fertilized egg. The earliest time for the appearance of these "pseudoblastulae" was 6 hr. after liberation, but their formation depended on the ripeness of the material. Ripe material, that is a batch of eggs giving a high percentage of mature eggs, would show complete cytolysis 24 hr. after liberation. In an unripe batch this was delayed, some fertilizable primary oocytes remaining 80 hr. after liberation.

These observations do not agree with the description of the material given by Hörstadius (1923). He considered that the primary oocytes were flattened and that, after breakdown of the germinal vesicle, the long diameter decreased and the breadth increased but the egg did not become spherical. The flattened forms are, however, not of one shape. Only extremely flattened eggs agree with Hörstadius's description of the primary oocyte. In no case did the mature egg resemble the stage described by Hörstadius. In the first place, it tended to become approximately spherical. Secondly, from both the figure and the description it should have shown a prominent perivitelline space; this could not be observed. By treating mature eggs and even fertilized eggs with hypertonic sea water the withdrawal of the egg protoplasm from the vitelline membrane could be watched, giving clear evidence that no perivitelline space existed before or after fertilization.

Hörstadius also stated that the eggs underwent a reduction in volume on reaching maturity. In view of the large variation in shape, such a conclusion cannot be arrived at from measurements of the long and short diameters of primary oocytes and mature ova.

Under the conditions of liberation which existed in these experiments the

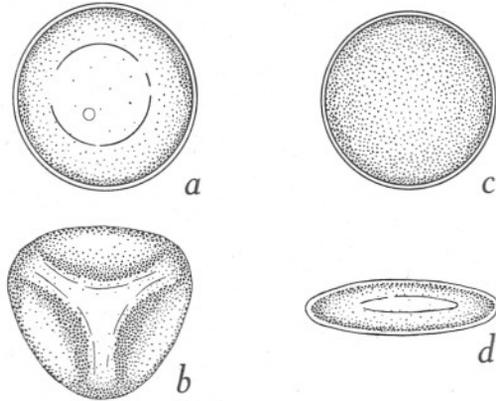


Fig. 1. *a*, flattened primary oocyte viewed from above, showing the germinal vesicle with excentric nucleolus; *b*, misshapen primary oocyte, this is the usual appearance of eggs after liberation; *c*, an egg which has matured; *d*, flattened primary oocyte viewed from the side, this corresponds to the form described by Hörstadius.

eggs were accompanied by a varying number of immature oocytes. The smallest of these are colourless, but as size increases the colour approximates to the light red of the primary oocyte. They can, as a rule, be distinguished from primary oocytes by a zone free from granules beneath the vitelline membrane.

The rounding off of eggs has been attributed to a change in permeability. In both *Asterias glacialis* (Dalcq, 1923, 1924) and *Sabellaria vulgaris* (Waterman, 1936), where such an explanation has been advanced, rounding off occurs immediately on contact with sea water and the germinal vesicles remain

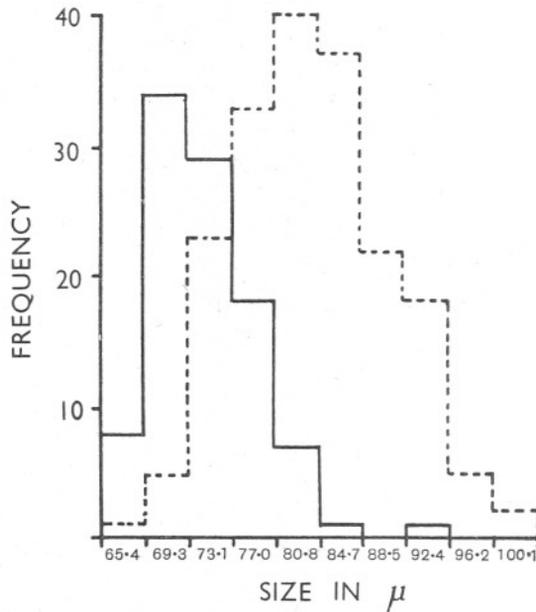


Fig. 2. A comparison of the variations in diameter of primary oocytes with eggs of the same batch which have matured. Mature eggs ———, primary oocytes - - - - -. A marked reduction in diameter is associated with this change and is due to the eggs rounding off. Primary oocytes $82.2 \mu \pm 6.8$. Mature eggs $72.8 \mu \pm 4.7$.

intact. In *Pomatoceros* conditions appear to be different. Mere liberation into sea water is not enough to produce rounding off; some eggs even after 24 hr. in sea water may still be misshapen. Here, rounding off is associated with the breakdown of the germinal vesicle. At first it appeared as though certain eggs with complete germinal vesicles also underwent the change, but, since such eggs were darker in colour than the unchanged primary oocytes, it is probable that the nuclear membranes whilst visible were not intact.

In animals such as *Pomatoceros*, where it is not an immediate phenomenon following liberation into sea water, rounding off can be explained without assuming a change in permeability. Hobson (1932) indicated that the vitelline membrane had elastic properties. If such a membrane were distorted owing

to the combined effects of the eggs being packed tightly in the body cavity and being forced out of a slit-like aperture on liberation, it would tend to return to its normal form unless some opposing force were exerted on it. Such resistance would exist if the egg protoplasm was in a very viscous condition. If, on maturing, the viscosity decreased or movements took place in the protoplasm, the elastic properties would result in the egg becoming spherical. Evidence from other species suggests that such changes might occur. Harris (1935) has recorded marked streaming movements and a large drop in viscosity following the breakdown of the germinal vesicle in eggs of *Sabellaria alveolata*. In *Nereis* (Hoadley, 1934) the internal changes associated with the disappearance of the germinal vesicle are so violent that distortions occur in the shape of the eggs.

THE NORMAL COURSE OF MATURATION AND FERTILIZATION

Maturation is not completed before fertilization. The egg can progress as far as the breaking down of the nucleus, but it must await insemination before the polar bodies are liberated. It is therefore like the majority of annelids, molluscs and echinoderms other than certain echinids.

In ripe eggs, the spermatozoon enters within 5 min. of adding the sperm, and, if it has not already done so, the germinal vesicle breaks down and the egg becomes spherical. Normally no membrane is raised on fertilization, although in exceptional cases a perivitelline space may appear at one or more points. No change was noticed in the attachment of the membrane to the egg surface until the polar bodies were liberated. Then the membrane was stretched in the region where the polar bodies lay and its internal and external limits could be clearly seen. There is nothing to suggest that the membrane is in any way different from the vitelline membrane of the unfertilized egg. An observation which pointed to the polar bodies being attached to the membrane was made when treating fertilized eggs with hypertonic sea water. The egg protoplasm withdrew from the membrane, but the polar bodies remained attached to both membrane and egg surface, being drawn out between them.

The minimum time for the disappearance of the nuclear membrane and the appearance of uniformity of colour in the egg was 6 min. at 16° C. The two polar bodies were liberated at about 30 and 50 min. respectively. There was no reconstitution of the pronucleus and the eggs reached the first cleavage after an interval of about 100 min. These figures are only approximations, for a considerable amount of variation occurs.

VARIATION IN THE MATERIAL

Variations in the physiological condition of the eggs liberated from different worms were often recorded. There was a wide range of variation in the percentage of unfertilized eggs which matured. In fertilized eggs there might

be present at the same time two- to eight-cell cleavage stages. In addition there might be a number of eggs into which sperm had penetrated, but which had not undergone division. It was an exception to find a batch of eggs which proceeded through the various cleavage stages as uniformly as is found in the majority of echinoderms. Evidence of variability was also brought to light in the experimental studies.

Variation in the Degree of Maturity

It was thought that the number of unfertilized eggs which matured would be a measure of the ripeness of the material. An attempt was made, therefore, to see whether the degree of maturity was directly related to fertilizability, as measured by the proportion of eggs undergoing cleavage. The procedure was to fertilize ten batches of eggs with sperm from the same male, an unfertilized control series being kept. These experiments failed to show that such a relationship existed.

Variation in Individual Egg Batches

The egg batch of one worm could be divided up by allowing the worm to remain in one dish only for a short time, after which it was transferred to another, and so on through a series of dishes. In general, it was found that the number of mature eggs was highest in the batches liberated last (Fig. 3). With the gradual rise in the number of mature eggs there was a sharp drop in the total number of eggs and immature oocytes (Table I).

TABLE I. TYPICAL SERIES OF RESULTS OBTAINED BY THE TRANSFER METHOD

The time of transfer given in column 1 is the time of removal of the worm from one dish to the next in the series.

Time of transfer	No. of eggs liberated	No. of immature oocytes	No. of eggs lib./sec.	Immature oocytes lib./sec.
15 sec.	8800	340	587	23
30 "	2400	440	160	29
40 "	780	180	78	18
1 min.	740	60	37	3
2 "	740	30	12	0.5
3 "	780	120	13	2
4 "	800	90	13	1
8 "	1160	100	5	0.4
16 "	1650	150	4	..
16+ min.	1150	200

Such transfer series, by providing fractions more uniform in themselves with respect to maturity than a complete egg batch, allowed further tests to be made regarding a relationship between maturity and subsequent cleavage. These experiments showed that the increase in maturity common in

transfer experiments could sometimes be associated with increased cleavage (Table II).

Whilst the difference in the degree of cleavage between the units of a transfer series might be negligible, it should be noted that on no occasion did a unit with few mature eggs give a cleavage value significantly higher than that given by a unit containing a greater proportion of mature eggs. In the experiments where whole batches were used it was quite common to find that

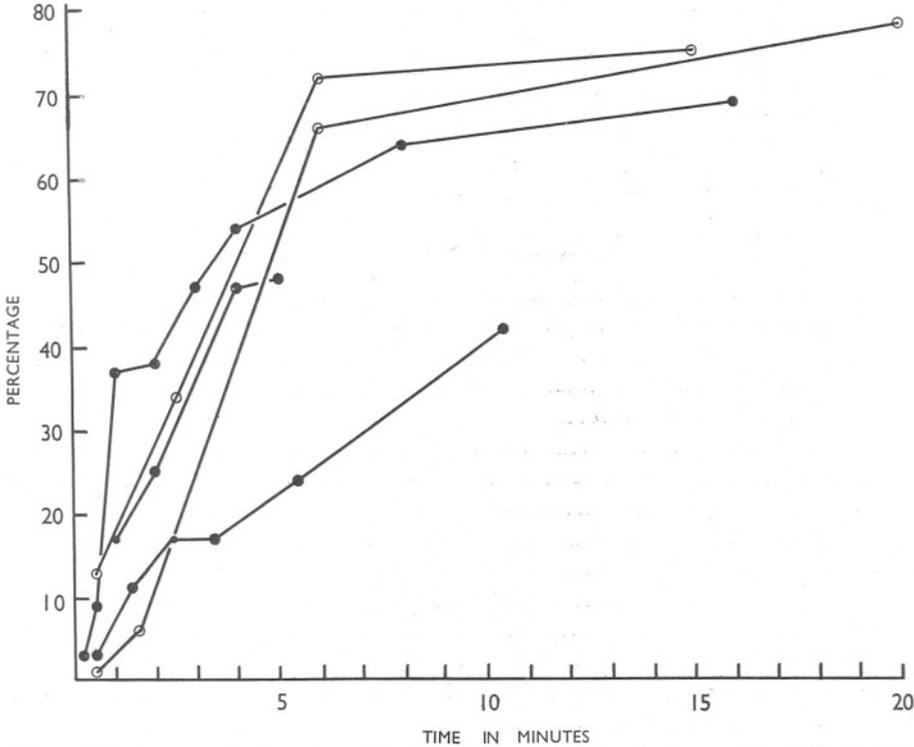


Fig. 3. The increase in the percentage of mature eggs shown when an egg batch is divided up into a number of units by the transfer method. The percentage of mature eggs in a unit is recorded against the time at which the worm was transferred from that unit.

batches with few mature eggs gave better fertilizations than others apparently more mature. It is evident from the difference in behaviour between units in a transfer series and complete egg batches, that it would be possible for fundamental physiological differences to be veiled by utilizing data gained only from experiments using the whole egg batch as a unit.

The mechanism whereby unripe material is liberated first has not yet been elucidated. It may be dependent on the relative positions of the unripe germ cells and the segmental openings. Certainly this liberation of unripe and then ripe eggs does not result from a linear distribution in the body cavity according

to ripeness. For in a series of nineteen experiments in which worms were cut in half, nine showed the anterior region to contain the higher percentage of ripe eggs and four the hinder region.

TABLE II. TRANSFER EXPERIMENTS SHOWING THE RELATIONSHIP BETWEEN MATURITY AND CLEAVAGE

The percentage of mature eggs in the unfertilized sample and the percentage cleavage after fertilization are given for each transfer unit.

	Transfer units					
Mature eggs	0	9	29
Cleavage	25	32	68
Mature eggs	1	2	1	24
Cleavage	8	9	14	24
Mature eggs	4	6	9	9	14	20
Cleavage	49	47	52	51	55	58

NATURAL SPAWNING

In view of the heterogeneity of the eggs liberated when the tube is opened, attention was turned to the possibility of collecting naturally spawned eggs. The aim was to see whether, in natural liberation, the eggs are more homogenous and whether the immature oocytes, which in artificial liberations are discharged first, are conserved.

Stones with one or more worms were kept in separate finger bowls, partially immersed in an aquarium tank and aerated. They were examined daily for signs of spawnings and, since none occurred, were opened after being two months in the aquarium. They contained high percentages of cytolysed eggs and yielded poor fertilizations. In one sample 50% were cytolysed and only 10% of the remainder were capable of fertilization; these, however, gave normal larvae. A characteristic of many of the eggs was the occurrence of vacuoles in the cytoplasm; as many as five were noted in some of the eggs. This condition was also shown by worms which had been kept in the aquarium tanks for the same period. The presence of large numbers of cytolysed eggs was common during the first three months of these artificial conditions. After that, some of the worms showed complete absence of germinal products, others showed a few normal primary oocytes and were quite free from cytolytic forms. After five to ten months a very significant change had occurred. Compared with the fresh material from the shore the egg content was about ten times as great; egg batches of 10,000-40,000 eggs being the rule. This tank material was quite normal and made possible experiments of a size which had hitherto been impracticable. Since all the worms kept in these artificial conditions contained a large store of eggs or sperm and since no natural spawnings were observed, the state must have arisen from continual production with non-liberation.

SEASONAL VARIATION

Very early in the work it was found that the possibility of obtaining a good batch of eggs, from the point of view of numbers and fertilizability, varied during the month. Daily records of maturity and cleavage were kept. In view

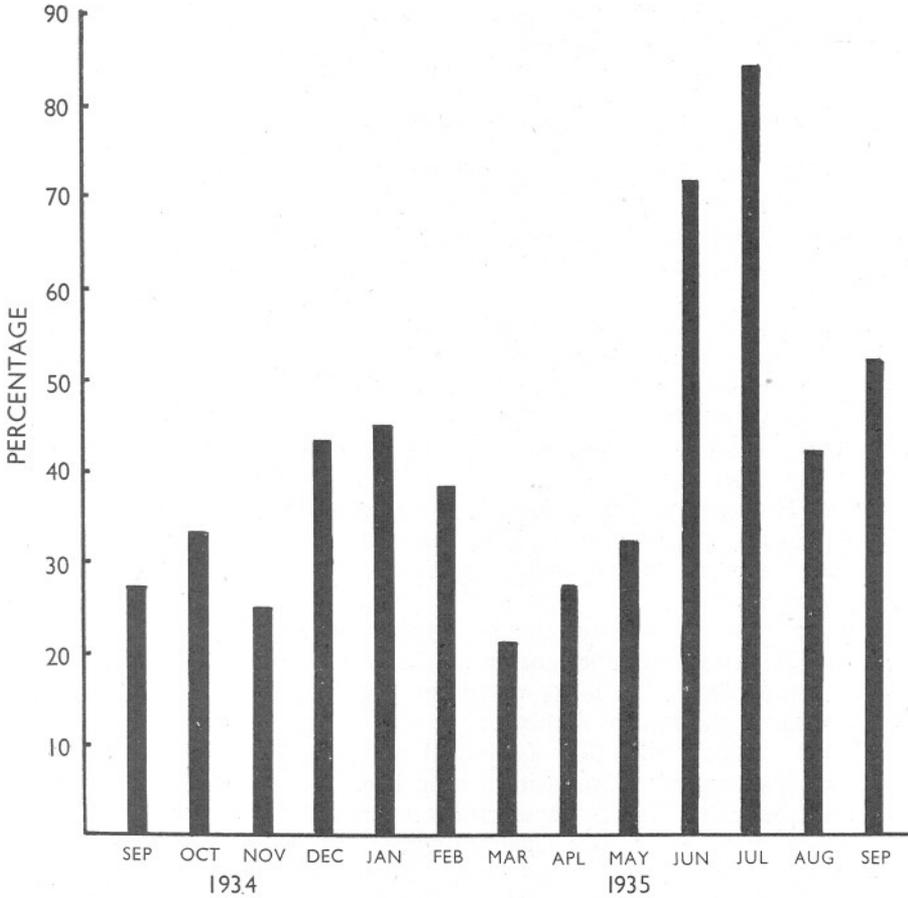


Fig. 4. The variation in cleavage throughout the year, as shown by the percentage of samples giving a cleavage value of or greater than 80%.

of the fact that the results did not reveal any definite periodicity the observations were extended to include the percentage of immature oocytes, the number of eggs, the relative proportions of the different cleavage stages, and the relative numbers of ripe and unripe females.

No definite lunar periodicity was found. The immature oocytes, which were only recorded over a period of three months, showed no signs of a periodicity.

As regards mature eggs and cleavage there was a slight tendency for the highest values to occur about the time of the full moon, but this was not definite. The cleavage results showed a period lying between the full moons of June and July of consistently high values. This June-July deviation cannot be attributed to the existing laboratory or sea temperatures for similar temperature variations occurred at other times. The general trend of the observations suggests it to be a period of special breeding activity, although the possibility of fertilization exists throughout the year (Fig. 4).

VARIATION IN MATURITY

Whilst the data referred to above failed to demonstrate a lunar periodicity, they served a more positive purpose in that the frequency distributions of percentages of immature oocytes and of mature eggs indicated the nature of the change undergone in the transformation of an immature oocyte into a mature egg.

In the maturity distribution (Fig. 5) it will be seen that the major class consists of egg batches containing 0-10% mature eggs. The distribution of immature oocytes (Fig. 5) is that of a skewed normal distribution. The skew is probably the result of the technique, for it was essential to obtain enough eggs for fertilized and unfertilized samples. When the number of immature oocytes was large, in particular over 90%, the material was rarely used. The difference between the two distributions, especially when it is remembered that the mature egg is only the later stage of the immature oocyte, is remarkable. There are two possible explanations for the peculiar form of the maturity distribution.

1. That eggs are liberated as they ripen, the actual time of liberation depending on certain variable conditions. In such circumstances most females opened would show a majority of unripe eggs and the immature oocytes would be fairly constant in number.

2. That complete liberation occurs just prior to maturity, the form of the frequency distribution of maturity arising from the nature of the reactions producing maturity. If the development of maturity were dependent on a series of chemical or physical changes whose rate after initiation gradually increased, then the interval passed by a batch in the 0-10 stage would be longer than that passed in the more mature conditions. Very mature batches would, therefore, be rarely found.

The second hypothesis appears to be the more probable. The frequency distribution of immature oocytes is in keeping with it and militates against the first hypothesis. Also, the total number of eggs liberated show marked fluctuations not in keeping with the idea of gradual liberation.

VARIATION IN MATURITY THROUGHOUT THE YEAR

Table III shows the monthly variations in maturity. There was a gradual decrease in the number of higher maturity groups present as the summer months approached, culminating in July where the 0-10 class predominated. In August the higher classes again increased.

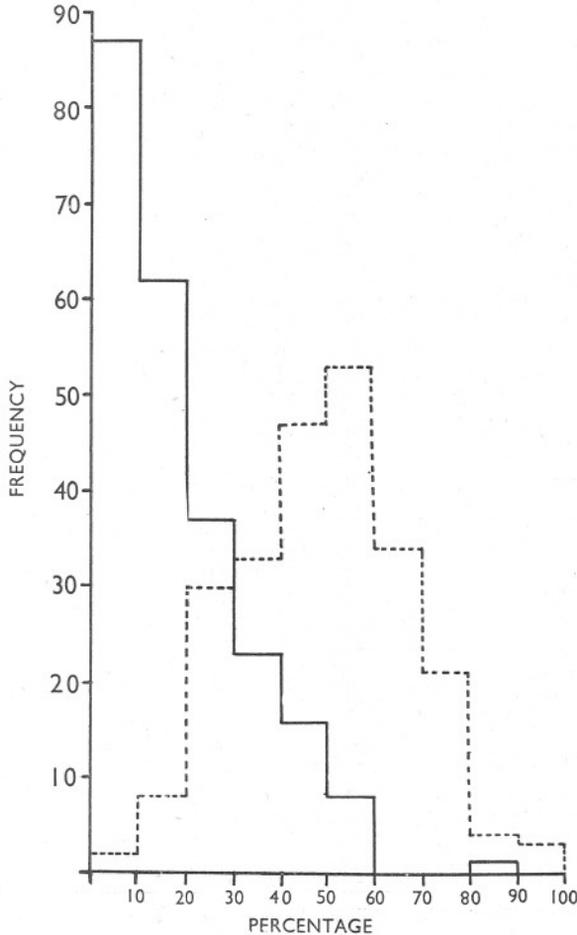


Fig. 5. A comparison of the immature oocytes and mature egg values given by the same material. Mature eggs ———, immature oocytes -----, Data collected June 21 to September 17 1935.

It is improbable that this monthly variation is wholly a result of the individual experiments being carried out at existing laboratory temperatures, which varied throughout the year. This conclusion is supported on the following grounds. Hörstadius has shown that up to 16° C. the percentage

of mature eggs increases with rise in temperature. Above that temperature there was a decrease and at 22° C. 81% of the eggs were deformed. If the present results arose principally from the effect of temperature at the time of the individual experiments, then the data when classified on a temperature basis should show the higher maturity values increasing with rise in temperature up to 16° C. and then decreasing. As will be seen by reference to Table IV, where the data have been reclassified, this is not so. Also, experiments to see the effect of allowing parts of the same egg batch to mature at different temperatures have shown that summer material can develop, without cytolysing, at higher temperatures than winter material (the degree of resistance of eggs to warmth depends on a number of factors and is at present being investigated). It seems reasonable to conclude, therefore, that whilst the temperature at which the experiment is conducted will undoubtedly influence the degree of maturity, it is not the principal factor concerned in producing the variation already outlined.

TABLE III. MATURITY DETERMINATIONS

This table gives the results of all maturity determinations made under standard conditions. The individual maturity percentages are classified in groups differing by 10%, the number of results in each group being expressed as a percentage of the total number of determinations made during the month.

Month	Percentage maturity groups										Total no. of results
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
Dec. 1934	22	15	30	11	15	4	4	..	27
Jan. 1935	19	21	17	15	15	6	4	2	52
Feb.	29	33	19	14	5	21
Mar.	24	51	18	3	3	33
Apr.	25	33	21	4	8	4	4	24
May	38	28	24	7	..	3	29
June	33	28	20	9	2	4	2	2	46
July	52	19	15	2	7	4	80
Aug.	34	24	19	13	9	1	79
Sept. 1-17	24	35	12	16	4	8	49

TABLE IV. MATURITY VALUES CLASSIFIED ON A TEMPERATURE BASIS

The results are classified according to the mean temperatures of the experiments. The number of results in each percentage maturity group has been expressed as a percentage of the total number of results in that particular temperature group.

Temperature C.	Percentage maturity groups										Total no. of results
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
9.9-10.8	32	48	4	4	4	..	4	4	25
11.0-12.9	24	24	22	14	7	4	2	1	90
13.0-14.9	25	24	32	7	5	7	1	..	76
15.2-16.8	27	31	17	13	6	3	1	96
17.3-17.8	45	26	11	6	10	2	62
19.0-21.0	43	23	14	7	9	4	91

On p. 492 a hypothesis has been advanced to explain the form of the maturity frequency distribution. The variation in the monthly classes would

signify an acceleration in the speed of the reactions concerned. Such an increase in the rate of production of ripe eggs would explain the gradual disappearance of the higher maturity values during the summer months.

DISCUSSION

The description of the eggs of *Pomatoceros* given here differs in certain respects from that of Hörstadius. Whilst the explanation might be that the earlier observations were based on a relatively small sample of eggs, the possibility of fundamental differences between the material at Cullercoats and at Kristineberg must be borne in mind.

From the viewpoint of cell permeability shape changes in eggs are of special importance, for it has been usual to treat the egg as spherical when calculating volume change. If the changes recorded here and by Hörstadius were of general occurrence then much of the work done on egg permeability would be vitiated.

The danger of static and dynamic variability leading to confusion in biological work, especially in cytological experiments, has been emphasized (Gray, 1931). Yet quantitative examinations of variability in populations of germ cells are few, although innumerable qualitative references occur in the literature of experimental cytology. A series of investigations by Goldforb has done much to record variability in the sea urchins, *Toxopneustes*, *Hipponeo* and *Arbacia*. The characters studied (Goldfarb, 1917) were size, shape, number of eggs with or without jelly layer, rate of membrane formation, and number and rate of cleaving eggs. These characters were held to be correlated and by knowing certain of them the behaviour of a batch of eggs could be forecast. Goldforb repeatedly asserts that exact forecasts of the physiological condition of eggs could be made, but his results do not always support this view. When studying the variation in agglutinating power of egg waters (Goldforb, 1929), the results given consideration are not the agglutinating times of sperm based on individual samples of egg water, but averages of the individual results. Fundamentally, it is equivalent to using mixed batches of eggs. The individual results (see tables IIa and IIb, Goldforb, 1929) used to give the averages often show marked differences. In one an average of 115% is obtained by averaging 300, 50, 112 and 0% (these figures represent percentage increase in agglutinating time after the egg batches have been kept for a common time). These average results are held to show "... progressive and marked increase in agglutination time with ageing of eggs". Such results indicate that whilst a trend towards "poor" and "good" might be obtained, individual batches could not be classified as such with any accuracy prior to carrying out the experiment.

Grave (1928) working on *Cumingia tellinoides* and Grave & Oliphant (1930) on *Hydroides hexagonis* record variations in the physiological condition of the germinal products, but no attempt at such a definite analysis as that of Goldforb is made.

Dalcq (1928) showed that in *Barnea candida* an increase in maturity could be produced by increasing the concentration of the potassium or calcium ion in the basic physiological solution. The relationship was in the form of a sigmoid curve. The explanation advanced was that the eggs leaving the ovary were not in a uniformly ripened series, but contained a major class which responded to a certain concentration.

Both Goldforb and Grave & Oliphant consider the poorest lot of eggs to be those giving the most rapid disintegration. This criterion is open to question. Grave & Oliphant use as their standard for disintegration two types of cytolysis. One of these, from their descriptions, bears a striking resemblance to the "pseudoblastulae" cytolysis of *Pomatoceros*. Now, in *Pomatoceros*, eggs which mature are the first to give rise to pseudoblastulae. A batch of eggs maturing rapidly after liberation will give rise to the cytolytic form before a batch of primary oocytes, although they need not be in an inferior physiological condition.

None of the work quoted offers any means whereby the effects of variation can be cut out or rendered more manageable. Fry (1936) describes a method for obtaining egg batches of common cleavage times in *Arbacia*. It amounts to carrying out tests on several batches of eggs and finding those with the same cleavage time. Such batches are then mixed and used for experiments. The problem is not so simple in *Pomatoceros*, for in this species it is clearly evident that the degree of variability among batches, especially where comparison is to be made among fertilized batches, is such as to prevent adequate knowledge of the processes leading to maturation being obtained from experiments on the whole egg batch. This variability is present in other marine worms also, for Grave & Oliphant record that for *Hydroides* "The variation in longevity within a single lot of eggs is greater than in the case of other species studied to date". Fortunately in *Pomatoceros* it is possible to divide up the egg content of a female into more uniform parts. The transfer series, as well as reducing the unwieldy extent of static variability, offers a means of studying eggs of different growth periods, which at least are uniform in that they have developed within a common environment. The use of this method can undoubtedly be advanced to the study of other worms. One drawback which exists in its application is the small size of the egg batch.

The problem of natural spawning is important, for it is in terms of the eggs so liberated that the results should be interpreted. In animals like *Pomatoceros* where primary oocytes can be successfully fertilized, it is not essential that naturally spawned eggs should be on the point of maturing. In view of the fact that the worm is capable of giving fertilizable eggs at all times of the year and that no knowledge of communal spawning exists, it is possible that liberation occurs before complete ripeness of the batch. In those circumstances the fertilizable life of the egg would be longer and the chance of fertilization greater than if it were liberated on the point of maturity.

The greater part of this work was carried out at the Dove Marine Laboratory, Cullercoats, during the tenure of a post-graduate studentship awarded by Armstrong (now King's) College, Newcastle-on-Tyne. I wish to acknowledge assistance given by the staff of that laboratory, especially Prof. A. D. Hobson under whose direction the investigation was made.

SUMMARY

The description of the material used in the present investigation does not conform with that of Hörstadius. Attention is drawn to this lack of agreement.

A hypothesis based on viscosity changes within the egg has been advanced to explain the rounding off of eggs in sea water.

By means of what is called the transfer series the egg batch has been divided into a number of parts; the last liberated eggs being the ripest. The occurrence of large numbers of egg batches giving low maturity values has been attributed to maturity resulting from a series of changes whose rate after initiation gradually increases.

Attention is drawn to seasonal changes in the reactions of the germinal products. No definite lunar periodicity is shown in the ripening of the eggs.

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