QUELQUES PARTICULARITES CONCERNANT LA PERMEABILITE ET LA STRUCTURE DU CYTOPLASME DE L'ŒUF DE PHOLADE *BARNEA CANDIDA*

PAR

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Au cours de recherches ayant pour objet l'étude de l'action des rayons gamma sur la morphologie et la physiologie de la membrane et du cytoplasme ovulaires, travail qui sera publié ultérieurement, j'ai été amenée à observer certaines particularités concernant les réactions de l'œuf de Pholade.

J'avais choisi l'œuf de ce mollusque comme test biologique sur les conseils de M. le Professeur Dalcq, à cause des avantages qu'il présente pour une observation directe sur matériel vivant. En effet, ses petites dimensions et sa transparence parfaite permettent en général de se rendre compte facilement et rapidement de l'action des agents utilisés. De plus, chaque femelle fournissant plusieurs milliers d'œufs, il est possible d'effectuer une expérience complète au moyen d'une seule ponte (ce qui est indispensable, étant données les variations individuelles considérables qui existent d'une femelle à l'autre) tout en utilisant, pour chaque point étudié, un nombre d'œufs suffisant à éliminer les variations statistiques.

La cytologie et l'embryologie de l'œuf de Pholade ont été étudiées en détail par le Professeur Dalcq et son assistant M. Pasteels, et j'ai pu bénéficier de leurs connaissances approfondies dans ce domaine. Qu'ils veuillent bien trouver ici l'expression de mes plus vifs remerciements pour les multiples indications qu'ils m'ont données.

Les animaux utilisés proviennent de la station biologique de Wimereux, et je tiens à remercier chaleureusement M. le Professeur Maurice Caullery et M. Robert Weill, qui ont bien voulu me laisser puiser à discrétion dans leurs réserves. Les frais de transport des animaux, ainsi que leur entretien dans l'aquarium d'eau de mer du laboratoire d'Embryologie, ont été couverts grâce à un subside qui m'a été accordé par la Commission du Radium et la Fondation Nationale du Cancer, auxquelles j'exprime ici toute ma reconnaissance.

I. — OBSERVATIONS MORPHOLOGIQUES

a) Œuf vierge.

Comme l'ont montré Dalcq en 1928 et Pasteels en 1930, l'œuf de Pholade est pondu à l'état d'oocyte de premier ordre et la maturation ne s'effectue qu'après la fécondation. Cependant, la rupture de la vésicule germinative peut se produire, dans une proportion plus ou moins élevée des œufs vierges, sous l'influence des sels de Ca et de K renfermés dans l'eau de mer; dans ce cas, Pasteels a montré que l'évolution de l'œuf s'arrête à la métaphase, soit de la première, soit de la seconde mitose de maturation. Il en résulte qu'après environ une demi-heure de contact avec l'eau de mer, une ponte non fécondée renferme généralement, à côté des oocytes pourvus de leur vésicule germinative, un nombre plus ou moins considérable d'œufs qui se trouvent en métaphase de première ou de seconde maturation; les expériences porteront donc toujours simultanément sur ces trois phases de l'évolution ovulaire et il ne sera pas nécessaire d'avoir recours à des artifices expérimentaux pour comparer leur mode de réaction.

L'examen microscopique direct ne me donnant que des renseignements assez grossiers sur la réaction des œufs (disparition de la vésicule germinative, cytolyse, courbe globale du gonflement dans les solutions hypotoniques) sans qu'il soit possible d'approfondir le mécanisme des phénomènes observés, j'ai eu recours à l'examen à l'ultramicroscope, suivant la technique décrite par Runnström en 1923. Ce sont les résultats obtenus par ce procédé que je me propose de rapporter dans ce travail (¹).

Examiné à l'ultramicroscope, l'oocyte de Pholade se présente sous l'aspect d'une sphère délimitée par une membrane anhiste, renfermant un noyau optiquement vide qui apparaît en sombre et une multitude de particules lumineuses agitées d'un mouvement brownien intense (fig. 1).

Immédiatement au-dessous de la membrane et sans limite de clivage visible, se trouve une couche corticale apparaissant comme une bande lumineuse continue, sans granulations et sans mouvement brownien. Elle est très semblable à la couche corticale décrite par Runnström dans l'œuf d'Oursin; mais elle conserve toujours une teinte d'un blanc jaunâtre et je n'ai jamais pu observer de variations de couleur analogues à celles décrites par cet auteur; cependant, son épaisseur est variable d'une ponte à l'autre et peut être modifiée par les conditions expérimentales. Dans une ponte donnée, son épaisseur est sensiblement la même pour tous les œufs et n'est modifiée ni par la maturation, ni par un séjour prolongé dans l'eau de mer.

(¹) Les expériences à l'ultramicroscope ont été effectuées au moyen du microscope confié par le Fonds National de la Recherche Scientifique au laboratoire d'Embryologie; l'instrument avait été muni à cet effet d'un condensateur cardioïde Zeiss et d'un objectif à immersion 90/1,25, avec iris.

La couche corticale n'existe généralement pas au moment où s'effectue la ponte; elle n'apparaît qu'après un délai variant de quelques secondes à un quart d'heure suivant les pontes; sa formation dépend peut-être de l'influence des sels de l'eau de mer et l'étude systématique de cette action sera effectuée ultérieurement. D'ores et déjà, il est intéressant de rapprocher ce fait d'une observation faite par DALCQ sur l'œuf d'*Asterias glacialis* : la semi-perméabilité de cet œuf n'atteint son état définitif qu'après un contact avec l'eau de mer; celui-ci s'effectue soit à l'intérieur de l'ovaire pendant les derniers jours précédant la ponte, soit directement dans le milieu extérieur lorsque la ponte est provoquée prématurément; dans ce cas, en effet, les œufs fraîchement pondus sont polyédriques, ont un aspect plasmolysé et n'acquièrent qu'ultérieurement leur turgescence normale. Les œufs de Pholade, au moment de leur émission, ont également un aspect irrégulier, et ce n'est qu'après quelques minutes de contact avec l'eau de mer qu'ils deviennent sphériques; la constitution de la couche corticale, qui s'effectue à peu près en même temps, est peut-être régie par les mêmes influences. Les différences observées à ce sujet d'une ponte à l'autre sont probablement dues au degré variable de maturation des différentes femelles.

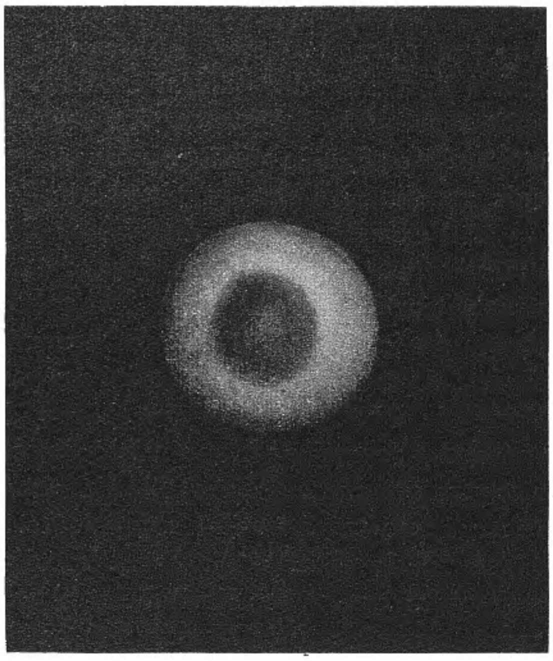
FIG. 1. — Oocyte normal de Pholade.

Les particules qui constituent la masse cytoplasmique sont de deux types différents. Les plus nombreuses apparaissent comme de petites masse sphériques ou polyédriques réfléchissant fortement la lumière diffusée, extrêmement lumineuses par conséquent. Les autres sont plus petites et ont un aspect de bâtonnets très courts; elles apparaissent en sombre, mêlées aux précédentes; elles semblent avoir une tendance à s'accumuler dans certaines régions, principalement dans le voisinage de la vésicule germinative. Quel que soit leur aspect, toutes ces particules sont animées d'un mouvement brownien intense qui semble démontrer que le cytoplasme se trouve dans une phase liquide. Il est impossible de dire si l'on a affaire à des éléments cytoplasmiques chimiquement différents ou s'il s'agit seulement d'aspects réversibles d'un même constituant cellulaire. Aucune structure n'est visible; on n'observe cependant aucun déplacement important des particules et il n'y a pas de courants à l'intérieur du cytoplasme en dehors du mouvement brownien.

La vésicule germinative se détache en sombre sur le fond lumineux du cytoplasme. Elle est située de façon légèrement excentrique, ce qui permet de distinguer dans l'oocyte un pôle animal et un pôle végétatif. La membrane nucléaire n'a pas de structure visible; elle paraît souvent être recouverte d'un revêtement constitué par une accumulation de particules cytoplasmiques sombres; mais lorsque, au cours de phénomènes de destruction de l'œuf, la vésicule germinative est isolée du cytoplasme, sa membrane se montre entièrement dépourvue de particules et son contenu est optiquement vide.

Lorsque, sous une influence quelconque (échauffement de la préparation, action d'une solution hypotonique, action de corps chimiques, etc.), l'œuf se gélifie au cours de l'examen, l'arrêt du mouvement brownien débute toujours au niveau des particules sombres qui avoisinent le noyau : ces particules s'accolent à la membrane nucléaire en un réseau continu, immobile. Après un intervalle plus ou moins prolongé, la région périnucléaire s'immobilise à son tour et la gélification se propage plus ou moins rapidement de la surface de la vésicule germinative vers la périphérie de l'œuf.

A l'exception de la disparition de la vésicule germinative, les œufs qui ont maturé spontanément dans l'eau de mer sont très semblables aux oocytes. La couche corticale a exactement le même aspect. Les particules cytoplasmiques sont animées d'un mouvement brownien dont la vitesse semble être la même. Aucun changement de viscosité ne paraît donc se produire à ce moment chez l'œuf de Pholade, alors que, dans d'autres œufs et en employant d'autres méthodes (centrifugation, microdissection, étude de la plasmolyse), différents auteurs, notamment **Lyon**, **Mc Clendon**, **Chambers**, **Dalcq**, ont admis l'existence d'une modification appréciable de la viscosité. Il convient cependant de noter que le procédé d'observation directe n'est pas suffisamment précis pour pouvoir affirmer avec certitude qu'il ne se produit aucune accélération du mouvement brownien après la maturation; elle serait en tous cas de peu d'importance au regard de celle observée après la fécondation. Mais bien qu'aucune modification du cytoplasme ne soit visible, nous verrons cependant ultérieurement que la réaction vis-à-vis des solutions hypotoniques est considérablement transformée, dans un sens qui indique un bouleversement de la structure.

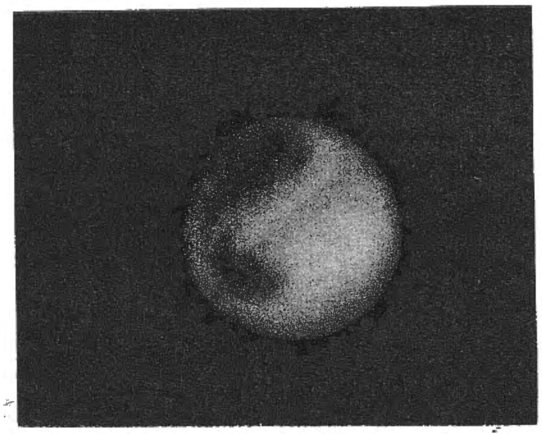

Fig. 2. — Disparition de la vésicule germinative et de la couche corticale après la fécondation.

b) Œuf fécondé.

Lorsqu'on effectue la fécondation sous l'ultramicroscope, on voit se produire rapidement un épaississement de la couche corticale. Le maximum d'épaisseur est atteint en général en moins de trois minutes. Il ne se produit aucun changement de couleur concomitant. Après un quart d'heure environ, la couche corticale disparaît et les granulations cytoplasmiques viennent directement au contact de la membrane (fig. 2). Cette évolution coïncide avec le flétrissement de la vésicule germinative : celle-ci s'allonge et prend une forme en croissant en même temps que sa membrane s'estompe et disparaît. Le mouvement brownien des particules cytoplasmiques est beaucoup plus intense que dans les œufs vierges de la même ponte, ce qui semble correspondre à un accroissement de la fluidité du cytoplasme sous l'influence de la pénétration du spermatozoïde et concorde avec les résultats obtenus par **Heilbrunn** en centrifugeant des œufs d'Oursin vierges et fécondés.

Au bout d'une demi-heure environ, la couche corticale s'est reconstituée; elle est plus épaisse que chez les témoins non fécondés. Lors de l'émission du second globule polaire (environ cinquante minutes après la fécondation), elle atteint son épaisseur maxima. Je n'ai pas suivi son évolution aux stades ultérieurs.

II. — COMPORTEMENT DES ŒUFS EN SOLUTION HYPOTONIQUE

L'étude du comportement des œufs vierges ou fécondés dans les solutions hypotoniques est un procédé d'analyse qui a été fréquemment utilisé avec des résultats extrêmement intéressants. Cette méthode, introduite par **Loeb** en 1892, a été reprise et mise au point par R. S. **Lillie** en 1916; utilisant de l'eau de mer diluée afin de conserver un certain équilibre de balance des sels et de s'éloigner le moins possible des conditions physiologiques, **Lillie** a mis en évidence, chez l'œuf d'Oursin, les différences de perméabilité à l'eau existant entre les œufs avant et après fécondation; il a montré que la vitesse de pénétration de l'eau dans la cellule vivante, en milieu hypotonique non toxique, varie : 1° avec le gradient de pression osmotique entre l'intérieur et l'extérieur; 2° avec la surface de la membrane semi-perméable; 3° avec la perméabilité de cette membrane à l'eau, celle-ci dépendant des conditions physiologiques. En 1918, **Konopacki** a étudié le mécanisme de la désagrégation cytoplasmique sous l'action mécanique de l'afflux aqueux. **Herlant**, en 1920, utilisant la méthode de Lillie, a montré sur l'œuf d'Oursin qu'en utilisant des dilutions suffisamment fortes (75 à 80 %), on observe des différences réactionnelles qui débutent immédiatement après la fécondation ou l'activation et donnent lieu à des courbes cycliques en relation avec les divisions cellulaires. **Dalcq**, en 1923, a confirmé la plupart de ces résultats pour l'œuf d'*Asterias glacialis* et a montré que la perméabilité à l'eau diminue au cours de la maturation, pour augmenter à nouveau après la fécondation. **Mc Cutcheon** et **Lucké**, dans une série de travaux publiés depuis 1926, ont étudié le mode de pénétration de l'eau dans l'œuf vierge d'*Arbacia* et ont proposé diverses équations de la perméabilité en fonction de la différence des pressions osmotiques et de l'état physiologique de la membrane. En 1929, **Page** a démontré l'existence d'une dissociation entre la rapidité de pénétration de l'eau et le volume atteint par l'œuf avant de se cytolyser : chez l'œuf d'*Arbacia* fécondé, ce volume est plus considérable pendant les phases d'imperméabilité relative que pendant celles qui correspondent à un maximum de sensibilité. Hobson, en 1932, étudiant l'œuf de *Psammechinus*, a utilisé une méthode d'observation cinématographique; ses résultats confirment ceux des auteurs précédents. En 1932 également, Dorfman, appliquant la méthode de Lillie à l'œuf de *Strongylocentrotus*, a retrouvé les mêmes phénomènes, mais il a constaté que l'augmentation de la perméabilité à l'eau se produit immédiatement après la fécondation; de plus il a dissocié la perméabilité à l'eau proprement dite des effets cytolytiques, ces derniers pouvant être rattachés à une déficience en oxygène et étant déterminés par les conditions où se trouve la solution hypotonique pendant les premières minutes de contact.

A mon tour, j'ai examiné attentivement quels étaient les effets de l'hypotonie sur le matériel nouveau et particulièrement favorable qu'est l'œuf de Pholade. Les œufs, pondus dans l'eau de mer normale, sont portés ultérieurement dans une solution d'eau de mer diluée dont la pression osmotique est déterminée par mesure cryoscopique. La vitesse de pénétration de l'eau est calculée, ainsi que cela a été fait par divers auteurs, à partir des variations du volume des œufs en fonction du temps. Dans ce but, on prélève des œufs à intervalles réguliers et l'on mesure leur diamètre au microscope à l'aide d'un oculaire à micromètre, dans des conditions de grossissement constantes; chaque point des courbes correspond à la moyenne des mesures faites sur un minimum de cinq œufs; de ce diamètre moyen on déduit la surface et le volume des œufs aux différentes phases de leur gonflement. Les mesures sont poursuivies jusqu'à ce que tous les œufs soient cytolysés ou que soit établi l'équilibre osmotique entre les œufs et le milieu extérieur.

Ce procédé de mensuration ne permet pas de suivre l'évolution d'un œuf déterminé. Mais, dans le cas de l'œuf de Pholade, le gonflement se fait de façon très homogène pour une ponte donnée et la valeur moyenne obtenue s'écarte très peu des mesures individuelles : dans une solution d'eau de mer diluée, les différences de diamètre de plusieurs œufs observés simultanément ne dépassent pas une à deux unités de l'échelle micrométrique, c'est-à-dire que l'erreur commise est de l'ordre de grandeur de 5 %, très inférieure aux variations observées au cours des expériences.

a) Œufs vierges.

Placé dans des solutions d'eau de mer de concentration donnée, l'œuf de Pholade absorbe de l'eau jusqu'à ce qu'il ait atteint un volume qui correspond à un état d'équilibre avec la pression osmotique du milieu extérieur. Ce volume final est sensiblement constant pour les œufs provenant de différentes pontes et n'est pas modifié par la maturation de l'œuf; il ne dépend que de la pression osmotique du milieu extérieur et de sa composition saline.

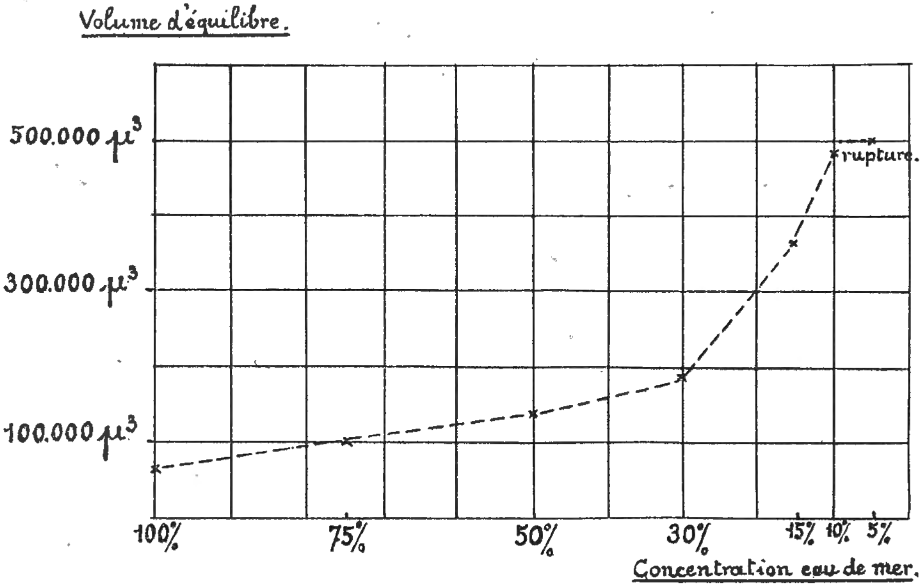

FIG. 3. — Courbe des volumes d'équilibre atteints par une même ponte dans des solutions d'eau de mer de concentration variable.

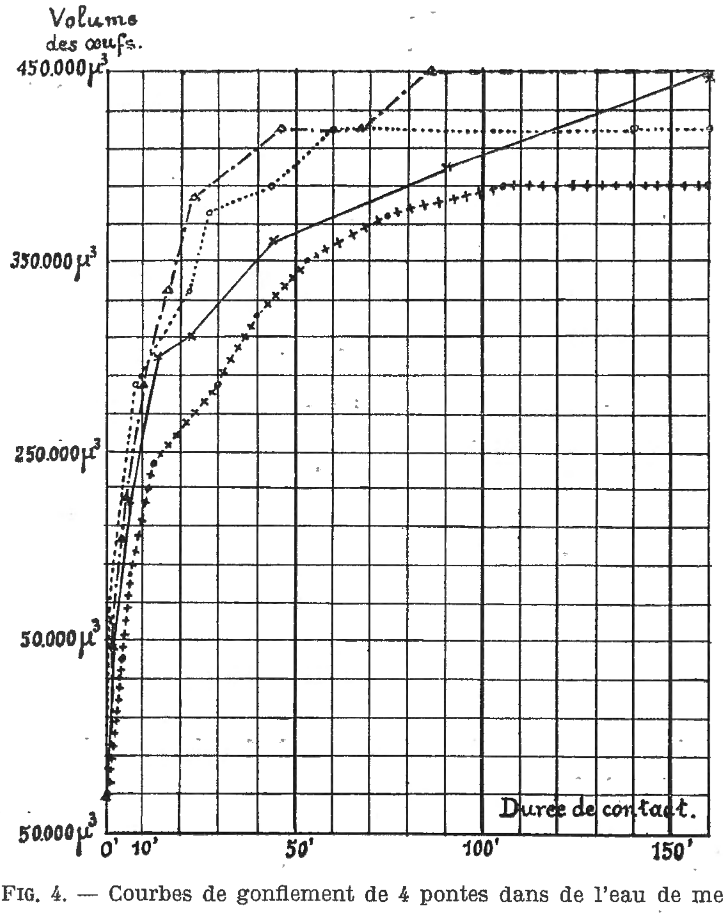

FIG. 4. — Courbes de gonflement de 4 pontes dans de l'eau de mer à 10 %.

Dans l'eau de mer normale, le volume de l'œuf de Pholade oscille autour de 68 000 μ^3. Le tableau I et la figure 3 donnent les volumes d'équilibre correspondant à différentes dilutions de l'eau de mer. La ponte qui a servi à établir cette courbe ne présentait pas de rupture de membrane après 2 heures 1/2 de séjour dans la solution à 10 %, alors que sa destruction était complète en 55 minutes dans la solution à 5 %. Il est à remarquer que le volume d'équilibre n'augmente pas suivant une progression régulière lorsque le gradient de pression osmotique entre l'intérieur de l'œuf et le milieu extérieur augmente, suivant la conception de Lillie, mais qu'il existe un point d'inflexion de la courbe très net situé aux environs de la dilution de 30 % : pour les dilutions plus fortes, le volume d'équilibre atteint est supérieur à ce que le début de la courbe permettait d'escompter, sans pourtant qu'il y ait rupture de la membrane ni dislocation du cytoplasme, comme le montreront les observations faites à l'ultramicroscope.

TABLEAU I

Concentration eau de mer.	Volume maximum.
100 %	68 000 μ^3
75 %	100 000 »
50 %	138 000 »
30 %	186 000 »
15 %	362 000 »
10 %	485 000 »
5 %	500 000 » et lyse.

Le temps nécessaire pour atteindre le volume d'équilibre est très variable d'une ponte à l'autre et peut être modifié par les conditions expérimentales. Mais l'allure générale de la courbe de gonflement reste toujours la même (cf. courbes de la fig. 4, faites chacune avec une ponte différente) : après avoir présenté au début une pente extrêmement accusée, elle s'infléchit peu à peu au fur et à mesure que l'on se rapproche du volume d'équilibre et se termine par un plateau presque horizontal. Cette allure peut être rapprochée de celle des courbes publiées par Dorman pour *Strongylocentrotus*. Elle dépend principalement de la variation progressive du gradient de pression osmotique existant entre l'intérieur de l'œuf et le milieu extérieur, gradient qui tend vers zéro au fur et à mesure que l'œuf se rapproche de son volume d'équilibre.

Si, s'inspirant des données de Mc Cutcheon et Lucké, on considère la quantité d'eau passant par seconde à travers l'unité de surface de la membrane ovulaire, en tenant compte de l'augmentation de surface qui s'effectue parallèlement à l'accroissement de volume, on constate que le coefficient de perméabilité est d'autant plus élevé que l'on est plus éloigné du volume d'équilibre final. Dans une solution à 10 % d'eau de mer, par exemple, la vitesse de pénétration de l'eau par unité de surface est très rapide pendant la pénétration de la première tranche de 100 000 μ^3 d'eau (il passe de 6 à 9 μ^3 d'eau par seconde et par micron carré), pour tomber très rapidement à des valeurs environ dix fois moindres : la seconde tranche de 100 000 μ^3 pénètre suivant un coefficient de perméabilité dont l'ordre de grandeur est environ 0,6 μ^3 par μ^2/sec. et qui tend progressivement vers zéro; il devient nul au moment où l'œuf est en équilibre osmotique avec le milieu extérieur.

La vitesse de pénétration de l'eau et le volume d'équilibre final sont exactement les mêmes, dans une ponte donnée, pour les oocytes et pour les œufs ayant maturé spontanément dans l'eau de mer avant d'être mis en contact avec la solution hypotonique. Les courbes correspondant aux œufs à ces différentes phases de leur vie cellulaire sont exactement superposables. A première vue, ce fait paraît en contradiction avec les observations faites par **Dalcq** sur l'œuf d'*Asterias glacialis* placé en solution hypotonique : considérant les modalités de la plasmolyse et de la cytolyse, **Dalcq** a, en effet, conclu à un renforcement progressif de la semi-perméabilité depuis la ponte jusqu'à la maturation; mais la méthode qu'il utilisait était différente et l'on ne peut déduire de ces divergences à l'existence d'une opposition entre ces deux espèces animales en ce qui concerne la vitesse de pénétration de l'eau.

L'introduction d'une quantité d'eau aussi considérable se fait en général sans qu'il y ait désintégration du cytoplasme ovulaire. En effet, remis dans l'eau de mer normale après séjour d'une vingtaine de minutes dans une solution à 10 % d'eau de mer (volume atteint : 325 000 μ^3), les œufs reprennent leur volume initial en moins de trois minutes et peuvent être fécondées : la formation des pronucléi s'effectue, en apparence normalement, dans les neuf dixièmes des œufs. L'œuf de Pholade semble, à ce point de vue, être particulièrement résistant; sur l'œuf d'Oursin en effet, **Lillie** a montré que le retour à la normale après séjour dans une solution hypotonique n'est possible que pour autant que la dilution de l'eau de mer ne soit pas trop forte; au delà d'une limite peu élevée, le cytoplasme se désagrège à l'intérieur de la membrane et l'œuf se cytolyse **Just**, en 1928, a confirmé les observations de Lillie.

Le développement ultérieur des œufs fécondés après pareil traitement est généralement troublé. Néanmoins, un certain nombre des œufs parviennent à se diviser en deux blastomères et poursuivent leur segmentation de façon parallèle aux témoins. Il y a lieu de noter que, lorsque ces œufs se segmentent, les deux premiers blastomères sont toujours inégaux; la pénétration d'une grande quantité d'eau et la perturbation de la balance des sels qui devrait logiquement en résulter ne sont donc pas suffisantes pour troubler l'action du facteur de division inégale qui caractérise l'œuf de Pholade; **Pasteels** a cependant pu démontrer que ce facteur, localisé dans le cytoplasme, est extrêmement labile et doit vraisemblablement se rattacher à un gradient de perméabilité.

L'examen à l'ultramicroscope des œufs placés dans une solution d'eau de mer diluée montre que la pénétration de l'eau s'effectue par toute la surface de l'œuf, tout en étant plus active à l'un des pôles. Bien que la vitesse de pénétration de l'eau soit la même dans les deux cas, les œufs ayant maturé réagissent différemment des oocytes à vésicule germinative et il convient de les étudier séparément.

Dans les oocytes, la couche corticale disparaît presque instantanément et les particules lumineuses arrivent au contact direct de la membrane. Ce phénomène correspond probablement à la dissolution de la couche lipoïdique dans les solutions hypotoniques telle qu'elle a été décrite par Runnström en **1923**. Il se pourrait cependant qu'il ne s'agisse pas d'une dissolution pure et simple : en effet, à un stade plus avancé du gonflement de l'œuf, on voit que l'emplacement de la couche corticale est occupé par une assise de particules formant une couche continue sur laquelle s'amorcent les mailles du réseau; on est dès lors en droit de se demander si la disparition apparente de la couche corticale ne correspondrait pas à une précipitation sous forme de granulations lumineuses. Dans cette hypothèse, on pourrait supposer que les disparitions et réapparitions physiologiques ou expérimentales de la couche corticale ne seraient que des

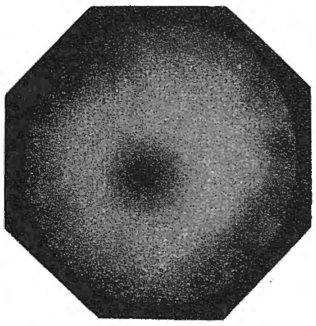

Fig. 5. — Début de pénétration de l'eau; la couche corticale a disparu (volume : 123.000 μ^3).

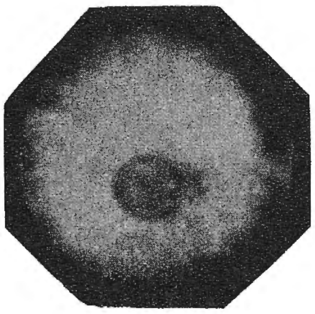

Fig. 6. — Stade plus avancé : réseau constitué à la périphérie (volume : 169.000 μ^3).

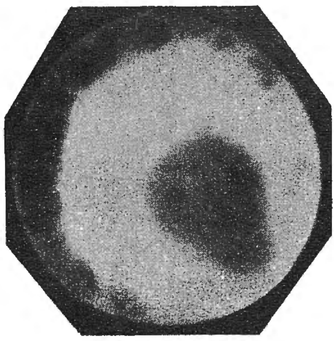

Fig. 7. — Réseau très développé au pôle végétatif, nul au pôle animal (volume : 245.000 μ^3).

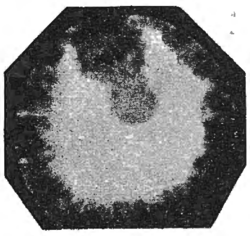

Fig. 8.
Stade plus avancé
(volume : 288.000 μ^3).

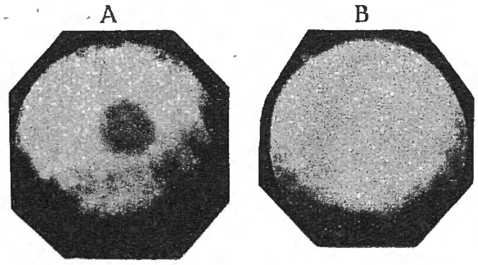

Fig. 9. — Le réseau gagne les couches profondes du cytoplasme (volume : 335.000 μ^3). *A* = impression faible; *B* = impression forte.

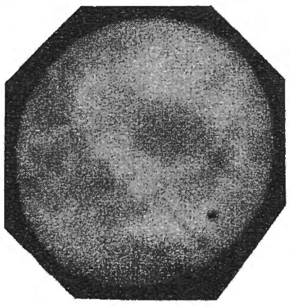

Fig. 10. — Œuf ayant atteint le volume d'équilibre osmotique : le réseau s'étend jusqu'à la vésicule germinative (volume : 485.000 μ^3).

changements de phases dus aux modifications physico-chimiques dont le cortex ovulaire est le siège.

En même temps que la couche corticale disparaît, l'eau pénètre à l'intérieur de l'œuf en distendant les mailles d'un réseau qui semble préexister dans le cytoplasme. Les figures 5 à 10 montrent les aspects successifs de la cellule au cours du gonflement. L'eau s'insinue par toute la surface, mais sa pénétration semble être minimale au pôle animal. On voit apparaître progressivement un réseau à larges mailles dans lequel circule l'eau. Ces mailles sont constituées par des files linéaires de particules cytoplasmiques; celles-ci continuent à être agitées de mouvements browniens rapides, mais conservent leur position réciproque sur les mailles du réseau; elles semblent être rendues solidaires les unes des autres par une substance non visible à l'ultramicroscope, non miscible à l'eau, dont la viscosité est insuffisante pour freiner le mouvement brownien, mais qui est capable de résister aux courants et aux remous qui accompagnent la pénétration rapide de l'eau. La figure 11 reproduit une photographie du réseau, vu à un fort grossissement et prise au stade d'équilibre osmotique; le cliché n'intéresse que la partie centrale de l'œuf et comprend la vésicule germinative qui a conservé son aspect optiquement vide. Ces différentes photos démontrent que le réseau progresse de la périphérie vers la zone nucléaire; pareille évolution ne concorde pas avec la théorie émise par Konopacki et suivant laquelle, chez l'œuf d'Oursin, la dislocation de la structure du cytoplasme ne s'effectuerait pas suivant un processus partant de la périphérie et gagnant le centre de l'œuf, mais bien par un remaniement intéressant l'ensemble du cytoplaseme et ne modifiant le cortex qu'à un stade terminal. Lorsque l'état d'équilibre est atteint dans les solutions très diluées (10 % d'eau de mer), tout l'œuf présente cette structure en réseau (fig. 10); à la périphérie, la membrane est doublée par une couche continue de particules cytoplasmiques qui a remplacé la couche corticale et d'où semblent partir les mailles du réseau; le mouvement brownien persiste sur les mailles. La vésicule germinative a également absorbé de l'eau, mais elle ne manifeste aucune structure et reste optiquement vide; elle ne conserve généralement pas sa forme sphérique, mais prend un aspect en bissac (fig. 8).

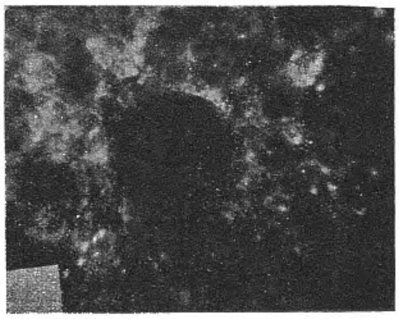

Fig. 11. — Réseau à un fort grossissement; au centre, la vésicule germinative.

Lorsque l'œuf est orienté dans le champ microscopique suivant l'axe de ses pôles (fig. 7 et 8), on voit que le pôle animal est dépourvu de réseau et que la vésicule germinative tend à être repoussée contre le cortex; la pénétration de l'eau se fait avec un maximum d'intensité au pôle végétatif, où se trouvent les mailles les plus larges du réseau. L'existence d'une polarité chez l'oocyte est à rapprocher de celle démontrée par Dalcq pour l'œuf d'*Asterias glacialis*; pour celui-ci cependant, Dalcq a admis l'hypothèse d'une perméabilité plus grande du pôle animal à l'égard de l'eau. Le gradient de polarité existe donc également chez l'oocyte de Pholade, mais il se manifeste en sens inverse de ce que pensait cet auteur.

Dans les solutions moins diluées (20 à 30 % d'eau de mer), le processus est le même, mais l'extension du réseau vers le centre reste limitée et il persiste, autour de la vésicule germinative, une zone plus ou moins épaisse dans laquelle la pénétration de l'eau s'est effectuée de façon discrète. La figure 12 montre l'aspect de l'œuf après 60 minutes de séjour dans une solution à 30 % d'eau de mer, alors que l'œuf a un volume de 170 000 μ^3 et a à peu près atteint son état d'équilibre. Le cytoplasme central conserve l'aspect habituel; mais il convient de remarquer que la superposition des couches cytoplasmiques ne permet de voir le réseau, et surtout de le photographier, que lorsque l'écartement des mailles est déjà très accusé.

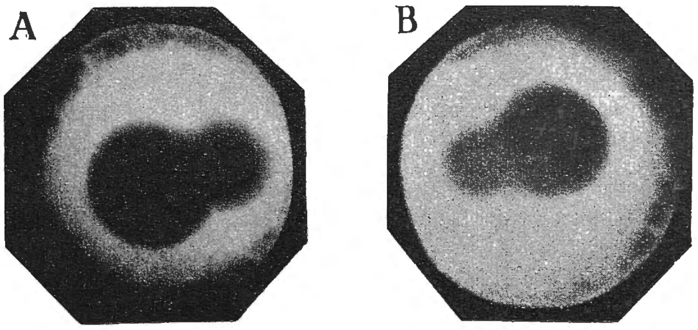

Fig. 12. — État d'équilibre dans une solution à 30 % d'eau de mer.
A = impression faible; *B* = impression forte.

Lorsque les œufs ont maturé spontanément dans l'eau de mer avant d'être placés dans la solution hypotonique, la pénétration de l'eau s'effectue avec la même vitesse, mais suivant un processus différent. Comme dans le cas des oocytes immatures, la couche corticale disparaît immédiatement. Mais il n'est pas possible de mettre en évidence une polarité de la cellule et l'eau semble s'introduire de la même façon par toute la surface cellulaire. Il ne se forme pas de réseau à mailles plus ou moins distendues dans lesquelles circulent des courants aqueux, mais l'eau imbibe rapidement l'ensemble du protoplasme qui conserve le même aspect global que chez les témoins; la dilution du protoplasme s'effectue de façon homogène et la périphérie de l'œuf réagit de la même façon que sa partie centrale. La maturation ne modifie ni la vitesse de pénétration de l'eau, ni le volume d'équilibre osmotique, ni la résistance mécanique de la membrane; mais elle détermine cependant un bouleversement des propriétés du cytoplasme tel que les relations entre l'eau et la substance cellulaire changent de nature. Ces phénomènes doivent probablement pouvoir être attribués à la diffusion du suc nucléaire dans le cytoplasme et aux modifications physico-chimiques en résultant, conformément à l'opinion déjà exprimée par **Dalcq** en 1923. De plus, une fois la vésicule germinative disparue, on n'observe plus de différence entre les diverses régions de l'œuf au point de vue de la pénétration de l'eau; celle-ci se fait de façon uniforme et le gradient physiologique, précédemment si net, s'est en quelque sorte effacé. Ces observations confirment donc celles de **Delage**, **Lillie**, **Morgan**, **Wilson**, **Yatsu**, **Mc Clendon**, **Dalcq**, etc., concernant le rôle primordial de la rupture de la vésicule germinative sur la physiologie de l'œuf vierge. Dans le cas particulier de la Pholade cependant, il semble que ces modifications intéressent uniquement le cytoplasme et ne touchent pas la membrane.

b) Œufs fécondés.

La fécondation entraîne chez l'œuf de Pholade, comme chez celui d'Oursin (**Lillie**, **Herlant**, **Dorfman**) et celui d'Astérie (**Dalcq**), une augmentation de la perméabilité à l'eau en même temps qu'une diminution de résistance de la membrane. Pendant les premières minutes qui suivent la pénétration du spermatozoïde, la perméabilité ne semble pas modifiée : la rapidité de pénétration de l'eau reste la même ainsi que le volume d'équilibre final, et l'on n'observe aucune accélération des processus de cytolyse. Mais à partir de la quinzième minute environ, la fragilité des œufs augmente : dans l'eau de mer à 10 %, le volume d'équilibre peut en général être encore atteint, mais il correspond au point de rupture de la membrane. Une demi-heure après la fécondation, tous les œufs sont détruits en une dizaine de minutes et la rupture de la membrane se produit, alors que le gonflement de l'œuf atteint environ les deux tiers du volume d'équilibre. Au moment de la fusion des pronucléi, la destruction des œufs est presque immédiate et se produit pour un volume sensiblement égal au quart du volume d'équilibre.

Dans l'eau de mer à 15 %, l'évolution des œufs est la même, mais la dilution moindre rend le détail du phénomène plus facile à suivre. Dans une telle solution, le volume d'équilibre atteint par les œufs vierges est égal à 362 000 μ^3, et l'on n'observe jamais de rupture de membrane. Aucune anomalie n'est visible pendant les vingt premières minutes qui suivent la fécondation. Vers la trentième minute, la vitesse de pénétration de l'eau est sensiblement doublée; les œufs ne dépassent plus le volume de 335 000 μ^3 et se détruisent lentement; il faut environ deux heures pour que la majorité des œufs ait éclaté. Après quarante-cinq minutes, l'accélération de la vitesse de pénétration de l'eau est telle qu'il devient difficile de faire des mesures précises; les œufs sont tous détruits en moins de vingt minutes et leur volume ne dépasse pas 200 000 μ^3.

La variation de la perméabilité à l'eau et l'augmentation de fragilité de la membrane sont donc progressives et ne débutent qu'environ un quart d'heure après la fécondation. Elles ne peuvent par conséquent être mises en rapport avec les variations d'épaisseur de la couche corticale, variations qui, comme nous l'avons vu précédemment, sont caractérisées par un épaississement immédiat suivi d'une disparition, puis d'une reconstitution de cette couche au cours de la première heure du développement. A première vue, ces faits semblent corroborer l'opinion d'HEILBRUNN (1920), qui, retrouvant sur *Cumingia* les phénomènes décrits par LILLIE, n'en discutait pas moins l'interprétation de ce dernier et attribuait l'augmentation de perméabilité qui suit la fécondation, non à une transformation de la membrane, mais à des changements dans l'état physique du protoplasme.

c) Anomalies provoquées expérimentalement.

La façon dont l'œuf de Pholade réagit à la pénétration de l'eau peut être modifiée par l'addition de certaines substances ou par les conditions expérimentales.

Le cyanure de potassium, à la concentration de M/2000, ne modifie en général pas la vitesse de pénétration de l'eau; ceci n'est cependant pas absolu : dans un petit nombre d'expériences et pour des raisons non élucidées, l'addition de cyanure s'est accompagnée d'une augmentation appréciable de la perméabilité à l'eau. La concentration utilisée est suffisante pour inhiber la consommation d'oxygène, comme le montrent des mesures de contrôle faites avec l'appareil de Warburg; elle est d'ailleurs dans la limite des concentrations admises généralement comme étant capables d'inhiber l'oxydation du cytochrome (KEILIN) et celle du glutathion réduit (HOPKINS et ELLIOT). Elle est inférieure à celle préconisée par LILLIE pour entraver l'augmentation de perméabilité qui suit la fécondation chez l'œuf d'Oursin (selon cet auteur, les dilutions inférieures à M/800 seraient inactives); HERLANT a cependant obtenu des modifications de la perméabilité chez *Paracentrotus* en employant une concentration de M/3000, mais il a montré que l'effet obtenu était fonction de la toxicité plus ou moins grande des ions contenus dans le milieu extérieur.

Si la courbe de gonflement des œufs en présence de cyanure à la concentration choisie est en général exactement superposable à celle des témoins, la réaction du cytoplasme est cependant différente. Le réseau cytoplasmique se dessine dans les mêmes conditions, mais sa propagation vers le centre de l'œuf est entravée : lorsque le volume d'équilibre est atteint, l'examen à l'ultramicroscope montre que la structure cellulaire est beaucoup mieux conservée que chez les témoins; la vésicule germinative a rarement disparu et le réseau à mailles ne couvre pas l'ensemble de la cellule; il persiste, autour de la vésicule germinative, une zone de cytoplasme compact dans laquelle la pénétration de l'eau est réduite à un minimum. Dans certains cas, la membrane nucléaire semble gélifiée et les particules sombres qui la recouvrent sont immobiles, alors que le mouvement brownien paraît normal dans les couches cytoplasmiques voisines.

Ce rôle préservateur du cyanure se manifeste également par une inhibition des cytolyses : dans les solutions à 10 % d'eau de mer additionnées de KCN à M/2000, la rupture de la membrane se fait beaucoup plus tardivement que chez les témoins. C'est ainsi qu'une ponte particulièrement fragile, qui était détruite en moins de vingt minutes dans la solution à 10 % d'eau de mer sans qu'aucun œuf puisse atteindre le volume d'équilibre, a pu être complètement protégée en ajoutant du cyanure à la concentration habituelle: après 160 minutes de contact avec la solution diluée, un quart seulement des œufs étaient détruits et le volume maximum atteignait 485 000 μ^3, alors que chez les témoins il n'avait pas pu dépasser 388 000 μ^3.

La même action s'observe si l'on étudie les œufs fécondés. Le cyanure ne modifie pas l'augmentation de la perméabilité à l'eau consécutive à la fécondation, mais il s'oppose, au moins partiellement, à la diminution de résistance des œufs à la cytolyse. Ce pouvoir protecteur a cependant une limite : une heure après la fécondation, dans les solutions à 10 et 15 % d'eau de mer, la fragilité des œufs est devenue telle que leur destruction s'opère en quelques minutes, que le cyanure soit ou non présent dans la solution.

L'action anticytolytique du cyanure a été signalée par **Loeb** dès 1906; cet auteur a montré que l'action toxique de nombreux corps sur les œufs fécondés d'Oursin était retardée par KCN et a attribué cet effet à la suppression d'oxydations anormales ou destructives. Ultérieurement, **Lillie**, au cours de ses études sur l'antagonisme existant entre sels neutres et anesthésiques, a confirmé l'existence d'une action anticytolytique du cyanure vis-à-vis des œufs d'Astéries et d'Oursins et a repris l'hypothèse de Loeb concernant une relation probable entre cette action de KCN et l'inhibition des oxydations, celles-ci jouant un rôle important dans les modifications de la perméabilité des cellules. Par la suite, de nombreux auteurs ont confirmé ces faits, en particulier **Ephrussi** (1933) en ce qui concerne la cytolyse de l'œuf d'Oursin par la chaleur; **Whitaker** (1933) a également observé des faits du même ordre. Au contraire, **Herlant** a observé que le cyanure, à la concentration de M/3000, rend plus précoce la plasmolyse des œufs fécondés de *Paracentrotus* mis en solution hypertonique; il interprète cette action comme correspondant à une diminution de la perméabilité sous l'influence du cyanure. Les rapports de la cytolyse avec les processus d'oxydation ont été mis en relief par **Dorfman**, en 1932, au moyen d'une technique très différente : si les œufs d'Oursin, mis dans une solution hypotonique, sont placés immédiatement en goutte pendante dans des conditions qui entraînent une déficience en oxy-

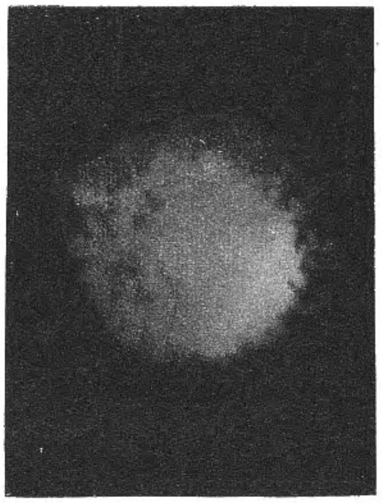

Fig. 13.

gène, la vitesse de pénétration de l'eau reste la même, mais on n'observe plus de cytolyse, alors que celle-ci est la règle chez les témoins dans les conditions des expériences.

D'autres corps, tout en n'ayant pas d'action apparente sur la perméabilité à l'eau, peuvent également agir directement sur le cytoplasme. C'est ainsi que, chez les œufs de Pholade colorés au préalable par le rouge neutre (solution à 1/500 000) et portés ultérieurement dans une solution d'eau de mer diluée, la vitesse de pénétration de l'eau n'est généralement pas modifiée (on peut cependant rencontrer exceptionnellement des pontes chez lesquelles pareil traitement détermine un accroissement de la perméabilité à l'eau). Mais si l'on examine les œufs à l'ultramicroscope, on ne voit pas se constituer de réseau : dans la zone périphérique, où devraient apparaître les mailles, on observe l'existence de gros blocs cytoplasmiques gélifiés, irréguliers, qui nagent indépendamment les uns des autres dans les courants liquides déterminés par la pénétration rapide de l'eau (fig. 13). Lorsque les œufs sont placés directement dans une solution hypotonique additionnée de rouge neutre à la même concentration, on observe un effet similaire, mais moins accentué et moins général; il semble que l'intoxication du cytoplasme par le rouge neutre nécessite un certain laps de temps, correspondant à une accumulation de ce corps dans la cellule.

III. — DISCUSSION

Runnström a conclu de ses expériences que la couche corticale de l'œuf d'Oursin était constituée par des lipoïdes qui, dans certaines conditions, ne sont pas miscibles au reste du cytoplasme et s'accumulent à la périphérie de l'œuf par un effet de tension superficielle. Il a également démontré l'existence d'une couche corticale similaire chez l'Étoile de mer et a pu en faire apparaître expérimentalement une dans les œufs d'*Arbacia.* Il semble bien que la couche corticale observée sur l'œuf de Pholade doive être interprétée de la même façon.

Par suite d'influences diverses (fécondation, action de corps chimiques, action du radium, solutions hypotoniques, etc.), cette couche lipoïdique peut modifier son épaisseur ou même disparaître complètement et se reconstituer ultérieurement. Ces changements morphologiques constituent vraisemblablement la manifestation visible des modifications de l'état physico-chimique de la cellule sous l'influence des agents extérieurs ou du métabolisme interne. Correspondent-ils à des transformations des propriétés physiologiques de l'œuf ?

La perméabilité ne semble pas être en relation directe avec l'épaisseur de la couche corticale. Les expériences faites avec les œufs fécondés ont montré une discordance complète entre les variations d'épaisseur de la couche corticale et l'augmentation de la perméabilité à l'eau. Les mesures faites sur les œufs vierges ne permettent pas de tirer de conclusions : dans une ponte donnée, la couche corticale est d'épaisseur sensiblement constante pour tous les œufs et la courbe de gonflement dans une solution hypotonique est très uniforme; mais on ne peut pas mettre en relation les variations d'épaisseur et les différentes vitesses moyennes de pénétration de l'eau qui s'observent entre les différentes pontes; dans de nombreux cas, une couche corticale épaisse correspond à une pénétration rapide de l'eau, tandis qu'une couche mince coïncide avec un temps de gonflement prolongé; mais cette règle n'a rien d'absolu et présente de nombreuses exceptions. La pénétration de colorants vitaux (rouge neutre, bleu de Nil) ne semble pas non plus être régie par la plus ou moins grande épaisseur de la couche corticale. Le taux des maturations spontanées s'effectuant dans l'eau de mer, phénomène qui correspond vraisemblablement à la pénétration dans l'oocyte de sels provenant du milieu extérieur, semble également indépendant de l'épaisseur de cette couche. Dans leur ensemble, ces résultats concordent avec ceux de RUNNSTÖM; celui-ci était, en effet, d'avis que la surface lipoïdique des œufs n'a pas une importance capitale en ce qui concerne leur semi-perméabilité.

Les expériences faites sur l'œuf de Pholade permettent également d'envisager le problème de la structure du protoplasme. Les nombreuses études faites dans ce domaine n'ont pas encore permis de résoudre définitivement cette question; il est d'ailleurs probable que l'on a affaire à des systèmes colloïdaux qui passent de façon réversible de l'état de sol à celui de gel, suivant les conditions physiologiques ou expérimentales, et que les données obtenues à partir d'un type cellulaire ne peuvent pas être généralisées à tous les cytoplasmes. En 1933, à la suite d'une analyse des conditions de déformation mécanique de l'œuf d'Oursin, VLES concluait à une impossibilité de résoudre le dilemme qu'il exposait dans les termes suivants : « Que l'œuf, dans une enveloppe élastique, » ait un médium fondamental liquide dans lequel roulent des hétérogénéités » d'une autre phase — l'œuf-pâte — ou qu'il ait une trame fondamentale solide » dans laquelle circule un médium liquide — l'œuf-gel — on a deux schémas » presque réciproques, qui sont d'autant moins éloignés l'un de l'autre que nous » connaissons des systèmes capables de passer réversiblement, suivant les conditions physico-chimiques, du premier au second ».

Placé dans une solution hypotonique, l'oocyte de Pholade absorbe de l'eau suivant un processus tel qu'il semble ne pas y avoir simplement imbibition du protoplasme : l'eau circule à l'état libre dans un réseau cytoplasmique dont les mailles se distendent sous l'action de cet afflux liquide. Pareil réseau n'est pas visible à l'intérieur de l'œuf normal placé en solution isotonique, et la question se pose de savoir s'il s'agit d'une néo-formation ou de la mise en évidence d'un système préformé invisible dans les conditions habituelles. Le mouvement brownien des particules qu'il supporte semble avoir la même rapidité que celui observé dans le cytoplasme normal, ce qui permet d'admettre que les viscosités sont les mêmes. Sa présence est une garantie de la survie de l'œuf : tant qu'il persiste et tant que ses mailles ne sont pas dilacérées par une distension trop considérable, les œufs, ramenés dans une solution d'eau de mer isotonique, reprennent leur volume normal et peuvent être fécondés. Le réseau disparaît lorsque des substances toxiques sont introduites dans l'oocyte ou en cas de désintégration du cytoplasme après rupture de la membrane.

L'hypothèse d'une structure cytoplasmique en réseau est à rapprocher des idées formulées par **Dalcq** en **1923**, suivant lesquelles l'œuf d'*Asterias glacialis* se comporterait comme s'il était constitué par une « trame cytoplasmique ou spongioplasme » formée de protéines et de lipoïdes avec un liquide insterstitiel chargé de sels dissous sous une pression osmotique considérable. **Herlant** a également supposé que le cytoplasme était constitué par une émulsion de protéines et de lipoïdes dans laquelle la phase continue serait tantôt albuminoïde, tantôt lipoïdique, suivant le stade cellulaire envisagé; cette hypothèse lui permettait d'expliquer les variations cycliques de la perméabilité au cours des divisions ovulaires, ainsi que l'action des différents agents extérieurs; mais sa théorie implique que la pénétration de l'eau est conditionnée par le pouvoir d'absorption de la phase continue du cytoplasme. Or l'observation ultramicroscopique des œufs de Pholade montre que la plus grande partie de l'eau qui pénètre à l'intérieur de l'oocyte reste libre dans les mailles du réseau et peut être expulsée très rapidement sans entraîner avec elle une part importante des constituants cellulaires; le facteur d'imbibition du cytoplasme ne doit donc jouer qu'un rôle secondaire dans l'interprétation des variations de la perméabilité. **Runnström** a également admis l'existence d'une structure en réseau dans le cytoplasme.

Dans certaines conditions expérimentales (chaleur, action prolongée du radium), on peut provoquer la gélification du réseau. Les oocytes placés dans la solution hypotonique forment alors un réseau qui a le même aspect morphologique que dans les conditions normales, mais dont les particules lumineuses sont immobiles par suite d'une gélification du substrat. En pareil cas, la pénétration de l'eau se fait plus difficilement et les mailles du réseau restent petites, même à la périphérie de l'œuf. Le volume d'équilibre osmotique avec le milieu extérieur est atteint beaucoup plus lentement que chez les témoins; il peut même être notablement inférieur à celui observé normalement dans les conditions de l'expérience. Bien qu'à première vue on semble avoir affaire à une imperméabilisation de l'œuf ainsi qu'à une diminution de l'élasticité de la membrane, il s'agit en réalité d'une action directe sur le cytoplasme et plus spécialement sur le milieu fondamental dont la viscosité est considérablement accrue et qui s'oppose, par résistance mécanique, à la pénétration de l'eau.

Dans le cas de l'oocyte de Pholade, par conséquent, l'aspect du réseau qui se forme dans les solutions hypotoniques et la persistance du mouvement brownien des particules semblent montrer que l'on a affaire à une phase colloïdale liquide qui constituerait le milieu fondamental de la cellule. Dans ce cas particulier, il semble donc que c'est la première des hypothèses proposées par Vlès (l'œuf-pâte) qui doit être adoptée.

En est-il de même après la maturation? Lorsque la vésicule germinative se rompt, on n'observe pas de modification apparente du cytoplasme auquel s'est mélangé le suc nucléaire, et le mouvement brownien des particules ne semble pas modifié. Dans les solutions hypotoniques, la vitesse de pénétration de l'eau dans les œufs maturés et dans les oocytes est la même et les courbes de gonflement sont superposables dans les deux cas. Mais l'examen sur fond noir montre que, dans les œufs maturés, il ne se forme pas de réseau visible et que l'eau se mélange au protoplasme par imbibition simple : on observe une dilution du cytoplasme, une raréfaction du nombre des particules lumineuses par unité de volume; le mouvement brownien est conservé. La maturation de l'œuf a-t-elle déterminé un changement de phase du cytoplasme, de telle sorte que l'on se trouverait dorénavant en présence d'une structure voisine de celle de l'« œuf-gel » proposée par VLES? Il est difficile de conclure de façon formelle dans ce sens, et ceci d'autant plus que l'observation du mouvement brownien permet d'éliminer l'hypothèse d'un accroissement notable de la viscosité.

Quel que soit le point de vue que l'on adopte à ce sujet, il n'en reste pas moins certain que la rupture de la vésicule germinative et la diffusion du suc nucléaire dans le cytoplasme jouent un rôle important dans la physiologie ultérieure de l'œuf et dans son comportement vis-à-vis des agents extérieurs. Comme nous l'avons signalé précédemment, cette conclusion est conforme à l'opinion émise déjà par plusieurs auteurs. Mais dans le cas particulier de l'œuf de Pholade, il convient de dissocier les propriétés de la membrane (perméabilité aux colorants vitaux, vitesse de pénétration de l'eau) qui ne semblent pas être modifiées, de celles du cytoplasme qui deviennent très différentes. Parmi ces dernières, il y a lieu de souligner la modification qui se produit dans la polarité physiologique : tant que la vésicule germinative est intacte, l'observation met clairement en évidence une plus grande perméabilité du pôle végétatif; après la rupture, ce gradient physiologique ne s'observe plus. Il y a lieu de suggérer qu'au moment où la membrane nucléaire disparaît, ce gradient joue un rôle dans la migration du fuseau vers le pôle animal, et de rapprocher ces faits de ceux observés par PASTEELS lors de la dépolarisation de l'œuf de Pholade, soit spontanément, soit sous l'influence de solutions calciques : dans ce cas, en effet, la figure mitotique de maturation n'émigre pas vers le cortex, mais reste située dans les couches profondes du cytoplasme.

RÉSUMÉ

1. Étude morphologique à l'ultramicroscope des caractéristiques de l'œuf de Pholade, avant et après maturation.

2. Comportement des œufs de Pholades (oocytes immatures, œufs vierges entrés en maturation, œufs fécondés) placés dans des solutions hypotoniques d'eau de mer diluée à des degrés variables. Analyse des courbes de gonfle-

ment et des conditions de rupture de la membrane. Examen à l'ultramicroscope des conditions de pénétration de l'eau; mise en évidence d'une structure en réseau et d'un gradient de polarité chez l'oocyte immature, caractéristiques qui disparaissent après maturation.

3. Étude à l'ultramicroscope des variations de la couche corticale chez les œufs vierges et après fécondation. Démonstration d'une absence de parallélisme entre ces variations et celles de la perméabilité.

4. Études de l'action préservatrice du cyanure sur la réaction des œufs vierges et fécondés placés en milieu hypotonique. Étude de son influence sur la structure du cytoplasme et en tant qu'inhibiteur des cytolyses.

5. Mise en évidence de la toxicité du rouge neutre qui, sans modifier la vitesse de pénétration de l'eau, altère considérablement la structure du cytoplasme telle qu'elle est visible à l'ultramicroscope.

6. Dissociation des phénomènes qui relèvent directement de la membrane (vitesse de pénétration de l'eau, perméabilité aux colorants vitaux, propriétés élastiques et résistance mécanique de la membrane en ce qui concerne la cytolyse en solution hypotonique) et de ceux qqui dépendent exclusivement de la structure du cytoplasme (gradient de polarité, liaison de l'eau avec le cytoplasme) et varient lors de la maturation.

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OBSERVATIONS AND EXPERIMENTS ON SEX-CHANGE IN THE EUROPEAN OYSTER, *OSTREA EDULIS* L.

PART V

A simultaneous study of spawning in 1927 in two distinct geographical localities

BY

J. H. ORTON (Plymouth and Liverpool).

CONTENTS

INTRODUCTION

The research herein described was carried out under the auspices and as part of the programme of research of the Marine Biological Association of the United Kingdom, to whom through the Director, Dr. E. J. Allen, I am indebted for permission to dedicate this work to our distinguished and honoured French colleague Professor Paul Pelseneer from whose labours I and many other naturalists at Plymouth derived inspiration and advantage.

During the year 1927, in continuation of a series of researches extending from 1920, a study was planned and made of the spawning of the European or flat oyster, *Ostrea edulis* L, in two widely separated localities in England, namely the Fal Estuary on the South coast and the more northerly situated Blackwater Estuary on the East coast. These are the two chief producing beds in England. At the same time more or less continuous records of sea-temperature were obtained on and about the respective oyster beds. A comparison can therefore be made of the correlation between temperature and spawning in these localities. It has already been shown (Orton, 1928) that owing to the insular position of the Blackwater Estuary the temperature range is there greater than in the upper Fal Estuary where a deep channel admits water, especially at the spring tides, direct from the English Channel. In summer this Channel water is cooler than the estuarine and in winter warmer.

By the permission and courtesy of the Truro River Committee samples of oysters were dredged throughout the closed (summer) season and examined generally on the beds in a temporary laboratory on the day the oysters were captured, but sometimes at Plymouth or West Mersea.

Samples of oysters were purchased regularly from the Blackwater beds by permission of the West Mersea and Tollesbury Native Oyster Co. : some of these were examined on the beds, but most were dispatched to Plymouth. Frequent travelling between Plymouth, Truro and West Mersea was necessary in order to examine all material as speedily after removal from the sea as was possible, and to secure temperature readings on the Fal beds where few previous records had been made. Regular temperature observations had been established at West Mersea for some years.

Methods.

In the collection of samples an endeavour was made to obtain at least one sample of 100 oysters per fortnight from each of the main sections of the Blackwater beds (West Mersea), each sample being carefully recorded. Unfavourable winds and scarity of oysters interfered a little with this plan but valuable material was obtained for the purpose of comparing the spawning behaviour of oysters from different habitats in this locality.

On the Truro grounds an effort was made to obtain *weekly* samples of about 100 oysters from each of the four main well-marked local beds. In all some 10,000 oysters were examined from the Truro beds and 5,000 from the Blackwater beds, and almost all of them were dredged. Material was examined continuously from the Truro beds from June to November, but cessation of dredging operations on the Blackwater beds deprived me of samples from August 19 to September 4 and samples were unobtainable from the beds between September 18 and October 3.

The sex-physiological condition of every oyster was noted and recorded in one or other of the 30 categories listed in Orton, 1928, p. 401. In this paper only the ripe or ripening females and those individuals carrying embryos or larvae will be noted, i.e. categories 0, 1, 2, 3; 4, 6 and 8. Other aspects of the study will be dealt with one by one in the future.

The methods employed in the study of temperature variation over these beds has been discussed in relation to rhythmic periods in shell growth and fattening in this species (Orton, 1928), and the monthly means of 5 years observations on the Blackwater beds are given in Orton and Lewis (1931).

A Chart of the oyster grounds on the Fal is given in (Orton, 1928, fig. 2), and of the Blackwater grounds in (Orton and Lewis, 1931, fig. 2); both are reproduced here by permission of the Council of the Marine Biological Association.

Salinity variations were investigated and are discussed with the environmental variations in the study on shell-growth (Orton, 1928, p. 394).

The weather of 1927.

The importance of temperature in relation to breeding in marine molluscs is stressed and summarized by Pelseneer (1935). In a study on the breeding of marine animals (Orton, 1920) the present writer arrived at the conclusion that normally *O. edulis* begins to spawn throughout its geographical range when or soon after the sea-temperature in its annual cycle of changes rises above about 15°-16° C, and moreover that this oyster *may* continue to spawn throughout the summer so long as the temperature remains above this level. It is known empirically that in a warm spring spawning will begin earlier than in a cold spring. It is manifest therefore that the weather must play an important role in the phenomenon of spawning, and the kind of weather experienced in 1927 is an important factor in the present study in its relation to normality, especially during the spawning period.

In the summary for the year 1927 the English Meteorological Office Report (1928) describes the general conditions as « a wet year with a dull and wet summer... the outstanding features being the persistent rain, thunderstorms, floods and lack of sunshine during the summer months and early autumn, June-September inclusive, and the excessive wetness of the year as a whole. To find a summer which in respect of persistent wetness and lack of sunshine can compare with that of 1927 it is necessary to go back to the black year of 1879. » Conspicuous general features of the seasonal variation in temperature during 1927 were « January on the whole mixed...; February; monthly mean temperatures being mostly about or above the normal; March unusually mild : in April the mean temperature was above the normal in most English districts. Unusually severe ground frost for the time of the year occurred during the last week of the month. May on the whole was warm in the South. Unusually severe ground frost for the time of the year occurred during the night April 30 to May 1. Unusually high temperatures were recorded during the period 4th. to the 9th. Low day temperatures occurred generally on the 10th. and 11th. and the 27th. and 28th. June was decidedly cold... Frost occurred in many districts during the early part of the month and some remarkably low screen temperatures for the time of the year were recorded between the 9th. and 15th. An exception to the generally low temperatures occurred on the 16th. 17th. and 18th; on the 16th. maxima in England generally exceeded 75°F at inland stations, a maximum of 85°F being recorded at London on this day. The mean temperature for July was above normal... in the western districts of England and Wales and about normal elsewhere. Monthly mean temperatures in August generally showed little divergence from the normal, the warmest days occurred at the beginning and end of the month... During the first few days of September temperature was above the normal..., but the mean temperature being 1° to 2°F below normal in all districts. The mean temperature for October was above normal in all districts... but during the third week lower temperatures (occurred) with severe frost about the 21st. Unusually high temperatures exceeding the highest on record for November at many stations were recorded during the first few days of the month, maximal temperatures of 60°F and above being recorded widely on the 2nd. and 3rd. On the 5th. temperatures began to fall and from the 7th. to the 14th. extremely cold wintry weather prevailed. »

These general observations afford a valuable criterion of probable local variation in sea-temperature conditions since it has been shown that in estuarine waters generally sea-temperature follows mean air-temperature with little or no lag-period (Orton, 1924, fig. 2, Orton, 1934, fig. 4) and that in such insular situations as the creeks and shallow waters of the Blackwater Estuary mean sea-temperatures remain above mean air-temperatures during most of the year and in close direct relationship.

General Observations on Spawning and Sex.

By spawning is meant the extrusion of ripe eggs or sperm from the genital aperture. As *O. edulis* incubates its ova in the mantle cavity until a free-living larval stage is attained, observation of the act of spawning in females is almost impossible. Normal extrusion of the larvae occurs after a variable period of incubation, but this extrusion or liberation of larvae is not a true spawning act and may be distinguished from the latter by the designation larval liberation.

The time and/or date at which a female-functioning oyster has spawned can be determined approximately by noticing the stage of development of the embryos or larvae, the sequence of whose development has been determined experimentally (Orton, **1922**, **1927***a*, **1933** and Spärck **1924**). It can be presumed that male-functioning oysters expel their sperm to the exterior as high percentages of mature males occur at the begunning of the spawning season and afterwards decline; morover one individual has been observed definitely to spawn in the laboratory in this way; partial spawning has been observed in others. No-one has yet described liberations either of larvae or male spawn from observations in the sea.

In dredged samples of oysters, individuals with eggs in one- to eight- celled stages are not infrequently found within a period of a few hours after capture, and especially after storage in a warm place overnight. There can be no doubt that most of these have spawned unnaturally or prematurely. Individuals which have spawned under these conditions are classed as « premature » spawners or « forced » spawners. It is not unlikely however that most of such spawners are ripe individuals almost ready to spawn under natural conditions on being subjected to the appropriate stimuli. The stimulus or stimuli which cause spawning are not definitely known but are discussed by the writer (**1926**); the subject will de dealt with again at a later date.

The problem of sex has been dealt with in a former paper (Orton, **1933**). The results of many years work lead to the conclusion that while high percentages occur of individuals which are predominantly female and function as such it is probable that pure females, that is, individuals which are entirely egg-bearing, are relatively rare on English beds. But purity in femaleness is largely dependent upon environment (*loc. cit.*) and possibly greater purity of females occurs normally on the Blackwater than on the Fal Estuary beds. Since the environment may vary from year to year it is inadvisable to make too sweeping assertions. Pure males are far more common on English beds than pure females, especially before and after the breeding season. At the begunning of the spawning season a high percentage (i.e. **40** to **50** %) of fully ripe males is normal. Femaleness is often superimposed rapidly upon a good male condition in the early part of the spawning season. It has been shown experimentally (*loc. cit.*) that such hermaphrodite individuals spawn their eggs, but without doubt produce fewer larvae than fuller females.

Intersexes or hermaphrodites therefore occur not uncommonly (Orton, **1926**, **1928**), with a variety of proportion of eggs and sperm; nevertheless such individuals probably mostly function as females.

It has been shown experimentally that female functioning individuals develop normally and rapidly into males from the *instant* of egg-spawning (Orton, 1927 *a*). This sex-change is very definite and affords a *point d'appui* for all experiments on sex-change in individuals. It occurs at all ages. The return from the male to the female stages has also been demonstrated experimentally (Orton, 1933) but this change of sex is probably entirely dependent upon external conditions. It may occur within a year or less or be delayed, if nutritional conditions are not good (*loc. cit.*).

The initial sex-condition in *O. edulis* is almost certainly maleness, but satisfactory proof of this point is very difficult to obtain (Orton, 1922). From the initial condition sex alternates throughout life as indicated above to an advanced age (Orton, 1933).

Fertilisation almost certainly always occurs in the oviduct as Hoek deduced long ago (1883). But it is still a matter of conjecture to what extent cross-fertilisation occurs. Since females on English beds nearly always carry some sperm morulae amongst the eggs, self-fertilisation will nearly always be possible. On the other hand pure males undoubtedly exist and spawn, and Hoek has described accumulations of sperm in diverticula of the renal duct of egg-bearing individuals. These sperm may be either collections from the individual itself or from some other individual so far as we know at present. Researches on this important aspect of oyster-culture are difficult but are urgently needed. It is clear therefore that self-fertilisation may occur; whether cross-fertilisation actually occurs is not known, but is biologically probable.

I. — BLACKWATER OYSTER BEDS

Categories of oysters with young.

The oysters examined from the Blackwater Oyster Beds varied in age from about 4 to 6 years old but were mainly about 5, being mainly 2 1/2 to 2 5/8 inches (=56 to 69 mm.) in mean diameter but with the deep heavy shells typical of this locality.

Experience had proved that spawning may begin in this locality about or after the middle of May and when the sea-temperature attained a level of about 15-16°C. A trial sample was examined on May 6 and no oyster found in spawn. Work on the beds was begun on May 25 when out of 1103 individuals dredged the previous day and stored overnight in a pit, two oysters were found oozing embryos from the shell in the 4-celled stage. Among 101 dredged on the 25th. and opened at once none were found in spawn, nor in similar samples dredged on the 26th and 30th. It was clear that spawning had not begun and that the two individuals which had been retained in the pits overnight on the 24the and extruded 4-celled embryos were in all probability premature or forced spawners. These are shown in Table 1, column 4, as premature spawners,

TABLE 1. — Blackwater Oyster Beds, 1927. — Percentages of various categories of mature female-functioning oysters, O. edulis, for periods (mainly weekly) beginning on the dates of the moon's quarters.

Date Week beging moon's quarters	N° of oysters examined	Sea-Temp. in °F (*)	Oysters carrying embryos or larvae					Total with embryos or larvae	Total ripe or nearly ripe unspawned females	Total females (All categories	Standard (**) % error of sampling of total females (11) ±
			Premature? White-sick	Embryos & larvae normal White-sick	Shelled No colour Greysick	Total columns 5 & 6	Coloured or Black-sick				
1	2	3	4	5	6	7	8	9	10	11	12
May 5	50	55.7	0	0	0	0	0	0	—	—	—
24	303	57.4	0.7	0	0	0	0	0.7	37.6	38.3	2.69
● 30	831	59.4	1.8	1.9	0.5	2.4	0	4.2	28.9	33.1	1.63
June 7	412	60.2	4.1	5.3	1.9	7.2	4.4	15.7	26.0	41.7	2.43
○ 15	309	60.6	6.1	2.9	3.6	6.5	12.9	25.5	17.9	43.4	2.82
22	416	58.4	3.1	0.5	4.6	5.1	5.0	13.2	15.9	29.1	2.23
● 29	517	61.2	3.1	0 4	4.4	4.8	6.0	13.9	19.4	33.3	2.07
July 7	305	65.6	6.6	1.6	1.6	3.2	8.5	18.3	16.5	34.8	2.73
○ 14	456	62.6	0.4	0.7	2.0	2.7	7.2	10.3	13.6	23.9	2.00
21	207	63.5	2.4	2.4	1.4	3.8	5.3	11.5	8.8	20.3	2.80
● 28	203	65.6	3.0	2.5	1.0	3.5	3.4	9.9	8.8	18.7	2.74
Aug. 5	102	67.2	1.0	0	2.0	2.0	2.9	5.9	6.8	12.7	3.30
○ 13	104	65.0	0	0	1.9	1.9	2 9	4.8	2.9	7.7	2.62
19	—	63.4	—	—	—	—	—	—	—	—	—
● 27	—	61.6	—	—	—	—	—	—	—	—	—
Sept. 4	—	63.3	—	—	—	—	—	—	—	—	—
○ 11	385	59.8	0	0	0	0	0.8	0.8	1.3	2.1	0.73
18	—	58.7	—	—	—	—	—	—	—	—	—
● 25	—	54.1	—	—	—	—	—	—	—	—	—
Oct. 4	132	54.2	0	0	0	0	0	0	1.5	1.5	1.06
○ 10	101	54.0	0	0	0	0	0	0	4.0	4.0	1.94
17	102	51.8	0	0	0	0	0	0	3.9	3.9	1.92
● 25	—	53.2	—	—	—	—	—	—	—	—	—
Nov. 2	—	53.0	—	—	—	—	—	—	—	—	—
○ 9	—	43.6	—	—	—	—	—	—	—	—	—
16	111	43 9	0	0	0	0	0	0	8.1	8.1	2.59
Total	5046	Aggregate %		18.2	24.9	43.1	59.3				

(*) Weekly means of readings daily at H. and L. W. excepting Sundays for the dates in column 1.

(**) Calculated on the formula : $\frac{100}{n}\sqrt{\frac{a(n-a)}{n}}$. See Fisher, 1928. Where n = number of individuals in the sample and a = number of females in the sample. I have to thank Mr G. M. Spooner and Mr E. Ford for this simplification of the full formula.

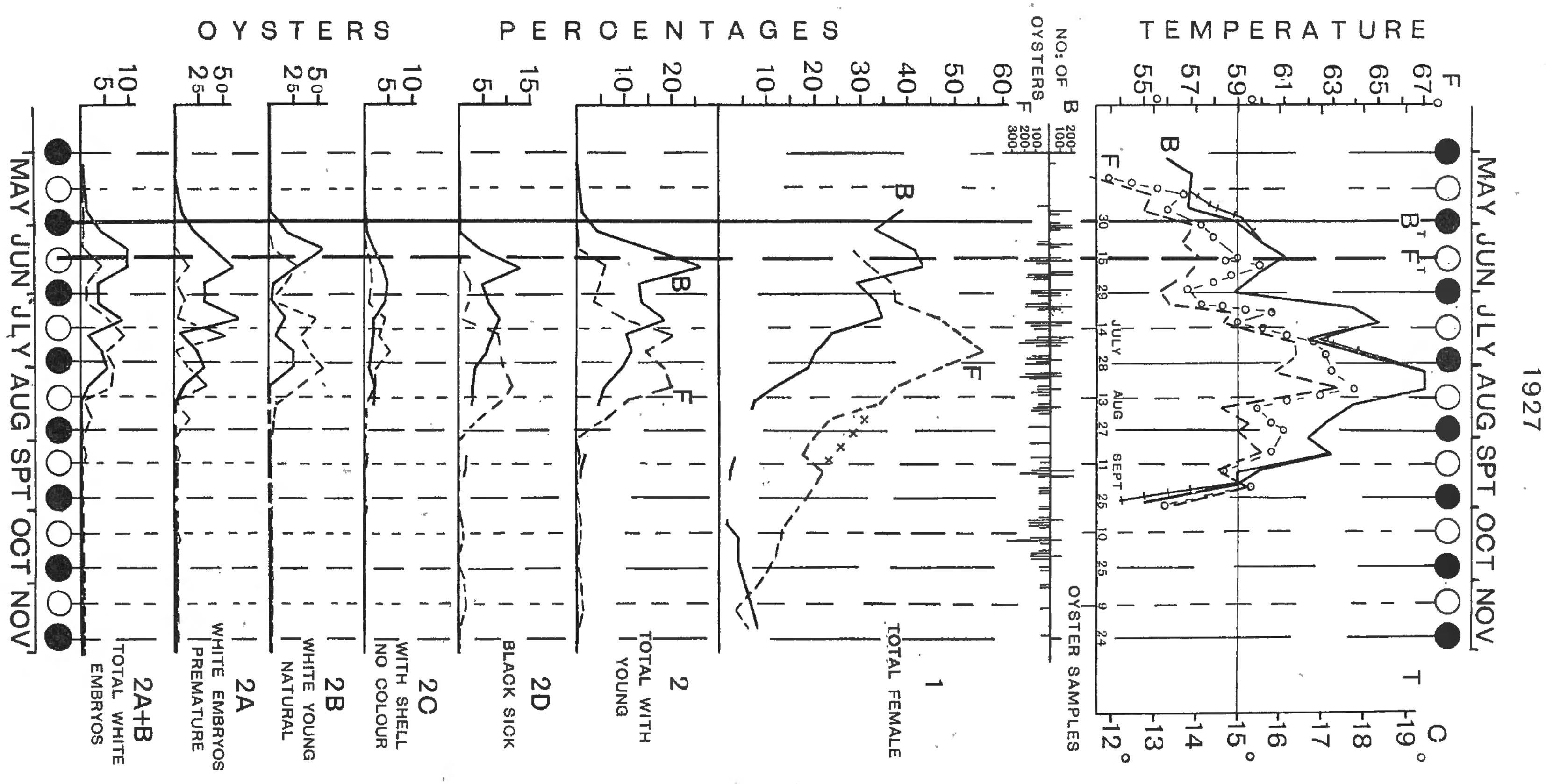

FIG. 1. — Data regarding the oyster beds in the Blackwater Estuary (B) and Truro Beds, Fal Estuary (F) for 1927 regarding: sea-temperature, T, size, date, and frequency of oyster samples examined, and mean group percentages (for mainly weekly periods beginning on dates of the moon's quarters) of 1, total female; 2, total with young; 2D, blacksick, 2C greysick; 2B, normal whitesick; 2A, premature (?) whitesick, and 2A+B, total whitesick.

The temperature records, T, given by the continuous thick-lined graph (B) are means for high and low water on the Blackwater; low water readings for this locality are given by the thin continuous line with short cross-strokes. Short period-means (i.e. a few days) and isolated daily readings for the River Station, Fal Estuary, are given by the broken-line graph with circles, and for the oyster banks by the plain broken-line. Vertical lines drawn from the origin of the respective graphs for normal whitesick for the Blackwater and Fal cut the corresponding temperature graphs at about the level of 59° F = 15° C.

as are all similarly spawning individuals. It was found that the proportion of premature spawners is often high in samples dispatched by rail to a distant destination. Whenever samples were examined either on the beds within a few hours of dredging or later, the state of development of all young embryos was determined microscopically. It was then possible to decide in most cases whether the spawning had occurred in the sea naturally, or subsequent to dredging, and in that case in all probability prematurely. All categories are given in mean percentage. Natural spawners are recorded in colums 5, 6 and 8 of Table 1. Oysters with segmenting embryos or trochospheres are shown in column 5; those with larvae which had developed more or less shell but no colour in the body in column 6, and those with shelled larvae with bodies coloured from slate to black in column 8.

The mean percentage of mature individuals with abundant ripe or nearly ripe eggs in the gonad are recorded in column 10, ond the percentage total of all mature female-functioning oysters, including those which are carrying embryos or larvae and those ready or almost ready to spawn, is given in column 11.

In all some 50 samples of 100 oysters were examined at different dates which have been grouped in Table I in weeks beginning on the date of the moon's quarters. Every sample was analysed macro- and micro-scopically into the categories shown in Table I, and some 20 other categories which will be dealt with later. 43 samples were examined between May 25 and August 17, but cessation of work on the beds then prevented examination of further samples until September 13 and also after September 15 until October 4.

The percentage of the classes denoted in colums 4 to 11 of Table 1 are plotted in fig. 1. In addition the classes « premature » and « normal white embryos and larvae » are plotted together in fig. 1 as 2 A + B.

Significance of the sampling.

The samples consisting of 100 to 831 individuals per week may be taken to represent the state of the population of the locality *or* localities *approximately* at the dates they were taken. They are grouped for weeks beginning on the date of the moon's quarters in Table 1 (p. 1004) and in periods centred on the dates of the moon's quarters in Table 2 (p. 1009). The succession of weekly samples increases the probability that the samples are representative of seasonal changes; while the alteration in the proportions of the different categories recorded as mean percentages — notwithstanding the range of variation in frequency observed — (Table 3, p. 1011) offers a test of the degree to which the samples represent the population and the change in sex-condition. The recurrence of similar general breeding phenomena in successive years to those recorded in Table 1 (see fig. 4, p. 1048) also adds to the probability that the observations offer an approximate picture of the sequence of changes in the population during the spawning season. No attempt is made in the present paper to analyse the results statistically, and only a broad comparison is made of the spawning in the two geographical localities. Evidence has emerged in the course of the investigation to indicate that the population examined on the Fal is not homogeneous.

On the Blackwater it seems probable that the populations of the various local beds are more homogeneous than those on the Fal : local differences in spawning behaviour of populations will be dealt with later.

Whatever may be the difference in local populations in one geographical locality, the comparison of the spawning behaviour in the two geographical localities as a whole brings out distinct and striking differences.

The difference in the sequence of spawning in the two main sets of populations on the Fal renders it desirable to draw at this stage only somewhat broad statistical generalisations from the results obtained. This is a pioneer study and will, without doubt, lead to improvements in the method of study as the factors conducing to a homogeneous population become better known.

Seasonal variation in percentage of potential females.

From Table 1 (p. 1004) and fig. 1, B (p. 1005), the following deductions may be made. The mean percentage of females ranges about 40 % at the beginning of the spawning season in June (see also Table 3). During June there occurs a diminution in the percentage correlated with a fall in temperature and during July a rise in the percentage correlated with a rise in temperature. The diminution is probably real due to evacuation of larvae by early spawning females and cessation of female production and the rise in early July is significant as it occurs with the observed superimposition of rapidly developing female phases upon a variety of male conditions, and the standard error of sampling as a percentage is small. After the middle of July the percentage of females diminishes gradually to the end of the spawning season when only about 2 % remain unexpended. Corroborative evidence will be given later that these results are statistically reasonable. In October-November a slight increase in total females occurs as has been observed on other occasions (See fig. 4, p. 1048).

The individuals in which a female phase is being rapidly superimposed on maleness can be recognised macro- and micro-scopically; they occur in fair proportion, and have been referred to a special category to be dealt with later along with other types.

It is now clear that a definite seasonal cycle of change occurs in the production and expenditure of females on all types of oyster bed (See Table 9, p. 1036, fig. 1 and p. 1046). Females begin to develop *mainly* in the pre-spawning period and especially in the spring, and reach a maximum proportion some time after the onset of the spawning season; they spawn and disappear as such from the population, while others are maturing. Although the production reaches a maximum about mid-summer, it may continue at a diminishing rate afterwards; during the later part of the spawning season the females are more or less gradually expended as they mature to reach a minimum about August or later, according — as will be shown — to the hydrographic characters of the particular oyster-bed and season.

Rate of development of the incubated young.

From observations and experiments made on the rate of development of oyster embryos and larvae by the writer (1926, p. 217 and 1927, p. 996) and Spärck (1924, p. 46) the following phases and approximate times may be allocated for the successive stages of development.

O. edulis extrudes white ova and retains and incubates them in the mantle cavity. So long as the ova and later developmental stages remain white the parent is said to be whitesick, when the larvae later become grey the term greysick is applied, and when these in turn become coloured in various hues from lilac to black, the parent is blacksick. The rate of development varies with temperature, but the following times may be regarded as approximately normal for English oyster-beds.

The embryos remain white for about 3 to 4 days; reaching the morula and early blastopore stage in about 20 hours; an elongate heart-shape, but not ciliated, stage in about 2 days; and becoming ciliated trochospheres in about 3 days. The colour begins to change at about 3 to 4 days to grey when the rudimentary shell appears. The grey stage is passed rapidly, while the trochosphere develops into a veliger and the shell attains a length of about 150 μ during the fourth or fifth day. On the 5th or 6th day the larvae become coloured by the development of pigment in the digestive gland. The coloured larva continues to develop and grow in the parent for a further period of a few days when the shell may acquire a length of 170 to 200 μ before being extruded.

The whitesick stage is thus normally of about 3 to 3 ½ days duration, the grey shelled stage about 1 ½ to 2 days or less, and the blacksick stage of variable duration, probably 4 days (Orton, 1926, p. 224) or less (Spärck, p. 46). It seems probable that the oyster larva becomes fully developed normally *in the sea* in a period of 6 or 7 days and is expelled at an age between 7 and 10 days (Orton, 1926, p. 217).

Percentage of population spawning as females in 1927.

In the samples from the Blackwater in 1927 (See Table 1) coloured larvae were not found (See fig. 1, 2D) until the week beginning June 7, and in the following week occurred in the maximum percentage of 12.9 of the population. Afterwards the percentage fluctuates around 5 to 8.5 until July 28 when the percentage fell to 3.4, and diminished afterwards until the small figure of 0.8 is found about September 14. The higher figure 8.5 again corresponds with the secondary maximum already noted in the graphs 1 and 2, fig. 1. None occur from October 4 to 19 and none were found in one sample examined on November 23.

TABLE 2. — **Blackwater Estuary, 1927. — Percentages of various categories of mature female-functioning oysters, O. edulis, in samples grouped to give maximal intervals between groups, and fortuitously, mainly centred on dates of moon's quarters.**

Dates of Moon's quarters		Period.	No. of oysters examined	Categories with young							
				Whitesick Prem.		Whitesick normal		Greysick		Blacksick	
				No.	%	No.	%	No.	%	No.	%
May	24	20 — 27	303	2	(0.7)	0		0		0	
●	30	28 — 3	814	15	(1.8)	16	(2.0)	4	(0.5)	0	
June	7	4 — 11	311	12	(3.9)	20	(6.4)	7	(2.3)	7	(2.3)
○	15	12 — 18	307	10	(3.3)	10	(3 3)	9	(2.9)	33	(10.7)
	22	19 — 25	306	22	(7.2)	3	(1.0)	18	(5.9)	22	(7.2)
●	29	26 — 3	415	5	(1.2)	0	(0.0)	27	(6.5)	22	(5·3)
July	7	4 — 10	311	16	(5.1)	2	(0 6)	0	(0.0)	26	(8.4)
○	14	11 — 17	305	20	(6.6)	5	(1.6)	5	(1.6)	26	(8.4)
	21	18 — 24	558	5	(0.9)	3	(0.5)	9	(1.6)	41	(7.3)
●	28	25 — 1	105	2	(1.9)	5	(4.8)	3	(2.9)	3	(2.9)
Aug.	5	2 — 9	306	7	(2.3)	5	(1.6)	4	(1.3)	10	(3.3)
○	13	10 — 15	—	—	—		—	—			—
	19	16 — 23	104	0		0		2	(1·9)	3	(2.9)
●	27	24 — 31	—	—		—		—			—
TOTALS			4145	116		69		88		193	
Aggregate %'s					34.9		21.8		27.4		58.7
							49.2				

The percentage of oysters with coloured larvae is the product of the percentages of those carrying younger larvae or embryos in about the preceding 5 or 6 days, and since the common interval between groups of samples is 5 to 7 days (see Table 3, p. 1011) it is seen that roughly the total of all oysters carrying embryos or non-coloured larvae in one weekly period should recur in the total carrying only coloured larvae in the following week. By comparison of columns 7 and 8 in Table 1, a rough correspondence is seen, but those in the former are on the whole less. The figures in column 7, however, do not include oysters presumed to have spawned prematurely. If columns 4, 5 and 6 be added together the total is on the whole somewhat higher than the percentage with coloured larvae in the following week. It is probable therefore that some of the oysters presumed to have spawned prematurely would have spawned naturally if they had remained undisturbed on the beds. The correspondence of percentage with early young in one week with older (coloured) young in the following week is sufficiently clear to be significant. It is possible although improbable that in some weeks the coloured larvae were retained longer than 5 or 6 days and therefore appear twice in the totals. On the other hand some may have been extruded before sampling occurred. These two sources of error probably balance to a considerable extent, except at the warmest periods when both development and extrusion is speeded up, and in the colder periods when the larvae are retained longer. On the whole the correspondence is reasonably good when considered in relation to all factors including variance in sampling. Adding up the totals of percentages with coloured larvae in column 8, Table 1, we obtain the figure 59.3 which represents approximately the mean percentage of the population which spawned as females from May 30 to September 11, since coloured larvae may be presumed to be evacuated in the interval between the groupings of the samples; any error likely to arise in this connection in this period of spawning would be slight. Moreover, there are good grounds for assuming that the populations examined are homogeneous or sub-homogeneous in spawning behaviour. A second independent estimate of the approximate percentage of the population functioning as female may be made from the maximum mean percentage of total females in any one week, and on the permissible assumption that individuals carrying coloured larvae extrude them in less than one week. In the week June 15, Table 1, 43.4 % of the population were female functioning and prior to that date 4.4 % were found with coloured larvae, giving a total to date of 43.4 + 4.4 = 47.8 % female. In the following week 5 % were blacksick but as they were younger in the previous week, they are not counted. In that week the total female reaches the first minimum of 29.1 %. In the week beginning June 29, 6 % blacksick occur and the percentage of females has risen to 33.3. In the following week a second maximum of females occurs, namely 34.8 %, including 8.5 blacksick. It has been noted that fresh acquisition of femaleness occurs in this period, and that this maximum is a real one, and not due to vagaries of sampling. In the interval between the minimum percentage of females (June 22) and the attainment of the new maximum 34.8 % (July 7) 6 % blacksick have been produced and dissipated and the percentage of females has been increased

TABLE 3. — Blackwater Estuary 1927. — Percentage frequencies of (4) recently normally spawned, 8a ripe or ripening unspawned female, and 8a plus prematurely spawned (= 8b) oysters (**O. edulis**) in samples grouped to give maximal time intervals (fortuitously in weeks centred on moon's quarters).

Periods centred on moon's quarters 1	Dates of samples (ca. 100 each) 2	Interval in days 3	% frequency of whitesick and greysick combined 4	Percentages (4)			8a % frequency of ripe and ripening unspawned females / 8b do + % prematurely spawned $8 = \frac{8a}{8b}$	Percentages (8)		
				min. 5	max. 6	mean 7		min. 9	max. 10	mean 11
May 20–28	25, 25, 26	4	0, 0, 0	0 0	0.0	0.0	$\frac{43.7}{45.6}$, $\frac{33.0}{33.0}$, $\frac{36.0}{36.0}$	$\frac{33.0}{33.0}$	$\frac{43.7}{45.6}$	$\frac{37.6}{38.2}$
28– 3	30, 31, 31, 1, 1, 2, 2, 3	5	0, 0.9, 1.0, 3.9, 1.0, 2.9, 5.0, 5.0	0.0	5.0	2,5	$\frac{42.0}{42.0}$, $\frac{27.1}{27.1}$, $\frac{31.0}{33.0}$, $\frac{20.4}{28.2}$, $\frac{24.0}{24.0}$, $\frac{28.8}{28.8}$, $\frac{28.0}{28.0}$, $\frac{31.0}{36.0}$	$\frac{20.4}{24.0}$	$\frac{42.0}{42.0}$	$\frac{29.0}{30.9}$
June 4–11	8, 8, 9	5	5.8, 10.7, 9.6	5.8	10.7	8.7	$\frac{29.8}{37.5}$, $\frac{24.3}{25.2}$, $\frac{27.9}{30.8}$	$\frac{24.3}{25.2}$	$\frac{29.8}{37.5}$	$\frac{27.3}{31.2}$
12–18	14, 15, 15	6	3.0, 8.6, 6.9	3.0	8.6	6.2	$\frac{21.8}{26.7}$, $\frac{16.2}{20.0}$, $\frac{25.7}{26.7}$	$\frac{16.2}{20.0}$	$\frac{25.7}{26.7}$	$\frac{21.2}{24.5}$
19–25	21, 22, 22	6	3.9, 5.0, 11,8	3.9	11.8	6.9	$\frac{11.7}{25,2}$, $\frac{16.8}{21.8}$, $\frac{11.0}{13.7}$	$\frac{11.0}{13.7}$	$\frac{16.8}{25.2}$	$\frac{13.2}{20.2}$
26– 3	28, 28, 30, 29	6	1.9, 1.9, 5.7, 17.0	1.9	17.0	6.6	$\frac{14.2}{17.9}$, $\frac{21.5}{22.4}$, $\frac{15.1}{15.1}$, $\frac{19.0}{19.0}$	$\frac{14.2}{15.1}$	$\frac{21.5}{22.4}$	$\frac{17.5}{18.6}$
July 4–10	5, 5, 6	6	0, 1.9, 0	0.0	1.9	0.6	$\frac{24.5}{33.3}$, $\frac{19.0}{20.0}$, $\frac{19.2}{25.0}$	$\frac{19.0}{20.0}$	$\frac{24.5}{33.3}$	$\frac{20.9}{26.1}$
11–17	12, 12, 13	5	0, 5.0, 4.9	0.0	5.0	3.3	$\frac{11.9}{19.8}$, $\frac{15.8}{18.8}$, $\frac{21.4}{30.1}$	$\frac{11.9}{18.8}$	$\frac{21.4}{30.1}$	$\frac{16.4}{22.9}$
18–24	18, 18, 19, 20, 20, 21	6	0, 4.8, 0, 5.1, 4.0, 0	0 0	5.1	2.3	$\frac{20.0}{20.0}$, $\frac{13.5}{14\ 4}$, $\frac{9.7}{9.7}$, $\frac{9.1}{9,1}$, $\frac{18.0}{20.0}$, $\frac{6.9}{9.8}$	$\frac{6.9}{9.1}$	$\frac{20.0}{20.0}$	$\frac{12,9}{13.8}$
25– 1	27	6	7.6	7.6	7.6	7.6	$\frac{10.5}{12,4}$	$\frac{10\ 5}{12.4}$	$\frac{10.5}{12.4}$	$\frac{10.5}{12.4}$
Aug. 2– 8	2, 3	6	0, 6,9	0.0	6.9	3.5	$\frac{8.8}{10.8}$, $\frac{8.9}{12.9}$	$\frac{8.8}{10.8}$	$\frac{8.9}{12.9}$	$\frac{8.9}{11.9}$
9–16	9	8	1.9	1.9	1.9	1.9	$\frac{6.8}{7.8}$	$\frac{6.8}{7.8}$	$\frac{6,8}{7.8}$	$\frac{6.8}{7.8}$
17–23	17	—	1.9	1.9	1.9	1.9	$\frac{2.9}{2.9}$	$\frac{2.9}{2.9}$	$\frac{2.9}{2.9}$	$\frac{2.9}{2.9}$
			TOTALS . . .	26.0	83.4	52.0				

34.8 - 29.1 = 5.7 %. Thus 6.0 + 5.7 are to be added to 47.8 = 59.5 % to give the approximate actual and potential femal production in the population at this date. Additional production of females after his date is probably slight. The minimum percentage of females spawning during the season is also given approximately by the accumulative weekly percentages of normal whitesick + greysick, since these phases will, with rare exceptions, be passed in the interval, 5 to 7 days, between sampling. When the samples are grouped to give a maximum interval between the weekly samplings (as occurs fortuitously when the weekly grouping is centred on the dates of the moon's quarters, see Table 2), the intervening period is found to be on the average 5.9 days. Therefore none of these categories will normally reappear as such; that is, they are expended in becoming or rather developing into the later category, the blacksick. The aggregate of these percentages (i.e. columns 5 and 6, Table 2) 49.2 %, will give approximately the minimum percentage of spawners during the season. Since, however, some of the premature would also have spawned naturally on the beds the actual percentage of spawners will be greater than denoted by the latter figure, and nearer the aggregate total percentage of blacksick. These arguments only hold if the population examined is approximately homogeneous for spawning behaviour, and there are good grounds for the view that the bulk of the population is homogeneous.

The three estimates are therefore as follows :

Accumulative mean percentage of blacksick (Tables 1 and 2) (1) . . . . =59.3-59.5
Accumulative mean percentage of normal whitesick plus greysick (do.). . =43.1-49.2
Maximum mean weekly percentage of total female with preceding and subsequent blacksick production (Table 1) . . . . =59.5

Notwithstanding the range of variation shown in samples in Table 3, the concordant results given above fully justify the broad conclusion that not less than 50 to 60 % of the oyster population functioned as females on the Blackwater in 1927.

It is interesting in comparison with the Fal grounds, to be noted later, that only 7.7 % potential females remained unspawned as early as August 19.

Incidence of spawning in relation to sea-temperature level.

The variation in temperature of the waters of the Blackwater Estuary in 1927 has been described and discussed (Orton, 1928) and is shown in the upper part of fig. 1, at B. The continuous thick-lined graph is the mean of daily readings at high and low water (excepting Sundays). The continuous thin-lined graph with transverse lines gives the means of low-water readings which are higher than the means of high water readings in the spring and lower in the autumn. Table 1 gives the weekly means, and Table 4, p. 1014, the daily observations during the critical pre- and early spawning period.

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(1) Note absence of 0.8 % blacksick, Sept. 11, in Table 2.

It has been shown that no evidence of natural spawning was found on the beds below low water before May 30. On May 31, 1 % had spawned naturally (white embryos) on the dredging ground and 1 % with trace of shell but uncoloured was taken by hand on the shore at low water. In the week beginning May 30, 831 oysters were examined and an average of 4.2 % found with young spawn, none being blacksick. Relatively heavy spawning occured in the following week, and also 4.4 % blacksick were found.

About this period « the temperature ranged between 47-50°F in the first fortnight of April, rose to 53°-54° on the 20th. to 23rd., but fell again on the 26th. to 50° and remained at this figure until May 1st. or 2nd. when it rose to 51-52°. From May 3rd. to 7th. the temperature rose to 59°-60° for 3 days, and then fluctuated between 57° and 54° until May 18th., but remained steady about 58° until the 21st., and then fluctuated between 57° and 59° until May 30th. » (Orton, 1928, p. 387).

The daily variations in this month and in June are given in Table 4 in detail.

In reviewing the temperature variation in May it is seen that the temperature rose rapidly from 51-52°F on May 2 to 59-61-60 in the period May 6th. to 10th., but no evidence of any spawning in this period was obtained. A sample of 50 oysters was dredged May 5 and examined at Plymouth on May 12. None of these carried spawn and there was no indication of any having spawned although at least 30 % were almost ready to do so. None were examined afterwards however until the 25th. We have no direct evidence therefore bearing on the spawning from May 6 to 10. None were reported to have spawned in the store. It is probable in view of what was observed later that if spawning did occur the reduction in temperature would cause the oysters to eject the eggs or embryos. It is also not unlikely that, if any spawning had occured without ejection of the spawn, the low temperature prevailing from the 10th. would have retarded development sufficiently for shelled larvae to have persisted until the 25th.

After May 18 the temperature varied little from 58° until the 30th. without any spawning amongst those oysters which were constantly immersed, as is proved by the examination of 403 oysters on the 25th. to the 30th. On and from the 31st. when the temperature ranged around 59-60° all samples contained naturally spawned oysters. Spawning among females therefore began when the temperature attained a level of 59°F (=15°C) or more and did not fall below this level, and continued with greater or less intensity until June 22.

The peak and cessation of spawning.

Coincidently with the fall in temperature on the full moon spring tides at the end of June occurred a marked fall in the percentage of newly spawning oysters to 0.5 and 0.4 in the last two weeks in that month.

TABLE 4. — Daily surface temperature readings (in °F at H and L.W.) on the Blackwater oyster beds during the pre- and early spawning period.

Date		Readings	Locality	Time in Hours	Temperature °F	Depth in Metres
May	2	2	Thornfleet	8–14.0	51–52	4–8
	3	2	do.	9–15.0	53–53	do.
	4	2	do.	9.30–15.30	55–55	do.
	5	2	M. P. and Thornfleet	10–16.0	57–56	–8
	6	2	D. M. and Thornfleet	6–11	58–59	–4
	7	2	do.	6–12	59–60	–4
	9	2	Thornfleet	7–13	60–61	8–4
	10	2	W. B. & do.	8–14	59–60	–4
	11	2	Thornfleet	9–15	57–56	8–4
	12	2	do.	10–15	57–56	8–5
	13	2	D. M. and Thornfleet	6–11	54–55	–8
	14	2	do.	6–12	54–55	–8
	16	2	Thornfleet	7–13	55–55	4–8
	17	2	do.	7.30–13.30	56–55	4–8
	18	2	do.	8–14	58–57	4–8
	19	2	do.	9–15	58–58	4–8
	20	2	do.	10–16	58–58	4–8
	21	2	D. M. and Thornfleet	6–11	57–58	7–4
	23	4	Th., mid-ch. Bl., Th.	6–14	56–57	4–14
	24	7	All parts	7–21	57–58	7–14
	25	3	Various	8–14	58–58	9–16
	26	5	do.	7–15	57–59	3–16
	27	3	do.	7–15	58–58.5	6–10
	28	3	do.	7–11	57–57	7–8
	30	3	do.	6–12	58–59	3–10
	31	4	do.	6.30–13	60–59	4–10
June	1	4	do.	7–14	60.5–60	4–13
	2	4	do.	7–15	59.5–59	8–16
	3	3	do.	7–14	59.5–59	6–10
	4	4	do.	7–12	60–60	4–13

TABLE 4. — Daily surface temperature readings (in °F at H and L.W.) on the Blackwater oyster beds during the pre- and early spawning period (continued).

Date	Readings	Locality	Time in Hours	Temperature °F	Depth in Metres
June 6	3	Various	6–11	59–59.5	6–14
7	3	All parts	7–13	59–59	8–14
8	3	W. B. and Thornfleet	8–14	59–60	8–4
9	3	Th. Bl.-Ch. Th.	7–15	59–61	6–14.3
10	3	W. B. and Thornfleet	7–15	59.5–61	9–8–4
11	2	E. B. & W. B.	7–11	60–60	7–10
13–18	12	All parts	7–21	60–62	4–12
19–25	13	do.	6–18	59–61	6–10
27	3	D. M. & Deeps	6–14	56–58	3–10
28	3	D. M. & Ch.	6–13	57–59	2½–10
29	3	Ch.	7–13	58–59	7–10
30	2	Deeps & Th.	8–14	59–59	8–8
July 1–2	5	Various	6–15	61–60	6–10
4–5	7	do.	6–16	62–63	4–8
6 9	12	do.	6–14	63–65	4–10

M. P.	Mell Pier	W. B.	West Boundary
D. M.	Dan's moorings	E. B.	East Boundary
Th.	Thornfleet	Ch.	Channel
		Bl.	Blackwater.

Early in July with a rise in temperature 1.6 % of fresh spawners was found, and rather fewer 0.4 % on the full moon tides, when temperatures again fell to a mean of 63.6 in the week beginning July 14th. In the latter half of this month with temperatures at first unchanged, and later rising, the percentages of new spawners rose respectively to 2.4 and 2.5 %.

By this time the mature unspawned females in the population had been reduced to 8.8 %. During early August 2 % and 1.9 % new spawners were found in samples of 100 examined weekly, 3 % oysters with shelled but uncoloured embryos in the week beginning August 13, and a decrease in the mature unspawned females to about 3 %.

As no samples were examined in the latter half of August and the first week in September when temperatures remained still high with weekly means 63.3 — 61.4 — 63.3, no direct evidence of spawning was obtained, but the occurrence of 0.8 % blacksick in the week beginning September 11th. among

385 oysters along with the reduction of mature unspawned females to 1.3 % indicates that spawning continued weakly, and finished during this period. The temperature fell rapidly on the full moon tide in September and in the week beginning September 18th. the mean fell to 58.7°.

The peak of spawning actually occurred in the week preceding the full moon tides in June, subsidiary peaks in the week preceding the full moon in July and round about the new moon tides at the end of July and early August. In each of these periods there was a distinct rise in temperature.

Spawning almost ceased in the middle of August when temperatures were about the maximum, but at this time there were only about 3.0 % of the population left as mature or maturing females. During late August a few of the latter spawned so that by the time of the September full moon tide, with temperatures at about 59°F. only 1.3 % of the oysters were potential females and none of these were found with new spawn.

Spawning, therefore, virtually ceased on the Blackwater oyster beds soon after the temperature had attained a maximum when few potential females remained to spawn. This type of spawning season appears to be characteristic of these beds.

Summary of spawning in relation to temperature variation.

Spawning began on the new moon tides early in June when the temperature rose to a level of about 59°F. and attained a maximum with the rising temperature (60 to 61°) in the week preceding the full moon in June. With the fall in temperature to about 59° coinciding with the period of the new moon tides at the end of June, spawning almost ceased, but with the rise of temperature to about 66° early in July, spawning increased but fell away again with a drop in temperature to about 64° on full moon tides, but increased again in the succeeding neap tides at about the same temperature. With temperature rising again to a mean of about 65° on the new moon tides at the end of July, the level of spawning was maintained.

Slight spawning continued at maximum temperatures of about 67° early in August. Spawning ceased during late August soon after the attainment of the maximum temperature, but when the stock of potential females was almost exhausted, that is, reduced to a figure between 1 and 2 %.

II. — THE FAL ESTUARY OYSTER BEDS

Categories of Oysters with young and seasonal variation in percentage of potential females.

The oysters obtained for examination from the Truro Lake, Fal Estuary, were more variable in age than those from the Blackwater. The actual size as estimated by length by breadth (=mean diameter) would be on the whole larger, but the shells are lighter and not so deep (see Orton, 1935). The range in age was about the same on the two beds, namely 3 to 6 years, but the average age of the Fal oysters is estimated to be rather less than those from the Blackwater. that is, about 4 years. Samples were examined continuously from June 7th to November 22nd, except for one week in November.

A study of spawning on these beds in 1925-6 indicated that spawning may be expected to begin some time during June. Regular sampling began on June 7th, though others had been examined in January, March, April and May negatively for spawning.

The results of the examination of material from this locality are shown in Table 5, p. 1020 and Fig. 1. Some 10,000 oysters were opened, the weekly samples varying usually from about 250 to 800. Individuals were allotted to the same categories as the Blackwater oysters, and comparable categories are shown in Fig. 1.

In this figure (at 1) is shown the weekly variation in total female functioning, which on the Fal contains more intersexual — definitely hermaphroditic — forms than on the Blackwater. As the weekly numbers are large the percentage of potential females may be considered to represent approximately the state of the population.

Unlike the Blackwater population, that on the Fal showed in 1927 a steady increase of females from about 28 % on June 7th to a maximum of about 56 % on July 21st, and thereafter a gradual diminution, except for a small rise in September to November. The paucity of spawning early in the season permitted an accumulation of females and the clear demonstration of the production of more than 50 % females in the population.

It is a remarkable fact that significant *effective* spawning ceased early in August, although about 20 % of the population were then ripe females which would have spawned under favourable conditions. It is undoubtedly significant also that water of high salinity invaded the beds during August and that the temperature over the main part of the beds was reduced suddenly to a level slightly below 59°F. (=15° C.) during the full moon spring tides in mid-August. The problem is discussed on page 1032 (see also *Nature* 1927*b*, p. 403). The attainment of the maximum proportion of females is coincident with maximal temperature and with the superimposition of effective female phases upon a variety of male conditions in a moiety of the total potential females. The weekly percentages of these (See Table 5 column 11) shows 55.7 % as the approximate maximum number of potential females existing in the week beginning July 21st. This figure is undoubtedly significant and is supported by the figures for the preceding and following weeks. If it be assumed as before that coloured larvae are not retained more than 5 days, they will not appear a second time in the weekly total percentages. Therefore the sum of the percentages of blacksick oysters found before the week of July 21st are additional members of the population which definitely spawned as females in 1927. Thus, if the popu-

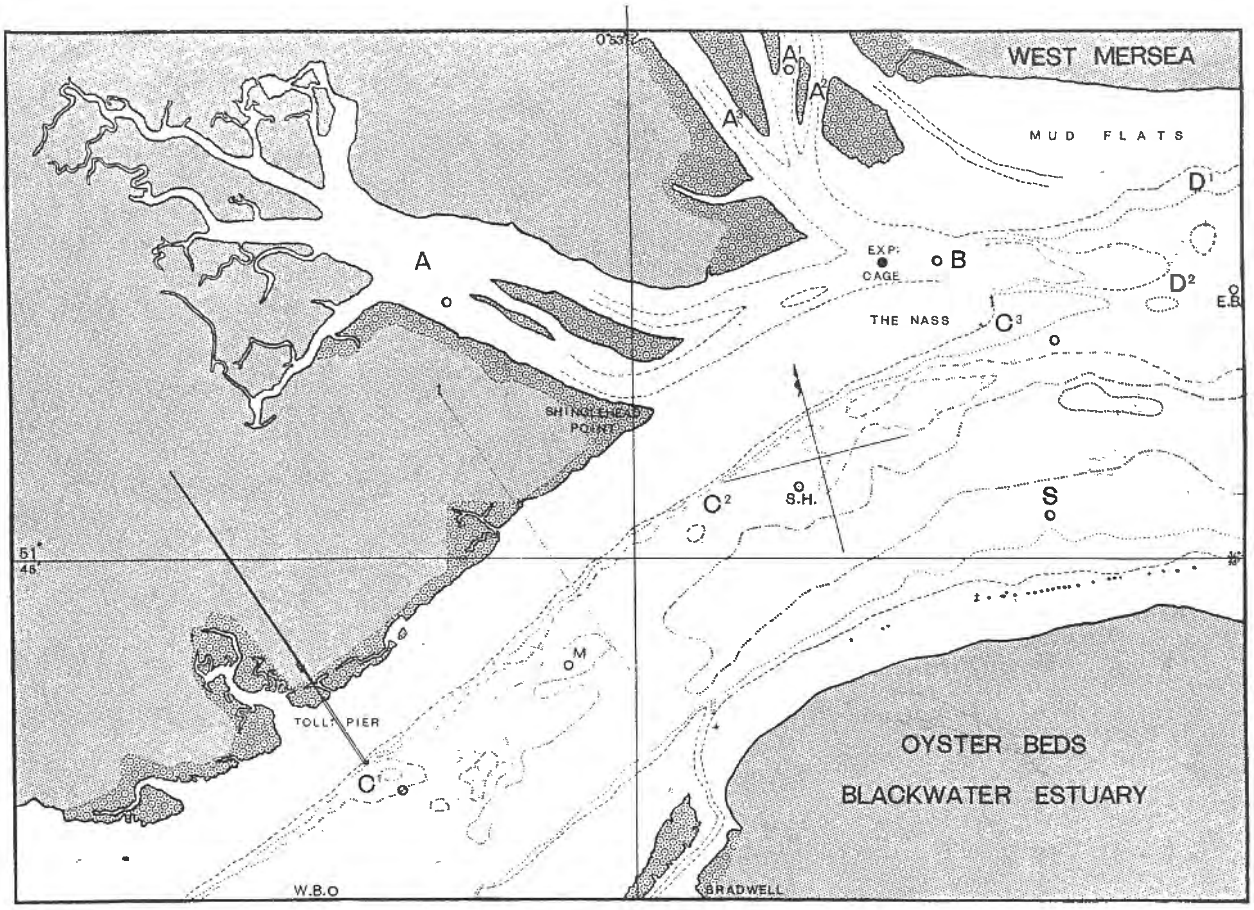

FIG. 2. — Chart of the West Mersea oyster beds and temperature stations in the Blackwater Estuary. (From Admiralty Chart 3740, 1918.)

A^1	Thornfleet beds.
A^2	Mersea Fleet beds.
A^3	Salcott Fleet beds.
A	Tollesbury Fleet and M. V. *Dan's* Moorings.
B	Deeps.
Exp. cage.	SITE OF EXPERIMENTAL OYSTER CAGE.
C^1	Grounds off Tollesbury Pier (= Toll. Pier).
C^2	Grounds below Marfleet (M.) and off Shingle Head.
C^3	Grounds off the Nass End.
D^1	North shore ground near East Boundary (E.B.).
D^2	Offshore northern grounds (Flat Ground).
S	South shore beds near watch boat.

Thirslet Creek lies a little above Tollesbury Pier and is not shown. The temperature stations (shown in circles) are denoted in the legend to Table 4, p. 1015).

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(1) Reproduced with fig. 3 by permission of the Council of the Marine Association, Plymouth.

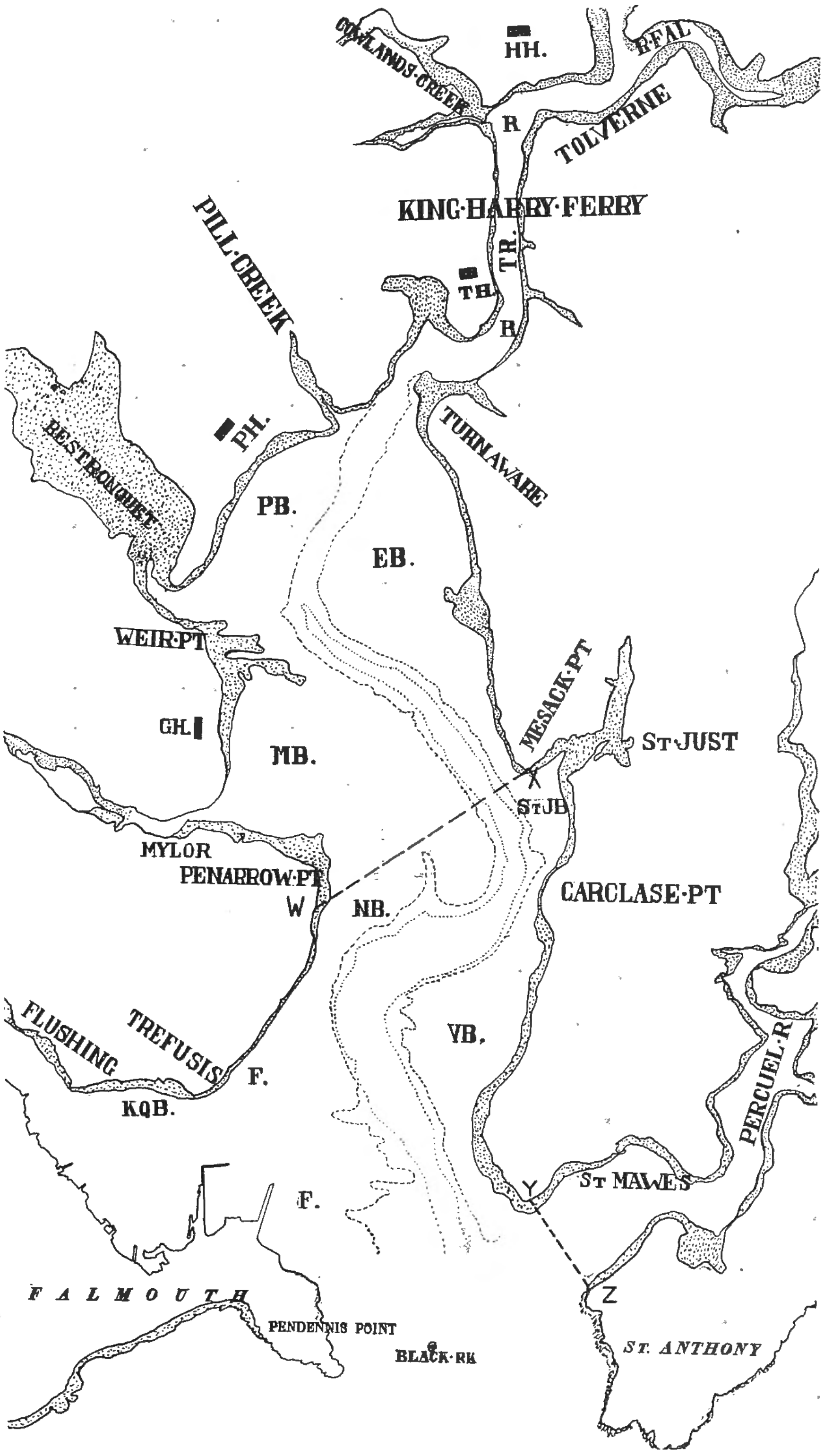

FIG. 3. — Chart of the Fal Estuary and River Fal showing the situation of the local oyster beds (scale : 1 inch = ca. 1,400 yds.). Reduced from Admiralty Chart No. 32, Falmouth Harbour.

The beds north of the line W-X are under the administration of the Truro municipality, while those south of the line W-X and west of the line Z-Y are administered by the Falmouth municipality, excepting private layings, which are situated mostly in the creeks and upper part of the river from Turnaware Bar, and are apparently always at, and above, low-water mark.

The chief oyster beds are as follows:

TRURO BEDS.	Depth in m.
M. B. = Mylor Bank	½ to 2½
E. B. = East Bank	½ to 2½
Turnaware Bar	0 to 2½
P. B. = Parson's Bank ...	½ to 1½
R. R. = The River beds ...	0 to 18
T. R. = Trelissick Reach .	0 to 18

FALMOUTH BEDS.	Depth in m.
N. B. = Falmouth North Bank	1½ to 3½
St. J. B. = St. Just beds... ...	2½ to 3½
V. B. = Vilt Bank or St. Mawes beds ...	1½ to 4
F. F. = Falmouth West Banks	4 to 6
K. Q. B. = Kiln Quay beds...	4½ to 5½

Other salient features are H.H., Higher Trelease; T.H., Trelissick House; P.H., Porthgwidden House; G.H., Great Wood House.

The chart is drawn to show the exposure of the beds at low water, ordinary spring tides, as at Turnaware Bar; the tongue of ground exposed on the East Bank is known locally as Brown Rose Bar. The Channel is marked by lines which denode the edge of the Banks where the depth is mainly 2-4 metres, but shelves rapidly in places to 6 m., and fairly rapidly everywhere to 10 m. The inner contour-line in the Channel, which ends opposite the middle of the East Bank, is a 10-fathom line (= ca. 20 m.) and gives an indication of the general steepness of the sides of the Channel.

TABLE 5. — Truro Oyster Beds, Fal Estuary, 1927. — Percentages of various categories of mature female-functioning oysters, O. edulis, for periods (mainly weekly) beginning on the dates of the moon's quarters.

Date Week begining moon's quarters	No. of oysters examined	Sea Temp. in °Fx	Oysters carrying embryos or larvae					Total with embryos or larvae	Total ripe or nearly ripe unspawned females	Total female
			Premature Whitesick	Embryos & larvae normal Whitesick	Shelled No colour Greysick	Total columns 5 & 6	Coloured or Blacksick			
1	2	3	4	5	6	7	8	9	10	11
June 7	331	58.2 (5)	0	0.3	0.3	0.6	0	0.6	27.5	28.1
○ 15	483	58.6 (5)	1.4	2.7	1.4	4.1	0.4	5.9	26.4	32.3
22	433	58.9 (5)	0	1.4	1.4	2.8	2 1	4.9	31.8	36.7
● 29	760	57.1 (3)	0.9	0.1	0.9	1.0	2.1	4.0	33 4	37.4
July 7	368	59.4 (10)	0.3	4.9	4.3	9.2	0.5	10.0	37.0	47.0
○ 14	667	62.6 (3)	4.9	3.6	3.3	6.9	8.1	20 0	31.0	51.0
21	456	62.4 (6)	0	4.2	5.3	9.5	5 3	14.8	41.9	55.7
● 28	934	62.2 (4)	1.4	5.2	2.2	7.4	9.6	18.4	28.9	47.3
Aug. 5	437	—	3.4	3.0	2.3	5.3	11.0	19.7	18.3	38 0
○ 13	503	59.6 (2)	0	0.6	0.6	1.2	9.3	10.5	23.7	34.2
19	292	60.0 (1)	1.4	0.3	0.3	0.6	3.8	5.8	17.5	23.3
● 27	185	61.0 (1)	0	0	0	0	0	0	20.0	20.0
Sept. 4	250	60.5 (2)	0.4	0	0	0	0	0.4	17.6	18.0
○ 11	614	58.3 (4)	0	0	0	0	0	0	21.7	21.7
18	267	59.5 (2)	0	0	0	0	0	0	19.9	19.9
● 25	271	55.5 (5)	0	0	0.4	0.4	0	0.4	16.6	17.0
Oct. 4	641	—	0	0	0	0	0.5	0.5	13.4	13.9
○ 10	630	—	0.3	0	0	0	0.3	0.6	12.3	12.9
17	714	—	0	0	0	0	0	0	12.0	12.0
● 25	265	—	0	0	0	0	0.8	0.8	8.3	9.1
Nov. 2	—	—	—	—	—	—	—	—	—	—
○ 9	91	—	0	0	0	0	1.1	1.1	2.2	3.3
16	97	—	0	0	0	0	0	0	6.2	6.2
TOTAL. . .	9689	Aggregate %		26.3	22.7	49.0	54.9			

Mean of observations (the number is given in brackets) for the River and Turnaware Bar Stations made in the period of usually a week; corresponding means for the Bank Stations were 1° tot 2.3° lower in June, 0.8° to 1.6° lower in July, 0.5° to 2.0° lower in August, but about the same in September until the last week when the banks gave a mean 0.8° F higher.

lation were homogeneous, the total potential females occurring in the Fal Estuary beds in that year was the aggregate of the percentages 55.7 + 8.1 + 0.5 + 2.1 + 2.1 + 0.4 = 68.9 % of the population. As however the population spawning in June is probably different from that on other parts of the beds, the aggregate percentage of the June spawnings, i.e. 8.5, should be deducted, giving a total of 60.4. Moreover, if individuals spawned twice in one year, this total would need to be modified. There is, however, no ground for such an assumption regarding oysters left undisturbed on the beds, although a second spawning might occur in nature rarely. When oysters are disturbed and spawn prematurely there is some evidence that an incomplete spawning may be followed later by a successful second spawning in a small percentage of such cases from the same batch of eggs (Orton and Amirthalingam, 1931).

We may safely accept the broad deduction that there was a total of at least 50 % of the oyster population (of the range of size examined on these beds) which were potential female spawners in 1927.

The actual percentages found in spawn in the weekly samples are again shown in Fig. 1, p. 1005.

Figure 1, 2 F, broken line, and Table 5 gives the total percentages of all categories of females : 2 gives the total percentage of those with young : 2 D gives the blacksick : 2 C with embryos developing shell but without colour : 2 B naturally spawned, with young white embryos or larvae : 2 A the prematurely spawned, with early segmentation stages, and 2 A + B the total with embryos or white larvae, i. e. trochosphere stages.

Since some 60.0 % were potential females and at the close of the normal season in October some 12 % remained unexpended, it is manifest that about 60.0 — 12 = 48 % spawned, that is, extruded eggs. But the accumulative percentage of blacksick individuals found throughout the season to this date is 53, and, excluding those spawned in June, is about 49. Again assuming that no blacksick persisted for more than 5 days, this result is concordant. The accumulative percentage of normal whitesick plus greysick — excluding those spawning in June — is about 40. The actual percentage is probably round about this figure. Slightly higher figures are obtained from the grouping in Table 6.

At the end of August some 20 % of the population existed as mature females which failed to spawn effectively, as the subsequent records show. Among these 20 %, however, ineffective spawning occurred, that is, ova were extruded but not retained within the shell for incubation, so that by the end of October about 8 % females remained. In the week beginning August 13th it is of great interest to note that about 20 % of females remained unspawned on the Fal beds, and only 2.9 % on the Blackwater.

In the peculiar temperature variations on these beds in 1927 are opportunities for a study of the conditions correlated with spawning, and also of those which are necessary for effective spawning, that is, to produce fully developed and viable larvae.

TABLE 6. — Truro Oyster Beds, Fal Estuary, 1927. — Percentages of various categories of mature female-functioning oysters, **O. edulis,** in samples grouped to give maximal intervals and mainly centred on the dates of the moon's quarters.

Dates of Moon's quarters		Period.	No. of oysters examined	Categories with young							
				Whitesick Prem.		Whitesick normal		Greysick		Blacksick	
				No.	%	No.	%	No.	%	No.	%
June	7	4 – 11	166	0	(0.0)	1	(0.6)	0	(0.0)	0	(0.0)
○	15	12 – 18	466	4	(0.9)	0	(0.0)	8	(1.7)	0	(0.0)
	22	19 – 25	462	3	(0.6)	18	(3.9)	2	(0.4)	8	(1.7)
●	29	26 – 3	423	1	(0.2)	2	(0.5)	9	(2.1)	6	(1.4)
July	7	4 – 10	490	6	(1.2)	0	(0.0)	2	(0.4)	13	(2.7)
○	14	11 – 17	468	9	(1.9)	18	(3.8)	24	(5.1)	5	(1.1)
	21	18 – 24	667	25	(3.7)	32	(4.8)	22	(3.3)	57	(8.5)
●	28	25 – 1	651	9	(1.4)	16	(2.5)	27	(4.1)	48	(7.4)
Augustus	5	2 – 7	639	4	(0.6)	44	(6.9)	10	(1.6)	60	(9.4)
○	13	8 – 13	533	15	(2.8)	15	(2.8)	11	(2.1)	55	(10.3)
	19	14 – 22	599	0	(0.0)	3	(0.5)	4	(0.7)	53	(8.8)
●	27	23 – 29	196	4	(2.0)	1	(0.5)	0	(0.0)	5	(2.5)
September	4	30 – 5	185	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
○	11	6 – 11	250	1	(0.4)	0	(0.0)	0	(0.0)	0	(0.0)
	18	12 – 17	614	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
●	25	18 – 25	267	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
October	4	26 – 4	453	0	(0.0)	0	(0.0)	1	(0.2)	1	(0.2)
○	10	5 – 10	459	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	17	11 – 16	630	2	(0.3)	0	(0.0)	0	(0.0)	2	(0.3)
		17 – 22	617	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
		23 – 29	181	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)
November		30 – 6	181	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)
		7 – 14	91	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.1)
		15 – 22	97	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
TOTALS			9785	83		150		120		316	
Aggregate %'s					16.0		26.8		21.7		56.6
							48.5				

Sea-temperature variation on the Fal Estuary oyster beds.

The variation in sea-temperature over the oyster beds in the Fal Estuary has been previously discussed (Orton, 1926 and 1928) on the information then available. In 1927 a special study was undertaken and an effort made to obtain continuous daily readings. Bottom temperatures were obtained by means of a specially made small Nansen-Petterson water bottle with a certified Richter thermometer (see Russell and Yonge, 1928). Surface readings were made with a certified (N.P.L.) Calderara sea-surface thermometer, Meteorological Office pattern. The results are shown in Tables 4 and 8, and plotted in Fig. 1 at T, by the brokenline graphs.

The conformation of the beds is well shown in Fig. 3 where PB, EB, and MB are placed in the middle respectively of the main oyster beds, Parsons Bank, East Bank and Mylor Bank; on which the depth of water at low water is 1 to 3 or 4 metres. The channel is shown zig-zagging across the beds and at the intersection of the dotted boundary line the depth at low water is about 30 metres : the inner contour line shown in the channel is about 20 metres. Temperature stations were established at or near the positions shown respectively by PB, EB, MB, off Turnaware Point, near R immediately above Turnaware over a depth of 16 metres, and in the Channel on the boundary line off Mesack Point (30 metres).

Temperature variation on these beds is not so simple as on those of the Blackwater. Water from the English Channel enters the beds and exerts a contrary influence especially during spring tides, being relatively warm in winter and relatively cold in summer. Water from the River influenced by mud-banks and extensive shallow creeks flows over the beds bringing relatively cold water in winter and relatively warm in summer, but varying directly with air-temperature. A similar influence is exerted on the shallow waters fringing all the beds as on the River water. Definite mixing of waters occurs off Turnaware Point. It was found that temperatures on the banks were usually similar, and that here surface waters differed from the bottom little usually and by a few tenths of a degree Fahrenheit. Temperatures over the middles of the banks were usually uniform grading towards Turnaware Bar on the East and Parsons Banks on the ebb tide, and towards the creeks and shores as the water shallows to about one metre deep. On May 18th, for example, temperatures over the beds ranged from 54° to 56.5 F., but about 20 metres from the shore of the East Bank was a belt of warmer water at 59°-60° at about 15 hours (L.W. = 13.30). From this point to about 200 metres off shore the temperature fell gradually to 55.5° and then remained almost constant to 55.4° at about 1000 metres off shore at the E.B. temperature station.

Thus temperatures taken at the Bank stations give a good mean for the banks, but in warm weather higher temperatures occur during the day in the River in the creeks and near the shores. The daily variation in the middle of

TABLE 7 (*). — Fal Estuary 1927. — Percentage frequencies of recently normally spawned (4), and ripe or ripening female (8a), and premature whitesick plus 8a = (8b) oysters, (**O. edulis**), in samples grouped to give maximum time intervals between sampling. (The periods marked thus 4-11* are fortuitously grouped around dates of the moon's quarters.)

Period	Dates of samples	Interval in days	% frequency of whitesick and greysick combined	Percentages			8a, % frequency of ripe and ripening unspawned females 8b, do. + % prematurely spawned $8 = \frac{8a}{8b}$	Percentages		
				min.	max.	mean		min.	max.	mean
1	2	3	4	5	6	7		9	10	11
June 4–11*	7, 8	6	0, 1.1	0.0	1.1	0.6	$\frac{35.9}{35.9}, \frac{30.7}{30.7}$	$\frac{30.7}{30.7}$	$\frac{35.9}{35.9}$	$\frac{33.3}{33.3}$
12–18*	14, 14, 15, 15, 16	5	0, 1.2, 6.6, 0, 1.0	0.0	6.6	1.8	$\frac{15.0}{15.0}, \frac{28.2}{28.2}, \frac{24.2}{24.2}, \frac{21.4}{22.3}, \frac{23.5}{26.5}$	$\frac{15.0}{15.0}$	$\frac{28.2}{28.2}$	$\frac{22.5}{23.2}$
19–25*	21, 21, 22, 22, 23	5	4.3, 10.2, 0, 0.8, 6.7	0.0	10.2	4.4	$\frac{24.5}{27.7}, \frac{39.8}{39.8}, \frac{33.3}{33.3}, \frac{25.4}{25.4}, \frac{34.8}{34.8}$	$\frac{24.5}{25.4}$	$\frac{39.8}{39.8}$	$\frac{31.6}{32.2}$
26– 3	28, 28, 29, 29, 30	4	5.6, 1.2, 0, 5.7, 0	0.0	5·7	2.5	$\frac{28.2}{28.2}, \frac{40.2}{40.2}, \frac{30.9}{30.9}, \frac{24.8}{24.8}, \frac{25.8}{26.8}$	$\frac{24.8}{24.8}$	$\frac{40.2}{40.2}$	$\frac{30.0}{30.2}$
July 4–10*	4, 4, 5, 5, 6	6	0, 0, 1.1, 0, 1.0	0.0	1.1	0.4	$\frac{48.5}{48.5}, \frac{30.7}{30.7}, \frac{35.8}{36.8}, \frac{40.4}{43.6}, \frac{30.1}{32.0}$	$\frac{30.1}{30.7}$	$\frac{48.5}{48.5}$	$\frac{37.1}{38.3}$
11–17*	12, 12, 13, 13, 14	4	10.0, 10.5, 4.3, 12.5, 8.0	4.3	12.5	9.1	$\frac{39.0}{39.0}, \frac{37.9}{37.9}, \frac{36.6}{37.6}, \frac{33.8}{33.8}, \frac{39.0}{47.0}$	$\frac{33.8}{33.8}$	$\frac{39.0}{47.0}$	$\frac{37.3}{39.1}$
18–24	18,19,19,20,20,20,21	5	12.9, 0, 3.3, 17.2, 4.3, 2.3, 16.0	0.0	17.2	8.0	$\frac{32.3}{35.5}, \frac{20.5}{33.7}, \frac{28.9}{28.9}, \frac{30.3}{31.3}, \frac{22.6}{24.7}, \frac{43.2}{52.3}, \frac{46.0}{46.0}$	$\frac{20.5}{24.7}$	$\frac{46.0}{52.3}$	$\frac{32.0}{36.1}$
25– 1*	26,26,27,27,28,29,29	4	7.0, 9.3, 10.1, 4.0, 10.1, 5.2, 1.0	1.0	10.1	6.7	$\frac{33.8}{33.8}, \frac{40.7}{40.7}, \frac{40.4}{40.4}, \frac{42.0}{42.0}, \frac{37.4}{41.4}, \frac{42.3}{42.3}, \frac{30.3}{35.4}$	$\frac{30.3}{33.8}$	$\frac{42.3}{42.3}$	$\frac{38.1}{39.4}$
Aug. 2– 7*	2, 2, 3, 3, 3, 4, 4	4	7.5, 8.4, 6.0, 7.6, 12.9, 11.4, 6.9	6.0	12.9	8.7	$\frac{23.7}{23.7}, \frac{30.5}{31.6}, \frac{19.0}{19.0}, \frac{28.3}{28.3}, \frac{18.6}{20.0}, \frac{19.3}{21.6}, \frac{34.7}{34.7}$	$\frac{18.6}{19.0}$	$\frac{34.7}{34.7}$	$\frac{24.9}{25.6}$
8–13	8, 9, 10, 10–11, 11	4	12.4, 1.1, 0, 6.1, 6.9	0.0	12.4	5.3	$\frac{22.7}{25.8}, \frac{17.4}{20.7}, \frac{15.8}{24.2}, \frac{21.2}{21.2}, \frac{14.9}{16.1}$	$\frac{14.9}{16.1}$	$\frac{22.7}{25.8}$	$\frac{18.4}{21.6}$
14–22	15,16,17,17,18,18,19	6	3.1, 2.4, 0, 0, 0, 1.2, 1.0	0.0	3.1	1.1	$\frac{25.0}{25.0}, \frac{23.8}{23.8}, \frac{22.4}{22.4}, \frac{27.3}{27.3}, \frac{26.2}{26.2}, \frac{17.4}{17.4}, \frac{17.7}{17.7}$	$\frac{17.4}{17.4}$	$\frac{27.3}{27.3}$	$\frac{22.8}{22.8}$

Aug. 23–29	25, 25	7	0, 1.1	0.0	1.1	0.6	$\frac{17.1}{17.1}, \frac{17.6}{22.0}$	$\frac{17.1}{17.1}$	$\frac{17.6}{22.0}$	$\frac{17.4}{19.6}$
30– 5	1, 1	6	0, 0	0.0	0.0	0.0	$\frac{19.8}{19.8}, \frac{20.2}{20.2}$	$\frac{19.8}{19.8}$	$\frac{20.2}{20.2}$	$\frac{20.0}{20.0}$
Sept. 6–11	7, 7, 9	3	0, 0, 0	0.0	0.0	0.0	$\frac{26.5}{26.5}, \frac{13.7}{13.7}, \frac{10.1}{11.4}$	$\frac{10.1}{11.4}$	$\frac{26.5}{26.5}$	$\frac{16.8}{17.2}$
12–17	12,13,13,14-15,14,14,15	5	0, 0, 0, 0, 0, 0, 0	0.0	0.0	0.0	$\frac{22.6}{22.6}, \frac{20.5}{20.5}, \frac{23.3}{23.3}, \frac{17.4}{17.4}, \frac{14.4}{14.4}, \frac{35.2}{35.2}, \frac{19.2}{19.2}$	$\frac{14.4}{14.4}$	$\frac{35.2}{35.2}$	$\frac{21.8}{21.8}$
18–25	20, 21, 22	6	0, 0, 0	0.0	0.0	0.0	$\frac{17.4}{17.4}, \frac{20.7}{20.7}, \frac{21.7}{21.7}$	$\frac{17.4}{17.4}$	$\frac{21.7}{21.7}$	$\frac{19.9}{19.9}$
26– 4	28, 29, 3, 4, 4	1	0, 0, 1.1, 0, 0	0.0	1.1	0.6	$\frac{19.8}{19.8}, \frac{14.7}{14.7}, \frac{15.6}{15.6}, \frac{8.6}{8.6}, \frac{11.2}{11.2}$	$\frac{8.6}{8.6}$	$\frac{19.8}{19.8}$	$\frac{14.0}{14.0}$
Oct. 5–10	5, 5, 6, 6, 7	4	0, 0, 0, 0, 0	0.0	0.0	0.0	$\frac{18.7}{18.7}, \frac{15.6}{15.6}, \frac{11.7}{11.7}, \frac{14.6}{14.6}, \frac{13.5}{13.5}$	$\frac{11.7}{11.7}$	$\frac{18.7}{18.7}$	$\frac{14.8}{14.8}$
11–16	11,11,12,12,12,12,14	3	0, 0, 0, 0, 0, 0, 0	0.0	0.0	0.0	$\frac{14.8}{14.8}, \frac{14.1}{14.1}, \frac{15.0}{15.0}, \frac{12.1}{12.1}, \frac{8.4}{8.4}, \frac{9.6}{9.6}, \frac{11.3}{13.8}$	$\frac{8.4}{8.4}$	$\frac{15.0}{15.0}$	$\frac{12.2}{12.5}$
17–22	17,18,18,19,19,20,20	4	0, 0, 0, 0, 0, 0, 0	0.0	0.0	0.0	$\frac{19.8}{19.8}, \frac{13.2}{13.2}, \frac{6.3}{6.3}, \frac{6.3}{6.3}, \frac{7.5}{7.5}, \frac{19.3}{19.3}, \frac{8.7}{8.7}$	$\frac{6.3}{6.3}$	$\frac{19.8}{19.8}$	$\frac{11.6}{11.6}$
23–29	24, 25	7	0, 0	0.0	0.0	0.0	$\frac{15.5}{15.5}, \frac{11.9}{11.9}$	$\frac{11.9}{11.9}$	$\frac{15.5}{15.5}$	$\frac{13.7}{13.7}$
30– 6	31, 1	10	0, 0	0.0	0.0	0.0	$\frac{5.7}{5.7}, \frac{10.4}{10.4}$	$\frac{5.7}{5.7}$	$\frac{10.4}{10.4}$	$\frac{8.1}{8.1}$
Nov. 7–14	10–14	8	0	0.0	0.0	0.0	$\frac{2.5}{2.5}$	$\frac{2.5}{2.5}$	$\frac{2.5}{2.5}$	$\frac{2.5}{2.5}$
15–22	22	—	0	0.0	0.0	0.0	$\frac{6.2}{6.2}$	$\frac{6.2}{6.2}$	$\frac{6.2}{6.2}$	$\frac{6.2}{6.2}$
			TOTALS . .	11.3	95.1	49.8				

(*) Note : the sum of the means of columns 4 and 8b sives the mean or the total potential females.

the banks was not great; occasional differences of 3.0° F. (on May 11th), 1.8°, 1.5° were obtained and more frequently of 1.0°, but usually in the summer of 1927 differences during the day were less than 1.0° F. In this locality low water of spring tides occurs about or soon after noon and midnight. The daily minimum of foreshore and River temperatures would ordinarily therefore occur after midnight when no readings were taken. Nevertheless at whatever time of day temperatures were taken during May-early September, River temperatures were never lower and usually higher than those on the banks, as is seen from Fig. 1. T. where short period means are plotted respectively for the means of all the Banks (continuous line) and for the River Station (broken line and circles). Temperatures at Turnaware Bar were very nearly the same as at the surface at the River Station, but at a depth of 4 to 6 metres off shore temperature on four occasions during the summer approached closely to those on the Banks.

Of the four main oyster beds the shallower part of the Turnaware bed which receives a large proportion of River water is covered by warmer waters than the middle and southern parts of the other three, namely, East, Parsons and Mylor banks (See Table 8). This hydrographic difference is associated, as will be seen, with the spawning behaviour of the population.

The incidence of spawning in relation to sea temperature level; Fal Estuary.

The results of the examination of the earlier samples from the Fal Estuary are given in Table 8 in detail, and in Table 5 in weekly analyses for the whole of the beds. In the latter it is seen that the weekly percentage of fresh natural spawners (column 5) during June was respectively 0.3, 2.7, 1.4 and 0.1, wheras during July these percentages ranged from 3.6 to 5.2. It is therefore clear that intermittent slight spawning occurred during June with a maximum in the second week, and that regular spawning occurred throughout July. In August the corresponding percentages of new spawning individuals were respectively 3.0, 0.6, 0.3 and 0.0. Spawning therefore continued during the first week in August, diminished in the second and third weeks, reaching zero in the last week. No fresh spawning individuals were found during September, October and part of November among some 4,000 oysters. That these fluctuations in spawning are closely related to temperature variation is fully demonstrated by the broken line graph in Fig. 1. A comparison of the graphs in 2A and 2A + B with F gives very close correspondence, the graph rising and falling very closely together on the whole. Moreover, spawning is closely related to temperature level, beginning when the temperature attains *about* 59° F. (=15° C.) or rises above the level.

When the problem is examined more closely it is found that although the variation in temperature over the beds complicates the investigation of the correlation between spawning and temperature, it actually increases the evidence for the correlation of spawning at and above a level of about 15° C., as will be seen.

The shallow mudbanks which are covered by the waters of the River and Pill and Restronguet creeks (see Fig. 3) and those on the periphery of the Banks are rapidly affected by sunshine, wind and the thermal state of the air, and quickly communicate their state to the overlying waters, which are also directly affected by the same conditions. It follows that these waters respond more quickly to warm (and cold) weather than those over the main banks and channels. Therefore the parts of the Banks affected by these waters, i.e., Turnaware Bar and the northern parts of Parson's and East Banks will receive rather warmer water than the main Banks, especially during neap tides in the spring and early summer. The St. Just Channel on the flood tide, and especially on spring tides, brings in relatively cold water in the spring and summer from the English Channel. These waters mixing with the River and creek waters produce intermediate temperatures on the main banks (see Orton, 1928, p. 396).

Temperature observations for different localities are recorded in Table 8 alongside the analyses of samples for individuals carrying embryos and larvae obtained from the corresponding localities. Weekly means are given in Table 5, p. 1020 and the daily observations in Table 8, p. 1028.

Temperatures on the East and Mylor Banks ranged about 57° to 58° until June 22 with no significant spawning among 471 individuals examined, two spawners only occurring. On the other hand, observed temperatures over the River, parts of Turnaware Bar and parts of Parsons Bank were found to range between 58° and 59° from about June 8 to June 22. Significant spawning occured on Parsons Bank about June 11 or 12, and again on 19 or 20, and heavy spawning occured on Turnaware Bar (10 %) on 19 and 20. Some spawning had also occured on the shallower parts of Turnaware Bar probably about 16 or 17 (See sample June 23rd with coloured larvae). The earlier spawning on these beds is correlated with a slightly higher temperature than occured on the others, and is related to a temperature level not less than 58° to 59° F., being higher on the ebb and lower on the flood tide. It is regrettable that no observations are available for the week-end June 9 to 12, the critical period, but it is improbable that the temperature in that period varied much, as mean air temperature at Falmouth remained steady at 54-55° and daily sunshine was respectively 8.7, 6.5 and 5.1 hours, and the full moon with its related spring tides did not occur until June 15th. The tendency would be for the temperature to rise slightly.

Spawning occurred on the East Bank about June 23 to 24, as samples examined on the 28 and 29 had 5 % shelled but uncoloured larvae aged about 5 days. Temperatures over the East Bank rose above 58° on the 22nd and were 60° or more on the River. No observations of temperature were made on the 23rd and 24th, but it is probable that the water warmed slightly, as mean air rose

TABLE 8. — Incidence of the spawning of **O. edulis** on various grounds
(The number of oysters (= N°) in each sample examined is given along with

Date 1927		Temperature² in °F River and Turnaware Bar	Turnaware Bar Oysters and categories with young					Temperature in °F Parsons Bank	Parsons Bank Oysters and categories with young				
			No.	0	1	2	3		No.	0	1	2	3
May	30	57.0 (3)	—	—	—	—	—	57.0 (1)	—	—	—	—	—
June	1	58.0 (3)	—	—	—	—	—	57.0 (2)	—	—	—	—	—
	6	57.0 - 58.0 (2)	—	—	—	—	—	57.0 (2)	—	—	—	—	—
	7	—	78	0	0	0	0	—	—	—	—	—	—
	8	58.0 - 58.3 (2)	—	—	—	—	—	57.0 - 57.2 (2)	—	—	—	—	—
	13	58.0 - 59.0 (3)	—	—	—	—	—	57.0 (2)	—	—	—	—	—
	14	—	85	0	0	1	0	—	—	—	—	—	—
	15	59.0 - 59.2 (2)	—	—	—	—	—	58.2 (1)	91	0	0	6	0
	16	—	98	3	0	1	0	58.0 (1)	—	—	—	—	—
	20	58.0 - 59.0 (2)	—	—	—	—	—	58.0 (1)	—	—	—	—	—
	21	58.0 (1)	88	0	9	0	0	58.0 (1)	94	3	4	0	2
	22	60.0 - 60.5 (2)	—	—	—	—	—	—	—	—	—	—	—
	23	—	89	0	4	2	4	—	—	—	—	—	—
	25	58.0 (3)	—	—	—	—	—	56.0 (2)	—	—	—	—	—
	28	—	82	0	1	0	2	—	—	—	—	—	—
	29	56.9 (1)	—	—	—	—	—	—	—	—	—	—	—
	30	—	—	—	—	—	—	—	—	—	—	—	—
July	4	57.0 - 57.5 (2)	97	0	0	0	2	56.0 - 56.5 (2)	—	—	—	—	—
	5	—	—	—	—	—	—	—	94	3	0	0	8
	6	—	—	—	—	—	—	—	—	—	—	—	—
	7	60.0 (2)	—	—	—	—	—	58.0 (1)	—	—	—	—	—
	9	61.0 (1)	—	—	—	—	—	59.0 (2)	—	—	—	—	—
	11	58.0 - 59.0 (2)	—	—	—	—	—	58.0 (2)	—	—	—	—	—
	12	59.0 (2)	100	0	9	1	2	59.0 (1)	—	—	—	—	—
	13	58.9 - 59.4 (3)	—	—	—	—	—	58.3 (1)	—	—	—	—	—
	14	—	100	8	0	8	3	—	—	—	—	—	—
	18	—	—	—	—	—	—	—	—	—	—	—	—
	19	—	—	—	—	—	—	—	90	0	3	0	10
	20	62.0 - 63.0 (2)	99	1	14	3	9	62.0 (1)	—	—	—	—	—

(1) Categories : 0 = premature; 1 = normal whitesick; 2 = greysick; 3 = blacksick.

in the Fal Estuary in relation to temperature variation in 1927.
the number of each of the different categories (¹) found with young (see footnote).

Temperature in °F East Bank	East Bank Oysters and categories with young					Temperature in °F Mylor Bank	Mylor Bank Oysters and categories with yong					Channel Temperature in °F
	No.	0	1	2	3		No.	0	1	2	3	
57.0 (1)	—	—	—	—	—	57.0 (1)	—	—	—	—	—	56.0 (1)
57.0 (2)	—	—	—	—	—	57.0 – 58.0 (2)	—	—	—	—	—	57.0 (2)
57.0 (1)	—	—	—	—	—	56 0 (1)	—	—	—	—	—	56.0 (1)
—	—	—	—	—	—	—	—	—	—	—	—	—
56.3 – 56.7 (3)	88	0	1	0	0	—	—	—	—	—	—	56.0 – 56.5 (2)
57.0 – 58.0 (3)	—	—	—	—	—	57.0 (1)	—	—	—	—	—	57.0 (2)
—	80	0	0	0	0	—	—	—	—	—	—	—
58.0 (1)	74	1	0	0	0	57.3 (1)	38	0	0	0	0	57.7 (1)
58.0 (1)	—	—	—	—	—	57.0 (1)	—	—	—	—	—	57.0 (1)
57.0 (1)	—	—	—	—	—	57.0 (1)	—	—	—	—	—	57.0 (1)
57.0 (1)	—	—	—	—	—	57.0 (1)	—	—	—	—	—	57.0 (1)
57.2 – 58.4 (2)	122	0	1	0	1	57.5 (1)	69	0	0	0	1	57.8(1), 54.4(30m.)(1)
—	—	—	—	—	—	—	—	—	—	—	—	—
56.0 – 57.0 (2)	—	—	—	—	—	56.0 (2)	—	—	—	—	—	56.0 – 57.0 (2)
—	71	0	0	4	1	—	—	—	—	—	—	—
55.2 – 56.7 (2)	105	0	1	5	0	55.2 – 55.8 (2)	—	—	—	—	—	54.5 (26m.) (3)
—	97	1	0	0	3	—	—	—	—	—	—	—
56.0 (1)	101	0	0	0	3	56.0 (1)	—	—	—	—	—	56.0 (1)
—	—	—	—	—	—	—	95	1	0	1	0	—
—	—	—	—	—	—	—	103	2	0	1	0	—
59.0 (1)	—	—	—	—	—	59 0 (1)	—	—	—	—	—	59.0 (1)
58.0 (1)	—	—	—	—	—	59.0 (1)	—	—	—	—	—	58.0 (1)
58 0 (1)	—	—	—	—	—	58.0 (1)	—	—	—	—	—	58.0 (1)
59.0 (1)	95	0	3	7	0	—	—	—	—	—	—	59.0 (1)
58 6 (1)	80	0	4	6	0	58.9 (1)	93	1	2	2	0	58.7 – 58.9 (2)
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	60.0 (1) (16 th)	93	3	1	11	7	—
—	83	11	0	0	10	—	—	—	—	—	—	—
62.0 (1)	88	8	2	0	2	—	93	2	4	0	12	62.3 (1), 60.6 (24m.)(1)

(²) The temperature records given are surface readings unless otherwise recorded. See Orton, 1928, for full details of surface and other records.

slightly on the 23rd when 11.3 hours of sunshine were recorded. After June 23rd or 24th until July 6th no further spawning occurred corresponding with a spell of cold weather which reduced the River temperatures to 57° to 58°. Warmer weather set in about the 7th and temperatures over the beds rose generally to 58-59, and to 60 or more on the River.

More or less general spawning then began, being especially heavy about the 10th to 12th at the change from the neap to the full moon spring tides. It is therefore seen that although the incidence of spawning varied on the different beds, it did so in correlation with an attainment of a temperature between 58 and 59° with a probability in all instances of a range to 59° or more on the ebb tide and a slightly lower temperature on the flood. It is not possible to fix the exact temperature range at the onset of significant spawning more definitely than this on the Fal beds in 1927.

The occasional spawners found at the approach of the spawning season, it will be noted, have been ignored, for the reason that it has been found empirically that oysters situated in inshore regions or at low water tend to spawn earlier than those on the main offshore beds. Isolated spawning individuals may have been taken in the dredge from an inshore or favourable thermal habitat. A percentage of 2 or more spawners in a dredged sample is a safer criterion for the area examined.

The peak and cessation of spawning.

From July 20th spawning continued mainly in warm weather which maintained the water temperature a few degrees above 60° until the first week in August. The heaviest weekly spawning occurred at the end of July. On August 3rd, however, the bottom (17 m.) temperature at the River Station at half-tide was only 60° and only 50° at 28 m. in the Channel at the southern end of the beds. On August 17th temperatures had fallen to 58-59° on the Banks and 60° at the surface in the River, whilst the bottom temperature in the Channel was only 57°. Cold weather with lack of sun coinciding with the full moon spring tides caused a quick fall in temperature to a low level. Few temperature observations were made during the period August 3rd to 17th, but the records on August 9th and 10th, supported by Meteorological Reports (Falmouth Observatory, 1928) indicate that temperatures had been maintained well above 60° until that date. A fall in temperature, however, soon followed due partly to cold weather and partly to the influx of cold water on the spring tides. Cold conditions were maintained until at least August 18th, but temperatures of 59-60° were general over the beds on the 23rd and 24th. On the 27th temperatures ranged from 59° to 61° over the beds except for a night reading of 58° on Mylor Bank. Similar conditions prevailed on September 5th and 7th but a general homothermous condition at 58° obtained on September 12th and a similar condition at about 58.5° on September 14th. On September 21st a slightly higher homothermous condition occurred at about 59.5°

at H. W. By September 29th, however, temperatures of about 56° were general and the downward trend of the temperature grade established.

The warm conditions in the week beginning August 5th were accompanied by a continuance of spawning, but the fall in temperature with the spring tides on the full moon of August 13th is correlated with the reduction of spawning in the following week to the low level of 0.6 %, and not a single oyster was found as a premature spawner. Still less spawning occurred in the week beginning August 19th although 1.4 % spawned prematurely and temperatures remained about 60°. After August 26th it is clear from the almost total absence of oysters with young that successful spawning ceased, nevertheless the percentage of ripe or nearly ripe females diminished gradually afterwards by some means from 20 % in the week beginning August 27th to 2.2 % in the week beginning November 9th.

The temperature conditions in August and September may be summarised as follows : in the first ten days the level was maintained at 61-63° but fell to about 58-59° during the spring tides centred about August 15th, afterwards rising to 59-60° by the 23rd and maintained about or slightly above this level until September 7th. After that date temperatures fell to about 58° during the full moon spring tides, rose in the following neaps to about 59° and fell again sharply, on the new moon tides at the end of the month, to about 56°.

An inspection of Table 5 and Fig. 1 shows that natural spawning virtually ceased on the beds on the full moon spring tides in the middle of August. This sudden stoppage is correlated with a sudden fall of temperature from about 63° to about 59° and an influx of high salinity water. It is interesting to note that at this time about 10 % of the population were carrying fully developed shelled black larvae ready to be extruded from the parent. The rise in temperature to or above 59° F. on the new moon spring tides at the end of August and in the following week did not result in successful spawning although the analysis of oysters demonstrates that mature ripe females occurred in the high proportion of 17 to 20 %. No spawning occurred on the September full moon tides when temperatures were again reduced nor in the following neap tides when temperatures again rose to about 59° F. During October with the approach of winter conditions no spawning occurred and the percentage of mature females gradually diminished to 8 %.

Successful spawning therefore ceased after the sudden fall in temperature on the full moon tides in August and did not occur afterwards in significant proportion during periods of August and September when the temperature remained at or above the level of 59° F. (=15° C).

Summary of spawning in relation to temperature variation.

Spawning began on the full moon tides of June contemporaneously with the rise in sea-temperature to about 59° F. and diminished to 0.1 % on the full moon tides along with a decrease in temperature to about 57°. Temperatures rose to 59°-63° in July and early August and spawning was continued throughout the period. A rapid fall in temperature from 63 to about 59° took place around the full moon tides in August and spawning afterwards ceased even though temperatures in August and September fluctuated in periods between 58° to 61°.

Incomplete spawning and incomplete fertilisation in relation to the spawning stimulus.

During the whole of the spawning season, but especially in August, on the Fal Estuary it was found that a large proportion of the newly spawning individuals (Category 1) carried from 20 to 60 % of the eggs in the mantle cavity in an unfertilised condition (Orton, 1927 *b*). A portion of each batch of eggs was fertilised and developed normally to a range of larval stages alongside the unsegmented eggs.

At the same time it was found that the percentage of females which failed to discharge *all* their eggs from the body in the spawning act was unusually high, and rose to 17 % (of the total oysters); whereas in a comparable sample (of 700 individuals) from the Blackwater beds the percentage of incomplete spawners was as low as 2 to 6 %, including, however, an abnormal sample from Thornfleet on August 2nd which had 12 %.

The two phenomena of incomplete spawning and incomplete fertilisation are probably related. It has been shown in an analysis of 1146 oysters with or recently with young (Orton, 1933) that high percentages of incompletely spent gonads occur among oysters which have embryos only a few days old, and much smaller percentages in those which have rather older embryos or larvae, and still smaller percentages one to two months after spawning. Unspent ova are therefore normally and mostly extruded from the gonad within a few days after spawning.

Formerly (Orton, 1927 *b*) it was pointed out that the coexistence of embryos and unfertilised eggs in an oyster might indicate insufficiency of sperm to fertilise all the eggs. But the discovery later that a large proportion of females, especially on the Fal Estuary, have a certain amount of sperm-morulae in the gonad renders this suggestion somewhat improbable if capacity for self-fertilisation be conceded. Moreover, the occurrence among the unfertilised eggs in many individuals of unsegmented eggs with many nuclei indicates that fertilisation had occurred in these cases but under conditions not favouring development. Incomplete fertilisation is therefore most probably a result of an incomplete spawning in response to the spawning stimulus, whereby a portion of eggs is extruded from the gonad with normal fertilisation, while the remainder of the eggs is extruded at some later period usually without but some times accompanied with fertilisation. In the latter event two ages of embryos or larvae occur in one oyster, a condition not uncommon in the Fal Estuary in 1927.

Among the causes of incomplete spawning itself may be over-ripeness with response to a minimal spawning stimulus, or under-ripeness with response to a maximal spawning stimulus. The nature of the stimulus causing spawning in *O. edulis* is, however, not known. Nevertheless the low level of the temperature on the Fal in 1927, along with the large fluctuations in temperature about the spawning level and other environmental factors coinciding with the marked tidal movements, doubtless provided a wide range of variation in environmental conditions among which must occur those inducing spawning. It is therefore inferred that the fluctuations in temperature around the minimum spawning level offer a partial explanation of the incomplete spawning in the Fal Estuary in 1927. But the continuance of incomplete spawning during July and early August when temperatures rose above the minimum spawning level points to some other factor or factors than temperature level affecting incomplete spawning. One such factor may possibly be absence of insufficient sperm, owing either to (1) paucity of mal oysters on the beds, or (2) incorrect timing of the spawning of the males in relation to the maturing of the females. It is, however, considered unlikely that these factors operated in view of the discovery of almost universal presence of a certain quantity of sperm in females of a sufficiency to effect fertilisation. Thus insufficiency of sperm is regarded as an unlikely explanation of the incomplete fertilisation. It is now realised that one other factor, namely, variation in salinity on the beds, is more probably significant, especially when accompanied by variation in temperature about the level of 59°F.

A considerable excess of rainfall over the average occurred at Falmouth in June, with heavy rains though a sub-normal average rainfall in July, and again a supra-normal average rainfall in August. Salinity observations were made and are recorded in Orton, 1928, Table V, p. 396. June salinities ranged from 33.22 to 33.87 $^{0}/_{00}$ at or near the surface at the River station while at the same time bottom salinities in the St. Just Channel ranged from 34.82 to 35.15 $^{0}/_{00}$, and intermediate values of 34.44 to 34.78 $^{0}/_{00}$ occurred on the Bank stations, e. g. East Bank. In July fewer observations were made but salinities were respectively about 34.1, 34.76, and 35.00 $^{0}/_{00}$ on the River station (surface), East Bank (bottom) and St. Just Channel (bottom). In August salinities were remarkably high at the St. Just Channel Station (bottom) on the three occasions they were investigated 35.28 (the 3rd) 35.23 (the 10th) and 35.24 (the 17th) $^{0}/_{00}$, with about 35 $^{0}/_{00}$ on the Banks and only slightly less at the River Station. As the rainfall was excessive in the month it would seem certain that alternations of very high salinity with undetected relatively low ones occurred. Such fluctuations in salinity may very well account for the correlated incomplete and ineffective spawning.

These high salinities in the upper part of the Estuary appear to be unusually abnormal, since Lumby in a recent survey of salinities in the English Channel (1935) estimates the mean monthly salinity of the upper water layer well off the mouth of Falmouth Estuary as about 35.10 $^{0}/_{00}$, and in August shows the easterly crest of the surface 35.35 $^{0}/_{00}$ isohaline in the middle of the mouth of the English Channel. Persistent rains continued in September when the rainfall was nearly 70 % above the normal, with reduction of salinities below those found in June (Orton, 1928). It is known that the oyster responds quickly to changes in salinity by absorbing water when transferred to solution of low salt content and by losing water in a solution of high salinity. Fluctuations in the water content of the ripe egg in the body would probably follow that in the body fluid. In this event it is also probable that the delicate mechanism of the maturing egg would be seriously affected and possibly destroyed.

Abortive spawning.

It is clear from the records displayed in Table 5 that after August 12th effective spawning virtually ceased. At this time the same records show that about one fifth of the adolescent and adult population were mature or nearly mature females ready to spawn. This percentage was maintained until the last week in September when reduction in the percentage began, so that by the end of October the percentage of females ready to spawn was reduced to 8.3 %.

During this period no oysters were found with eggs or embryos in the mantle cavity and only four with prematurely extruded ova. The reduction in the percentage of mature females can therefore be accounted for only by abortive spawning during which eggs were extruded from the gonad directly to the exterior. In this period mature females were not infrequently found with a lax gonad with contents in a somewhat fluid state, and were regarded as pathological. There can be little doubt that these individuales were in a phase of abortive spawning.

They occurred as follows : — May, June and July rarely; August., 10.8 % among 406; Sept., 15.4 % among 299; Oct., 30.7 % among 278, and in Nov., 11 individuals out of only 15 mature females found. Similar phases have, however, been observed at the end of normal spawning seasons on both the Fal and Blackwater beds.

III. — COMPARISON OF SPAWNING IN THE FAL AND BLACKWATER BEDS

Comparison of incidence of spawning.

A comparison of the incidence of spawning on the Blackwater and Fal beds in the same season is peculiarly instructive (See Table 9).

Spawning began on the Blackwater beds early in June contemporaneously with the rise in temperature to about 59° F. and increased in the second week, beginning June 7th, to 5.3 %. At the same time on the Fal beds none were found in spawn during the first week and only 0.3 % in the week beginning June 7th when the temperature fluctuated between 58-59 at the River station, but lower temperatures obtained on the main banks. The discrepancy between total new spawners (i.e. natural and presumed premature) on the two beds in the same period is still more striking, being 3.7 and 9.4 % on the Blackwater in comparison with an unknown small percentage, and only 0.3 % on the Fal (See Table 9, D). On the full moon tides for the week beginning June 15th the percentage of new spawners is about the same 2.7 to 2.9 on both beds, but the total new is 9.0 % on the Blackwater and 4.1 % on the Fal. In this week temperatures ranged about 60.6° F. on the former and 58.6 (River stations) on the latter. In this week 12.9 % of the population were blacksick, i.e. with fully developed coloured and shelled larvae on the Blackwater against only 0.4 % on the Fal.

Spawning therefore began on the Blackwater about a fortnight earlier than on the Fal in correlation with the rise in sea temperature to about 59° one fortnight earlier on the Blackwater than on the Fal beds.

Comparison of the seasonal course of spawning.

A comparison of the course of spawning throughout the season in the two localities can quickly be made from a perusal of Table 9, Sections B, E and D and Fig. 1.

Section B gives the percentage of oysters with undoubtedly normal white embryos, E the percentage of whitesick plus greysick, and Section D the percentage with young embryos from a probable spawning after capture. It has been mentioned that the latter females were probably mostly on the point of spawning and might soon have spawned under an appropriate stimulus had they remained on the beds. Thus the actual percentage of new spawners is not known, but probably lies nearer the total of Sections D and E than that of undoubtedly normal spawners in Section E.

In the first instance it is seen that spawning was at its height in the Blackwater in June, with heavy spawning also during July, except in the week beginning the 14th on the full moon spring tides. On the other hand after a small maximum of spawning on the Fal on the full moon tides in the middle of June, the height of spawning is concentrated in July and early August.

Spawning virtually ceased on both beds about the middle of August. The closest correspondence in spawning on the two beds occurred about June 15 and during July. The accumulative percentage of total newly spawned (Table 9, E) is 43.1 on the Blackwater and 49.0 on the Fal, while that of normal whitesick (Table 9, B) amounts only to 18.2 on the former and 26.3 on the latter beds.

Comparison of percentage of oysters about to extrude larvae, i.e., blacksick.

The percentage of oysters blacksick, i.e., on the point of extruding larvae is of great importance economically, as it is possible to predict closely from this information when the spat may begin to settle. The data are set out in Table 9,

TABLE 9. — Comparison of female spawning of **O. edulis** and sea-temperature variation on the

Dates of moon's quarters 1927	MAY	JUNE				JULY			
	●		○		●		○		●
	30	7	15	22	29	7	14	21	28
A. — NUMBER OF OYSTERS.									
Blackwater	831	412	309	416	517	305	456	207	203
Fal	—	331	483	433	760	368	667	456	934
B. — PERCENTAGE NORMAL WHITESICK.									
Blackwater	1.9	**5.3**	2.9	0.5	0.4	1.6	0.7	2.4	2.5
Fal	—	0.3	2.7	1.4	0.1	**4.9**	3.6	4.2	**5.2**
C. — WEEKLY MEAN OF TEMPERATURE (°F).									
Blackwater	59.4	60.0	**60.6**	58.4	60 7	**65.6**	63.6	63.5	65.3
Fal (*) (River)	—	58.2	58.6	**58.9**	57.1	59 4	**62.6**	**62.4**	62.2
D. — PERCENTAGE PREMATURELY SPAWNED.									
Blackwater	1.8	4.1	6.1	3.1	3.1	6.6	0.4	2.4	3.0
Fal	—	0.0	1.4	0.0	0.9	0.3	4.9	0.0	1.4
E. — PERCENTAGE NORMAL WHITESICK AND GREYSICK.									
Blackwater	2.4	7.2	6.5	5.1	4.8	3.2	2.7	3.8	3.5
Fal	—	0.6	4.1	2.8	1.0	9.2	6.9	9.5	7.4
F. — PERCENTAGE BLACKSICK.									
Blackwater	0.0	4.4	**12.9**	5.0	6.0	**8.5**	7.2	5.3	3.4
Fal	—	0.0	0.4	2.1	2.1	0.5	**8.1**	5.3	9.6
G. — TOTAL WITH EMBRYOS OR LARVAE.									
Blackwater	4.2	15.7	**25.5**	13.2	13.9	**18.3**	10.3	11.5	9.9
Fal	—	0.6	5.9	4.9	4.0	10.0	**20.0**	14.8	18.4
H. — PERCENTAGE MATURE AND NEARLY MATURE FEMALES. (UNSPAWNED.)									
Blackwater	28.9	26.0	17.9	15.9	**19.4**	16.5	13.6	8.8	8.8
Fal	—	27.5	26.4	31.8	33.4	37.0	31.0	**41.9**	28.9
I. — PERCENTAGE TOTAL FEMALE-FUNCTIONING.									
Blackwater	33.1	41.7	**43.4**	29.1	33.3	**34.8**	23.9	20.3	18.7
Fal	—	28.1	32.3	36.7	37.4	47.0	51.0	**55.7**	47.3

(*) Fal temperature means cover only such observations as were made in the period (see Orton, 1928, p. 390) and

Blackwater and Fal Estuaries, 1927. — (Weekly means from the days beginning the moon's quarters.)

AUGUST				SEPTEMBER				OCTOBER				Accumulative Total %
5	○ 13	19	● 27	4	○ 11	18	● 25	4	○ 10	17	● 25	
102	104	—	—	—	385	—	—	132	101	102	—	
437	503	292	185	250	614	267	271	641	630	714	265	
0.0	0.0	—	—	—	0.0	—	—	0.0	0.0	0.0	—	18.2
3.0	0.6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.3
67.4	64.8	63.3	61.4	63.3	59.7	58.7	54.6	54.2	54.0	52.0	53.2	
—	59.6	60.0	61.0	60.5	58.3	59.5	55.5	—	—	—	—	
1.0	0.0	—	—	—	0.0	—	—	0.0	0.0	0.0	—	32.3
3.4	0.0	1.4	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0	14.4
2.0	1.9	—	—	—	0.0	—	—	0.0	0.0	0.0	—	43.1
5.3	1.2	0.6	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	49.0
2.9	2.9	—	—	—	0.8	—	—	0.0	0.0	0.0	—	59.3
11.0	9.3	3.8	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.0	0.8	53.8
5.9	4.8	—	—	—	0.8	—	—	0.0	0.0	0.0	—	
19.7	10.5	5.8	0.0	0.4	0.0	0.0	0.4	0.5	0.6	0.0	0.8	
6.8	2.9	—	—	—	1.3	—	—	1.5	4.0	3.9	—	
18.3	23.7	17.5	20.0	17.6	21.7	19.9	16.6	13.4	12.3	12.0	8.3	
12.7	7.7	—	—	—	2.1	—	—	1.5	4.0	3.9	—	Max. % 59.5
38.0	34.2	23.3	20.0	18.0	21.7	19.9	17.0	13.9	12.9	12.0	9.1	do. 68.9

are not regular bi-daily observations as were those on the Blackwater (see p. 1014).

Section F and Fig. 1, 2 D. On the Blackwater the percentage of oysters blacksick rose to 12.9 as early as the middle of June when only 0.4 % were present on the Fal. As the parent oyster normally extrudes its larvae soon after the black stage is reached and the larvae may develop to the settling stage after about 10 days of free life in the sea, it follows that a heavy spatfall may be expected within 10 days to a fortnight after 5 to 10 % of the oysters are found blacksick. Larvae would therefore be expected to be ready to settle in abundance on the Blackwater at the end of June in 1927. On the other hand a corresponding abundance of larvae in the sea did not occur in the Fal until July-August. Good percentages of blacksick oysters persisted on the Blackwater until the middle of August and until the third week in the same month on the Fal.

These differences are characteristic of these two localities as is shown by investigations in other years, except that in a normally warm season abundance of larvae may persist on the Fal Estuary until as late as the beginning of September (See Orton, 1926, p. 205). The main spatting period on the Blackwater extends therefore from about the end of June to about the middle of August, but from the middle of July to about the middle of September on the main Fal beds. It is probable, however, that breeding begins earlier, i.e. in early June, on the upper beds of the Fal River proper, which could not be investigated, so that the length of the breeding season in the whole of the Fal River and Estuary is actually longer than on the Blackwater.

Comparison of percentage of potential females in the populations.

The weekly percentages of mature and nearly mature unspawned females are given in Table 9, Section H and those of total female-fuctioning, i.e. including those about ready to spawn and those with embryos and larvae in Section I.

As the eggs after extrusion from the body require to be incubated from including those about ready to spawn and those with embryos and larvae, in about 7 to 10 days, according to the level of the sea-temperature, before the resulting larvae are liberated, it follows that at the lower temperatures some larvae will be retained more than one week and will therefore be recorded twice in the statistics given above, *but in successive weeks.* The rate of development of oyster embryos *in situ* in the parent in relation to temperature is not known with sufficient accuracy to permit of more than this general statement. At the end of the season on the Fal embryos may be retained two weeks or more, and one instance was noted in 1927 of retention of larvae by an oyster in the Plymouth tanks until December 1st. The retention of larvae therefore tends to be prolonged at low temperatures.

Thus the total percentage of female-functioning is not given at once by the weekly proportions shown in Section I. The highest weekly percentages of total female-functioning on the Blackwater grounds were 43.4 in the week beginning June 15th and a secondary peak of 34.8 in the week beginning July 7th. Allowing for the oysters which spawned before the latter maximum was reached it has been shown herein (p. 1010) that approximately 59.5 % of the population were females or had produced larvae in or before the week beginning July 7th. On the Fal weekly percentages of total females rose gradually to a maximum of 55.7 in the week beginning July 21st, and afterwards slowly declined. Prior to this date it has been shown (p. 1021) that 13.2 % had spawned and shed their larvae, therefore 68.9 % of the population were potential females in 1927. The proportion of the population developed as females was higher on the Fal than on the Blackwater by 68.9 — 59.5 = 9.4 %. It has however been shown that the Fal oysters spawning in June may constitute different populations from those on the main beds, and that therefore it is advisable to deduct the spawnings in June, 8.6 %, from the total to avoid a possible over-estimation. When this is done, the totals estimated for the two beds are about the same. There is no doubt that when all the different sex-categories (intersexes) have been analysed the percentage of potential females will be somewhat increased for both the Blackwater and Fal (see Fig. 4, p. 1048).

It is noteworthy that on the Blackwater the increase in percentage of females corresponds with an increase in the sea-temperature, and that the secondary maximum on the Blackwater coincides with a secondary maximum in the trend of the seasonal rise in temperature. Similarly the maximum percentage of females on the Fal is attained after a sustained rise in temperature. This correspondence is considered causally significant, and it has been shown in unpublished work that female phases are superimposed rapidly on various male phases, especially at the beginning of the spawning season. The cause of the production of eggs in association with the increase in temperature is being investigated.

The weekly percentage of mature or nearly mature females, i.e. those almost ready to spawn, corresponds roughly with that of the total females, but varies with the amount of spawning in each week. There is a maximum on the Blackwater of 28.9 at the beginning of the spawning season and a secondary maximum in the week beginning June 29th. On the Fal there is a gradual rise from the beginning of the spawning season to 37 % in the week beginning July 7th and a secondary maximum of 41.9 in the week beginning July 21st. The figures on the Fal are much higher at this date than those on the Blackwater, and since far fewer had spawned the females ready to spawn on the receipt of the appropriate stimulus had accumulated, whereas those on the Blackwater had spawned mostly soon after becoming ripe.

The mature females on the Blackwater had become almost exhausted (2.9 %) by the middle of August, whilst as has been noticed already, the percentage at that time on the Fal ranged about 20 % and did not decline significantly until the end of September and October. Thus about 20 % of the population functioning as mature females failed to produce young on the Fal in 1927, as against only little more than 1 % on the Blackwater.

Total percentage with young (embryos or larvae).

The percentage of oysters with young, i.e. embryos or larvae, is given in Table 9, Section G, and well shown in Fig. 1, graph 2. The latter graphs bring out in a striking manner the difference in the seasonal spawning in the two localities in 1927. On the Blackwater the percentage, already 15.7 in the week beginning June 7th, rises to a maximum of 25.5 in the following week. A secondary maximum occurs in the week beginning July 7th and thereafter the percentage declines steadily to the middle of August and probably until middle September. On the Fal a sudden rise to 5.9 % occurs in the week beginning June 15th followed by slightly lower percentages until July 7th when the percentage rises to 10 % and in the following week to a maximum of 20 %. Afterwards a slight decline occurs followed by a secondary maximum in the period July 28th—August 12th. There is then a sharp decline to zero at the end of August with only a trace of spawning in September-October. There is a marked similarity in the graphs of the two sets of data with a lag of the Fal on the Blackwater of about one fortnight corresponding to the initial lag in the full attainment of the spawning temperature.

Percentage with colourless shelled embryos.

The graphs depicting percentages with colourless shelled embryos Fig. 1, 2 C, show little correspondence with either natural or total new spawning on the Blackwater; there is, however, in the beginning of the spawning season an indication of a lag of about a week in the rise to a maximum of about 5 %. On the Fal the lag is hardly perceptible at the beginning of the season, and shows little relation to either natural or premature spawners later. At the end of the effective spawning season there is a lack of this category corresponding with the normal spawners. The lack of correspondence of frequencies of this category to either the younger embryos or the older ones is explained by the rapidity through which this phase of development is passed.

The shelled stage is reached in about 3 ½ to 4 days according to temperature level and the black stage in about 5 or 6. The uncoloured shell stage may therefore be completed in about 1 ½ to 2 days.

At the end of the spawning season with low and falling temperatures the uncoloured stage may, however, persist more than a week, and it would appear that the blacksick stage is acquired very slowly and with difficulty under these conditions.

Spawning in relation to tidal phases.

It is readily seen from Table 9, p. 1036, Sections B and E, that spawning is indiscriminate in the various tidal phases on both the Fal and Blackwater grounds when the samples are grouped in periods beginning on the dates of the moon's quarters. On the Blackwater individuals carrying embryos and larvae aged one to about four days old (Section E) show a continuous periodical percentage for June and July varying from 2.7 to 7.2. Similar figures for the Fal occur in July and early August, showing a continuous range from 9.2 to 1.2. Therefore it is evident that no marked periodicity in breeding occurs when the samples are grouped in periods each beginning on the date of the moon's quarter.

The samples have also been grouped in periods centred on the date of the moon's quarters in Tables 2 and 6 and are summarised with those given in Table 9 in Table 10, p. 1042. In the upper part of this Table the percentages respectively of normal whitesick (=NW) and normal whitesick plus greysick (=NW+Gr) spawning in each of the four tidal phases, i.e. neaps preceding full moon, full moon, neaps preceding new moon, and new moon, are given for the Blackwater and Fal; the percentages for groupings on dates beginning the moon's quarters are given in block type (Series A) and those centred on the moon's quarters in the Series B in ordinary type. Weekly or short period means of temperature are given and those ranging near the lower spawning limit are shown in italics.

The aggregate percentages for each series of comparable tides, e.g. neaps, 2nd Quarter=N^2, is given below to offer a criterion of comparison. These aggregate percentages are arranged in the lower part of the Table (**10**, 3) in numerical order for both the A and the B method of grouping for both Blackwater and Fal.

The frequencies of the numerical order of the four tidal phases are collected into tabular form for both A and B and also for A and B combined.

From this analysis it is clear that when the samples are grouped in periods beginning on the dates of the moon's quarters, that is the A series, there is shown a distinct tendency for a greater proportion of spawning to occur in the neap tides preceding the full moon spring tides. On the groupings centred on the moon's quarter days, series B, there is no preponderance of spawning on any phase of the tide. On combining the results of the two methods of grouping there still remains the indication of preferential spawning on the neaps preceding the full moon tides.

Thus in 1927 there is no indication of such periodicity of spawning in the Fal on the full moon tides as was found in 1925 (Orton, 1926). The peculiar weather and hydrographical conditions in 1927 no doubt affected the sequence of spawning as is clearly shown in Fig. 1. There is however no ground for concluding that lunar periodicity in spawning does not occur on the Fal in favourable seasons. The spawning behaviour of many marine species, it is now becoming clear, depends upon the local general hydrographical and biological conditions, therefore a difference in behaviour in the same locality when conditions are abnormal is to be expected. The spawning of *Mytilus edulis* in

TABLE 10 (¹). — New spawning of **O. edulis** in relation to tidal phases on the Blackwater and Fal
B (figures in smaller type) weekly periods contred on date of moon's quarters. — The percentages of
given for the total of presumed premature spawners and normal whitesick in 10 (²). Mean tempera-

	10 (¹)		NEAPS (= N²)			FULL MOON		
			N. W. %	N.W.+Gr.%	T. in °F.	N. W. %	N.W.+Gr.%	T. in °F.
MAY	Blackwater	A	—	—	—	—	—	—
		B	—	—	—	—	—	—
	Fal	A	—	—	—	—	—	—
		B	—	—	—	—	—	—
JUNE	Blackwater	A	5.3 *(26.0)*	7.2	60.0	2.9 *(17.9)*	6.5	60.6
		B	6.4 —	8.7	—	3 3 —	6.2	—
	Fal	A	0.3 *(27.5)*	0.6	*58.2*	2.7 *(26.4)*	4.1	*58.6*
		B	0.6 —	0.6	—	0.0 —	1.7	—
JULY	Blackwater	A	1.6 *(16.5)*	3.2	65.6	0.7 *(13.6)*	2.7	63.6
		B	0.6 —	0.6	—	1.6 —	3.2	—
	Fal	A	4.9 *(37.0)*	9.2	59.4	3.6 *(31.0)*	6.9	62.6
		B	0.0 —	0 4	—	3.8 —	8.9	—
AUGUST	Blackwater	A	0.0 *(6.8)*	2.0	67.4	0.0 *(2.9)*	1.9	64.8
		B	1.6 —	2.9	—	— —	—	—
	Fal	A	3.0 *(18.3)*	5.3	—	0.6 *(23.7)*	1.2	59.6
		B	6.5 —	8.1	—	1.5 —	3.8	—
Aggregate percentages for NW, and N W + Gr.	Blackwater	A	6.9 —	12.4	—	3.6 —	11.1	—
		B	8.6 —	12.2	—	4.9 —	9.4	—
	Fal	A	8.2 —	15.1	—	6.9 —	12.2	—
		B	7.1 —	9.1	—	5.3 —	14.4	—
	10 (2) N.W.+P.							
Aggregate percentages for N W + P.	Blackwater	A	—	18.6	—	—	10.1	—
		B	—	19.9	—	—	14.8	—
	Fal	A	—	11.9	—	—	13.2	—
		B	—	9 5	—	—	10.7	—

Estuaries in 1927 for : A (figures in large type) weekly periods beginning on date of moon's quarters; normal whitesick, and normal whitesick + greysick, are shown in detail; aggregate percentages only are tures are given in °F, and percentage unspawned ripe and ripening females in brackets in italics.

Neaps (= N^4)			New Moon			Sum of aggregate percentages	
N. W. %	N.W. + Gr. %	T. in °F.	N. W. %	N.W. + Gr. %	T. in °F.		
0.0 (37.6)	0.0	*58.2*	1.9 (28.9)	2.4	59.4		
0.0 —	0.0	—	2.0 —	2.5	—		
—	—	—	—	—	—		
—	—	—	—	—	—		
0.5 (*15.9*)	5.1	*58.4*	0.4 (*19.4*)	4.8	60.7		
1.0 —	6.9	—	0.0 —	6.5	—		
1 4 (*31.8*)	2.8	58.9	0.1 (*33.4*)	1.0	*57.1*		
3.9 —	4.3	—	0.5 —	2.6	—		
2.4 (*8.8*)	3.8	63.5	2 5 (8.8)	3.5	65.3		
0.5 —	2.1	—	4.8 —	7.7	—		
4.2 (*41.9*)	9.5	62.4	5.2 (*28.9*)	7.4	62.2		
4.8 —	8.1	—	2.5 —	6.6	—		
— —	—	63.3	—	—	61.4		
0.0 —	1.9	—	—	—	—		
0.3 (*17.5*)	0.6	60.0	0 0 (*20.0*)	0.0	61.0		
0.2 —	0.8	—	0.5 —	0.5	—		
2.9 —	8.9	—	4.8 —	10.7	—	18.2	43.1
1.5 —	10.9	—	6.8 —	16.7	—	21.8	49.2
5.9 —	12.9	—	5.3 —	8.4	—	26.3	48.6
8.9 —	13.2	—	3.5 —	9.7	—	24.8	46.4
—	9.1	—	—	12.7	—	—	50.5
—	10.3	—	—	11.7	—	—	56.7
—	7.3	—	—	7.6	—	—	40.0
—	13.2	—	—	7.1	—	—	40.5

TABLE 10 (3). — Aggregate percentages for normal whitesick and normal whitesick plus greysick

A								
Numerical order	Blackwater		Fal		Totals for tidal periods			
	NW	NW + Gr.	NW	NW + Gr.	NM	N^2	FM	N^4
1	6.9 N^2	12.4 N^2	8.2 N^2	15.1 N^2	—	4	—	—
2	4.8 NM	11.1 FM	6.9 FM	12.9 N^4	1	—	2	1
3	3.6 FM	10.7 NM	5.9 N^4	12.2 FM	1	—	2	1
4	2.9 N^4	8.9 N^4	5.3 NM	8.4 NM	2	—	—	2

NW = Normal whitesick. Gr. = Greysick. P = Prematurely spawned.

Passamaquoddy Bay, Bay of Fundy, in 1930 tended to occur monthly after the new moon spring tides from June to September (Battle, 1932). In England the general tendency is for *Mytilus edulis* to spawn once during the year in April-May. Recently Marshall and Stephenson (1933) adduced evidence that the coral, *Pocillopora bulbosa*, on Low Isles, Great Barrier Reef, in 1928-29 breeds discontinuously about the time of the new moon from December to April, but at the full moon during the months of July and August.

Since a species may vary in spawning behaviour in different localities, there is good ground for anticipating variation in spawning behaviour in one species in one locality in correlation with varying general biological conditions in different spawning seasons. Continuous observations over a period of years are thus generally desirable in investigations on spawning behaviour.

Rate of development of egg to veliger in oysters in the sea.

It is clear from the observations on the Blackwater that eggs develop in the mantle cavity of the oyster in the sea to the mature coloured bivalve larva in about a week at a mean temperature of about 59.4° F.

In the first week of spawning, beginning May 30th, there were 3.7 % total new spawners, i.e. whitesick (including 1.9 % normal), 0.5 % greysick, i.e. uncoloured larvae, with more or less development of the shell, but none blacksick. In the second week 4.4 % blacksick were found. There can be no doubt that the latter blacksick are the representatives on the beds of the whitesick for each tidal phase arranged in numerical order for periods A and B for Blackwater and Fal.

B								Totals A + B for tidal periods			
Blackwater		Fal		Totals for tidal periods							
NW	NW + Gr.	NW	NW + Gr.	NM	N^2	FM	N^4	NM	N^2	FM	N^4
8.6 N^2	16.7 NM	8.9 N^4	14.4 FM	1	1	1	1	1	5	1	1
6.8 NM	12.2 N^2	7.1 N^2	13.2 N^4	1	2	—	1	2	2	2	2
4.9 FM	10.9 N^4	5.3 FM	9.7 NM	1	—	2	1	2	—	4	2
1.5 N^4	9.4 FM	3.5 NM	9.1 N^2	1	1	1	1	3	1	1	3

NM = New moon. N^2 = Neaps, 2nd quarter. FM = Full moon. N^4 = Neaps, 4th quarter.

and greysick of the preceding week, since spawning began after May 30th (See Table 1, p. 1004). Similarly, a percentage of 0.3 normal whitesick plus 0.3 % greysick on the Fal in the week beginning July 7th was succeeded in the following week by 0.4 % blacksick; and 2.7 % normal whitesick plus 1.4 % greysick in the week beginning June 15th, followed by 2.1 % blacksick in the following week, and there can be no doubt that these blacksick are the representatives on the beds of the white and greysick taken during the previous week. Sea-temperature on the Fal during this period ranged between the weekly means of approximate 58.6° and 58.9° F.

The lag of one week on the appearance of blacksick on whitesick is shown in the graphs 2B and 2D in Fig. 1, p. 1005, especially well on both beds at the beginning of the spawning season, and throughout the season on the Fal grounds. The succession of blacksick after whitesick is not so well shown in the graphs for the Blackwater after the middle of July, when irregular spawning is related with fluctuations in temperature. As this fluctuation in temperature occurred at a high level, it is probable that development to the blacksick stage was more rapid than at lower temperatures, and followed by quicker extrusion of larvae.

Period of incubation of young in the sea.

The examination of large samples of oysters in successive weeks during the spawning period renders it possible to deduce approximately the length of time oyster larvae are retained within the parent in the sea.

The graphs of whitesick given in Fig. 1 at 2 B and 2 A and B in relation to the blacksick in 2 D give not only the period elapsing between these two stages but also the approximate time the larvae are retained within the parent. For example the 10 % whitesick (including at least 5 % normal) found on the Blackwater beds in the week beginning June 7th is followed by 12.9 % blacksick in the following week, but only 5.0 % a week later. It is clear that most if not all of the 12.9 % blacksick discharged their larvae soon after the blacksick stage was reached, as the 5.0 % are clearly the outcome of the 6.5 % new spawners in the previous week. On the Fal grounds the graph of the blacksick shows a close lag of one week in rise and fall on the natural whitesick proving that the average discharge of the larvae occurs approximately after a week of incubation.

These results agree entirely with those found in investigations in 1925 (Orton, 1926). As the latter results were based on single weekly samples it was possible to estimate the period of incubation at 7 to 10 days, but with the more frequent sampling in the present investigation, it is now possible to estimate the average period of incubation more closely at about 7 days, with, however, indications that the larvae when mature may be retained for a few days longer.

These results confirm empirical observations and are valuable for economic purposes.

IV. — DISCUSSION ON SEX-CHANGE IN OYSTER POPULATIONS

The examination of large numbers of individuals weekly throughout the spawning season of 1927 has established the broad fact that about 50 % or more of the population (ca. 60 % on the Fal and ca. 60 % on the Blackwater, See p. 1039) functioned as females or were potential female spawners. The percentage of females increases at the approach and during the beginning of the spawning season to a maximum and afterwards diminishes as the animals reach full maturity and spawn. The figures given in Tables 1 and 5 prove that many of the females become nearly mature early in the spawning season, but reach full maturity at various intervals — extending to many weeks — later. On the Blackwater in 1927 the females matured rapidly and spawned except for a small remnant during August, whereas on the Fal 20 % of the population which were then maturing or mature females had not then spawned, but did spawn ineffectively later (See p. 1034). In addition to these definite percentages of females small increments will be made later when several categories of intersexes (herein omitted) have been fully analysed.

With the establishment of the important facts that 50 % or more of the population of oysters on the Fal and Blackwater beds were females in one year, and that the females reach a maximum and afterwards decline by successive spawnings, it is now possible to utilise observations made on this aspect during other years for information on population sex-change. In investigations on the Fal grounds in 1925 it was found that samples of oysters from Turnaware Bar and East Bank on July 8th and 15th respectively each showed about 46 % females either ripe or with young (Orton, 1926). There can be no doubt that other members of these populations had functioned as females before this time, and it is probable that more females would be produced later. Thus in 1925 it is certain that at least 50 % of the population functioned or were matured as females. In this year spawning was normal and continued to the end of September, when the percentage of unspawned ripe females was reduced to 4 to 7.6 %. In 1925, therefore, about 50 % of the population did function as females.

In 1926 examinations of samples from the Fal are recorded in Orton, 1926, p. 979, and show already in June an average of 32.2 % mature or nearly mature unspawned with a few spawned females and 12.7 % of mixed sexes, which would mostly spawn as females, giving a total of 45 % potential females. Later observations (unpublished) show that the percentage of potential females rose well above 50 % at the end of June (See Fig. 4, p. 1048).

Therefore in three successive years on the Fal grounds there has recurred in each spawning season a total of 50 % or more females in the population. It has also been shown that in 1925 and 1927 most of these females spawned either effectively or ineffectively, so that there could only remain a small percentage of unspawned females at the end of the spawning season. In 1926 it was found (See Fig. 4, p. 1048) that the unspawned females at the end of the season amounted to about 5 % of the population.

In the three successive seasons the percentage of unspawned females was reduced to a level of about 5 %. It is, however, improbable that eggs retained until the winter are held over to be spawned in the following summer. Absorption or rejection of the eggs usually occurs with assimilation of the absorbed products into the tissues (Orton, 1924 and 1933).

The same sequence of 50 % females which are almost expended during the spawning season can be shown to occur in successive years on the Blackwater beds (Orton, 1926, p. 979). In June 1922 48 % were female-functioning; in July 1924 after many females had spawned and shed their larvae, at least 32 % females still remained; and in 1926 in June, 45 % were female although many females had shed their larvae as is shown by the records of earlier samples (See Orton, 1926, p. 979).

It is therefore established that on English Oyster beds i.e. Blackwater and the Fal, there is a succession year after year of at least 50 % or more females in the populations. These females develop in the spring, become expended during the summer and autumn except for a remnant of about 5 %, which in all probability do not pass over into the following season as mature females. Half of the population (at least) develop into females each year. As it is known

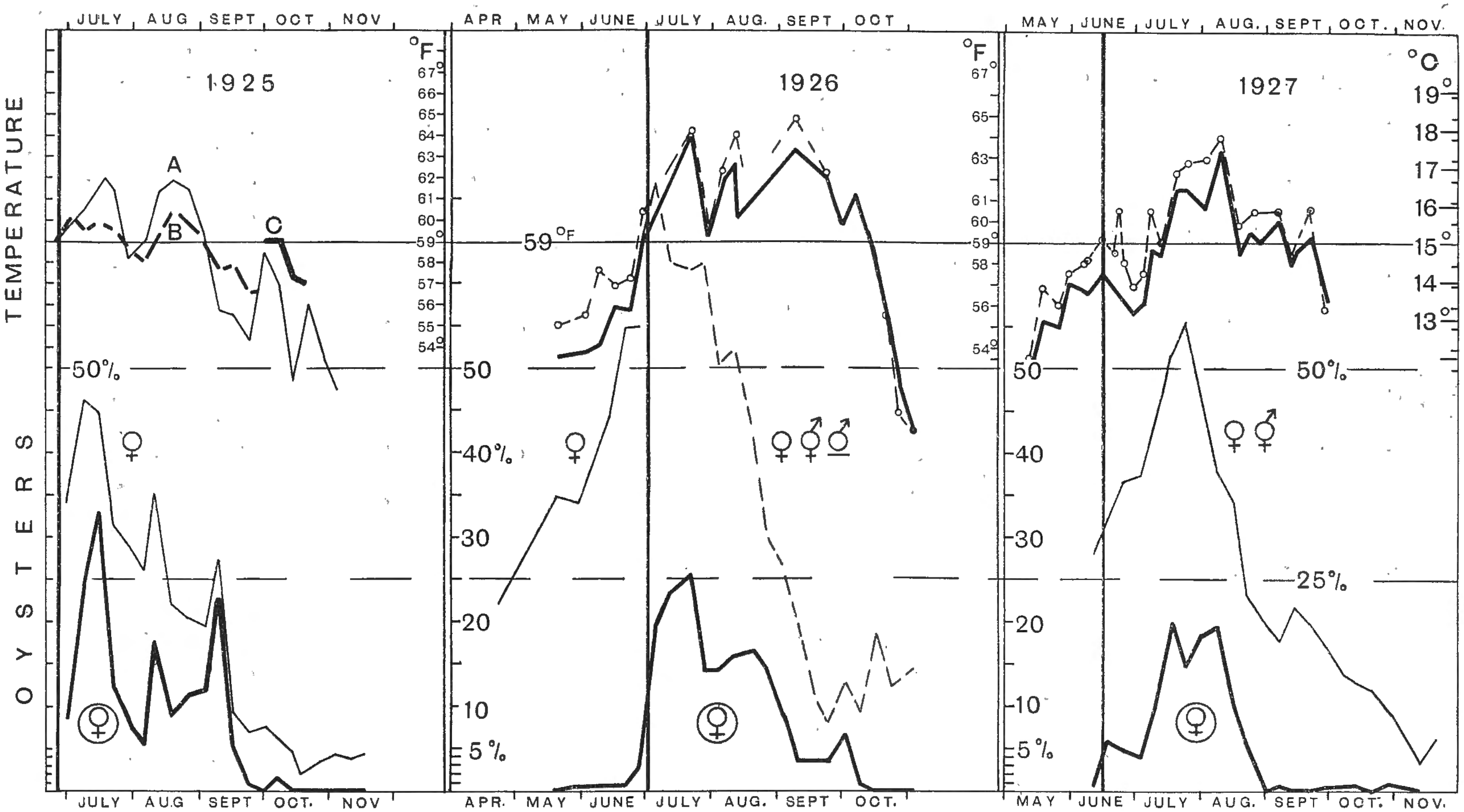

FIG. 4. — Seasonal variation in sea-temparture, percentages of total female-functioning and total (normal) oysters with young (♀) on the Truro beds, Fal Estuary, for the years 1925, 1926 and 1927.

Mean sea-temperature for short periods of a few days or isolated readings for the Bank Stations (see Orton, 1928) are given by the thick continuous line graphs. Similar readings for the River Station are given by the thin broken-line graph with circles. In 1925 sea-temperatures, C, are available only for October, and the graph A is of mean air-temperature and B an estimate (probably low) of sea-temperature. Total female (virtually pure) is given by the continuous thin-line graph in 1925 and 1926, but in 1927 includes ⚥ forms which would function as ♀'s. The thin broken-line graph for 1926 includes besides ♀ and ⚥ some female-like males indistinguishable from females by the naked eye, and not identified microscopically; the sex of all other oysters was confirmed microscopically.

A thick vertical line is drawn at the first sharp rise in the graph for total spawning oysters, and cuts the temperature graph in 1926 and 1927 at about the level of 59° F = 15° C.

The percentages recorded are derived from samples examined and grouped in short periods of 1 to 5, and usually 3, days in successive weeks.

(Orton, 1927) that after spawning the female oyster reverts to a male phase, and as also in the early spawning season rather more than 50 % of the population may be mature males, it follows that *Ostrea edulis* on the English oyster beds named is female one year and male in the following, reverting to the female phase every other year on the average. Since, however, more than 50 % of the population may be female in successive years, it follows that a proportion of reversions to the female phase must occur in less than one year. These quicker reversions probably occur in the well-nourished older oysters.

It is emphasized that these observations appertain to the adolescent and adult section of the population, which includes on English beds oysters mainly three or more *years* old.

GENERAL DISCUSSION

The study of the incidence of spawning on the Blackwater and Fal Estuaries in 1927 has elicited virtual proof that oysters, *O. edulis*, on these beds begin to spawn, if the general biological conditions are otherwise favourable, when the sea-temperature in its normal gradual seasonal rise has attained a level of about 59° F. = 15° C. The evidence is clear in this instance for the Blackwater, but absence of records in a critical week-end on the Fal leave it possible that spawning began when sea-temperature was about 58°-59 F. The records for the Blackwater indicate that a sudden rise to 59-60° from 51-52° F. in four days early in May apparently did not induce spawning. On the other hand a fall in temperature after the spawning season had begun arrested spawning at the end of June on the Fal, where it is probable that temperature fluctuation was the cause of the arrest in spawning, as salinity observations (Orton, 1928) showed no greater range than in the preceding two weeks when spawning occurred. Observations of this kind should, however, be repeated over a number of years before the relationship can be regarded as clearly established. These facts are of importance in assessing the role of temperature in the maturing and spawning of female *O. edulis*. There can be little doubt that egg-development is a function of time and temperature as advocated by Helland-Hansen (1908), but there is equally little doubt that egg-development does not proceed at the lower levels of temperature to which oysters are subjected in certain habitats. The temperature below which egg-development is inhibited is, however, not yet known, but as feeding activity is probably inhibited below about 5° C. and little occurs below about 10° C. according to Savage's work (1925) on *O. edulis* in the sea, and as feeding also ceases at about 5° C. in *O. virginica* as is shown by the work of Nelson (1923) and Galtsoff (1928) and in *O. lurida* by Hopkins (1931) it is probable that metabolism of reproductive elements ceases above this minimum temperature. Spärck concludes that egg-development does not occur below 10-12° C. (1924). From the probable absence of spawning observed on the Blackwater in early may when the temperature rose suddently to 59-60° it would seem that either no individuals had then reached maturity or that a somewhat sustained period below a temperature of 59° is necessary for maturation. When the relationship between the minimum effective anabolic temperature and egg-development is established, it may be possible to calculate the period required for the attainment of female maturity from a given initial phase of female potentiality.

It is certain, however, that the mature female will not spawn naturally until invoked by the spawning stimulus, or stimuli, amongst which a temperature level of at least 58-59° F. seems to be essential. Information on the development of females is given in Fig. 1, p. 1005, but it cannot be dealt with adequately until the seasonal variations in categories of individuals other than those given in this paper are discussed.

The factors which may possibly contribute to the spawning stimulus have been previously discussed (Orton, **1926**) when the suggestion was made that decrease of pressure with increase of temperature at low water may be the most important. In view of the occurrence of periodicity in spawning of many marine animals which live below low water mark and in some cases (e.g. *Centrechinus setosus*) in the Mediterranean Sea where tides are almost negligible, it would seem that other factors are of greater importance. Fox (**1923**) has discussed the factors likely to operate in the curious case of *Centrechinus*, which spawns in the full-moon period, without arriving at a satisfactory conclusion.

In **1925** *O. edulis* exhibited periodicity in spawning at the full moon periods on the Fal Estuary, as is shown in Fig. **4**, p. **1048** (see Orton, **1926**), so that the spawning stimulus in *O. edulis* may very well be of the same general kind as that operating in other marine animals which exhibit this phenomenon.

In a reconsideration of the factors likely to operate in periodical spawning, it would seem that some internal as well as external factors are important. Such an internal factor may be bound up with oxygen consumption as Fox surmised. Egg-development is accompanied by increased metabolism and, it may be presumed, a high rate of oxygen consumption. It seems probable that when a phase of egg-development is being completed, the oxygen consumption will decline. Thus, as the female becomes sexually mature the oxygen consumption will fall, and the animal may reasonably be expected to be susceptible to slight changes in oxygen content in the medium. Thus, alternations of excess and deficiency in the oxygen content of the medium such as may be brought about by the conditions accompanying exposure and immersion of intertidal animals, may provide a spawning stimulus. In animals living below low water a threshold of excess oxygen may operate as such. If this is indeed the case, it should not be difficult to obtain experimental evidence with suitable animals.

The facts presented with regard to the percentage of the adult population which develops femaleness in one season yield the broad deduction that at least

50 % pass through the female stage. This is most clearly shown in the Fal records where the female stages were accumulated in the early part of the season owing to the arrest of spawning by the unusually slow rise in sea-temperature. On the Blackwater the proportion reaching the female stage is somewhat obscured by the removal of a moiety, i.e., the early spawners, while others are developing femaleness. It follows, therefore, that a sequence of analysis of samples may be necessary on certain types of beds to determine the proportion of the population which becomes female. On beds which have the hydrographic characteristics of the Fal Estuary, namely, a slow rise in sea-temperature and a relatively low minimum temperature extending over a considerable part of the spawning season, females will tend to accumulate during the early stages of the spawning season. The Danish, German and some of the Norwegian habitats probably approach this type. Möbius (1877) long ago inferred that 44 % of the population were females from the following records :

Date.	Oysters.	Whitesick.	Blacksick.	Total.	%
June 16, 1873 . .	112	5	4	9	8
July 6, 1873 . .	63	7	6	13	20.6
Aug. 12–17, 1869 .	480	—	—	72	15.8

Although Möbius' figures are erroneously derived from different seasons, they do nevertheless indicate that 50 % may probably be female, as his observations would not cover the whole of the spawning season in either year, and additional spawners may reasonably be inferred. Of the Danish oyster beds in the Limfjord Spärck (1924) records the examination of about 100 oysters in the pre-spawning period in 1920 and the recognition of nearly 50 % female stages. He also gives occasional records of examinations of samples for oysters with young and concludes that at the beginning of the breeding season, i.e. usually in June, 15 % of the stock will have young and that a decreasing percentage will be found with young until the middle of August. Thus there seems to be little doubt that 50 % of the oyster stock in the Limfjord will breed in some years as females. Hagmeier's observations (1916) on the weekly percentages of oysters with young in the tanks at Sert in Sylt in 1912 give indications of a relative small percentage of female development in that particular population, which occupied an abnormal habitat.

On the other beds which have similar hydrographic characters to those on the Blackwater, namely, rapid rise in temperature to a high maximum (See Fig. 1, and also Orton and Lewis, 1931) with a rapid fall in the autumn to a low winter minimum, some females begin to spawn early while others are maturing rapidly, and there will be a tendency for all individuals with the potentiality for femaleness to develop relatively early in the season. Thus the females overlap in spawning and development and become exhausted soon after midsummer. It is probable that the Dutch, some of the warmer Norwegian oyster-pols and many French beds are of this or a similar type. Hoek's records (1902) of the examination of samples, mainly out of the spawning season, show a high proportion of females, namely 46 %, and very high percentages among what he called the good and very good, or well-fished classes. In another sample of 190 oysters examined at the end of June, early in the spawning season, Hoek (1884) records 31 with young and 49 female, that is, 42 % total female in the sample *at that date*.

On the French beds Dantan (1913) from examination of isolated samples concluded that only 25 % of the population of three year old oysters attain the female stage at Arcachon, but it would appear that further seasonal observations are desirable. Gerbe's (1870) records for one year old oysters are interesting and can now probably be interpreted more accurately. He examined 435 brood in nine or ten lots of 40 to 50 over the period June 15 to July 31, 1870, at intervals of about five days, and gives only a summary of his findings as follows : 35 with young, 127 ripe females, 189 males, 6 hermaphrodite, and 78 doubtful, which may have emitted spawn. It would however be incorrect to assume that he found $\frac{35+127}{4.35}$ % females, since it will be seen from the investigation described herein that females mature during the early part of the season (and especially also during the season in the case of very young individuals (Dantan, 1913 and Orton, 1922) and spawn and become expended gradually. Thus the maturing females reappear in a succession of samples taken at short intervals of time as is shown in Tables 1 and 5. The actual percentage of females in Gerbe's samples is therefore probably small. In Table 11 is given an analysis of the spawning intensity among the population (column 5) and among the unspawned ripe and ripening females (column 10) on the Blackwater in 1927. On the assumption of the same kind of intensity amongst Gerbe's material, it is possible to estimate the percentage of females in the population he sampled at round about 15 to 20 %. It is important that Gerbe does not state that his material was taken at random. In 1922, the year following an unusually hot summer when a good spatfall occurred on the Blackwater, about 10 % of a single sample of *selected* large 1921 spat were found with young in August (Orton, 1922). Dantan also (1913) found 6.7 % female in a single sample of 133 brood examined in August. High percentages of females among older oysters may therefore be predicted to occur on the warmer French grounds, e.g. Arcachon and Morbihan, and a similar rapid exhaustion may occur to that recorded for the Blackwater beds. New observations are however desirable.

It would be highly interesting to know what occurs on the Italian beds at Taranto, where winter temperatures are relatively high (ca 13° C., as is shown by the charts of the London Meteorological Office 1912-1915) and spawning is recorded from April to October (Dean, 1891). It is possible that the population may be entirely different from those found in England. Helland-Hansen (1907) is of the opinion that the whole stock of *O. edulis* breed (as famales) in one season in the warmer oyster-pols of Norvay.

TABLE 11. — Spawning intensity among the population and among mature females on the Blackwater oyster beds in 1927.

Dates of moon's quarters 1	Population 2	Interval in days between sampling 3	N.W.+Gr. % spawned and removed 4	Accumulative % spawned as female and removed 5	% unspawned as female 6	Intensity of ♀ spawning in populat^{n} $\frac{4}{6}$ 7	Temperature °F. iu weekly means 8	% Potential available ♀s 9	Intensity of spawning among potential ♀ $\frac{4}{9}$ 10
May 24	100	3	0.0	0.0	100.0	.000	57.8	38.3	.000
30	100	6	2.5	2.5	97.5	.026	59.4	33.3	.075
June 7	100	5	8.7	11.2	88.8	.098	60.0	39.0	.223
15	100	5	6.2	17.4	82.6	.075	60.6	30.6	.203
22	100	7	6.9	24.3	75.7	.091	58.4	27.1	.255
29	100	5	6.5	30.8	69.2	.094	60.7	25.3	.257
July 7	100	6	0.6	31.4	68.6	.009	65.6	26.7	.022
14	100	5	3.2	34.6	65.4	.049	63.6	26.2	.122
21	100	6	2.1	36.7	63.3	.033	63.5	15.4	.136
28	100	6	7.7	44.4	55.6	.138	65.3	20.0	.385
Aug. 5	100	6	2.9	47.3	52.7	.055	67.4	15.3	.190
13	100	8	0.0	47.3	52.7	.000	64.8	9.7	.000
19	100	—	1.9	49.2	50.8	.037	63.3	4.8	.396
27	—	—	—	—	—	—	61.4	—	—
Sept. 4	—	—	—	—	—	—	63.3	—	—
11	—	—	—	—	—	—	59.7	—	—
18	100	—	0.0	49.2	50.8	.000	58.7	1.3	.000

The percentage of the population which develops femaleness will in all cases give some criterion of the rate of sex-change. Continuous yearly percentages above 50 clearly prove that a section of the population is reverting to the female phase in less than two years, while continuous percentages below 50 would show that reversion is taking longer than two years. As all workers find that the female phase is assumed in only small proportions by young oysters, it is probable that the more rapid reversions to the female phase occur among the older and better nourished individuals on beds of the same type as the Blackwater and Fal. Adequate nourishment is however probably more important than age, since even one year old young may under optimal conditions develop femaleness.

Thus in this review of the simultaneous study of one species in the same year in different environments the interdependent relation between the organism and its environment is clearly shown with respect to spawning behaviour. In a previous communication (Orton, 1928) an equally definite and different habit of shell growth has been shown to be dependent on the difference in the environment in the same two localities, and later (Orton, 1935) that Shell-shape also differs in these localities.

SUMMARY

In a comparison of the spawning of *O. edulis* on the Blackwater and Fal Estuaries in 1927 from the examination of large weekly samples throughout the spawning season, it was found that spawning began on the former beds coincidently with the attainment of a sea-temperature of 59-60° F. on May 31, and on parts of the latter beds on June 11 or 12 when temperatures ranged between at least 58° and 59° F. On other parts of the Fal beds where temperatures were lower at that time no spawning occurred.

Heavy spawning occurred on the Blackwater in the middle of June. Spawning was arrested on the Fal at the end of June correlated with a fall in mean temperature to about 57°, but general spawning began during the second week in July correlated with a general rise in temperature above 59° F. Spawning was continuous on the Blackwater from May 31 to the middle of August, and on the Fal from early July to the middle of August, when temperature was maintained above the level 59-60°7.

No other periodicity in spawning occurred than a tendency towards a greater amount of spawning on the neap tides of the moon's second quarter in both localities. On the Blackwater the females matured and spawned relatively rapidly and were expended except 3 % by the middle of August, one week after the attainment of the maximum temperature. On the Fal the females matured relatively slowly and accumulated in July to percentages higher than 50, and some 20 % remained unexpended in August when effective spawning ceased, correlated with a fall in temperature towards the spawning limit. Incomplete and abortive spawning occurred on the Fal in correlation with high and probably fluctuating salinities, but with temperatures above the spawning limit. 20 % of the population on the Fal existing as mature females in August failed to spawn effectively.

12.9 % of the Blackwater population were blacksick in mid-June giving prospects of a big spatfall at the end of June. Comparable percentages of

blacksick did not occur on the Fal until mid-July with prospects of a big spatfall at the end of July. Data were obtained indicating that eggs develop in the oyster in the sea to the coloured veliger stage in one week, at a temperature of about 60° F, and that the total period of incubation of the young is 7 to 10 days.

The time and temperature range for egg-development is discussed in relation to Helland-Hansen's theory of time-calorie period. It is pointed out that more information is required of the temperature level for the incidence or arrest of egg-development.

The spawning stimulus is discussed, and from the similarity in behaviour of the oyster to other rhythmically spawning marine animals the general suggestion is made that the reduced oxygen consumption, presumed to follow from completion of egg-development, may render the female especially sensitive to such fluctuations of oxygen deficit and excess as occur in tidal habitats, and that these supply a spawning stimulus; or that a threshold of oxygen excess might, in situations below low water, function as the spawning stimulus, or a contributary to the spawning stimulus.

Data are given showing that 50 % or more of the population mature as females in successive years on both the Blackwater and the Fal. Since all females change to males at once on the extrusion of eggs, these facts prove that reversion to the female phase must occur on the average every other year, and that a small proportion revert more quickly than every other year.

Comparable proportions of females are shown to have occurred at some time on Danish, German, Dutch and probably on French grounds. Reversion to the female stage every other year therefore appears to be normal in Northern Europe, but more information is required especially from the French and Italian beds and the warmer Norwegian oyster-pols.

This study demonstrates the dependence of the organism studied upon the environment, with respect to time and period of spawning, sex-proportion of the population, and to some extent the rate of change of sex.

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