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EXPERIMENTS AND OBSERVATIONS ON SWARMING, PELAGIC LIFE AND SETTING IN THE EUROPEAN FLAT OYSTER, OSTREA EDULIS L.

BY

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CONTENTS

III Hydrographical conditions

V Methods

VI Spawning

INTRODUCTION

Periodical decline in the productiveness of oyster beds is a world-wide feature. Practically every oyster producing region has not only seen prosperity but also adversity. Overfishing, enemies, unusual mortality and inadequate spatfalls, may rapidly decrease the population of the oyster beds almost to extinction point. At such times, when the future looks very gloomy and uncertain to the oyster farmers, the Government usually will lend a helping hand. Scientific investigations will be made to find the way to restore normal conditions.

A very important change was effected in the second half of the nineteenth century by stopping the free fishery on the natural oyster beds and by beginning a real oyster culture, of which the principal features are the regulation of the fishery and the provision of a suitable cultch for the young fry. The oyster grounds were withdrawn from the free fishery and a leasing system passed these grounds on to private use. The declining production rendered these measures necessary and scientists in many countries recommended the measures to be taken and urged to take prompt action. France was the first country to effect this change of paramount importance to a real oyster culture, which was to result in an unexpected revival of the oyster industry. About 1860 Professor COSTE (1861) was the champion in France, rediscovering the Italian methods of spat-collection and applying them on a large scale. The unexpected good results were the cause of a rapid change in oyster culture in many countries of Europe and in America, and there are but few places in civilised countries nowadays, where the oyster industry still clings to the old free fishery without some form of oyster culture.

This change did not solve all problems, for it soon appeared that a decline of oyster beds was still possible. Thus the oyster producing regions of France as well as those of England were up against a very high mortality, which led to an almost complete exhaustion of the oyster grounds in the year 1921.

Moreover the countries in the northern parts of the range of the oyster often suffered from inadequate spatfall. Continued failures of spatfall over several consecutive seasons is disastrous for oyster culture.

An increase in the production of spat is the best way to get out of these difficulties. Such an increase can be achieved by more intensive and more efficient methods of spat-collection, which require a thorough knowledge of the biology of the oyster and the oyster larvae and of the demands these organisms make upon environmental conditions.

In those countries where the natural conditions do not regularly ensure a sufficient spatfall attempts were made to effect an artificial production in enclosed sea-ponds or in specially prepared pits. It does not seem very probable that artificial oyster culture will soon be carried out on a large commercial scale, for though it proved to be possible, after a great many vain attempts, thus to produce a profuse settlement, this system requires so much labour and expense that competition with the natural system does not seem possible as yet. Importation of seed oysters from regions where the breeding grounds are more suitable is as yet more economical.

It has been shown that one of the most successful methods to intensify the natural spatcollection is the providing of the cultch at the right time. Clean cultch laid out just at the moment when the mature oyster larvae are on the point of setting ensures a spatfall many times larger than a cultch immersed many days previous to the setting. Already Winslow (1884) declared that "thousands of dollars would be saved annualy by the oystermen if they would determine with any approximate accuracy the date when attachment of the young oysters would occur".

Especially in France, since the crisis in 1921, and in America extensive inquiries have been made into the periodicity in the production of oyster larvae and into the way of forecasting with some accuracy the moment of spatfall, if possible on long term. Each season these investigations are carried out in many important seed producing centres and bulletins are issued to communicate the right time for the planting of cultch and collectors.

The years following 1930 were extremely hard for the oyster farmers in Holland. An extraordinarily rapid propagation of the slipper limpet, *Crepidula fornicata*, on the oyster grounds in Holland, where it has found a very congenial home, made it impossible to continue with the application of *Cardium*-shells as cultch-material. After the tremendous extension in the English oyster grounds, where *Crepidula* was imported with oysters from America in about 1880, the slipper-limpet came to the waters of Holland in about 1925, probably on the sea currents, attached to wreckage or sea-weed. I found a piece of wreckage covered with many large Actinia and about twenty living slippers on the beach near Zandvoort in 1926. The disastrous extension in Holland began about 1930. The slipper did not reach the French oyster regions, as the direction of the prevailing seacurrents safeguard the French shore against such an unwelcome invasion.

The *Cardium*-shells, which were yearly sown out in large quantities on the oyster grounds in Zeeland, proved to be an excellent cultch-material for the young slippers, too, which soon overcrowded the oyster spat. It was necessary to return to the old tile-collector system used in the years about 1885, which requires far more labour and expense than the sowing out of the *Cardium*-shells. These limed tiles are immersed in the water shortly before the spatfall can be expected and by regular cleaning and by detroquage in the next winter an efficient combatting of undesirable rivals and enemies is made possible. Moreover a disease caused an enormous mortality during these years (HAVINGA 1931, KORRINGA 1939) and most of the surviving oysters were in a bad condition.

The most important measures taken are repeated cleaning of the oyster grounds in order to keep the slippers within bounds, the importation of French seedoysters in order to obtain many oyster larvae as well as grown-up oysters of good quality in the following winter-season and the prediction of the spatfall by studying the quantity of oyster larvae in the plankton during the season of propagation.

In 1935 Dr. HAVINGA (1938, 1939) carried out these studies for the first time in Holland, using a more perfect and better quantitative method than the French investigators and moreover Dr. GRIJNS checked the spatfall, so that afterwards it could be seen if the forecasts were right.

As the study of the oyster larvae in daily plankton samples requires a good deal of time and effort, Dr. HAVINGA soon found it impossible to carry out these investigations in addition to his other work in the Governmental Institution for Biological Fishery Research. Since 1936 I have pursued these investigations every summer, the first year at the laboratory of Dr. HAVINGA in Amsterdam and after that in the laboratory of the Fishery Board of the Zeeland Streams at Bergen op Zoom. Moreover I carried out many investigations on the biology of the oyster larvae and on the setting behaviour of our native oyster, *Ostrea edulis*. The results of these studies as well as those of the daily plankton samples will be discussed in the following sections. As far as possible the results obtained by other investigators, often with other kinds of oysters, will be compared with those of *Ostrea edulis*.

ACKNOWLEDGEMENTS

The data obtained by Dr. HAVINGA in 1935 as well as those on the spatfall by Dr. GRIJNS in 1935 and 1936 have been placed at my disposal in behalf of these studies, for which I am very grateful. I am particularly indebted to Dr. GRIJNS for having had an important part in the spatcollecting operations. Without his collaboration it would have been impossible to

me to study the larval stages and the setting process simultaneously.

I wish to express my thanks to Dr. HAVINGA, director of the section Inshore Fishery of the Governmental Institution for Biological Fishery Research for introducing me to the problems of oysterbiology and for his constructive criticism. It is a pleasure to express my thanks to the Fishery Board of the Zeeland Streams for giving me hospitality in their laboratory at Bergen op Zoom and for placing at my disposal their police-vessels in behalf of these investigations. The punctual assistance of commanding officers and crew of these vessels, who often worked at night and in bad weather, has contributed much to the success of these experiments. Finally I wish to thank Mr. BAKKER, the skilful laboratory attendant, for the construction of much apparatus and for the preparation of the material used.

I. THE PROPAGATION OF THE OYSTER

General information concerning the propagation of the different species of the genus *Ostrea* and the state of our knowledge of the morphology and anatomy of the oyster larvae.

It is outside my scope to give here a detailed description of the morphology and anatomy of the reproductive organs of *Ostrea edulis*. HOEK (1884) made an elaborate study of this subject and elucidated his paper with clear figures. So far no better study of the reproductive organs of the native oyster has come to my knowledge.

I consider the protandric alternating hermaphroditism of this kind of oyster to be generally known.

In the reproductive season the sperm and ripe eggs are liberated from the gonaducts of the spawning oyster and are extruded into the suprabranchial chamber (syn. cloacal chamber, syn. exhalent chamber). The sperm leaves this suprabranchial chamber, which is situated right under the adductor muscle and above the junction of the gill-bases, in the same way as the outgoing stream of the water current; so it follows the same course as the waste products from rectum and kidneys.

The ripe eggs do not leave the mother oyster in this simple and easy way. They first pass into the branchial chamber (syn. mantle chamber, syn. inhalent chamber.) Further particulars about the spawning act will be discussed in the section on spawning. After this passage to the branchial chamber the eggs are either extruded at once into the surrounding sea water or (in other species of oysters) the eggs are held for some time in the branchial chamber adjacent to the gills and labial palps. Here they then develop for a considerable period before they leave the mother oyster. Such species of oysters are often called viviparous, though it would be better to call them incubatory or embryophorous. This difference in the time during which the larvae are held in the branchial chamber is very important. Though this study deals with *Ostrea edulis*, an embryophorous species, many remarks will be made about other kinds of oysters, for many papers on those oysters will be discussed. Therefore I herewith give a table of the two reproductive types of oysters, only including species of economic value.

Incubatory and larviparous	*Non-incubatory, spawning directly into the seawater*
Ostrea edulis L, Huître plate European flat oyster.	*Ostrea virginica* Gmelin American oyster, Bleu-Point.
Ostrea lurida Carpentier Olympia Oyster, British Columbian Oyster.	*Gryphaea angulata* Lamarck Portuguese Oyster.
Ostrea denselamellosa Lischke Japanese Oyster.	*Ostrea gigas* Thunberg Common Japanese Oyster.
Ostrea Angasi Sowerby Australian mud-oyster.	*Ostrea commercialis* Iredale and Roughley Australian rock-oyster.
Ostrea lutaria Hutton New Zealand Oyster.	*Ostrea cuculata* Born Indian Oyster.

It is not very certain that all these forms are really different species. Oysters are difficult subjects to the systematist, as the shell-shapes are to a high degree dependent on environmental conditions.

For the greater part the studies discussed deal with two incubatory species of oysters, viz. *Ostrea edulis* and *Ostrea lurida*, and moreover with *Ostrea virginica* and *Ostrea gigas* as non-incubatory species.

The process by which the reproductive elements leave the gonaducts is always called the spawning act. The final release of the larvae from the material brood-chamber in larviparous

Ostrea virginica.

Our knowledge about the morphological and anatomical features of the larvae of *Ostrea virginica* at various stages of development was completed till it formed a whole by itself at an earlier date than the knowledge of these features in the larvae of *Ostrea edulis.* After the descriptions of the main points of larval development by BROOKS (1880), RYDER (1882, 1884) and JACKSON (1888) other scientists elaborated these studies. A fine paper on the development of *Ostrea virginica* is that by STAFFORD (1913) in which he gives a full account of the anatomy of both fully developed larvae and recently settled spat. In an earlier paper (1910) STAFFORD described for the first time the appearance of the foot in mature oyster larvae. WELLS (1927) gives a description of the alimentary organs of the larva of *Ostrea virginica.* NELSON (1924 c, 1926) reports the possibility of metamorphosis without attachment in the larvae of the oyster and of the mussel. He suspended mature oyster larvae in a cradle of bolting cloth close to the surface of an aquarium. A few of these larvae showed metamorphosis, with loss of velum and foot and development of the dissoconch shell, though into an unusual shape. Such a metamorphosis without attachment may occur, though rarely, even in nature, as has been shown in two instances in which such unique pelagic dissoconchs, "floating spat", have been found in plankton catches in Barnegat Bay. (New Jersey, U.S.A.)

I have never met with such floating spat in plankton samples containing larvae of *Ostrea edulis.*

There is still difference of opinion concerning the nature and function of the so-called pigment spot occurring in mature larvae. NELSON (1926) advocates the photosensitive character of these pigment spots which corresponds with the opinion of ERDMANN (1934) about the nature of the pigment spot ("eye") in the mature larvae of *Ostrea edulis.* PRYTHERCH (1934 a), on the contrary, denies those pigmentspots a light-sensitive character, but conceives them as leucocyte-generating tissues, from which cells migrate to the blood-stream during metamorphosis. The discussion of this controversy will be continued in the section on the influence of light on the setting process.

Less extensive is the literature on the morphology and anatomy of the larvae of other species of oysters.

Ostrea lurida.

The general embryology of *Ostrea lurida* (Carpenter) and incubatory species has been described well by STAFFORD (1914) and is essentially the same as in *Ostrea virginica.*

HORI (1933) was able to rear the larvae of *Ostrea lurida* to maturity in vitro and setting under artificial conditions was successfully accomplished. Some figures of the larvae of *Ostrea lurida* can be found in this paper of HORI.

Ostrea gigas.

FUJITA (1929, 1934) provides us with a description of the larval development of the Japanese oyster, *Ostrea gigas* Thunberg. YOKOTA (1936) mentions his studies on the development of the various organs of this oyster, but these studies have not been published so far.

A description of the larval development of *Ostrea denselamellosa,* an incubatory species, closely related to *Ostrea edulis,* is to be found in the paper of SENÔ (1929), while some remarks about another incubatory species, *Ostrea angasi,* the larvae of which likewise show a close resemblance to those of *Ostrea edulis,* are made by ROUGHLEY (1925). In another paper ROUGHLEY (1933) gives a description of the larvae of the non-incubatory species *Ostrea commercialis* and deals briefly with the anatomical reorganisation at fixation in this species.

As far as the latter species is concerned, these processes are in general so similiar to those described by STAFFORD for *Ostrea virginica* that ROUGHLEY did not consider it necessary to give a detailed description.

II. THE RANGE OF OSTREA EDULIS

General demands on environmental conditions to obtain an adequate propagation.

Though *Ostrea edulis* can be found from 66° N. lat. in Norway as far as in the Mediterranean, the regions where nature makes a really intensive and extensive oyster culture possible are limited.

The occurrence of oysters in a certain place does not imply the possibility of oyster farming in that locality. It only indicates that the oysters can keep up the struggle for life in that place, but the equilibrium with the natural conditions may be so unstable that a very slight interference on the part of man may be the cause of a rapid decline of such natural oyster banks. "It is the last straw that breaks the camel's back" is a very appropriate remark in this connection. An intensive oyster culture is only possible in those regions where a sufficient yearly seed-production is assured. The demands on the natural conditions for a successful propagation are fairly high and cannot be satisfied in the northern parts of Europe. Practice and science (ORTON, 1929 a, 1929 b, 1937 a) prescribe the following properties as essential to producing grounds:

A. Waters sufficiently enclosed, if possible with a relatively narrow communication with the open waters. Only such bodies of water ensure an adequate retention of the larvae within the environs of the locality during the free-swimming stage and counteract the dispersing action of the sea-currents on the larval herds.

B. A local seasonal temperature-range giving frequent probabilities of a maximum temperature in the bulk of the sea-water above 18° C, preferably above 20° C for some time. There is a fair margin for variations in salinity.

C. A suitable subsoil, not too soft mud, no moving sands, but preferably a hard sandy mud or muddy shell-gravel. Peat-soil is considered to be very suitable, too.

D. Some other factors, which are to a certain degree under human control, such as a sufficient stock of larvae-producing oysters, the provision of a suitable cultch-material and the combating of enemies.

The most important producing centres of the European oyster are: in France the region called le Morbihan, especially the rivers Auray-le-Bono, Saint-Philibert and Crach and in Holland the Oosterschelde. A good description of the nature, character and history of the French oyster beds can be found in papers by HINARD and LAMBERT (1928) and by LAMBERT (1935, 1936, 1938). A very suitable locality in France is moreover the Basin of Arcachon, where formerly oyster farming used to take place on a large scale. Another kind of oyster, however, *Gryphaea angulata* Lam., has ousted *Ostrea edulis* here to a considerable extent. In recent years *Ostrea edulis* has been gaining ground in the basin of Arcachon (BORDE and BORDE 1938). Of less importance are the breeding-grounds in the Mediterranean, in Norway and in England.

That temperature is a very important factor was already known to HERDMAN (1893), who stated that the watertemperature in the oyster producing regions in France exceeded 21° C in the open waters (Arcachon). He added: "However, it may be hoped that, although temperatures like this may be favourable, they are not necessary for successful oyster breeding".

Later it appeared that water-temperatures above 20° are very favourable indeed for a profuse settlement of the spat, though some propagation is possible at any temperature above 16° C. A comparison of the results obtained under different temperature conditions is very instructive.

The duration of the season of propagation is dependent on the space of time that the water-temperature is above a certain level. Though production of larvae can take place at temperatures of 15 to 16° C, a successful spatfall can only be expected when the water-temperature rises above 18° C, the more the better. So it goes without saying that the propagation is distributed over a much longer period in the Mediterranean than in the Northern parts of the oyster range.

BRACH (1689) told us that the oysters in the Adriatic Sea near Venice carried larvae in the mantle cavity all summer and in the beginning of autumn. In the Mare Piccole in the Gulf of Tarent in Italy the oysters breed from April to October (ORTON, 1920). MAZZARELLI (1924) informs us that reproduction takes place from March till in August in the lake of Fusaro near Naples. It is remarkable that, though the water-temperatures are very favourable in the Mediterranean, oyster culture never assumed such proportions here as on the West-coast of France. Various other conditions are less favourable in the Mediterranean, such as the nature of the sub-soil, which is often steep and rocky, consequently unsuitable for dredging operations, while perhaps quality and quantity of food-organisms differ to a high degree from those in the Atlantic coastal waters. The higher salinity is not responsible for checking the oyster industry in the Mediterranean.

The farther we go northward the shorter the season of propagation will be, till we reach at last the northern boundary of the range of *Ostrea edulis,* where propagation is exceptional, alternating with many years without spatfall. It will be understood that no effective oyster culture based on the natural spatfall will ever be possible in these regions. Only localities with very favourable hydrographic conditions ensure an adequate spatfall in the countries around the North Sea. There is but one place that has appeared to be an excellent seed-producing locality on the North-Sea coast and that is the Oosterschelde in Holland.

The estuaries of the English coast are not sufficiently enclosed to ensure a statisfactory retention of the free-swimming larvae and the water-temperature usually does not rise enough to counteract this disadvantage to a sufficient extent. In my opinion only a very abundant production of larvae can bring about a satisfactory spatfall in the English waters. A considerable decreasein the number of adult oysters, especially owing to the mortality of 1921, has since then made such an abundant production of larvae impossible there. Though it is not impossible that the Fall Estuary can produce a moderate amount of seed-oysters when science and practice join in a great effort (ORTON, 1927 a), it is not likely that the English coast will ever be reckoned among the very important seed-producing grounds.

The reason why the Oosterschelde may be considered as the most important centre farthest North for the production of seed-oysters will be discussed in a separate section on the hydrographical conditions of this water.

The German oyster grounds in the Wattenmeer, the "fiskalische Austernbänke", cannot ensure a profuse yearly spatfall, because the water-temperature does not rise enough and the sea-currents disperse the planktonic larvae to a high degree.

Though the Limfjord in Denmark may be considered as a nicely enclosed water, the average summer temperature is too low here. The temperature does not only affect the spatfall in a direct way, but also in a more indirect way, as oysters become mature at a far older age in colder waters than in warmer, which accounts for a far smaller percentage of larvae-producing oysters in such colder waters (SPÄRCK 1925). Although some spatfall can be found even in the colder years, the oyster culture in these regions is dependent on the importation of seed-oysters from other localities. Adult oysters are found to thrive in those colder waters. Though the rate of feeding is correlative to temperature, the checking of growth and fattening in the summer-months in behalf of the production of the elements of propagation, is much shorter or may even be absent in colder waters. To a certain extent one may say that the colder the water in the summer months, the better the quality of the oysters and the worse the spatfall.

It will be understood that especially countries which are not so fortunate as to possess rich seed-producing grounds, but which do possess excellent localities for growth and fattening, have a great interest in the possibilities of obtaining a sufficient spatfall in enclosed sea-ponds or oyster pits. Many attempts have been made in England (DODGSON, COLE 1936, 1938 b, 1939, BRUCE and PARKE, 1935–1938), Germany, especially at Heligoland (HAGMEIER, 1916, ERDMANN, 1933) and Denmark (SPÄRCK, 1927). Recently it proved to be possible to ensure an adequate spatfall in limited quantities of water, even in glass-houses (Heligoland), but it requires far too much labour and expense to make large scale operations on a commercial basis practical as yet. Though I shall not describe all these attempts, I shall frequently discuss data on the biology of the oyster larvae obtained in the course of these investigations. From a scientific point of view I consider these attempts highly interesting, for they offer the possibility of entering the new and vast, hitherto unexplored field of the heredity of the oyster, which may yield important practical results in the future.

In Norway a self-reliant oyster culture exists, though on a relatively small scale, which produces its seed-oysters in the so-called pollen. These pollen are pools, a few hundred meters wide and 4 to 8 meters deep, which can be completely closed off by a temporary damming-up of the communication with the fjord-water. Their suitability for oyster culture was discovered about 1880 (RASCH, 1880 a, b), but the reason why a profuse spatfall is possible so far north was discovered much later. There are but a few suitable pollen in Norway, so that seed-production cannot be extended very much. The first investigators ascribed the high water-temperatures in these pollen to the nature of the bottom, which consists of a soft black mud. Later it appeared that the pollen present very peculiar hydrographical conditions. There are no important currents, so stratification is possible. The upper layers of the water are fresh or brackish, flowing in from little brooks, while the lower layers consist of the salt fjord water. These fresh upper layers make the vertical exchange of warmth impossible, as the warm salt water remains heavier than the colder fresh upper layers. The fresh water acts like the glass of a glass-house by transmitting a good deal of the sunrays and by functioning as a cover during the night (HELLAND-HANSEN, 1908, GAARDER and SPÄRCK, 1932, GAARDER, 1933, GAARDER and BJERKAN, 1934, GAARDER, 1938). The temperature in the salt lower layer may rise to 25 to 30° C during a short period in summer. GAARDER (1933) is right when he states: "Das Pollwasser bildet somit eine kleine südlandische Welt für sich".

In respect to the duration of the season of propagation in the different localities of the range, the oyster species in America (*Ostrea virginica* and *Ostrea lurida*) do not differ essentially from *Ostrea edulis*.

Ostrea virginica can be found on the East-coast of North America from Cape Cod to the Rio Grande. *Ostrea virginica* makes high demands on the breeding-temperature (20° C at least) and the spawning season here too is correlative with the duration of such higher temperatures.

While in the South (Texas) spawning oysters occur from April till in October and the greater part of the setting-season extends between May 1 and October 1 (MOORE, 1907, 1915; HOPKINS, 1931), the propagation in the extreme north of the range takes place during a few weeks in July or August or fails to take place at all (NELSON, 1928 c). In the North one generally finds a slow growth, an unreliable reproduction, but a better quality of the oyster meat. The inner parts of the bays contain the warmest water here, and are therefore the most suitable for reproduction, though the subsoil is relatively soft. The mouths of the bays are generally colder, so less suitable for seed production, but have a better subsoil and are more suitable for the cultivation of a superior quality. The farther north, the more a well conducted culture is indispensable.

All this holds good for *Ostrea lurida*, too, which is cultivated on the West-coast of North America. The breeding temperature of this species lies above the 16° level. In the North, in British Columbia, propagation takes place in July and August (STAFFORD 1913). In the main culture centre, the Puget Sound, setting occurs during the months of June, July and August (HOPKINS, 1937), while in South-California (La Jolla) this species propagates during seven months, as long as the water-temperature remains above 16° C (COE 1932 b).

That the young oyster reaches maturity at an earlier age as it lives farther southwards is a rule that applies to *Ostrea lurida* as well (COE, 1931).

NELSON (1928 c) very rightly states, considering the dependence of the duration of the spawning-season on temperature, that these rules hold good for all parts of the range, with no adjustment to the extremes of its distribution. No such adjustment to the environmental conditions has taken place, which a priori was not to be expected either.

III. HYDROGRAPHICAL CONDITIONS

General Description of the Region

The production of oyster brood in the Dutch waters takes place practically exclusively in the Oosterschelde. The Oosterschelde, a bay which penetrates far into the land, is situated in the South-West of Holland, in the province of Zeeland. Centuries ago it was the estuary of the river the Schelde. Gradually a new breaking, the Westerschelde, gained in importance. Finally, in 1869, the communication with the river the Schelde was entirely cut off by the construction of a railway-dam between the mainland and the island of Zuid-Beveland. Since then an other railway-dam, between the islands of Walcheren and Zuid-Beveland, has moreover completely closed off the fresher water of the Westerschelde from the Oosterschelde.

As far as oyster culture is concerned, the eastern part of the Oosterschelde, the bag-shaped widening, bounded on the West by the narrowing between Gorishoek and Kijk-uit (fig. 1) is the most important region. By far the greater part of the oyster beds are situated in this basin, which will be called henceforth in this paper the basin of the Oosterschelde.

An important part of the basin of the Oosterschelde belonged to the inhabited land of Zeeland but some hundreds of years ago. In the beginning of the 16th, partly only in the 17th century, this land was flooded in consequence of dike-bursts. Many local denominations still remind us of those times.

The amount of fresh water that flows into the Oosterschelde is insignificant. Since the communication with the Westerschelde was cut off in 1869, no stream of any importance discharges into the Oosterschelde. Only a few little brooklets from the mainland and some drained-off water from some polders in the Zeeland islands enter the Oosterschelde. Westward the Oosterschelde is continuous with the Northsea. Between the islands of Tholen and Duiveland we find the Keeten, a communication with the Grevelingen. In the Grevelingen fairly important variations in salinity occur, caused by the admixture of fresh water coming from the great rivers (the Rhine and the Meuse). Especially when these rivers discharge extraordinarily big volumes of water low salinities will be stated in the Grevelingen. As may be seen on the chart (fig. 1), a greater volume of water moves north-eastwards than south-westwards through the Keeten during a tidal cycle, or in other words: the Keeten shows a surplus of flow. Consequently variations in salinity in the Grevelingen will have little influence upon the salinity in the Oosterschelde.

These properties of the Oosterschelde offer many advantages: no variations in salinity dangerous to oyster culture will ever occur here.

Much of the water that flows away westwards from the basin of the Oosterschelde during ebb, will return to the basin during the next flow. This oscillating movement is possible since no inflowing fresh water regularly forces a considerable portion of this body of water westwards to the Northsea. This oscillating movement prevents a rapid dispersion of the planktonic oyster larvae by the tidal movements and makes possible a rapid warming-up of the water in the basin during fine weather. Especially warmth-absorption by the vast tidal lands is helpful in effecting a rapid rise of the water-temperature. The reverse, a rapid falling of the water-temperature during cold weather, obviously also occurs here.

In cold winters drift-ice will be formed fairly soon in the basin of the Oosterschelde. In consequence of the force of the tidal movements the water will never be frozen over completely here, however.

The bottom configuration of the basin of the Oosterschelde is rather irregular and complicated, as may be seen from the chart (fig. 1). There are deep creeks and channels, often deeper than eight metres (e.g. the Channel of the Oosterschelde and the Lodijksche Gat), and vast tidal lands. These tidal lands consist partly of sand-banks, not suitable for oyster culture, partly of a rather hard sandy mud. The oyster beds are situated mainly on a subsoil of such sandy mud and of peat-soil, which

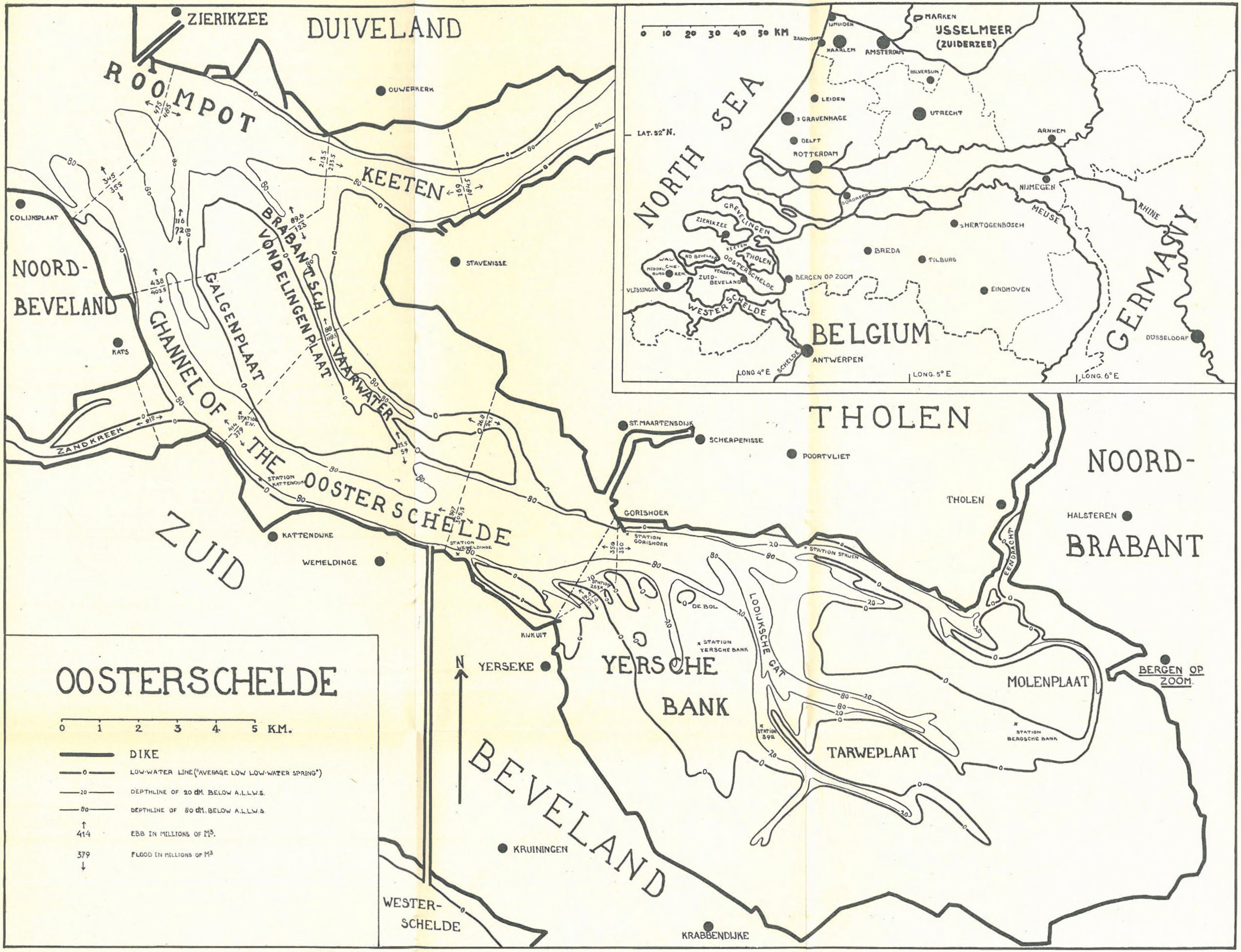

Fig. 1. Hydrographical Chart.

is found in many places, reminding us of the times that there was land or in any case fresh-water marshland in those places.

Tidal movements

By courtesy of Rijkswaterstaat[1]) I am able to give some information about the tidal movements and the water-currents in the Oosterschelde. Ir. P. Ph. Jansen was so kind as to submit to me for inspection the data of his hydrographic measurements and to compute for me the degree of water-renewal during a tidal cycle. For this I wish to express my thanks.

Moreover data on observations with the aid of floats for the sake of investigating the direction and the speed of the local current swill be found in the reports by Fokker (1905) and by Hubrecht (1884).

The range of the tide in the basin of the Oosterschelde varies between 3 and 4 metres. During an average low water the basin of the Oosterschelde (western boundary Gorishoek-Kijk-uit) contains 275 000 000 cubic metres of water. During an average high water the basin contains 675 000 000 cubic metres of water. Consequently an amount of 400 000 000 cubic metres of water regularly passes the narrowing Gorishoek-Kijk-uit during each ebb, to return for the greater part during the next flow.

These enormous tidal movements, attended with strong tidal currents, cause such an intensive mixing of the water that any permanent stratification in this body of water is thereby prevented. In fact we shall never find a difference in salinity or water-temperature of any importance at different depths in the Oosterschelde that is maintained there till the next tide. The tidal mixing of the water in the basin is thorough, which makes it easier to study the biology of the oyster larvae here than in places where marked stratifications complicate hydrographical conditions. The Eendracht, a shallow water between the mainland and the island of Tholen may be left out of account, as the relatively small volume of water it contains may be considered to perform a continuously oscillating movement. Consequently all the tidal water passes the narrowing Gorishoek-Kijk-uit.

It is very important to know how much of the water passing this narrowing during each ebb, re-enters at the next flow. West

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[1]) Ministry of Public Works, Department for the maintenance of dikes, roads, bridges and the navigability of canals and rivers.

of the basin (fig. 1) we can distinguish the "area of the conducting channels", of the Brabantsch Vaarwater and the Channel of the Oosterschelde, both of which conduct the waterstreams to and from the basin. The Zandkreek, a water situated between the islands of Noord-Beveland and Zuid-Beveland, may be left out of account as its content performs an oscillating movement during a tidal cycle.

The area situated northwest of these conducting channels may be distinguished as the "outlying district". The volumes of water which pass through the Brabantsch Vaarwater and the Channel of the Oosterschelde are not the same during ebb and flow. In the Brabantsch Vaarwater we shall find more water passing during flow than during ebb. In several places on the hydrographical chart (fig. 1) we shall see two numbers, accompanied by little arrows, all of them situated in the centres of the channels. These numbers indicate the volume of water which passes these channels during one average flow and during one average ebb, expressed in millions of cubic metres, the left indicating the flow. A difference between two such numbers indicates a surplus of flow or a surplus of ebb in that channel.

On the chart it may be seen that the Brabantsch Vaarwater shows a surplus of flow of 30 000 000 cubic metres. The Channel of the Oosterschelde consequently shows a surplus of ebb of 30 000 000 cubic metres.

So 30 000 000 m^3 of "new" water are conducted via the Brabantsch Vaarwater in south-eastern direction during each tide (12h 25m). According to Rijkswaterstaat we may speak in this case of really "new" water, because it is derived from the area called "outlying district" (the Roompot). Of course the water in this "outlying district" contains a fraction of the water originally derived from the basin, brought there in consequence of the surplus of ebb in the Channel of the Oosterschelde. This basin-water, however, is considered to be diluted with "new" water to such a high degree on reaching the Roompot, that its relative value may be regarded as negligible. Consequently oyster larvae originating from the basin will be practically absent in the "new" water of which the surplus of flow of the Brabantsch Vaarwater consists. We are not justified, however, in deducing from the foregoing that the tidal renewal of the water in the basin of the Oosterschelde amounts to 30 000 000 m^3.

The Brabantsch Vaarwater discharges itself into the eastern part of the Channel of the Oosterschelde and not directly into the basin. Consequently a certain part of the 30 000 000 m^3 surplus of flow does not reach the basin at all, but streams back westwards via the Channel of the Oosterschelde. According to Rijkswaterstaat the average tidal renewal of the water of the basin may be estimated at about 25 000 000 cubic metres. The different phases of this tidal renewal are sketchily represented in the series of small charts. (fig. 2).

Unlike the watermovements in the "conducting channels", where the greater part of the waterparticles follow the direction of these channels, the streams in the basin itself are not surveyable in consequence of the irregularity and complexity of its bottom-configuration.

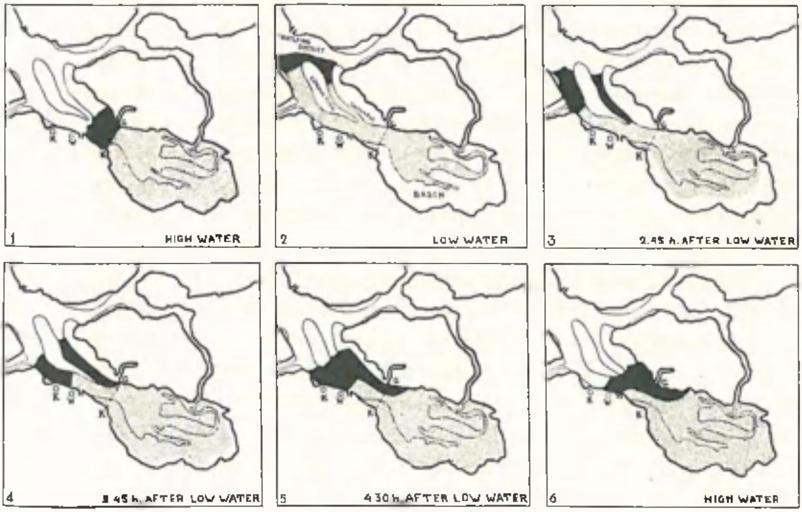

Fig. 2. Tidal movements.

It is certainly possible that a waterparticle, which reaches the basin only near the end of the flow, does not leave the basin during the beginning of the ebb. According to Rijkswaterstaat it is even admissible to assume that all the waterparticles which reach the basin during flow have an equal chance of reaching a certain point after a couple of days. In other words, each waterparticle in the basin, irrespective of its whereabouts in this basin, has an equal chance of remaining in the basin during a couple of days, at least if we do not consider too short a period.

Consequently the 25 000 000 cubic metres of "new" water, which reach the basin at each tide may be considered to drive out from the basin an equal volume of 25 000 000 m^3 of the water present in the basin during the preceding tide.

This water streams away in north-western direction, via the Channel of the Oosterschelde (which shows a surplus of ebb) and after this, much diluted, in north-eastern direction via the Keeten, which shows a surplus of flow.

So the water originating from the basin never reaches the western part of the Oosterschelde (West of Zierikzee), but takes its way via the Keeten to the Grevelingen. Strong winds from any direction are considered to have no appreciable influence on the degree to which the water is renewed in the basin. If the period under consideration is not too short, and it is not too short considering the duration of the pelagic stage of the oyster larvae, the influence of the differences between springtides and neap tides on the degree to which the water of the basin is renewed may be considered to be of no importance either.

The foregoing is of paramount importance with regard to the oyster larvae in the Oosterschelde. In the first place it will be clear, assuming that by far the greater part of the oyster larvae are set free on the oyster beds in the basin of the Oosterschelde, that it is of little importance to know during which part of the tidal cycle the larvae are liberated. Even in case of a swarming during ebb, practically all these larvae will return to the basin again during the next flow. Then they start their game of chance: each tide a greater part of these larvae leave the basin and a certain percentage of them never return.

At an average high water the basin contains 675 000 000 m^3 of water. Each tide approximately 25 000 000 m^3 of this water are renewed, so about 96,3% of the original water returns.

After two tides $(0{,}963)^2 \times 675\,000\,000$ m^3 return and after n tides $(0{,}963)^n \times 675\,000\,000$ m^3.

It may be computed that for instance after 14 days less than about 35% of the original water at high tide is still present in the basin. The rest has streamed away via the Channel of the Oosterschelde and the Keeten, carrying many oyster larvae along with it. The longer the pelagic period, the smaller will be the percentage of the number of larvae originally present in the basin that will remain there till the mature stage. In the section on the horizontal movements of the larvae we shall see to what degree the movements of the larval herds correspond with the tidal movements.

As will be discussed later on, there are several factors which cause a loss of larvae during their pelagic life. It is often difficult or even impossible to estimate the relative importance of these factors. So it is an advantage that we do know the degree to which the water in the basin is renewed, this being one of these factors.

Apart from the degree of water renewal in the basin during a tidal cycle, there is another factor brought about by the tidal movements, which is often considered of importance with regard to the biology of the oyster larvae. This factor is the velocity of the currents.

In the section on the vertical movements of the larvae and in that on the influence of the currents on the setting process further particulars concerning this factor will be discussed. Rijkswaterstaat investigated the direction and the velocity of the tidal currents during entire tidal cycles at several stations in this region. These stations are situated in the channels as well as in shallower places. Observations were carried out in the surface layers as well as 10 cm and 60 cm above the bottom. It is outside my scope to reproduce here all these data by Rijkswaterstaat.

An important station in connection with my experiments on the setting process is a fairly shallow place on the Yersche Bank called "the Bol" (fig. 1). During low water the depth is 1,50 to 2 metres here, during high water some 3 to 4 metres more. The velocity of the tidal currents at the surface exceeds a maximum of 30cm/sec. here, but does not reach 50 cm/sec. Near the bottom the velocities are about one third less. Another important station is, for instance, the eastern part of the deep Channel of the Oosterschelde near Gorishoek (fig. 1). The velocity of the current in the surface layers in the middle of this Channel reaches a maximum of about 150 cm/sec., the maximum velocity near the bottom exceeding 100 cm/sec.

The station situated in the middle of the Channel of the Oosterschelde near Wemeldinge yields the same figures. In general we may state that in the surface layers of the deep channels maximum current velocities of 100 to 150 cm/sec. may be expected, while the maximum velocities near the bottom are about one third less. The channels within the basin show maximum current-velocities of 50 to 100 cm/sec., while maximum current-velocities below 50 cm/sec. will only be found in shallow places.

In correlation with the irregular bottom-configuration in the basin, directions and velocities of the tidal currents often show a less regular course in shallow places than in the deep channels.

Salinity and water-temperature

Since March 1921 the commanding officer aboard one of the policeboats of the Fishery Board of the Zeeland Streams has

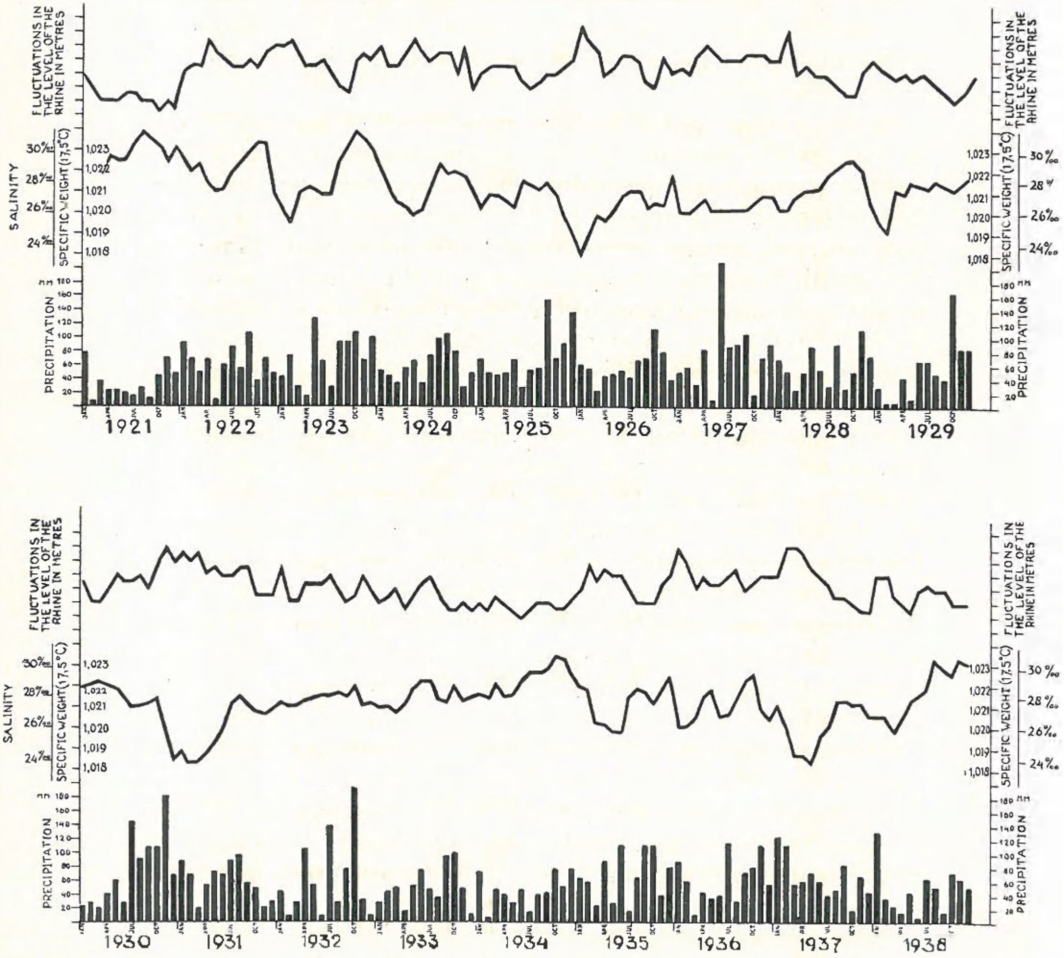

Fig. 3. The salinity in the Oosterschelde.

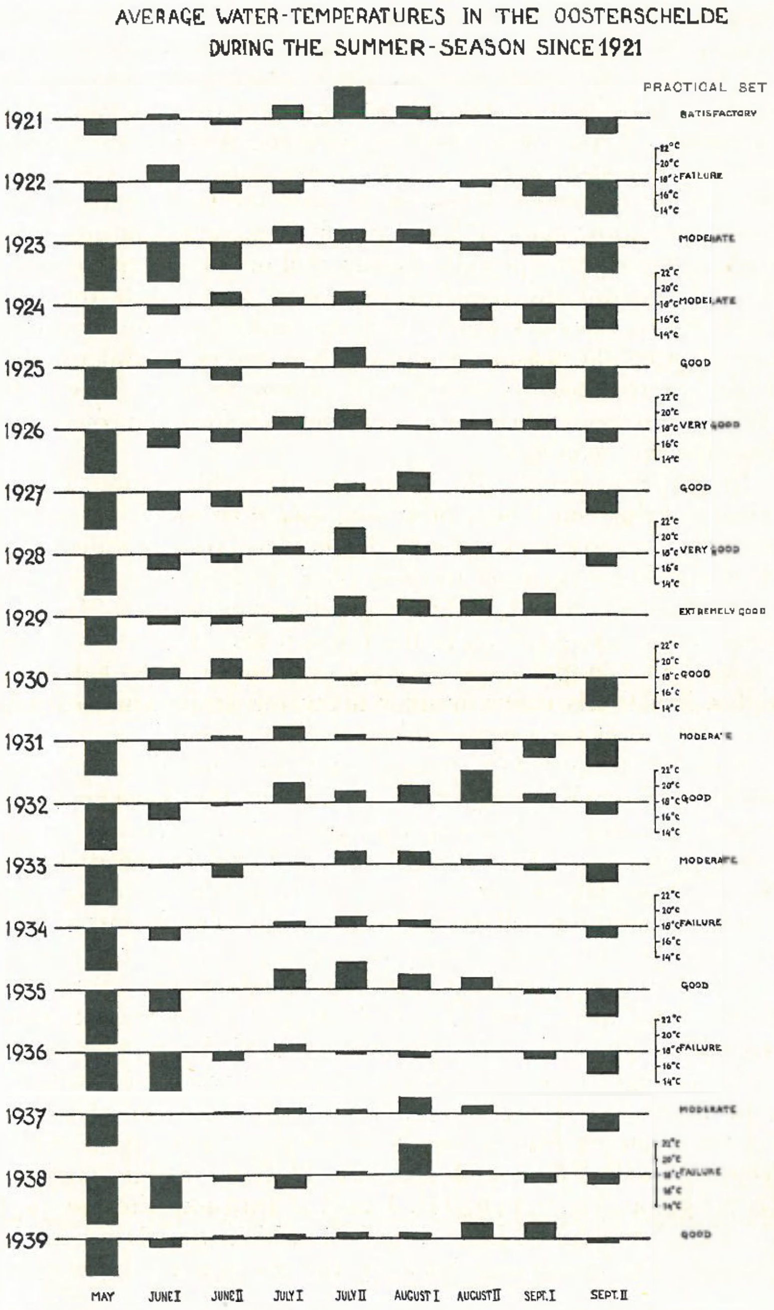

Fig. 4. Watertemperatures during the summermonths.

ascertained twice a day, at high slack water and at low slack water, the temperature and the salinity of the surface-water in the basin of the Oosterschelde. These temperature-readings are exact to half degrees. The salinity is measured by means of an areometer. I used these data to compute monthly averages of temperature and salinity for the years since 1921 (table 1, 2 fig. 3). Temperatures taken on or near the tidal lands at low water may show marked deviations from the temperature in the bulk of the water, especially during cold or very warm weather. So I sorted out the temperatures taken on or near the tidal lands during low slack water. As in this study the water-temperatures during the season of reproduction are of special interest, I have represented the data for the summermonths (June, July, August and September) in a more detailed form by computing half-monthly averages.

In a special graph (fig. 4) to elucidate this matter I have tried to bring out when, how long and how much the water-temperatures rose above the 18 degrees level during these years. As I shall have occasion to show later on there exists a certain connection between temperature conditions and the rate of development of the oyster larvae. In the tables it may be seen that these halfmonthly temperature averages have never exceeded 22° C so far. Practically every summer the water temperature exceeds the 18° C level for a reasonable period of time.

Apart from these data, temperatures were taken daily at low slack water at Kattendijke as well as on the Yersche Bank, (exact to tenths of degrees) during the summer seasons of the years 1936-1939. These data are to be found in the diagrams on the oyster larvae (fig. 7, 8, 9, 10). Generally speaking, low-water temperatures will show extreme values. The deviation from the average daily water temperature is not very great, however, especially not at Kattendijke. On the Yersche Bank, rather close to the tidal lands, the water-temperature at low slack water may show values of 1° or sometimes even 2° above or below the daily average, especially during extremely warm or extremely cold weather. During the summerseasons of 1938 and 1939 thermograph records of the bottom-water were taken on the Yersche Bank. These data may also be found in the diagrams on the larvae (fig. 7, 8, 9, 10). I used a timepiece thermograph (by Fuess), contained in an iron box, which was placed at the bottom. The differences between the low-water temperatures (also taken near the bottom) and the daily averages may be studied from the diagrams on the larvae. Generally speaking, we may state that during summer the low-water temperatures at the Yersche Bank anticipate the average daily water temperatures, with a difference of about one degree. So we see for instance that in the beginning of August 1938 low-water temperatures reached high values sooner than the daily averages and conversely that in the second half of August 1938 low-water temperatures were lower than the daily averages during a period of falling temperature. All these thermograph records have not been reproduced here. They generally show a very regular course with slight peaks during low water. These peaks practically never exceed 1° C. The thorough water mixing by the tides make it impossible for differences between water-temperatures at the surface and near the bottom to subsist throughout a tidal cycle.

As regards the salinity I assume that the areometer-readings effected twice a day since 1921 suffice to form an adequate idea about the salinity in the Oosterschelde. The lowest monthly value of the specific weight recorded was 1,0179 (at 17,5° C) during January 1926. The highest specific weight recorded was 1,0239 during August 1921 and October 1923. On an average the specific weight in the Oosterschelde is 1,0212 (at 17,5°C), which corresponds with a salinity of 27,75 $^0/_{00}$.

Several factors have their influence on the salinity. In the first place we think of precipitation and evaporation as predominating factors. I mentioned before that no volume of fresh water of any importance is discharged into the basin of the Oosterschelde, so only rainwater that has fallen directly into the basin itself has to be taken into account. The salinity of the adjacent waters should not be overlooked, however.

The salinity of the coastal surface water of the North-Sea shows more or less regular seasonal fluctuations. VAN RIEL (1929) states that the 34 $^0/_{00}$ isohaline is to be found at an average distance of 22 km off the shore of Hook of Holland during winter, 44 km during spring, 42 km during summer and 27 km during autumn. The discharge of the great rivers (the Rhine and the Meuse, of which the former is the most important) and the supply of Atlantic water exercise their influence on the salinity of the coastal surface water of the North-sea.

In order to investigate which factor has the greatest influence

TABLE I

AVERAGE WATER-TEMPERATURES IN THE OOSTERSCHELDE SINCE 1921

Computed from temperature-readings twice a day, at high water and

Year	January	February	March	April	May	June I	June II	July I
1921				10,0	15,9	18,5	17,2	19,5
1922	2,0	1,5	5,8	6,6	15,5	20,1	16,3	16,3
1923	3,4	4,9	6,0	10,0	11,9	12,9	14,5	20,0
1924	0,5	1,7	3,3	6,3	14,4	16,8	19,3	18,8
1925	4,6	5,0	4,9	8,8	14,2	18,5	16,7	18,4
1926	3,2	5,0	6,7	9,2	12,4	15,8	16,7	19,4
1927	3,9	2,9	6,5	9,6	13,3	15,6	16,1	18,3
1928	3,2	5,1	6,0	9,9	12,7	15,9	16,8	18,8
1929	0,5	0,2	3,4	7,1	14,6	17,0	17,0	17,3
1930	5,3	3,3	5,1	9,0	14,3	19,2	20,3	20,4
1931	4,0	2,6	4,0	8,6	13,5	16,8	18,3	19,2
1932	4,2	2,1	2,9	7,5	11,8	15,8	17,6	20,3
1933	3,4	1,9	4,7	9,4	13,0	17,7	16,3	18,1
1934	1,3	2,0	4,4	9,2	12,3	16,3	17,8	18,6
1935	3,3	2,7	4,2	7,6	10,9	15,2	17,8	20,4
1936	3,3	1,4	3,6	7,6	12,9	12,9	16,9	18,7
1937	1,8	3,2	4,2	7,6	14,0	18,1	18,2	18,6
1938	3,4	3,5	6,3	8,1	12,1	13,9	17,4	16,5
1939	2,9	3,8	5,6	9,3	13,6	17,1	18,2	18,5
Total monthly averages	3,0	2,9	4,9	8,5	13,3	16,5	17,3	18,7

at low water. Observations at low water on or near the tidal lands excluded. *Observations exact to half degrees. Surface-water used.*

Half-monthly averages have been computed for each summer-season.

No observations on account of ice-drift: Dec. 17–23 1927, Febr. 8–March 11 1929, Jan. 23-31 and Dec. 4–21 1933, Jan. 5-16 1934, Dec. 17–28 1938. The real averages for these months may be somewhat lower.

July II	August I	August II	Sept. I	Sept. II	Oct.	Nov.	Dec.
22,0	19,4	18,5	18,1	16,0	14,8	4,4	3,2
17,5	18,0	17,1	16,1	13,6	9,7	5,3	4,8
19,5	19,6	17,0	16,5	13,9	11,9	4,9	2,4
19,5	18,7	15,9	15,7	14,8	12,6	7,5	5,1
20,3	18,5	18,7	15,2	14,2	12,3	5,8	1,1
20,3	18,4	19,1	19,1	16,7	12,6	6,9	5,2
18,9	20,5	17,8	18,1	15,2	12,6	9,6	3,3
21,1	19,0	18,8	18,5	16,4	12,3	9,3	5,1
20,5	19,9	20,1	20,7	18,0	13,0	7,6	5,1
17,5	17,4	17,6	18,3	14,3	11,9	8,6	4,6
18,4	18,2	16,9	15,8	14,6	12,8	8,2	5,7
19,4	20,2	22,1	19,0	16,5	11,8	7,5	3,9
19,7	19,7	18,6	17,3	15,8	12,5	6,2	0,1
19,1	18,7	17,9	18,0	16,6	12,0	6,4	6,1
20,6	19,8	19,4	17,4	14,3	10,6	7,0	1,9
17,6	17,1	18,0	17,0	15,2	9,2	5,3	2,0
18,5	20,0	19,0	18,0	15,8	12,7	7,3	3,1
18,5	22,1	18,6	17,2	17,0	12,7	9,8	4,5
18,7	18,7	19,8	20,0	17,6	11,4	7,9	2,1
19,5	19,1	18,5	17,7	15,6	12,1	7,1	3,6

TABLE II

MONTHLY AVERAGES OF THE SPECIFIC WEIGHT OF THE WATER IN THE OOSTERSCHELDE SINCE 1921

Specific weight at 17,5° C.

Year	January	February	March	April	May	June	July
1921			1,0218	1,0227	1,0226	1,0226	1,0232
1922	1,0227	1,0220	1,0223	1,0214	1,0211	1,0212	1,0219
1923	1,0202	1,0194	1,0209	1,0212	1,0211	1,0209	1,0209
1924	1,0220	1,0211	1,0206	1,0204	1,0198	1,0202	1,0213
1925	1,0202	1,0208	1,0209	1,0206	1,0203	1,0215	1,0214
1926	1,0179	1,0188	1,0199	1,0197	1,0201	1,0208	1,0211
1927	1,0201	1,0201	1,0204	1,0206	1,0202	1,0203	1,0203
1928	0,1202	1,0202	1,0209	1,0211	1,0211	1,0212	1,0219
1929	1,0195	1,0191	1,0212	1,0210	1,0215	1,0214	1,0213
1930	1,0218	1,0220	1,0222	1,0221	1,0218	1,0214	1,0209
1931	1,0188	1,0182	1,0183	1,0187	1,0193	1,0200	1,0212
1932	1,0212	1,0211	1,0211	1,0215	1,0216	1,0217	1,0217
1933	1,0211	1,0211	1,0208	1,0212	1,0218	1,0223	1,0223
1934	1,0217	1,0216	1,0222	1,0216	1,0217	1,0223	1,0227
1935	1,0220	1,0218	1,0202	1,0201	1,0198	1,0198	1,0216
1936	1,0220	1,0202	1,0206	1,0215	1,0218	1,0204	1,0206
1937	1,0211	1,0201	1,0187	1,0187	1,0184	1,0197	1,0202
1938	1,0207	1,0207	1,0199	1,0206	1,0215	1,0216	1,0220
1939	1,0230	1,0221	1,0212	1,0202	1,0208	1,0210	1,0207
Total Monthly Average	1,0209	1,0206	1,0207	1,0208	1,0209	1,0211	1,0214
Salinity °/oo	27,4	27,0	27,1	27,2	27,4	27,6	28,0

Computed from daily areometer-readings. (Surface-water used).

No observations on account of ice-drift:

Dec. 17–23 1927, Febr. 8–March 11 1929, Jan. 23–31 and Dec. 4–21 1933, Jan. 5–16 1934, Dec. 17–28 1938.

August	September	October	November	December	Year-average	Rain-fall (total)
1,0239	1,0234	1,0231	1,0223	1,0231	1,0229	405 mm
1,0225	1,0230	1,0223	1,0223	1,0209	1,0220	757 mm
1,0225	1,0232	1,0239	1,0236	1,0228	1,0217	860 mm
1,0223	1,0216	1,0217	1,0215	1,0209	1,0211	736 mm
1,0212	1,0215	1,0210	1,0198	1,0190	1,0207	874 mm
1,0212	1,0202	1,0205	1,0204	1,0218	1,0202	696 mm
1,0203	1,0202	1,0206	1,0208	1,0208	1,0204	915 mm
1,0223	1,0225	1,0226	1,0222	1,0208	1,0214	736 mm
1,0216	1,0214	1,0212	1,0214	1,0217	1,0210	693 mm
1,0210	1,0212	1,0215	1,0202	1,0183	1,0212	906 mm
1,0215	1,0211	1,0207	1,0206	1,0209	1,0199	702 mm
1,0218	1,0215	1,0220	1,0210	1,0212	1,0214	735 mm
1,0213	1,0212	1,0220	1,0214	1,0216	1,0215	601 mm
1,0227	1,0228	1,0235	1,0233	1,0227	1,0224	592 mm
1,0219	1,0217	1,0212	1,0218	1,0224	1,0212	802 mm
1,0215	1,0223	1,0226	1,0209	1,0205	1,0212	733 mm
1,0214	1,0214	1,0212	1,0211	1,0207	1,0202	790 mm
1,0233	1,0229	1,0227	1,0233	1,0232	1,0219	586 mm
1,0209	1,0218	1,0215	1,0196	1,0186	1,0210	
1,0218	1,0218	1,0218	1,0214	1,0213	**1,0212**	
28,5	28,5	28,5	28,0	27,9	**27,75**	

on the salinity in the basin of the Oosterschelde I have composed a diagram (fig. 3). The average monthly values of the salinity of the surface water in the basin of the Oosterschelde and the corresponding values of the specific weight at 17,5° C are recorded in the intermediate graph of the diagram. (N.B. No observations on account of ice-drift: Dec. 17–23 1927, Febr. 8–March 11 1929, Jan. 23–31 and Dec. 4–21 1933, Jan. 5–16 1934, Dec. 17–28 1938). The total monthly precipitation in mm at Bergen op Zoom is recorded below. These data were placed at my disposal by the Koninklijk Nederlandsch Meteorologisch Instituut at De Bilt. (N.B. The observations during Dec. 1925, Febr. 1929, March 1929, April 1933 and October 1938 were carried out at Poortvliet, 12 km N.W. of Bergen op Zoom, and the observations during December 1938 at Krabbendijke, 14 km S.W. of Bergen op Zoom). The graph at the top of this diagram shows the fluctuations in metres of the waterlevel of the river Rhine. The Rhine is the most important fresh water discharging river in Holland. I assume that the height of the waterlevel recorded for instance some 100 km from the shore, may be regarded as an approximate standard for the volume of water discharged. The average monthly values of the waterlevels can be found in the "Jaarboeken der Waterhoogten" by Rijkswaterstaat. So the topmost graph may be considered to indicate fluctuations in the discharge of the river Rhine. I cannot demonstrate variations in the intensity of the evaporation in this diagram, as I have no data about them at my disposal.

Studying this diagram, we shall find that regular seasonal fluctuations in salinity do not occur here. The local precipitation does not show a marked relation with the variations in salinity. Often sharp fluctuations in the rainfall fail to evoke a reaction of the salinity, sometimes the salinity even increases during a very wet month. Fluctuations in the discharge of the Rhine, on the other hand, have a well-marked influence on the salinity in the basin of the Oosterschelde. Generally speaking, we may state that the graph on the waterlevel of the Rhine may be considered to be approximately the reflected image of the graph on the salinity.

So I want to conclude that the discharge of the Rhine has a marked influence on the salinity in the basin of the Oosterschelde and that the local precipitation is of much less importance in this respect.

IV. AIMS OF INVESTIGATION

In connection with the enforced return to the old approved system of tile-collectors in the Zeeland waters and with the very difficult position of the local oyster industry in the years following 1930 support was rendered to the oyster farmers by elaborating a system of forecasting the periods of spatfall in order to increase the chance of obtaining a sufficient spatfall on the tiles (HAVINGA, 1938, 1939).

The primary purpose of these investigations was to make an analysis of the maxima and minima of swarming in the course of the season of reproduction and of the pelagic life and the setting habits of the oyster larvae with reference to environmental conditions and the demands on the nature of the collector-material.

Especially our knowledge of the free-swimming stage of the larvae of *Ostrea edulis* showed many gaps. Though many investigations have been carried out on the biology of the larvae of other kinds of oysters during the pelagic stage, especially in America, it should not be assumed that the larvae of *Ostrea edulis* will act in quite the same way as those of the foreign species. It is impossible to understand the correlation between swarming and setting without a thorough knowledge of the interjacent stage, in casu the pelagic stage. So special attention was paid to the biology of the free-swimming larvae. Because but few reliable scientific data concerning the demands that mature larvae of *Ostrea edulis* make upon the nature of the cultch material are available, this subject was also inquired into. Although larval life and the setting process were studied simultaneously, I shall discuss these different subjects separately. The correlation between swarming and setting, the most important point in connection with the aims of these investigations, can only be discussed after an elaborate review of the methods and results of the inquiries into these separate subjects.

Spawning and swarming determine when and where young larvae can be expected, while still other factors determine when and to what degree spawning will occur. Therefore a discussion of the processes preceding spawning should not be lacking here for a full understanding of the entire process.

V. METHODS – INVESTIGATION ON THE PELAGIC STAGE OF THE LARVAE

Recognition of the larvae

A conditio sine qua non in the study of planktonic organisms like oysterlarvae, is the possibility of an infallible recognition of such larvae among the herds of other planktonic organisms, which will be found to include many larval stages of other kinds of Lamellibranchs. Notwithstanding the fact that the larvae of Bivalvia form one of the few groups of planktonic organisms, about which no extensive systematic literature[1]) exists, the recognition of the pelagic larvae of *Ostrea edulis* does not present any difficulties. Morphology and colour of the larvae of *Ostrea edulis* are very characteristic. Moreover the larvae of this incubatory species of oyster do not make their appearance in the plankton till they have attained a clearly recognizable stage, as the first part of their larval live is spent in the maternal mantle chamber.

Though investigators in the nineteenth century (e.g. HORST 1884) did not succeed in finding oysterlarvae in the plankton, no recent complaints about difficulties in identifying the pelagic larvae of *Ostrea edulis* are known to me. Without doubt living larvae, which show their natural colours, are easier to recognise, but well-trained eyes can also identify colourless preserved larvae. Though at first the simultaneous appearance of larvae of *Ostrea edulis* and of *Gryphaea angulata*, the Portuguese oyster, in the basin of Arcachon, presented some difficulties, BORDE (1930), the local oyster investigator, soon detected the difference between the larvae of those two kinds of oysters. The larvae of *Gryphaea angulata* closely resemble those of *Ostrea virginica*. With the aid of the figures of the larvae of *Ostrea virginica* in WELLS' paper (1927) BORDE was able to bear out his statement.

The identification of the pelagic larvae of some other kinds of oysters does not seem to present serious difficulties. After the paper of STAFFORD (1912) on the recognition of bivalve larvae, many authors (e.g. NELSON, 1921, WELLS, 1927) have discussed the features by which the larvae of *Ostrea virginica* can be identified. SENÔ (1929) and SEKI (1930) tell us how the larvae of *Ostrea denselamellosa* are to be recognized.

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[1]) Recent contributions to the systematics of the larvae of Lamellibranchs are e.g. the papers by KÄNDLER (1927), LEBOUR (1938) and WERNER (1939).

Quantitative plankton-samples:

It is easy to understand that only a comparison of comparable plankton- samples can give reliable information about the amount of oysterlarvae in the water, about the growth of those larvae, their movements and the amount of mature larvae.

Ostrea edulis.

When we consider the methods used in studying the larvae of the common European oyster, *Ostrea edulis*, our attention is in the first place directed to France, where planktonic oyster-larvae have been investigated since 1922.

In France samples are invariably collected by means of a plankton-net. After preliminary studies in the first years (LEENHARDT 1922, 1924) a uniform plankton-net and a uniform sampling method were introduced at several stations on the French coast. A description of how these investigations have developed in the French oyster localities is given by LAMBERT (1935) and by LADOUCE (1938 c).

In France a standard plankton-net, of a standard model, of standard measurements and made of a standard bolting silk (since 1924 no. 130, in the first years no. 140), is used. The construction of this net is rather primitive, however, and lacks, for instance, a tap to draw off the plankton. To procure comparable samples it is necessary to try to eliminate as many of the inconstant factors as possible. To eliminate the influence of the tidal cycle the samples in France are preferably taken at the same stage of the tide (at half flow). To filter off with the net approximately the same volume of water the French investigators tow the net against the stream for a certain time (five minutes) at approximately the same speed. The volume of filtered water is not known. The towing is carried out along the surface of the water ("à une vitesse telle qu'il restait au surface sans sortir de l'eau et faisant un sillage"). As the surface-layers of the water show the most extreme variations in salinity (rain-showers) and temperature, a possible migration of the larvae in a vertical direction will show a maximum amplitude in those surface-layers. In their endeavours to eliminate such migrations some investigators in France have sampled with a double net in recent years, one along the surface and one somewhat deeper (VOISIN, 1932). This problem will be discussed in the chapter on vertical migrations. To avoid floating eelgrass at Arcachon, the net was towed about 10 to 15 cm below the surface in 1937 and in 1938 (LADOUCE, 1938 a, 1938 b).

Reliable observation of the emission of larvae into the plankton and of their subsequent growth is only possible when samples are collected very frequently. Daily sampling is very desirable. Although the French investigators aim at daily sampling, they are handicapped by practical and technical difficulties. The consequence is that in some localities sampling takes place only once or twice a week, which results in a less reliable comparison of the data obtained.

The plankton samples collected in this way are diluted with water to a certain volume and in known quantities of this thoroughly shaken mass the oyster larvae are counted with a microscope. With the aid of the data obtained in this way the total amount of oysterlarvae in the original plankton sample is computed. BOURY (1928) tells us that countings which seem too extreme are eliminated! Such a proceeding is open to criticism.

In France the oyster larvae are not measured regularly. In recent years however, some French investigators distinguish and count the so-called larvae in the second stage. This division is equivalent to the classification "straight-hinge-" and "umbo-larvae" given by other writers. I wonder with KÄNDLER (1928) why the French investigators omit measuring the oyster larvae in the plankton-samples, which procedure may give very important information concerning growth, age and loss of those larvae. Where the larvae of *Ostrea edulis* and *Gryphaea angulata* occur simultaneously, as in the bassin d'Arcachon, the totals of both kinds of larvae in the samples are often added up. Diagrams composed of such data (BORDE) cannot possibly give clear information.

The results of the French investigations are to be found in the papers of LEENHARDT (1922, 1924), BOURY (1928, 1929 a, 1930), RAPHENNE (1930, 1931), VOISIN (1931, 1932), TACLET (1932, 1935), HERMAN (1935, 1936, 1937, 1938 a, 1938 b) all of them on the waters in the Morbihan. Further CHAUX-THÉVENIN (1931–1938) (*Gryphaea angulata*) at Marennes and at Arcachon BORDE (1929–1937) and LADOUCE (1938 a, 1938 b).

Other quantitative studies on the planktonic larvae of *Ostrea edulis* in open waters (I leave out the frequent checking of the amount of larvae in enclosed ponds or pits that went with the attempts in England, Germany and Denmark to effect "artificial" propagation) can be found in the papers by Kändler (1928) and Hagmeier and Schubert (1930) on investigations in the German oystergrounds, by Spärck (1925) on the Limfjord-plankton and by Gaarder (1932) on studies in the Norwegian oyster pools.

Spärck (1925) found but very few larvae in the Limfjord waters and in fact only once detected a mature larva in the samples obtained with his plankton-net.

The German investigators use a plankton-net of a special construction. The large quantities of detritus in the Wattenmeer often cause a rapid obstruction of the meshes of the bolting silk and make it impossible to obtain quite reliable quantitative plankton-samples. Kändler (1928) likewise judges it impossible to estimate approximately the volume of water filtered during the five minutes' towing. The German plankton-investigations were carried out in the summers of 1926, 1927 and 1928, after the importation of Dutch seed-oysters in the German oystergrounds. The larvae in the plankton samples were counted and measured without preliminary dilution. The sampling did not take place very frequently, but only a few times during the season of reproduction. Though the volumes of filtered water in the German samples cannot be compared with those in the French samples and in either case cannot even be estimated approximately, it will be clear that during the years of investigation in the Wattenmeer far fewer larvae per unit of water could be found there than at the French sampling-stations. In France thousands of larvae occur in one sample and in the Wattenmeer seldom more than a hunderd, both samples being obtained by five minutes' towing; in the Wattenmeer even with a larger net than in the French waters.

Gaarder (1932) studied quantitative plankton samples from two Norwegian oyster pools (Espevik-poll and Saelø-poll). The exceptional hydrographic conditions in those pools (no tidal cycle and a marked stratification of the water) present other possibilities. The oyster larvae are only present in the deeper salt layers. Sampling was carried out by pumping up ten litres of water from the deeper layers and filtering them with a plankton-net. Samples from different depths were taken five times during the season of reproduction. All the larvae were counted and measured.

Before describing the methods of plankton-sampling in Holland I will briefly discuss the methods used in America. Very curious data have been obtained by some American investigators.

On the Atlantic coast samples of the larvae of *Ostrea virginica* are collected quantitatively. The first quantitative sampling was carried out by filtering a pail of water through a plankton-net (NELSON, 1917). Though some investigators obtain quantitative samples by towing a plankton-net (GALTSOFF, 1930), in most cases a known quantity of water (from 50 litres to 200 gallons) is pumped up from a known depth and filtered through a plankton-net (CHURCHILL, 1921, NELSON, 1921, PRYTHERCH, 1929, PERKINS, 1931). In the southern part of the range of *Ostrea virginica* HOPKINS (1931) carried out investigations. A quantitative method of measuring the abundance of oyster larvae in plankton-collections was not employed, for while such measuring might be feasible during a few weeks, it would take too much time to continue it over a period of several months. A crude method (plankton-net-tow) was devised, which gave results of relative value.

In some localities the plankton-samples always contain numerous larvae during the season of reproduction. Some investigators sieve off the oysterlarvae with monel-metal screens before counting them (CHURCHILL, 1921, PRYTHERCH, 1929); sometimes they are counted and measured (CHURCHILL, 1921); often the larvae are only counted, the occurrence of mature larvae being recorded (NELSON). It is very curious that, though often thousands of larvae may occur in 100 litres of water in some localities (Barnegat Bay, Great South Bay), other investigators fail to detect any larvae in many samples collected in other places (Wareham River, Onset Bay, Milford Harbor), only a few larvae being found in the other samples (GALTSOFF, PRYTHERCH, MC.MILLAN, 1930, PRYTHERCH, 1929). This is not a question of rich and poor breeding localities, for even in those places where few larvae are found in the plankton-samples an extraordinarily profuse setting may occur. The cause of this remarkable difference will be discussed in the chapter on vertical migration.

No quantitative studies on the amount of the larvae of *Ostrea lurida* have been published so far.

The methods of plankton-sampling that have been in use in the Oosterschelde (Holland) since 1935, have been worked out by HAVINGA (1938, 1939). A quantitative sample is obtained by pumping up 100 litres of water from a known depth and by filtering them at once through a specially constructed planktonnet (bolting silk Nr. 130). The plankton is drawn off from the net by means of a tap. The sampling is carried out daily at low slack water to eliminate as far as possible the influence of the tides. A total elimination of the influence of the tidal movements would be impossible, as one cannot get round the difference between spring-tides and neap-tides and the influence of strong winds on the currents.

A discussion of the possible influence of the depth of sampling can be found in the section on vertical migrations.

The sampling is carried out at two stations of different character, the first being the centre of larvae-production, while the second, an important spatfall-centre, receives all its larvae through the tidal watermovements. During the most important part of the reproduction-season daily sampling is carried out. In the beginning and during the last part of the season the sampling takes place every second day. The daily samples are studied alive, as living oysterlarvae are more easily recognisable than preserved ones. It has appeared to me that no mortality or loss of oysterlarvae occur in such living samples, if we let for instance 24 hours elapse between sampling and microscopical examination. With the aid of a counting glass and a counting table the entire sample is searched for oyster larvae, which are all of them counted and measured (the greatest length of the shell parallel to the hinge), while the presence of larvae bearing a pigment-spot (mature larvae) is recorded.

Movements of the larvae under the microscope are prevented by means of a coverglass.

Apart from the daily sampling many groups of other quantitative plankton-samples have been collected during the season of reproduction in the years 1937 and 1938 for the sake of inquiries into movements of oyster larvae in horizontal and vertical directions.

As the study of the daily samples requires a good deal of time, it was impossible to count and measure the oyster larvae in these special samples alive. Therefore I preserved these samples of larvae in alcohol; corrosion of the shell-edges of the larvae, which often takes place in formaline-solution, does not occur in alcohol. Alcohol causes precipitation of the calciumsulphate in seawater as a thick whitish mass. Before microscopical investigation is proceeded to, dilution with water will dissolve this troublesome gypsumprecipitate.

In my opinion the pump-method gives better quantitative results than plankton-net-towing: the depth of sampling and the volume of filtered water are absolutely under our control in this way.

I am aware that objections can be raised against the use of a pump and suctionhose. Excellent apparatus for the collection of indisputably reliable quantitative watersamples at a known depth exists, but these methods are only practicable in the study of minute and abundant plankton-organisms. Samples of 100 litres cannot easily be collected with this apparatus.

As regards the pump-method we can find a discussion of the use of this kind of gear in the paper by GIBBONS and FRASER (1937); they compared the results obtained with the pump-method with those of several kinds of plankton-nets.

They prefer a pump for the study of non-motile and minute forms, but nets are considered better for the collecting of samples of fish-larvae and other motile forms.

Limnologists know that many little Crustacea tend to swim against the direction of the current stirred up around the mouth of the suction hose. The speed with which the hose sucks in the water will vary with the power of the pump. We may imagine a number of concentric spherical zones around the mouth of the suction-hose, in which the speed of the flowing water will vary. The nearer to the hose-mouth the greater the speed. It will be impossible for little plankton organisms in the inner zones to counteract this current by their own motive force. In the outer zones the speed of the flowing water will be so tardy that those animals which tend to swim against the stream are able to escape the suction-hose, which will make the sampling unreliable.

These arguments only apply to stagnant waters. When the mouth of the suction-hose moves in respect to the water with a sufficient speed, those zones do not endure long enough in one place to make such an escape possible and the sampling will be more reliable. So pumps can be used in streaming water and also from a moving boat in stagnant water.

Though hitherto it was not known how oysterlarvae will behave in this respect, there is a possibility that their behaviour will be similar to that of the said Crustacea, of which the consequence would be unreliable sampling in stagnant water. As the tidal movements of the waters in the Oosterschelde cause strong currents (see hydrographical conditions), these objections against the pump method cannot be raised here. Later on I shall prove that even during slack water pump-sampling of oyster larvae yields trustworthy quantitative results (see horizontal movements). In how far a reliable comparison of the daily plankton-samples is justified will also be discussed in that section.

To prevent the whirling of bottom material in case of a too close proximity of the hose-mouth to the bottom, which would render microscopical examination of such plankton-samples practically impossible, a leaden plate, about a square foot in size, is attached horizontally a few inches under the mouth of the suction hose. The mouth is protected against the invasion of undesirable material, such as seaweed and jelly-fishes, by means of a piece of copperwire with fairly wide meshes.

VI. SPAWNING

Spawning act

Female spawning involves the discharge of the eggs from the gonad into the suprabranchial (= cloacal) chamber.

In non-incubatory species of oysters an extrusion of the eggs from the inhalent chamber into the free seawater immediately follows on spawning, while in the incubatory species of oysters this extrusion is postponed till the larvae have reached a certain stage of development. It is a very remarkable fact that those non-incubatory species extrude the eggs via the inhalent chamber instead of following the seemingly much easier way direct from the exhalent chamber (= suprabranchial chamber) into the outer world, i.e. the way followed by the exhalent water, the sperm and the waste products of rectum and kidneys.

This points to a close relationship of those two types of oysters. The only difference between incubatory and non-incubatory oysters as regards the way in which the offspring leaves the mother-oyster lies in the time required for the entire process.

The second stage of this event, the discharge of the eggs from the inhalent chamber into the seawater, has been observed more than once in *Ostrea virginica*. NELSON (1921) gives a good description of his observations; he was, however, not the first to witness the spawning of this kind of oyster. Each female, in spawning, relaxes the adductor-muscle until the shells gape to their widest extent and then by a quick contraction of the adductor muscle expels the water within the shells and with it a great quantity of eggs. With each expulsion, at intervals of about 30 seconds, the eggs are driven out in a fan-shaped cloud.

In another paper by NELSON (1922) figures of spawning oysters will be found, together with the reproduction of a chimograph-tracing of the shell movements of a spawning female oyster. The shells of these oysters were attached to a recording apparatus used for observing the water current caused by the gills and by good fortune the rhythmic contractions by which the eggs are shot out could be recorded. Similar chimograph records of the spawning actions of *Ostrea virginica* and *Ostrea gigas* will be found in the papers by GALTSOFF (1930 a, 1932). While the eggs are discharged by violent shell movements, the males show no shell movement whatever, but remain quiet while spawning, with the shells gaping, while on the dorsal or upper side about midway between the hinge and the tips of the shells (exhalent chamber!) a steady stream of milt issues from between the shells (NELSON, 1921, figures in NELSON, 1922). GALTSOFF (1930 a, 1932) describes a similar action, the washing out of the sperm through the cloaca with the water pumped by the gills, both in *Ostrea virginica* and *Ostrea gigas*.

The first stage of the spawning process, however, the action by which the eggs pass from the gonad to the suprabranchial chamber and thence from the suprabranchial chamber to the branchial chamber, has never been observed directly. So far the only plausible explanation is the passage of the eggs from the suprabranchial chamber through the water-tubes and gill-slits to the branchial chamber.

In accordance with this explanation is the discovery of ELSEY (1935) that the gillslits (or ostia) of *Ostrea lurida* and *Ostrea gigas* have a diameter proportional to that of their eggs. It is difficult to understand in what way the eggs are forced through the gill-apertures. STAFFORD (1915) suggests pressure, but perhaps the opinion of GALTSOFF that suction caused by opening the valves during spawning draws the eggs through the ostia is more satisfactory. Just as the first stage of the spawning process in

Ostrea virginica has escaped observation so far, no investigator has as yet observed the extrusion of the eggs into the branchial chamber in incubatory species, such as *Ostrea edulis* and *Ostrea lurida*. Attention has been called to this gap in our observations by HOPKINS (1937) as far *Ostrea lurida* is concerned and by HAGMEIER (1931) and ORTON (1937 a) for *Ostrea edulis*. The latter again suggests pressure as effecting the passage through the gill-slits, by "contraction of the posterior mantle and gill-muscles, which obliterate the cavity of the exhalent chamber and forces the eggs into the inhalent".

The delayed second stage of the spawning-act in the incubatory species will be described in the section on "swarming".

Periodicity of spawning

Oysters show a more or less regular periodicity in spawning. The spawning season may vary in length to a high degree, according to the local climatological conditions. The length of the season of reproduction has been discussed already in the section on the range of the oysters. As regards the two types of oysters different methods of observation of this periodicity have to be applied.

Spawning in non-incubatory species can be observed by following the quantity of oysterlarvae in the plankton, which often shows marked increases. In some of the culture-regions of these oysters (e.g. *Ostrea virginica*) nearly all the spawning of the season tends to be concentrated in one or two days, while in other regions the spawning of the same kind of oyster is not limited to a few days, but is distributed over many spawning-days of different importance. As far as incubatory kinds of oysters are concerned, it is clear that a certain space of time separates spawning from the increase of larvae in the plankton; the length of this time is dependent on the duration of the incubation. The only reliable method of studying the periodicity of spawning in incubatory oysters directly is a regular examination of the percentages of adults carrying young in comparable samples of oysters. The spawning periodicity in *Ostrea edulis* has been investigated especially by ORTON.

This periodicity is brought about by the stimulating activity of two factors of a different nature. Temperature is one of these factors, while a stimulation of a chemical nature may be exercised by the sex-products of other oyster individuals, at least in some species of oysters. The mature egg or sperm must practically be ready for extrusion for any kind of stimulation of spawning to be possible.

There is often much difference in the stage of development of the gonads in different individuals of *Ostrea edulis*. This complicated matter can only be elucidated by a preliminary treatise on the phenomenon of sex-change. So I shall have to deal with the internal and external influences on the periodicity of spawning separately.

VII. INTERNAL FACTORS GOVERNING THE PERIODICITY OF SPAWNING

Ostrea edulis.

In the days of DAVAINE (1853) and LACAZE-DUTHIERS (1855) the first discoveries about the hermaphrodetic character of *Ostrea edulis* were made. MOEBIUS (1871) records the development of sperm after the shedding of the eggs.

The beautiful microscopical studies by HOEK (1884) provided us with further information. He isolated a number of oysters carrying larvae and picked these oysters up again after one to four weeks. Microscopical investigation showed development of sperm in all of them. The eggs in one female oyster always show the same stage of development, so most probably all the eggs are extruded simultaneously. The stages in the development of the sperm, on the other hand, will differ. The presence of ripe sperm usually accompanies the appearance of earlier stages of sperm-development. So HOEK declared that the shedding of sperm will take place during a much longer period than the extrusion of the eggs. HOEK rejects the possibility of self-fertilization.

Though this study meant an advance in our knowledge, HOEK was not aware of the entire sex-cycle and the laws governing sex in *Ostrea edulis* were not to be discovered till forty years later.

Recent researches by ORTON and SPÄRCK cleared up a great deal of the confusion. Both ORTON and SPÄRCK realized that no observation of the actual stage of the gonad could ever give clear information about a succession of sex-phases, no matter the amount of oysters examined. Repeated examination of the gonad of the same oyster individuals, for instance in the course of one year, would be necessary. They discovered that the boring of a hole in one of the shells and the extraction of a portion of the gonad-tissue for microscopical examination did not do the oyster any harm.

While ORTON sorted out a certain amount of oysters showing the same stage of sex and isolated these identified oysters in the sea in cages (1922 a), SPÄRCK isolated single examined individuals in aquaria (1925). The assertion of DAVAINE (1853) that the first sexual phase of the oyster is the male one was confirmed (ORTON, 1921).

The change from female to male was investigated in oysters of ages varying between one and six or seven years. Female functioning oysters of any of these ages appeared to change their sex at the time of spawning from female functioning to maleness (ORTON, 1921, 1922 a, SPÄRCK, 1925). The fact that practically every oyster shows a development of sperm shortly after the shedding of the eggs prevents the production of a second batch of larvae during the period of femaleness, at any rate as a rule. When some eggs happen to remain in the gonad after the general extrusion, they will be absorbed or eliminated as an excretion-blister (ORTON, 1927 d).

The change from female to male sets in very quickly after the shedding of the eggs and at the time of swarming practically every oyster contains mature sperm-morulae (ORTON, 1927 c, 1927 d). The way in which this change takes place resembles an automatic reaction. The culminating point in the production of sperm is reached about a month after this sex-change (ORTON, 1927 d). When the extrusion of the eggs takes place towards the end of the season of reproduction and the temperature is falling rapidly, the subsequent development of the sperm fails to take place and gonad-action comes to a standstill till the next spring sets in (SPÄRCK, 1925). In the paper by ORTON and AMIRTHALINGAM (1931) some figures on the development of sperm during incubation can be found.

The next point to be investigated was whether these individuals which have changed from female functioning to maleness remain permanently male or change back to femaleness. If all the females change into males, the proportion of males should increase with age, unless a reversion to the female stage occurs. A study of the proportion of the two sexes at various ages (ORTON, 1936) clearly shows that maleness does not increase with age and establishes indirectly that sex-change from male to female must occur. The occurrence of this sex-change was proved with the aid of the boring-device. ORTON (1933) isolated his male oysters in cages in the sea, while SPÄRCK (1925) observed isolated individuals in aquaria. Though many of ORTON's oysters did not reach the date of examination in the next year alive, the presence of a certain amount of indubitable females among the surviving oysters proved the occurrence of a sex-change from male to female, even under unfavourable conditions. These oysters were left unexamined during a year and we do not know what happened in the course of that year. ORTON states (1933) that he did not expect a rapid reversion and that probably many of these oysters passed the female stage undetected. His experiments were not conducted with a view to detecting a rapid development of the female phase. He presumes that under wholly natural conditions it is highly probable that the percentage of males changing into functioning females within the period of one year is much greater than that found under his experimental conditions.

SPÄRCK (1925) succeeded in obtaining some data on the rate of change from male to female. Though this reversion does not take place as quickly and automatically as the change from female to male, he found that this change may be completed within a few weeks. Thus SPÄRCK observed for example a male oyster (June 6) which contained sperm as late as July 8 and which was found to be female on July 15. Another individual was male on June 20, female on July 4 and carried young on July 25.

As the eggs develop, the oysters remain functioning as males for a considerable time and often sperm-production continues till about ten days before the discharge of the eggs! (SPÄRCK 1925). When we consider that a few days after the shedding of the eggs the gonad again contains sperm-morulae, we may conclude that the purely female phase can be very short (about three weeks).

Knowing this, we shall not be astonished at finding far more males than females in practically every sample of oysters.

As the first sex-phase in an oyster is always the male one, which is reached in its second summer (ORTON (1922 a) once met with a male at the age of 23 weeks), the first change which will take place is the change from male to female. After many contradictory statements in the past concerning the age at which an oyster can reproduce as a female it was proved in recent years that the farther north, the later the first sex-change will occur.

Spärck (1925) states that in the Limfjord no female oysters younger than three or four years can be found and that a first reproduction as a female at the age of four is more common than at three.

Orton (1922 a) tells us how he once found an oyster carrying young in his second summer after the exceptionally fine summer of 1921. After the fine summer of 1935 the occurrence of female oysters in their second summer in the English waters was established (Dodd, Mc.Cloy, Davies, Edmonds and Orton, 1937). In the course of my investigations in the Oosterschelde I noticed more than once an oyster in his second summer carrying larvae. In Holland a fine preceding summer is not a necessary condition for such a rapid female development, but all the same I consider female reproduction in the second summer as exceptional in the Zeeland waters.

In the warmer waters of France female reproduction in the second summer is no exception (Gerbe, 1876, Dupain, 1932). The result of this difference in the age at which the first sex-change takes place is a much higher percentage of male oysters in samples taken in the northern waters, at least if young oysters are not excluded. We do not possess adequate information about the natural frequency of sex-change and about the period normally required for an entire cycle in the various regions of oyster culture. The frequency of sex-change is probably affected by the temperature and possibly by the amount of nourishment. (Spärck, 1925 and Orton, 1936).

In winter the sex-change is checked. Spärck (1925) states that the development of sex-cells is practically interrupted from September 1 till the beginning of April in the Limfjord. The warmer the water, the shorter the interruption will be, which corresponds with the greater length of the season of reproduction in the southern regions.

A fall in temperature in autumn will check the sex-phenomena, though as yet we do not exactly know when. The stage of the gonad at the moment of interruption will be the stage during hibernation. When the temperature rises in spring, sex will revive at a certain temperature. The oyster recommences at the sex-stage at which it left off in autumn. Indeed a certain percentage of the oysters will hibernate as females, the gonads containing eggs, others as males, the gonads containing sperm, while again others do not show any sex-products at all during that period (SPÄRCK, 1925; ORTON, 1927 d). The last category probably consists mainly of oysters that spawned the last time as males. Before egg-development begins a certain period of rest and fattening is often assumed (ORTON 1927 c, ORTON, 1937 a). Though the change from male to female is certainly less rapid than the reversed one, the occurrence and length of such a period of recuperation has not yet been established. We know that the change from male to female can take place fairly quickly (SPÄRCK, 1925), but we know too little as yet about the influence of temperature and nourishment on this change.

SPÄRCK made an attempt to study the influence of temperature on the sex-change in aquaria with heated water. He was hampered in this by a high mortality of his oysters and great difficulties with feeding, especially during the winter-months.

A very frequent examination of the gonad under conditions as natural as possible might give further information about the frequency of sex-change in different localities, so under different conditions of temperature and nourishment.

ORTON (1924) informs us that he observed an oyster which passed the female stage twice during one season of reproduction. This oyster spawned again as a female six weeks after it was last found to be in a male condition. ORTON (1926) with the aid of data on the percentages of oysters carrying young (in weekly samples) computes that each adult oyster will function as a female at least once every year. His data (collected in 1925) show that more than 100% of the adult oysters carried larvae during that season, so that a certain amount of oysters must have passed the female phase twice. I believe that this phenomenon, i.e. the functioning as female for the second time during one reproduction-season, is not exceptional in the Oosterschelde. I base this assertion on the fact that production of larvae can take place in the Oosterschelde for a considerable period every summer, often showing several optima, as well as on the fact that the average temperature conditions are more favourable in the Oosterschelde than in England. So when ORTON concludes from his data on the percentage of oysters carrying larvae that a second female phase may occur during one season, I deem this phenomenon still more probable in the Oosterschelde. I did not observe the sex-change in isolated oyster individuals.

A treatise on the causes of the phenomenon of sex-change and on the nature of its regulating power is to be found in a paper by ORTON (1927 c).

Sex-chromosomes are apparently not all-powerful in oysters. As regards the intermediate factor between sex-chromosomes and sex-determination Orton is inclined to think in the first place of sex-hormones. The presence of sex-hormones has not yet been established in oysters or allied animals (KOLLER, 1938).

ORTON suggests a connection between metabolism and sex. He suggests a metabolic rhythm as an alternative to the ordinary sex-hormone theory. He believes that sex-change is brought about by a rhythmical change in the nature of metabolism. At the female stage of the oyster the proteïn-metabolism is considered to be predominant and the glycogen-metabolism during the male phase. An excess of unusable metabolic products, characteristic of one sex, is believed to induce a reversal of the sex-metabolism and the sex-manifestation to that of the other sex (ORTON, 1927 d). ORTON points to the predominancy of the glycogen-metabolism during autumn, which according to him induces the male phase and he thinks that the predominancy of proteïn in May and June induces the female phase. It should be borne in mind, however, that these maxima are of a quite different nature. The glycogen-maximum in autumn means a real increase of the glycogen content, but the proteïn "maximum" in June is caused by a decrease of the amount of glycogen, so that we find a higher percentage of proteïn, notwithstanding the fact that the amount of proteïn shows a decrease as well at this time (GAARDER, 1938). This metabolic rhythm is considered to be characteristic of this organism. ORTON points to the connection between metabolism and sex in bees, Cladocera, Rotifera and the influence of Sacculina on a crab. ORTON quotes in his support the following passage from one of GOLDSCHMIDT's papers: "The action of the hormones probably calls forth a specific type of metabolism, and this is the ultimate and direct cause of the morphological differentiation of the sexes".

Unfortunately our knowledge about the changes in the chemical composition of oysters in connection with sex-change is very limited. We know (RUSSELL, 1923, GAARDER, 1938) that the dry weight of the adult oysterbody increases during autumn simultaneously with the increase of carbo-hydrates (glycogen) during the main storage of food-reserves (fig. 5). In spring and early summer the food reserves are largely used up in breeding, with a consequent decrease of carbo-hydrates and dry weight. The percentage of proteïn shows a maximum when the oyster is in a poor condition, because of the using up of the glycogen-supply. Though the proteïn-percentage shows a maximum at that time, the total amount of proteïn shows no increase at all. In summer, on the contrary, it shows a decrease as well, though not to the same extent as the glycogen. The production of eggs and sperm makes high demands on the metabolic processes. ORTON (1927 d) uses these data in support of his hypothesis. We should remember, however, that these analyses by RUSSELL are of oysters of unknown sex-stage, and we have to take into account the above remarks about the different nature of the two predominancies.

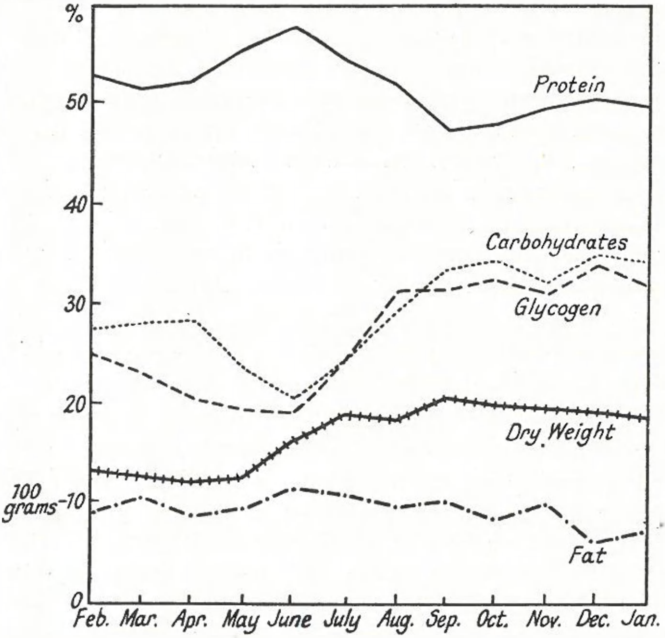

Fig. 5. Variations in the chemical composition of the oyster. After RUSSEL 1925. (Calculated on dry weight).

Considering the interference of the storage of reserves in autumn and the using up of these supplies in the next breeding season, which lead to the above-mentioned changes in chemical composition, a phenomenon that will no doubt be found in allied genera of Molluscs which show no sex-change at all, I do not believe that these analyses can be used in support of ORTON's theory.

GAARDER's analyses (1938) show a sharp decrease of glycogen-content and a decrease of the proteïn-content, but an increase of the protein-percentage during the breeding season. The oysters GAARDER used were of exactly the same age and had lived before the beginning of the sampling under exactly the same conditions. This led to the remarkable fact that practically all his oysters showed simultaneously the same stage of sex at the beginning of this breeding-season, in casu the female one. Most probably RUSSELL's oysters did not show the same sex-stage simultaneously, nor did the oysters (*Ostrea circumpicta*) of OKAZAKI and KOBAYASHI (1929). Nevertheless both these groups show exactly the same decrease of glycogen and dry weight during the breeding-season as that of GAARDER. I am inclined to think that the preparation of the functional male stage will result likewise in a decrease of glycogen. Perhaps the demands on the food-supplies are not *so* high as during the preparation of a female spawning, but I do not see the possibility of an essentially different kind of metabolism (proteïn versus glycogen) with regard to the building up of the different sex-products. I believe that, if GAARDER had also begun sampling from adult oysters that begin the reproduction season in the male phase, he would have obtained the same results as he actually did with his female-oysters. I should prefer a frequent sampling during the breeding season from two groups of oysters, laid out, if possible, in a cold locality as well as in a warmer one, to study the influence of the rate of sex-change. One of these groups should consist of female oysters, the other of oysters beginning the season in the male phase. Only a difference between such groups would point to other types of metabolism during the male and female period. As yet I don't believe we are justified in considering the male phase as a kind of famished female phase (GAARDER, 1938); male oysters in the beginning of the breeding season do not look like famished oysters. GAARDER's oysters lived under unfavourable circumstances as regards nourishment during the season of reproduction; many of them died towards the end of the summer. I do not reject the idea that there may be some chemical influence which governs sex-change as an intermediate factor, but I should prefer to think of a kind of hormonal cycle and not of an influence of the kind and quantity of the materials stored.

Until we know more about the chemical composition of oysters in correlation with the stage of sex, the theory of a metabolic change as the causation of sex-change must be regarded as a mere hypothesis.

It was necessary for me to expatiate on the phenomenon of sex-change in *Ostrea edulis* to demonstrate the possibilities of periodicity in larval production during the season of reproduction.

When the external factors stop checking reproduction, the mature females will spawn their eggs; in other words: the new season of reproduction has begun. This first spawning brings on the first supply of oysterlarvae in the plankton after the incubation-period has passed.

Oysters which function first as males, because they are in the male phase at the moment of revival, will spawn during a certain time as males and after this change their sex and then spawn as females, which causes a second increase of oyster larvae after the incubation-time. When the external factors governing reproduction remain favourable for a considerable time, the oysters that spawned as females early in this season will show a reversion to the female sex once again after passing through the male phase, which sets in immediately after the first female spawning. These oysters will spawn as females for the second time in one season near the end of the summer. As the external influences, such as temperature and nourishment, will differ more or less in the various parts of an oyster-ground, it is self-evident that the above-mentioned spawning- groups will overlap one another more or less.

What I wanted to bring out is that external factors governing in a direct way the periodicity of spawning only affect oysters which contain at that particular moment mature sex-products and that the occurrence of mature sex-products is dependent on the cycle of sex-change. The rate at which this cycle is completed cannot be ascertained till we know more about the influence of temperature and nourishment on sex-change. So the influences considered by me as internal ones are in their turn dependent on external factors, such as temperature and possibly nourishment.

It is self-evident that we are as yet unable to forecast how the spawning will be distributed over the entire breeding-season. Even when we know the external factors directly governing the periodicity of spawning, we can forecast only approximately the moment at which the first spawning in the new season will take place, but we shall not know beforehand when further spawning may be expected during the time that external factors remain favourable, until we know more of the rhythm of sex-change in *Ostrea edulis*. It will be impossible to attain this aim without lengthy and large-scale operations.

Before we pass on to a discussion of the external factors governing directly the periodicity of spawning, it will be interesting to have a look at the phenomenon of sex-change in other species of oysters. Of course we are especially interested in the behaviour of *Ostrea lurida*, an incubatory species closely related to our *Ostrea edulis*.

Ostrea lurida.

STAFFORD (1913) made the first observations on the hermaphroditism of *Ostrea lurida*. He noticed oysters containing male reproductive elements as well as female features. STAFFORD was not aware of the sex-change phenomenon in *Ostrea lurida*. It was COE (1931, 1932 c) who thoroughly investigated the sexual rhythm in *Ostrea lurida*. A fairly frequent sampling, beginning with very young oysters, clearly showed the development of the gonad, its first functioning and the sex-change cycle. COE worked at la Jolla in South-California, the southern part of the range of *Ostrea lurida*, where propagation continues for about seven months. COE could determine exactly how old his oysters were; he collected them on his concrete experimental blocks laid out in the sea at regular intervals for the sake of studying sedentary marine organisms (COE, 1932 b, COE and ALLEN, 1937). At an age of eight weeks the first signs of the development of the gonad could be observed. At about fifteen weeks the presence of the primitive ovogonia as well as the spermatogonia can be shown. The spermatogonia proliferate more rapidly than the ovogonia. *Ostrea lurida* is always protandric. Proliferation of the ovogonia and the formation of ovocytes begins before the extrusion of the spermballs takes place. When the sperm is discharged (at an age of about five months) the oyster is in the female phase. The ovocytes build up their yolk material in the secondary gonad, the ovarian tubules, which intrude in the underlying connective tissue. At an age of about six months the first eggspawning occurs in South-California. During the incubation-period the new sperm-development can be observed. At the time of swarming the oyster is ready again to function as male and this time we shall find far more spermballs in the oyster than during the first male period. After the second male period COE noticed a period of rest, the recuperation period, during which the oyster restores its food-supplies. The duration of this period is probably dependent on metabolic conditions. If the external conditions remain favourable a second female stage occurs, after which a third male period will follow. COE is inclined to think that in colder regions the rhythm is likely to be less rapid. Perhaps we may expect an annual or even a biennal rhythm there. The alternation will stagnate when the temperature has dropped too low (± 16° C). The alternation rebegins in the next season at the phase where the oyster left off in autumn. About 25% of the oysters will be found to contain eggs simultaneously in early spring.

COE (1931) states that: "Fertilized eggs are all alike in regard to their primary sexual inheritance, with an associated hereditary mechanism, perhaps metabolic in nature, which is responsible for the rhythmical alternation of the sexual phase".

External conditions cannot upset the sexual alternation, but they may retard or quicken the various stages and affect the quantity of gametes produced. From the moment that external conditions allow reproduction to begin, early in the new season, the sex-change will govern the periodicity of spawning in *Ostrea lurida*. *Ostrea lurida* reaches the mature stage at an earlier age and shows (at least in South-California) a more rapid completion of the cycle than *Ostrea edulis*, but other important differences are not yet known to us.

Ostrea virginica.

Like other kinds of non-incubatory oysters *Ostrea virginica* was considered to be dioecious until quite recently.

Although many years ago STAFFORD (1913) found indications that this American oyster is protandric on the Canadian coast, it was Burkenroad (1931, 1937) who, carrying out his investigations in Louisiana, provided us with data on the correlation of size and sex in this kind of oyster. The smaller the oyster, the higher the percentage of males. (E.g.: < 20 mm 50 ♂ — 5 ♀, < 40 mm 220 ♂ — 58 ♀, > 40 mm 151 ♂ — 315 ♀, < 80 mm 7 ♂ — 48 ♀). If we reject a differential growth-rate or a differential death-rate, this important difference in sex-percentages must indicate that sex-change is likely to occur, with a strong tendency towards protandry. It was Miss Needler (1932 b, c) who isolated oysters of a known sex and examined them in the next season. Though but few of her oysters survived, a few of her initially male oysters proved to be female in the next year and a few initially female oysters showed a male gonad. These experiments actually proved that American Atlantic oysters change their sex. In order to determine more precisely the sequence of this change in sex and the histological activities which accompany them Coe (1932 a, 1932 d) examined the gonads of a large number of oysters at frequent intervals during the first two years of their life. His histological studies were made from serial sections. Coe describes the development of the primary bisexual gonad in young oysters, which is transformed into a spermary in the vast majority of individuals. Nevertheless one year old females may be found. Coe proved that these females had not functioned as males before; they had only shown an abortive male phase which precedes the transition of the intersexual gland into an ovary. 3% to 30% of the individuals in different localities may be such one year old females. Sex-change may take place in the interval between two breeding-seasons. Sex-change appears to be more or less facultative in *Ostrea virginica*, for it is an established fact that in at least some individuals the same sex-phase may be retained for several years. Coe assumes the possibility of two genetically distinct types of males, true males and protandric males, the latter changing into females later on.

Although evidence concerning the sequence of sex-change in the extreme southern part of the range of this oyster is insufficient, it seems very probable that sex-change in *Ostrea virginica* takes place far less frequently and less automatically than in *Ostrea lurida* and *Ostrea edulis*. This difference in frequency will have a marked influence on the periodicity of spawning. As a great many oysters will show the same stages of development of the gonads at the beginning of the breeding-season, we shall not find the same degree of overlapping of the various stages of development as in species of oysters with a more frequent sex-change. This implies the possibility of a simultaneous spawning of the vast majority of females in *Ostrea virginica*, which is quite unlike the continuous spawning (albeit with certain maxima) we find in *Ostrea lurida* and *Ostrea edulis* in consequence of their frequent sex-change. GALTSOFF (HIGGINS, 1938) has been continuing sex-change experiments with *Ostrea virginica*. He eliminates the unknown effect of injury (unavoidable when the boring device is used) by using a method which consists in determining the sex of the oyster by inducing ovulation or ejaculation by increased temperature and chemical stimulation.

Ostrea gigas.

Just like other non-incubatory species of oysters *Ostrea gigas* was formerly regarded as dioecius. AMEMIYA (1928 a, 1929) demonstrated the occurrence of a sex-change in this kind of oyster. He used the boring device, separated the oysters according to the sex-stages, isolated them in cages in the sea and examined them again after about a year. The change from male to female as well as the change from female to male was demonstrated in this way. AMEMIYA obtained no data on the frequency of the change in this manner. It is remarkable that the idea of a sex-change cycle or of a certain rhythm of alternation did not occur to this investigator. Though AMEMIYA has not adduced proofs so far, he is inclined to think that "at the beginning of every new spawning season the sex differentiates independently to the sex of the preceding season, so that the sex-change, if it appears, occurs only once in the season or in a year at the very stage when the gonad differentiates sexually". AMEMIYA does not assume protandry in *Ostrea gigas*.

As far as I can see the experiments on *Ostrea gigas* have not yet shown the latter to differ essentially from other kinds of oysters as regards sex-conditions. Until we know more about this I am inclined to assume the occurrence of a sex-change cycle.

VIII. EXTERNAL FACTORS GOVERNING THE PERIODICITY OF SPAWNING

Ostrea edulis

The external factors which have or may have a direct influence on the periodicity of spawning in oysters can be divided in biotic and a-biotic ones.

A-biotic factors

It is an indisputable fact that *temperature* is a very important factor in the biology of *Ostrea edulis*. The regulation of reproduction is to a high degree dependent on temperature conditions, the latter determining in an indirect way the periodicity of spawning during the season of reproduction by governing the frequency of sex-change and by regulating in a direct way the beginning and the end of the breeding season.

The moment at which spawning, that is to say the extrusion of the eggs into the mantle-chamber, occurs in *Ostrea edulis* can be determined by opening fairly large samples of oysters at regular intervals.

Moreover ERDMANN (1934) succeeded in observing spawning in a direct way without killing the oyster. ERDMANN noticed that oysters, placed in aquaria, will spill a few eggs in the spawning-act. In this way he could determine exactly when his oysters spawned, be it under artificial conditions.

ORTON (1920) states that a temperature stimulus of some kind is the normal impulse inducing sexual activity in marine animals, normal biological conditions being assumed. European investigators are agreed on the temperature at which spawning starts in *Ostrea edulis*. The oyster begins to breed at about 15° C and continues to breed as long as the temperature remains above that figure (ORTON, 1920, 1926, 1936, 1937 a, HAGMEIER, 1931, 1932).

This does not imply, however, that spawning always begins at the moment at which this temperature is reached! The course of the temperature during the weeks that precede this moment is of the greatest importance.

Development of the eggs starts probably at about 10° to 12° C (ORTON, 1927 b, HAGMEIER, 1931), and the time required for the maturation of the eggs is most probably mainly dependent on temperature conditions. So the maturation-period of the eggs is a function of time and temperature. If the temperature increases rapidly after a cold spring, we cannot expect maturation to be completed already at the moment when 15° C is reached. Indeed we shall find the first spawning in the new season at a somewhat higher temperature after such a rapid increase

(SPÄRCK, 1925). On the other hand, it is possible that after a relatively warm spring a prolonged temperature between 12° and 15° may be the cause of a spawning before 15° C. SPÄRCK mentions a spawning at 13° to 14° C in the Limfjord in 1921 after such a warm spring. Such data cannot be said to have been clearly specified, however, as it remains to be seen whether the temperatures given are actually averages or merely approximations, maxima and minima not being stated, so that it is difficult for us to judge of the exactness of the conclusions arrived at. MAZZARELLI (1924), on the other hand, did state maxima and minima of the temperature of the bottom-water in the lake of Fusaro (Naples), recorded by a thermograph. His observations make it clear that a considerable spawning is certainly possible there before the maximum temperature reaches 15° C. (N.B. Observations on *Ostrea lurida* (HOPKINS, 1937) have shown that minimum temperatures are the most important with regard to spawning).

ORTON (1927 b) criticizes SPÄRCK's methods of temperature-recording to defend his own 15° C limit, but none the less he states that egg-development is a function of time and temperature! I believe that, this being so, it will certainly render spawning below 15° C possible, it being assumed that 15° C is the average beginning-temperature. Both HAGMEIER (1932) and VOISIN (1933) assume that the maturation of the eggs requires a certain amount of warmth and so is a function of time and temperature. In a recent paper (1936) ORTON admits the probability of the influence of a time-caloric period. The degree at which spawning starts is dependent on the previous course of the temperature and will usually be about 15° C. Of course the oysters that spawn first in the new season are those which have hibernated in an advanced female stage and among these the individuals living under particularly favourable temperature conditions will be the very first. ORTON (1928 a) states that the oysters that spawn first of all are to be found in shallow water, which shows an increase of temperature at an earlier moment. The majority of the oysters mature and spawn in the course of the spawning season.

Lengthy and large-scale operations may make it possible to obtain exact data on the nature of this time-caloric-period, which will enable us to forecast the dates at which spawning begins, if the temperature-conditions at the oyster beds are known precisely. Though this would be interesting, it will never be of such great practical importance as it is in the case of the non-incubatory *Ostrea virginica.* I shall explain below that the beginning of the spawning period of *Ostrea edulis* holds no clue as to the dates at which a maximum of setting may be expected.

Though the periodicity of the swarming of *Ostrea edulis* has been frequently subjected to investigation, especially in France, and the correlation of this periodicity with external conditions has often been studied, there exist but very few papers on the periodicity of the spawning of *Ostrea edulis* during the season of reproduction. Such investigations by means of collecting samples of oysters at regular intervals during the entire season have only been carried out by ORTON, chiefly during 1925 and 1927. ORTON nowhere mentions a *direct* correlation of the periodicity of spawning with the *actual* water-temperature during the spawning season. Such a direct correlation is hardly to be expected, for we know that sex-change governs the periodicity of egg-maturation in the course of the spawning season.

Just as temperature determines the beginning of spawning, it will regulate its end in autumn. We have not many data at our disposal about the end of the breeding season. A gradual decrease of the percentage of oysters carrying young is often observed near the end of the spawning season, often before the temperature drops below 15° C. It may be that a fall of the temperature below the same 15° C level will check reproduction. I believe that the beginning of this interruption in the breeding-process does not set in at a well-defined limit. ORTON's graphs (1936) show that a few oysters, carrying young, may still be found a considerable time after the fall of the water-temperature below the 15° figure, e.g. in the beginning of November 1927. I am in a position to confirm these data by ORTON. In the Zeeland waters I noticed a very occasional gravid specimen long after a fall of the temperature below 12° C; e.g. 14 October 1937, 11 November 1937, 21 November 1938, the last one even with white embryos. The black-sick oyster, found 11 November 1937 contained living larvae of a normal size.

A close study of the diagrams will show the occurrence of an undeniable decrease in the intensity of the swarming activities near the end of the season of reproduction, in spite of the fact that the water-temperature still remains favourable or at least rather favourable. This phenomenon of "weariness" may perhaps be attributed to a protraction of the period of recuperation, which period elapses between a phase of male spawning and the subsequent female reproductive phase. Metabolic conditions are probably among the most important factors governing the length of the period of recuperation.

Mazzarelli (1924) carried out his investigations in the lake of Fusaro near Naples, where spawning often starts in March already, attaining its optimum in the months of April and May. After this the intensity of spawning tends to decrease, although temperature conditions remain favourable. A small percentage of spawning oysters will be found there till late in the summer season. So the longer the season of reproduction, the more conspicuous the phenomenon of "weariness" near the end of the season will be.

If no investigator advocates a direct correlation between the temperature curve and the occurrence of certain maxima in spawning in the course of the breeding season, cannot there be other a-biotic factors governing the periodicity of spawning?

Changes in salinity have never been mentioned in connection with this periodicity, at least not when they remain within normal bounds (Voisin 1933).

A very remarkable paper is that by Orton (1926) on lunar periodicity in the spawning of oysters. During the breeding season in 1925 Orton examined weekly samples of 100 Fal-Estuary oysters of no less a size than about 2 inches. The ages of these oysters were not exactly known and it is doubtful if these samples can be considered as absolutely comparable. The percentage of oysters carrying young was recorded, whitesick oysters with embryos upwards to about 2 days old being recorded apart. No exact records of the water-temperature were made in the course of that season. Orton states that "there is an undoubted, although slightly irregular periodicity in the spawning; the maximum spawning occurs at about the time of full moon" (figure 6).

Orton's graph on the weekly samples shows 3 maxima in spawning during the breeding season in 1925: July 6, August 11 and September 2. The dates of full moon were July 6, August 4 and September 2. Unusually cold weather during August delayed the spawning for about a week, according to Orton.

A simultaneous study of the spawning in „dumpy" oysters did not show this correlation with the moonphases, however. There *exist hydrographical factors which show a rhythmic* variation in accordance with the tidal and lunar cycles and perhaps one or more of these factors may cause a lunar periodicity in spawning. Orton declares that perhaps a thorough investigation of the local hydrographical conditions can tell us something of the nature of the influence of the moon. Nevertheless Orton passes in review beforehand the factors which may possibly be the cause of this lunar periodicity. He thinks for example of the influence of water-pressure, which will show

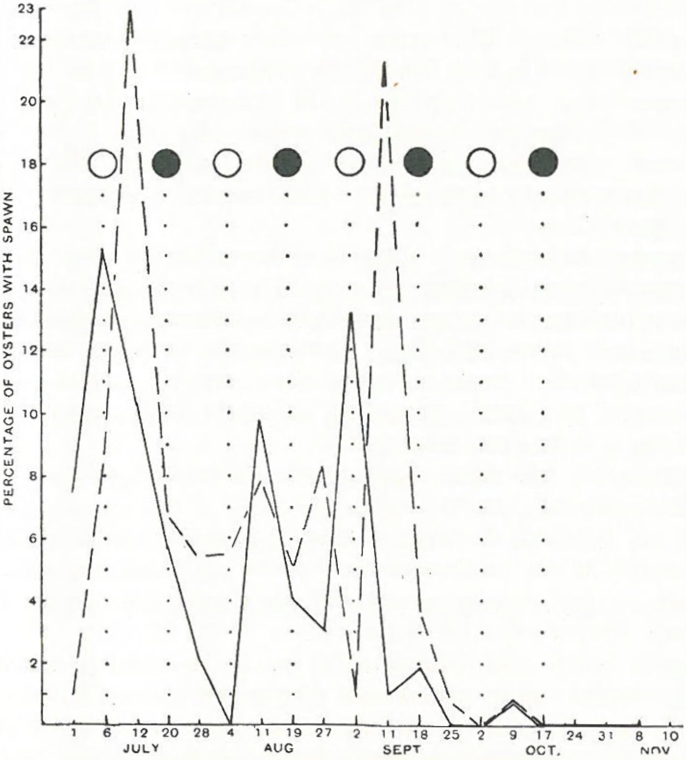

Fig. 6. Lunar periodicity in spawning. After Orton 1926. (Continuous line graph: whitesick oysters with embryos upwards to about 2 days old; dashes: oysters carrying young more than 2 days old).

extreme values during spring tides. It must be remarked that here the most extreme range of the tides occurs about two days after full-moon and after new-moon. Though full-moon spring-tides show a slightly greater range of tide than newmoon spring-tides in this part of the North-sea-coast (Admirality Tide Tables for 1925), I think it hardly conceivable that this relatively minute difference in water pressure between full-moon spring-tides and new-moon spring-tides can be the cause that spawning occurs during a full-moon spring-tide and is not observed during new-moon spring-tides. SPÄRCK (1929) never observed this lunar periodicity in the Limfjord. He thought that this might be attributed to the practical absence of tidal influences there.

Another hypothesis of ORTON is the influence of a variation in intensity and duration of moonlight, which may be thought of as an influence of the moonlight on the rate of feeding and the amount of nourishment! The absence of lunar periodicity in the Limfjord (SPÄRCK 1929) does not give support to this hypothesis, for, although there is no tidal cycle in the Limfjord, there is a moonlight-cycle!

Believers in the direct influence of the moonlight have a fine opportunity to carry out investigations in the breeding tanks, used in England for artificial propagation. In these tanks the influences of the tidal cycle have been eliminated. COLE's diagrams (1939) do not show any lunar periodicity in these tanks during 1937 and 1938, however.

Again another hypothesis of ORTON is the rapid development of the female stage round and about full moon. If, however, spawning takes place at full moon, we must expect the maturation-process to begin at least about 10 days earlier. So this influence of the full moon on the rate of development of the gonad is only conceivable in oysters which are already in an advanced female stage.

This paper (1926) makes the impression of being premature. ORTON even points to a "maximum" of spawning in October, a week after full moon: a maximum of 1 % occurring in a sample of 100 oysters! (fig. 6). As I said before, ORTON records the percentage of whitesick oysters carrying larvae upwards to about 2 days old apart. It will be clear that we are not justified in establishing periodicity in the occurrence of larvae upwards to 2 days old by means of weekly sampling. Lots of young larvae will escape such examination and entire maxima may be overlooked.

ORTON's investigations during 1927 (1936) did not show any lunar periodicity in spawning.

American investigators like NELSON (1928 c) and PRYTHERCH (1929) assume the possibility that changes in temperature are the cause of ORTON's lunar periodicity. During springtides the tide-lands are exposed to a high degree. In fine weather the warming-up of the water will advance rapidly during springtides. But as prolonged fine weather is exceptional on the English and Dutch shores we shall seldom find a temperature curve with neat peaks at every spring-tide. ORTON gives only the air-temperatures for 1925. But it must be remembered that air temperatures do not necessarily show a direct correlation with water-temperatures.

ERDMANN (1934) observed his oysters in tanks, which spawned at any time of the day or night and at any time of the moon's cycle.

In a recent publication (1937 a) ORTON admits that he never observed this lunar periodicity again and that "this phenomenon has not yet been generally confirmed". He states that: "it seems possible that *Ostrea edulis* may tend to spawn normally at full moon or at some other definite phase of the moon in different localities, but that the regularity may be upset by local variations in seasonal conditions".

Although the periodicity of spawning in *Ostrea edulis* has not been investigated much, the periodicity of swarming has been studied more extensively, especially in France and Holland. Although spawning and swarming are separated by the incubation-period, there is certainly some correlation between these two events. From what we know of the periodicity of swarming, lunar periodicity in ORTON's sense is out of the question, as will be discussed below. As long as lunar periodicity in the spawning of oysters has not actually been established by means of a very frequent sampling of oysters of a known age and a known past, with a precise recording of the environmental conditions and if possible by a comparison of natural conditions with the conditions in a basin, I feel inclined to regard the results obtained by ORTON in 1925 as a mere coincidence.

Biotic factors

The possibility of the influence of biotical factors on the periodicity in spawning of *Ostrea edulis* has not yet been demonstrated.

American investigators, especially GALTSOFF, showed by beautiful experiments the occurrence and importance of a stimulation of spawning by means of the sex-products of other oyster individuals, as will be discussed presently.

HAGMEIER (1931) and VOISIN (1933) point to the possibility of such a stimulation in *Ostrea edulis,* although no data to support this hypothesis are as yet at our disposal. ORTON (1937 a) states that "simultaneous spawning may occur in 15 to 20 percent of the population, so that there can be no doubt of the occurrence of a spawning stimulus". We should remember, however, that ORTON's data are based on weekly samples. So it is not certain to what degree a strictly simultaneous spawning in the sense of the American investigations may occur in *Ostrea edulis*, but I believe with ORTON that the marked spawning maxima, which are often observed in *Ostrea edulis*, may point to the occurrence of a spawning stimulus in this kind of oyster.

Ostrea lurida

COE (1932 a, 1932 b, 1932 c) informs us that spawning in *Ostrea lurida* begins at about 16° C and that the season of reproduction continues as long as the temperature is above this level; in casu at La Jolla, in South-California, for about 7 months. HORI (1933) investigated Olympia oysters introduced in Japan. He found that this oysters initiated spawning at 14° C.

These data cannot be said to have been clearly specified, however, for it is not stated whether the temperatures given are actually averages or merely approximations, maxima and minima not being stated.

HOPKINS (1937) thoroughly investigated the spawning activities of *Ostrea lurida* in the Puget Sound. As far as I know these are the most accurate and exact observations of the spawning periodicity of incubatory oysters. In the first place his hydrographical observations have been carried out very extensively. The Puget Sound shows peculiar hydrographical conditions. Most of the bays are generally rather deep and oyster culture is carried out on beds surrounded with low dikes, so that the oysters remain submerged at low tide. On the sloping banks the diked grounds are arranged in terraces. The range of tide during a spring-tide period is about 18 to 19 feet; during neap-tides, however, the range may be only 10 or 12 feet. While the highest water-temperature is to be found within the dikes at low-tide during a period of spring-tides, the highest high-tide temperature may frequently occur in the neap-tide period, for during spring-tides the colder water of the channels in the deeper parts of the bays reaches the oystergrounds at high tide.

A correct estimate of the spawning activity of functional females was obtained by opening 100 adults two or three times weekly throughout the season on several selected typical beds. The oysters were opened on the beds to eliminate a confusion which might arise from spawning or abortion during transport. This mode of sampling has been applied in two bays during four consecutive years.

The water-temperature during the tidal cycle is subject to wide fluctuations (e.g. L.W. 28°, H.W. 18° c). HOPKINS has proved that it is certain that it is not the low-tide temperature which initiates spawning in spring, for on many days preceding the moment of spawning, the water inside the dikes reached 20 or 25° C and remained at this level for several hours. HOPKINS shows by means of fine graphs that, although maximum and average water-temperatures may be different at the moment that spawning begins in different years and in different places, the minimum-temperature will always be 12,5 to 13° C when spawning sets in. Spawning continues at a considerable rate for about six weeks in the Puget Sound. Spawning slowly increases to a maximum, then gradually diminishes until gravid individuals are found only occasionally. Sometimes there is a late secondary wave of spawning. During most years approximately 100% of the adults bear larvae (sex-change!); in 1932 a much greater spawning activity was noticed. "After the minimum water-temperature reaches the critical level for spawning, there appears to be no connection between further spawning and tides".

HOPKINS' diagrams show that there is no direct correlation between the course of the water-temperature and the amount of spawning oysters. No lunar periodicity was noticed in the Puget Sound. "Alternation of sexual phases probably is responsible for the rather slowly developed wave of spawning, for different individuals are at any time in different stages of maturity".

Whether sperm will stimulate the discharge of eggs by functional females has not been demonstrated in this species, but is considered probable by HOPKINS (1937) and COE (1931).

It will be clear that we possess more adequate information about the spawning activities of *Ostrea lurida* than about those of its near relation *Ostrea edulis*. Extensive investigations of hydrographical conditions, including their rhythmical change during the tidal cycle, combined with a frequent examination of samples of comparable oysters (like those of HOPKINS), have not yet been made as far as *Ostrea edulis* is concerned. Neither species shows a close correlation between the actual water-temperature and the waves of spawning in the course of the season of reproduction, it being assumed that the temperature does not fall beneath the critical level. Sex-change determines the degree and the date of spawning in the middle of the season.

Ostrea virginica

It is easier to study the spawning activities of non-incubatory oysters like *Ostrea virginica* and *Ostrea gigas* than those of incubatory species, which spawn more secretly. A few hours after spawning an increase of oysterlarvae in the plankton can be noticed. Moreover in laboratory-experiments to determine the exact moment of spawning use is made of the occurrence of the violent valve-movements during the spawning-act. These movements can be recorded by means of a chimograph (NELSON 1922). *Ostrea virginica* requires a higher minimum-temperature to spawn than *Ostrea edulis* and *Ostrea lurida*. This critical level is 20° C. (STAFFORD, 1913, CHURCHILL, 1920, GUTSELL, 1924, NELSON, 1928 a, b, c, PRYTHERCH, 1929, GALTSOFF, 1930 a, 1932). NELSON (1928 b) complains, that "*Ostrea virginica*, the most valuable mollusc in the world[1]) is barred from most of the otherwise favourable coastlines of the earth, since the waters there rarely attain a temperature of 20° C for a sufficient period to permit spawning, while the inferior species[1]) *Ostrea lurida* and *Ostrea edulis* may thrive there, since much of the coastline of the northern hemisphere rises to 15° C, or slightly above, for the time necessary to permit these species

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[1]) Spacings are mine. So far I have never met a connoisseur of oysters knowing both *Ostrea edulis* and *Ostrea virginica* who asserted that *Ostrea virginica* is the better of the two.

to spawn". We shall see later on that although *Ostrea edulis* will spawn at these low temperatures, a setting of commercial magnitude can only be expected when the water reaches temperatures of 20° C and higher. NELSON (1921) states that the development of sex-products starts at a temperature of about 10° C and that oysters begin spawning when the temperature of the water reaches 20° C. A more detailed study of the gonad showed later on (NELSON, 1928 c) that sperm-production begins at about 10° C, deposition of yolk-material at about 15° C and the definite maturation of the eggs at about 18 to 20° C, though a wide individual variation will always be noticed. In case of a too rapid rise of temperature the critical level for spawning will be reached in advance of the maturation of the gonad (HOPKINS, 1931). Therefore it is always necessary to investigate both the development of the gonad and the phenomenon of spawning (GALTSOFF, 1934). If, in extreme cases, in the northern part of the range, the temperature never, for any length of time, attains or exceeds 20° C, the spawn may not be thrown out at all, but will be reabsorbed by the oyster (NELSON, 1921). The length of the latent period, which extends from the moment 20° C is reached till spawning, bears a definite relation to the slope of the temperature curve. NELSON showed (1927) that spawning of *Ostrea virginica* may be expected to take place approximately 160 degree-hours after the temperature of the water reaches 20° C and remains at this level. Thermograph records of the water temperatures obtained during these "latent periods" in the years 1924, 1925, and 1926 were measured by a planimeter and the area enclosed between the temperature curve and the 20° C line was replotted in the form of a smooth triangle on the same base-line of elapsed time. The areas of these three triangles show a definite relation to the length of the baseline of elapsed time. NELSON computed mathematically that, if the temperature does not rise above 20° C, spawning may be expected to occur approximately 100 hours after the temperature reaches 20° C.

Local conditions may affect the initiating of spawning. So PRYTHERCH (1929) in his study on the spawning and setting behaviour of *Ostrea virginica* near Milford, Conn., concluded that spawning begins when the temperature at high tide reaches 20° C. He considered that the lower pH (7,2) of the water at low tide, when the temperature was much higher, was the factor preventing spawning. At high tide, on the other hand, when the water reached about 20° C and the pH was about 8,2, the oysters spawned.

Spring-tides are a factor in determining the moment at which the 20° C level is reached, owing to the warming-up of the tidal lands during extremely low tides, seeing that the latter may cause a rapid rise of the water-temperature (PRYTHERCH, 1929, 1934 b).

It will be clear that we can only count on a regular rise in water-temperature during spring-tides in a region where prolonged fine weather prevails. On the Northsea coast e.g., with its very changeable weatherconditions, such a constant connection between the water-temperatures and the tides is not to be expected.

GALTSOFF (1930 a, 1932) placed single oysters in tanks (30 litres), controlled factors like oxygen-percentage, pH and salinity, and was able to vary the temperature with the aid of a thermoregulator. The valves of his oysters were attached to a chimograph in order to register valve movements and spawning action. GALTSOFF found it impossible to effect spawning by merely raising the water-temperature as long as it remained below 24, 5°C. The latent period, which had to elapse before spawning begins, showed wide individual fluctuations (22 to 217 minutes). However, *there appeared to be another method of initiating* spawning even below 24,5° C, but above 20° C, viz. the addition of oyster-sperm to the water of the tank! GALTSOFF's fine experiments showed that the action of sperm on a female individual must be considered as a stimulation, for this action shows the all-or-none principle. The minimum amount of sperm causing stimulation proved to be about 150 sperms per cc. of water. Addition of sperm provokes no immediate spawning, but there must elapse a latent period of 6 to 38 minutes, independent of the amount of sperm, before spawning begins. The duration of the spawning-act proved to be 3½ to 74 minutes, likewise independent of the amount of sperm and of the duration of the latent period. GALTSOFF found no difference between the character of a spawning-act provoked by a mere raise of the water-temperature (above 24,5° C) and one by sperm-stimulation (above 20° C). The occurrence of a latent period indicates the indirect nature of this reaction. Sperms of Mya and Mytilus proved to be inactive and collodion filtration as well as a heating to 55° C eliminates the stimulative power of the sperm. Below 20° C no sperm-stimulation was possible.

A single male oyster spawned at about 22 to 23° C by a mere increase of the temperature. Addition of egg-suspension (only eggs of the same oyster-species proved to be active!) provokes a direct spawning reaction without any latent period. This agens is proof against boiling and filtration. It is remarkable that sperm-addition may also induce spawning in male oysters, but in that case a latent period has to elapse before spawning begins. GALTSOFF assumes that the influence of sperm on both males and females operates via the intestinal tract, as is indicated i.a. by the length of the latent period.

It is clear that this chemical stimulation of spawning has a great influence on the periodicity of spawning in *Ostrea virginica.* While single oysters will not spawn until a temperature of 24,5° C is reached, this stimulation may cause the simultaneous spawning of all the oysters in the neighbourhood that contain mature female sex-products at that moment. A few spawning males may initiate a wholesale spawning at any temperature above 20° C. This is the reason why investigators in various places may notice a prolific spawning on one particular day (PRYTHERCH, 1929). Of course the gonads must be at the required state of maturity before spawning will take place. Where external conditions are about the same all over an oyster bed, a simultaneous maturity of a considerable part of the stock may be expected, for no rapid sex-change interferes, as in *Ostrea edulis* and *Ostrea lurida.*

Indeed we may expect a simultaneous spawning on one particular day in such localities where the entire stock lives at an equal depth, so in about equal external conditions, e.g. in Little Egg Harbor (NELSON, 1921), Milford Harbor (PRYTHERCH, 1929) and other shallow bodies of water.

After eggspawning the individual will not respond to any sperm-stimulation for some days (refractory period). When the ripe eggs are spawned out, others pass down from the tubes of the gonad and take their place (no sex-change into the male phase immediately after female spawning in *Ostrea virginica!*), so that the spawning of an oyster may continue over a considerable part of the summer season (NELSON, 1921). So subsequent waves of spawning, likewise induced on one particular day by the action of sperm-induction, may occur when temperature remains favourable. It depends on local conditions whether we can expect more than one wave of spawning. Thus NELSON (1929) states that four main broods of larvae were produced in Barnegat Bay during 1927.

The peaks in the graphs that record the amount of larvae are often found to coincide with those in the temperature curve. Owing to the time required for the development of eggs into shelled larvae, we shall always expect the increase in the amount of larvae some 24 hours after the moment of spawning. Such a correlation of temperature and spawning, though it certainly cannot always be shown, is conceivable, seeing that the individuals that spawn first of all require a mere rise in temperature as a stimulation. This spawning will thereupon induce the chemical stimulation, resulting in the subsequent simultaneous spawning of those individuals which are mature at that particular moment. According to PRYTHERCH (Long Island Sound) a relatively light first spawning during spring tide (rapid rise in water-temperature!) will generally be followed about two weeks later (during the next period of spring-tides, when the water is warmer) by a more general spawning. In other localities spawning conditions are more complicated. When the oyster beds are not situated in shallow water, but when the oysters occur at different depths, it is clear that these oysters do not all live under the same temperature-conditions. In the latter case maturation cannot be expected to occur simultaneously. Oysters in shallow water spawn before those at a greater depth, so that in any body of water where oysters may be found at a depth from 1 to 30 feet and more, spawning will be found to occur over a considerable part of the spawning season. In such places spawning may be prolonged throughout the greater part of summer, with an occasional sharp increase of short duration, e.g. in Delaware Bay (NELSON 1921).

Summarizing we may state that there is much difference between *Ostrea virginica* and the two incubatory species *Ostrea lurida* and *Ostrea edulis* as regards the periodicity of spawning. This difference is not in the first place due to the phenomenon of incubation, but must be attributed to the less rapid and more irregular sex-change in *Ostrea virginica*. This makes it possible for the bulk of females to show the same stage of gonad-development. In such cases a chemical stimulation by means of the mutual sex-products may induce a strictly simultaneous spawning on one particular day. This will be found to be the case in shallow bodies of water. Oysterbeds with a more irregular bottom-configuration, and consequently with different temperature conditions, interfere with this phenomenon and will show a prolonged spawning, with an occasional sharp increase, thus resembling the spawning in *Ostrea edulis* and *Ostrea lurida*, with the difference that in this case the prolonged spawning is not due to the overlapping of the stages of sex-change, as it is in the two latter species!

It is not certain that sex-change has no influence at all on the spawning periodicity in *Ostrea virginica*, especially in the southern part of the range.

Many data on the periodicity of spawning in *Ostrea virginica* obtained both by gonad inspection and observations on the occurrence of oysterlarvae in the plankton can be found in the papers of HOPKINS (1931, Galveston Bay Tex.), NELSON (1917, 1922, 1923 b, 1924 b, 1925 a, 1926, 1927, 1928 a, 1929, 1930, 1931, 1932, New Jersey) and PRYTHERCH (1929, Milford Harbor), many of them illustrated with graphs and diagrams.

Ostrea gigas

Although we do not know very much about the natural periodicity of spawning in this non-incubatory oyster, because no extensive investigations on samples of adult oysters and on the appearance of the larvae in the plankton have been carried out, there are reasons to assume that *Ostrea gigas* acts very similarly to *Ostrea virginica* in this respect.

GALTSOFF (1930 a, 1932) found that by merely raising the water-temperature *Ostrea gigas* will spawn at about 30° C. Just as in *Ostrea virginica* the addition of sperm will induce mature females to spawn at a considerably lower temperature, in casu at about 25° C (GALTSOFF 1930 a, 1932), or perhaps even at lower temperatures (ELSEY 1933). The nature of this stimulation proved to be quite the same as of that in *Ostrea virginica*.

Very interesting is the practical application of this knowledge as described by ELSEY (1933, 1936). Japanese oysters imported into the Canadian Pacific waters near Ladysmith Harbor often fail to spawn there, owing to a too low water-temperature. Sometimes they spawn very late in the season; the result of this is that probably no suitable setting will be obtained and that the adult oysters will begin the winterseason in a bad condition, which is a disadvantage from a commercial point of view.

ELSEY placed a lot of adult oysters in live-boxes and anchored them above the oyster beds on a suitable day as regards temperature and maturity conditions.

High slack water has proved to be the right moment to set to work. The sperm of a few dozens of oysters is shed into the live-boxes. This induces these oysters to spawn. When they have all been put into action, the bottoms of the sinkfloats are opened. Favoured by the practical absence of currents at high slack water, all the oysters in the beds in the neighbourhood are soon stimulated to spawn. So this intervention may result in a good setting as well as a good quality of the adult oyster in the next winter-season.

IX. FERTILIZATION

It is fairly certain that fertilization in non-incubatory species like *Ostrea virginica* and *Ostrea gigas* takes place in the free water after spawning. Fertilization in vitro succeeds easily in these species when sperm is added to seawater containing mature eggs. Relatively little is known about fertilization in non-incubatory species. No recent investigator has advocated self-fertilization.

Ostrea edulis

HOEK (1884) assumed that fertilization takes place *before* the eggs are spawned. Though HOEK occasionally discovered sperms in the renal tubulus of the females, he could never demonstrate a functional spermatheca. HOEK never actually saw an oyster spawning, but he referred to the observations of WAALEWIJN (1885), who declared that he once saw eggs in segmentation, issuing from the genital aperture.

ORTON (1927 a) continued the studies of HOEK and searched for a spermatheca. He concluded that the precise situation of a functional spermatheca has not yet been defined. He thought it incredible that such a spermatheca, in which sufficient sperm to fertilize about a million eggs of a female oyster can be stored, will ever be found in *Ostrea edulis*. ORTON observed that sometimes a fairly considerable percentage of the eggs of *Ostrea edulis* appear to be unfertilized, which indicates that sperm has not been abundant enough at the right time.

SPÄRCK (1925) states that he once found unsegmented eggs in the mantle-chamber of an oyster. ERDMANN (1934) kept his oysters in tanks. He could observe the exact moment at which his oysters executed their secret spawning-act, for a few eggs are always spilt by a spawning oyster, which eggs could be detected on the bottom of his tanks right under this oyster. ERDMANN concludes that the eggs are fertilized after spawning and that the sperm is brought into the mantle chamber (where the eggs are held) with the water pumped up by the gills. ERDMANN succeeded in observing microscopically the act of fertilization in eggs found on the gills immediately after spawning: "Oft konnte ich diesen Vorgang (spawning) direkt, und bei sofortiger, mikroskopischer Untersuchung sogar noch den Befruchtungsakt beobachten". Yet there have been writers in recent years (e.g. BIERRY and GOUZON, 1939, BORDE and BORDE 1938) who assume that fertilization takes place before spawning. The sperms are assumed to enter the mantle chamber with the inhalent water and thence to pass through the gill-slits into the genital aperture, which procedure would render a spermatheca superfluous. However, I have never yet come across a recent description of observations on fertilized eggs in the ducts of the gonad.

Though it has not been proved that fertilization before spawning never occurs, I am inclined to think that the difficulty in finding unfertilized and unsegmented eggs on the gills (owing to the very short duration of the unfertilized and unsegmented period after spawning) has been the main reason to assume fertilization before spawning. I am inclined to attach more value to the accurate observations of ERDMANN and SPÄRCK than to the vaguer and more speculative data of earlier writers.

In non-incubatory species the sperm will find the eggs in the free water, most probably through chemotaxis, as has been proved to be the case in so many other acts of fertilization. It would be interesting to know to what degree chemotaxis plays a part in fertilization in incubatory oysters. How does the sperm reach the mantle chamber: is it carried along with the watercurrent brought about by the action of the gills, or does it reach the chamber on its own account through mere chemical allurement? I assume that the speed at which the sperms move on is far too little for them to be able to counteract watercurrents of some importance, such as the current brought about by the action of the gills and the tidal currents in the free sea-water. So I am inclined to believe that the first part of their way to the eggs will be covered passively, the sperms being carried along with the inhalent water. After the mantle chamber has been reached, the sperms on the latter part of their way may be guided by chemical allurement. I have to remark, however, that so far no experiments have been made to determine the rate of flow of inhalent water in oysters during incubation.

Ostrea lurida. Ostrea denselamellosa

Fertilization after spawning and the entrance of sperm with the water-current caused by the gills is assumed likewise for *Ostrea lurida* (Coe 1931, 1932 c) and *Ostrea denselamellosa* (Senô 1929).

X. INCUBATION

The eggs are held in the mantle or branchial chamber adjacent to the gills and labial palps, where in incubatory species of oysters they develop for a considerable period. It is clear that the inhalent stream of water will keep them in their place on the gills during the period of incubation.

Ostrea lurida

Stafford (1914) was the first to study the duration of the incubation period in *Ostrea lurida*. He would periodically prise the valves of a gravid specimen partly open and take a sample of the larvae. Such handling of the specimen may, however, easily cause a disturbance of the normal function and interfere with larval development. Stafford computed that the duration of the incubation period of *Ostrea lurida* in the waters of British Columbia is about 16½ days. Coe (1931) estimated that the larvae of this oyster develop normally for a period of about 10 to 12 days in Southern California.

Hopkins (1937) moreover used the data yielded by his frequent samplings of oysters during several seasons of reproduction to fix the duration of the period of incubation. As a single brood was found to consist of larvae at approximately the same stage, within relatively narrow limits, Hopkins found it possible to organize the results in such a way that the gravid specimens, bearing broods at the same stage of development, could be grouped and thus followed through the various stages, as in subsequent samples one group would continue to recur until the larvae reached the size at which they are discharged. HOPKINS concluded that the normal duration of the incubation period is about 9 to 11 days, which accords with COE's estimate (1931). STAFFORD's 16½ days' period may be correct for the locality in which *he* worked, or it may due to his method of analysis.

Ostrea edulis

What do we know about the duration of the incubation period in *Ostrea edulis*?

ORTON (1926, 1936, 1937 a) estimated that under natural conditions oyster larvae are retained in the mantle cavity for a period of about 7 to 10 days from the date of spawning. He founded this estimate on a comparison of his graphs on the percentage of oysters containing white and conchiferous larvae. We should remember, however, that ORTON sampled only once a week. So HOPKINS (1937) is quite right when he states: "Apparently his samples were not taken with sufficient frequency to permit analysis in the manner described above. Nevertheless it is probable that the period of larval development within the maternal brood chamber is not greatly different in the two species".

Further information was obtained partly by isolating individuals carrying embryos or larvae at a known stage and observing the subsequent stages by opening the shells or inducing the oyster to throw out his larvae and partly by taking embryos and larvae from the parent and keeping them in dishes at known temperatures; but development tends to become irregular under artificial conditions. ORTON states (1936): "The whitesick stage is thus normally of about 3 to 3½ days duration, the grey-shelled stage about 1½ to 2 days or less, and the black-sick stage of variable duration, probably 4 days or less".

SPÄRCK (1925) collected some data on the duration of incubation during his regular gonad-examinations of oysters which were kept in tanks in his sex-change experiments. Though, apparently, his oysters did not live under natural conditions, he concluded that the first part of the development (till the black-sick stage) requires about 3 to 4 days and that the second part of incubation requires about 5 to 6 days at a temperature of 14° to 16° C and about 2 days at an temperature of 18° to 20° C.

ERDMANN (1934) likewise kept his oysters in tanks under controlled temperature-conditions. At a temperature of about 13° to 14° C his oysters proved to incubate the larvae for about 18 days. At 17° to 18° C for about 14 days, and at 23° C for about 6 to 8 days. ERDMANN stated that low temperature will not only delay swarming to the above-mentioned extent, but that it will also cause these larvae to show larger dimensions and a more advanced stage of development at the moment they are discharged. If this phenomenon occurs regularly in nature, continued bad weather will cause a delayed swarming and an increasing size of the freshly spawned larvae with a decreasing water-temperature. We shall see in the next chapters in how far, under natural conditions, this assumption corresponds with the facts.

XI. THE PERIODICITY OF SWARMING

STAFFORD used the word "swarming" to designate the final release of the larvae from the maternal brood-chamber in contrast with the original spawning whereby the eggs are released from the gonad. It has been remarked before that swarming is only a delayed completion of the spawning process.

Ostrea lurida

The Olympia oyster has not yet been taken in the very act of swarming. Even HOPKINS (1937) declares that he does not know whether the discharge of the larvae is accomplished in the same manner as the discharge of the eggs in oviparous oysters. As HOPKINS observed that during abortion the embryos are forcibly ejected by violent shell movements, he is inclined to believe that natural swarming takes place in about the same manner.

The assumption of BONNOT (1936), who presumes that the larvae leave the mantle cavity of the adult in the outgoing water current, must be wrong, for this would require a passage of the developed larvae through the gill-slits, as the outgoing water-current comes from the exhalent chamber (suprabranchial = cloacal chamber) and the larvae develop in the inhalent chamber (= branchial chamber). The amount of larvae of *Ostrea lurida* in the water has not been determined quantitatively. Observation of the increase of young oysterlarvae in the water is the only exact way to determine directly the rate of swarming. Now HOPKINS computed the periodicity of swarming from the graphs on the percentages of oysters incubating larvae, so that his data are indirect ones.

As changes in the percentage of oysters carrying larvae in an advanced stage of development may be attributed to losses owing to swarming as well as to gains owing to a further development of larvae of younger stages, it is always difficult to estimate the actual extent of swarming only from such graphs on the percentage of oysters carrying larvae.

I did not come across any discussion of a possible influence of external conditions on the rate of swarming in *Ostrea lurida*.

Ostrea edulis

As far as I know only ERDMANN (1934) has described observations of the act of swarming in *Ostrea edulis*. His swarming oysters, kept in tanks, opened their shells widely, and then ejected a cloud of larvae by a violent contraction of the adductor muscle. This action was repeated several times, with longer or shorter intervals. The entire swarming-act may be accomplished in a few hours. ERDMANN states that he made this observation more than once. Although this action is essentially the same as that by which oviparous oysters discharge their eggs, there is some difference in the rate at which it is accomplished. Instead of a succession of rapid rhythmical closing movements, as can be observed in *Ostrea virginica* (NELSON, 1922), ERDMANN observed several intermittent violent shell-movements, spread over a fairly considerable lapse of time.

Indirectly some figures about the periodicity of swarming can be computed from data on the percentage of oysters carrying larvae. ORTON's graphs on such data about *Ostrea edulis* (1926, 1936) make an approximate computation even more difficult and less reliable than the same kind of data from HOPKINS' investigations on *Ostrea lurida* (1937), because of ORTON's less frequent sampling. ORTON does not mention any direct influence of external conditions on the moment of swarming. He confines himself to the statement (1937 a) that the larvae are retained longer in cooler and shot out earlier in warmer conditions, which may be understood from the influence of temperature on the duration of the period of incubation, as has been discussed before.

Figures about the periodicity of swarming in *Ostrea edulis* can be obtained directly by means of quantitative and semi-quantitative studies on the amount of oysterlarvae in the plankton. PETERSEN (1908) stated that oysterlarvae can be found in the water already at 15° C. German investigators (KÄNDLER, 1928, HAGMEIER and SCHUBERT, 1930) made some observations on the amount of oyster larvae in the plankton after the sowing-out of a certain amount of Dutch seed-oysters near List in the Wattenmeer. Their method of sampling can hardly be called quantitative. Detritus often stopped up the meshes of their plankton-net. Moreover the sampling took place only a few times during the season, with fairly long intervals. After swarming they noticed many young larvae in the neighbourhood of the sown-out mother-oysters. An observation on a profuse swarming shortly after a period of stormy weather led to the conclusion that unfavourable weather prevents swarming and that the mother-oysters await more favourable circumstances before they eject their larvae. In 1931 and 1932 we still find HAGMEIER expressing the same opinion: "Es findet dann, wie dies auch von KÄNDLER im Freien beobachtet wurde, beim ersten Eintritt günstiger Verhältnisse ein massenhaftes Ausstoszen von Brut statt".

I do not believe that a single observation on swarming after a period of bad weather justifies the conclusion that oysters await favourable weather to eject their larvae. More frequent and better quantitative sampling is necessary if we want to obtain adequate information justifying such a conclusion.

More or less frequent sampling of plankton has been carried out in the most important French centres of oyster-production since 1921, with a view to forecasting the setting. Appreciation should be expressed for the inestimable initiative of the French investigators, who have been the first in Europe to try to build up a regular advisory service in order to render assistance to the oyster farmers in raising spat-production. LAMBERT (1935) describes the development and the methods of these investigations. Many publications of these investigations contain graphs showing the amount of oyster larvae in the plankton. Water-temperature and salinity are recorded likewise: LEENHARDT (1924), BOURY (1928, 1929 a, 1930), RAPHENNE (1931), VOISIN (1931, 1932), HERMAN (1935, 1936, 1937, 1938 a, 1938 b), BORDE (1929, 1930, 1932, 1935, 1936, 1937), LADOUCE (1938 a, b). (The latter two at Arcachon, the former at le Morbihan). I have already described the methods used by these French investigators in a previous chapter. I have stated that the towing of a plankton-net cannot be considered an ideal quantitative method of sampling, and it is especially unsatisfactory for the special reason that it does not enable us to estimate, even approximately, the volume of filtered water. Many of these graphs on the amount of larvae show a very steep course.

It is noteworthy that such a steep course appears especially in those graphs that reflect the greatest frequency of sampling. The less frequent the sampling, the more quiet the course of the graphs generally is.

So the graphs of LEENHARDT (1924), for instance, are "quiet" probably mainly in consequence of *infrequent sampling*.

An extreme of the other kind is presented by the steep graphs on the larvae in the river d'Auray in 1935 (HERMAN, 1936). A steep course does not necessarily mean, however, that the method of sampling is quite unsuitable, for graphs on really quantitative samples may show such a steep course as well.

When we collect plankton-samples right above the place where the larvae-producing mother-oysters live, we may expect to find enormous amounts of young larvae during swarming. A sample taken a few days later in the same place, under the same tidal conditions, may contain but a comparatively small fraction of such an amount. This does not point to an enormous mortality, but it only means that the young larvae are distributed more uniformly over a larger volume of water now. It is clear that a graph on the number of larvae per unit of water near the oyster beds will show a much steeper course than a graph on samples taken at some distance from the place where the young larvae are set free.

Consequently very steep graphs may be due to the local occurrence of larvae that have been liberated very recently as well as to an unreliable method of sampling. Larvae which have been in the plankton already for several days, however, will be distributed more equally than these newcomers. So if we find that a graph on the amount of older larvae in the same water is likewise very steep, our method of sampling is most probably not quantitative. When, however, the amount of older larvae remains fairly constant in the daily samples, coinciding with enormous variations in the amount of young larvae, we may conclude that our method of sampling is all right and that swarming takes place.

It is a pity that the graphs of the French investigators indicate only the total amount of larvae in the samples, so that we cannot see from these graphs the degree of variation in the amount of older larvae; consequently there is no possibility of checking the reliability of their method of sampling.

Partly the steep graphs will no doubt be caused by the proximity of adults ejecting young larvae. A less steep graph may be the result of sampling in a place fairly remote from the swarming centre as well as of a less frequent sampling! That such steep graphs do not necessarily point to a tremendous mortality, but that they may be due to a dispersion of the newly-liberated larvae, is not always realized by the French investigators. So BOURY (1928) declares: "De l'existence des variations assez brutales dans les quantités de larves nageantes, on déduit que celles-ci périssent en grand nombre".

In their commentaries on the graphs the French authors sometimes declare that the peaks of the temperature-course coincide with peaks in the course of the amount of larvae, from which they concluded a direct influence of the temperature on the rate of swarming (BORDE, 1935, 1936, LEENHARDT, 1924). LEENHARDT even states that swarming is a direct function of temperature: "Nous disions (1922) que l'huître peut pondre à partir de 15° (si les mères ont suffisamment incubé); nous devons ajouter que la ponte est fonction de la température, et subit, à peu de chose près, les mêmes variations. Le nombre de larves libres augmente avec la température". VOISIN (1933) still states that no considerable emission of larvae will take place below 18° C.

A close study of the graphs in all these papers will make it clear, however, that a correlation between the actual water-temperature and the periodicity of swarming certainly does not exist! Though sometimes a considerable swarming will be observed during high water-temperatures, there are a great many data from observations of swarming at much lower temperatures, even below 18° (BOURY, 1928; Cuhan en '27). So LEENHARDT's conclusion has not been confirmed by the numerous data from subsequent French investigations.

Holland

In the course of my investigations in recent years I have obtained much information about the periodicity of swarming in the

Oosterschelde. The frequent sampling (daily at the height of the season), at the same stage of the tide, of known quantities of water make it possible to get a good impression of the amount of oyster larvae in the two places where the samples are collected. At first I used to record my data in the form of a graph on the total amount of larvae in 100 litres. There soon appeared to be a marked difference in character between our two sampling-stations. Though the average number of larvae did not differ very much, the two graphs differed greatly in the steepness of their course.

The reason of this will be clear, for the first station (Yersche Bank) is situated in the middle of the most important oyster-beds of the Oosterschelde and may be considered to represent the centre of larvae-production, while the second station (Kattendijke) is situated fairly remote from the important oysterbanks, and receives its larvae by the tidal movements. At the first station the occurrence of swarming will be observed immediately. On days of swarming we shall notice high peaks in the course of our graphs, while a rapid decline may be found the next day owing to the dispersion of these young larvae through a larger volume of water. At the second station,

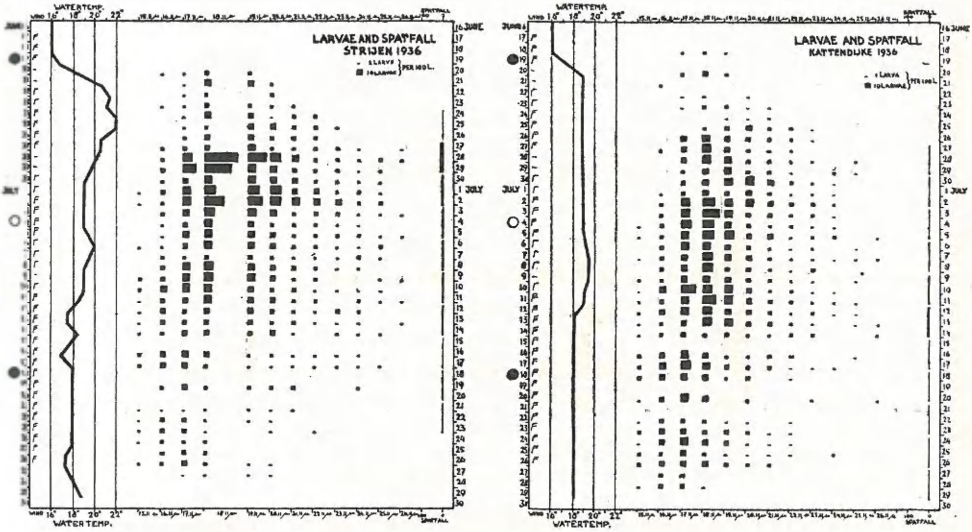

Fig. 7. Larvae and spatfall. Strijen and Kattendijke 1936.

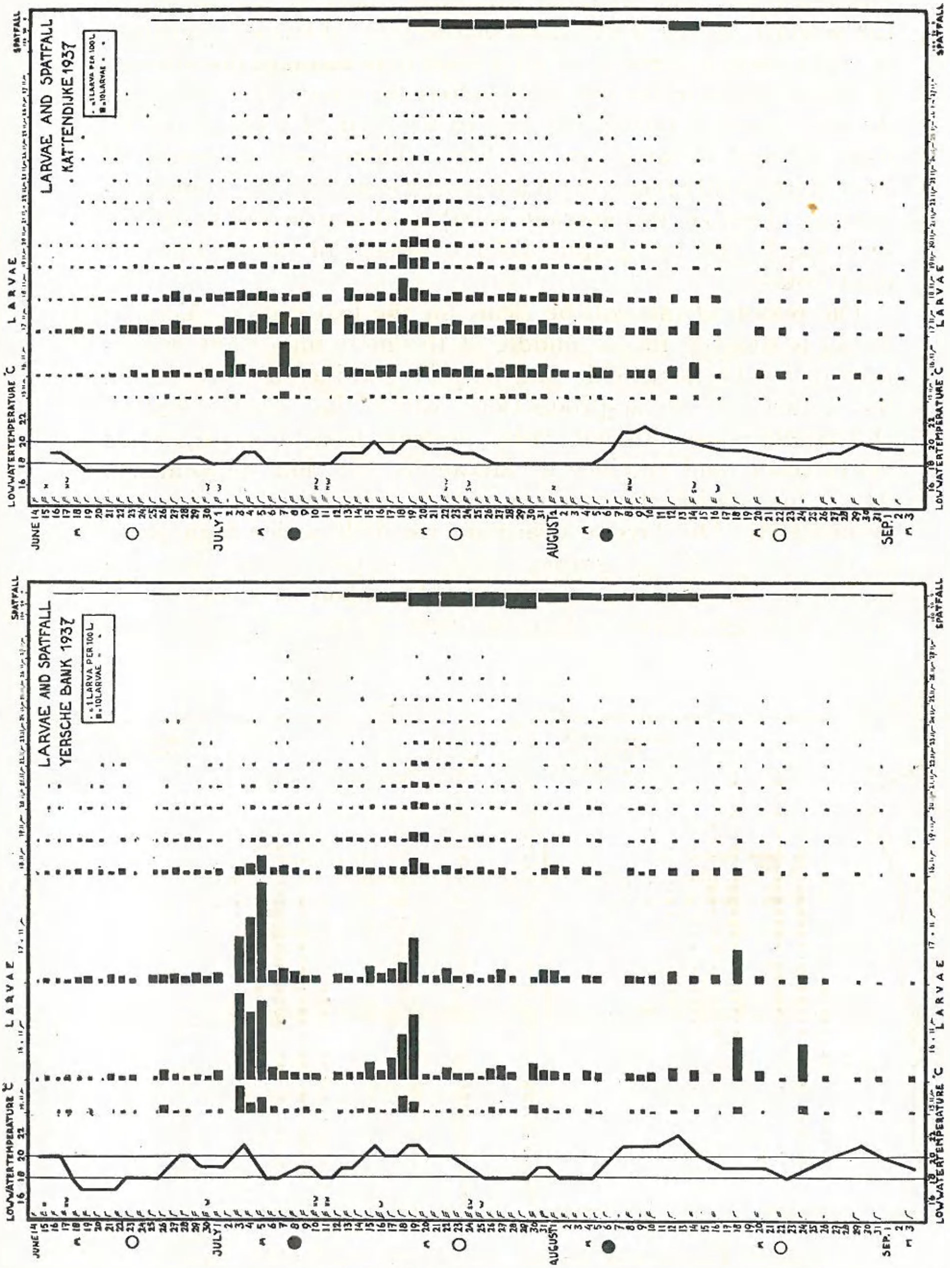

Fig. 8. Larvae and spatfall Yersche Bank and Kattendijke 1937.

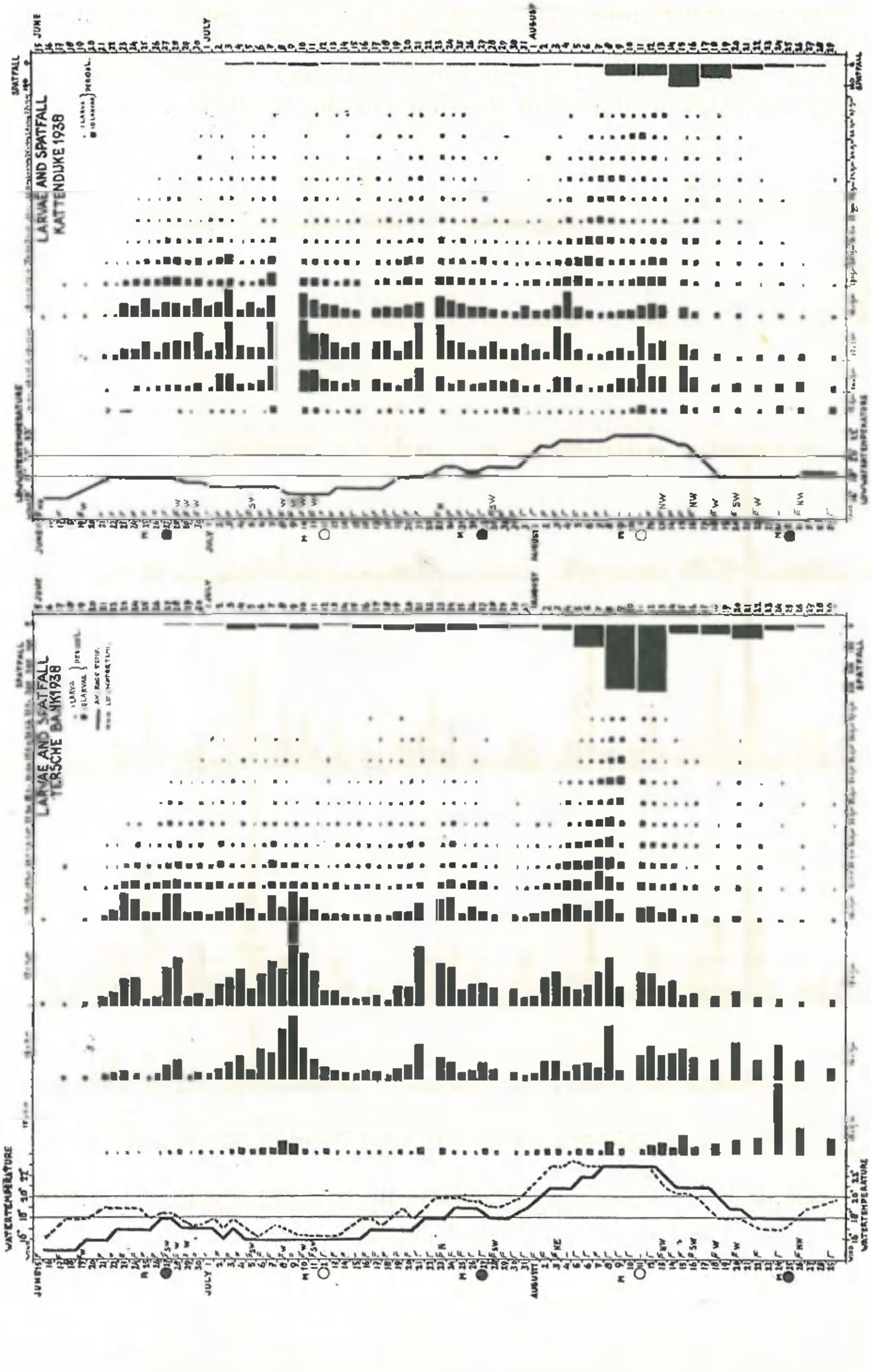

Fig. 9. Larvae and spatfall Yersche Bank and Kattendijke 1938.

however, things take place more gradually. We shall seldom observe sharp increases there. The number of larvae will rise fairly gradually after days on which swarming of some importance has taken place and decreases in the number of larvae

Fig. 104. Larvae and spatfall Yersche Bank 1939.

will also take place gradually. It will be clear that we shall have to focus our attention on the data of the centre of larvae-production, in studying the periodicity of swarming.

These data are exhibited in the figures 7, 8, 9, 10.

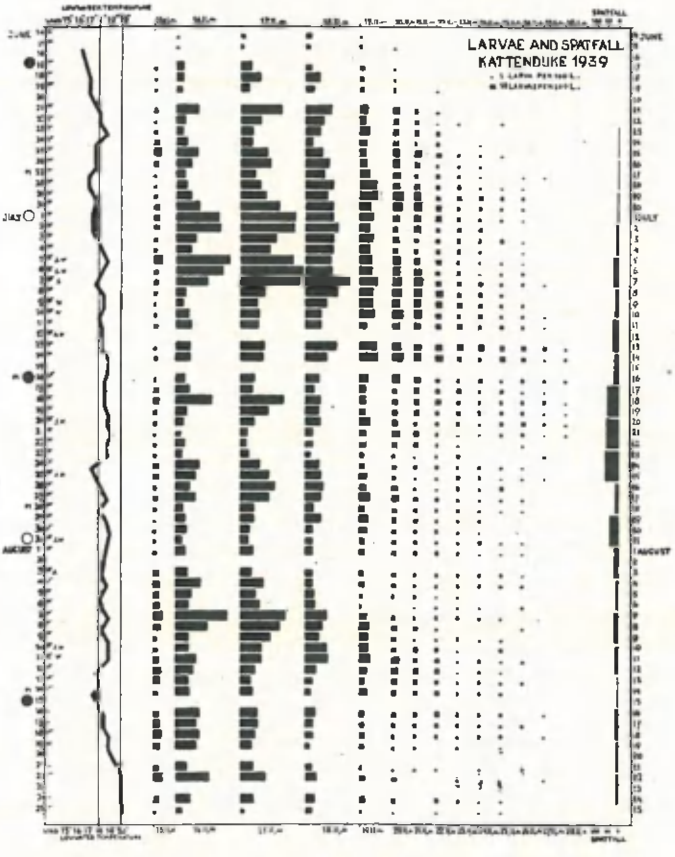

Fig. 10*b*. Larvae and spatfall Kattendijke 1939.

I succeeded in finding a kind of diagram that made it possible to record the number of larvae per unit of water, the size of these larvae, the setting of spat, and some a-biotic factors, such as water-temperature, wind and the phases of the moon. I devided the larvae in groups according to the size of their shells. One degree of my micrometer corresponds with 11 μ in the combination of lenses used, so each group consists of larvae 11 μ larger on an average than those of the preceding group. The diagram on the number of larvae at the Yersche Bank (centre of larvae-production) shows many marked increases in the number of young larvae, which points to swarming. The swarming is not limited to a few days, but is distributed over several weeks, showing more and less important peaks.

I want to point out, however, that we are not justified in deducing the absolute value of swarming directly from the amplitude of the number of newly-liberated larvae in the diagrams. The degree of proximity of the liberating adults will not always be the same. When we want to compare the values of swarming we must consider likewise the number of larvae during the next days and the subsequent increase of larvae in a more distant place, like Kattendijke.

These diagrams do not reveal any marked direct correlation between the periodicity of swarming and the course of temperature. Sometimes intensive swarming occurs during a high water-temperature (e.g. July 3, 4, 5: 1937, July 18, 19: 1937, early in August 1937); sometimes during an increase of the temperature (e.g. August 18: 1937) and sometimes during relatively low water-temperatures, even below 18° C (e.g. July 8, 9: 1938). I want to emphasize the heavy swarming during the days early in July 1938, when the mean water-temperature (recorded by a thermograph) was 16° C and the maximum temperature during low-water did not reach 18° C! This important swarming under these temperature conditions proves that the opinion of LEENHARDT (1924) and VOISIN (1933), that no important swarming will be observed at lower temperatures, does not hold good for the Oosterschelde. MAZZARELLI (1924) stated likewise that considerable swarming may occur before the water reaches 18° C. The view of the German investigators, who assume that the young larvae are held in the mantle-chamber till the weatherconditions are more favourable, does not hold good either. For not only did low water-temperatures occur during swarming in July 1938, but also stormy weather.

It is my belief that swarming will take place when the larvae have been sufficiently incubated. So the periodicity in swarming is mainly governed by the periodicity in spawning.

The period of incubation may be protracted somewhat by lower and shortened somewhat by higher water-temperatures, but as the influence of temperature on the periodicity in spawning is rather complex, the periodicity in swarming cannot possibly be a simple function of the actual water-temperature.

There is *no direct correlation* between the dates of swarming and factors like the actual water-temperature, the strength of the wind, rain, air-pressure, etc. The salinity varies but very little in the Oosterschelde and as these variations do not coincide with swarming, I do not believe that they have any influence on the periodicity of swarming in the Oosterschelde.

Is there perhaps some influence of the phases of the moon, either in a direct way by regulating the dates of swarming; or in the way assumed by ORTON, by regulating spawning and thereby causing a swarming maximum about 7–10 days after full-moon?

It is evident that swarming is not limited to the days of full and new moon or to the days, immediately following these phases, on which the spring-tides occur. This has never been observed in France either. Many swarming-maxima occur about 10 days after one of the extreme phases of the moon, e.g. 3–5 July 1937, 18–19 July 1937, early in July 1938, 21–23 July 1938, early in August 1938, 25–26 June 1939, 11 July 1939 and 12 August 1939, which points to the possibility of spawning-maxima during these extreme phases.

I would, however, emphasize the fact that, even if a maximum of spawning during the extreme phases of the moon (so during spring-tide) should occur, there is certainly no difference between the full-moon spring-tide and the new-moon spring-tide. So I can not bear out ORTON (1926), who assumes a maximum of spawning during full moon only. I dare not yet decide if the periodicity of swarming in the Oosterschelde justifies the conclusion that most of the spawning occurs during the spring-tide. My data show, however, that spawning certainly is not limited to spring-tides, and in no case to the full-moon spring-tide. Moreover the diagrams show that spawning, which most probably occurs about 7–10 days before swarming, does not require high water-temperatures. We may state, on the contrary, that often a profuse spawning must have taken place during fairly low water-temperatures.

So summarizing our knowledge about the periodicity of swarming of *Ostrea edulis*, especially based on the observations in the Oosterschelde and on the graphs of the French investigations, I can state that:

1. Swarming is not a simple function of the actual water-temperature. The graph on the number of free-swimming larvae does not run strictly parallel to that on the water-temperature.

I would have it clearly understood, however, that this does not preclude all influence of the water-temperature on the periodicity of swarming. This influence is of a rather complex nature, however, as has been discussed in previous chapters. The mother-oysters do not await favourable weather before ejecting their larvae.

2. Slight variations in salinity apparently have no influence on swarming.

3. Swarming takes place during fine weather as well as during rainy weather. Strong winds do not prevent swarming.

4. Swarming is dependent on the periodicity of spawning. The duration of the period of incubation will vary to a certain degree with the water-temperature.

5. Spawning is not limited to the full moon spring-tide in Holland and France. There are indications, however, that a considerable part of the spawning tends to be concentrated at both of the springtides.

6. Spawning does not exclusively show its maxima during very high water-temperatures.

7. The periodicity of spawning is governed mainly by the periodicity of sex-change, which is in its turn dependent on temperature and probably on metabolic conditions.

It is not yet known if a chemical stimulation of some kind plays any part by causing a strictly simultaneous spawning of that percentage of oysters which is at that very moment ready to spawn. Temperature-conditions being propitious, we may expect 100 % or even more than 100 % of a population of *Ostrea edulis* to function as females every year, even in the countries around the North-Sea.

So far the only reliable method to record the rate of swarming is a frequent observation of the number of oysterlarvae in the water. I consider a frequent examination of fairly large samples of adult oysters in order to find the measure of spawning less suitable to ascertain the periodicity of swarming, for we shall always have to take into account that oysters at different levels of the beds, so living under different conditions, and oysters of different age-classes, will probably show a different periodicity of spawning. By observing the number of larvae per unit of water this difficulty may be eluded.

No investigator in Europe has succeeded so far in deducing a reliable mathematical formula, exclusively built up of easily observable factors, such as water-temperature, for the purpose of forecasting swarming. Such a formula would render the time-consuming plankton-investigations superfluous.

In view of the complexity of the processes which precede swarming and the fitful weather-conditions on the Atlantic coasts of Europe, I do not expect that such a really reliable formula will soon be found.[1])

XII. THE EXTENT OF THE ANNUAL PRODUCTION OF LARVAE

The total amount of larvae which is produced annually on certain producing grounds is not always the same. There are several factors which govern the quantity of this production and it is interesting to find out to what degree these factors vary in the Oosterschelde. Some of them will be fairly constant, while others will show wide fluctuations. The latter will be mainly responsible for differences in the annual production of larvae.

These factors are:

a. The age at which oysters reach sexual maturity in the localities concerned.

b. The percentage of oysters participating in female reproduction every year.

c. The number of larvae produced by one oyster in the female reproducing phase.

d. The number of sexually mature oysters on the producing grounds and the proportion of the strength of the age-classes of which that number is composed.

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[1]) In a recent paper LOOSANOFF (1939) tells us about observations of a spawning by *Ostrea virginica* at water-temperatures below 20° C. Even the maximum bottomwater-temperatures in the entire region remained below this figure. These observations show that even the methods for predicting the time of spawning in *Ostrea virginica* are not yet infallible.

I believe that the age at which the oyster reaches sexual maturity will be fairly constant in one special locality, at least in localities like the Oosterschelde where the yearly differences in temperature are not very great. In waters where female reproduction takes place only at a fairly advanced age we need especially these older oysters to obtain an adequate production of larvae, while in other localities younger oysters will play an important part in reproduction, which is an advantage of paramount importance.

At what age does *Ostrea edulis* reproduce as a female?

Investigators in the South of France (Arcachon) stated that oysters of one year old, so in their second summer, are often found to carry larvae there (GERBE 1876, DANTAN 1913). DUPAIN (1932) is of opinion that the production of larvae by one-year old oysters, attached to the collectors ("piquetage") used in the estuary of the river Charente (farther north in France) is by no means negligible.

ORTON (1937 a, 1922 a) states that after exceptionally fine summers, like that of 1921, the female phase may be reached in 12 months in the English waters, but even there only exceptionally. This is confirmed by DODD, etc. (1937), who in 1936 detected some oysters containing larvae amongst a number of selected well-grown oysters, born in the fine summer of 1935.

SPÄRCK (1922, 1925) stated that in the Limfjord he never found an oyster carrying larvae in his second summer and that even the completion of egg-development in the course of the third summer is exceptional. As a rule the oysters in the Limfjord start female reproduction in their fourth summer, so when the animal is three years old. It will be clear that such a great difference between the ages at which the oyster reaches maturity in France and Denmark will govern the extent of the production of larvae to a high degree.

I examined many oysters in the Oosterschelde (Holland) and I observed that we may find a small percentage of one-year old oysters carrying larvae in their second summer (1937, 1938, 1939). It is not necessary for the preceding summer to be exceptionally fine, for even in 1937 (after the cold summer of 1936) I detected some one-year old oysters with larvae.

As it is, I consider the share in larvae-production of these one-year old oysters of so little importance, not only because of the small percentage participating, but also in view of the small number of larvae in each of those oysters, that their influence on the total number of larvae produced is not likely to be of any importance in the Oosterschelde.

A discussion of the percentage of oysters participating in female reproduction is to be found in the sections on the phenomenon of sex-change.

There are many data available on the percentage of oysters that carry larvae simultaneously. The greater the frequency of sampling, the more valuable these data will be. From the latter the approximate percentage of oysters participating in female reproduction in one special year may be computed, especially when the frequency of sampling is fairly high and a reliable sampling of comparable oysters is carried out. However, I want to emphasize that such investigations will only yield data concerning the age-class from which samples are collected. Simultaneous sampling from several age-classes and in several places will be necessary if we want to form an adequate idea of the extent of the spawning-activities in the entire population.

SPÄRCK (1925) states that in the Limfjord about 14 % of the mature oysters may be expected to carry larvae simultaneously in the beginning of the season. This percentage decreases as the season advances.

HAGMEIER (1916) at List found about 10 to 15 %, that carried larvae simultaneously. ORTON (1926, 1928 a) states that in the English waters the maximum percentage of oysters carrying larvae simultaneously is usually about 25 % at the height of the season. Once he even recorded 33 %. ORTON computed that especially during fine summers more than 100 % of the adult oysters will function as females in the English waters.

HOPKINS (1937) investigated the closely related *Ostrea lurida* in the Puget Sound and found that a percentage of 15 to 20 % oysters carrying larvae simultaneously is common there, though peaks of 35 % and even 45 % may be found, especially in the beginning of the season.

Though I did not carry out frequent samplings of oysters in order to examine the percentage carrying in the Oosterschelde, I made some observations during the summer season on the percentage of incubating oysters, examining native oysters as well as oysters imported from France. I carried out these investigations in order to find out if subsequent swarming of some importance was still to be expected; for instance, after the failure in setting of the larvae of the first important maximum of swarming in the beginning of July 1938. In that particular case I forecast subsequent swarming, for both the native and French oysters proved to be carrying larvae from 20 to 25 % on July the 15th (1938).

Very interesting are the data obtained in 1939. As may be seen from the diagram (fig. 10 a) heavy swarming occurred on June 25/26 and 29/30, July 5, 11 and 24 and August 7, 11/12 and 22. Moderate swarming took place on June 19 and August 4. Of less importance is the swarming on June 13.

In 1939 I ascertained three times in samples of 50 oysters the percentage of oysters carrying larvae, each time in the same three parcels of oysters. One parcel consisted of native oysters of about 50 kg a 1000, the second parcel of newly-imported French oysters of about 25 kg and the third parcel of French oysters imported the year before (1938), weighing about 45 kg.

I recorded:

	Natives			French 1939			French 1938		
	white	black	total	white	black	total	white	black	total
June 20	20%	22%	42%	14%	4%	18%	6%	8%	14%
July 12	4%	6%	10%	4%	0%	4%	4%	0%	4%
August 2	6%	4%	10%	16%	8%	24%	20%	0%	20%
			(62%)			(46%)			(38%)

Though the samples contain but 50 oysters I will yet assume that these figures do not deviate too much from the real percentages. The examination of 12 July showed smaller percentages, which is in accordance with the scanty swarming during the days following on the 12th of July. The smaller percentage of black-sick oysters on the 2nd of August is in accordance with the moderate swarming on August 4, while the higher percentage of white-sick oysters on that date accounts for the heavy swarming on the 7th of August.

It will be clear that the incubation actually observed during these investigations can, on a liberal calculation, only have contributed to the swarming-maxima on June 25/26 and 29/30, August 4, 7 and perhaps also to that on August 11/12.

I actually observed that about 50 % of the oysters incubated. As these 50 % can only account for about half of the total swarming (probably for still less), I conclude that about 100 % of the total population must have been functioning as females in the Oosterschelde in 1939. It is my belief that the swarming towards the end of August, observed in 1938 and 1939, must be ascribed to oysters functioning as females for the second time during that season.

In any case my data on the periodicity of swarming, in combination with some data on the percentage of oysters carrying larvae indicate that at least 75 % of the adult oysters function as females annually, and sometimes probably more.

We already discussed that the frequency of sex-change will govern the percentage of oysters functioning as females during one season and that the frequency of sex-change is governed in its turn by the water-temperature and probably by the amount of nourishment. So cold summers may be expected to cause a decrease in the production of larvae by decreasing the percentage of oysters that function as females (e.g. 1936). Fine summers will produce the opposite effect.

There are many data on the number of larvae that may be found in one incubating oyster.

LEEUWENHOEK (1722) was the first to form an estimate, but he dared not mention the number, for he said they would not believe him anyhow! DAVAINE (1853) estimated about 1.125.000 larvae in one incubating adult and MOEBIUS (1883) found by weighing about 1.000.000 larvae in the mantle chamber of one adult. DANTAN (1913) carried out more detailed investigations at Arcachon in the South of France. He computed that one-year old oysters produce about 100.000 larvae, two-year old oysters about 250.000 larvae and older oysters about 750.000 larvae. As he found no difference between the numbers of "white" larvae and the number of "black" larvae, he concluded that no mortality or losses of any importance are likely to occur during incubation.

ORTON found 525.000 larvae in small three or four year old oysters from the river Blackwater and once 3.000.000 in a giant oyster.

All these data indicate that the usual estimate that marketable oysters (four or five years old) produce about 1.000.000 larvae during each female reproduction will be about correct. Stress must be laid on the fact that the proportion of the strength of the age-classes is of importance, as younger oysters produce far fewer larvae than older ones.

Ostrea lurida

Marketable Olympia oysters (*Ostrea lurida*), which are much smaller than *Ostrea edulis*, produce about 250.000 to 300.000 larvae, according to HOPKINS (1937).

Ostrea virginica

Non-incubating oysters produce far more eggs than incubating oysters, but the number of their larvae will have decreased considerably by the time they reach the stage at which the larvae of incubating oysters swarm, while in incubating oysters the number of swarming larvae is about equal to the number of fertilized eggs. NELSON (1921) estimates a spawning of 16.000.000 to 60.000.000 eggs in *Ostrea virginica.* The total annual production by one female of this species (which does not show a frequent sex-change!) is estimated by NEEDLER (1932 a) at about 500.000.000 eggs and by PRYTHERCH (1934 b) at about 100.000.000 to 500.000.000 eggs.

Ostrea gigas

GALTSOFF (1930 b) stated that the non-incubating *Ostrea gigas* will spawn 55.000.000 eggs at a time.

Though investigators are agreed on the average number of larvae produced by one female of *Ostrea edulis*, many investigators state more or less important annual fluctuations in this number, brought about by differences in external conditions.

SPÄRCK (1925) believes that not only the actual summer temperature governs the amount of eggs produced, but that temperature-conditions in the preceding autumn are also of influence, as egg-development often sets in towards the end of the preceding season.

HAGMEIER and SCHUBERT (1930) are also of opinion that the water-temperature during the preceding autumn affects reproduction. That the condition of the adult oysters will influence the number of eggs produced by these oysters is highly probable. Thus it is stated by **Gaarder** and **Spärck** (1932) that feeding-conditions in the Norwegian pollen may show wide fluctuations. In summers with scanty sunlight there is but little algal growth in these pollen; bacteria and Peridinea will predominate then. This will result in a bad condition of the adult oysters; many of them will even die. Such summers are characterized by a decrease in the production of larvae, mainly owing to malnutrition of the adults.

Orton (1937 a) is inclined to neglect the differences in the production of larvae: "There is no doubt that larvae occur in abundance in the water every year".

Voisin states (1931): "Nous sommes donc encore obligés de constater toute l'obscurité qui entoure les différents facteurs d'influence qui régissent la ponte des huîtres. C'est pourquoi toute tentation pour prévoir à longue échéance l'époque et l'amplitude[1]) des pontes est, en état de nos connaissances, vouée à un échec".

Ostrea virginica

American investigators made more elaborate studies of the influence of external conditions on the amount of eggs produced by female oysters, especially by estimating and measuring the thickness of gonad-tissue shortly before spawning. **Nelson** (1928 a) states that a cold spring (e.g. 1926) results in a bad development of the gonads for lack of nourishment.

Prytherch states (1929 a, 1934 b) that there is a marked correlation between the water-temperature from April to June and the production of larvae. The thickness of gonad-tissue varies from about 1,5 cm (e.g. 1925) to about 0,5 cm (e.g. 1926, 1927), resulting in important differences in the number of eggs produced in these years. During 1925 the water-temperature from April to August was above the normal. **Prytherch** says that "it is likely that the fullness of gonad development is dependent on the amount of food consumed by the oyster". **Prytherch** (1929) points to the investigations by **Galtsoff** (1928) on the correlation between the water-temperature and the volume of water sieved off by the gills

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[1]) Spacing is mine.

(*Ostrea virginica*). With the aid of these data obtained by laboratory-experiments and of data on the water-temperatures PRYTHERCH computes how many litres of water were sieved off by an average oyster during the months of April, May, June and July in the years 1922 to 1927 in Millford Harbor (Conn.). According to PRYTHERCH the normal averages are: April 25 litres, May 408 litres, June 816 litres, July 995 litres, total 2244 litres. A comparison of these years shows that the maximum was attained in 1922: 2551 litres and the minimum 1926: 2040 litres.

According to PRYTHERCH these relatively slight differences between the volumes of filtered water are responsible for the enormous annual differences in egg-production, the extent of which is directly deducible from the thickness of the gonad-tissues.

He does not mention the possibility that the amount of nourishment per litre may have varied, too, in those years. Unless we are given the amount of nourishment per litre, I do not believe that PRYTHERCH will ever convince us that only the slight differences in the litres of filtered water computed by him are really responsible for the differences in egg-production.

He estimates moreover the extent of egg-production during the years 1922–1927 from differences in the spatfall observed during these years, starting from the assumption that there is a direct correlation between egg-production and spatfall.

I disagree with PRYTHERCH when he states that the amount of eggs produced is the main factor governing spatfall, for the percentage of eggs becoming spat will vary according to external circumstances at the pelagic stage. Spatfall is no reliable standard for egg-production.

Ostrea edulis

What do we know about the variation in the production of larvae in the Oosterschelde? Though the water-temperatures since 1921 are known to me and I have also rough estimates of the spatfall during the years 1921–1935 at my disposal, I shall not try to compute from these data the influence of the water-temperature during the preceding autumns and springs on the extent of the production of larvae, as I am convinced that the spatfall is no reliable standard for the production of larvae.

However, these data do permit me to state that a relatively poor condition of the oysters in the preceding autumn does not necessarily result in a poor spatfall in the next summer season.

Since 1935 plankton investigations have made it possible to form an adequate idea of the amount of larvae produced in in the Oosterschelde. These data can be computed from the diagrams by studying in particular fluctuations in the number of the youngest larvae. In comparing e.g. the diagrams for 1936, 1937, 1938 and 1939 we should bear in mind that in 1936 the samples were not collected exactly in the centre of larvae-production (the Yersche Bank) but at the station Strijen. A comparison of the data of these years at the station Kattendijke convincingly shows that the production of larvae in 1936 was much smaller than in subsequent years.

There has been a marked increase in larvae-production from 1936 to 1939.

Before considering the possible influence of temperature and feeding on the production of larvae, we shall try to estimate the number of adult oysters (oysters from their third summer) that were present on the oyster grounds in these successive years:

1936	about	14 500 000	adult	oysters
1937	,,	24 000 000	,,	,,
1938	,,	30 000 000	,,	,,
1939	,,	36 000 000	,,	,,

The increase in larvae-production appears to run parallel to the increase in the number of adult oysters. This does not mean, however, that differences in temperature and feeding-conditions may not also influence the production of larvae, but I believe the influence of the number of adult oysters to be very great.

When we compare the water-temperatures in the springs of these years (table 1), we find that it is impossible to show a marked correlation between the water-temperatures and the extent of the annual productions of larvae. The years when the larvae were very abundant (1938 and 1939) had cold springs, with water-temperatures below the normal. The year 1937 shows a spring with water-temperatures above the normal; still this year does not show an abundant production of larvae, not even when we take into account the above-mentioned increase in the number of adult oysters. The springs of 1935 and 1936 show approximately the same temperature-conditions, but in 1935 the number of larvae produced was higher than that in 1936. The production of larvae in 1936 was extremely poor, even when the number of adult oysters is taken into account, but temperature-conditions in that spring did not deviate much from those in the rich years 1938 and 1939. The years 1938 and 1939, with their abundant production of larvae, were preceded by autumns with rather favourable temperature-conditions, while 1936 and 1937 were preceded by autumns with lower water-temperatures.

This does not suffice, however, to convince me of the correctness of the assumptions of HAGMEIER and SPÄRCK, who state that the water-temperatures in the preceding autumn greatly influence the extent of the production of larvae.

I have no data about the influence of factors like quality and quantity of nourishment on the annual production of larvae in the Oosterschelde.

I agree with VOISIN that we have to await how many larvae will appear in the plankton each year. There is not yet a reliable formula to predict it. The larger the number of adult oysters, however, the greater the chance of an adequate production of larvae.

XIII. THE SIZE OF THE PELAGIC LARVAE

Ostrea edulis

MOEBIUS (1883) informs us that the larvae of *Ostrea edulis* measure from 0,15 to 0,18 mm (the greatest length of the shell parallel to the hinge) at the moment when they are set free. HAGMEIER (1916) organized his measured larvae (experiments on propagation in an enclosed oyster-pit) in three groups: newly-liberated larvae measuring 0,17 to 0,21 mm, grown-up larvae 0,22 to 0,26 mm and mature larvae 0,27 to 0,30 mm. HAGMEIER made several observations on larvae measuring about 0,21 mm, while still held in the mantle chamber of the adult. Moreover he obtained some indications that the size of the newly-liberated larvae tends to decrease towards the end of the season of reproduction: "Es hat den Anschein als ob gegen Ende der Brutzeit die Grösse der frisch ausgestossenen Larven abgenommen hätte, doch reichen meine Messungen nicht aus um diese Tatsache einwandfrei festzustellen".

BOURY (1930) and VOISIN (1931) divide their larvae in larvae in the first stage and larvae in he second stage, the former being straight-hinged, the latter being provided with an umbo. Straight-hinge larvae measure from 165 μ to 228 μ, umbo-larvae from 200 μ to 295 μ; so both groups overlap. According to BOURY fixation takes place at a shell-length of about 270 μ, larvae larger than 270 μ being rare. ERDMANN (1934) informs us that the larvae of *Ostrea edulis* are set free normally at a shell-length of 0,16 to 0,18 mm. In the course of his experiments he found that a high water-temperature shortens the period of incubation, whilst decreasing the size of the freshly-liberated larvae, and conversely. A water-temperature of 23° C resulted in an incubation period of 6 to 8 days and in a shell-length of the liberated larvea of 0,16 to 0,17 mm. A water-temperature of 13 to 14° C resulted in an incubation period of 18 days and in a shell-length of the swarming larvae of 0,20 to 0,21 mm. So when HAGMEIER finds a decreasing shell-length of the swarming larvae towards the end of the season, this may be due to a higher water-temperature during incubation in the second part of the season. ORTON (1937 a) records that the ovarian egg measures 150 μ, the spherical segmenting embryo 130 μ, and the swarming larvae from 170 to 190 μ (l.c. p. 41); occasionally

TABLE III

THE SIZE OF NEWLY-LIBERATED OYSTERLARVAE

Date of swarming	Average temperature during incubation	Temperature at swarming	Distribution of the newly-liberated larvae over the size-classes			
			15×11μ	16×11μ	17×11μ	18×11μ
23 June 1938	16° C	18° C	1%	12%	41%	**46**%
28 June 1936	20° C	21° C	5%	13%	**52**%	30%
27 June 1938	17° C	18° C	1%	17%	**46**%	35%
13 June 1939	18° C	16,5°C	0%	15%	**49**%	36%
21 July 1938	17,5°C	18° C	6%	29%	**50**%	15%
19 June 1939	17° C	18° C	4%	28%	**46**%	22%
26 June 1939	18° C	18° C	4%	28%	**41**%	27%
11 July 1939	18,5°C	18,5°C	4%	39%	**50**%	7%
29 June 1939	18° C	17,5°C	5%	35%	**46**%	14%
9 July 1938	17° C	17° C	6%	35%	**45**%	14%
5 July 1939	18° C	18,5°C	5%	42%	**43**%	10%
7 Aug. 1939	19° C	19° C	14%	37%	**42**%	7%
8 Aug. 1938	20° C	23° C	9%	**40**%	35%	16%
4 Aug. 1939	19,5°C	19° C	8%	**42**%	41%	9%
24 July 1939	19° C	18,5°C	5%	**45**%	43%	7%
19 July 1937	20° C	21° C	10%	**43**%	34%	13%
18 Aug. 1937	20° C	19° C	9%	**47**%	35%	9%
22 Aug. 1939	19,5°C	20,5°C	11%	**50**%	29%	6%
3 July 1937	19° C	21° C	16%	**52**%	28%	4%
12 Aug. 1939	18,5°C	18° C	16%	**54**%	24%	6%
24 Aug. 1938	19° C	18° C	**64**%	30%	4%	2%

they may be found in the incubating mother-oyster with shells as long as 210 to 220 μ (N.B. on page 118 l.c. ORTON declares that "the usual size at which they are set free is when the shell measures about 190 to 200 μ long by about 170 μ broad". This is not in accordance with his assertion on page 41!). According to ORTON mature larvae measure from 270 to 290 μ.

COLE (1939) states that at liberation his larvae measured from 0,16 to 0,20 mm, predominantly from 0,18 to 0,19 mm. Larvae from the same parent did not vary in size more than 0,015 mm. Recently set spat measured from 0,29 to 0,31 mm in his tank, the pigmentspot developed at a diameter of 0,27 to 0,28 mm. COLE mentions aberrations in both directions: large larvae of 0,33 to 0,35 mm, at a size of 0,33 mm often without any trace of a pigmentspot (tank-experiment 1937) and a case in which all the mature larvae remained under 0,285 mm and showed pigmentspots at 0,255 mm already (Helford River, Cornwall, 1938). COLE assumes that external conditions may be responsible for these large poorly differentiated larvae and these small completely differentiated larvae.

What about the size of the oysterlarvae in the Oosterschelde? From the diagrams we can derive data about the size of the larvae at liberation as well as at setting. So I sorted out a lot of marked swarming dates. A considerable swarming (on the Yersche Bank) causes a sharp rise in the number of larvae in consequence of the liberation of new larvae. Then the large majority of the larvae of the smaller size-classes may be considered as newly-liberated larvae. So we can form an idea of the size of the larvae at swarming by comparing the number of larvae in each of the smaller size-classes on the dates of swarming, although I admit that a slight interference by larvae that are already present before swarming sets in is unavoidable.

These data are arranged in order of a decreasing size of the newly-liberated larvae in table III. From this table we can deduce that in the majority of cases the size of the larvae at liberation varies between 0,175 mm and 0,185 mm. Larvae measuring from 0,165 to 0,175 mm are often recorded and are sometimes even abundant. I seldom or never found larvae of a size smaller than 0,165 mm in the plankton-samples. Sometimes a fairly considerable part of the liberated larvae measure from 0,185 to 0,200 mm, but so far I have never recorded during swarming a marked increase in the number of larvae of a size larger than 0,200 mm.

Further we may state that apparently temperature is not all-powerful in this respect. Although in general high water-temperatures during incubation coincide with a smaller size of the liberated larvae, there are so many exceptions that I cannot endorse unconditionally ERDMANN's assumption that temperature during incubation regulates the size of the swarming larvae. During the low water-temperatures in the first part of July 1938 (16 to 17° C) I measured the larvae of many incubating oysters, but I never recorded larvae larger than 0,200 mm in the mantle chamber. I did not measure larvae incubated below 16° C.

It is a striking fact that the liberations in June are all included in the first part of the table, so among the young larvae of a fairly large size, while the swarming in August, especially in the second part of August, will always be found in the second part of the table among the smaller larvae. Generally the water-temperatures are somewhat higher in August, but this certainly cannot always account for the smaller size of the August larvae (e.g. 12 August 1939, incubated at 18,5° C). Moreover I recorded the appearance of large larvae in the plankton during high water-temperatures as early as 28 June 1936. In my opinion the water-temperature during incubation probably has some influence on the size at which the larvae are liberated, especially when the differences are great, but moreover I am inclined to assume that HAGMEIER was right when he supposed that the size of the larvae tends to decrease near the end of the season. So far I have recorded too few liberations in August during low water-temperatures to be able to state with certainty that the date of liberation is more important than the water-temperature at the time of incubation, at least when the variations in temperatures are not very great.

Larvae provided with a pigmentspot (so-called mature larvae) usually measure from 0,260 to 0,300 mm in the Oosterschelde. Most of them are of a size from 0,275 to 0,285 mm. Mature larvae somewhat smaller than 0,260 mm were only recorded during the month of August. Larvae larger than 0,300 mm. occur very rarely in the plankton of the Oosterschelde; the largest planktonic larva I ever observed measured 0,315 mm. It is my belief that the large larvae of COLE in one of his tanks are certainly aberrant, but his larvae from Helford River are of a quite normal size, compared with the larvae in the Oosterschelde.

A comparison of the number of oysterlarvae that may be found in 100 litres of water in the different centres of spat-production in Europe is hardly possible. The French investigators do not know the volume of water they filter off with their plankton-nets. Though the German and Danish data also refer to net-towing, it is clear that larvae are far scantier in the Wattenmeer and Limfjord than in the French waters. GAARDER and SPÄRCK (1932) counted 20 to 60 oyster larvae in 10 litres of poll-water (Norway), which corresponds with my data from the Oosterschelde. In the Oosterschelde a few hundreds of oysterlarvae per 100 litres may occur at the height of the season, even in places fairly remote from the swarming centre. During swarming quantities of 1000 larvae and more per 100 litres of water may be counted in the centre of larvae-production.

XIV. FOOD AND FEEDING OF THE LARVAE

ORTON's statement (1937 a) that the eggs of the European and kindred oysters are supplied with such a large store of reserve materials that the larvae will not need much actual food before settlement, must be taken with a grain of salt. The yolk-material of the eggs indeed renders active nourishment unnecessary for some time after the spawning of the eggs. Thus it has been stated by PRYTHERCH (1924) for *Ostrea virginica* and by FUJITA (1934) for *Ostrea gigas* that active nourishment only begins when a certain degree of larval development has been reached. Active nourishment in the larvae of incubatory species of oysters starts before swarming takes place, so during incubation. It was DANTAN (1916) who noticed that larvae show a stomach content during incubation, which statement was confirmed by ERDMANN's investigations (1934): "Ich konnte einwandfrei feststellen, dass sie während dieser Zeit schon aus dem die Mantelhöhle durchströmenden Kiemenwasser Nahrung zu sich nehmen".

YONGE (1926) has described in detail how the larvae feed. By feeding it carmin grains, east indian ink and iron-saccharate he was in a position to follow the entire process. From these experiments we may conclude that the young larvae ingest anything of an adequate size. So the actual observations of the ingestion of a certain food material (HAGMEIER 1931: detritus, yeast-cells, starch grains, SPÄRCK 1927: Chlorella) does not prove that this kind of food can be digested by the larvae, at least not before the possibility of rearing the larvae with this food till fixation has been proved. KÄNDLER (1930) did not succeed in rearing the larvae by feeding them a pure culture of Chlorella, a non-motile alga.

The authors agree in stating that the larvae are unable to ingest food-particles larger than 8 to 10 μ. SPÄRCK (1927) observed that even *Nitzschia minutissima* is too large to be ingested.

It has appeared to be possible to rear oysterlarvae in vitro till settlement by supplying cultures of certain micro-organisms as food material (COLE 1936, 1938 b, 1939, ERDMANN 1933, HORI 1933, BRUCE and PARKE 1938, 1940). In co-ordination with his large-scale tank-breeding COLE (1939) carried out laboratory experiments, by which he proved that his original hypothesis (1936) that the larvae of *Ostrea edulis* require nude nannoplankton flagellates as food material is correct. So far he has only succeeded in obtaining setting in vitro by feeding the larvae cultures of such small nude flagellates. Non-motile green or blue algae of a suitable size proved to be useless to the larvae as food. BRUCE, KNIGHT and PARKE (1938, 1940) succeeded likewise in rearing the larvae till settlement by feeding cultures of several species of small flagellates (1–7 μ). Some species of flagellates proved to be more efficacious than others. In their most successful experiments the number of larvae reared from liberation to settlement exceeded 90%, in one case even 99% of the number introduced in the experimental vessel. The larvae are probably unable to digest the firm cell-walls of those non-motile algae, possibly because of the rapid passage of the food through the intestinal tract. COLE (1936) confirms the statement of SPÄRCK that non-motile algae of a suitable size are, however, readily ingested by the larvae. After settlement digestion of firm cell-walls becomes possible, so that non-motile algae can be used as food; meanwhile the quantity of food required increases considerably after metamorphosis.

COLE succeeded in stimulating the growth of these flagellates in his tanks and in preventing a predominance of undigestable non-motile algae by the use of off-shore water and by an organic enrichment in the shape of a daily addition of a certain amount of ground crab to the water. In this way he obtained a rapid multiplication of small nanno-planktonic flagellates, so that adequate food-conditions are created for the larvae, which will then develop and settle in the tanks.

HORI (1933), however, succeeded in rearing the larvae of *Ostrea gigas* in vitro till settlement by feeding a culture of the non-motile alga *Chlorella pacifica*. It has not been made clear whether the larvae of this oyster are able to digest the cellwalls of such algae or whether it is the very thin cellwall of this species of alga (UYEDA 1927) which renders its digestion possible.

The Norwegian pollen have been closely examined (GAARDER 1932, GAARDER and SPÄRCK 1932, GAARDER 1933), especially the Espevik-poll and the Saelø-poll. Owing to the exceptional hydrographic conditions in the pollen (discussed already in a previous section) the danger of a shortage of suitable food is not imaginary. Especially summers with scanty sunlight may be the cause of a very poor algal growth, causing a poor condition and even starvation of adult oysters.

The investigators inquired into the productivity of these pollen and effected an inorganic enrichment (nitrogen, phosphor and copper) to ameliorate the conditions for algal growth. They paid special attention to the occurrence of small non-motile algae, as they supposed that these might be a suitable food-material for oysterlarvae. This assumption was not based upon experiments in vitro. GAARDER and SPÄRCK did not actually prove that non-motile algae can be digested by the larvae, although they readily ingest them. I will not absolutely deny that the high temperature of the poll-water (± 25° C) may enable the oyster larvae to digest non-motile algae, but this remains still to be proved. GAARDER and SPÄRCK cannot point to marked ameliorations in setting as the result of their experiments. Inorganic enrichment may cause rather dangerous changes in hydrographic conditions, as for instance an alarming rise of the pH by an increase in assimilation. Oysterlarvae die when the pH exceeds 9.0. Addition of copper counteracts algal growth and assimilation and thus the rise of the pH; additions of more than 0,040 mg Cu per litre are injurious to oysterlarvae, according to these investigators. GAARDER and SPÄRCK added copper to the poll-water, as they thought it possible that it might contain too little copper to render a good development of the larvae possible (Prytherch 1931, 1934 a), although a real shortage of copper has never actually been demonstrated in the European oysterculture regions. The favourable effect of the addition of copper to the poll-water, as mentioned by Gaarder, was probably not apparent enough to allow of demonstration by numbers. .

I did not carry out experiments to investigate this matter in the Oosterschelde. As general food conditions have appeared to be remarkably favourable to adult oysters here during the last few years, I am inclined to believe with Orton (1937 a) that the absence of sufficient suitable food at the right time in the open sea is a possible, though perhaps not a probable factor Of course this problem is worth special investigation. I regret having to admit that we know but very little about the productivity of the Oosterschelde. We know practically nothing about the local variations in quantity and about the cycles of nitrogen, phosphor, manganese and other important elements, which in co-operation with other factors, like temperature and light, govern the productivity of the water.

XV. DISTRIBUTION OF OYSTERLARVAE DURING THEIR PELAGIC LIFE

Oysterlarvae are somewhat heavier than seawater. By ciliar action of the velum they are able to counteract the influence of gravitation. So by swimming or ceasing to swim they can to some extent control their vertical level in the water.

When we observe oysterlarvae in a small glass jar, we shall see that part of them are swimming actively and that another part is lying on the bottom, while again others may be found hanging quietly on the surface-film. When we stir the water vigorously, thus causing severe turbulences, all the larvae retract their velum and sink to the bottom.

It would be interesting to know whether oysterlarvae exhibit any rhythmical movements in the sea, whether they tend to accumulate near the surface at one time of the day or at a certain stage of the tide and to sink at other times.

We know beforehand that it is probably not necessary for larvae of *Ostrea edulis* to rest on the bottom or on the surface-film during their pelagic life. This is what we learn from the experience in the Norwegian oyster-pools. The bottom of these pollen is rich in hydrogen sulphide (GAARDER and BJERKAN 1934), while oxygen is lacking. So the larvae cannot rest on the bottom there. The surface-layers are fresh or almost fresh, so that resting on the surface-film is also impossible. Nonetheless oysterlarvae develop till settlement in the pollen, from which we may conclude that resting on bottom or surface-film is most probably not necessary during pelagic life.

I shall discuss separately various factors which may affect vertical migrations, such as differences in temperature, salinity, light, current-velocity and the influence of wave-action on the vertical distribution of the oysterlarvae.

In another section special attention will be paid to the vertical distribution of mature oysterlarvae.

The influence of light on vertical distribution

PETERSEN (1908) told us about his observations in the Limfjord

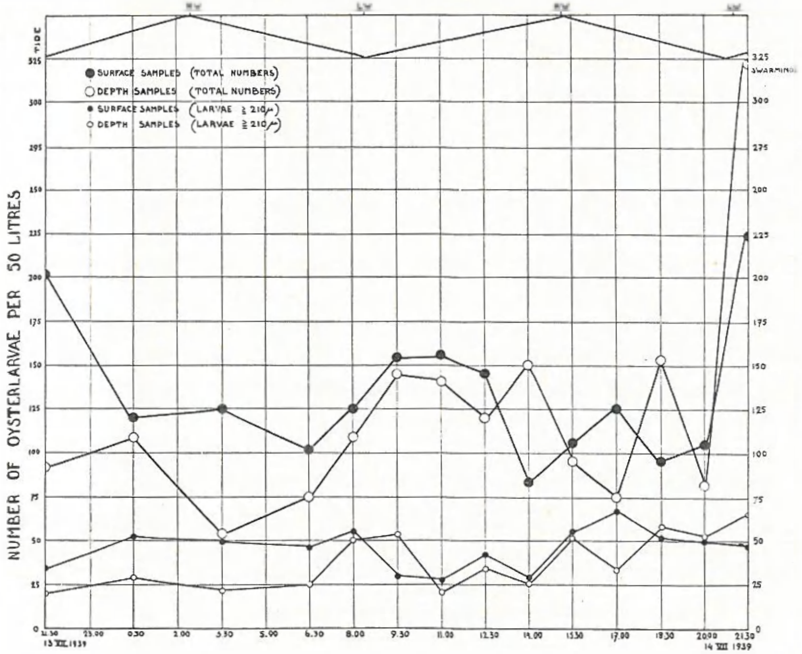

Fig. 11. Number of oysterlarvae in the course of the tidal cycle. Station Yersche Bank.

"Both night and day, in bright, sunny and in dull weather, the spat was found on the surface, but constantly also in deeper water".

German investigators did not succeed in finding any appreciable influence of light on the movements of oysterlarvae in the course of their experiments in vitro (HAGMEIER 1932). Such observations in vitro (in a large container) had been made before by MAZZARELLI (1922), who did not observe any phototactical movements either: "O che il vaso sia tenuto nell' oscurità, o in piena luce, ovvero che sole un lato del vaso stesso sia colpito da raggi luminosi, restando nell' oscurità il resto, le larve dell' ostrica continuanno sempre ad aggirarsi in tutta la massa acquea, nella quale seguitano a distribuirsi in maniera affatto uniforme".

I did not come across other observations on the influence of light on the vertical movements of oysterlarvae. Nor did I

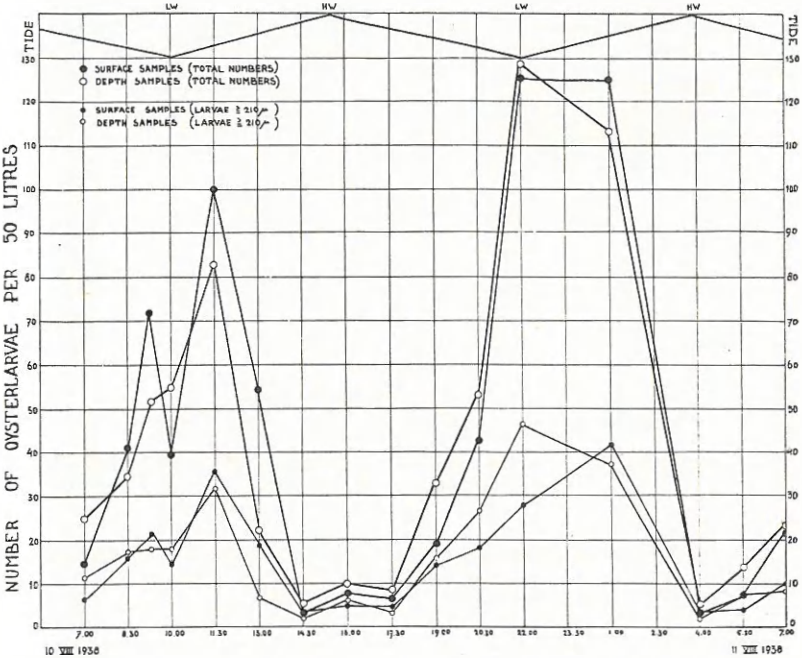

Fig. 12. Number of oysterlarvae in the course of the tidal cycle. Station Kattendijke.

find any comparisons of the number of oysterlarvae near the surface and in deeper layers during night and day.

In the summers of 1937, 1938 and 1939 I collected many special plankton-samples at different stages of the tides, sometimes continuing sampling for 24 hours in one station. Some of these series of samples were collected in the station Yersche Bank. During low water the depth is about 2,00 metres here, during high water about 5,00 metres. The samples were collected about 0,30 m below the surface and about 0,50 m above the bottom.

Other series of samples were collected at the station Kattendijke, where the depth is about 15 metres. Here I collected samples about 0,30 m below the surface and at a depth of about 5 metres below the surface. The bottom could not be approached here, as the length of the suction-hose did not allow of this.

The results of some of these investigations have been visualized graphically (fig. 11, 12). I shall often refer to these data.

In night-samples of 50 litres from the station Yersche Bank I counted e.g.:

Date	Hour	Surface	Bottom		
14 VII 1937	23.15	64	69	larvae	
19 VII 1937	24.00	106	156	„	
22 VII 1937	24.00	50	44	„	
22 VII 1938	24.00	112	124	„	
23 VII 1938	1.30	113	60	„	
23 VII 1938	4.30	155	175	„	
15 VII 1939	0.30	118	109	„	(fig. 11)
15 VII 1939	3.30	127	55	„	(fig. 11)
		845	792		

There appears to be no appreciable difference in the number of larvae in the surface-layers and in the bottom-layers during the night.

The data on couples of night-samples collected at Kattendijke show a still closer similarity in the number of larvae in the surface-layers and at a depth of 5 metres (fig. 12). As will be discussed below, we shall always expect a somewhat irregular course of the number of larvae at the station Yersche Bank, owing to the proximity of liberating adults.

Some data on samples during daylight are (also Yersche Bank, 50 litres):

Date	Hour	Number of larvae		
		Surface	Bottom	
15 VII 1937	10.00	43	60	
20 VII 1937	12.00	120	117	
23 VII 1937	12.00	50	72	
23 VII 1937	18.00	56	31	
22 VII 1938	7.30	298	189	
22 VII 1938	9.00	188	170	
22 VII 1938	10.30	75	158	
22 VII 1938	12.00	123	113	
22 VII 1938	15.00	149	102	
22 VII 1938	16.30	255	128	
22 VII 1938	17.15	192	227	
22 VII 1938	18.00	93	123	
15 VII 1939	8.00	126	112	(fig. 11)
15 VII 1939	9.30	158	144	(fig. 11)
15 VII 1939	11.30	153	138	(fig. 11)
15 VII 1939	12.30	147	120	(fig. 11)
15 VII 1939	14.00	82	150	(fig. 11)
15 VII 1939	15.30	109	93	(fig. 11)
15 VII 1939	17.00	123	75	(fig. 11)
15 VII 1939	18.30	97	148	(fig. 11)
		2637	2470	

So there is no considerable difference in the number of larvae near the bottom and in the surface layers during daylight either. In this case, too, the couples of samples collected at Kattendijke (fig. 12) show a still closer resemblance as regards the number of larvae at the surface and at a depth of 5 metres.

So far my field observations have shown that the enormous difference in illumination between day and night does not seem to have any influence on the vertical distribution of the larvae of *Ostrea edulis*. Variations in illumination of less importance, caused e.g. by differences in cloudiness never appeared to have an appreciable influence on the vertical distribution of oysterlarvae in the Oosterschelde. This does not surprise me, since I failed to detect an influence of the difference in illumination between day and night.

All this in perfect agreement with the observations in vitro by MAZZARELLI and with those in Heligoland.

The influence of temperature on vertical distribution

There are only a few investigators who tell us something about the influence of temperature on the vertical distribution of oysterlarvae. MAZZARELLI (1922) noticed that his larvae in vitro sometimes rose to the surface in groups and thereupon sank to the bottom again in a way that strikingly resembled the movements of convection-streams. He assumed differences in temperature to be the cause of this phenomenon. HAGMEIER (1932) told us that such movements in vitro have also been observed in the Heligoland laboratory. The German investigators ascribed them likewise to convection-streams and to differences in temperature. When the temperature in the basin is perfectly equable, this phenomenon does not occur.

PERKINS (1931), inquiring into the vertical distribution of the larvae of *Ostrea virginica* in Barnegat Bay (U.S.A.), tells us that the part played by temperature in bringing about vertical migrations of the larvae is insignificant there. In Barnegat Bay the temperature of surface- and bottom-layers differed 1° or 2° C. There appeared to be no marked relation at any time between temperature and vertical distribution (field observations).

The tidal mixing of the water in the basin of the Oosterschelde is so thorough that marked differences in temperature between surface-layers and bottom-layers practically never occur. The differences in temperature between bottom-water and surface-water, read off by me whilst collecting the series of samples at the station Yersche Bank, always remained below 1° C and often were hardly perceptible. The temperatures at Kattendijke (surface and 5 metres below the surface) showed the same phenomenon. The insignificance of the differences is probably the reason why I cannot deduce from my data any apparent relation at any time of day or night between water-temperature and vertical distribution. This does not imply, however, that the larvae of *Ostrea edulis* are unable to react upon marked differences in temperature. The possibility remains that the vertical distribution is not the same during low water-temperatures (e.g. 16 to 17° C) as during high temperatures (e.g. 22° C). Although my special series of samples were collected during temperatures from 18 to 20° C, I do not believe in such a difference, however, for the diagrams on my daily samples do not provide the slightest indication of its occurrence.

Moreover it has been proved by MAZZARELLI (1922) that the larvae of *Ostrea edulis* show a uniform distribution in vitro at water-temperatures far below 16° C.

The influence of wind and wave-action on vertical distribution

BOURY (1930) compared the number of oysterlarvae in samples collected by net-towing along the surface and somewhat deeper. He collected some samples on a day with moderate wind and another number during slight air.

In spite of the fact that the towing of a plankton-net is not a reliable method of collecting quantitative samples, while moreover the number of samples collected was rather scanty and the differences in the number of larvae were not very considerable, BOURY concluded that wave-action expels the larvae from the surface layers: "Lorsque l'eau est calme les larves nagent donc surtout près de la surface; mais pour peu qu'il ait de vagues elles descendent vers les couches tranquilles". His compatriot BORDE (1931, 1932) tried to find out whether BOURY's assumption held good for the basin of Arcachon. He compared many samples collected by net-towing in different water-layers in various weatherconditions. BORDE was unable to confirm BOURY's data. He did not find relatively fewer larvae in the surface layers during rough sea than during smooth sea.

The data on my special series of samples allow me to inquire into this matter, too. I sorted out the couples of samples that were collected either during rough sea, caused by strong winds, or during smooth sea or slight rippling, during calm or slight air. The other samples were collected during intermediate degrees of wind and sea and are therefore less suitable for a comparison of the vertical distribution of oysterlarvae during smooth sea and rough sea.

These data show that in the Oosterschelde there is no appreciable difference between the vertical distribution of oysterlarvae during calm weather with a smooth sea and during rough weather. On an average the vertical distribution during smooth sea is similar to that during rough sea. During rough sea the oysterlarvae are not expelled from the surface-layers into the quieter deeper layers.

Station	Date of sampling.	Hour	Wind	Water	Number of larvae in 50 litres.	
					Surface	Bottom
Yersche Bank (L.W. 2,00 m) (H.W. 5,00 m)	10 VII 1937	18.30	strong	rough	92	47
	22 VII 1937	24.00	strong	fairly rough	50	44
	23 VII 1937	6.00	strong	rough	73	35
	23 VII 1937	12.00	strong	rough	50	72
	23 VII 1937	18.00	strong	rough	56	31
	23 VII 1938	6.00	fairly strong	rough	124	141
	15 VII 1939	3.30	fairly strong	rough	127	55
	15 VII 1939	15.30	fairly strong	rough	109	93
	15 VII 1939	17.00	fairly strong	rough	123	75
					804	593
	14 VII 1937	23.15	calm	smooth	64	69
	15 VII 1937	10.45	calm	smooth	43	60
	19 VII 1937	6.00	slight air	rippling	68	28
	19 VII 1937	24.00	calm	smooth	106	156
	22 VII 1938	7.30	slight air	rippling	289	189
	22 VII 1938	9.00	slight air	rippling	188	170
	22 VII 1938	10.30	slight air	rippling	75	158
	22 VII 1938	12.00	slight air	rippling	123	113
	22 VII 1938	15.00	slight air	rippling	149	102
	22 VII 1938	16.30	slight air	rippling	255	128
	22 VII 1938	17.15	slight air	rippling	197	227
	22 VII 1938	18.00	slight air	rippling	93	123
	22 VII 1938	21.00	slight air	rippling	150	93
	22 VII 1938	22.30	slight air	rippling	225	56
	22 VII 1938	24.00	slight air	rippling	112	124
					2137	1796
					Surface	5 metres
Kattendijke (L.W. 12,00 m) (H.W. 15,00 m)	10 VIII 1938	7.00	fairly strong	rough	14	25
	10 VIII 1938	8.30	fairly strong	rough	41	34
	10 VIII 1938	9.15	fairly strong	rough	73	52
					128	111
	6 VIII 1937	10.30	calm	smooth	27	40
	6 VIII 1937	16.30	calm	smooth	17	15
	10 VIII 1938	10.00	slight air	rippling	38	55
	10 VIII 1938	11.30	slight air	rippling	100	83
	10 VIII 1938	20.30	slight air	rippling	43	53
	10 VIII 1938	22.00	calm	smooth	127	130
	11 VIII 1938	1.00	calm	smooth	126	114
					478	490

The influence of salinity on vertical distribution

As far as I know only **Leenhardt** (1924) mentions a possible influence of differences in salinity on the vertical distribution of the larvae of *Ostrea edulis*. **Leenhardt** states that the larvae are expelled from the surface-layers during rainy weather. He does not, however, adduce facts to prove his statement.

My data do not point to any influence of rainy weather on the vertical distribution of oyster larvae[1]), but the possibility of such an influence in a very thin layer, immediately under the surface, remains, for I collected my surface-samples some 20 or 30 cm below the surface. In any case such an influence of rainy weather in such a thin layer will be negligible and it will have no appreciable effect on the distribution of all the larvae beneath this level.

Influence of salinity of another kind is discussed by American investigators for *Ostrea virginica*.

Julius Nelson (1916) noticed differences in vertical distribution at different stages of the tide. He believed that the larvae rise into the tide early in flow, and settle to the bottom before ebb begins and are thus able to migrate landwards and to avoid a rapid dispersion by the tidal streams. His son, **Thurlow C. Nelson**, assumed some years later (1921) that the youngest stages of the larvae do not show any marked difference in their vertical distribution; consequently they are carried seaward from the beds (surplus of ebb). Towards the end of the first week of larval life a distinct active reaction to the ebb and flow of the tide is evidenced: "within one hour after the tide has begun to fall the great majority of the oysterlarvae in the later stages have sunk to or upon the bottom. There they remain until the tide has begun to flow, when they swim upwards and are carried towards headwaters by the incoming tide. By repeating this performance with each ebb and flow the larvae are able by progressive stages to move upstream for considerable distances" (Delaware Bay).

Nelson carried out many investigations in Barnegat Bay

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[1]) The samples from the station Kattendijke collected on the 19th of August 1938 (fig. 2) were practically all procured in rainy weather.

(N.J.), which confirmed the occurrence of marked differences in the vertical distribution during different stages of the tide. Moreover he succeeded in finding the cause of this phenomenon. (1926, 1927, 1928 a, 1931).

Marked differences in salinity may occur there in the various water-layers. During flood-tide the heavier water of relatively high specific gravity creeps up along the bottom. The zone of transition may be very sharp (differences in sp. gr. from 1,010 to 1,018 for instance). In this zone, which lies immediately on the surface of the layer of dense bottom-water, the larvae of oysters and other animals may be found in comparatively enormous numbers. This discontinuity layer, where the upper more brackish water is in contact with the heavier more saline water, is called the halicline. NELSON states that it should not be assumed that the larvae "seek out" a zone of optimum salinity and congregate there. On the contrary, the effect of the salinity gradient appears to be one of purely relative salinity change. According to NELSON the congregation in the halicline is probably the result of a stimulus arising from rapid osmotic changes in passing from water of low salinity into that of higher salinity and vica versa. Once caught in the halicline, the larvae cannot easily escape from it: if they tend to sink into the heavier saline water, the rapid osmotic changes stimulate the larvae to swim upwards and when they swim into the more brackish layers, the larvae shrink back from the rapid osmotic changes in the other direction. As a rule this stratification disappears during ebb. Rough weather furthers a rapid mixing of the water. As the current-velocities may differ in the various waterlayers, the presence of the halicline and its influence on the vertical distribution of oyster-larvae may very well cause a passive migration in some horizontal direction.

NELSON often states that the larvae tend to accumulate near the bottom, when there is no halicline. His data do not prove this, however. When there is little difference in specific gravity between the water of the surface and the bottom, the larvae appear to be about uniformly distibuted (1928 a: fig. 2, diagram 2 and 3). The number of larvae near the bottom is then about equal to that near the surface. When there is a halicline, very few larvae will be found near the bottom in the layers of high salinity.

SEKI and TANAKA (1931) often noticed marked differences in the number of larvae of *Ostrea denselamellosa* in samples from the surface and from deeper layers. Although their numbers of larvae (practically always less than 100 per 100 litres) are, strictly speaking, too small to base conclusions on, these differences may possibly be attributed to the differences in salinity that have been recorded by these authors, although differences in temperature, which occurred likewise, may also have exercised their influence.

I do not know whether the larvae of *Ostrea edulis* tend to react to marked stratifications of the water in the same way. The water in the Oosterschelde gets so thoroughly mixed by the tidal currents that any difference in salinity between the surface-layers and the bottom-water is hardly perceptible. The absence of a halicline in the Oosterschelde simplifies the study of the oysterlarvae there, as no interference on the part of the salinity is to be feared. It would be interesting to inquire into the behaviour of the larvae of *Ostrea edulis* in respect of salinity in a place where hydrographic conditions correspond with those in Barnegat Bay (N.J.).

The influence of the current-velocity on vertical distribution

The papers by American investigators mention two entirely different ways in which the current-velocity influences the vertical distribution of the larvae of *Ostrea virginica*.

In the first place there are the data on plankton-sampling in Milford Harbor (Conn.) and adjacent waters (PHYTHERCH 1929, GALTSOFF, PRYTHERCH and MAC MILLAN 1930). The investigators collected plankton samples by towing a plankton-net and by pumping up known quantities of water and filtering them through a plankton-net. Although profuse settlement occurs in the places where they sampled, they found but very few larvae in their samples. Many samples contained no larvae at all. They tried to find out the cause of this scarcity of larvae in the samples in these places, where settlement is abundant. The majority of a series of samples, collected during an entire tidal cycle, both from the surface and from the bottom-layers, proved to contain no larvae at all, while the number of larvae in other samples was very small. It should be remarked that the majority of these larvae were found to be in the later stages of development.

The largest number was counted in the samples collected at low slack water (e.g. 120 per 200 gallon). This led PRYTHERCH to assume that the larvae only swim roundabout slack water and rest on the bottom for the remainder of the tidal cycle. At high slack water the number was also very small, however, and the number of larvae at low slack water is minute in comparison with the numbers observed elsewhere on the east coast of North America.

PRYTHERCH assumes that the larvae drop to the bottom when the current-velocity exceeds a certain degree. Indeed he succeeded in finding some larvae in bottom material, collected at a moment of considerable current-velocity. Yet I believe that the number of larvae he found on the bottom is rather small in comparison with the heavy spatfall in that locality. PRYTHERCH tried to imitate the current-velocity in a large elliptical tank (1.000 gallon). The larvae in this container dropped to the bottom when the artificial current-velocities exceeded 0,3 to 0,5 foot per second. (8,5 to 14 cm per second). I want to remark, however, that an experiment in a tank like that does not reproduce natural conditions. The circular water movement in the tank introduces a centrifugal force, which complicates the situation. Moreover, water movements in containers are accompanied by more vigorous turbulences than watermovements in the open sea. Experiments in smaller jars have shown that the larvae soon drop to the bottom when vigorous turbulences occur.

The results of the investigations by other scientists are not in agreement with PRYTHERCH's assumption that the larvae of *Ostrea virginica* drop to the bottom when the current-velocity exceeds 0,5 foot per second. PERKINS (1931, 1932) for instance, found that the larvae are swimming actively during current-velocities that sometimes exceed 0,7 foot per second. In many places the larvae are abundant during all stages of the tide. It is still an unsolved problem why PRYTHERCH found so few larvae in the samples collected near Milford Harbor.

What do we know about this influence of the current-velocity on the larvae of *Ostrea edulis?* The fact that the vertical distribution of oysterlarvae in the Norwegian oyster-pollen is fairly uniform (GAARDER 1932) clearly shows that the absence of any current-velocity does have as a result that the larvae drop to the bottom-layers, as is sometimes supposed. HAGMEIER (1931, 1932) states that the larvae of *Ostrea edulis* do not tend to drop to the bottom in quiet places in the open sea, as is often assumed. Surface-samples collected in such quiet places do not show far fewer larvae than samples collected from the bottom-layers.

My series of samples collected in the Oosterschelde enable me to inquire into this matter, as I collected several series of samples during two entire tidal cycles at the station Yersche Bank. One of them is visualized in fig. 11. The current-velocity in the surface-layers exceeds 25 cm/sec about one hour after slack water, about two hours after slack water the velocity is from 30 to 40 cm/sec. One hour before the next slack water the velocity is again 25 cm/sec. So far I have never observed that the larvae *tend to drop to the bottom when the current velocity attains* these figures. It may be seen (fig. 11) that no marked peaks in the number of larvae occur during slack water. The bottom samples were collected about 50 cm above the bottom, where the current-velocity (measured by Rijkswaterstaat) is but a trifle smaller than that at the surface. About 10 cm above the bottom the current-velocity attains 20 to 25 cm/sec at the height of the tide. The greatest number of larvae (300 per 50 litres) in the series collected at this station on 22 July 1938, was counted in the surface-sample, collected at half flow (at 7.30 h.),

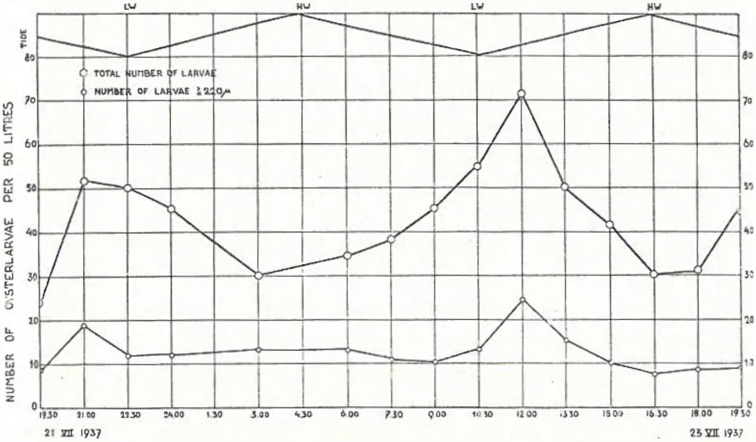

Fig. 13. Number of oysterlarvae in the course of the tidal cycle. Station 263.

when the velocity of the tidal current was about 40 cm/sec. The largest number I ever met with in the Oosterschelde (2000 per 50 litres) occurred in a sample collected likewise at half flow (at 18.00 h.) on the 14th of July 1937 at the same station. This enormous number must be attributed to the proximity of liberating adults.

In series of samples collected in station 263, somewhat farther westward (fig. 13), where current-velocities exceed 50 cm/sec for some time, I did not notice any dropping to the bottom either.

Even at Kattendijke (fig. 12) such a dropping does not occur, although here the current-velocity exceeds 50 cm/sec from one hour after slack water till one hour before the next slack water, often exceeding 100 cm/sec at the height of the tide. These samples do show marked peaks in the number of larvae during a certain stage of the tide (at low water), but it is clear that this has nothing to do with the current-velocity, for the peaks do *not* occur at *high* slack water. This phenomenon will be explained presently.

Although the current-velocities in the Oosterschelde far exceed 0,5 foot per second (the limit for active swimming, as assumed by PRYTHERCH), I never noticed that the larvae of *Ostrea edulis* tend to drop to the bottom during these strong tidal currents.

The larvae of *Ostrea denselamellosa* were collected by SEKI and TANAKA (1931) in large numbers during a current-velocity of 1,45 miles per hour. The surface-samples did not contain fewer larvae than the bottom-samples, so these larvae do not drop to the bottom either.

The other way in which the current-velocity influences the vertical distribution of oysterlarvae is discussed by PERKINS (1931, 1932). PERKINS worked in the same locality as NELSON: viz. Barnegat Bay N.J. He stated that often there is no apparent correlation between salinity and the distribution of larvae: "In fact the curve of vertical distribution was practically the same as when sharp stratification was evident". "When we see that concentrations of larvae occur at levels other than where salinity changes are great and that the distributional curve is practically the same whether a halicline is present or not, it is clear that here salinity is not playing a part. On the other hand the distributional curve does not follow the tidal velocity curve in its general aspects". This he stated in his first paper (1931), after he had carried out some measurements of the tidal velocities at different depths, but without a simultaneous sampling of oysterlarvae at these various levels.

PERKINS does not believe that salinity is all-important in bringing about an orientation of bivalve larvae, but he assumes the current-velocity to be the cause of the inequal vertical distribution; according to him the increase of the number of larvae is commensurate with the increase in current-velocity.

PERKINS emphasizes that the larvae do not actively seek out the zone of greatest current-velocity, but that they are swept passively into the layer of increased current-velocity.

PERKINS explains the mechanism as follows: "It is a fact generally known to physicists that particles carried by a stream of liquid through a cylindrical tube become aggregated in the axis of the stream. This is due to friction against the walls of the tube which slows up the peripheral layer. Similarly in a shallow body of water such as Barnegat Bay there is friction against the bottom and at the surface water-air interface so that particles carried in the current tend to become aggregated in the axial stream and form, not a cone, as in the cylindrical tube, but a wedge. In this way the vertical distribution not only of oysterlarvae but of all other small larvae and lifeless particles may be accounted for".

The amount of suspended lifeless matter shows likewise a maximum in the zone of greatest current-velocity. In order to show that he was right in his suppositions, PERKINS collected in the next year some series of plankton-samples at different depths, at the same time measuring the current-velocity (fig. 14). It is his opinion that these data suffice to prove the correctness of his assumptions. In this second paper (1932) PERKINS admits that the larvae are found in the halicline, whenever current-velocities are small and salinity changes relatively great.

In the first place I want to make some remarks about his diagrams (fig. 14):

PLATE 1. Oysterlarvae responding to salinity changes, which according to PERKINS only occurs when the current-velocity is negligible.

Fig. 1. Larvae congregated in the halicline; the current-

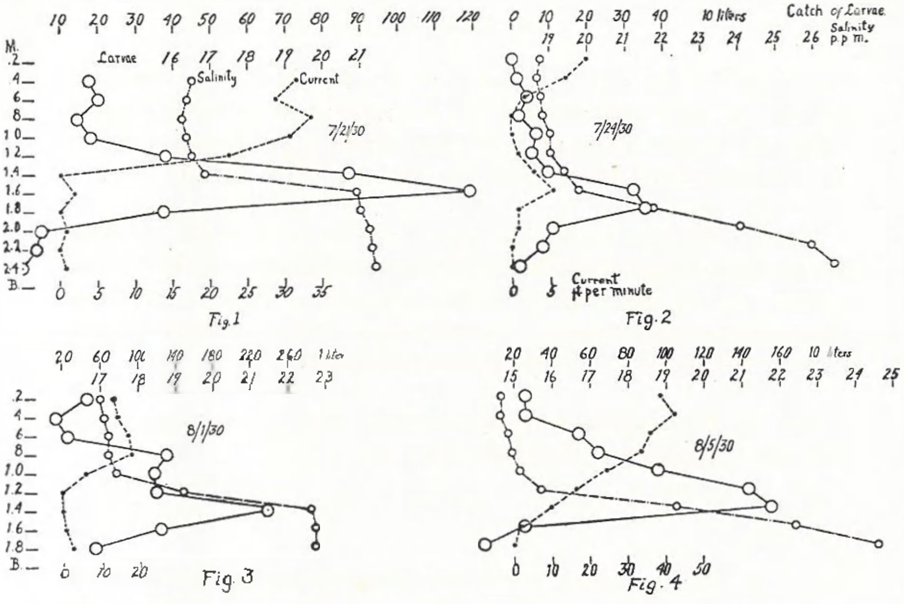

Fig. 1 Fig. 2 Fig. 3 Fig. 4

PLATE 1. OYSTER LARVAE RESPONDING TO SALINITY CHANGES

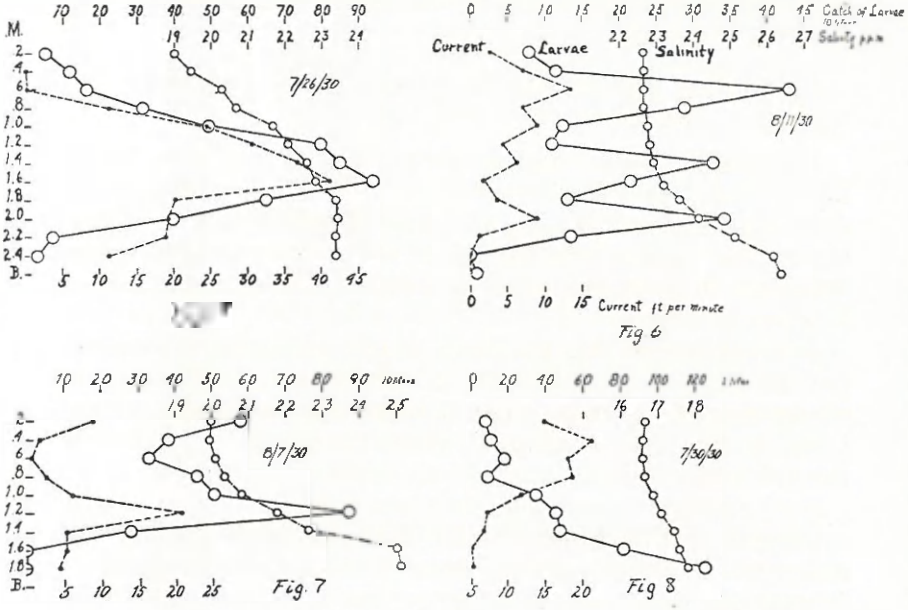

Fig 6 Fig. 7 Fig 8

PLATE 2. OYSTER LARVAE RESPONDING TO CHANGES IN CURRENT VELOCITY. NO HALICLINE PRESENT

Fig. 14. The influence of the current-velocity on the vertical distribution of oysterlarvae. After PERKINS, 1931. (Plate 1. Oysterlarvae responding to salinity changes; plate 2. Oysterlarvae responding to changes in current-velocity).

velocities in the upper layers, to 40 cm above the halicline, exceed 30 feet per minute! (The maximum current-velocity recorded in his diagrams does not attain 45 feet per minute). The current-velocity is certainly not negligible in this case.

Fig. 3. PERKINS ascribes a slight peak in the number of larvae above the maximum in the halicline to an increase of the current velocity in the layer in question. This current velocity remains below 20 feet per minute, so that action of the current velocity is more likely in the case visualized in fig. 1.

Fig. 4. The current-velocity exceeds 40 feet per minute and still the larvae respond to the halicline.

PLATE 2. Oysterlarvae responding to changes in current velocity.

Fig. 6. "Remarkable correlation between variations in current velocity and vertical distribution of oysterlarvae". The current velocity does not attain 15 feet per minute and in my opinion the number of larvae is not so remarkably commensurate with the current velocity.

Fig. 8. The current-velocity exceeds 20 feet per minute and might be expected to come into operation.

These figures show that the larvae are seldom abundant in layers with a salinity above 23 °/₀₀. The only case of an abundance of larvae close to the bottom is represented by fig. 8, where the salinity remains below 18 °/₀₀ to near the bottom. I believe that salinity plays a greater part than PERKINS is inclined to admit.

I do not believe that the forces which, according to PERKINS, are the cause of the difference in the vertical distribution, are strong enough to bring about a marked accumulation of the larvae in the layer of greatest current-velocity in the course of the few hours that the currents are rather considerable.

It is possible to compute the force exercised on a particle suspended in a medium of which the current-velocities in the different layers are not the same. TAYLOR (1928 a) gives a formula for that force. If we know the force, the size of the particle and the viscosity of the medium, it is possible to compute the velocity with which the particle tends to move towards the layer of greatest current-velocity. This force is strong enough to bring about an accumulation of the larvae in a few hours, when the current-velocities are about 40 feet per minute.

In the case of oysterlarvae the particles are, however, not suspended, but they move along with the medium. The force exercised on a particle in this case is many times smaller than that in case of a suspended particle. Though TAYLOR gives the energy-function for this system water/particle (1928 b), it is very difficult to calculate how great the force really is, as some of the coefficients are difficult to compute.

Therefore I do not believe that the force in question is great enough to bring about the effect observed by PERKINS. PERKINS did not collect series of samples during an entire tidal cycle, so it is impossible to infer from his data in how far differences in the horizontal distribution of the larvae play a part in the vertical distribution. For if the larvae are transported by the currents from the centre of larvae-production to a station where the larvae are rather scarce and the current-velocities are different in the various water-layers, it is very well possible that we shall find a far greater number of larvae in the layer of greatest current velocity during a certain part of the tidal cycle.

The currents in the Oosterschelde are many times stronger than those observed by PERKINS. Consequently, if PERKINS' hypothesis is correct, an accumulation of the larvae in the layer of greatest current-velocity is bound to occur in the Oosterschelde. I never observed, however, a difference between the number of larvae near the surface (layer of greatest current-velocity) and near the bottom at the height of the tide and at slack water. I therefore conclude that the force exercised by a difference in current-velocity in the various water-layers is not strong enough in the Oosterschelde to bring about a marked difference in the vertical distribution of the larvae. As the currents were far slower in PERKINS' case, this force was most probably too small to have brought about the differences observed by him.

Movements in horizontal direction

PERKINS states (1931): "A knowledge of horizontal distribution is of extreme importance in determining the location of setting areas, because it has been known for many years that sets are heaviest in localities where the larvae had been most abundant." It is easy to understand that the presence of an adequate number of mature larvae is the most important condition in procuring a spatfall of commercial magnitude. Several investigators have proved indeed that, generally speaking, the heaviest spatfalls are to be expected in those localities where larvae are most abundant (CHURCHILL and GUTSELL 1921, NELSON 1923 b: *Ostrea virginica*). It is impossible, however, to determine the horizontal distribution, unless vertical maxima are known.

Only PRYTHERCH (1929) assumes that the oysterlarvae are but to a negligible degree subject to the dispersing action of the tidal currents and that they wander only a few hundred yards from their place of origin. According to him the larvae have to rest on the bottom during by far the greater part of the tidal cycle to avoid dispersion. PRYTHERCH's assumption does not receive any support from other American investigators. Since my data concerning the larvae of *Ostrea edulis* in the Oosterschelde enabled me to prove that strong tidal currents do not cause the larvae to drop to the bottom, I do not think that it is necessary for me to take PRYTHERCH's views into account.

NELSON (1926, 1927, 1928 a, 1931) discusses a possible influence of vertical distribution (as brought about by marked differences in salinity) on horizontal distribution. When oysterlarvae tend to congregate in a special layer and the various layers show moreover different current-velocities, it is possible and even probable that horizontal distribution is affected by this state of affairs. It is doubtful, however, whether this influence is so efficacious as it was assumed to be by NELSON in a previous paper (1921), in which he supposed that the larvae migrate upstream. The influence of the salinity discussed above makes a movement upstream by progressive stages in principle possible, but the combination of the influence of salinity and that of different current-velocities may as well have a resultant in another or even in the oppostite direction.

There are not many data available on the horizontal distribution of the larvae of *Ostrea edulis*. German investigators (KÄNDLER 1928, HAGMEIER and SCHUBERT 1930) made some observations on the horizontal distribution of the larvae produced by the seed-oysters imported in the Wattenmeer. They found larvae in places fairly distant from the place of liberation and concluded that the larvae had been dispersed by the tidal streams. Many of them were washed away into the Northsea whence they but seldom returned.

BORDE (1932) stated that the larvae of *Ostrea edulis* are produced in a certain part of the basin of Arcachon, but that they appear to be distributed over the entire basin after a couple of days. HAVINGA (1932) and ORTON (1937 a) assume likewise that the larvae are probably moved up and down in the tide and that many larvae are carried away from the beds never to return.

My special series of samples collected in various stations in the Oosterschelde, where I often sampled for 24 hours at a stretch, enable me to inquire into this matter.

I have already stated that the vertical differences in temperature and salinity are always negligible in the Oosterschelde, so that complications in the vertical distribution of the larvae, brought about by these factors, are not to be expected here. Moreover the vertical distribution proved to be essentially the same at slack water and at the height of the tide, when the current-velocity exceeds 100 cm per second in many places. From this it appears that the larvae of *Ostrea edulis* refrain from dropping to the bottom when the current-velocity exceeds e.g. 100 cm per second.

As differences in illumination and wave-action likewise appeared to have no influence on the vertical distribution of the larvae, we may conclude that in the Oosterschelde the larvae are about uniformly distributed in a vertical sense at any time of the day and the night, in all kinds of weatherconditions and at all stages of the tide. As a result of this uniform vertical distribution we may expect that the larvae are absolutely at the mercy of the tidal currents. As to the direction and the force of the tidal currents, I refer to the description in the chapter on hydrographical conditions (fig. 1, 2).

The larvae are liberated in the basin of the Oosterschelde, so that the very first larvae in the beginning of each new season may be expected to appear at the station Yersche Bank. All the swarming takes place in this region, which causes sharp peaks in the number of larvae at this station. The tidal currents disperse these larvae and distribute them over a large area. So we shall find that the number of larvae at a station, situated at some distance from the centre of swarming, tends to increase some time after swarming; the greater the distance, the later this increase is to be expected. It is self-evident that this dispersion causes a decrease of the great number of young larvae counted on the day of swarming in the centre Yersche Bank. The real extent of swarming on one particular day can be deduced from the degree with which the number of larvae increases at a more distant station and from the number of larvae in the centre after dispersion. The amplitude of the number of larvae during swarming in the centre itself is a less reliable measure, as it is affected by the degree of proximity of the liberating adults. Thus the enormous number of larvae (2000 per 50 litres) in a sample collected on the 14th of July 1937 at the station Yersche Bank, for instance, did not point to a heavy swarming, for the diagram (fig. 8) clearly shows that it must be attributed to the immediate proximity of one (or more) liberating adult(s).

Very instructive is the number of larvae at the station Kattendijke in the course of a tidal cycle (fig. 12). As there are no adult oysters in the neighbourhood, Kattendijke receives all its larvae from the basin by the action of the tidal currents.

From the series of small charts (fig. 2) it may be seen that the water which fills the basin at high-tide moves westwards during ebb and reaches Kattendijke about two or three hours before low slack water. Then for some time it moves still farther westwards to return during flow. So the water at the station Kattendijke during a few hours roundabout low slack water consists of water that fills the basin of the Oosterschelde at high water. About high tide the water at the station Kattendijke consists of water derived from the "outlying district", situated N.W. of this station. As the Channel of the Oosterschelde shows a surplus of ebb, part of the low-tide water derived from the basin will not return to the basin during the next flow, but will mix with the high-tide water from the outlying district.

Two series of samples collected during 24 hours at this station both yielded the same results. In fig. 12 the data from the sampling from 10 to 11 August 1938 are visualized. They show a marked correlation between the tidal cycle and the number of larvae.

During low tide, when for some hours the water is made up of the water that fills the basin during high tide, the number of larvae is great, while the number of larvae counted in the samples collected during high tide, when the water is derived from the outlying district, is relatively small. The larvae present during high tide at this station originate likewise from the basin of the Oosterschelde, however; they are introduced with the water of the successive surpluses of ebb which are mixed with a great deal of practically "barren" water from the outlying district. It will be clear that this will result in differences in the composition of age-classes at high-water and at low-water. As a matter of fact we do find that the high-water larvae are on an average of a larger size than the low-water larvae. At this station newly-liberated larvae are practically never met with during high water. It may be seen in fig. 12 that at Kattendijke the percentage of earlier larvae is much smaller during high-tide than during low-tide. This series of samples provides at the same time an instance of an almost negligible difference between the samples collected near the surface and those collected at a depth of 5 metres.

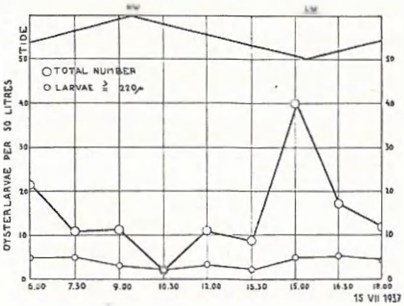

Fig. 15. Number of oysterlarvae in the course of the tidal cycle. Station E.V.

When we collect a series of samples, still more distant from the basin (e.g. station E.V. fig. 15), we shall expect a priori (provided we do not cross the low-tide boundary of the basin-water) a shorter period with ample larvae during low water than at the station Kattendijke. Fig. 15, which represents such a series, shows at the same time that the maximum number of larvae during low-water (80 per 100 litres) remained considerable below the maximum at the station Kattendijke on that day (about 150 per 100 litres). On 20 July 1936 we made a trip from Bergen op Zoom to the outlying district (Zierikzee), on which occasion we collected plankton-samples in many places. A sudden drop in the number of oysterlarvae indicated that we had crossed the boundary of the basin-water. This boundary appeared to be situated between Wemeldinge and Kattendijke, about 4 hours after low-water. The next day, when we made the trip in the reversed direction, we traced the boundary somewhat N.W. of Kattendijke (near the station E.V.) about 4 hours after high water. This is perfectly in accordance with the data on the movements of the basin-water supplied by Rijkswaterstaat, visualized in the series of small charts (fig. 2). The point of the tongue of basin-water which intrudes in N.W. direction during ebb appears to be less rich in larvae than the rest of the tongue.

The point of the tongue is made up, generally speaking, of the water that enters the basin during the very last stage of the flow and which does not penetrate far into the basin. When the point of the tongue is less rich in larvae, we shall also expect fewer larvae in the western part of the basin during high-water. That this is really the case is shown by a series of samples taken in the western part of the basin (station 263, fig. 13). As here we are quite close to the centre of larvae-production the difference in the number of larvae between high water and low water is much slighter. As is to be expected, the average size of the larvae is largest during high tide, for during low tide we find more newly-liberated larvae.

I procured many series of samples during an entire tidal cycle and others during 24 hours at the station Yersche Bank, the same station where one of the daily samples is collected. I invariably noticed that the course of the number of larvae is not so neatly gradual as, for instance, at the station Kattendijke.I often met with peaks of greater or lesser importance and with differences between the number of larvae at the surface and the bottom.

A comparison of all these series of samples proved, however, that these peaks are not correlative with the tidal cycle and that the differences in vertical direction have nothing to do with variations in factors like illumination, temperature, salinity, wave-action and current-velocity. In fig. 11 such a series of samples is visualized. From this it may be seen that there is no regularity in the occurrence of the vertical differences in the distribution of the larvae.

In my opinion all these deviations from the "normal" course and from the "normal" vertical distribution, observed at this station, may be attributed to the proximity of liberating adults. This station is situated in the centre of the most important oyster-beds. The water that passes this place at all stages of the tidal cycle has flowed for some time over these oyster-beds.

Though swarming tends to be concentrated to a considerable degree, single liberating adults are practically never absent. The nearer the station of sampling is to the adults, the greater the chance that the larvae of one or more liberating adults will interfere with the normal regular course of the graph on the number of larvae.

I mentioned before that at this station I once counted 2000 larvae per 50 litres on a day on which the average number of larvae was about 100 per 50 litres. If my supposition that these irregularities at this station are mainly due to the proximity of liberating adults is right, we may expect these peaks to be caused by a sudden abundance of the youngest planktonic larvae, in other words, we may expect the graphs on the older larvae not to run parallel to the graph on the total number. This appears to be the case: when tracing a separate graph for each size-class I find that only the youngest larvae are guilty of the irregularities. Generally speaking we may state that the number of larvae at the station Yersche Bank does not show any marked tidal fluctuations.

Summarizing, I believe I may state that the larvae of *Ostrea edulis* are passively moved up and down in the tide.

The tidal currents disperse them and transport them. It has been shown that the data on these transports perfectly fit in with the data on the tidal movements supplied by Rijkswaterstaat. The water in the centre of basin of the Oosterschelde contains an approximately equal number of larvae throughout a tidal cycle.

Stations situated west of the basin are characterized by a smaller number of larvae at high-tide; the farther west the greater the difference between the high-water and the low-water number. The farther west the shorter the period with many larvae and the smaller the total number of larvae during the entire tidal cycle.

The larvae are apparently unable to counteract dispersion. This inability clearly shows the enormous advantage of sufficiently enclosed breeding-grounds. An adequate retention of the larvae is only to be expected in places where the tides perform a more or less perfectly oscillating movement, which enables the majority of larvae to return to the grounds after each tidal cycle.

In connection with the points discussed above I want to make a few additional remarks about the reliability of my daily samples (fig. 8, 9, 10). At the station Kattendijke the daily samples are procured at low slack water. Roundabout low water the number of larvae is great there. The moment of sampling is seldom more than half an hour removed from the moment of low slack water. Consequently errors caused by untimely sampling are not to be expected. The depth of sampling is always 5 metres here. As no variations in vertical distribution occur here, errors owing to differences in illumination, salinity, temperature, wave-action or tidal currents are not to be expected. Consequently (and this may be deduced likewise from the diagrams) the abrupt transition from sampling at evening low water to that at morning low water (indicated by "m" in the diagrams) has no influence whatever on the number of larvae collected. Pump sampling at low slack water does not yield unreliable results, for I never noticed fewer larvae in the samples collected just at slack water (fig. 11, 12). Hence it follows that the larvae are most probably not able to counteract perceptibly the suction of the pump by their own locomotive force.

At the station Yersche Bank samples are procured about half an hour after low slack water and about two feet above the bottom. Irregularities owing to the proximity of liberating adults may occur here. The occurrence of swarming is soon noticed at this station. The extent of each swarming can safely be deduced from the number of larvae counted in the samples collected at both stations on the day after swarming.

Weatherconditions, tidal currents, differences in salinity and the abrupt transition to the morning-tides apparently have no influence on the reliability of the sampling at this station either.

In order to explain the occurrence of fairly large numbers of spat in places fairly distant from the spawning-grounds, several investigators suggest a secondary accumulation of larvae. This accumulation is not ascribed to differences in vertical distribution by these authors.

They speak of eddies and of bights where the currents are negligible. In places like these the larvae are supposed to accumulate by an unexplained mechanism (CHURCHILL and GUTSELL, 1921, NELSON, 1921, KÄNDLER, 1928, 1930). In places where the same body of water maintains itself during an entire tidal cycle the dispersion by the currents will be less effective. This phenomenon is not observed in the Oosterschelde, however, owing to the thorough tidal mixing and the considerable tidal range (3 to 4 metres).

This is not what these writers have in view, however; what they do think of is a secondary accumulation in such places after a previous dispersion: "Anderseits bewirkt die Bodengestaltung im Verein mit Stromstauungen und Wirbelbildungen wiederum Anhäufungen von Austernlarven an einzelnen Stellen, die sich darum durch einen reichen Brutfall auszeichnen". (KÄNDLER, 1930). According to NELSON (1921) eddies act as a sort of "trap" for the larvae and he, too, mentions a heavy spatfall in such places.

They never state the real difference between the number of larvae in the bulk of the water and in such eddies. It is my opinion that the heavy spatfall in such places is what led to these suppositions. It will be discussed below that the proportion between the number of full-grown larvae and the intensity of setting is not the same at each station. Consequently there may be places, though remote from the centre of larvae-production, which may yet show an abundant spatfall. We need not assume a previous accumulation of the larvae to account for this phenomenon.

Unless we suppose that the larvae rest on the bottom during part of the tidal cycle or that circular currents cause centrifugal forces of some importance, I know of no other kind of mechanism capable of accumulating again the dispersed larvae. Since such a resting on the bottom does not occur in the Oosterschelde and as I have never met with such an accumulation, I do not believe that this phenomenon occurs here.

XVI. DURATION OF THE PELAGIC STAGE

Apart from the quality and quantity of the available nourishment, which factor has already been discussed in a previous chapter, there are some others which determine or are supposed to determine the general growth-conditions for the larvae and through this the duration of the pelagic stage. I shall respectively discuss the factors salinity and water-temperature.

The effect of salinity on the development of oyster-larvae

Many experiments in vitro have been carried out by several scientists in order to ascertain the optimum salinity for the development of oysterlarvae. Larvae of non-incubatory species of oysters lend themselves admirably to this purpose, for artificial fertilization succeeds easily in these species. The following data have been obtained in this way:

Species	Salinity limits between which development is possible	Optimum salinity for early developmental stages	Salinity in the locality where these oysters grow wild	Author
Ostrea circumpicta Pilsby		28–38$^{\circ}/_{oo}$	23–35$^{\circ}/_{oo}$	Amemiya (1928*b*)
Ostrea denselamellosa Lischke	20–35$^{\circ}/_{oo}$	30–33$^{\circ}/_{oo}$	26–34$^{\circ}/_{oo}$	Amemiya (1921, 1928*b*)
Ostrea gigas Thunberg	8–35$^{\circ}/_{oo}$	15–25$^{\circ}/_{oo}$	10–32$^{\circ}/_{oo}$	Amemiya (1928*b*)
		18–27$^{\circ}/_{oo}$		Amemiya (1921)
		23–25$^{\circ}/_{oo}$		Seno, Hori and Kusakabe (1926)
		20–22$^{\circ}/_{oo}$		Fujita (1929)
Ostrea gigas var. *sikamea* Amemiya		15–25$^{\circ}/_{oo}$	8–30$^{\circ}/_{oo}$	Amemiya (1928*b*)
Ostrea rivularis Gould		15–25$^{\circ}/_{oo}$	10–30$^{\circ}/_{oo}$	Amemiya (1928*b*)
Ostrea spinosa Quoy.		30–38$^{\circ}/_{oo}$	25–33$^{\circ}/_{oo}$	Amemiya (1928*b*)
Ostrea virginica Gmelin.	15–39$^{\circ}/_{oo}$	25–29$^{\circ}/_{oo}$		Amemiya (1926)
	14,5–39$^{\circ}/_{oo}$	23$^{\circ}/_{oo}$		Clark (1935)
Gryphaea angulata Lam.	21–43$^{\circ}/_{oo}$	28–35$^{\circ}/_{oo}$		Amemiya (1926)

Development often tends to become irregular and often shows a considerable retardation in cases when salinity is unfavourable.

It may be seen that there is in many cases a marked relation between the optimum salinity for development in vitro and the salinity in places where the species under consideration grow wild. During their larval life, too, littoral species are more eurihaline.

As regards *Ostrea virginica* some data on field-observations are available. Nelson (1921) states that oyster culture is possible between salinities of 10 to 31$^{\circ}/_{oo}$. Hopkins (1931) made some observations in Galveston Bay (Texas), where considerable variations in salinity frequently occur. He assumed a correlation between increases in salinity and the intensity of setting. Although "quantitative methods of measuring the abundance of oysterlarvae in planktoncollections were not employed" and his methods of measuring the intensity of setting are not very reliable, he stated that "the results indicate that setting periods followed risings in salinity above approximately 20°/₀₀, although it is certainly impossible to state an exact limit". As a matter of fact his data show that setting also occurred with salinities of 8 to 16°/₀₀. A profuse spatfall was even observed at 12°/₀₀ (l.c. p. 70 fig. 12).

As regards *Gryphaea angulata* it may be remarked that RANSON's (1938) observations are not in agreement with AMEMIYA's (1926) data. RANSON stated that no spatfall is to be expected in places where the salinity exceeds 24°/₀₀. The natural beds of the Portuguese oysters are always situated in places with a salinity below this figure. In dry years the salinity in the French estuaries tends to increase to such a high degree that no spatfall of *Gryphaea angulata* takes place. BORDE and BORDE (1938) assume likewise that high salinities are unfavourable for the reproduction of the Portuguese oyster.

As artificial fertilization has been unsuccessful in *Ostrea edulis* so far and it is even impossible to rear young embryos taken from the maternal mantlechamber, the optimum salinity for development in vitro cannot be ascertained in the same way as in the non-incubatory oysters.

AMEMIYA (1926) tried to do so, but he stated that his results are not sufficient to base conclusions on.

GAARDER (1932, 1933) and GAARDER and BJERKAN (1934) assume that the larvae of *Ostrea edulis* require a salinity of 24°/₀₀ or more for successful growth and presume that the optimum salinity will be about 30 to 35°/₀₀. MAZZARELLI (1924) observed a normal larval development in the Lago Fusaro with salinities from 34 to 39,5°/₀₀. The salinity during COLE's (1939) successful experiments in tanks varied from 30,5 to 32,5°/₀₀. ORTON (1937a) assumes that the salinity of the water in the sea is probably rarely unsuitable for larval development.

Though the salinity in the Oosterschelde varies but slightly in the course of the summer, there may be some difference in salinity in different years.

The table on page 134 clearly shows that in the Oosterschelde there is no correlation between the intensity of the setting and the average salinity during the season of reproduction.

It is my opinion that variations in salinity between 25°/₀₀ and

Year	Average Summer-salinity	Spatfall.
1922	29% ± 1	complete failure
1925	28% ± 1	good
1926	27% ± 1	profuse
1928	28% ± 1	profuse
1929	28% ± 1	very abundant
1931	28% ± 1	moderate
1934	29 to 30% ± 1	failure
1935	28% ± 1	good
1936	27% ± 1	complete failure
1937	26% ± 1	moderate
1938	28 to 29% ± 1	good
1939	27% ± 1	good

35°/oo probably have little or no influence on larval growth and development in *Ostrea edulis*. So I agree with VOISIN (1933) who states: "La salinité, si elle se maintient dans des limites normales, paraît ne jouer qu'un rôle assez effacé." Excellent spatfalls occurred in the Oosterschelde with salinities from 27 to 29°/oo, but other factors are probably responsible for the enormous annual variations in spatfall. I never detected any variation in the rate of development of the oysterlarvae in the Oosterschelde correlative with these slight annual differences in salinity.

The effect of water-temperature on the development of oysterlarvae

Just as in the case of salinity, it is easier to investigate the influence of the water-temperature on the development of larvae of non-incubatory oysters than of incubatory species. The study of the influences of temperature during early developmental stages in vitro is possible after artificial fertilization. The strictly simultaneous spawning in *Ostrea virginica* and *Ostrea gigas* facilitates the determination of the duration of pelagic life in the open sea.

Ostrea gigas

Fertilization takes place most vividly and the rate of development of the young larvae of *Ostrea gigas* proved to be most rapid at temperatures from 23 to 27° C (FUJITA 1929, SENO, HORI and KUSAKABE 1926). ELSEY (1936) and ELSEY and QUAYLE (1939) observed in Ladysmith Harbor that the duration of the pelagic stage of this oyster is about 18 days at temperatures of 21 to 22° C.

Ostrea virginica

Miss CLARK (1935) showed by experiments in vitro that the rate at which early development takes place varies considerably at different temperatures. The first swimming stage is reached 25 hours after fertilization at 15° C and after 3 hours at 30° C.

When the day on which the simultaneous spawning of *Ostrea virginica* occurs is known, it is possible to ascertain the length of the pelagic stage by determining the moment at which the first spatfall takes place.

JULIUS NELSON (1908) presumed that the pelagic stage lasted from one week at 24 to 27° C to three weeks at 21 to 24° C. CHURCHILL and GUTSELL (1921) mentioned a free swimming stage of 12 to 14 days at 21° C. NELSON (1923 a) stated 15 days of pelagic life in Barnegat Bay, while the temperature varied from 21 to 24° C; later on (1928 a) he stated 13 days at temperatures of 23 to 25° C and 17 days when the water was 20° C. According to NEEDLER (1932 a) the free-swimming stage often lasts three weeks in the colder water of Canada. PERKINS (1932) states that low water-temperatures are often the cause of a protracted free-swimming period. When high water-temperatures are recorded (25° C and more), fixation will be observed from the twelfth day after spawning. MEDCOF (1939) carried out investigations in Canada (Bideford River). He stated that the duration of the pelagic stage there is about 30 days at 19° C, about 26 days at 20° C and about 24 days at 21° C, which figures are considerably higher than those of CHURCHILL and NELSON. It is doubtful, however, whether his dates are absolutely reliable; MEDCOF admits that his constructions are partly conjectural.

The determination of the length of the free-swimming periods presents more difficulties in incubatory species of oysters, because these oysters do not show a strictly simultaneous swarming. It is only possible to speak of maxima of swarming, for during the season of reproduction hardly a day passes with no swarming at all. Swarming usually initiates with the appearance of a small number of larvae in the plankton, practically none of which may reach the fixation stage, because of their small number. The measuring of the duration of the free-swimming period by ascertaining the period of time elapsing between the appearance of the first larvae in the plankton and the observation of the first spatfall must therefore be regarded as an altogether unreliable method. It is necessary to ascertain the interval between a maximum of swarming and a maximum of setting in order to know the duration of the pelagic stage in the field. For this a very frequent examination of larvae and setting is required. Often the spatfall does not show clearly marked maxima, which makes the determination of the length of the pelagic stage practically impossible in such cases.

Ostrea lurida

STAFFORD (1914) estimated the length of the free-swimming period in the Olympia oyster at about one month.

It was GALTSOFF's (1929) opinion that the length of this period is about 14 days. COE (1932 c) even presumed that the free-swimming stage is so short in *Ostrea lurida* that the chances of a dispersion of larvae by the currents are limited.

Neither of these authors had many reliable data at their disposal.

It was HORI (1933) who reared these larvae in vitro till fixation in 22 days at 20° C by feeding them ground sea-lettuce.

HOPKINS' (1937) investigations enabled him to establish the dates of spawning and thus to ascertain the maxima of swarming in Olympia oysters. Quantitative investigations on the abundance of larvae in the water were not made, however, so it was impossible for him to follow the larval age-groups. HOPKINS examined the spatfall, too, and it is possible to read from his diagrams (e.g. fig. 40) that the period which elapses between spawning and setting exceeds one month (17 to 18°C). So HOPKINS' conclusion that „the free-swimming period appears to be 30 to 40 or more days, depending largely perhaps on water-temperatures, so that the total larval life is at least 40 days" will not be far from the truth. This shows that STAFFORD's first estimate was about right.

Ostrea edulis

Petersen (1908) observed that the larvae of *Ostrea edulis* swim actively in water of 13° C. He detected the presence of larvae in the plankton at 15° C. Hagmeier's (1916) data on tank-breeding enabled him to state that the free-swimming period lasts from 10 to 14 days at 18 to 21° C. Mazzarelli (1922) succeeded in rearing the larvae of *Ostrea edulis* in vitro till settlement. The water-temperature in his containers was not constant during his experiments. He stated that the duration of the free-swimming period is about 16 to 17 days at a water-temperature from 15 to 16° C. The water-temperature increased during his experiments, so that the temperature in the first part of the free-swimming period during which, in my opinion, the larvae are most sensitive, must have been fairly low (14 to 15° C.)

Mazzarelli found viable larvae in the water of the Lago Fusaro at any temperature between 13° C and 30° C. He assumes that the larvae of *Ostrea edulis* can stand any variation in temperature between these figures.

Boury (1928) concluded that he could not state a limit below which larval development and fixation are not possible.

Kändler (1930) stated that the larvae require a temperature of about 20° C to grow and settle: "Erst etwa von 18° C ab gestatten sich die Entwicklungsbedingungen günstig und die Wassertemperaturen müssen sich eine Zeitlang um 20° C und darüber halten, damit die Austernbrut heranwächst und sich festsetzt."

There are more statements to this effect, for instance that by Gaarder (1933) who says that the larvae require 20° C for normal development and that by Lambert (1935) who tells us that no fixation takes place below 18° C.

I want to emphasize that it is wrong to argue on the assumption that no development and no fixation are possible below these temperatures, if this assumption is based on the fact that one does not succeed in finding spat after swarming during a cold spell. When the larvae are not very abundant and the pelagic stage is protracted owing to low water-temperatures, the number of larvae that survives till fixation may be so small that it is extremely difficult to find some spat. The experiments by Mazzarelli prove that larval development and spatfall are certainly possible at fairly low water-temperatures.

Chaillé (1938) expresses himself more cautiously when he states: "Dans nos eaux on peut dire qu'au dessous de 18 degrés la récolte est pratiquement nulle; les huîtres pondent, les larves sont émises, mais elles périssent avant d'avoir atteint leur stade de fixation."

Erdmann (1934) observed that fixation in vitro is still possible after very long periods of pelagic life (e.g. 50 days). Cole's experiments in tanks (1936, 1939) yielded some further data. He observed fixation 9 to 10 days after swarming and concluded that the pelagic stage was much shorter than had been assumed by Hagmeier and Mazzarelli. Cole forgot to mention, however, that the water-temperature in his tanks was 21 to 22° C, which temperature is much higher than that in the experiments of the above-mentioned authors. From the data in his second publication it may be seen that the length of the free-swimming period in his tanks was about 10 to 11 days at temperatures from about 19 to 20° C.

Summarizing, we find that the following figures have been stated:

temperature	Duration of pelagic stage	Author
15–16° C	16–17 days	Mazzarelli (1922)
18–21° C	10–14 days	Hagmeier (1916)
19–20° C	10–11 days	Cole (1936)
21–22° C	9–10 days	Cole (1939)

The length of the pelagic stage in the Oosterschelde can be approximated by comparing in the diagrams the maxima of swarming with the maxima if setting.

It should be borne in mind that these comparisons are somewhat arbitrary, for the setting was not ascertained daily, but in periods of three days, as will be discussed below. The centre of such a period may be assumed to represent about the right place of the maximum of setting. Sometimes no marked maxima of setting occur. Maxima of swarming are also often not clearly marked and secondary waves of swarming frequently interfere. (See table on page 139.)

These data do not differ essentially from those of other investigators collected in the above table.

Temperature during the first 5 days	Temperature during the next days	Duration of pelagic stage	Dates	
			swarming	setting
16° C	17° C	14 days	8–9 VII 1938	22 VII 1938
17° C	16° C	13 days	27 VI 1938	10 VII 1938
17° C	17° C	12 days	23 VI 1938	4 VII 1938
19° C	18–19° C	12 days	17–18 VII 1939	29 VII 1939
20–21° C	18–19° C	9–10 days	18–19 VII 1937	29 VII 1937
21° C	21° C	7 days	21 VI 1936	28 VI 1936
22,5° C	22° C	7 days	11–12 VII 1935	18–19 VII 1935
22° C	23° C	6 days	2–3 VIII 1938	8–9 VIII 1938

I want to conclude that the length of the pelagic stage is largely dependent on the water-temperature and that Cole underestimates this influence when he suggests that the lengthening of the free-swimming period by water-temperatures as low as 15 to 17° C is not likely to exceed one day or at most two.

Though we know the length of the free-swimming period and also the measure in which the size of the oysterlarvae increases during this period, we are not justified in computing the daily growth-rate by a simple division of these two figures. It is very well possible that the daily growth-rate varies considerably in the course of the pelagic period.

As far as I know only Nelson (1923 b) and Medcof (1939) published some data about the daily growth-rate of the larvae of *Ostrea virginica*. Nelson measured regularly a number of larvae from eggs spawned simultaneously on one particular day in Barnegat Bay. The growth-curve proved to be of a sigmoidal shape; so the growth-rate appears to increase during the first part of the pelagic stage and to decrease during the second part. I doubt, however, whether the last part of the curve is quite reliable, for fixation, unlike spawning, is not quite simultaneous in *Ostrea virginica*. Nelson measured a certain number of larvae (10) and not the larvae from a certain volume of water; consequently we do not know how many of the original larvae had settled already. However, setting will no doubt influence the average size of the larvae during the very last part of the pelagic stage. In case we observe a "decrease in growth-rate" shortly before setting, by measuring a certain number of larvae, there is a possibility that such a decrease must be attributed to fixation of the most precocious larvae. For these precocious larvae disappear from the plankton by attachment, so that only the late-comers get measured.

MEDCOF (1939) constructed his growth-curves with the aid of frequency polygons for larval measurement, plotted on a percentage basis. He did not sample daily and secondary waves of spawning interfered. Though his constructions are partly conjectural, we may yet conclude from them that the growth-rate increases in the course of the pelagic period.

A close study of my diagrams (fig. 8, 9, 10) may yield some data on the daily growth-rate of the larvae of *Ostrea edulis* during their pelagic life. It should be borne in mind that the division in size-classes in the diagrams does not imply a division in age-classes! There is an apparent difference in the proportions between the numbers of larvae in the different classes of the smaller sizes (165 μ to 210 μ) and between the classes of the larger sizes (210 μ to 300 μ). The decrease in the number of larvae in the course of the pelagic stage appears to be far most considerable in the groups of the smaller sizes. This phenomenon is not due to the dispersal of newly-liberated larvae, for the diagrams for Kattendijke show the same facts. Such a phenomenon may be attributed to the operation of two factors, a differential death-rate and a differential growth-rate. In this particular case I mean by the word "death" a disappearance of the larvae from the plankton in the basin, which may be caused by real death or by dispersion of the larvae to other bodies of water. The larvae of all size-classes appeared to be equally subject to the dispersing action of the currents (see vertical distribution).

A great many plankton-eating animals are the cause of the untimely death of countless oysterlarvae and although it is possible that some of those animals prefer to feed on the youngest stages or are not able to ingest the larger larvae, I do not believe that such a preference can be the only cause of the considerable difference in the proportion of the numbers of larvae in the various size-classes. It is my opinion that this difference in casualities points to the occurrence of a differential growth-rate. If we assume that the daily "death"-rate through dispersion and devouring is about the same in the course of pelagic life, but that the growth-rate of the larvae increases (so that a very rapid growth occurs after the size of 210 μ has been attained), we shall expect a difference in the proportions of the larval groups as deducible from the diagrams.

It is my belief that my data point to the probability that the growth-rate increases considerably in the course of the pelagic life of *Ostrea edulis*, so that growing from 210 μ to 300 μ requires far less time than growing from 170–180 μ to 210 μ. Such an increasing growth-rate is in agreement with the result of the American investigations on *Ostrea virginica*; my diagrams do not point to any decrease of the growth-rate near the end of the pelagic stage, however.

XVII. DESTRUCTIVE AGENCIES

If all the larvae which are produced in one single year in the basin in the Oosterschelde should reach the adult stage, the basin would be filled up with them to above the high-water mark. A very high percentage of the larvae disappears from the plankton before the setting stage is reached. Only part of the mature larvae succeed in finding a suitable cultch to attach themselves and only a small percentage of the newly-settled spat will reach the adult stage.

The destructive agencies which cause the disappearance of so many oysterlarvae from the plankton may be divided in abiotic and biotic ones.

Abiotic destructive agencies

Tidal currents

All things considered, the tidal currents cannot be classed among the destructive agencies in a narrower sense, for they do not cause an untimely death of planktonic oysterlarvae. Nevertheless the disappearance of a considerably part of the oysterlarvae from the plankton above the oyster-grounds is due to the action of the tidal currents. Many of the larvae which are swept away during ebb do not return during the next flow. Hydrographic conditions determine what percentage of the larvae shall be carried away from the oyster-grounds by the tidal currents. It has been discussed in a previous chapter that the water renewal during each tidal cycle in the basin of the Oosterschelde is but slight. The larvae which do not return to the basin are still alive when they arrive in other bodies of water and probably growth-conditions there do not differ much from those in the basin. Those larvae are dispersed to such a degree, however, that the number of larvae per 100 litres of water is so small in the outlying district, that the planting of cultch material there would not be justified from a commercial point of view. As a great part of the subsoil in the outlying district is absolutely unsuitable for the fixation of oysterlarvae and natural cultch material is very scarce, by far the greater part of the oysterlarvae which are carried away by the tidal currents to the outlying district will not be able to find a suitable place to settle. Consequently those larvae have not only disappeared from the basin, but they should be considered as lost for the greater part.

In the section "Hydrographical conditions" it has been stated that about 4% of the water of the basin of the Oosterschelde disappears during each tidal cycle, carrying its plankton along with it. After 14 days about 35% of the original water is still present in the basin. The toll levied each tide of the oysterlarvae by the tidal currents is consequently not very great in the Oosterschelde. When the pelagic stage is protracted by unfavourable temperature-conditions, the percentage that remains in the basin will be smaller than that which remains when the pelagic stage is short. We are, however, not justified in deducing from the foregoing that, in case of a pelagic period of 14 days, about 65% of the larvae initially present in the basin disappear from the plankton by the action of the tital currents, for there are still other factors causing loss of larvae. Consequently the 4% loss by the tidal water-renewal forms only a part of the total tidal loss, so that the losses during the next tide must be substracted from a figure smaller than 96% of the original number of larvae. Consequently the total loss caused by the tidal currents in the cause of the pelagic stage is not $(100 - 0{,}963^n \times 100)\%$ of the original number, but far less. How much it is in reality will be discussed presently.

Water-temperature

Churchill (1920) and Nelson (1920, 1921) assume that the larvae of *Ostrea virginica* are extremely sensitive to sudden decreases in the temperature of the water. "A drop within 24 hours of from 3° to 5° may be followed almost immediately by the disappearance of a large part of the larvae from the water" (Nelson 1921).

Prytherch (1929) and Galtsoff, Prytherch and McMillan (1930), on the contrary, stated that their studies had shown that oysterlarvae can stand sudden changes in temperature. **Prytherch** does not even take into account the effect of biological conditions during the pelagic stage on the percentage of larvae that reaches maturity. He assumes that especially the number of eggs produced determines whether the crop of spat will be sufficient or not. I believe that this view should be attributed to the circumstance that **Prytherch** could not follow the fate of the larval herds, because of the scarcity of larvae in his samples.

Miss **Clarck** (1935) observed that the larvae of *Ostrea virginica* (in vitro) can stand considerable differences in temperature. Decreases in temperature do not so soon cause a considerable mortality.

Mazzarelli (1924) stated that the larvae of *Ostrea edulis* can stand temperatures from 12° to 30° C at least. Sudden changes in temperature do not cause a heavy mortality. The larvae swim actively at 13° C. **Spärck** (1927) transported oysterlarvae in thermoflasks with ice (0° C). The changes from 18° to 0° and later on from 0° to 18° did not do any harm to the larvae of *Ostrea edulis*. Later on (1929) he proved that the larvae can stand a temperature of 0° for 24 to 48 hours, even of −2° C for a short time and of about 5° C for several weeks. So **Cole** (1939) is right when he states that "once shed, the larvae are exceptionally hardy". **Cole**'s view that after liberation the food supply rather than temperature is the critical factor, is, however, only applicable to tank conditions. One of the principal advantages of spat-production in an enclosed basin is the increased measure of protection it affords to the larvae. Tidal currents and a great many animal enemies are thus shut out from the tanks. Consequently the length of the pelagic period has not much influence on the percentage of larvae that reach the setting stage. In the open sea, on the contrary, animal enemies and the tidal currents exact daily a heavy toll of the larvae, so that every protraction of the pelagic stage will cause a decrease in the percentage of larvae that reaches maturity. And although low water-temperatures do not directly cause an untimely death of oysterlarvae, they do bring about a protraction of the pelagic stage and thus become the cause of heavy losses.

As the protraction of the pelagic stage may be considerable (22° C: 7 days, 16° C: 16 days of pelagic life), it is my opinion that, although oysterlarvae are exceptionally hardy and can stand very low temperatures, the influence of the water-temperature on the extent of the larval losses during the pelagic stage is very great.

Other abiotic factors are seldom considered as destructive agencies during the pelagic period. NELSON (1921) states that strong winds, which stir up large amounts of sediment, destroy many larvae: the larvae fill their digestion tracts with dirt instead of with their accustomed food, which may cause their death.

GAARDER (1932) states that a pH above 9.00 is injurious to oysterlarvae. Anorganic enrichment caused high pH values in the pollen. His experiments in vitro showed moreover that a copper content of more than 0,04 mg per litre is injurious to oysterlarvae.

Biotic destructive agencies

A great many marine animals that naturally feed on plankton-organisms exact a very heavy toll of oysterlarvae during the pelagic period. It is probable that practically all kinds of animals which strain the water for their food ingest oysterlarvae. Such animals can be divided in bottom-dwellers and sessile forms, such as barnacles, sea-squirts, other bivalves, certain worms, hydroids, etc., pelagic forms, such as most kinds of young fish, e.g. herring and anchovy, and some representatives of the macroplankton, such as *Aurelia aurita* and Ctenophora.

In several cases the ingestion of oysterlarvae has actually been observed and their presence in the stomach-content of various freshly-caught animals has been demonstrated.

Thus ORTON (1922 b) tells us that the jelly-fish *Aurelia aurita* greedily ingests larvae of *Ostrea edulis*, which are also to be found in the stomach-content of these animals when caught in the open sea. DODGSON (1922) describes observations made by SHERWOOD: *Noctiluca*, put together with young oysterlarvae (*Ostrea edulis*) in an aquarium, appeared to cause a rapid diminution in the number of these larvae. The actual ingestion of these oysterlarvae was repeatedly observed. HORI and KUSAKABE (1926) tell us that the larvae of *Ostrea gigas* are eaten by *Noctiluca* and by the larvae of Actiniae.

In some cases one kind of depredator is so numerous that but very few of the oysterlarvae can escape it. The spatfall will be negligible in such cases. Thus NELSON (1925 a, 1925 b, 1927, 1929) tells us that there have been years in which myriads of the Ctenophore *Mnemiopsis leidyi* (Agassiz) decimated the larvae of *Ostrea virginica* in Barnegat Bay. As many as 125 early larvae were found in the stomodeum of a single specimen of this Ctenophore and as sometimes 15 Ctenophores occur in one cubic metre of water, the ravages caused by these animals may assume alarming proportions.

Other kinds of Ctenophores, such as *Pleurobrachia*, are likewise classed among the enemies of oysterlarvae (KINCAID 1915).

NELSON (1921) reckons the adult oysters among the depredators, for he found sometimes as many as 62 mature larvae in the stomach of one adult oyster. YONGE's (1926) classing the oyster as wholly herbivorous does not correspond with the facts (NELSON 1933), for *Ostrea virginica* ingests animal forms (Nematodes) as well and is able to digest them, too.

Although some authors assume that adults of *Ostrea edulis* likewise ingest a great many oysterlarvae (BIERRY and GOUZON 1939, CHAILLÉ 1938), the rightness of this assumption has not been sufficiently proved. I often examined the stomach-content of the adults of *Ostrea edulis* in the Oosterschelde in the course of the season of reproduction. Although I occasionally met with some oysterlarvae in the stomach-content, I never found such great numbers of ingested larvae as NELSON did in *Ostrea virginica*. It is my belief that other animals consume far more oysterlarvae than adult oysters do. Sea-squirts, barnacles and plankton-eating fishes abound in the Oosterschelde. *Noctiluca* is often very numerous here.

Especially in the summer of 1938 I noticed myriads of *Noctiluca* at the station Kattendijke. The Ctenophore *Pleurobrachia* is often numerous in the Oosterschelde. It is my belief that the toll levied by animal depredators is the most important cause of the diminution of the larval herds in the Oosterschelde. It is very difficult to estimate the part played by each of the various animal enemies.

Although the number of certain enemies is probably liable to annual variations, I have never yet found a sudden abundance of one kind of enemy causing a failure of the spatfall in the Oosterschelde. The daily toll exacted in the open sea is very heavy and every protraction of the pelagic period (e.g. by low water-temperatures) will decrease the percentage of the larvae reaching maturity. This is the reason why high water-temperatures are so favourable to the propagation of oysters.

It is not possible to compute the exact percentage of the larvae that reach maturity, but as the number of mature larvae in 100 litres of water rarely exceeds a few tens, I believe that under normal conditions less than 10% of the larvae reach maturity in the Oosterschelde, probably even far less than 10%. If I estimate roughly from the data collected in my diagrams the percentage of the larvae that reach the full-grown stage, I find the following figures:

water-temperature	duration of the pelagic period	percentage reaching maturity
22° C	6– 7 days (13 tides)	10 %
20° C	10 days (19 tides)	5 %
18° C	12 days (23 tides)	2,5%

These figures, which are partly conjectural, enable me to calculate the daily losses (assuming that the larvae are about equally subject to devouring and dispersion during their entire pelagic life). After n tides the remainder is: $A\ (1 - 1/p)^n$ (A: original number of larvae, 1/p: decrease during one tidal cycle). Thus it can be computed from the figures in the table above that 1/p, the loss during one tidal cycle, amounts to about 13 or 15%. As the water renewal causes a loss of about 4% during each tidal cycle, the remaining 10% are caused by other factors, probably mainly by animal enemies.

Consequently we may conclude that in the Oosterschelde, in consequence of favourable hydrographical conditions, the losses caused by the tidal currents amount to less than one third of the total losses. I do not doubt that this proportion will contrast favourably with that in many other breeding-grounds of *Ostrea edulis*.

XVIII. VERTICAL DISTRIBUTION OF FULL-GROWN LARVAE

Although it may be assumed that the oysterlarvae in the Oosterschelde are, generally speaking, uniformly distributed in a vertical sense (as has been discussed in a previous section), this does not imply that full-grown larvae, ready to settle, also show a uniform vertical distribution. Differences in the vertical distribution of full-grown larvae will not appreciably affect the general vertical distribution, for the number of mature larvae of *Ostrea edulis* forms but a small percentage of the total number of oysterlarvae in the plankton.

Full-grown larvae are provided with a pigment-spot, which is often credited with a photosensitive character. It is often supposed that the pigment-spot enables the larvae to react to differences in light-intensity. Moreover, it is often assumed that mature larvae show a general inclination to make for the bottom regions when the moment of fixation draws near.

Not many observations have been made on the vertical distribution of full-grown oysterlarvae so far. MAZZARELLI (1922) who reared larvae of *Ostrea edulis* in vitro, tells us that larvae, on reaching maturity, cease hanging on the surface-film of the water: "Ad un dato momento sparice dal pelo dell' acqua lo strato formato dalle larve in posizione di riposo, le quali si sommergono del tutto e si mescolano alle altre." Very gradually "like a gentle rain", the mature larvae make for the bottom. The full-grown larvae, which continue swimming, are still uniformly distributed in the water: "E quindi lentissimamente, como una pioggerella sottile, le larve stesse cominciano a scendere al fondo. Le quali si accumulerabbero poi verso il fondo, ma pur restando sempre le larve regolarmente distribuite in tutta la massa acquea, il loro numero va scemando a poco a poco, man mano, che molto larve vanno e posarsi sul fondo, finchè rare restano le larve natanti."

COLE and KNIGHT JONES (1939) investigated the vertical distribution of oysterlarvae in tanks. They sampled in the daytime. Eyed larvae were abundant in the tank, but their distribution appeared to be similar to that of earlier larvae.

Not much is known about the vertical distribution of full-grown larvae of other kinds of oysters. NELSON (1931) states that his investigations have shown that mature larvae are much more abundant close to the bottom than in the upper layers, but he does not give any figures or details.

SEKI and TANAKA (1931) state that full-grown larvae of *Ostrea denselamellosa* are more numerous in the lower layers than at the surface of the sea. It is difficult, however, to infer the correctness of their statement from the figures in their tables. Both full-grown larvae and earlier larvae are more numerous in the bottom-samples, probably owing to differences in salinity.

Moreover the samples but rarely contain more than ten mature larvae, in most cases even far fewer. Such numbers are too small to base conclusions on.

I invariably measure all the larvae in the plankton-samples collected in the Oosterschelde. Moreover I note down which of them are provided with a pigment-spot. Consequently my special series of samples in particular enable me to find out whether or not full-grown larvae show a vertical distribution similar to that of earlier larvae.

The special samples were filtered off from 50 litres of water. I but rarely met with more than 10 full-grown larvae in these samples. As the bottom samples are collected about half a metre above the bottom, it is possible, though not probable, that an accumulation of mature larvae occurs in layers still closer to the bottom.

A series of samples collected at the station Yersche Bank (14 July 1939, fig. 11) seems to point to the possibility that mature larvae are relatively more abundant near the surface during the night and that they are distributed more uniformly in the day-time. In 9 couples of samples collected at this station during darkness I counted 46 full-grown larvae in the surface samples and 25 in the bottom samples. In 33 couples of samples collected at the same station in the day-time I counted 115 mature larvae in the surface samples and 127 in the bottom samples.

At Kattendijke I found in 4 couples of night-samples 42 mature larvae in the surface samples and 42 in the samples collected 5 metres below the surface. In 12 couples of day-samples I found 27 full-grown larvae in the surface samples and 25 in the samples procured from a depth of 5 metres.

Although these numbers are quite small, I believe that they suffice to justify the conclusion that full-grown larvae show, at any rate in the day-time, an uniform vertical distribution. I dare not decide whether mature larvae are really more abundant in the surface layers during darkness, as fig. 11 seems to indicate; the numbers are far too small to base conclusions on. Moreover at the station Kattendijke this phenomenon was not observed (fig. 12). In any case, I never noticed a marked accumulation of mature larvae in the bottom layers, which is in accordance with the data obtained by MAZZARELLI and COLE.

XIX. THE PROCESS OF ATTACHMENT

> "The setting period, though of relatively short duration in the development of the oyster, is of particular importance, as at this time its existence as a sedentary organism begins, its future location is selected and its possibilities for survival are determined by the ability of the larva to obtain a favourable place for attachment." (PRYTHERCH, 1934 *a*).

In many accounts of the life history of the oyster the belief is frequently expressed that fixation occurs when the shell becomes so heavy that the larvae are no longer able to swim continually and consequently sink to the bottom (e.g. MAZZARELLI 1922).

HAGMEIER rejected this view in 1916 already. COLE and KNIGHT JONES assert that the old view is totally erroneous, as the swimming powers of the larvae are undoubtedly greatest during the period immediately preceding attachment.

The first part of this period, during which the larva searches for a suitable substratum to attach, is called the searching stage (PRYTHERCH 1934 a). At this stage the larva is swimming with its foot protruding (COLE and KNIGHT JONES 1939: *Ostrea edulis*; PRYTHERCH 1934 a: *Ostrea virginica*). PRYTHERCH observed that the mature larva of *Ostrea virginica* often produces a thin byssus-thread in the searching phase, in many cases of a considerable length. The entire setting behaviour may be interpreted as an effort to find the ecological norm: i.e. the environmental conditions that are necessary for their well-being (RUSSELL 1934).

NELSON (1931) says that oysterlarvae are positively stereotropic during the searching period: that is they react strongly to contact with surfaces, with a tendency to cling to them and to crawl about on them.

YOKOTA (1936)¹) tells us how the full-grown larva of *Ostrea gigas* reacts, when, descending, it comes into contact with an object. It will descend about another 0,4 cm and will then rise as high as the object which gave the stimulus and cling to it or crawl about on it.

HOPKINS (1937) suggests that the chance of the foot touching an object decreases according as the angle of surface of the object

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¹) I wish to express my thanks to Mr. S. Nakano of the Imperial Fisheries Experimental Station for his kindness to translate Yokota's papers for me.

departs more and more from the "under horizontal", seeing that in the normal swimming position of the larva of *Ostrea lurida* (and of other species of oysters) the velum and the foot project upwards, although HOPKINS admits that the foot is extensible in all directions. HOPKINS puts forward this suggestion in explanation of his observations on the influence of the angle of surface on attachment, which phenomenon will be discussed below. Mature larvae of other kinds of oysters swim in the same way, but often show other reactions with regard to the angle of surface; so it is my belief that HOPKINS' explanation is too simple.

The second part of the period immediately preceding attachment is called the crawling phase. During the crawling phase the larva crawls over the surface of the substratum with the velum retracted, the foot extended in front and dragging the shell. Crawling has been observed in *Ostrea virginica* (NELSON 1923 a, 1924 a, 1924 b, PRYTHERCH 1934 a), in *Ostrea edulis* (COLE and KNIGHT JONES 1939) and in *Ostrea gigas* (YOKOTA 1936 b). Crawling may alternate with periods of normal swimming. NELSON (1924 b) states that during crawling the larvae describe circles of ever-decreasing diameter, but the other investigators have not confirmed this statement. COLE and KNIGHT JONES tell us that the larvae of *Ostrea edulis* crawl backwards and forwards rather irregularly over a few centimetres of surface, making "smaller excursions" in every direction. During the crawling phase the foot of both *Ostrea edulis* and *Ostrea virginica* gradually changes from a long slender shape to one that is short and broad.

Both PRYTHERCH (1934) and YOKOTA (1936) state that during crawling the larva stabilizes its body by sending out byssus. These byssus-threads, which are at first cylindrical and about 0.004 mm thick (PRYTHERCH), prevents the larvae from being washed off from the substratum during crawling. YOKOTA describes how the larva of *Ostrea gigas* crawls towards a current produced with a syringe. If the current is stronger some of the larvae cease crawling and swim away, while others firmly stabilize the body on the crawling surface. The body is supported by the byssus and slightly rocks from side to side against the current. If the current becomes still stronger the byssus snaps off. This experiment can be repeated several times; it is the simplest way to show the existence of a byssus-thread.

Such experiments have not yet been carried out on the larvae of *Ostrea edulis*. COLE and KNIGHT JONES (1939) regard it as possible that such a fine byssus-trail is laid by the European oyster during the final phases of crawling. HORST (1884) tells us that he repeatedly observed fine threads, probably byssus-threads, attached to recently set spat. I also often observed such fine threads attached to the spat on my test-plates.

When crawling is finished, the real fixation takes place. Formerly it was generally assumed that the larva attaches itself to the substratum by starting the production of the dissoconch shell. It was assumed that to this end a contact was effected between the secreting edge of the mantle and the substratum (HORST 1883, RYDER 1883, NELSON 1923 a, PRYTHERCH 1924: "by means of a shelly secretion of the left lobe of the mantle").

It was STAFFORD (1913) who found that the space between the left valve of the spat and the substratum was almost completely filled with a cement-like substance. Anatomical investigations of the fully developed larvae of *Ostrea virginica* led to his discovery of the byssus-gland. He came to the conclusion that this cement is poured out in liquid form from the byssus-gland. The same cement-like substance between the left valve of the shell and the substratum was found by HORI (1926) in *Ostrea gigas*. As to *Ostrea edulis* I frequently observed the occurrence of the cement between the left prodissoconch shell of the spat and the substratum; consequently it certainly is not only the new dissoconch shell which attaches the spat to the collector.

STAFFORD's assumption has been confirmed by the direct observations of NELSON (1924 b) and PRYTHERCH (1924) on *Ostrea virginica* and of COLE and KNIGHT JONES (1939) on *Ostrea edulis*. The cementing fluid is forced out of the byssus-gland by one or two vigorous contractions of the valves against the base of the foot. With the aid of the foot the left valve is pressed against the substratum in the place where the cementing fluid has been exuded. COLE and KNIGHT JONES (1939) tell us that the behaviour of the larva during the fixation act is curiously reminiscent of that of a dog preparing its beds: "The larva rocks the shell backwards and forwards and to some extent from side to side while, we suppose, the byssus cement is squeezed out, then comes to rest, and the shell twists over on its side, the left valve with to bigger umbo undermost, completing the setting process."

The cementing fluid hardens sufficiently in a minute or two to prevent the newly-set spat from being washed off by a jet of water from a pipette (COLE and KNIGHT JONES 1939, PRYTHERCH 1934). According to NELSON (1924 b) the newly-set spat of *Ostrea virginica* is inclined to the surface of the substratum at an angle of about 30 degrees; according to PRYTHERCH (1934 a) at an angle of about 45 degrees. I observed that the angle of inclination between the spat and the substratum is about 45 degrees in *Ostrea edulis*. The growth of the dissoconch shell soon renders this angle of inclination less conspicious.

Both ERDMANN (1934) and COLE and KNIGHT JONES (1934) tell us that larvae, dislodged immediately after setting, are not observed to attempt to set a second time, although these larvae may remain alive for a considerable time. ERDMANN came across a few mature larvae with an empty byssus-gland. He assumes that these larvae are "over-ripe": "Allem Anschein nach ist somit die Sezernierungsfähigkeit der Drüse auf einen bestimmten Zeitpunkt beschränkt." With ERDMANN "maturity" of the larvae denotes a physiological phase: the ripeness of the byssus-gland.

His "beschränkter Zeitpunkt" is an elastic term, however, for COLE and KNIGHT JONES (1939) state that "crawling alternating with periods of normal swimming, may extend over several days, if no suitable surface for attachment is presented; this happens frequently if fully developed larvae are kept in clean smooth glass vessels without any other object to which they may attach. This ability to delay attachment, i.e. metamorphosis, is likely to be of considerable value in aiding survival of larvae carried to places where there are no suitable surfaces for attachment." It is the opinion of several American authors (PRYTHERCH 1929, MEDCOF 1939) that the full-grown larvae of *Ostrea virginica* settle without any considerable delay. The larvae from a single spawning appeared to attach within a period of two days at most. It is my belief that the enormous difference between the number of full-grown larvae and the number of successful spat (section XXII) justifies the conclusion that the period during which *Ostrea edulis* is able to attach is limited, which is in accordance with ERDMANN's view.

XX. THE ORIENTATION OF THE SPAT

> "It is probable that the settling down of pelagic larvae is a much more precise and complex affair than we imagine, involving specific behaviour-acts or trains of behaviour." (RUSSELL, 1934).

In the course of my examination of the spat on my test-plates it struck me that the umbo of a high percentage of the spat points more or less in the same direction. This phenomenon has been noticed before by other investigators. Thus HORST (1883) tells us that the spat on his test-collectors was fixed with the hinge uppermost (*Ostrea edulis*). COLE and KNIGHT JONES (1939) state that the majority of the larvae of *Ostrea edulis* set with the dorsal margin (i.e. the hinge) uppermost on inclined or vertical surfaces.

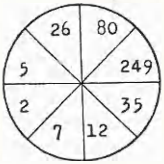
PRYTHERCH states (1934): "When oyster larvae set upon a vertical surface, they invariably place the shell with the dorsal edge or hinge uppermost" (*Ostrea virginica*). YOKOTA (1936 b) tells us that on vertical surfaces it is normal for the spat to attach with the umbo to the left (posterior part turned upwards), when the spat is seen from the surface of setting (*Ostrea gigas*). Only transparent collectors allow us to see the spat from the surface of setting. On any other kind of substratum we shall see the spat from the opposite side. If he had observed it from this side, YOKOTA would have stated the umbos of the majority of his spat as turned to the right. Of 113 of his spats 70 umbos were thus turned to the right (posterior part turned upwards), 26 umbos pointed upwards, 10 pointed downwards and 7 were turned to the left (anterior part turned upwards).

My inquiries into this matter have shown me that these observations of YOKOTA hold good for *Ostrea edulis*, too. I noted down these data in a kind of compass-card. The direction "North" indicates the side of the test-plate that was uppermost during attachment (i.e. the projection of the vertical).

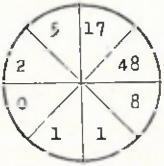
YOKOTA's data visualised in this manner yield the following diagram:

I used eight sectors instead of four, however. I have analyzed the orientation of the spat I found on the test-plates that I used for studying the effect of the angle of surface:

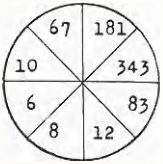
angle of surface
90°



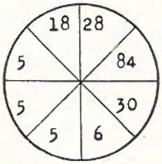
angle of surface
112½°



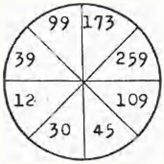
angle of surface
67½°



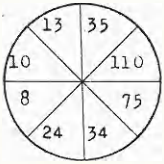
angle of surface
135°



angle of surface
45°



angle of surface
157½°



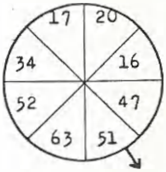
angle of surface
22½°

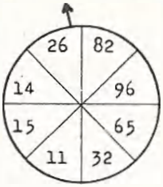
These data clearly show that the larvae of *Ostrea edulis* prefer to attach with the umbo pointing in a definite direction. This direction is not simply "upwards" or "to the right", as the above-mentioned authors believed, although the majority of the umbos certainly point to the right as well as upwards.

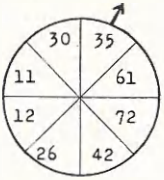
The direction they prefer apparently forms an angle facing to the right of about 30° with the horizontal axis. In other words: there is an angle of 60° between the direction of preference and the projection of the vertical (direction "North"). Not all the larvae succeed in attaching in the preferred direction; a certain percentage point in other directions, but for the greater part in adjacent directions and but seldom in the opposite direction. The distribution over the compass-card is remarkably similar on test-plates that have been exposed at angles of surface of 90°, 112½°, 67½° and 135°. For the sake of uniformity I have adopted the notation of angles of surface used by other investigators. The upper surface of a plate inclined at an angle of 45° is considered as being at an angle of 135° to the horizontal and other surfaces accordingly. When the angle of surface is 157½°, the distribution is essentially the same, but a greater percentage of the umbos point in the adjacent directions and the number that point in the opposite direction is somewhat greater, too. In cases where the angle of surface was 45° and especially in those of $22\frac{1}{2}$°, the percentage that point in the opposite direction is still greater, while moreover the angle of preference appears to be somewhat smaller than 30°, as the adjacent sector situated under the horizontal axis (E.S.E.) shows more spat than the sector (N. N.E.) placed immediately above the sector of preference (E.N.E.).

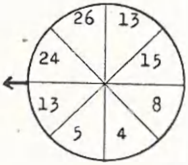
Moreover I analyzed the spat on many test-plates that had been exposed horizontally. As will be discussed below, these test-plates have been placed in containers of reinforced concrete. These containers are placed on the seabottom. It was not possible to place such a container in a precisely horizontal position. So the "horizontal" test-plates have not been exposed in a precisely horizontal position, but at slight angles in an unknown direction.

The oysterlarvae are apparently extremely sensitive to the angle of surface, for although the deviation from the horizontal will have been very slight in most of these cases, the analyses of the spat practically always show which side of the test-plate has been uppermost. The more equal the distribution over the compass-card, the slighter the deviation from the horizontal will have been. The side which has apparently been uppermost is indicated by an arrow (60 degrees to the left from the direction of preference).









I herein reproduce the compass-cards of two cases which did not show a distinct direction of preference. The test-plates in question will have been exposed at an angle, deviating little from the horizontal:

The pull of gravitation is apparently the only factor that governs this remarkable orientation.

This apparent influence of gravitation on the setting process is not in accordance with NELSON's view (1921) that the pull of gravitation has practically no influence in determining the position of attachment of oysterlarvae. Whether in this respect *Ostrea virginica* reacts in the same way as *Ostrea edulis* still remains to be seen, however.

Test-plates exposed both in light and darkness show the same phenomenon. Another series of experiments provided me with test-plates along which the tidal currents swept from one and the same side during the entire period of exposure. The orientation proved to be the same whether the current came from the "left" (direction W) or from the "right" (direction E).

It is very remarkable that the orientation is essentially the same on surfaces inclined at an angle of 0 to 90° and on those inclined at angles of 90 to 180°, although the axis of the spat points in quite different directions in all these cases.

XXI. METHODS OF DETERMINING THE INTENSITY OF SETTING

No data on field-observations on the intensity of setting throughout the season of reproduction in *Ostrea edulis* are available. COLE (1939) measured the frequency of setting in tanks (so under semi-natural conditions) by exposing smooth slates for 24 hours. Afterwards the spat was counted with a binocular microscope.

More is known about the intensity of setting in other species of oysters. It is my opinion, however, that the methods generally used cannot exactly be called smart.

Gryphaea angulata

CHAUX-THÉVENIN (1933, 1934) immersed some test-collectors every fortnight. He did not compare the numbers of spat of *Gryphaea angulata* on these collectors till the next year. As the percentage of the newly-set spat surviving till the next year is but small and moreover not always the same, this method can hardly be called quantitative.

Ostrea virginica

NELSON (1923 b, 1924 b, 1927, 1928 a, 1930, 1931) determined the time and intensity of setting by placing two shells in a wire basket, which shells he intended to replace by a new couple every 24 hours. In practice, however, the period of exposure was almost invariably considerably longer than 24 hours. The shells were not exchanged very regularly. Although the outer surfaces of the shells may frequently bear a good deal of spat, NELSON "for ease and accuracy" only counted the spat in the concave or inner faces.

It is my belief that shells are not suitable for the purpose of determining the intensity of setting quantitatively. As will be discussed below the angle of surface has a great influence on the frequency of setting. Both the irregular shape of the shells and the impossibility of exposing them every time in exactly the same position prevent a reliable measuring of the spatfall. NELSON placed one of his shells with the inner surface upwards and the other downwards, but I do not believe that this is an effective method of getting round this difficulty. PRYTHERCH (1929) and HOPKINS (1931) used wire bags with shells to determine the frequency of setting, while MEDCOF (1939) used one single shell, which he exposed for 24 hours.

Later on PRYTHERCH (1934 a), in a special experiment, used a more quantitative method. He immersed cement-coated partition-collectors at regular intervals. He allowed the spat to grow somewhat before he proceeded to counting. Errors owing to the influence of the angle of surface need not be feared with this device.

Ostrea lurida

COE (1932 b) and COE and ALLAN (1937) obtained some data on the season of attachment of *Ostrea lurida* in the course of their studies on sedentary organisms.

Both BONNOT (1936, 1937 a) and HOPKINS (1937) used wire bags filled with shells of *Ostrea gigas* to determine the intensity of setting. The shells remained in the water for 7 days. HOPKINS used two series of bags at each station, so that the one series overlapped the other. The spat on the inner surfaces of the shells was counted with a binocular microscope. The outside of the shells is too rough and lamellate for the attached spat to be counted with sufficient accuracy. According to these investigators the inner surfaces carry on an average about 30 to 35% of the total number of spat on the shell. The differences between the shells from one bag in the number of spat caught on the inside surfaces were enormous, which is obviously due to differences in the angle at which the shells were held, as well as to their size and their position in the bag. I do not believe that HOPKINS is right in assuming that any error traceable to the angle of surface is eliminated by the fact that the shells are held at every possible angle in the bag.

The method of determining the intensity of setting used in the Oosterschelde has been described briefly in HAVINGA's papers (1938, 1939). Since 1935 the periodicity of the spatfall has been determined here throughout the seasons of reproduction. In 1935 two spatfall-stations were established, in 1936 five, of which the stations Yersche Bank and Kattendijke are the most important for our purpose, seeing that the daily plankton-samples are procured at these stations. The spatfall is measured by regularly exposing sets of 3 specially prepared glass plates for periods of 3 days at each station. The plates are placed in containers of reinforced concrete, weighing about 40 kg, of which the side-walls have oblong narrow holes let into them (fig. 16). The plates are held in the containers at angles of 45°. A regular record has been kept of the places in the container in which the plates have been exposed and of the sides that have been uppermost. To keep off floating sea-weed the entire container is covered with wire-netting. The containers are placed on the sea-bottom in places which are not exposed during low water. The plates, which measure 13 × 18 cm, are of glass ground on both sides. The grounding causes the coating to adhere firmly to the plates. The thin coating is composed of a mixture of equal parts of cement, lime, fine sand and water. This coating provides the oysterlarvae with an excellent substratum to attach on.

Before the prepared plates can be used it is necessary that they should have been dried thoroughly. When the plates have not been dried long enough, they will get covered with a thin slimy layer when exposed in seawater; this layer catches many sand-particles and such plates are unsuitable as collectors. The same phenomenon is observed when limed tile-collectors have not been thoroughly dried. French oyster farmers speak of such tiles as "caillée". If there should be too little time for thor-

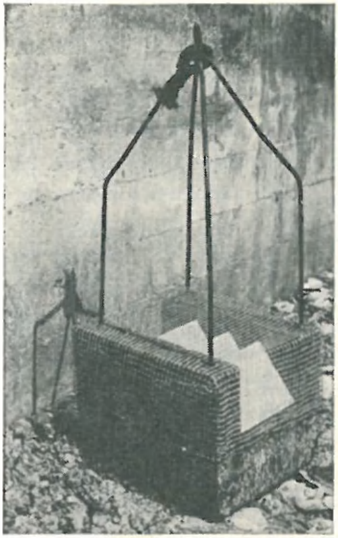


Fig. 16. Container for the determination of the intensity of the spatfall. (Photo Havinga).

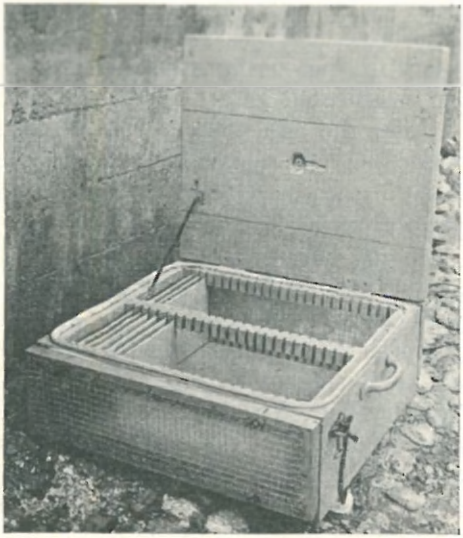


Fig. 17. Floating container for the growing up of the spat. (Photo Havinga).

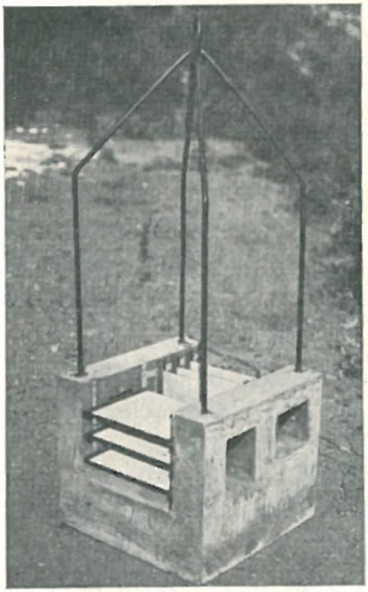


Fig. 18. Container with horizontal and vertical plates. (Photo Havinga).

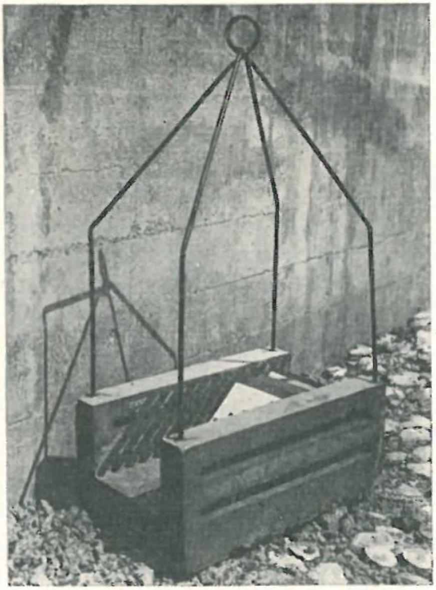


Fig. 19. Container for 12 plates. (Photo Havinga).

ough drying, bathing in a solution of sodium-bicarbonate may be substituted as a way of eliminating this drawback (CO_2!).

After the plates have been exposed in the container for three days they are replaced by another set. The plates are carried in a zinc container, filled with seawater, to an oysterpit near the laboratory at Bergen op Zoom.

As the counting of the spat with a microscope is very time-devouring, we let the spat grow for another 6 days, so that they can easily be detected with a lens magnifying 6 times. For this purpose the plates are placed for 6 days in a wooden box floating in an oysterpit (fig. 17). To keep off further oysterlarvae and enemies and to admit organisms on which the newly-set spat can feed two of the side-walls of the box have been made of bolting silk, which is regularly exchanged and cleaned. It appeared to me that practically none of the newly-set spat died during this 6 days' period. At the end of it the plates are carried to the laboratory, where they are exposed to the air to dry. When the plates are dry, the counting of the spat may be proceeded to at any time. The shells of the spat measure from about 0,6 to 0,8 mm at the time they are dried. This size is reached in 6 to 9 days of growth after attachment. The largest specimen often reach 1,0 mm in 9 days of sedentary life.

If the plates are exposed for 6 days, which is often the case with special series of plates, the oldest spat will be about 12 days when they are taken from the water. The largest spat on such plates measure from 1,2 to 1,4 mm.

The young spat is easily recognizable. Sometimes the plates will also be found to carry small mussels (*Mytilus edulis*) and small specimens of *Tapes pullastra*, which differ not only in size and colour from oyster-spat, but which are moreover attached by means of byssus-threads only and can consequently easily be moved to and fro with a needle.

Sometimes profuse settings of barnacles will be found to occur on the plates. The intensity of the setting of barnacles showed marked maxima and minima.

The spat is counted with the aid of a large kind of counting-table on which the plates are moved in a systematical manner under a lens, which is held in a support.

Special series of plates used in other experiments on the spatfall are treated in the same way. The plates can be used more than once; they can be cleaned by scrubbing them with an abrasive.

XXII. THE CORRELATION BETWEEN THE NUMBER OF FULL-GROWN LARVAE AND THE INTENSITY OF SETTING

Although it is self-evident that the presence of full-grown larvae is a conditio sine qua non for setting, many authors studying the problems of spatfall omit to investigate the number of full-grown larvae quantitatively.

The waves of setting are determined by an optimum combination of certain environmental factors. The suitability of a particular place for spatfall is dependent on several factors, as will be discussed below. These factors may cause enormous differences in the suitability for collecting oysterspat. Though the number of full-grown larvae in different places, with different environmental conditions, may be the same, this does not imply that the number of spat caught will also be the same. The intensity of setting in one and the same place, however, is correlative with the number of full-grown larvae present there. In other words: the proportion between the number of full-grown larvae and the intensity of the spatfall is fairly constant for each particular place, but the value of this ratio may be different for each place.

I share KÄNDLER's view (1928) that it is not sufficient to count the total number of oysterlarvae in the water on investigating the periodicity of setting, but that it is also necessary to measure the larvae and thus to determine the number of full-grown larvae.

The French investigators do not measure the larvae, but they divide them in larvae in the first stage (straight-hinge larvae) and larvae in the second stage (umbo larvae). BOURY (1930) suggested a comparison of the number of larvae in the first stage with the number in the second phase, so as to get an idea about the average degree of development of the larvae. It was BORDE (1931, 1932, 1935, 1936, 1937) who established the "fixation-coefficient":

$$\frac{\text{number of larvae in the second stage}}{\text{total number of larvae}}$$

This "fixation-coefficient" is assumed to indicate what percentage of the larvae approaches the setting stage. It is my belief, however, that it is not the percentage of full-grown larvae which is proportionate to the intensity of setting, but the number of full-grown larvae per unit of water. BORDE (1935) tells us that in the basin of Arcachon the total number of larvae decreases according as the samples are collected at greater distances from the centre of larvae-production, but that the "fixation-coefficient" increases proportionately in such series of samples. The same phenomenon may no doubt be observed elsewhere, for instance in the Oosterschelde. In the centre of larvae production the number of newly-liberated larvae is greater than that at other stations. Assuming that the number of full-grown larvae is about the same at the various stations, the "fixation-coefficient" will be by far the smallest in the centre of larvae-production! The spatfall prospects are, however, certainly not least favourable in the centre of larvae-production! If the number of umbo-larvae remains constant for some days at a particular station, and with it the spatfall prospects, a new wave of swarming may cause a considerable decrease of the "fixation-coefficient"! Therefore it is my belief that this „fixation-coefficient" is of no use in the study of the intesity of the spatfall. Though high values of this coefficient will often be followed by a heavy spatfall, its application is no safeguard against disappointments (HERMAN 1938 a, LADOUCE 1938 a).

If we want to know something about the spatfall prospects, we shall have to determine the number of full-grown larvae (if desired: umbo-larvae) per unit of water.

NELSON (1927, 1928 a, 1929, 1930) visualized in his diagrams by means of solid black circles the samples in which mature oysterlarvae (*Ostrea virginica*) formed a portion of the total catch. The number of mature larvae per unit of water has not been recorded by NELSON, however.

His diagrams show that the duration of setting closely paralleled the period during which eyed larvae were found in plankton-collections.

PRYTHERCH (1929, 1931) collected a series of samples (each sample from 200 gallons of water) throughout a tidal cycle. The majority of the oysterlarvae (*Ostrea virginica*) in these samples were found to be at later stages of development and within a few days of setting. Roundabout low water he counted about 100 larvae in his samples, during the remainder of the tidal cycle less than 10. PRYTHERCH measured the spatfall by immersing cement-coated partition-collectors from hour to hour. The collectors exposed roundabout low water caught far more spat than the others, which fits in with the data on his plankton-samples.

SEKI and TANAKA (1931) conclude that the intensity of setting of *Ostrea denselamellosa* depends upon the number of full-grown larvae, for they found that the time at which full-grown larvae abounded was the most suitable moment to place collectors.

The diagrams (fig. 7, 8, 9, 10) on the data obtained in the course of my investigations in the Oosterschelde (in the years 1936, 1937 1938 and 1939) show both the number of larvae in the different size-classes per 100 litres of water and the number of spat collected on one plate (i.e. the total number caught by the entire set divided by 3) at the same station in periods of 3 days. The correlation between the number of full-grown larvae and the intensity of setting strikes the eye, especially if the left part of the diagram is covered by a piece of paper, so that only the data on the largest size-groups (from 24 × 11 μ to 27 × 11 μ) and those on the spatfall remain visible. It is interesting to note the close parallelism between the intensity of setting and the periods during which full-grown larvae abound.

Very striking are the events in the month of August 1938. High water-temperatures favoured the growth of the larvae to such an extent that the numbers of full-grown larvae counted in the samples from 5 to about 14 August were unprecedentedly high. Consequently the spatfall was very profuse during the same period. A slight peak in the number of mature larvae in the second part of July in the same season corresponds with a slight increase of the spatfall shortly after. In 1939 the greatest numbers of full-grown larvae as well as most of the spatfall were recorded in the middle of July. In 1937 it was the second part of July that yielded the largest numbers.

Both the station Yersche Bank and the station Kattendijke show this correlation between the number of full-grown larvae and the intensity of setting. The diagrams clearly show, however, that the proportion between the number of full-grown larvae and the number of spat is not same at these two stations. The same number of mature larvae yields far more spat at the station Yersche Bank than at the station Kattendijke. It should be remembered, however, that the number of larvae is fairly constant in the course of the tidal cycle at the station Yersche Bank, while the number of larvae at the station Kattendijke shows its maximum roundabout low water (the daily samples are taken at low water there). Moreover there are still other factors which influence the proportion between the number of mature larvae and the intensity of setting at different stations, as will be discussed below.

It is not easy to estimate what percentage of the mature larvae succeed in accomplishing attachment.

Bierry and Gouzon (1939) estimate that about 400 out of 1 000 000 newly-liberated larvae attach on an average.

It is interesting to estimate the proportions between the number of young larvae, mature larvae and spat in the Oosterschelde. The following are the figures for the year 1939 (partly conjectural):

The number of adult oysters in the Oosterschelde was about 36 000 000 in 1939, while the percentage of functional females in the course of the season of reproduction may be estimated at 75% to 100%, as has been discussed above. The number of larvae produced by one oyster in the female stage varies from 500 000 to 1 000 000. Hence the total production of larvae in 1939 may be estimated at 10 000 000 000 000 at least. This figure is certainly not too high, for I very often counted from 200 to 300 larvae in 100 litres of water at a station situated far from the centre of larvae-production (Kattendijke). From this it follows that the water that fills the basin during high tide (675 000 000 m^3) contains more than one larva per litre, which comes to more than 1 000 000 000 000 larvae in the entire basin on one particular day!

How many larvae reached the full-grown stage in the Oosterschelde in the course of 1939?

On several days the number of full-grown larvae exceeded 10 per 100 litres (fig. 10) at both stations, which corresponds with at least 50 000 000 000 full-grown larvae in the entire basin on one particular day. I believe that the total number of full-grown larvae in the course of this season of reproduction may be estimated at 250 000 000 000 at least (i.e. 2,5% of the original number of larvae). In a previous section I estimated the percentage of larvae reaching maturity in the Oosterschelde at less than 10%, probably even far less. Only in periods characterized by very favourable water-temperatures, such as the middle of August 1938 and July 1935, does the percentage probably exceed 10%.

I examined the number of spat on a good many samples of tile-collectors and of sown-out shells in the autumn of 1939. Knowing the total amount of collector-material, I estimate that the number of spat caught in the Oosterschelde in 1939 and surviving till in October amounted to at least 250 000 000.

Perhaps only 1 out of 10 newly-attached spat survives till in October. The other 9 perish, partly by the action of animal enemies, partly by smothering. The most notorious enemies are *Carcinides maenas* and *Asterias rubens*. Small specimens of the latter sometimes occur in great abundance on the tile-collectors in some places. Smothering is caused by a rapid growth of other sessile invertebrates (e.g. *Ascidiella*, *Botryllus*, Sponges and Bryozoa) or by the deposition of silt or sand. I base the proportion 1 out of 10 on the following facts: The surface of the collectors is only for a limited period suitable for fixation. Soon a thin slimy layer, formed by a growth of microscopical algae and bacteria, renders attachment impossible. It is my belief that the suitable period does not exceed 12 days. My test-plates, which were exposed in the most favourable part of the season in 4 successive periods of 3 days, at two stations where many tile-collectors are placed as a rule (Wemeldinge and Kattendijke), caught from 100 to 150 spat in the aggregate. The surface of a tile-collector is about 8 times as large as that of a test-plate. So I assume that the number of spat caught by one tile-collector during the most favourable part of the season 1939 may be estimated at 500 or 1000. On many tiles from these stations I counted from 50 to 100 spat in October, which shows that about 9 out of 10 spat had disappeared. This proportion is of course subject to fluctuations in different years.

As at least 250 000 000 spat survived till in October, the total number of larvae that accomplished fixation will have been at least about 2 500 000 000. So we may say that about 250 out of every 1 000 000 liberated larvae settled. This number does not differ essentially from the estimate by BIERRY and GOUZON (i.e. 400).

When we compare the number of settled spat with the number of full-grown larvae in the course of the season of reproduction, we shall find that only about 1 % of the mature larvae succeed in accomplishing fixation!

Though my figures are partly conjectural, I am of opinion that indeed but a small percentage of the full-grown larvae succeed in finding a suitable collector and in attaching on it. This is probably due to lack of collector-material. The surface of the collectors is only for a short period in a condition suited to fixation. Moreover there are vast areas where the larvae, which are at the mercy of the tidal streams, practically never meet with any suitable piece of collector-material.

Interference on the part of man may effect an increase in the percentage of mature larvae that accomplish attachment. To that end the oysterfarmer will have to lay out his cultch in adequate amounts in suitable places and at the most propitious moment. Nature largely governs the values of the other proportions and percentages.

The smallness of the percentage of mature larvae accomplishing fixation is the reason why the number of spat per collector does not perceptibly decrease when collectors are planted together in great numbers in one place, if only care is taken that all of them are easily accessible for the larvae-bearing water. In my experiments there was no perceptible difference between the number of spat per plate in containers with 3 plates and in those with 12 plates, placed at the same station. The oysterfarmer in placing his collectors need not take into account that" where the tiles are many, the spatfall is poor".

From the foregoing it may be concluded that the "useful effect" is not very great in the propagation of *Ostrea edulis*. Only about 25 out of a million larvae are found back on the collectors in autumn.

This percentage of survival is not only small when considered by itself, but also when compared with the results in the propagation of other kinds of marine Molluscs. SMIDT (1938), for instance, estimates that about one eighth of the larvae of the Gastropod *Rissoa membranacea* succeed in accomplishing metamorphosis!

XXIII. THE EFFECT OF TEMPERATURE AND SALINITY ON THE SETTING PROCESS

LEENHARDT (1922, 1924) assumed that mature oysterlarvae are incapable of attachment, unless the temperature of the water is at least 18° C: "La fixation ne devant s'opérer que si la température était d'au moins 18°". MAZZARELLI (1924), however, assures us that in the lake of Fusaro fixation is certainly possible at temperatures below 18° C. Later French investigators (e.g. BOURY 1928) did not succed in establishing a temperature-limit below which attachment is impossible (field-observations).

COLE (1939) observed that fixation in his tanks was not affected by sudden falls in temperature. He describes several cases in which a sudden drop in water-temperature had no visible effect on the larvae that at this time were on the point of setting. Under tank-conditions no correlation between temperature and intensity of setting can be established.

I fully suscribe to COLE's view. A comparison of the number of full-grown larvae and the intensity of setting in the Oosterschelde has led me to the conclusion that fluctuations in water-temperature do not affect the value of this proportion. Although stormy spells of weather frequently occurred, often accompanied by sudden drops in water-temperature (e.g. 22–26 July 1937, early in July 1938, 12–22 August 1938, early in August 1939), they produced no visible effect on the correlation between the number of full-grown larvae and the intensity of setting.

Though the intensity of setting was very slight in the first part of July 1938, owing to the small number of mature larvae, the diagram clearly shows that the proportion between the number of mature larvae and the number of spat caught on the plates was essentially the same during the stormy days early in July and in the subsequent calmer period.

It is my belief that the process of attachment is practically not liable to varying water-temperatures and stormy weather (of course only as far as temperatures recorded in the field in the summer-months are concerned). This is in accordance with HOPKINS' view (1937) that "local weather conditions appear to have little or no influence upon the setting of larvae, save in their effect upon water-temperature which controls spawning and rate of larval development."

It has been supposed by some that salinity influences the intensity of setting. In the Southern parts of the range of *Ostrea virginica* (Louisiana, Galveston Bay) fluctuations in salinity are often considerable. MOORE and POPE (1910) and MOORE (1913) observed no spatfall during the period in which the crevasse water from the river Mississippi was pouring over the beds. HOPKINS (1931) concluded that setting periods coincided with increases in salinity above about 20 parts per 1000, although no exact limit could be stated. He assumes that "completion of larval development is not attained, unless the water reaches these higher concentrations." As no exact data on the number of mature larvae have been provided by these authors, we cannot be sure whether this influence of salinity is due to an insufficient development of the larvae or to failure in fixation.

PRYTHERCH (1934 a) ascertained the time required by the larvae to complete the setting process under various salinity conditions (experiments in vitro). The larvae of *Ostrea virginica* were found to complete fixation in 12 to 19 minutes when the salinity was 16–18,6 °/₀₀. Higher or lower salinities appeared to protract the setting process (the limits were: 5,6 °/₀₀ : 140 minutes; 32,2 °/₀₀ : 144 minutes). PRYTHERCH ascribed the effect of salinity on the setting process to a physical change in the byssal fluid, in consequence of which the time required for its complete discharge is altered.

Observations in Long Island Sound (HIGGINS 1938) have shown that the salinity of the bottom water there is only liable to slight seasonal changes, so that this factor cannot be held responsible for the success or failure of the spatfall.

The same conclusion may be drawn from the data concerning the Oosterschelde. The salinity in the Oosterschelde is only subject to slight seasonal changes in the year's course and remains virtually the same from year to year (table 2, fig. 3). So this factor cannot be held responsible for the success or failure of the spatfall in this case either. Any difference in the proportion between the number of full-grown larvae and the intensity of setting, owing to changes in salinity, cannot be shown here. Experiments in vitro, like those of PRYTHERCH, have not yet been carried out with the larvae of *Ostrea edulis*, but it is an established fact (MAZZARELLI 1924) that fixation of *Ostrea edulis* is certainly possible with salinities of even 34 to 39 °/₀₀.

XXIV. THE INFLUENCE OF COPPER ON THE SETTING PROCESS

PRYTHERCH (1931, 1934 a) ascertained the intensity of setting in the course of the tidal cycle in Milford Harbor (*Ostrea virginica*). The setting there appeared to be by far most abundant roundabout low water. He concluded: "It is evident, therefore, that attachment of the larvae is not a haphazard process, but is a definite biological reaction in response to some environmental stimulus." PRYTHERCH ascribed the tidal periodicity in setting to differences in the chemical composition of the water in the course of the tidal cycle.

The salinity appeared to show considerable differences at different stages of the tide, owing to inflowing river-water. The river-water contains in relatively large amounts several elements which are rather scanty in sea-water. Consequently these elements show their maximum at about low water at his station. PRYTHERCH assumes that the presence of adequate amounts of one of these elements is required to initiate the process of attachment. He came to the conclusion both by laboratory experiments and field observations that the required element is copper. He presumes that copper dissolved in river-water is precipitated in the form of copper-oxychloride when the river-water is discharged in the sea. According to PRYTHERCH fine particles of this precipitate are ingested by mature oysterlarvae. He supposed that the larvae cannot complete attachment, unless adequate quantities of copper have been ingested by them.

PRYTHERCH tries to prove the correctness of his assumption by pointing to the neat parallelism between the course of a graph on the intensity of setting and one on the copper content of the water for the same station. (fig. 20).

Personally, I do not agree with PRYTHERCH's interpretation.

In the first place it is the chemical side of the question that is liable to exception. Ir. F. LIEBERT, Director of the Governmental Institution for Chemical, Microbiological and Hydrographical Fishery Research informed me that the few reliable determinations of copper in sea-water yielded from 0,005 to 0,01 mg per litre. PRYTHERCH's figures are very high; they amount from 0,25 to 0,50 mg per litre at low water. A reliable determination of copper with sodiumdiaethyldithiocarbaminate is only possible after the element iron, which is always contained in relatively large amounts in river-water, has been eliminated. PRYTHERCH does not describe if and how he eliminated iron and he does not tell us whether or not he filtered his samples of water. Reliable determinations of the copper-content in coastal water have not been carried out so far.

In the second place it is the biological aspect that is subject to exception. I doubt whether oysterlarvae really ingest particles of copper-oxychloride. Some preliminary experiments with young

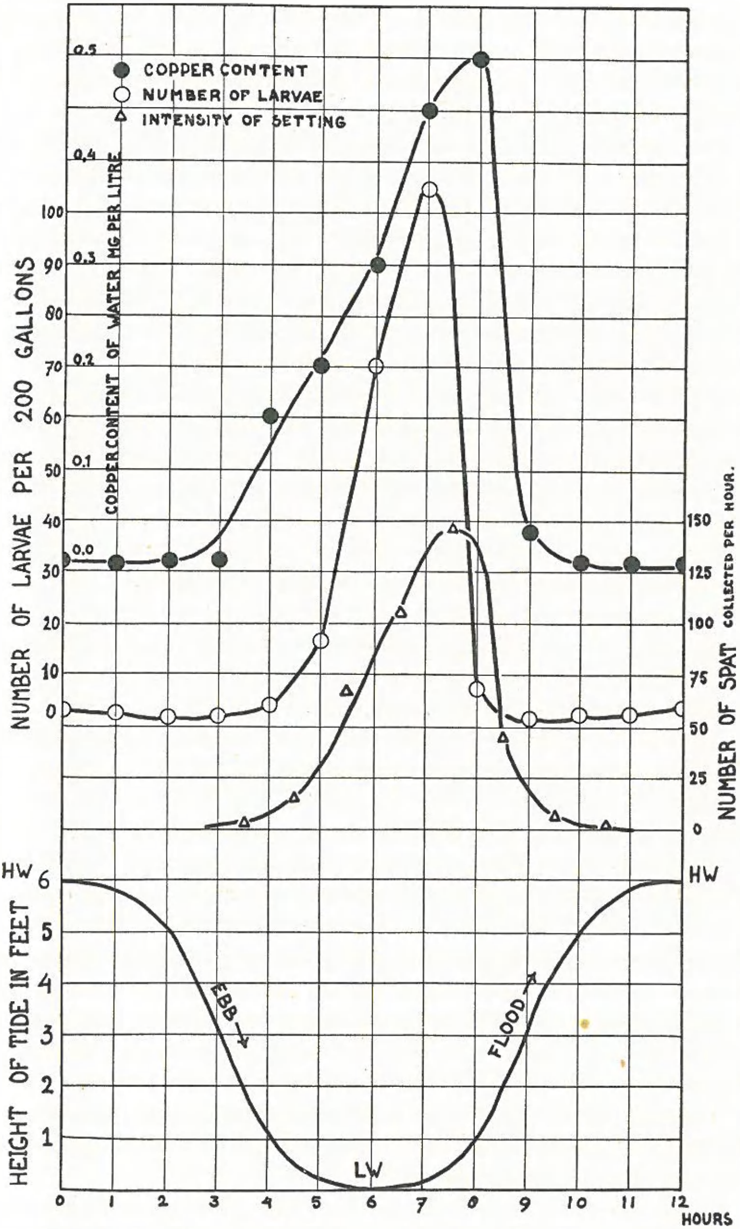


Fig. 20. The influence of copper on the setting process. After PRYTHERCH, altered by me. The graph on the number of larvae has been traced by me.

oysters and mussels have shown us that they do not ingest copper-oxychloride. The particles of copper-oxychloride are rejected be them as pseudo-faeces. In a little time they close their shells and stop feeding.

PRYTHERCH does not describe in his papers how he expects his mature larvae to swim. If the larvae are carried along with the currents, they will remain in the same body of water during the entire tidal cycle, so that they are not subject to differences in the chemical composition of the water in which they swim. It is only when they remain in the same place throughout the tidal cycle that at low water they may find themselves in water of another chemical composition than at high water. PRYTHERCH's views on the vertical distribution of the larvae have so far received no support whatever, as has been discussed above.

PRYTHERCH neglects one of the most important factors governing the intensity of setting, i.e. the number of full-grown larvae per unit of water! If there is any factor influencing the intensity of setting during a certain part of the tide, it is to be expected that the proportion between the number of mature larvae and the frequency of setting will vary in the course of the tidal cycle. The determination of the intensity of setting is not sufficient by itself to prove the influence of such a factor. In a previous paper (1929) PRYTHERCH stated the number of larvae (which practically all of them happened to be ready or nearly ready to settle) per 200 gallons of water in the course of the tidal cycle. This number showed a marked peak at low water. I am inclined to ascribe the differences in the number of his larvae to an influence of the currents on their distribution. PRYTHERCH gives no details about the currents, however. In his diagram (1934) I traced a graph on the number of larvae recorded in the above-mentioned paper (fig. 20), taking as my starting-point the average number of larvae per 200 gallons in 3 series of his samples, collected in 3 successive days (PRYTHERCH 1929). This graph closely parallels that on the intensity of setting! It shows that the proportion between the number of mature larvae and the intensity of setting remained constant in the course of the tidal cycle. This proves that other factors, such as the copper content, had no influence on the intensity of setting!

From all this it will be clear that PRYTHERCH's original diagram cannot be used in support of his assumptions!

I do not mean to say that oysterlarvae may not require some copper in the course of their development. I only want to demonstrate that PRYTHERCH's conclusions have not been borne out by the results of his field-experiments. His conclusions that the location of natural oysterbeds depends on the local occurrence of adequate amounts of copper and that failures in tank-breeding can be ascribed to a deficiency of copper, are very premature.

Nor have PRYTHERCH's assumptions been supported by experiments carried out by other investigators. NELSON (1931) tried to obtain a more intensive setting by adding copper to the cultch-material. Neither in Barnegat Bay nor in Delaware Bay was there any evidence, however, that the treatment of cultch with copper salts or the addition of metallic copper served to increase the spatfall. Basing himself on PRYTHERCH's conclusions, HOPKINS (1937) ascribed a small difference in the vertical distribution of the spatfall of *Ostrea lurida* to very slight differences in salinity (i.e. in the chemical composition of the water). I believe, however, that there is a simpler way of accounting for these differences in spatfall, for which it would be necessary, however, to measure the current velocities and to count the larvae.

GAARDER (1932, 1933) considered the possibility of a deficiency of copper in the water of the enclosed Norwegian pollen. He added copper to the water of one of the pollen, but he did not observe a clearly marked difference between the intensity of setting in the treated poll and in a neighbouring poll to which no copper had been added.

VOISIN (1933) does not absolutely deny that some copper may be required by oysterlarvae. If it should be required, he thinks it very probable that coastal waters contain this element in sufficient quantities. This is also my view of the matter. I have never yet made any field-observation which showed that copper affects the spatfall in the Oosterschelde. As the salinity of the water in the Oosterschelde remains constant in the course of the tidal cycle, and along with it the content of chemical components, it is impossible to ascribe periodicities in spatfall here to an influence of copper on attachment.

XXV. THE CORRELATION BETWEEN THE PERIODICITY IN SWARMING AND THE PERIODICITY IN SETTING

The importance of a sufficient knowledge of the events in the course of the pelagic life of oysterlarvae is often underestimated. In previous sections we discussed how variations in environmental conditions will affect the percentage of larvae that reach maturity.

Several authors neglect the influence of the lot that befalls the larvae and assume that the periodicity in setting is proportionate to the periodicity in swarming. Thus ORTON states (1937 a): "The density of the subsequent fall will be proportional to the percentage of blacksick oysters if healthy growth and settlement occurs."

HOPKINS (1937) carefully ascertained the frequency and intensity of spawning in *Ostrea lurida* by opening oysters periodically. HOPKINS made no quantitative investigations on the number of larvae in the water, for he considered this superfluous in case the periodicity of spawning is precisely known. In his discussion of the correlation between spawning and setting he has to admit, however, that the record of setting resembles that of spawning in some respects only. He regards considerable differences in mortality of the larvae of the various spawning maxima as a possible explanation of the imperfect resemblance between his graphs on spawning and setting. As he did not have at his disposal any data on the course of events in the long period of pelagic life (about 30 days in *Ostrea lurida*), he was unable to demonstrate the occurrence of such differences in mortality.

PRYTHERCH (1929) ascribes success or failure in setting of *Ostrea virginica* entirely to success or failure in spawning. According to him the quantities of eggs and sperm developed annually by adult oysters govern the intensity of setting. He assumes that the extent of spawning and with it the extent of setting are governed by temperature conditions. He shows that in those summers between 1922 and 1927 that were characterized by a successful setting the air-temperatures were above the normal. Although his conclusion that the warmest summers yield the best spatfalls holds good for the Oosterschelde as well, as will be shown below, I shall demonstrate that here it certainly is not mainly the increase in the extent of spawning (brought about by favourable temperatures) that is responsible for the satisfactory results in warm summers.

Other authors sometimes exaggerate in the opposite direction by denying all correlation between extent and periodicity of spawning and the intensity of setting. Thus STAFFORD (1913) stated: "Spatfall is the all-important event. The value of the oyster harvest does not depend upon the number of eggs spawned, nor upon the number of larvae in the water, but upon the number of successful spat."

Miss CLARK (1935) came nearer to the truth. She observed that variations in the setting of *Ostrea virginica* are often produced by variations in the percentage of the larvae that reach maturity. Although her experiments in vitro showed her that low water-temperatures bring about a protraction of the pelagic period, she failed to see that it is the protraction itself which is the cause that but few larvae reach maturity at low water-temperatures. She stated that the variations in temperature and salinity, recorded in the field, do not reach values at which they become injurious to oysterlarvae by causing untimely death. It is, however, the protraction of the pelagic period itself that is the dangerous factor, as this exposes the larvae for a longer time to the many dangers besetting them.

Both SPÄRCK (1929) and HAGMEIER (1931) declare that low water-temperatures, by protracting the pelagic stage, will often decrease the percentage of larvae reaching maturity considerably.

From my diagrams I shall discuss separately the course of events in the Oosterschelde in the years 1935, 1936, 1937, 1938 and 1939.

1935 [1]) (fig. 21).

The number of adult oyster was rather small in 1935. Therefore the total number of larvae produced in the course of the season of reproduction was not very great. Swarming showed a marked maximum from 10 to 12 July. Just then the water-temperature happened to be very favourable (above 22° C), so that a high percentage of these larvae reached the full-grown stage, which resulted in a marked maximum of setting in the period from 17 to 20 July. Further maxima of swarming of some importance were not observed in 1935. The intensity of

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[1]) In 1935 the larvae were counted by Dr. HAVINGA. Dr. GRIJNS determined the periodicity of setting in 1935 and 1936. The data visualized in the diagram for 1935 (fig. 20) concern the station Strijen.

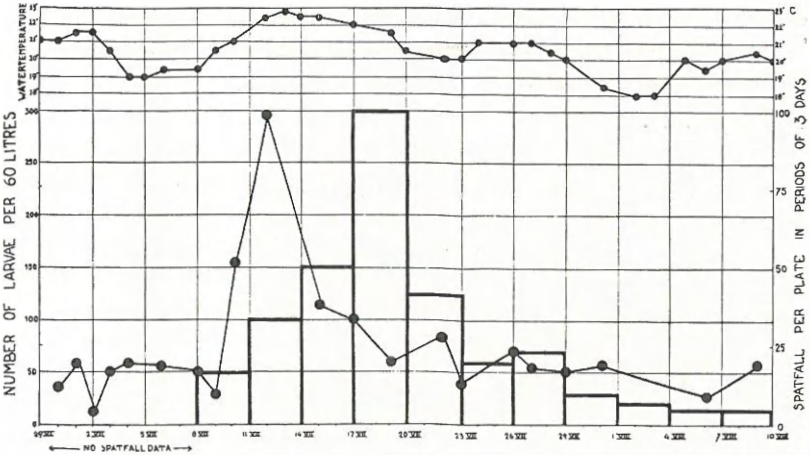

Fig. 21. Larvae and spatfall. Strijen 1935.

setting in the remainder of the season was of no importance either. 1935 is an instructive instance of a season with a relatively small production of larvae and which yet yielded a good spatfall, which may be put down to the extremely favourable conditions during pelagic life. Most oysterfarmers had placed their tiles just before the maximum of setting occurred, which led to a set of commercial magnitude.

1936 (fig. 7).

From the diagram it can be deduced that the production of larvae was smaller in 1936 than in 1935. The production of larvae in 1936 was unsatisfactory, which may be ascribed to the relatively small number of adult oysters (14 500 000) and possibly to less favourable temperature-conditions in June as compared with June 1935 (fig. 4, table 1). It may be seen from the diagram on temperature-conditions in the summer seasons (fig. 4) that the water-temperature was not nearly so favourable in 1936 as in 1935. The percentage of the larvae that reached maturity in 1936 consequently fell far short of that in 1935. A comparison of the diagram for 1936 with the diagrams on the data obtained in later years shows that the percentage of larvae which reached the full-grown stage in 1936 was about "normal". The total number of larvae and consequently the number of full-grown larvae was quite unsatisfactory, however. In 1936 swarming was moreover not concentrated into a few days, so that the spatfall was spread over quite a long period. The combination of all these factors resulted in a complete failure of the 1936 set.

1937 (fig. 8).

Since 1937 plankton-sampling has been carried out at the stations Yersche Bank and Kattendijke. It is, however, possible to compare the production of larvae in 1937 with that of 1936, as one of the diagrams for 1936 also refers to Kattendijke. The spatfall may be compared without more ado, as the spatfall-stations Kattendijke and Strijen were used in each season in the course of the investigations.

The diagram for the year 1937 (fig. 8) clearly shows that the production of larvae was greater in that year than in 1936 and 1935, which may be ascribed to the increased number of adult oysters (24 000 000) and possibly to a more favourable temperature in June (fig. 4).

Although temperature-conditions were somewhat more favourable in 1937 than in 1936, they were not nearly so favourable as in 1935 (fig. 4, table 1).

A clearly marked liberation occurred from 3 to 5 July. Unfortunately the water-temperature was dropping at that time. In consequence of this the pelagic phase was protracted and the percentage of larvae that reached the full-grown stage was rather small. The water-temperature was somewhat higher in the second part of July, which resulted in a higher percentage of larvae that reached maturity. As a consequence of this setting was most intense in the second part of July. Most oyster-farmers, however, had immersed their tiles in the first days of July in the hope of catching spat from the larvae that swarmed in the first days of that month. Adverse weather-conditions interfered, however, while at the time that the spatfall prospects ameliorated (in the second part of July) clean tiles were rather scarce. This was the cause that the practical spatfall, i.e. the harvest obtained by the oyster farmers, was less satisfactory in 1937 than in 1935, although the potential spatfall in the course of the entire season of reproduction did not differ much from that of 1935.

1938 (fig. 9).

Very instructive are the results that were obtained in 1938. The first thing that strikes us in the diagram is that the total number of larvae produced in 1938 exceeded that produced in 1937, probably owing to a further increase in the number of adult oysters (30 000 000). The swarming observed on 23 and 24 June and on 27 and 28 June was of little importance. This led to small setting maxima in the periods from 3 to 5 July and 9 to 11 July. These numbers of larvae and spat were far too small to be of practical importance.

Heavy swarming occurred early in July, at which time enormous numbers of larvae were liberated. These larvae met with extremely unfavourable temperature-conditions, owing to a spell of adverse weather. The temperature of the water remained below 17° C. during the greater part of their pelagic life. Consequently the pelagic stage was protracted considerably, so that only a very small percentage of all these larvae reached the full-grown stage. This was the cause that the spatfall produced by the larvae liberated early in July turned out a complete failure. Dutch oysterfarmers used to immerse their collectors in the first part of July. When they learned that the production of larvae was great early in July, most of them immersed their tiles in spite of unfavourable temperature-conditions and in spite of our prediction that no satisfactory spatfall was likely to occur in the first part of July. The result was that the practical spatfall was a failure in 1938, especially at Kattendijke.

The occurrence of a fairly large percentage of oysters carrying larvae in the second part of July indicated that another liberation of some importance could be expected. This prediction proved to be correct. The number of larvae liberated towards the end of July and in the first days of August was rather large, though not so large as it had been during the swarming early in July. The water-temperature increased rapidly early in August and soon temperatures of 23° C were recorded. The larvae liberated just then consequently met with extremely favourable temperature-conditions, so that the percentage of those that reached the full-grown stage was uncommonly high, which resulted in an unprecedented intensity of setting. I caught about 300 spat per plate during two successive periods at the station Yersche Bank!

Only an insignificant percentage of this large number of full-grown larvae (fig. 9) succeeded in finding suitable collector material. Clean tiles were very scarce, as most of the tiles had been immersed long before. The few oysterfarmers that placed some tiles early in August caught an incredible number of spat on them.

So the season of 1938 was characterized by a complete failure in the setting of the larvae of the first considerable maximum of swarming, owing to extremely low water-temperatures in the first half of July, and by an uncommonly intense setting in the first part of August, owing to swarming under extremely favourable temperature conditions. The partical set was a failure in 1938, as but few oysterfarmers had profited by this intense setting. The others had not dared to wait long enough with the placing of their collectors, as we could not guarantee beforehand a spell of extremely fine weather in August to favour the larvae of the second important wave of swarming.

1939 (fig. 10).

The season of reproduction of the year 1939 was characterised by an equable water-temperature. Till the middle of July the water-temperature was about 18° C, after that about 19° C for quite a long period. These temperatures are not very favourable for the development of oysterlarvae The total number of larvae produced in 1939 was still larger than that produced in 1938, which may be ascribed to a further increase of the number of adult oysters (36 000 000).

The numbers of larvae that were liberated towards the end of June and in the first half of July were uncommonly large. The percentage of these larvae that reached the full-grown stage was rather small, however, owing to the rather low water-temperatures.

The larvae liberated in July met with a slightly higher water-temperature than those liberated in June. The setting showed a diffuse maximum in the middle of July, from which it may be deduced that of the larvae produced in July a somewhat greater percentage reached maturity than of those liberated in June. I ascribe this to the small difference in water-temperature mentioned above. The production of larvae early in August led to a small maximum of setting in the second part of August.

Although the maximum of setting in the middle of July was not very large (it was even insignificant in comparison with the setting in August 1938), it yet resulted in a satisfactory practical spatfall, as most of the tiles were in a clean state at the time this setting took place. Several oysterfarmers obtained harvests amounting to about 50 spat per tile (counted in autumn). On many tiles exposed during the most favourable part of the season the number of spat even exceeded the figure 50.[1])

So the season of 1939 was characterized by an uncommonly large production of larvae, of which only a rather small percentage reached the full-grown stage owing to rather low water-temperatures. But as the intensity of setting was greatest in the middle of July, the oysterfarmers succeeded in obtaining a satisfactory spatfall. The practical set is comparable to that obtained in 1935. It should be remembered, however, that the number of larvae produced in 1935 was but a fraction of that produced in 1939.

Summarizing, it may be stated that the intensity of setting is not always commensurate with the production of larvae. The results obtained in the year 1935 clearly demonstrate that even a small number of larvae may yield a spatfall of commercial magnitude, if only the larvae are liberated within a short period and provided liberation is accompanied by extremely favourable temperature conditions.

The larger the number of larvae, however, the greater the chance that at least part of them will meet with favourable temperature conditions and thus lead to a successful spatfall. The results obtained in 1939 demonstrate that although the percentage of larvae which reaches maturity may be small, owing to rather low water-temperatures, the practical spatfall may be satisfactory, if only the total number of larvae produced is large enough. The greater the number of adult oysters, the greater the chances of a satisfactory spatfall will be.

Immersion of collectors at water-temperatures continuing below 18° C proved to be ineffective, as in consequence of such low temperatures the percentage of larvae reaching maturity is too small anyway to render a satisfactory spatfall possible.

From what I have said, it will be clear that I do not agree with those authors who believe that setting is commensurate with spawning, nor with those who reject all correlation between the periodicity and extent of swarming and of setting.

The annual extent of the potential spatfall determined with our methods was (number of spat per plate):

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[1]) Owing to the severe winter 1939–1940 by far the greater part of the spat on the tiles died, alas.

Seasons	Stations				
	Kattendijke	Wemeldinge	Strijen	Yersche bank	Bergsche bank
1935 8 VII– 3 IX	361		357		
1936 24 VI–17 VIII	39	111	56	180	49
1937 16 VI– 2 IX	272	342	175	447	86
1938 24 VI–29 VIII	357	697	383	1053	171
1939 23 VI–31 VIII	385	433	351	629	109

From these figures it appeared that the potential spatfall was most intense in the year 1938 and that the setting in 1936 was a failure indeed. The most satisfactory harvests were obtained by the oysterfarmers in the years 1935 and 1939.

The reason why the practical set was not very satisfactory in the season of 1937 and was even a failure in 1938, in which year the potential spatfall was greater than in any of the other years under consideration, has been discussed above.

From the foregoing it may be concluded that the water-temperature is a very important factor in the reproduction of *Ostrea edulis*. Although it has been shown that data on the extent of the practical set do not always enable us to determine the extent of the potential setting, we will yet briefly discuss the results obtained by the farmers in the years from 1921 till 1935.

I do not have at my disposal any reliable data on the number of adult oysters that were present in the basin of the Oosterschelde in these years. Some data on the extent of the spatfall with other information on oyster-culture in the Zeeland Streams in the years since 1921 can be found in Jaarverslagen omtrent den toestand der Visscherij op de Zeeuwsche Stroomen, issued by the Fishery Board of the Zeeland Streams at Middelburg [1]). Some of these data have been collected in table IV. This table gives a brief survey of the course of events in the years since 1921.

In a special diagram (fig. 4) I have visualized the temperature of the water in the basin of the Oosterschelde in the summers since 1921.

I should like to discuss the years from 1921 to 1931 first.

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[1]) The annual report of 1928 was published in Verslagen en Mededeelingen van de Afdeeling Visscherijen Nr. 13 's Gravenhage.

TABLE IV

Years	Numbers of tiles (rounded off)	m^3 of shells (rounded off)	Practical set	Remarks
1921	120 000	6 000	satisfactory	mortality
1922	185 000	15 000	complete failure	
1923	300 000	14 000	moderate	
1924	300 000	22 000	moderate	
1925	140 000	47 000	good	
1926	100 000	46 000	very good	
1927	100 000	46 000	good	
1928	100 000	48 000	very good	
1929	70 000	50 000	extremely good	
1930	40 000	40 000	good	
1931	45 000	50 000	moderate	mortality
1932	45 000	33 000	good	mortality
1933	40 000	31 000	moderate	slippers become abundant
1934	350 000	14 000	failure	mortality
1935	1 000 000	2 000	good	
1936	2 000 000	– –	failure	
1937	3 300 000	– –	moderate	
1938	5 000 000	– –	failure	potential spatfall good!
1939	6 000 000	4 000	good	

During these years the greater part of the spat was caught on shell-collectors, mainly old shells of *Cardium edule*. Profuse spatfalls occurred in the years 1929, 1928 and 1926, all of which years were characterized by high water-temperatures during the summer-months (fig. 4).

A complete failure was recorded in the year 1922, in which year the water-temperatures in the summer were extremely low. Moderate spatfalls occurred in 1923, 1924 and 1931, years with moderate water-temperatures in the season of reproduction. In 1923 the month of June was uncommonly cold. Although a considerable mortality was recorded in the year 1921, which probably was the cause of a decrease in the production of larvae, the spatfall was nevertheless satisfactory then, probably owing to the favourable temperature conditions.

In the following years other factors interfered. The first slippers (*Crepidula fornicata*) were observed in the Zeeland Streams in 1929. An uncommonly rapid propagation of the slipper-limpet soon made it impossible to continue with the sowing-out of

Cardium-shells, as the oyster-spat got overcrowded by slippers. The volume of shells sown out decreased rapidly in the years following 1933, while commensurately the number of tile-collectors increased from year to year.

Moreover a considerable mortality was caused by shell-disease in the years following 1930 (HAVINGA 1931, KORRINGA 1939) and most of the surviving oysters were in a bad condition. In spite of this the spatfall in 1932 was quite good, no doubt owing to the very favourable temperature conditions in the summer of that year.

The results obtained in the last five years have already been discussed above. It may be seen in the diagram (fig. 4) that temperature conditions were far more favourable in 1935 than in the years following, which may partly account for the satisfactory setting by which this year, notwithstanding the small number of larvae, was characterized.

Summarizing, I believe that the data on the years from 1921 to 1934 clearly show that my conclusion (based on the data from 1935 to 1939) that water-temperature is a very important factor in the propagation of *Ostrea edulis*, is correct. If no other factors interfere and provided there is a large number of healthy adult oysters, we may expect warm summers to be productive of profuse potential spatfals and cold summers to result in partial or even complete failures. This may be ascribed to the influence of temperature on the duration of the pelagic stage and thus on the percentage of larvae reaching maturity. Moreover, favourable temperature conditions may incraese the production of larvae, as has been discussed above.

In the next section it will be discussed how science may help the oyster farmers to obtain a satisfactory practical set, whenever the potential setting in good.

XXVI. THE PREDICTION OF THE SPATFALL

> "Thousands of dollars would be saved annually by the oystermen if they would determine with any approximate accuracy the data when attachment of the young oysters would occur." (WINSLOW, 1884).

The oystergrowers are faced with the problem of deciding when to plant cultch, so that it shall not be silted over or covered with organic growth before the larvae are able to attach. In many cases the cultch is laid out far too early, with the result that the maximum catch of spat is not obtained. Sometimes collectors are immersed when the potential maximum of setting is over, which does not increase the chances of obtaining a satisfactory practical set either. In the years before 1935 Dutch oysterfarmers in deciding when to plant cultch had to consider the dates on which the collectors were ready to be planted and had to trust to the experience gained in former years, but most of all to their good luck. The desire for more reliable indications induced several oysterfarmers to plant "test-collectors". Small numbers of tiles were planted at intervals and as soon as these tiles were found to be covered with spat, the farmer started to plant his entire supply of collectors. As the spat can only be seen with the naked eye about a week after attachment, it goes without saying that this method frequently led to disappointments, the best time for setting often being over when the cultch was planted.

The first attempt to predict the spatfall was made by JULIUS NELSON (1907). He examined plankton-samples and tried to predict the probable date of setting from the stage of development of the larvae. The simultaneous spawning, discussed above, accounts for the fact that often all the pelagic larvae of *Ostrea virginica* show the same stage of development, which makes it easy to predict the date of setting with reasonable accuracy. THURLOW C. NELSON (1917) states that it is possible to predict the time of setting within two days by means of the technique developed by him.

It is not only necessary to predict the date of setting, but also the intensity of the spatfall. The latter problem is more difficult, as the percentage of the larvae reaching maturity is largely dependent on environmental conditions. The solution of this problem requires extensive quantitative observations.

In the main centres of spat-production in France, le Morbihan and the basin of Arcachon, oysterfarmers have for several years been assisted in choosing the right moment for cultch-planting. Each summer-season the French investigators issue bulletins in which they publish the number of larvae counted in their plankton-samples, the water-temperatures recorded and often the "fixation-coefficient". It has been discussed above why I refuse to believe in the reliability of the "fixation-coefficient". Moreover the time elapsing between the observation of a favourable "fixation-coefficient" and the occurrence of the spatfall is rather short. I am of opinion that with the Dutch methods and frequency of sampling the dates and intensity of setting can be predicted with a higher degree of accuracy than with the French. It should be remembered that *Ostrea edulis* does not show simultaneous spawning, so that the prediction of the spatfall of *Ostrea edulis* presents greater difficulties than that of *Ostrea virginica*.

The enforced return to the system of tile-collectors in the Oosterschelde led to the establishment of a system of predicting the spatfall in Holland. Once or twice a week bulletins are issued in which are given the number of larvae per 100 litres of water, its composition in size-classes, the water-temperatures recorded and the setting prospects. We not only predict the dates of setting, but also the intensity of the spatfall. When predicting the setting, I always compare the number of spat that attached under similar conditions in previous seasons. The high frequency of our sampling, the regular determination of the potential spatfall and the division of the larvae in size-classes have proved to be of the greatest use for this purpose.

When a considerable maximum of swarming occurs under very favourable temperature conditions, we advise oysterfarmers to plant soon as many of their collectors as possible. If no swarming occurs or if it occurs under extremely unfavourable temperature conditions, as was the case in the first half of July 1938, I make it known that no setting of commercial magnitude is likely to occur. When moderate swarming takes place under rather favourable conditions or when a considerable swarming occurs under fairly unfavourable conditions, I announce that some spatfall is likely to occur within about 10 days, provided the water-temperature does not drop. As soon as the number of larger larvae is found to be increasing considerably, the setting prospects are announced with greater precision.

When the season is well on its way already and no favourable predictions are forthcoming (as was e.g. the case in 1938), the oysterfarmers get into a difficult position. If they plant their cultch in spite of unfavourable predictions, the odds are that they will obtain an unsatisfactory set. If they wait any longer, the setting prospects may ameliorate, i.e. if the water-temperature and the number of larvae should happen to increase, but it is also possible that no favourable predictions follow, in which case the chance of obtaining a satisfactory spatfall is still smaller. It is advisable in such cases not to put all one's eggs in one basket. When the larvae of a considerable maximum of swarming have not led to a spatfall of some importance, we proceed to predicting if a subsequent swarming of some importance is likely to occur. For that purpose we examine fairly large samples of adult oysters from different oysterbeds in order to ascertain the percentage of those that is incubating. So far it has been impossible, however, to predict so long before whether a spell of fine weather is likely to occur at the time that this subsequent swarming may be expected.

COLE (1939) does not think it impossible that *Ostrea edulis* is cleverer than our meteorologists: "It is remarkable that during the last three seasons the major liberation of the season has occurred just after the beginning of the first lengthy hot spell, as if the breeding oysters had sensed in some way the indicence of a prolonged spell of fine weather during which the larval development could be completed satisfactory."

Unfortunately Dutch oysters appear to be unable to sense this, for liberation has but too often occurred during adverse weather or during a falling water-temperature.

Many attempts have been made to predict the spatfall at long notice. It is partly the absence of quantitative data on larval development that has compelled investigators to predict the spatfall long before and partly these attempts have been made to meet the wishes of the oyster farmers, who want to be informed as soon as possible of the expectations about the date of the spatfall and the intensity of setting. HOPKINS, ORTON and PRYTHERCH belong to the first category.

HOPKINS concluded (1937) that the periodicity in spawning parallels the periodicity in setting in some respects only. HOPKINS did not follow the development of the pelagic larvae. It is my belief, however, that quantitative observations in the course of the pelagic stage are especially indispensible in species of oysters with such a long pelagic period as *Ostrea lurida* (30 to 40 days). HOPKINS has not provided himself with data from which to predict the intensity of setting and as for the time of setting he states that attachment is likely to show a maximum in the third tidal period following on that during which spawning starts. HOPKINS often observed maxima in the frequency of setting about the time of the greatest tidal range, but he does not yet know the exact reason for this phenomenon.

PRYTHERCH (1929, 1934 b) does not base his predictions on observations on quality and quantity of pelagic larvae either, although *Ostrea virginica* shows a less complicated composition of larval age-groups in the plankton (owing to simultaneous spawning) than the gradually spawning incubatory species of oysters. PRYTHERCH observed that the local hydrographical conditions are the cause of a rapid warming up of the water during spring-tides, which is in its turn the cause of maxima of spawning at spring-tides. PRYTHERCH bases his predictions of the time of spawning on observations of the water-temperature, for which he uses a long-distance thermograph. It has been stated before that PRYTHERCH neglects the influence of environmental conditions on the percentage of larvae reaching maturity which omission I am inclined to ascribe to a lack of quantitative data on the pelagic larvae. Before spawning begins PRYTHERCH is able to predict its intensity from studies on the thickness of gonad-tissues, which according to him is liable to considerable annual differences, resulting in proportional differences in the spatfall.

It may be seen from HIGGINS' papers (e.g. 1933, 1937) that announcements about the condition of the oyster, changes in water-temperature and the dates at which spawning and setting may be expected are being continued in the district of Long Island Sound.

NELSON (1926, 1928 c) too, predicts the dates at which spawning of *Ostrea virginica* will start, for which he bases himself on observations of the water-temperature. His methods have been described in a previous section. He also predicts the dates of setting, for which he bases himself on quantitative observations of larvae.

As regards the prediction of the spatfall ORTON states (1937): "An examination of samples of oysters on each bed each year is therefore desirable to find out the rate of spawning and from this information the most propitious time for the laying of cultch." "The density of the subsequent fall will be proportional to the percentage of black-sick oysters." "It is suggested that a reasonable course is to lay cultch when about 5% of the stock are black-sick, and there is in addition at least 5% of younger stages of sickness." As this method requires the sacrifice of a considerable number of adult oysters (**Anon.** 1928), **Orton** suggests (1927 e, 1928 b) a more economical method of testing adult oysters. The oysters can be exposed to the air till they open their shells a little, which enables us to see whether they are incubating. After this they are thrown back into the water. It will be clear from the foregoing that the fitful weatherconditions on the North-sea coast preclude a fixed proportion between the intensity of spawning and the intensity of setting. Consequently it is my opinion that the application of **Orton**'s methods of prediction cannot lead to reliable results here.

Nevertheless a determination of the percentage of oysters carrying larvae is useful, if we want to know whether subsequent spawning of some importance is still to be expected, in case the larvae of the first maximum of spawning should have failed to yield a commercial spatfall (as, for instance, in the Oosterschelde in 1938).

As regards the prediction of setting at long notice I agree with **Voisin** (1931, 1933) who states that, owing to the fitful weather-conditions, such predictions must always be unreliable on the west coast of Europe.

A reliable prediction of setting at short notice, on the contrary, appeared to be very well possible in Holland.

As the Dutch oystermen began to realize that those who followed our advice generally caught the best sets of spat, their interest in the predictions has increased rapidly. With our method we try to synchronize the practical setting with the largest maxima in the potential setting. In this way we try to reduce the enormous difference between the number of mature larvae occurring in the course of the season of reproduction and the number of successful spat on the collectors.

XXVII. THE INFLUENCE OF LIGHT ON THE SETTING PROCESS

The pigment-spots or pallial eyes of mature oysterlarvae have been the subject of considerable controversy. There can, however, be no doubt about the eye-like structure of these organs in the larva of *Ostrea edulis* (**Erdmann** 1934, **Cole** 1938 a). The black colour of these spots is due to heavy pigmentation. The pigment-spots disappear soon after attachment. Phagocytes appeared to play a part in the process of desintegration. **Cole** (1938 a) observed phagocytes heavily loaded with pigment-grains leaving the fading pigment-spots in recently-set spat of *Ostrea edulis*.

PRYTHERCH (1924, 1934 a) has not observed any response to changes in light-intensity or colour by the larvae of *Ostrea virginica*. He came to the conclusion that the pigment-spots are not light-sensitive organs at all, but fulfil a quite different function. It is his belief that they are leucocyte-generating tissues. PRYTHERCH probably made the same observation as COLE did and based his conclusion on the fact that shortly after attachment he observed many phagocytes in the proximity of the pigment-spot. COLE's interpretation of this fact is the more plausible, however.

As the eye-like structure of the pigment-spot has been established (at least for *Ostrea edulis*), we should not be surprised to find that light plays some part in the selection of a site for attachment. Only a few investigators made deliberate experiments to investigate this matter, however. Others based their conclusions on accidental field-observations. Thus PETERSEN (1908), FOLPMERS (1924) and ORTON (1937 a) found more surviving spat of *Ostrea edulis* in situations where the light is subdued, for instance, on the shaded sides of collectors. ORTON and PETERSEN admit, however, that this fact does not necessarily indicate that oysterlarvae prefer dark places for attachment. For it is possible that a growth of plants, macroscopical or microscopical, on the light side of the collectors will soon hinder attachment, while the shaded sides may remain longer in a condition suitable for attachment. Moreover it is possible that the death-rate of the spat on the two sides of the collectors is different, for the light sides are as a rule also more exposed to enemies and to silting. To eliminate such factors it is necessary to expose test-collectors for short periods and to count the spat a few days after attachment.

PRYTHERCH (1924) states that the setting of the larvae of *Ostrea virginica* occurred as heavily on the light sides of the collectors as it did on the darkened sides, it being assumed that both sides were equally clean. In a later paper (1934) he tells us that during eight years of experimentation he did not observe any influence of light on the setting process. He does not describe, however, what kind of experiments he made to investigate this matter.

NELSON (1926), on the other hand, states that the pigment-spot of *Ostrea virginica*, "although unable to form an image like a true eye, is sensitive to light." "In the presence of light the eyed larvae of the oyster are stimulated and continue moving until they get into a shaded place where they become quiescent."

Consequently the shaded under-surfaces of the collectors are supposed to catch more spat than the upper-surfaces during daylight. It is my belief that it was the observation of a heavier fall of spat on under-surfaces that induced NELSON to form his hypothesis of a stimulated swimming-activity in illuminated places. Whenever he found more spat on the undersides of collectors (1927, 1934), he used this hypothesis in explanation of this phenomenon. NELSON neglects, however, the influence of the angle of surface on the intensity of setting, so that we are not justified in concluding from his observations that light plays a part in the setting process.

Deliberate experiments to investigate the influence of light on the setting process have been carried out by YOKOTA (*Ostrea gigas*), HOPKINS (*Ostrea lurida*) and COLE and KNIGHT JONES (*Ostrea edulis*). YOKOTA (1936) placed full-grown larvae of *Ostrea gigas* in a glass filled with seawater and screened off half the glass (source of light: electric lamp 100 volt, 60 watt, 36 cm above the water). The distribution of the spat on the bottom of the glass appeared to be almost uniform in spite of the great difference in illumination. YOKOTA concludes from these results that at the time of setting *Ostrea gigas* does not react negatively to light.

HOPKINS (1935, 1937) placed wire frames containing plates of clear glass on an oysterground, so that the plates were held in a horizontal position. The upper-surfaces of the plates of one set were painted black, the others were left clear. He found that the numbers of larvae setting on the under-surfaces of clear and darkened glass were more or less the same, which shows that under field-conditions the larvae of *Ostrea lurida* do not select shady places for attachment. HOPKINS concludes that light is not an orienting factor in the setting behaviour of this species.

COLE and KNIGHT JONES (1939) used the same device in the tanks that are in use for spat-production in England (*Ostrea edulis*). They observed a marked preference on the part of the larvae for the undersides of the dark plates (in 18 sets: under-surfaces of clear glass 228 spat, under-surfaces of dark plates 735 spat). This selection of shady places appeared to be confined to the daylight hours, while plates exposed at night showed no difference in the number of spat caught on the undersides of the plates. They concluded that during daylight the larvae of *Ostrea edulis* undoubtedly tend to select a shady situation for attachment.

In the same season that COLE and KNIGHT JONES made their observations in tanks (1938), I used HOPKINS' device in the Oosterschelde. Experience had previously taught me that plates of smooth glass exposed in the field are far less suitable to catch spat of *Ostrea edulis* than plates of various other materials. This phenomenon will be discussed more amply in one of the following sections. Therefore I used ground glass for this experiment, this being a little more suitable for attachment and yet pervious to light. The plates were placed horizontally in containers of reinforced concrete. Clear plates and plates with a black coating on their upper surfaces were exposed together in one container. In the field ground glass catches somewhat more spat than smooth glass, but the number of spat counted on the undersides of the plates appeared to be too small to base conclusions on, for, owing to the influence of the angle of surface on the intensity of setting, the undersides of the plates caught far less spat than the uppersides, as will be discussed presently. The total catch of the undersides of 3 series of 3 plates, each series exposed during 6 days, was:

upper-surfaces clear: 11 spat
upper-surfaces black: 43 spat

There are, however, many data available on other series of spatfall experiments in the Oosterschelde, which might show that light influences the setting process. In the containers I often placed three coated plates in a horizontal position, the one right above the other with an interspace of 3 cm. Consequently the uppersides of the two lower plates caught far less light than the uppersides of the topmost plates. The numbers of spat I counted on the uppersides of the plates of these series are recorded in the table on page 191.

These data do not allow me to conclude that under field-conditions the larvae of *Ostrea edulis* tend to select shady situations for attachment.

In the summer of 1938 I shaded one half of a group of tile-collectors, seeing to it that factors other than light did not change

Series	Topmost plate	Second plate	Third plate
4 series July 1938	191	161	206
3–12 July 1938	69	30	40
12–21 July 1938	53	23	36
2–8 August 1938	224	–	70
8–14 August 1938	1194	–	1031
8 series 1939	903	744	723
8 series 1939	760	476	504

under the cover. In September I counted about an equal number of spat on the tiles under the cover and on the tiles that had been exposed to the sunrays.

As the poor spatfall on smooth glass and ground glass prevented me from obtaining reliable data on the influence of light on the setting process in the field with **Hopkins**' device, I constructed an other apparatus to investigate this matter. The factor light alone had to be changed, while the other factors, especially the accessibility for the water and with it for the oysterlarvae, had to remain constant. I used one of my large containers, in which there is room for 12 plates. I numbered the places in the container (fig. 22) and I made a sluice of clear glass in places 1 and 2, put test-plates in places 3, 4 and 5, making a sluice of glass coated with black paint (but for the rest similar to the first

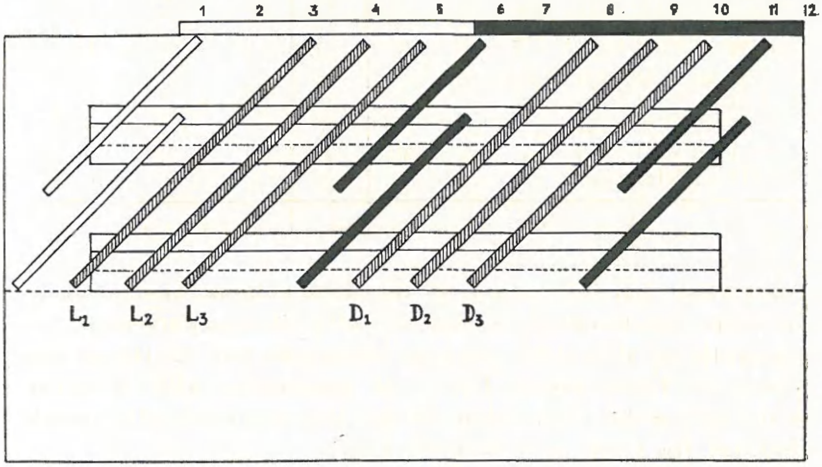

Figure 22. Container with light and dark compartment.
Longitudinal section.

sluice) in places 6 and 7, putting test-plates in places 8, 9 and 10 and making another dark sluice in places 11 and 12. The container was covered with a glass plate of which one half was coated with black paint. I did not shut the long narrow apertures in the sidewalls of the container (cf. fig. 19), but I constructed sluices of zinc in them, so that no lightrays could penetrate into the inside of the container through these apertures while the larvae-bearing water could flow in freely through them.

In this way the container was divided in two compartments with test-plates. In one compartment the light could enter freely and in the other complete darkness prevailed, while the larvae-bearing water could enter both compartments equally well. Moreover I regularly interchanged the places of the dark and the light compartment in the container. To this end the plates of the sluices and the covering glass plate were made to fit in brass rails, so that they could easily be exchanged. The results obtained with this apparatus are the following:

Series	L. I	L. II	L. III	L. Av	D. I	D. II	D. III	D. Av.
2 VII– 8 VII 1939	38	21	22	27	43	13	16	24
11 VII–14 VII 1939	9	18	22	16	17	14	10	14
14 VII–20 VII 1939	79	46	37	54	47	30	39	39
20 VII–26 VII 1939	36	20	15	24	29	16	22	22
26 VII– 4 VIII 1939	60	47	31	46	34	43	56	44
4 VIII–10 VIII 1939	30	27	22	26	65	26	16	36
10 VIII–16 VIII 1939	19	13	38	23	26	19	16	20
16 VIII–22 VIII 1939	31	29	32	31	49	38	28	38
Total	302	221	219	247	310	199	203	237

No more spat was caught in the dark compartment than in the light compartment. So far my field-observations have not demonstrated that light plays an important part in the setting process of *Ostrea edulis*. This is in agreement with HOPKINS' field-observations on *Ostrea lurida*, but not with the results obtained by COLE and KNIGHT JONES.

Although light appears to be no orienting factor in the setting behaviour of *Ostrea edulis* under field conditions, it is not impossible that light influences the setting process, in cases when the intensity of the light exceeds a certain degree. The water in the Oosterschelde is rather turbid and above the containers we find from 2 to 6 metres of water, so that the intensity of light on the bottom, on which the containers are placed, is not very great.

COLE placed the plates in his tanks about one metre below the surface and as the water in the tanks was moreover very clear and completely free from silt, his plates received far more light than HOPKINS' and mine. This problem calls for further experiments under controlled laboratory conditions. YOKOTA's laboratory experiments have not yet been carried out on the larva of *Ostrea edulis*.

Summarizing, I conclude that light evidently does not play any part in the setting process of *Ostrea edulis* under field-conditions in the Oosterschelde, but that COLE's experiments in tanks point to the possibility that oysterlarvae tend to select shady situations for attachment, when the intensity of the light or the difference between "light" and "dark" is great enough. It is doubtful, however, whether this influence is great enough to play any part in setting under field-conditions, especially when the water is rather turbid and the range of the tide is great, as is the case in the Oosterschelde. It is not probable that light-perception by the pigment-spot is indispensible in the setting process of *Ostrea edulis*.

The comparison of the spatfall during day and night is closely bound up with the problem of the influence of light on the setting process.

If NELSON's hypothesis (1926) of a stimulated swimming activity in the presence of light is correct, we may expect a greater intensity of setting during darkness than in daylight. NELSON did not investigate this matter, however. HOPKINS (1937) demonstrated that the setting of *Ostrea lurida* often proceeds equally well at night as during the day. COLE and KNIGHT JONES (1939) compared the setting in tanks during 7 hours of darkness with that obtained during a period of daylight of the same length. Their figures show a marked tendency of the larvae to attach themselves during daylight hours, for the average number of larvae setting per daylight period was about three times that which set during a period of darkness of the same length. They do not explain how this phenomenon is to be reconciled to their conclusion that the larvae of *Ostrea edulis* tend to select shady situations for attachment. Is it perhaps the contrast between light and darkness that creates favourable setting conditions? Or is it possible to interpret the selection of shady places in the tanks as a tendency to avoid too intense an illumination and can the difference in setting during night and day be correlated with differences in the swimming behaviour of the mature larvae?

I placed 3, sometimes 6 plates in a container at the station Yersche Bank during dark night-hours. As I knew beforehand that the setting is not intense enough to obtain an adequate number of spat in one night, I placed the plates in a zinc container filled with filtered sea-water during the day and put the plates back in the concrete container on the sea-bottom for the next night. Thus the plates were exposed for three consecutive nights. I placed the daylight set in the same container at the same station for a period of the same length. To eliminate the influence of the tidal cycle on the intensity of setting, I placed the daylight set in the container during exactly the same part of the tidal cycle as the night set. Some series caught too little spat. One of the series (i.e. 8 VIII-11 VIII 1938) caught a great number of spat, as it had been exposed during the most favourable part of the setting season and as on that occasion I used a container in which the plates were held in a horizontal position.

Period	Exposure		Spatfall			
	Night-Set	Daylight-Set	Night-Set	Av.	Daylight-set	Av.
8 VIII–11 VIII 1938	20.30–5.30	8.30–17.30	82–61–75 60–78–68	71	200–362–356 332–362–304	320
11 VII–14 VII 1939	22.00–5.00	10.00–17.00	1–2–3	2	3–4–2	3
16 VIII–19 VIII 1939	21.00–6.00	9.00–18.00	5–4–4 0–7—	4	18–18–5 15–17–2	12

The above results seem to confirm the conclusion of COLE and KNIGHT JONES that the setting of *Ostrea edulis* is more intense during daylight than during the night.

I cannot yet give any plausible explanation of this phenomenon. I have never observed a marked difference in the vertical distribution of full-grown larvae during night and day (fig. 11). Other conditions, such as current-velocity, salinity and water-temperature, are practically the same during night and day. Only the factor illumination differs. However, I did not catch more spat in the light than in the dark compartment of the apparatus with which I investigated the influence of light on the setting process. This apparatus was placed at the same station and at an equal depth as the container with the plates in the experiments described above.

Consequently I do not yet see how the difference in illumination can bring about a difference in setting between night and day.

I have remarked in a preceding section that full-grown larvae sometimes seem to show a slight difference in their vertical distribution during night and day (fig. 11), but that the numbers of mature larvae I counted in my samples were too small to base conclusions on. Perhaps it is this factor that holds the key to the problem.

Cole and Knight Jones suppose that the difference between the setting intensity during night and day is possibly due to a decrease in the swimming activities of the larvae at night, resulting in a general sinking towards the bottom (which has not been observed by them, however), but my investigations clearly demonstrate that such a general sinking towards the bottom during the night most certainly does not occur in the Oosterschelde (fig. 11, 12).

XXVIII. THE INFLUENCE OF DIRECTION AND VELOCITY OF THE CURRENTS ON THE SETTING PROCESS

We can distinguish a favourable and an unfavourable influence of the currents on the setting process. In the first case we will consider how the currents continuously supply full-grown larvae to the places where the collectors have been planted. The larvae-bearing water flows along and through the masses of cultch material and provides them with the required larvae. Only when the cultch has been planted in too compact masses, we shall observe that the supply of larvae has been smaller in the centre of the masses than in the outer zones. Thus it has often been stated that as a rule less spat is found per shell in the centre of the wire bags filled with shells, which are in use in America [1]).

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[1]) The same experience has been gained in the Oosterschelde with wire-covered trays filled with shells.

BONNOT (1937 b) also mentions a less intense setting of *Ostrea lurida* in the centre of his batteries of wooden collectors, which resemble the "plateaux collecteurs" of the French oystermen. It is an important problem in the production of seed-oysters to find adequate methods to utilize the natural supply of larvae to the best advantage. The same quantity of cultch can be supplied in different ways. The best method is that which makes it easy for the larvae to reach the surfaces. It has been discussed in a preceding section that many collectors may be planted successfully in one place, owing to the enormous difference between the number of full-grown larvae and the number of successful spat. Accumulation of collectors may yield good results, provided the larvae-bearing water can flow freely through the cultch masses. Generally speaking, we may say that the more easily the larvae can reach the surface, the more spat will be caught. Thus COE and ALLAN (1937) tell us that more spat of *Ostrea lurida* settled on the outer surfaces of their experimental collectors than on the other surfaces.

HOPKINS (1935, 1937) placed glass plates parallel and perpendicular to the direction of the flow of the tide. The plates were placed either vertically or at an angle of 45°. In either case the plates that had been exposed parallel to the current definitely caught more spat (*Ostrea lurida*), presumably because more larvae-bearing water comes into contact with the surfaces of these plates. HOPKINS' conclusion that setting may be proportional to the rate of current is not justifiable, however, for the currents may just as well have an unfavourable influence on the setting, especially when the current is strong, as will be discussed below.

SCHAEFER (1937) obtained the same results with *Ostrea gigas*, using the same device as HOPKINS.

Independently of these experiments I tried to find out whether the direction of the current has any influence on the setting of *Ostrea edulis* in the Oosterschelde. Here it is impossible to catch an adequate number of spat during the short period of flood or ebb. I constructed an apparatus that holds the plates in the desired position for several days. An iron axis was erected on a solid foot of reinforced concrete, which could be placed on the sea-bottom (fig. 23). A structure of wood and iron, provided with a zinc rudder, could revolve freely round the axis. Brass frames were attached vertically to this construction, either parallel with the rudder or perpendicular to it. In these frames the coated glass plates were held. A rope and a buoy were attached to the iron ring at the top of the axis in order to make it possible to find back the apparatus in the field.

The results obtained with this apparatus are the following:

Series	Parallel to the current				Perpendicular to the current			
5 VIII–11 VIII 1938	40	17	36	40	19	16	25	22
11 VIII–23 VIII 1938	21	20	30	15	13	18	11	11

Other series caught less spat.

Although these numbers are not very great, which is mainly due to influence of the angle of surface (vertical plates catch far less spat than those held at other angles), they suffice to show that the plates placed parallel to the direction of the current catch more spat than those perpendicular to the tide. This is in accordance with the results obtained by Hopkins and Schaefer with other kinds of oysters.

However, the currents may also have an unfavourable influence on the setting process. Yokota (1936) observed the setting behaviour of *Ostrea gigas* in vitro. When by means of a syringe he produced currents in his vessel, the mature larvae often appeared to be able to continue crawling on the substratum, as the body was supported by byssus-threads. When the current was strengthened, the byssus snapped off, however. Then the mature larva could only start crawling again after the current-velocity had been sufficiently decreased. From this we see that when the current is too strong the larvae are washed off from the substratum and that attachment will be impossible for the greater part of the tidal cycle in places where strong currents prevail.

Nelson's view (1921), though not based on such experiments, is in accordance with the observations of Yokota. Nelson states that the setting process of *Ostrea virginica* "cannot be carried out successfully except in relatively quiet water, hence in places where swift currents prevail, the larvae attach during the period of slack water between tides, or they creep in between shells or other objects, where the current is practically nil."

Prytherch's observation (1929) that the larvae of *Ostrea virginica* mainly attach themselves to the leeward sides of the collectors also accords with these data.

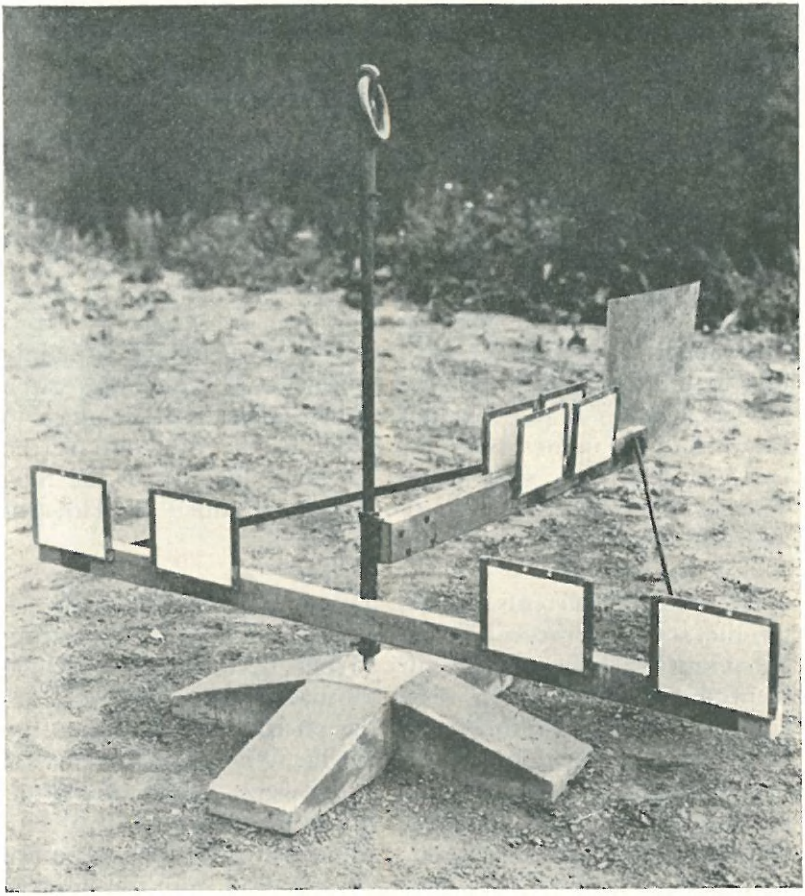


Figure 23. Apparatus for investigating the influence of the current on the setting process. (Photo Havinga).

In investigating this matter I used the apparatus described above (fig. 23). I changed, however, the position of the brass frames. All the frames were placed horizontally. Four of the eight frames were sheltered from the current in front and at the sides with zinc screens. The other frames remained exposed to the currents as fully as possible.

For the results obtained with this apparatus see page 199 top.

The fully exposed plates apparently caught less spat (16 per

Period	Small shelter		Large shelter		Fully exposed			
5 VII–11 VII 1939	18	29	43	37	11	14	9	6
11 VII–17 VII 1939	55	61	81	73	19	19	16	10
17 VII–23 VII 1939	–	13	38	36	19	–	6	9
23 VII–29 VII 1939	19	–	19	36	7	18	8	1
29 VII– 7 VIII 1939	27	–	60	22	14	17	9	–
7 VIII–13 VIII 1939	18	13	19	–	18	12	6	–
13 VIII–19 VIII 1939	28	33	69	75	29	21	34	–
19 VIII–25 VIII 1939	60	–	48	36	43	42	21	–
On an average	31		45		16			

plate on an average) than the sheltered ones (31 and 45 per plate on an average). The plates that were exposed behind the larger shelters showed an equal distribution of the spat over their entire surface. The majority of the spat on the plates behind the smaller shelters, however, appeared to have congregated on that side of the plate that had been close to the zinc shelter. The side farthest away from the screen was apparently not sufficiently sheltered, which accounts for the smaller number of spat on these plates.

The current-velocity at the station where I placed this apparatus amounts from 30 to 50 cm/sec. for the greater part of the tidal cycle.

My data clearly indicate that in the Oosterschelde the spatfall is more intense in sheltered places. I dare not decide whether all the spat on the fully exposed plates attached during or about slack water, but I am inclined to think so.

From the foregoing I conclude that strong currents hinder the spatfall considerably, because the larvae are washed off from the substratum by them. In places where the tide is rather swift, attachment is probably only possible during slack water and during the tide only in those parts of the collectors that are sufficiently sheltered from the currents.

If the currents are slow their favourable effect (i.e. the continual supply of larvae) will preponderate, but if the currents are strong, the unfavourable effect (i.e. the washing off from the substratum) will become operative. HOPKINS' assumption that setting may be proportional to the rate of current only holds good in the case of slow currents.

XXIX. THE SPATFALL IN THE COURSE OF THE TIDAL CYCLE

The distribution of the spatfall over the different stages of the tidal cycle has not often been thoroughly investigated.

In a preceding section I have related already how PRYTHERCH (1929) found that in the Long Island Sound the setting of *Ostrea virginica* occurs mainly roundabout low slack water and during the early flow (fig. 20). PRYTHERCH states that the setting gradually becomes less intensive as the velocity of the currents increases and finally ceases altogether on the current attaining a velocity of 10 cm/sec. As I have stated above (fig. 20) it is my opinion that the main factor causing a concentration of the spatfall during low slack water in the Long Island Sound is the variation in the number of larvae per unit of water during the tidal cycle. It is my belief that other factors, such as the copper-content of the water and the current-velocity, exercised less influence on the intensity of setting in this particular case.

HOPKINS (1937) ascertained in several stations in the Puget Sound the number of spat of *Ostrea lurida*, caught hourly per unit of cultch. He used batteries of glass plates supported in wire frames. He invariably found that the least spat was caught at low tide, when the dikes are exposed and the water-temperature is high. The maxima of setting in different stations did not always occur at corresponding stages of the tide. HOPKINS tried to establish a correlation between the intensity of setting and variations in factors, such as the rate of current, temperature, salinity and pH. He concluded that in some cases there seemed to be a correlation between the rate of current and the frequency of setting. HOPKINS neglected, however, the most important factor, i.e. the variation in the number of full-grown larvae per unit of water!

HOPKINS writes that: "Chance is a large factor in determining whether the water in the particular place happens to contain larvae. For this reason the error involved in the tests is considerable."

I tried to ascertain the intensity of setting in the course of the tidal cycle in the Oosterschelde. I knew beforehand that it is impossible here to obtain adequate numbers of spat on plates exposed during one hour. Even if we divide the tidal cycle in four sections: high water, ebb, low water and flow, each of three hours' duration, adequate numbers of spat can only be caught when the spatfall is exceptionally intense. Under normal conditions a three hours' period of exposure is too short. Therefore I placed the plates during three hours in the containers on the sea-bottom, replaced them by an other set of plates during the next section of the tide and put the removed plates temporarily in a zinc container, filled with freshly filtered sea-water. In this way I used four sets of three plates. The first three plates were put back in the container when the three other sets had had their turn. I continued exchanging the plates during 3 × 24 hours. As the exchanging of the plates during night and not infrequently under unfavourable weather-conditions is by no means a sinecure, it was not easy to obtain many of such series. The results were rather disappointing. The total number of spat on all of the 12 plates was in most cases insignificant, although the number of spat caught by the plates of the normal series, placed at the same station during the same three days, was rather considerable. The same phenomenon, i.e. the decrease in the total number of spat caught according as the period under consideration is subdivided in an increasing number of shorter periods, has been observed by HOPKINS (1937). HOPKINS suggests that "those larvae which had not completed the setting process released their hold when the plates were withdrawn from the water, so that possibly only those that began to set soon after the plates were immersed were able to attach permanently."

In the course of my investigations this exchanging of plates during 3 × 24 hours has been carried out five times, viz. four times at the station Yersche Bank and once at the station Kattendijke. Only one of these five series caught a moderate number of spat, although the total catch of the entire set remained far below the number of spat caught on the ordinary series of plates, which remained constantly in the water during those three days. This was not caused by the death of the spat during the hours spent in the zinc container, for in that case the empty shells of the dead spat could have been detected on the plates. Moreover I obtained excellent results with other series of plates which also remained in the zinc container for a considerable time. HOPKINS' explanation is probably correct, but the problem calls for further investigation.

Series exposed from 14 to 17 July at the station Yersche Bank:

	Period of exposure	Number of spat on the plates			Average
Low water	(1½ h. before L.W.–1½ h. after L.W.)	3	8	6	6
Flood	(1½ h. after L.W.–1½ h. before H.W.)	4	4	1	3
High water	(1½ h. before H.W.–1½ h. after H.W.)	16	23	19	19
Ebb	(1½ h. after H.W.–1½ h. before L.W.)	6	7	4	6

These results seem to indicate that at the station Yersche Bank the setting is most intense during high water.

I have tried to find other methods to investigate this matter, as the exchanging of the plates during 3 × 24 hours is no trifle and as such series of plates often bore little spat. In one of the preceding sections I have shown that the great majority of the spat of *Ostrea edulis* attaches with the umbos pointing in about the same direction, provided the substratum is not held in a horizontal position. An analysis of an adequate number of spat enables us to infer which side of the collectors has been uppermost at the time of setting.

If we imagine a collector that changes its position in the course of the tidal cycle, so that it is not always the same side of the collector that is uppermost, we might be able to deduce by analyzing the orientation of the spat which side of the the collector has been uppermost at the time of the greatest intensity of setting. As, alas, the umbos of the spat do not all point precisely in the same direction, we shall need a fairly great number of spat, if we want to use a method of ascertaining the intensity of setting in the course of the tidal cycle, based on the revolving of collectors.

I constructed an apparatus based on the idea explained above (fig. 24). The centre of it was formed by a solid time-piece capable of running for several days. This time-piece was shut up in a water-tight iron box, which was placed on a solid foot of reinforced concrete. Only the axis of the time-piece protrudes from the iron box at an angle of 45° with the horizontal surface. A set of three coated plates could be attached perpendicular to the axis, for which purpose the plates had been pierced in the centre. Consequently the plates were exposed at an angle of 45° with the horizontal surface. This angle ensures the maximal

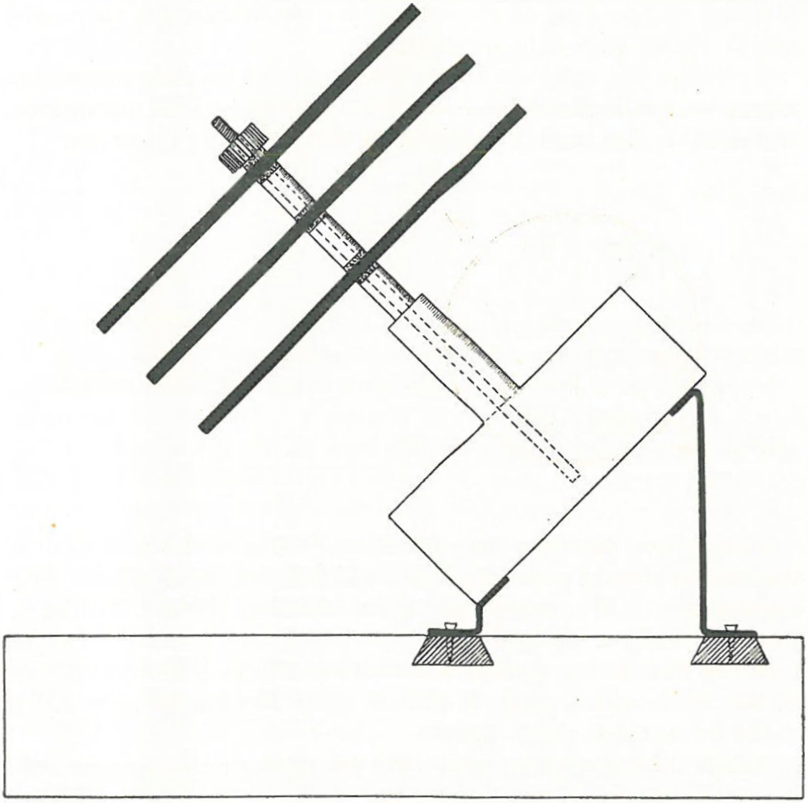


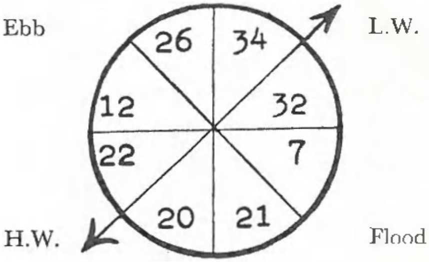
Figure 24. Time-piece apparatus. (Schematic).

orientation of the spat and a far better spatfall than may be expected if the angle is greater (e.g. 90°).

As the axis completes one revolution in 12 h. 25 m., the plates turn round once during each tidal cycle. If we place the plates on the apparatus with one particular side uppermost, we shall find the same side uppermost when we return the next day or after some days at the same stage of the tidal cycle, leaving out of account the rather slight differences between the duration of the actual tide and the average duration of the tidal cycle (i.e. 12 h. 25 m.). The entire apparatus was covered with wire netting to keep off floating sea-weed.

Though several series caught but little spat and some series miscarried on account of the water that penetrated into the box through the packing of the axis, I yet obtained some successful sets of plates with this apparatus.

I caught 174 spat on three plates placed on this apparatus at the station Kattendijke from 8–14 July 1939. The orientation of this spat has been visualized in the following diagram:



Kattendijke
8—14 July 1939
Time-piece apparatus

The arrows indicate the direction in which the majority of the umbos should point if all the spat has attached during low slack water (L.W.) or during high slack water (H.W.). Although this was only a preliminary experiment, the results seem to indicate that at the station Kattendijke setting is most frequent at low slack water, while a maximum of less importance seems to occur at high slack water.

As not all the spat attaches with the umbo pointing precisely in the direction of preference, my data are as yet insufficient to enable me to determine the precentage of spat attaching at low slack water; so it is only possible for me to indicate a maximum of setting during the last-named part of the tidal cycle.

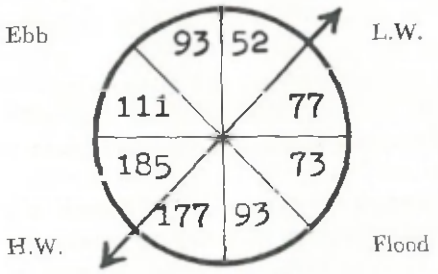
These results are in accordance with the other data concerning this station. At Kattendijke the number of larvae is several times greater in the hours roundabout low water than roundabout high water, as has been discussed in a preceding section (fig. 12). The number of full-grown larvae is likewise greatest at low water, but their number does not vary so much in the course of the tidal cycle as that of the smaller larvae, seeing that the larvae present here during high water are practically all of them in later developmental stages (fig. 12).

The currents are fairly strong at this station; during the height of the tide velocities from 100 to 150 cm/sec. may be recorded. This hinders attachment during an important part of the tidal cycle. Attachment during these stages of the tide is only possible in sheltered places. Consequently maxima of setting are to be expected here during slack water; the largest maximum during low slack water, as the number of full-grown larvae is greatest then. The results obtained with the time-piece apparatus are exactly those that were expected.

I collected other series with this apparatus the at station Yersche Bank. The results obtained indicate that the intensity of setting is not uniform during the entire tidal cycle at this station either.

Period	Direction of Umbos (8 sectors)							
	NNE	ENE	ESE	SSE	SSW	WSW	WNW	NNW
26 VII– 1 VIII 1939	11	25	20	18	34	64	29	24
16 VIII–19 VIII 1939	8	17	12	19	19	26	29	21
19 VIII–25 VIII 1939	24	24	34	51	109	81	45	40
25 VIII–28 VIII 1939	9	11	7	5	15	14	8	8
Total	52	77	73	93	177	185	111	93

The total numbers recorded in another form yield the following diagram:



Yersche Bank
Time-piece apparatus

These preliminary data indicate that at the station Yersche Bank the intensity of setting is greatest at high water. This is in accordance with the results of the exchanging-experiment carried out at this station.

What factors may be the cause of there not being a marked setting maximum at low slack water?

The number of full-grown larvae per unit of water does not vary much here in the course of the tidal cycle (fig. 11), but the total volume of water and consequently the total number of full-grown larvae is much greater at high water than at low water at this station, which may account for a difference in setting at high slack water and low slack water. Moreover in shallow places there is a tendency on the part of the current-velocity near the bottom to be insignificant for a shorter period at low water than at high water, which is connected with the height of the moving water-masses. I dare not yet decide whether these two factors are in themselves sufficient to account for the absence of a marked setting maximum at low slack water.

It is my intention to carry out further experiments with the time-piece apparatus in the next season.

XXX. VERTICAL DISTRIBUTION OF THE SPATFALL

In recent years several investigators have investigated the vertical distribution of the spatfall. The results of their investigations clearly show how injustifiable it is to base general conclusions and practical advice on accidental field observations.

Statements like that of KÄNDLER (1930): „Die Austernlarve heftet sich meist an der Unterseite der Gegenstände fest und tut dies mit Vorliebe in Nähe der Wasseroberfläche falls sie hier Ansatzkörper vorfindet", and that of ORTON (1937a): „Larvae of all kinds of oysters will therefore settle when they are ready at any depth in the water or on the bottom and although tradition and economic necessity may have imposed bottom collection, there is no reason why good spat-collections may not be made in these regions on suitable material suspended in the water at suitable depths", are not based on the results of deliberate experiments.

HOPKINS (1937), experimenting on *Ostrea lurida*, filled wire baskets with clean shells, suspended them in series one above the other and hung the entire series from a float. In all cases the samples from the surface (0–15 cm) caught least spat, possibly, in HOPKINS' opinion, because of the scouring action of the waves.

The samples from depths of 0,15 to 2,00 metres contained about equal numbers of spat. Although the bags suspended near the surface appeared to have caught somewhat more spat than those at greater depths, I do not believe that the differences are great enough to admit of reliable measurement with the methods used by HOPKINS (shell-collectors).

BONNOT (1936) stated that in Humbolt Bay there appeared to be no appreciable difference between the spatfall of *Ostrea lurida* near the surface and somewhat deeper.

Floats filled with shells or manufactured collectors (e.g. egg-case fillers) are in use on a commercial scale in the Puget Sound, not only because of the somewhat greater spatfall near the surface, but also owing to a shortage of suitable breeding-grounds for the planting of collectors. The deep channels here appeared to be unsuitable for spat-collection.

Other investigators have recorded or recommended the use of floats filled with collector-material for other kinds of oysters. Thus NEEDLER (1933) describes the use of floats in the spat-collection of *Ostrea virginica* in Malpeque Bay, which were introduced there after he had convinced himself (1932 a) that the optimum setting occurs here just below the low-water mark. NEEDLER tells us that the advantages attending the use of floats are the absence of starfish and of smothering silt and sand.

BONNOT and MAC MILLAN (1931) advised oyster-farmers to make attempts to collect the spat of *Ostrea gigas* with the aid of tarred rope suspended from floats after the example of the Japanese culture-methods practised on this kind of oyster.

SEKI and TANAKA (1931) hung tile collectors from the surface to the bottom and found that the most favourable layer for attachment of *Ostrea denselamellosa* was situated 0,6 metre above the bottom.

VILLALUZ (1939) ascertained the vertical distribution of the spatfall in Bacoor Bay (Philippines). He does not tell us which of the 18 species of oysters occurring in the Philippines was studied. He used string collectors consisting of oystershells strung on a piece of galvanized iron wire. As the shells differed somewhat in size and shape and were moreover not held at precisely the same angle of surface and as the periods of exposure were generally quite long, his methods can hardly be called quantitative. The bottommost shells caught no spat, probably because of a contact with the soft mud-bottom. The best spatfall was obtained about halfway between bottom and surface. VILLALUZ does not give any information about the local hydrographical conditions.

PRYTHERCH (1929, 1934) compared the vertical distribution of the spatfall of *Ostrea virginica* in several localities. In Great South Bay the optimum spatfall occurs near the bottom, in Beaufort (Sth. Carol.) from 3 to 5 feet above the low-water mark and in Long Island Sound from 1 foot above to 1 foot below the low-water mark. PRYTHERCH points to the great difference in hydrographical conditions in these different localities. He concludes that the heaviest spatfalls may be expected to occur at the levels where at the moment the current reaches its minimum velocity the larvae are most abundant. No other investigators mention the great influence current-velocities may have in determining the vertical distribution of the spatfall in co-operation with factors, such as the distribution of the larvae. Attachment of *Ostrea virginica* is even possible at a depth of 70 feet, but it is usually heavier in shallower water (HIGGINS 1937).

Apart from the remarks of KÄNDLER and ORTON quoted above, it is only COLE and KNIGHT JONES (1939) who inform us about the vertical distribution of the spatfall of *Ostrea edulis* (under semi-natural conditions). Their towers of curved limed tile-collectors, built up from the tank bottom (depth 6 feet) to the surface, showed no constant variation in the intensity of setting from bottom to surface, which led them to the belief that larvae settle equally readily at all depths in the tank.

In 1938 they hung out shells at three different levels and caught far more spat on the shells at the surface than on those at the other levels. They were, however, able to reconcile those two facts by considering that oysterlarvae swim vertically upwards only and that lateral movements of any importance, which are only produced by the motion of the medium, are practically absent in tanks. In consequence of this it must be difficult for the larvae to reach the piled-up tiles. Although COLE and KNIGHT JONES admit that the number of larvae reaching the collectors may be commensurate with the height of the column of water below the collectors (owing to the vertical swimming habit) and that moreover the larvae are somewhat more abundant in the upper half of the tank than in the lower half, these investigators yet assume that these phenomena are not in themselves sufficient to account for the high intensity of setting observed on the under-surfaces of shells held near the surface. They conclude that the larvae of *Ostrea edulis* probably prefer to attach near the surface and that „on the natural beds, where there is every reason for supposing that larvae will behave in the same way[1]), recognition

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[1]) Spacings are mine.

of the fact that spat set most readily near the surface opens the possibility of utilizing floating trays of cultch for the collection of spat." They even say that, as larvae are abundant on the British oysterbeds (according to Orton), heavy spatfalls might be obtained there on shells held in floating trays.

I will presently show how injustifiable it is to draw general conclusions from results obtained under semi-natural conditions.

I used the following device to investigate this matter in the Oosterschelde. A long pole (8 metres), such as are in use as sea-marks here, was fixed into the sea-bottom. Brass frames, in which the coated glass plates had been made to fit, were attached in a horizontal position to a stout lath, carrying a heavy (20 kg) foot of reinforced concrete. The plates were placed in the frames, the lath was planted on the sea-bottom right next to the sea-mark and the topmost part of the lath was attached to the sea-mark with a piece of rope. The plates were exchanged regularly.

One of these devices was planted at the Yersche Bank in the station 392 (fig. 1), where the depth is about 3 metres at low water, so that one or two of the plates became exposed during low water. The plates were fully exposed to the currents.

Periods of exposure	Distance of the plates from the bottom							
	25cm	75cm	125cm	175cm	225cm	275cm	325cm	375cm
15 VII–24 VII 1938	17	2	7	6	2	0	0	0
24 VII–30 VII 1938	30	6	4	4	0	0	0	0
30 VII– 5 VIII 1938	11	2	1	4	0	1	0	0
5 VIII–11 VIII 1938	326	76	98	54	89	57	8	1
11 VIII–23 VIII 1938	64	14	35	23	18	7	4	1
5 VII–11 VII 1939	21	6	4	1	–	0	0	0
11 VII–17 VII 1939	38	22	–	0	–	0	0	0
17 VII–23 VII 1939	26	12	–	1	0	0	–	–
23 VII–29 VII 1939	20	2	–	2	0	0	–	–
29 VII– 7 VIII 1939	39	13	23	–	0	–	–	–
7 VIII–13 VIII 1939	26	11	3	–	1	–	–	–
13 VIII–19 VIII 1939	22	13	4	–	0	0	–	–
19 VIII–25 VIII 1939	23	15	14	–	0	0	–	–
Totals	663	194	193	95	110	65	12	2

A similar apparatus was placed at the station Kattendijke, in a place where the depth is 4 metres at low water, so that the topmost plate seldom or never became exposed at low tide.

Periods of exposure	Distance of the plates from the bottom							
	25 cm	75 cm	125 cm	175 cm	225 cm	275 cm	325 cm	375 cm
5 VII–11 VII 1939	14	15	9	1	0	1	0	0
11 VII–17 VII 1939	27	23	7	9	15	5	2	0
17 VII–23 VII 1939	27	20	9	5	6	7	5	1
23 VII–29 VII 1939	10	11	8	2	4	1	2	1
29 VII– 7 VIII 1939	30	5	10	8	9	5	–	–
7 VIII–13 VIII 1939	14	10	5	5	12	6	4	0
13 VIII–19 VIII 1939	2	2	4	2	0	3	1	0
Totals	124	86	52	32	46	28	14	2

A similar device was placed at the station Bergsche Bank (fig. 1), where only the topmost plate became exposed at low water.

Periods of exposure	Distance of the plates from the bottom						
	25 cm	75 cm	125 cm	175 cm	225 cm	275 cm	325 cm
5 VII–14 VII 1939	6	13	2	5	3	0	0
14 VII–17 VII 1939	6	1	3	0	0	0	0
17 VII–23 VII 1939	4	1	1	0	0	0	0
23 VII–29 VII 1939	7	2	0	3	0	0	0
29 VII– 7 VIII 1939	12	3	2	4	1	0	0
7 VIII–16 VIII 1939	6	3	2	0	1	–	1
16 VIII–22 VIII 1939	11	8	10	1	7	–	0
Totals	52	31	20	13	12		1

I also used another kind of device at the station Yersche Bank 392, consisting of a floating buoy [1]) from which a stout lath, carrying 6 frames attached to it in a horizontal position, had been suspended. The end of the lath carried a heavy foot of reinforced concrete, weighing about 20 kg, with a view to keeping the lath in a vertical position. In this way the plates were held at a constant distance from the surface, while the other device ensured a constant distance from the bottom. With

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[1]) The buoy was anchored to prevent it from being carried away by the currents.

this floating apparatus I obtained 11 series of plates in the course of the summers of 1938 and 1939. Only one of these series appeared to have caught a moderate number of spat, viz. the series that had been exposed during the very intense spatfall in August 1938.

Periods of exposure	Distance of the plates from the surface					
	25 cm	75 cm	125 cm	175 cm	225 cm	275 cm
5 VIII–11 VIII 1938	15	17	35	32	45	63

My field observations in the Oosterschelde clearly demonstrate that the heaviest setting occurred near the bottom. Floating collectors that had been fully exposed to the currents yielded only a mere sprinkling of spat. This phenomenon cannot be ascribed to differences in the vertical distribution of the full-grown larvae, as my data convincingly show that the vertical distribution of the larvae is quite uniform during the entire tidal cycle (fig. 11). Hence it follows that the larvae do not sink to the bottom in consequence of their growing weight, as has often been supposed.

I ascribe the differences in the vertical distribution to the influence of the currents on the setting process. I have shown in a preceding section that setting is more intense when the plates are sheltered from the currents. The current-velocity is far slower near the bottom than in the other water-layers. In the Oosterschelde the greatest current-velocities are recorded near the surface. Owing to the friction against the bottom the current slackens in the bottom layers. Consequently the period favourable for attachment is far shorter in the upper layers of the water than near the bottom, as in situations remote from the bottom crawling on exposed surfaces is possible during a very short part of the tidal cycle only, owing to differences in current-velocity.

My data support the view of PRYTHERCH that a heavy spatfall is likely to occur at those levels where larvae abound at the moment that the currents are negligible.

It will be clear from the foregoing that the occurrence of intense spatfalls near the surface in tanks does not imply that the same phenomenon will be observed under field-conditions, except perhaps in bodies of water where the currents are negligible throughout the tidal cycle.

As the larvae in the Oosterschelde are distributed in the water uniformly, I think it would be possible to catch more spat than I did in places remote from the bottom by sheltering the cultch from the currents, for instance by using wire bags with shells, but this does not alter the fact that current conditions are more favourable near the bottom.

In 1938 we constructed a solid raft, attached limed tiles on it and bags of shells and anchored it above the oysterbeds. The results were disappointing. All the cultch-material appeared to be covered with a heavy layer of barnacles and the oyster-spat was very scarce. I ascribe these poor results not merely to the influence of the current-velocity, but I am inclined to believe that especially the exceptionally heavy set of barnacles must have hindered the spatfall and have overcrowded the spat.

The fact that in the Puget Sound Hopkins did not find a distribution of the spatfall similar to that in the Oosterschelde does not necessarily point to a different behaviour of the larvae of *Ostrea lurida*, but may perhaps be ascribed to different current conditions there. Moreover it should be remembered that Hopkins does not tell us how the larvae are distributed in the water of the Puget Sound. The practical absence of spatfall in the deep channels in the Puget Sound is perhaps also to be explained by the influence of the currents on the spatfall.

XXXI. THE HORIZONTAL DISTRIBUTION OF THE SPATFALL

It is a striking fact that practically every field investigator states that there are places where the spatfall may be profuse, while other places, though often quite near the former, yield only a mere sprinkling of spat.

The suitability for setting is evidently determined by an optimum combination of environmental factors. The number of mature larvae in the water is one of the most important of these factors. Several investigators neglect the influence of other factors on the horizontal distribution of the spatfall and try to correlate the observed differences with differences in the horizontal distribution of the larvae. Thus Nelson (1921) states that the intensity of the spatfall is commensurate with the number of larvae present in the places concerned. He advises that the number of larvae per unit of water should be ascertained in order to determine the suitability for attachment of the places in question. NELSON observed profuse setting in places where the currents are slow or where eddies occur. The same observation has been made by CHURCHILL and GUTSELL (1921). Neither they nor NELSON have interpreted this phenomenon as an effect of the influence of the currents on the setting process, but merely as an outcome of differences in the horizontal distribution of the larvae. The fact that there are places, which, though fairly remote from the centre of larvae-production, yet show an abundant spatfall, has been ascribed by them to a local secondary accumulation of the previously dispersed larvae. They do not make clear, however, the nature of the mechanism that may be supposed to bring about such an accumulation and they have not yet supplied any data to prove the actual occurrence of the accumulation assumed by them.

It will be clear from the foregoing that the slow currents in places where eddies occur bring about favourable setting conditions, so that setting may be profuse there, while in places near by, where the number of larvae may be the same, we shall find but a mere sprinkling of spat, as strong currents hinder the setting for the greater part of the tidal cycle there.

The number of larvae per unit of water is a factor that should not be neglected, however, and I do not agree with authors like BONNOT (1936), who assume that the number of mature larvae in the water cannot give us any indication of the number of spat that is going to settle.

In the preceding section I have already discussed PRYTHERCH's statement (1929, 1934) that the heaviest setting is likely to occur in places where the larvae are plentiful at the moment that the current reaches its minimum velocity. PRYTHERCH also states that the best spatfall occurs in the centre of the natural oysterbeds, but perhaps it would be more exact to say that the centre of natural oysterbeds is situated in those places where the combination of all the factors concerned ensures the most favourable conditions for setting and survival.

Eddies and other bodies of relatively still water also appeared to be favourable for the setting of *Ostrea edulis*. KÄNDLER (1928, 1930) and ORTON (1937 b) did not ascribe this to the influence of the current-velocity on the setting process, but they suppose that the larvae are accumulated in such places. BOURY (1929 b) tried to account for the fact that some parts of the beds are more favourable for spatfall than others, but he did not compare the number of larvae, nor did he ascertain the current conditions, so that we are not surprised at his inability to explain the difference.

Just as in other centres of spatproduction marked differences in the spatfall were found to occur at different stations in the Oosterschelde. I soon found out, however, that the occurrence of young oysters is no reliable standard for the intensity of setting, as marked differences in the local death-rate were found to occur. In some places with a heavy spatfall we find but little spat in autumn, as the conditions for survival are unfavourable there owing to smothering by silt or sand or to an abundance of enemies, such as young starfish.

In using the method of ascertaining the spatfall with the aid of coated glass plates placed in containers of reinforced concrete the influence of the death-rate is eliminated. With this device we caught the following number of spat (the plates were exposed for periods of three days):

Periods of exposure	Yersche Bank	Strijen	Bergsche Bank	Gorishoek	Kattendijke	Wemeldinge
8 VII– 3 IX 1935		357			361	
24 VI–17 VIII 1936	180	56	49	28	39	111
16 VI– 2 IX 1937	447	175	86		272	342
24 VI–29 VIII 1938	1053	383	171		357	697
23 VI–31 VIII 1939	629	351	109		385	433

There is not much difference in the number of full-grown larvae in the course of the tidal cycle at the stations Yersche Bank, Strijen, Gorishoek and Bergsche Bank.

Current conditions are, however, by far the most favourable at the station Yersche Bank, a shallow place in the centre of the basin of the Oosterschelde, where the currents are far slower than in the deeper channels near by. Strong currents prevail at the station Bergsche Bank, so that the period during which attachment is possible is very short there. Hence the poor spatfall. The station Strijen is situated on the slope of a deep channel in which swift currents prevail, but as the container is sheltered to some extent behind a small breakwater, the number of larvae caught is rather satisfactory here, though far smaller than at the station Yersche Bank. The number of mature larvae at the station Gorishoek does not differ much from that at the other stations in the basin, but as the currents are very strong here this station is nonetheless unsuitable for spat collection. Just in the lee of a dike at Gorishoek the water is relatively still and tile-collectors placed there in 1939 caught a great number of spat.

At the stations Wemeldinge and Kattendijke conditions are different. The number of larvae at these stations is far greater during low water than during high water, as has been described above. As the station Wemeldinge is not so remote from the basin as the station Kattendijke, the period during which the basinwater (containing a great number of larvae) is present is of longer duration at the station Wemeldinge than at the station Kattendijke. This may explain the difference in spatfall, current conditions being the same at these stations.

From the foregoing I conclude that the two most important factors in determining the suitability for spatfall are the number of mature larvae in the course of the tidal cycle and the current-velocities during that period. This view, which is in accordance with that of PRYTHERCH, is supported by what we know of the distribution of the spatfall in the Oosterschelde, which spatfall was observed both on practical collectors and on the plates placed in containers for periods of three days. In giving advice as to places suitable for spat-collection on a commercial scale, it is not sufficient to know the current conditions and the relative abundance of the larvae, but we also ought to know if the bottom is suitable for the planting of collectors and if smothering by silt or sand or ravages by animal enemies are not too considerable.

XXXII. PROPERTIES OF THE COLLECTOR-MATERIAL AND THEIR INFLUENCE ON THE FREQUENCY OF SETTING

The influence of the angle of surface

Oyster-growers have often noticed that most of the spat of *Ostrea virginica* and *Ostrea lurida* is to be found on the under-surfaces of collectors. NELSON (1926, 1927) compared the frequency of setting on uppersides and undersides of shells and found more than 10 times as much spat on undersides than on uppersides. His shells were exposed for short periods, so that this phenomenon cannot be explained by a decreased suitability for setting on the upper-surfaces caused by a deposition of silt or by algal growth. Nelson ascribed this difference to the influence of light on the setting process. He assumed that light stimulated the larvae to swim and that they become quiescent and proceed to set in shady situations, such as the undersides of the collectors. Nelson stated in a previous paper (1921) that his observations had shown him that the pull of gravity has practically no influence in determining the position of attachment of oysterlarvae.

Prytherch (1934 a) studied the spatfall in vitro and observed that the vertical surfaces of his containers carried far more spat than their horizontal bottom-surfaces.

The first deliberate field-experiments to investigate this matter were carried out by Hopkins (1935, 1937), who experimented with *Ostrea lurida*. He eliminated the influence of sedimentation and algal growth by exposing his glass test-plates for short periods (24 h.) He exposed his plates at different angles, which have been referred to as follows:

0°	under-horizontal
180°	upper-horizontal
90°	vertical
45°	under-surface of a 45° plate
135°	upper-surface of a 45° plate

Hopkins obtained the following results with *Ostrea lurida* (number of spat per 2400 square inch):

0°	45°	90°	135°	180°
1195	181	11	3	1

These results led to the construction of a special type of collectors of cement-coated cardboard. This collector provided a great many horizontal surfaces and was moreover less subject to silting of its partitions than egg-case fillers, another collector of cement-coated cardboard. Hopkins' special design appeared to be more than three times as effective per unit of surface than the standard egg-case fillers introduced in oyster-culture by Prytherch. In consequence of the rather rough surfaces of the cement-coated cardboard the vertical walls have a rather large horizontal component, so that the numbers of spat on the surfaces exposed at different angles are less divergent than in the case of the smooth glass plates.

Hopkins does not ascribe the enormous difference in spatfall on the surfaces held at different angles to some geotropic reaction of the larvae or to a discriminating selection of the angle of surface by the larvae, but he puts foreward a purely mechanical explanation based on the accidental contact of the foot of the larvae with the substratum as a result of their vertical swimming habit. The larvae swim with velum and foot turned upwards and therefore HOPKINS assumes that "it is most likely that the swimming larva, as it comes into contact with a surface from below, is able to hold on it with the foot, while on coming down upon a surface it is the hinge portion of the shell that touches. In this manner, as the angle of surface departs more and more from the under-horizontal, there is constantly less chance of its foot touching."

If this interpretation is correct, it is difficult to explain why surfaces held at an angle of 45° catches far less spat than under-horizontal surfaces, instead of the proportion being about 3 : 4 (sin. 45°)!

Other observations on *Ostrea lurida* have been made by COE and ALLAN (1937), who found more spat on the undersides of their collectors than on the vertical sides and by BONNOT (1937 a, 1937 b). BONNOT ascertained the spatfall on a special type of manufactured collector consisting of strips of cement-coated plywood placed above each other in a horizontal position, with interspaces of 3/4 inch. The largest spatfall occurred on the upper-surfaces. BONNOT explains this phenomenon by ascribing it to the considerable friction in the interspaces of his collectors, which causes the water to roll and swirl. The larvae are unable to maintain their normal swimming position, many of them are even presumed to be turned over, so that "larvae are carried along in all positions and it would seem logical that the slight pull of gravity would cause more of them to rest on the surface which was below them."

SCHAEFER (1937) investigated the influence of the angle of surface on the intensity of setting in *Ostrea gigas*, using the same device as HOPKINS. He counted per 2400 square inches of smooth glass:

0°	45°	90°	135°	180°
346	119	35	21	6

These results are essentially the same as those obtained by

Hopkins for *Ostrea lurida*, although for *Ostrea gigas* the proportions are less excessive. Schaefer is inclined to believe that Hopkins' explanation does not cover all the facts and that a negative geotropism is likely to play a part in the setting of these kinds of oysters. Schaefer's results do not tally, however, with those obtained by Yokota (1936) and Miyazaki (1938). Yokota compared the spatfall of *Ostrea gigas* on shells held at different positions and found that upper-surfaces catch more spat than under-surfaces. Shells are, however, less suitable to this purpose, as they do not provide plane surfaces. Yokota also observed that more spat settled on the bottom of a glass container (180°) than on its vertical walls (90°). Miyazaki exposed calcareous plates in a horizontal position at different depths in order to study the season of attachment of various sedentary organisms. He found that 4 to 8 times as many spat of *Ostrea gigas* settled on the upper-surfaces of his plates than on the under-surfaces.

Spat of *Ostrea edulis* has often been observed in the field attached to under-horizontal surfaces (Kändler, 1928, Havinga, 1929, Hagmeier, 1930, Gaarder and Bjerkan, 1934). As the objects to which this spat was attached had been in the water for a considerable time, we are not justified in concluding from these observations that *Ostrea edulis* sets more frequently on under-surfaces than on upper-surfaces, seeing that factors like algal growth, sedimentation and differences in the death-rate have not been eliminated.

Cole and Knight Jones (1939) used Hopkins' device in their tanks. They used slates instead of glass plates, as it is difficult to induce larvae of *Ostrea edulis* to set on smooth glass. Their results were:

0°	45°	90°	135°	180°
12407	6123	119	316	232

These results seem to tally with those of Hopkins and Schaefer concerning *Ostrea lurida* and *Ostrea gigas*. Vertical surfaces appeared, however, to catch less spat of *Ostrea edulis* than surfaces held at angles of 135° and 180°, which was not the case with the other kinds of oysters. Cole and Knight Jones do not believe that Hopkins' explanation, based on the vertical swimming position of the larvae, covers all the facts, for the plates held at an angle of 45° caught only about half the number of spat that was counted on under-horizontal surfaces (0°), instead of about 3/4 (sin. 45°). They are inclined to suppose that the crawling larvae must excercise a more or less discriminating selection of the angle of surface.

I have gathered many data on the influence of the angle of surface in the Oosterschelde. All the plates I used for ascertaining the intensity of setting in the course of the season of reproduction were exposed at angles of 45°, so that from the data thus obtained I can also study the difference in spatfall between surfaces at 135° and 45°. Moreover I constructed a special container in which 3 plates could be held in a vertical position and 3 in a horizontal position (fig. 18). This device was used in the seasons of 1938 and 1939. In 1939 I used moreover a container in which 12 plates could be exposed at different angles, so that the entire set was arranged in the shape of a fan. I adopted the same notation as has been used by the other authors, i.e. the notation by which the upper-surfaces of a plate inclined at an angle of 45° are considered as being at an angle of 135° with the horizontal and other surfaces accordingly.

The plates used for ascertaining the intensity of setting yielded the following data:

Seasons	Yersche Bank		Kattendijke		Wemeldinge		Strijen		Bergsche Bank	
	45°	135°	45°	135°	45°	135°	45°	135°	45°	135°
1935			147	541			233	413		
1936	120	343	30	82	125	220	65	101	43	104
1937	260	1181	173	551	342	650	188	329	88	166
1938	554	2607	324	747	748	1338	475	682	264	249
1939	231	1656	288	866	393	886	306	757	107	220
Totals	1165	5787	962	2787	1608	3094	1267	2282	502	739

In 1938 the coated plates from the container with horizontal and vertical plates yielded the following figures:

Periods of exposure	Horizontal plates						Vertical plates					
	topm. pl.		2nd. pl.		3rd pl.							
	0°	180°	0°	180°	0°	180°	90°	90°	90°	90°	90°	90°
3 VII-12 VII	10	69	15	30	25	40	5	2	6	7	5	7
12 VII-21 VII	12	53	7	23	7	36	3	2	4	8	8	18
2 VIII- 8 VIII	45	224	–	–	37	70	18	9	7	16	27	14
8 VIII-14 VIII	136	1194	–	–	71	1031	89	59	96	99	124	78
Totals	203	1540			140	1171	115	72	113	130	164	117

Averages:

0°	90°	180°
171	118	1358

In 1939 the same device yielded:

Periods of exposure	Horizontal plates						Vertical plates					
	topm. pl.		2nd. pl.		3rd pl.							
	0°	180°	0°	180°	0°	180°	90°	90°	90°	90°	90°	90°
2 VII- 8 VII	23	75	15	35	19	30	3	10	9	4	13	9
8 VII-14 VII	33	127	31	40	36	28	4	13	10	14	13	12
14 VII-20 VII	33	136	43	139	38	109	9	15	16	12	15	16
20 VII-26 VII	18	113	16	36	39	48	4	8	14	17	9	21
26 VII- 4 VIII	27	75	29	76	39	103	12	9	15	8	27	11
4 VIII-10 VIII	38	82	35	23	19	17	20	11	5	11	7	5
10 VIII-16 VIII	23	48	21	29	43	64	17	8	8	10	10	6
16 VIII-22 VIII	34	104	27	98	42	105	34	20	41	17	44	26
Totals	229	760	217	476	275	504	103	94	118	93	138	106

Averages:

0°	90°	180°
240	109	580

The fan-shaped set of plates caught in 1939:

Periods of exposure	0°		22½°		45°		67½°		90°				112½°		135°		157½°		180°	
2 VII – 8 VII	20	10	22	13	13	7	2	5	6	1	10	4	23	34	54	61	34	38	24	35
8 VII – 14 VII	12	10	16	16	18	23	9	5	7	4	7	0	37	21	20	45	59	41	27	58
14 VII – 20 VII	35	13	32	27	17	11	10	4	16	12	13	6	56	25	106	48	130	70	29	57
20 VII – 26 VII	27	27	26	22	22	22	10	7	13	7	10	8	39	26	46	60	55	32	27	25
26 VII – 4 VIII	5	14	28	37	15	21	9	14	8	11	1	2	50	31	76	104	77	87	26	15
4 VIII – 10 VIII	15	3	23	17	5	6	8	6	4	3	7	5	28	18	48	15	41	30	7	10
10 VIII – 16 VIII	11	6	17	22	9	14	6	4	5	5	11	6	29	25	19	35	25	38	9	12
16 VIII – 22 VIII	26	34	20	34	17	11	4	6	11	4	12	4	26	42	72	21	52	72	31	12
Totals	151	117	184	188	116	115	58	51	70	47	71	35	288	222	441	389	473	408	180	224

Averages:

0°	22½°	45°	67½°	90°	112½°	135°	157½°	180°
134	186	115	55	56	250	415	441	202

During the same periods that the container with horizontal and vertical plates was used, the ordinary container at the same station caught per plate:

	135°	45°
1938	633	140
1939	478	67

The following are the data obtained at the station Yersche Bank, combined in one table:

	0°	22½°	45°	67½°	90°	112½°	135°	157½°	180°
Ordinary plates (1936–1939) .			1165				5787		
H.V. plates + Ord. plates 1938	171		140		118		633		1358
H.V. plates + Ord. plates 1939	240		67		109		478		580
Fan-container 1939	134	186	115	55	56	250	415	441	202
Special horizontal series 1939.	771								2370

I conclude from these data that under field-conditions in the Oosterschelde upper-surfaces collect more spat than under-surfaces and that vertical surfaces are least suitable for attachment.

These data differ greatly from those obtained by HOPKINS, who experimented likewise under field-conditions, albeit with *Ostrea lurida*. As the larvae of *Ostrea edulis* show the same swimming position as those of other kinds of oyster, viz. with the velum upwards, HOPKINS' interpretation, based on the chance of the foot touching an object, cannot be correct. The great difference between the number of spat on surfaces held at 0° and at 45° in his experiments points in the same direction. It is not easy to give a plausible explanation of this difference in setting of these closely related species.

COLE and KNIGHT JONES obtained results which are quite different from mine, but then they carried out their experiments in tanks (so under semi-natural conditions), where horizontal currents are practically absent, so that the larvae can practically move in a vertical direction only, while under field-conditions horizontal movements are far more considerable than vertical. In consequence of the absence of horizontal currents in tanks it is very difficult for the larvae to reach the uppersides of objects, especially when these objects are exposed near the surface. If the conclusion from my experiments, that the larvae of *Ostrea edulis* prefer to attach on upper surfaces, is correct, it remains possible that in tanks under-surfaces collect far more spat than upper-surfaces, owing to the limited possibilities of movement in them. It is my belief that the fact that vertical surfaces caught less spat than upper-surfaces in COLE's experiments indicates that his results are in better accordance with mine than with those of HOPKINS.

The preference of *Ostrea edulis* for certain angles of surface is by no means so pronounced as in *Ostrea lurida*, although I was able to prove in the section on the orientation of the spat that the full-grown larvae of *Ostrea edulis* are extremely sensitive to the pull of gravitation.

The difference in spatfall on upper- and under-horizontal surfaces varied somewhat. On an average upper-surfaces of horizontal plates caught from 2 to 3 times as much spat as under-surfaces (under field-conditions). Upper-horizontal surfaces often caught more spat than surfaces exposed at an angle of 135°.

It is my belief that in the container in which the plates were held in a fan-shaped arrangement other factors must have interfered. The plates in this container stood close together and although the side-walls were amply provided with oblong holes (c.f. fig. 19), it remains possible that current conditions were not quite the same for the various plates. Consequently I do not think that we are justified in deducing from the data obtained with this device that surfaces exposed at an angle of 157½° are more suitable than those held at an angle of 180°.

Although current-velocities are somewhat greater in the Oosterschelde than in the Puget Sound, it is impossible to ascribe the differences between Hopkins' data and mine to this. For also in those cases where the plates were exposed in such a way that current conditions were exactly the same for upper-surfaces and under-surfaces (I am referring to the plates used for ascertaining the vertical distribution of the spatfall) I found more spat on upper than on under-surfaces (1190 : 479).

From my first table in this section it may be seen that the proportion between the intensity of setting on surfaces held at an angle of 135° and of 45° was about 5 : 1 at the station Yersche Bank, 3 : 1 at Kattendijke, 2 : 1 at the station Wemeldinge and less than 2 : 1 at the stations Strijen and Bergsche Bank.

The strongest currents occur at the stations Bergsche Bank and Strijen, while the currents are relatively slow at the station Yersche Bank. It is possible that the correlation between the current-velocity and the proportion of spat on upper- and under-surfaces of plates held at an angle of 45° is an indication that under-surfaces are somewhat better protected from the currents than upper-surfaces, though we tried to eliminate this factor by piercing the side-walls of the containers (fig. 16).

It may also be seen from one of my tables in this section that the upper-surface of the topmost of a set of three horizontal plates caught but slightly more spat than the upper-surfaces of the other two plates, although the upper-surface of the topmost plate was more easily accessible for the larvae than were the other two. It should be borne in mind, however, that the active vertical movements of the larvae are far slower than the passive horizontal, so that under field-conditions the chance of their reaching the second and the third plate is not so much smaller than the chance of their reaching the topmost plate.

Summarizing, I can state that my experiments demonstrate that the larvae of *Ostrea edulis* do not show the same behaviour with regard to the angle of surface at which the collectors are exposed as those of *Ostrea lurida*. Upper-surfaces catch more spat than under-surfaces and vertical surfaces collect least spat. HOPKINS' interpretation of the behaviour of *Ostrea lurida* cannot be correct. The difference between my data and those obtained by COLE and KNIGHT JONES may be ascribed to the limited possibilities for horizontal movements in the still water of the tanks.

I am inclined to ascribe the difference in spatfall on surfaces held at various angles to a discriminating selection on the part of the larvae and I reject a purely mechanical explanation. This selection may be interpreted as a result of the efforts of the mature larvae to attain their ecological norm.

COLE says that the larvae of *Ostrea edulis* set most frequently near the upper free edges of the undersides of slates held at an angle of 45°, which, according to him, points to a tendency to crawl upwards against gravity on inclined surfaces. I have never yet observed this phenomenon in the Oosterschelde.

The influence of the colour of the substratum

FOLPMERS (1924) presumed that oysterlarvae prefer a dark substratum for attachment: „Het zwemmende oesterbroed zoekt toch bij voorkeur het donker op en is afkeerig van veel licht." He compared the spatfall on black and white tile-collectors, but he counted only 3 to 4 spat per tile, which numbers are far too small to base conclusions on.

Another experiment with coloured tile-collectors has been carried out by THIEBLEMONT (HERMAN 1937). He counted the number of spat on his tiles at the end of the season of reproduction and found on an average 7,7 spat on white tiles, 6,8 on blue tiles, 3,7 on green tiles, 3,6 on red tiles, 3,1 on violet tiles, 2,2 on black tiles and 1,7 on yellow tiles. He did not eliminate differences in the death-rate, for he only counted the spat that had survived till in autumn, when already about 9 out of 10 of the spat that originally attached will have disappeared.

ORTON (1937 a) assumes that oysterlarvae prefer to set on dark surfaces and recommends the use of dark-coloured cultch. ORTON does not tell us how he came to the conclusion that dark-coloured surfaces catch more spat than white; he only mentions a small-scale application of black-varnished shells.

To investigate this matter I used glass plates frosted on both sides and coated with a coloured paste. Twelve of these plates were placed at an angle of 45° in oblong containers of reinforced concrete (fig. 19). I eliminated differences in the death-rate by applying the same methods as were used with the plates in the experiments described above. The places of the coloured plates in the container were regularly interchanged.

The coating of the plates was composed of dye, cement, lime, sand and water; the proportions of the components were modified emperically and we compared the tints obtained after the dry plates had been immersed in seawater. Several plates appeared to show a less intensive colour after immersion in seawater, some dyes even changed their colour. At last we succeeded in finding suitable compositions of the coatings, so that finally we obtained plates which showed bright colours in seawater.

We tried to avoid dyes with poisonous components, such as lead and mercury.

The coating of the white plates contained lime and fine white sand, but no cement. The yellow paste contained yellow ochre, cement and sand. All the other coatings contained sand, cement, lime and dye. I used ultramarine (blue), Swedish black, an unidentified green dye of the group of the crystal-greens and a red analine-dye of which the colouring component is "litholechtscharlach".

I obtained the following results 1) (station Yersche Bank):

Periods of exposure	White			Red			Yellow		
	upper-side	under-side	total	upper-side	under-side	total	upper-side	under-side	total
3 VII-12 VII 1938	35	20	55	36	15	51	24	16	40
15 VII-21 VII 1938	12	6	18	9	7	16	14	10	24
21 VII-27 VII 1938	37	21	58	26	21	47	22	15	37
27 VII- 2 VIII 1938	4	4	8	10	5	15	8	5	13
2 VIII- 8 VIII 1938	59	33	92	75	33	108	65	32	97
8 VIII-14 VIII 1938	362	120	482	364	108	472	253	113	366
Totals	509	204	**713**	520	189	**709**	386	191	**577**

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1) For each colour I divided the total number of spat caught by the number of plates used.

Periods of exposure	Green			Blue			Black		
	upper-side	under-side	total	upper-side	under-side	total	upper-side	under-side	total
3 VII-12 VII 1938	16	7	23	40	15	55	34	21	55
15 VII-21 VII 1938	6	3	9	13	7	20	10	10	20
21 VII-27 VII 1938	17	8	25[1]	23	16	39	17	13	30
27 VII- 2 VIII 1938	6	2	8[1]	9	1	10	9	5	14
2 VIII- 8 VIII 1938	47	10	57	58	29	87	54	27	81
8 VIII-14 VIII 1938	231	54	285	353	117	470	288	96	384
Totals	323	84	**407**	496	185	**681**	412	172	**584**

The differences between the number of spat caught under field-conditions on substrata of different colours are not great. Only the number of spat on the green plates is considerably smaller than that on the plates of other colours. I am sure, however, that it is not the colour green which causes this difference, but the chemical nature of the dye. I counted the spat 6 to 9 days after attachment, just as I did the spat of other series. The spat then measured 0,6 to 0,8 mm, but the spat on the green plates showed little or no growth after attachment; it measured 0,37 mm on an average. During the periods from 21 VII to 27 VII 1938 and 27 VII to 2 VIII 1938 I used green plates that had been used once before. These plates caught about the same number of spat as the plates of the other colours and the size of the spat appeared to be about the normal. Probably the poisonous character of the dye had decreased after having been immersed for 12 days. The green dyes of the group of the crystalgreens are known to have a bacteriostatical effect, so that we were not surprised to find that it affected other living beings.

In 1939 I placed black and white plates in an container in a horizontal position. Two series of three plates in vertical order: white-black-white and black-white-black. The results are stated in the table on page 227 (station Yersche Bank).

Just as in 1938 the white plates appeared to have caught somewhat more spat than the black ones, but the difference is not great, perhaps even negligible. It is not impossible that this difference was caused by the far heavier setting of barnacles on the black plates. I also observed in 1938 that black plates

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[1] The green plates of these series had been used once before.

Periods of exposure	Black plates									
	topmost			intermediate			bottommost			averages
	upp.	und.	tot.	upp.	und.	tot.	upp.	und.	tot.	
2 VII- 8 VII 1939	43	12	55	50	15	65	57	17	74	65
8 VII-14 VII 1939	65	17	82	41	24	65	45	14	59	69
14 VII-20 VII 1939	105	33	138	94	22	116	71	44	115	123
20 VII-26 VII 1939	10	6	16	9	7	16	11	6	17	16
26 VII- 4 VIII 1939	70	15	85	42	25	67	67	20	87	80
14 VIII-10 VIII 1939	21	18	39	23	9	32	19	29	48	40
10 VIII-16 VIII 1939	30	17	47	25	20	45	30	17	47	46
19 VIII-22 VIII 1939	27	16	43	32	10	42	35	5	40	41
Totals	371	134	**505**	316	132	**448**	335	152	**487**	**480**

Periods of exposure	White plates									
	topmost			intermediate			bottommost			averages
	upp.	und.	tot.	upp.	und.	tot.	upp.	und.	tot.	
2 VII- 8 VII 1939	50	12	62	49	11	60	52	12	64	62
8 VII-14 VII 1939	140	25	165	72	14	86	70	18	88	113
14 VII-20 VII 1939	105	15	120	142	19	161	119	31	150	144
20 VII-26 VII 1939	7	4	11	10	4	14	10	4	14	13
26 VII- 4 VIII 1939	99	18	117	88	20	108	70	19	89	105
4 VIII-10 VIII 1939	50	22	72	10	20	30	30	17	47	50
10 VIII-16 VIII 1939	46	22	68	27	5	32	29	15	44	48
19 VIII-22 VIII 1939	35	9	44	30	6	36	8	11	19	33
Totals	532	127	**659**	428	99	**527**	388	127	**515**	**568**

caught a heavier set of barnacles than white plates. This is in accordance with ZOBELL's statement (1938) that red-brown and black substrata catch more barnacles than white, yellow and blue substrata.

Summarizing I conclude that under field-conditions little or no influence of the colour of the substratum on the intensity of setting could be shown. There is no reason to recommend the application of collectors of another colour than those commonly used, as for instance, white tiles ¹).

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¹) Tile-collectors are coated with a mixture of lime and sand. This is not done to obtain a white colour, but to make it possible to detach the spat after some time ("detroquage").

It should be remembered that the water in the Oosterschelde is rather turbid and that the column of water above my container varied from 2 to 6 metres, so that it is not impossible that the influence of the colour of the substratum on the intensity of setting is greater when the plates are illuminated more intensely. Larvae of barnacles are in any case more sensitive to colour than oysterlarvae.

The influence of cleanness and roughness on the intensity of setting

Successful attachment is only possible when the surface of the substratum is clean enough to fix the cement from the byssus-gland on it. Many authors state that clean surfaces are undoubtedly the most suitable for settlement (e.g. PETERSEN 1908, MAZZARELLI 1922, KÄNDLER 1930, GALTSOFF, PRYTHERCH and MAC MILLAN 1930, HINARD 1932, NEEDLER 1932 a, ORTON 1937 a, HOPKINS 1937).

HOPKINS (1937) tested roughly the decrease in efficiency of cultch after it had been in water for some time. Shells exposed in wire bags appeared to have lost a good deal of their efficiency as spat-collectors in 9 days. Local conditions have much influence on the rate at which the suitability of cultch-material decreases. Algal growth shows great differences at various depths and the deposition of silt is not the same everywhere. The plates of my "ordinary series", with which I ascertained the intensity of the spatfall, remained in the water for 3 days and the plates of my special series for 6 days. In the containers with which I tested the influence of the roughness of the surface on the intensity of setting I placed i.a. plates with a "normal" coating at an angle of 45° (the "normal" angle) for 6 days at the station Yersche Bank.

Periods of exposure	2 × 3 days	1 × 6 days
3 VII–12 VII 1938	48	64
15 VII–21 VII 1938	29	28
21 VII–27 VII 1938	67	41
27 VII– 2 VIII 1938	30	24
2 VIII– 8 VIII 1938	123	85
8 VIII–14 VIII 1938	610	523

A comparison of the number of spat attached to these plates with the number on the two ordinary 3-days series from the same station will show whether or not the plates have lost much of their efficiency as spat-collectors after three days (see page 228).

We may conclude from these data that, although a slight decrease in efficiency of the spat-collectors can be observed after 3 days of immersion, the plates maintain their suitability during a period of at least 6 days. After about 10 days the suitability decreases rapidly, while a growth of organisms, for instance of algae, may then be observed with the naked eye. I stated above that in the year 1938 the potential spatfall in August was enormous, but that the partical spatfall was a failure owing to the fact that the tile-collectors had been immersed many days too early.

The roughness of the substratum often appeared to influence the intensity of setting. Horst (1884) exposed in the Oosterschelde plates of smooth glass, frosted glass and limed glass. The limed glass appeared to catch far more spat than the smooth glass and the frosted glass. Lees (1930) in his attempts to invent new kinds of cultch-material found that the larvae of *Ostrea edulis* attach intensively on limed glass. Hinard (1932) stated that the larvae prefer to attach on substrata which contain lime: "Elles semblent avoir une prédilection pour les supports calcaires"; but Voisin (1933) tells us that lime is not indispensible and that the cultch-material ought to be clean and firm in the first place. Cole (1938 a) and Cole and Knight Jones (1939) tell us that it is difficult to induce larvae of *Ostrea edulis* to set on smooth glass. They caught far more spat on slates (tank-observation).

Similar observations have been made on other kinds of oysters.

Ostrea virginica

Nelson (1930) compared the setting on smooth glass, ground glass and objects (glass and shells) coated with a mixture of lime and cement. The limed objects caught far more spat than the others. Prytherch (1934 a) observed that the cement coating of the partition-collectors, which is very rough, is ideal for attachment of the larvae of *Ostrea virginica*. Smooth glass slides and china placed near and inside these collectors gathered far less spat per unit of surface than the partition-collectors. Ground glass was nearly as effective as the cement surface of the partition-collectors.

Ostrea gigas

Hori (1936) observed that the spatfall on frosted glass was poor, although mature larvae of *Ostrea gigas* were abundant at that time. Yokota (1936) states that it is impossible for the larvae of this kind of oyster to attach on objects coated with paraffin.

Ostrea lurida

Coe and Allan (1937) caught far more spat of *Ostrea lurida* on wood and cement than on smooth glass. The spatfall was more intense on glass that had been used once before. They presume that it is perhaps organic matter in decomposition that brings about this difference. In connection with this I mention Zobell (1938), who tells us that several sessile organisms attach more intensely on objects of which the surface is covered with a film of bacteria than on sterile surfaces.

To investigate this matter I placed in a long container plates with different surfaces: smooth glass, ground glass and glass with a coating of lime, cement and sand. I used fine sand (the same as I used for my ordinary plates) as well as sand of fairly coarse and of very coarse grain. In a dry state the coating with fine sand is very smooth to the naked eye, while the other

Periods of exposure	Smooth glass	Ground glass	Smooth coating	Rough coating	Very rough coating
3 VII–12 VII 1938	1	2	64	54	60
15 VII–21 VII 1938	0	0	21	20	17
21 VII–27 VII 1938	0	1	42	38	26
27 VII– 2 VIII 1938	0	3	24	30	35
2 VIII– 8 VIII 1938	3	13	85	90	95
8 VIII–14 VIII 1938	18	101	523	585	690
Totals	22	120	759	817	923

coatings are respectively rather and very rough. I placed two or three plates of the same kind in the container and regularly interchanged the places of the different materials. The results obtained are stated in the table on page 230 (station Yersche Bank).

The coated plates appeared to catch far more spat than the plates of smooth glass and frosted glass. Smooth glass is very unsuitable for attachment of *Ostrea edulis*. The numbers of spat caught on the coated plates of different roughness did not diverge very much. Consequently macroscopical roughness is probably not essential to attachment. These small differences may probably be ascribed to the enlargement of the surface owing to roughness.

Although the coating of the ordinary plates looks smoother than ground glass, the former proved to be more suitable as spat-collector. In fact the surface of the coating is much rougher than that of ground glass, as the microscope will show. I dare not yet decide whether the chemical composition of the substratum may not play some part, but it is not impossible that it is merely the microscopical roughness which brings about the difference in the intensity of setting discussed above. The larvae seem to require or at least to prefer microscopical roughness. Perhaps it is not only the suitability for fixation that plays a part, but also the suitability for crawling, for it is possible that the larvae are more easily washed off from one kind of substratum than from another.

SUMMARY

1. A crisis in oyster-culture in the years following 1930, caused by an extremely rapid propagation of the slipper-limpet and an aggravation of shell-disease compelled Dutch oyster-farmers to abandon spat-collection on sown-out shells and to revert to tile-collectors. They were assisted by the Government in various ways, one of which was the prediction of the time of setting, by which the chances of a good spatfall on the tiles are increased.

2. The water in the basin of the Oosterschelde performs an almost perfectly oscillating movement, which has a favourable influence on the water-temperature and at the same time guarantees a satisfactory retention of the larvae.

3. The Oosterschelde has a rather high and constant salinity.

The fluctuations in salinity are correlative with fluctuations in the discharge of the big rivers. Local rainfall has no perceptible influence on the salinity of the Oosterschelde.

4. Owing to the great tidal range the water in the basin is regularly mixed very thoroughly, which prevents stratification.

5. At high water the basin contains 675.000.000 m^3, at low water 275.000.000 m^3. At each tide about 3,7 % of the water of the basin (25.000.000 m^3) is replaced by water from the districts situated farther west.

6. At several stations the current-velocity in the surface layers attains 100 to 150 cm/sec. at the height of the tide; about 50 cm/sec. in shallow places. Near the bottom the velocity is about 1/3 less.

7. Practically every summer the water-temperature rises above 18° C. for a considerable period of time. Temperatures above 22° C. occur only sporadically. Owing to the thorough mixing of the basinwater the daily fluctuations in water-temperature are small.

8. The Dutch methods for the numerical determination of oysterlarvae have been modelled on the French methods, but important modifications have been introduced, so that the Dutch plankton-samples are undoubtedly better quantitative than the French.

9. In the course of the summer season plankton-samples are procured daily, under comparable circumstances, from 100 litres of water at two stations in the Oosterschelde. The one station is situated in the centre of larvae-production (Yersche Bank), the other in an important tile-centre (Kattendijke). All the larvae in the samples are counted and measured.

10. In consequence of the frequent sex-change the spawning of *Ostrea edulis* is spread over a greater number of days than that of non-incubatory oysters.

11. As our knowledge of the influence of temperature and nutrition on sex-change is as yet insufficient, we are unable to predict the way in which egg-production will be distributed over the season. In practice it is of less importance to know the moment of the beginning of spawning than the date at which spawning reaches its height.

12. Spawning in August may probably be largely ascribed to oysters that pass through the female stage a second time.

13. As egg-maturation requires a certain amount of warmth,

it is not possible to fix a clearly defined limit below which spawning does not take place.

14. The intensity of spawning decreases as the season advances. It is not possible to indicate the exact limit at which in autumn spawning ceases altogether. Some oysters bearing larvae may still be found long after the temperature has fallen below 18° C.

15. Although there are indications that spawning attains its greatest maxima at the spring-tides, we are not justified in saying that spawning preferably shows its greatest maxima at full moon.

16. There cannot be a strict parallelism between the periodicity of spawning and the actual water-temperature, as *Ostrea edulis*, owing to frequent sex-change, does not show a simultaneous occurrence of ripe eggs in a great part of the stock.

17. Although the water-temperature influences the duration of incubation, we cannot expect the periodicity in swarming to run strictly parallel to the course of the actual water-temperature, as the intensity of swarming is entirely dependent on the intensity of the spawning preceding it.

18. The larvae are liberated after having been sufficiently incubated. The mother oysters do not await favourable weather-conditions for this. Unfortunately a considerable part of the larvae will therefore be found to swarm under unfavourable circumstances.

19. In the centre of larvae-production liberation causes sharp increases in the number of larvae. These increases are mostly followed by sharp drops, which, however, are not a sign of a great mortality of the larvae, but are a consequence of the dispersion of the larvae through a greater volume of water.

20. The annual extent of larvae-production is determined by the number of mature oysters on the banks, the ages of these oysters, the age at which the oyster reaches sexual maturity in the district concerned, the percentage of oysters participating in female reproduction every year and the number of larvae produced by one oyster.

21. Female reproduction in the second summer may occur in the Oosterschelde, but is of little practical importance.

22. In favourable summers probably about 100 % of the population takes part in female reproduction. Many oysters probably produce larvae twice a year.

23. Larvae-production in the Oosterschelde steadily increased

from 1936 till 1939 owing to an increase in the number of adult oysters.

24. Oysterlarvae at swarming measure 0,165 to 0,200 mm, the majority 0,175 to 0,185 mm. Temperature is not all-powerfull in regulating the size of the swarming larvae. The size of the newly-liberated larvae tends to decrease towards the end of the season. Full-grown larvae measure 0,260 to 0,300 mm, in most cases 0,275 to 0,285 mm.

25. The vertical distribution of the oysterlarvae in the Oosterschelde is essentially the same in daylight and at darkness.

26. In the Oosterschelde temperature and salinity have no influence on the vertical distribution of the larvae, as strong tidal currents prevent all stratification of the water here.

27. The vertical distribution of the oysterlarvae in the Oosterschelde is essentially the same in stormy weather with rough sea and in calm weather with smooth water.

28. Although the current-velocities at many stations exceed 50 cm/sec during a great part of the tidal cycle, it must not be thought that at these velocities the larvae drop to the bottom.

29. During strong currents the larvae of the water-layers near the bottom are not passively swept to the surface layers.

30. As the oysterlarvae do not drop to the bottom at any stage of the tide or of a space of 24 hours, they are quite at the mercy of the tidal streams. The greater the distance between a given station and the basin, the shorter the period during which the basinwater and with it a large number of larvae will be present at that station.

31. Owing to the proximity of liberating adults, the course of the number of larvae is less even in the centre of larvae-production than at other stations. The number of older larvae, however, does show an even course here.

32. Water-temperature has a very great influence on the duration of the pelagic stage. The differences in salinity are too small here to influence the duration of the pelagic stage. Any protraction of the pelagic stage decreases the percentage of the larvae that reach the full-grown stage.

33. As regards the Oosterschelde only about one third of the losses during the pelagic stage is attributable to the tidal water-renewal.

34. Wholesale dropping to the bottom of full-grown larvae is out of question.

35. The spat prefers to attach with the umbo pointing in a special direction. The pull of gravity is the orienting factor in this.

36. At several stations here the intensity of the potential spatfall is measured quantitatively in the course of the season of reproduction.

37. It is possible to demonstrate a distinct correlation between the number of mature larvae and the intensity of spatfall. The proportion between the number of full-grown larvae and the intensity of spatfall is different for each station, but constant for one station.

38. Less than 10 % of the larvae reach maturity; about 1 à 2 % of the mature larvae succeed in attaching; 90 % of the newly-set spat already dies before winter. About 250 out of every 1.000.000 produced larvae attach.

39. The oysterfarmer by selecting the right place and time for the planting of his collectors should try to raise the percentage of mature larvae accomplishing fixation to the highest possible degree.

40. Environmental conditions, such as temperature, salinity and copper-content have little direct influence on the process of fixation in the Oosterschelde.

41. In considering the correlation between swarming and setting it should be known what has become of the pelagic larvae.

42. Prediction of time and intensity of setting at short notice has proved to be very well possible here and is practised with success.

43. It has been shown that light has no perceptible influence on the fixation process under field-conditions. It is, however, possible that oysterlarvae may show a predilection for shaded situations in case the intensity of light at fixation happens to be great.

44. The current may have a favourable influence on the spatfall by providing a regular supply of larvae, but also an unfavourable influence, by washing off the crawling larvae from the substratum. Places much exposed to currents are consequently less suitable for fixation.

45. Most of the spat settles roundabout still water in consequence of the influence of the current on the setting process. The number of larvae in the course of the tide determines whether at a given station the intensity of setting will be greatest at high water or at low water.

46. In the Oosterschelde more spat settles near the bottom than near the surface. This is not attributable to differences in the vertical distribution of the full-grown larvae, but to differences in current-velocity.

47. The suitability of a place for spatfall is mainly determined by the number of mature larvae present in the course of the tidal cycle and by the course of the current-velocity. Other factors determine what percentage of the settled spat shall survive.

48. Under natural conditions more spat settles on upper-surfaces than under-surfaces in the Oosterschelde, vertical surfaces being the least suitable for attachment.

49. Under field-conditions the colour of the substratum has little influence on the intensity of setting.

50. The degree of roughness of the substratum has a great influence on the intensity of setting. Microscopical roughness is much more important than macroscopical roughness.

Practical applications

A. In consequence of its special hydrographical conditions the Oosterschelde is no doubt very suitable for the production of oyster-spat.

B. Owing to differences in watertemperature and in the course of the production of larvae the setting-maxima do not occur in the same part of the season every year.

C. By far the best results are obtained with collectors when they are placed just at the moment that a large setting maximum is to be expected.

D. A great setting-maximum may be expected when a considerable production of larvae occurs at a high water-temperature. At water-temperatures below 18° C. no spatfall of any importance is to be expected, even though the supply of larvae should be considerable.

E. Prediction of the spatfall at long notice is not possible in Holland, owing to the fitful weatherconditions and the intricacy of the problem of the periodicity in larvae-production.

F. Prediction of the spatfall at short notice is very well possible. Besides registration of the water-temperature this requires frequent determinations of the number and size of the oysterlarvae per volume of water. Care should be taken that the plankton-samples are quite comparable.

G. The best spatfall may be expected in places where the number of full-grown larvae is large at the moment that the current-velocities are small. These factors are largely governed by the tidal movements.

H. Collectors can be placed close together in large numbers without any objection, if only care is taken that the masses are not too compact, as then they would not be sufficiently accessible for the larvae-bearing water.

I. Near the bottom the current is less unfavourable for fixation than near the surface. Consequently there is no reason to recommend spat-collection by means of floating collectors. The number of mature larvae is about the same at any depth.

J. The results of the inquiries into the influence of the angle of surface on the intensity of setting have shown that there is no reason for the construction of special types of collectors. Upper-surfaces catch more spat than other surfaces, but at the same time the former catch more silt, so that more spat is smothered on them. Cemented cardboard collectors cannot be used here, as wave-action and currents would soon smash them to pieces.

K. There is no reason why collectors should be protected against light, as in practice there is little evidence of a difference in setting in light and in dark places.

L. There is no reason why another colour than the usual should be used for collectors.

M. The roughness of surface of the collectors commonly used here is highly conducive to fixation.

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