perimental evidence, the whole question of the factors influencing the behavior of oyster larvae at the time of setting needs to be examined.

TO THE ANGLE OF SURFACE

French oyster growers take advantage of the preference of oyster larvae for the under surfaces of submerged objects and use special spat collectors made of tiers of tiles set one upon the other with their concave surfaces underneath. New spat is always found in larger numbers on the lower surfaces. According to Cole and Knight-Jones (1939) the larvae of O. edulis reared in large tanks in Conway, Wales, set more intensely on the under surfaces of test shells. A study of the effect of the angle of a flat surface on the attachment of larva was made by Hopkins (1935) in his work on O. lurida of the Pacific Coast. He used glass plates, each 2,400 sq. in., placed at different angles over the oyster grounds. The under horizontal surface was designated as 0° and the upper horizontal as 180°. Other plates were set at 45° intervals between the two extremes. The average number of larvae attached to each surface were:

<table>
<thead>
<tr>
<th>Angle</th>
<th>Number of Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0° (under horizontal)</td>
<td>1,195</td>
</tr>
<tr>
<td>45°</td>
<td>181</td>
</tr>
<tr>
<td>90°</td>
<td>11</td>
</tr>
<tr>
<td>135°</td>
<td>3</td>
</tr>
<tr>
<td>180° (upper horizontal)</td>
<td>1</td>
</tr>
</tbody>
</table>

Similar observations were made by Schaefer (1937) with the larvae of C. gigas. He set 150 glass plates in positions varying by 45°; some of the plates were parallel to the direction of the tidal current while others were transverse to it. The plates were left for 5 days before the spat were counted. There is a functional relationship between the intensity of setting and the angle of the surface on which the larvae set, the number of larvae being greatest on the under horizontal surface (0° angle) and lowest as the angle approaches 180°. The curve shown in figure 354 is drawn between the points taken as the average numbers of larvae attaching during 5 days on a glass surface held at different angles. The curve is hyperbolic. Schaefer attributes the setting behavior of C. gigas to the upward position of the foot of the swimming larvae and possibly to negative geotaxis. No experimental evidence is given to substantiate either point.

The behavior of oyster larvae does not differ from that of many other fouling organisms which were found by Pomerat and Reiner (1942) to attach in greatest abundance to the under surfaces of plates held in a horizontal position.

Contradictory results were obtained, however, in experiments with cement covered boards held at several angles either suspended in water or placed near the bottom. These tests made by Butler (1955) on oyster bottoms near Pensacola, Fla., showed a preponderence of spat on the upper surfaces of the boards. Setting on upper surfaces (135° and 180°) comprised 78 percent of the total number set, and only 18 percent were counted on the lower surfaces. The remaining 4 percent were found on vertical boards. Butler was not able to confirm the results of Pomerat's and Rienner's tests made earlier at Pensacola in which frosted glass plates suspended in water attracted the greater percentage of oysters (and barnacles) to the lower surfaces. According to Cole and Knight-Jones (1939), the larvae of O. edulis reared in large tanks in Conway, Wales, set more intensely on the under surfaces of test shells. A study of the effect of the angle of a flat surface on the attachment of larva was made by Hopkins (1935) in his work on O. lurida of the Pacific Coast. He used glass plates, each 2,400 sq. in., placed at different angles over the oyster grounds. The under horizontal surface was designated as 0° and the upper horizontal as 180°. Other plates were set at 45° intervals between the two extremes. The average number of larvae attached to each surface were:

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<td>1</td>
</tr>
</tbody>
</table>

Figure 354.—Effect of angle of the surface of plate glass on the number of spat of C. virginica which attach to it within 5 days. Glass area 2,400 sq. in.; 0° is under horizontal surface. (Figure 1 from Schaefer, 1937).
responsible for the greater number of larvae setting on the upper surfaces.

A lack of consistency in observations of various investigators in different environments indicates that it is impossible to ascertain the effects of a single factor of the environment while testing under complex and variable natural conditions. Real progress in the study of the reaction of oyster larvae may be achieved if further observations are made under controlled conditions.

Larvae of *C. virginica* that are grown artificially in culture jars and not disturbed by stirring or aeration are more or less uniformly distributed. Eyed larvae frequently congregate on the surface, swimming with their vela uppermost and touching one another with the tips of the cilia. They form groups or "rafts" visible to the naked eye. Some of them close their valves, fall rapidly to the bottom, and after a short time resume swimming. Falling to the bottom should not be confused with negative geotaxis, which has not been demonstrated for oyster larva. In the laboratory, larvae often attach themselves to the sides of plastic or glass containers and apparently do not discriminate between light and dark surfaces.

**TO THE PROPERTIES OF SURFACE**

Oyster larvae attach themselves to many kinds of hard and semihard surfaces. They are found on rocks, gravel, cement, wood, shells of other mollusks, on stems and leaves of marsh grass, and on a great variety of miscellaneous objects such as tin cans, rubber boots and tires, glass, tar paper, and pieces of plastic that may be accidentally thrown on the bottom or deliberately used as spat collectors. There is no evidence that the larvae are selective in finding a suitable place to set, provided the surface is not covered with a slimy film, detritus, or soft mud. Under natural conditions they are never found on shifting sand or on a bottom covered with loose sediment. Success of setting always depends primarily on the availability of clean surfaces rather than on other factors. Shells covered with oil and greasy substances in polluted areas are not suitable for the attachment of larvae. Cole and Knight-Jones (1939) found that it is difficult to induce *O. edulis* to set on smooth glass, but the larvae of *C. virginica* raised in the laboratory readily attach to polished glass. In fact live preparations of spat may be obtained for microscopic examination of small oysters by suspending glass slides in a tank with fully grown larvae.

**GREGARIOUSNESS**

An interesting gregarious tendency has been observed by Cole and Knight-Jones (1949) among the larvae of *O. edulis*. During experiments in large rearing tanks they found that larvae set more readily on shells already bearing 50 to 100 spat than on shells bearing fewer spat. They suggest that a substance secreted into the surrounding water by the spat, and possibly by the fully developed larvae, encourages the setting. No attempts were made to isolate the substance and test its effect. The authors make another observation which may throw some doubt on the validity of their interpretation. They state (p. 36) that "Larvae set more readily on shells which had remained uncleaned in the tanks for 2 or more weeks, and which bore a visible film of bacteria or diatoms, than on similar shells which were cleaned daily." In their study of gregariousness they placed shells of uniform size and shape in pairs in a tank containing fully developed larvae, and the number of spat attached to them was counted daily. One shell of the pair was considered a control and was cleaned every day, and the other (experimental) remained uncleaned. By the end of the setting period the total spat settled on the experimental shells significantly exceeded the total spat settled on the controls by a ratio of 2.5 to 1. The figures suggest that the observed differences may be due to the attraction of larvae by those which had already settled on the shell, but the conclusion cannot be accepted without further verification. The possibility is not excluded that some other unknown factor, such as the position of the controls in relation to the experimentals, affected the results or that handling and removal of spat from the control shells caused changes to the surface which made them less attractive to the larvae. It would be profitable to conduct a series of tests designed to eliminate bias by placing experimental and control shells at random and making a statistical analysis of the significance of the differences.

Yonge (1960) expresses no doubt "that larvae (of *O. edulis*) settle more readily on surfaces to which others are already attached," and points out that this tendency aids in reproductive efficiency and is, therefore, a major benefit to attached animals. In view of the fact that a single oyster
shell has sufficient space for only a few spat to grow to maturity; heavy concentrations of spat are of doubtful value for reproduction and may even be harmful by creating overcrowded conditions.

**ARTIFICIAL REARING OF OYSTER LARVAE**

Early attempts to rear oyster larvae under artificial conditions produced uncertain results. Sometimes a small number of spat were obtained, but the experiments could not be repeated under similar conditions. At that time oyster larvae were placed in 5-gallon carboys, and the water was aerated and circulated. At 2-day intervals the larvae were concentrated by centrifuging and transferred into fresh sea water (Wells, 1920). In another method, tried with only partial success, the larvae were reared in slowly running sea water which was filtered through a 2-inch layer of white sand or porous stone (filtrose) placed on the bottom of a container. The rates of filtration and of addition of new water were regulated by a valve placed below the filtering layer (Frytherch, 1924). In both types of experiments no food was added to the containers under the assumption that enough was present in the water. Then Gaarder and Spärck (1933) and Gaarder (1933) studied the food of the larva of O. edulis in Norwegian oyster ponds and made what may be considered the first significant step toward solving the problem of rearing larvae under artificial conditions. Spärck observed that the water of the ponds contained considerable numbers of a small green unicellular alga which later on was isolated and cultured in the laboratory. It appeared to be a species of Chlorella which was consumed by the larvae. Studies by these investigators revealed also that nannoplankton of the ponds consisted principally of small green algae and flagellates measuring from 2μ to 3μ. Fertilization of experimental tanks by the addition of liquid manure greatly increased the production not only of Chlorella but also of various diatoms, chiefly Nitzschia, flagellates, various large unicellular green algae, and bacteria. In this enriched water a few larvae grew to a size of 300μ but failed to attach (Spärck, 1927). After it was found that Chlorella is present in the Norwegian oyster ponds, in experimental tanks in Conway, Wales, and in certain experimental basins in Denmark, Kándler (1933) attempted to grow oyster larvae on a diet of this alga alone but had little success. This led him to conclude that oyster larvae are unable to digest Chlorella, which left the intestine apparently unchanged. Feeding experiments with Carteria and Chlamydomonas were also unsuccessful. More critical experiments conducted at Conway, Wales, showed that the larvae are unable to utilize nonmotile green algae such as Chlorella and Collomyza but that yellow-brown chrysomonads (not identified but designated as flagellate C) gave satisfactory results (Cole, 1937).

The Conway experiments demonstrated that organic enrichment of the water of the large tanks was consistently successful in giving rise to a good crop of flagellates with the resulting good growth and setting of larvae (Cole, 1939). The most satisfactory fertilizer was the meat of the shore crab Carcinus ground with sand and heated to the boiling point. The suspension of meat was added to a 90,000-gallon tank at the average rate corresponding to 12.5 medium-sized crabs per day for a period of 3 to 4 weeks. Production of nannoplankton was judged by pH readings, and as soon as the readings reached 8.3 to 8.4 and the tank had a distinct slight cloudiness, no more crab meat was added.

Evidence presented by Cole showed that growth and attachment of O. edulis larvae in tanks were significantly increased by organic enrichment which stimulated the development of the nannoplankton. Under laboratory conditions the oyster larvae grew and set satisfactorily in the water containing cultures of Platymonas tetrahele. The larvae of oysters and other bivalves apparently are not able to swallow microorganisms which exceed 8μ, but according to Thorson (1950) the size of nannoplankton normally devoured by larval forms is smaller (2μ to 3μ).

Difficulties in obtaining reproducible results from using organic enrichment for rearing larvae suggested that variations in the composition and quantity of nannoplankton may be responsible. To determine the food requirements of O. edulis larvae, Bruce, Knight, and Parke (1940) isolated from sea water six flagellate organisms ranging in size from 1.5μ to 7μ in diameter. A known number of oyster larvae were introduced into glass vessels filled with 16 l. of uncontaminated, sterile sea water which was stirred and aerated. The water was changed continuously by a drop feed; the loss of larvae was prevented by covering the outflow tubes with bolting silk. The larvae were
fed pure cultures of flagellates grown in the so-called "Erdschreiber" medium of the following composition (Gross, 1937):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nitrate (NaNO₃)</td>
<td>0.1 g.</td>
</tr>
<tr>
<td>Sodium orthophosphate (Na₂HPO₄)</td>
<td>0.02 g.</td>
</tr>
<tr>
<td>Soil extract</td>
<td>50 ml.</td>
</tr>
<tr>
<td>Sea water</td>
<td>1,000 ml.</td>
</tr>
</tbody>
</table>

Soil extract is made by boiling 1 kg. of good potting or garden soil with 1 l. of distilled water in an autoclave for 1 hour. The flask is set aside for 2 or 3 days, and the muddy dark fluid is decanted and sterilized by heating to the boiling point. After standing 3 to 4 weeks the suspended particles settle on the bottom, and the transparent brown or red fluid is poured into another container and boiled for a short time. Boiling of the medium should be avoided once the required quantities of nitrates and phosphate have been added.

Since the six flagellates used in these experiments (Bruce, Knight, and Parke, 1940) were not identified and were labeled only by letters, inconsistencies in the results reported may be attributed to the appearance in the culture of other species, or, as the authors state, "to the supervision of factors outside experimental control." The authors suggest that one of these conditions may be the fact that larvae from different oysters are not equally viable.

The feeding of oyster larvae (O. edulis) with pure cultures of nannoplankton was repeated by Walne (1956). In this case the larvae were kept in vessels of 1 l. capacity without change of sea water, and the species of flagellates grown in cultures were identified. Among the Chlorophyceae, only Pyramimonas grossii Parke gave consistently good results. Tests made with Chlorella stigmatophora Butcher seemed to indicate that those chlorococcales which have a thick cell wall are poor food for oyster larvae. The best results were obtained with Isochrysis galbana Parke, a chrysophycean of about 5 μ to 6 μ in length. Prymnesium parvum Carter was found to be toxic to larvae. So far there is no proof that the species of flagellates used in these experiments form a significant component of the natural population of nannoplankton and that their presence in estuaries is necessary for larvae living under natural conditions.

Imai and Hatanaka (1949, 1950) reported that the larvae of C. gigas can be reared on a culture of colorless flagellate, Monas sp., which abounds in brackish waters of Japan. The authors believe that the flagellate of the Monas type plays an important role in the production of oysters in Japan. The possibility remains, however, that in their experiments other flagellates were present in the culture of Monas enriched with glucose, cane sugar, nitrates, and phosphates.

The pelagic life of C. virginica and C. gigas, and probably of all oviparous oysters, is longer than that of larviparous O. edulis and O. lurida. Consequently, the rearing of these oviparous larvae under artificial conditions presents additional difficulties. Considerable advances in the rearing of larvae of various bivalve species were made by Loosanoff, Davis, and their collaborators at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn. Phases of the work are summarized by Loosanoff (1954) and Loosanoff and Davis (1963a, 1963b). Oysters were induced to spawn by increasing the temperature and by adding sperm suspension (see p. 305, Chapter XIV). The fertilized eggs were freed from debris by passing the water through a series of fine screens and placed in 5-gallon earthenware jars until freeswimming larvae emerged. Then the water was changed every 24 to 48 hours by straining it through fine sieves which retained the larvae. The sea water in which the larvae lived was filtered through cotton to remove detritus and zooplankton. Aeration and mechanical agitation were considered unnecessary if the water was changed every other day. The larvae were given measured amounts of cultures of various micro-organisms. In general the results obtained in Milford corroborate the findings of British investigators. Davis (1953) established that oyster larvae can utilize as food the following species of flagellates: Dicrateria inornata, Chromulina pleiades, Isochrysis galbana, Hemiselmis rufolescens, and Pyramimonas grossii. Chlorella sp. was used only by advanced larval stages and not by young veligers.

The utilizable flagellates were added to the rearing tanks at the rates of 15,000 and 25,000 cells per ml. per day but no toxic effects were noticed in these heavy concentrations, and the larval oyster population of approximately 5,000 per 1. showed satisfactory growth. The actual number of flagellates ingested by the larvae was not determined, but the inference was made that "the rate of growth of oyster larvae had an inverse relation to the number of larvae per unit volume" (Davis, 1953). Cole (1939) states that a population of 20,000 to 30,000 small flagellates per 1 ml.
is adequate to promote growth of larvae of *O. edulis* but is insufficient for the spat.

With the exception of the toxic *Prymnesium parvum*, the naked flagellates provided better food for young oyster larvae than the organisms with heavy cell walls, which can be utilized only by older larvae. The best single foods were found to be *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, *Dicrateria inornata*, and some other unidentified species of *Dicrateria*. Since the cultures used in these experiments were not free of bacteria, the question naturally arises whether the marine bacteria are utilized as food.

Davis (1953) states that none of the 13 species of marine bacteria tested by him were used by the larvae. The species of bacteria have not been identified. However, the probability that larvae may derive a certain amount of food from some bacteria is strengthened by the observation reported by Davis (1953) and Loosanoff (1954) that larvae kept in cotton-filtered sea water without algal food continued to grow for as long as 14 to 18 days. The role of marine bacteria in the feeding of oyster larvae needs further experimental study.

Apparently the best results in rearing larvae under artificial conditions are obtained with a mixed food of *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, and *Dicrateria* sp. With such a diet and at 30 °C, the larvae of *C. virginica* begin setting between the 10th and 12th days after fertilization; at 24 °C, the sibling larvae are ready to set on the 24th to 26th day; at 20 °C, only a few of the larvae set by the 38th day. Setting of larvae of *O. lurida* at a temperature of 22 °C takes place on the 7th day after release of larvae from the brood chamber (Loosanoff and Davis, 1963a).

Under laboratory conditions in Woods Hole the young larvae of *C. virginica* are often found on the bottom of vessels entangled in lumps of several individuals. These larvae never recover and usually die within the next 24 hours. Sometimes the larvae of oysters and clams are attacked and killed by a fungus which has been tentatively identified by the workers at the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., as belonging to the genus *Sirolpidium zoophorum* Vishniac (Davis, Loosanoff, Weston, and Martin, 1954; Johnson and Sparrow, 1961). There are undoubtedly other bacteria and possibly viruses which inflict epizootic mortality on larval populations in the laboratory and in natural waters.

The technique of rearing oyster larvae has progressed sufficiently to be applicable to practical purposes of oyster culture. Details of techniques, organization, and operation of a mollusk hatchery are summarized by Loosanoff and Davis (1963b).

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