trochophore stages, and then become so " sticky " that they adhere to each other and to the walls of the containers, particularly at the

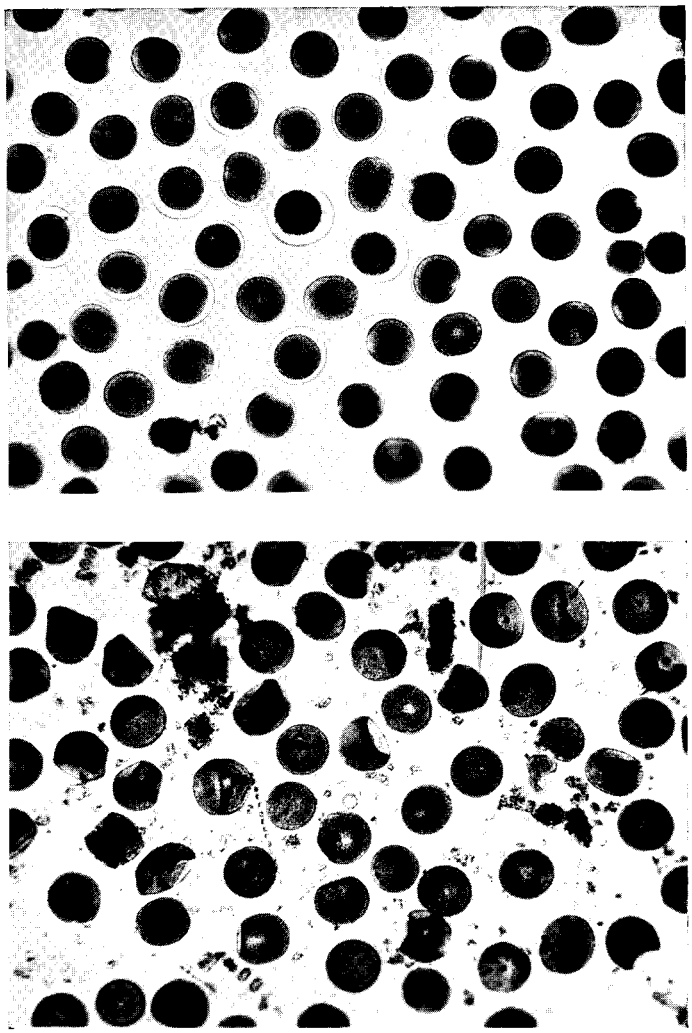

FIG. 15. Largely normal (*above*) and abnormal (*below*) eggs of *Ensis directus*. Abnormal eggs were discharged by a female compelled by strong stimulation to spawn before eggs were ripe. Normal eggs vary from 64 to 73 μ in diameter.

air-water-glass interface where they normally congregate in large numbers.

With somewhat more advanced, but still not entirely ripe, oyster

eggs the larvae develop more normally but are quite small, measuring only 60 to 70 μ at the 48-hr stage (Davis, 1949). Finally, in induced spawning of oysters late in the season after resorption of their gonads has begun, embryonic development of eggs is frequently abnormal and only a low percentage of them develop into healthy straight-hinge larvae.

Subjecting eggs and spermatozoa to temperatures higher than 30°C may injure or even kill them. Maintaining recently discharged eggs in heavy concentrations, a condition that leads to formation of a thick layer of them on the bottom of rearing vessels, may result in a sufficient

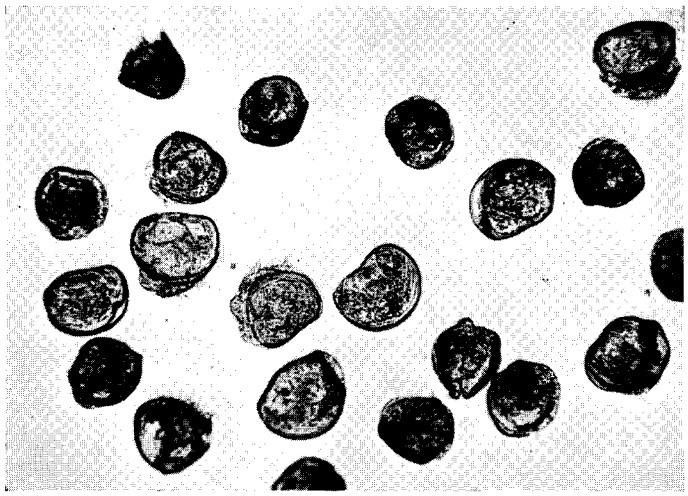

FIG. 16. Abnormal 4-day-old larvae of *Mercenaria mercenaria* from crowded culture Largest larvae in photomicrograph are approximately 105 μ long.

depletion of oxygen and accumulation of catabolic products that will affect the eggs and their further development. If zygotes and early embryos are badly overcrowded, their shell development usually proceeds only as far as the shell gland stage. Thus, instead of shells being fully formed 48 hr after fertilization, as occurs in normal larvae which, at that time, can retract their soft bodies completely within the new shell, overcrowded larvae have a small, dark oval area denoting the position of the shell gland or a small band of shell material not more than twice the diameter of the shell gland.

In less-crowded cultures larvae form enough shell to protrude from their bodies to give the appearance of small wings (" winged larvae "). Under somewhat better conditions a larger, but still incomplete shell

is formed, but the hinge line, instead of being straight, is concave, characterizing "saddleback larvae", or convex, typical of "humpback larvae" (Fig. 16). In both of these abnormalities much of the ventral portion of a larva's body will extend beyond the shell. The results of overcrowding on development and growth of larvae will be more fully described in a later section of this article.

Occasionally, in some cultures many larvae have abnormally small vela. This abnormality may, sometimes, be due to mechanical injuries to the velum when larvae are screened before their shells are fully developed to protect the soft parts. In other cultures it has been associated with the presence of numerous ciliates. It is possible that velar deformities in these cases were the results of injuries by ciliates, but it is more probable that the ciliates were feeding on particles of vela cast off by larvae in response to adverse conditions, such as artificially-created concentrations of certain chemicals.

The same type of abnormalities, as observed in overcrowded cultures, occurs when eggs are cultured in sea water in which adult oysters have previously been kept. Probably because of the same reason, eggs carried along with water from tanks or trays in which a mass spawning has occurred seldom develop into normal larvae unless the original water is greatly diluted with fresh sea water.

Failure of larvae to develop normal shells when overcrowded, or when grown in water in which adult oysters had previously been kept, may indicate a depletion of certain substances, normally present in sea water, that are needed for shell formation. In experiments devised to verify this possibility some of the eggs discharged by a single female were placed in fresh sea water, while others, fertilized with sperm from the same male, were placed in sea water taken from an aquarium in which adult oysters had been kept. The latter group of eggs gave a much lower percentage of normal larvae.

One interesting class of abnormal larvae consists of those that do not feed, even though they do not show clear-cut anatomical malformations. These apparently normal larvae, which developed to straight-hinge stage under our standard conditions and are kept in the same cultures with other larvae which are feeding and growing normally, seem unable to feed, do not grow, and eventually die. Before death, the larvae become emaciated so that most of the space inside their shells is empty with only the retractor muscles, a small velum and a shrunken visceral mass remaining. In some cultures this abnormality is found in more than 25% of the larvae.

In several experiments this type of abnormality has been associated with the kind of food given. In these instances approximately 50%

of larvae of *M. mercenaria* receiving *Chlamydomonas* sp. displayed this abnormality, while the remaining 50% fed and grew at a normal rate, as did all of the sibling larvae in other cultures receiving other foods. A similar phenomenon was observed in American oyster larvae fed *Phaeodactylum tricornutum* (Davis and Guillard, 1958).

While some anatomical abnormalities may interfere with the ability of larvae to gather or ingest food, resulting in poor growth and eventually death, other abnormalities, such as badly deformed shells, may still permit larvae to feed and grow. Sometimes, as they grow, such larvae gradually become more normal in appearance, but usually remain distinguishable even when they are nearing metamorphosis.

Experiments on tolerance of eggs and larvae of bivalves to such factors as turbidity and salinity, and to chemicals, such as pesticides, antibiotics, and bacteriostatic compounds, have also shown that if any one of these falls outside of the tolerated limits, embryonic development becomes affected, resulting either in death of the zygotes or in abnormal larvae. These matters will be discussed in more detail later in the article.

Some of the larvae, particularly those of the clam, *M. mercenaria*, that are abnormal because of overcrowding, exposure to low temperatures or high turbidity during early stages of development often grow to metamorphosis if returned to favorable conditions and given good food.

Dense algal blooms may also cause abnormal development. We have frequently observed, during blooms of dinoflagellates in Milford Harbor, that in our laboratory cultures only a small percentage of clam or oyster eggs developed into normal straight-hinge larvae. Eggs placed in water from which algae were removed by Millipore filters showed only a slightly higher rate of normal development than eggs grown in unfiltered water containing dinoflagellates. Eggs from the same spawnings but cultured in sea water collected prior to the bloom gave considerably higher percentages of normal larvae (Davis and Chanley, 1956b).

It may be added that plankton samples collected in Long Island Sound during or immediately following heavy algal blooms are usually characterized by the scarcity or even complete absence of early straight-hinge stages of bivalve larvae (Loosanoff, 1958a). We believe that reduction in numbers of normally developing bivalve eggs and larvae in the above instances is primarily caused by highly toxic metabolites of algae that may persist for several days after the blooms have ended. It is possible, however, that this phenomenon is due, at least in part,

to removal by algae of certain chemicals from sea water that are essential to larval development.

C. *Methods of cultivation of eggs and larvae*

Methods of culturing eggs and larvae of bivalves under laboratory and small-scale hatchery conditions have been tested by many workers for over 100 years. Costé, a Frenchman, was probably the first to attempt this around 1858. In the United States a number of extremely capable men, including Brooks (1880), Ryder (1883) and Winslow (1884), continued these efforts on *C. virginica*, but were unsuccessful. Perhaps the best summary of these efforts is given by Winslow, who states, " But after my experience of the past spring and summer I am convinced that it will require a series of painstaking experiments, extending over considerable time and conducted under many dissimilar conditions, before the artificial production and culture of the oyster is made a matter of practical importance."

Interest in artificial propagation of bivalves was revived when Prytherch (1924) and Wells (1920, 1927) succeeded in carrying oyster larvae to metamorphosis. This success was probably due to the practice of renewing the water in which oyster larvae were kept. Wells used a milk separator for this purpose, while Prytherch used filtros plates. Other successful workers in this field included Hori and Kusakabe (1926), Cole (1936), Bruce *et al.* (1940), Lindsay and Woelke (1960), Woelke (1960) and, especially, Imai *et al.* (1950b). In our case some oysters were carried to metamorphosis as early as 1932, but efforts to repeat this success usually failed until about 1946–1947 when we began to develop and improve the methods used at our laboratory (Loosanoff and Davis, 1950; Davis, 1953; Loosanoff, 1954).

It is our practice to fertilize eggs as soon as they are discharged. Usually it happens automatically because since we use a sperm suspension to stimulate spawning, spermatozoa are already present in the water when females begin to discharge eggs. Fresh, actively-moving sperm are used to assure normal fertilization of eggs and development of zygotes. Sufficient quantities of sperm are always added, but when working with small eggs, such as those of *C. virginica*, which cannot be retained even by fine screens, we limit the quantity of suspension. In this way excessive quantities of sperm are not carried into our culture vessels, and the undesirable effects of decomposing sperm on developing eggs are avoided.

The bivalves are usually spawned in Pyrex glass dishes containing about 1·5 liters of water (Figs. 10 and 11). As has already been mentioned, to separate the eggs from the debris and, later, from the excess sperm,

blood cells and body fluids accompanying the spawning, we use a series of stainless steel sieves with screens containing different numbers of meshes per linear inch (Fig. 17). The finest screen that was found practical in our operation has mesh openings averaging 44 μ, but since many meshes are actually larger and exceed the diameter of an oyster egg, which is about 50 μ, many eggs pass through the sieve. It is true that by using No. 25 bolting silk a smaller size mesh is available but in that case the openings are so small that they easily get clogged,

FIG. 17. Screening bivalve eggs through 100-mesh sieve to remove detritus. Similar screens, of 3 in diameter and of smaller mesh opening, are used to collect bivalve eggs and to retain larvae during changes of sea water in culture vessels.

rendering the sieves useless. As a result, in our practice we use a series of sieves, the finest of which has a nominal opening of about 44 μ, followed, when necessary, by any of the coarser screens with openings of 53, 62, 74, 88, 120, 125, 149 and 177 μ.

In species having eggs too small to be retained even by our finest screen, the eggs can be partially freed of body fluids, sperm, etc., by letting the eggs settle on the bottom of a dish and then syphoning or decanting most of the fluid. By repeating this procedure several times most of the undesirable substances that are dissolved or suspended in the water will be discarded.

We have used a variety of containers to culture larvae. Some of them were large glass vessels, including Downing and McDonald jars used in fish hatcheries for incubation of semi-buoyant eggs, lobster jars, 5-gal earthenware jars, and 75-gal polyethylene and Fiberglas containers. We have also grown larvae in large, outdoor, concrete tanks containing several thousand liters of sea water (Loosanoff, 1954). In all these instances the larvae were grown successfully.

For precise experimental work Pyrex glass beakers of 1000- to 1500-ml capacity are perhaps the most satisfactory because they are not toxic and are readily cleaned and sterilized (Fig. 5). Polyethylene and other plastic containers are also convenient and non-breakable and some can be sterilized. However, some of them are permeable to certain insecticides and, perhaps, to other substances and are known to adsorb a variety of toxins. Because of these considerations polyethylene and some other plastic containers, while convenient as culture vessels, cannot be used in experiments involving certain toxic substances, such as insecticides.

New, soft glass vessels may contain substances which are toxic to eggs or larvae of oysters and clams. Even though these vessels are conditioned in sea water for several days, culturing larvae in them is always haphazard because, although larvae in different vessels are presumably grown under identical conditions, their rates of growth are often distinctly different and they show mortalities unrelated to the treatment. At the same time sibling larvae grown in earthenware jars or Pyrex glass containers suffer no mortality and different cultures receiving the same treatment show good duplication in their rate of growth.

Although we could not identify the substances responsible for poor growth of larvae grown in soft glass jars, we found, nevertheless, that young bivalve larvae, especially those of *C. virginica*, are sensitive to presence in the water of even minute quantities of certain chemicals. For example, we noticed that washing of glassware and other implements with tap water that passed through a pipeline containing copper unfavorably affected larval development. Apparently, even minute quantities of these metals are sufficient to interfere with normal development of eggs and larvae.

Our experience in growing bivalve larvae has shown that they cannot be kept in recently-built concrete tanks and that, usually, it is necessary to age the tanks with sea water for a long time before this can be done.

Since most of our studies are quantitative, as well as qualitative, definite numbers of eggs or larvae per ml of culture are needed from the start. This is achieved in the following manner: eggs are placed

in a tall, narrow glass jar and the water in it is thoroughly agitated with a perforated plastic plunger to distribute the eggs uniformly. A sample is then taken with a volumetric pipette, and the number of eggs or larvae per ml is determined by counting them on a Sedgwick-Rafter cell. After that the eggs or larvae are again agitated, to ensure their even distribution in the vessel, and the necessary volumes of water carrying larvae are transferred to culture vessels.

We usually begin our experiments with 10 000 to 15 000 straight-hinge larvae per liter of sea water. However, because not all fertilized eggs develop into normal larvae, it is our practice to place approximately twice this number of eggs into each culture vessel to produce a sufficient number of larvae. Accordingly, about 30 000 eggs per liter are used in starting cultures. These eggs are placed in containers filled with sea water that is first filtered through an Orlon filter and then subjected to the sterilizing action of ultraviolet rays. The eggs are then left to develop undisturbed for 48 hr and no food is added during this period.

All culture vessels are immersed in a common water bath table, the temperature of which is controlled within $\pm$ 1·0°C (Figs. 5 and 6). Usually, neither aeration nor mechanical agitation is employed because we have established that clean, well-attended cultures do not require aeration if the water is changed every second day. In special experiments requiring mechanical agitation we use a number of devices, including a rotating wheel, paddle-agitator (Fig. 5), or regular shaking machine.

At the end of 48 hr young larvae, now protected by fully formed shells, are collected by screening the cultures through sieves having 325 meshes per lineal inch (44 μ opening). Larvae retained by the screen are gently washed and placed in a tall jar. Using the same method as that employed with eggs, the number of larvae per ml is determined, and the appropriate volumes of water containing larvae are placed in each culture jar to create desired concentrations.

Samples of larvae are taken whenever needed, usually at 2-day intervals. This is again accomplished by collecting all larvae from a container on a 325-mesh screen and then transferring them to a graduated cylinder of 1-liter capacity from which, after proper agitation, required samples are taken, while the remaining larvae are returned to the culture vessel.

D. *Larval period*

Rate of growth of veligers from straight-hinge stage to metamorphosis is affected by many conditions. In our laboratory work

the chief controlling factors have been food and temperature. The role of these conditions will be more fully discussed in special sections later on, and also in the sections dealing with development and growth of larvae of different species; therefore, here it will be sufficient to mention only general observations.

Our experiments have shown that larvae of different bivalves display different food requirements (Loosanoff and Davis, 1951). Until they reach a length of about 125 μ larvae of *C. virginica*, for example, are quite restricted in types of food they can utilize (Davis, 1953). Certain naked flagellates are the only organisms, thus far tested, that may be included in this category. *Chlorella* is one of the many genera of algae having thick cell walls that oyster larvae either cannot utilize or utilize only to a very limited extent during early stages, although it seems to be quite a satisfactory food for older larvae (Davis and Guillard, 1958). Thus, if during the early stage of development of oyster larvae specific food organisms are either entirely absent or are uncommon, the larval free-swimming period may be greatly prolonged or the larvae may never reach metamorphosis. For example, during our earlier efforts of raising larvae of *C. virginica*, when little was known about their food requirements, approximately 50 days were required before the most advanced individuals began to metamorphose in some cultures. In similar cases, with larvae of *C. gigas*, the cultures were discarded after 53 days because the largest larvae at the time measured only about 100 μ. Now, using good food organisms, such as *Isochrysis galbana* and other naked flagellates, and maintaining the temperature at about 23°C, larvae of *C. virginica* have been reared to metamorphosis in our laboratory in 18 days. This is, probably, the approximate time required by larvae to grow to setting size under natural conditions in Long Island Sound (Loosanoff and Engle, 1947). At 30°C well-fed oyster larvae, grown under laboratory conditions, began to metamorphose 10 days after fertilization.

The importance of the second factor, water temperature, on length of larval period of bivalves has also been well demonstrated in our studies of larvae of *M. mercenaria* (Loosanoff *et al.*, 1951). These studies showed that, under identical conditions, larvae kept at a temperature near 30°C began to set as early as the 7th day after fertilization, while cultures maintained at 18°C contained the first metamorphosing individuals only after 16 days.

In the case of larvae of all the species we reared it has been clearly demonstrated that even though larvae originate from the same spawning and, sometimes, from the same parents, and are kept in the same vessel under identical conditions, individuals grow at widely

different rates and, therefore, metamorphose at different times (Fig. 18). For example, in a recent experiment a healthy culture of larvae of *C. virginica* fed a mixture of *I. galbana* and *Monochrysis lutheri* and kept at about 23·5°C began to set 18 days after fertilization. Setting gradually increased in intensity and remained heavy for the first 17 days, but some larvae continued to swim, before metamorphosing, for another 10 days. Thus, setting of this, presumably, homogeneous culture continued uninterrupted for a period of 27 days.

A number of similar observations on larvae of *M. mercenaria* obtained from the same parents and grown under identical conditions, but showing considerable individual variations in rate of growth and in

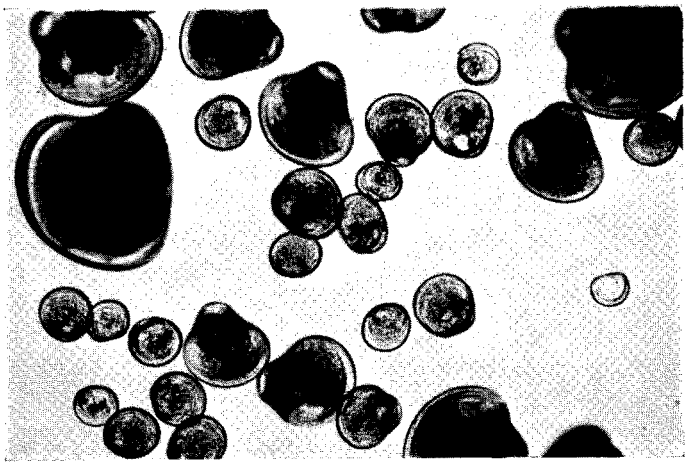

FIG. 18. Differences in sizes of larvae of *Crassostrea virginica* of the same age and grown under identical conditions in the same culture vessel. Some of the largest larvae are approximately 295 μ long and are nearing metamorphosis.

time needed to reach metamorphosis can be given. Perhaps the most detailed description of this phenomenon appears in the paper of Loosanoff *et al.* (1951) on growing clam larvae at five constant but different temperatures. These authors gave the minimum and maximum sizes of larvae recorded in each culture every 2nd day from time of fertilization until the majority of the larvae metamorphosed. During the early life of a culture, on the 2nd or 3rd day, the larvae differed in size by only a few microns, but several days later, depending upon the temperature, the size ranged from small straight-hinge larvae of approximately 100 μ long to full grown, ready-to-metamorphose individuals. For example, sizes of larvae grown at 30°C ranged on the 8th day from 107 to 226 μ. In a culture kept at 21°C the minimum and

maximum sizes recorded on the 21st day were 107 and 221 μ. Our colleagues working in the same field, especially Imai *et al.* (1954), fully share our experiences.

At present, no well-based explanation can be advanced for these differences. Perhaps, as has been suggested in connection with survival and growth of certain fish, vitality of the individual eggs and larvae that emerge from them depends to some extent upon the position of the eggs in the ovaries and the amounts of nutritive materials that have been stored in the individual eggs before they are discharged.

Chanley (1955) assumed that differences in sizes of larvae in the same cultures must be due, at least in part, to inherited characteristics. He also reported evidence of significantly different rates of growth of larvae originating from eggs of the same female crossed with different males and larvae grown from eggs of two females individually crossed with the same male. He tentatively concluded that inherited differences from either parent may be responsible for differences in rate of growth of different larvae.

In some cultures, especially those kept at comparatively high temperatures, the range of larval sizes usually diminishes several days after beginning of metamorphosis. This is due to the disappearance of larger individuals because of setting and, partly, because abnormal, undeveloped, slow-growing larvae are rapidly dying.

There were periods in our practice, for example, in growing larvae of *Ostrea edulis*, when regardless of all efforts they would not grow at all or ceased growing soon after reaching a size of about 220 μ. The reasons for cessation of growth still remain an enigma because, at times, these larvae refused to grow even when given foods on which, in previous experiments, they grew well.

E. *Hardiness of eggs and larvae*

According to Nelson (1921) larvae of *C. virginica* are extremely sensitive to a sudden change in water temperature. A drop of only 3° to 5°C within 24 hr may be followed by the disappearance of a majority of the larvae. According to the same author rain storms, as well as strong winds, cause death of large numbers of bivalve larvae. Nelson, however, failed to offer experimental evidence to support his contention of the unusual sensitivity of bivalve larvae to relatively minor changes in their environment. Our observations, reported partly in this section and partly in the sections to follow, lead us to disagree with Nelson's point of view because they have clearly demonstrated that bivalve eggs and larvae, if protected against disease-causing organisms and toxic substances, are rather hardy.

Laboratory and field observations lead us to believe that oyster eggs that are still in the ovaries are hardy and capable of withstanding sharp physical changes in their environment. For example, on several occasions oysters with mature gonads have been kept for various periods of time in the refrigerator at about 2°C to delay their spawning. Some of them, kept at this low temperature for 7 days, have spawned copiously later on, when subjected to proper stimulation, and larvae from these spawnings have been reared to metamorphosis. Other groups kept in the refrigerator for 15 days also spawned normally and produced healthy larvae. However, oysters that were kept in the refrigerator for 30 days spawned feebly, and only a portion of the eggs developed into normal larvae. This semifailure was probably due to severe dessication of the oysters and their gonadal tissue. This conclusion is supported by the observation that the best spawnings occurred when refrigerated oysters, prior to attempts to spawn them, were kept in running sea water at room temperature for at least 6 hr. During this recuperation period they probably restored their water loss.

Another experiment on effects of low temperature upon ovarian eggs of *C. virginica* of Long Island Sound was performed only last winter (1961–62). Oysters measuring from 3 to 6 in long were brought into the laboratory early in January from their natural beds and placed in conditioning trays to be ripened for spawning. After conditioning at about 20°C for about 1 month, these oysters, now ripe, were transferred to outdoor tanks where the water temperature was near 0°C and where, at times, a layer of ice was formed.

Twenty days later, on 26 February, the first group of oysters was returned to the laboratory and placed in water of the same temperature as that outdoors. Then the temperature was slowly raised to about 17°C for 2 days. Following this recovery period fifteen oysters were placed in spawning dishes and our usual method of inducing spawning was applied. Eight of fifteen oysters responded, of which five were females and three were males. Spawnings were light to medium with a total of 27 million eggs released. These eggs were cultured by our usual method, but only a comparatively small number of larvae developed to straight-hinge stage.

On 6 March another group of the oysters was brought into the laboratory from the outdoor tanks and later induced to spawn. Four out of eight oysters spawned, three of which were females. The total number of eggs discharged by these females was 57 800 000. The majority of the eggs were normal in appearance, although a few were deformed and some were small.

In one of the containers, in which 375 000 eggs from this spawning

were placed in 5 gal of water, a count of straight-hinge larvae was made 48 hr later and showed that the culture contained 155 000 normal, straight-hinge larvae, 11 000 abnormal ones and 2 000 dead individuals. Therefore, approximately 168 000, or about 45% of the eggs placed in the culture developed into larvae. In another culture, where approximately 750 000 of these eggs were placed in 10 gal of water, the count made 48 hr later gave 424 000 normal, 46 000 abnormal and 32 000 dead larvae; therefore, approximately 502 000, or 67% of all the eggs that were originally placed in the culture developed to straight-hinge stage. From then on, however, larval development was poor, showing high mortality.

On 19 March, 43 days after the ripe oysters were placed in the icy water, another group was brought in and, after being kept for 4 days in running sea water at a temperature ranging between 14° and 17°C, was induced to spawn. Both males and females spawned. One of the females released 18 million and another, 27 million eggs. Larvae obtained from eggs of one of the females grew well, increasing approximately 10 μ in length per day.

This experiment demonstrated the remarkable fact that oysters artificially ripened in the middle of winter can be transferred abruptly from warm to freezing water, retained there for over 40 days, and then returned to warm water and induced to spawn, producing viable eggs and sperm that eventually develop into normal straight-hinge larvae. Even though mortality among larvae obtained in this unusual manner was relatively high and many larvae were abnormal, the experiment demonstrated, nevertheless, the remarkable power of oysters to retain their ripe sex cells under extremely adverse conditions. Results of histological studies of gonads of oysters involved in these experiments will be described later in a special publication.

Regardless of the ability of ovarian eggs of *C. virginica* to withstand exposure to low temperatures for long periods, recently fertilized eggs, in the polar-body stage of development, do not display the same tolerance. This was demonstrated by an experiment in which eggs, within 1 or 2 hr after fertilization, were placed in a refrigerator maintained at a temperature of about 2°C, and kept there for 6, 24 and 48 hr. Samples were then returned to room temperature and further development of the eggs subsequently observed. In all samples a few eggs developed into abnormal ciliated blastulas, but practically all of them failed to develop further and soon disintegrated.

Healthy shelled larvae of oysters, *C. virginica*, are, nevertheless, capable of withstanding sharp changes in temperature of the surrounding water. In a special series of experiments, designed to verify Nelson's

conclusion of extreme sensitivity of these larvae to temperature changes, beakers of 1-liter capacity containing larvae grown at a temperature of about 22°C and measuring about 200 μ in length were placed in a refrigerator at 2°C and returned to room temperature following 6, 12 and 24 hr of refrigeration. Within a few hours the predominating majority of larvae exposed to the low temperature for 6 and 12 hr were swimming and feeding normally. However, many of the larvae which were refrigerated for 24 hr lost a portion of the velum and eventually died. The mortality in each of the above groups at the end of one week after return to room temperature was: control, 4·2%; 6-hr chilling, 6·5%; 12-hr chilling, 4·9%; and 24-hr chilling, 44·9%. Thus, even though exposed to a near freezing temperature for a 24-hr period, more than half of the larvae survived and continued to develop.

In still another experiment oyster larvae lived and grew when subjected every 48 hr to a sharp drop in temperature, from 20° to 10°C, for periods of 15 to 30 min, followed in a few minutes by an equally abrupt return to 20°C. A majority of these larvae subsequently reached metamorphosis. It would seem unlikely, therefore, as claimed by Nelson (1921), that ordinary short-term temperature fluctuations of only a few degrees, occurring in natural waters, could be responsible for an appreciable, sometimes total mortality of larvae.

It is also certain at this time that bivalve larvae may survive long periods with little or no food. In many of our experiments several control cultures survived from 2 to 3 weeks with little or no mortality, even though they did not receive any food except that which was present in the filtered sea water where they were kept. Moreover, in our earlier experiments, before such good food forms as naked flagellates became available, many oyster larvae cultures were kept for more than 40 days, although they did not show any growth. As already mentioned, in some of these cultures setting began only after 50 days. These observations demonstrate that bivalve larvae may tolerate comparatively long periods of semi-starvation and some may even reach setting size and metamorphose regardless of poor feeding conditions. It is improbable, therefore, that under natural conditions larval populations of such mollusks as oysters will die within 2 or 3 days because of a lack of sufficient quantities of food. It is clear, however, that lack of food will prolong the larval period, thus increasing the loss of larvae because of predation and dispersal.

Larvae are also able to tolerate very low oxygen concentrations, at least for short periods. For example, on several occasions a number of

larvae were accidentally left overnight in a small pipette of sea water, yet they were found alive and healthy the following day.

Recent studies of Davis (1958) have clearly demonstrated that eggs and larvae of at least some estuarine species, such as *C. virginica*, can endure sharp changes in salinity. This matter will be discussed more extensively in the section dealing with the general aspects of changes in salinity upon development of eggs and larvae.

Studies of effects of turbidity upon eggs and larvae of *C. virginica* (Davis, unpublished) and those of *M. mercenaria* (Davis, 1960), which will be discussed later in greater detail, have demonstrated that larvae of these two species can endure and even continue to grow in water that is quite turbid. For example, Davis has shown that larvae of *C. virginica* may survive for at least 14 days in a concentration of 2 g of silt per liter of sea water. Such a heavy concentration seldom occurs in nature.

Fertilized eggs and larvae of many bivalves can also withstand vigorous mechanical disturbances without ill effects. For example, to obtain a representative sample of the population from our culture vessels, the water is strongly agitated by means of a plunger to assure a homogeneous distribution of larvae. Such relatively rough treatment, usually performed every day or every second day, does not cause an increase in mortality or decrease the rate of growth of larvae. Observations on the behavior of larvae in nature also support this conclusion because, as shown by our studies of plankton samples and by observations on intensity of setting of oysters on natural beds, it has been definitely established that strong winds accompanying New England hurricanes and churning the water of Long Island Sound steadily for several days do not noticeably diminish larval populations. This was especially well demonstrated in August 1955, when a marked increase in intensity of setting of oysters occurred immediately after hurricane "Connie". This increase continued for 2 weeks, thus showing that larvae of all ages survived the hurricane. It is also of interest that setting of oyster larvae occurred during the hurricane, thus indicating that strong water turbulence does not easily destroy larvae or seriously interfere with their metamorphosis.

Recent studies have repeatedly demonstrated the sensitivity of bivalve larvae to traces of certain substances in the water. These observations showed that sea water, in which our larval cultures are grown, sometimes contains substances, so far unidentified, which determine whether larvae will grow normally (Loosanoff *et al.*, 1951; Davis, 1953). Wilson (1951) found similar differences between natural sea water collected from widely separated areas of the ocean. We are

still not certain whether it is the presence of deleterious materials or absence of growth-promoting substances in sea water that slows growth or prevents normal development of larvae.

We have observed that some substances which interfere with normal development of larvae may originate from sources to which we have previously paid little attention. Under certain conditions these substances may be released mechanically from bottom soil. This was noticed during a winter when a deep channel was dredged in Milford Harbor, from which our laboratory obtains its water. During that period the water acquired certain properties which strongly interfered with normal development of eggs and larvae. These substances were apparently in solution or in fine colloidal suspension because they were still present in the water after it was filtered. Neither aeration nor ageing appreciably improved the quality of the water.

Sensitivity of eggs and larvae to different substances dissolved in sea water was further demonstrated by Davis and Chanley (1956b) in a series of experiments which showed that, while low concentrations of antibiotics may increase rate of growth of larvae, even a slight excess of them reduces rate of growth. Progressively increasing concentrations of these substances correspondingly decrease rate of growth of larvae and eventually cause their mortality. This matter will be discussed in greater detail in the section devoted to larval diseases and their treatment.

Recently, extensive studies on effects of numerous insecticides, weedicides, oils, organic solvents and detergents on mollusks have been undertaken at Milford Laboratory. While these studies are still in progress, it has already been found (Davis, 1961) that within each group of these compounds there are great differences in toxicity of individual chemicals to eggs and larvae of bivalves. For example, DDT was found to be one of the most toxic of the commonly used insecticides because even at a concentration of 0·05 parts per million it caused almost total mortality of oyster larvae. On the other hand, another common insecticide, Lindane (1, 2, 3, 4, 5, 6 hexachlorocyclohexane), even at a concentration of 10 ppm, which is essentially a saturated solution in sea water, caused no appreciable mortality of larvae. On the contrary, growth of clam larvae in 5 ppm of Lindane was somewhat faster than that of larvae in control cultures.

Certain concentrations of phenol, chloramphenicol and Dowicide "A", among the antibiotic, bactericide and disinfectant compounds also appreciably improved rate of growth of bivalve larvae. This is attributed to the action of these compounds which inhibits growth of

bacteria toxic to larvae. Other compounds which, in certain concentrations, probably improve rate of growth of larvae by partially inhibiting growth of toxic bacteria are acetone and trichlorobenzene among the organic solvents, Monuron and Fenuron among the weedicides, and Guthion among the insecticides. Davis (1961) appropriately suggested that, in some phases of shellfish culture, a sufficient concentration of such insecticides as Lindane may be maintained to destroy all undesirable crustaceans, while not affecting growth of bivalve larvae or their food organisms.

Our laboratory and field observations have shown that metabolites released by some microorganisms, especially dinoflagellates, seriously affect not only adult bivalves (Loosanoff and Engle, 1947), but also development of their eggs and larvae (Loosanoff *et al.*, 1953). Such toxicity of external metabolites and their physiological effects on aquatic organisms have been recognized by many biologists, some of these studies having been summarized by Lucas (1947, 1961) and Korringa (1952). More recently, Loosanoff (1955) reported that a heavy bloom of dinoflagellates in Milford Harbor caused abortion of embryos and immature larvae of gravid European oysters, *O. edulis*. Davis and Chanley (1956b) found that a dense bloom of dinoflagellates caused abnormal development of eggs and larvae of the clam, *M. mercenaria*, and oyster, *C. virginica*. Under these conditions only a few developed into shelled veligers. During that summer concentrations of dinoflagellates in some areas of Milford Harbor were as high as 300 000 cells per ml. Placing eggs of clams or oysters in this water, even after it was passed through a Millipore filter to remove dinoflagellates, resulted in only a slight increase in the percentage of clam or oyster eggs that developed normally.

Although we assume that the effects noted above were due to external metabolites emitted by dinoflagellates, it is possible that they were caused by removal from sea water, by these cells, of certain substances necessary for normal development of clam or oyster eggs and larvae. Another possibility is that the presence of a certain substance, favoring rapid growth of dinoflagellates and preventing normal development of larvae, was simultaneously responsible for both phenomena.

F. *Effects of temperature on eggs and larvae*

Certain observations and experiments devoted to studies of effects of sudden and extensive changes in temperature on eggs and larvae of several bivalves have already been described in the preceding section.

Here, we shall briefly discuss the results of observations on effects of temperature within a much more limited range.

Larvae of most of the species cultured at our laboratory were grown under routine conditions, i.e. at room temperature, which was normally near 20°C. Because of this no extensive information is available as to temperature ranges within which larvae of different species may survive or their optimal growing temperatures. In a few species, nevertheless, rather extensive observations on effects of temperature on development of their eggs and on growth of the larvae were undertaken. Studies of this nature on *M. mercenaria* and *C. virginica* have been the most complete.

M. mercenaria has been grown from egg to metamorphosis at constant temperatures ranging from 18° to 30°C (Loosanoff *et al.*, 1951). If, within 3 hr after fertilization, eggs of these clams were placed in water of 15°C, virtually none of them developed normally to straight-hinge stage. If eggs were kept at room temperature from 6 to 9 hr after fertilization and then subjected to a temperature of about 15°C, some developed into straight-hinge larvae. The majority of these larvae, however, were abnormal and many of them soon died, although some continued to grow at a very slow rate.

If eggs and, later, larvae developing from them were kept at room temperature for the first 2 days after fertilization, until straight-hinge stage was well formed, and then placed in water of 15°C, some of the larvae survived for 12 days or even longer. It is possible that if given good food, some of the individuals might eventually reach metamorphosis. However, if larvae grown at room temperature for the first 2 days of their existence were placed in water of 10°C (Fig. 19), they would not grow.

At the other end of the temperature range, at about 33°C, abnormal development and heavy mortality usually occurred if recently fertilized eggs were transferred to water of this temperature. However, if eggs and, later, larvae developing from them were kept at room temperature for the first 48 hr after fertilization and then transferred to water of 33°C, rapid normal development, similar to that observed in cultures kept at 30°C followed. Thus, our observations on development of eggs and growth of larvae of *M. mercenaria* at temperatures from 15° to 33°C support the view expressed by Pelseneer (1901) that normal early cleavage stages of molluscan eggs are limited to a narrower temperature range than can be tolerated by more advanced stages of the eggs or larvae.

Larvae of *M. mercenaria*, developing from eggs within the temperature range of 18° to 30°C, grew to metamorphosis, growth being

generally more rapid at higher temperatures. At 30°C larvae began to set as early as the 7th day after fertilization. Sometimes, the entire population grown at this temperature would metamorphose within

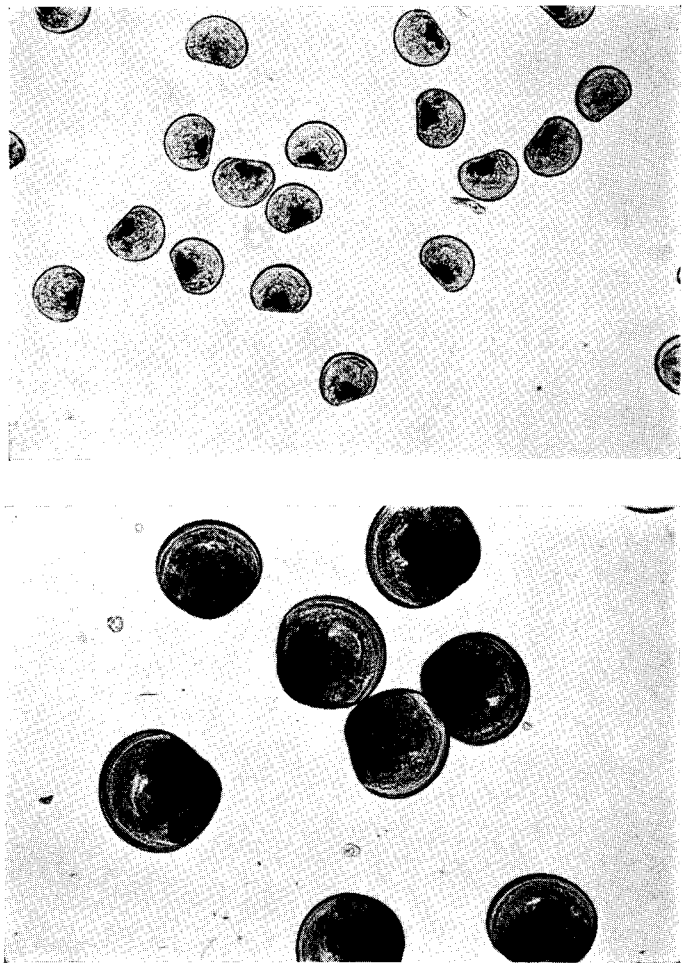

FIG. 19. Differences in sizes of 12-day-old larvae of *Mercenaria mercenaria* grown at 10°C (*above*) and 30°C (*below*). Average lengths of larvae were 105 and 195 μ, respectively.

5 to 7 days. When grown at 18°C the first metamorphosing individuals were noticed 16 days after fertilization, although in some cultures this event did not occur until after 24 days. Other factors, such as quantity and quality of food, density of larval population, etc., are no doubt

responsible for these variations. However, by maintaining the cultures at a constant temperature of 24°C and providing the larvae with good food, such as *Isochrysis galbana*, we consistently bring cultures of

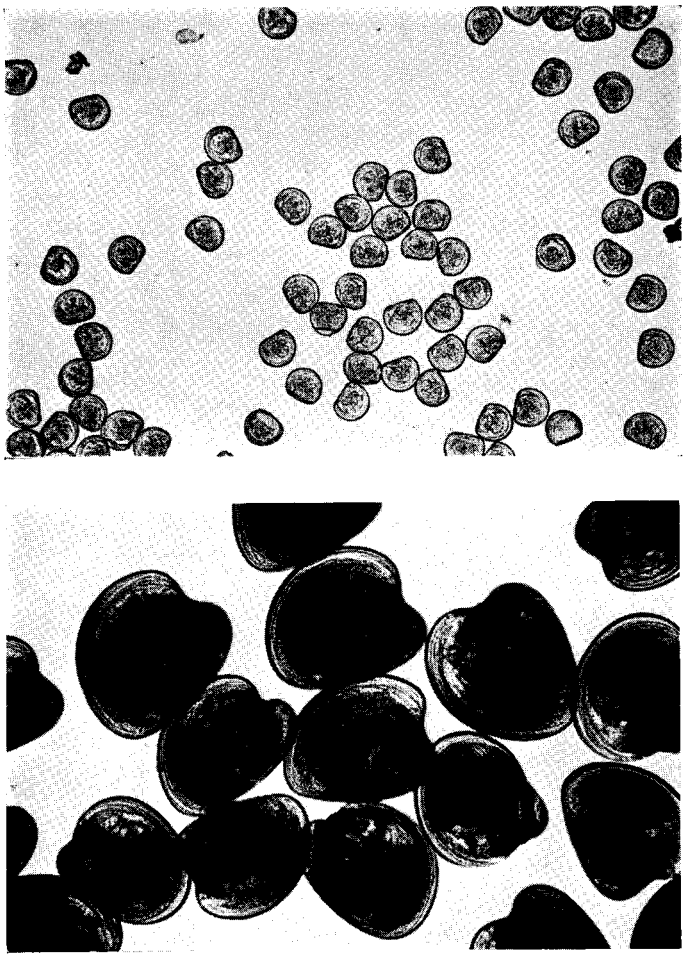

FIG. 20. Differences in sizes of 12-day-old larvae of *Crassostrea virginica* grown at 10°C (*above*) and 33°C (*below*). Average lengths of larvae of cold and warm water groups were 77 and 203 μ, respectively.

M. mercenaria to the beginning of setting 12 days after fertilization.

In experiments designed to determine temperature limits for development of eggs of *C. virginica* it was found that at 17·5°C as many as 97% of the eggs may develop to normal straight-hinge stage.

At 15°C, however, none of the eggs reached this stage although a few developed as far as early shelled larvae.

In some experiments 100% of recently fertilized oyster eggs transferred directly to 30°C developed into normal straight-hinge larvae, but, at 33°C, only 48% or less reached this stage. The abnormal larvae of this group were unable to feed or grow even when returned to a temperature of 24°C.

Although 2-day-old larvae placed in water at constant temperatures of 10° and 15°C for 12 days did not grow (Fig. 20), their rate of mortality during this period was comparatively low. The larvae kept at 10°C for 12 days could not feed even after being returned to a temperature of 24°C. However, some of the larvae kept at 15°C for the same length of time and then returned to 24°C fed, but their growth was negligible. Larvae kept at 17·5°C took some food, but also showed little growth. The majority of these larvae, however, began to grow rapidly when returned to water of 24°C.

At temperatures of 20°C and higher growth of oyster larvae was, to a large extent, dependent upon the food given. When fed *Chlorella* sp. (580), which is a relatively poor food, the larvae grew less rapidly than they did at the same temperatures when given better foods. Nevertheless, even when fed *Chlorella* sp. growth of larvae within the range from 20° to 33°C increased progressively with each increase in temperature.

Recent experiments suggest that one of the ways in which low temperature may affect growth of bivalve larvae is through inactivation of certain enzymes. For example, clam larvae kept at 10°C can ingest food organisms but are apparently unable to digest them. This is well shown in the upper photograph of Fig. 19. Larvae kept at 15°C can digest and assimilate naked flagellates and grow slowly, but are unable to utilize *Chlorella* sp. Those kept at 20°C were able to utilize both the naked flagellates and *Chlorella* sp. Similarly, larvae of *C. virginica* kept at a temperature of 20°C or lower cannot utilize *Chlorella* sp. However, at 25°C these larvae receiving *Chlorella* sp. showed some growth and at 30°C they were apparently able to utilize *Chlorella* sp. much more efficiently and, as a result, grew quite rapidly.

Larvae receiving *Dunaliella euchlora*, a moderately good food organism, showed a sharp increase in growth between 20° and 25°C. However, within the temperature range from 25° to 33°C the rate of growth remained virtually the same.

Larvae given a mixture of our best food organisms, *M. lutheri* and *I. galbana*, together with *Dicrateria* sp. (BII) and *Chlorella* sp. (580), grew better at the same temperatures than when fed only *Chlorella*

sp. (580) or *Dunaliella euchlora*. In general, through the range from 20° to 30°C growth increased parallel with the increase in temperature. At 30° and 33°C, however, the larvae grew virtually the same and metamorphosis at both temperatures began consistently between the 10th and 12th days.

G. *Effects of salinity on eggs and larvae*

Bivalves, even though they belong to the same class of mollusks, display extremely wide differences in their salinity requirements and in ability to withstand sharp or gradual changes in salt content of sea water. Therefore, in determining minimum, maximum and optimal salinities for their existence each species, especially those populating estuarine regions, must be studied individually. For example, deltas of rivers, where salinities are relatively high most of the time, may be populated by both *C. virginica* and *M. mercenaria*, while a short distance above this line, where the salinity of the water is considerably lower than 20 parts per thousand, only oyster beds can be found because clams are unable to survive under such brackish conditions. To demonstrate the differences that may exist between two species that often may be found in the same environment, we shall briefly discuss the differences in salinity requirements of larvae of *C. virginica* and *M. mercenaria*.

Loosanoff (1952) found that the lowest salinity at which normal development of gonads of *C. virginica* of Long Island Sound may proceed is near 7·5 ppt. Continuing the study of various aspects of variations in salinity on propagation of American oysters, Davis (1958) demonstrated that 22·5 ppt was the optimum salinity for development of eggs of oysters that had grown in Long Island Sound and had developed gonads at a salinity of about 27 ppt. Some normal larvae developed, nevertheless, in salinities as low as 15 ppt and as high as 35 ppt. At salinities below 22·5 ppt the percentage of eggs that developed to straight-hinge larval stage steadily decreased until, at 15 ppt, only 50 to 60% of the eggs developed normally. At 12·5 ppt practically none of the eggs developed into normal shelled larvae.

In another experiment Davis used Maryland oysters that had grown and developed gonads in the upper parts of Chesapeake Bay where the salinity, at the time the oysters were collected, was only 8·7 ppt. These oysters were spawned at Milford Laboratory in salinities of 7·5, 10 and 15 ppt. Under these conditions some eggs developed into normal larvae even at 10 ppt and 7·5 ppt although, in the latter, slightly smaller than normal larvae were common. In general, the optimal salinity for normal development of eggs of these oysters from

very brackish water was between 12 and 15 ppt, while a salinity of 22·5 ppt was the upper limit.

When oysters that had developed gonads at a salinity of 27 ppt were used as parents, the optimal salinity for growth of their larvae, after they had reached straight-hinge stage, was 17·5 ppt. Good growth was also recorded at a salinity of 15 ppt, but at 12·5 ppt growth was appreciably slower, although some larvae grew to metamorphosis. At 10 ppt growth was practically at a standstill and it is doubtful that any larvae could reach setting stage at this salinity. The older the larvae, however, the better they withstood the salinity of 10 ppt. Larvae that were reared almost to setting stage at our normal salinity of about 27 ppt continued to grow and even metamorphosed when transferred to a salinity of only 10 ppt.

Davis (1958) also showed that the optimal salinity for development of eggs of *M. mercenaria* of Long Island Sound was about 27·5 ppt. No normal larvae developed at salinities of 17·5 ppt or lower. The upper salinity limit for development of clam eggs was 35 ppt, but only an occasional normal larva developed at that concentration of salt.

Straight-hinge clam larvae grew reasonably well at 17·5 ppt and many reached metamorphosis, but at 15 ppt none of them reached that stage, although some lived for 10 or more days and showed a slight increase in size. At 12·5 ppt straight-hinge clam larvae showed no growth and all were dead by the 10th day.

As can be seen from this brief comparison, eggs and larvae of *C. virginica* can normally develop and grow to metamorphosis in a much lower salinity than those of *M. mercenaria*. Undoubtedly, using present methods of cultivation of larvae, similar studies will soon be performed on other species of bivalves and prove to be as informative and useful as those reported in the recent article by Davis and Ansell (1962) on development of eggs and growth of larvae of *O. edulis* in water of different salinities.

H. *Effects of turbidity on eggs and larvae*

One of the least studied factors of molluscan environments is that of turbidity (Loosanoff and Tommers, 1948; Jørgensen, 1949; Loosanoff, 1962a). A review of the literature in this field (Jørgensen, 1960) shows that even though some work has been performed on adult mollusks, until the recent contributions of Davis (1960, and unpublished), virtually nothing was known of the ability of bivalve eggs to develop or larvae to survive in turbid waters. Davis employed a rotating wheel, to which culture vessels were attached, to maintain turbidity at definite constant levels. The turbidity-creating substances

used in his experiments were the same as those employed by Loosanoff and Tommers (1948) in their studies on behavior of adult oysters. They were fine silt collected from tidal flats, kaolin (aluminium silicate), powdered chalk and Fuller's earth.

Davis showed that silt was considerably more harmful to eggs of oysters, *C. virginica*, than to those of clams, *M. mercenaria*. For example, in concentrations of 0·25 g/l of silt only 73% of oyster eggs survived, while more than 95% of clam eggs developed to straight-hinge stage. Practically all clam eggs developed to straight-hinge stage in concentrations of 0·5 g/l of silt, while only 31% of oyster eggs survived.

In a suspension of kaolin and Fuller's earth, on the other hand, clam eggs showed much higher mortality than eggs of oysters. Thus, in concentrations of 1 g of these substances per liter of sea water, practically all oyster eggs developed to straight-hinge stage, while only 37% and 57% of clam eggs survived.

Strangely enough, of the materials which were tested in these experiments, silt, a natural substance, was more harmful to oyster eggs than either kaolin or Fuller's earth. While practically none of the eggs exposed to 1 g/l of silt reached straight-hinge stage, some eggs developed normally even in concentrations as heavy as 4 g/l of kaolin or Fuller's earth.

As in the case of eggs of these two species, silt was more harmful to oyster larvae than to clam larvae. At a concentration of 0·75 g/l of silt growth of oyster larvae was markedly decreased while, as a striking contrast, clam larvae grew normally even in 1 g/l of silt. Moreover, the majority of the clam larvae survived for 12 days and even showed some growth in 3 and 4 g/l although it is doubtful that they could reach metamorphosis under these conditions.

Kaolin and Fuller's earth were considerably more harmful to clam larvae than to oyster larvae. Concentrations of 0·5 g/l of kaolin caused about 50% mortality of clam larvae in 12 days and, while practically no clam larvae survived in concentrations of 1 g/l of either kaolin or Fuller's earth, growth of oyster larvae was not appreciably affected by 1 g/l of kaolin. Davis also found that some oyster larvae may live as long as 14 days in concentrations of 2 g/l of silt and up to 4 g/l of either kaolin or Fuller's earth. These observations demonstrated an unusual ability of larvae of *C. virginica* to withstand highly turbid water, a situation often existing in summer at or near mouths of rivers where natural oyster beds are found.

In several experiments, where small quantities of turbidity-creating materials were added to water containing straight-hinge larvae

of oysters or clams, growth of these organisms was stimulated, often becoming considerably more rapid than in control cultures. Possibly, this was a result of adsorption, by particles of such materials, of toxic substances formed in larval cultures. It is also possible that some of these materials that were added to the water contained a positive growth factor, as do certain soil solutions.

In summarizing the observations on the effect of turbidity on oyster and clam larvae, it may be concluded that larvae, as well as adults, are affected by turbidity-creating substances, although larvae seem to display a considerable tolerance towards some of these materials. Moreover, larvae of different species react differently, one species may be more tolerant or less resistant to the same material under the same conditions. It is significant, nevertheless, that in some instances comparatively light concentrations of common silt may strongly interfere with normal development of eggs of some bivalves.

I. *Effects of foods on growth of larvae*

In any laboratory, where large numbers of adult and juvenile mollusks are kept, these animals, because of limited space, are often crowded, receiving insufficient quantities of water and, therefore, food. To improve these conditions we add large quantities of artificially-grown plankton to the water flowing through troughs and trays.

Because of the scope of our work several hundred gallons per day of relatively rich phytoplankton are often needed. Obviously, it is impractical to grow such large quantities of plankton in glass flasks using common laboratory techniques. Fortunately, by merely adding complete commercial fertilizers to sea water, rapid growth of phytoplankton can be initiated and later maintained in heavy concentrations. Our experiments have shown that fertilizers designated by formulas 5-3-5 and 6-3-6, both used by Connecticut tobacco growers, gave the best results, although lawn fertilizer, 10-6-4, was also good (Loosanoff and Engle, 1942). Using these fertilizers, mass cultures of rich, mixed plankton have been continuously grown at our laboratory since 1938. A wooden, 2000-gal oval tank is used for this purpose, although on several occasions the cultures have been grown in outdoor, concrete, 10000-gal tanks. The sea water used in these tanks was passed through a sand filter.

To supply the laboratory in winter with a sufficient quantity of plankton we designed a special enclosure, resembling a greenhouse, in which a plankton-containing tank is installed (Fig. 21). By providing artificial light, when needed, and maintaining the temperature of the

enclosure at the desired level, the laboratory is now supplied with rich, mixed phytoplankton on a year-round basis.

A common difficulty experienced in growing phytoplankton in open tanks of several-thousand-liter capacity, as is done at Milford, is invasion of these cultures by zooplankton organisms. In our mass cultures the most common offenders are crustaceans, especially copepods. These forms multiply so rapidly in rich phytoplankton that they soon consume most of the plant cells, rendering the culture useless.

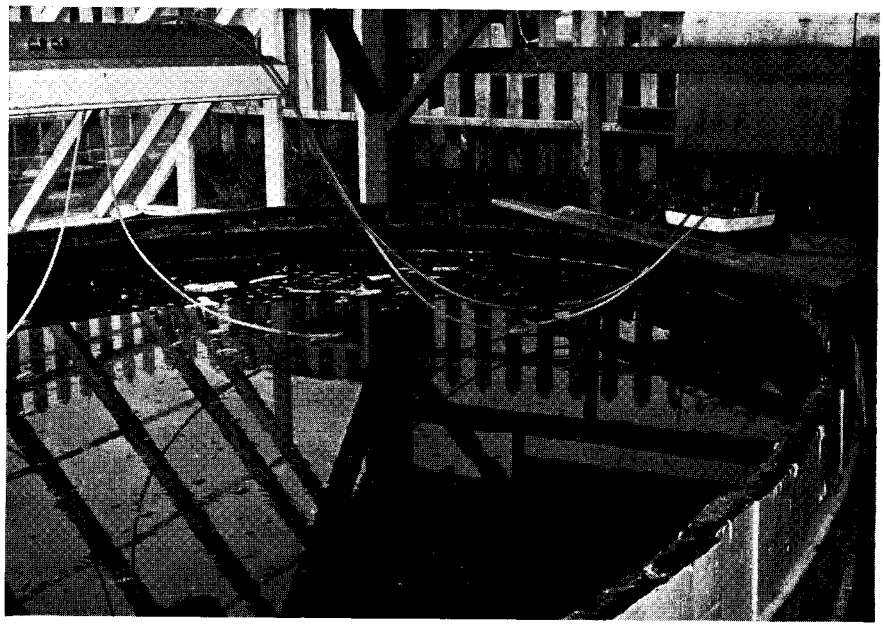

FIG. 21. Mass culture of mixed phytoplankton grown in large wooden tank of about 2 000-gal capacity under semi-outdoor conditions. Description in text.

In the past, several methods were tried to prevent such contamination of open-air algal cultures, but they usually were unsuccessful because some crustacean eggs were always left behind and eventually hatched, reinfesting the cultures. Recently, we developed an extremely simple and safe method to control these infestations by merely adding to our cultures, when necessary, small quantities of insecticides (Loosanoff *et al.*, 1957). Several of these substances have been tried and found successful in concentrations as low as 0·1 ppm. At present, we use a commercial preparation known as TEPP, which contains 40% of tetraethyl pyrophosphate. The advantages of using TEPP are

that it hydrolyzes within 24 to 48 hr and it has no permanent effect on algae; therefore, it does not impair the usefulness of the culture as a food for mollusks.

The mass culture grown in our tank is not a single species but a mixture, the composition of which varies from day to day or even hour to hour. This culture usually contains various species of *Chlorella*-like organisms, but because *Chlorella* is not one of the best foods for larval

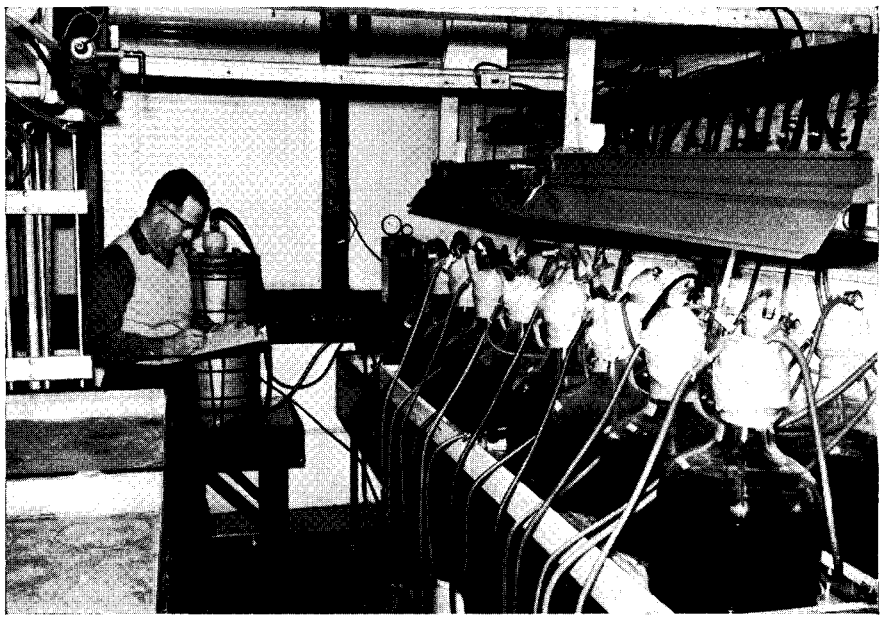

FIG. 22. Battery of specially-fitted, 5-gal Pyrex carboys serving as growth chambers for mass culture of photosynthetic microorganisms. Description in text.

and juvenile mollusks, it has become necessary to grow on a large scale such forms as have been recently found to assure good growth and low mortality of larvae. Moreover, because it was found by our European colleague (Walne, 1956, 1958) and at our laboratory (Davis, 1953; Davis and Guillard, 1958) that bacteria in sea water, especially in algal cultures, may seriously affect lamellibranch larvae, sometimes not only slowing their growth but causing complete, or almost complete mortalities, we made special efforts to develop a method by means of which virtually bacteria-free algal cultures can be grown. To achieve this an apparatus was devised and placed in operation at our laboratory

approximately 3 years ago and has since consistently given satisfactory results (Davis and Ukeles, 1961).

The culture apparatus consists of sixteen 5-gal Pyrex carboys as growth chambers (Fig. 22). Vigorous agitation, by bubbling a mixture of air and CO_2 through the cultures, keeps the contents of the chambers thoroughly mixed. This prevents stratification and helps to expose all cells to equal periods of strong illumination. The carboys are immersed to a depth of 3 or 4 in in a water bath kept at a desired temperature, usually $19° \pm 1°C$. About 3 liter of the algal culture from each growth chamber are harvested each day, yielding about 1·5 ml of packed wet cells. The present system, thus, produces daily approximately 50 liter of algal suspension, averaging 0·5 ml of packed cells per liter.

A volume of sea water, nutrient salts and antibiotics, equal to the volume of culture drawn off, is added daily to each growth chamber. The sea water used is first passed through Orlon filters, previously described, to remove larger particles. Nutrient salts are then added and this solution is forced through a ceramic bacteriological filter (Selas No. FP-128-03, maximum pore size 0·6 μ) into the growth chambers. Recently, we have been adding to the sea water and nutrient salts, prior to final filtration, 0·002% Acronize (approximately 10% chlorotetracycline) to reduce bacterial growth on the ceramic filters. This concentration of Acronize does not interfere with algal growth and helps prolong the life of the cultures.

Although *Chlorella* sp. and a number of other algae will grow on a media made from 5-3-5 or 6-3-6 fertilizers, a more elaborate media is needed for our bacteria-free cultures. Since the requirements of each of the more than eighty species of marine algae maintained at our laboratory have not been determined, we use the following as " universal " media, although it is recognized that the necessity for the various ingredients has not been ascertained. Two stock solutions of nutrient salts are prepared and 1 ml of each is used per liter of sea water.

Solution A

Dissolve in 1 liter of distilled water

NaH_2PO_4	20·0 g
Thiamine HCl	0·2 g
Biotin	0·001 g
B_{12}	0·001 g
Pyradoxine HCl	0·1 g
Calcium pantothenate	0·2 g

Solution B

Dissolve in 1 liter of distilled water

$NaNO_3$	150·0 g
*NH_4Cl	50·0 g
Ferric sequestrine	10·0 g

* Media for *Isochrysis galbana* should omit the NH_4Cl.

Experiments conducted at our laboratory have shown that some species of bacteria are harmless, others are strongly pathogenic, and still others produce toxic metabolites. It is quite possible, therefore, for some bacterized cultures of algae to be good larval foods, while the same algae, with a different bacterial population, may be acutely toxic. Consequently, for critical evaluation of any phytoplankton organism as a food for larvae it is necessary to use a bacteria-free culture of it. Furthermore, in using mass cultures of algae, where there is always danger of bacterial contamination, it should always be ascertained that the cultures have not become contaminated with toxic or pathogenic bacteria.

As already mentioned, certain species of algae also produce metabolites that are toxic to bivalve larvae, while other species produce little or none (Fig. 23). Some of the algae produce so much toxic material that they are useless as foods because their toxins kill larvae even when concentration of algal cells is too light to satisfy larval demand for food. Others, such as *Chlorella* (580), produce some toxic products but are still usable foods, provided that their concentrations are not too high.

Recently, Davis (1953) and Davis and Guillard (1958) concluded that presence and thickness of cell walls and degree of toxicity of metabolites are important factors in determining usability of photosynthetic microorganisms as larval food. They showed that the naked flagellates, *I. galbana* and *M. lutheri*, were of approximately equal value as food for larvae of the American oyster and induced more rapid growth than any of the other species tested. Davis also believes that *I. galbana* and *M. lutheri* produce little, if any, toxic external metabolites which unfavorably affect larvae. This is supported by his observations that the optimal concentrations of the two forms for either clam or oyster larvae were at least double the optimal concentrations of *Chlorella* sp. (Lewin's isolate).

The food value of microorganisms also depends, in part, upon how completely they meet the food requirements of larvae. It was found, for example, that a mixture of *I. galbana*, *M. lutheri*, *Platymonas* sp. and *Dunaliella euchlora* induced more rapid growth of both clam

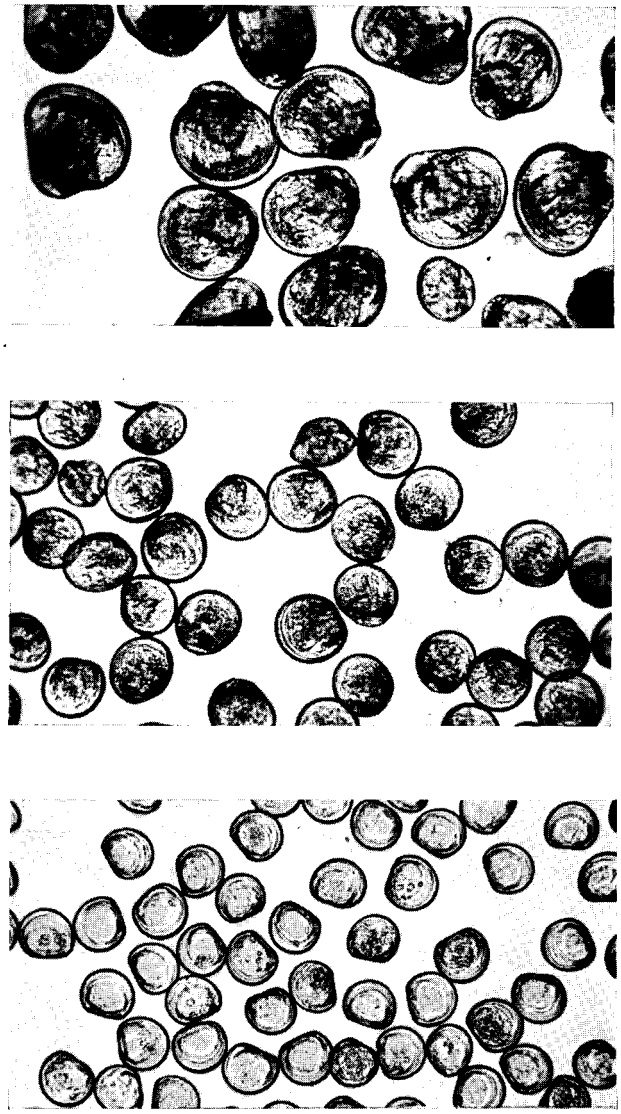

FIG. 23. Twelve-day-old larvae of *Crassostrea virginica* given different algae as food. Group fed *M. lutheri* (*upper photograph*) averaged 169 μ in length, while the middle group, serving as control and receiving no supplementary food, averaged 96 μ. The lower group, containing only dead larvae, was given *Stichococcus* sp. isolated from Great South Bay, Long Island, New York, which produces toxic metabolites.