and oyster larvae than did equal quantities of any of these foods separately.

D. euchlora and *Dunaliella* sp., both naked flagellates, as are *I. galbana* and *M. lutheri*, also induced better growth of oyster larvae during the first 6 days of their development than did any of the forms having cell walls. Thus, Davis concluded that, with the exception of *Prymnesium parvum*, which is toxic, even the poorest of the naked flagellates is a better food for young larvae of *C. virginica* than any of the organisms with cell walls. This conclusion supports the earlier one of Cole (1939) and Bruce *et al.* (1940), that nanoplankton may differ in their value as food for larvae of *O. edulis*. Davis found, nevertheless, that *Chlorella* (580), *Platymonas* sp., *Chlorococcum* sp., and *Phaeodactylum tricornutum*, all forms having cell walls, were utilized by larvae of *C. virginica*, but growth, particularly of younger individuals, was slow and might have occurred because of presence in the water of other food materials.

Observations on behavior of larvae of approximately twenty different species of bivalves grown at our laboratory have shown that, in general, as far as their qualitative food requirements are concerned, they can be roughly divided into two or, perhaps, three groups. The first group, well-represented by larvae of oysters of the genus *Crassostrea*, is able to utilize, during early straight-hinge stages, only a few of the many food forms (Davis, 1953; Davis and Guillard, 1958). The second group includes larvae of such species as *Mercenaria mercenaria* and *Mytilus edulis*, which seem to be able to utilize most of the microorganisms, provided that they are small enough to be ingested. The third, an intermediate group, also can be tentatively recognized. This group includes such larvae as those of larviparous oysters of the genus *Ostrea*, which are much less restricted in their qualitative food requirements than larvae of the genus *Crassostrea*, yet they are unable to grow quite as well on some of the foods as larvae of *M. mercenaria*.

The food requirements of the two marginal groups were clearly shown in our experiments where larvae of the oyster, *C. virginica*, and clam, *M. mercenaria*, were placed simultaneously and kept together in the same laboratory culture vessels or in large outdoor tanks. All cultures were given the same food, which consisted of mixed phytoplankton in which small, green algae, such as *Chlorella*, normally predominated. Under these conditions larvae of *M. mercenaria* grew rapidly and metamorphosed approximately in 12 days, while larvae of *C. virginica*, after attaining straight-hinge stage, showed virtually no growth and eventually died. The results of these early experiments were confirmed by Davis (1953), who showed that while young larvae of

C. virginica are unable to utilize forms having cell walls, such as *Chlorella*, older larvae of the same species become able to do so after they reach a larger size of approximately 110 μ.

In general, these studies have demonstrated several important points. One of them is that larvae of *M. mercenaria* can live and grow to metamorphosis on a very restricted diet, consisting of a single species of algae, such as *Chlorella*, and that unlike larvae of *C. virginica*, they can utilize these algae during all stages of development. Our conclusions, therefore, disagree with those of Cole (1936) who maintained that bivalve larvae, in general, do not possess the enzymes needed for digestion of cellulose, of which the cell walls of algae, such as *Chlorella*, are made.

As our techniques improved and we were able to evaluate the food value of different forms of phytoplankton, we found that larvae of *M. mercenaria* can be grown not only on a pure culture of *Chlorella*, reaching metamorphosis in some cases in 12 days, but that they can also be grown to metamorphosis on pure cultures of any one of the following three flagellates: *Chlamydomonas* sp., *Chromulina pleiades* or *I. galbana* (Davis and Loosanoff, 1953).

Our studies have also shown that organic detritus, at least of the types tested, cannot be utilized by larvae of clams, *M. mercenaria* (Loosanoff *et al.*, 1951), or oysters (Davis, 1953).

Larvae of *M. mercenaria* seem to be capable of both mechanical, or quantitative, and chemical, or qualitative, selectivity in feeding. They are able to regulate the amount of food ingested and thus survive in heavy concentrations of food cells, often containing less food in their stomachs than larvae kept in lighter food concentrations. Apparently, clam larvae are not merely mechanical feeders, but possess a mechanism by means of which they can control the food intake by rejecting algal cells, when necessary. However, if kept in heavy concentrations of algae for a long time, the larvae lose this regulating ability, become choked with food cells and, eventually, die.

We also observed that larvae can select certain food organisms from a mixture of several forms of phytoplankton. For example, when given a mixture of *Porphyridium* sp. and *Chlamydomonas* sp. larvae of *M. mercenaria* ingested the much larger cells of *Chlamydomonas*, while rejecting the cells of the smaller *Porphyridium* (Loosanoff *et al.*, 1953).

An important problem faced in connection with cultivation of bivalves was to ascertain the effects of different concentrations of food organisms upon larval survival and rate of growth. The first series of experiments was conducted with larvae of *M. mercenaria* in

concentrations of approximately seven larvae per ml, but fed different quantities of food, consisting principally of small *Chlorella* measuring only about 3 μ in diameter. These cells were fed to larvae in concentrations ranging from 6 500 to 1 million cells per ml of water in culture vessels. Simultaneously, another series of larval cultures was fed a unialgal strain of another *Chlorella*, the cells of which were about 8 μ in diameter (Loosanoff *et al.*, 1953).

Results showed that optimal concentrations of food cells clearly depended upon their size. When large *Chlorella* was given, the optimal concentration of this form for best survival and growth of larvae of *M. mercenaria* was approximately 50 000 cells per ml, while approximately 400 000 cells per ml of the smaller *Chlorella* were needed to achieve the same results. This suggests that the food value of 400 000 small *Chlorella* cells closely approached that of 50 000 cells of the larger form, both concentrations being near optimum. If the cells are considered as perfect spheres, the volume of 400 000 cells, 3 μ in diameter, is approximately equal to the volume of 50 000 cells, 8 μ in diameter.

A concentration of approximately 750 000 cells per ml of small *Chlorella* was already above the optimum because, when given so much food, the larvae grew more slowly than when given only 400 000 cells per ml of small *Chlorella*.

Larvae of *M. mercenaria* and many other bivalves can be killed if concentrations of food cells, such as *Chlorella*, become too heavy. Again, these concentrations depend upon the size and kind of cells. For example, approximately 90% of clam larvae were killed within a few days and those that survived grew very slowly or not at all when given approximately 300 000 cells of large *Chlorella* per ml of water. When the concentration was increased to 500 000 or more cells per ml, all clam larvae were killed within 24 hr. However, when given the much smaller form of *Chlorella*, which measured only about 3 μ, the larvae grew comparatively well in concentrations as high as 750 000 cells per ml.

It is of interest that larvae that managed to survive in heavily overfed cultures usually displayed certain anatomical abnormalities which, probably, made the larvae unable to ingest food. Perhaps these abnormalities were responsible for survival of these larvae under the conditions where normal individuals were killed.

We realize that, in studying the food requirements of bivalve larvae, the quantity of algal cells in the surrounding water constitutes only one factor needed to determine the adequacy of a food because this value may be subject to considerable variations according to the

age of the algal cultures, their density, chemical composition, bacterial flora and, of course, the media in which they are grown. These difficulties have been eliminated, to some extent, in our more recent experiments where production of food cells has been standardized (Davis and Ukeles, 1961).

As has previously been shown for adult bivalves (Loosanoff and Engle, 1947), their larvae can be killed either by a heavy concentration of algal cells alone, by the filtrate of algal cultures or by a combination of the two (Loosanoff *et al.*, 1953). In other words, dense concentrations of certain food organisms, such as *Chlorella*, affect larvae of *M. mercenaria*, as well as those of several other species, both mechanically, by interference of food cells with larval swimming and feeding mechanisms, and chemically, by producing external metabolites which are toxic to larvae. As an illustration, the larvae grew comparatively well when control cultures received approximately 100 000 cells per ml of large *Chlorella*, even though this concentration was somewhat above the optimum for this strain of algae. However, cultures of larvae receiving cells of *Chlorella*, that had been removed from the culture medium by Millipore filters and later resuspended in sea water at the rate of 1 million cells per ml, were rapidly killed. Similarly, larvae receiving the filtrate only from a certain volume of algal culture originally containing one million cells of *Chlorella* per ml also quickly died. These studies further showed that a filtrate containing heavy concentrations of metabolites of *Chlorella* cells may be even more detrimental to larvae than heavy concentrations of the resuspended cells themselves. As has already been mentioned, the ecological effects of external metabolites have long been recognized by aquatic biologists (Lucas, 1947).

Recently, Davis and Guillard (1958) conducted extensive experiments to determine the relative value, as larval food, of representatives of ten different genera of microorganisms. *I. galbana* and *M. lutheri* were the best foods and were approximately of equal value. In some experiments, nevertheless, *Chlorococcum* sp. was the best food for larvae of *M. mercenaria*. Clam larvae were also able to utilize several species of *Chlorella*, *Dunaliella euchlora*, *Dunaliella* sp., *Platymonas* sp., *Chlamydomonas* sp. and *Phaeodactylum tricornutum*. However, they could not utilize one species of *Stichococcus* or *Prymnesium parvum*.

Experiments also demonstrated that, as in the case of larvae of *C. virginica*, a mixture of *I. galbana*, *M. lutheri*, *Platymonas* sp. and *D. euchlora* promoted somewhat more rapid growth of clam larvae than did equal quantities of any of these foods separately.

Some of the algae tested at Milford Laboratory are given below with

the species listed in their approximately descending order of value as foods for larvae of *M. mercenaria* and *C. virginica*. Several other species tested are not listed here because they were either poor foods or were toxic.

M. mercenaria

A. Good Foods:
- *Monochrysis lutheri*
- *Isochrysis galbana*
- *Dicrateria* sp. (B II)
- *Chlorococcum* sp.
- *Platymonas* sp. (1)

B. Medium Foods:
- *Carteria* sp.
- *Chlamydomonas* sp.
- *Cyclotella* sp. (O-3A)
- *Chlorella* sp. (580)
- *Stichococcus* sp. (O-18)
- *Phaeodactylum tricornutum*
- *Skeletonema costatum*
- *Chlamydomonas* sp. (D)
- *Rhodomonas* sp.
- *Dunaliella* sp.
- *Olisthodiscus* sp.
- *Dunaliella euchlora*

C. virginica

A. Good Foods:
- *Monochrysis lutheri*
- *Isochrysis galbana*
- *Chromulina pleiades**
- *Dicrateria inornata**
- *Pyramimonas grossi**
- *Hemiselmis refescens**

B. Medium Foods:
- *Dunaliella euchlora*
- *Platymonas* sp. (1)
- *Cyclotella* sp. (O-3A)
- *Dunaliella* sp.
- *Chlorococcum* sp.
- *Chlorella* sp. (UHMC)
- *Phaeodactylum tricornutum*
- *Cryptomonas* sp.

* Not tested on clam larvae.

The most recent contribution to our knowledge of food requirements of the European flat oyster, *O. edulis*, was made by Walne (1956) who also reviewed efforts in this field by earlier investigators. Walne conducted over 200 tests using many species of algae. Although he states that many of his experiments were failures, often for no apparent reason, his results showed that the Chrysophyceae, especially *Isochrysis galbana* and *Chromulina pleiades*, gave good results, notwithstanding that *P. parvum* was toxic to larvae. Members of the Chlorococcales, which have a thick cell wall, were found to be poor food for the larvae of *O. edulis*. Of the Chlorophyceae, only *Pyramimonas grossi* was a good food.

In our experiments we found that larvae of *O. edulis* can be grown on a species of *Chlorella*, even though it has a thick cell wall. In this respect our results disagree with those of Walne and some of the earlier investigators. We agree, however, that naked flagellates,

especially *I. galbana* and *M. lutheri*, are the best foods for larvae of this oyster.

Imai *et al.* (1953) mentioned in several articles the successful use of the colorless naked flagellate, *Monas* sp., as a food for oyster larvae. Through the kindness of Dr. Imai we received a sample of a culture of this organism and grew it on a large scale. However, we have consistently been unable to raise larvae on a diet of this flagellate alone. In our controlled experiments larvae of *M. mercenaria* and *C. virginica* grew and survived better in unfed controls than when given 4 000 cells of *Monas* sp. per ml of water in culture vessels. Use of the same flagellate by oyster biologists of the State of Washington (Lindsay, personal communication), who also obtained these flagellates from Dr. Imai, always resulted in failure. Our conclusion that colorless flagellates are not a good food for bivalve larvae is also supported by recent observations of Walne (1956), who could not raise larvae of *O. edulis* by using another colorless flagellate, *Bodo* sp.

Growing of algal food for larval or juvenile mollusks, even if these cultures do not need to be kept unialgal and bacteria-free, presents a number of difficulties and, naturally, an easier method of producing food for bivalves, including adults, is desirable. Our experience, in the late 'thirties, in attempting to increase the glycogen content of adult oysters by feeding them pulverized dried algae, such as different species of *Ulva* and *Laminaria*, led us to try the same approach in feeding larvae with dried algae. In the initial experiments dried and pulverized *Ulva*, which we preserved and kept in a glass jar for over 20 years, was used. Surprisingly enough, many cultures of larvae of *M. mercenaria*, given this food, grew to metamorphosis. However, several problems were encountered in connection with this method. First, it was found difficult to grind these algae into particles small enough to be ingested by larvae. Secondly, the material quickly settled on the bottom, thus becoming unavailable to swimming larvae. Finally, because of rapid decomposition of unconsumed algae, the larval cultures became fouled and the bacterial flora in them quickly increased.

Fortunately, we have been able to overcome these difficulties. Through the courtesy of Dr. Hiroshi Tamiya of The Tokugawa Institute for Biological Research in Tokyo, Japan, and Dr. Hiroshi Nakamura of the Japanese Nutrition Association, we received samples of dried *Scenedesmus* sp. and *Chlorella* sp. Because the average size of the individual cells of both of these algae is small, they can be easily ingested by larvae. By feeding proper quantities of dried, ground *Scenedesmus* in larval cultures, which are continuously agitated by

plungers or horizontal-action paddles (Fig. 5), and by controlling the bacterial flora in the cultures by the addition of Sulmet, our associate, H. Hidu, to whom these studies were suggested, has been

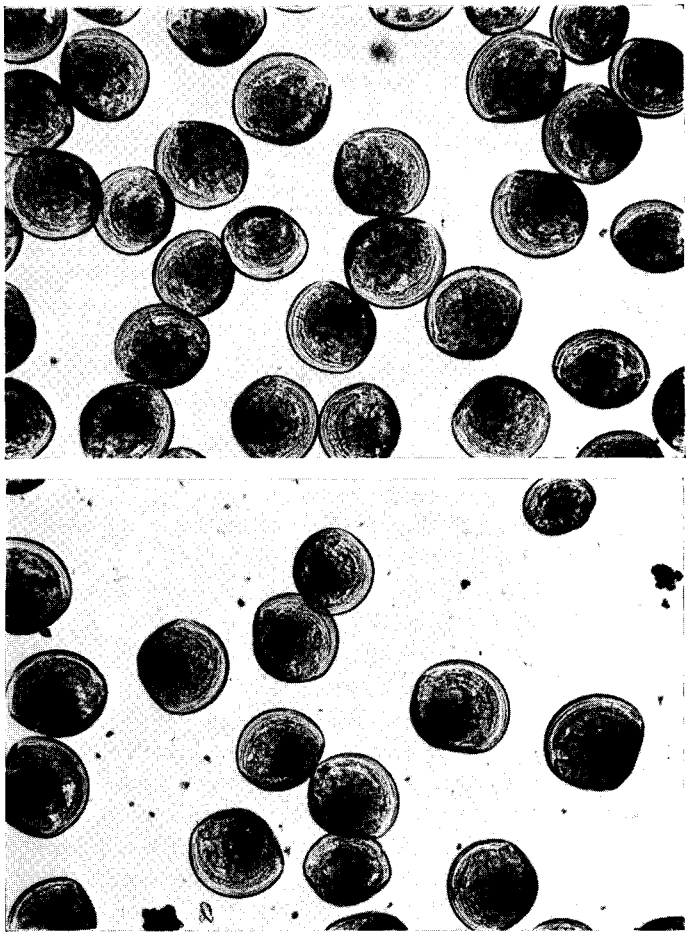

FIG. 24. Twelve-day-old larvae of *Mercenaria mercenaria*. Larvae shown in upper photomicrograph were fed freeze-dried cells of *Isochrysis galbana*, while those in the lower picture were given the same quantities of live cells of the same algae. Largest larvae are about 215 μ long.

able to grow larvae of *M. mercenaria* almost as effectively as when they are fed our best live food organisms, such as naked flagellates. In some experiments clam larvae fed freeze-dried *I. galbana* grew as rapidly as larvae in the control cultures given the same quantities of live cells of the same form (Fig. 24).

When fed *Scenedesmus* clam larvae grew best in concentrations of 0·02 ml packed food per liter of water per day. However, dosages as low as 0·005 ml packed food per liter gave just as good results in feeding larvae during their first 4 days of existence (Hidu, personal communication). Agitated cultures treated with Sulmet gave 50 to 100% better growth of larvae than identical unagitated cultures. The type of motion and size of agitating devices appeared to be of little significance to larval growth and survival. Mortality in all agitated cultures was negligible. In some situations, nevertheless, agitation may not be desired.

Experiments on growing larvae of American oysters on dried *Scenedesmus* have been unsuccessful thus far. We are, however, continuing experiments in this field using, at present in a dried state, such organisms as *I. galbana* and *M. lutheri* which, when alive, are readily utilized by larvae of *C. virginica*. Results of these and related studies will be reported in the near future by Mr. Hidu. It should be realized, nevertheless, that this sphere of research is now only in its initial phase and, therefore, it is too early to form general conclusions. We hope, however, that it will be extremely productive.

In discussing larval foods we would like to emphasize that color of the larvae themselves depends, to a large extent, upon color of food consumed. By feeding larvae differently colored food organisms we were able, especially during early straight-hinge stages, to change their color within a few hours.

Although the color of the shell proper was not always affected, the soft parts of the body quickly changed their coloration which, of course, reflected on the color of the larvae as a whole. It may also be added that according to our observations even the color of shells of recently set oysters growing in running water is darker than that of oysters of the same age that are kept in filtered sea water and fed naked flagellates. However, if these pale young oysters are transferred to natural water containing color-carrying phytoplankton, they quickly develop a much darker color. It seems logical to expect that in nature, where combinations of forms constituting phytoplankton change frequently, correspondingly frequent and rapid changes in the general color of larvae may also occur. We disagree, therefore, with workers who maintain that color is a dependable criterion for identification of bivalve larvae.

J. *Effects of crowding*

An important problem that faced us in early attempts to rear molluscan larvae was to determine maximum concentrations at which

they could survive and optimal concentrations for their growth. We expected these concentrations to depend upon species and quantity and quality of food, but soon found that such hardy varieties as *M. mercenaria* could be grown in concentrations of 50 to 100 larvae per ml of water. If these cultures were properly attended and fed, the majority of the larvae survived and, despite their slow rate of growth, they could, nevertheless, be reared to metamorphosis.

Although these were much heavier concentrations than those advocated by other investigators, who usually emphasize the danger of overcrowding, we sometimes reared larvae to metamorphosis even under more crowded conditions. However, since these observations were of a general nature, special quantitative experiments were undertaken (Loosanoff *et al.*, 1955). In these experiments larvae of *M. mercenaria* were grown in concentrations of about six, thirteen, twenty-six and fifty-two individuals per ml. Each culture received the same quantity of food, namely, 100 000 cells of a small strain of *Chlorella* per ml of water in the rearing vessels.

Larvae in all cultures showed good survival and grew to metamorphosis, although the mean rate of growth in the different cultures had an inverse relationship to population density. For example, on the 10th day the mean length of larvae in the series of cultures containing six, thirteen, twenty-six and fifty-two individuals per ml was 162, 156, 151 and 144 μ, respectively. Experiments showed that, as reported earlier (Loosanoff *et al.*, 1951), overcrowding clam larvae may not be as dangerous as formerly believed but, nevertheless, rate of growth in overcrowded cultures is slower and time of metamorphosis is correspondingly delayed.

Slower growth in the more crowded cultures may be attributed, in part, to more frequent collisions between the larvae and, in part, to the deleterious effect of greater concentrations of excretory products of larvae. Finally, the fact that there was less food per larva in the more heavily populated cultures was suspected as the most probable cause of slower growth. If this were true, rate of growth of larvae in densely populated cultures should be brought up to normal by a proportional increase in amount of food supplied. Accordingly, a new series of experiments was conducted in which number of cells of *Chlorella* was increased in proportion to increase in larval population. This was achieved by adding daily to the culture vessels containing six, thirteen, twenty-six and fifty-two larvae per ml of water, approximately 100 000, 200 000, 400 000 and 800 000 cells of *Chlorella* per ml.

After 10 days larvae in the least crowded culture averaged 162 μ in length, while larvae in the culture containing thirteen larvae per ml

averaged only 148 μ. The two other cultures, containing twenty-six and fifty-two larvae and given 400 000 and 800 000 *Chlorella* cells per ml, died within a few days. This and similar experiments showed, therefore, that crowding of larvae beyond certain limits cannot always

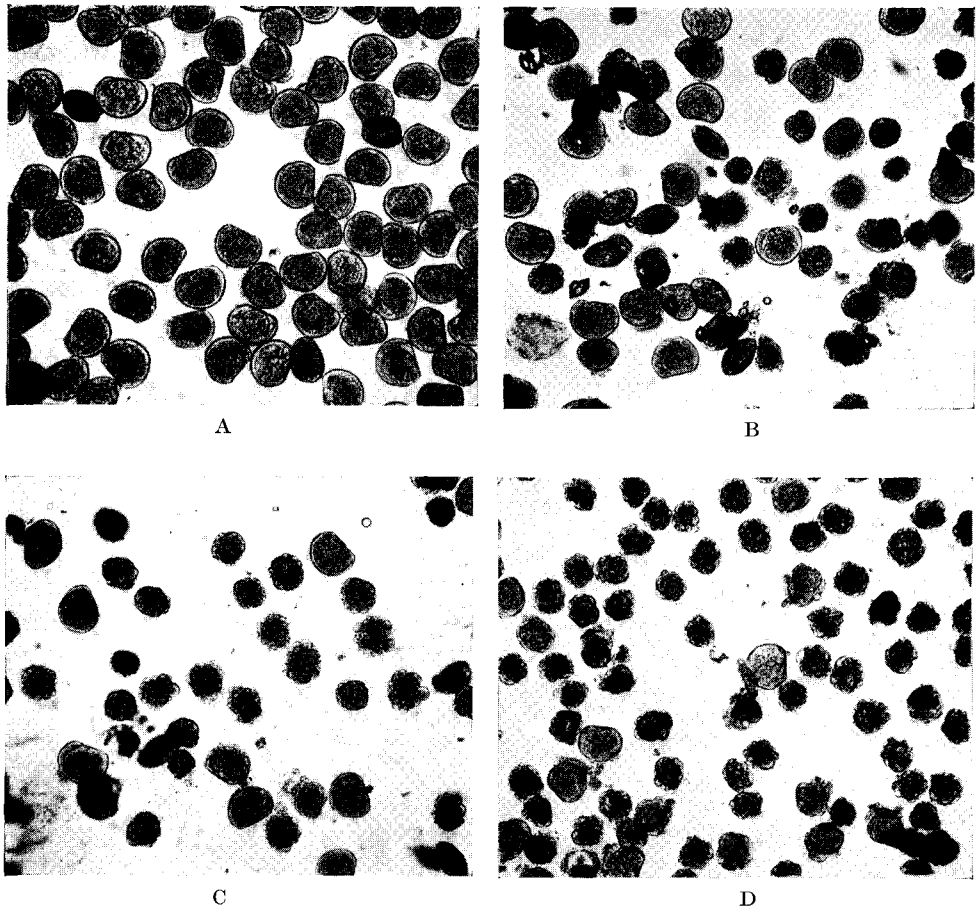

FIG. 25. Forty-eight-hour larvae of *Mercenaria mercenaria* developing from eggs cultured at different densities. A, 250 eggs per milliliter of water; B, 500 eggs per ml; C, 1000 eggs per ml; and D, 3000 eggs per ml.

be remedied by a proportional increase in quantity of food. It was also demonstrated that lethal concentrations of food organisms, as was the case when 400 000 and 800 000 cells of *Chlorella* per ml were given, cannot always be reduced to a safe level by the large number of larvae that presumably would rapidly consume these cells.

Still another experiment showed that eggs and larvae of *M. mercenaria* can develop under even much more crowded conditions than we have indicated thus far. In this experiment cultures were begun by placing in rearing vessels 250, 500, 750, 1000 and 3000 eggs per ml of water. The cultures were incubated at a temperature of about 24°C and examined at the end of 48 hr. The majority of eggs reared at a density of 250 per ml developed into normal straight-hinge larvae (Fig. 25), while in cultures that were begun with 3000 eggs per ml, most of the eggs failed to develop past morula stage and those few that managed to develop to shelled stage were abnormal. In intermediate concentrations of 500, 750 and 1000 larvae per ml some eggs managed to develop into normal straight-hinge larvae but their numbers decreased proportionally with each increase in the initial concentration of eggs.

Experiments on effects of crowding on growth of larvae of the American oyster, *C. virginica*, were described by Davis (1953). He used 640, 2 785, 18 518 and 32 918 48-hr-old larvae per liter and gave all of these cultures the same quantity of food, namely, 50 000 cells of *Chlorella* per ml of water. At the end of 14 days larvae from all cultures were examined and measured. Results showed that there was an inverse relationship between concentrations and rates of growth of larvae. Thus, observations on larvae of *C. virginica* were in agreement with similar observations on clam larvae.

On the basis of our results we can conclude that the danger from overcrowding larvae in well-maintained cultures may not be as acute as believed. Nevertheless, crowding is undesirable because it decreases rate of growth of larvae and, obviously, overcrowded larvae are more susceptible to diseases than are larvae in less densely populated cultures.

K. *Metamorphosis*

In such forms as oysters the end of the free-swimming and crawling periods is clearly terminated by attachment of larvae to some clean, hard surface. However, in most of the other bivalves, whose larval development has been studied at our laboratory, the precise or, often, even approximate moment of metamorphosis is difficult to determine because metamorphosis in such forms as *M. mercenaria*, as we have already indicated (Loosanoff *et al.*, 1951), is a gradual process. It is preceded by a stage in which advanced larvae have both a functional velum and a foot, and alternately swim about and crawl on the bottom. This stage, which has been appropriately termed " pediveliger " by Carriker (1956), may last, under certain conditions, for several hours

or, perhaps, even days. This opinion is indirectly supported by the suggestion of Thorson (1946), Wilson (1958) and other investigators that pediveligers of many bivalves are able to postpone metamorphosis if conditions of environment are unfavorable for completion of this important stage.

Literature contains few references to anatomical changes accompanying metamorphosis of bivalves. This is understandable because, until recently, few forms were successfully and repeatedly grown to this stage. Prytherch's (1934) description of metamorphosis in *C. virginica*, Cole's (1938) in *O. edulis* and, especially, Quayle's (1952) in *Venerupis pullastra* should be mentioned as contributing to our knowledge in this field. However, since a description of the major anatomical changes occurring during metamorphosis does not constitute a part of this paper, no references will be made to the details of this phenomenon in larvae of the mollusks studied by us.

As already mentioned, the number of days required for larvae of the same species to reach metamorphosis depends upon a complex combination of ecological factors, of which temperature and quantity and quality of food are the most important. Each species, of course, has its optimal temperature range for larval development, as well as minimum and maximum levels within which development is possible. Since all species that we cultured were grown to metamorphosis at room temperatures ranging from 18° to 24°C, it is quite certain that this part of the temperature range is comparatively favorable for development of most bivalves of our waters, as well as some of the exotic species with which we worked. However, information as to complete temperature range within which development of each of these species is possible is still lacking in most instances.

We have obtained such data for some species. For example, as already mentioned, in the case of *M. mercenaria* it has been ascertained that larvae grown at 18°C began to metamorphose 16 days after fertilization and continued to set as late as the 30th day. Thus, at this temperature, the period between beginning and completion of metamorphosis of larvae of the same cultures extended for 14 days. At a temperature of 24°C the earliest metamorphosing larvae were noticed on the 11th day, and the latest, 22 days after fertilization. At the considerably higher temperature of 30°C setting of clam larvae began as early as the 7th day after fertilization and was normally completed within 5 to 7 days, or by the 14th day, although in some of the more erratic cultures the last setting larvae were observed on the 16th day after fertilization (Loosanoff *et al.*, 1951).

Similar observations on larvae of *C. virginica* showed that, at a

temperature of 30°C setting usually began on the 10th or 12th day but, again, it extended for several days, as was the case with clam larvae. At lower temperatures the beginning of setting was delayed and its duration prolonged. For example, in one culture kept at a temperature of 24°C the larvae metamorphosed continuously for 27 days.

The number of days needed for larvae of the same cultures to reach setting stage is, therefore, not sharply defined even for a culture reared under standard, well-controlled conditions. In poorly fed, crowded or generally neglected cultures the beginning of setting is usually delayed and the time difference between beginning and end of setting is considerably extended. Sometimes, in cultures kept under poor conditions, towards the end of the experiment large numbers of larvae were found which were apparently unable to grow beyond a certain stage and eventually died without metamorphosing.

In earlier studies (Loosanoff and Davis, 1950) we were already aware that at the time of metamorphosis the size of larvae varied, even among those of the same species. At first, the smallest metamorphosing larvae of *M. mercenaria* were thought to be 210 μ long but, later, larvae measuring only about 175 μ but already going through this process were seen in the cultures. However, setting occurs most commonly when larvae measure between 200 and 210 μ in length.

The largest larvae do not always metamorphose first. Often, in the same cultures comparatively small individuals only about 180 μ in length will complete metamorphosis, while many larger larvae, exceeding 220 μ in length, still continue to swim.

Since there are several suggestions in the literature that invertebrate larvae reach a larger size before metamorphosis if they develop at low temperatures, we conducted a number of experiments to verify these suggestions (Loosanoff, 1959). This information was desired because if it were true that there was a definite relationship between setting size and temperature at which the larvae were grown, then it should be possible to devise a formula to predict setting size for each temperature.

The studies were conducted with larvae of *M. mercenaria* at temperatures ranging from 18° to 30°C. Within this temperature range there were no significant differences with respect to *mean lengths* of larvae at time of setting. Thus, although larvae grown at lower temperatures required more time before reaching metamorphosis, in all cases the larvae attained approximately the same mean lengths before setting. Furthermore, there was no apparent relationship between

maximum lengths of larvae at time of setting and water temperature. Finally, no relationship was found between shape of larvae (ratio of mean lengths to mean widths) at the time of setting and water temperature.

Incidentally, these studies gave us an opportunity to verify the opinion expressed by some authors that forms living in warmer waters have just as long a pelagic life as related species living in northern waters, and that eggs and larvae of southern species develop more slowly than those of northern species of the same genus if both species are kept under identical temperature conditions. We conducted these studies with *M. mercenaria* of Long Island Sound and *M. campechiensis* imported from the Gulf of Mexico. Both groups were ripened in our laboratory under identical conditions and induced to spawn by the usual methods. Eggs and larvae of both species were grown at a temperature of 21°C in triplicate cultures, and random samples of 100 larvae from each culture were taken for length and mortality measurements every two days from fertilization to metamorphosis.

Results clearly indicated that rates of growth and mortality of larvae of the two species were practically identical and that setting of larvae of both groups began simultaneously. On the basis of these observations we may conclude that the belief that eggs and larvae of southern species, when grown at the same temperatures, develop more slowly than those of the northern species of the same genus is not true in all cases.

As has already been mentioned, in some bivalves the beginning of metamorphosis may be delayed because of unfavorable conditions. In addition, in at least one case, namely, that of *Anomia simplex*, we observed what appeared to be partial or interrupted metamorphosis (Loosanoff, 1961). While in larvae of this species metamorphosis commonly occurs at a length of approximately 200 μ, hundreds of individuals considerably larger than 215 μ were seen in some cultures crawling on the bottom by means of a powerful ciliated foot. One of these individuals measured $577 \times 514\ \mu$.

Partial metamorphosis was characterized by disappearance of the velum but retention of a functional foot. Moreover, examination of these crawlers showed another common characteristic, namely, a definite narrow band on their shells, which probably indicated the edge of prodissoconch, or larval shell, and the beginning of dissoconch, or post-larval shell. We assume that the band dividing the two portions of the shell was formed during an important event in the life of the young mollusk, possibly when the velum had entirely disappeared or some other equally important anatomical or physiological change

had taken place. This conclusion is supported by measurements of the inner prodissoconch shells surrounded by the band, which were usually approximately 200 μ in length. This is the size at which setting of the majority of normal larvae of *A. simplex* occurred.

L. *Diseases of larval and juvenile mollusks and their treatment*

Even under the best conditions that can now be maintained in the laboratory there have been occasional heavy mortalities of larval and juvenile mollusks that cannot be accounted for by experimental treatment or errors in technique. Although in most instances this mortality was light, weak or dying larvae could usually be seen harboring heavy populations of bacteria or protozoans; in some cases the mortality was epizootic, killing nearly all young bivalves within a few days.

The field of diseases and parasites of larval and juvenile mollusks is entirely new because studies of this nature were impossible until successful methods for rearing larvae were developed. No information was available, therefore, as to whether the bacteria, protozoans, etc., observed in weak and dying larvae, were the primary cause of their death or merely secondary invaders and, perhaps, only scavengers. Although Davis (1953), in attempts to feed oyster larvae on bacteria, had shown that bacteria retarded growth of larvae and eventually killed them, it was not until 1954 that a fungus, *Sirolpidium zoophthorum* (Fig. 26), was found responsible for some of the epizootic mortalities in our cultures of clam larvae (Davis *et al.*, 1954; Vishniac, 1955). So far, fungus has been found to affect larvae of *M. mercenaria*, *M. campechiensis*, the hybrids of these two species, *Teredo navalis*, *Pecten irradians*, *Tapes semidecussata* and *C. virginica*. However, since it was observed and identified only recently, we have not established whether it can infect larvae of those bivalves which were reared at our laboratory prior to its discovery.

Not all of the forms mentioned above are equally susceptible to fungus. For example, while larvae of *M. mercenaria* and *Teredo navalis* are easily infected, the infection often assuming epizootic proportions, infection of larvae of *C. virginica* is extremely rare. The only case of this type occurred when a large number of fungus-infected clam larvae was introduced into a vessel containing young oyster larvae. Larvae of other forms, such as the bay scallop, *Pecten irradians*, and the Japanese clam, *Tapes semidecussata*, frequently became infected, but the disease seldom reached epidemic proportions.

In *M. mercenaria* and several other species, larvae of all ages, from very early free-swimming stage to those ready to undergo metamorphosis, can be parasitized by fungus. Juvenile clams up to 400 μ in

size have also been found severely infected. On many occasions hundreds of thousands of young clams of this size were found dead or dying within a day or two after the first signs of infection were noticed.

Our observations indicate that fungus is introduced into larval cultures with untreated sea water or, perhaps, more often with food organisms, such as phytoplankton grown in our outdoor tank in which the media is not sterilized. It may be the same organism that infects and kills small crustaceans, such as copepods, which are sometimes present in our outdoor cultures of phytoplankton.

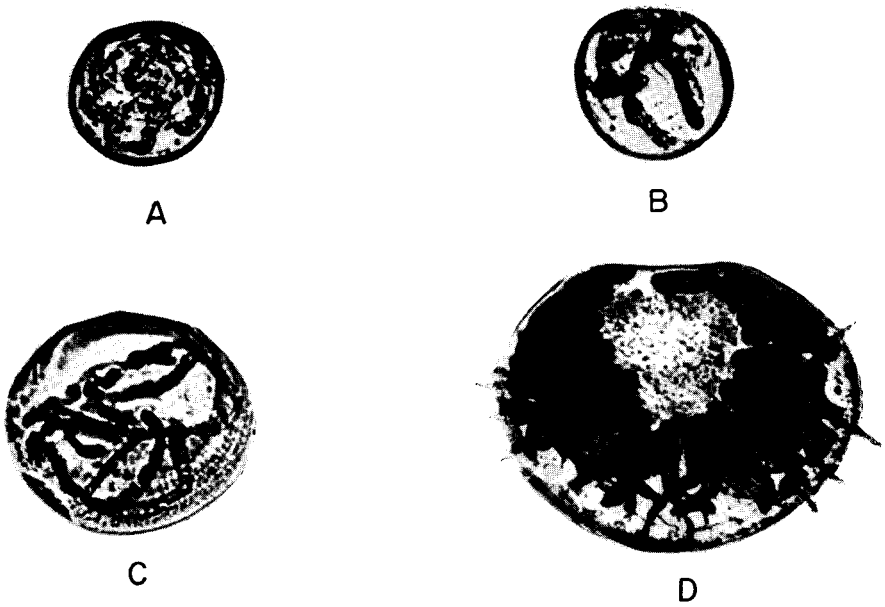

FIG. 26. Larvae infested with fungi. A and B, young larvae of *Crassostrea virginica* stained with Neutral Red; C, young larvae of *Mercenaria mercenaria* stained with Neutral Red; D, old larvae of *Mercenaria mercenaria* stained with Cotton Blue. × 225.

In seeking a method to control fungus infections in our larval cultures we studied the effects of many fungicides, including most of those used in fish hatcheries. So far, not a single promising compound has been found. As a rule, the chemicals unfavorably affected the larvae at concentrations lower than those at which the fungus was affected. However, precautionary measures, consisting largely in maintaining a general cleanliness of all utensils coming in contact with larvae and in using germicidal ultraviolet rays to treat all water, gave promising results. Plankton food, if it comes from impure mass

cultures, should also pass over the ultraviolet tubes. This measure, in addition to controlling the fungus, also protects larval cultures against invasion by undesirable forms, such as small crustaceans, larvae of different worms, rotifers and protozoa, which compete with bivalve larvae for space and food. In some instances raising the water temperature to approximately 32·5°C for several hours may kill fungus without causing serious injury to young mollusks.

Ultraviolet treatment of sea water for purification of shellfish has been described by several workers in Japan, and by Wood (1961) in England. Waugh (1958) and Walne (1958) also used ultraviolet-treated sea water for rearing larvae of *O. edulis*. To prevent fungus diseases of young clams we began using ultraviolet light in 1954 to treat sea water and soon found that this treatment was helpful in preventing mortality of juvenile clams kept in trays of running water. In the summer of 1955 it was found that larval cultures receiving ultraviolet-treated sea water, but untreated phytoplankton from the outdoor tank, developed fungus, while cultures receiving the same phytoplankton and sea water, both of which were treated, did not become infected.

Because of these observations and because it had long been recognized at our laboratory that many mortalities in larval cultures were caused by bacteria (Loosanoff, 1954; Davis and Chanley, 1956b), it became our standard practice, since the summer of 1955, to use filtered and ultraviolet-treated sea water in all larval cultures and in standing water trays containing recently set clams.

Walne (1958) also reported that bacteria may seriously affect larvae and Guillard (1959) demonstrated, while working at our laboratory, that certain bacteria produce toxins that can retard growth of larvae or kill them. Guillard isolated two virulent clones, one of which appeared to be a species of *Vibrio*, and the other, of *Pseudomonas*. His experiments showed that larvae of *M. mercenaria*, when exposed to virulent bacteria and treated simultaneously with antibiotics, such as penicillin and streptomycin sulfate, remained as healthy as the control cultures. High temperature, near 30°C, favors growth of bacteria, thus emphasizing some of the disadvantages of growing larvae at comparatively high temperatures. Bacterial metabolites, when present in high concentrations, may stop larval growth entirely. Guillard also noticed that bacterial contamination of algal food cultures sometimes caused a sharp decrease in growth of larvae without, however, causing immediate extensive mortality.

More recently our associates, Chanley and Tubiash, again isolated bacteria from moribund and dead clam larvae. When these pathogens were seeded into cultures of clam or oyster larvae they caused almost

complete mortality of larvae within 12 hr (Fig. 27). Later, these strains were reisolated from moribund larvae to satisfy Koch's postulates. These studies are still in progress and it appears that the recent isolation deals with different clones from those observed by Guillard.

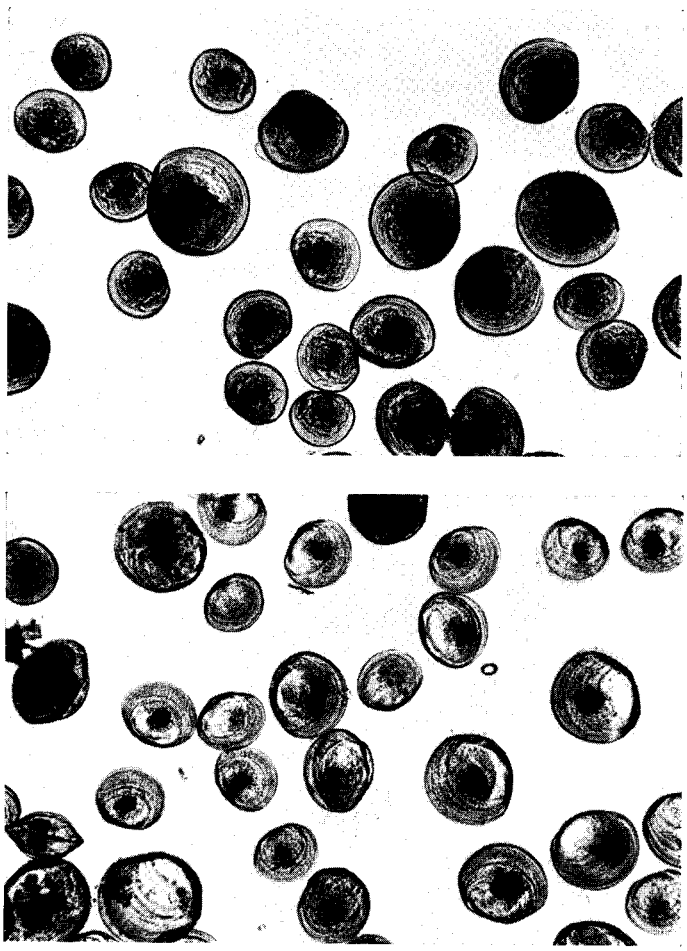

FIG. 27. Effect of pathogenic bacteria upon larvae of *Mercenaria mercenaria*. Healthy larvae (*above*); larvae from the same culture 12 hr after inoculation (*below*). Largest larvae in this photomicrograph are approximately 150 μ long.

Following recognition of the pathogenicity of fungi and bacteria we began testing a number of antibiotics and fungicides, in addition to using physical measures for prevention of diseases, to determine their effects on survival and growth of larvae (Davis and Chanley,

1956b). It was found that at low concentrations streptomycin, aureomycin, Combistrep (a mixture of dihydrostreptomycin and streptomycin sulfate), and Sulmet (sulfamerazine) increased the rate of growth of clam larvae but that, as concentrations were increased beyond certain limits, the rate of growth was retarded and, at still higher concentrations, the larvae were killed.

The most rapid growth of clam larvae occurred in cultures containing about 100 ppm of streptomycin or Combistrep, in about 33 ppm of Sulmet and in only 3 ppm of aureomycin. The increase in rate of growth of clam larvae in cultures receiving 3 ppm of aureomycin was not as great as in cultures receiving optimum concentrations of other antibiotics, such as streptomycin, Combistrep or Sulmet. The commercial preparations of terramycin, sulfathiazole and sulfanilamide were somewhat toxic in minimum concentrations tested and retarded growth of larvae. Combistrep, even at 100 ppm, which is the optimal concentration for clam larvae, retarded the rate of growth of oyster larvae.

In some tests streptomycin did not reduce larval mortalities but, for certain pathogens a closely related compound, Combistrep, in concentrations ranging from 125 to 200 ppm has proved to be quite effective. Acronize (chlorotetracycline HCl) at 10 ppm and chloromycetin at 20 ppm have also been found effective for certain pathogens. Furthermore, cultures of clam or oyster larvae, routinely receiving 33 ppm of Sulmet with each change of water, consistently showed either better survival or more rapid growth, or sometimes both, than larvae in cultures receiving no treatment. Although at this concentration Sulmet did not appreciably decrease the number of bacteria, in some instances, where larvae in untreated cultures grew poorly, treatment with Sulmet improved their growth remarkably. In these cases, we believe, poor growth of larvae in untreated cultures was caused by toxins produced by bacteria introduced with food cultures. The much better growth of Sulmet-treated larvae, therefore, must have been due either to a reduction in number of the bacteria that produced the toxins or to a removal of the toxin from solution by forming a nontoxic complex with the Sulmet.

We find that, in addition to antibiotics, several substances, including such unrelated compounds as phenol, the insecticides Lindane and Guthion, the weedicide Endothal and such presumably inert substances as silt and kaolin, when present in proper concentrations cause a similar improvement in rate of growth of larvae. We suspect that it is the result of removal of toxins from the water in larval cultures. This is, probably, accomplished either by adsorption on particles, such as

silt and kaolin, or by formation of nontoxic complexes with large organic molecules of antibiotics and pesticides.

We have also found that routine use of certain chemicals can improve survival of larvae and aid in control of many fouling and competing organisms. One of these extremely useful compounds is polyvinylpyrrolidone-iodine, known commercially as PVP-Iodine. Recently set clams will remain open and feed for at least 24 hr in concentrations of 100 ppm of this compound, and they can tolerate 250 ppm of it for 24 hr, although in this concentration they will remain closed most of the time. We have also found that as little as 50 ppm of this compound can protect juvenile clams from certain pathogenic bacteria and that, in cultures free from organic debris, 250 ppm of PVP-Iodine will kill bacteria, free-swimming and attached ciliates, some annelid worms and even crustaceans.

Good survival and growth of juvenile clams can also be helped by the following treatment: The cultures are treated overnight, for approximately 18 hr, with 100 ppm of PVP-Iodine. During this time the flow of water is stopped; the young bivalves, therefore, remain in standing water. Upon completion of this part of the treatment the water is turned on for a period of 48 hr, and then the second 18 hr of treatment with 250 ppm of PVP-Iodine in standing water is applied.

Pentachlorophenol at 1 ppm, formalin at 40 ppm, and dichlorophene at 10 ppm are other compounds that have been found useful in controlling certain bryozoans, ciliates and ascidians that sometimes infest trays or other containers of hatchery-reared juvenile mollusks.

M. *Selective breeding and hybridization*

Development of reliable standard methods for cultivation of larvae opened fields of studies of heredity of bivalves and their selective breeding. Using these methods it is now possible to cross individuals showing specific characteristics and attempt to develop strains, or races, of oysters, clams, mussels, scallops, etc., with such desirable characteristics as rapid growth, high glycogen content in their bodies, resistance to certain diseases and, finally, ability to propagate and exist under less favorable conditions. For example, the ability to reproduce at lower temperatures, say, within the range of 13°–16°C, may extend the oyster beds of such species as *C. virginica* several hundred miles northward of their present geographical limits.

Davis (1950) and Imai *et al.* (1950b) were among the first to start cross-breeding experiments of commercial bivalves. Davis found that crosses of *C. virginica* ♀ × *C. gigas* ♂ and *C. gigas* ♀ × *C. virginica* ♂ showed a lower proportion of eggs developing to veliger stage than did

non-hybrids. Moreover, neither group of hybrid larvae could be reared to metamorphosis, while under the same conditions non-hybrid larvae of *C. virginica* and *C. gigas* grew to setting stage. In both hybrid crosses fertilization and early larval development apparently progressed normally but about 5 days later, after reaching straight-hinge stage, the larvae died.

More recently, Imai and Sakai (1961) presented results of their extensive studies of crossing different strains of Japanese oysters, *C. gigas*. According to these authors the hybrids displayed a greater adaptability to environmental conditions than the inbred strains. Imai and Sakai also reported that hybrid oysters can be grown from crosses of *C. gigas* and *C. angulata*, while in the case of crossing *C. gigas* with *C. virginica*, *C. echinata* or *C. rivularis*, cross-fertilization might take place but hybrid larvae will not survive.

It has also been demonstrated that two closely related species of the hard shell clam, *M. mercenaria*, of Long Island Sound and *M. campechiensis* from the Gulf of Mexico can be successfully crossed (Loosanoff, 1954). Larvae resulting from these crosses were often grown to metamorphosis, perhaps as a result of hybrid vigor, at a faster-than-normal rate.

Many thousands of juvenile clams from these crosses, after they reached sizes ranging from $\frac{1}{8}$- to $\frac{1}{4}$-in, were distributed to a number of marine laboratories along our Atlantic and Gulf coasts for observations on their survival, behavior and growth under different conditions. Usually, together with the two groups of hybrids, non-hybrid juveniles of *M. mercenaria* and *M. campechiensis* of the same age as the hybrids were also included. A number of papers, including those of Haven and Andrews (1957), and Chestnut *et al.* (1957), described the observations on survival and growth of these four groups of young clams.

Chanley (1961) using the techniques for rearing larvae from crosses of selected clams showing certain peculiarities demonstrated that shell markings of *M. mercenaria notata* were inherited as a simple Mendelian character with incomplete dominance. In another series of experiments he crossed two unselected clams and used the faster growing offspring obtained from that cross as parents for the next generation. He compared growth of progeny of these clams with growth of progeny of two randomly selected individuals. When grown under the same conditions for 15 months progeny of the fast growing individuals were 60% larger than progeny of the randomly selected individuals.

V. Rearing of Different Species

Since much of the material discussed earlier in this article was

based on our observations and experiments on larvae of *C. virginica* and *M. mercenaria*, these species will be considered first. However, to avoid repetition of the material already discussed, information on rearing of larvae of these two bivalves will be presented in the form of a comparatively brief review.

A. *Crassostrea virginica* (Gmelin)

A discussion of the literature on various aspects of propagation of this species has already been given in the earlier part of this article.

By using different methods these oysters can be conditioned for spawning throughout the year and, if necessary, several times annually (Loosanoff and Davis, 1952a). Spawning is induced by raising the temperature within the range from 20° to 32°C, depending upon the previous temperature régime. Addition of suspensions of sex products helps to stimulate the spawning. Eggs can be stripped and fertilized.

Eggs of *C. virginica* vary in size from 45 to 62 μ, but the majority measure between 50 and 55 μ. Costello *et al.* (1957) stated that the size of the oyster egg varied between 45 and 54 μ. The size of the egg is not influenced by the size of the mother oyster. For example, eggs discharged by females 30 to 40 years old and over 9 in long averaged 50·4 μ and ranged between 48 and 54 μ in diameter, while the eggs from much younger and smaller females, approximately 6 years old and 4 in long, averaged about 51 μ, the difference being insignificant.

Under favorable conditions 90 to 95% of ripe, properly handled eggs can develop within 24 hr to shelled veliger stage. The smallest normal straight-hinge larvae measure approximately 68 $\times$ 55 μ. In 48 hr, at a temperature of about 22°C, the larvae measure about 75 $\times$ 67 μ. At this stage they already begin to take in particulate food.

The length of the larvae, measured parallel with the hinge line, continues to be approximately 5 to 10 μ greater than the width, measured from the hinge to the ventral edge of the shell, until the larvae reach the size of 85 $\times$ 80 μ. At 95 to 100 μ the length and width are approximately equal, but after that stage the increase in width is more rapid. When the length reaches 125 to 130 μ the width already exceeds it by about 10 μ. This 10 μ disparity between the two measurements remains virtually constant until the larvae reach metamorphosis.

The length–width ratio of larvae of *C. virginica* is almost linear, except for the section roughly confined to the length between 105 and 130 μ where it displays a tendency to form a flat curve. It is of interest that the length–width curve of the closely related species, *C. gigas*, the Japanese oyster, clearly shows the same tendency.

The majority of larvae of *C. virginica* metamorphoses between 275 and 315 μ in length, but occasionally free-swimming larvae may be 355 μ long. Larvae were grown to metamorphosis at temperatures ranging from about 17° to 33°C; at the latter temperature some larvae metamorphosed in about 12 days. Naked flagellates, such as *I. galbana* and *M. lutheri*, are the best foods, whereas some of the green algae with thick cell walls, such as certain *Chlorella* species, are not readily utilized by early veligers, especially at comparatively low temperatures.

Stafford (1912), in his paper on recognition of bivalve larvae in plankton collections, offered a description and drawings of larvae of *C. virginica* of different stages. His description and, especially, the length–width relationships of the larvae are widely different from ours. For example, Stafford gives the following length–width (depth) measurements, in microns, as typical for oyster larvae: 82·8 × 69·0; 110·4 × 96·6; 124·2 × 110·4; 151·8 × 131·1; 165·6 × 144·9; 207·0 × 193·2; 310·5 × 276·0; and 345·0 × 303·6. A comparison with our data shows that the majority of the above length–width ratios cannot apply to larvae of *C. virginica*. For example, we found that among the 130 of our larvae measuring 110 μ in length the width varied only from 105 to 125 μ. In other words, none of these larvae were only 96·6 μ wide, as indicated by Stafford. His discrepancies in length–width ratio are even greater when he gives measurements of larger larvae. Finally, his larvae always measure more in length than in width (depth) although, as has already been mentioned, when a length of about 125 μ is reached, the width of the larvae begins to exceed the length by about 5 to 10 μ and continues to do so until metamorphosis. Sullivan's (1948) measurements and photomicrographs of larvae of *C. virginica* are quite accurate.

The color of larvae varies somewhat with the food, but healthy larvae are usually a golden brown, particularly in the area of the digestive diverticulum. Young larvae are usually pinkish-orange *en masse*, but this color changes to brown and, later, to almost black as the larvae grow larger.

Larvae are highly active and remain in suspension throughout the free-swimming period. Large larvae, 200 μ or more in length, have a tendency to gather on the surface of the water and may appear as small " rafts " floating just beneath the surface. A pronounced " eye " spot develops, when larvae are about 270 μ in length, and is present throughout the remainder of the free-swimming period.

Larvae of *C. virginica* may be affected by fungus, *Sirolpidium zoophthorum*, and die, but they are much less susceptible to this disease than larvae of *M. mercenaria* and some other forms.

B. *Mercenaria* (= *Venus*) *mercenaria* (Linné)

Literature pertaining to the natural history and propagation of this species has already been reviewed.

These clams can be conditioned and induced to spawn throughout the year. As in the case of oysters, both chemical stimulation, by the addition of suspensions of sex products, and physical methods, by increasing the water temperature, are effective. However, stripped eggs, if not treated with certain chemicals, cannot be fertilized.

Eggs proper of these clams measure between 70 and 73 μ in diameter and differ from eggs of some other lamellibranchs in that they are surrounded by a thick membrane, the diameter of which ranges from

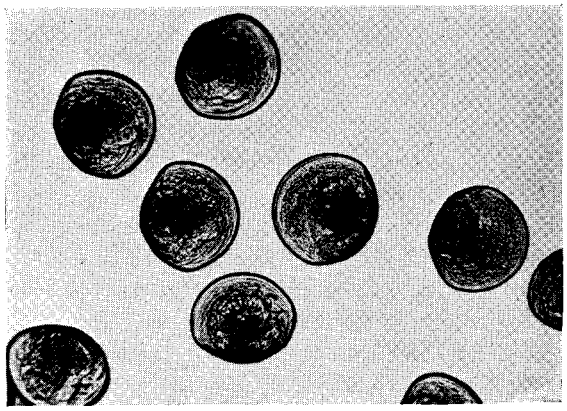

FIG. 28. Larvae of *Mercenaria mercenaria* approaching metamorphosis. Largest individuals in this group are about 200 μ in length.

163 to 170 μ. These clams are prolific. A single female was recorded to produce about 24 million eggs in one spawning.

The smallest but apparently normal straight-hinge larva measured 86 $\times$ 64 μ, although, usually, the size of early veligers is considerably larger, being approximately 105 $\times$ 80 μ and, at a favorable temperature, the majority of normal larvae are from 110 to 115 μ in length.

The size at which metamorphosis occurs varies considerably. Larvae ranging from 175 to 240 μ were seen at this stage. Nevertheless, metamorphosis occurs most commonly when larvae are between 200 and 215 μ in length (Fig. 28). Jørgensen (1946) found that metamorphosing veligers of the closely related species *Venus gallina* are from 210 to 225 μ in length. In our case, as indicated above, variations in the maximum size of prodissoconch of *M. mercenaria* are even greater than those indicated by Jørgensen. However, in our extensive experi-

ence larvae of these clams have never approached the size of 320 μ, as reported by Sullivan (1948). In a letter to us Miss Sullivan explained that she had confused larvae of *Pitar morrhuana* with those of *M. mercenaria* and ascribes the error to the circumstances that newly set spat of both species were present in her collection (Loosanoff *et al.*, 1951).

Our observations on maximum size of larvae reached before metamorphosis also show that it is highly improbable that Stafford (1912) was actually describing larvae of *M. mercenaria* because he stated that veligers of this clam grew to a size of 448·5 μ before setting. Furthermore, when the shapes of the larvae shown in Stafford's figures are compared with those of larvae grown from eggs at our laboratory, it becomes clear that Stafford mistook larvae of some other bivalve for those of *M. mercenaria*.

Food requirements of larvae of *M. mercenaria* are less restricted than those of some other species, such as *C. virginica*. Clam larvae can be grown to metamorphosis on a diet consisting almost exclusively of a unialgal culture of *Chlorella* sp. However, such foods as *I. galbana* and *M. lutheri* promote better growth.

Larvae have been grown at temperatures ranging from 15° to 33°C. At the latter temperature setting occurred 6 to 8 days after fertilization.

During early stages the color of the larvae can be significantly changed by differently colored foods. However, as the larvae grow and their shells become darker, this effect becomes less noticeable. Larvae of the same cultures greatly differ in size, especially if they are not properly attended or are too crowded.

Larvae of *M. mercenaria* are not too selective with regard to the substratum on which their metamorphosis is completed. They metamorphose in containers made of a variety of substances, including glass, plastics, earthenware, Plexiglas, concrete and several other materials. This is contrary to observations on other forms, such as echinoderms (Mortensen, 1938) and polychaetes (Wilson, 1948), where it was found that mature larvae postponed metamorphosis until they located a substratum possessing the necessary physical and chemical characteristics. In our cultures setting usually began when some larvae were approximately 180 μ in length, regardless of the size of containers or material of which they were made.

Setting of clam larvae occurred at approximately the same time, whether they were grown in containers from which all light was excluded or in transparent glass vessels. Turner and George (1955) found that clam larvae were rapid swimmers capable of moving upward at the rate of 8 cm/min. However, they showed no detectable reaction to light.

C. *Arca transversa* Say

No description of larval development of *A. transversa* or any references to its spawning could be found in the literature.

Our attempts to condition and spawn *A. transversa* in winter met with failure or only partial success. Later in the season, about the middle of May, some of the " arks " brought in from Long Island Sound and placed directly in water of 20°C spawned lightly within 2 or 3 hr. No normal larvae, however, could be grown from these eggs. Nevertheless, by placing the same " arks " in running sea water at 15°C from 3 to 7 days to condition them, spawning was easily induced by raising the water temperature to 27° or 28°C. Both males and females

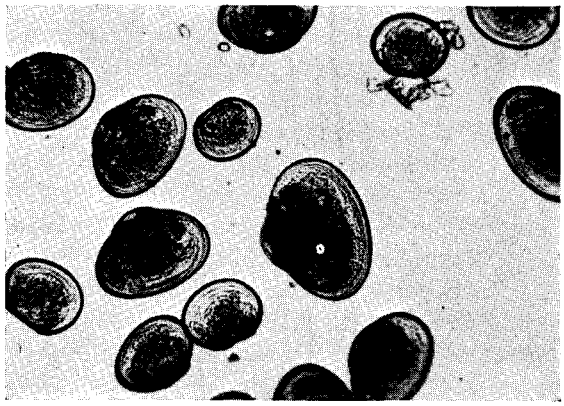

FIG. 29. Larvae of *Arca transversa*. Largest larva, approximately 230 μ in length, may be approaching metamorphosis. Note differences in sizes of larvae, all of which are of the same age.

spawned profusely, releasing gametes in streams similar to streams of sperm ejected by male American oysters.

The eggs of *A. transversa* are pinkish-orange in color and average approximately 52 μ in diameter. They are surrounded by a very thin, almost indistinguishable membrane. The sperm head is roughly triangular in shape, with a broad centerpiece. The anterior part of the head is " pinched " off in a short, nose-like structure. The head and centerpiece of the spermatozoa are between 3 and 4 μ in length, while the tail is from 47 to 55 μ long.

Larvae were grown at room temperature, which was approximately 20°C, and fed mixed phytoplankton composed principally of *Chlorella* and closely related forms. Under these conditions the larvae set from 27 to 37 days after fertilization.

Larvae of *A. transversa* are quite distinctive in appearance resembling, in general, their adults, particularly as they grow larger. The smallest normal straight-hinge larva measured 70 μ long and 55 μ wide. Thus, even in the youngest larvae, the difference between length and width is quite considerable. However, when larvae are near setting, the length may exceed the width by about 70 μ (Fig. 29).

The larvae are dark brown in color. As they grow larger, their color becomes darker and one end of their shells becomes more pointed than the other. Under the microscope larvae appear quite variable in outline because their odd shapes cause them to rest in a variety of positions.

The first indication of an umbo begins to appear soon after the larvae pass 105 μ in length, and at 130 μ it usually is quite conspicuous. When the larvae reach approximately 140 μ in length the pointed, or less rounded end of the shell becomes distinctly reddish-brown, while the opposite, more rounded end remains almost brown. This color difference is quite pronounced in many individuals.

An " eye " spot appears when the larvae are approximately 225 μ long. In some individuals this spot is not too conspicuous at first, but as the larvae approach metamorphosis it becomes quite prominent. Slow-growing larvae may develop the " eye " spot at a much smaller size. For example, in at least one case it was observed in larvae that were only 205 μ in length.

As is usual in rearing larvae of bivalves, considerable variation was noticed in setting sizes. The smallest, virtually metamorphosed individuals measured only 215 μ in length, while one larva in the same culture measured about 310 μ and, although it had a large foot, was observed actively swimming. This may have been a somewhat abnormal individual because a majority of the normal larvae in our cultures set when only 240 to 260 μ long.

Upon reaching metamorphosis recently set clams develop a strong byssus by which they attach to particles of sand, shells or other material.

Unlike larvae of *M. mercenaria* and several other species, which are easily affected by fungi, no epizootics that could be ascribed to these organisms were observed in our cultures of *A. transversa*.

D. *Modiolus demissus* (Dillwyn)

M. demissus, the common ribbed mussel of our waters, is also referred to by some authors as *Volsella demissa*, *Volsella plicatula* and *Modiolus plicatulus*. A review of the literature shows that, except

for Sullivan's (1948) article, there are no other references to larval development of this species. Considerable literature, nevertheless, exists on larvae of *Modiolus modiolus* and closely-related forms. These references can be found in the papers of Jørgensen (1946) and Rees (1950).

Various methods used to stimulate spawning of other bivalves proved unsuccessful for ribbed mussels. On two occasions, however, mussels kept in aquaria spawned without artificial stimulation. In both cases spawning occurred at night and, when the eggs were found in the morning they were already undergoing segmentation. Thus, no measurements of recently discharged eggs were made. Jørgensen

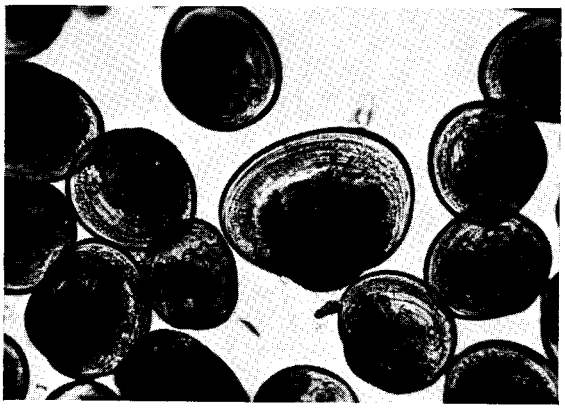

FIG. 30. Larvae of *Modiolus demissus*. Note differences in sizes of larvae that originated from the same group of eggs and were reared in the same vessel. Largest larva in center is approximately 300 μ long.

(1946) states that the diameter of an egg of a closely related species, *Modiolus modiolus*, is about 100 μ.

Larvae developing from eggs released during two normal spawnings were reared to metamorphosis. At room temperature straight-hinge stage was reached in about 24 hr. The majority of the larvae at that time measured from 110 to 115 μ in length and 85 to 90 μ in width. The smallest normal straight-hinge larva was only 105 μ long.

If kept at a temperature of about 22°C and given good food, such as *I. galbana*, many larvae reached a length of 200 μ by the 10th day. A few, upon reaching this size, developed a prominent " eye " spot and functional foot. Nevertheless, although completely metamorphosed juveniles of a size slightly above 220 μ in length were observed in some cultures, the majority of the larvae did not lose their vela until they

reached 275 μ, and a few individuals measuring between 295 and 305 μ in length still retained a functional velum.

While some larvae metamorphosed in about 12 to 14 days after fertilization, many others in the same cultures were still free-swimming when the experiment was discontinued after 43 days (Fig. 30). In one of these cultures, while there were still free-swimming larvae from 250 to 295 μ long, there were also several thousand completely metamorphosed juvenile mussels that ranged up to 1 mm in length. Why so many of the larvae were still in the free-swimming stage, while others in the same cultures had metamorphosed and showed considerable post-larval growth, remained unexplained.

According to Sullivan (1948) the smallest straight-hinge larvae of *M. demissus* measure approximately 112 $\times$ 90 μ. This size quite closely agrees with our measurements. However, the maximum size given by Sullivan, 205 $\times$ 180 μ, is considerably smaller than that observed in our cultures, which was about 303 $\times$ 260 μ. We also differ with Sullivan on the color of ribbed mussel larvae because she states that these larvae are a deep yellow in the early stages, whereas we found that throughout their development they are brown.

Jørgensen (1946) discussed larvae of closely-related species. In his references to *Modiolus modiolus* Jørgensen stated that larvae of this species have not been identified with certainty in plankton samples taken in Danish waters. He thinks that this is probably due to the very close resemblance of this species to veligers of *Mytilus edulis*. We disagree with Jørgensen since, in our studies of larvae of these two species, we found them to be distinctly different in their length–width measurements. Furthermore, the difference in their general appearance, especially during later stages of development, is too great to confuse these two groups of veligers.

Rees (1950) stated that in the smallest veligers of *Modiolus* and *Mytilus* the shape is similar, although in the former the narrow end is less pointed. Nevertheless, in later stages the shapes of the two genera become more differentiated. Rees also thought that the small specimens of the two mussels are distinguished more easily by their texture than by their shape. He concluded that Jørgensen's drawing (Fig. 167d) is probably of *Modiolus modiolus*, instead of *Mytilus edulis*, as claimed by Jørgensen. However, examination of the photomicrographs of, presumably, *Modiolus modiolus* offered by Rees clearly shows that their shape significantly differs from that of *Modiolus demissus* grown in our cultures from known parents. This is especially true of his larvae, approximately 250 μ in length, which have very little resemblance to our larvae of the same length.

E. *Mytilus edulis* Linné

A study of the literature on lamellibranch larvae shows that veligers of *M. edulis* have been described more frequently than those of other bivalves. Borisjak (1909) was probably the first to describe these larvae while, more recently, descriptions of presumably the same veligers were given by Jørgensen (1946), Sullivan (1948), Rees (1950) and others.

Mussels with apparently morphologically-ripe gonads are found in our waters in early spring, but under laboratory conditions, using our method of maturing animals in warm water, ripe mussels can be made available in January.

Field (1922) reported that rough handling of mussels, such as shaking them in a dish of sea water, will induce spawning within an hour, but Costello *et al.* (1957) found this method unsuccessful. In our case, during studies conducted for 3 yr, except for brief periods in April and early May, all efforts usually employed to stimulate bivalves to spawn failed to work with *M. edulis*. These included sharp increases or decreases in temperature, addition of sex products to the water, keeping mussels in air overnight, placing them in refrigerators for various periods, increasing or decreasing salinity of the water, and even applying light electrical shocks. This was true also of mussels that were conditioned in the laboratory for a considerable time and whose gonads appeared to be morphologically ripe.

Fortunately, it was noticed by two of our associates, first, W. Arcisz, and several years later, J. Uzmann, that some ripe female mussels released large numbers of eggs after their adductor muscles were cut.

These observations suggested a number of experiments on inducing spawning in mussels. The first attempt consisted of opening a large number of these mollusks and placing each individual in a separate vessel. The results were rather gratifying because, in the majority of cases, the mussels discharged large quantities of normal sex products. The method worked even on mussels that were not laboratory conditioned. For example, on 26 April 1951, mussels were brought in from Milford Harbor, where the water temperature was 8°C. The adductor muscles of twelve females were severed and the mussels were placed in water at room temperature. The first two females spawned within 12 hr; four others deposited masses of eggs within 24 hr, and six remaining females released eggs toward the end of the 48-hr period. This experiment was repeated on several occasions giving virtually the same results.

Eggs released by females with severed muscles were usually discharged in " strings ", as occurs in normal spawning. The majority