REARING OF BIVALVE MOLLUSKS

VICTOR L. LOOSANOFF* AND HARRY C. DAVIS

U.S. Bureau of Commercial Fisheries Biological Laboratory, Milford, Connecticut

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* *New address:* U.S. Bureau of Commercial Fisheries, Shellfisheries Laboratory, Tiburon, California.

I. Introduction

Until recently rearing of larvae and juveniles of marine bivalves, on a basis where repeatable results could be expected, was virtually impossible because of the lack of satisfactory, reliable methods. Thus, although culturing of larvae of bivalves was first attempted in the last century, few workers succeeded in rearing them to metamorphosis and, as a rule, they were rarely grown beyond early straight-hinge stage. Even though, in the twenties, Wells (1927) was able to rear the American oyster, *Crassostrea virginica*, from artificially-fertilized eggs to spat, and Prytherch (1924) raised larvae of the same species in large numbers, their results could not be consistently repeated by other investigators. The failures were usually due to poor culture methods and want of good food for the larvae, especially when they were grown in heavy concentrations. It is also possible that diseases, including those caused by fungi, were responsible for the persistent failures.

Attempts to rear larvae of bivalves were not confined, of course, to *C. virginica*. Cultivation of larvae of several other species was also tried by early workers. For example, Belding (1912) attempted to raise larvae of clams, *Mercenaria mercenaria* (formerly *Venus mercenaria*), but without success. He concluded that there was no practical method for raising clam larvae to straight-hinge stage because of the small size and delicate nature of the egg. Wells (1927), however, was more successful and carried the clam larvae in his cultures until they metamorphosed.

Even in more recent years the situation remained practically the same. This is well demonstrated by the work of Yoshida (1953) who, in his attempts to identify larvae of Japanese bivalves, had to depend upon obtaining the larvae from plankton, instead of trying to grow them from fertilized eggs under controlled laboratory conditions where their identity would be assured. The difficulties experienced as recently as 1953 by Nikitin and Turpaeva (1959), in their attempts to raise larvae of some bivalves of the Black Sea by using old methods, vouch for the inefficiency of these now obsolete approaches.

Obviously, as the general studies of marine organisms progress, the necessity for methods by means of which bivalve larvae can be reared successfully becomes more and more urgent. The availability of such methods would immediately offer the opportunity to study the effects of numerous environmental factors, singly and in combination, upon the growth of larvae, thus helping to determine the physiological requirements of these organisms. It would also offer the means for studying the genetics of bivalves and initiating properly controlled experiments on selective breeding of these mollusks. Moreover, by

growing larvae under different conditions their diseases and parasites could be studied and methods for their control developed. Finally, because the larvae of many species of bivalves are much alike in size and appearance, it was virtually impossible to identify them, with any degree of accuracy, in plankton collections. With the recent development of methods of rearing larvae in the laboratory, however, this difficulty should soon disappear because larvae found in plankton can now be easily and accurately compared with preserved samples and photomicrographs of larvae grown from known parents under controlled conditions.

By using successfully conditioning and rearing methods, many aspects of which were developed at Milford Laboratory (Loosanoff and Davis, 1950; Loosanoff, 1954) and are described in this article, larvae of approximately twenty species of bivalves have been cultured at Milford. Not all of these species are indigenous to New England waters or even to our Atlantic coast. Several are native to the Pacific and one species came from Europe. The non-indigenous forms were representatives of commercially important species in which we were interested. The bivalves, the larvae of which have been reared from fertilization to metamorphosis, included the transverse arc clam, *Arca transversa*; the ribbed mussel, *Modiolus demissus*; the common mussel, *Mytilus edulis*; the bay scallop, *Pecten irradians*; the jingle shell, *Anomia simplex*; the European oyster, *Ostrea edulis*; the native Pacific coast oyster, *Ostrea lurida*; the American oyster, *Crassostrea virginica*; the Japanese oyster, *Crassostrea gigas*; Morton's cockle, *Laevicardium mortoni*; the hard shell clam, *Mercenaria* (—*Venus*) *mercenaria*, and its relative, *Mercenaria* (—*Venus*) *campechiensis*; hybrids of these two species; the Japanese clam, *Tapes semidecussata*; the small clam, *Pitar* (—*Callocardia*) *morrhuana*; the rock borer, *Petricola pholadiformis*; the razor clam, *Ensis directus*; the surf clam, *Mactra* (—*Spisula*) *solidissima*; the soft shell clam, *Mya arenaria*; and the common shipworm, *Teredo navalis*.

Of the above species the larvae of *Crassostrea virginica* and *Mercenaria mercenaria* have been studied most intensively and, as a result, we have accumulated an extensive knowledge of their physiological and ecological requirements (Loosanoff and Davis, 1950; Loosanoff *et al.*, 1951; Loosanoff and Davis, 1952a; Loosanoff and Davis, 1952b; Davis, 1953; Loosanoff, 1954; Loosanoff *et al.*, 1955; Davis and Chanley, 1956a; Davis, 1958; Davis and Guillard, 1958; Loosanoff, 1958a; Loosanoff, 1958b; Loosanoff, 1959; and Davis, 1960). Several other species, such as the European oyster, *Ostrea edulis*, and the Olympia oyster, *Ostrea lurida*, have also received much attention.

Most of the other species, however, were studied less intensively, work on them being confined to culturing their larvae and observing the appearance and general behavior of the latter. Naturally, our knowledge of the requirements of larvae of such species is still fragmentary but, nevertheless, we shall present the information already available even though it is admittedly incomplete.

II. Equipment

The rearing of larval and juvenile bivalves requires an adequate supply of sea water of proper salinity and free of substances that may interfere with their normal development. The water used at Milford Laboratory is pumped from the Wepawaug River at a point about 100 yd from its entrance into Long Island Sound. Because the tidal rise and fall in this area is from 6 to 10 ft, the flushing rate of this comparatively narrow and shallow inlet is relatively high.

The sea water is pumped into a 6 000-gal wooden storage tank located in the laboratory attic. Because pumping normally takes place 1½ hr before and after the high tide stage, the salinity of the water is usually near 27 parts per thousand, which is virtually the same as in Long Island Sound, where the majority of the forms, the larvae of which are described in this article, exists. To assure a supply of water of high salinity the intake of the salt water system is located approximately 4 ft below the mean low water mark; therefore, it is at a safe distance from the surface layers which, after periods of heavy rains, may be greatly diluted.

The main pump providing the laboratory with salt water is rubber-lined. The intake and distribution lines, as well as the check and cutoff valves, are made of lead. The faucets, however, are of hard rubber. The storage tank is of cypress wood and is painted inside with asphalt paint.

We prefer lead pipes because, although pipes made of several new plastics are nontoxic, light and inexpensive, they possess several important disadvantages. One of them is that since it is often necessary to reduce fouling inside of the pipes by treating them with hot water or steam, this treatment, commonly used with lead pipes, cannot be employed in systems containing plastic parts as it may cause damage, especially at the joints of the pipeline.

Another serious disadvantage in using plastics is that they adsorb and absorb many chemicals, including insecticides, and once contaminated can themselves become a source of later contamination of the sea water. Moreover, since some plastics are permeable to insecticides and other compounds, these materials might enter from the sur-

rounding soil into pipes carrying sea water. Finally, some laboratories that have plastic sea water systems have complained that since these pipes are not electrically self-grounded, they present a serious element of danger in laboratories with wet floors.

We are finding an increasing usage for plastic pumps and pipes, especially in our temporary installations. We have also found that tanks made of Fiberglas, instead of wood, can be advantageously used, especially in areas where wood-boring organisms, such as *Teredo*, are common.

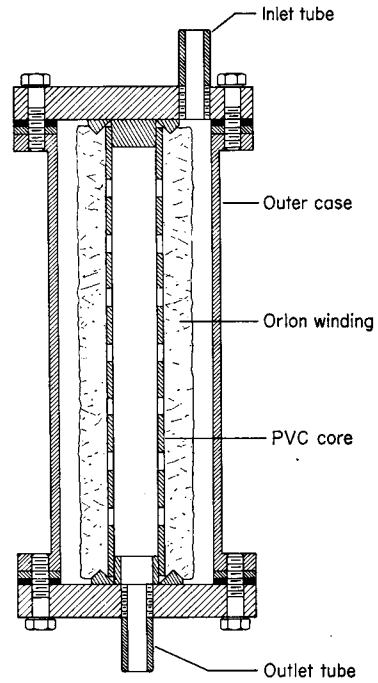

FIG. 1. Diagram of water filter designed to remove all particulate matter larger than 15 μ in diameter. Description in text.

Normally, in addition to small algae on which larval and juvenile mollusks feed, sea water contains many large diatoms, free-swimming crustaceans, gastropods, worms, etc., and their eggs and larvae. Many of these forms compete with bivalve larvae for food, prey on them or may even harbor diseases or parasites that could be transmitted to larvae. We prevent undesirable organisms of larger sizes from entering our larval cultures by filtering the water and later killing the smaller forms with ultraviolet light.

The filter element consists of a polyvinylchloride (PVC) core wound with Orlon. The complete unit (Fig. 1) is manufactured by Commercial Filters Corporation, Melrose, Massachusetts (filter no. CFXl-10-5

with an O15-R10X filter element). These filters, designed to remove all particulate matter larger than 15 μ in diameter, are made with a variety of core and winding materials. We chose the PVC core because it is nontoxic, and the Orlon winding because it is inexpensive, nontoxic and does not support bacterial growth.

To prevent fungus diseases in clam larvae and juveniles we began treating sea water with ultraviolet light in 1954 and, within a short time, had some evidence that such treatment, even of running, unfiltered sea

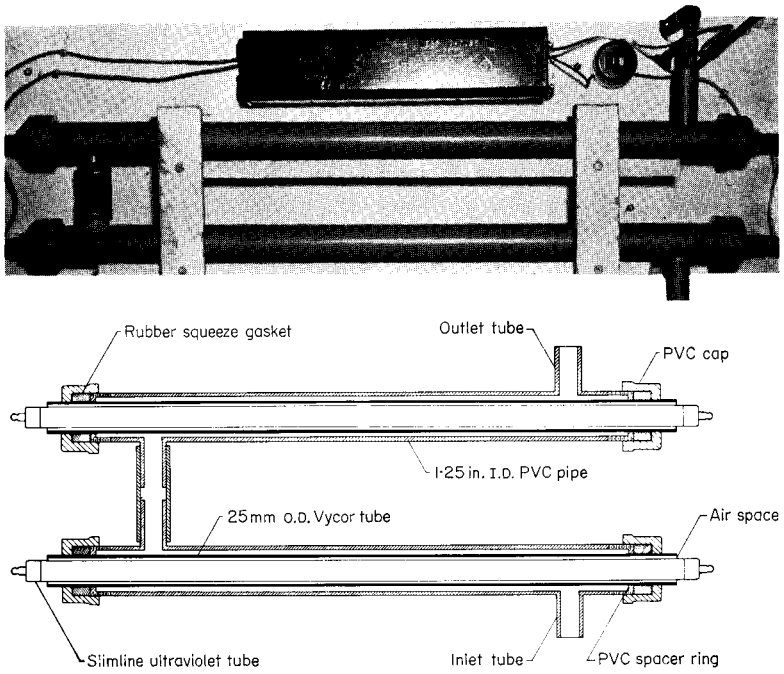

FIG. 2. Photograph (*above*) and drawing (*below*) of ultraviolet water treatment unit used at Milford Biological Laboratory. Description in text.

water, was helpful in preventing mortality of juvenile clams. In the summer of 1955 it was definitely demonstrated that larval cultures, receiving treated water and untreated phytoplankton from the outdoor mass culture, developed fungus, whereas larval cultures in which phytoplankton and sea water were both treated did not. Since that time, it has become a routine practice to treat with ultraviolet light all sea water used for our larval cultures and for keeping recently-set clams and oysters. Moreover, we are attempting to supply ultraviolet-treated running sea water to all containers in which later stages of juvenile clams are grown.

Ultraviolet treatment of sea water for purification of shellfish has been described by several workers in Japan (Sato, 1954; Satoh, 1960) and Wood (1961) in England. As is the practice in our laboratory, Waugh (1958) also used ultraviolet-treated sea water for rearing larvae of the European oyster, *O. edulis*. Several of these authors have described the equipment used but, because of certain considerations, we constructed our own units, a description of which is offered here.

The ultraviolet water treatment unit consists of a $1\frac{1}{4}$-in inside diameter PVC pipe, 30 in long, threaded at each end for caps (Fig. 2). A small ring of PVC is cut to fit inside of each end of this pipe and reamed to act as a spacer for a 25-mm Vycor tube. A squeeze gasket is used to make a water-tight seal between the Vycor tube and the end of the PVC pipe. An inlet tube is located on the side at one end of the PVC pipe and an outlet tube is located on the opposite side at the other end. The 33-in-long, slimline ultraviolet tube lays free in the $32\frac{1}{4}$-in-long Vycor tube and extends slightly beyond at each end.

In practice we use two such units connected in a series so that the water passes the length of both tubes. Since there is only about a $\frac{1}{8}$-in layer of water surrounding the Vycor tube, this apparatus, when used with filtered sea water, should give practically sterile water at the rate of flow of about 10 gal per min. With unfiltered sea water the efficiency is not expected to be as great, but our experience has shown that even then the treatment is of considerable help in reducing mortality of juvenile clams and in preventing fouling by tunicates, worms and bryozoa.

To condition mollusks for out-of-season spawning it is necessary to keep them in running sea water at temperatures of 18° to 20°C or sometimes higher. Warm sea water is also needed for rearing larvae and juveniles during the cold season. Since the water must not contact toxic metals, conventional water heaters cannot be used. Therefore, to heat the water we use a type of heat exchanger (Loosanoff, 1949). The sea water is heated as it passes through a coil of lead pipe immersed in hot fresh water, which fills the tank of a conventional gas water heater that has had the top removed to permit insertion of the lead coil (Fig. 3). However, because the thermostatic controls of a conventional water heater are not sufficiently accurate, the gas flame is controlled through a solenoid gas valve by a Minneapolis-Honeywell thermostat (T415A323XA3). The thermostat-sensing bulb is encased in a lead well in the warm sea water line and maintains the temperature at 37°C $\pm$ 0·5°C.

By mixing varied amounts of cold and heated sea water any temperature between that of the unheated water and 37°C can be main-

tained. In our winter work, when the temperature of the water in Milford Harbor is near freezing, we often simultaneously employ streams of water at 5°, 10°, 15°, 20°, 25°, 30°, and even 35°C. Of course, any other temperature within this range can also be maintained by using constant level jars of cold and warm water and regulating the

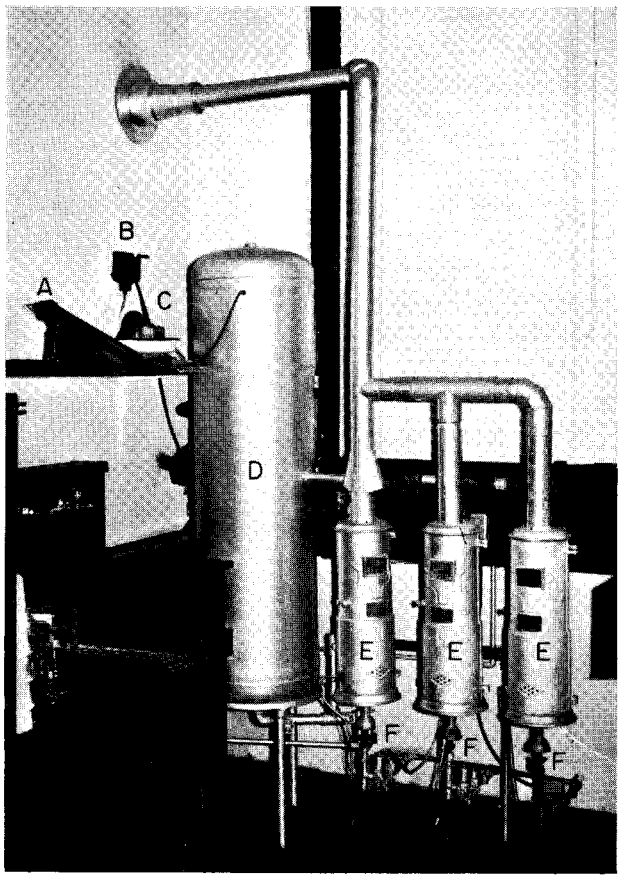

FIG. 3. Heat exchanger to provide laboratory with warm sea water. A, thermostat-sensing bulb; B, thermostat; C, air pump to prevent stratification of fresh water in tank; D, tank; E, gas water heaters; F, solenoid gas valves.

flow from these jars into a mixing chamber from which water of desired temperature flows into trays or aquaria where experimental animals are kept (Fig. 4).

To keep larval cultures at desired temperatures various constant temperature devices are used. Since a temperature of about 24°C is

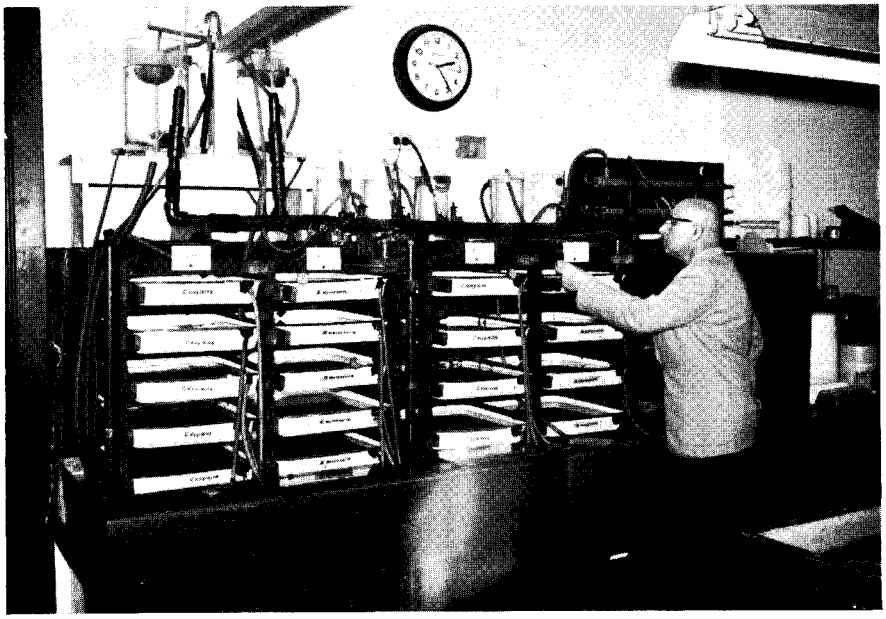

FIG. 4. Racks of trays for conditioning bivalves for spawning. Racks are provided with running sea water of different temperatures. Constant level jars for warm and cold water are seen in upper left corner. Water from these two jars is mixed in required proportions in the smaller glass jars located on lower shelf. Ultraviolet unit for treatment of water is located at right.

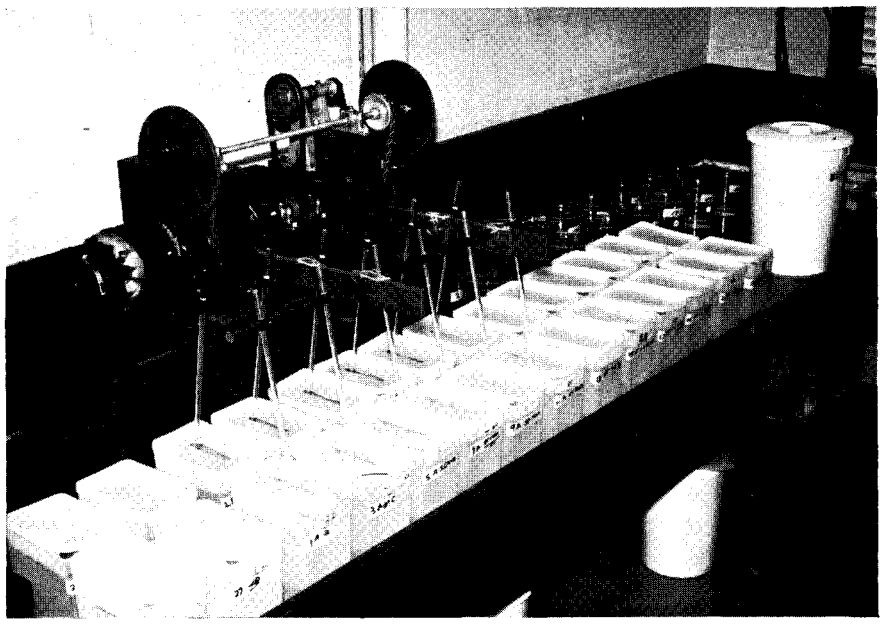

FIG. 5. Constant temperature water bath used to maintain desired temperature in culture vessels, which are partially immersed in water circulating in bath.

near the optimum for growth of algae, such as *Isochrysis galbana* (Ukeles, 1961), which are the best larval foods, this temperature level is often maintained. However, because 24°C is somewhat above normal room temperature, our simplest and most commonly used temperature control devices are lead-lined water tables, 3 ft × 12 ft, that serve as constant temperature water baths (Fig. 5). These tables are filled to a depth of 3 to 4 in with tap water, which is kept in constant circulation by a pump that takes water at one end of the table and discharges it

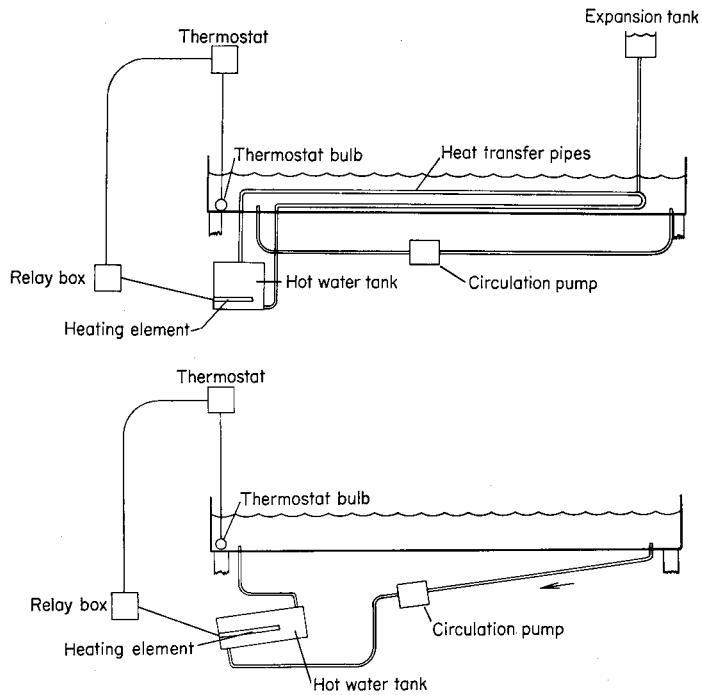

FIG. 6. Two methods of heating water for constant temperature baths. Diagram of closed system above, and open system below.

at the other end. A Minneapolis-Honeywell thermostat (T415A323XA3) with the sensing bulb enclosed in a lead pipe, immersed in the water on the table, controls a 1000- or 1500-W immersion heater. The heater may be either inserted in the pipeline, through which the circulating pump transfers water from one end of the table to the other (open system) or it may be inserted in a small hot water tank and the heat transferred to the water on the table by passage of the water from the tank through a lead coil or loop immersed in the water on the table (closed or heat exchange system) (Fig. 6).

Whenever the larval cultures are to be kept at about 24°C any type of container, the lower part of which is immersed in the bath, will maintain this temperature because convection currents within vessels prevent temperature stratification. Even in containers of

FIG. 7. Constant temperature apparatus consisting of 6 units. Temperature of each unit can be adjusted independently and maintained at any desired level within the range from 5° to 37°C. If necessary, all units may be maintained at the same temperature.

different sizes and shapes the temperature will vary only slightly, while in a series of individual containers of the same type the water will be maintained at almost precisely the same temperatures.

A similar arrangement can be used to maintain temperatures below that of the room by employing a liquid cooler, instead of a

heater. Units that combine a heater and cooler and will maintain temperatures above or below room temperature are also available. However, when cooling devices are used it is necessary to keep the water in cultures continuously agitated to prevent temperature stratification.

To study the effects of different temperatures on development of eggs and larvae of bivalves another apparatus was devised (Fig. 7). This apparatus, which can also be used for studies of many other forms, consists of a series of six lead-lined tanks, each 15 in wide by

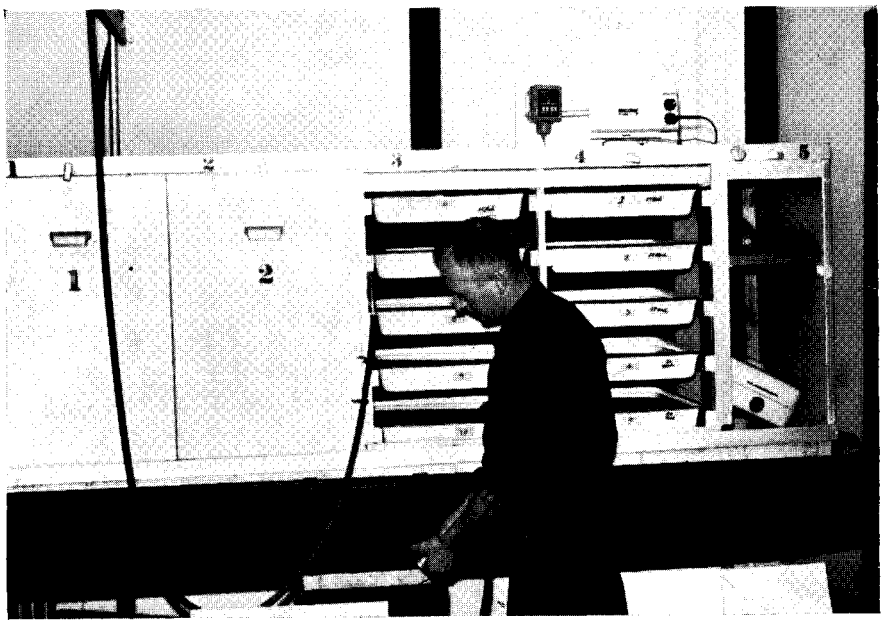

FIG. 8. Constant temperature air chambers in which trays or other containers with larval or juvenile mollusks are held. Electric heater and fan for circulating the air can be seen in right hand corner of unit.

26 in long and 13 in deep and filled with fresh water to a desired level. Vessels containing experimental animals are immersed in these tanks.

To maintain water in the tanks at desired temperatures each tank is equipped, along its walls, with loops of tubing to circulate cold and hot water. The amount of water passing through each of these tubes is controlled by double-action thermostats which activate solenoid valves so that if the temperature in any tank falls below the thermostat setting, the valve in the line, through which hot water circulates, opens, allowing hot water to flow through the loop. If, on the other

hand, the temperature exceeds that indicated by the thermostat, the solenoid valve in the cold water line opens, allowing circulating cold water to reduce the tank temperature. To have the entire mass of water at uniform temperature a circulator pump is employed. The temperature controls of the entire unit are so arranged that all tanks may be maintained at the same temperature, within the range from 5° to 37°C or, if necessary, at different temperatures.

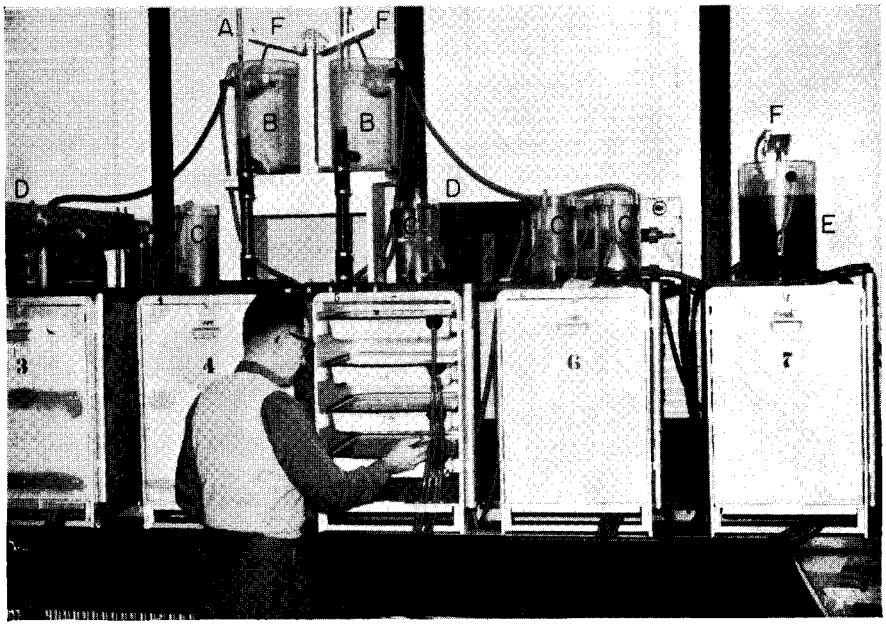

FIG. 9. Temperature apparatus for simultaneous studies of certain aspects of behavior of juvenile mollusks in running water of different but constant temperatures. A, escape tube for air trapped in sea water lines; B, cold and warm water constant level jars from which water in different proportions enters mixing jars (C). Each jar may be maintained at any temperature ranging from 5° (in winter) to 35°C; D, ultraviolet units through which all sea water passes to constant level jars; E, constant level jars from which phytoplankton is added at a definite rate to running sea water; F, floats controlling levels in constant level jars.

To control the temperature of the air chambers in which culture vessels are kept in some experiments thermostatically-controlled electric heaters are used. For example, in experiments, where a series of four banks of five trays each of standing water are used to hold juvenile clams, a uniform constant temperature is maintained by enclosing all twenty trays in a chamber where thermostatically-controlled electric heaters are installed (Fig. 8). When heated air is used, however,

special precautions are necessary to prevent its stratification. To achieve this in the enclosure where our racks of trays are kept a large fan forcing air through has been found sufficient.

Still another temperature apparatus is used at our laboratory for simultaneous studies of growth of juvenile mollusks in running water of different but constant temperatures (Fig. 9). The entire apparatus consists of seven independent units, each insulated so as not to be affected by outside temperatures. As many as five trays may be placed in each chamber. By mixing, in winter, different proportions of warm and cold sea water, temperatures ranging from about 5° to 35°C can be maintained quite accurately.

The amount of water entering each tray can be adjusted to a desired rate and, when necessary, the trays in all seven chambers may receive the same quantity of water and plankton food per hour. As a rule, sea water and food, before entering trays containing juvenile mollusks, are passed through the special unit where they are sterilized by ultraviolet rays.

In addition to various apparatus and devices discussed in this section there are several others that have been used in special studies. A description of these will be given elsewhere.

III. Conditioning Mollusks for Out-of-Season Spawning

Before the present method of providing laboratories with warm water in winter was developed, experiments on most of the bivalves and, especially, their larvae were confined, in New England waters and similar areas, almost exclusively to the short periods of natural propagation, usually lasting for only the $2\frac{1}{2}$ or 3 summer months. However, since it was found that in many bivalves, by using proper conditioning methods, normal development of gonads can be stimulated and spawning induced during late fall, winter and spring, the experimental period has been greatly expanded (Loosanoff, 1945).

Conditioning of bivalves to develop mature gonads during the cold part of the year is relatively simple. It consists of placing mollusks, brought from their natural environment where water temperature may be near freezing, into somewhat warmer water and then gradually increasing the temperature several degrees each day until the desired level is reached (Loosanoff and Davis, 1950). Sometimes, especially towards the spring, instead of a gradual conditioning the mollusks can be placed directly in water of about 20°C. As a rule, the gametes obtained from these mollusks were no less viable than from those conditioned gradually. We have often employed this more rapid

approach, thus shortening by several days the length of the conditioning period which, for oysters kept at 20°C, is approximately 3 to 4 weeks.

The conditioning period can also be shortened by keeping mollusks at temperatures higher than 20°C (Loosanoff and Davis, 1952b). For example, *Crassostrea virginica* kept at 25°C developed ripe spermatozoa and fertilizable eggs by the 5th day, and light spawning could be induced on the 7th day. When kept at 30°C ripe spermatozoa and fertilizable eggs were found in oysters which, only 3 days before, were brought from the ice-covered harbor where they were hibernating. Some oysters of this group were induced to spawn on the 5th day.

Obtaining spawn from another common bivalve, the hard shell clam, *Mercenaria mercenaria*, is also relatively simple in summer. It is often accomplished merely by raising the water temperature a few degrees and by adding a sperm suspension (Loosanoff, 1937a). Previously, as already mentioned, this could be accomplished only during a short period, whereas, using our recently-developed methods, it is now possible to obtain ripe gametes and raise larvae of this species on a year-round basis (Loosanoff and Davis, 1950, 1951).

The method for conditioning clams for spawning in winter is the same as that described for oysters. The entire conditioning period takes approximately 2 to 3 weeks, but can be made even shorter towards or during spring. On several occasions clams brought directly from natural beds during early spring could be induced to spawn without any preliminary conditioning. However, this method often failed and cannot be considered reliable. Usually, only males responded on such occasions. As a rule, some conditioning of clams is necessary, even towards spring, to have a reliable source of eggs and sperm.

Our studies have demonstrated that bivalves can be conditioned for late fall and early winter spawning only after they recover from the natural spawning activities of the preceding summer. This recovery consists of many complex physiological processes leading, in general, to accumulation of reserve materials, of which glycogen is probably the most important (Loosanoff, 1937a, 1942). Since many species of bivalves of Long Island Sound, including oysters and clams, sometimes continue to spawn until late August or even the middle of September and are not completely recovered from these activities until the end of November, they cannot be conditioned for spawning during these months.

We solved the problem of supplying ripe mollusks during the period from late August to late November by delaying their gonad development and spawning until late fall (Loosanoff and Davis, 1951). Clams,

M. mercenaria, and oysters, *C. virginica*, are taken from Long Island Sound early in the season, usually late in May, long before the beginning of their natural spawning, and transplanted to the waters of Maine, where the summer temperature averages about 7° lower than in our waters. This temperature, while permitting slow development of gonads, is, nevertheless, low enough to prevent spawning. Thus, when oysters and clams in Long Island Sound are already spent, those transplanted to Maine still retain their spawn. In the fall, small groups of these mollusks are routinely shipped back to our laboratory, where they are easily induced to spawn, providing normal gametes which are unobtainable locally during that time of the year.

By using the above method spawning of *C. virginica* can be postponed only for 6 or 8 weeks after oysters of Long Island Sound are completely spent. After that period the oysters, even if they are still kept in the waters of Maine, begin to resorb undischarged gonad material and, thereafter, become useless as spawners. We overcame this difficulty by developing another useful method, which postpones early gonad resorption. It consists of conditioning oysters early in the spring and spawning them at Milford by early June. After that they are transferred to the colder waters of Maine. Oysters treated in this manner must resorb old gonads and build up glycogen before developing new gonads. Because they are compelled to go through these processes, these oysters reach ripeness much later in the season than those that are planted in Maine without spring conditioning and spawning and, as a result, they do not begin to resorb gonad material as early as do unspawned oysters transferred to Maine at the same time. Taking advantage of this situation we have been obtaining normal larvae from October to January from spawn of oysters so treated.

Because *M. mercenaria* does not resorb undischarged gonad material in the fall, as oysters do, transferring them to the colder waters of Maine in the spring proved to be a highly satisfactory method of delaying spawning. Under these conditions the clams retain sperm or eggs throughout the summer and, as a result, can be induced to spawn throughout the next fall, winter and even during the following spring, always producing gametes which develop into normal larvae.

We have also delayed spawning of clams and oysters by taking these mollusks early in the summer from their natural habitat and keeping them in insulated boxes through which mechanically-cooled sea water flowed. Usually, only a comparatively small number of adult mollusks could be conveniently kept under these conditions and, as a rule, bivalves so treated were in much poorer condition than those kept under natural surroundings in the waters of Maine. More-

over, a failure of the artificial refrigeration system may cause the entire stock to spawn prematurely.

By combining our two methods, one consisting of conditioning mollusks for spawning during the cold periods and the other of delaying gonad development and preventing spawning during their normal reproductive season, ripe bivalves may now be available throughout the entire year.

We have also found (Loosanoff and Davis, 1952a) that *C. virginica* and *M. mercenaria* are able to reproduce several times a year, provided that changes in ecological conditions, especially temperature, are so controlled that these mollusks can rapidly recover from spawning, accumulate in their bodies material needed for gonad development, and begin the cycle again. As a result of the discovery of these new approaches and methods, as much can now be accomplished in one year in certain fields of the biology of bivalves as could formerly be done in three or four.

It should be emphasized that our conditioning methods are not equally successful or applicable to all groups of oysters, *C. virginica*, and, perhaps, certain other species of bivalves of our Atlantic and Gulf coasts. This is probably because populations of these species are not genetically homogeneous, but consist of different physiological races. We began to suspect the existence of such races in *C. virginica* as early as 1937 (Loosanoff and Engle, 1942). Stauber (1950), in reviewing the literature on spawning of the American oyster, also came to the conclusion that oysters from different areas along our Atlantic coast may belong to different geographical races. Our experiments in this field strongly supported this assumption by demonstrating that, even though all these oysters belong to the same species, the temperature requirements for gonad development and spawning of the northern populations are definitely lower than those of the southern groups (Loosanoff and Nomejko, 1951).

The results of our later, more extensive studies, in which several thousand specimens representing populations of different areas of the oyster-producing belt extending from the Gulf of Mexico to Cape Cod were used, fully supported our original conclusions (Loosanoff, 1958a). The oysters used in these experiments and observations were from Florida (Gulf of Mexico), South Carolina, Virginia, New Jersey and New England. They were received in the fall, after they had completely spawned in their native environment, and were kept in Milford Harbor throughout early winter. Some time in January the first groups of these oysters were transferred to the laboratory to be conditioned for spawning.

We employed two criteria to evaluate ripeness of the oysters. The first was to ascertain the number of days needed for 50% of the oysters constituting a sample to develop active spermatozoa or fertilizable eggs. Secondly, we had to determine the length of the conditioning period before spawning in 50% of the oysters could be induced by our usual method. Each sample contained fifty adult individuals.

The experiments showed conclusively that Long Island Sound oysters develop gonads and can be induced to spawn after considerably shorter conditioning periods than those required by southern oysters. When kept at the same temperatures oysters from New Jersey, although slower than those of Long Island Sound, showed, nevertheless, much faster gonad development than oysters of Virginia, South Carolina and Florida. In averaging the results of the experiments it was found that 50% of Long Island Sound oysters, conditioned at 21°, 24° and 27°C, contained mature gametes after only 15, 8 and 5 days, respectively. The corresponding groups of New Jersey oysters reached this stage only after 55, 32 and 22 days, thus requiring three or four times as long at the three above-mentioned temperatures as did the northern race.

In certain experiments we were able to induce spawning in 50% of Long Island Sound oysters after only 18 days of conditioning at 21°C. To achieve the same results with New Jersey oysters 78 days were needed. The more southern groups kept under the same conditions failed, as a rule, to produce 50% spawners.

The most striking differences were noticed when oysters of different geographical regions were kept at relatively low temperatures. For example, after 68 days of conditioning at 12°C, 67% of Long Island Sound oysters contained mature eggs or spermatozoa. In this group we were able to induce spawning in one male and, 10 days later, in one female. Oysters of the other groups kept at the same temperature contained not a single individual with mature gonads, even after 78 days. Moreover, in the majority of New Jersey and Virginia oysters and in all of those from South Carolina and Florida the gonads were so poorly developed that the sexes could not be distinguished, even by microscopic examination of the raw gonad material.

The method of inducing spawning of oysters and clams in summer has already been described in detail (Galtsoff, 1930, 1932; Loosanoff, 1937a, 1954). The same method, as a rule, has also been used to induce spawning in other bivalves. In general, our present method can be described as follows: After the proper conditioning period ripe bivalves are placed in glass spawning dishes, each containing approximately 1 liter of sea water of the same temperature as that at which mollusks

FIG. 10. Ripe oysters in spawning dishes. Male oyster is shown spawning in center dish.

FIG. 11. Inducing spawning of clams, oysters and other bivalves by immersing dishes of sea water containing animals in warm water on spawning table.

were conditioned (Fig. 10). These dishes are partly immersed in a large tray or sink, which is filled with hot water, thus quickly raising the temperature in the dishes to the desired level (Fig. 11).

In some instances thermostimulation alone is sufficient to induce spawning. In other cases, however, mollusks need additional stimulation, which consists of adding to the water small quantities of sperm or egg suspension made from gonadal material of ripe individuals of the same species. Many forms quickly respond to combined thermal and chemical stimuli; others, such as the common mussel, *Mytilus edulis*, do not usually respond to this method but can, nevertheless, be induced to spawn by other means, which will be discussed later.

In a special series of experiments we tried to cause artificial discharge of reproductive elements by injecting weak solutions of Mn_4OH and other chemicals into the bodies of bivalves that could not be spawned by other means. The results were usually not gratifying, except in the case of *M. edulis*, when injection was made in its adductor muscle.

At the time of our experiments to induce spawning in ripe bivalves we were already aware of the success of Japanese workers in inducing spawning in mussels by giving them a mild electric shock. We repeated these experiments but, unfortunately, with indifferent results.

In still other species, for example, *Modiolus demissus*, all our methods, including those that were successful in the case of *Mytilus edulis*, proved to be ineffective in inducing spawning. Therefore, unless ribbed mussels spawn naturally, thus providing normally fertilized eggs, no other means, except perhaps stripping, are left for obtaining their spawn.

Fertilizable eggs of many species, including those of *C. virginica* (Brooks, 1880), can be obtained by stripping mature females but, since many of these forms spawn so readily in response to chemical and thermal stimulations, it is seldom necessary to resort to this means. However, when working with other species, especially those that cannot be spawned by conventional methods, stripping may be the only way to obtain ripe eggs. It is a simple process and is carried on as follows: After removing the outer membrane that covers the gonads, the mollusk is gently rinsed in sea water. This action separates from the gonad large numbers of eggs without serious injury to them. Using a series of sieves of proper size mesh the eggs are later freed of blood cells, pieces of tissue, etc., and then placed in sea water to which sperm is added. The fertilized eggs can then be placed in culture vessels.

This approach is possible only for eggs of those forms in which the germinal vesicle dissolves after stripping. In many species, however,

including *Mercenaria mercenaria* and *Pitar morrhuana*, attempts to fertilize stripped eggs usually fail because in these eggs the germinal vesicles remain intact and, as a result, fertilization does not occur. Under normal conditions the germinal vesicles in eggs of such species dissolve while they are still in the ovaries of the female, just before they are discharged in the process of spawning. Upon dissolution of the germinal vesicle the germinal spindle is formed and the discharged egg is ready for fertilization (Loosanoff, 1953).

Recently, following the suggestion of Mr. David Tranter of Australia, we used a weak solution of ammonium hydroxide to break the germinal vesicle of eggs of certain bivalves. By employing this method we succeeded in raising normal larvae from eggs stripped from *Mercenaria mercenaria*, *Tapes semidecussata* and several other species.

After the eggs were washed from a gonad they were passed through a coarse, 50-mesh screen to remove debris, large pieces of tissue, etc. Later, they were washed on a 325-mesh screen which retained the eggs but let pass the body fluids that might pollute the water in culture vessels. After that, 3 ml of 0·1 normal solution of ammonium hydroxide were added to every 100 ml of the prepared suspension of eggs in sea water. After the eggs were in this solution for some time they were washed again on a 325-mesh screen, being finally ready for fertilization. A more detailed description of handling fertilized eggs will be given in the section on methods of cultivation of eggs and larvae.

The length of exposure to the solution of ammonium hydroxide may vary somewhat from species to species. The following table shows the ratio between length of exposure and percentage of normally developing eggs of *M. mercenaria*:

15 minutes—32%
30 minutes—16%
45 minutes— 9%
60 minutes— 3%
75 minutes— 2%
90 minutes— 0%

Even after 90 minutes of exposure to the solution of ammonium hydroxide some eggs became fertilized, but their development was not normal.

The percentage of normal larvae obtained from chemically-treated eggs was low compared to that of naturally spawned eggs but, nevertheless, it was high enough to permit successful culturing of larvae of those species in which we were not able to induce spawning. Perhaps by changing the concentration of ammonium hydroxide, using other

chemical agents, or by improving the methods of stripping even better results may be obtained.

Finally, there were several species of bivalves which we could neither spawn artificially nor collect their normally discharged eggs. Moreover, eggs stripped from some of these forms could not be fertilized regardless of various preparatory measures, which included the chemical treatment described above.

Fecundity of many lamellibranchs, especially those of commercial importance, has been speculated upon for a long time. Brooks (1880) estimated that *C. virginica* could produce between 18 750 000 and 125 000 000 eggs. He based his estimate upon volume of material removed from the ripe female, but stated that this figure should be reduced by approximately 50% because of other matter that was measured together with eggs. Churchill (1920) stated that a large oyster may discharge 60 million eggs, while Galtsoff (1930) estimated that the number of eggs released in a single spawning may range between 15 and 115 million. He concluded that the maximum number of eggs that can be released by a single female during the entire spawning season is approximately half a billion. Burkenroad (1947), without offering any experimental observations of his own, suggested that Galtsoff's estimate was approximately ten times too high. Belding (1912) estimated that *M. mercenaria*, $2\frac{1}{2}$ in long, produces an average of 2 million eggs, a figure not substantiated by experimental studies.

Since reliable information on the fecundity of even the most commonly studied pelecypods was unavailable, experiments were undertaken by Davis and Chanley (1956b) to determine total numbers of eggs actually produced by individual oysters, *C. virginica*, and clams, *M. mercenaria*, under natural and artificial conditions.

The first series of observations was made on seventy-five oysters, measuring from $3\frac{1}{2}$ to $4\frac{1}{2}$ in long, and on the same number of clams approximately 3 to 4 in long.

The experiments were conducted in the laboratory during the winter, a most convenient period for proper conditioning of both clams and oysters. Each bivalve was individually numbered and a complete record was kept of its behavior during the entire experiment. The first group of oysters composed of twenty-five individuals was spawned at 3-day intervals, the second group at 5-day intervals and the third at 7-day intervals. In clams, which were also divided into three groups of twenty-five individuals each, spawning was induced at 3-, 7- and 14-day intervals. Spawning of these groups of clams and oysters was continued at the specified intervals for more than 2 months.

Experiments have shown that, as a rule, an individual oyster or clam does not discharge all its eggs or sperm in a single spawning, but will continue to spawn at intervals over extended periods. One female oyster spawned on sixteen occasions and a clam, eleven times. The number of spawnings per female oyster ranged from two to sixteen. The highest number of eggs was produced by an oyster that spawned nine times, while a female that spawned sixteen times ranked second. The lowest total number of eggs released by an oyster was by an individual that was induced to spawn seven times.

No significant difference was observed in the average number of eggs released during the entire experimental period, whether the oysters were induced to spawn at 3-, 5- or 7-day intervals, although the average number of spawnings per female oyster decreased progressively as the intervals between spawnings were increased. It was also determined that female oysters having larger numbers of eggs tended to spawn more frequently than did females with smaller numbers.

The highest number of eggs released by any female clam in a single spawning was 24·3 million and the total number released by individual clams during the entire experimental period of about 2 months ranged from 8 million to 39·5 million, with an average of about 24·6 million.

There was no significant difference in the average number of eggs released in a season, whether the clams were spawned at 3-, 7- or 14-day intervals. It was also found that correlation between the number of times a female clam spawned and the number of eggs produced was not significantly different from zero.

An auxiliary experiment consisted of observations on spawning of fifty oysters taken from Milford Harbor early in April, brought into the laboratory and placed in conditioning trays at temperatures of about 20°C. Three weeks later these oysters were induced to spawn daily for 5 consecutive days, and seventeen females and twenty-four males responded during the first day. Altogether, this group contained twenty-four females and twenty-six males. Of the twenty-four females, fourteen spawned on 2 or more consecutive days, eight spawned on 3 or more consecutive days, five spawned on 4 or more consecutive days, and three females spawned on each of the 5 days of the experiment. Eight males spawned each day.

The important contribution of this experiment was the clear-cut demonstration that there is no 2- to 5-day refractory period during which female oysters cannot be induced to spawn, as maintained by Galtsoff (1930). On the contrary, the results suggest that upon proper

stimulation both male and female oysters can spawn any time they have physiologically-ripe sex cells to discharge.

The final experiment consisted of observations on nine female oysters developing gonads under normal conditions in Long Island Sound and induced to spawn at the end of June. The total number of eggs discharged by these oysters ranged from 23·2 million to 85·8 million and averaged 54·1 million eggs per female. Thus, both average number of eggs and maximum number per female of the summer

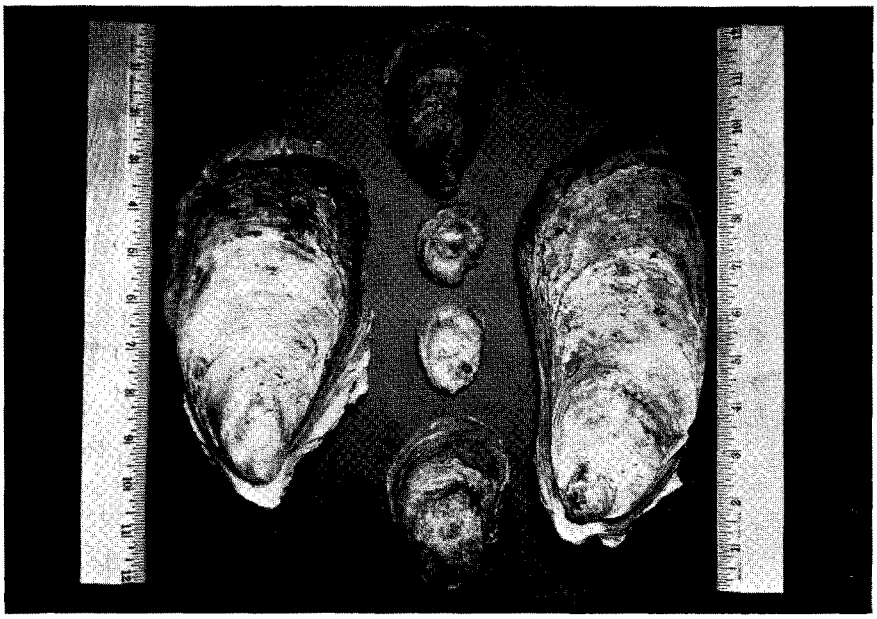

FIG. 12. Representatives of three groups of oysters of different ages and sizes used in studies to determine viability of their gametes. Members of oldest group were estimated to be between 30 and 40 years.

spawning group were about 20 million higher than found in the winter experiment. Nevertheless, none of these oysters discharged as many as half a billion eggs, as suggested by Galtsoff (1930). The larger number of eggs developed by oysters of this group, as compared with production of eggs by oysters conditioned in the laboratory during early winter, may be ascribed to larger reserves of glycogen possessed by oysters developing gonads under natural conditions.

A question that had long been of interest to biologists was, At what age do oysters and clams produce the best, most viable sexual products? Until recently, no answer could be given because no reliable methods

were available to conduct critical experiments on development of eggs and growth of larvae to setting or post-setting stage. Since development of these methods, such studies have become possible and recently were undertaken at our laboratory.

Three groups of oysters of different ages and sizes were conditioned for spawning (Fig. 12). The average age of individuals of the oldest group was estimated to be between 30 and 40 years, some of them being over 9 in long and over 4 in wide. The intermediate group of oysters of marketable size was from 5 to 7 years old, while the youngest group was composed of small oysters approximately 2 years old. These groups were conditioned and induced to spawn under controlled conditions, their larvae grown to setting stage, and rates of survival and growth of larvae from the three size groups compared.

The results showed no significant difference between oysters of the different age groups in the time needed to develop ripe gonads. We were somewhat surprised, however, to find that oysters of the oldest group responded to spawning stimuli more rapidly than individuals of the two younger groups.

There was also no significant difference in percentage of fertilizable eggs because almost 100% of the eggs of all three groups became fertilized. Furthermore, the percentage of fertilized eggs developing to straight-hinge larval stage showed no consistent variation that could be ascribed to size or age of parent oysters. Finally, no consistent difference was found either in the sizes of the early straight-hinge larvae originating from eggs of different age-group oysters or in survival and rate of growth of their larvae.

Similar studies on hard shell clams, *M. mercenaria*, measuring from 37 to 110 mm in length, also showed that there was no significant difference in viability of spawn produced by clams of different sizes and ages. Often the differences between the progeny of individuals of the same size groups were as great as the differences between those of different ages and sizes. Larvae grown from eggs of clams of all three sizes were successfully carried to setting stage.

On the basis of the above-described experiments we came to the conclusion that since there was no significant difference in the quality of spawn developed by individuals of different ages or sizes, mature oysters and clams of all age groups may be safely used as spawners.

Of special biological interest was the observation that the sexes among the oldest oysters were about evenly divided. This discovery was contrary to the old conception that in the oldest groups females should decidedly predominate in numbers. We also noticed that many of the largest and oldest oysters, while kept in the laboratory to be

conditioned for spawning, formed normal, new shell growth, thus indicating that even at that age and size the oysters did not lose their ability to grow (Fig. 12).

IV. Cultivation of Eggs and Larvae of Bivalves

A. *General description of the development*

Eggs of bivalves differ in many respects, including their size, color and specific gravity. They also differ in thickness of the membrane surrounding them (Costello *et al.*, 1957). In oysters and certain other forms this membrane is only a few microns thick. In others, however, such as *M. mercenaria*, the egg proper measures only 70 to 73 μ, while the total diameter of the egg and surrounding gelatinous membrane is about 170 μ. This membrane, in many instances, continues to surround the embryo past blastula stage and, on some occasions, until late trochophore stage is reached (Loosanoff and Davis, 1950).

We shall describe specific characteristics of the eggs later on, when discussing each of the species studied. Here, because the description of a typical bivalve egg and its development to straight-hinge stage or, as it is often called, early veliger has been given on many occasions, including Brooks (1880), MacBride (1914) and others, we shall present only a general picture of changes occurring from the moment the egg is discharged, or stripped, until it becomes a straight-hinge larva. This description is based upon observations made on eggs and early embryos of *Mactra* (= *Spisula*) *solidissima*, the surf clam, which is the largest bivalve of our Atlantic coast. It measures up to 7½ in long and can be found in considerable numbers from Labrador to Cape Hatteras. Additional information on spawning of these clams and rearing of their larvae is included in the section dealing with rearing of larvae of different species.

The diameter of a mature egg of *M. solidissima* averages 56·5 μ (Fig. 13*a*). Costello *et al.* (1957) give the diameter of the unfertilized ovum of the same species as ranging between 53 and 56 μ, thus agreeing with our measurements. According to Cahn (1951), who bases his conclusions on the work of the Japanese investigators, Kinoshita and Hirano (1934), whose paper was not available to us for consultation, the diameter of the egg of a closely related form, *Spisula sachalinensis*, is only 50 μ. Another group of Japanese workers (Imai *et al.*, 1953), studying the same species reports that the diameter of the mature egg of this clam varies from 70 to 75 μ, thus being considerably larger than the size given by Cahn. Jørgensen (1946) states that eggs of *Spisula subtruncata* of European waters vary in diameter from 50 to 55 μ.

Early development of the egg of *M. solidissima* is basically the same as that of many other bivalves. After dissolution of the germinal vesicle (Fig. 13*b*) the size and shape of the egg remain the same. If

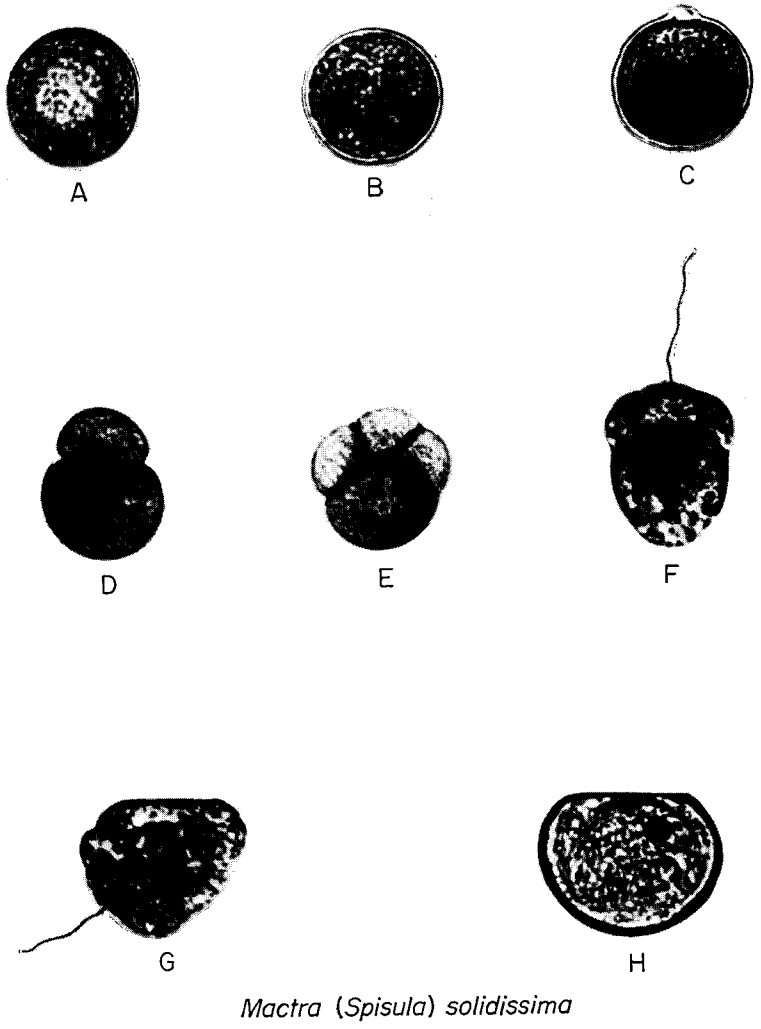

FIG. 13. Development of *Mactra* (= *Spisula*) *solidissima* from unfertilized egg (A) to straight-hinge larva (H). Diameter of egg is about 56 μ, while length of early straight-hinge larva is about 79 μ. Detailed description in text.

the fertilized egg is kept in water of about 20°C, the polar body is formed in about 45 min (Fig. 13*c*) and the two-cell stage, measuring about 65 μ along the longest axis, is reached in 90 min (Fig. 13*d*).

Development of the egg of a bivalve, as described above, is typical only of a group in which the germinal vesicle breaks upon discharge

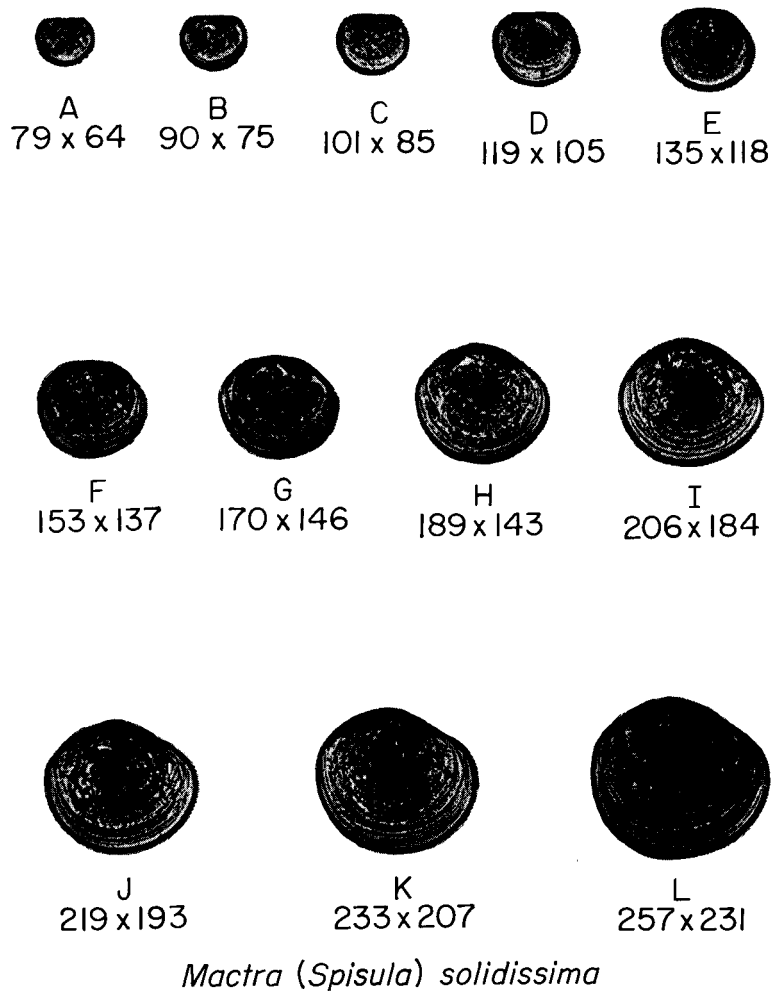

FIG. 14. Development of *Mactra* (= *Spisula*) *solidissima* from straight-hinge stage (A) to metamorphosis (L). Measurements of length and width of larvae of different stages are given in microns.

or upon stripping, thus rendering the egg ready for fertilization. Eggs of *Crassostrea virginica* and many other species belong to this category. In the other group stripped eggs continue to retain their

germinal vesicles intact and fertilization does not occur. However, as already mentioned, eggs of such species can be chemically treated and thereby become ready for fertilization.

As cleavage progresses and more micromeres are formed (Fig. 13*e*), the embryo gradually develops into a swimming, ciliated larva which eventually reaches trochophore stage (Fig. 13*f*). Under favorable conditions this stage may be reached in 12 to 16 hr, depending upon the original condition of the eggs, culturing methods and, of course, water temperature.

During late trochophore stage (Fig. 13*g*) the cell gland begins to secrete the shell. When the shell completely encloses the soft parts the larva has reached early straight-hinge stage (Fig. 13*h*).

Development of larvae of *Mactra solidissima*, from early straight-hinge stage until metamorphosis, and their length—width measurements during this entire period are shown in Fig. 14. Very early, normal straight-hinge larvae measure only about 79 μ in length and usually between 63 and 65 μ in width. In some cultures, composed mostly of abnormal individuals, somewhat smaller, slightly deformed straight-hinge larvae can be seen occasionally, but it is doubtful that they survive to metamorphosis.

Individual larvae of *M. solidissima* display considerable variations as to the size at which certain organs of their bodies begin to develop and at which metamorphosis occurs. For example, in some individuals the foot can be seen when they are only about 160 μ long. Approximately 80% of the larvae show a well-developed foot by the time they are 215 μ long, and at a length of 240 μ practically all possess this organ.

Disappearance of the velum is another step in larval development that is not strictly correlated with a definite size. In some larvae measuring only 219 μ in length the velum was already completely resorbed, while in extreme cases a diminishing, but still functional velum was seen in larvae about 257 μ long.

A few larvae begin to metamorphose when they are about 220 μ long, but the majority are between 230 and 250 μ before metamorphosis occurs. At this time the velum is resorbed, rudimentary gills develop, and a powerful ciliated foot, which when expanded is as long as the young clam itself, serves as the only means of locomotion. Individuals measuring 262 μ in length were the largest true larvae recorded. In this respect our observations are in agreement with those of Imai *et al.* (1953), who found that in *Mactra sachalinensis* the foot begins to develop at a length of about 200 μ and that larvae set at about 270 μ.

B. *Abnormal eggs and larvae*

Abnormal development of eggs and larvae of bivalve mollusks may be due to any one of a variety of factors or to a combination of such factors. It is our practice, however, to discard cultures in which less than 50% of the eggs develop into normal straight-hinge larvae. This is done because batches of eggs giving a low percentage of larvae may be abnormal in some respects, and these abnormalities may lead to aberrant experimental results and wrong conclusions. We do not know, as yet, what factors are responsible for poor eggs and feeble embryos. In some instances abnormal larvae, or failure of eggs to develop to straight-hinge larval stage, may be the result of incompatible genetic combinations. Our experience indicates, however, that such combinations are comparatively rare.

Some abnormalities of larvae may be ascribed, no doubt, to the poor physical condition of spawners. Several investigators have believed that eggs released late in the season were less viable and produced less vigorous larvae than those from earlier spawnings. Loosanoff and Davis (1950) were under the impression that the last batches of eggs discharged by virtually spent females gave feeble larvae that grew slowly and showed high mortality. Cole (1941) offered evidence that the brood strength of *Ostrea edulis* may decline during the course of a breeding season and he believed that this was due to a depletion of food reserves in the bodies of parent mollusks. Walne (1956) thinks that the lack of " vigour " in larvae may result from poor condition of the parent oyster and believes it possible that the vigour of larvae may be affected by the quantity of food reserves laid down in the eggs.

More recently, Davis and Chanley (1956b) have shown conclusively that the last batches of eggs of both clams, *M. mercenaria*, and oysters, *C. virginica*, discharged by virtually spent females were cultured with no apparent diminution of either percentage of eggs developing into straight-hinge larvae or rate of growth of these larvae. Accordingly, we now believe that abnormal or feeble larvae do not occur more frequently in later spawnings than in other spawnings throughout the season. As has already been mentioned, our experiments have shown that there is no correlation between viability of spawn and age of parents.

There is evidence that bivalves can be induced, by strong chemical and thermal stimulations, to abort eggs even though they are not fully ripe (Fig. 15). In some cases such spawnings appear to be quite normal and a large number of eggs may be released. More often, however, comparatively few eggs are shed. In the case of *C. virginica* immature oyster eggs usually develop only to late gastrula or early

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

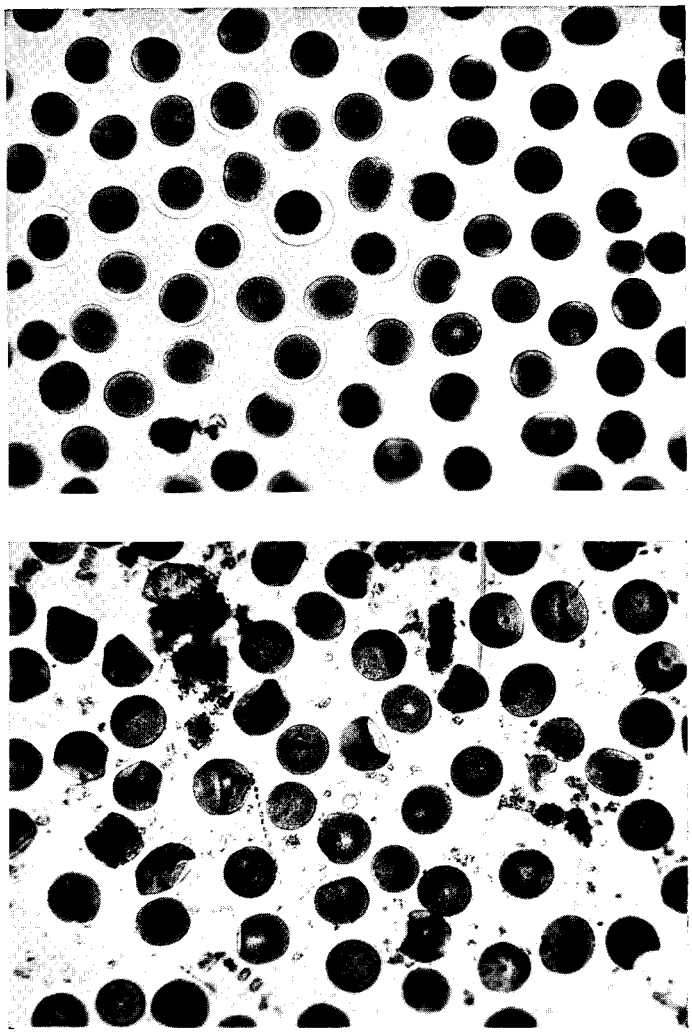

FIG. 15. Largely normal (*above*) and abnormal (*below*) eggs of *Ensis directus*. Abnormal eggs were discharged by a female compelled by strong stimulation to spawn before eggs were ripe. Normal eggs vary from 64 to 73 μ in diameter.

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

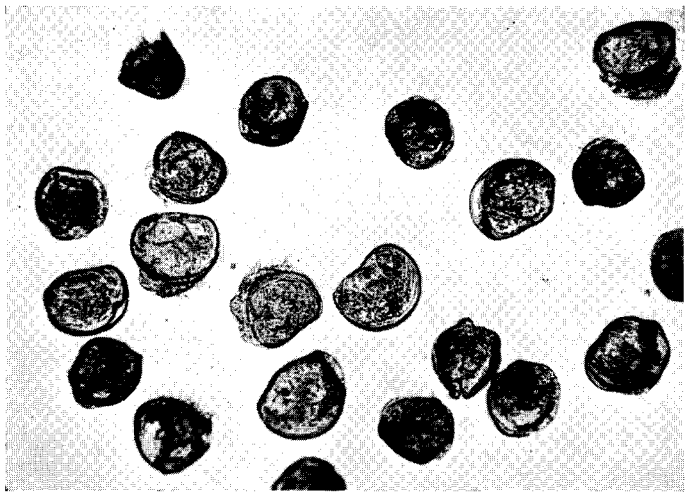

FIG. 16. Abnormal 4-day-old larvae of *Mercenaria mercenaria* from crowded culture Largest larvae in photomicrograph are approximately 105 μ long.

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

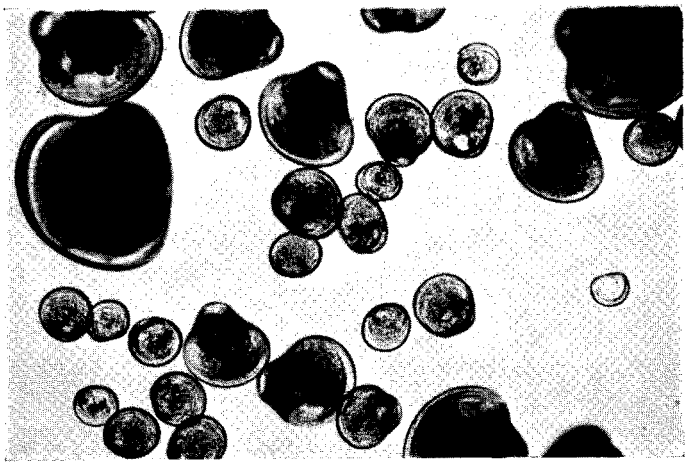

FIG. 18. Differences in sizes of larvae of *Crassostrea virginica* of the same age and grown under identical conditions in the same culture vessel. Some of the largest larvae are approximately 295 μ long and are nearing metamorphosis.

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

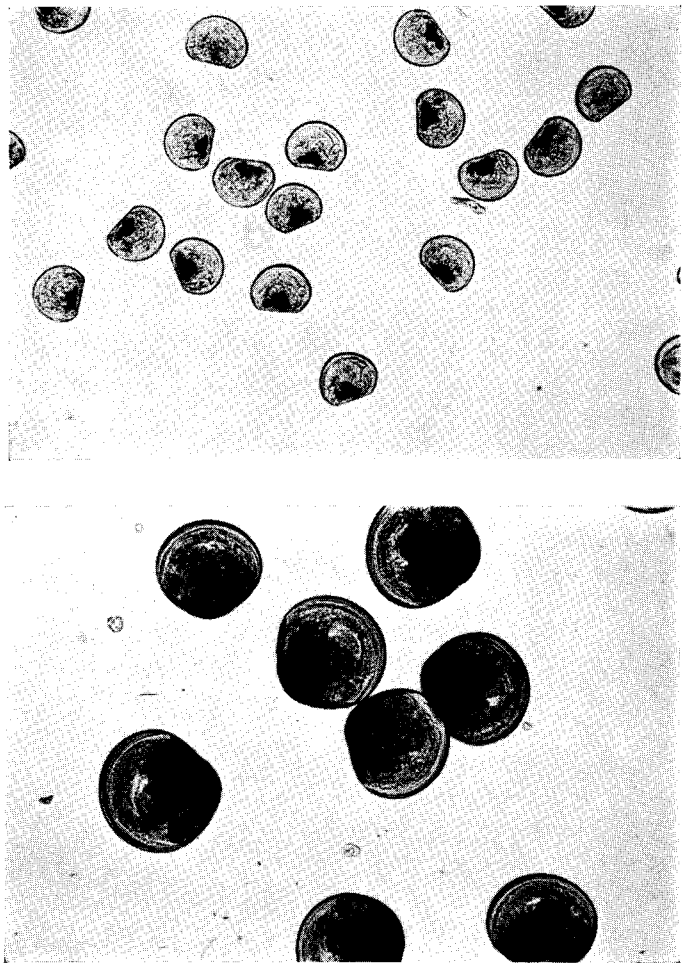

FIG. 19. Differences in sizes of 12-day-old larvae of *Mercenaria mercenaria* grown at 10°C (*above*) and 30°C (*below*). Average lengths of larvae were 105 and 195 μ, respectively.

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

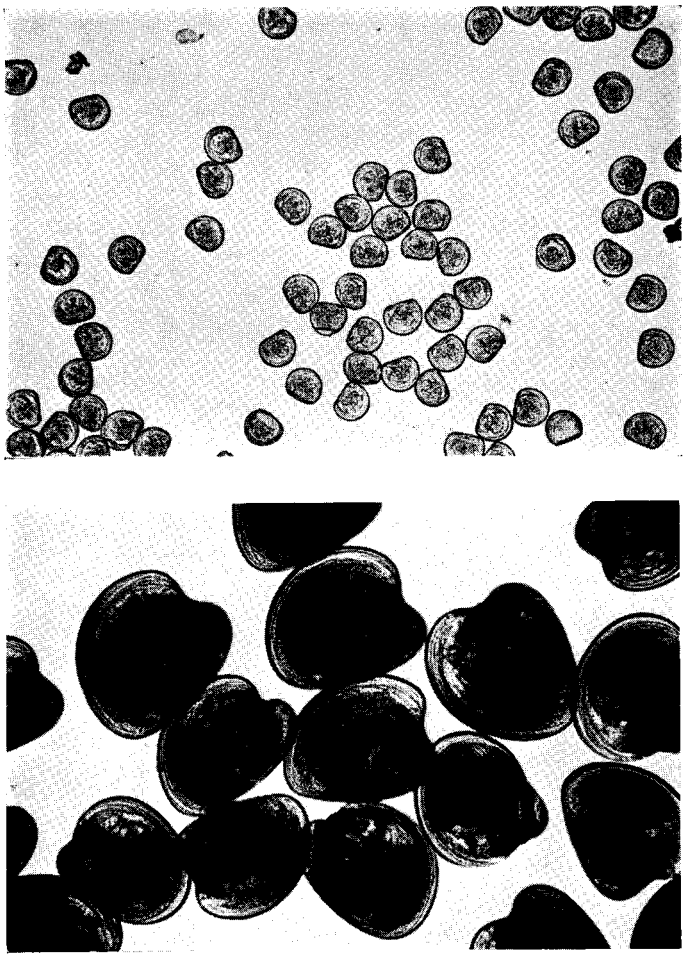

FIG. 20. Differences in sizes of 12-day-old larvae of *Crassostrea virginica* grown at 10°C (*above*) and 33°C (*below*). Average lengths of larvae of cold and warm water groups were 77 and 203 μ, respectively.

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.

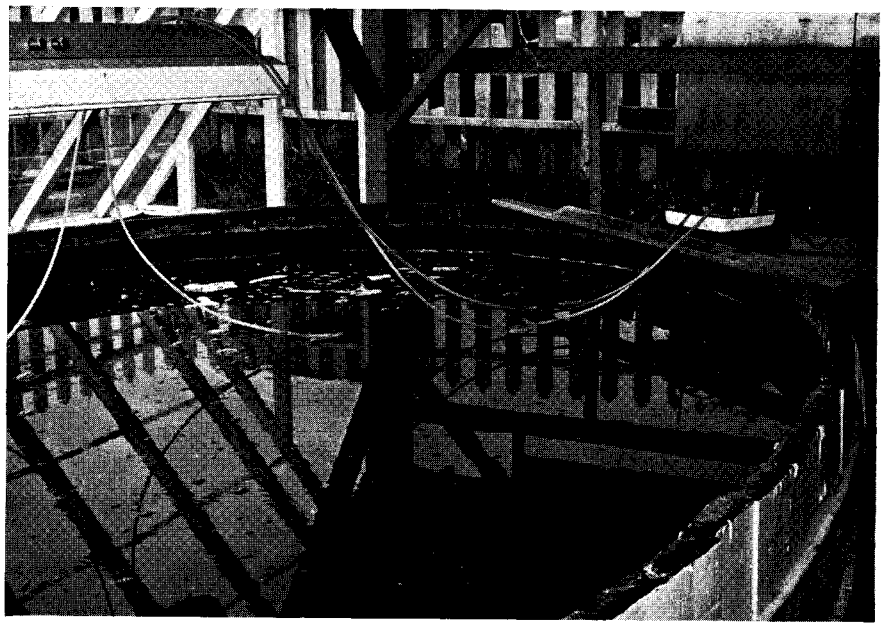

FIG. 21. Mass culture of mixed phytoplankton grown in large wooden tank of about 2 000-gal capacity under semi-outdoor conditions. Description in text.

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

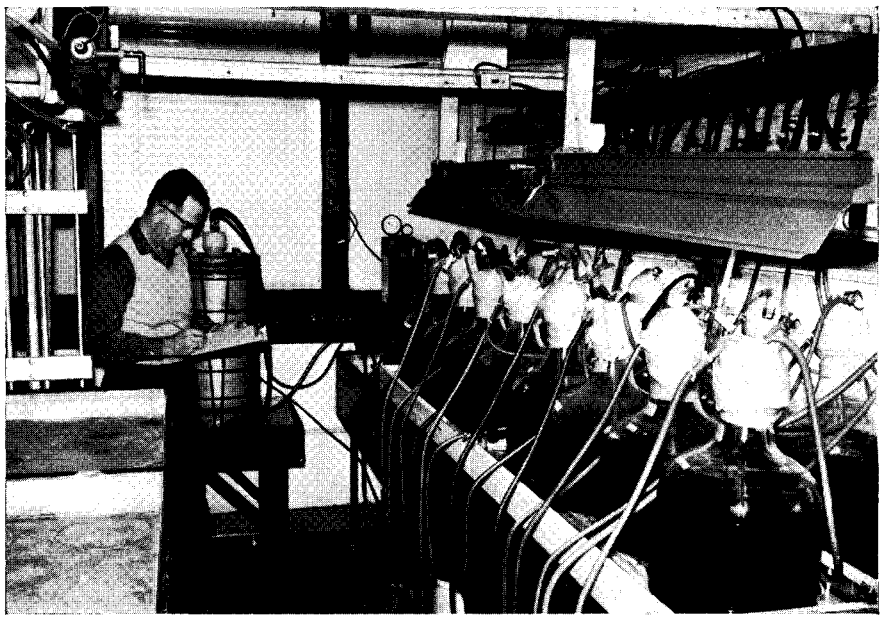

FIG. 22. Battery of specially-fitted, 5-gal Pyrex carboys serving as growth chambers for mass culture of photosynthetic microorganisms. Description in text.

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

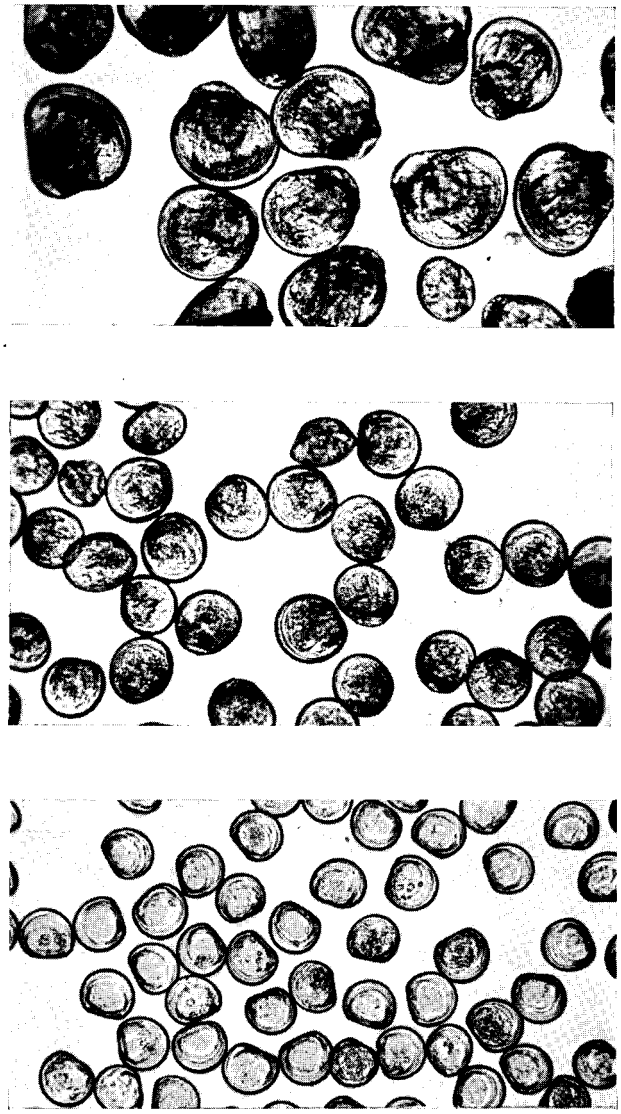

FIG. 23. Twelve-day-old larvae of *Crassostrea virginica* given different algae as food. Group fed *M. lutheri* (*upper photograph*) averaged 169 μ in length, while the middle group, serving as control and receiving no supplementary food, averaged 96 μ. The lower group, containing only dead larvae, was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

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

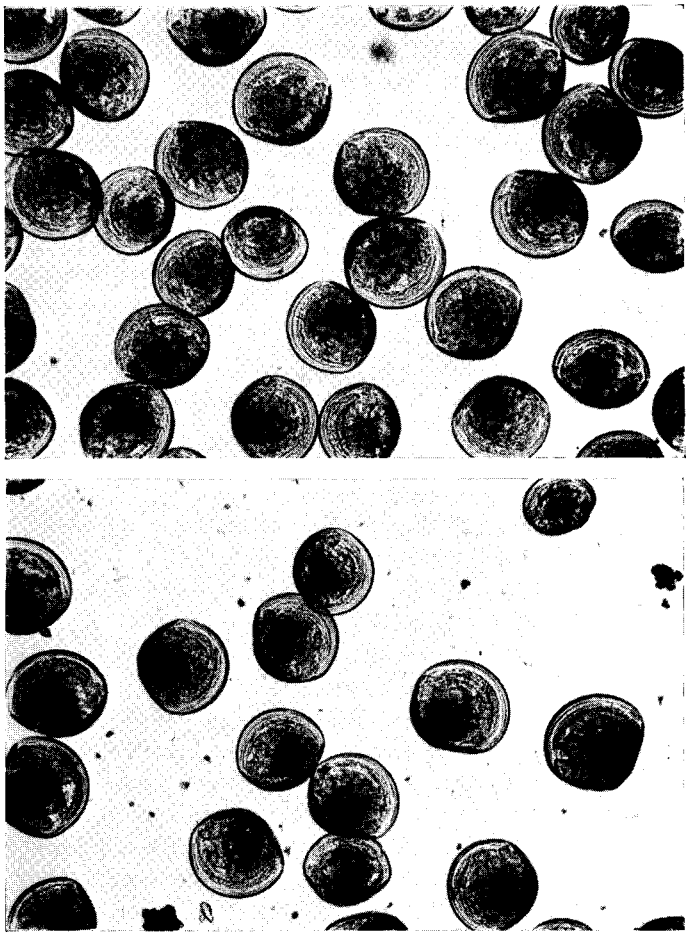

FIG. 24. Twelve-day-old larvae of *Mercenaria mercenaria*. Larvae shown in upper photomicrograph were fed freeze-dried cells of *Isochrysis galbana*, while those in the lower picture were given the same quantities of live cells of the same algae. Largest larvae are about 215 μ long.

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

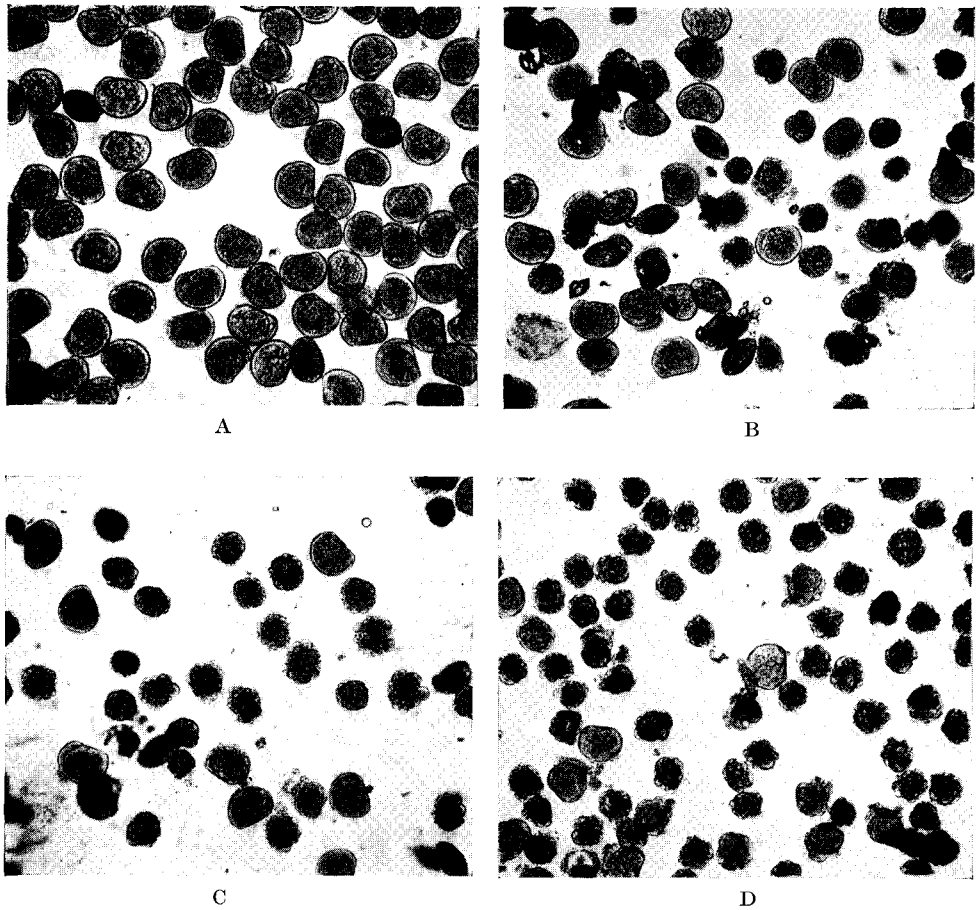

FIG. 25. Forty-eight-hour larvae of *Mercenaria mercenaria* developing from eggs cultured at different densities. A, 250 eggs per milliliter of water; B, 500 eggs per ml; C, 1000 eggs per ml; and D, 3000 eggs per ml.

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.

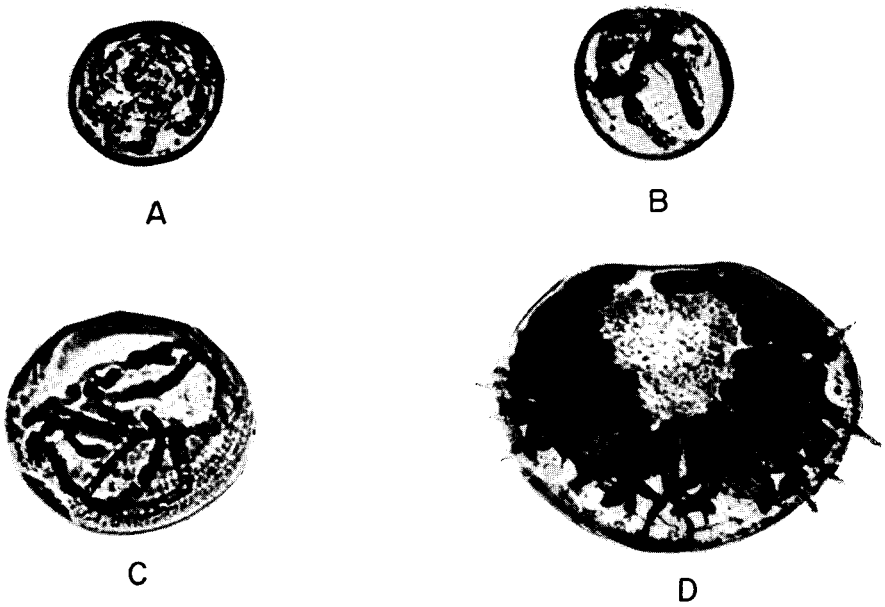

FIG. 26. Larvae infested with fungi. A and B, young larvae of *Crassostrea virginica* stained with Neutral Red; C, young larvae of *Mercenaria mercenaria* stained with Neutral Red; D, old larvae of *Mercenaria mercenaria* stained with Cotton Blue. × 225.

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.

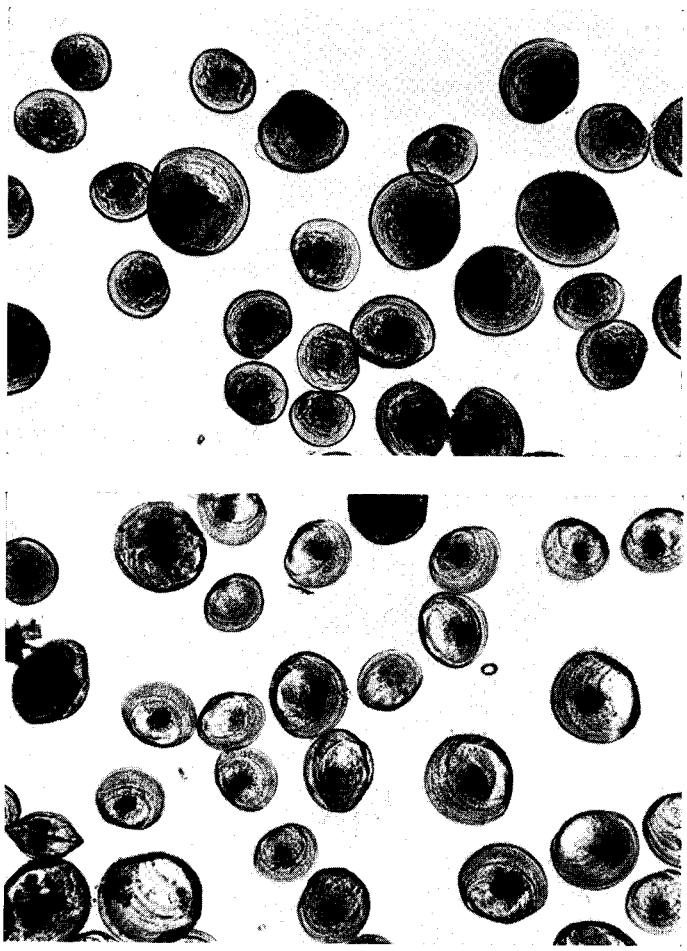

FIG. 27. Effect of pathogenic bacteria upon larvae of *Mercenaria mercenaria*. Healthy larvae (*above*); larvae from the same culture 12 hr after inoculation (*below*). Largest larvae in this photomicrograph are approximately 150 μ long.

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

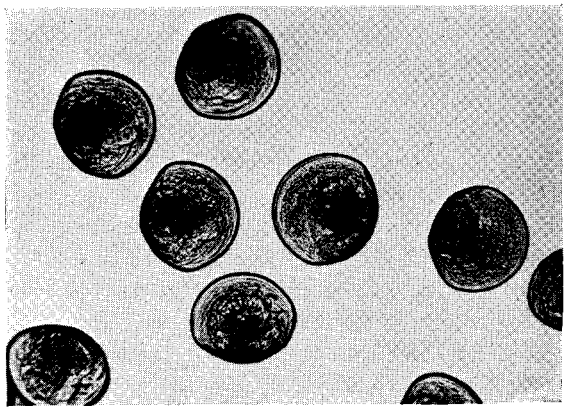

FIG. 28. Larvae of *Mercenaria mercenaria* approaching metamorphosis. Largest individuals in this group are about 200 μ in length.

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

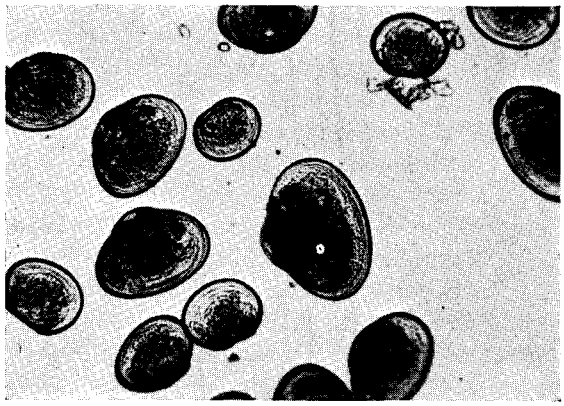

FIG. 29. Larvae of *Arca transversa*. Largest larva, approximately 230 μ in length, may be approaching metamorphosis. Note differences in sizes of larvae, all of which are of the same age.

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

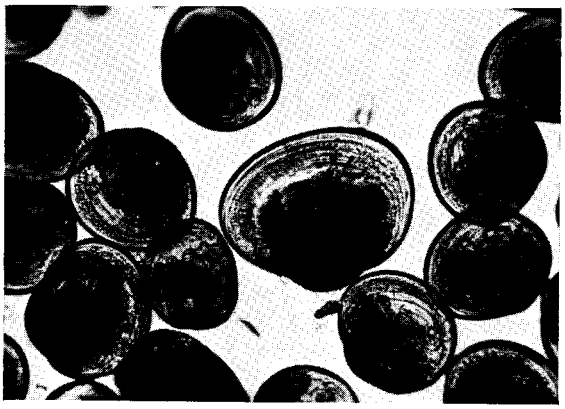

FIG. 30. Larvae of *Modiolus demissus*. Note differences in sizes of larvae that originated from the same group of eggs and were reared in the same vessel. Largest larva in center is approximately 300 μ long.

(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

of the eggs released appeared normal, but many abnormal larvae were found later (Fig. 31). However, such larvae were also common in cultures grown from eggs discharged in normal spawnings. Mussel eggs are orange-yellow in color. They are quite heavy and usually settle quickly on the bottom.

We also noticed that mussels which were dying from some undetermined cause often spawned soon after they relaxed their shells, even when their muscles were not cut. This observation suggested that the stimulating center, which induces discharge of eggs or sperm, is probably located in the adductor muscles. If these muscles are abnormally relaxed or stimulated, spawning follows. This reasoning led to another series of experiments designed to obtain eggs and sperm from ripe

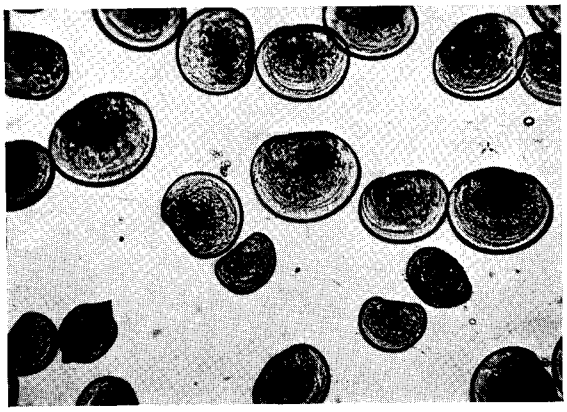

FIG. 31. Larvae of *Mytilus edulis*. Largest larvae are approximately 170 μ long. Several small, abnormal individuals can be seen in lower right corner.

mussels without killing them by cutting their muscles. This modification of the method would prevent fouling of eggs with blood cells, decomposition products, large numbers of bacteria, etc., that usually accompanies collection of eggs from females whose muscles are cut.

The new method consisted of inserting a small wooden wedge between the mussels' shells, thereby stretching the adductor muscle so that the two shells would be forced open about $\frac{1}{8}$- to $\frac{1}{4}$-in. In experiments where this principle was used the majority of pegged mussels spawned, while control mussels kept under identical conditions did not. Some began spawning within 2 hr after pegs were inserted and several spawned three times during a single week.

No mortality was noted among pegged mollusks, even when the pegs remained between the shells for 10 days. Mussels fed in an

apparently normal way, forming true feces and pseudo-feces. Mussels that were placed together in large trays with running sea water eventually attached to each other by their byssus.

Since the second series of experiments has also showed that discharge of spawn in mussels of both sexes can be induced by stretching the posterior adductor muscle, it became more certain that the nerve locus causing the discharge is located there. Accordingly, it was decided to stimulate this region, by touching or pricking it with a sharp needle, and note whether this type of stimulation would also induce spawning reaction.

In the middle of March two groups of mussels, each composed of ten individuals, were placed in running water at a temperature of 19°C. The first group, the control, contained individuals that were not stimulated. In the second group each individual was gently stimulated by pricking the muscle. The first male in the stimulated group spawned in about 2 hr. An hour later the first female spawned and continued to discharge eggs for almost 75 min. The eggs were discharged in orange-colored rods, 3 to 4 mm long. These rods were often expelled with such force that they travelled 4 to 5 in away from the spawning female.

Seven hours after the stimulation another male spawned, and after 9 hr two more females began to discharge spawn. Within 12 hr every individual in the stimulated group had spawned, while none in the control group had done so. Thus, it was demonstrated that needling the adductor muscle is an effective method of inducing spawning in *M. edulis*.

All mussels that had spawned were later placed in running sea water to see whether they would survive the stimulation treatment. All of them lived for over a month and were then discarded.

Although the previously-described experiment had demonstrated that pricking of the adductor muscle stimulated spawning of mussels, the fact that all ten animals were kept in the same dish left some doubt as to the exact conditions that caused spawning during the latter part of the experiment. This doubt existed because toward the end both sperm and eggs were present in the water and, therefore, might have helped to stimulate the mussels that had not yet spawned. To clarify this matter another experiment was designed in which we kept each mussel in a separate dish. Again, ten mussels were used in the control group and ten in the group that was stimulated. The first male spawned 30 min after stimulation and the first female began to discharge eggs 1 hr after its muscle had been gently touched several times with the point of a needle. The second female spawned only

2 or 3 min after the first one. Both individuals discharged eggs in large numbers for a considerable period. Within 12 hr eight of ten stimulated mussels spawned, while none of the control group discharged any sex products.

The experiment was repeated four times involving, altogether, 120 mussels. Of the sixty stimulated mussels, fifty-four responded, while none of the sixty control animals spawned. All mussels of the stimulated group remained alive for at least 2 weeks, after which they were discarded. Thus, a very simple method for stimulating ripe mussels to spawn has been developed. To make the method even easier we filed a small notch at the edge of the shell through which the needle was inserted.

According to Jørgensen (1946) one of the most remarkable peculiarities of larvae of *M. edulis* is " its excessive variability both as regards the color of the shell, its shape, and stage of development as compared with larval size, in which respects it is not surpassed by any of our other common Lamellibranch veligers " (p. 287).

Our cultures of *M. edulis* were grown during the early period of our studies of molluscan larvae; in other words, before the good food organisms, *I. galbana* and *M. lutheri*, became available to us. The larvae were fed an algal mixture, consisting chiefly of *Chlorella* and other green forms, and grew remarkably well. Strangely enough, it was virtually impossible to injure them by adding to the water large quantities of food microorganisms, a situation that often arises in culturing larvae of other species, such as clams and oysters.

The smallest normal straight-hinge larvae measured approximately $93 \times 64\ \mu$, and the largest, about $300 \times 286\ \mu$. Thus, our measurements are very different from those given by Sullivan (1948), who stated that larvae of *M. edulis* range in size from $155 \times 120\ \mu$ to approximately $355 \times 320\ \mu$. The smallest size given by Sullivan is, therefore, almost $60\ \mu$ greater than that of the smallest larvae in our cultures, and the maximum size is considerably larger than that which we ever recorded among swimming larvae.

Jørgensen also apparently mistook other larvae for advanced stages of *M. edulis* because he speaks of individuals which are $400\ \mu$ long. Werner (1939), whose length and width measurements of larvae of *M. edulis* somewhat resemble ours, still fails to give correct measurements for the smallest straight-hinge stage larvae. In his growth curve for these organisms he gives the size of the smallest larvae as approximately $112\ \mu$ long and $84\ \mu$ wide, which is considerably larger than the size of the smallest larvae ordinarily found in our cultures.

The so-called " eye " spot usually begins to appear in larvae when

they reach 215 μ in length. This spot is usually located approximately in the center of the larvae and measures from 5 to 7 μ in diameter. In one individual measuring only 205 μ in length a well-defined " eye " spot was found, but this was the only case of an " eye " in such a small larva recorded after examining thousands of individuals. When a length of 230 μ was reached the " eye " spot was present in all larvae.

The size at which mussel larvae metamorphosed varied by almost 90 μ. The smallest metamorphosed individual, attached by a byssus to an oyster shell laid in one of our culture jars, was only 215 $\times$ 201 μ. Individuals measuring 225 μ in length, but fully metamorphosed and attached by a byssus, were not uncommon. On the other hand, some larvae continued to swim until they were almost 300 μ in length. Large variations in the size of *M. edulis* larvae at the time of metamorphosis were also recorded by Jørgensen (1946). He thinks that variable size at metamorphosis is not entirely due to the fact that veligers may pass into the bottom stage after attaining a different stage of organization, but also because of the varying ratio between the rate of differentiation of the tissues and rate of growth in different individuals.

The factors which govern appearance of the foot and disappearance of the velum in larvae of *M. edulis* are rather baffling. In some instances a well-developed foot was observed in animals as small as 185 μ in length. Such cases, however, were not too common and, usually, the appearance of a developing foot occurred as the larvae approached 210 μ in length. Most larvae possessed a well-developed foot by the time they reached 230 μ in length, but a functional velum has been observed in larvae as large as 288 μ.

The great differences in the state of organization attained at a certain size by larvae of *M. edulis* are also emphasized by Jørgensen. He gives examples of larvae measuring 250 μ long and nearing metamorphosis with well-developed gills and foot and with a velum in a reduced condition, while in other cases larvae of the same size will have only a vestige of a foot but a large velum, thus indicating that they are still far from metamorphosing.

The variation in size of larvae at the time of metamorphosis suggested a more critical analysis of the data on growth of larvae in the same cultures. Fortunately, among our cultures there was one in which all larvae originated from eggs of the same female and were fertilized by sperm of the same male. Regardless of this, 2 days after fertilization the larvae varied in length from 93 to 120 μ, with an arithmetic mean of 106 μ. When the culture was 8 days old the length varied from 107 to 162 μ, the average being 130 μ. At 14 days some of the

larvae were already metamorphosing and the mean length was 181 μ, but some larvae only 128 μ long were still present.

This experiment demonstrated, as we have pointed out on many occasions, that it is obviously incorrect to relate the size of larvae to their age because variations in size, even among larvae that originate from the same parents, are of considerable magnitude. In cultures that are grown at different temperatures and fed different foods these differences are usually even more striking.

Some workers explain variations in size of larval fish originating from eggs produced by the same female as dependent upon development of these eggs while still in the ovaries, including proximity of an egg to large blood vessels which provide the best feeding conditions for a single egg. Others explain the different rates of survival and growth of fin fish larvae as dependent upon quantities of yolk in different eggs. Possibly, the same considerations hold true and, in part, explain variations in rate of growth of bivalve larvae that originate from eggs discharged by the same female in the same spawning.

As is usually the case with larvae of most bivalves, their color varies greatly in accordance with the color of the food microorganisms that they are able to obtain from water. Larvae of *M. edulis* given heavy doses of purple bacteria rapidly acquired a pinkish color, which was most pronounced in the area of their digestive organs. If these larvae were later fed green *Chlorella*, their coloration changed to a greenish tint and the livers became dark green. Contrary to Sullivan's (1948) contentions, no purple tint was observed around the shoulders of the shell, even in advanced larval stages.

F. *Anomia simplex* D'Orbigny

A. simplex is a common bivalve of Long Island Sound, where it propagates at approximately the same time as *C. virginica*. Because of its more rapid growth immediately after setting, however, this mollusk is a dangerous competitor, since it overgrows and completely covers large numbers of recently set oysters (Loosanoff and Engle, 1941).

Descriptions of larvae of several species of *Anomia* are given by students working in widely separated parts of the world. Stafford (1912) gave a description of *A. aculeata*; Miyazaki (1935) described the larvae of the Japanese species, *A. lischkei*, and Lebour (1938), as well as Jørgensen (1946), described those of *A. squamula*. More recently, Sullivan (1948) also described larvae of *A. aculeata*, while Rees (1950) made a brief reference to three species of *Anomia*. One

of these, *A. ephippium*, is a synonym for *A. simplex*. Other references can be found in the above-mentioned articles of Jørgensen and Sullivan.

Large *A. simplex* can be conditioned in December and induced to spawn during the first part of January. Some conditioned groups were induced to spawn several times between the middle of January and the beginning of August. To induce spawning presented no problem because, usually, ripe animals began to spawn as soon as the temperature of the water was raised only a few degrees above 20°C. Even in summer it was not necessary to raise the temperature much above 25°C. In many instances spawning began without addition of any sex products, a circumstance which is unusual in our experiences with spawning of other bivalves.

Artificial fertilization of *A. simplex* eggs is possible. Stripping can be easily accomplished by gently washing the light yellow gonad tissue of a female in sea water. The eggs separate easily and many remain uninjured. Therefore, a high percentage of them ordinarily develop into normal larvae.

Compared with eggs of other bivalves, eggs of *A. simplex* are comparatively small, ranging only from 42 to 45 μ in diameter. Because of their small size the eggs are difficult to handle, as they easily pass through the mesh of the smallest screen used for egg retention. Because eggs and early larvae cannot be strained and, thus, separated from the surrounding water, as is done with larger eggs, in fertilizing eggs of *A. simplex* care should be taken not to use too much sperm so as not to pollute later cultures of developing eggs and early larvae.

In our cultures of *A. simplex* the smallest larva with completely formed shells measured approximately 58 $\times$ 47 μ. Young larvae are more fragile and transparent than those of *C. virginica* of corresponding stages. The " straight-hinge " is actually sloped about 5° so that it is somewhat convex. This is difficult to observe under a microscope unless larvae are seen precisely in profile.

The most common size at which larvae of *A. simplex* metamorphosed was at a length of 195 to 210 μ. The largest swimming larvae measured approximately 215 $\times$ 220 μ. We cannot compare the dimensions and shapes of our larvae with those given by Stafford (1912) because his drawings and measurements bear little resemblance to the larvae of *A. simplex* in our cultures.

Sullivan (1948) gave the smallest size of *A. aculeata* as 110 $\times$ 100 μ, and the largest as 285 $\times$ 280 μ. Jørgensen (1946), in referring to his own observations on *A. squamula*, reported that the smallest larvae measured 65 to 75 μ in length, and the largest attained a length of

250 to 270 μ. Lebour (1938) stated that *A. squamula* may metamorphose at a length of only 180 μ, which coincides with our observations on *A. simplex*, while Stafford maintained that the veliger of *A. aculeata* is still pelagic at about 350 μ.

The size of larvae of *A. simplex*, when the " eye " spot appears, is difficult to indicate. In a few cases the " eye " was observed in individuals measuring only 136 μ in length although, as a rule, it appeared when the larvae were near 160 μ. The " eye " seems to be composed of from four to six dark bodies grouped together. In many individuals, including some that were near setting, no " eye " spot could be distinguished.

Even in early straight-hinge stages there is considerable difference in the shape of the two shells. The right shell, or the one that will

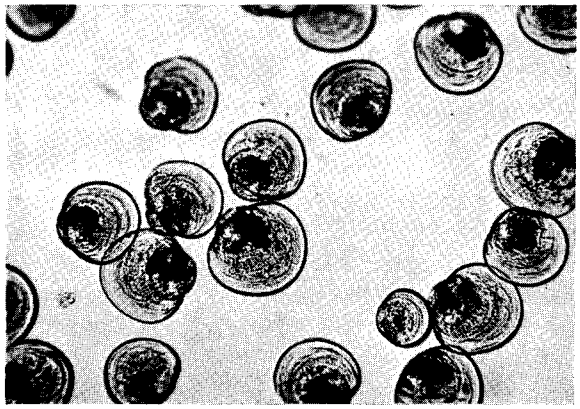

FIG. 32. Larvae of *Anomia simplex*. Largest larvae in photomicrograph are about 160 μ long. Note transparency of larval shells.

eventually be the upper, is conspicuously rounded, while the left, or the one that will become the lower shell is virtually flat. This distinction remains throughout larval development. Jørgensen (1946) reported that in *A. squamula* the umbo of the right shell is not developed. We made the same observation on the shell of *A. simplex*.

An observation made during the rearing of larvae of *A. simplex* concerned the inconsistency of the appearance on its shell of the indentation known as the byssus notch which, presumably, is diagnostic of larger larvae of this species. Since this was one of the features which we expected to be rather prominent and to appear in all older larvae, we were somewhat perplexed not to find this notch in many individuals. It is true that in some individuals this notch was present

in larvae only about 180 μ long, but often it was only a thickening of the shell edge on the side opposite the foot. In our extensive studies of living material and numerous preparations of larvae imbedded in balsam, in permanent slides, we were unable to find such " deformed " larvae as those shown by Sullivan (1948).

Early larval stages of *A. simplex* are very light, almost transparent in color (Fig. 32). Even larvae nearing metamorphosis remain silvery transparent except, of course, their digestive organs which, as a rule, take on the color of the food that they contain. In this respect our observations are rather different from those of Sullivan (1948), who stated that, at first, *Anomia* larvae are pale yellow but, later, become deep yellow, except for the viscera, which are dark gray.

Since larvae of *A. simplex* may be mistaken for those of oysters, especially in advanced stages when umbones in both species are quite prominent, one way to distinguish the two species is to remember that in *A. simplex* the digestive organs are situated much higher, lying almost under the umbones.

In the early stages of our work on cultivation of *A. simplex* larvae, which was conducted in 1950 and 1951, the only food that was regularly available was a mixture of green algae composed largely of *Chlorella*. Although the larvae reached metamorphosis when fed this food, their growth, as a rule, was slow and mortality heavy. Later, when cultures of *I. galbana* and *M. lutheri* became available, the larvae responded well to the new food, growing much faster and showing lower mortality. When fed these flagellates at room temperatures, many larvae of *A. simplex* began to metamorphose after the 12th day although, in the same cultures, some larvae were still swimming 33 days after fertilization.

As already mentioned, one of the most interesting observations made on cultures of *A. simplex* was what appeared to be partial metamorphosis, or metamorphosis without attachment to substratum (Loosanoff, 1961). Partial metamorphosis was characterized by disappearance of the velum, but retention of a functional foot. Moreover, these partially metamorphosed individuals were unable to attach to the substratum, and their shells showed a distinct demarcation line between larval and post-larval portions.

G. *Pecten irradians* Lamarck

The early embryonic development of the bay scallop, *P. irradians*, also called *Aequipecten irradians*, has been described by a number of authors, including Fullarton (1890), Drew (1906), Belding (1910),

Gutsell (1930) and, more recently, by Costello *et al.* (1957). The later development of the larvae, beginning with the formation of the straight-hinge stage, however, has never been adequately described because virtually no one succeeded in growing these larvae to metamorphosis. The only exception was Wells (1927), who grew scallop larvae past setting stage, but gave only a superficial description of them, although he supplied good photographs showing the different stages of development.

Descriptions of eggs and early stages of several European Pectinidae were offered by Jørgensen (1946). However, as in the case of the American bay scallop, virtually none of the European workers, except Odhner (1914), gave photomicrographs of older veliger stages that may be helpful in identifying the larvae.

The scallops used in our studies were brought, in February, from Long Island, where they are found in a number of bays, harbors and inlets. They were placed in running sea water, the temperature of which was increased within a few days to 20°C. The first spawning was attempted after 23 days of conditioning. The scallops responded readily to a temperature stimulus of about 30°C and spawned profusely.

Scallops usually spawn first as males and, later, as females. During one spawning, fourteen scallops started spawning as males. However, 10 or 15 min later seven of these fourteen individuals began to discharge eggs, but within a few minutes two of these seven again reverted to spawning as males, releasing large numbers of spermatozoa. On one occasion the same scallop was observed releasing sperm and eggs simultaneously.

Scallop eggs obtained in our experiments measured from 55 to 65 μ in diameter, the average size being near 60 μ. Belding (1910) and Gutsell (1930) gave the size of the scallop egg as 63 μ. Fullarton (1890) reported that the egg diameter of a related European species, *Pecten opercularis*, is 68 μ. The scallop egg is surrounded by a thin membrane and is usually of a pale orange color. Brief remarks on fertilization, cleavage and rate of development of the fertilized ovum were given by Costello *et al.* (1957), who based his descriptions on the works of Belding (1910) and Gutsell (1930).

The smallest straight-hinge scallop larvae observed in our cultures were 80 μ long and 65 μ wide, and the largest swimming individuals, approximately 200 $\times$ 195 μ. Some larvae metamorphosed upon reaching 175 μ in length. Jørgensen (1946) stated that the largest planktonic stage of the European scallop, *P. opercularis*, is approximately 250 to 260 μ long, while the larvae of another European scallop, *Pecten striatus*, approaching metamorphosis may be only 200 to 210 μ

in length, which is in close agreement with the measurement of larvae of our species.

Scallop larvae were grown at room temperatures varying between 20° and 23°C. They were fed either mixed cultures of plankton consisting principally of *Chlorella*-like forms or a mixture of flagellates, such as *I. galbana* and *M. lutheri*. They grew well on either diet, but cultures fed flagellates grew better. When well fed the growth of larvae is comparatively rapid, averaging more than 10 μ per day. In some of our cultures setting began 14 days after fertilization.

Larvae are susceptible to fungus diseases, but fungus does not appear to be as devastating to scallop larvae as it is to larvae of

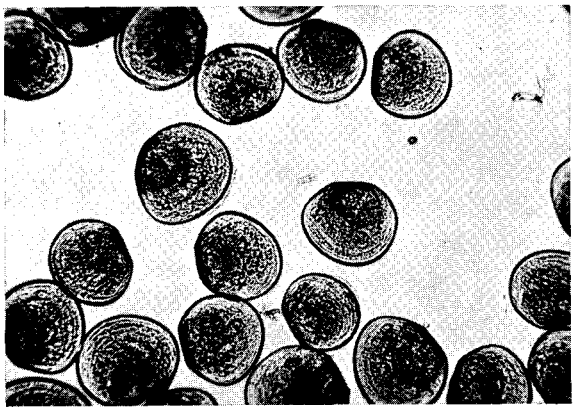

FIG. 33. Larvae of *Pecten irradians*. Largest larvae are about 180 μ long.

M. mercenaria or *T. navalis*. Larvae are also highly susceptible to bacterial infections.

Scallop larvae are quite pale and, although the color changes with the food contained in their digestive system, normal scallop larvae are invariably paler than those of *M. mercenaria*. At all stages they appear slightly asymmetrical, this condition becoming more pronounced in larger and older larvae (Fig. 33). In those measuring over 125 μ in length a slight notch is evident at the base of the shell, on the less pointed end of the larvae. Although this mark is not too conspicuous, it does appear to be characteristic.

Healthy larvae are active and energetic swimmers. Some of them display a small inconspicuous " eye " spot when they reach about 150 μ in length. Although the " eye " can be seen in most larvae about 170 μ in length, in larger larvae this spot is virtually invisible, probably because of the thickening of the shell.

H. *Ostrea edulis* Linné

We are interested in this species because its northern races propagate at somewhat lower temperatures than the American oyster, *C. virginica*. Because of this we imported *O. edulis* into the United States in 1949 in the belief that certain areas along the shoreline of Maine and our Pacific coast states were promising for natural propagation of these oysters (Loosanoff, 1951, 1955, 1962b). Since then, *O. edulis* have grown and propagated naturally in New England waters and have become established in Maine. Young oysters reared at Milford, Connecticut and sent to the State of Washington have also grown well there.

Literature on the sexuality and propagation of the European flat oyster, *O. edulis*, has been well reviewed by Orton (1937), Korringa (1941), Walne (1956) and Yonge (1960) and, therefore, need not be discussed here in detail. Using our methods for ripening mollusks out of season we have obtained larvae of *O. edulis* from the end of January to the end of the normal spawning period which, in Milford Harbor, extends into September. Our attempts to induce spawning of ripe oysters were not always successful. On several occasions, nevertheless, by raising the temperature of the water containing conditioned oysters and simultaneously adding a suspension of gonad material, spawning has been initiated in both sexes. This was ascertained by finding sperm balls or recently discharged eggs on the bottom of the aquaria. Well-developed eggs ranging in size from 114 to 126 μ were often lost when, during the process of spawning, they were released from the gonad to enter the gill chamber where, normally, they are incubated until larvae are released at swarming.

The manner in which eggs are lost was observed on several occasions during normal spawnings. These eggs were released from the exhalant chamber in a thin stream, almost in "single file". Because only a weak current was carrying them, the eggs settled next to the shells of the mother oysters, eventually forming a small mound. By a gentle moving of the spawning oysters from one area of the aquarium to another and observing the formation of new piles of eggs, it was established that a spawning female may continue a steady discharge of eggs for as long as 4 hr.

Since we could not always induce spawning in conditioned oysters, we depended quite often on unprovoked normal spawnings and subsequent release, or swarming of the larvae. Therefore, when a gravid female was detected, by the presence of a small pile of eggs near its excurrent side, it was placed in a separate aquarium with aerated standing water and kept there until larvae were released. The

water was changed daily and the oysters were given sufficient quantities of food to keep them and the larvae alive.

At room temperatures swarmings took place from 6½ to 10 days after spawning. The size of larvae released by a single female in the same swarming varied considerably. On one occasion samples of 100 larvae were collected from swarms released by each of six females which had been kept in separate aquaria. Larvae released by female (A) ranged in length from 142 to 199 μ, with a modal size of 186 μ; by female (B), from 164 to 192 μ, with a modal size of 184 μ; by female (C), from 164 to 203 μ, with a modal size of 188 μ; by female (D), from 149 to 203 μ, with a modal size of 187 μ; by female (E), from 164 to 192 μ, with a modal size of 180 μ; and female (F), from 164 to 195 μ, with a modal size of 183 μ. Occasionally, broods of smaller larvae measuring 175 $\times$ 160 μ were released. The average size of such broods closely resembled those reported by Boury (1928), Voisin (1931) and Korringa (1941).

In rare cases the modal sizes of larval broods were unusually large, over 200 μ in length. Because all female oysters incubating larvae were kept at the same temperature, it is improbable that the difference in size of the liberated larvae of the different broods was governed by the temperature at which incubation occurred. It seems more reasonable that size of larvae at liberation depends upon how long larvae are retained in the mantle cavity. This may be governed by the physiological condition of the parent oysters or the conditions in the water in which oysters are kept, the larvae being retained longer and, hence, are larger at time of release when these conditions are not at their optimum. This is supported by observations on liberation of larvae by two females that appeared sick. The first was used in an experiment where induced spawning was attempted by quickly raising the temperature to 37°C. The oyster did not spawn but was apparently injured by this treatment because it gaped for several hours after its return to sea water at room temperature. It did spawn the next day, however, but the spawning was not normal because too many eggs were found on the bottom of the aquarium near the oyster. The eggs, nevertheless, were fertilized.

Ten days later this oyster discharged, simultaneously, a brood of larvae and many empty larval shells, indicating that many larvae had died within the mantle cavity during incubation. There was also a considerable number of abnormal larvae with defective vela. Normal larvae, which still were in the majority, measured from 199 to 212 μ in length, with a modal size of about 208 μ. Regardless of some abnormality observed in the development of this brood, the larvae were reared to metamorphosis.

The second release of larger-than-average larvae, measuring between 200 and 210 μ in length, was also observed in an oyster that was kept at high temperature for several hours and was probably injured by this treatment.

Another possibility is that the size upon release may be governed by the number of incubating larvae in the mantle cavity, with the larvae growing faster if the number is small.

Swarming or, more correctly, release of larvae by parent oysters was observed on numerous occasions. In general, it resembled discharge of eggs by a spawning female of *C. virginica*. The larvae were expelled in cloud-like masses by a forceful closing motion of the shells. Discharge of larvae by a single female may continue for 3 or even 4 days. Usually, during the 1st day only a few larvae are released. The chief masses of larvae are expelled during the 2nd day and then the number of newly released larvae sharply decreases. Larvae that had just been released usually contained in their stomach large quantities of algal cells which had been added to the water during their incubation.

In many of our experiments, including the early one when larvae were fed phytoplankton composed principally of *Chlorella*, the veligers grew and survived well. At temperatures ranging between 18° and 20°C " eyed " larvae measuring approximately 270 μ, with a prominent pigmented spot, began to appear about the 9th or 10th day. Setting usually began on the 15th or 16th day, although in several cases, when fed *I. galbana* and *M. lutheri*, the larvae began to set between the 7th and 8th days.

Setting size of the larvae in our cultures was most commonly between 280 and 300 μ. In this respect our observations agree with those of Cole (1939), who reported that metamorphosing larvae in his experiments measured from 290 to 310 μ. However, we have never observed larvae as large as 350 μ which Cole found in some of his tanks. Imai *et al.* (1953) reported that none of the larvae of *O. edulis* in their cultures reached 300 μ in width, and most of the individuals in Walne's (1956) broods also set before reaching 300 μ.

Throughout the experiments the rate of growth of individual larvae of the same broods showed considerable variations. For example, at the time of setting, when some larvae in a culture already measured 300 μ in length and were undergoing metamorphosis, other larvae still measured only about 175 μ, thus being of approximately the same size as many larvae at the time of swarming.

When recently released larvae were placed in culture jars many of them ascended to the surface and formed large, brown, floating masses,

often measuring several square millimeters in area. This congregating tendency apparently did not unfavorably affect larvae. If these masses of larvae were disturbed, the larvae would separate and continue to swim for some time but, later, would again form floating groups. Similar floating masses of larvae are frequently observed among older larvae of other species, including *C. virginica*.

I. *Ostrea lurida* Carpenter

The native oyster of our Pacific coast is the Olympia oyster, *O. lurida*. It is considerably smaller than its close relative, *O. edulis*, or the Atlantic coast oyster, *C. virginica*, seldom exceeding a length of $2\frac{1}{2}$ in. Unlike *C. virginica* which, when adult, are of separate sexes, the Olympia oyster is hermaphroditic. Its reproduction is also different from *C. virginica* in that the eggs are not discharged directly in the water but, as in *O. edulis*, they remain within a special brood chamber in the mantle cavity of the mother oyster.

Gonad development and spawning of *O. lurida* can be induced out of season by keeping them in water at about room temperature for several weeks. Spawning can be detected, as in *O. edulis*, either by clouding of the water with discharged sperm or by presence on the bottom of a few eggs that were lost during spawning. The eggs measure from 100 to 110 μ in diameter.

According to Coe (1931) Olympia oysters begin to spawn when the temperature reaches 16°C. Hori (1933) indicated the temperature as 14°C and Hopkins (1937) thought that spawning occurred soon after the minimum daily water temperature reached 13°C.

In our experiments the incubation period lasted from 7 to 9 days after which swarms of larvae were discharged in the water. Stafford (1914) estimated that in *O. lurida* the period between spawning and swarming was $16\frac{1}{2}$ days. Coe (1931) reported this period as 10 to 12 days, and Hopkins (1937), as about 10 days. The difference in time is probably due to environmental conditions, especially temperature, under which the different observers conducted their studies.

We were unable to rear successfully recently fertilized eggs that were occasionally lost by females. However, embryos that were lost after they had reached the ciliated blastula stage were cultured to straight-hinge stage.

In one of our experiments larvae as small as $160 \times 149\ \mu$ were released at swarming, but these larvae appeared immature as they were too transparent and unable to withdraw the velum completely. It was possible, nevertheless, to rear even these small larvae to metamorphosis.

The size of normal larvae, at the time of swarming, was about 185 μ, but on several occasions groups of smaller larvae, measuring from 165 to 170 μ were released. Hori (1933) reported that larvae at the time of release are between 175 and 185 μ. Hopkins (1937) gave 180 μ as the average. Imai *et al.* (1954), in culturing *O. lurida* in Japanese waters, found that the length of larvae immediately after swarming ranged from 174·6 to 189 μ. In general, all these figures closely agree with ours.

The larvae are quite dark, even when first released, and appear black when seen *en masse* (Fig. 34). When given good food and kept at a temperature of about 24°C growth of these larvae is quite rapid, some of them beginning to set only 7 days after swarming. At 18°C,

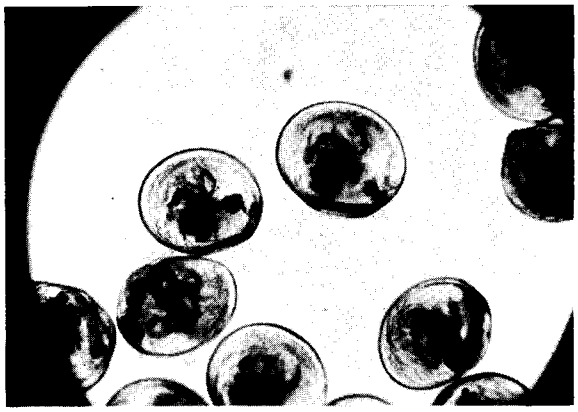

FIG. 34. Larvae of *Ostrea lurida*. Largest larva, near center, is 204 × 185 μ.

however, setting does not begin until the 16th day (Davis, 1949). Imai *et al.* (1954) reported that in their best cultures setting began on the 10th or 11th day at temperatures averaging 21·8°C.

Unlike larvae of the genus *Crassostrea*, larvae of *O. lurida* can utilize *Chlorella* from early straight-hinge stage and, in general, appear to be less restricted in types of phytoplankton organisms that they can utilize as food.

Larvae of *O. lurida*, particularly the larger ones, when seen under a microscope appear as thick wedges. Individuals 250 to 300 μ in length may measure as much as 200 μ in thickness if measurements are taken near the hinge, tapering rapidly to the sharp ventral or " bill " side. This makes it difficult to obtain accurate width measurements.

The setting size of larvae grown in our laboratory cultures was approximately 300 μ, which is not significantly different from setting

sizes of larvae of *C. virginica* and *C. gigas*. No free-swimming larvae measuring more than 312 μ in length were observed. Growth after metamorphosis was, for a short period, somewhat faster in *O. lurida* than in *C. virginica*.

J. *Crassostrea gigas* (Thunberg)

C. gigas, the common commercial species of Japan, was introduced to the waters of the State of Washington in 1902. During the last few decades it has become one of the most important mollusks of our Pacific northwest. Although the industry still depends largely upon seed oysters imported from Japan, a natural set of *C. gigas* occurs occasionally in such areas as Quilcene, Willapa and Dabob Bays and Hood Canal.

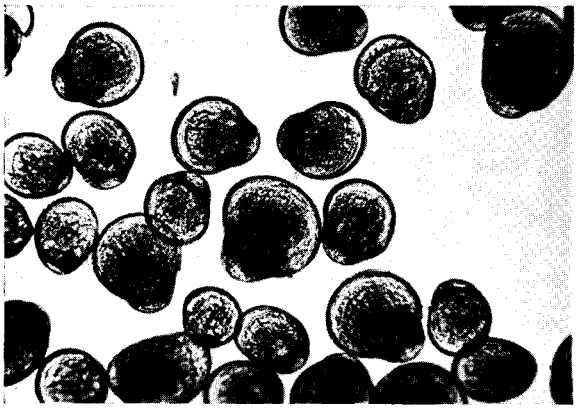

FIG. 35. Larvae of *Crassostrea gigas*. Largest larva, near center, is approximately 175 μ long.

Extensive studies conducted by Japanese workers on early development and propagation of *C. gigas* are reviewed at length by Cahn (1950). We may add to this review the articles of Hori and Kusakabe (1926) describing their successful efforts in growing larvae of *C. gigas*. More recently, Imai and Hatanaka (1949) and Imai *et al.* (1950b) grew larvae of these oysters on a much larger scale.

Our experience with *C. gigas* received from the State of Washington showed that their spawning behavior, egg development and the shapes of their larvae seem to be identical to those of *C. virginica* (Fig. 35). The similarity in shapes and sizes of the larvae of these two species is so great that we doubt if even an experienced investigator, familiar with both species, would be able to differentiate them in a mixed sample.

The general behavior, food requirements and growth rate of larvae of *C. gigas* also appear to be identical to those of larvae of the American oyster. Even the size of the eggs and the size at which larvae of the two species begin to metamorphose are alike. A comparison of the length–width relationship of larvae of the two groups will still further indicate their similarity. Yet, regardless of this similarity, the attempts of Davis (1950) and Imai *et al.* (1950b) to hybridize these two species were unsuccessful. Although fertilization occurred and the larvae developed into veligers, they all died before reaching metamorphosis.

Gonad development and spawning of Japanese oysters kept at Milford have not been as thoroughly investigated as those of the American oyster. Nevertheless, there appear to be several important differences. First, Japanese oysters require higher temperatures to begin spawning. Groups of these oysters kept in the laboratory all summer did not spawn, although the temperature at times was over 25°C. Furthermore, *C. gigas* either does not resorb the gonad material that remains undischarged at the end of the spawning season or does so at a much later date and at lower temperatures than *C. virginica.* Japanese oysters, brought to Milford from Puget Sound in October with undischarged gonads, held this material far into the winter and active sperm was found in some oysters late in December.

In our waters growth of post-setting stages of Japanese oysters is much more rapid than that of the local species. *C. gigas* that set in the laboratory in the spring and then were planted in Milford Harbor were $2\frac{1}{2}$ to 3 in long by the end of the growing season, while native oysters, which had set at the same time and were grown under identical conditions, were only 1 to $1\frac{1}{2}$ in long.

K. *Laevicardium mortoni* (Conrad)

This bivalve, which is called *Cardium mortoni* by some authors and is also known as Morton's cockle, is quite common in the comparatively shallow waters of Long Island Sound. If brought into the laboratory in late January and conditioned at temperatures between 15° and 20°C for several weeks, these cockles are easily induced to spawn by temperature stimulation alone. On several occasions spawning of ripe cockles began in less than 5 min after the water temperature was raised only 1° or 2° above 20°C. The majority spawned within $\frac{1}{2}$ hr and, as a rule, all began spawning before the water reached 30°C. Some cockles spawned at least three times during a single week.

The hermaphroditic cockles often acted first as males and then as females in the same spawning. Sometimes, after first discharging sperm, they released large quantities of eggs and finally ejected sperm

again. Probably because of this condition, which resulted in large quantities of sperm in the spawning dishes, many cases of polyspermy were seen.

The eggs of *L. mortoni* measure from 60 to 65 μ in diameter. Lovén (1848) reported that eggs of the related species, *Cardium exiguum*, have a diameter of 64 μ and are deposited in a thick gelatinous mantle within which development goes on until the shell-bearing veliger has attained a length of 90 μ. Orton (1924), who performed successful artificial fertilization of a closely related species, *Cardium fasciatum*, and Jørgensen (1946) reported that eggs of that bivalve measure about 80 μ in diameter, thus being considerably larger than those of our species. In still another closely related form, *Cardium edule*, according to Lebour (1938), the egg diameter is only 50 μ.

The smallest normal straight-hinge larvae in our cultures measured about 85 $\times$ 70 μ. Sullivan (1948) stated that very small larvae of *Cardium pinnulatum* are 90 $\times$ 80 μ, a size closely corresponding to our smallest normal larvae. In early stages the margin of the larval shell is outlined by a dark band, similar to that seen in *Teredo* larvae, but not quite as prominent. Usually, one end of the shell is slightly more pointed than the other.

The umbo begins to appear when larvae are between 135 and 150 μ in length and is usually quite prominent when a length of about 160 μ is reached.

The larvae were grown at room temperature of about 20°C and fed a mixture of *I. galbana* and *M. lutheri*. The first metamorphosing individuals were observed 8 to 10 days after fertilization. The smallest individual, having a definite foot but no velum, measured only 205 μ, while the largest swimming larvae with a still well-developed velum measured 245 μ. Some metamorphosed cockles with well-developed gills and a siphon were only 220 μ long. No " eye " was observed in any of the larvae.

The size of the largest swimming larva ever recorded in our cultures was 250 $\times$ 220 μ, but only one such individual was seen. The maximum size of our larvae, therefore, closely agrees with that described by Sullivan (1948) for *C. pinnulatum*, which measured 250 $\times$ 230 μ. Jørgensen (1946) mentioned that Lebour grew larvae of *Cardium echinatum* which, at metamorphosis, measured about 480 μ in length. We accept this figure with considerable reservation. Jørgensen also assumed that the maximum length of larvae of *C. exiguum* is about 250 μ, although he never observed this veliger with certainty in the Sound, where he carried on his experiments. His observation on *C. fasciatum* indicated that the largest larval stages

of this species found in plankton were about 300 μ long. However, he added that the veliger may leave the plankton at a length of about 255 μ, which is not too different from our maximal size.

Jørgensen (1946) also offered extensive observations on the length of larvae of *C. edule*. He stated that in rearing experiments at Kristineberg the length at metamorphosis varied from about 275 to 345 μ. Length measurements of prodissoconchs in oyster ponds near Limfjord varied from 200 to 275 μ, averaging 240 μ. Jørgensen was under the impression that larvae of this species are distinguished by an excessive variation in size at the time of metamorphosis. This condition coincides with our observation that usually in cultures of larvae approaching metamorphosis there is a great variation in size. For example, in our

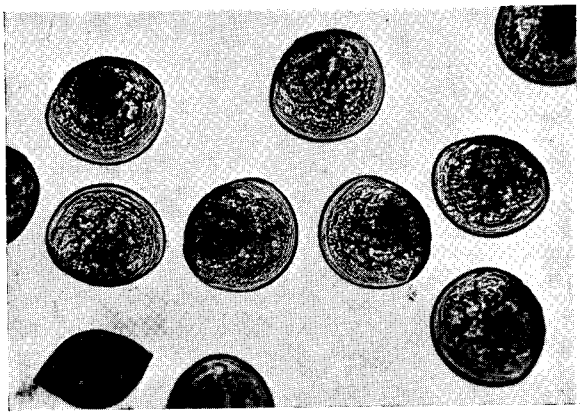

FIG. 36. Larvae of *Mercenaria campechiensis*. Largest larvae are approximately 185 μ long.

cultures of *L. mortoni*, while some of the larvae had already set and others approaching metamorphosis measured 245 $\times$ 205 μ, the smallest individuals in the same culture, grown under identical conditions, were only 140 $\times$ 120 μ.

L. *Mercenaria* (= *Venus*) *campechiensis* (Gmelin)

These clams, commonly known as southern quahogs, were brought to Milford from the Gulf of Mexico, near Apalachicola, Florida. Although they appear to differ somewhat from northern quahogs, *Mercenaria* (= *Venus*) *mercenaria*, their spawning behavior and appearance of their eggs and larvae are identical and, therefore, they will not be described (Fig. 36).

On several occasions groups of *M. mercenaria* and *M. campechiensis*

were conditioned for spawning and later induced to spawn simultaneously but in separate dishes. The eggs obtained from these spawnings were cultured under identical conditions. Observations showed that egg development and rate of growth of larvae of these two species were virtually identical throughout the experiment and that metamorphosis in both groups began at the same time.

Reciprocal crosses of the two species produced viable larvae, which grew to metamorphosis; at times these hybrids showed somewhat more rapid growth, possibly as a result of hybrid vigor. Many of these hybrids have been grown to maturity in several locations along our South Atlantic coast and also in the Gulf of Mexico, but not in cold areas, such as Milford Harbor, where the low water temperature in winter usually caused their death.

M. *Tapes semidecussata* Reeve

T. semidecussata is one of the most important commercial species of clams in Japan, where it is known under the common name of Asari. It was accidentally introduced to our Pacific coast when Japanese oysters, *C. gigas*, were brought there early in the century. It spread rapidly and is now one of the most important species of clams in the State of Washington, where it is called the Japanese little neck, or Manila clam. In the literature *T. semidecussata* appears under a variety of names, including *Venerupis semidecussata*, *Venerupis philippinarum*, *Paphia philippinarum*, *Tapes japonica*, and others.

The first group of *T. semidecussata* was received in Milford from Puget Sound in December 1955. Upon arrival the clams were conditioned at about 20°C for 2 to 3 weeks and then induced to spawn. Cultures of larvae were successfully grown from January until the end of the summer. According to Cahn (1951) the spawning period of *T. semidecussata* of the Hokkaido region of Japan extends from June to late August, when the water temperature is between 20° and 23°C.

In our spawning experiments ripe clams quickly opened and pumped vigorously when the temperature of the water was increased to about 25°C. However, raising of the temperature even to 30°C was often insufficient to induce spawning, and addition of sperm or egg suspension was usually necessary. Even then, on some occasions, although their gonads were filled with mature gametes, the clams did not respond to the combination of stimuli.

Spawning usually occurred at temperatures between 20° and 27·5°C. The act of spawning closely resembled that of *M. mercenaria*.

Sperm or eggs were released in a constant stream from the excurrent siphon, sometimes continuously for 30 min or longer.

Eggs of *T. semidecussata* range from 60 to 75 μ in diameter, including the transparent membrane around the eggs that measures from 5 to 10 μ in thickness. The average diameter of the egg, including the membrane, is approximately 69·6 μ. According to Cahn (1951) Japanese workers found that eggs of this clam measured between 63 and 66 μ in diameter with a perivitelline space measuring from 3·3 to 4·6 μ in width and surrounded by a gelatinous coating approximately 23 μ in thickness.

Most of the cultures of *T. semidecussata* were grown at room temperature. They were fed a variety of algal cultures, but no systematic

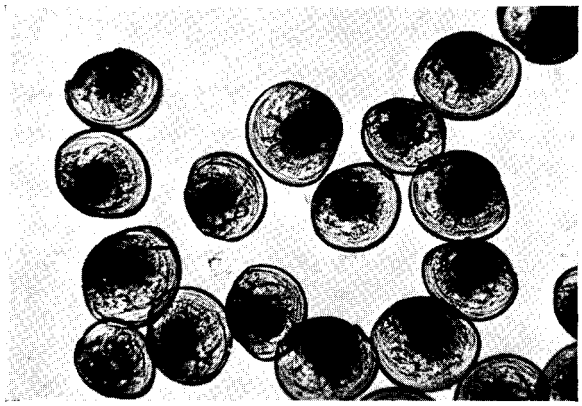

FIG. 37. Larvae of *Tapes semidecussata*. Largest larvae in photomicrograph are approximately 175 μ long.

studies of their food preference were made. Nevertheless, it was noticed that, while sometimes they grew well on mixed green algae consisting mostly of *Chlorella*, at other times, when given practically the same food, growth was poor. When given a mixture of *Chlorella* sp. and *P. tricornutum*, their growth also varied on different occasions. When fed *Chlorococcum* sp. the larvae usually grew well. Best growth was achieved when they were given *I. galbana* or *M. lutheri*.

Very early stages of straight-hinge larvae of *T. semidecussata* are remarkably similar to those of *M. mercenaria*. Some of the smallest straight-hinge larvae in our cultures were only about 90 μ long, but such individuals were uncommon; the majority measured approximately 95 $\times$ 70 μ. The umbo begins to develop when the larvae are approximately 120 μ long and becomes fairly prominent by the time

the larvae are about 140 μ in length although, even then, the hinge line may appear straight when seen from certain angles. As the larvae grow, the umbo becomes more prominent and in older larvae it is somewhat more pronounced than in *M. mercenaria* (Fig. 37).

When fed *Chlorella* sp. larvae reached metamorphosis in about 2 weeks. However, by giving them better food, such as naked flagellates, and by increasing the temperature of the water, setting size could be reached several days earlier.

The smallest metamorphosed individuals with a large, functional foot and fully resorbed velum measured about 175 μ in length, or about the same as that reported for *M. mercenaria*. The majority of the larvae metamorphosed when they were between 200 and 220 μ in length, but some much larger larvae measuring about 235 μ in length still possessed a functional velum and were able to swim. Yoshida (1960) gave the size of fully grown veligers of *T. variegata* as 215 $\times$ 195 μ.

As in most of our larval cultures, the color of the larvae depended, to a large extent, upon the food given. Furthermore, differences in size among larvae of the same cultures were of about the same magnitude as in cultures of other species. It has also been definitely established that larvae of *T. semidecussata* are susceptible to fungus diseases.

Because of our prolonged studies of *T. semidecussata* we have had an opportunity to observe their adaptability to changes in their surroundings. These clams were able to survive considerable variations in temperature and salinity, which occur over the tidal flats of Milford Harbor in front of our laboratory. During one winter, when the temperature of the water remained below zero for several weeks and the shores were covered with a thick layer of ice, only about 3% of the clams planted on the flat, near the mean low water level, died. The clams also survived periods of freshets, such as were caused by hurricane "Donna" in 1960, when salinity of the water in Milford Harbor was reduced almost to zero and remained low for several days.

The clams spawned and their larvae set normally in our experimental ponds. The small clams grew to a size of about 1 cm by the middle of September.

N. *Pitar* (= *Callocardia*) *morrhuana* Gould

This small mollusk, which somewhat resembles *M. mercenaria*, is found along the northern section of our Atlantic shore, roughly from Prince Edward Island to Cape Hatteras. In some sections of this range, as in Narragansett Bay, it is quite numerous.

Recent literature contains few references to propagation or larval

stages of *P. morrhuana*. Sullivan (1948) is virtually the only author to devote some attention to veligers of this bivalve. Costello *et al.* (1957), calling the same form *Callocardia convexa*, gave a good description of procuring gametes of these mollusks.

Our own experience with *P. morrhuana* has been extensive but not always satisfactory because this clam is one of the few bivalves that we could not induce to spawn. All the methods known to us, including increasing of the water temperature, addition of ripe sex products, changes in pH or salinity, and use of mechanical and electrical stimulation failed to induce spawning. Moreover, we have never observed natural spawning among the hundreds of these clams that have been kept in our laboratory under different conditions, sometimes for periods of several months.

Animals brought into the laboratory from their natural beds in January already contained what appeared to be morphologically-ripe gametes. The color of the female gonad was usually creamy white and that of the male a light yellow. Even this early in the season active sperm could be taken from male gonads. The spermatozoa resembled those of *M. mercenaria* in shape. Eggs could be easily obtained by stripping, but they could not be fertilized so early in the season. This was true even when *P. morrhuana* was slowly conditioned in the laboratory by day-by-day increases in temperature. However, as the season progressed, the eggs became more responsive and the germinal vesicle in some of them dissolved soon after introduction of spermatozoa.

Our larval cultures were grown from eggs obtained by stripping. The size of the eggs taken from females with well-developed gonads ranged in diameter from 49 to 60 μ, with the modal size-group between 50 and 55 μ. These measurements, however, refer only to the egg proper; if the entire diameter of the ovum, including its thick, gelatinous membrane were measured, it would vary between 92 and 128 μ, with the modal class near 117 μ.

On several occasions we tried to improve the condition of stripped eggs by adding 3 ml of 0·1 N ammonium hydroxide to every 100 ml of egg suspension. After 25 min of this treatment the eggs were screened to discard the fluid. The eggs were then rinsed with sea water, resuspended, and spermatozoa added in proper quantities. In some instances, after this treatment about 40% of all eggs underwent cell division, but usually not more than 5% developed to normal straight-hinge larvae.

Early development of eggs and larvae of *P. morrhuana* is virtually identical to that of other mollusks of the same group. Progress of development, as usual, depends upon the temperature at which the

organisms are grown. In some cases straight-hinge larvae were formed after 24 hr at a temperature of about 21°C.

The size of the smallest normal straight-hinge larvae was approximately 78 × 64 μ. Several smaller larvae measuring only 75 μ in length were also observed, but appeared to be abnormal. Some larvae began developing an umbo when 95 μ long, and the majority possessed a well-developed umbo by the time they reached a length of 125 μ. No " eye " spots were observed in either larvae or recently set juveniles. Larvae of *P. morrhuana* closely resembled those of *M. mercenaria*, but were considerably less active (Fig. 38).

Metamorphosis occurred when the larvae were still comparatively small. The smallest young clam possessing a functional foot and

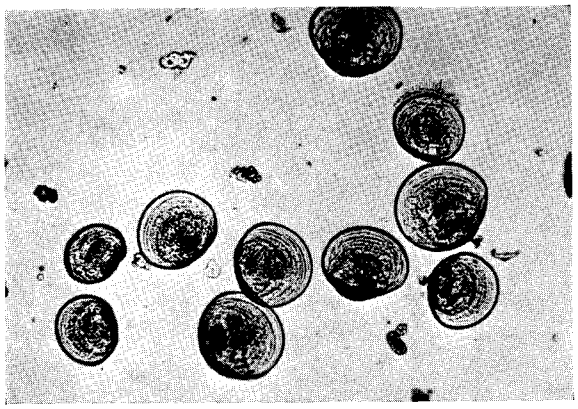

FIG. 38. Larvae of *Pitar morrhuana*. Largest larvae shown are approximately 150 μ long. Small, abnormal larvae can be seen in the group.

attached by a byssus thread measured only 160 μ. However, a majority of the larvae did not begin to metamorphose until reaching a length of approximately 180 μ. The largest free-swimming larvae still possessing a functional velum measured 192 × 179 μ.

According to Sullivan (1948) the smallest larvae of *P. morrhuana* were 120 × 95 μ. This size is considerably larger than that of the smallest larvae in our cultures. The maximum size offered by Sullivan, 220 × 210 μ, was much larger than ever recorded in our cultures. It seems improbable, therefore, that the larvae considered by Sullivan to be those of *P. morrhuana* were actually of this species.

Growth of larvae in different cultures varied considerably, depending upon temperature and quality of food given. The majority of the cultures fed a mixture of plankton, composed mostly of several species

of *Chlorella*, and kept at room temperature began to set about 14 days after fertilization. Probably if given better foods, such as *I. galbana* and *M. lutheri*, which at the time of rearing *P. morrhuana* were not available at our laboratory, a better rate of survival and growth could have been achieved.

Many cultures suffered mass mortality, which usually occurred during the late stages of larval growth. These mortalities were probably caused by the same fungi that were later found responsible for heavy mortalities of larvae in cultures of *M. mercenaria* and several other species.

O. *Petricola pholadiformis* Lamarck

Even though the range of this clam extends from Prince Edward Island to the West Indies and Texas and their larvae should be common in plankton samples during the reproductive period, Sullivan (1948) seems to be the only author who has described them. According to this author the first larvae of *P. pholadiformis* normally appear in the waters of Malpeque Bay, Prince Edward Island, about the 2nd week of July and, after reaching peak numbers in early August, disappear from plankton in early September.

In our laboratory, groups of *P. pholadiformis* were conditioned for spawning from the middle of December until summer. In the early winter these efforts were not as successful as later in the season and conditioning required a much longer time. For example, clams placed in conditioning trays at about 20°C in December required 4 to 6 weeks before the majority responded to stimulation and spawned. In the middle of April, however, only a week of conditioning at about 20°C was sufficient to bring these clams to spawning condition.

Conditioned clams were induced to spawn by our usual method, consisting of subjecting them to a quick increase in the water temperature from about 20° to 27°C and by the addition of a sperm suspension. Later in the season spawning, especially of males, could be induced on some occasions at a temperature as low as 15°C. Raising the water temperature to about 32°C always affected clams unfavorably.

Early in the season several hours at the increased temperature were often required before the first individuals, usually males, would respond, whereas late in March and in April spawning was usually provoked within the first hour. Even young clams, less than 1 yr old, and individuals with partially damaged shells responded to stimulation and discharged large quantities of spawn. Often the same individual would spawn for a while, cease spawning and then resume it. Many individually-marked clams spawned several times during the season.

Attempts at artificial fertilization of eggs of *P. pholadiformis* were partially successful, indicating that this can be done. As usual in these cases, the majority of stripped eggs were somewhat injured.

Ripe eggs measured immediately after discharge varied in diameter from 51 to 58 μ, but the modal size was quite clearly defined at 52 μ. No special studies were made as to the number of eggs produced by a single female during the season. However, in one case a large female discharged approximately 1 125 000 eggs in a single spawning.

According to Sullivan (1948) straight-hinge larvae of *P. pholadiformis* are quite large, measuring 115 $\times$ 100 μ. We found, however, that young straight-hinge larvae are considerably smaller than reported by Sullivan. With the exception of one, apparently an abnormal larva

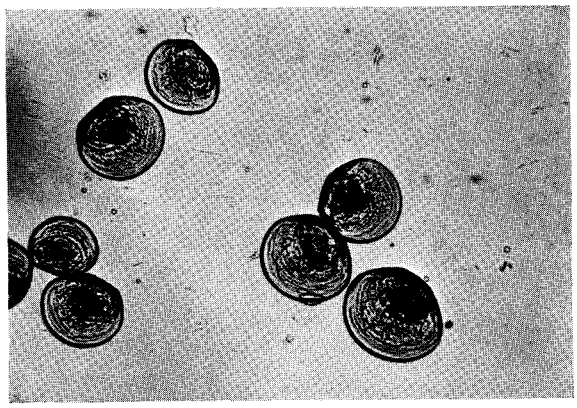

FIG. 39. Larvae of *Petricola pholadiformis*. Largest larva in this photomicrograph is about 164 $\times$ 143 μ.

which measured approximately 71 $\times$ 67 μ, the majority of normally formed young larvae were 79 $\times$ 65 μ in size. The largest free-swimming larva was about 193 $\times$ 174 μ, although many underwent metamorphosis before reaching this size, being approximately 186 $\times$ 164 μ (Fig. 39). These dimensions are somewhat larger than indicated by Sullivan (1948).

Early straight-hinge larvae are very light in color and even their digestive organs are comparatively pale. In general, they are more transparent and thinner than larvae of most of the species described in this paper. Some of them remain transparent up to the time of metamorphosis. However, when given purple sulphur bacteria belonging to the genus *Chromatium*, larvae quickly acquire a pinkish color. If they are given such foods as *Chlorella*, their digestive glands rapidly become a greenish color.

Since studies on larvae of this species were carried on when good food organisms, such as the naked flagellates, were not available, the larvae were fed mixed cultures of microorganisms in which *Chlorella* was usually the predominating form. Larvae grew well on this alga, although it is quite certain that they would have grown faster if given better foods.

Larvae of *P. pholadiformis* were grown at temperatures ranging from 18° to 30°C. The best cultures were observed at room temperatures ranging from 20° to 25°C. At somewhat lower temperatures, between 17° and 18°C, eggs developed and larvae grew, but growth was slow. At 30°C egg development was rapid, but larvae were feeble and the majority died within 2 to 4 days. Perhaps mortality was not due exclusively to temperature alone, but to the heavy bacterial flora that quickly established itself under these conditions.

The shortest period from fertilization of the egg to metamorphosis at room temperature was 13 days. In several instances, however, the larvae of these cultures did not begin to set until the 20th or 21st day. The differences were probably due to variations in quality and quantity of food.

Two characteristics were noticeable in young clams soon after metamorphosis. First, the color of their shells becomes much denser and, secondly, the edges of the shells opposite the umbo acquire a serrated, roughened appearance.

P. *Ensis directus* (Conrad)

The general characteristics of larvae of *E. directus* were given recently by Sullivan (1948). Costello *et al.* (1957) gave a brief description of early stages of egg development, while Lebour (1938) had studied larvae of *Ensis siliqua*. In general, however, larvae of *E. directus* have been less intensively studied than those of other common bivalves of our coast.

In our laboratory, observations on *E. directus* and its larvae were conducted for three winters. Each year these clams were brought from the field and placed in conditioning trays in late December. It was found, however, that when these clams are removed from the soil they soon begin to die because the powerful hinge pulls the clam shells apart. This can be prevented by placing tight rubber bands around each clam to counteract the action of the hinge and keep its shells closed. Using this simple method these clams have been kept alive without any soil for periods as long as 6 months.

Attempts to induce spawning of *E. directus* were made during the middle of January after 2 or 3 weeks of conditioning. Although at

that time their gonads contained some eggs that appeared morphologically ripe and active sperm, the clams would not spawn. Even after 6 or 8 weeks of conditioning, attempts to spawn these bivalves often met with failure. In those cases, however, when stimulation was successful, spawning proceeded normally. Some females were observed spawning almost continuously for a period of approximately 45 min, and males that spawned for periods sometimes as long as 2 hr were common.

When the clams were ripe they usually spawned within an hour after the temperature was increased to about 25°C. Males continued to spawn profusely even after being returned to water of only 13°C. Clams became sluggish at 30°C but, nevertheless, would spawn even

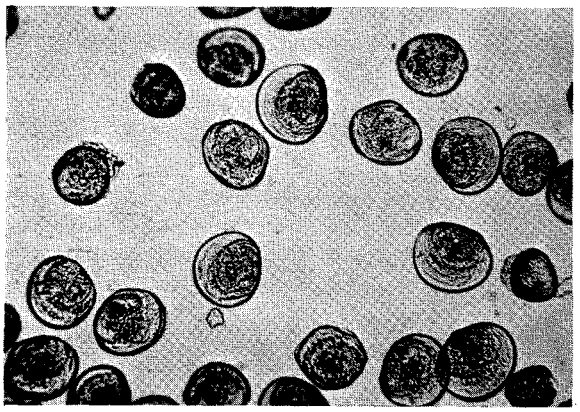

FIG. 40. Larvae of *Ensis directus* grown from eggs obtained by stripping. Note that many larvae are abnormal. Largest normal larvae shown are about 125 μ long.

at this temperature. Spawning activity was definitely accelerated by the presence of sperm. Both males and females released gametes in a thin, steady stream from the excurrent siphon.

Several clams spawned at least three times, at weekly intervals. The greatest number of eggs released by one female in a single spawning was 12 585 000. The greatest number of eggs released by one female in three spawnings was 20 535 000.

Unprovoked spawning of razor clams kept in conditioning trays occurred on many occasions. It took place at temperatures ranging from about 15° to 22°C. It has not been determined what stimulus induced spawning in those instances. Histological studies showed that on many occasions gonads were almost completely spent in a single spawning effort.

Success of fertilizing eggs stripped from conditioned females depended upon the degree of their ripeness. Early in the season, when the largest of the eggs were only 66 μ, none were fertilizable. Later, when the clams were riper, a large percentage of stripped eggs underwent cleavage but, as is often the case in using eggs obtained in this manner, most of the larvae were abnormal (Fig. 40). Costello *et al.* (1957) also reported that eggs of *E. directus* can be fertilized artificially and they remain fertilizable for about 5 hr.

The egg of a razor clam is surrounded by a narrow perivitelline space enclosed in a thin vitelline membrane. Measurements of eggs discharged in a normal spawning showed that they varied in diameter from about 64 to 73 μ, the majority being 71 μ, thus indicating that this is the modal size of normal eggs. Lebour (1938) gave the diameter of eggs of *Ensis siliqua* as about 70 μ, which closely resembles measurements of eggs of our species. Jørgensen (1946) gave references to other workers who studied eggs and larvae of *Ensis* and related forms.

Eggs and larvae were usually cultured at about 22°C. The smallest straight-hinge larvae were 80 μ in length, but only two individuals this small were observed. Even larvae as small as 85 μ in length were scarce, while those measuring approximately 92 $\times$ 78 μ were common. This size is considerably smaller than the 140 $\times$ 120 μ indicated by Sullivan (1948) as the smallest larvae of this species.

The larvae are light in color and more transparent than those of *M. mercenaria*. The umbo appears in larvae only 115 μ in length and becomes well developed in larvae 135 μ long. Larvae of this size have definitely passed the straight-hinge stage. The umbo is so pronounced in larger larvae that it is impossible for them to lie flat. As a result, considerable variation in the length–width ratio is obtained when large larvae are measured under a microscope.

Setting of *E. directus* began soon after some larvae reached a length of about 210 μ. At 24°C this often occurred only about 10 days after fertilization. However, some larvae remained swimming until they reached a much larger size before undergoing metamorphosis. For example, in one culture fed *Chlorella* sp. larvae measuring 250 to 260 μ in length were still swimming 27 days after fertilization and possessed both a powerful velum and a well-developed foot. The largest swimming larva measured 270 μ, while in the same culture many fully metamorphosing individuals showing a well-developed foot, gills and siphons were only 220 μ long.

The appearance of the " eye " was irregular and it was not located where this structure is usually found in most bivalve larvae. The smallest individual possessing this structure was only 126 μ long, but

many other larvae, even those nearing metamorphosis, did not display it. Sullivan (1948) did not mention the presence of an " eye " in *E. directus* larvae and Werner (1939), working with a species that he called *Cultellus pellucidus*, a form closely related to *Ensis*, stated that its larvae do not possess " eyes ". This observation is quite important because, according to Rees (1950), the description by Werner of *C. pellucidus* was really that of *Ensis*. Our observation that the " eye "-like structure is sometimes present in larvae of *E. directus* and Werner's, that it is always absent in the species that he studied, may indicate that Rees' assumption is not correct and that the *C. pellucidus* of Werner was not an *Ensis*.

One of the characteristics of larvae of *E. directus* which may be of diagnostic value is the presence of a clear area around the edge of the shell. This area, which does not occur in larvae of other species that we have studied, is well defined and clearly seen. It appeared in larvae of about 120 μ long and persisted until they reached a length of 180 to 190 μ. Later this area became less definite and more or less merged with other parts of the shell. Near setting, however, a purple cast became apparent along the edge of the larval shell.

In rearing of *E. directus* the presence of many abnormal larvae was quite common, not only in cultures that were started from stripped eggs, but also in those that were grown from normally discharged ones.

In several cultures, usually those where larvae were approaching setting stage, mortalities on an epizootic scale were recorded. In several instances practically all of the larvae died within a short period, usually less than one day. Since this happened before we learned the devastating effect of larval diseases caused by fungi or bacteria, we did not establish the cause of death of these larvae but strongly suspect that these mortalities were principally the result of fungus infestations.

Q. *Mactra* (= *Spisula*) *solidissima* Dillwyn

The clams used in these studies were taken from the sandy beaches of the south shore of Long Island, New York. They were shipped to Milford and kept for many months, either in the laboratory or in the harbor, without any appreciable mortality, although the salinity at Milford is about 27 ppt, or approximately 8 ppt lower than in the areas where the clams were dredged. The clams survived equally well whether they were allowed to dig into sand or were kept in wire baskets containing no soil. To prevent mortality, however, it was necessary to expel bubbles of air which were trapped inside the clams while they were out of water. Clams placed on their sides cannot expel these bubbles and, eventually, die.

Our experience in growing larvae of *M. solidissima* was confined to the period from the middle of January to the end of June. During that time morphologically-ripe eggs and spermatozoa were always present in the gonadal follicles. Possibly, equally well-developed sex cells could have been found in the gonads several weeks earlier than January because, apparently, the most important period of gametogenesis in *M. solidissima* begins immediately after the end of spawning, as in *M. mercenaria*, in which gonads of both sexes present a ripe appearance in December (Loosanoff, 1937b). Allen (1953) obtained ripe eggs of *M. solidissima* from early spring until late autumn.

Spermatozoa, taken from clams brought from their natural beds early in January, when placed in a drop of water at room temperature quickly began to move. However, eggs taken from clams of the same groups usually could not be fertilized, probably because they were not physiologically ripe. Nevertheless, placing female clams in water of about 15°C for only a few days was sufficient to ripen them. Later in the season, when the temperature of the water in Milford Harbor increased to 12°C, no conditioning was needed.

Fertilizable eggs could be obtained either by stripping or by inducing spawning. Sometimes, stripped eggs obtained from a ripe female were as viable as those discharged during a normal spawning. More often, however, a culture started with stripped eggs contained many abnormal individuals. Costello *et al.* (1957) suggested a different method of obtaining gametes, which consisted of straining the eggs through a cheesecloth.

Spawning was induced by the usual method of thermal and chemical stimulation. On several occasions thermal stimulation alone was sufficient. Normally, addition of a suspension of sex cells was required and, even then, many conditioned clams did not spawn.

Spawning was induced at temperatures ranging from 18° to 25°C. Temperatures of 30°C and over unfavorably affected the clams. They gaped and abnormally extended their feet which, like the rest of their bodies, became limp and soft. If, after exposure to such high temperatures the clams were quickly returned to cooler water of about 25°C, they often began to spawn, but the eggs obtained in such a manner did not develop past the formation of polar bodies. Apparently, exposure of clams to temperatures over 30°C caused serious injuries to their eggs. Our observations in this respect agree with those of Imai *et al.* (1953) who, while working on a closely-related species, *Mactra sachalinensis*, found that at a temperature of about 32°C none of the eggs underwent normal cleavage.

Observations were made on the growth of larvae of *M. solidissima*

at two different temperatures. Eggs obtained from several females and fertilized with spermatozoa of three ripe males were mixed together and then divided into two groups, one to be grown at a temperature of about 22°C and the other at 14°C. The concentration of eggs per ml of water was the same in both cultures. During the experiment the larvae were fed equal quantities of phytoplankton composed largely of *Chlorella* sp.

Eggs placed in water at 14°C were in the trochophore stage at the end of 40 hr. After 72 hr the culture contained normal and active straight-hinge larvae averaging 85 μ in length, which were quite uniform in size and appearance. At 22°C this stage was reached after only 28 hr. Young straight-hinge larvae of *M. solidissima* were usually

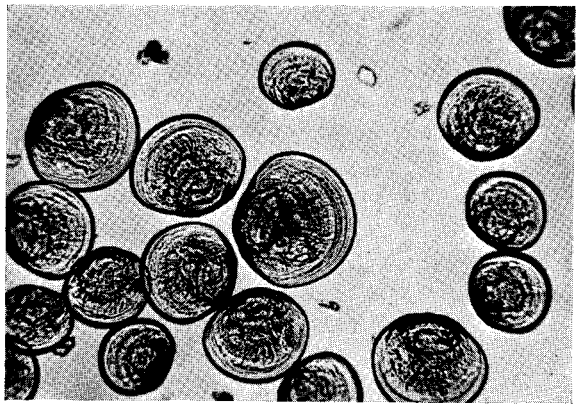

FIG. 41. Larvae of *Mactra solidissima*. Largest larva, 219 μ long and 193 μ wide, is approaching metamorphosis. Note differences in sizes of larvae that originated from the same group of eggs and were reared under identical conditions.

almost transparent and had a granular appearance with the internal organs at this time not too well defined. Later, as in the case of most larvae, the color began to deepen.

On the 19th day, when larvae in the warmer culture began to metamorphose, the length of the modal class of larvae grown at 14°C was only 117 μ, and the larger individuals in the cultures were only 153 μ long. The first metamorphosing clams were observed in this culture after 35 days. When the experiment was discontinued on the 50th day the remaining larvae showed a wide range in size, the smallest being only 109 μ long (Fig. 41).

A partial description of development and dimensions of larvae of *M. solidissima* was given earlier in this article. Here we may add

that the most recent description of what were assumed to be larvae of *Mactra* (= *Spisula*) *solidissima* was reported by Sullivan (1948). She gave the minimum size of straight-hinge larvae as about 95 × 80 μ, which is approximately 15 μ longer than usually found in our cultures, while the maximum size of 270 × 245 μ closely agreed with our measurements. In both instances the length–width relationship given by Sullivan falls near the median line of length–width relationships which we found for larvae of *M. solidissima.*

In describing larvae of a related form, *Spisula subtruncata*, Jørgensen (1946) reported that veligers are about 400 μ at the time of metamorphosis. Kändler (1926) stated, however, that the length of this species at metamorphosis is only 310 μ. Considering that Imai *et al.* (1953) found the maximum size of larvae of *Mactra* (= *Spisula*) *sachalinensis* to be about 270 μ, virtually the same as we found for *M. solidissima*, and because Imai's conclusions and ours are based on measurements of larvae of a known origin, we think that Kändler's measurements are more realistic than Jørgensen's and that the latter was probably describing larvae other than those belonging to the genus *Mactra* (= *Spisula*). The same consideration leads us to believe that the descriptions and measurements offered by Rees (1950) of advanced stages of larvae presumably of the superfamily Mactracea, including *Spisula solida* (360 μ) and *Spisula elliptica* (355 μ), are really those of some other species.

R. *Mya arenaria* Linné

Our efforts to induce spawning of *M. arenaria* were confined largely to the period extending from March until the middle of July. Several groups of these clams were also conditioned and spawned in winter. Moreover, to induce spawning of even well-conditioned and apparently ripe *M. arenaria* is difficult, nevertheless.

In developing a method to induce spawning of these clams we tried many approaches, including sudden and gradual changes in water temperature, changes in pH, salinity, hydrostatic pressure, light intensities and the addition of sex products. Usually, none of these worked. The only method that proved to be successful with some regularity consisted of subjecting ripe clams to water of relatively high temperature, of about 26° to 28°C, for long periods often extending from 6 to 8 hr and adding, during this time, a suspension of sex products. Many clams spawned profusely when this method was employed and discharged a large number of eggs, but a high percentage of these eggs usually developed into abnormal larvae.

Belding (1931) reported the diameter of the average egg of the

soft shell clam as 62·5 μ, while Battle (1932) gave the egg size as varying from 70 to 80 μ. Our measurements of hundreds of eggs discharged by different females and on different occasions showed that the majority were between 68 and 73 μ in diameter, with a modal size of 70·5 μ.

Belding (1931) expressed the opinion that artificial cultivation of *M. arenaria* is virtually impossible because the eggs either fail to develop normally or else never pass the young veliger stage. Nevertheless, Belding was able to show that, unlike other pelecypods, eggs stripped from *M. arenaria* can be artificially fertilized.

The smallest normal straight-hinge larvae recorded in our cultures measured only about 86 × 71 μ. These were, however, extremely uncommon and normal, fully-formed straight-hinge larvae were usually about 93 × 77 μ. As in most pelecypod larvae, they were light in color at this stage and their internal organs were not well defined. They remained quite light, almost transparent, until a length of about 110 μ was reached. As the larvae grew, they became darker. Nevertheless, as mentioned on several occasions, these larvae do not possess characteristic colors that would help to distinguish them from members of other genera or species of bivalves. In our experiments, where larvae of this species were fed different foods, their color ranged from a dark reddish-brown to dark green. We cannot, therefore, agree with Sullivan (1948) that brown pigmentation in large larvae of *M. arenaria* is diagnostic of that species.

In older larvae measuring about 175 μ and longer we noticed the presence, in the margins of the mantle, of irregular opaque spots varying in size from 5 to 15 μ. These granules occurred with such regularity that we are inclined to consider them as characteristic of the species, at least during late larval stages. Jørgensen (1946) noticed a somewhat different pigmentation of the soft parts of larvae of *M. arenaria* measuring about 200 μ and larger. He also suggested that this may be a reliable specific character.

The size of larvae of *M. arenaria* at setting is extremely varied. Metamorphosis may occur at any length from 170 to 228 μ. The latter is the size of the largest free-swimming larva ever recorded in our cultures. The majority metamorphosed at a length between 200 and 210 μ.

The smallest larva in which the foot was present was about 165 μ long but many of the larvae had a well-developed foot by the time they reached 175 μ in length. The presence of a large foot does not necessarily indicate that the velum has already become non-functional. Larvae as long as 210 μ have been seen at times swimming about using both the velum, which still appeared to be of normal size, and also

the large foot. However, the velum normally begins to disappear soon after a length of 172 to 175 μ is reached and sometimes even earlier. In most individuals 200 μ in length the velum is already resorbed. Some larvae measuring only 175 μ in length, and having no velum, were seen actually crawling, using their feet.

The balancing organ, the otocyst, can be clearly seen at the base of the foot of larvae measuring about 175 μ in length. The byssus gland also can be seen in larvae less than 200 μ in length, and the gills may be clearly discerned in some individuals of about the same size. The byssus thread is strong, and our attempts to break it by directing a strong jet of water from a pipette caused the larvae to sway from side to side, but did not break the thread.

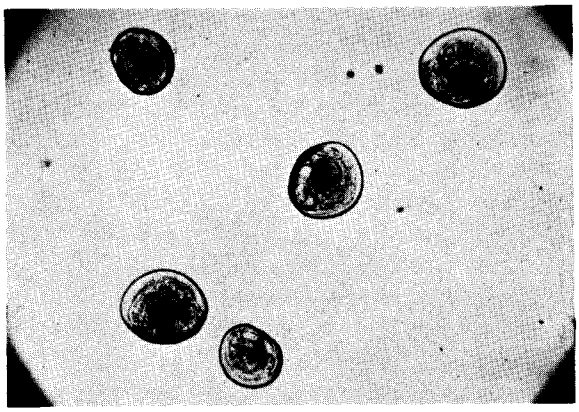

FIG. 42. Young larvae of *Mya arenaria*. Largest larva shown is about 140 μ long.

No systematic studies on the rate of growth of larvae of *M. arenaria* at different temperatures, such as those conducted with larvae of *M. mercenaria*, were made. Our attempts to grow larvae at low temperatures ranging from 12° to 15°C were usually unsuccessful because of slow growth. For example, at a temperature of about 14°C larvae, even after 15 days, were only about 110 to 115 μ long. Larvae grown at low temperatures, probably because of the slow rate of growth, were usually of extremely uniform size.

Most of our cultures were grown at room temperatures which ranged from about 19° to 24°C. Under those conditions the rate of growth was quite rapid, although it varied, of course, from culture to culture, depending upon the temperature, concentration of larvae, and quality and quantity of food given. At about 23°C the average length of larvae 2 days after fertilization was approximately 109·5 μ

and the maximum 117 μ. After 5 days larvae averaged 120 μ in length and the largest individuals were 140 μ long (Fig. 42). After 10 days some of the largest larvae approached a length of 180 μ at which setting is possible. By the 15th day many individuals had already set, and the average size of the larvae in the cultures had increased to about 175 μ. A few large larvae, measuring about 225 μ in length but still free swimming, were also found in our samples. In some cultures setting began when the larvae were about 10 days old and continued until the end of the 35th day.

As has already been mentioned, the smallest straight-hinge larvae of *M. arenaria* in our cultures measured about 86 $\times$ 71 μ. The largest free-swimming individuals were 228 $\times$ 207 μ, although most of them metamorphosed before that size was reached. Our measurements, therefore, differ from those given by Stafford (1912), who stated that the smallest straight-hinge larva of *M. arenaria* that he found was only 75·9 $\times$ 62 μ, while the largest measured 414 $\times$ 345 μ. Of the latter he said, "The largest measurement I have is 64 $\times$ 53, and I have seen them attached by a byssus-thread, their siphons protruded, and the big hinge-tooth on the left valve." Since, according to Stafford, each unit of his measurements was equal to 6·9 μ, the dimensions of his larvae were as given above. It is quite possible, if his measurements were correct, that Stafford was working with larvae of a species other than *M. arenaria* and, possibly, his large individuals were already juvenile mollusks and not free-swimming larvae. Another possibility is that Stafford's microscope was not correctly calibrated.

The dimensions of larvae in our cultures were not too different from Sullivan's (1948). Nevertheless, her smallest size of 105 $\times$ 90 μ is considerably larger than we found and her largest, 250 $\times$ 230 μ, also somewhat exceeds ours. Yoshida's (1938) observation that larvae of *M. arenaria* in Japanese waters metamorphose upon reaching a size ranging from 240 to 300 μ also disagrees with ours because, while the length of 240 μ does not differ radically from the measurements of our largest larvae, the maximum size of 300 μ given by Yoshida exceeds ours by about 70 μ.

The length of early straight-hinge larvae of *M. arenaria* given by Jørgensen (1946) is similar to ours. This is to be expected because his early larvae were laboratory-reared, as were ours, and, therefore, there is no doubt that we and Jørgensen were working with the same species. However, we disagree with Jørgensen that larvae of *M. arenaria* may reach 300 μ in length before metamorphosis. His conclusion regarding the setting size is based not on laboratory-reared *M. arenaria*, but on specimens collected in the field and only assumed to be this

species. The fact that Jørgensen stated on several occasions that metamorphosis of *M. arenaria* may occur upon attainment of a much smaller size than 300 μ strongly supports our opinion. For example, Jørgensen mentioned that in Ringkbing Fjord the size of larvae at metamorphosis in shallow water varies between 200 and 225 μ, thus being within the size range observed in our cultures.

S. *Teredo navalis* Linné

Larvae of *T. navalis* have been described by many authors, including Jørgensen (1946), Sullivan (1948) and, also, Imai, Hatanaka and Sato (1950), who gave a good description of the method of rearing them.

In our laboratory adult *T. navalis* were conditioned to spawn as early as the first part of December. This was done by placing pieces of wood, containing wood-borers, in sea water maintained at a temperature between 15° and 20°C. Spawning occurred at temperatures of 14°C and higher, and larvae were released at temperatures ranging from about 16° to 20°C. Grave (1928) reported that spawning of *T. navalis* began when the water temperature reached 11° to 12°C. Imai *et al.* (1950b), however, found that spawning begins when the water temperature reaches 18°C. Sullivan's (1948) data closely agree with ours, that spawning and swarming may occur at approximately 15°C.

Although *T. navalis* is naturally larviparous, both recently-fertilized eggs and immature larvae removed from the gill chambers of the parents developed normally past metamorphosis. The diameter of unfertilized eggs varied between 50 and 60 μ, agreeing with measurements given by Jørgensen (1946) and Costello *et al.* (1957). In the dissected adults, however, most of the eggs found were either already fertilized or immature, thus presenting difficulty in obtaining reliable egg measurements.

The smallest larvae released in natural swarmings at our laboratory measured only $80 \times 70\ \mu$, while the largest larvae found in the gill chamber of the mother were 90 μ long, or approximately 10 μ longer than reported by Jørgensen (1946). Evidently, the length of larvae at the time of release may vary by at least 20 μ. Imai *et al.* (1950b) indicated that the mean size at the time of release is $85 \times 72\ \mu$. Our observations that the average size of just-released *Teredo* larvae is between 85 and 95 μ are in agreement with those of Sullivan (1948), Jørgensen (1946) and Imai *et al.* (1950a). We cannot, however, accept the conclusion of Lane *et al.* (1954) who maintained that larvae are about 250 μ in size when released from the gill chamber.

Eggs, in early stages of development, taken from the gill chamber

of a female *Teredo* were cultured to metamorphosis in 28 days at a temperature of about 20°C. Grave (1928) thought that the entire period of development of *Teredo* from the moment of fertilization to metamorphosis takes about 5 weeks. Judging by data offered by Imai *et al.* (1950a), setting in their cultures occurred between the 24th and 34th days.

A brief description of early stages of development of eggs and larvae of *T. navalis* are given by Costello *et al.* (1957) and of later stages, by Sigerfoos (1908). Imai *et al.* (1950b) gave a good account for all stages. In our cultures the shells of straight-hinge larvae appeared heavy and thick. The larvae were also characterized by a dark band

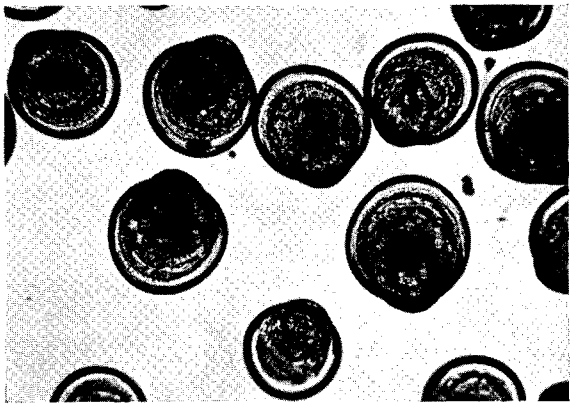

FIG. 43. Late larval stages of *Teredo navalis*. Largest larva in the group is about 185 μ long and 200 μ wide. Note dark band around edge of shell characteristic of larvae of this species.

around the edge of the shell from one end of the hinge to the other (Fig. 43). A light band was quite conspicuous inside of this dark band. Although these bands are probably optical illusions resulting from curvature of the shell seen under the microscope, they are well pronounced. The bands are quite narrow and less conspicuous in larvae smaller than 90 μ in length but, nevertheless, they are present even in these small individuals. As larvae approach setting size, the bands become less sharply delineated, although they remain quite prominent.

The color of larvae begins to darken soon after they reach 100 μ in length. Imai *et al.* (1950a) also came to the same conclusion. However, while Imai reported that neither the foot, otocyst or gill filament appears before larvae reach the size of 200 $\times$ 215 μ, we observed their appearance in larvae at least 15 μ smaller.

Imai *et al.* (1950b) gave a table showing growth of *T. navalis* larvae from day to day, indicating length–width relationships during different stages of growth. These data closely resemble ours. Other observations of these authors on appearance and behavior of larvae are also in close agreement with ours.

Larvae of *T. navalis* are extremely active and usually swim vigorously and virtually continuously. This is particularly true of younger stages. We noticed that the larvae have some substance on the outside of their shells by means of which they adhere readily to glassware and, as a result, it is extremely difficult to rinse them from beakers, pipettes, slides, etc.

Larvae began to metamorphose soon after a length of 200 μ was reached. However, several fully-metamorphosed individuals measuring only 190 μ in length and 206 μ in width were seen. The largest swimming larvae were approximately 200 $\times$ 231 μ. Our maximum size of free-swimming larvae of the wood-borer is, therefore, somewhat smaller than the 220 $\times$ 250 μ reported by Sullivan (1948), but closely approaches that given by Imai *et al.* (1950).

Larvae of advanced stages do not develop an "eye" that is characteristic of larvae of corresponding stages of other species, such as *C. virginica*. The foot of recently set borers is extremely slender and worm-like. The set attach themselves to the substratum by means of a byssus.

The time required for larvae in our cultures to reach metamorphosis varied. Early in our work, before good food organisms became available, the first metamorphosing larvae were observed 20 days after swarming, when grown at room temperature. If better growing conditions were provided, the free-swimming period could undoubtedly be shortened. Nevertheless, we strongly disagree with the conclusions of Lane *et al.* (1954) that the normal free-swimming period of *Teredo* larvae does not exceed 4 days.

Teredo larvae are quite susceptible to fungus diseases. Such infections were observed on numerous occasions and were probably responsible for the complete mortalities of *Teredo* larvae in some of our cultures.

VI. Acknowledgments

Our studies, which provided most of the material for this article, have been continuous since 1944 and, naturally, during this time many people have participated either directly or indirectly. We want to express our appreciation to all biologists of our laboratory, especially to Mr. Paul E. Chanley, who grew larvae of the several species of

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