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

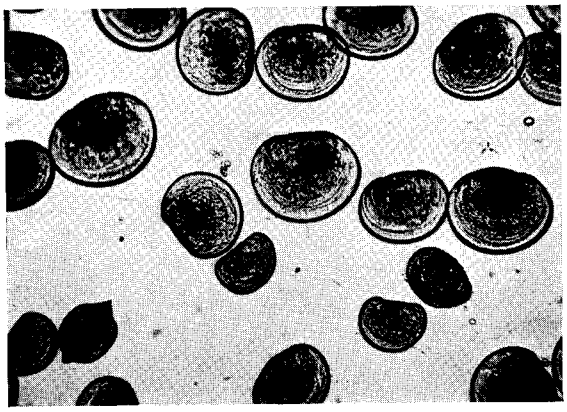

FIG. 31. Larvae of *Mytilus edulis*. Largest larvae are approximately 170 μ long. Several small, abnormal individuals can be seen in lower right corner.

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

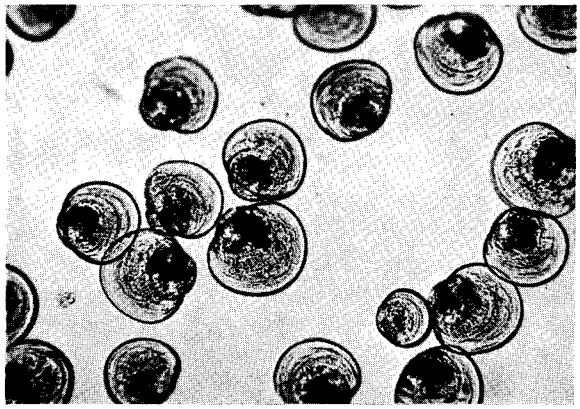

FIG. 32. Larvae of *Anomia simplex*. Largest larvae in photomicrograph are about 160 μ long. Note transparency of larval shells.

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

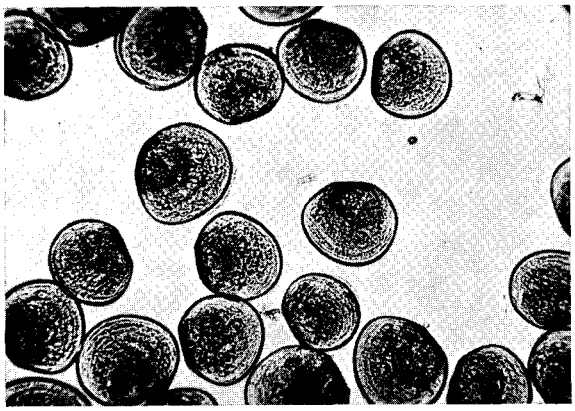

FIG. 33. Larvae of *Pecten irradians*. Largest larvae are about 180 μ long.

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,

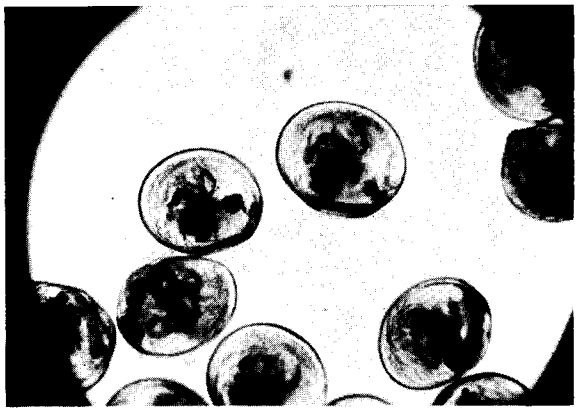

FIG. 34. Larvae of *Ostrea lurida*. Largest larva, near center, is 204 × 185 μ.

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.

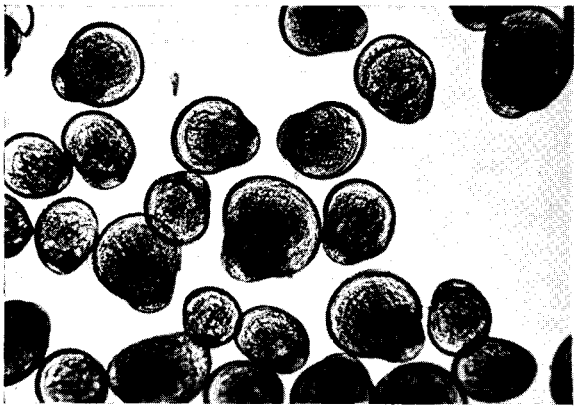

FIG. 35. Larvae of *Crassostrea gigas*. Largest larva, near center, is approximately 175 μ long.

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

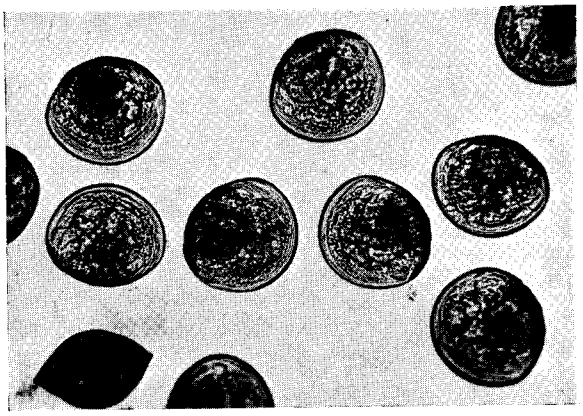

FIG. 36. Larvae of *Mercenaria campechiensis*. Largest larvae are approximately 185 μ long.

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

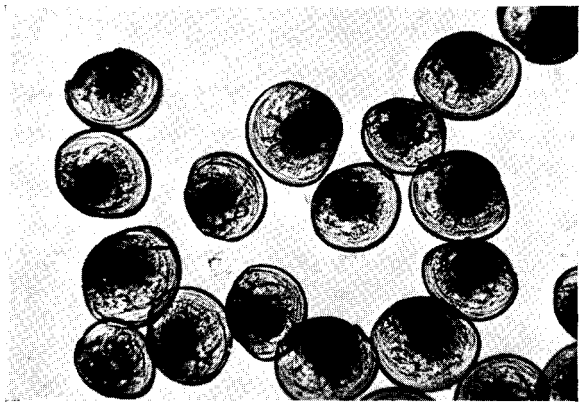

FIG. 37. Larvae of *Tapes semidecussata*. Largest larvae in photomicrograph are approximately 175 μ long.

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

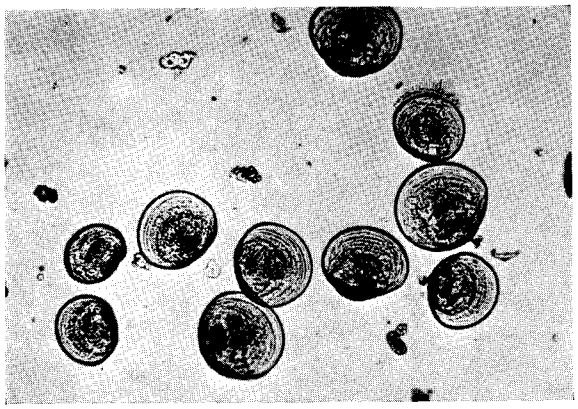

FIG. 38. Larvae of *Pitar morrhuana*. Largest larvae shown are approximately 150 μ long. Small, abnormal larvae can be seen in the group.

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

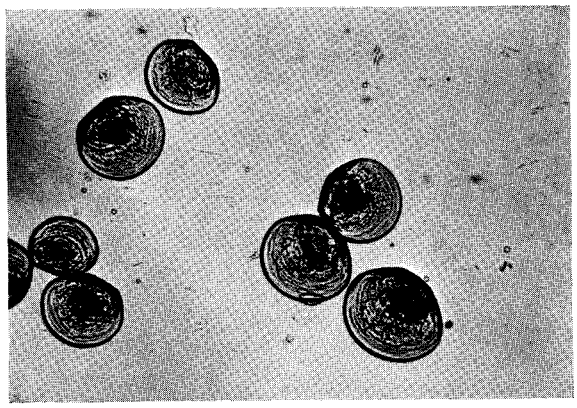

FIG. 39. Larvae of *Petricola pholadiformis*. Largest larva in this photomicrograph is about 164 $\times$ 143 μ.

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

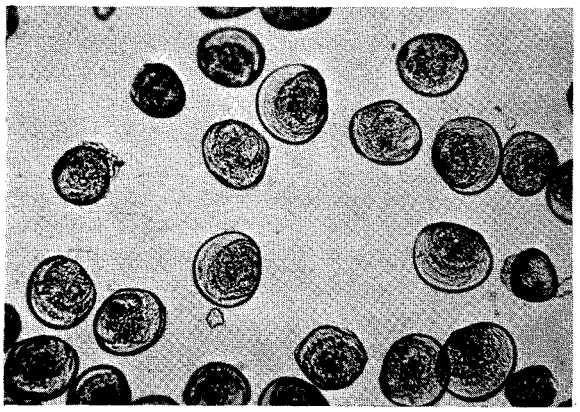

FIG. 40. Larvae of *Ensis directus* grown from eggs obtained by stripping. Note that many larvae are abnormal. Largest normal larvae shown are about 125 μ long.

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.