STATE OF CALIFORNIA DEPARTMENT OF NATURAL RESOURCES
DIVISION OF FISH AND GAME BUREAU OF MARINE FISHERIES
FISH BULLETIN No. 56
Development of the Eggs and Early Larvae of Six California Fishes[1]

By
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1940

[1] A thesis submitted to the Department of Zoology and the Committee on Graduate Study of the Leland Stanford Junior University in partial fulfillment of the requirement for the degree of Master of Arts, June, 1937.

FOREWORD

The California Division of Fish and Game is pleased to present in bulletin form Mr. Budd's work carried on at the Hopkins Marine Station and presented by him to Stanford University as a thesis in partial fulfillment of the requirement for the degree of Master of Arts.

The eggs and larvae of very few marine fishes of this coast have been described. Many fish eggs remain unidentified so that each description narrows the field of unknowns, and by elimination assists in further identifications.

In the present work the eggs and larvae of six species are described: our most important flatfish, three turbots and two cottids. The first species, the pointed-nosed sole, makes up half of the State's commercial catch of flatfishes with an average take of about five and a half million pounds. The next three species considered are the so-called turbots of the genus Pleuronichthys, which are highly prized food fishes, accounting for a catch in this State of roughly 75,000 pounds per year. The two cottids are not utilized commercially.

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May 1, 1940.

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1. INTRODUCTION

The study of the embryonic and larval development of the fishes of the west coast of America has been almost entirely overlooked in favor of work pertaining to other fields of biology. A scant half dozen or so of the many eggs and early larvae that may be taken in one plankton haul are identifiable. This paper is an attempt to fill in some small part of the information lacking in this great field. It adds nothing new to the study of embryology itself, but is intended as a contribution to the urgently needed information which will make it possible to identify and determine the age of some of the eggs and early larvae of fishes found in the coastal waters of California.

The research covered by this paper was done at the Hopkins Marine Station of Stanford University at Pacific Grove during the summer months of 1935, and from April, 1936, to May, 1937, inclusive. It includes a short general discussion of the eggs of the bony fishes, descriptions and figures of the eggs and early larvae of the flatfishes, Parophrys vetula, Pleuronichthys decurrens, Pleuronichthys verticalis, Pleuronichthys coenosus; and the cottids, Artedius lateralis and Clinocottus analis; and finally, a list of references.

The work was based entirely upon living material and the discussion and figures contain only those details apparent to workers laboring under the same conditions. Where more detailed description was needed the reference source is given.

At this point I would like to express my thanks for the many favors and the kindly interest of the members of the Hopkins Marine Station staff. Their advice and encouragement made the problem much easier than it otherwise could have been. In particular I can not voice too strongly the sincere debt of gratitude I owe Dr. Rolf L. Bolin for his never flagging guidance, his intense interest and his friendly counsel in my problems. It is a pleasure to express my appreciation of the courtesies extended by Mr. G. H. Clark of the California Division of Fish and Game, which enabled me to obtain much of the material upon which this paper is based. Several trips made on the Division of Fish and Game boat *Albacore* made available material which otherwise would have been very difficult if not impossible to obtain.

Most work on fish eggs has been very recent. Very little was known even of the spawning seasons and habits of common fishes a half century ago. In 1864, G. O. Sars discovered that the eggs of the cod, haddock and gurnard were pelagic. When he first took cod eggs in a fine meshed net, Sars was under the impression that he had captured a new species of planktonic animal. However, he observed

them carefully and said that he "* * * succeeded in following their development step by step until the tender little fish slipped out of the shell and swam about in the water."

From the time of this auspicious start, an increasing number of workers have lent their energy and faculties to furthering the knowledge of teleostean embryology. Particularly helpful in pursuing this research have been the many well illustrated contributions of Alexander Agassiz dealing with various fish eggs, the excellent piece of detailed work by John Ryder on the development of the cod (Gadus morrhua), and the no less outstanding study of Henry Wilson on the embryological development of the sea-bass (Serranus atrarius). Two comprehensive and very valuable works dealing with the embryology and early larval forms of many different fishes are "The life-histories of the British marine food-fishes", produced by the research staff of the St. Andrews Marine Laboratory in England, and particularly "Eier und Larven von Fischen" by Ehrenbaum.

2. THE EGGS OF BONY FISHES

Since the fishes under discussion in this paper are all true bony fishes, it might be well at this point to make a brief survey of the general characteristics of teleostean ova, so that the eggs which will be dealt with in detail later may be readily oriented in the complex scale of known modifications.

Among the marine bony fishes we find that a few types such as the Embiotocidae are truly viviparous, and many including the Scorpaenidae are ovoviviparous. Such forms are particularly characteristic of the California fauna, but these fishes lie beyond the field of the present discussion. By far the great majority of bony fishes are oviparous, and it is with these that the following is concerned.

The eggs of oviparous fishes have several features that may be discussed in a broad manner. Many of the differences occurring in the eggs seem to be adaptations to fit their environment. This is most clearly shown by contrasting pelagic and demersal eggs. In the former category the specific gravity of the eggs is equal to or slightly less than that of sea water and they float about usually at or near the surface of the sea. The demersal eggs with a specific gravity greater than that of sea water remain upon the bottom where they are deposited by the parent fish.

A great majority of fishes discharge their sex products into the surrounding water where the eggs float as free individuals. Here they are fertilized and wash about at the mercy of waves and currents which are often adverse. Frequently they are swept into unfavorable environments which cause their total destruction. Aside from these dangers inherent in their natural environment, the eggs fall easy prey to voracious plankton feeders. McIntosh and Masterman (1897, p. 21) have pointed out that, since there is no appreciable increase in the number of cod fishes, all but two of the 5,000,000 eggs produced by an average female in one season must meet an untimely death before becoming mature adults. Such evidence of wholesale destruction indicates the importance of the production of many eggs as a means of insuring the survival of the species, and we find, in fact, that most fishes laying pelagic eggs liberate them in enormous numbers. A single

ling (Molva) has been found to have 28,361,000 eggs in the ovaries (Norman, 1931, p. 283), and most fishes produce hundreds of thousands of eggs. While this is the general rule, many demersal eggs which are deposited in protected places beneath rocks or in crevices which are guarded by the parents, are produced in moderate numbers, their chance of survival being much greater than that of pelagic eggs. Rusciculus rimensis, a small cottid which lays a mass of about one or two hundred eggs, may serve as an example.

The eggs of fishes in general and particularly pelagic eggs are usually small and spherical. It is partly due to their unobtrusive size that they find escape from their enemies, and it is to their roundness that they owe their great mechanical strength. A few pelagic eggs such as those of the Engraulidae depart from the spherical shape, being slightly elongate in one axis and bluntly ovoid. Demersal eggs, guarded as they are from predatory enemies and maintained in a favorable environment, are assured of a protected development for an extensive period and are consequently larger in size. Although the fundamental shape of demersal eggs is round, they become more or less flattened in the areas where they press against each other or the substrate. A few depart markedly from the spherical form, those of the Gobiidae being club-shaped.

The shell or zona radiata of fish eggs is very tough even though sometimes quite fragile in appearance. It requires great pressure to break the most delicate egg between the thumb and forefinger. Pelagic eggs requiring buoyancy have comparatively thin and light shells. These floating eggs act almost as part of their fluid environment and do not need great protection against wear and tear. On the other hand, the shells of demersal eggs are thicker and stronger, and their membranes are usually sticky when first deposited, causing the eggs to adhere to each other or to foreign objects. This serves as protection against the wave shock, currents and scouring, typical of the shallow benthonic habitat where most of them are spawned.

The shells are usually clear and transparent, but a few species have sculptured patterns or are pierced by many tiny, radiating pores which tend to render the shell slightly translucent. As a rule the membranes are smooth. However, in some forms elaborations of the shell occur. In the Macrouridae, the egg bears conical projections which are suspected of acting as organs of flotation, holding the egg at certain depths in the water during critical periods in its development, and in some of the Clupeidae, long filaments serve for anchorage.

A mass of yolk usually occupies most of the space within the shell. Generally, but by no means always, oil globules are present, occurring in more or less definite numbers depending on the species of the fish. There may be from one to several large globules and whole groups of tiny droplets. In pelagic eggs, the yolk is almost always completely colorless and transparent. The consequent almost perfect invisibility of the eggs contributes largely to their survival. Anyone who has tried to locate pelagic eggs, floating even in the confines of a finger bowl, will recognize this fact. Although the shell is transparent and colorless in demersal eggs, the yolk usually is brightly colored. The eggs of Artedius lateralis are cherry red, those of Clinocottus analis are tannish yellow, while others are bright green

or lavender. It is surprising how effectively these colors function as a protective device, causing the egg masses to blend with the colors of the sessile organisms which form their background.

There is a decided difference in the rate of development between pelagic and demersal eggs. The mortality of pelagic eggs and early larvae due to plankton eaters and the sometimes adverse environmental conditions is tremendous, and rapid growth is essential to their survival. Eggs taken in plankton hauls well illustrate this great mortality, in that the younger stages outnumber those only 24 hours older by almost ten to one. Some pelagic eggs have an incubation period of only 24 hours, but most require two or three days and sometimes longer to complete their embryonic development. Demersal eggs, more or less protected during their incubation period, are permitted a longer time in which to develop; these usually take two or three weeks. However, some forms are known which have an incubation period of months.

The difference in the time of incubation results in marked differences in the newly hatched larvae. The larvae of pelagic eggs are thrust upon their watery world in an extremely immature condition. I have noticed the newly hatched larvae of a number of species floating upside down for several days, absolutely helpless due to the buoyancy of their unabsorbed yolk sacs. Again, an almost absolute lack of coloration comes to their rescue, their chromatophores usually being few in numbers and unexpanded. The larvae from demersal eggs hatch in an advanced state and can meet their new environment much more competently. They are comparatively large and well formed, heavily pigmented, and immediately upon leaving the shell they swim rapidly about apparently looking for food.

Fish eggs and larvae play an important role in the economy of the sea. In addition to their primary function of perpetuating their own species, they form a great source of food for other organisms. It is possible that the presence of huge masses of eggs may cause whole migrations of fishes which come solely to feed upon them. Examination of sardines taken at San Pedro during the spring has disclosed that their stomachs are often crammed with anchovy eggs. On occasion I have noticed in the material collected in a plankton haul, many small medusae containing partially engulfed larval fishes. Lebour (1923) made a study in a plunger jar in which she observed thirteen different planktonic animals feeding upon fish larvae. She concludes that newly hatched and very young fishes forming part of the natural food of most of the common coelenterates and pelagic worms have little chance against all these enemies.

3. METHODS OF PROCEDURE

The material used in this research was obtained in two ways. Obviously the best means of studying the complete embryological development of fish eggs is to strip them from ripe females, artificially fertilize them with milt stripped from males, and carefully observe all subsequent developments. This method proved successful for the pointed-nosed sole (Parophrys vetula) and the two cottid fishes described in this paper. All attempts to fertilize artificially the eggs of the three species of turbot (Pleuronichthys) failed, although many attempts were made during the spawning seasons of 1936 and 1937.

The greatest difficulty was encountered in obtaining both ripe males and females at the same time. For these fishes the method of taking naturally fertilized eggs from the sea by frequent plankton tows was resorted to. This proved only partially successful as none of the early segmentation stages was ever found.

The fertilized eggs were kept in the laboratory in clean finger bowls of fresh sea water. The water was changed frequently during incubation, and the eggs were kept as free as possible from contamination. The temperature of the water was kept as constant as possible since slight variations cause radical changes in the rate of development. Microscopic examinations were made at frequent intervals to check the time and stages of development.

The real difficulties arose after the eggs had hatched. To date, very little success has attended the efforts to raise larval fishes through their critical period which extends from the time when the yolk sac is absorbed until the larval fish has completely adapted itself to a maintenance on the food in its environment. The experiment has been carried through to completion in several laboratories in Europe and on a commercial scale with flatfishes on the Isle of Man, but to the best of my knowledge all attempts in this country have failed. This present research suffered the same fate, and only the early larval stages have been studied.

Upon hatching, the larvae were transferred to stirring jars. This apparatus was slightly modified from that used by Fabre-Domergue and Biétrix, Anthony and others, the chief difference being in the use of celluloid instead of glass for the stirring parts. Celluloid dissolved in acetone proved to be a very easily worked material, and the completed units were much more durable than their glass counterparts which originally were tried. The apparatus consisted of six one-gallon jars, each stirred by a celluloid disc fixed obliquely to a celluloid axle which was rotated by a small electrical motor. The disc revolved like a propeller giving a helicoidal movement. Various speeds were obtained by inserting different sized pulleys between the axles and the motor.

The function of such an apparatus is to keep the larvae afloat by constantly agitating the water. Preventing the larvae from settling to the bottom saves them from destruction by bacteria which quickly appear on the bottom and sides of vessels of sea water kept in the laboratory. Further, the movement of the water circulates the food and keeps it available to the fish.

As a safeguard against bacteria, the agitating apparatus proved very successful as none of the larval fishes ever showed signs of infection. However, in all cases the larvae died during their critical period, apparently through starvation. Various types of food in different sizes and quantities were tried. Nitzschia, Dunaliella, the freshly hatched larvae of Strongylocentrotus purpuratus, Artemia, and Tigriopus fulvus failed. Even carefully sifted plankton brought in daily from the sea failed to offer the proper sustenance to the larval fishes. I regret to say that this part of the experiment was of necessity left unfinished as no solution was found during the many months of experimentation devoted to this problem.

4. PAROPHRYS VETULA GIRARD POINTED-NOSED SOLE

Examination of fish collected at several different times by the California Division of Fish and Game vessel *Albacore* in the southern part of Monterey Bay, and of many other specimens in the Monterey fish markets, indicates that Parophrys vetula has a spawning season extending from January to May and reaching a maximum in late March or early April. This statement is based on data gathered in 1937, but according to G. H. Clark of the California Division of Fish and Game, the spawning is usually a month or two earlier.

4.1. Eggs

The very buoyant eggs of this species are pelagic, their specific gravity being considerably less than that of sea water. They are spherical and transparent, with a diameter of 0.9 (0.89–0.93) mm. The egg membrane is thin, slightly less than 0.014 mm. in thickness and marked by minute wrinkles, visible only upon the closest inspection, which form a delicate vermiculated pattern over the entire surface of the egg. An attempt has been made to illustrate this texture in the drawing of the unfertilized egg (see Fig. 1), but in all other figures this detail is omitted for the sake of clarity, although the wrinkles persist until hatching. The yolk is clear and transparent and contains no oil globules.

4.1.1. Blastodisc

The formation of the blastodisc is initiated almost immediately upon fertilization. Protoplasm which has hitherto invested the yolk in an invisible layer, slowly accumulates at the lower pole of the egg. As it concentrates beneath the yolk it begins to lose some of its transparency and becomes slightly whitish. The accumulation of the protoplasm at the animal pole is completed in about one hour. The actual streaming motion of the protoplasm in the formation of the blastodisc so well described by Ryder (1884) could not be seen in the case of Parophrys vetula. However, it was clearly observed in the eggs of the two cottids to be described in this paper and will be discussed more fully later.

The completed blastodisc is of a lenticular shape with fairly abrupt edges where its protoplasm merges into an extremely thin and invisible layer that continues to surround the entire yolk sphere. (See Fig. 2.) The surface of the yolk immediately opposite the blastodisc has become flattened, and the slight compression of the yolk caused by the accumulated protoplasm brings the perivitelline space into evidence.

4.1.2. Cleavage

About 30 minutes after the blastodisc is fully formed, the first cleavage begins. The plane of cleavage is meridional and divides the blastodisc into two rounded blastomeres of approximately the same size. The division is initiated by a slight lengthening of the blastodisc in the axis perpendicular to the plane of cleavage. The first sign of actual division is a slight protuberance of the yolk as it flows into the transverse furrow made by the dividing protoplasm. This is followed

almost immediately by a definite furrow cutting the outer surface of the protoplasm in the same plane, and as this deepens, the inner furrow slowly retreats pressing the yolk back into place. This continues until only the outer furrow remains, dividing the two blastomeres. It does not, however, cut completely through the blastodisc, but leaves a thin connection of protoplasm joining the two blastomeres at their surfaces adjacent to the yolk. At first the sides of the blastomeres are gently sloping, but they gradually steepen and at the end of two hours they have assumed the well-rounded dome shape shown in figure 3.

After the completion of the first cleavage, there is a resting period of about three-quarters of an hour. The second division then takes place and is usually complete in 15 minutes, the process being the same as described above. The second plane of cleavage is meridional and at right angles to the first, and the establishment of four smaller blastomeres restores the equality of the blastodermal axes. After the cells have been outlined, their sides gradually steepen until there is a temporary constriction at their bases, after which the sides of the blastomeres assume a more gentle slope. (See Fig. 4.)

Some eggs divide slightly more rapidly than others, but the average interval between cleavages is approximately one hour; the third cleavage is completed three and a half or four hours after fertilization. It is accomplished by two furrows appearing simultaneously, one on either side of and parallel to the first plane of cleavage. The resulting blastoderm again has one axis elongated, and its eight cells are arranged in two parallel lines of four cells each. (See Fig. 5.)

The fourth cleavage taking place about five hours after fertilization once more restores the equality of the axes. Again two simultaneous furrows appear, but this time they lie on either side of and parallel to the second plane of cleavage. Most of the cleaving eggs display variations departing from a strictly symmetrical pattern, but the result is always a fairly well-rounded, 16-cell blastoderm, one cell thick. (See Fig. 6.)

It will be remembered that the cleavage furrows have not cut entirely through the blastodisc, but have left a thin layer of protoplasm adjacent to the yolk joining the cells at their bases. This protoplasmic layer is called the central periblast and plays an important part in later development. By the time the fourth cleavage is completed, the four central cells of the blastoderm become detached from the underlying central periblast. The resulting space between the blastomeres and the central periblast is the segmentation cavity. This can not be seen in living eggs, but has been described carefully from prepared material of the sea-bass (Serranus atrarius) by Wilson (1891, pp. 211–212).

The fifth cleavage is extremely difficult to follow. As all of the furrows of this cleavage seldom occur simultaneously and more often than not depart from the strictly symmetrical pattern of cleavage, the planes of segmentation can be accurately determined in few cases. In ideal cases the four corner cells are cut by meridional planes, the remaining peripheral cells by planes parallel to the blastodermal margins, while the four central cells are cut by a horizontal plane of

cleavage. This horizontal plane can only be observed by means of cross sections. The result of the fifth cleavage as seen in living material is a round blastodermal cap usually made of 28 visible cells, the other 4 of the 32 forming a two-layered central portion of the blastoderm.

Cleavage continues and at approximately 10 hours a well-rounded, dome-shaped blastodermal cap with very abrupt edges has been formed. (See Fig. 7.) Subsequent cell divisions result in a large number of minute cells so small that it is no longer possible to trace the individual cell outlines, and the previously abrupt walls of the blastoderm assume a more gentle slope.

4.1.3. Blastodermal Cap

The blastodermal cap, fully developed at approximately 23 hours, is a rounded lenticular dome hanging below the surface of the yolk. (See Fig. 8.) The blastoderm at this stage may easily be confused with the blastodisc as both have approximately the same shape, size and color. The blastodermal cap of Parophrys vetula has the distinguishing feature of being very large, its outer surface forming a gentle curve which is almost a continuation of the outline of the yolk. Its inner surface, pressed against the yolk, is almost flat and displays a very irregular face of tiny undulations. Around the periphery of the blastodermal cap a narrow band of protoplasm, the periblast, forms a collar surrounding the proximal portion of the yolk; the perivitelline space is very small.

4.1.4. Periblast

All that is visible of the periblast in living material is the marginal region around the blastodermal cap. It may be well to give here a few details of this interesting structure as determined by other workers with the aid of stained sections. The peripheral cells of the blastodermal cap are not well defined on their outer borders, the protoplasm remaining continuous with the thin layer of protoplasm which still invests the yolk sphere. This peripheral protoplasm concentrates into a slight ridge that forms the periblast. The ill defined, marginal blastodermal cells contribute nuclei to the syncytial periblast. These multiply and migrate centripetally into the thin layer of protoplasm, already mentioned as the central periblast, which intervenes between the adjacent surfaces of the yolk and blastoderm. (Kuntz and Radcliffe, 1918, p. 93.) During the formation of the peripheral periblast the process of segmentation has continued and finally the marginal cells of the blastodermal cap are cut off from the periblast. The free nuclei which had been derived from the blastoderm become scattered evenly throughout the entire periblast. In all probability the ultimate fate of these free nuclei is associated with the later disappearance of the yolk material. Thus the periblast nuclei have some special physiological function in making the yolk easily assimilable by the developing embryo. (Wilson, 1891, p. 217.)

4.1.5. Segmentation Cavity

During the next few hours the segmentation cavity is fully developed. As a preparatory step to invagination, the blastodermal cap slowly begins to change shape. Its outer surface becomes more convex while its inner surface, opposite the central periblast, becomes more

concave. In this way the space between the blastodermal cap, already designated as the segmentation cavity, grows in size as the blastoderm pulls away from the periblast in changing shape. This cavity, lenticular in shape, does not occupy the exact center of the blastoderm but is slightly excentric in position. Thus the blastoderm at one side of the segmentation cavity is somewhat thicker than on the side opposite. This is the first indication of the main axis of the future embryo, the thicker area marking the posterior pole. At 25 hours the blastodermal cap resembles a bowl with one wall slightly thickened and suspended by its brim from the yolk above. (See Fig. 9.)

4.1.6. Invagination

Continued growth and development of the segmentation cavity causes a thinning of the central portion of the blastodermal cap and a thickening of its periphery. This thickened peripheral area is the early germ ring. Wilson (1891, pp. 219–220) gives an excellent description of the development of the germ ring under his discussion of the formation of the "rand wulst."

The cells forming the surface layer of the blastodermal cap become flattened and form the ectodermal layer which does not take part in invagination. The cells at the edge of the blastoderm start to grow in centripetally and form the actual thickened area of the germ ring. This occurs along the entire periphery of the blastoderm. Figure 10 shows the developing germ ring as a marginally thickened band encircling the yolk sphere, with the slightly granular-textured, interior face of the blastoderm showing through the walls of the blastodermal cap.

4.1.7. Embryonic Shield

Once the germ ring has been established, the embryonic shield starts to form. The first evidence of this is the appearance of a small tongue of cells protruding from the germ ring into the segmentation cavity. This occurs at the thickened portion of the wall already designated as the posterior pole. The more rapid proliferation and centripetal growth of cells here than elsewhere causes more pronounced invagination at this point. The invaginating tip of the embryonic shield marks the anterior end of the embryo. With continued growth the embryonic shield becomes a broad area of cells as shown in figure 11. This view, depicting the extent of the embryonic shield about an hour after its appearance, is drawn looking directly down upon the top of the blastodermal cap as seen through the transparent yolk. Lateral views fail to show the extent of its growth. A remarkable feature of the embryonic shield of Parophrys vetula is the flatness of its advancing edge. Rather than the usual tongue of cells, one whole side of the germ ring appears to advance into the segmentation cavity.

Shortly after the embryonic shield has advanced past the mid point of the segmentation cavity, it begins to thicken along its median line. The general body outline of the embryo is now unmistakable, its ventral surface being pressed into the yolk and its dorsal ridge raised into the perivitelline space.

4.1.8. Migration of the Germ Ring

So far there has been only a centripetal growth of the germ ring, and particularly the embryonic shield. Continuation of this phenomenon

would eventually cause the invaginating edges of the germ ring to meet, forming a layer of cells cutting off the segmentation cavity from the central periblast. This, however, does not happen. After the embryonic shield has advanced well into the segmentation cavity, and the main body axis has become slightly thickened, the anterior edge of the germ ring opposite the tip of the embryonic shield begins a centrifugal growth. This causes the germ ring to migrate anteriorly away from the end of the embryonic shield. In Parophrys vetula the embryo lengthens at about the same rate as the germ ring advances until, at about 30 hours after fertilization, when the germ ring is almost equatorial in position, an extremely long and thin embryo with a definitely thickened cephalic region has been formed which extends almost half way around the yolk. (See Fig. 12.) The movement of the germ ring may be likened to that of a tight hoop which is pivoted at the posterior pole and swings away from the anterior end of the embryo. Once past an equatorial position, the diameter of the ring grows increasingly smaller as its anterior edge now migrates toward the posterior pole. It finally pinches together at the caudal end of the embryo, leaving behind it a cellular sheath over the entire yolk sphere.

As the germ ring passes the equator, it rapidly draws away from the anterior end of the embryo and by 40 hours is approaching closure. (See Fig. 13.) Its pinching action causes the uncovered portion of the yolk to bulge prominently outward to form the yolk plug. This is gradually forced inside as the blastopore closes. There has been a great thickening of the entire embryo, most pronounced in the cephalic region. With careful manipulation of the light, the eyes may be vaguely made out. Ventrally and near the posterior end of the embryo, a small transparent sphere has made its appearance. This is Kupffer's vesicle which will be discussed later. In the mid dorsal region and particularly on the nape of the developing embryo, a few scattered minute specks give the first indication of pigmentation.

The blastopore closes at a point immediately posterior to the tail end of the embryo. The lips of the ring meet and merge forming a dimple with faint radiating wrinkles. Then, complete union of the edges takes place and no trace of the closed blastopore is left. According to Ryder (1884, pp. 564–565), the germ ring, which at this stage he terms the caudal plate, merges with the caudal mass of the embryo and contributes to the tail. This occurs shortly before the 50-hour stage shown in figure 14.

The head of the embryo by this time is quite well formed. The eyes in which the pupils have developed can be easily distinguished. The heart is indicated by a slight bulge of the body wall into the yolk just below the nape. The body has increased in bulk, and Kupffer's vesicle has apparently reached its full size. The pigment spots are now much larger and darker, and are scattered along the dorsal surface of the embryo from the nape to the posterior end. The embryo remains a faint whitish color and is fairly transparent.

4.1.9. Completion of Embryonic Development

After the closure of the blastopore, there begins a rapid growth of the tail as new somites are added. Because of the extreme transparency

of the living embryos, the somites can be made out only with the greatest of difficulty.

It will be remembered that Kupffer's vesicle had reached its full growth by the time the blastopore closed. Its location was adjacent to the caudal mass which now becomes the area of most intense metabolic activity. According to Sumner (1900), Kupffer's vesicle is a post anal gut and is largely responsible for certain phases of embryonic growth. As mentioned earlier in this discussion, the periblast nuclei have the physiological function of making the yolk easily assimilable. The free nuclei of the periblast are more concentrated about Kupffer's vesicle than elsewhere, and no doubt contribute largely to the almost fluid yolk, partially assimilated, that fills the vesicle. This supply of food in Kupffer's vesicle, at the area where the tail begins to develop, probably contributes greatly to the rapidity of its growth.

At approximately 70 hours, the contour of the head develops slight curves following the lobes of the brain which can now be made out in good light. (See Fig. 15.) The eyes are well rounded and when viewed from above can be seen to protrude beyond the general outline of the head. The heart has assumed fairly large proportions and occasionally pulsates. Shortly before the stage figured, the auditory capsules make their appearance just posterior to the eyes and slightly above the mid line. It is not until much later that the otoliths become visible. In good light the notochord can be seen to extend from below the posterior portion of the eyes to the tip of the tail which now bears the tiny continuous fin fold. There has been a further increase of melanophores along the dorsal surface of the body, but none is apparent in any other region. About this time the embryo shows the first signs of movement, occasionally twitching its tail.

From this stage until the time of hatching at about 90 hours, few further changes occur. The tail continues to grow until the embryo almost encircles the yolk; the pigmentation becomes slightly heavier. Movement becomes more vigorous, the tail being swung violently from side to side; twitchings of the body often cause the embryo to turn completely over in its shell.

4.1.10. Hatching

Several hours before hatching, the egg, probably due to the increased bulk of the embryo which has gained weight from the water absorbed during its development, begins to sink slowly, and the movements of the embryo become increasingly violent. The force exerted by attempts to straighten the curved tail seems to be transmitted to the point where the crown of the embryo's head meets the shell and eventually results in the rupture which liberates the larva. The whole process of hatching as observed in the laboratory covered a period of about an hour. This was no doubt considerably longer than the time consumed under normal conditions. The embryo had only its own struggles to rely upon in freeing itself from the shell, whereas if floating in its natural environment, the motion of the waves might have helped in rolling the egg about and stimulating the embryo in its hatching struggle. The larvae hatched at approximately 90 hours, having been kept in water of about 13.0° C. during the entire period of incubation.

4.2. Larvae

Because of the buoyancy of their large yolk sacs, the newly hatched larvae float helplessly upside down at the surface of the water. By energetic flutterings of their tails and a rapid beating of their small pectoral fins, the larvae may swim to the bottom of a shallow finger bowl, but as soon as their efforts cease they rise to the surface.

The average standard length of the newly hatched larvae is 2.8 mm. Upon leaving the shell the body of the larva immediately straightens out except for a slight cranial flexure which soon disappears. (See Fig. 16.) The gently rounded head shows no indication of a mouth. The eyes are large, and the choroid fissure may be seen in good light. The otoliths are now definitely developed. The body of the embryo tapers gently from the nape to the caudal tip, and the large ovoid yolk sac extends from beneath the snout to the rectum which is situated slightly anterior to the mid point of the body. The broad transparent fin fold arises from the crown of the head and extends to the anus. It is broadest just posterior to the level of the anus where the dorsal fold is about equal to, and the ventral fold 1.5 times as wide as the body. The pectoral fins are transparent fan-like structures just posterior to the auditory capsules and usually escape notice until the larva is viewed from directly above.

There is no sign of pigmentation on either the yolk sac or fin folds. The body displays scattered groups of melanophores on the head and sides of the body, and the rectum is marked by several black pigment spots. An area of pale amber color, due to greatly expanded chromatophores, borders the eyes posteriorly and extends to the auditory capsules. The rectum and the body immediately above it show the same coloration, and a very definite band of light amber encircles the tail about half way between the anus and the tip of the tail. Dorsally the body shows an occasional patch of the same color.

Four days after hatching the larvae average 3.8 mm. in standard length. The head has become heavy and angular with a slightly pointed snout, and the jaws are developed and functional. (See Fig. 17.) Just behind the chin the strongly pulsating, two-chambered heart is a prominent feature. The auditory capsules have increased in size. The yolk sac is almost completely absorbed and the liver has become a conspicuous structure. Due to the rapid growth of the posterior part of the body, the anus now occupies a position at the end of the anterior third of the body.

Marked changes in pigmentation have occurred. The pupils of the eyes are jet black and the iris has assumed a silvery, blue-green sheen. The head is almost opaque, the expanded chromatophores giving it a general amber color. The amber yellow patch above the rectum has become more pronounced, while the rectum itself has assumed a deeper hue. The band of amber chromatophores that lies half way between the anus and the posterior edge of the fin fold is now quite dark, and a new faint band of the same color has appeared about midway between this area and the rectum. Most of the melanophores are now concentrated in a row along the ventral side of the body, a few occur dorsally and others are scattered in the amber colored areas.

Evidently due to the lack of proper food, the larvae showed little subsequent gain in size, and after complete absorption of the yolk

sac about the sixth day after hatching, they became emaciated in appearance. A nine-day larva, 4.0 mm. in standard length, is shown in figure 18. The stomach area is caved in and evidently contains no food. The eyes and body show actual shrinkage as the larva has apparently been calling upon its own tissues for nourishment.

However, a few developmental characteristics can be discerned. The sharp contours of the head are not all due to emaciation, as it is quite evident that the lower jaw has lengthened. The auditory capsules are enlarged and the cells of the notochord can be seen along its entire length. The tail has begun to develop rays and to change shape. The melanophores remain concentrated along the ventral side of the body, and a number of new ones have appeared along the ventral mid line of the gut. A few greatly expanded melanophores remain in the amber pigmented patches, particularly in the original band between the anus and the tip of the tail. The amber yellow area above the rectum has disappeared and the next band posteriorly has almost vanished. The head has assumed a more general amber color and a few chromatophores remain scattered along the dorsal surface of the body.

Unfortunately, it was impossible to carry the larvae of Parophrys vetula beyond this stage; all of the specimens died, probably due to lack of proper food. However, a larval fish was taken in a plankton tow and so closely resembled the last stages of Parophrys vetula that I have little hesitation in using it as a later developmental stage of this species. There was a close similarity of body shape and proportions except for those changes obviously due to more advanced development. (See Fig. 19.) The larval fish was 6.3 mm. in standard length and probably well over a week older than the last stage discussed. The head showed the same general contours and proportions but was not emaciated. The jaw had lengthened and was well formed. The eyes were rounded and the auditory capsules had reached a much larger size. The liver, granular in texture, filled the entire anterior end of the peritoneal cavity, and the gut seemed full of food and well extended. The most interesting feature of this stage was the extreme broadening of the body posterior to the anus, a feature typical in the development of all flatfishes. The somites were well defined and the body had become almost opaque, but the notochord could still be made out. The tip of the tail had been damaged so that it was impossible to accurately determine its shape.

The eyes were pigmented as in the previous stage. The head, nape and gut were of a soft amber tone. This color had almost completely disappeared from the other portions of the body. of the melanophores along the ventral surface of the gut, only a single greatly expanded spot remained at the level of the transverse septum. One very large pigment spot remained laterally above the mid gut. The series of about 30 melanophores extending along the ventral margin of the tail had arranged themselves one to each somite at its posterior edge, and the dorsal surface of the rectum had become darkly pigmented.

It is to be regretted that this single specimen did not survive the handling in the plankton tow, but died soon after examination under the microscope.

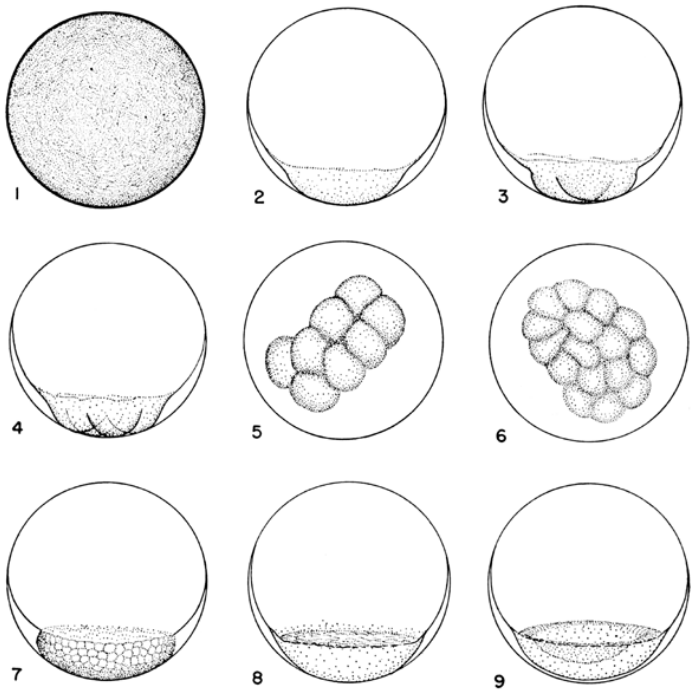

PLATE I

Parophrys vetula

FIG. 1. Unfertilized egg.
FIG. 2. Blastodisc. 1 hour.
FIG. 3. Two cells. 2 hours.
FIG. 4. Four cells. 3 hours.
FIG. 5. Eight cells. 4 hours.
FIG. 6. Sixteen cells. 5 hours.
FIG. 7. Early blastodermal cap. 10 hours.
FIG. 8. Completed blastodermal cap. 23 hours.
FIG. 9. Segmentation cavity. 25 hours.

PLATE I
Parophrys vetula

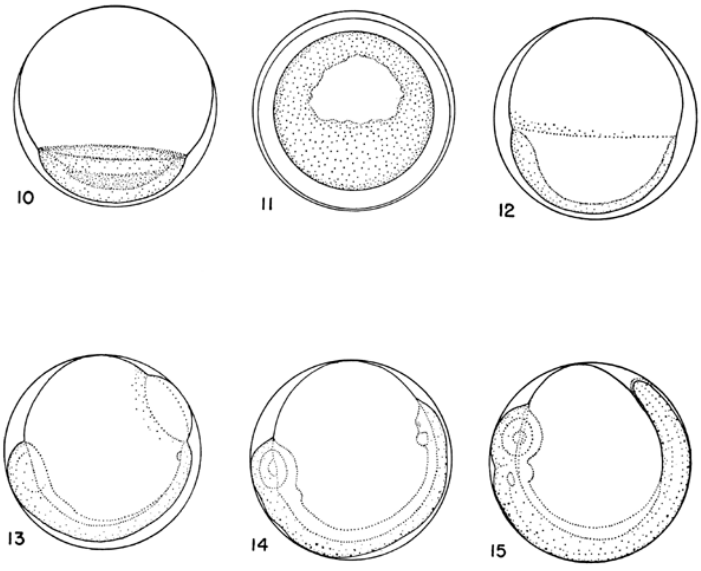

PLATE II

Parophrys vetula

FIG. 10. Early germ ring. 27 hours.
FIG. 11. Embryonic shield. 28 hours.
FIG. 12. Germ ring approaching equator. 30 hours.
FIG. 13. Blastopore nearing closure. 40 hours.
FIG. 14. After closure of blastopore. 50 hours.
FIG. 15. Advanced embryo. 70 hours

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PLATE II
Parophrys vetula

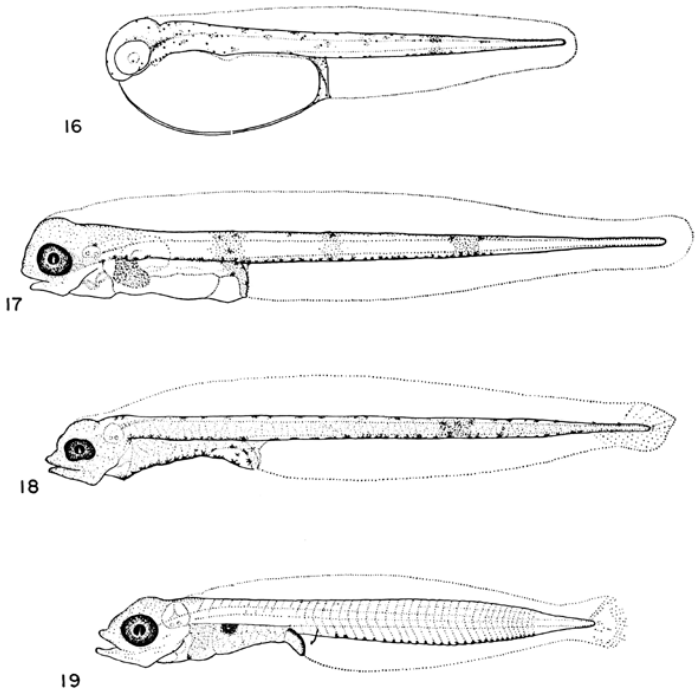

PLATE III

Parophrys vetula

FIG. 16. Larva just after hatching. 2.8 mm.
FIG. 17. Larva four days old. 3.8 mm.
FIG. 18. Larva nine days old. 4.0 mm.
FIG. 19. Larva. 6.3 mm.

PLATE III
Parophrys vetula

5. PLEURONICHTHYS VERTICALIS JORDAN AND GILBERT SHARP-RIDGED TURBOT

5.1. Spawning

Attempts to strip eggs from Pleuronichthys verticalis were initiated in April, 1936, but only a small proportion of the fish caught in Monterey Bay proved to be ripe. It was not until well past the middle of the month that the majority of the females were found to be bearing mature eggs. Shortly after this an abnormally warm current of water found its way into the Bay with the consequence that no eggs were to be obtained for a considerable period. Apparently the influx of warm water caused a sudden shedding of all eggs and spawning was not resumed until normal temperatures had been restored. A few eggs were taken in plankton tows as late as August, and after this none was taken until the early spring of 1937. In light of the above, it appears that Pleuronichthys verticalis has an extended spawning period from March until August, with an optimum from late April to June.

All attempts at artificial fertilization failed, and the material for study was collected by means of frequent plankton hauls. Unfortunately, the earliest eggs taken had a fully developed blastodermal cap. Estimating from their later rate of development, these eggs were approximately 24 hours old and must have been carried by currents a considerable distance from the spawning area. Every effort was made to locate this spawning area so that earlier stages might be taken for observation, but although hauls were made in many different localities, at various depths from the surface to 20 fathoms, and at all hours of the day and night, no earlier stages were taken. It is with regret that the segmentation stages are of necessity omitted, but there is little reason to assume that they differ essentially from the typical teleostean cleavages already discussed under Parophrys vetula.

5.2. Eggs

The eggs of Pleuronichthys verticalis are pelagic, with a specific gravity approximately equal to or very slightly less than that of sea water. They are spherical with a diameter of 1.07 (1.03–1.11) mm. The thin membrane averages 0.018 mm. in thickness. To the naked eye, the egg appears slightly translucent. Examination under the microscope shows that this is due to a hexagonal pattern extending through the entire thickness of the membrane. This is shown in the drawing of the unfertilized egg (see Fig. 20), but is omitted for the sake of clarity in the later stages. The hexagons are of approximately equal size, measuring about 0.042 mm. across, and their regular arrangement gives the shell the appearance of a sphere made up of tiny hexagonal tiles. The yolk is perfectly clear and transparent.

5.3. Embryonic Development

The blastodermal cap (see Fig. 21) has a slightly granular texture in the central area which fades out peripherally to an even translucent margin. In obtaining the texture desired in the drawing, the effect of the transparency which really exists is lost. The blastodermal cap

of Pleuronichthys verticalis is considerably smaller than that of Parophrys vetula, and is straw yellow in color instead of transparent. When viewed from the side, the lenticular dome of blastoderm has quite abrupt edges where it meets the yolk sphere, contrasting markedly with the gentle roundness displayed by the blastodermal cap of Parophrys vetula.

Since the blastodermal cap was the earliest stage observed, it will be used as "zero hours" as a basis of timing future development. An addition of 24 hours would approximately fix the time after fertilization.

Soon the segmentation cavity begins to form and is completed after four hours. When viewed from above, the thickened rim of the blastodermal cap in contact with the yolk and representing the early germ ring is not as transparent as the central area of the blastoderm which has pulled away from the yolk in forming the segmentation cavity. (See Fig. 22.)

In the case of Pleuronichthys verticalis no eccentricity in the position of the segmentation cavity is immediately discernible. However, shortly after the definite establishment of the germ ring, its centripetal growth becomes more rapid at one point than elsewhere, and at nine hours after the blastodermal cap stage, the resulting widened portion of the germ ring develops into a bluntly pointed tongue of cells extending into the segmentation cavity. (See Fig. 23.) Its shape is strikingly different from that of the broad intrusion in the egg of Parophrys vetula.

The germ ring now commences its centrifugal migration. In approximately 14 hours it has reached an equatorial position. (See Fig. 24.) The slightly tapered body and the thickened area of the head are well defined, the clear straw yellow color persisting. The diameter of the germ ring is usually slightly less than that of the yolk so that it more or less squeezes the yolk at its edge of contact. However, it is after the germ ring passes the equatorial position that its pinching action becomes more pronounced. By about 20 hours (see Fig. 25), the eyes have formed and bulge prominently from the sides of the head but are without pupils. The notochord can be faintly discerned, and Kupffer's vesicle is making its first appearance. The embryo at 24 hours and shortly before the close of the blastopore is represented in figure 26. At this stage the body of the embryo has becomes less transparent and has deepened in its general amber color. Kupffer's vesicle has reached its full development and the germ ring has almost closed. At 26 to 28 hours the blastopore closes.

With the closure of the blastopore, the pupils of the eyes become outlined, and slightly posterior to them the auditory capsules become evident. The heart appears as a slight ventral bulge of the body wall, and soon the first signs of pigmentation are apparent. The chromatophores show at first only as minute black dots in the vicinity of the nape with a slight scattering over the body. None appears at this stage upon the yolk sac.

By 36 hours (see Fig. 27) the pigment spots are well defined although small. They seem to be present in almost their full numbers. Although the larval fish becomes much more heavily pigmented, the darker coloration seems largely due to the expansion of already existing

chromatophores rather than to the addition of new ones. After the appearance of the chromatophores, the embryonic and particularly the larval stages present many contrasting appearances. At one moment the developing fish is quite lightly colored because of tightly contracted pigment spots, and an hour later the same specimen will be dark and almost opaque due to their expansion. It seems to be characteristics of the early pigmentation of Pleuronichthys verticalis that the pigment spots on the body are almost entirely of a tannish color. While contracted in their early stages, these chromatophores give the appearance of being melanophores, however, at later stages when expanded most of these spots are seen to be light yellow brown. On the other hand, the pigment spots which first appear on the yolk sac seem to be made up almost entirely of melanophores. Later there is an invasion or development of amber colored chromatophores on this structure. The fin fold is transparent and colorless.

After the closure of the blastopore, there is a rapid growth of the tail and a widening of the continuous fin fold. Shortly before the 48-hour stage (see Fig. 28) Kupffer's vesicle disappears. In good light the lobes of the brain may be made out. The otoliths can be clearly seen in the auditory capsules and the heart is beating regularly.

At 72 hours after the formation of the blastodermal cap, the tail of the embryo encircles the yolk sac, the body has increased greatly in bulk, and the fin fold has widened considerably. (See Fig. 29.) Pigmented areas of light amber with scattered melanophores have appeared on both dorsal and ventral fin folds just posterior to the anus and send streaks half way to the end of the tail. This pattern becomes more clearly defined later and serves as the main identifying characteristic of the larvae. The pectoral fins appear as small fans pressed flat against the yolk sac.

From this stage until the time of hatching, very few changes are evident except a slight increase in size and length of tail, a deepening of pigmentation, and the appearance of scattered amber chromatophores on the yolk sac. The movements of the embryo become increasingly vigorous.

The embryo immediately before hatching is shown in figure 30. In water about 13.8° C. hatching occurs at approximately 86 hours after the blastodermal cap stage. The struggles of the embryo result in the rupture of the shell opposite the head. The aperture, just large enough for the head to be thrust through, is usually so small that as the embryo struggles for freedom, the edges of the opening press sharply into the nape and yolk sac. (See Fig. 31.) As the fish continues to twitch from side to side, the opening is slightly enlarged, and the thrusting of the tail against the inner wall causes the embryo to emerge slowly.

5.4. Larvae

The newly hatched larva (see Fig. 32) measures about 3.16 mm. in standard length. It is hampered somewhat in its movements by the buoyant yolk sac, but not to the same extent as Parophrys vetula.

The head is distinguished by having a very high crown, concave anterior profile, and a bluntly pointed snout. The body tapers gently back to the tail. There is no indication of a mouth, and the ovoid yolk

sac extends back to the rectum which is located at about the mid point of the body. The continuous fin fold rises over the crown of the head in a peculiar crest of varying size. The combination of high crown and crest is quite distinctive of Pleuronichthys verticalis. The fin fold extends around the body to the anus. It is widest at a point slightly posterior to the anus, the dorsal and ventral folds each being almost twice as wide as the body. The body is well covered with amber chromatophores and sparsely sprinkled with melanophores. The rectum is light amber with a scattering of dark pigment spots. The anterior third of the yolk sac is clear and colorless. This is probably the pericardial cavity in an exaggeratedly large condition; its size varies from specimen to specimen. The most distinguishing feature of the larva which serves to make it at once identifiable is the color pattern on the continuous fin fold. A scattering of several small amber colored areas arises from the dorsal edge of the body slightly posterior to the anus. Further scattered areas of the same color extend posteriorly along the mid region of the fin fold at least half way to the end of the body. A few melanophores are present in the broken amber patches. The same pattern is present in the ventral fin fold but arises from the ventral body wall slightly more posteriorly than its dorsal counterpart.

By six days (see Fig. 33) the larva measures about 3.35 mm. in standard length. Although there has been little actual increase in length, the larva has gained appreciably in bulk and solidity. The jaws are now well formed and functional, and the crown of the head has become still more abruptly elevated. The yolk sac is almost completely absorbed and the gut has assumed a solid appearance. The fin fold still rises in a prominent crest over the crown of the head and keeps its former proportions around the body to the anus.

A considerable change has taken place in the pigmentation. The head, nape and anterodorsal areas of the body display a light yellow amber color, with very few greatly expanded melanophores overlying the surface. The eyes have a silvery greenish sheen and are almost covered with contracted melanophores. The pupils are black. The pericardial cavity which was colorless in the earlier stage is now covered with greatly expanded melanophores, and the heart can be seen beating within it just behind the jaw. The body and gut appear a smoky gray, the color being due to many small expanded melanophores almost obscuring the underlying amber color of these areas.

The pigmented pattern on the fin fold is now confined to more restricted areas. Just posterior to the vertical of the anus, a triangular shaped patch of melanophores rises to an apex near the dorsal margin of the fold. There is included in the pattern a few greatly expanded yellow chromatophores. The same color pattern occurs in the ventral fin fold but is located posterior to the vertical of its dorsal counterpart. Greatly expanded melanophores are also present in the ventral fin fold, lying close to the rectum and extending along the body between the anus and the colored area of the fin.

The larvae of Pleuronichthys verticalis were not carried any further than six days after hatching. They proved to be the most delicate of the three turbots discussed in this paper and required extremely careful handling.

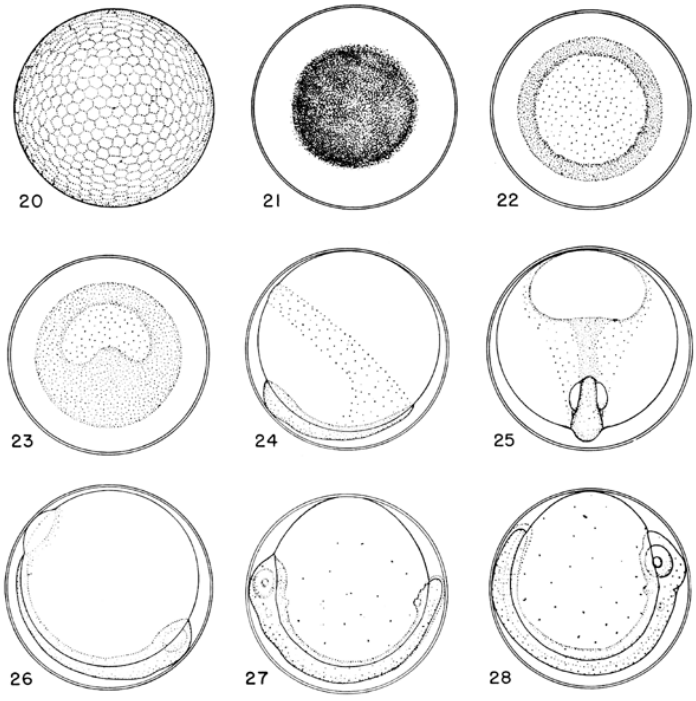

PLATE IV

Pleuronichthys verticalis

FIG. 20. Unfertilized egg.
FIG. 21. Late blastodermal cap. 0 hours.
FIG. 22. Segmentation cavity and early germ ring. 4 hours.
FIG. 23. Embryonic shield. 9 hours.
FIG. 24. Germ ring at equatorial position. 14 hours.
FIG. 25. Germ ring past equatorial position. 20 hours.
FIG. 26. Blastopore nearing closure. 24 hours.
FIG. 27. After closure of blastopore. 36 hours.
FIG. 28. Embryo with tail free of yolk sac. 48 hours.

PLATE IV
Pleuronichthys verticalis

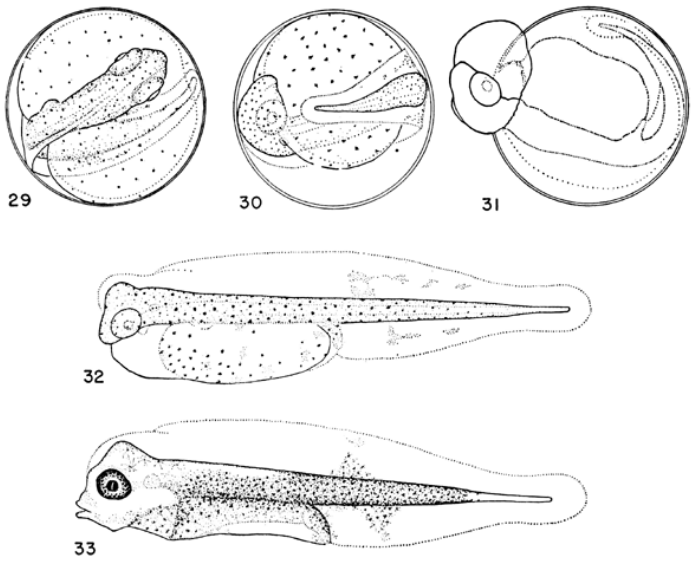

PLATE V

Pleuronichthys verticalis

FIG. 29. Embryo encircling yolk sac. 72 hours.
FIG. 30. Embryo immediately before hatching.
FIG. 31. Hatching. 86 hours.
FIG. 32. Larva just after hatching. 3.16 mm.
FIG. 33. Larva six days old. 3.35 mm.

PLATE V
Pleuronichthys verticalis

6. PLEURONICHTHYS DECURRENS JORDAN AND GILBERT CALIFORNIA TURBOT

6.1. Spawning

The spawning season of Pleuronichthys decurrens corresponds closely to that P. verticalis, ripe females of the same species being taken at the same times, and the eggs of both forms being found in almost equal numbers in the same plankton hauls.

6.2. Eggs

The pelagic eggs, with a specific gravity about equal to that of sea water, are spherical and have a diameter of 1.44 (1.31–1.50) mm. They are decidedly larger than those of P. verticalis, and there is no overlapping of sizes. The membrane is slightly thinner, being 0.014 mm. in thickness, and is marked by the same hexagonal pattern, but the hexagons, measuring approximately 0.037 mm. in width, are slightly smaller than in P. verticalis. The yolk is clear and transparent and contains no oil globule. In general appearance, the unfertilized egg closely resembles that of P. verticalis. (See Fig. 20)

6.3. Embryonic Development

The same difficulties with artificial fertilization occurred that had been encountered with P. verticalis, so eggs were again obtained by frequent plankton tows. Once more, although every effort was made to find the early cleavage stages, the youngest stage found was that of the early blastodermal cap in which the large cells of the blastoderm could still be distinguished. (See Fig. 34.) This stage, probably about 10 hours after fertilization, will be used as "zero hours." Fourteen hours later, the blastodermal cap reaches its full growth. (See Fig. 35.)

The blastodermal cap now begins to broaden. At the time that invagination is initiated, its size is slightly smaller than in P. verticalis. Its color is pale yellow. The segmentation cavity develops rapidly and by 22 hours (see Fig. 36) the blastodermal cap shows the marginal thickening of the germ ring, with the embryonic shield indicated by a slightly widened portion at one pole. As development continues, the diameter of the blastodermal cap increases and the migration of the germ ring soon causes the outlines of the cap to blend more evenly with the contours of the yolk sphere.

At 34 hours the germ ring, which is of a much heavier texture in this species than in P. verticalis, is approaching the equator. (See Fig. 37.) At the margin of the yolk where the germ ring appears in profile, an actual thickness of the ring can be seen. It is interesting to note that the germ ring displays a much more marked pinching action than in P. verticalis. In the case of the latter, the pinching of the yolk by the germ ring does not become very apparent until this structure has passed the equatorial position. In P. decurrens the constricting action of the ring is seen from the beginning of its migration. This is probably due to its heavier structure and to the fact that it arises from a relatively smaller blastodermal cap. Another marked

feature of this stage is the extreme thickness of the body of the embryo, particularly in the cephalic region.

At approximately 42 hours, the eyes are well defined, Kupffer's vesicle has assumed quite large proportions, and the body is thickened throughout. (See Fig. 38.) By 62 hours, shortly after the closure of the blastopore, the eyes and their pupils have become almost completely differentiated, the auditory capsules have put in their appearance, and the lobes of the brain may be readily seen. (See Fig. 39.) Shortly before this stage tiny pigment spots had appeared on the body; now they may also be seen on the yolk sac. In the proper light, the almost completely transparent somites and notochord are evident.

By 86 hours, the choroid fissure of the eyes can be easily seen and the auditory vesicles have become clearly defined. (See Fig. 40.) The heart is now pumping regularly and because of the rapidly growing tail, the embryo encircles about three-quarters of the yolk sac. A generous scattering of yellow chromatophores can be made out upon the body. The pigmentation of this stage closely corresponds to the 72-hour stage of Pleuronichthys verticalis, but the coloration may be slightly lighter. Except for the size, it would be very difficult at this stage to differentiate the egg of P. decurrens from that of P. verticalis.

Except for a slight lengthening of the tail and an increase in the bulk of the body, the embryo at 114 hours (see Fig. 41) shows little change. The pigment spots remain quite small, and the fin fold shows no signs of color. By 140 hours, the embryo has encircled the yolk and there has been a decided darkening of pigmentation. (See Fig. 42.) The body can be seen to be evenly covered with yellow and black chromatophores, both kinds occurring in about equal numbers. A sparse scattering of chromatophores is also present on the yolk sac. About two-thirds of the way between the anus and the tip of the tail, a slight coloration occurs near the body, appearing as if the chromatophores of the body had crept out into the fin fold. This is entirely different from the pigment pattern found on the tail of P. verticalis and serves as the main differentiating character of the larvae to be discussed below. Hatching occurs at about 150 hours after the early blastodermal cap stage and probably about 160 hours after fertilization. Although the eggs of both P. verticalis and P. decurrens were kept in water of approximately the same temperature (13.8° C.), the rate of development in P. decurrens was much slower than in the case of P. verticalis, the eggs requiring approximately one week instead of five days for hatching.

6.4. Larvae

The newly hatched larvae of Pleuronichthys decurrens (see Fig. 43) measure about 3.88 mm. in standard length. Although quite active, their movements seem to be poorly directed, due probably to the bulk and buoyancy of the large yolk sacs. In proportions, color and general appearance, these newly hatched larvae are similar to those of P. verticalis but may be readily distinguished by the following consistent differences.

The head is more evenly rounded than in P. verticalis, and the anterior end of the dorsal fin fold never displays a sharp supracephalic crest. The general body melanophores are more numerous, while those of the yolk sac are few, widely scattered and expanded instead of being

numerous and contracted. The rectum, in its curved course past the posterior margin of the yolk sac, shows no indication of the heavy pigmentation characteristic of P. verticalis, but is transparent except at its dorsal extremity where it becomes light amber. The dorsal and ventral fin folds, at a point adjacent to the body and about midway between the anus and the tip of the caudal fin fold, bear a few very definite melanophores. These black pigment spots, usually greatly expanded, are surrounded by large amoeboid chromatophores which give the area a faint yellow color. Occasionally one or two melanophores occur in the middle of the ventral fin fold between this pigmented area and the anus. This pigmentation of the fin folds is the most striking feature which may serve to distinguish the larvae of P. decurrens from P. verticalis, and is diagnostic for all stages discussed in this work. A comparison of figures 32 and 42 will indicate the differences.

Four days after hatching, the larvae measure about 4.35 mm. in standard length. The crown of the head has risen in a large crest and the jaws have developed into functional structures. (See Fig. 44.) Ventrally and posterior to the jaws, a clear well-rounded area marks the pericardial cavity in which the heart can be seen pulsating evenly. The yolk sac has been almost entirely absorbed, a large opaque mass at its anterior end is the liver, and the intestine is well differentiated. The dorsal fin fold has widened anteriorly. Striking changes have occurred in the pigmentation. The eyes have become very dark, the general color of the head is now light amber with a scattering of expanded melanophores on the crown and nape, and the body gives the appearance of real solidity due to its heavy pigmentation. The background of amber has become greatly darkened, and there is an even arrangement of closely spaced melanophores. These are almost perfectly round but with ragged edges, and give the entire body the appearance one associates with a leopard skin. The dorsal and lateral surface of the gut are marked by the same coloration, but its lower portion is light amber fading out ventrally. The pigmented pattern on the tail has grown in size and deepness of color. The large semi-circular area of light amber on both dorsal and ventral fin folds bears a few greatly expanded melanophores, and has apparently moved slightly posteriorly. A few scattered melanophores form a diffuse pigmented area along the edge of the dorsal fold just above the crown of the head.

The larvae have reached an average length of 4.62 mm. by seven days. (See Fig. 45.) The crown of the head has become much more prominent, and the lower jaw protrudes beyond the upper giving the head a pointed appearance. The amber coloring of the cephalic region remains the same, but the melanophores appear darker. The iris of the eye is now black green. The opercle is clearly defined and the area of the auditory capsule immediately dorsal to its end is conspicuously dark. While the amber color of the body and dorsolateral surfaces of the gut have remained the same, these areas appear more heavily pigmented due to the development of additional melanophores which are smaller but much more closely spaced. The pigmented area of the fin folds has also darkened, and posterior to the anus occasional melanophores can be seen extending out a short distance into the dorsal and ventral fin folds.

By nine days the larvae have increased in length to about 4.75 mm. Due to further lengthening of the lower jaw, the head has assumed a still more pointed appearance. (See Fig. 46.) The pigment spots of the body have diffused and give the tiny fish a very dark appearance. The pseudopodlike extensions of the melanophores which had previously appeared in the fin folds close to the body have developed rapidly. Many new ones have made their appearance along the entire length of the fin fold anterior to the pigmented area of the tail. Each sends out many fine filaments giving the pigmented areas of the fins an almost lace-like appearance. The small pigmented area on the fin fold just above the crown of the head has become slightly more extended and darker.

At eleven days the last larva died, and no figure could be made before distortion took place. To all outward appearances, however, very little change had occurred except a continued increase in the coloration of the fin folds following the same pattern already described.

The success in carrying the larvae for a period of eleven days when all other attempts had ended in failure at nine days or less, was probably due to the utilization of mineralized water. Despite the constant agitation of the water, larvae kept in ordinary sea water still had to combat bacterial invasion to some extent. Mineralized sea water mitigated this factor, and thus favored the larvae enough to enable them to exist a few days longer.

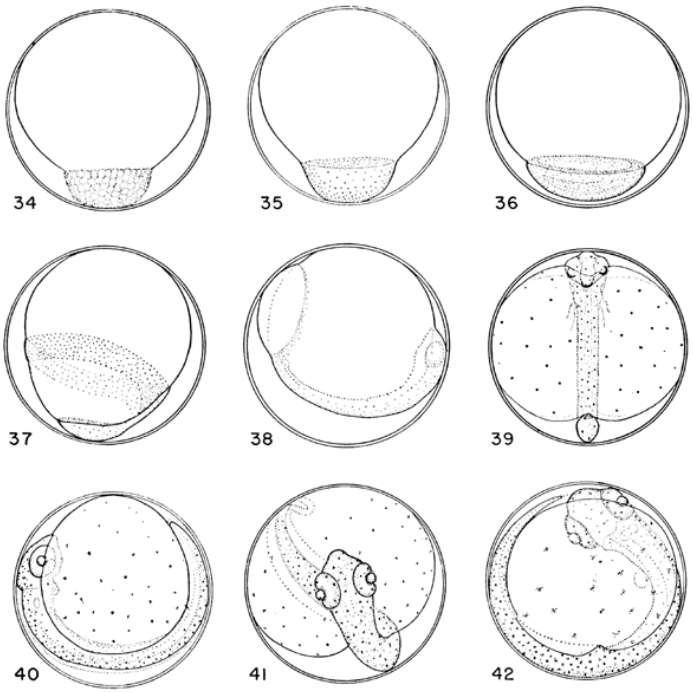

PLATE VI

Pleuronichthys decurrens

FIG. 34. Early blastodermal cap. 0 hours.
FIG. 35. Completed blastodermal cap. 14 hours.
FIG. 36. Segmentation cavity and early germ ring. 22 hours.
FIG. 37. Germ ring approaching equatorial position. 34 hours.
FIG. 38. Germ ring past equatorial position. 42 hours.
FIG. 39. Shortly after closure of blastopore. 62 hours.
FIG. 40. Tail free of yolk sac. 86 hours.
FIG. 41. Tail encircling yolk sac. 114 hours.
FIG. 42. Late embryo. 140 hours.

PLATE VI
Pleuronichthys decurrens

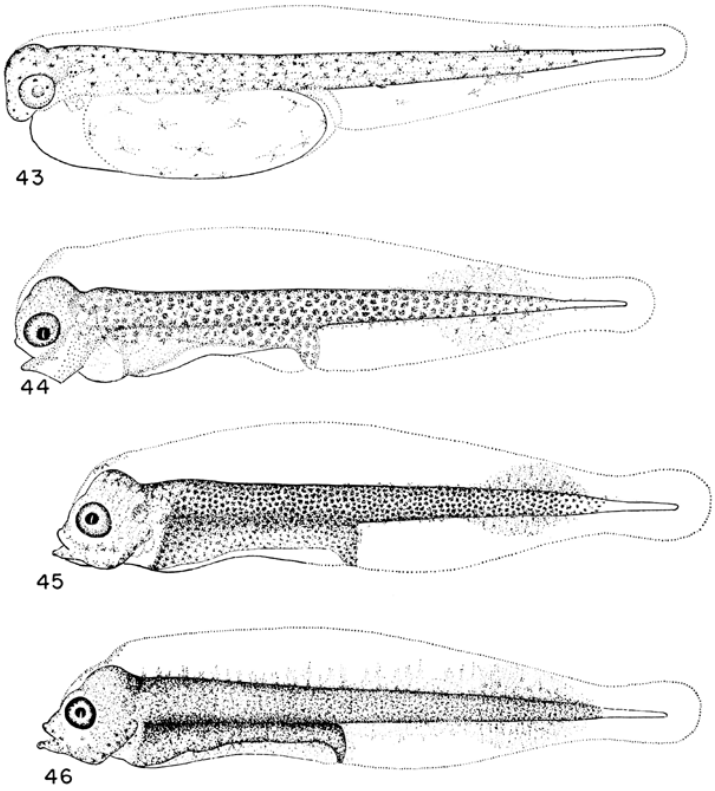

PLATE VII

Pleuronichthys decurrens

FIG. 43. Larva just after hatching. 3.88 mm.
FIG. 44. Larva four days old. 4.35 mm.
FIG. 45. Larva seven days old. 4.62 mm.
FIG. 46. Larva nine days old. 4.72 mm.

PLATE VII
Pleuronichthys decurrens

7. PLEURONICHTHYS COENOSUS GIRARD MOTTLED TURBOT

As Pleuronichthys coenosus is not common in Monterey Bay, very few specimens could be obtained, and no estimate of the spawning season could be made. Only a half dozen eggs of this species were taken in all of the plankton hauls made in the vicinity of Hopkins Marine Station. One was obtained in November, 1936, and contained an embryo at the stage of the closure of the blastopore. This egg was observed during the remainder of its incubation period and the larva was kept alive for eight days after hatching. In April, 1937, several more eggs were found which were in the early blastodermal cap stage. Their development was carefully followed and checked against that of the first egg.

7.1. Eggs

The eggs of Pleuronichthys coenosus closely resemble those of P. verticalis and P. decurrens in all respects except size. They average 1.88 mm. in diameter, so greatly exceeding the size of the largest P. decurrens egg that they can be distinguished at a glance. The membrane is 0.018 mm. in thickness and the hexagonal figures measure 0.042 mm. across. Both of these measurements are about the same as in P. verticalis. The membrane and yolk are clear and transparent and no oil globule is present. The specific gravity of the eggs is just about equal to that of sea water as they are not particularly buoyant.

7.2. Embryonic Development

The early blastodermal cap (see Fig. 47) is comparatively small and of a well-rounded dome shape. Its color is a clear straw yellow. Approximately 15 hours after this stage, the blastodermal cap (see Fig. 48) has reached its full development, the component cells becoming so small that their outlines can not be distinguished, but the cap itself changes very little in size and shape. During the next few hours the blastodermal cap slowly spreads until it represents a very shallow bowl whose expanding brim embraces more and more of the yolk sphere. When invagination starts, the germ ring grows only slightly in a centripetal direction, and the embryonic shield never attains any great size. The result of the limited growth of these structures is that by 33 hours, the greatly expanded germ ring has attained an almost equatorial position while the embryo remains comparatively small because of the limitations of the embryonic shield. (See Fig. 49.) For comparison, see the corresponding figure of Parophrys vetula (Fig. 12) representing the opposite extreme.

By 41 hours after the first stage observed, the germ ring has migrated about three-quarters of the way around the yolk sphere, and the embryo has gained a little in length and bulk. (See Fig. 50.) While in the other flatfishes already discussed, the eyes and Kupffer's vesicle were clearly defined by the time the germ ring had attained this position, no definite structural details can be made out in Pleuronichthys coenosus. The embryo remains a clear straw color, while the germ ring, similarly colored, and the extra-embryonic area show a slightly granular texture.

The blastopore closes at about 50 hours, and at this time the embryo takes on more definite characteristics. The greatly thickened cephalic region contains poorly defined eyes, the body tapers back to the slightly enlarged caudal mass, and the embryo cradled in the yolk sphere has lost some of its former transparency.

It is not until after the blastopore has closed that Kupffer's vesicle becomes evident. By 58 hours (see Fig. 51) the vesicle has attained considerable size. The eyes are well formed but are as yet without pupils. The head is well defined and in good light somites can be seen. Pigment spots have appeared, particularly in the mid region of the embryo and along the dorsal ridge, and seem to be small contracted melanophores of varying degrees of darkness, causing the embryo to look as if it had been sprinkled with pepper.

By 82 hours (see Fig. 52) the head has taken on the characteristic shape due to the formation of the lobes of the brain which can now be easily made out. The pupils have appeared in the eyes and the auditory capsules have become evident. The heart is indicated by a slight bulge below the nape. Kupffer's vesicle has reached its full proportions and the tail has started to grow free of the yolk sac. A liberal sprinkling of melanophores can now be seen on the dorsal half of the yolk sac, and the body shows both melanophores and small yellow chromatophores evenly scattered along its entire length.

The tail continues to grow rapidly and by 136 hours (see Fig. 53) has almost encircled the yolk sphere. The broad continuous fin fold has developed, and the pectoral fins have made their appearance. The whole body has gained considerably in bulk, the eyes are large and better defined, and the heart is beating slowly. The color of the embryo has deepened to amber yellow and the pigment spots are slightly expanded.

From now until hatching the development of the embryo is marked by a continued growth of the tail and a slight gain in bulk. The structures already mentioned become more clearly defined, and the embryo shows increasing signs of activity. Figures 54 and 55, at 177 and 250 hours respectively, show this development. The most marked change during this period is in the pigmentation. The entire body becomes completely covered with an even scattering of melanophores and yellow chromatophores, the dark yellow of the chromatophores making a general amber background, over which the melanophores expand. The fin fold comes to be covered with expanded melanophores both dorsally and ventrally, and the gut shows the same pigmentation but to a lesser degree. The eyes become increasingly dark. As a result, the embryo as it remains curled up in its shell has a dark gray appearance with two tiny black dots for eyes.

Hatching occurs in the usual manner at approximately 264 hours after the early blastoderm stage if the eggs are kept in water of about 13.8° C. Estimating by the early rate of development, this is probably about 280 hours, or approximately 12 days after fertilization. A remarkable feature of the development of Pleuronichthys coenosus, as compared to that of the other fishes of the same genus which have already been dealt with, is the much longer period of incubation. Whereas P. decurrens, with a considerably slower rate of development than P. verticalis, required only 150 hours after the early blastodermal

cap stage to hatch, it took P. coenosus 264 hours to complete its embryonic development from the same stage. All were kept in water of approximately the same temperature.

7.3. Larvae

The greatly increased length of the incubation period has a marked effect on the larva which is large, well-developed, extremely active and able to swim about at will immediately after hatching. There is a very slight tendency for the larva to float on one side, but its unabsorbed yolk sac is comparatively small and does not to any appreciable degree hamper its movements. It is comparable in its degree of organization to the week-old larvae of Parophrys vetula and Pleuronichthys verticalis. The rapid changes in size and form which take place in these species have already in the case of Pleuronichthys coenosus been passed within the egg and modifications occurring in the first few days of larval existence are very slight.

The newly hatched larva of P. coenosus, 5.54 mm. in standard length, has the head well formed and not deflected over the yolk sac. (See Fig. 56.) The jaws are large and functional. The body is thick and tapers gently back to the tip of the tail. The gut is small, and the yolk sac bears little resemblance to the bulbous structure possessed by P. verticalis and P. decurrens. The broad continuous fin fold runs from the crown of the head to the anus. The eyes are large and black, and the head is colored pale amber with a few greatly expanded melanophores on the crown and nape. The pigmentation is quite heavy over the entire body, the background being a dark amber covered with closely spaced, partially expanded melanophores. The tip of the tail is colorless and transparent. The dorsal surface of the gut has the same color pattern of pigment spots as the main body, but its lateral and ventral surfaces display only occasional greatly expanded melanophores. The large rectum curving around the posterior end of the yolk sac bears the same color pattern as the body.

The most striking feature of the pigmentation of P. coenosus, and one that serves for immediate specific identification, is the coloration of the dorsal and ventral fin folds. Expanded melanophores cover the entire surface of the fin between the verticals of the nape and the transparent area of the tail. They give the fin a grayish appearance, and their pseudopodial extensions form a fine network. Anteriorly at the nape, and posteriorly around the tip of the tail, the melanophores fade away and leave transparent areas.

Although the larvae were kept alive for eight days after hatching, very little change was evidenced. An initial slight increase in length was soon counteracted by an actual shrinkage which may have been due to a natural consolidating of tissues, or to the fact that the larvae had to resort to their own body tissues for nourishment. Figure 57 shows a larva shortly before it died. Emaciation is not only evident in the collapsed condition of the gut but in the shrunken appearance of the head and eyes. The tail has begun to take on a more definite entity and incipient rays can be seen. Pigmentation has become considerably heavier over the entire body and fin fold, and the eyes have taken on a dark bluish sheen. The pupils remain jet black.

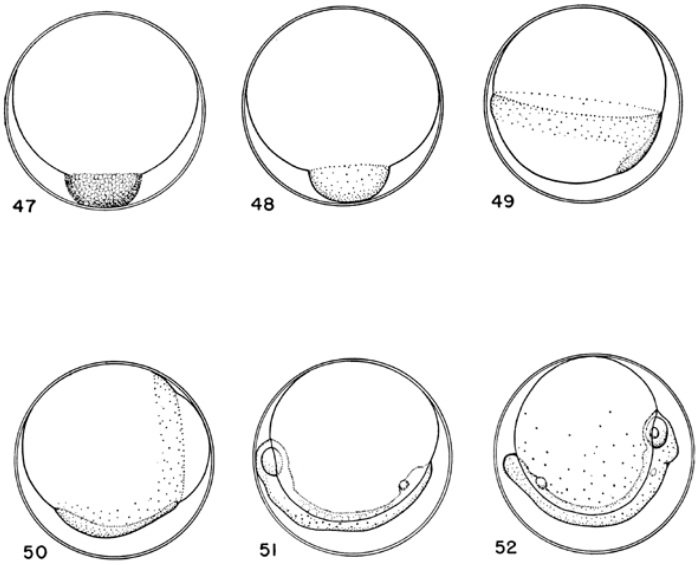

PLATE VIII

Pleuronichthys coenosus

FIG. 47. Early blastodermal cap. 0 hours.
FIG. 48. Late blastodermal cap. 15 hours.
FIG. 49. Germ ring approaching equatorial position. 33 hours.
FIG. 50. Germ ring past equatorial position. 41 hours.
FIG. 51. After closure of blastopore. 58 hours.
FIG. 52. Tail starting free of yolk sac. 82 hours.

PLATE VIII
Pleuronichthys coenosus

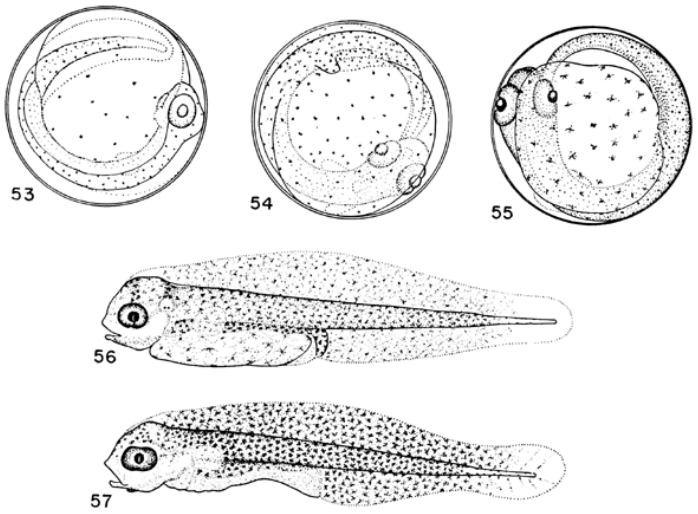

PLATE IX

Pleuronichthys coenosus

FIG. 53. Tail almost encircling yolk sac. 136 hours.
FIG. 54. Tail encircling yolk sac. 177 hours.
FIG. 55. Embryo shortly before hatching. 250 hours.
FIG. 56. Larva just after hatching. 5.54 mm.
FIG. 57. Larva eight days old.

PLATE IX
Pleuronichthys coenosus

8. ARTEDIUS LATERALIS (GIRARD) TIDE-POOL COTTID

Specimens of Artedius lateralis captured in the tide pools at Pacific Grove in January, 1937, spawned in an aquarium at the Hopkins Marine Station on February 16. The eggs when examined under the microscope were found to have been naturally fertilized.

8.1. Eggs

The eggs of Artedius lateralis are demersal and adhere firmly to one another and to the substrate. They are almost spherical in shape but are slightly flattened at their points of contact. There is a considerable variation in egg diameter, but the average size is very close to 1.07 mm. The heavy shell membrane measures approximately 0.031 mm. in thickness. It is clear and transparent and is pierced by many fine radiating canals giving the surface of the egg a pin-pricked appearance. The most striking characteristic of the egg is the bright cherry red color of the yolk. A single large oil globule appearing lighter cherry red in color and approximately 0.22 mm. in diameter is present. An irregularly shaped mass of granular material, blackish red in color, occurs in the yolk opposite the oil globule. This is shown in the drawing of the newly fertilized egg (see Fig. 58) but for the sake of clarity, it is only outlined in all other figures.

8.2. Embryonic Development

During the following two hours the blastodisc forms. In the case of Artedius lateralis and Clinocottus analis discussed below, the actual streaming of protoplasm can be seen. Long ripples extend over the surface of the yolk as the protoplasm slowly concentrates with an almost amoeboid movement. This phase of development in the case of Gadus morrhua is excellently described by Ryder (1884, pp. 470–471, 553). The large blastodisc is fully formed in two hours (see Fig. 59), extending in width over almost a third of the yolk sphere. It is of a flat tan color.

Cleavage follows in the typical teleostean manner, and one hour later the two-cell stage is complete. (See Fig. 60.) The blastomeres are very large, well rounded, with abrupt edges at their point of contact with the yolk. By four hours the second cleavage has resulted in four similar but smaller cells (see Fig. 61), and at six hours the third division establishes eight cells in two well-defined rows of four each. (See Fig. 62.) Subsequent divisions, reducing the size of the individual blastomeres, occur at approximately two-hour intervals, forming the 16-cell stage (see Fig. 63) by eight hours, and the early blastodermal cap at approximately sixteen hours. (See Fig. 64.)

Twenty-four hours after fertilization, a large blastodermal cap very similar in appearance to the blastodisc has been formed. (See Fig. 65.) The blastodermal cap at this stage is tan in color and appears to be of a slightly granular texture, particularly when viewed from directly above. A slightly lighter band on the periphery of the blastoderm at its circle of contact with the yolk marks the periblast.

At about 30 hours, a large segmentation cavity forms in the usual manner, leaving a comparatively thick wall. (See Fig. 66.) Due to

the opacity of the colored yolk, the subsequent formation of the embryonic shield and the migration of the germ ring are very difficult to make out. By 40 hours (see Fig. 67) the germ ring has migrated to an almost equatorial position and the long embryo is well established with a substantially thickened cephalic region. This cephalic thickening is extremely pronounced by the time of the closure of the blastopore at approximately 50 hours.

At 73 hours (see Fig. 68), the eyes are well formed but are as yet without pupils. Kupffer's vesicle has finally made its appearance. This does not correspond to the time of development in the pelagic eggs already discussed where it appears before or immediately following the closure of the blastopore. Large somites can be made out at this time with great difficulty. The body color is light amber. It is interesting to note that during the following stages the segmentation cavity, which has come to invest the yolk sphere, is very apparent in most eggs as a clear space between the yolk and blastoderm, usually just anterior to the head.

The embryo at approximately 97 hours has made rapid progress in its development. (See Fig. 69.) The pupils have appeared in the eyes and the head is definitely delimited by a constriction at the nape. The auditory capsules have developed but no otoliths are present, and the heart makes a prominent bulge into the yolk sac just below the nape. The tail has lengthened so that the embryo now encircles more than half the circumference of the yolk. The notochord can be easily distinguished and Kupffer's vesicle has disappeared just a short time prior to this stage.

By 197 hours (see Fig. 70), the heart is beating regularly and the embryo commences to twitch. The head is considerably broadened, and just posterior to the auditory capsules which now contain the otoliths, the body narrows abruptly and tapers evenly to the tip of the tail. The iris and pupils of the eyes have turned a dull gray color but no other actual pigmentation is evident. The body of the embryo has, however, become more opaque and appears to be slightly mottled. The cherry red of the yolk sac has deepened. This is probably because it has shrunken greatly in size so that the darker granular material is more in evidence. Soon blood vessels appear on the yolk sac below the cephalic region, and others extending below the notochord carry blood to the tail and back to the heart.

At approximately 242 hours (see Fig. 71), the head of the embryo has become greatly widened and the body has gained in length and solidity. The eyes have become almost black and there has been a deepening of the general color of the yolk and body. Many extremely small, closely massed, scarlet chromatophores have made their appearance covering a small area of the dorsal surface of the yolk sac on either side of the body. This pigmented area forms the characteristic color pattern which makes the later embryonic and early larval stages easily identifiable. Soon this scarlet field expands slightly and becomes evenly spotted by many contracted melanophores.

Figure 72 at 362 hours shows the embryo shortly before hatching. The cephalic region now fills a great part of the egg, the eyes are larger and the auditory capsules have increased greatly in size. The tail with its broad fin fold has become so elongated that it swings to

one side and wraps itself completely around the yolk. The yolk sac is considerably shrunken and the notochord and somites are clearly visible. The body remains light amber and the yolk changes to a similar but darker color as the deep red granular material of the yolk disappears. The oil globule which has remained unchanged in size throughout embryonic development also appears clear amber at this stage and rests within the yolk sac just below the head. The melanophores on the previously mentioned scarlet area have become larger and now almost obscure the red field. Large melanophores have appeared ventrally on the tail, one occurring on the posterior edge of each somite. The iris of the eye is blue black in color and the pupil jet black.

Several days before hatching, a patch of minute nodules appears on the crown of the embryo's head. Eigenmann, Breder and others have reported observing this phenomenon in the embryos of several different species of fishes. The roughened region is believed to aid as a rasp in breaking through the shell, thus allowing the larva to escape anterior end foremost. The nodules disappear soon after hatching. While they were seen in both Artedius lateralis and Clinocottus analis described below, no similar structures were observed in any of the flatfishes studied.

The average incubation period was 380 hours or about 16 days in a temperature of about 15.5° C. However, the time of hatching was extremely varied, the larvae escaping from the eggs of the same mass over a period of several days.

8.3. Larvae

The well-developed larvae swim about actively immediately after hatching. Their movements are perfectly directed and they show none of the difficulty in maintaining an upright position experienced by the early larvae from pelagic eggs.

Figures 73 and 74 show a one-day larva, 4.10 mm. in standard length, in lateral and dorsal view. The head is large and quite evenly rounded with well-developed functional jaws. The eyes are prominent and the auditory capsules are large. The body at the nape above the gut is thick and solid and tapers gently to a long pointed tail. Large somites are easily distinguishable. The heart can be seen beating regularly in the relatively transparent pericardial cavity between the jaw and the comparatively small yolk sac which still contains the large oil globule. The liver appears as a diverticulum of the gut.

The general color of the larva, particularly that of the head, is light amber. The eyes are bluish black. Overlying the body cavity is the regularly shaped area of scarlet in which the melanophores, now greatly expanded, almost obliterate the underlying color. Along the ventral margin of the body, starting on the fourth or fifth segment behind the rectum, a series of about 20 melanophores, one at the posterior margin of each somite, extends almost to the tip of the tail. Here they produce pseudopodia-like extensions that push out into the ventral surface of the fin which otherwise is always devoid of pigmentation.

Although the larvae were kept alive for a week after hatching, no further changes except for the collapse of the gut through starvation were observed.

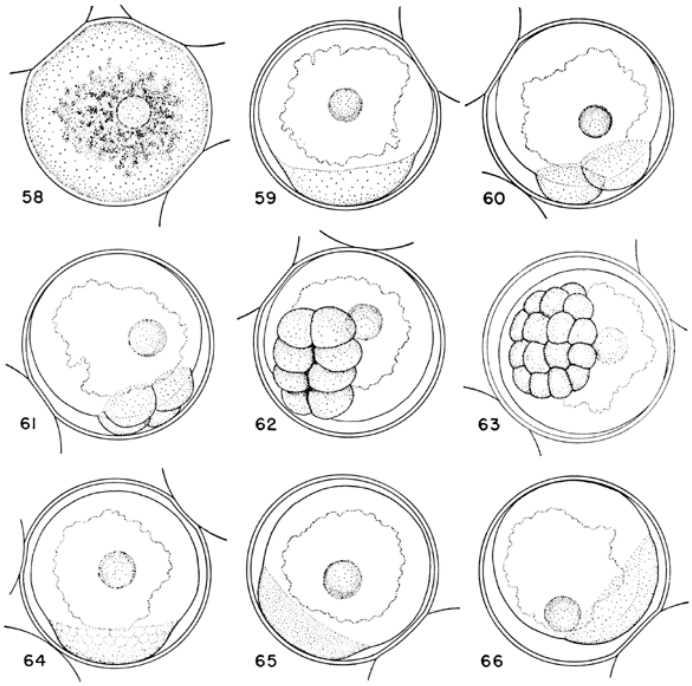

PLATE X

Artedius lateralis

FIG. 58. Egg shortly after fertilization. 0 hours.
FIG. 59. Blastodisc. 2 hours.
FIG. 60. Two cells. 3 hours.
FIG. 61. Four cells. 4 hours.
FIG. 62. Eight cells. 6 hours.
FIG. 63. Sixteen cells. 8 hours.
FIG. 64. Early blastodermal cap. 16 hours.
FIG. 65. Late blastodermal cap. 24 hours.
FIG. 66. Segmentation cavity. 30 hours.

PLATE X
Artedius lateralis

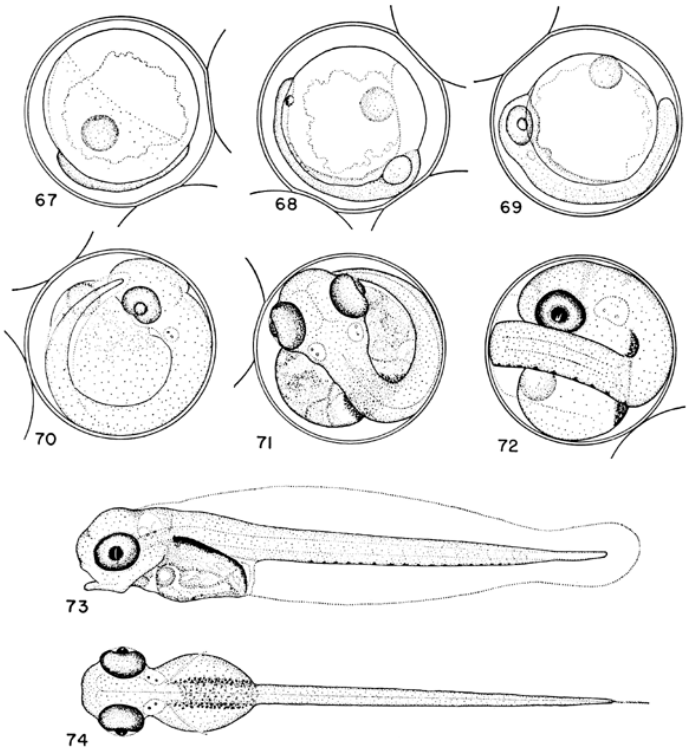

PLATE XI

Artedius lateralis

FIG. 67. Germ ring at equatorial position. 40 hours.
FIG. 68. After closure of blastopore. 73 hours.
FIG. 69. Tail growing free of yolk sac. 97 hours.
FIG. 70. Tail encircling yolk sac. 197 hours.
FIG. 71. Late embryo. 242 hours.
FIG. 72. Embryo shortly before hatching. 362 hours.
FIG. 73. Larva just hatched. Lateral view. 4.10 mm.
FIG. 74. Larva just hatched. Dorsal view.

PLATE XI
Artedius lateralis

9. CLINOCOTTUS ANALIS (GIRARD) TIDE-POOL COTTID

The gross embryology of this species has already been described by Eigenmann (1892) under the name of Oligocottus analis. While his description is good, it is very brief and the accompanying figures are few in number, small and very sketchy. The following description does not differ essentially from Eigenmann's but is more complete, calling specific attention to the various growth stages and illustrating each with a figure drawn as accurately as possible.

9.1. Eggs

The fertilized eggs of Clinocottus analis were obtained in the same manner and at the same time as those of Artedius lateralis. Two distinctly separate egg masses had been introduced under a rock, each mass being of a different color. Eigenmann describes the color of the eggs as being brownish yellow. One of the masses discovered in the aquarium matched this description closely. The other mass was light lavender and the eggs which composed it had a smaller average diameter, but in all other respects were identical to the brownish yellow eggs. A careful examination of the fish in the tank proved all of them to be without doubt Clinocottus analis. The two kinds of eggs followed the same development and the larvae produced were all alike. There is the possibility that this was a freak spawning or that such a difference might be due to a natural variation. It may also be possible that two extremely similar species are masquerading under the name of Clinocottus analis. Time did not permit the writer to investigate this interesting matter.

The egg masses consist of several hundred eggs adhering closely to each other but not to the substrate as do those of Artedius lateralis. Their generally spherical shape is slightly distorted by flattening at their points of contact. The eggs of the brownish yellow mass averaged 1.30 mm. in diameter, and the lavender eggs measured 1.25 mm., slightly greater than the diameter of 1.20 mm. given by Eigenmann. The shells, of approximately the same thickness, measure 0.037 mm. and are slightly heavier than those of Artedius lateralis. They are clear and transparent and display the many tiny perforating canals already described in the previous form. There are always several fairly large oil globules present, the largest seldom exceeding 0.18 mm. in diameter. Eigenmann states that aside from the one large oil globule there are from five to nine smaller ones. I found this number to be extremely variable and in all cases there were also present scattered groups of many tiny droplets. The translucent yolk mass bore the coloring. Figure 75 shows a newly fertilized egg.

9.2. Embryonic Development

The formation of the blastodisc and the cleavages which result in the formation of the blastodermal cap are typical of teleostean development. The blastodisc (see Fig. 76) formed about one hour after the eggs had been discovered. The first cleavage took place about one and a half hours later and subsequent cleavages occur at one and a half hour intervals. Figures 77 to 81 show this development. The blastodisc, blastomeres and the completed blastodermal cap are considerably

smaller than those of Artedius lateralis as a comparison of the figures will show. Whereas it requires 24 hours for Artedius lateralis to reach the fully completed blastodermal cap stage, Clinocottus analis takes twice as long. (See Fig. 82.)

The segmentation cavity is developed at 72 hours (see Fig. 83) and the embryo proper develops in the usual manner, but in smaller proportions than that of Artedius lateralis during the same stages. The germ ring is well defined and its migration can be followed with comparative ease. It reaches the equatorial position at about 82 hours. (See Fig. 84.) The blastopore closes at approximately 100 hours, the embryo at this time being still quite small and showing only a slight thickening in the cephalic region.

By 130 hours, Kupffer's vesicle has appeared. Eigenmann mentions that at this stage, the embryo ends posteriorly in a mass of large cells or vesicles, the larger of which represents Kupffer's vesicle. This may possibly be so, as in several other embryonic forms I have seen several vesicles located in the area usually occupied by the single Kupffer's vesicle. However, I believe that in the case of Clinocottus analis, the structures called large cells or vesicles by Eigenmann are merely a mass of small oil globules more or less obscuring the real Kupffer's vesicle. Such seemed to be the case in all of the eggs of this stage that I examined, one of which is shown in figure 85 at approximately 130 hours after fertilization. As may be seen in this figure, recent development has been rapid. The body has taken on definite shape, the eyes and pupils have formed, and the heart is evident in the ventral bulge of the body wall below the nape.

By 180 hours (see Fig. 86), the auditory vesicles have made their appearance, but the tail has lengthened only slightly. By 305 hours, the body has increased greatly in bulk and the embryo almost encircles the yolk while the tail has developed a fairly wide continuous fin fold. (See Fig. 87.) Up to this time the development has closely paralleled that outlined by Eigenmann. He states that by approximately 264 hours (11 days) the pigmented areas appear. It was not until well after the stage shown at 305 hours that this occurred in the eggs now under discussion. The difference is probably due to incubation in water of different temperatures. Although Eigenmann makes no reference to the temperature in which his eggs were kept, the fact that his work was pursued in San Diego, which is characterized by a much warmer climate and higher oceanic temperatures than Pacific Grove, suggests that appreciable differences occurred in the incubation conditions. This is further indicated by the fact that my specimens required several days longer, maintained as they were in a temperature of about 15.5° C., to complete their embryonic development than did his.

By 420 hours (see Fig. 88), the embryo has become very large and pigmentation is well established. The iris and pupils of the eyes have darkened slightly and the nape bears a group of greatly expanded melanophores. In most cases there appears to be an isolated pigment spot on the crown of the head or over each eye. A black pigmented area, surrounded by faint and greatly expanded yellow chromatophores, comes to lie over the body cavity. Shortly before hatching at about 580 hours or approximately 24 days, the eyes are large and dark, the black area on the nape has become better defined and the dark area

over the body cavity has assumed a shield shape when viewed from above. As seen in figure 89, this area appears like a curved black bar with a yellow margin. A row of melanophores has appeared along the ventral side of the body, one melanophore at the posterior border of each somite. The general color of the embryo and yolk sac at this stage is pale amber. The heart has been functioning for several days and the blood can be seen to course through the vessels spread over the yolk sac and through the larger vessels of the body.

Some eggs hatched at about 24 days after fertilization but other eggs in the same mass required as much as six days longer. Eigenmann noted the same difference in time, his material requiring from 18 to 24 days for hatching.

9.3. Larvae

The newly hatched larvae of Clinocottus analis measured about 4.50 mm. in standard length. This is slightly larger than the largest measurement of 4.20 mm. given by Eigenmann but is not surprising since the eggs were somewhat larger than his. Figures 90 and 91 show lateral and dorsal views of the newly hatched larva. The little fish are extremely active and have no difficulty in maneuvering at will. Their motions are perfectly coordinated, the flutterings of the tail propelling them about with remarkable rapidity and the fanning of the pectorals keeping the body perfectly balanced. The head and snout are slightly more pointed than in Artedius lateralis, and the yolk sac not quite so well rounded. However, the body proportions of the two species are very similar. Below and behind the jaw, the heart can be seen pumping regularly and just posterior to it the large grayish mass of the liver can be seen through the body wall. Imbedded in the liver, a small green sphere indicates the gall bladder. The oil globule lies at the anterior end of the yolk sac. Many blood vessels extend over the surface of the yolk sac, and the dorsal aorta can be made out running ventral to the notochord.

The most distinguishing characteristic lies in the pigmentation. The eyes are well colored. The general tone of the iris is green and bordering it, particularly on the dorsal surface, are many closely massed melanophores. The pupil is black. The head bears several greatly expanded yellow chromatophores, giving it a yellow appearance, and there is usually an expanded melanophore on the crown or above each eye. A group of very black pigment spots lies on the nape, sometimes one or two of the anterior melanophores being more or less detached from the rest. A broad black shield-shaped area of melanophores lies above the body cavity, surrounded by chromatophores which add a yellow tone to this area. This area presents many different aspects according to the degree of expansion of the individual melanophores. A series of ventral melanophores align themselves one to each body segment, starting at about the sixth somite posterior to the rectum and extending almost to the tip of the tail. Sometimes the posterior melanophores of this series send pseudopodia out into the ventral fin fold.

Although the larvae were kept alive for nearly two weeks, very little change could be noted. The body lengthened slightly, coloration became a little darker but no radical developmental changes occurred.

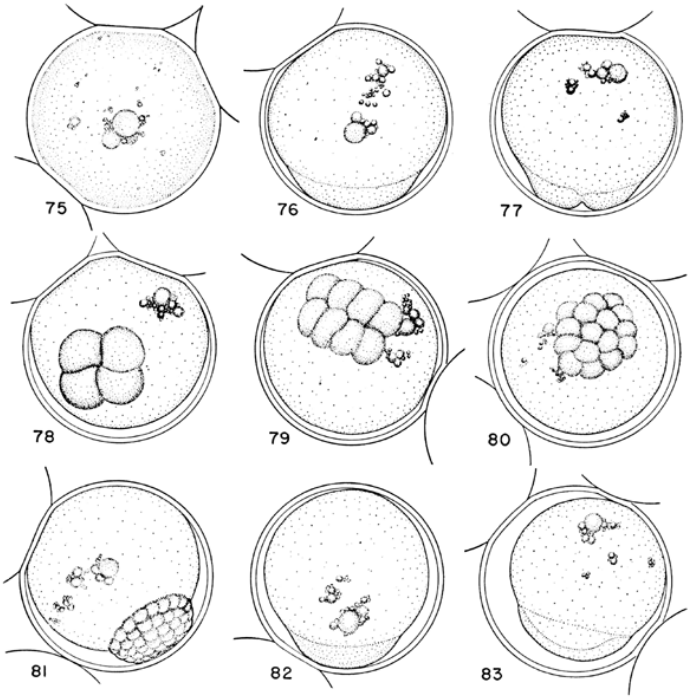

PLATE XII

Clinocottus analis

FIG. 75. Egg shortly after fertilization. 0 hours.
FIG. 76. Blastodisc. 1 hour.
FIG. 77. Two cells. 2.5 hours.
FIG. 78. Four cells. 4 hours.
FIG. 79. Eight cells. 5.5 hours.
FIG. 80. Sixteen cells. 7 hours.
FIG. 81. Very early blastodermal cap. 16 hours.
FIG. 82. Late blastodermal cap. 48 hours.
FIG. 83. Segmentation cavity. 72 hours.

PLATE XII
Clinocottus analis

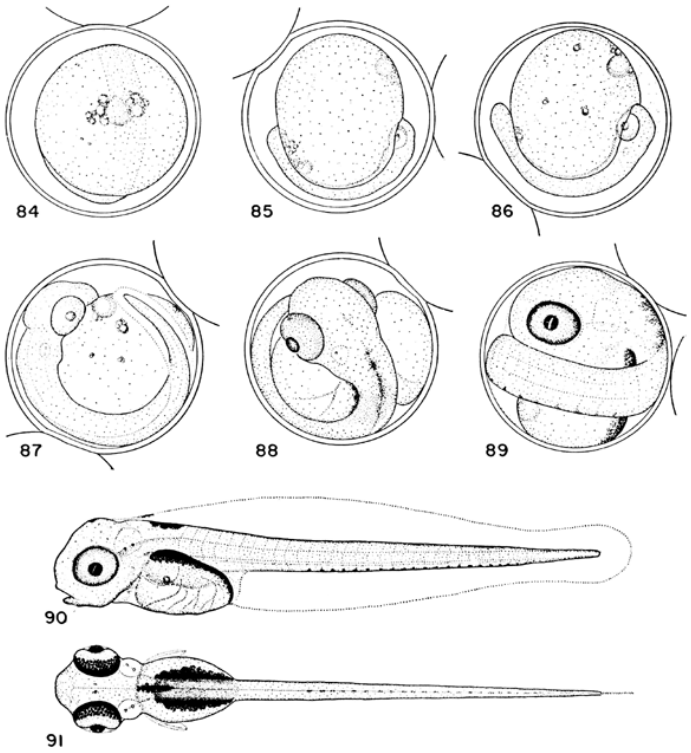

PLATE XIII

Clinocottus analis

FIG. 84. Germ ring at equatorial position. 82 hours.
FIG. 85. After closure of blastopore. 130 hours.
FIG. 86. Tail growing free of yolk sac. 180 hours.
FIG. 87. Tail encircling yolk sac. 305 hours.
FIG. 88. Late embryo. 420 hours.
FIG. 89. Embryo shortly before hatching. 575 hours.
FIG. 90. Larva just hatched. Lateral view. 4.5 mm.
FIG. 91. Larva just hatched. Dorsal view.

PLATE XIII
Clinocottus analis

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