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REPRODUCTION IN *CLEVELANDIA IOS* (JORDAN AND GILBERT), WITH AN ACCOUNT OF THE EMBRYONIC AND LARVAL DEVELOPMENT

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ABSTRACT

The paper deals with the size at first maturity, breeding habits and sexual dimorphism, embryonic development and larval stages of the goby, *Clevelandia ios*.

It was noticed that 23 per cent of females start maturing at a standard length of 29.0 mm. The percentage increases gradually and all females 34.0 mm. and above are mature.

The spawning season extends over a period of nine months with the heaviest spawning taking place from March to June. The fish lays about 750 to 1,000 eggs but actual counts of ripe ovarian ova vary from 800 to 1,200 according to the size of the fish.

There is no parental care among *Clevelandia* and the eggs are laid over a considerable area either singly or in small groups. Sexes can be separated easily, in specimens larger than 19 mm. by the examination of the genital papilla. There is no significant difference in standard length between the two sexes, but the length of head and length of maxillary are significantly different in the males and females for a given standard length, they being greater in the males than in the females.

The embryonic development and larval stages of *Clevelandia* up to the tenth day after hatching have been described from eggs artificially spawned in the aquarium. At temperatures varying from 15.0 to 15.5°C the period of incubation extends from ten to twelve days. Descriptions of post larval stages are also given. Attempts to rear the larvae in the laboratory for more than ten days proved to be impossible and the details of the methods tried are given.

INTRODUCTION

The members of the family Gobiidae are exceedingly numerous in the tropical and temperate zones, both in species and individuals, but they are of little economic importance in most parts of the world. This is true largely because most species of this family are small in size. In only one known instance do the members of this family enter the commercial fisheries. The goby fishery in the Philippines is extremely interesting and an account of this has been given by Manacop (1941). Gobies have also been used as bait fish (Weisel, 1947) in the United States of America. Presumably because of their relative economic unimportance no serious attempt seems to have been made to study the complete life history of any species of goby. The author, therefore, undertook to gather as much data as possible on the life history of *Clevelandia ios*, a goby which lives both free and commensally in the burrows of *Urechis*, *Upogebia* and *Callianassa*. The following account deals only with the size at first maturity, breeding habits, sexual dimorphism, embryonic development and larval stages of *C. ios*[2] and accounts on the other aspects of the investigation on the life history of this goby will be published elsewhere.

The material for this study was collected from Elkhorn Slough, a tributary of Monterey Bay during 1946-48 and the details regarding the locality, methods of

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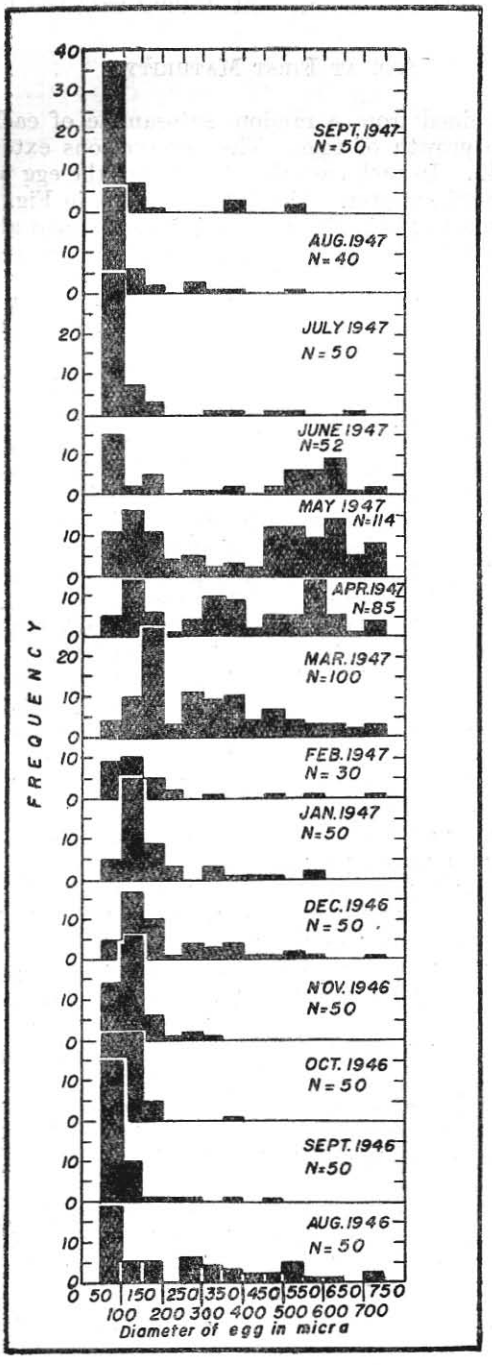
[2] Recently a series of papers on the life history of Japanese gobies have been published by Dôtu (1954, 1955*a*, *b* and *c*, 1956*a* and *b*), Dôtu and Mito (1955*a* and *b*) and Dôtu, Mito and Ueno (1955).



TEXT-FIG. 1.

Ova diameter frequency distribution showing the growth of eggs to maturity.

collection, etc., will be given in the account dealing with the habitat and habits of *Clevelandia*.

Size at First Maturity

Ovaries were examined from a random sub-sample of each monthly sample in order to follow the growth of eggs. The observations extended from August 1946 to September 1947. In each case the diameter of the egg was measured with the aid of an eye-piece micrometer. The data are given in Fig. 1. In constructing this figure only the largest eggs in the ovary are included and also fish of less than 20.0 mm. are not taken into account. These observations have led to the following conclusions.

A group of eggs measuring up to 200 micra in diameter is seen throughout the year in every adult female. A larger group of eggs, ranging approximately from 250 to 450 micra in diameter, is the maturing group. They start appearing in December and are well represented in the months of March and April : a few also are found from May to August. A still larger group of eggs, ranging from 500 to 732 micra in diameter, are the ones which are to be spawned immediately. This igroup is predominant from April to June and is also found in a few individuals dn March. It is presumed that the intermediate group of eggs, found in individuals ouring the peak of the spawning season, will grow to the maximum size by the end if the same season. Since the first group of eggs is present throughout the year in all adult females it is considered to be a group of immature eggs. Moreover, the mmature eggs are absolutely transparent with large nucleus (Pl. III, Fig. 1). On the other hand, as the ova start maturing, granules of yolk begin to appear in the cytoplasm and the transparency of the eggs is gradually lost.

Before considering the question of the size at first maturity, it is advisable to define as to what is meant by "mature" and "immature". The word "immature" is sometimes used to refer to young fish which have never spawned and also to designate older fish which have spawned previously but as yet show no indications of the onset of maturity for the next spawning season. Similarly, the word "maturity" has a double meaning.

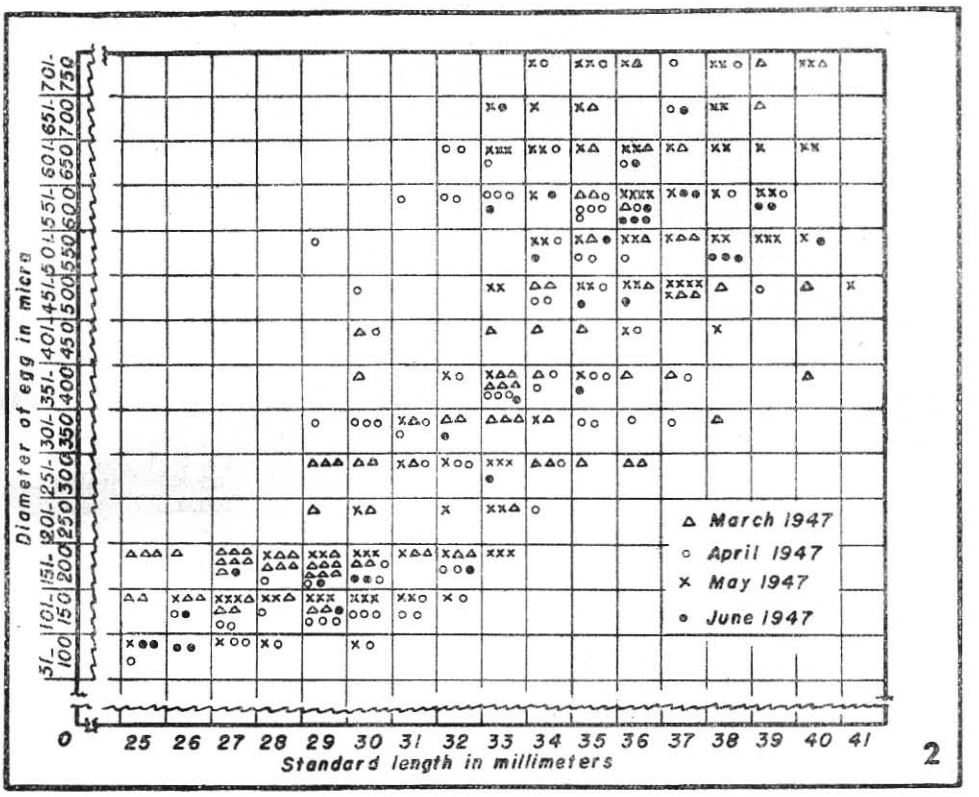
In this report, fish which have never spawned are designated as "immature". "Maturing" refers to individuals having eggs larger than 200 micra but not yet developed to a stage which is ready for spawning. Mature individuals are those having completely ripe eggs which are ready for liberation. Such mature fish have eggs ranging from 715 to 732 micra in diameter.

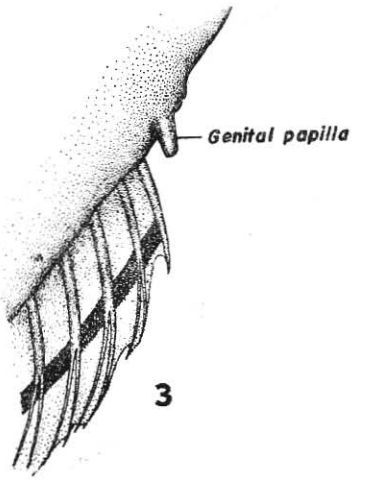
The limit of 200 micra is thus fixed to separate the immature from the maturing eggs. Any increase in size beyond this point is regarded as an indication of the beginning of growth toward maturity. The size at first maturity was determined from the data collected during March 1947 to June 1947 (Fig. 2). The percentage of females maturing in each length unit is given in Table 1. For these calculations, data at the onset and the close of the spawning season have been omitted because of the rather irregular and sporadic cases of spawning taking place before and after the maximum spawning period. Thus the data computed is from the heaviest spawning time.

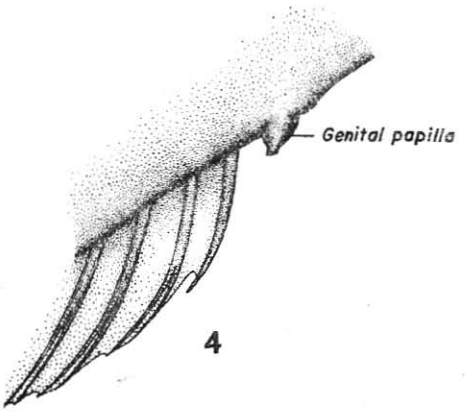
From these data it is concluded that all females smaller than 29.0 mm. in length are immature and that 23 per cent of the fish are found maturing in the 29.0 mm. group ; approximately 39 per cent in the 30.0 mm. group ; 50 per cent in the 31.0 mm. group ; about 62 per cent in the 32.0 mm. group ; about 91 per cent in the 33.0 mm. group and all the females at and above 34.0 mm. are found maturing.

Spawning Season

From the data presented graphically in Fig. 1 it is clear that the peak of the spawning season extends from March to June ; it is chiefly during these months







TEXT-FIG. 2.

Fig. 2. Distribution of different size groups of eggs in each millimeter of standard length. Class designated as '25' includes all measurements recorded as 25.00, 25.25, 25.50 and 25.75 mm.

Fig. 3. The genital papilla and the black pigment band on the anal fin of a male *Clevelandia*

Fig. 4. The female genital papilla.

TABLE 1

Number and percentage of fish maturing in each millimeter of body length. Data collected from March 1947 to June 1947.

Body length in millimeters[1]	Total numbers	Number maturing	Percentage maturing
25.00	9	0	0
26.00	8	0	0
27.00	19	0	0
28.00	13	0	0
29.00	26	6	23.0
30.00	28	11	39.3
31.00	16	8	50.0
32.00	21	13	61.9
33.00	36	33	91.4
34.00	26	26	100.0
35.00	31	31	100.0
36.00	31	31	100.0
37.00	21	21	100.0
38.00	17	17	100.0
39.00	12	12	100.0
40.00	9	9	100.0
41.00	1	1	100.0

[1] Class designated as "25.00" includes all lengths recorded as 25.00, 25.25, 25.50, and 25.75 millimeters.

that mature eggs are found in the ovaries of the females. Apparently, however, some spawning takes place both before and after the maximum period because females with mature or maturing eggs are observed in December, January and February and also in July and August. In Fig. 1 the frequency distribution for August 1946 indicates that there were quite a number of spawners, whereas that of August 1947 shows no individuals containing eggs more than 500 micra in diameter. This discrepancy is believed to be apparent rather than real. Fig. 2 shows that the size of the eggs during the spawning season depends also on the length of the individual fish. It is probable, therefore, that the difference in the relative numbers of mature and immature fish in 1946 and 1947 is due to a difference in the collecting technique—that for 1946 selecting the larger individuals.

An effort was made to locate naturally spawned eggs in the field as an indication of the spawning season but without conclusive results. A few eggs were collected in May 1947 at the height of the spawning season as indicated by the study of ovarian eggs.

The fish kept in the aquarium started spawning in March 1947 ; the first one spawned on 20th March. The second one, which was ready to spawn, was stripped on 8th April and the eggs were artificially fertilized to study the development. A third female spawned on 1st May Occasional plankton tows were made in the Slough with a view to collecting larvae. A group of twenty larvae, measuring 4.75 mm. in total length and 4.55 mm. in standard length, were collected on 7th July 1947. In general appearance these larvae resemble the larvae reared in the laboratory although there is a slight difference in the arrangement of the pigment pattern. This is presumably due to the fact that the ones taken in the plankton tow are of a slightly more advanced stage of development than those reared in the laboratory. Unfortunately it proved to be impossible to rear those taken from the Slough until they can be positively identified ; nor was it possible to rear the larvae that hatched in the laboratory to a stage comparable to those taken in the Slough. Since there is no other goby found commonly in large numbers in the Slough, it seems safe to assume that these larvae are of *Clevelandia*

ios in spite of the minor differences in pigment pattern, which, as already mentioned, may very well be due to the difference in the stage of development.

On 9th March 1948 five larvae of *C. ios*, ranging in size from 4.25 to 4.50 mm. in length were collected from the Slough with the aid of a plankton net.

From these results it is concluded that *Clevelandia ios* has a protracted spawning season, extending over a period of possibly nine months, but with the heaviest spawning taking place from March to June. Isolated cases of spawning apparently occur as early as December and as late as July and August. There is only a short period, at the most three months, September, October and November when there is no spawning.

Clevelandia lays from 750 to 1,000 eggs at a time. The number of ripe ovarian ova, as shown by actual counts, varies from 800 to 1,200. The size of the ovaries, as well as the number of eggs they contain, varies with the size of the fish. The largest mature ovaries examined (15.0 mm. long and 5.25 mm. wide) were from a fish 50.5 mm. in standard length and contained 1,200 mature ova.

An examination of the ripe ovary reveals eggs of three different sizes (Pl. III, Fig. 2). The largest eggs range in size from 715 to 732 micra while the intermediate group measures between 200 and 250 micra and the smallest from 30 to 95 micra in diameter. Many other species of fish show more than one distinct size of eggs in the ovary (Calderwood, 1892 ; Thompson, 1914 and Clark, 1925 and 1934). These different classes of eggs may indicate a multiplicity of spawning as demonstrated by Clark (1925 and 1934) in *Leuresthes tenuis* and *Sardinops caerulea* respectively or the intermediate and the small eggs may be resorbed after the spawning season as happens in many species of fish. An examination of the cross section of a spent ovary of *Clevelandia* soon after spawning does not show any indication of degeneration, whereas that of a later stage shows signs of disintegration indicating thereby that the unspawned ripe eggs degenerate and are resorbed. The group of intermediate eggs also seems to degenerate while the group of smallest eggs seems to remain unchanged (Pl. III, Fig. 3). This does not mean that the possibility of a multiplicity of spawning in *Clevelandia* may be completely excluded.

Breeding Habits and Sexual Dimorphism

MacGinitie (1935, p. 748) remarks, "*Clevelandia* lays eggs in the spring, fifteen to twenty-five eggs at a laying. The eggs, which are laid singly, are allowed to settle to the bottom, where they adhere to the sand. The eggs are 735μ long and 570μ wide ; the yolk is 645μ long and the same width as the whole egg." This is all that has previously been known about the breeding habits and eggs of this fish. Great difficulty was experienced in making observations of their breeding habits in the field and hence the following observations were made on specimens kept in the aquarium.

The females during the breeding season display slight changes in their colour pattern. This is especially marked just a few days prior to the spawning time. This change is purely transitory and disappears soon after spawning. The mature female, ready to spawn, can be easily recognized by its greatly distended abdomen (due to the enormous increase in the size of the eggs) and a bright yellow colour, caused by the underlying eggs, is visible through the distended abdominal wall. The fish becomes sluggish and rather inactive. In some of the mature females it is observed that a streak of black pigment developes on the anal fin. In addition to this there is a great increase in the melanophores on the two dorsal fins and a considerable increase in the number of xanthophores all over the body.

In the males there is as much colour change during the breeding season as in the females. A black streak appears on the anal fin of all mature males. This fades out to a faint streak after milting. Yellow pigments develop chiefly on the ventral surface and two streaks of melanophores appear on the lower jaw. The

melanophores on the dorsal fins increase in number and the upper half of the pectoral shows black pigmentation. Contrary to the behaviour of the females, the males are quite active during the spawning time. There is no distension of the abdominal wall in the males as the testes are extremely small, measuring about 3.0 mm. in length and 0.75 mm. in breadth.

Parental care, in various forms, is a trait often observed amongst gobies and the males or the females, or both, protect the eggs during the period of incubation. *Clevelandia* shows no sign of parental care. This is probably explained by the fact that it lays eggs over a considerable area, either singly or in small groups,[1] whereas those forms which provide parental care deposit their eggs at one place and are generally found attached to some foreign material, such as empty molluscan shells, pieces of wood, etc.

Many species of gobies exhibit marked sexual dimorphism and characters associated with sex have been mistakenly considered as specific. *Clevelandia* does not exhibit striking sexual dimorphism and there are no easily noticeable secondary sexual characters. As already pointed out, mature females can be distinguished from males by the nature of their belly, considerably distended and yellowish in the females. The dark band of pigments on the anal fin of the male is not always a reliable character since it is observed in some of the females, too. However, as in many other gobies, the shape of the genital papilla is a character that can be used with confidence to separate the sexes. In both sexes the papilla is minute and can be differentiated only with the help of a low-power microscope. In the males the genital papilla is straight and tubular (Fig. 3) whereas in the females it is a fleshy bulbous tubercle with a short spout-like opening (Fig. 4). This character, even though readily separates the sexes in the larger fish, cannot be used for those of about 19.0 mm. or less in standard length, in which the difference is not generally marked.

There is no significant difference in standard length between the two sexes. This was determined by a study of the larger fish, above 19.0 mm. in standard length, taken in two collections. A sample collected in October 1946 contained 258 males and 350 females greater than 19.0 mm. in length. Another taken in November 1946 had 273 males and 424 females. The sexing was done by the examination of the genital papilla and in several of the smaller fish this was checked by the examination of the gonad. In the October sample the arithmetic mean (with its standard error) for the males is 26.607 ± 0.191 mm. and for the females 26.677 ± 0.175 mm. In the November sample the corresponding figures for the males are 25.673 ± 0.236 mm. and for the females 25.945 ± 0.207 mm. The *t*-test was applied to each sample after calculating the standard error of the difference between the means. In both samples the difference in the standard length of the two sexes is not statistically significant, $P > 0.05$.

Other characters such as the length of head, length of maxillary, length and width of snout were also studied for possible differences. For these studies a total of fifty specimens were measured from each sex, their standard length ranging from 20.0 mm. to 40.0 mm. Scatter diagrams were prepared for these—the respective characters plotted against the standard length (Figs. 5, 6, 7 and 8). Both males and females show a very high positive correlation in all these characters and except in one, the length of head, the differences between the correlation coefficients are not statistically significant (Table 2). Even the significance of the difference in

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[1] In a personal communication from Dr. Bolin on 17th January 1955, he writes that zoology students from the University of California "found goby eggs, undoubtedly those of *Clevelandia*, attached to the mucus-cemented sand just inside the mouth of the burrow of *Urechis*. I have looked for more on the couple of occasions that I have taken my class over to Elkhorn Slough but have had no luck."

the correlation coefficients of the length of head is rather doubtful as P is greater than 0.02. These tests were carried out by the z-test of R. A. Fisher as given by Simpson and Roe (1939, p. 242).

TABLE 2

Data for the test of significance of the difference between the correlation coefficients

Character studied	♂		♀		σd_z	t	P
	r	z	r	z			
Length of head	+0.973	2.15	+0.989	2.60	0.206	2.18	>0.02
Length of maxillary	+0.993	2.80	+0.989	2.60	0.206	0.97	>0.05
Length of snout	+0.966	1.82	+0.949	2.03	0.206	1.01	>0.05
Width of snout	+0.965	2.01	+0.973	2.15	0.206	0.68	>0.05

In an attempt to ascertain whether any of the characters considered above show sexual dimorphism the significance of the difference between the two regression coefficients for each character of the two sex groups was tested adopting the method given in Simpson and Roe (1939, p. 274).

The given values of P (Table 3) indicate that the differences in the regression coefficients of length of head and length of maxillary are significant whereas those of the length of snout and its width are not. This means that in *Clevelandia ios*, within the range of size studied, length of head and maxillary of the males for a given standard length are significantly greater than those of the females (Figs. 5 and 6). The length of snout and width of snout, on the contrary, are not significantly different (Figs. 7 and 8).

TABLE 3

Data for the test of significance of the characters compared in the males and females of Clevelandia ios.

Character studied	♂ r	♀ r	♂ $b_{y \cdot x}$	♀ $b_{y \cdot x}$	σd_b	t	P
Length of head	+0.973	+0.989	0.296	0.250	0.011	4.22	<0.01
Length of maxillary	+0.993	+0.989	0.227	0.195	0.006	5.32	<0.01
Length of snout	+0.966	+0.949	0.083	0.093	0.005	1.76	>0.05
Width of snout	+0.965	+0.973	0.172	0.158	0.009	1.62	>0.05

EMBRYONIC DEVELOPMENT AND LARVAL STAGES

The development of *Clevelandia ios* up to the tenth day after hatching was followed in the eggs spawned artificially on 8th April 1947. The eggs were stripped into a clean finger bowl which had been rinsed with fresh sea water. Owing to

there is no visible change in the size or shape of the yolk-mass except that at the top it is slightly flattened (Fig. 13). The oil globules remain the same.

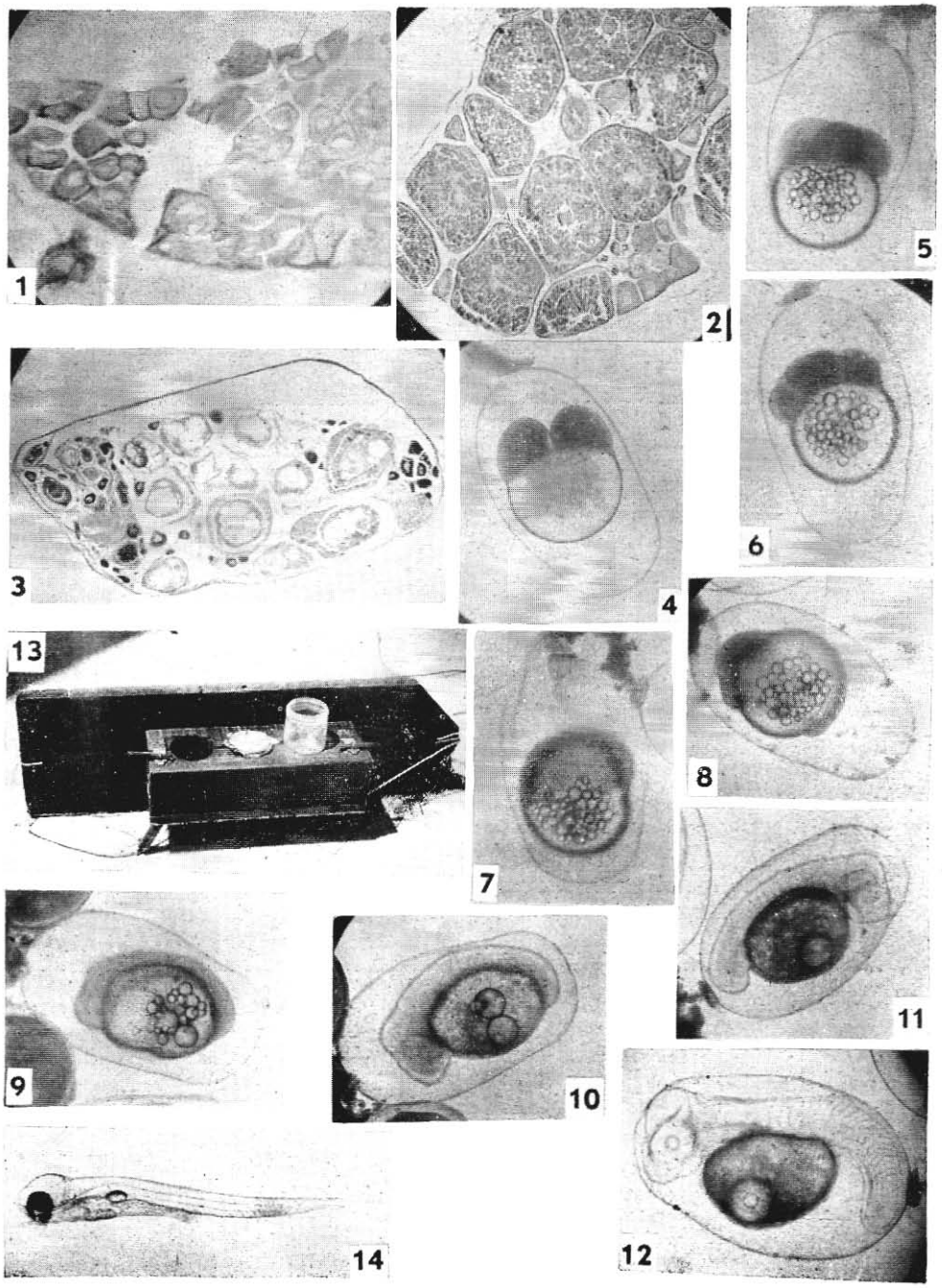
Segmentation : The first sign of cleavage starts at approximately two and half hours after fertilization. When cleavage starts, in most cases, the yolk with the blastodisc migrates gradually to the distal end of the egg capsule (away from the attachment). In a few of the eggs this process may start a little later and consequently the yolk with the dividing blastodisc reaches the narrow end of the egg-case only when it is in the eight- or sixteen-cell stage. The oil globules show a tendency to accumulate under the blastomeres. By the end of three hours the first cleavage is completed resulting in two huge blastomeres (Fig. 14 and Pl. III, Fig. 4). The first cleavage plane is meridional and divides the blastodisc into two blastomeres equal in size and which, when viewed from above, appear almost circular in outline. This results in the axis of the blastoderm being elongated at right angles to the cleavage plane. The subsequent cleavages take place at hourly intervals. The second cleavage which is also meridional and at right angles to the first, results in four equal blastomeres standing out as more or less isolated rounded elevations (Fig. 15 and Pl. III, Fig. 5). The four blastomeres are still large and as a result of the second cleavage the axis of the blastoderm is restored to symmetry.

The third cleavage, resulting in two parallel rows of four symmetrical blastomeres, takes place on each side of, and parallel to, the first plane of cleavage. At the end of this cleavage the axis of the blastoderm is once again lengthened and a marked diminution in the size of the individual blastomeres is observed (Fig. 16 and Pl. III, Fig. 6).

The result of the fourth cleavage, resulting in sixteen cells, is that the blastoderm once again becomes almost circular in outline. As cleavages advance not only is the size of the individual blastomeres reduced but they exhibit a great degree of irregularity in size, shape and position. With the fifth cleavage and the formation of thirty-two cells the blastoderm becomes two cells in thickness and from here on it is practically impossible to follow the course of cleavage in the living material. The size of the yolk-mass is reduced gradually with the onset of further cleavage.

After nine hours the blastoderm has assumed the form of a thick dome-shaped cap with sharply defined edges but still the individual cells can be made out under the microscope (Fig. 17). As development proceeds it becomes more and more difficult to distinguish the individual cells as they become progressively smaller until finally at the end of about twenty-one and half hours the blastoderm somewhat resembles in appearance the stage just before the first cleavage took place except that the dome-shaped blastoderm has already begun to lose its steepness and to thin out as it spreads around the yolk (Fig. 18).

Segmentation cavity : The segmentation cavity appears soon after the blastoderm has assumed the stage when the individual cells are indistinguishable, about twenty-two hours after fertilization. It first becomes visible as a narrow central area beneath the blastoderm and in about twenty-four hours the cavity is well represented (Fig. 19). In the living eggs of *Clevelandia ios* it is impossible to make out the periblast. According to Kuntz (1916) the periblast of the eggs of *Gobiosoma bosci* appears relatively thick, but cannot be satisfactorily observed in the living material by reason of the opacity of the yolk. In the early stages of the formation of the segmentation cavity the solid cellular wall is of uniform thickness. As growth proceeds the roof of this cavity becomes thinner, while the cavity itself becomes larger, but narrower and spreads more over the yolk-mass. The margin of the blastoderm grows down around the yolk-mass. Due to this growth the yolk-mass, covered over by the blastoderm, slightly elongates (Fig. 20 and Pl. III, Fig. 7). As a result of the centripetal migration of the peripheral cells of the blastoderm, a thickened cellular rim is formed around the edge of the blastoderm.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This is the germ ring which, until the closure of the yolk blastopore, marks the advance of the blastoderm. The yolk-mass at the level of the germ ring is slightly constricted.

This growth by the proliferation of the marginal cells continues uniformly around the yolk until the blastoderm covers almost three quarters of the surface of the yolk-mass. But with the appearance of the germ ring the proliferation of the peripheral cells, which resulted in the formation of the germ ring, is much more rapid at one pole than around the rest of the margin of the blastoderm. This place denotes the posterior pole of the future embryo. An extremely rapid proliferation of cells occurs here resulting in the formation of a broad tongue which grows forward into the segmentation cavity. This is the embryonic shield. Along the median line of the embryonic shield a thickening soon appears (Fig. 21) which represents the axis of the future embryo and this comes to lie parallel to the major axis of the egg-capsule. The thickening referred to above soon grows forward (Fig. 22 and Pl. III, Fig. 8) and projects into the perivitelline space. It produces a deep V-shaped groove in the yolk, in which the early embryo lies cradled.

The yolk blastopore remains open until forty-one hours after fertilization but in another hour it is completely closed. At about the same time the blastopore is closed, the embryo extends about three quarters of the way around the remaining yolk-mass and the V-shaped groove has deepened considerably. Kupffer's vesicle has put in its appearance and is seen embedded in the yolk at the posterior end of the embryo (Fig. 23).

The formation of the optic vesicle and the appearance of the first pair of mesoderm somites occur almost simultaneously at about forty-six hours. The first pair of somites appear almost near the middle of the embryo (Figs. 24, 25 and Pl. III, Fig. 9). Along with these changes the oil globules gradually decrease in number but at the same time there is a tendency for those remaining to become larger. This is brought about by the fusion of two or more oil globules. By now the yolk-mass presents a granular appearance.

About fifty-nine hours after fertilization the embryo has developed considerably. The optic cup, well developed and with a prominent choroid fissure, has increased in size. The auditory capsules have made their appearance as a pair of small oval vesicles posterior to the eyes. The notochord can be seen and there are ten fully formed somites. Kupffer's vesicle is still persistent but has been reduced in size (Fig. 26 and Pl. III, Fig. 10). Very soon the tail of the embryo shows indications of getting detached from the underlying yolk-mass. This is very well seen in Pl. III, Fig. 11. The quantity of yolk has been reduced considerably and the yolk appears to be more opaque. The three primary divisions of the brain can be made out. The number of oil globules is reduced to two or three. In most cases the embryo lies with the head toward the free end of the egg-capsule but a few are found to be the other way about.

About sixty-three hours after fertilization Kupffer's vesicle has completely disappeared and shortly after this the tail becomes free from the yolk. The eyes are now provided with lens. In another six hours the pericardial cavity appears. Growth takes place rapidly at the tail region and there are fourteen fully formed somites.

The embryo has completely filled the egg-capsule by about seventy-one hours. The tail is still blunt and bent downwards. On the ventral side of the embryo where the tail bends, a pit is seen which is the anal pit. A few cells can be made out in the pericardial cavity and soon indications of the formation of the heart are seen. At about the same time the olfactory capsules appear as a pair of horse-shoe-shaped depressions below the eyes. In another six hours the tail, still bent downwards, has grown down further and the tip is in level with the tip of the head. The head has slightly increased in size and some of the lobes of the brain can be made out. The heart is seen as a simple tube containing a few corpuscles. The

otoliths are clearly visible, so is the posterior part of the alimentary canal (Fig. 27 and Pl. I, Fig. 12).

About eighty hours after fertilization the heart is seen pulsating slowly. There remains a single oil globule in the yolk.

The growth proceeds rapidly and by the fifth day the embryo has begun to move in the egg-case. It rotates on its longitudinal axis ; twitches its tail, and exhibits wriggling movements. The yolk has become practically spherical. As the embryo lengthens, the tail becomes more active and moves constantly. It is kept bent either to the right or to the left side of the embryo. The fin fold develops as a continuous fold running from the nape around the tail to the posterior margin of the yolk-mass (Fig. 28).

Pigmentation in the eyes appears very early on the sixth day and the eyes become increasingly darker as development progresses. About six hours after pigmentation first appears in the eyes, melanophores can be observed developing along the dorsal and ventral sides of the posterior half of the body. They appear as small black dots along the region where the fin fold and the body meet. By now the tip of the tail (which is bent forward) extends as far as the middle of the eye and the rudiments of the pectoral fins are visible. The fin fold has become slightly wider (Fig. 29).

The stomodaeum becomes apparent by the seventh day. In the embryo of the eighth day the eyes have the silvery sheen. Four stellate melanophores appear on the yolk-mass. The quantity of yolk has been considerably reduced and the yolk-mass is about 455 micra in diameter. There is the first indication of the air bladder which appears just above the yolk-mass at the level of the pectoral fin. The embryo exhibits occasional violent jerking movements.

On the ninth day the pectoral fins are well developed and the embryo has started using them actively. Pigmentation has increased on the yolk-mass. The air bladder is very well seen and pigments have developed on them. The movements of the embryo are more vigorous. By the end of the ninth day the melanophores have become larger. The embryo has by now a well developed mouth.

As the tenth day approaches the embryo becomes more and more active. The yolk-mass is reduced to about 325 micra in diameter and there is still one oil

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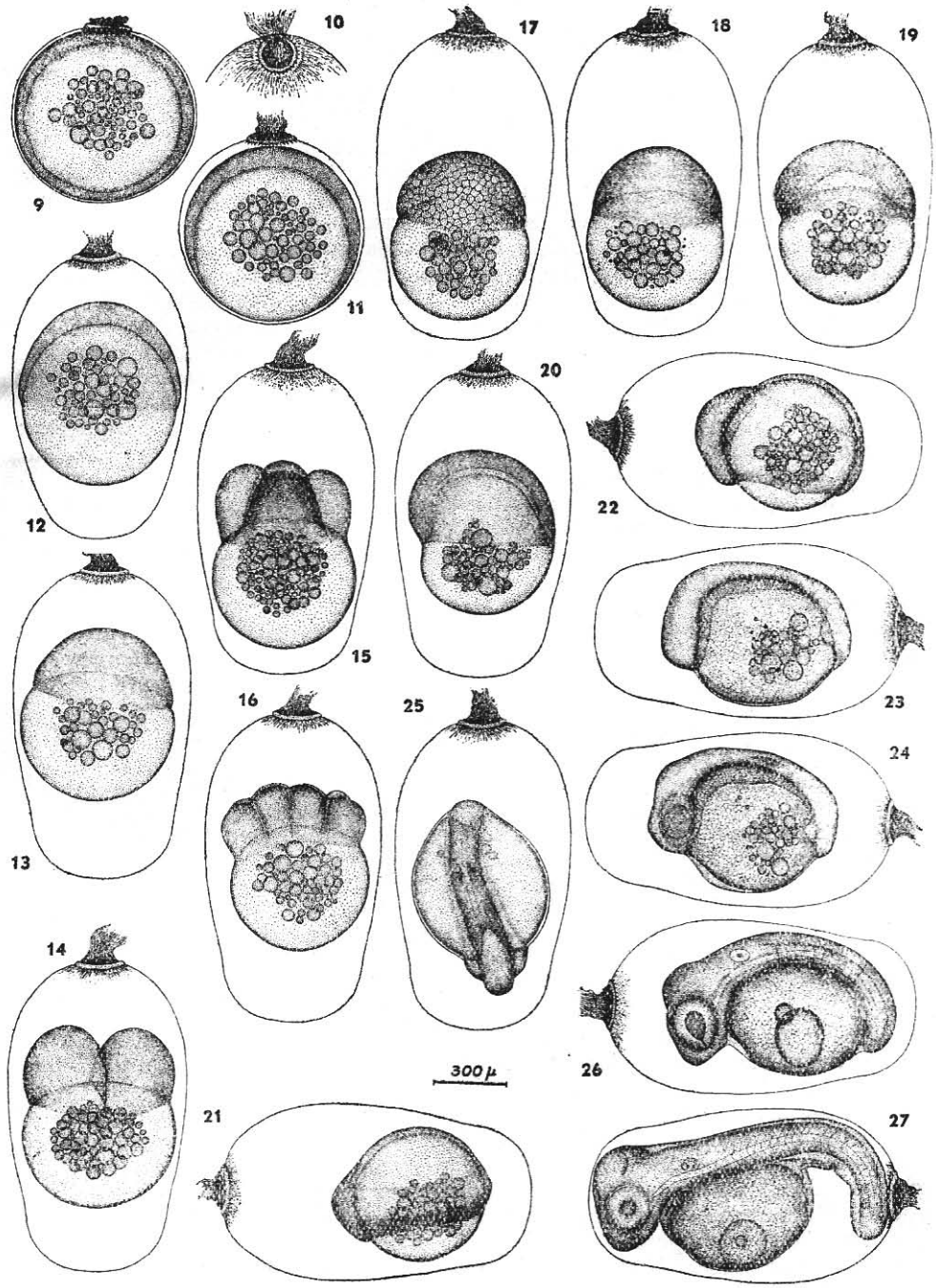
TEXT–FIG. 4.

Fig. 9. An unfertilized egg after the formation ov the adhesive threads.
Fig. 10. The circlet of adhesive threads at the base of the egg-capsule.
Fig. 11. An egg five minutes after fertilization showing the change in the distribution of the protoplasm.
Fig. 12. An egg showing the elongated egg-capsule and the increased perivitelline space.
Fig. 13. Egg with fully developed blastodisc.
Fig. 14. Egg showing two blastomeres.
Fig. 15. Egg with a blastoderm of four cells.
Fig. 16. Egg in the eight cell stage.
Fig. 17. Egg with a many celled blastoderm.
Fig. 18. Egg showing the dome-shaped blastoderm resembling the blastodisc of Fig. 13.
Fig. 19. Egg showing the segmentation cavity.
Fig. 20. Egg with increased segmentation cavity and slightly elongated yolk-mass.
Fig. 21. Egg showing the embryonic axis.
Fig. 22. Egg showing an advanced stage in the differentiation of the embryonic axis.
Fig. 23. Egg showing embryo with Kupffer's vesicle.
Fig. 24. Embryo with well developed optic vesicle and a pair of mesoderm somties.
Fig. 25. Dorsal view of the embryo shown in Fig. 24 showing the V-shaped groove in which the embryo lies.
Fig. 26. Egg with an advanced embryo showing well developed optic cup with choroid fissure, auditory vesicles, notochord and mesoderm somites. Notice the reduction in the size of Kuffer's vesicle.
Fig. 27. The embryo showing olfactory capsule, tubular heart, anal pit and posterior part of the alimentary canal.



TEXT-FIG. 4.

globule persisting ; but it has become much smaller. Along the dorsal side can be seen three melanophores, the last one being the largest. On the ventral side are two rows of melanophores, one along the edge of the body, composed of about eleven stellate melanophores and the other on the lower part of the alimentary canal, composed of about six small pigment spots. On the yolk can be made out about seven melanophores. The arrangement and number of melanophores seem to be fairly constant for the species. Associated with the fourth and eighth melanophores on the ventral side are two xanthophores. A series of xanthophores extend from the region of the mid brain up to the last melanophore on the dorsal side. But these xanthophores seem to be transitory. On careful examination corpuscles can be seen moving in the blood vessels but they are still colourless. On the evening of the tenth day, 228 hours after fertilization, the first larva hatched. A series of jerking, wriggling and lashing movements of the embryo rupture the egg membrane. The tail comes out first in most cases and gradually the larva wriggles out of the egg-case. The time of hatching varies considerably. The first one hatched at 9 p.m. on the 17th April and the last one hatched on the 19th at 11 a.m.

Larvae : At temperatures varying from 15° to 15.5°C the incubation period extends from ten to twelve days. This seems to be in agreement with the incubation period of many other species of gobies, both American and European. For the blind goby, *Typhlogobius californiensis*, the incubation period is ten to twelve days at temperatures ranging from 17° to 20°C (MacGinitie, 1939). Weisel (1947) assumes that at about 18°C the period of development prior to hatching for *Gillichthys mirabilis* is ten to twelve days. For those European species such as *Gobius microps*, *G. minutus* and *G. pictus*, Shann (1910) mentions that the period of incubation is about fourteen days. But according to Kuntz (1916) the incubation period of *Gobiosoma bosci*, an American species, at "laboratory temperature" is approximately five days whereas for *Ctenogobius stigmaticus* the period of incubation is not over eighteen hours.

The newly hatched larvae of *Clevelandia ios* (Figs. 30 and 31) are pelagic. They vary in size from 2.75 mm. to 3.25 mm. in total length. They are slender, delicate and transparent. The head is rounded with well-developed mouth which is horizontal and inferior. The eyes are very prominent. The auditory vesicles and the notochord are clearly seen. The yolk-sac, which is almost round, ranges in diameter from 260 to 325 micra. The oil globule is still present even though reduced in size. The fin fold is rather broad and on the dorsal side it starts from opposite the middle of the yolk-sac and is continuous with the ventral fin fold which stops at the vent. From the vent there is another short fold extending up to the posterior margin of the yolk-sac. The tail is rounded. The vent is situated almost at the middle of the body, being slightly closer to the tip of the tail.

The larvae exhibit a characteristic pattern of pigmentation that is common to many of the larvae of gobies. The xanthophores which were present on the head region have disappeared. All the melanophores which were described in the previous stage are still present. On the dorsal side are three melanophores, the last one being the largest. With it is associated a xanthophore. On the ventral side are eleven melanophores and associated with the fourth and eighth are xanthophores. Along the ventral side of the alimentary canal, at the place of junction of the fin fold and the alimentary canal are distributed six melanophores and usually seven or eight melanophores can be seen on each side of the yolk-sac. The upper part of the air bladder is covered with melanophores and a few xanthophores thus presenting the characteristic crescent shaped black area on the upper half. The larvae are exceedingly active and are positively phototropic.

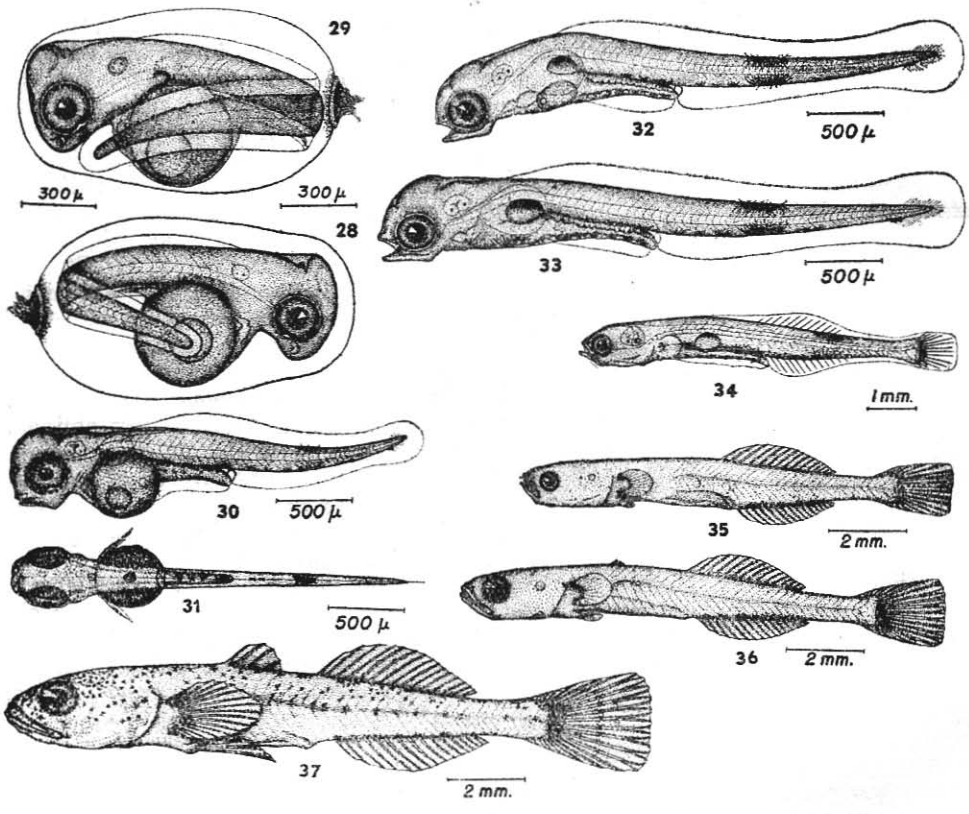
This detailed description of the larvae is made, in part, to facilitate the identification of the larvae of *Clevelandia ios* that may be taken in nature in future studies.

The larvae survived in the laboratory for ten days. Different methods were tried to keep them alive longer but all attempts failed. They were kept at a fairly constant temperature ranging from 15° to 15.5°C. The water was changed every twenty-four hours. In one lot the water in the bowl was kept gently stirred with the aid of a plunger, while the other lot was left undisturbed. The result was the same in both cases. Different types of food, such as, algal spores, nauplii, sea urchin blastulae and diatoms of different species were provided. Occasionally plankton hauls were made and the various small organisms present in it were also given as food. An examination of these larvae showed that they were not feeding. Their alimentary tracts were completely empty except for one specimen. In another attempt to rear the larvae, six of them were left in a bottle covered with fine bolting silk, which permitted small organisms to get in but at thes ame time prevented the larvae from escaping. This was left floating on the surface of the sea with the help of a wooden raft. The complete equipment is shown in Pl. III, Fig. 13. This was left approximately 200 yards from the shore. The larvae were periodically examined. Although this method provided the larvae, all the time, with fresh sea water and presumably enough food, all of them died within a period of nine to eleven days. Owing to many difficulties in keeping such equipment in Elkhorn Slough no attempt was made at rearing the larvae in the Slough although it might have proved more successful. Two stages in the growth of the larvae are described below. The larva shown in Fig. 32 is six days after hatching and measured 3.5 mm. in total length. It has undergone only slight changes from the newly hatched larva. The general shape of the head has changed and has become more pointed. The position of the mouth shifted slightly to terminal and more or less oblique. The quantity of yolk has been reduced considerably. The oil globule, even though still persistent, is extremely small. Owing to the more rapid growth of the tail region the vent is much nearer to the head than to the tip of the tail. The tail is still rounded and the continuous fin fold remains broad. Of the three melanophores on the dorsal side, two of the smaller ones have disappeared.

Figure 33 and Pl.III, Fig. 14 show a larva measuring 3.9 mm in total length. No great advance over the preceding stage is shown. The larva is ten days old at which age all of them died. The yolk is completely absorbed. The air bladder has slightly increased in size and so also have the pectoral fins, which the larva uses quite actively. During this period the larva increased approximately 1.2 mm. in total length.

The next developmental stage obtained was post larval, averaging about 7.00 mm. in standard length and 7.75 mm. in total length (Fig. 34). Three of these were collected from Elkhorn Slough, with the aid of a plankton net, on 7th July 1947. The larvae are still transparent. The mouth has become quite oblique and the skeletal elements are fairly well developed. The fish are still transparent enough so that the vertebral column can be seen and thirty-five vertebrae can be counted. Through the body wall the air bladder is still perceptible. All fins except the first dorsal and the ventral fins are well developed. The rudiments of the ventral fins show as light thickenings on the ventral surface immediately below the base of the pectorals. The spinous first dorsal fin is not yet formed. The caudal fin has become truncated and has a straight posterior margin. Twelve rays can be counted in the second dorsal fin, twelve also in the anal fin and fourteen in the caudal fin. The hypural bone and the urostyle can be clearly made out. The membraneous fold of skin on the ventral side in front of the anus has begun to decrease in width. The dorsal fin fold is still continuous with the posterior half of the ventral fin fold, but at certain places it has begun to decrease in width. Yellow pigments are found in association with melanophores especially with the one on the dorsal side and the ventral group behind the anus. Even though the pigment pattern remains basically the same, slight alterations have taken place. The individual melanophores on the ventral side behind the anus have fused to

form a streak whereas those below the alimentary tract still remain separated. There has appeared a pigment spot on the chin below the middle of the eye and the pigments on the caudal fin are clearly seen. The auditory vesicles are still visible from the outside and it is interesting to note that the posterior otolith has grown to be larger than the anterior one. The same condition has been described in the post larval stage of *Chasmichthys gulosus*, measuring 6.7 mm. in length, by Nakamura (1936).



TEXT FIG. 5

Fig. 28. A well developed embryo showing the fin fold.
Fig. 29. An advanced embryo with the rudiment of the pectoral fin and melanophores in the tail region.
Fig. 30. A newly hatched larva.
Fig. 31. The dorsal view of the newly hatched larva.
Fig. 32. A larva six days after hatching.
Fig. 33. A ten day old larva.
Fig. 34. A post larva 7.0 mm. in standard length.
Fig. 35. A post larva 9.75 mm. in standard length.
Fig. 36. A specimen 10.75 mm. showing the development of the first dorsal fin and the two separate ventral fins.
Fig. 37. A juvenile 14.00 mm. in standard length showing all the essential characters of the adult.

It is not long after this before the adult characters are established. A specimen 9.75 mm. in standard length is shown in Fig. 35. The body is still more or less transparent and such internal structure as the vertebral column and the air

bladder are still clearly visible as in the previous stage. The fin fold which was persistent and continuous in the earlier stage is no longer continuous. The first dorsal fin has not yet developed. It is interesting to note that the fin fold at the place where the first dorsal fin is to appear, is absorbed completely, while at the same time it persists caudad and all the three fins viz., the second dorsal, caudal and anal, are developed from it. The same has been observed in *Chasmichthys dolichognathus* by Nakamura (1936). The fin-rays in the three fins just mentioned are quite definite. The pectoral fins show indications of the development of rays. The ventral fins, however, are still in the form of two buds. The auditory vesicle is no longer clearly visible from the outside.

The pattern of distribution of pigments has changed. There are no pigments on the dorsal margin of the body. Along the ventral side there are two black pigment spots in the head region, about nine from the base of the operculum to anus and about six along the base of the anal fin.

Fig. 36 represents a fish measuring 10.75 mm. in standard length. The important changes from the previous stage are, (1) the appearance of the spinous dorsal fin which has only three spines at this stage. (2) the ventral fins have developed further and are visible as two separate fins in close proximity, (3) the eyes have moved slightly dorsally. The remaining characters are almost the same as in the previous stage except that between the ventral fins and the anus there are fewer melanophores.

All of the essential adult external characters are developed in a juvenile measuring 14.0 mm. in standard length (Fig. 37). The fins are fully developed with the characteristic number of fin-rays. The ventral fins have fused to form a single fin. The distribution of the integumentary papillae has assumed more or less the typical form. The eyes have practically moved to the position taken in the adults. Thus, at this stage the species can be identified with certainty and, in appearance, the juveniles resemble the adults except that they are less heavily pigmented.

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