On the Buoyancy of the Egg of Alaska Pollack, *Theragra chalcogramma**

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The eggs of some kinds of fishes are floating about after the spawning. They are called pelagic eggs. Hitherto little attention has been paid to the nature and mechanism of such phenomenon.

While studying the problems concerning the osmotic regulation of fish egg, Krogh et al ('38) have observed the eggs of plaice floating at first in the water of 25 or 34 ‰ salinity but beginning to sink after a while (1 1/2−3 minutes later). As will be discussed later, there still remain insufficiencies in their explanation for this behaviour. The egg of cod (*Gadus macrocephalus*) which is closely allied to the Alaska pollack, was reported by some authors to be pelagic; while by others to be demersal. However, Tamura ('52) is of opinion that the egg with a diameter larger than 1.35 mm may be floating because the specific gravity of such egg may become smaller than that of the ordinary sea water.

The nature of the egg of Alaska pollack (*Theragra chalcogramma*) has been already recognized as pelagic since Kamiya '24. Recently Yusa ('52) has found, while studying normal development, that the specific gravity of the egg is smaller than that of sea water and still decreases gradually in the course of the development, and thus the fry hatches out in the floating state. On the other hand, for the practical purpose of the propagation of this important market fish in our country, artificial fertilization has been made and the people have often believed that the eggs which have not been fertilized sink in sea water immediately after insemination, while the floating ones are those successfully fertilized. If so, why does the egg float when fertilized? Since there has been no experimental datum nor any exact observation, the matter has been left as an unsolved problem.

In the present study the behaviour and the morphological changes both of unfertilized and fertilized eggs of Alaska pollack in sea water were examined. The specific gravity of the egg was also measured. It will be shown later that when the egg attains to maturity its specific gravity decreases and it floats in sea water whether fertilized or not. Then if the egg remains unfertilized, it begins to sink after a while. Taking into consideration the morphological changes which occur in this case, it is highly probable that the unfertilized egg decreases gradually in regard to its vital activity, losing the osmoregulating capacity against the surrounding sea water. Consequently exosmosis of

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a light substance, namely water, ensues gradually, resulting in the increasing of the specific gravity of the egg, though the egg is activated without insemination (in other words, it is activated parthenogenetically) merely on contact with sea water and behaves for a while as the fertilized egg does.

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II

The study was carried out in winter, during January and February, eggs taken from Alaska pollack (*Theragra chalcogramma*) in the breeding season being employed. The eggs were placed in a small syringe tube (2--5 cc) containing sea water with known specific gravity determined previously by ordinary pycnometer, and then by noting whether they were heavier or lighter than this sea water, their specific gravity was estimated. Sea water was always used after filtration and by adding distilled water diluted sea waters such as 1/2—1/64 were obtained.

Observations were made in most cases with living eggs. However, for the examination of detailed structure fixed materials were also used. Allen-Bouin's fluid was used as the fixative and sections were made by means of paraffin method. They were stained with Delafield haematoxylin and eosin. In addition, Bauer's and Ciaccio's tests for polysaccharide and lipoid were employed in order to ascertain the nature of the droplets existing in the egg.

III

The mature egg of this species is colourless and very transparent, being spherical in form with about 1.4 mm diameter. A funnel-shaped micropyle is found penetrating the chorion at one pole. There are no distinct oil drops, but many small droplets ranging from 5 to 10 microns in diameter are found in the egg cortex (Figs. 1 & 2). These droplets, according to the observation on sections, are embedded in cortical cytoplasm and not stained with Sudan IV by Ciaccio's method, but show positive reaction to the Bauer's test for polysaccharide. Accordingly, they do not consist of lipoid, but contain polysaccharide. It is supposed therefore that they must not be oil droplets but what are called cortical alveoli, because it is obvious that cortical alveoli in fish egg correspond to the 'goutte claire' named by Konopacka ('35) in her cytochemical investigation of oogenesis in fish egg and said to be composed of mucoproteid.

When such mature eggs were put into sea water of 1.0260 specific gravity (temp. 10° C), from the first they were floating on the surface of the water. Thus, the mature

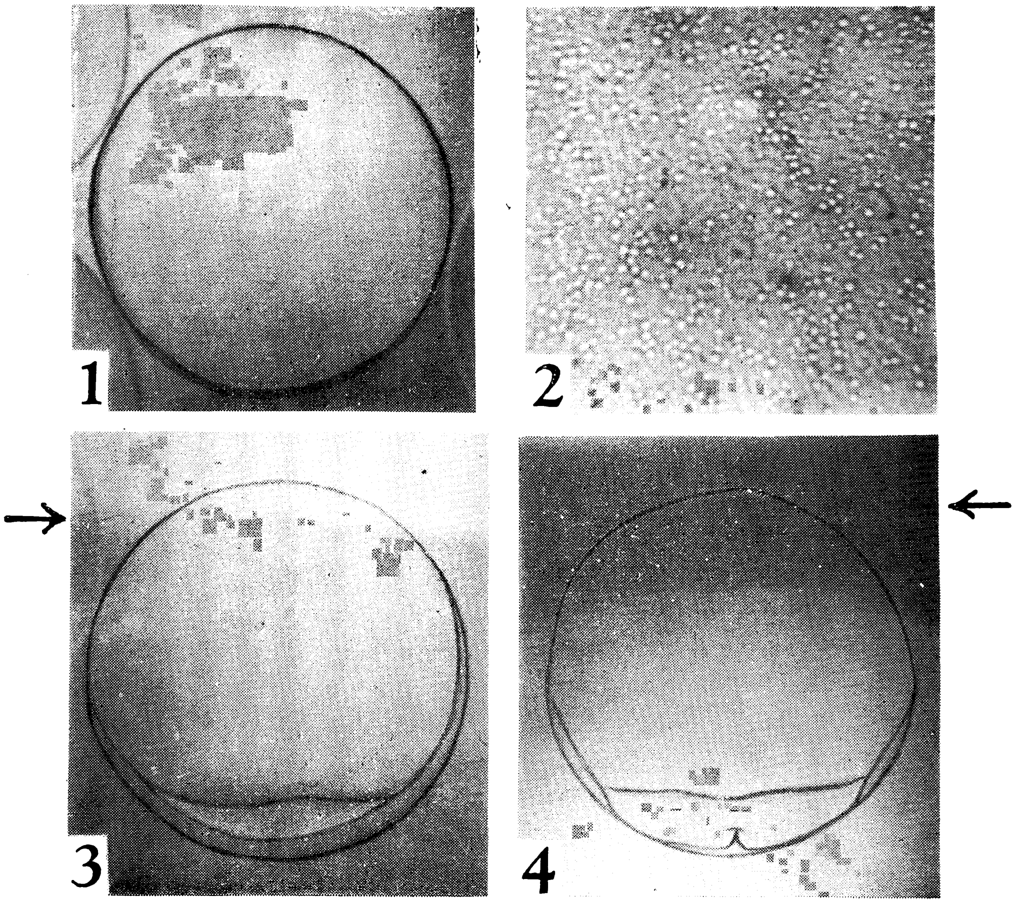

Fig. 1. Intact mature egg. 35 X
Fig. 2. Cortex of unfertilized mature egg. 130 X
Fig. 3. Parthenogenetically activated egg, 90 minutes after immersion in sea water lateral view). Arrow indicates surface of water. 35 X
Fig. 4. 4 cell-stage in floating state in sea water (lateral view). 35 X

egg was found to be lighter than the sea water, that is, its specific gravity was less than 1.0260. In this case its actual specific gravity was estimated as 1.0222. Furthermore, if such eggs were inseminated and fertilized, chorion was thrown off the egg proper progressively, forming the perivitelline space, and then the ooplasm was condensed gradually at one pole to form a raised blastodisc which was continuous at its periphery with the rest cortical plasm. At this stage, the egg was, as a matter of course, floating. The egg proper was biased to the upper pole in the chorion, maintaining the blastodisc in the lower position, and accordingly the perivitelline space was recognized only beneath the egg proper. In this state the development went on passing two-cell, four-cell, eight-cell, morula, blastula stages and so on as in other fish eggs (Fig. 4). Finally fries hatched out also in floating condition. Therefore, it is evident that fertilized eggs are also lighter than the sea water and this state continues till the hatching stage, as Yusa has already pointed out.

Contrary to the foregoing fact it happened occasionally that some eggs sank to the bottom, in spite of their mature appearance, and remained incapable of being fertilized, when inseminated. Observations on the sections prepared from such eggs revealed, however, that there exists a thin unicellular layer outside of the chorion, that is to say, the chorion is covered with a thin layer, the follicle epithelium, which is hardly distinguishable in the living state. This means that they were actually not mature eggs. Consequently, the eggs which sink to the bottom of the sea water and remain unfertilized even after insemination are not eggs incapable of being fertilized in spite of the mature condition, but immature eggs not yet having the capacity of fertilization.

As the actual mature eggs are, whether fertilized or not, lighter than the sea water, they can float. Infertilizable, immature eggs are heavier than the sea water and accordingly sink from the first. Herewith, a question arises how the mature egg behaves in unfertilized condition in the sea water. Then the mature eggs were put into the sea water without insemination and observed. As expected from the results observed already, they were floating at first, but about 2 or 3 hours later some of them began to sink and within about 6 hours all of them had sunk to the bottom (Temp. 9°—11° C). It becomes evident that an unfertilized mature egg is, at first like the fertilized one, lighter than the sea water. However, differing from the fertilized, it becomes heavier with the lapse of time so that it finally sinks if it remains unfertilized. Moreover, morphological changes were observed to occur as follows: When put into the sea water without insemination, the egg (mature egg) exhibits similar morphological changes to those of the fertilized one, in other words, accompanying with the formation of a raised blastodisc, perivitelline space begins to appear. This shows clearly that, merely on contact with the sea water, mature egg is parthenogenetically activated without insemination (Fig. 3). However such activated egg does not undergo cleavage but after a while the raised blastodisc flattens down and disintegrates. Further, it has been found that the sinking of the egg begins with this flattening of the blastodisc. In the sunk egg the perivitelline space is shifted to the upper pole and the egg proper is pushed downwards. The disintegrating appears several hours later. The floating egg has a raised blastodisc but in the sunk one no marked blastodisc exists. Since the flattening of the blastodisc without division is regarded to be in the course of disintegration, the lowering of the vitality in the activated egg may be interpreted as a cause of the sinking of the egg, increasing the specific gravity.

As the causes for the increase of specific gravity, generally speaking, three cases may be considered: viz., (1) penetration of a heavy substance into the egg interior, (2) diffusing out of a light substance from the interior, (3) simultaneous occurrance of (1) and (2). The change of the permeability of the plasma surface of the egg proper*

* Permeability of the chorion is in this case out of the question, for the egg proper rests in contact with the upper surface of the chorion when the egg is floating; on the other hand when it sinks, the egg proper falls downwards to rest on the bottom of the chorion. Furthermore the chorion is in most fish eggs regarded to be freely permeable both to water and salts (Gray '32, T. YAMAMOTO '36, Aoki '39), which view seems to be substantiated in the present material.

is, therefore, to be considered.

As has been already discussed in the previous paper (Kanoh '52) it is supposed nowadays that in unfertilized fish eggs the surface of the egg proper is not an absolutely impermeable membrane, that is, impermeable to electrolytes while permeable to water though to a small degree. After fertilization, however, the permeability to water may decrease more slowly or may be lost. From this the increasing of specific gravity in question is highly probable due to the diffusing out of a light substance, that is, water in this case, from the egg interior. Since mature egg was found to be osmotically equivalent to M/4.5 NaCl solution as measured by Barger's method, the sea water employed is apparently hypertonic to the egg, accordingly diffusing out of water from its interior may take place gradually when the egg is immersed in the sea water. This would not happen in fertilized eggs, in other words the permeability would not remain simple in this case. The fertilized eggs may become resistant against the hypertonicity of the surrounding medium, floating without increasing specific gravity, that is, the specific gravity of the egg may not be changed after fertilzation even in hypertonic medium, namely the sea water. The same may be proved to occur to some extent in the case of activated eggs. When activated merely on contact with the sea water without insemination, eggs (unfertilized) may be also resistant against the hypertonicity as fertilized ones, and float at least at first without increasing the specific gravity. But when they are in the course of disintegration, they lose gradually the resisting capacity, may be, owing to the lowering of vitality, then they, giving off water from interior, begin to sink with increasing specific gravity. The fact that the raised blastodisc of unfertilized but activated eggs flats down and then the eggs sink may support the explanation.

On the other hand, mature eggs were immersed in hypotonic sea water of various concentrations such as 1/4—1/64 sea water and specific gravity was measured 5 hours later. It was found that the specific gravity decreases gradually in proportion to the hypotonicity of the surrounding medium without marked visible changes, for instance, appearance of a space between chorion and the egg-proper or gathering of ooplasm at one pole did not occur. This may be, contrary to the case of hypertonic medium, due to the endosmosis of water, probable because the diluted sea waters used did not induce egg-activation but were unsuitable media (see the next paragraph), consequently the vitality of the egg might become low from the beginning and so the resistance to the surrounding hypotonicity would not be gained.

Therefore, the increasing of specific gravity of unfertilized mature egg in the sea water may be in the first place* due to the diffusing out of a light substance, namely water, from its interior which ensues on the lowering of its vitality.

IV

* In the second place, penetration of heavy substances, probably salts, may happen, because impermeability to electrolytes may be lost sooner or later as the result of progressive disintegration.

As has been described already, the egg of Alaska pollack is considered to be easily parthenogenetically activated when it comes in contact with the sea water. The same phenomena have also been reported in a brackish water minnow, *Fundulus heteroclitus*, by Kagan ('35) and in a flounder, *Limanda schrenki*, by K. Yamamoto ('51); in the case of *Fundulus*, parthenogenetic activation is induced by immersion in sea water, but not in hypotonic water (fresh and distilled water); also, such activation of the *Limanda*-egg is found to be affected by salt concetration of medium in such way that in concentrated solutions above 1/4 b.s.s. (balanced salt solution, osmotically equivalent to M/4 NaCl) the egg is activated. However in more diluted solutions this is not the case.

Accordingly an attempt was made to discover by what concentration of the medium the egg of Alaska pollack is activated. The results show that the sea water induces the activation in any of the eggs tested and even 1/2 sea water can activate a few of them; more diluted sea water and distilled water can not do so and in non-electrolytic solution such as sucrose, independent of its concentration, egg-activation does not occur. Since 1/2 sea water is, roughly speaking, osmotically equivalent to M/4 NaCl solution which is nearly isotonic to the egg, it is concluded that, as in the egg of *Limanda* or probably of *Fundulus*, parthenogenetic activation of the egg of Alaska pollack is affected by salt concentration of medium and its critical concentration is nearly isotonic to the egg interior.

Thereupon, a question may arise whether the development of the egg is also affected by salt concentration of medium. To examine this point, eggs at the blastula stage were put into sea water of various concentrations (from 1/1 to 1/64). It was found that in an hour after putting into each solution no marked changes occur. However, three hours later in 1/1 sea water the eggs develop to the early gastrulae, whereas in 1/2 sea water the development is suppressed; in 1/4 the once raised blastoderm flats down and the perivitelline space shifts to the upper pole; in 1/8 the perivitelline space almost disappears and nearly all of the eggs are cytolyzed, and in 1/16 to 1/64 sea water all the eggs tested are cytolyzed. Therefore it is clear that the development is also affected by salt concentration of the medium and can not proceed in hypotonic sea water. Thus, the isotonic and also hypotonic sea water are supposed to be unsuitable media for the egg of Alaska pollack.

V

As the mature eggs used in this study had specific gravity of 1.0222 (10° C) which is a much smaller value than that of ordinary sea water, from the date given the following general deductions may be drawn (Fig. 5).

Immature eggs which are incapable of being fertilized are heavier than ordinary sea water and so they can not float but sink in it. On the contrary, when they attain to maturity, their specific gravity become smaller than that of ordinary sea water, and consequently they can float at the surface of water. To the intact eggs ordinary sea water is, needless to say, hypertonic but fertilized or developing eggs gain a resisting

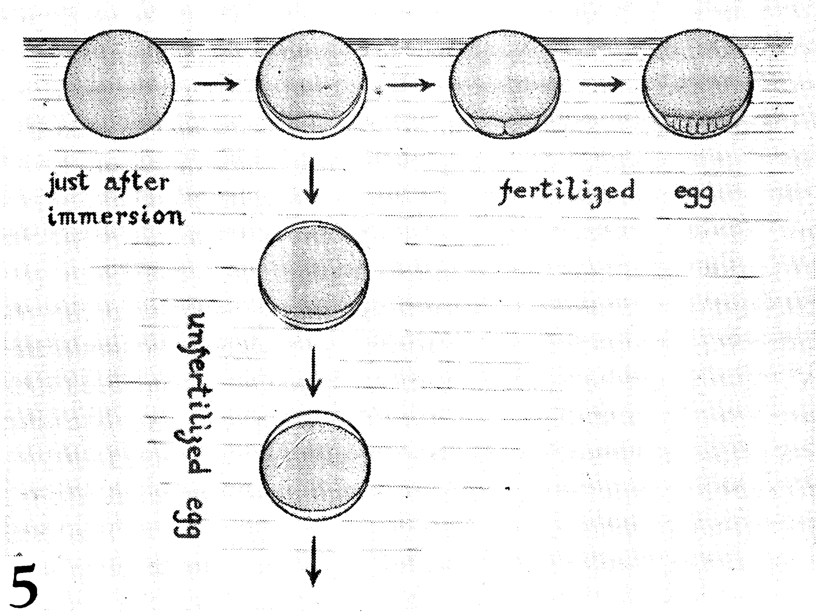

Fig 5. Illustration of behaviour of fertilized and unfertilized egg in sea water. In case of fertilized egg, development proceeds throughout in floating state, on the contrary, unfertilized egg begins to sink after a while with flattening down of the once raised blastodisc, though the egg was once activated to behave as a fertilized egg and was floating for some time at first.

capacity to the surrounding hypertonicity upon fertilization and accordingly they do not lose, as Yusa has reported, floating property at least till the hatching stage.

Thus, the eggs which sink in sea water immediately after discharge must be not yet fully matured eggs, i. e. immature eggs incapable of being fertilized.

Furthermore, unfertilized mature eggs are able to float for some time in sea water since they are parthenogeneitcally activated without insemination when they come in contact with sea water, undergoing the same changes as the fertilized though formed blastodisc does not begin to divide, and they also gain a resistance to hypertonicity of the surrounding sea water. However, with lowering of the vitality they begin afterwards in the course of disintegration to sink as a result of the loss of the resistance to hypertonicity. That is, water diffuses out from the egg interior and the specific gravity increases. Shrinkage of the egg-proper which might happen when water escaped from its interior was not clearly observed in the present study, probably on account of the complicated course of the morphological changes occurring in the process of disintegration.

The fact that the eggs of plaice are floating in water of 25 or 34 ‰ salinity but in such water they become heavier after 1 1/2—3 minutes has been attributed by Krogh et al ('38) to the permeability change of eggs both to water and to salt because the diameter decreases at first, but increases again returning at least approximately to the initial value. The assumption made by Krogh et al is seemingly similar to that of the present writer. However, it is questionable in what stage of egg——unfertilized or fertilized or parthenogenetically activated eggs——their observations and experiments were performed, as no detailed morphological changes of the egg-proper were described in their paper. In respect to these points further investigation is necessary.

Summary

When the egg of Alaska pollack, *Theragra chalcogramma* attains to actual maturity, it decreases in specific gravity and floats in the sea water, whereas the specific gravity of immature egg is smaller than that of the ordinary sea water. Consequently, it may be admissible to consider that the eggs which sink in sea water immediately after discharge are not yet fully matured, i. e. that they are immatured eggs having no capacity for normal fertilization.

Fertilized or developing eggs float at least till the hatching stage, because, upon fertization, they have gained a resistance against the hypertonicity of the surrounding sea water. The unfertilized mature egg is parthenogenetically activated without insemination when it comes in contact with sea water, undergoing the same changes as the fertilized though formed blastodisc does not begin to be divided, and it gains a resistance against the hypertonicity of the surrounding medium; accordingly it can remein for some time floating in the activated state in sea water. But such an egg begins to sink afterwards from loss of resistance to hypertonicity because of the lowering of its vitality. That is, the diffusing out of water from egg interior occurs and increase of specific gravity of the egg results.

Egg-activation and development are much affected by the salt concentration of the medium and it may be considered that isotonic and also hypotonic sea water are unsuitable media for activation and development of the egg.

References

AOKI, K. 1939. Über die Wasseraufnahme der Lachseier I. J. Fac. Sci. Hokkaido Imp. Univ., Ser. VI (Zool), 7, p. 27.

GRAY, J. 1932. The osmotic properties of the eggs of the trout (*Salmo fario*). J. Exp. Biol., 9, p. 277.

KAGAN, B. M. 1935. The fertilizable period of the eggs of *Fundulus heteroclitus* and some associated phenomena. Biol. Bull., 69, p. 184.

KAMIYA, N. (神谷尚志) 1924. On the pelagic eggs and their youngs found in the Hokuriku coastal waters of Japan 北陸沿岸に於ける浮性魚卵並に其の稚魚 (in Japanese). 水講試報 (J. Imp. Fish. Inst.,) 21 (3), p. 86.

KANOH, Y. (狩野康比古) 1952. Über Wasseraufnahme und Aktivierung der Lachseier III. Die Permeabilität der Eioberfläche. J. Fac. Sci. Hokkaido Univ., Ser. VI (Zool), 11, p. 95.

KONOPACKA, B. 1935. Recherches histochimiques sur le dévelopment des Poissons. 1. La vitellogénèse chez le Goujon (*Gobio fluviatilis*) et la Carpe (*Cyprinus carpio*). Bull. Acad. pol. Sci. Let., 62, p. 163.

KROGH, A., A. KROGH & C. WERNSTEDT 1938. The salt concentration in the tissues of some marine animals. Skand. Arch. Physiol., 80, p. 214.

TAMURA, O. (田村 修) 1952. 真鱈卵の比重. The specific gravity of matured egg of cod (*Gadus macrocephalus*) (in Japanese, with English résumé). Commemoration volume of the 3rd anniversary of the foundation of the Japan Sea Regional Fisheries Research Laboratory (日本海区水産研究所創立3周年紀念論文集), p. 271.

Yamamoto, K. (山本喜一郎) 1951. Studies on the fertilization of the egg of the flounder 1. Effects of salt concentration in the fertilization. J. Fac. Sci. Hokkaido Univ., Ser. VI (Zool), 10, p. 253.

Yamamoto, T. (山本時男) 1936. Shrinkage and permeability of the chorion of *Oryzias* egg, with special reference to the reversal of selective permeability. J. Fac. Sci. Tokyo Imp. Univ., Sec. IV (Zool), 4, p. 249.

Yusa, T. (遊佐多津雄) 1952. On the normal development of the egg of *Alaska Pollack* (*Theragra chalco gramma*) (inJapanese). スケトウダラの正常発生 Report of the survey of fisheries resources from the Hokkaido Regional Fisheries Research Laboratory (北海道区資源調査要報), 3, p. 106.

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of lower jaw blackish. Teeth are present only on jaws, small, rather slender (being fairly widely separated from one another) and arranged in a few rows anteriorly which taper laterally.

Sclaes are cycloid, large, soft and very deciduous. Lateral line high; there are only ½ 2 rows of sclaes between dorsal base and lateral line. Scales along lateral line *ca.* 50.

Testes attenuated and narrow. Peritoneum not black. Posterior part of pharynx has a pair of hard sacs which are internally provided with papillae bearing teeth. Nostrils paired on each side, located near anterior end of snout.

The general appearance, and more especially that of the head region, is sugestive of *Icticus* and *Tetragonurus*. Color in farmalin is greyish brown, lighter below. The spinous dorsal is much darker than the other fins.

ii) *A young example from Manazuru.* During July-August 5, 1952, Mr. Masaji Hirai, Manazuru Branch Station, Tokai Regional Fisheries Research Laboratory, collected a young example of *Ariomma lurida* taken by a trap net at Takaura (near Manazuru), and presented it to the writer for study. The specimen measures 113 mm in total length, 103 mm in fork length and 95 mm in standard length. The following measurements are given in hundredths of the standard length: Length of head 20 0, greatest depth of body 26.3, greatest breadth of body 14.7, least depth of caudal peduncle 5.8, diameter of eye* 7.6, interorbital (not bony) breadth (above centers of orbits) 10.5, length of snout 10.5, length of highest (3rd) dorsal spine 12.6, length of highest (2nd) dorsal fin-ray 7.9, length of highest (2nd) anal spine 4.2, length of highest (1st and 2nd) anal fin-ray 6.8, pectoral fin damaged, length of ventral spine 9.5, length of longest (3rd, counted from outside) ventral fin-ray 15.8, length of longest caudal fin-ray *ca.* 20.0.

D. XII 15 (anteriomost 2 or 3 fin-rays unbranched); A. II 15 (anteriormost 1 fin-ray unbranched); P. 23 on each side; V. I 5 (all fin-rays branched; received in a long and narrow groove which reaches anal origin).

Pseudobranchiae very well developed. Inner fold of branchiostegal membrane not covering its partner proximally Branchiostegals 6 on each side. Gill-rakers 9/1/19 (left) and 11/1/19 (right). Shape of tongue as in the larger specimen mentioned above. Tongue and palatines with extremely fine teeth. Jaw teeth are as in the larger specimen.

Scales have been rubbed off. The coloration differs considerably from the adult mentioned above. The tint of the back and inner side of the opercle are as in the adult, but there are three large violet (or dark brown) blotches on the side of the trunk and much smaller markings of irregular shape on the back. Spinous dorsal fin has blackish spots of small size. The ventral fin is mostly black. The caudal, pectoral and anal fins have no markings.

(*continued on p. 255*)

* The adipose eyelid is fairly well developed, but not covering the greater part of the eye.