

ONTOGENESIS OF **EUGERRES BRASILIANUS** (CUVIER, 1830)
(PISCES-GERREIDAE) OBTAINED BY FERTILIZATION "IN VITRO"

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ABSTRACT. Ontogenesis of *Eugerres brasiliianus* (Cuvier, 1830) (Pisces-Gerreidae) obtained by fertilization "in vitro".

The ontogeny of embryos, vitelinic larvae and larvae of *Eugerres brasiliianus* (Cuvier, 1830) (Pisces-Gerreidae) is studied. The eggs were artificially fertilized "in vitro" and the fecundity as well as the hatching rates are calculated. The main diagnostic features for the identification of the early stages of this species are established using descriptions and measurements of living and fixed eggs and larvae: mean egg diameter of 0,65mm; total lenght of the larvae at hatching 1,14mm; vacuolized and segmented yolk; a single non pigmented oil globule at the vegetative pole migrating to the anterior of the yolk before hatching; ventral longitudinal pigmented stripe in the larvae; pigmented eye just before it becomes functional; anus formed 24 hours after hatching and the ratio of preanal to post anal lenght changing with development.

Key words: Artificial fertilization - Ontogeny - fish - Gerreidae.

RESUMEN Ontogénesis de *Eugerres brasiliianus* Cuvier, 1830 (Pisces-Gerreidae) obtenida por fertilización "in vitro".

La fecundación artificial fue realizada con gametos obtenidos de ejemplares de la especie *Eugerres brasiliianus* Cuvier, 1830 (Pisces-Gerreidae). Se calcularon los valores porcentuales de huevos fecundados y de eclosión de las larvas. El principal propósito del presente trabajo fue registrar los caracteres morfológicos y merísticos en los distintos estadios embrionarios

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y larvales (en muestras vivas y fijadas), con la finalidad de crear bases para la identificación de los huevos y larvas de esta especie, encontrados en el plancton. Las principales características que deben ser utilizadas para el diagnóstico de esta especie en las primeras etapas de desarrollo ontogénico son: el diámetro medio de los huevos (0,65mm); la longitud total de las larvas recién eclosionadas (1,14mm); el vitelo dividido en segmentos y con numerosas vacuolas; una gota oleosa única no pigmentada en su polo vegetativo que migra a la región anterior del vitelo poco antes de la eclosión; una línea de pigmentos en la región ventral de la larva; los ojos presentan pigmento poco antes de ser funcionales; el ano se forma 24h después de la eclosión y la relación de tasas de longitud preanal y postanal se alteran durante el desarrollo de la larva.

Palabras Clave : Fecundación artificial - Ontogenia - Peces - Gerreidae.

INTRODUCTION

Eugerres brasiliianus (Cuvier, 1830), an economically important fish, is the biggest species of the family Gerreidae occurring from the Antilles to the South of Brazil, mainly in lagoons and estuaries (Menezes & Figueiredo 1980) and frequently in mangrove areas with muddy and sandy muddy bottoms. Other species of the same family, occurring in the same region are *Eucinostomus melanopterus*, *E. gula*, *E. argenteus* and *Diapterus rhombeus* (Menezes & Figueiredo 1980).

The aim of the present study is to describe morphological and morphometric characteristics of *Eugerres brasiliianus* from mature oocytes up to the larval stage which follows the total yolk absorption. For some species it is difficult to establish a series of type specimens, for identification purposes,

from eggs and larvae collected in the wild. This is because adaptations to their environment may generate confusion between closely related groups (Mito 1961). Thus, Matsuura (1977), Matsuura & Nakatani (1979) and Sinque et al (1982) registered the occurrence of Gerreidae in the Ichthyoplankton collected at the Brazilian coast between 22.5° and 25.5° South, but they were not able to identify the species of the larvae.

Therefore, we decided to collect eggs and sperm from identified mature specimens and, after artificial fertilization, rear the eggs and larvae. In this way, a definitive series of eggs and larval development stages, for this species, could be described.

MATERIAL AND METHODS

Sexually mature males and females of *Eugerres brasiliensis* (Cuvier, 1830) were collected by trawl nets at Pontal do Sul (25° 35' South and 48° 22' West) (Paraná, Brazil), near the shoreline, during the late afternoon, in November. After the identification of the gonad maturity stage, according to Angell (1976), the living specimens were measured (L_t) and weighed (W_t). The oocytes were analysed, counted and measured from a sample of 1ml. Thereafter, eggs and sperm were obtained by manual extrusion and fertilization was effected in a glass container by the dry method.

The eggs were transferred to a small glass incubator with 15 liters of well aerated and filtered sea water. Through the analysis of small samples, the fertilization and the hatching rates were determined after one hour. The hatching success was calculated taking into account the number of individuals that did not emerge from the eggs.

The larvae were successively washed with filtered sea water treated with G-Kallium Penicillin (40 UI/ml), and transferred to 180 liters aquaria,

containing filtered sea water with G-Kallium Penicillin (20 UI/ml). The day hours were maintained constant (12L/12D), and the aquaria protected from direct sunlight. The salinity was maintained at 31‰ (as in the environment where the adults were collected), and the temperature of 24°C was maintained constant with thermostatically controlled heaters.

As soon as the mouth was open and the intestine formed, the larvae were fed *Nannochloris oculata*, *Tetraselmis striata* and *Dunaliella tertiolecta*.

Some samples were taken periodically for observation and fixed in 4% formaldehyde for subsequent description along their development.

The nomenclature was based on Ahlstrom & Ball (1954) and the determination of embryonic stages was the same as used by Ciechomski & Weiss (1973).

Measurements were made with a Wild-Leitz Microscope with eyepiece micrometer, and drawings with a camera lucida adapted to the microscope.

RESULTS

1. OOCYTES AND FERTILIZATION:

Before fertilization, the eggs have transparent and internally smooth envelopes, closely applied to the yolk. Each egg contains a single dark grey oil globule (0.19mm diameter).

In 1 ml of gonad products, 650 mature oocytes were counted. One hour after the contact with the male gametes, 87.3% of the oocytes had been fertilized (activated). This time was considered as the starting point (T_0) for the description of embryonic development.

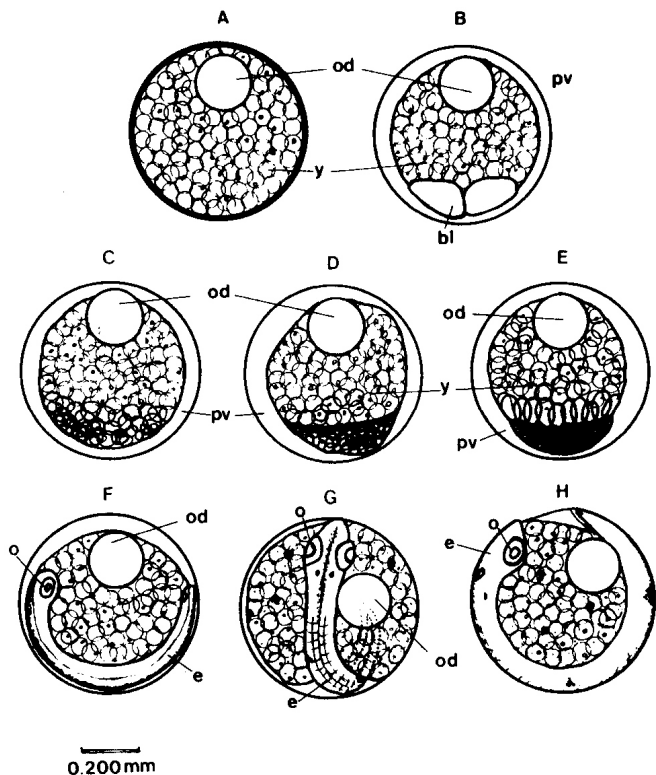


Fig. 1 Embryonic development of *Eugerres brasiliensis*. (A) minutes, (B) 1 hour, (C) 1 hour 30 minutes, (D) 2 hours 30 minutes, (F) 6 hours, (G) 10 hours, and (H) 11-12 hours after fertilization - (od) oil globule; (y) yolk; (pv) perivitellinic space; (e) embryo; (bl) blastomeres.

II. EMBRYONIC DEVELOPMENT:

1st phase - From fertilization to the beginning of gastrulation.

Immediately after fertilization, the egg diameter increases to 0.65mm by hydration (Fig. 1A). The oil globule lies at the vegetative pole, having the same diameter and color as in the oocyte, and sometimes with small oil globules adhering to it. A perivitelline space of 0.01mm is formed 15 minutes after fertilization. The egg envelope is thin, smooth, transparent and colorless. The yolk is spherical with mean diameter of 0.64mm, totally segment and with many small vacuoles.

Some minutes later, the blastodisc begins to form with the accumulation of protoplasm at the pole together with space. The diameter of the eggs remains the same. Now, 1 hour after T_0 , the first cleavage takes place: the blastodisc divides into two oval shaped blastomeres (Fig. 1B). The process can be followed until 16 blastomeres are formed. Thereafter, the cleavages are frequent and the cells so small that they can no longer be counted. As a result of unequal cleavages, smaller cells are observed at the animal pole region (Fig. 1C) in later stages.

2nd phase - From gastrulation to the blastopore closure..

About two hours after T_0 , the animal pole becomes narrow, and the development of the blastodermal cap-shaped shield begins (Fig. 1D). This shield is wide and the periblast is formed at its margins, close to the yolk. As soon as fully developed, this

structure is identified as the germ ring.

The invagination of the blastoderm shield (Fig. 1E) starts 30 minutes later. The germ ring grows enclosing the yolk and, as soon as this process is complete, the development of the embryonic shield takes place.

As a product of the germ ring, a row of cells will proliferate, increasing the invagination. By this time, the embryo is ventrally close to the yolk and dorsally to the perivitelline space. The blastopore closure is at the embryo's posterior end. At this time, the head region is slightly wider than the remainder of the body (Fig. 1F).

3rd. phase - From blastopore closure to tail detachment.

Six hours after T_0 , the embryo is longer than half the internal circumference of the egg. Now, nonpigmented eyes can be seen, as well as the Kupffer vesicle that lies ventrally, close to the end of the embryo. Along the whole dorsal surface, mainly at the anterior third of the embryo, very small pigment spots can be seen for the first time.

Around 10 hours after T_0 , the embryo is nearly formed (Fig. 1G) and the myomeres are detected. The eyes without pigmentation are dorsally flattened, the longer axis measuring 0.09mm. Two punctiform pigment spots are present where the auditory capsules will later be formed. The oil globule is now close to the embryo tail.

4th phase - From tail detachment to hatching.

Twelve hours after T_0 , the embryo starts to move forcefully inside the still spherical egg, causing the external membrane rupture. Auditory capsules begin to develop and are without otoliths. The oil globule, which remains with the same size throughout the development, lies near the embryo tail as in phase 3. At the end the tail a small continuous embryonic fin can be seen. At this stage the Kupffer vesicle disappears. The embryo is never as long as the internal contour of egg capsule (Fig. 1H).

From this moment on, the larva starts to hatch. After the rupture of the egg membrane, the embryo tries to escape from the capsule and membrane through violent bursts of movements. Thereafter the larva swims around carrying the yolk sac that will provide its endogenous food until the structure for external feeding and digestion are completed.

III. LARVAL DEVELOPMENT:

1. Vitellinic larva.

Just after hatching the larvae remain close to the surface, floating with the help of the oil globule and yolk. The tail beats make them swim.

Within 3 hours of the start of hatching, all the larvae had hatched. This point at which 97.5% of the larvae had hatched, occurred 14 hours after fertilization, and was considered as the starting time (T_0) for the

description of the vitellinic larvae.

A just hatched yolk sac larva (1.10mm L_t) has a straight body, only the cranial and caudal flexions persisting (Fig. 2A). The yolk sac extends anteriorly beyond the head. It is segmented and vacuolized, oval in shape and greater than half the total length of the larva (yolk length 0.63mm). The oil globule situated at the anterior end of the yolk sac maintains the original diameter of 0.19mm. The head measured as the distance between its anterior part and the posterior margin of the auditory capsule (head length 0.16mm); its height is 0.13mm and the snout length 0.03mm. The eyes (0.09mm diameter) have now the coroidal fissure. No otoliths are visible at this stage. The myomeres lie along the body but are not yet well formed and cannot be counted.

Six hours after T_0 , the larvae (1.24mm total length and 1.20mm standard length) are active, swimming mainly near the surface, but sometimes in mid water. The yolk begins to be absorbed (yolk length 0.62mm). Inside each auditory capsule there is an otolith. (Fig. 2B).

About 24 hours after T_0 , the larvae swim throughout the aquarium, mainly propelled by the caudal fin. They return slowly to the surface as soon as their movements stop. The larvae have grown to total length of 1.73mm (1.63mm standard length) and the yolk is reduced to 0.50mm (Fig. 2C). The preanal distance is 0.69mm. The head (0.27mm length, 0.22mm width) protrudes anteriorly to the yolk, and the length of the snout is 0.07mm. Olfactory

vesicles are present. From this point on the larvae grow rapidly but without significant morphological changes.

48 hours after T_0 (Fig. 2D), the larvae swim through the whole aquarium. The yolk sac larva measures 2.02mm total length and 1.90mm standard length. The preanal distance (0.71mm) is still shorter than the postanal distance (1.31mm) what means that the digestive tube will open closer to the anterior region of the body. The head size increases (0.33mm length and 0.28mm height). The snout measures 0.10mm and the nostrils are evidente. A row of melanophores follows the anterior margin of the eyes, while, close to the auditory capsules, the first signs of opercular formation can be seen.

72 hours after T_0 , the larvae swim normally throughout the aquarium. Besides the tail movements, transparent and non rayed pectoral fins become active. Two melanophores are at the dorsal anterior region. There is a great yolk reduction (Fig. 2E). The preanal distance (0.72mm) is more or less 1/3 of the total body length (2.16mm). From this stage on, the head length is measured as the distance between the anterior margin of the snout and the operculum (0.36mm). The urogenital duct can be seen close to the intestinal posterior margin. A deep invagination of the anterior margin of the snout leads to mouth differentiation.

90 hours after T_0 , the oil globule shrinks to a diameter of 0.18mm. 108 hours after hatching the yolk is totally absorbed and the oil globule

diameter is only 0.06mm (Fig. 3)

114 hours after T_0 , the yolk sac larvae reaches the last stage of development. Only a small part of the oil globule remains (Fig. 2E), its color and position being the same as at the beginning of the larval development. The larvae (2.35mm total length and 2.23mm standard length) migrate to the bottom and swim normally. The preanal distance is 1/3 of the total length and individuals up to 2.36mm total length can be found. The head is now as long as its height (0.42mm). The snout length is 0.18mm. The mouth is totally differentiated, with maxillae and mandibles. The operculum and preoperculum as well as the digestive tract are formed. The eyes (0.20mm) are mostly pigmented presenting a coroidal fissure. For the first time a row of stellate pigment cells extends from the anterior to the posterior region, ventrally, along the body.

From this stage on, the yolk sac larva absorbs the remaining oil, the last endogenous food reserve. At this time the eyes become fully pigmented and the mouth and digestive system are functional (Fig. 2F). After these transformations, the animal is referred to as a larva.

2. Larva:

132 hours after hatching, the larvae swim actively searching for food. The body length decreases (Fig. 4). The preanal distance (0.74mm) still corresponds to 1/3 of the total length (2.32mm). Rays begin to develop in the caudal fin. Ventrally, along the body,

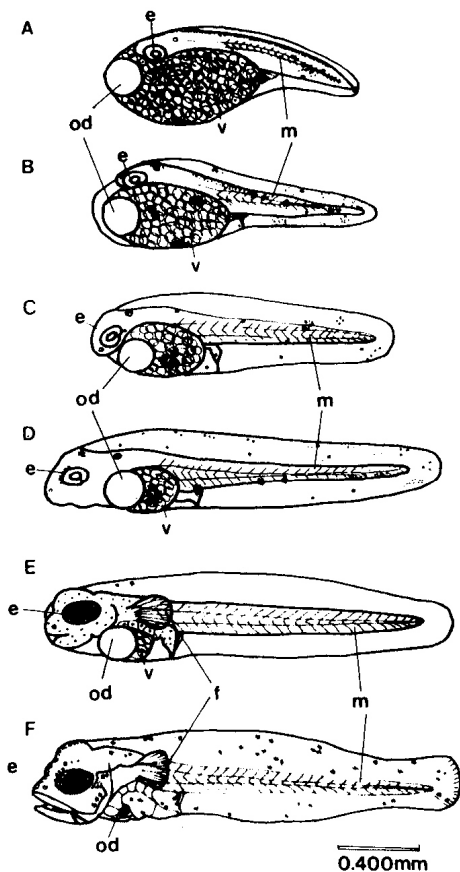


Fig. 2 Development of the vitelinic larva of *E. brasiliensis*. Times after hatching: (A) 10 or recently hatched, (B) 6 hours, (C) 24 hours, (D) 48 hours, (E) 72 hours, (F) 114 hours (m) myomeres; (od) oil globule; (v) yolk sac; (f) fin; (e) eye.

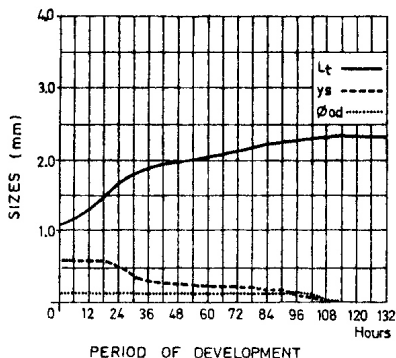


Fig. 3 Mean size of all stages from egg to larva, of the oil globule, and of the yolk, along the development, obtained from *Eugerres brasiliensis* fertilized "in vitro". (L_t) total length; (\varnothing_{od}) oil globule diameter; (ys) yolk sac length.

the row of stellate pigment cells remains.

The larva feeds on algae, which can be seen in the digestive tract of the animal, thus confirming the transition

from yolk sac stage to larval stage.

The growth in total length during the entire development of the larva is represented in Fig. 3.

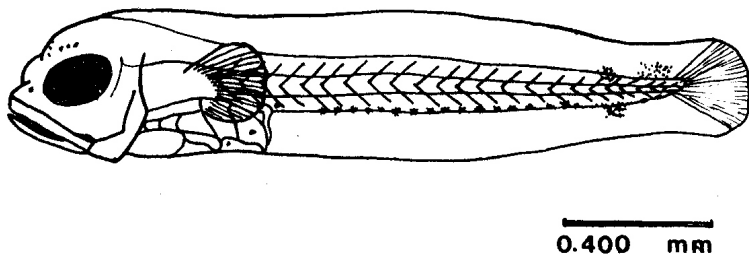


Fig. 4 Larva of *Eugerres brasiliensis* around 132 hours after hatching.

DISCUSSION

The most difficult steps concerning fertilization "in vitro" are the obtention of mature adults as well as the detection of the daily spawning rhythm. The oocyte maturation in the ovaries is very fast and good material for fertilization can only be obtained at the right time (Ciechomski 1965 and 1968; Ciechomski & Weiss 1973; Delsman 1931). When eggs are collected from the plankton these times can be detected (Kuntz 1914; Delsman 1929; Nichols 1939; Simpson 1959). This was not possible for *Eugerres brasiliianus* as eggs and larvae were not identified at the species level (Singue et al 1982). Mature males and females were only obtained from the open sea in the evening or early night, leading to the conclusion that spawning only occurs at that time.

From observations in the aquaria both the eggs and yolk sac larvae are obviously planktonic.

According to Ahlstrom & Ball (1954), species in which the embryonic development is quick and relatively undifferentiated are mostly species which produce a large number of small eggs. Usually the determination of species according to the size of their eggs is not a convincing diagnosis, as smaller and bigger eggs may be related to the parents size (Rannack 1958; Toom 1958; Blaxter & Hempel 1961; Arbault & Boutin 1968). But yet in *E. brasiliianus* the egg diameter can be considered as a diagnostic character, as it is different from the egg diameter of other species. The egg diameter (ϕ =

0.65mm) and the total length of the recently hatched larvae (L_t = 1.14mm) are very small, even originating from females with sizes as different as 32.4cm (W_t = 455g) and 49.4cm (W_t = 1400g). The first measurement represents the mean size of the species, and the second one is the biggest ever found (in nature and in the literature).

Segmented yolk is considered a primitive characteristic (Delsman 1926) rarely described for Percoidel (Ahlstrom & Ball 1954). *Eugerres brasiliianus* with the segmented and vacuolized yolk, being a Percoidel, seems to be one of the exceptions for this group.

Some fish species have various oil globules at the beginning of the embryonic development which, just before hatching, fuse into one (David 1939; Joseph et al 1964); in others, oil globules have not a uniform distribution (Arbault & Boutin 1968); thus their diameters are not important for identification (Joseph et al 1964). But *E. brasiliianus* has only one oil globule whose position and diameter are important for diagnosis. During the embryonic development, the mean diameter is constantly 0.19mm. The position of the oil globule during embryonic development and its change when the larvae hatch is described for some species (Delsman 1926; Ahlstrom & Ball 1954; Ahlstrom & Counts 1955; Matsuura 1971). The position of the oil globule of *E. brasiliianus* during the early embryonic stages is the same as

for other species, but from egg to yolk sac larva the change is specific. Just before hatching, oil globule is close to the embryo tail. However, at the same time it is not far from the head region, as the embryo lies curved inside the egg. At the moment of hatching, the oil globule is at the anterior extreme of the yolk sac and this is an important feature for identification of this species.

The number of myomeres is a useful character for the identification of many species. However for other species (Deisman 1931; David 1939; Snyder et al 1977) including *E. brasiliianus*, myomeres cannot be clearly distinguished.

The main cause of the external egg capsule rupture at the moment of eclosion is discussed by many authors, and may be related to the strong movements of the embryos (Orcutt 1950) and to their excessive size inside the egg (Simpson 1959) as well as to a hatching enzyme. In *E. brasiliianus* never the embryo was sufficiently long to have the tail overpassing the head, and the larvae did not surround the yolk completely, being the egg diameter the same throughout the development. Thus, since the embryo is never excessively large as compared to the egg size, hatching may be the result of an enzymatic action, helped by the strong movements of the embryo.

The relative position of the anus changes during larval development in many systematic groups (Mito 1966). Up to 6 hours after hatching there is no sign of the anus, but during the next 24 hours it not only appears but also migrates rapidly to the anterior region

of the body, with the preanal distance in relation to body size quickly reduced from 44.6% to 37.6%. This reduction slows down and the end of the yolk sac stage it is 31.6%. This change in the relative position of the anus becomes an important diagnostic character when the larvae shrink.

The shape and position of the yolk in the larva just after hatching are important features for certain groups. In *E. brasiliianus* the yolk sac is oval throughout the larval development. Just after hatching the yolk extends anteriorly beyond the limits of the larval head, as was also observed for *Trachurus picturatus australis* by Ciechomski & Weiss (1973).

The absorption of yolk and oil globule is different for different species. This absorption can be detected through the measurement of the large horizontal axis of the yolk sac (Hussain et al 1981). In *E. brasiliianus*, the absorption of the yolk starts just after hatching, but the oil globule remains for a further 90 hours. After that, its size suddenly diminishes. The yolk is totally absorbed before the oil drop disappears. This was also described for *Caranx kurra*, *C. crumenophthalmus* and *C. macrostoma* (Deisman 1926), *Sardinops caerulea*, *Sardina pilchardus* (Blaxter 1969) and *Parona signata* (Phonlor 1978).

In *E. brasiliianus*, pigmentation occurs only during the last stages of embryonic development, as for most fishes (Simpson 1959), and is restricted to the embryo and the yolk. For identification purposes, only the row

of pigment cells along the ventral region, the black pigment of the anterior margin of the eyes before they become functional, and the absence of pigmentation of the oil globules are important.

Fabre-Dornegue & Blétrix (1897) were the first authors that described a "critical point" during the larval development, just after the absorption of the yolk, when a change in growth occurs. Budd (1940) suggests that this phenomenon is caused by a natural consolidation of the tissues in the larva or even to the use of these tissues for the larval nutrition. According to Phonlor (1978) the

interruption of larval growth must be related to the quality or amount of food, so that larvae possibly start using their own energetic reserves, causing a shrinkage. In *E. brasiliensis*, a shrinkage was observed just after yolk absorption, even in larvae that had ingested food from the environment possibly due to the fact that only algae were offered. So it seems that for the larvae it is not easy to adapt to exogenous feeding. Even with a reduction in the total larval size, no anatomic alterations are observed, nor is formation of new structures interrupted, as also observed in *Caranx hippos* (Subrahmanyam 1964).

CONCLUSIONS

1. *Eugerres brasiliensis* has a reproductive period from the end of spring to summer, in open sea, during the evening and the first hours of the night, along the coast of the state of Paraná, Brazil.

2. Eggs are spherical, with an extremely small mean diameter (0,65mm) which remains unchanged during the embryonic period. The eggs are pelagic due to their size and spherical shape, the short period observed for the development, and the smooth, transparent, thin external capsule, as well as the immaturity of the recently hatched larva.

3. Even pertaining to the sub-order Percoidae, this species has a segmented and vacuolized yolk. The yolk is oval shaped, anteriorly located, extending

anteriorly beyond the head of the animal. It begins to be absorbed slowly about 12 hours after hatching, being consumed quickly after that. The yolk is absorbed before the oil globule absorption.

4. A single oil globule (0.19mm mean diameter) is observed during all the developmental period and only 90 hours after hatching it starts to be consumed. From the early embryonic stages on it is located at the vegetative pole. Just before hatching, it migrates to the anterior region of the yolk, remaining there until its total absorption.

5. The pigmentation appears only at the last embryonic stages, being restricted to the embryo and the yolk. There is a characteristic row of pigment cells

along the ventral region of the yolk sac larva and the following stage after the absorption of the yolk. Black pigment is found at the anterior margin of the eyes before they become functional in the 48 hours vitellinic larva. The oil globule is unpigmented during the whole embryonic and larval development.

6. The myomeres are poorly distinguished in the embryonic and larval stages, and therefore are not useful for diagnosis.

7. Hatching occurs after more or less 12 hours, at a temperature of 24°C, salinity 31‰ and a 12/12 hours photoperiod. The rupture of the egg capsule may be due to enzymatic action and the hatching is mainly a result of strong active movements of the embryo.

8. The vitellinic larva hatches with a mean total length of 1.14mm, poorly developed, without pectoral and caudal fins and with a big yolk mass making swimming movements quite difficult.

9. After the yolk absorption the larva

shrinks even after eating. Probably this shrinkage is related to the time needed for the adaptation to an exogenous food.

10. Up to 6 hours after hatching the anus cannot be detected in the vitellinic larvae. From this point on, its position changes along larval development. 30 hours after hatching the preanal distance is suddenly reduced from 44.56mm to 37.60mm, and to 31.60mm as soon as the yolk was totally absorbed. Thus, the shrinkage of the larva, during the "critical period" seems to be more marked at the preanal region.

11. The main features for diagnosis and identification of this species are: diameter of the eggs, morphology of the yolk, oil globule (amount, diameter and position during the stages of embryonic and vitellinic larval development), pigmentation of some structures at different developmental stages, total length of recently hatched vitellinic larvae, timing after hatching when the anus is formed and its migration during larval stage.

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