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Embryonic Developmental Stages in Cultured Rabbitfish (*Siganus guttatus*, Bloch 1787)

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Abstract

In the Philippines, the rabbitfish (Siganus guttatus, Bloch, 1787) has significant aquaculture potential because of its high demand and increasing value. Studies on breeding and behavioural patterns including aspects on larval rearing and juvenile production are available for rabbitfishes. However, there are relatively few studies on the early development especially, on the description of eggs and embryonic development hence, this study was conducted. The present study describes the embryonic development of Siganus guttatus. It highlights the changing spectrum of developmental processes that occur under desirable conditions. The ripe egg diameter is around 550 µm and egg colour is transparent and filled with fat globules. In this study the process of embryonic development was divided into five (5) periods: zygote, cleavage, blastula, gastrula and segmentation until it reached the larval stage. Asynchronous pattern of cleavage starts after the 32 cell stage and become progressively more variable in the later stages. The different stages that are recognizable for each period were documented and time lapsed of each embryonic development was recorded. This study will serve as baseline information for future studies on rabbitfishes, useful for optimization of large scale culture to enhanced captive production.

Keywords: *Siganus guttatus,* embryonic development, rabbitfish, aquaculture

Introduction

Studies on the embryonic development of fishes are a useful tool for the morphological and chronological characterization of events. The description of eggs and embryonic development is available for relatively few species of fish but none is found in *Siganus guttatus.* In the Philippines, the rabbitfish (*Siganus guttatus*) has significant aquaculture potential because of high demand and its increasing value. Moreover, culture of rabbitfishes is found to be promising because of many favorable traits such as tolerance toward crowded conditions, ready acceptance of artificial diets and its low trophic level that require less protein or inexpensive feeds. One of the major concerns of any seed production and hatchery system however, is to produce the maximum number of quality fingerlings from the available broodstock for aquaculture[1]. In this connection, studies on embryonic development become consequential to the successful rearing of larvae for large scale seed production and aquaculture[1,2]. In addition, such studies on any fish species can be useful in directing efforts of the fish farmer to the specific state and requirements of each larval stage as this information is also essential to optimize larval growth and survival. It is also possible to group same age of embryos to treat them with the same environmental conditions to hasten development and to further improve harvesting of fries with the same age for greater profitability. Embryonic studies will also determine the possibility of prolonging embryonic development[4], to delay hatch and the moment of hatching to ensure synchronous development[5].

Material and Methods

A total of 300 fertilized eggs were collected from a newly spawned female Rabbitfish (figure-1) cultured in a 400L fiberglass tank that has been prespawned by male rabbitfishes (figure-2). Two male rabbitfishes were placed in a tank to ensure successful external fertilization of eggs. Fertilized eggs were collected to ensure consistency throughout the study. Eggs were measured using stage micrometer (in µm). These eggs were observed "*in situ*" using Leica light microscope for the different developmental stages per hour until it hatches[6]. Description of embryonic stages were made and captured with 12.1 megapixel Sony camera. A total of 10 set ups containing 30 eggs per dish were aerated and incubated with the following conditions: 30ppt seawater, pH 9 at 27°C. The number of individuals was counted for each stage including non-viable eggs (dead, undeveloped eggs, milky opaque eggs). Results were tabulated.

Figure -1
Mature female *Siganus guttatus* (golden rabbitfish) ready to spawn

Figure-2
Female (leftmost) and males (center and rightmost) rabbitfishes prespawning in 400L fiberglass tank

Results and Discussion

Morphologically, the ready to spawn female fish can be identified from swollen abdomen, loss of appetite, and continuous movement. The female laid tiny spherical transparent eggs that are externally fertilized by the sperm. The eggs can be seen attached to an egg jelly in a grapelike manner composed of glycoprotein meshwork which functions to attract or activate sperm[6]. Once successful fertilization occurs, fertilized eggs attaches to the substrate such as the sides of the tank. The unfertilized eggs however, float or sink to the bottom. The surface of the egg was smooth, with adhesive and demersal property, and contains many fat globules. The ripe egg diameter is around 550 µm. The temperature and pH conducive for egg development was around 27-29ºC at pH 9. Under desirable conditions, the early stages of *Siganus guttatus* (rabbitfish), development were characterized by meroblastic discoidal cleavage. Initially, cell division occurs only in the blastodisc, which is a thin region on the animal cap at the animal hemisphere region of the egg. This blastodisc contains all of the egg's cytoplasm and organelles and it is where the embryo develops while the rest of the egg is filled with yolk. The egg is surrounded by a thick envelope. One distinct characteristic observed in rabbitfish's developing embryo is the presence of a transparent membrane that encloses the embryo as it develops throughout the different stages. This unique feature is relatively not observable in any other species of fishes such as zebrafish.

The phases of egg development were divided into different stages based on the morphological characteristics of the developing embryo.

Zygote Period: This period is observable an hour after the fertilization period. The non-yolky cytoplasm begins to stream toward the animal pole thereby, segregating the blastodisc from the clearer yolk granule-rich vegetal cytoplasm[7]. In this stage the animal pole (blastodisk) had the form of a semispherical cavity. Rabbitfish eggs appeared to be telolecithal meaning that most of the egg cell is occupied by yolk [6] with an accumulation of vitellus in the vegetal pole (figure-3). Fat globules appeared in the middle of the egg.

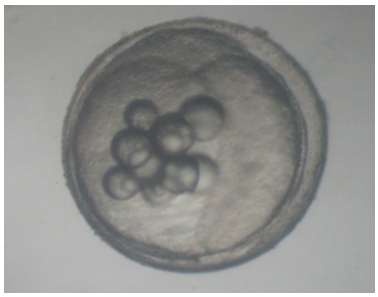

Figure - 3
The zygote period of *Siganus guttatus* (golden rabbitfish) under 400x magnification (550 µm). The zygote, within its uplifted chorion and fat globules found in the center, 1 hr. after fertilization

Cleavage Period: The type of cleavage observed was a meroblastic discoidal cleavage wherein, the first cleavage divides the blastodisc into two blastomeres. The first cleavage furrow is usually vertically oriented and this is usually the case until the 32-cell stage. The furrow arises near the animal pole and it progresses rapidly toward the vegetal pole [7]. It passes through only the blastodisc and not the yolky region of the egg. After a few minutes, the second cleavage (4 cell stage) occurred. This was followed by successive divisions producing 8 cell stage, 16 cell stage, 32 cell stage and 64 cell stage (figure-4). It was noted that complete cleavage occurs approximately near the end of the 16-cell stages. This is accounted by the way the cleavage furrows undercut the blastodisc from the center, going outward toward the blastodisc margin. Moreover, as successive cleavage occurs blastomeres become reduced in size and morula stage was reached. In addition, at the 16-cell stage cleavage patterns becomes more complex such that a solid hemisphere of 16 blastomeres is produced and blastomeres tend to overlap each other. After the 32-cell stage, in the later stages the cleavage patterns becomes progressively asynchronous and the number of blastomeres are somewhat, more variable. Since, it follows asynchronous pattern of cleavage, the cleavage patterns were observed 2-3hrs. after fertilization.

Blastula Period: This is described as the period wherein, blastodisc begins to look like a ball that is at the 128-cell stage until the time of the onset of gastrulation. During blastula stage important processes happened. The embryo enters midblastula transition (MBT), the yolk syncytial layer (YSL) forms, and epiboly begins[7]. Formation of these three distinct cell populations can be observed then. Firstly, due to movement of the mid-blastula transition that is made up of the most superficial cells from the blastoderm forming a single cell layer thick of epithelial sheet known as Enveloping layer (EVL). Secondly, YSL mentioned which serves an important function for directing some of the cell movements of gastrulation. The YSL is formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell. This becomes the periderm, an extraembryonic protective covering that is shed off at later development. Thirdly, deep cells found between YSL and EVL gives rise to embryo proper[6].

In the blastula period, the blastocoele and embryonic shield is formed. The epiboly continues until the gastrula period. Figure 5 shows some stages observed during the blastula period. In the earlier part of the period the cells continue to divide synchronously producing the 256K cell stage, 512K cell stage then the high stages comprising the 1k and the 2k cell stage this progresses until, the YSL surface bulges or "domes" toward the animal pole, thus resulting in a change in shape. Somehow, this is an apparent sign that epiboly is about to begin. Epiboly actually is the stage in the last part of the blastula. This is usually indicated by the thinning and spreading of both YSL and the blastodisk over the yolk cell [8]. During this stage, it was observed that there is considerable thinning of the blastodisc hence, a change in cell form from a high-piled mound to a multi-layer cell of nearly uniform thickness called a blastoderm. Here, it consists of a flattened monolayer and a deep multilayer about four cells thick. However, it is noted that blastoderm thickness is not exactly uniform for all observed embryos at this stage. The different stages were observed within 2-6hrs. post-fertilization.

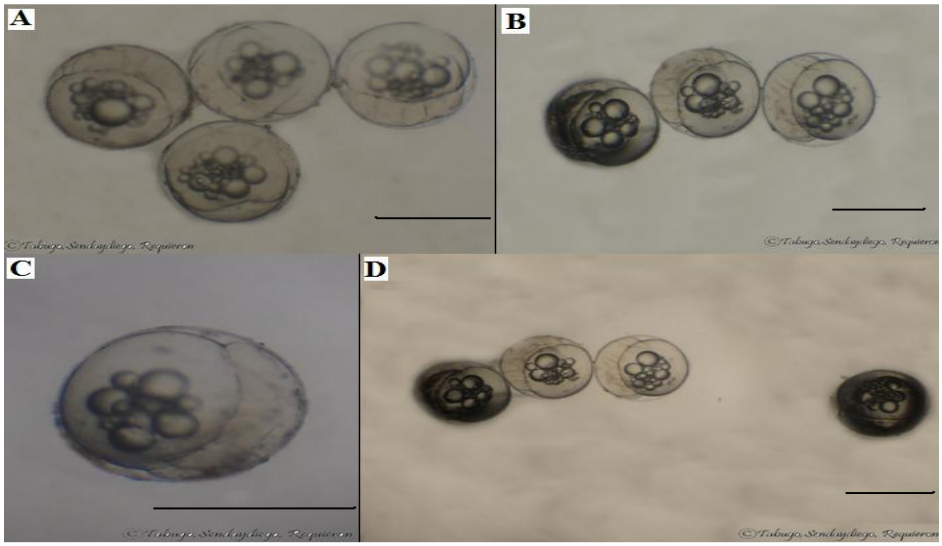

Figure - 4

***Siganus guttatus* (golden rabbitfish) embryos during the cleavage period. Face views. A) eight-cell stage; B) 16-cell stage; C) sixty-four cell stage; D) embryos still in the 16-cell stage. Each scale bar = 550µm (taken at different magnifications), Observed 2-3hrs. after fertilization**

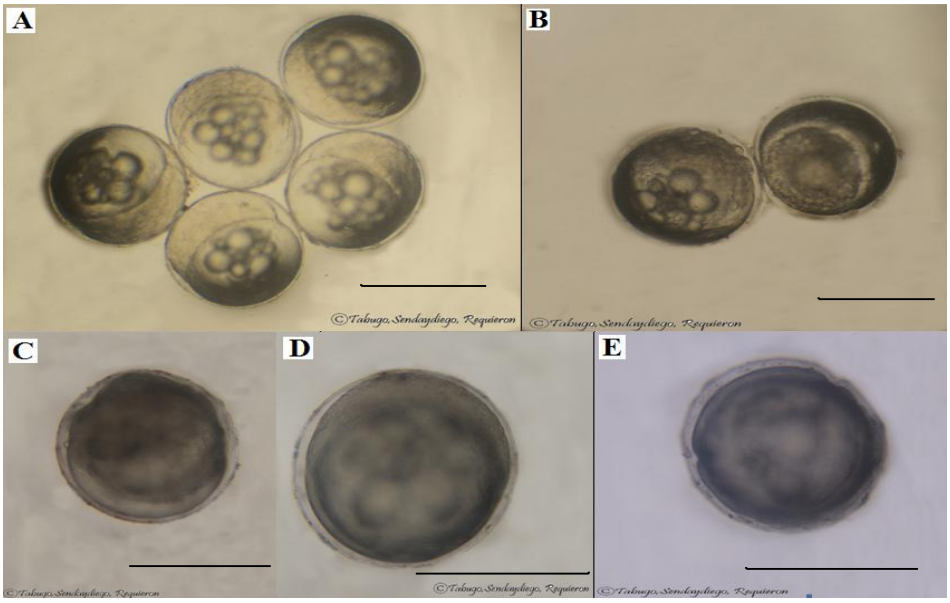

Figure -5

Observed face views of *Siganus guttatus* (golden rabbitfish) embryos during blastula period. A.) 512k-cell stage; B) high stage; C.) transition between the high and oblong stage; D.) dome stage; E.) 30% Epiboly stage. Each scale bar = 550µm (taken at different magnifications). Observed 2-6 hrs. after fertilization

Gastrulation Period: In this stage epiboly continues and morphogenetic cell movements of involution, convergence and extension occur thereby, producing the primary germ layers and the embryonic axis. The beginning of the involution is defined the onset of gastrulation, this occurs at 50% epiboly wherein, a thickened marginal region termed the germ ring appears (figure-6), nearly simultaneously all around the blastoderm ring. Then, certain convergent movements almost rapidly result to accumulation of cells at one position along the germ ring producing the embryonic shield. In this step the epiboly temporarily arrests but after the shield formation epiboly continues, so at a nearly constant rate the margin of the blastoderm advances around the yolk cell to cover it completely. Gastrulation is characterized as a phase in which the cells of the blastodisk shift and separate into epiblast and the lower, the hypoblast. In this stage the blastomeres extends towards the vegetal pole[7].The number of fat globules greatly reduced. As the blastomeres moved more from the animal pole to cover the part of the yolk called the epiboly, the fat globules begins to disappear. Afterward the embryo began to form the head region and the neural ectoderm. This step also included the conformation of the embryo body, which occurred. At this time, the neural line was also recognized, whereas the cephalic region became distinct from the caudal (figure-7). The different stages were observed within 3-10hrs. after fertilization.

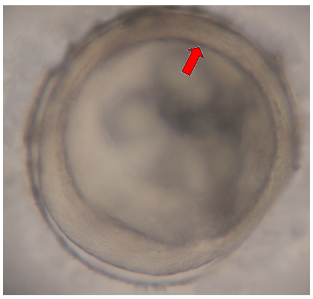

Figure - 6
Development during gastrula period of *Siganus guttatus* (golden rabbitfish); the Germ ring stage; the arrow indicates the germ ring; the embryonic shield will probably develop from the flattened region of the ring

Segmentation Period: In this period a variety of movements occurred. The somites develop wherein, the rudiments of the primary organs become visible and the tail bud becomes more prominent and the embryo starts to elongate. This period is also called the tail bud period (figure-8, A-D), because the tail bud becomes more prominent in the caudal end. In the one-somite stage, the first somatic furrow forms. In the five-somite stage, the brain primordium has distinctively thickened into the neural keel. The Neural keel formation occurs basically in the anterior trunk between the six and ten somite stages[9]. In the 14-somite stage the notochord becomes more distinguishable from the ventral part of the neural keel. By the 18-somite stage about ten (10) neuromeres have developed. Segmentation appeared as early as the 4th hr. post-fertilization and they continue to appear until the 20th hr.

Newly Hatched Larvae: The newly-hatched (day 0) rabbitfish larvae appeared transparent and 1.7 mm in total length. Body parts are prominent (figure-9). At day 1 after hatching, total length reaches 2.0 mm and the size of yolksac reduced. On day 2 (2.1mm), larvae start to open the mouth and eyes, the digestive tract develops, and the gills, pectoral fins and tail appear. The yolk sac disappears.

Asynchronous pattern of cleavage starts after the 32 cell stage and become progressively more variable in the later stages. The cleavage patterns were observed 2-3 hrs. post fertilization. Intra-specific variation of cleavage pattern was reported in the embryo of Atlantic cod [10] and this is true with other fishes such as the rabbitfish. Naturally within species, the timing of division events is based on temperature[11]. But it was also found out that changes in pH may also alter the rate of development of embryos [12] by hastening it under optimum temperature or delaying it under acidic environments of pH 4-5. In this study somehow, it was determined what particular period and time lapse when the asynchronous pattern of cleavage is predominant, it basically appears during 2-3hrs. post-fertilization. Hence, given the fact that temperature and pH have astounding effects on rate of development of embryos for this species then, it is possible to adjust optimum temperature and pH that can hasten development and achieve healthy embryos to ensure maximum survivability. From here, there is the possibility of grouping same stage of embryos to treat them under the same environmental conditions to hasten development and to further improve harvesting of fries with the same age to ensure maximum profit.

Conclusion

The series of developmental stages of the embryo of rabbitfish *Siganus guttatus,* were described and characterized based on morphological features identified by examination of live embryo using LEICA light microscope. The identified periods include the zygote, cleavage, blastula, gastrula, and segmentation. The ripe egg diameter is around 550 µm. The rate of embryonic development was observed under desirable conditions of temperature around 27-29ºC and pH 9. The early stages of *Siganus guttatus* (rabbitfish), development were characterized by meroblastic discoidal cleavage. One distinct characteristic observed in rabbitfish's developing embryo is the presence of a transparent membrane that encloses the embryo as it develops throughout the different stages. This unique feature is relatively not observable in any other species of fishes such as zebrafish. Asynchronous pattern of cleavage starts after the 32 cell stage and become progressively more variable in the later stages. The cleavage patterns were observed 2-3 hrs. post fertilization. Results suggests the possibility of grouping same stage of embryos to treat them under the same environmental conditions to hasten development and to further improve harvesting of fries with the same age for greater profitability. Moreover, data obtained imply that qualitative and early morphological criteria can be routinely used in several marine

fishes of economic importance as predictive measures of larval viability under standard commercial and experimental purposes.

It is recommended that a study on the effect of different pH levels and temperature should be done and documenting each stage under various conditions.

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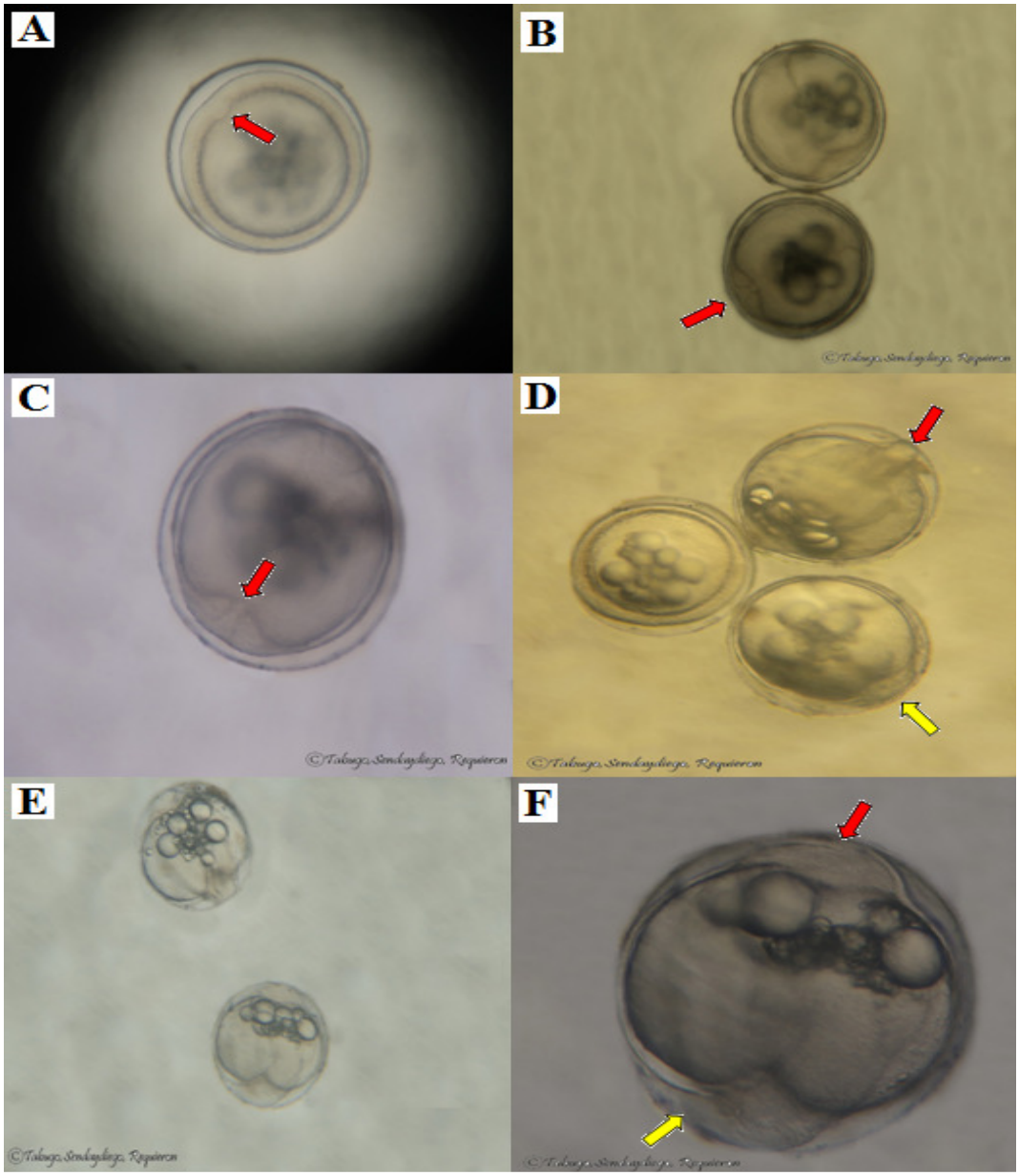

Figure - 7

Gastrula Period. A.) Shield Stage; the embryonic shield indicated by the red arrow marking the dorsal side, is visible as the thickening of the germ layer to the left; B-C.) The arrow indicates the embryonic shield at 70% epiboly stage; D.) 80% epiboly stage as indicated by the yellow arrow; 90% epiboly stage indicated by red arrow, the tail bud (arrow) becomes visible for some embryos in this stage; E.) Bud stage; F.) The tail becomes more visible (yellow arrowhead shows the tail bud); the red arrowhead points to the area that is most likely will develop into the cephalic region. Observed 3-10 hrs. post-fertilization

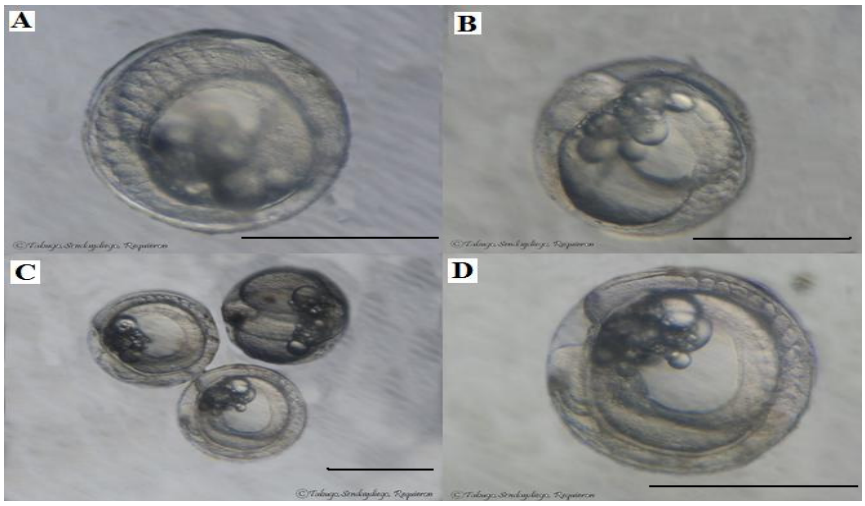

Figure -8 A-D
Segmentation period (taken at different magnifications). Somites, pharyngeal arch primordial and neuromeres develop; primary organogenesis; earliest movements and tail appears. Each scale bar = 550µm

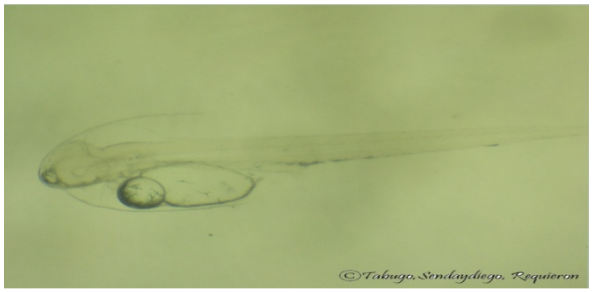

Figure – 9
Newly hatched larvae of *Siganus guttatus* (golden rabbitfish), The eyes, the head and tail region are highly observable

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