

ARTICLE

Embryonic development of Peruvian grunt *Anisotremus scapularis* (Perciformes: Haemulidae)

Desarrollo embrionario de la chita *Anisotremus scapularis* (Perciformes: Haemulidae)

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Resumen.- La chita *Anisotremus scapularis* se distribuye desde Ecuador a Chile y se considera un importante recurso acuícola en el Perú. El conocimiento del desarrollo embrionario es crucial porque es parte de la biología básica de una especie. El objetivo de este estudio fue describir las etapas embrionarias de la chita. Los huevos se obtuvieron por desove espontáneo y se cultivaron a 19 °C en condiciones de laboratorio. Se evaluaron las características morfométricas del huevo: diámetro ($0,752 \pm 0,025$ mm) (media \pm desviación estándar) and diámetro de la gota oleosa ($0,165 \pm 0,014$ mm). La primera división se observó aproximadamente 45 min después de la fertilización. La etapa blástula comenzó después de 4 h y la gástrula media 12:30 h después de la fecundación. La neurula temprana se observa 17 h después de la fertilización. Los latidos cardíacos y los movimientos de la cola embrionaria libre se registraron después de 30 h de incubación. La eclosión se produjo entre las 31 a 41 h y la longitud de las larvas recién eclosionadas fue de $2,558 \pm 0,051$ mm. El desarrollo embrionario de esta especie es similar a estudios previos con respecto a otros peces marinos. Este estudio es el primer reporte del desarrollo embrionario de *A. scapularis*, lo cual es una información valiosa que proporciona una línea base de referencia para los esfuerzos en el cultivo de esta especie.

Palabras clave: Huevo, segmentación, blástula, gástrula, faringula y eclosión

Abstract.- Peruvian grunt *Anisotremus scapularis* is distributed from Ecuador to Chile and it is considered an important aquaculture resource in Peru. Knowledge of embryonic development is crucial because it is part of the basic biology of a species. The aim of this study was to describe the embryonic stages of Peruvian grunt. The eggs were obtained by natural spawning and reared at 19 °C under laboratory conditions. Morphometric characteristics of the egg were evaluated: diameter (0.752 ± 0.025 mm) (mean \pm sd) and oil globule diameter (0.165 ± 0.014 mm). The first division was observed approximately 45 min after fertilization. Blastula stage started after 4 h and the middle gastrula stage after 12:30 h. Early neurula was observed 17 h after fertilization. Cardiac beats and movements of the free embryonic tail were recorded after 30 h of incubation. Hatching occurred between 31 to 41 h and length of newly hatched larvae was 2.558 ± 0.051 mm. The embryonic development of this species is similar to previous studies regarding other marine fish. This study is the first report of embryonic development of *A. scapularis*, which is a valuable information that provide a baseline reference for the efforts for the culture of this species.

Key words: Egg, cleavage, blastula, gastrula, pharyngula, hatching

INTRODUCTION

Marine fish farming has experienced an important increase in recent years, in 2012 represented 12.6% of total production of finfish for an estimated value of 23,500 million USD (FAO 2014). Aquaculture in Peru has been increasing at a rate of 20% annually, according to the National Aquaculture Development Plan -(DS No. 30-2001-PE) and the National Program of Science, Technological Development and Innovation in Aquaculture 2013-2021 (C+DT+i). The objectives of both programs are to support and guide the research related to technological development in aquaculture based on a list of main species (PRODUCE 2009, 2012). One of this species is the Peruvian grunt *Anisotremus scapularis* (Tschudi, 1846), that belongs to the Haemulidae family, which includes 17 genera and

150 recognized species (Nelson 2006). This fish family is distributed around the world in coastal areas and waters with rocky or sandy bottom of the Atlantic, Indian and Pacific Ocean, and it is considered an important species both economically and ecologically (Barden *et al.* 2014). The Peruvian grunt is one of the six species of *Anisotremus* registered for Peru (Chirichigno & Cornejo 2001). This fish is a benthopelagic marine species, carnivorous and is distributed from Manta in Ecuador to Antofagasta in Chile and Coco y Galapagos island (Chirichigno & Velez 1998). Also, this species is important in the interaction of marine coastal communities because uses trophic resources of both sandy and rocky environments where it feeds preferably on invertebrates as *Chiton cumingsii* and *Semimytilus algosus* (Iannacone & Alvariano 2009).

In the last few years, different investigations have been carried out for the knowledge of this species, including the description of the parasites associated with this species (Iannacone & Alvarino 2012, Chero *et al.* 2014) and studies to improve the transport of juvenile (Rosado *et al.* 2016). Also, there are a few studies related to the physiological and zootechnical aspects, such trials of commercial diets for juveniles (Dionicio-Acedo *et al.* 2017), thermal tolerance studies (León-Palomino *et al.* 2017). Also, Carrera *et al.* (2018) presented a manual for rearing and reproduction of this species.

Research on embryological development is an important aspect in the study of any species, which allows for morphological and physiological understanding during early development (Honji *et al.* 2012). Also, it is a helpful tool for determining egg quality, based on fertilization capacity of the egg and its subsequent development (Bobe & Labbé 2010). Currently, there are no studies on eggs and larvae of Peruvian grunt, however these studies are necessary for identifying morphological events and establish the techniques of incubation and larviculture (Valbuena-Villareal *et al.* 2012). Therefore, the aim of this study was to describe the embryonic development of *A. scapularis* under culture conditions, which provide information needed for seed production of this species.

MATERIALS AND METHODS

This study was carried out under laboratory conditions at the Fish Culture Laboratory of the Aquaculture Research Center Alexander von Humboldt at the Peruvian Marine Institute Research (Instituto del Mar del Perú- IMARPE).

BROODSTOCK MANAGEMENT

Broodstock were collected in Pisco, Chincha and Callao (Peru) and placed in two recirculating aquaculture systems at the Fish Culture Laboratory (IMARPE). Daily physicochemical parameters such as temperature (19.02 ± 0.60 °C), dissolved oxygen (7.06 ± 0.69 mg L⁻¹), pH (7.60 ± 0.36) were monitored with a portable multiparameter (Thermo Scientific Orion Star A329). In addition, total ammonia nitrogen (0.28 ± 0.11 mg L⁻¹), nitrite (0.39 ± 0.20 mg L⁻¹), nitrate (11.05 ± 7.29 mg L⁻¹) and carbon dioxide (4.75 ± 1.07 mg L⁻¹) were measured weekly with colorimetric tests to ensure adequate water quality. The broodstock were maintained under a constant temperature and photoperiod of 12L:12D in order to stimulate their maturation in captivity. The feeding was with pieces of frozen anchovy *Engraulis ringens* at a feed rate of 4.5-5% of the total biomass of each culture tank. Additionally, biometric sampling and

assessment of gonadal maturity were performed once a month (Carrera *et al.* 2018). For females, a sample of oocytes was obtained by ovarian biopsy or cannulation (Mylonas *et al.* 2010) and maturation was classified taking into account the diameter, characteristics, and proportion of each type of oocyte (*i.e.*, pre-vitellogenic, vitellogenic and mature) (Perea *et al.* 2015). In males, a semen sample was collected by abdominal pressure and sperm quality was evaluated by measuring sperm concentration and sperm motility (Lanes *et al.* 2010). According to gonadal maturity stage, three females in stage IV or spawning with hydrated egg and four males with good sperm quality (sperm motility > 80%) were selected. The selected individuals were 542.99 ± 124.97 g and 28.40 ± 1.67 cm and kept in a fiberglass tank of 1,500 L provided with constant aeration, in a static culture system, in order to obtain spontaneous spawning. Natural spawning generally occurred in the evening hours.

EGGS SAMPLING AND DESCRIPTION

The monitoring of the embryonic development began from 18:00 h by verifying the presence of eggs in the tank by using a sieve of 600 µm. To describe the morphology and dimensions of eggs, samples in 2 L jars were collected, then a small sample (n= 45-50 eggs) was taken to observe the development stages, which were determined when all eggs were in the same stage. The monitoring was conducted at intervals of 15 to 30 min, in order to observe and describe the morphological changes in the egg. The characteristics of the different embryonic stages were recorded under an optical microscope (LEICA DM 1000 LED) with a 10X objective coupled to a camera and computer. Morphometric data were documented using the LAS software version 4.3. The mean, standard deviation and confidence interval (significance level 5%) were calculated for each variable. The length of yolk sac was measured according to the morphometric traits described by Park *et al.* (2013). The embryonic development was detailed according to the description for *Danio rerio* (Kimmel *et al.* 1995) and *Lutjanus colorado* (Abdo de la Parra *et al.* 2014).

INCUBATION OF EGGS

Eggs were collected in the gastrula stage and washed with seawater sterilized by ultraviolet (UV) and disinfected with a solution of iodine and seawater 0.005%. The 32,689 eggs were incubated in a 300 L tank at 19 °C to monitor the morphological changes. The fertilization and hatching rate was 83.45 and 80.89%, respectively.

RESULTS

MORPHOMETRIC ASPECTS OF EGG AND LARVA

The fertilized eggs were spherical, completely smooth and pelagic, without projections, with transparent chorion and uniform yolk with one oil globule. The morphometric characteristics of egg and newly hatched larvae of *A. scapularis* are presented in Table 1.

EMBRYONIC DEVELOPMENT DESCRIPTION

The embryonic development stages were cleavage (first division, second division, etc.), blastula, gastrula (middle, late), pharyngula (structures as somites and Kupffer vesicle) and hatching, the time of occurrence of each stage is described in Table 2.

Cleavage stage: is the phase when cell divisions occurred. The fertilized egg (Fig. 1A) began the segmentation process and a small perivitelline space due to the separation of the chorion membrane egg cell was observed. The first division (Fig. 1B) occurred 45 min after fertilization and two blastomeres were observed. At 1 h 15 min, the second division with four blastomeres was observed (Fig. 1C). The third division (Fig. 1D) occurred 1 h 30 min after fertilization and 8 blastomeres were formed, the 4th division with 16 blastomeres (Fig. 1E) took place after 30 min and the 5th division with 32 blastomeres (Fig. 1F) happened 30 min later. Finally, 3 h after fertilization the morula was observed (Fig. 1G).

Table 1. Morphometric characteristics of egg and newly hatched larvae of Peruvian grunt *Anisotremus scapularis* / Características morfológicas del huevo y larva recién eclosionada de chita *Anisotremus scapularis*

Morphometric characteristics	Length (mm)	Standard deviation	Confidence interval	Range
Egg				
Diameter	0.752	0.025	0.0005	0.7515 - 0.7765
Oil globule diameter	0.165	0.014	0.0003	0.1647 - 0.1787
Hatched larvae				
Total length	2.558	0.051	0.0010	2.5570 - 2.6093
Yolk sac length	0.553	0.033	0.0007	0.5528 - 0.5862
Oil globule diameter	0.160	0.015	0.0003	0.1597 - 0.1747

Table 2. Embryonic development of Peruvian Grunt *Anisotremus scapularis* at 19 °C / Desarrollo embrionario de chita *Anisotremus scapularis* a 19 °C

h: min	Embryonic development
0:00	Fecundation
0:45	First division
1:15	Second division
1:30	Third division
2:00	Fourth division
2:30	Fifth division
3:00	Morula
4:00	Blastula
12:30	Middle gastrula
15:00	Late gastrula
17:00	Early neurula
17:30	Neurula with Kupffer vesicle
19:00	Embryo with somites
20:45	Pigmentation (appearance of melanophores)
24:00	Beginning heart differentiation
31:45	Start of hatching
40:40	Larvae hatched

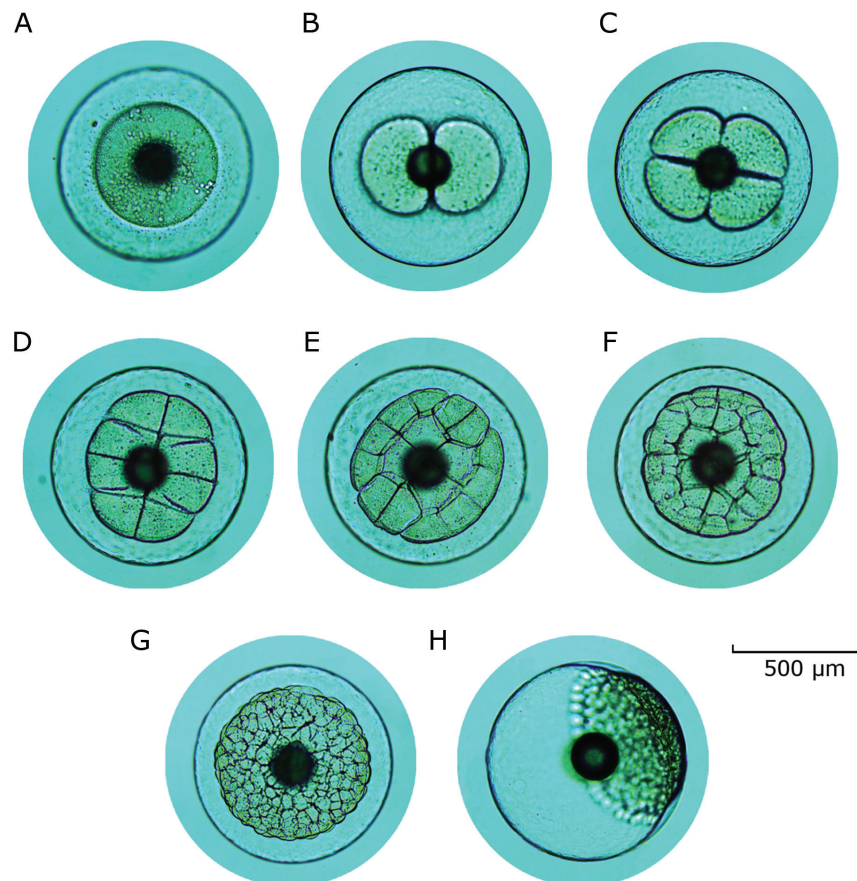


Figure 1. Embryonic developmental stages of *Anisotremus scapularis* at 19 °C. A) fertilized egg, B) 2-cell stage, C) 4-cell stage, D) 8-cell stage, E) 16-cell stage, F) 32-cell stage, G) morula, H) blastula stage / Desarrollo embrionario de *Anisotremus scapularis* a 19 °C. A) huevo fecundado, B) 2 blastómeros, C) 4 blastómeros, D) 8 blastómeros, E) 16 blastómeros, F) 32 blastómeros, G) mórula, H) blástula

Blastula stage: 4 h after fertilization, a solid mass of blastomeres, constituted the blastoderm that had the shape of a convex disc toward the animal pole (blastodisc) (Fig. 1H).

Gastrula stage: started at 11 h 30 min after fertilization with the appearance of the germinal ring. Three stages were observed which were defined by percentage of epiboly. At 12 h 30 min after fertilization, middle gastrula stage was observed and characterized because the germinal ring covered the half of the yolk and there was a thickening of the anterior and posterior regions of the germinal ring (Fig. 2A). The late gastrula was defined when $\frac{3}{4}$ of the yolk were covered by the germinal ring at 2 h 30 min later (Fig. 2B). At 17 h after fertilization after the closing of the blastopore, the early neurula stage was identified by the formation of the embryonic axis (Fig. 2C).

Pharyngula stage: the embryo had showed defined structures such as notochord and somites. The neural tube as well as the head, tail and Kupffer vesicle were observed,

which were clearly distinguishable at 17 h 30 min after fertilization (Fig. 2D). Somites were distinguished in the body of the embryo at 19 h after fertilization, (Fig. 2E); 1h 45 min later, the embryo's pigmentation began with the appearance of melanophores (Fig. 2F). After 24 h from fertilization, the heart began to differentiate and it was observed as a slight bulge behind the head and the heartbeats were recorded (Fig. 2G).

Hatching: After 28 h from fertilization, the larvae had completed its development and the embryo is distinguished within the chorion (Fig. 2H). The heartbeat ratio was 28 beats/min at 30 h after fertilization and 1 h 45 min later hatching began with the rupture of the chorion and liberation of the embryo. The hatched yolk-sac larvae had an average of 84 heartbeats/minute and the hatching finished, when 100% of the sample totally hatched, which occurred at 40 h 40 min.

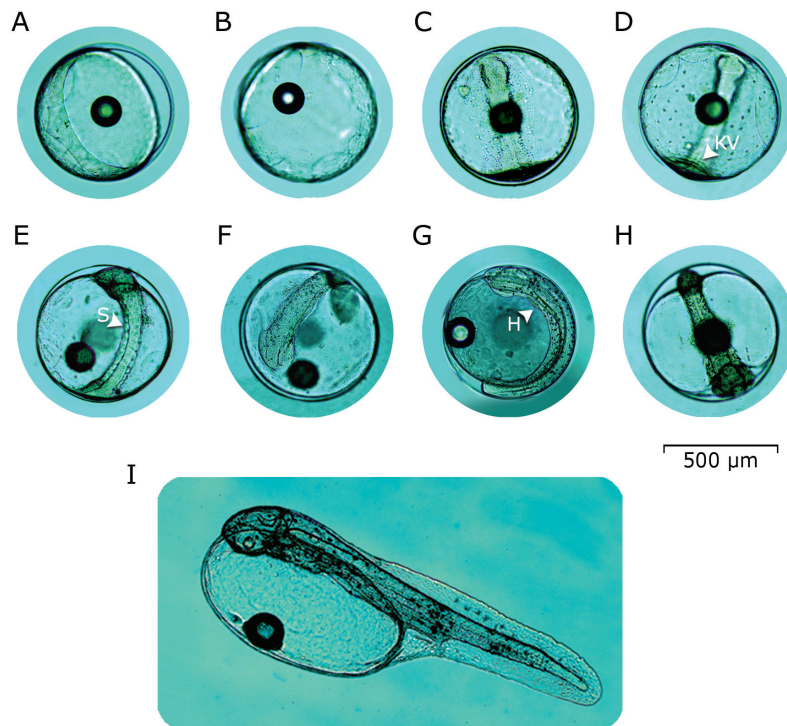


Figure 2. Embryonic developmental stages of *Anisotremus scapularis* at 19 °C. A) middle gastrula stage, B) late gastrula stage, C) early neurula stage, D) early pharyngulation with Kupffer vesicle (KV), E) embryo with differentiated somites (S), F) begins pigmentation, G) the heart (H) is differentiated, H) begins hatching, I) hatched larvae / Desarrollo embrionario de *Anisotremus scapularis* a 19 °C. A) gástrula media, B) gástrula tardia, C) néurula temprana, D) inicio de faríngula con vesícula de Kupffer, E) embrión con somitas diferenciados, F) inicios de pigmentación, G) corazón diferenciado, H) inicio de eclosión, I) larva eclosionada

Early larvae: The newly hatched larvae had an average total length of 2.558 ± 0.051 mm (Fig. 2I); yolk sac (0.553 ± 0.033 mm) spanned almost half the length of the body and the oil droplet was located anteriorly and was 0.160 ± 0.015 mm. Mouth and anal pore were closed at the time of the hatching and their opening were observed two days post hatch (dph). The digestive tract and eyes were undifferentiated and not distinguished.

DISCUSSION

The present study described the embryonic development of Peruvian grunt *Anisotremus scapularis* under laboratory conditions and the results were similar to those reported in other teleosts: *Haemulon bonariense* (Cuartas *et al.* 2003), *Gadus morhua* (Hall *et al.* 2004), *Sparus aurata* (Kamacı *et al.* 2005), *Lutjanus colorado* (Abdo de la Parra *et al.* 2014), *Lutjanus peru* (Peña *et al.* 2014).

A. scapularis develop via small and pelagic egg as other marine fishes such us cod, turbot, halibut, sea bream and sea bass (Falk-Petersen 2005). The egg diameter was in the range reported for marine teleost fish from 0.5 to 5.5 mm (Mandić & Regner 2014). The results were similar to other species such us black grunt *Haemulon bonariense*

0.80 ± 0.05 mm (Cuartas *et al.* 2003), red snapper *Lutjanus campechanus* 0.82 mm (Papanikos *et al.* 2003), yellow snapper *Lutjanus argentiventris* 0.75 mm (Muhlia-Melo *et al.* 2003), and russel snapper *Lutjanus russellii* 0.71-0.84 mm (Leu & Liou 2013). Other species of the same order have higher egg diameter such us European sea bass *Dicentrarchus labrax* with 1.162 ± 0.004 mm (Saka *et al.* 2001) and gilthead sea bream *Sparus aurata* with 1.001 ± 0.005 mm (Kamacı *et al.* 2005).

Most of pelagic eggs possess only one drop as Peruvian grunt, however there is species with no oil drop or those that have from two to a large number of oil droplets (Mandić & Regner 2014). Some authors explain that the number of oil globule are related to the deficiency in essential fatty acids (Watanabe *et al.* 1984) or to the decrease of free aminoacids (Rønnestad *et al.* 1999). The diameter of oil globule was similar to other species such us *Lutjanus campechanus* with a diameter of 0.16 mm and *Lutjanus guttatus* with 0.115 ± 0.013 mm (Rabalais *et al.* 1980, Papanikos *et al.* 2003, Boza-Abarca *et al.* 2008). However, it was smaller than those reported for black grunt *Haemulon bonariense* 0.20 ± 0.02 mm (Cuartas *et al.* 2003) and red porgy *Pagrus pagrus* 0.18-0.20 mm (Machinandiarena *et al.* 2003).

In relation to Peruvian grunt newly hatched larvae (2.558 ± 0.051 mm) there were larger than those reported for pond perch *Diplectrum radiale* 1.33 ± 0.02 mm (López *et al.* 2002), *H. bonariense* $1.42 + 0.05$ mm (Cuartas *et al.* 2003) and colorado snapper *Lutjanus colorado* 1.9 mm of length (Abdo-de la Parra *et al.* 2014). There are some studies about the size of hatched larvae in which it had been observed a positive correlation between larval size length and egg size, for example *Acipense baeri* and *Acanthopagrus schlegeli* (Gisbert *et al.* 2000, Kang *et al.* 2014).

Peruvian grunt as all teleosts have telolecithal egg with a discoidal meroblastic cleavage pattern, where cell division is restricted to a small area at the animal pole (Hall *et al.* 2004, Falk-Petersen 2005). The embryonic development of Peruvian grunt followed the same pattern as the one described for zebra fish *Danio rerio*. The authors defined each stage or period as a reference to identify part of the continuum development and named the stages as zygote, cleavage, blastula, gastrula, segmentation, pharyngula, hatching and early larvae (Kimmel *et al.* 1995). Similar phases were used to describe the embryonic development of spotted rose snapper (Boza-Abarca *et al.* 2008) and colorado snapper (Abdo-de la Parra *et al.* 2014). The duration of each phase, stage or period is species-specific and had a close relationship with incubation temperature (Radonic *et al.* 2005, Peña *et al.* 2014). For instance, the cleavage process at 19 °C including cell divisions and morula took 3 h for Peruvian grunt, while in *P. pagrus* (20 °C), *D. labrax* (17 °C) and *S. aurata* (18.5 °C) lasted 3.5, 4.30 and 4.15 h respectively (Saka *et al.* 2001, Kamacı *et al.* 2005, Radonic *et al.* 2005). Although for *H. bonariense* lasted only 1 h 20 min at 27 ± 1 °C (Cuartas *et al.* 2003).

The blastula stage identified by the mound of cells similar to a solid half ball in the animal pole (Muhlia-Melo *et al.* 2003) was observed 4 h after fertilization in Peruvian grunt. Just as cleavage process, the blastula stage occurred at different hours after fertilization in each species for example at 8.30 h European sea bass (Kamacı *et al.* 2005), 6 h gilthead sea bream (Abdo-de la Parra *et al.* 2014) and 2.14 h Pacific red snapper *Lutjanus peru* (Peña *et al.* 2014). The gastrula stage is divided in three phases, of which middle and late gastrula are observed in *A. scapularis*. The difference between each one lay in percentage of epiboly, had been middle gastrula with 50%-epiboly and late gastrula ~75%-epiboly (Kimmel *et al.* 1995). The pharyngula stage included the formation of structures such as optical capsules, Kupffer vesicle and somites, and the appearance of melanophores for pigmentation. All of these structures were observed for Peruvian grunt and matched with those described for *D. radiale* (López *et al.* 2002), *H. bonariense* (Cuartas *et al.* 2003), *L. campechanus* (Rabalais *et al.* 1980), *L. guttatus* (Boza-Abarca *et al.* 2008), *L. peru* (Peña *et al.* 2014).

The incubation time for of Peruvian grunt was 31-40 h at specific temperature of 19°C, this time is specie-specific and several authors documented that the temperature is one of the main environmental factors that influence the time and percentage of hatching in marine fish (Bobe & Labbé 2010). In some species, this influence was observed for example spotted rose snapper *L. guttatus* were 15 h at 26.3-28.2 °C (Boza-Abarca *et al.* 2008) and 21 h at 25-26 °C (Alvarez-Lajonchère *et al.* 2012), for Pacific red snapper *L. peru* were 23, 20 and 18 h at 26, 28 and 30 °C, respectively. These results showed that the time elapsed from fertilization to hatching is inversely proportional to temperature (Peña *et al.* 2014).

Knowledge of different embryonic stages, the sequence of divisions, the shape and size of the blastomeres, the number and size of the oil drop and the time of formation of the body were investigated in the present study. Embryonic development of Peruvian grunt was morphologically similar to other species of the same order. The results obtained are the first report for this species. The demonstration of the feasibility of incubating eggs and normal development in laboratory conditions demonstrate its potential for aquaculture. Likewise, the present investigation could be used as a tool for the quality assessment of future batches of eggs and as a basis for future research taking into considerations the environmental factors. Finally, the description of embryological stages provide an important reference point for the reared conditions during larviculture.

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