

# The Cleavage and Embryonic Phase of Gilthead Sea Bream (*Sparus aurata* Linnaeus, 1758) Eggs

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**Özet:** *Çipura* (*Sparus aurata* Linnaeus, 1758) yumurtalarının bölünme ve embriyonik safha gelişimi. Bu çalışmada, çipura (*Sparus aurata* L.) yumurtalarının bölünme ve embriyonik safha gelişimleri incelenmiştir. Denemeler 18.5°C sıcaklıkta yapılmıştır. Yumurtaların ve yağ damlalarının ortalama çapları sırasıyla 1.001±0.005 mm ve 0.217±0.001 mm olarak tespit edilmiştir. Yumurtalardaki embriyolojik gelişimleri erken gelişim safhalarında 15 dakikada sonrasında ise saatte bir gözlenmiştir. Ayrıca temel morfolojik gelişim farklılıkları ayrıntılı fotoğraflar ile belirlenmiştir. Açılım oranı, 18.5°C de %84 ve %89 aralığında tespit edilmiştir. Larvalar bu sıcaklıkta 53:00 saatte yumurtadan çıkmışlardır.

**Anahtar Kelimeler:** Çipura, yumurta, hücre bölünmesi, embriyonik faz.

**Abstract:** The cleavage and embryonic phase of gilthead sea bream (*Sparus aurata* L.) eggs has been investigated. The experiments have been carried out at 18.5°C. The average diameters of eggs and oil globule were determined approximately 1.001±0.005 mm and 0.217±0.001 mm, respectively. Embryonic development was observed every fifteen minutes until morula stage and then hourly interval. Also basic morphological development changes were examined by detailed photographs. The hatching rates were determined between 84% and 89% at 18.5°C. Hatching of larvae took 53:00 hours at this temperature.

**Key Words:** Gilthead sea bream, egg, cleavage, embryonic phase.

## Introduction

The sea bream has a wide distribution from the Mediterranean to Atlantic Ocean and this teleost fish has recently become an important subject of aquacultural researches because of its economic value. At present, the commercial production of gilthead sea bream has become an important aquacultural resource in the Mediterranean countries.

Various studies have been conducted on the properties and the development of sea bream eggs. It was reported that survival rate predominantly depended on egg size and eggs obtained by hormonal treatment were relatively smaller in size (Boulineau, 1974; Nash va Kuo, 1975). Feeding of the broodstock under favourable conditions has a major impact on the quality of egg (Bromage ve Roberts, 1995). It was also reported that an increase of 2-3‰ in salinity during incubation would be risky (Freddi, 1985).

The main factor affecting the rate and the quality of the embryonic development is temperature. Going beyond optimal limits during incubation leads to the deterioration of the cellular symmetry and the breaking of the oil globule; it also causes mass mortality and consequently a drop in the rate of larvae production during gastrulation (Jennings and Pawson, 1991). In this study, cleavage and embryonic phase of 18.5°C, which is accepted as the optimal limit of temperature for incubation, have been investigated.

## Materials and Methods

This study was conducted at Ege University Fisheries Faculty Aquaculture Department. The eggs were obtained from wild breeders and hormonal applications were not employed. The eggs were stocked in the incubator with a volume of 50 l (mesh size, 425µ) as 2500 egg/l. The experiment was carried out at 18.5°C. During the experiment, the flow rate into the tanks was 5% per hour. Experiments took place in complete darkness and were triplicate.

Before the eggs used during the experiment were placed in the incubators, 30 samples were taken, and the mean egg and oil globule diameter was measured and standard error calculated. After statistical analyses were done, eggs were distributed among the incubators. In order to determine the common embryonic developments, 30 eggs were taken from each incubator in every fifteen minutes until morula stage and then hourly intervals. Whenever there was an evident difference during the embryonic development, the photographs were taken before or after the due time. At the end of the study, the volumetric method was used to determine the survival rate and stocking of eggs. Also during the study, most basic morphological changes such as formation of otoliths, rhythmic heartbeat, observation of optic lens etc., were taken detailed photographs.

Each group were compared between them by the significance test of the difference between two percentages in

independent groups and given within a 95% confidence interval (Koray, 1993). When the difference between the mean values was  $p > 0.05$ , it was accepted as insignificant.

## Results

During the incubation, the salinity of natural seawater was 36.6–37.4‰. The oxygen levels ranged between 6.6 and 7.8 mg/l. The diameter of the average egg was determined as  $1.001 \pm 0.005$  mm.

During the incubation a two-cell stage was observed at 18.5°C, 1:15 h after fertilization (Figure 1-1A). Four-cell stage was observed at 1:45 h (Figure 1-1B). An 8-cell stage appeared after 2:00 h (Figure 1-1C). At 2:30 and 3:00 h, 16-

cell and 32-cell stage were recorded respectively. (Figure 1-1D, 1E). It was difficult to observe the other symmetrical divisions, but they continued to divide. During the incubation, morula stage was observed at 4:15 h after fertilization (Figure 1-1F). Then, high blastula stage started at 6:00 h (Figure 1-1G). The starting of gastrulation stage was determined 10:00 h after fertilization (Figure 1-1H). Also, gastrulation 1/2 was determined after 12:00 h. (Figure 1-1J). After 16:00h from fertilization, neurula stage was observed clearly (Figure 1-1K). Then, the embryo started to become denser and, after 18:00 h, the embryo profile was barely visible (Figure 1-1L). The closing of blastopore was established 19:00 h after fertilization (Figure 1-1M).

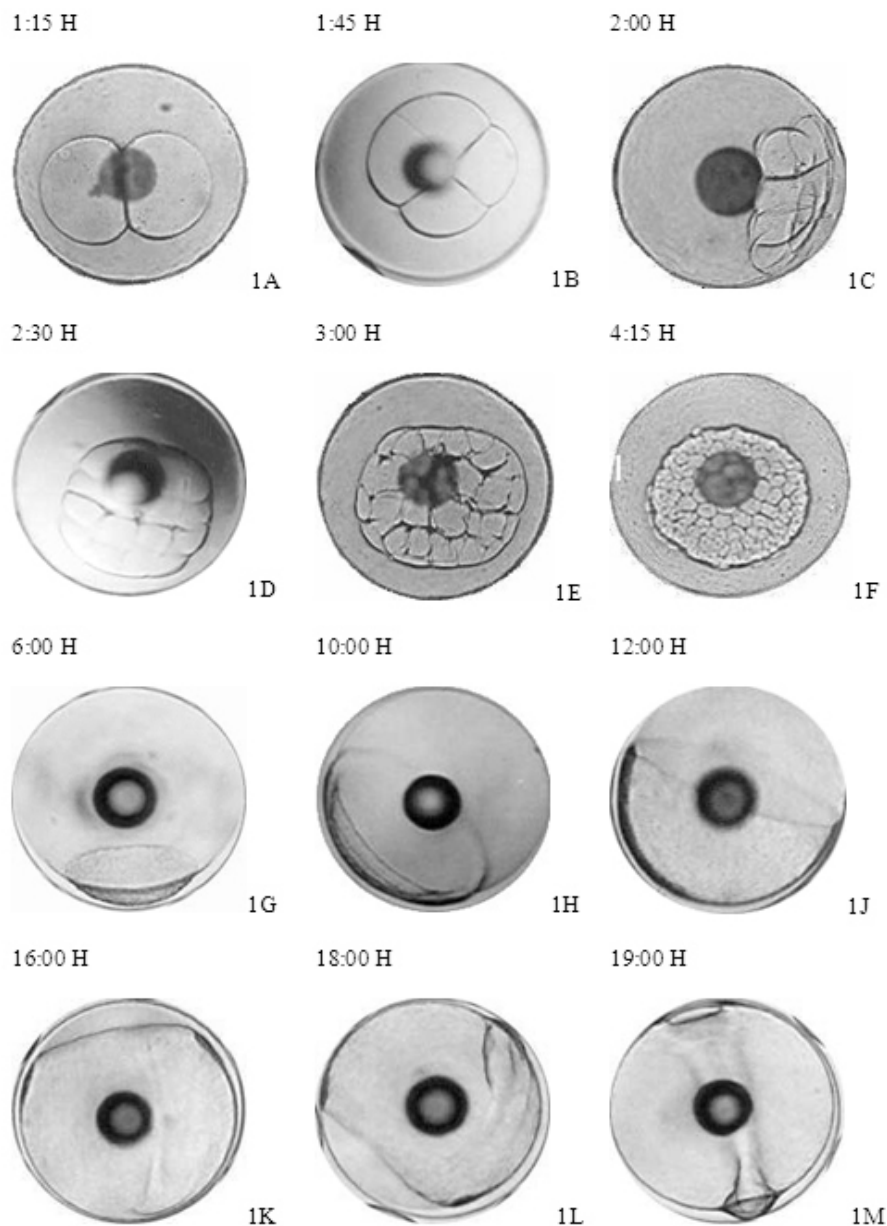


Figure 1. The embryonic development of sea bream between 00:00 h and 19:00 h (Original).

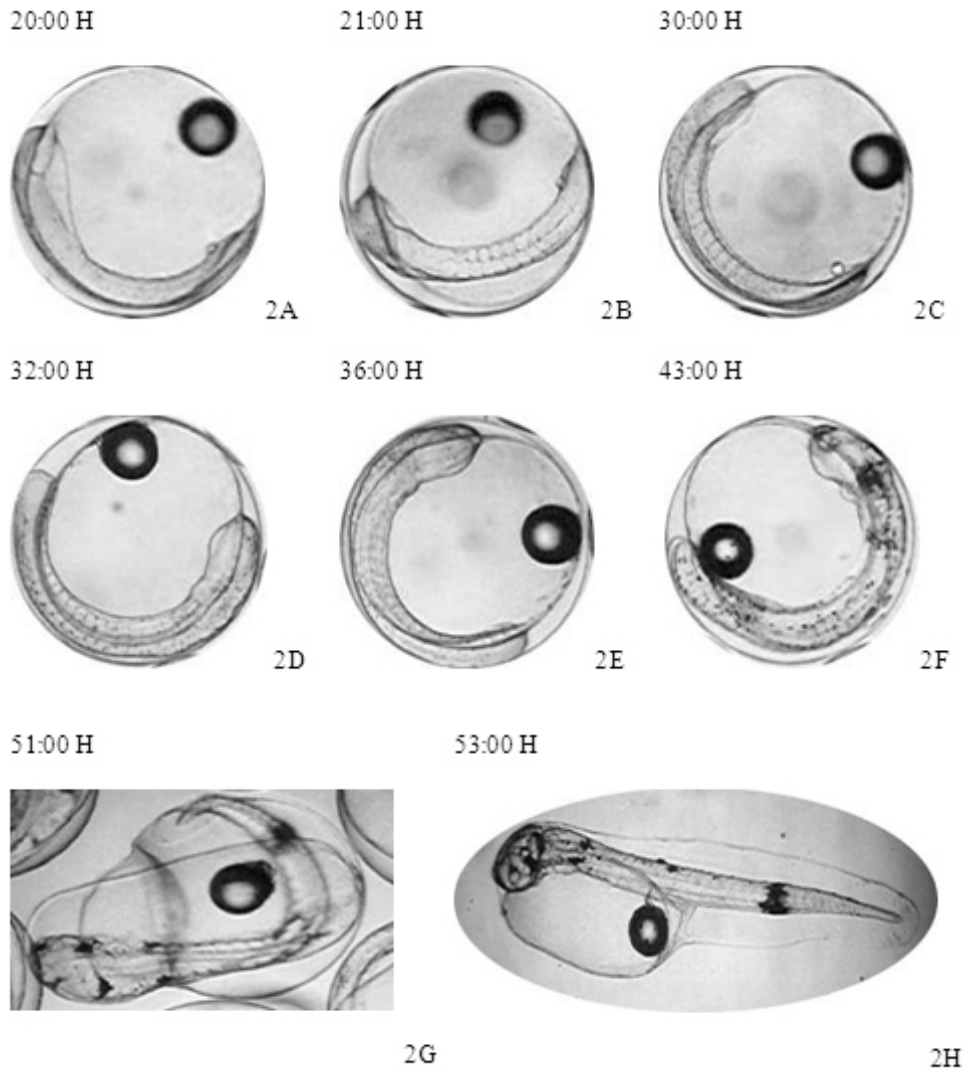


Figure 2. The embryonic development of sea bream between 32:00 h and 53:00 h (Original).

20:00 h after the fertilization, 5-6 couples of somits and kupffer apparatus were observed (Fig.2-2A). The appearance of first pigmentation was determined at 21:00 h after fertilization (Fig.2-2B). The first appearance of heart was observed at 30:00 h (Fig.2-2C). 32:00 h later, the primordial fin started to form (Fig.2-2D). Then, the formation of optic lens was determined at 36:00 h (Fig.2-2E). Besides, increasing of pigmentation was observed clearly at 43:00 h after fertilization (Fig.2-2F). 51:00 h after the fertilisation of the 10% of the larvae, and 53:00 h later, 100% of them splitted the corion with the help of the enzyme secretion, excreted from the cranium of larvae and released from the egg (Fig.2-2G, 2H). Morphogenesis developments (head, optic cup, somits and kupffer apparatus, primordial fin, otoliths, rhythmic heartbeat) during the study were shown in Figure 3. Hatching rates were determined 84, 86 and 89% at 18.5°C. There were no significant statistically differences on embryonic developments and hatching rates between groups at 18.5°C ( $p>0.05$ ).

## Discussion

The ripe sea bream egg is a typical sparidae egg. The average diameters of eggs and oil globule were determined approximately  $1.001\pm 0.005$  mm and  $0.217\pm 0.001$  mm, respectively. Egg and oil globule diameters measured in this study were relatively similar with the other author's results (Uçal, 1983; Glamuzina *et al.*, 1988; Camus and Koutsikapoulos, 1984). It might be thought that natural conditions (temperature, food, geographic location) and broodstock management (salinity, temperature, nutrition) in captivity could be effected broodstock and egg quality. The temperature levels varied between 18.3°C and 18.7°C. Also oxygen rates were measured and determined minimum 6.6 mg/l and maximum 7.8 mg/l during incubation. Salinity levels have never been decreased up to the 36‰. Temperature, oxygen and salinity differences in this study were kept within the values at which embryonic developments

in nature usually occurred, and thus it was attempted to prevent their adverse effects on the incubation of the eggs. As a result of these preventions, there have no observed abnormalities during incubation. When the egg completed its embryonic development, the initial hatching period for 10% and 100% hatching observed after 51:00h and 53:00h at 17°C. Polo et al. (1990) and Uçal, 1983 explained that 100% hatching was observed after 52:00h and 46:00h at 18°C and

20°C, respectively, 88:00h at 14°C (Polo et al., 1990), 50:10h at 18°C (Glamuzina et al., 1988), 50:33h at 19.5°C (Dujakovic and Glamuzina, 1989), 70:00h at 18.5°C (Camus and Koutsikopoulos, 1984) and 60:00h at 16°C (Alpaz et al., 1989) (Table. 1). Hatching time was found differentially with Camus and Koutsikopoulos (1984) despite of incubated at same temperatures.

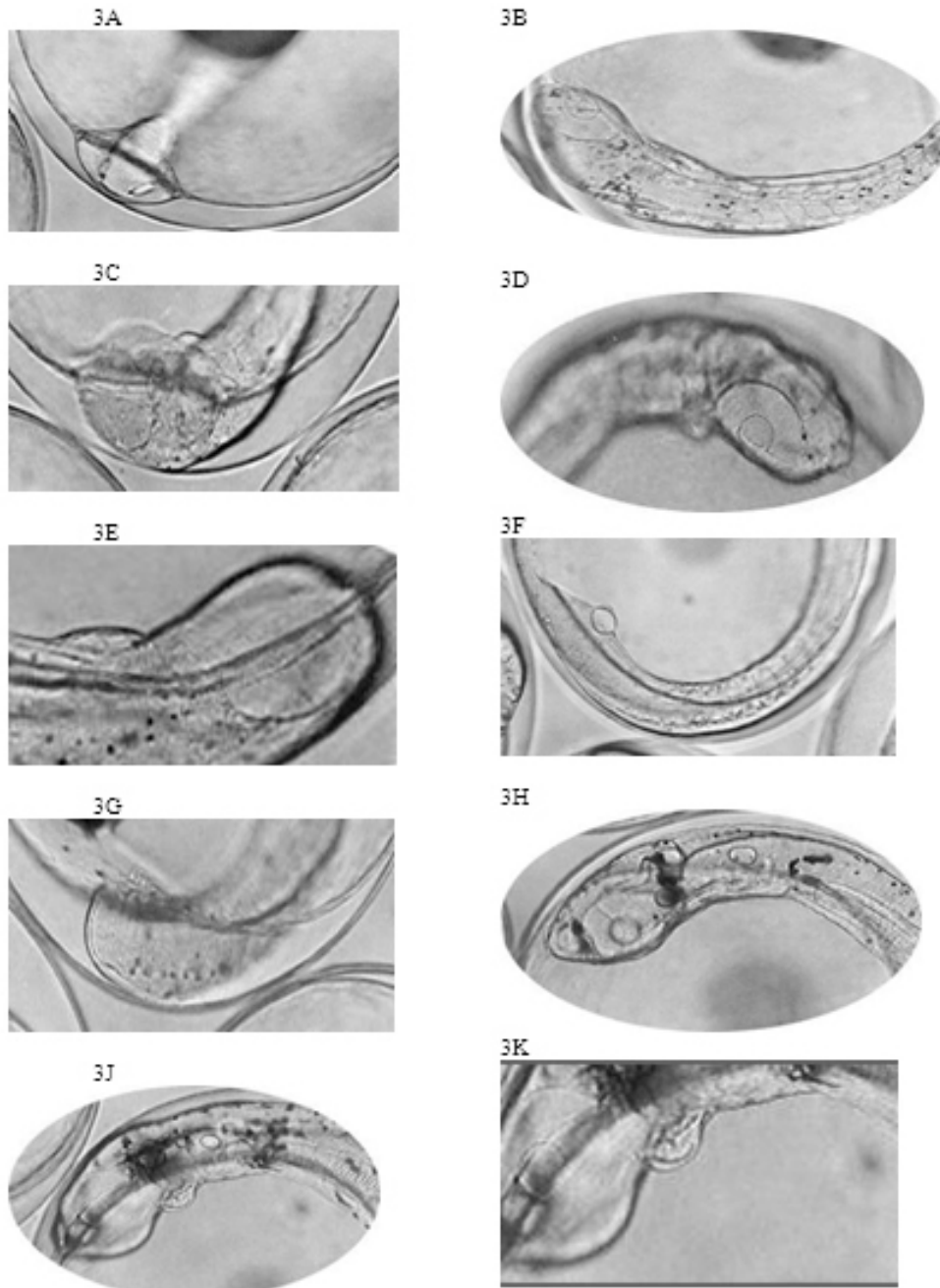


Figure 3. Basic morphological development changes of eggs. 3A: formation of head; 3B: appearance of optic cup; 3C: optic cup; 3D: somits and kupffer apparatus; 3E: first appearance of heart; 3F: optic lens; 3G: primordial fin; 3H: appearance of otoliths; 3J: increasing pigmentation of head and 3K: rhythmic heartbeat.

Finally, embryonic development of gilthead sea bream eggs at culture conditions was investigated and development stages were photographed hourly intervals. Generally, gilthead sea bream spawns sequentially during the day in spawning period. For this reason, whenever careless cleaning

of the collector, remaining egg would be mixed with freshly spawned led to differences hatching time. To determine of hatching time, it is important that quite stages of eggs should be known for transfer and/or successfully hatching.

Table 1. The comparison of embryonic developments. Results\* (Results of this study), 1\*. Uçal (1983), 2\*. Camus and Koutsikopoulos (1984), 3\*. Glamuzina and Dujakovic (1988), 4\*. Dujakovic and Glamuzina (1990), 5\*. Polo and Yufera (1991). 6\* Albaz (1988).

Stages of Embryonic Development	Resources and Experimental Temperatures								
	Results*	1*		2*		3*		4*	
	18.5	18	20	18.5	18	19.5	14	16	
2 <sup>nd</sup> Blastomere	1:15	0:45	0:45		1:25	1:28		0:45	
4 <sup>th</sup> Blastomere	1:45	1:00	1:00		1:35				
8 <sup>th</sup> Blastomere	2:00	1:45	1:45					1:45	
16 <sup>th</sup> Blastomere	2:30								
32 <sup>th</sup> Blastomere	3:00				2:20				
Morula	4:15	3:15	2:45		3:35	5:39		3:15	
High Blastula Stage	6:00	5:30	4:30						
Starting of Gastrulation	10:00	8:00	6:30	9:00	6:10	12:38		10:00	
Gastrulation ½	14:00	11:00	9:15					11:00	
Neurula	16:00								
Observation Embryo Profile	18:00			28:00	23:50	23:44		25:00	
Closing of Blastopore	19:00	25:00	23:30						
Formation of Somits and Kupffer	20:00	28:00	26:30					28:00	
Appearance of Pigmentation	21:00	32:00	30:00						
Appearance of Heart	30:00								
Formation of Primordial Fin	32:00								
The Formation of Optic Lens	36:00								
Increasing of Pigmentation	43:00	36:00	34:00						
Hatching (% 10)	51:00								
Hatching (% 100)	53:00	52:00	46:00	70:00	50:10	50:33	88:00	60:00	

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