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Research Article

Embryonic Development of Common Dentex (*Dentex dentex* L.) Eggs

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Abstract: The embryonic development of common dentex eggs was investigated in detail in aquacultural conditions at constant temperature. The experiments were carried out at 18 ± 0.2 ºC. The average diameters of the eggs and oil globule were 1.032 ± 0.008 mm and 0.233 ± 0.002 mm, respectively. Embryonic development was observed every 15 min until the morula stage and then at hourly intervals. The morula stage, gastrula stage, neurula stage and formation of somite and kupffer's vesicle were determined 04:15 h, 10:00 h, 17:00 h and 23:00 h after fertilization, respectively. The hatching rates were 82%-86% at 18 ± 0.2 ºC. Hatching of the larvae took 56.30 h at 18 ± 0.2 ºC.

Key Words: Common dentex, egg, embryological development, incubation

Sinagrit (*Dentex dentex* L.) Yumurtalarının Embriyonik Gelişimi

Özet: Sinagrit (*Dentex dentex*) yumurtalarının embriyolojik gelişimleri akuakültür ortamında sabit sıcaklıkta ilk defa detaylı olarak incelenmiştir. Deneme 18 ± 0,2 ºC de sıcaklıkta yapılmıştır. Yumurtaların ortalama çapları 1,032 ± 0,008 mm., yağ damlası çapı 0,233 ± 0,002 mm olarak tespit edilmiştir. Yumurtalardaki embriyolojik gelişim erken safhalarda 15 dakikada sonrasında ise saatte bir gözlenmiştir. Morula, gastrula, neurula ve somit-kupfer şekillenmesi döllenmeden sonra sırası ile 04:15, 10:00, 17:00 ve 23:00 saatlerde tespit edilmiştir. Yumurtaların açılım oranı 18 ± 0,2 ºC de %71,6-78,4 arasında saptanmıştır. Larvalar 18 ± 0,2 ºC de 56,30 saatte yumurtadan çıkmışlardır.

Anahtar Sözcükler: Sinagrit, yumurta, embriyolojik gelişim, inkübasyon

Introduction

Dentex dentex is a Sparidae fish of high commercial value, which is newly introduced in aquaculture and has attracted wide interest of many scientists in respect to its reproduction and physiology (1,2), larval rearing (3,4), nutrition (5-7), and morphological and osteological features (8,9). Embryonic development has been described, but the demonstrated results were not detailed (3,10-12). Information on the embryonic development stages is lacking.

The main factor affecting the rate and the quality of embryonic development is temperature (13). Going beyond optimum 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 an oil globule in the rate of larvae production during gastrulation (14). In related studies, different hatching rates of the larvae have been investigated (15).

This paper describes the embryonic development of common dentex (*Dentex dentex*) eggs. The objectives were to describe the embryonic development stage, thus contributing to marine culture studies (progress in the identification of the planktonic stages) and to the introduction of this valuable species into commercial aquaculture.

Materials and Methods

The eggs were taken from wild breeders in natural season. Common dentex broodstock, 8 females (2.4 ± 0.4 kg mean body weight) and 8 males (1.3 ± 0.2 kg mean weight), were selected from wild breeders and stocked in an 8 m^3 tank with a seawater supply of 35 l/min. Frozen cuttlefish (*Sepia officialis*) and leander

squilla (*Palaemon elegans*), the primary food source, were provided daily. Eggs spawned by fish group were immediately collected in a recuparator. Following the fertilization the viable buoyant eggs were separated from the dead sinking eggs. After statistical evaluations, the eggs were placed in single incubators (Pyrex beakers) (mesh size, 300 µ) of 15 l water volume as 2000 egg/l. The incubators were placed in the tank of 1 m^3 water volume. Water flow rate was adjusted in a way as to change 15% of the total volume of the beakers in an hour. Temperature was kept at 18 ± 0.2 °C, and the salinity at a level of 37‰ during the experiment. The aeration rate was 40 ml/min.

Incubation took place in complete darkness and were triplicated. Diameter of the egg and oil globule was measured to the nearest 0.025 mm using an ocular micrometer. To determine the common embryonic developments, 100 eggs were taken from each incubator and were determined every 15 min until the morula stage and then at hourly intervals. Whenever there was an evident difference during the embryonic development, photographs were taken before or after the due time. The volumetric method was used to determine the hatching rate and stocking of the eggs. After a homogeneous mixture of larvae and eggs in the incubators was obtained at the end of the experiment, 5 samples were taken with a 10 ml pipette and the average of the 3 samples was found to determine the survival rates of the larvae. Each group were compared between them by Fischer chi-square test (the significance test of the difference between 2 percentages in independent groups) and given within a 95% confidence interval. When the difference between the mean values was $P > 0.05$, it was considered insignificant.

Results

Spawning commenced on April 3 2003 and continued to June 5 2003, with a peak from April 24 to May 28, corresponding to temperatures ranging from 16 to 19 ºC. Oxygen concentration was 6-7 mg/l and pH was around 7.9. Ammonia and nitrite were always <0.01 mg/l. The total number of eggs collected during the spawning season was estimated at 24.55 million, of which 4.68 million (19.08%) were sinking eggs and 19.87 million (80.92%) were buoyant eggs.

Common dentex eggs are buoyant, transparent, and typical of sparid fish. Eggs ranged in diameter from 0.947 to 1.062 mm with a mean of 1.032 ± 0.008 mm eggs containing a single unpigmented oil globule were positively buoyant (Figure 1-1A). Oil globules ranged from 0.233 ± 0.002 mm to diameter. Fertilized eggs have a small periviteline space. Most fertilized eggs floated at 37‰ salinity and 18 ºC at the surface.

During the incubation a 2-cell stage was observed at 18 °C, 1.15 h after the fertilization. The two blastomeres are highly rounded and symmetrical just after cleavage. The dimensions of each blastomere were 444 x 266 µm. (Figure 1-1B). The second division occurred 1.45 h after the fertilization and a 4-cell stage was observed. The dimensions of each blastomere were 266 x 222 µm (Figure 1-1C). An 8-cell stage appeared after 2.15 h. The dimensions of blastomeres were 115-158 µm (Figure 1-1D). 16-cell stages were observed at 2.45 h and 3.15 h after fertilization, respectively, and the dimensions of blastomeres were 88-112 µm (Figure 1-1E, 1F). It was difficult to observe the other symmetrical divisions, but they continued to divide. The morula stage was observed after 4.15 h (Figure 1-1G). High blastula and flat blastula stages were observed and 6.30 h and 8.30 h, respectively (Figure 1-1H, 1J). The gastrulation started 10.00 h after the fertilization and gastrulation 1/2 appeared after 16.00 h (Figure 1-1K, 1L). The neurula stage was observed after 17.00 h (Figure 1-1M). Two hours after this period, observation embryo profile appeared (Figure 2-2A). Closing of the blastopore was observed after 22.00 h (Figure 2-2B). 23.00h after the fertilization, 5-6 somite stage, kupffer vesicle and appearance of pigmentation were observed (Figure 2-2C). 31.00 h later, the formation of the heart started (Figure 2-2D). The primordial fin started to form 34.00 h later, and 36.00 h later, the optic cup formed (Figure 2-2E, 2F). Increasing pigmentation was observed 41.00 h after fertilization (Figure 2-2G). 49.00 h after the fertilization 10% of the larvae and 50.00 h later 100% of them split the corion with the help of the enzyme secretion, excreted from the cranium of larvae and released from the egg fertilization (Figure 2-2H, 2J, 2K).

Hatching rates were 82%, 85% and 86% in incubators at 18 °C. There were no significant statistically differences in embryonic development stages and hatching rates between the incubators at 18 °C ($P > 0.05$).

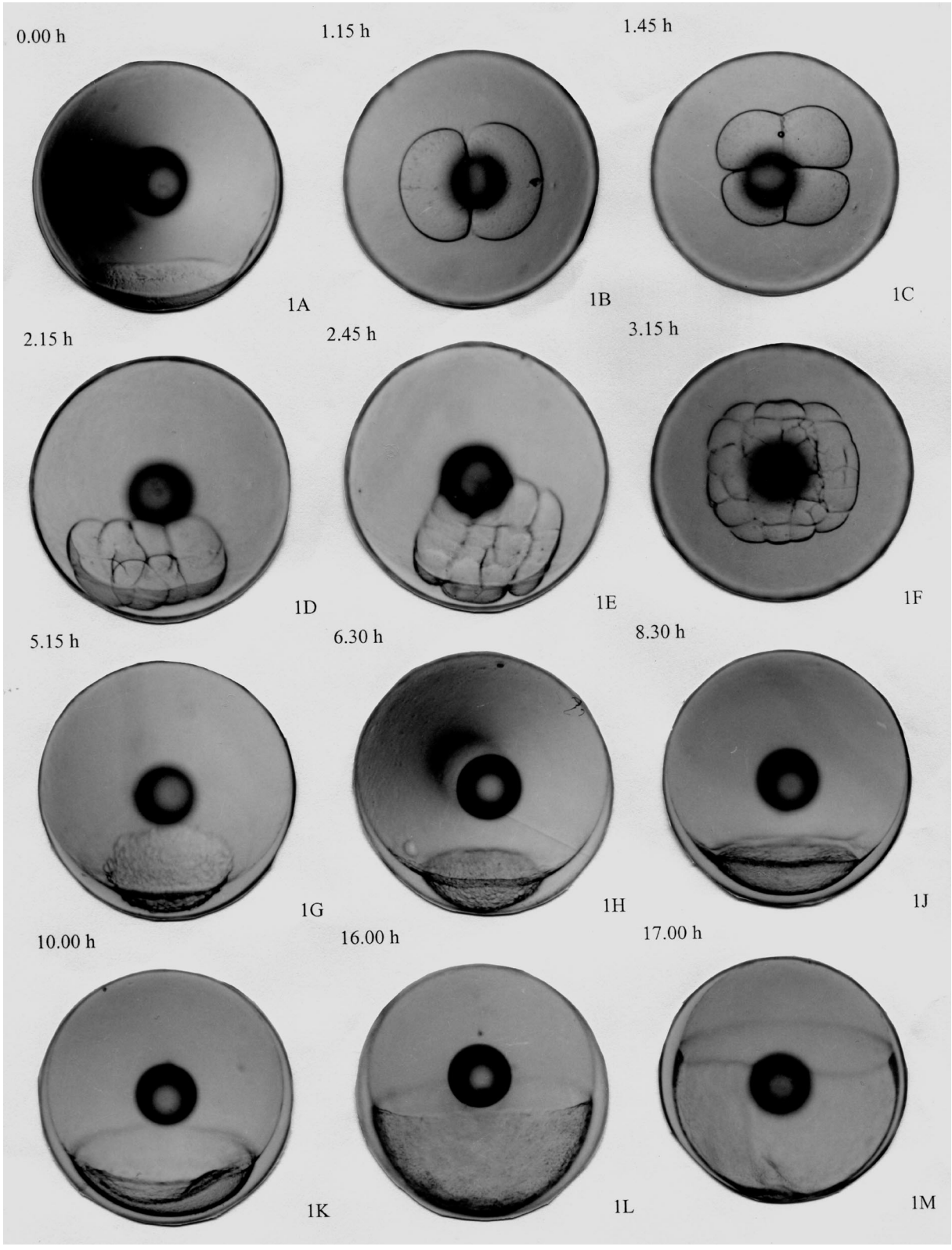

Figure 1. The embryonic development of common dentex (*Dentex dentex* L.) eggs at 18 °C.

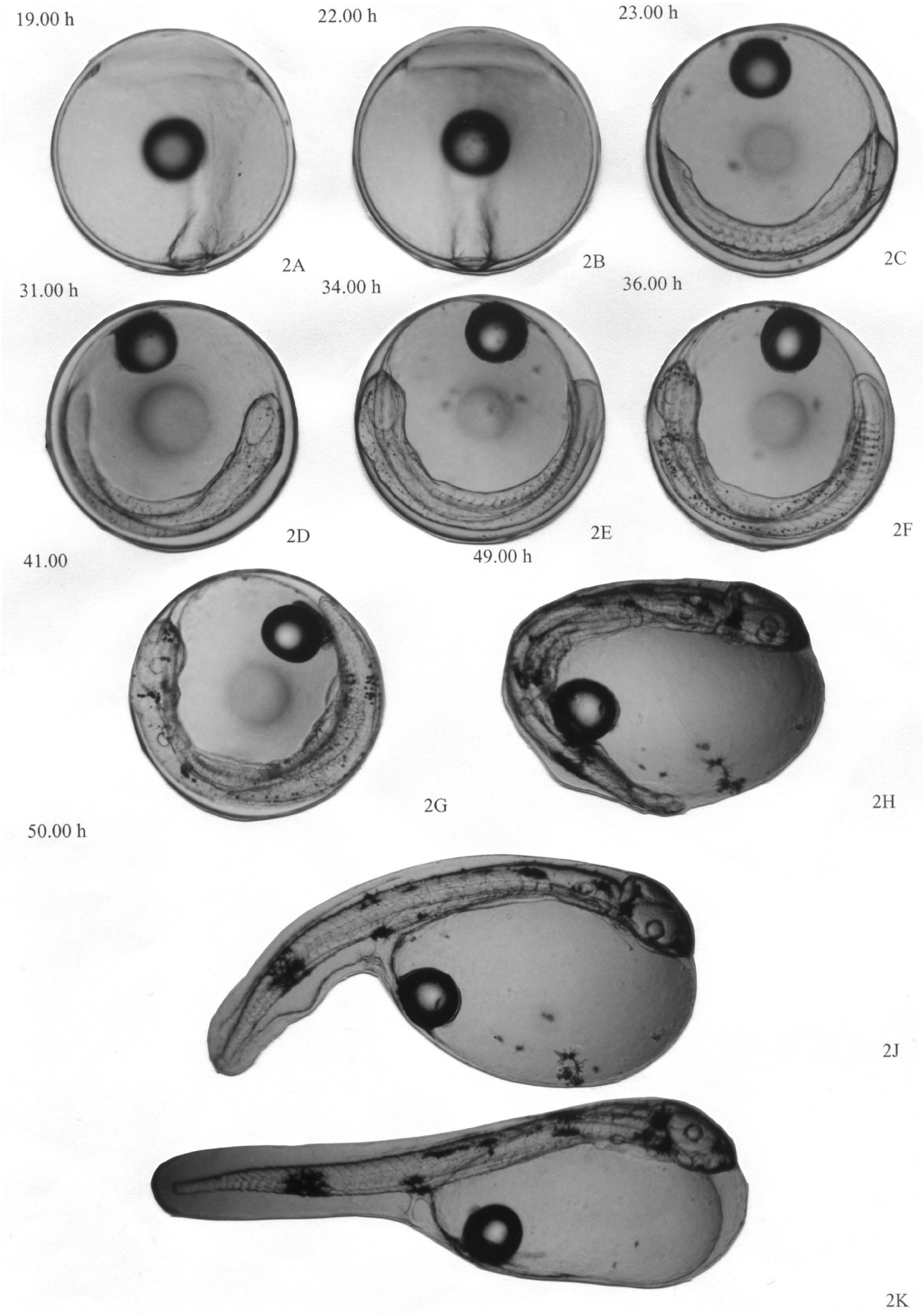

Figure 2. The embryonic development of common dentex (*Dentex dentex* L.) eggs at 18 °C.

Discussion

The early development of fish is strongly affected by incubation temperature. Generally, lower temperature retards the rate of embryonic development of fish and higher temperature accelerates it. Production of viable larvae is a better indicator of temperature effect than total hatch because total hatching rate, which includes abnormal larvae, does not provide the information required for prediction of the percentage of larvae that may achieve exogenous feeding and successive normal development. The temperature levels were 17.8-18.2 °C during incubation. These values were different from some studies (10,12,15), which used 18 °C.

The minimum oxygen rate in the incubators was determined as 6.4 mg/l and maximum 7.2 mg/l. It has been reported that oxygen levels of 6-7 mg/l in the incubation of marine fish eggs have affected the embryonic development in a positive way (13,14). In this study, the oxygen level in all the incubators was kept within specified limits. Oxygen, salinity, ammonia and nitrite differences in this study were kept within the values at which embryonic developments in nature usually occurred, in an attempt to prevent adverse effects on the incubation of the eggs.

The embryonic development of common dentex eggs was investigated in detail for the first time in this study. When the egg completed its embryonic development, the initial hatching period for 10% was determined as 55.30 h at 18 °C. Hatching of 50% occurred after 56.30 h at 18 °C. Jug-Dujakovic et al. (10) reported 79.10 h and 80.30 h for hatching rate at 17 ºC of 10% and 50%, respectively. Hatching of 50% was observed after 55:00 h at 16-18 °C (12), and 61 h at 16.6 °C (16) (Table).

Table. The comparison of embryonic developments. Result* (Result of this study); 1*, 10; 2*, 12; 3*, 15.

Stages of Embryonic Development	Resources and Experimental Temperatures			
	Result*	1*	2*	3*
	18 ˚C	17 ˚C	16-18 ˚C	16.6 ˚C
2-Cell Blastomere	1:15 h	1:45 h		
4-Cell Blastomere	1:45 h	2:26 h		
8-Cell Blastomere	2:15 h	2:50 h		
16-Cell Blastomere	2:45 h			
32-Cell Blastomere	3:15 h			
Morula	4:15 h	5:10 h		
High Blastula Stage	6:30 h			
Flat Blastula Stage	8:30 h			
Starting of Gastrulation	10:00 h	10:30 h		
Gastrulation 1/2	16:00 h			
Neurula	17:00 h			
Observation Embryo Profile	19:00 h	30:00 h		
Closing the blastopore	22:00 h			
The Formation of Somite and Kupffer's vesicle	23:00 h	44:30 h		
The Appearance of Pigmentation	23:00 h			
The Appearance of Heart	31:00 h			
The Formation Primordial	34:00 h			
The Formation of Optic Cup	36:00 h			
Increasing of Pigmentation	41:00 h			
Hatching (10%)	49:00 h	79:10 h		
Hatching (100%)	50:00 h	80:30 h	55:00 h	58-61 h

In this study, the hatching rates were determined between 82% and 86%. When these results were compared with the hatching of *S. aurata, P. major* (17) and *D. labrax* (13) the hatching rates were observed to be similar. Therefore, it is important that this photograph series provides certain results to hatcheries taking eggs from different farms, and supplies them with information on which period the eggs are at and when they are expected to hatch. Finally, this paper were to describe the detailed embryonic development of common dentex eggs and assist in the identification of planktonic stages.

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