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RESEARCH PAPER

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Embryonic Development Stages of Pink Dentex *Dentex gibbosus* (Rafinesque, 1810) Eggs in Aquaculture Conditions

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Abstract

In this study, the embryonic development of pink dentex (*Dentex gibbosus* Rafinesque, 1810) eggs were investigated in detail by using groundwater in aquacultural conditions. Groundwater at 35‰ salinity and 19.5°C temperature was used during the experiment. The eggs were obtained by applying hormone to the *D. gibbosus* broodstocks which were caught in nature and grown in a hatchery. The average diameters of the eggs and oil globule were 0.7252±0.020 mm and 0.1517±0.015 mm respectively. In order to investgate the embryonic developmental stages of the eggs, photographs were taken in every 15 minutes until the stage of the morula, and then, at hourly intervals. Following the fertilization of the eggs, the morula stage, the gastrula stage, the neurula stage, the somit formation and the kupffer vesicle formation were occured at 3.42th, 8.30th, 14.21th, 18.13th hours, respectively. Hatching occured after 39.41 hours from fertilization. Hatching rates were determined as 86%, 8%, and 89% at 19.5±0.3°C. The length of the newly hatched prelarvae were measured around 2.52±0.045 mm.

Introduction

Searching for new potential species is still keep going in order to increase the diversity of cultivated marine fish species, since the decrease in prices of two species, gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentarachus labrax*), which are heavily raised in Mediterranean aquaculture (Stephanis & Divanach, 1993). The pink dentex *Dentex gibbosus* (Rafinesque, 1810), a member of the sparidae family, is seen among the potential species to be cultivated. It is thought that this species is more advantageous than other species in terms of survival rate and growth potential which are biologically compared to other siparidaes (Katavic, Grubisic, & SkakelJa, 2000).

It is possible to see pink dentex in the stalls in Israel, Greece, Sicily, Egypt and Turkey. *D. gibbosus* is a demersal marine fish, disseminated ranging from 20 to 400 meters deep on rocky and sandy grounds, and is seen in the Mediterranean and Eastern Atlantic as well as from Portugal to Angla (Alves, Faria, Reis, Pinto, & Vieira, 2011). *D. gibbosus* which is a carnivorous, is mostly fed with crustacean, cephalopod and fish. It was seen in the studies done that sexual maturation occurred around age 4 at the rate of 50% (Alves & Vasconcelos, 2012). Although it is not seen in large individuals, the 3rd and 4th rays on the dorsal fins of juveniles are longer (Grubisic, Jelic-Mrcelic, Skakelja, Katavici, Ticina, & Siliskovic, 2007). That kind of rudimentary is described as hermaphrodites (Buxton & Garratt, 1990; Alves *et al.*, 2011). The spawning period of this species occurs during the summer months around Madeira and the most intensive spawning take place in May and June (Alves *et al*., 2011). This fish can be 1.2 m in length and 25 kg in weight (Jardas, 1996). While several studies done concerning the pinx dentex

were about reproductive biology, its production was possible to be made in controlled conditions (Fernandez-Palacios, Montenero, Socorro, Izquierdo, & Vergaram, 1994).

Eventhough the research of the embryonic and larval development of *D. gibbosus* was done in sea water, there is no studies carried out by using underground water. This study aims to determine whether pink dentex can be grown by using groundwater. For this purpose, obtaining quality eggs from broodstock of the pink dentex under controlled conditions by using groundwater and the embryonic development of the eggs, were investigated in detail.

Materials and Methods

The study was carried out in May-2016, in Muğla, Olivka Aquaculture Inc., Turkey. Groundwater from a depth of 125 meter was used in the experiment. Although the oxygen level of groundwater was initially very low, it was increased by ventilation. The broodstocks that were used in the study were caught in the North Aegean Sea 3 years ago as 4 females (3.8 kg mean weight) and 2 males (3.2 kg mean weight). The water at the rate of 83.3 l/min was added to 30 m^3 broodstock tank which was kept in the natural photoperiod (14 h light: 10 h dark). Broodstocks were fed by frozen cuttle fish (*Sepia officialis*), shrimp (*Palaemon elegans*), mackerel (*Scomber scombrus*), anchovy (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) in every other day during the year. LHRH-A (D-Ala^6, des-Gly^{10} LHRH ethylamide analog, Sigma) was applied to the broodstocks to get eggs. LHRH-A was dissolved in 0.9% sodium chloride (NaCl). Hormone was prepared a few hours before injection. Fish were anesthetized in 0.05% 2-phenoxyethanol. Fish were injected with 5 µg/kg a single doses of hormone. Hormone was aplied into dorsal musculature by using syringes. Eggs were obtained at 19.8±0.4°C. After fertilized eggs were collected in the collector, viable buoyant eggs and dead sinking eggs were separated from each other. Fertilized eggs were stocked in 350 liter incubators to be 2100 eggs per liter. 300 µm mesh size was used in the incubators was set to 100% of the volume of the incubators was changed an hour. Incubators were kept in the darkness. Aeration at a rate of 40 ml per minute was applied. The eggs were kept at 19.5±0.3°C temperature and 35‰ salinity during the incubation period, and the water in the incubation unit was controlled in terms of physical and chemical parameters.

The experiment took place were triplicate. Measurement of the diameters of egg and oil globule performed by using 0.025 mm millimetric ocular. 100 eggs were randomly taken from each incubator for the measurements. In order to follow-up the embryonic development, eggs were taken in every 15 minutes until the phase of the morula, after this they were taken in every one hour and photographed. The hatching rate and stock density of the eggs were determined by means of the volumetric method. At the end of the experiment, 5 homogenous mixture samples were taken from each incubator with a 10 ml pipette by ventilation, and the survival rates of prelarvae were determined. The obtained data were analyzed using ANOVA (analysis of variance). The results were evaluated by Duncan Multiple Comparison Test. The SPSS statistical package program was used to test whether there is any difference between the application groups (IBM SPSS 2012).

Results

The physical and chemical parameters of groundwater source used in experiment were 35‰ salinity, 19.5°C temperature, ammonia ($NH4^+$) <0.04 mg/l, nitrite (NO_2) <0.16 mg/l, pH 7.31, iron 20 mg/l, copper <0.1, turbidity <1 NTU, carbon dioxide 165.94. The eggs were taken from the broodstock that were inside the unit of broodstock between April 28, 2016 and June 27, 2016 with hormonal treatment. After 168 hours applying hormone spawning was seen. Spawning peaked from 5 June to 18 June when the temperature increased from 19.5°C to 20.03°C., pH at around 8.0, ammonium and nitrite at the rate of <0.012 mg/l. Oxygen concentration was measured 7-8 mg/l. Approximately 11.5 million eggs were collected during the spawning season. 8.7 million of these eggs (75.65%) were buoyant eggs while 2.8 million of these (24.35%) were sinking eggs. The eggs of the pink dentex was pelagic, transparent and the chorion was seen clearly (Figure 1-1). The mean diameter of the egg with a single oil globule was 0.725±0.020 mm, and varied ranging from 0.676 mm to 0.787 mm, and the diameter of the oil globule was 0.151±0.015 mm, and varied ranging from 0.126 mm to 0.188 mm. The fertilized eggs had a small perivitelline spaces, and most of them floated at 19.5°C temperature, 35‰ salinity at the surface. (The development of the egg is shown in Table 1).

The 2-cell stage was observed during the incubation at 19.6°C temperature after 1.25 h from fertilization. The two blastomeres were divided in a way of highly rounded and symmetrically. The dimensions of each blastomere were measured as 336x244 µm (Figure 1-2). The second division formed after 1.35 h from fertilization, and 4-cell stage was observed. Each blastomere was determined to be 240 x 139 µm (Figure 1-3). The 8-cell stage occured after 1.52 h from fertilization, and the dimensions of blastomeres were 180 x 137 µm (Figure 1-4). 16-cell stages were observed after 2.30 h, and the dimensions of blastomeres were 102 x 91 µm (Figure 1-5). After that, the blastomeres continued to divide symmetrically but it became very difficult to measure divisions (Figure 1-6). Morula stage appeared after 3.42 h (Figure 1-7,1-8). High blastula and

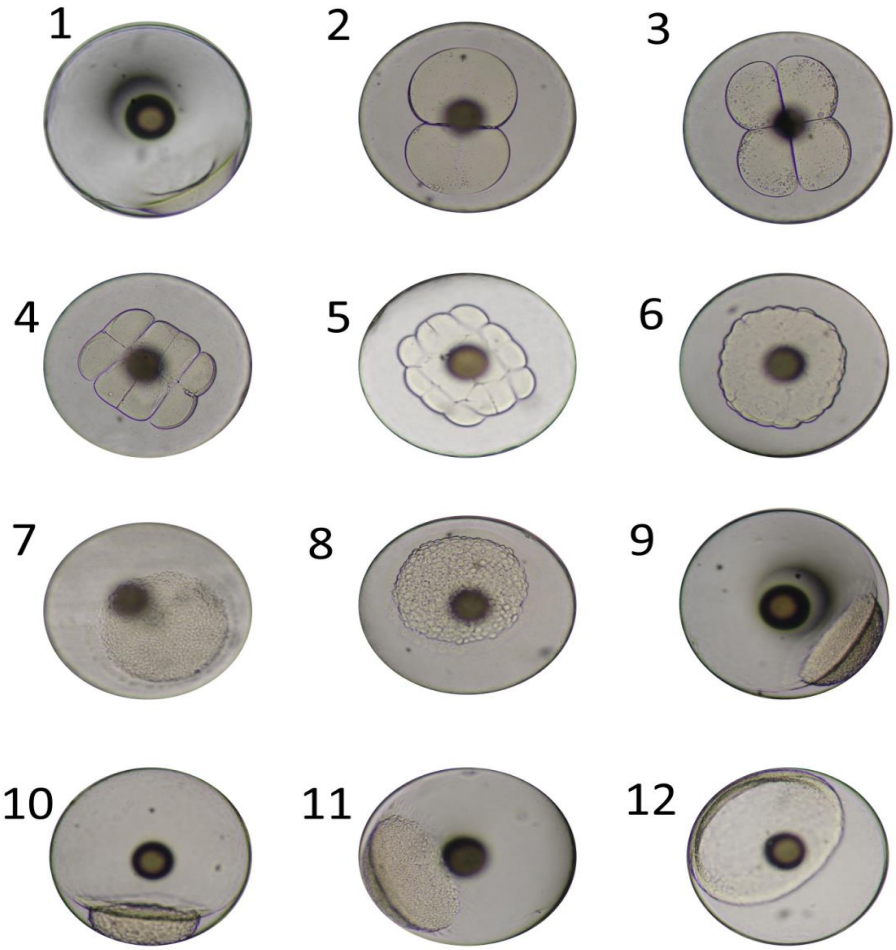

Figure 1. Embryonic development of *Dentex gibbosus* eggs (1-12))(Original).

Table 1. Chronology (in hours) of the embryonic developmental stages in *Dentex gibbosus* eggs at 19.5±0.2°C

Stage	Description	Time(hours)
Fertilization		00:00
2-Cell Blastomere	First cleavage	1:25
4-Cell Blastomere	Second cleavage	1:35
8-Cell Blastomere	Third cleavage	1:52
16-Cell Blastomere	Forth cleavage	2:30
32-Cell Blastomere	Fifth cleavage	2:45
Morula	Much more cells	3:42
High Blastula Stage	Blastodisc formation	4:22
Flat Blastula Stage		5:08
Starting of Gastrulation		8:30
Gastrulation ½		12:36
Neurula		14:21
Observation Embryo Profile		15:05
Closing the blastopore		16:00
The Formation of Somite and Kupffer vesicle		18:13
The Appearance of Pigmentation		18:15
The Appearance of Heart		21:46
The Formation Primordial		25:00
The Formation of Optic Cup		33:34
Increasing of Pigmentation		36:50
	otoliths and kaudal fin	37:15
Hatching (20 %)	Embryo tears the corion and hatching	39:00
Hatching (100 %)		39:41

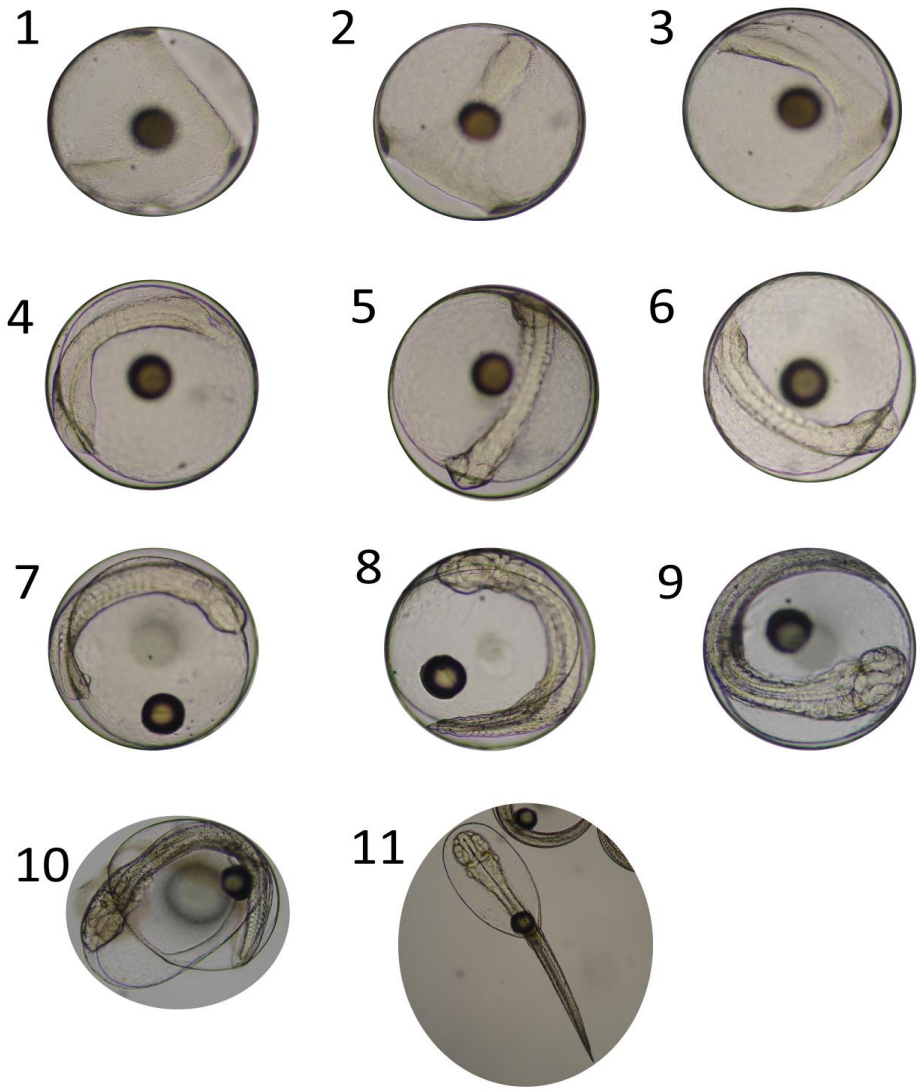

Figure 2. Embryonic development of *Dentex gibbosus* eggs (1-11)(Original).

flat blastula stages were determined after 4.22 and 5.08 h from the fertilization, respectively (Figures 1-9, 1-10). Gastrula stage was started after 8.30 h and gastrulation ½ was observed after 12.36 h. (Figure 1-11,1-12). At this phase, it was seen that blastoderm covered ½ of the egg yolk. It was passed to the nerula stage after 14.21 h from fertilization (Figure 2-1). Observation of embryo occurred after 15.05 h (Figure 2-2). Closing of blastopore stage occurred after 16.00 h (Figure 2-3). 5-6 somite stage was appeared after 18.13 h, and formation of the kupffer vesicle and pigmentation were observed.

The appereance of heart was started after 21.46 h (Figure 2-6). The promordial fin formation was observed clearly after 25.00 h from fertilization, and after 33.34 h optical cup formation occured (Figure 2-7, Figure 2-8). There was an increase in pigmentation after 36.50 h (Figure 2-9). Otoliths and caudal fin formation were clearly observed after 37.15 h. The embryo covered the ¾ of yolk-sac, and the activity began in the embryo (Figure 2-10). After 39.00 h from fertilization, 20% of the embryo and after 39.41 h from fertilization, 100% of embryo hatched out from the egg (Figure 2-10, Figure 2-11). The releasing of larvae from the egg occured with cracking and corroding of the shell by the enzyme called corion that was secreted from the head of the larvae (Figure 2-10). The image of the larvae that was emerged by cracking of the egg was in curved shape, and it took the form of a plane in the first 30 minutes of hatching from the egg. (Figure 2-2H, 2J, 2K). The mean length of the newly hatched larvae from the egg was measured as 2.52±0.045 mm.

Hatching rates of the eggs in the incubator were determined as 86.4%, 87% and 89%, respectively at 19.5±0.2°C. It was not seen any statistically significant difference between embryonic development phases and hatching ratios in incubation at this temperature range ($F=1.057$; $P=0.378$).

Discussion

In many marine hatcheries, fresh marine organisms are used in addition to commercial feeds to increase egg productivity of broodstock (Tandler, Harel,

Koven, & Kolkovsky, 1995). Minced mackerel and cuttle fish diet was given to pink dentex several days and eggs were obtained naturally from 22 broodstocks ranging in weight from 2.5 to 3 kg. Floating eggs per kg female (58200) was obtained at day 26 (Fernandez-Palacios *et al.,* 1994). In our study, 6 broodstocks ranging from 3.2 to 3.8 kg were used and they were fed with frozen cuttle fish, shrimp, mackerel, anchovy and sardines in every other day throughout the year. Eggs were obtained from broodstock by applying LHRH-A hormone. Buoyant eggs per kg female (572000) was optained in the course of spawning season.

A mature pink dentex (*Dentex gibbosus)* egg cannot be distinguished from the other sparidae eggs. The Eggs of the species such as gilthead sea bream (*Sparus aurata),* common dentex *(Dentex dentex),* sharpsnout sea bream *(Diplodus puntazzo),* red porgy *(Pagrus pagrus),* common pandora *(Pagellus erythrinus)*, white sea bream (*Diplodus sargus*) and striped sea bream *(Lithognathus mormyrus)* which are generally involved in the sparidae genus in terms of morphologically, have similar features to each other (Divanac, 1985; Firat, Saka, & Coban, 2003; Jug-Dujakovic, Dulcic, & Katavic, 1995; Klimogianni, Kalanji, Pyrenis, Zoulioti, & Trakos, 2004).

Eventhough most of the sparidae species have similar or approximate egg diameters, there may still be differences in the total lengths of the newly hatched larvae (Table 2). Although it is not a reliable method to determine which sparidae belongs to which egg by looking at the egg diameters, these differences stem from temperature, nutrition, broodstock size and geographical distribution (Chambers, Leggett, & Brown, 1989; Buckley *et al.,* 1991). Fertilized eggs were transparent, with a diameter of 0.956±0.02 mm, and a central oil globule of 0.184±0.005 mm diameter (Fernandez-Palacios *et al*., 1994). The mean diameter of the egg with a single oil globule was 0.725±0.020 mm, and the diameter of the oil globule was 0.151± 0.015 mm in present study. These describes different from ours egg size.

Temperature is one of the most important factors for fish ontogeny and directly affects the growth and development. The effects of the temperature on embryo development, eggs hatching time and ratios, nutrition and larval growth, mortality, deformity and metamorphosis are significant (Polo, Yufera, & Pascual, 1991; Koumoundouros, Divanach, Anezaki, & Kentouri, 2001). The eggs were obtained from the broodstock while the water temperature was at 19.9±0.3°C in our study. This temperature showed similarity to the previous study which is same species (Fernandez-Palacios *et al.,* 1994). These outhors described that the first cellular division took place 60 min, second cell division was apparent 80 min, the heart was seen 24 h and 40 min, the embryo showed sudden movements, with no apparent periodicity after 33 h, after fertilization. In this study, the 2-cell stage was observed after 85 min, the second division formed after 95 min, the appereance of heart was started after 21 h and 46 min, the activity began in the embryo after 39 h after fertilization. These results different from the previous study.

According to Saka, Fırat, and Çoban (2006) while the low incubation temperature decelerates and the growth in the eggs, the high temperature accelerates it and the incubation temperature is an important indicator in terms of a healthy larvae formation and high hatching ratio. Fernandez-Palacios *et al*. (1994) first reported that the hatching occurred 35 h after fertilization, at a water temperature of 20°C. Maximum hatching and survival rates *D. Gibbosus* were 94.74% and 67.82%, respectively. Newly hatched larvae were 2.097±0.12 mm in body length. In this study, during the incubation temperature was around 19.5±0.2°C, the hatching of the eggs was observed after 39 h 41 min and the hatching rates of larvae varied in the range of 86% to 89%. The length of the newly hatched larvae was measured as 2.52±0.045 mm. When these results are compared with the same and other sparidae species, i.e., *S. aurata* (Kamacı, Saka, & Fırat, 2005), *D. puntazzo* (Klimogianni, Kalanji, Pyrenis, Zoulioti, &

Table 2. Egg, oil globule and yolk-sac larvae of different sparid species

Species	Egg diameter (mm)	Oil droplet diameter (mm)	TL_0 (mm)	Reference
Sparus aurata	0.920 - 1.120	0.180 - 0.260	2.23 - 2.53	(Kentauri, 1985; Polo *et al*., 1991)
	1.001 ± 0.05	0.217 ± 0.01	_	(Kamacı *et al*., 2005)
Dentex dentex	0.958 - 0.99	0.208 - 0.240	2.17 - 2.47	(Fernandez-Palacios *et al.*, 1994)
	1.032 ± 0.008	0.233 ± 0.002	2.476 ± 1.02	(Saka *et al*., 2006)
Diplodus puntazzo	0.760 - 0.88	0.160 - 0.240	1.69 - 2.20	(Divanac, 1985; Kentouri, 1985)
	0.868 ± 0.009	0.237 ± 0.007	2.179±0.019	(Klimogianni *et al.,* 2011)
Pagrus pagrus	991 ± 1.09	0.24	3178 ± 85	(Mihelakakakis *et al.,* 2001)
Diplodus sargus	0.900 - 1.160	0.180 - 0.260	3.0	(Divanac, 1985; Kentouri, 1985)
Lithognathus mormyrus	0.700 - 0.820	0.160 - 0.220	1.6 - 1.7	(Divanac, 1985; Kentouri, 1985)
	0.71 ± 0.128	0.18 ± 0.134	1.74 ± 0.03	(Firat *et al.,* 2005)
Dentex gibbosus	0.956 ± 0.020	0.184 ± 0.005	2.097 ± 0.12	(Fernandez-Palacios *et al.,* 1994)
	0.7252±0.020	0.1517±0.015	2.52 ± 0.045	Present paper

*TL_0: Total lenght

Table 3. Hatching time of different sparid species

Species	Temperature	Hatching rate(%)	Hatching time(hours)	Reference
Sparus aurata	18.5°C	84 - 89	53	(Kamacı *et al.,* 2005)
Diplodus puntazzo	21°C	50	41	(Klimogianni *et al*., 2011)
Pagellus erythrinus	18 - 21°C	75 - 86	39 - 68	(Klimogianni *et al*., 2004)
Dentex dentex	17°C	_	81	(Jug-Dujakovic *et al*., 1995)
	18±0.2°C	71.6 - 78.4	56:30	(Saka *et al*., 2006)
Pagrus pagrus	15 - 25°C	79.7	37-60	(Radonic *et al.*, 2005)
Dentex gibbosus	19.9-21.7°C	67 - 95	35	(Fernandez-Palacios *et al*.,1994)
	19.5±0.2°C	87	39:41	Present paper

Trakos, 2011), *Pagrus pagrus* (Radonic, Lopez, Oka, & Aristizábal, 2005), *D. dentex* (Saka *et al*., 2006), and *P. erythrinus* (Klimogianni *et al*., 2011), it is seen that their hatching rates and the length of the newly hatched larvae are different from ours (Table 2) (Table 3).

The oxygen levels at the incubation phase varied in the range of 6.9 to 7.6 mg/l. The fact that the oxygen values are between 6-7 mg/l in the incubation of marine fish eggs, positively affects the embryonic development (Jennings & Pawson, 1991: Saka, Fırat, & Süzer, 2001). The oxygen levels in each of the three incubators were kept at specific limits in this study, and the salinity value did not exceed the rate of 35‰ during the study period. Ammonium and nitrate values did not become at the limits that will negatively affect the embryonic development.

As a result, this paper was to describe the detailed embryonic development of *D. gibbosus.* It demonstrates that it is possible to produce pink dentex in the hatchery conditions by using groundwater. *D. gibbosus* broodstock acclimates well to captive conditions showing high fecundity and hatching rates. Producing this species can be evaluated as a candidate species in order to overwhelm the problem of limited species in the Mediterranean aquaculture.

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