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Research Article

Early Life History of Cultured Common Dentex (*Dentex dentex* L. 1758)

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Abstract: The early life history of common dentex (*Dentex dentex* L. 1758) was investigated under culture conditions. The percentage of fertilized buoyant eggs was generally high, between 80 and 85%. Egg diameters ranged from 0.938 to 1.089 mm. Larval development, growth, and some morphological changes were described from day 0 to day 32. Larvae were reared intensively in 6 m^3 tank under a constant photoperiod (24 h light), and fed initially on rotifers, followed by *Artemia* nauplii and *Artemia* metanauplii. A green water technique was applied using *Nannochloropsis* sp., *Chorella* sp., and *Isochrysis* sp. at a density of 150,000–200,000 cells ml^{-1}. First feeding began on day 6. Larvae completed yolk absorption on day 8 after hatching at 136 ºC cumulative temperature (day/degrees). Swimbladder inflation occurred between day 5 and day 7 post-hatching. Notochord flexion started on day 23 at 7.01 mm total length. Metamorphosis from larval to juvenile stage occurred between days 23 and 32 post-hatching at a mean total length of 12.7 mm.

Key Words: *Dentex dentex*, common dentex, larval development, metamorphosis, morphology

Kültüre Alınan Sinagrit (*Dentex dentex* L. 1758) Larvalarının Erken Dönem Özellikleri

Özet: Sinagrit (*Dentex dentex* L. 1758) balıklarının kültür koşulları altında larval dönem özelikleri incelenmiştir. Döllenmiş yumurta oranı yüksek olup, % 80-85 arasındadır. Yumurta çapı 0,938 mm'den 1,089 mm'ye kadar değişim göstermiştir. Larval gelişim, büyüme ve bazı morfolojik değişimler 32. güne kadar tanımlanmıştır. Larvalar intensif koşullarda 6 m^3'lük tanklarda 24 saatlik aydınlık periyotta yetiştirilmiş, ilk yem olarak rotifer daha sonra ise *Artemia* nauplii ve *Artemia* metanauplii ile besleme yapılmıştır. Yeşil su tekniği olarak *Nannochloropsis, Chorella,* ve *Isochrysis* türleri 150,000–200,000 hücre ml^{-1} olarak kullanılmıştır. Larvalar besin kesesini yumurtadan çıktıktan sonra 8. günde toplam 136 ºC' de tüketmişlerdir (gün-derece). İlk besleme 6. günde başlamıştır. Notokorda bükülmesi 7,01 mm toplam boyda ve 23 günde başlamıştır. Larval dönemden jüvenil döneme geçiş, 23-32 günler arasında toplam boyun 7,01 mm olduğu dönemde başlamıştır. Hava kesesi gelişimi 6. ve 7. günler arası meydana gelmiştir.

Anahtar Sözcükler: *Dentex dentex*, sinagrit, larval gelişim, metamorfoz, morfoloji

Introduction

There is an increasing interest in the farming of new marine species in the Mediterranean aquaculture to supplement the intensive production of sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). One of the main strategies to ensure future expansion of this industry, besides production cost reduction and market enlargement, is that of species diversification.

Since the first report on the feasibility of experimental rearing of common dentex (*Dentex dentex*) by Glamuzina et al. (1), this species has become one of the most attractive candidates for diversification (2). Besides being a highly valued table fish in all Mediterranean countries, it was reported to exhibit higher growth rates than those for the sea bream (2-4) and other sparids such as red porgy (*Pagrus pagrus)*, sharpsnout sea bream (*Diplodus puntazzo)* and *Diplodus sargus*, all of which are potential candidates for Mediterranean aquaculture. Another advantage of common dentex is that its natural spawning takes place in April-May, which does not coincide with those of gilthead sea bream (November-February) and sea bass (February-March). This advantage permits a better utilization of the hatchery infrastructure as regards to production planning and management.

However, high mortality rate of dentex throughout

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larval and juvenile stages is a significant obstacle for its farming, larval rearing being the most crucial bottleneck for the commercial production of this species (5,6). Early life history is a complex process of growth and differentiation of body for animals especially for fish. During that differentiation larvae undergo extreme changes in body shape, morphology, metabolism and behavior in order to transform into juveniles while remaining a functioning organism. The change in body shape, which results from the growth of its components at different relative rates, reflects the close relationship between ontogeny of morphology and functions and is associated with acute changes in internal anatomy, etiology, physiology, metabolism and feeding preferences (7,8). Knowledge of the ontogeny of a species is important not only for basic embryology but also for fishery and aquaculture applications (9).

A numerous studies have been carried out on its reproduction and physiology (10,11), larval rearing (12), nutrition (13) morphological and osteological ontogeny (14). Literature about its early life history in intensive rearing conditions is scarce, either fragmentary or related to the external morphology of a few individuals caught in the wild (15), or limited to embryonic and yolk-sac larval development under captivity (16,17).

An understanding of normal larval morphology is critical, as it may be used to evaluate culture conditions for mass production of high quality juveniles. The aim of this paper is to describe the early life stages (morphological and functional development) of common dentex under intensive rearing conditions with focus on the age and size at transformation.

Materials and Methods

Broodstock and egg incubation

Common dentex broodstock, 8 females (2.4 kg mean weight) and 8 males (1.3 kg mean weight), were selected from wild breeders and stocked in 8 m^3 tank with a seawater supply of 35 l min^{-1}. Frozen cuttlefish (*Sepia officinalis*), Leander squilla (*Palaemon elegans*) were provided daily as the primary food source. The fish were subjected to natural photoperiod of natural rearing seasons (16 h light: 8 h dark), and the water temperatures varied throughout the experimental period between 15.5-21.0 ºC. Eggs spawned by fish group were immediately collected in recuparator. Following the fertilization, the viable buoyant eggs were separated from the dead sinking eggs.

Eggs were incubated in 50 l incubators at an initial density of 2500 eggs l^{-1} with a gentle flow of seawater of 15.5 ± 0.5 ºC. Oxygen saturation was over 85%, salinity was 37 ppt and pH was around 7.65. Ammonia and nitrite components were always < 0.012 mg l^{-1}.

Larval rearing

Larvae were stocked at density of 80 ind l^{-1} in a cylindrical tank (6 m^3). The color of the tanks was dark-gray. Larval rearing was carried out in a closed sea water system. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature was maintained between 15.5 and 21.0 ºC (temperature increased day by day from 15.5 to 19 ºC between 0 and 7^{th} days, 19 to 20 ºC between 8 and 26^{th} days, from 20 to 21 ºC between 27 and 32^{nd} days). During larval culture period, oxygen, salinity and pH were maintained at > 85%, 37‰ and 7.6, respectively. Ammonia and nitrite were kept constant always below 0.01 mg l^{-1}. The water in the tank was static during the first 3 days of the rearing period. From day 4 to 12, the tank water was partially replaced (5–6% daily) by draining through a 160 µm mesh. Water exchange rate was increased gradually with the age of the larvae. Light was supplied by fluorescent tubes, with a power of 80-100 lux at water surface. Photoperiod was set on a 24 h light cycle daily until the end of larval rearing period.

Newly hatched larvae fed from day 4 (when the mouth opened) to day 12-14 with rotifers (*Brachionus plicatilis* but mainly with *Brachionus rotundiformis*) cultured with algae and enriched (DHA Protein Selco, Artemia Systems SA, Ghent, Belgium) at a density of 10–15 ind ml^{-1} plus green-water composed of *Nannochloropsis* sp., *Chorella* sp., and *Isochrysis* sp. at a density of 150.000–200.000 cells ml^{-1}. From day 9 to day 17, *Artemia* nauplii grade (AF480 INVE Aquaculture) at 4–7 ind ml^{-1} and from day 15 until day 32, *Artemia* metanauplii at 2-4 ind ml^{-1} (EG, Artemia Systems SA, Gent, Belgium), both enriched with Protein Selco (Artemia Systems SA, Ghent, Belgium).

Observations and measurements of larvae and juveniles

20 individuals were taken from medial water column of each tank at the same times every day. The yolk sac

(V_{ys}) and oil globule (V_{og}) volumes were calculated using the formulae produced as follows. $V_{ys}=4/3\pi*(L/2)*(H/2)^2$ (L, major axis; H, minor axis) and $V_{og}=4/3\pi*(d/2)^3$ (d: diameter).

The following metrical characters were measured: Total length (TL), from the tip of the lower jaw to the posterior margin of the caudal fin; Notochord length (NL), from tip of snout to posterior margin of the notochord; Preanal length (PL), from the tip of the snout to the anus; Head length (HL) from tip of snout to the margin of gill cover; Body height (BH) from anus to the upper limit of the myomere excluding dorsal fin fold area and eye diameter (ED), parallel to the longitudinal axis of the body. The maximum length of the swimbladder was also measured. Observations and measurements were made on anaesthetized (phenoxy-2 ethanol, 0.5 ml l^{-1}) specimens using a binocular microscope with an ocular micrometer. Curled larvae were not used for morphometric measurements. Total length (TL) was used as a measure of development and growth with respect to other morphometric characteristics. The allometric equation $Y=aTL^b$ of notochord length (NL), preanal length (PL), head length (HL), body height (BH), and eye diameter (ED) on TL was estimated (18).

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 ºC to 19 ºC. During the spawning period, a total of 24,555 eggs.kg^{-1} of broodstock were collected and 19,870 eggs.kg^{-1} were viable.

Eggs, ranging in diameter from 0.938 mm to 1.089 mm with a mean of 1.032±0.008 mm and containing a single unpigmented oil globule, were positively buoyant. Oil globule volume ranged from 0.00662 mm^3 on day 1 to 0.00008 mm^3 on day 9 (Table). Fertilized eggs had a small perivitelline space. Most fertilized eggs floated at 37 ppt salinity and temperature 15.2ºC at the surface.

Larvae were hatched at 59-60 h post-fertilization. Mean total length of the larvae at the time of the hatching was 2.452 ± 0.024 mm. Dendritic melanophores, which were first visible in the embryo head, were clearly visible in each yolk-sac larvae. The mouth and digestive tract of the larvae were functional on day 6 after hatching; first feeding and eye pigmentation occurred on the same day. Yolk sac was completed on day 8. Oil globule was completely resorbed on day 10 when larval size was 3.958 ± 0.256 mm (Table).

Table. Total length, yolk sac, oil globule and temperature data obtained during the first 10 days of culture of common dentex larvae.

Days after hatching	Total length (mm)	Yolk sac volume (mm^3)	Oil globule volume (mm^3)	Temperature (ºC)	Cumulative temperature (day-degrees) (ºC)
0	2.452 ± 0.024	0.47762 ± 0.021	0.00662 ± 0.00022	15.2	15.2
1	2.957 ± 0.021	0.20336 ± 0.017	0.00479 ± 0.00019	15.4	30.6
2	3.011 ± 0.012	0.06245 ± 0.008	0.00301 ± 0.00026	16	46.6
3	3.244 ± 0.019	0.02669 ± 0.095	0.00257 ± 0.00018	16.8	63.4
4	3.304 ± 0.064	0.01210 ± 0.086	0.00191 ± 0.00012	17.2	80.6
5	3.478 ± 0.048	0.00810 ± 0.041	0.00158 ± 0.00008	17.8	98.4
6	3.554 ± 0.112	0.00390 ± 0.158	0.00104 ± 0.00015	18.5	116.9
7	3.678 ± 0.164	0.00080 ± 0.019	0.00060 ± 0.00019	19.1	136
8	3.660 ± 0.109	-	0.00036 ± 0.00017	19	155
9	3.448 ± 0.181	-	0.00008 ± 0.00021	19	174
10	3.958 ± 0.256	-	-	19	193

The larvae grew exponentially and their growth is represented by the equation $y=2.7697e^{0.0409x}$, (r = 0.9322), where y is total length in mm and x is days after hatching (Fig.1 A). The light-reflective bubble indicating initial inflation was observed in 35% of larvae on day 5. The percentage of larvae with inflated swim bladder increased to 70% on day 6 and to 100% on day 7. The relationships and proportional changes of several body parts with growth against total length are shown in Fig.1 (B, C, D, and E) and Fig. 2 (A, B, C, D, E, and F). Notochord flexion started on day 23 at 7.01 ± 0.96 mm total length. Transformation from larval to juvenile stage occurred between days 23 and 32 after hatching, at a mean TL of 12.7 ± 1.2mm.

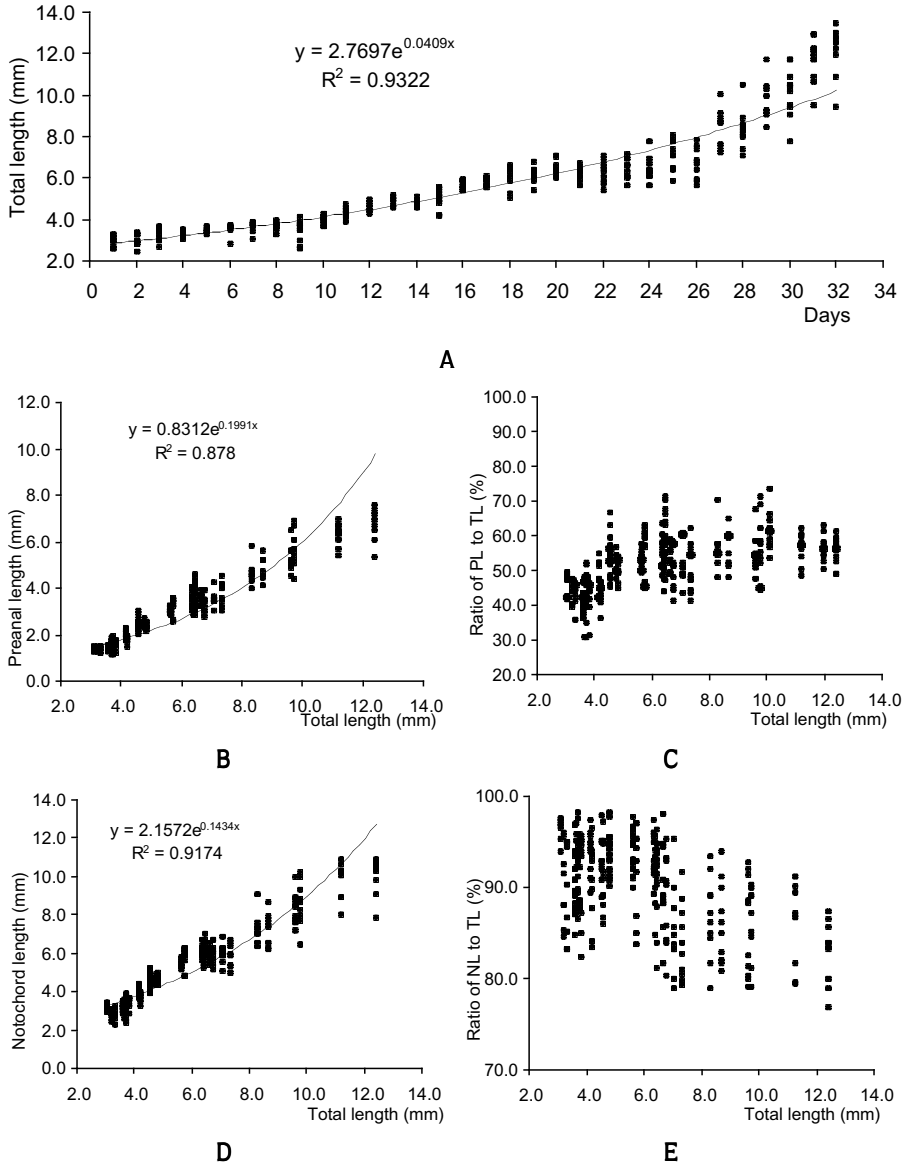

Figure 1. (A) Growth in total length, (B) relationship between preanal length (PL) and total length (TL), (C) proportional change of preanal length against total length, D) relationship between notochord length (NL) and total length (TL), and (E) proportional change of notochord length against total length in common dentex during the first 32 days post-hatching.

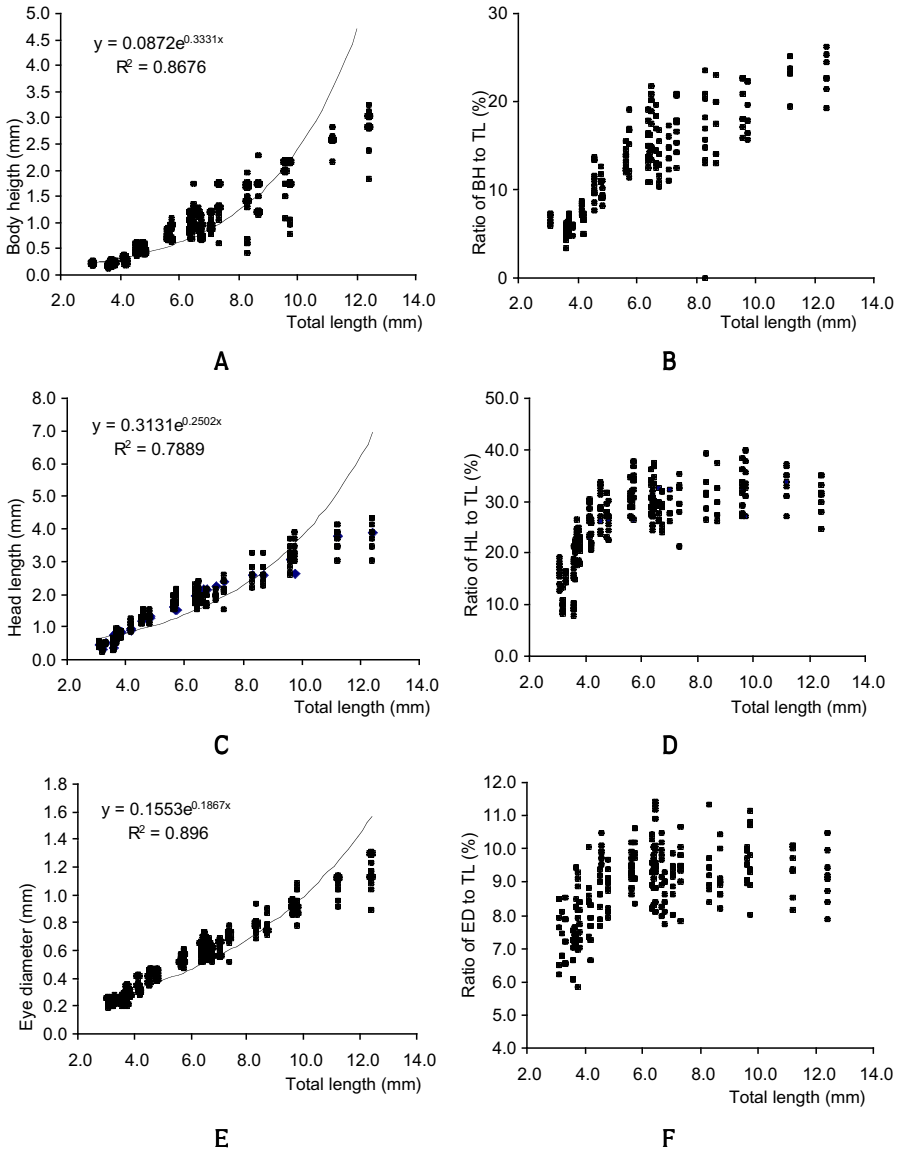

Figure 2. (A) The relationship between body height (BH) and total length (TL), (B) proportional change of body height against total length, (C) relationship between head length (HL) and total length (TL), (D) proportional change of head length against total length, (E) relationship between eye diameter (ED) and total length (TL), (F) proportional change of eye diameter against total length in common dentex during the first 32 days post-hatching.

Discussion

Early life history of fishes is a complex phenomenon of growth and differentiations. From an aquacultural field of view, the understanding of normal larval morphology may be used to utilize rearing conditions in terms of influential production of high quality larvae and juveniles. The purpose of this study was to describe the early life stages (morphological and functional development) of common dentex under intensive rearing conditions with focus on the age and size at transformation. The results confirm that the study has reached its aims.

In our study, spawning commenced on April 3, 2003, and continued until June 5, 2003, with a peak from April 24 to May 28, corresponding to temperatures ranging

from 16 ºC to 19ºC. Spawning period and temperature found in the present study are in line with those reported by Pastor et al. (12). In the present study spawning naturally took place at spawning period without requiring hormone treatment. Natural spawning was also reported by Loir et al. (19). Moreover, Pavlidis et al. (10) reported that GnRHa implants effectively induced final oocyte maturation and female ovulation, causing a greater than 10-fold production increase in the common dentex. The total number of eggs collected was estimated to be 24.5 million, of which 80.9% were viable. In addition, the broodstock produced between 21,000-23,000 eggs kg^{-1} per day while Riera et al. (2) reported this to be 20,000-35,000 eggs day^{-1}. This quantity is relatively higher than our findings. This difference can be explained with the differences in natural conditions (temperature, food, geographic location) and broodstock management techniques (salinity, temperature, nutrition etc.).

Feeding of larvae in leicthotrophic phase is provided by absorption of yolk sac and oil globule (20,21). First feeding of larvae is important for survival and improving the organogenesis. In the present study, absorption of yolk sac was completed in 7 days at a total length of 3.68 mm in common dentex. In Sparidae, generally, yolk sac absorption is completed in about 5 days at a total length of 3.22 mm in common dentex (11), 3 days at a total length of 2.6-2.8 mm in gilthead sea bream (20) and in 4 days at a total length of 3.93 mm in red porgy (22). It was observed that at the start of leichoexotrophic phase, larvae had about 28% of their oil globules. In gilthead sea bream, approximately 10% of the oil globule volume was still present at the onset of exogenous feeding (23). Most larvae start feeding before the yolk-sac is completely absorbed. Absorption of yolk sac and oil globule in the present study took longer than those reported in the literature (24). The reason for this was thought to be due to low water temperatures applied during the early larval culture period. The lower temperature also delayed the mouth and digestive tract formation in this species.

Generally, in physoclistous fishes, swimbladder inflation and the establishment of hydrostatic regulation in larvae occur at the onset of external feeding. Swimblader inflation in common dentex larvae occurred between days 5 and 7 post-hatching at total lengths ranging from 3.48 mm to 3.68 mm. The initial gas in the swim bladder of red sea bream (*Pagrus major)* and gilthead sea bream has been shown to originate from swallowing air (22,25) and the elimination of oily surface films can greatly increase swim bladder inflation rates in intensively cultured larvae. In red sea bream, the swim bladder begins to inflate and to be functional in larvae at about 3.5–4.0 mm TL during the period 5 to 10 days post-hatching (26). Inflation of swimbladder occurs at a total length of 4-5 mm in gilthead seabream and 5-9 days post-hatching in sharpsnout seabream (27,28).

Water temperature is known to be the most important environmental factor affecting larval development and metamorphosis. Optimal water temperature is species-specific and supports organogenesis to be completed successfully. In the present study, it is found that, under intensive conditions, the metamorphosis of common dentex begins 27 days after hatching at a cumulative temperature of 444.3 °C $days^{-1}$. This period was reported as 23 days for red porgy (*P. pagrus*) (29) and 26 days for red sea bream (*P. major*) (24).

The size at which notochord flexion begins in sparids depends on species, size of newly hatched larvae, rearing conditions, and preservation methods in case of preserved specimens. There are differences in the rates of caudal fin ontogeny between sparids, e.g. the caudal fins of common dentex and red sea bream developed more rapidly compared to the caudal fin of gilthead sea bream (14). By the flexion stage (started at 5.0–5.5 mm and present in 50% of the larvae at 5.5–6.0 mm) the notochord curved upward at the end (14). The development of the caudal complex began with the formation of the hyparalia (Hy), which was closely related to the flexion of the notochord (14). In this investigation, we observed similar findings about caudal fin development of common dentex larvae.

In conclusion, it is suggested that in addition to the present results and those available in the literature more studies should be carried out to optimize larval culture conditions (i.e. light intensity, photoperiod, stocking density, salinity etc.) in order to improve the reliability of the present protocols for larval rearing of common dentex, which then in turn will help Mediterranean Aquaculture industry to increase species diversity.

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