Mar. Life 1996 - Vol. 6 (1-2) : 41 - 50

Embryonic and yolk-sac larval development of *Dentex dentex* Linnaeus 1758 (Osteichthyes, Sparidae)

Développement des embryons et des larves vitellines de Dentex dentex *Linnaeus 1758 (Osteichthyes, Sparidae)*

George Koumoundouros*/**, Pascal Divanach*, Maroudio Kentouri*/**

*Institute of Marine Biology of Crete, P.O. Box 2214, 71003 Iraklio, Crete, Greece, Fax + 3081-241543, Tel. + 3081-242022, e-mail koumound@imbc.gr

**Biology Department, University of Crete, Greece.

Key-words: Dentex dentex, *embryo, yolk-sac larvae, development.*

Mots clés : Dentex dentex, *embryons, larves vitellines, développement.*

ABSTRACT

Koumoundouros G., P. Divanach, M. Kentouri, 1996 - Embryonic and yolk-sac larval development of Dentex dentex *Linnaeus 1758 (Osteichthyes, Sparidae).* Mar. Life, **6** (1-2): 41-50.

Embryonic and yolk-sac larval development of Dentex dentex *was morphologically and chronologically studied from the initial temperature of 16.6±0.3°C prior to hatching, to 16.9±0.3°C during the yolk-sac larval stage. The eggs, measuring on average 0.99±0.01 mm in diameter, were spherical and pelagic with a transparent yolk and a single lipid globule. Hatching occurred 59:30 hours after fertilisation. Newly hatched yolk-sac larvae had a mean total length (TL) of 2.47±0.03 mm. The yolk-sac larval stage lasted 121 hours after hatching. At the end of this stage, the lipid globule was still present and larvae measured 3.63±0.03 mm TL.*

RÉSUMÉ

Koumoundouros G., P. Divanach, M. Kentouri, 1996 - [Développement des embryons et des larves vitellines de Dentex dentex *Linnaeus 1758 (Osteichthyes, Sparidae].* Mar. Life, **6** (1-2) : 41-50.

Le développement des embryons et des larves vitellines de Dentex dentex *est étudié à la température moyenne de 16.6±0.3°C (avant éclosion), puis 16.9±0.3°C (après éclosion). Les œufs, de diamètre moyen égal à 0.99±0.01 mm, sont des sphères pélagiques munies d' une seule gouttelette d'huile. L'éclosion a lieu 59:30 heures après fécondation. Les larves vitellines qui viennent d'éclore ont une longueur totale (TL) moyenne égale à 2.47±0.03 mm. 121 heures après l'éclosion, les réserves vitellines sont entièrement résorbées, le globule lipidique est presque intact et les larves mesurent 3.63±0.03 mm TL.*

INTRODUCTION

The common dentex (*Dentex dentex* L. 1758) is a Sparidae species of high commercial value which inhabits the Mediterranean, the Atlantic from the Bay of Biscay to Madeira, and, rarely, the Black Sea. It is a protandrous hermaphrodite species, but sex reversal does not seem to be obligatory (Bauchot and Hureau, 1986). In the Mediterranean spawning takes place between March and July (Lo Bianco, 1909; Bauchot and Hureau, 1986; Glamuzina *et al.*, 1989). It is a partial spawner with a fecundity of more than 1 million eggs per Kg of female.

Literature about the development of *D. dentex* is rather scarce. The majority of related studies describe a few lone individuals caught in the wild (Lo Bianco, 1909; De Gaetani, 1938). Only one offers a description of continuous development (Jug-Dujakovic *et al.*, 1995). This, however, targeted only some embryonic stages.

A detailed knowledge of the early development of a species is very important not only from the

embryological point of view, but also for fisheries biology and aquaculture. The identification of the early life stages in ichthyoplankton surveys is based on a developmental series of specimens, especially in the case of families or species with close morphology and overlapping spawning seasons and habitat (Russell, 1976; Blaxter, 1984). From an aquacultural point of view, precise developmental knowledge is a prerequisite for the early detection and elimination of morphological abnormalities common under rearing conditions (Divanach *et al.*, 1996), and the subsequent cost effectiveness of hatcheries.

The present study describes the embryonic and yolk-sac larval development of *D. dentex*.

MATERIAL AND METHODS

Egg collection

The eggs used in this study were obtained from breeders matured under natural photoperiod and temperature conditions (35° latitude North). Spawning was spontaneous and uninduced. Egg collection was conducted from a water overflow-pipe using a 10 m^3 tank (water exchange rate of 100% per hour) equipped with a cylindro-conical planktonic mesh. To collect the eggs immediately after spawning and fertilisation, the collector was continuously (every 5 min) examined.

Incubation conditions

Eggs and yolk-sac larvae were kept in a 500 l cylindro-conical tank, with an initial concentration of 50 eggs l^{-1}. The incubator was supplied with filtered (sand filter) sea water from the base of the tank at an average hourly rate of 25-30%. The water of the incubator was gently aerated by an air diffuser (150 ml min^{-1}, bubble diameter of 150-200 µm). Water temperature during the embryonic stage was 16.6±0.3°C, reaching 16.9±0.3°C during the yolk-sac larval stage. Oxygen saturation was over 90%, salinity was 40, and pH was around 8.1. Ammonia and nitrite were always less than 0.05 mg l^{-1}.

Sampling

For the study of the embryonic development, samples of eggs were taken every 5 min up to the 6th cell division, and every 1-1/2 hours thereafter. In order to describe all the developmental stages, sampling was continuous throughout 24 hours. General observations and photographs for detailed description of the developmental stages were made *in vivo*, from both dorsal and lateral positions, using a stereoscopic microscope. Reflected and transmitted light was used for this observation. Initially, 30 eggs were sampled on each occasion to determine the dominant development stage. However, as it was soon determined that development was synchronous throughout the egg population, the sample size was reduced to 10. Definition of the stages during the embryogenesis followed Cassie (1956) and Divanach (1985).

At the yolk-sac larval stage, 15 specimens per eight hours were anaesthetised (Ethylenglycol-monophenylether, 0.2-0.5 ml l^{-1}) and photographed under the conditions previously described. Subsequently, yolk-sac larvae were fixed individually in phosphate buffered 5% formalin (Markle, 1984). Morphological evolution of the yolk-sac larvae was studied *in vivo* and verified on the fixed specimens. Pigmentation development was studied both *in vivo* and on the fixed specimens. Formalin preserves the melanophores well but not the chromatophores, which disappear (Russell, 1976). Therefore, melanophores were studied in the fixed specimens, and any observed differences with the *in vivo* state were attributed to the chromatophores. This method of pigmentation study avoided *in vivo* artifacts, which were made by special light effects (shadows) and by the overlapping of melanophores with chromatophores in some regions.

During the whole study, 100 specimens per day were sampled to estimate the occurrence of developmental abnormalities. The incubator bottom was cleaned daily to estimate possible mortality.

Measurements and statistics

Measurements of egg and lipid droplet diameters were taken from the photographs to the nearest 0.01 mm. All time intervals were measured from fertilisation (t_0=0 hours) and expressed both as absolute times and as relative times (Divanach, 1985).

During the yolk-sac larval stage, total length (TL) measurements were taken from the photographs, to the nearest 0.01 mm and parallel to the longitudinal axis of the body. All time intervals were measured from approximately 50% of hatching (t_0=0 hours) and expressed both as absolute times and as relative times. The growth curve was estimated by fitting (Systat 5 for the Macintosh) the mean TL data on the Gompertz model (Ricker, 1979):

$$TL = ae^{-be^{-ct}}$$

where TL in mm; a= asymptote (mm); c= instantaneous growth rate at the inflexion point (hr^{-1}); t= hours from hatching and b= a dimensionless parameter, such that b.c is the instantaneous growth rate at hatching (t=0). For the measurement of TL just after hatching, 20 eggs, ready to hatch, were put into a 1 l beaker and each yolk-sac larva was photographed immediately after hatching.

RESULTS

Embryonic development

Common dentex eggs are pelagic, spherical in shape and transparent with a colourless, unsegmented vitellus that fills the shell completely.

Chorion diameter ranges between 0.97-1.00 mm (0.99±0.01 (±SD), n=38). They have one lipid globule 0.24-0.25 mm in diameter (0.24±0.00 (±SD), n=38), positioned at the periphery of the egg. In the first developmental stages, and up to the stage of epiboly, the perivitelline space is very narrow and can be clearly seen only when laterally viewed in the area of the animal pole (figures 1a-1i). After epiboly, the perivitelline space increases as parts of the vitelline reserves are utilised.

Fifty five minutes after fertilisation (A.F.), the protoplasm concentrates at the animal pole opposite the lipid globule, forming a cytoplasmic "cap" (figure 1a). Cleavages are incomplete, occurring only at the animal pole (i.e., meroblastic eggs). The first cleavage occurs 1 hour and 10 min A.F. (figure 1b). Up to, and including, the 16 cell stage, (3:05 hours A.F., figure 1e), the first cell divisions are synchronous and symmetrical, whereas from the 32 cell stage (3:45 hours A.F.) they become asymmetric. The cells are clearly visible up to the stage when the germ ring is formed (14:10 hours A.F.), which appears as a slight thickness at the periphery of the inner layer of the blastoderm (figure 1i).

The end of blastulation and the beginning of gastrulation takes place 15:00 hours A.F., forming the embryonic shield which can easily be seen in dorsal view. Epiboly starts just after the end of blastulation. The beginning of neurulation takes place 24:10 hours A.F., leading to the appearance of the neural plate and the future anterior region of the embryo (figures 1j-1k). The outline of the eyes and the vesicle of Kuppfer appear at the anterior and on the posterior ventral part of the embryo, respectively, 27:15 hours A.F., a short time before the blastopore closes. At this stage, the blastopore diameter is approximately 0.15-0.20 mm and the first somites begin to form, although they still are not clearly visible.

The blastopore closes 28:20 hours A.F. Twenty nine hours and fifty minutes A.F., the embryo occupies the periphery of the lower 1/2 of the egg and has, at its middle, seven well-formed somites. The first pigment cells appear in the form of black dots at the dorsal anterior half of the body (figure 1l). As embryonic development continues, these cells migrate to the sides of the embryo and the surface of the vitellus, between the lipid globule and the cephalic region. Consequently, punctuate pigments appear on the surface of the lipid globule (37:00 hours A.F.). The number of somites increases, the Kuppfer's vesicle gradually disappears and the crystalline lenses, auditory vesicles and heart begin to form (40:50 hours A.F.).

When the embryo occupies 2/3 of the egg's periphery, (43:40 hours A.F., figure 1m), the vesicle of Kuppfer is no longer present. The auditory vesicles, the mouth cavity and the olfactory lobes can now be seen in the area of the future head (47:30 hours A.F.). Furthermore, the crystalline lenses and the heart are well formed by this stage and the caudal bud is separate from the vitellus. The primordial marginal finfold can be seen surrounding the main part of the body but it is not yet completely formed. At the end of this stage, the primordia of otoliths are visible in the auditory vesicles. The pigmentation of the body is provided by punctuate and stellate black melanophores and by yellow chromatophores of irregular shape, all of which are situated mainly in the anterior and posterior regions of the embryo and on the surface of the oil droplet. The heart commences beating at approximately 47:30 hours A.F. The embryo starts to move 48:20 hours A.F. When it occupies approximately 3/4 of the periphery of the egg (52:25 hours A.F., figure 1n), the pigment cells are concentrated in patches in the areas where they will occur in the newly hatched yolk-sac larvae, i.e., in front of and behind the eyes, behind the auditory vesicles, above the intestine (where it is separated from the body), on the yolk and lipid globule surface, and just before the caudal bud, forming a ring (figure 2a). The primordial marginal finfold is well formed and colourless.

Immediately prior to hatching, the embryo occupies approximately the whole of the egg periphery (figure 1o). It moves regularly, making complete rotations at irregular time intervals. Hatching occurs between 58:10-61:25 hours A.F. During this process the embryo, using a series of contortions, pushes its head strongly against the chorion. Eventually the chorion breaks locally and the yolk-sac larvae exit (figure 1p).

Total mortality during the embryonic development was at non detectable levels (<1%). The chronological development of all these stages of the embryonic development is shown in table I.

Yolk-sac larval development

At hatching (figure 2a), the yolk-sac larvae of *D. dentex* are 2.47±0.03 mm in TL (2.42-2.51 mm TL, n=12) and their morphology does not differ from that just prior to hatching. The massive yolk sac is situated at the anterior ventral part of the body. The lipid globule has a mean diameter of 0.25±0.00 mm (0.25-0.26 mm, n=12) and is located at the posterior ventral part of the yolk sac. The head is bent downwards. The intestine cuts the primordial marginal finfold vertically at approximately 56% of the TL. The primordial marginal finfold is the only formed fin. On its surface it has well-like formations in random arrangement. Eyes lack retinal pigmentation and the mouth is not formed.

Twenty four hours after hatching (A.H.), the first signs of retina pigmentation are apparent, especially in the fixed state. The lipid globule is positioned at the posterior margin of the yolk sac (figure 2b).

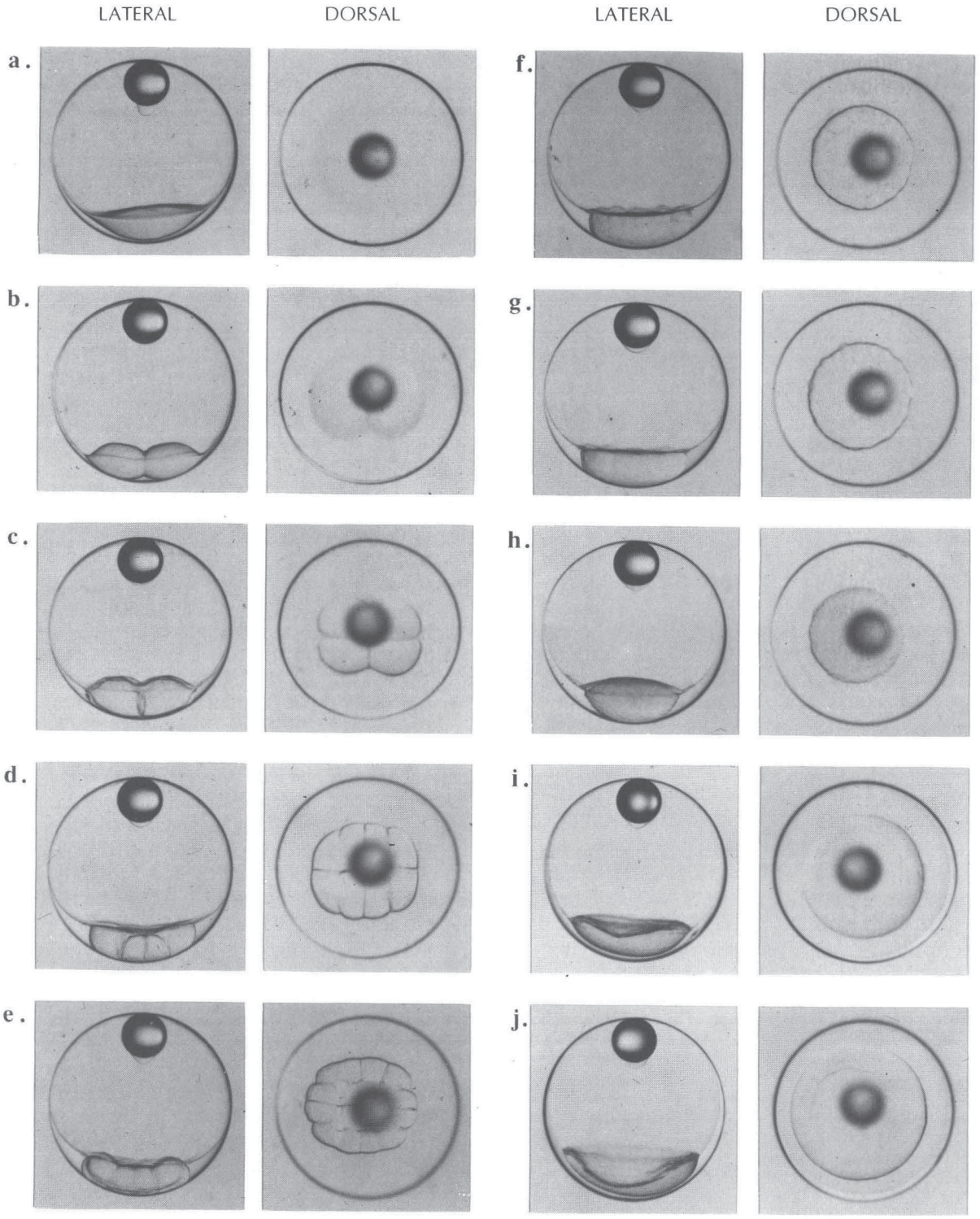

Figure 1 - Embryonic development of *D. dentex* at 16.6±0.3°C. (a) Protoplasm concentration at the animal pole; the following incomplete cleavages give rise to two (b), four (c), eight (d), sixteen (e), thirty two (f), and sixty four cells stage (g); (h) Morula; (i) Germ ring is present at the periphery of the blastoderm ; (j) Epiboly onset, with clearly defined embryonic shield; (k) 3/4 Epiboly; (l) Embryo 1/2: the outline of the eyes, Kuppfer's vesicle, first somites and pigment cells are present; (m) Embryo 2/3: auditory vesicles, mouth cavity, olfactory lobes, lenses, and heart are well formed; (n) Embryo 3/4; (o) Embryo 4/4; (p) Hatching. Scale equals to 1.00 mm./*Développement embryonnaire de* D. dentex *à la température de 16.6±0.3°C. (a),Concentration du protoplasme au pôle animal. Les divisions cellulaires qui suivent donnent naissance à deux (b), quatre (c), huit (d), seize (e), trente-deux (f), et soixante-quatre cellules (g); (h) Morula; (i) Disque germinal présent à la periphèrie du blastoderme; (j) Début d'épibolie avec disque embryonnaire bien défini; (k) Epibolie 3/4; (l) Embryon 1/2 : les traces des yeux, de la vésicule de Kuppfer, de premiers somites et des chromatophores sont présentes; (m) Embryon 2/3 : les vésicules auditives, la cavité de la bouche, les lobes d'olfaction, les lentilles et le coeur sont formés; (n) Embryon 3/4; (o)Embryon 4/4; (p) Eclosion. Echelle égale à 1.00 mm.*

Although yolk-sac larvae react to mechanical stress 16 hours A.H., neuromasts are first seen 33 hours A.H. There are 9 bilaterally symmetrical pairs of neuromasts, 4 cephalic and 5 somatic. Somatic neuromasts are arranged between the 5th myomere and the onset of the undifferentiated posterior part of the notochord. The first and 5th (caudad counted) somatic pair is located dorsally and ventrally to the notochordal axis, respectively. The second, 3rd and 4th somatic pairs of neuromasts are located on the notochordal axis. Cephalic neuromast pairs are distributed as follows: 1) one pair anterior to the yolk sac and ventral to the snout, 2) one pair on the snout, 3) one pair dorsal to the upper margin of the eye, 4) one pair posterior to the eye.

The rudiments of the pectoral fins start to form 33 hours A.H. (figure 2c). The eyes are clearly brown, and the ureter and the pectoral fins are clearly visible 57 hours A.H. Alimentary tract development becomes clear with the intestinal loop (64 hours A.H.), which in later stages leads to the development of the stomach (figures 2d-2g). The rudiments of the lower jaw have begun to form. Eighty hours A.H., gill cover starts to form and the vitelline reserves are considerably absorbed, resulting in the head detaching from the yolk sac. The mouth starts to develop as a small hole at the ventral part of the cephalic region (Figure 2e). Eighty eight hours A.H., liver formation is evident inside the yolk sac and near the lipid globule, while gill cover is fully formed.

As a first sign of completion of the yolk-sac larval stage, the eyes become black (105 hours A.H.). Remaining development consists of the anus

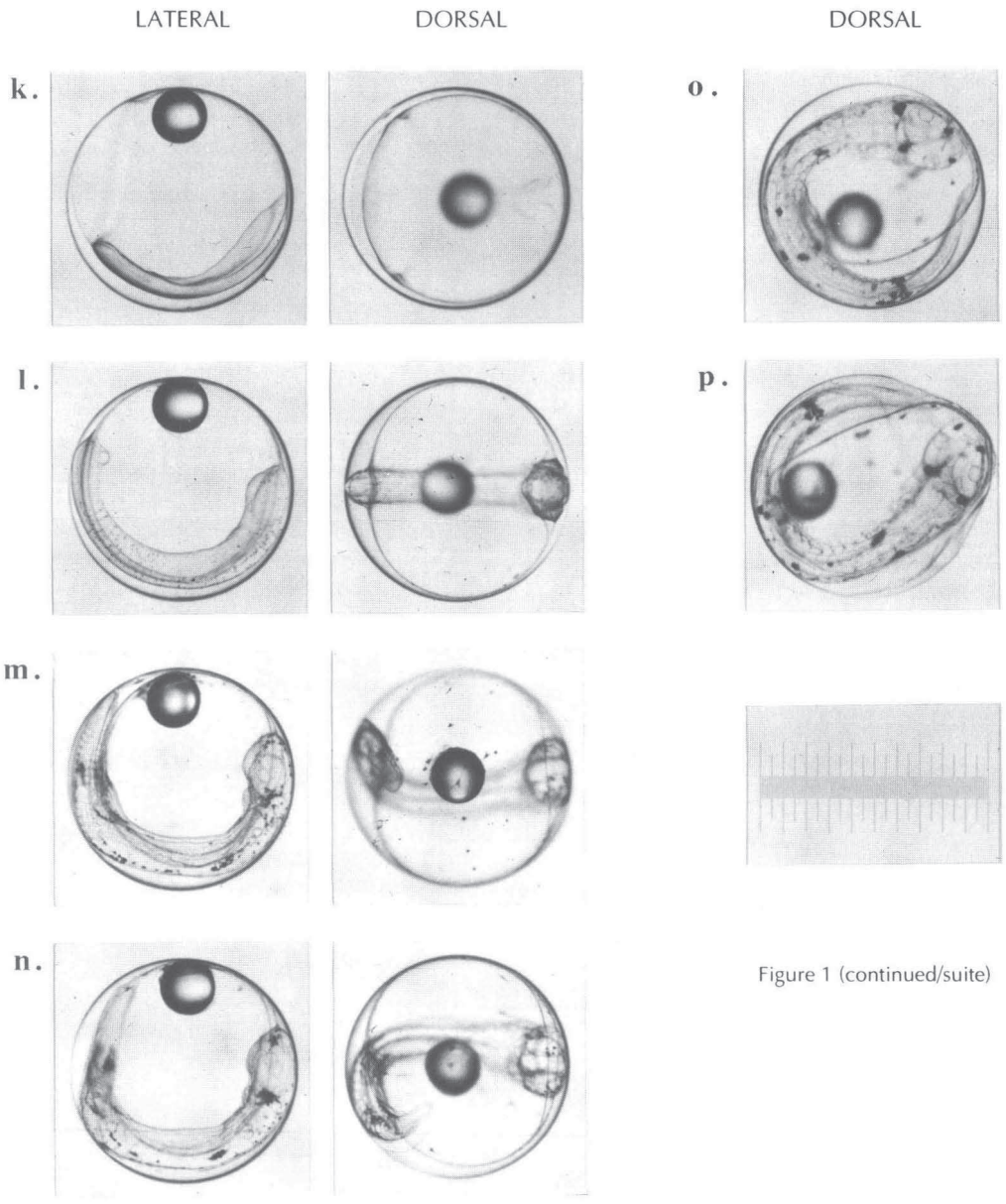

Figure 1 (continued/suite)

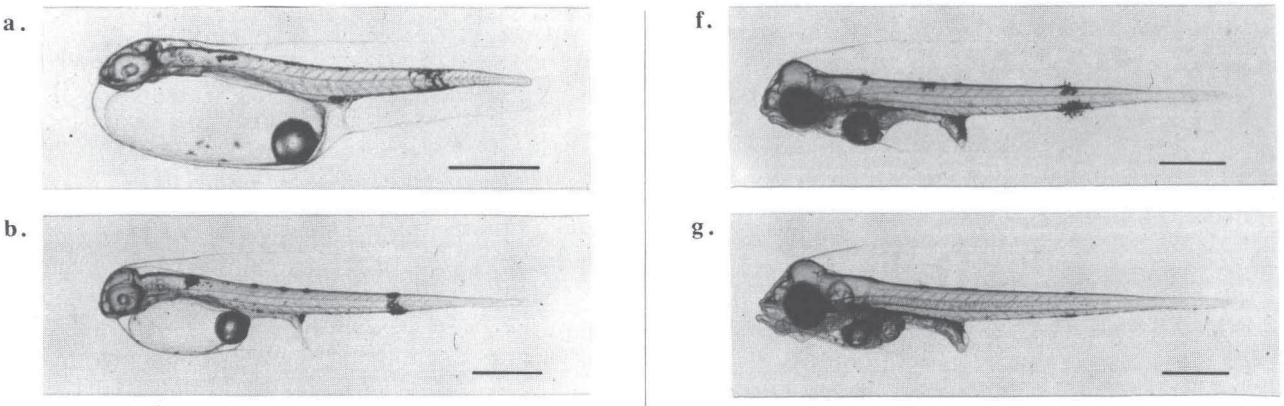

Figure 2 - Yolk-sac larval development of *D. dentex* at 16.9±0.3°C. (a) At hatching, the eyes lack retinal pigmentation and mouth is not yet formed (t=0 hr, TL=2.49 mm); (b) t=24 hr, TL=3.24 mm; (c) The rudiments of the pectoral fins are clearly visible (t=40 hr, TL=3.43 mm); (d) Alimentary tract development becomes clear with the intestinal loop, the eyes are clearly brownish (t=73 hr, TL=3.61 mm); (e) The vitelline reserves are considerably absorbed and the mouth starts to develop (t=80 hr, TL=3.57 mm); (f) The liver can be observed near the lipid globule, and the gill cover is formed (t=96 hr, TL=3.63 mm); (g) The vitelline reserves are completely consumed, and the eyes, liver, stomach and mouth are well developed (t=124 hr, TL=3.65 mm). Scale bars equal to 0.50 mm.

Développement des larves vitellines de D. dentex *à 16.9±0.3°C. (a) A l'éclosion, la réfine n'est pas développée et la bouche n'est pas encore formée (t=0 hr, TL=2,49 mm); (b) t=24 hr, TL=3,24 mm; (c) Les rudiments des pectorales sont bien visibles (t=40 hr, TL=3,43 mm); (d)- Le développement du tractus alimentaire est clair avec la courbure de l'intestin, les yeux sont brun clair (t=73 hr, TL=3,61 mm); (e) Les réserves vitellines sont considérablement absorbées et la bouche commence à se développer (t=80 hr, TL=3,57 mm); (f) Le foie est observé près du globule lipidique et les opercules sont formés (t=96 hr, TL=3,63 mm); (g) Les réserves vitellines sont complètement consommées ; les yeux, le foie, l'estomac et la bouche sont bien développés (t=124 hr, TL=3,65 mm). Echelle égale à 0,50 mm.*

opening, complete formation of the liver and of the stomach, and the functionality of the mouth (112 hours A.H.). The autotrophic stage ends when vitelline reserves are completely consumed (121 hours A.H.). In contrast, the lipid globule is not completely absorbed, measuring 0.15±0.02 mm (n=17) in diameter (figure 2g). Yolk-sac larval development of *D. dentex* is summarised in table I.

Total length increases rapidly up to 33 hours A.H. (3.41±.0.05 mm), followed by a low growth rate up to 65 hours A.H. (3.58±.0.03 mm), and remaining constant during subsequent development. The estimated Gompertz growth curve is :

$$TL = 3.608e^{-0.387e^{-0.054t}} \quad (r^2=99.67).$$

During the yolk-sac larval stage, abnormalities appeared at a frequency of approximately 2%. They mainly involved displaced lipid globule, notochord distortions, cranial deformities and necrosis of the periblast which surrounds the lipid globule. Total mortality did not exceed 5%.

Yolk-sac larval pigmentation

At hatching, the majority of the melanophores are distributed in the preanal dorsal part of the body. The rest are distributed as follows: 1-4 on the yolk, on the lipid globule surface, behind the auditory vesicles, 2 ventrally to the myomeres and in contact with the posterior of the gut, 1-5 on the postanal myomeres, and 2 ventrally to the myomeres and above the lipid globule. Melanophores are punctate or branched. Chromatophores are situated anteriorly and posteriorly to the eyes, posteriorly and dorsally to the auditory vesicles, and on the postanal myomeres, behind or overlapping the melanophore zone. The last chromatophore zone is predominantly observed *in vivo* on the postanal myomeres.

During the first 16 hours after hatching, the dorsal melanophores migrate to the sides of the body and on the snout. Subsequently, all of them are located in the following regions: dorsal to the intestine (internal melanophores), internal between the eyes, dorsal to the eyes, on the snout, around the lipid globule, on the anteriormost part of the yolk, and ventral to the postanal myomeres. Up to 56 hours, 5-7 snout melanophores were counted. At 64 hours A.H., the melanophores dorsal to the eyes (2-7 in number) join the snout pigment cells,

which cannot be counted because they overlap each other. The melanophores located ventrally to the postanal myomeres number 2-7, 5-12 and 12-18 in the time interval 16-64, 72-96 and 105-120 hours after hatching, respectively.

DISCUSSION

In their main morphology, *D. dentex* eggs and yolk-sac larvae are very similar to those of the other Sparidae species (Divanach, 1985; Faranda *et al.*, 1985; Fernandez-Palacios *et al.*, 1994). The size variation among the eggs of most of the Sparidae species is distributed among approximately the same or overlapping ranges, while the variation between the total length of the larvae at hatching and at the end of the yolk-sac larval stage is higher (table II). However, if the effects of the spawning season, spawning group, diet and geographic distribution of the breeders, and of the female size on the egg size and subsequently on the yolk-sac larvae are considered (Blaxter and Hempel, 1963; Chambers *et al.*, 1989; Buckley *et al.*, 1991), the dimensions of the eggs and yolk-sac larvae are not characters of high systematic value for the Sparidae family.

In ichthyoplankton surveys, melanophore distribution is of high diagnostic value (Russell, 1976). Although larvae under captivity are pigmented more heavily than those in the wild, the number and location of the melanophores are the same (Hunter, 1984). The presence of melanophores and chromatophores in the yolk-sac larval stage is characteristic of all the Sparidae species that have so far been described (Ranzi, 1933; De Gaetani, 1936, 1938; Divanach, 1985; Faranda *et al.*, 1985; Fernandez-Palacios *et al.*, 1994). According to the present study, the development of *D. dentex* pigmentation follows a

Table I - Embryonic (at 16.6±0.3°C) and yolk-sac larval (at 16.9±0.3°C) development of *D. dentex*. R.T.i: relative time of i developmental event; TL: Total length./*Développement des embryons et des larves vitellines de* D. dentex *à la température de 16.6±0.3°C et 16.9±0.3°C respectivement. R.T.i: temps relatif d'apparition de l'événement i.*

DEVELOPMENTAL EVENTS						
EGGS			YOLK-SAC LARVAE			
	TIME (hr)	R.T.i		TIME (hr)	R.T.i	TL (mm)
Fertilisation	0:00	0	Hatching	0	0	2.47±0.03
Animal pole differentiation	0:55	1.5	Eyes' pigmentation onset	24	20	3.27±0.04
2 cells	1:10	2.0	Intestinal canal	33	27	3.41±0.05
4 cells	1:55	3.2	Pectoral fins' buds	33	27	
8 cells	2:30	4.2	Neuromasts	33	27	
16 cells	3:05	5.2	Ureter	57	47	3.53±0.05
32 cells	3:45	6.3	Pectoral fins formation	57	47	
64 cells	4:30	7.6	Brown eyes	57	47	
Morula	10:45	18.1	Intestinal loop	64	53	3.58±0.03
Germ ring	14:10	23.8	Lower jaw's appearance	64	53	
Embryonic shield	15:00	25.2	Mouth opening	80	67	3.58±0.04
1/2 Epiboly	20:40	34.7	Gill cover's formation onset	80	67	
3/4 Epiboly	24:10	40.6	Liver's formation onset	88	73	3.60±0.04
Kuppfer appearance	27:15	45.8	Gill cover formed	88	73	
Blastopore closure	28:20	47.6	Black eyes	105	87	3.59±0.04
Embryo 1/2	29:50	50.1	Anus opening	112	93	3.62±0.02
Pigment cells	31:10	52.4	Stomach formed	112	93	
Kuppfer disappearance	40:50	68.6	Functional mouth	112	93	
Embryo 2/3	43:40	73.4	Liver formed	112	93	
Crystalline lens formed	47:30	79.8	Absorption of vitelline reserves	121	100	3.63±0.03
Heart beats	47:30	79.8	End of the stage	121	100	
Embryo start moving	48:20	81.2				
Embryo 3/4	52:25	88.1				
Hatching onset	58:10	97.8				
Embryo 4/4	59:25	99.9				
Hatching >50%	59:30	100.0				
Hatching complete	61:25	103.2				

Table II - Chorion and oil droplet diameter of the eggs of some Sparidae species. TL_0: Total Length at hatching; TL_f: Total Length at the end of the yolk-sac larval stage; Ref.: reference./*Diamètre du chorion et de la gouttelette d'huile des oeufs de certaines espèces de Sparidés. TL_0: Longueur totale à l'éclosion; Tl_f Longueur totale à la fin du stade vitellin. Ref: Références bibliographiques.*

Species	Egg diameter (mm)	Oil droplet diameter (mm)	TL_0	TL_f	Ref.
Boops boops	0.921 (0.860-1.000)	0.200 (0.180-0.220)	-	-	1
Dentex dentex	0.958±0.007 0.99±0.01	0.208±0.006 0.24±0.00	2.17±0.20 2.47±0.03	3.48±0.11 3.63±0.03	2,3 *
Dentex gibbosus	0.956±0.020	0.184±0.005	2.097±0.12	3.35±0.15	4
Diplodus annularis	0.759 (0.710-0.810)	0.184 (0.180-0.220)	-	-	1
Diplodus sargus	1.004 (0.900-1.160)	0.224 (0.180-0.260)	3.0	4.1	1,5
Diplodus vulgaris	0.958 (0.880-1.040)	0.217 (0.180-0.260)	-	-	1
Lithognathus mormyrus	0.752 (0.700-0.820)	0.188 (0.160-0.220)	1.6-1.7	2.94	1,5
Pagellus erythrinus	0.917 (0.880-0.960)	0.200 (0.180-0.220)	-	-	1
Puntazzo puntazzo	0.806 (0.760-0.880)	0.202 (0.160-0.240)	2.0	3.12±0.19	1,5
	0.845 (0.720-0.969)	0.18-0.20	1.69	3.17	6
Sparus aurata	1.020 (0.920-1.120)	0.215 (0.180-0.260)	2.23-2.53	3.59-3.93	1,5,7
Spondyliosoma cantharus	1.09 (1.0-1.2)	0.21 (0.20-0.25)	2	-	8

1. Divanach, 1985; 2. Glamuzina *et al.*, 1989; 3. Jug-Dujakovic *et al.*, 1995; 4. Fernandez-Palacios *et al.*, 1994; 5. Kentouri, 1985. 6. Faranda *et al.*, 1985; 7. Polo *et al.*, 1991; 8. Camus and Besseau, 1986; * Present paper.

certain pattern of distribution. However, the *in vivo* distinction of chromatophores from melanophores requires certain observational conditions.

Lipid globule position in the yolk sac is a character of high value for systematics. In aquaculture, mechanical stress can lead to reversible or irreversible lipid globule detachment from the normal position, with subsequent developmental abnormalities (Kentouri, 1985), or even lipid absorption with a significantly lower rate than normal (unpublished data). In the present study, this abnormality was rare and did not result in any of these effects.

With respect to the duration of the two developmental stages, the egg dimensions, the mortality during the two stages, and the size of the yolk-sac larvae, the results of the present study differ from the results of other studies on the development of *D. dentex* (Glamuzina *et al.*, 1989; Jug-Dujakovic *et al.*, 1995). The different egg dimensions are not contradictory if the effects of the conditions of broodstock maturation, the use of hormones for the induction of spawning (applied by the other studies) and the phase of the spawning period on these characters are considered (Divanach, 1985). In addition, the longer total length at hatching observed by the present study can be explained by the effects of the egg diameter (Chambers *et al.*, 1989) and temperature (Polo *et al.*, 1991).

According to Glamuzina *et al.* (1989) and Jug-Dujakovic *et al.* (1995), embryonic and yolk-sac larval development of *D. dentex* at 17°C lasts 20 hours and 30 hours, respectively, longer than in the present study, which was conducted at a lower temperature. This difference is the opposite of what was expected (Divanach, 1985; Polo *et al.*, 1991) and cannot be explained unless there is an effect of egg diameter or of hormonal induction on this duration, which however, has not so far been proven. The continuous monitoring of development throughout 24 hours in the present study leaves no doubt about its estimation of developmental duration under certain conditions.

The present study, through continuous monitoring of morphological characters, significantly contributes to the developmental

biology of *D. dentex*. As the survival rate of the eggs and yolk-sac larvae was higher than 95% and there was an extremely small incidence of abnormalities among the larvae, it is felt that the results can be used as a reference for normal development for the control of egg and yolk-sac larval quality, allowing the early identification of possible abnormalities, and the avoidance of artifact or wrong traits under systematic considerations. It may also be used as a tool for determining each developmental stage and estimating the degree of hatching synchronicity and the time remaining before hatching and onset of feeding. The similarities observed between *D. dentex* eggs and yolk-sac larvae, and those of the other Sparidae, underline the importance of future extensive studies on the egg and larval development for the definition of a systematic key for use in ichthyoplankton surveys.

ACKNOWLEDGEMENTS

This research was financed by the Greek Ministry of Development, "General Secretariat of Research and Technology" (IPER 94 YP: 17). We wish to express our thanks to M. de Wilde for her advice concerning the English.

BIBLIOGRAPHY

Bauchot M.L., J.C. Hureau, 1986 - Sparidae. In: *Fishes of the North-eastern Atlantic and the Mediterranean*.. P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen, E. Tortonese (eds), UNESCO, Great Britain, pp: 883-907.

Blaxter J.H.S., 1984 - Early life history stages of fishes and their characters. In: *Ontogeny and Systematics of Fishes*. H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall, S.L. Richardson (eds), *American Society of Ichthyologists and Herpetologists*, Special Publication no. 1, Lawrence USA, Allen Press Inc, pp: 1-6.

Blaxter J.H.S., G. Hempel, 1963 - The influence of egg size on herring larvae (*Clupea harengus*). *J. Cons. C.I. E. M.*, **28**: 211-240.

Buckley L.J., A.S. Smigielski, T.A. Halavik, E.M. Caldarone, B.R. Burns, G.C. Laurence, 1991 - Winter flounder *Pseudopleuronectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae. *Mar. Ecol.-Prog. Ser.*, **74**: 125-135.

Camus P., L. Besseau, 1986 - Sparidae, *Spondyliosoma cantharus*. *Fiches Ident. Plancton*, **177**, 4 pp.

Cassie R.M., 1956 - Early development of the Snapper, *Chrysophrys auratus* Forster. *Trans. R. Soc. N. Z.* **83**: 705-713.

Chambers R.C., W.C. Leggett, J.A. Brown, 1989 - Egg size, female effects, and the correlations between early life history traits of capelin, *Mallotus villosus*: an appraisal at the individual level. *Fish. Bull.*, **87 (3)**: 515-523.

De Gaetani D., 1936 - Uova, sviluppo embrionale e stadi post-embrionali negli Sparidi. 4. *Pagrus vulgaris* C.V. *Mem. R. Com. talassogr. ital.*, **226**, 16 pp.

De Gaetani D., 1938 - Uova, sviluppo embrionale e stadi post-embrionali negli Sparidi. 6. *Dentex vulgaris* Cuv. *Mem. R. Com. talassogr. ital.*, **255**, 10 pp.

Divanach P., 1985 - Contribution à la connaissance de la biologie et de l'élevage de six sparidés mediterranées: *Sparus aurata, Diplodus sargus, Diplodus vulgaris, Diplodus annularis, Lithognathus mormyrus, Puntazzo puntazzo* (Poissons, Téléostéens). Thèse d'État, Université des Sciences et Techniques de Languedoc, 479 pp.

Divanach P., C. Boglione, B. Menu, G. Koumoundouros, M. Kentouri, S. Cataudella, 1996 - Abnormalities in finfish mariculture: An overview of the problem, causes and solutions. In: *Seabass and Seabream culture: problems and prospects*. B. Chatain, M. Saroglia, J. Sweetman, P. Lavens (eds), International Workshop, Verona, Italy, October 16-18, 1996, European Aquaculture Society, Oostende, Belgium, pp: 45-66.

Faranda F., A. Cavaliere, G. Lo Paro, A. Manganaro, A. Mazzola, 1985 - Preliminary studies on reproduction of *Puntazzo puntazzo* (Gmelin, 1789) (Pisces: Sparidae) under controlled conditions. *Aquaculture*, **49**: 111-123.

Fernandez-Palacios H., D. Montero, J. Soccoro, M.S. Izquierdo, J.M. Vengara, 1994 - First studies on spawning, embryonic and larval development of *Dentex gibbosus* (Rafinesque, 1810) (Osteichthyes, Sparidae) under controlled conditions. *Aquaculture*, **122**: 63-73.

Glamuzina B., J. Jug-Dujakovic, I. Katavic, 1989 - Preliminary studies on reproduction and larval rearing of common dentex, *Dentex dentex* (Linnaeus, 1758). *Aquaculture*, **77**: 75-84.

Hunter J.R., 1984 - Synopsis of culture methods for marine fish larvae. In: *Ontogeny and Systematics of Fishes*. H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall, S.L. Richardson (eds), *American Society of Ichthyologists and Herpetologists*, Special Publication no. 1, Lawrence USA, Allen Press Inc, pp: 24-27.

Jug-Dujakovic J., J. Dulcic, I. Katavic, 1995 - Embryonic and yolk-sac larval development of the sparid *Dentex* (*Dentex*) *dentex* (Linnaeus, 1758). *Fish. Res.*, **24**: 91-97.

Kentouri M., 1985 - Comportement larvaire de 4 sparidés mediterranéens en élevage *Sparus aurata, Diplodus sargus, Lithognathus mormyrus, Puntazzo puntazzo* (Poissons Téléostéens). Thèse d'État, Université des Sciences et Techniques de Languedoc, 492 pp.

Lo Bianco S., 1909 - Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli. *Mitt. Zool. Sn. Neapel.*, **19**: 513-761.

Markle D.F., 1984 - Phosphate buffered formalin for long term preservation of formalin fixed ichthyoplankton. *Copeia*, **2**: 525-528.

Polo A., M. Yufera, E. Pascual, 1991 - Effects of temperature on egg and larval development of *Sparus aurata* L. *Aquaculture*, **92**: 367-375.

Ricker W.E., 1979 - Growth rates and models. In: *Fish Physiology, Vol. VIII.* W.S. Hoar, D.J. Randall, J.R. Brett (eds), New York, Academic Press, pp.: 678-743.

Russell F.S., 1976 - *The eggs and the planktonic stages of British marine fishes.* Academic Press, London-New York-San Francisco, 510 pp.

Reçu en août 1996 ; accepté en juillet 1997.
Received August 1996; accepted July 1997.