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1 **Egg quality variability in common dentex (*Dentex dentex*, L.): comparison of different quality**
2 **indexes**

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11 **Abstract**

12 The egg quality of two common dentex captive broodstocks were monitored for two consecutive
13 years during their natural spawning season. Volume of spawned eggs, volume of buoyant eggs,
14 fertilization rate, egg weight, hatching rate and mortality of larvae were recorded.

15 According to the volume of spawned eggs, the ratio of buoyant eggs spawned, the number of
16 spawning days and the fertilization rate pointed to an improvement from Year 1 to Year 2. But data
17 on hatching rate and larval mortality lead to the opposite conclusion.

18

19 **Keywords**

20 Common dentex, *Dentex dentex*, egg quality, spawning quality

21 **Introduction**

22 Common dentex (*Dentex dentex* L.) was one of the species identified as suitable for Mediterranean
23 aquaculture diversification, mainly after a market crisis occurred in the early 2000's (Abellán &
24 Basurco 1996, Basurco & Lovatelli 2003).

25 Common dentex is a Sparidae finfish, gonochoristic, with a bisexual period during its juvenile stage.
26 It reaches sexual maturity and sexual differentiation at 1 year old (Jug-Dujakovic, Dulcic & Katavic
27 1995; Pavlidis, Loir, Fostier, Mölsa & Scott 2000). In wild populations, the sex ratio is 1:1 (Ramos
28 & Bayle, 1991; Morales-Nin & Moranta, 1997) and, during Spring, its natural reproductive season
29 (Glamuzina, Jug-Dujakovic & Katavic 1989), pelagic eggs are spawned daily at nightfall or at dawn
30 (Abellán, 2000). In captive populations, hormones have been successfully used for the maturation of
31 gonads (Greenwood, Scott, Vermeirssen, Mylonas & Pavlidis 2001), but the most used and successful
32 method is the photothermal induction (Abellán, 2000; Rueda & Martínez, 2001); i.e. a gradual
33 increase of daylength and temperature, mimicking the shift from Winter to Spring in the wild.
34 Broodstocks are kept in groups of various females and males, which makes difficult to control and
35 assess their reproductive performance because the proportion and timing of spawning females are
36 highly unpredictable (Pavlidis, Loir, Fostier, Mölsa & Scott 2000; Grau, Morales-Nin, Quetglas,
37 Riera, Massuti & Pastor 2001; Loir, Le Glac, Somarakis & Pavlidis 2001), leading to high variability
38 in the number and quality of spawns among farms and stocks (Bodington, 2000).

39 The three basic quality criteria used for finfish species with pelagic eggs are buoyancy of the eggs,
40 fertilization rate, and hatching rate. Good quality eggs are buoyant, while unfertilized and / or
41 damaged eggs sink (Moretti, Pedini, Cittolin & Guidastrì 1999). Fertilization rate identifies the
42 amount of fertilized eggs from the total buoyant eggs, which include highly hydrated, non-fertilized
43 mature oocytes (Fabra, Raldúa, Power, Deen & Cerdá 2005). Finally, it requires 24-48h to determine
44 the hatching rate in common dentex, depending on the incubation temperature; this criterion identifies
45 the amount of fertilized eggs that can complete the embryonic development and hatch successfully
46 (Manning & Crim, 1998; Nissling, Larsson, Vallin & Frohlund 1998; Nocilado et al., 2000;

47 Morehead, Hart, Dunstan, Brown & Pankhurst 2001; Salze, Tocher, Roy & Robertson 2005). These
48 criteria do not provide information on the quality of the yolk reserves, which determines the fitness
49 and eventual survival of common dentex larvae in the early stages (first feeding period). Some authors
50 have suggested the use of additional data on larval performance for the assessment of spawning
51 quality in finfish species (Carrillo, Zanuy, Oyen, Cerdá, Navas & Ramos 2000).

52 In the present work, the spawning quality of two common dentex captive broodstocks, photothermally
53 induced, has been monitored for two consecutive years during their natural spawning season. Criteria
54 based on buoyancy, fertilization rate, hatching rate and larval performance have been compared.

55 **Materials and methods**

56 Common dentex juveniles were fished in the Western Mediterranean Sea and acclimated to captivity.
57 They were kept at natural temperature and photoperiod until sexual maturation, 4 years after the
58 capture. Afterwards, they were kept at natural temperature and photoperiod, and fed with a
59 commercial feed for gilthead sea bream (Skretting, Norway). One month before their natural
60 spawning season, which is usually between March and June, 9 females and 13 males of *D. dentex*
61 were identified, separated into two groups with a ratio 1:1.4 (Giménez, Estévez, Lahnsteiner, Zecevic,
62 Bell, Henderson, Piñera & Sánchez-Prado 2006) and reared in 4000 L circular tanks connected to a
63 recirculation unit with controlled temperature and photoperiod (Carbó, Estévez & Furones 2002).
64 Females in broodstock 1 had an average weigh of 1449g, males had an average weigh of 1146g.
65 Females in broodstock 2 had an average weigh of 1561g, males had an average weigh of 1482g.
66 Once reared in the 4000 L circular tanks, feed regimes changed and photothermal induction started.
67 On year 1 they were fed *ad libitum* with a semi-moist pellet with the following composition: fresh
68 minced fish (*Boops boops*; 41%), fish meal (Skretting, Spain; 41%), fish oil concentrate (TG0525,
69 Croda, UK; 15%), and vitamin premix (Skretting, Spain; 2%). On year 2 they were fed *ad libitum*
70 with a commercial dry diet for broodstock (Vitalis Repro©, Skretting, Spain) with the following
71 composition: fish meal (65%), wheat gluten (11%), fish oil (11%), bean meal (8%) and wheat (4%).

72 Photoperiod was increased 0.5 h of light per week from 12hL:12hD (hours of Light : hours of
73 Darkness) to 14hL:10hD. Temperature was increased 1°C per week from 14°C to 18°C. During the
74 spawning season, photoperiod was kept at 14hL:10hD and temperature was left to naturally rise up
75 to 20°C.

76 Eggs were collected every morning from a cylindroconical tank covered with a 500 µm net and
77 immersed in a 100 L holding tank, located under the outflow of each 4000 L tank, and connected to
78 the same recirculation system. The volume of buoyant and non-buoyant eggs was measured with a
79 measuring cylinder. A sample of buoyant and fertilized eggs was plated onto a 96-well cell culture
80 plate (EIA plate), one egg per well containing UV-filtered seawater. Plates were incubated in the
81 darkness in a refrigerated incubator at 19°C, and monitored daily in order to record the hatching rate
82 and larval survival. Subsamples of at least 50 floating eggs were used to obtain wet weight (WW),
83 dry weight (DW) and water percentage (W%): about 20-30 mg eggs were counted and weighed to
84 the nearest 0.1 mg (wet weight, WW); then they were kept at 100°C for 24h and re-weighed (DW).
85 The estimation of water percentage (W%) was based on WW and DW data.

86 Data for WW, DW, W%, fertilization of buoyant eggs (%), hatching rate (%), larval mortality at 3
87 dph (%) and larval mortality at 5 dph (%) were statistically analysed by a Tukey's test ($P < 0.05$)
88 using a StatgraphicsPlus 4.1 program (StatPoint Inc., Virginia, USA), in order to compare the results
89 obtained from each broodstock between the two monitored spawning seasons.

90 **Results and Discussion**

91 Variability of egg quality within the same spawning season has been already described for common
92 dentex captive broodstocks (Giménez, Estévez, Lahnsteiner, Zecevic, Bell, Henderson, Piñera &
93 Sánchez-Prado 2006). In the present work, variability between broodstocks kept under the same
94 management conditions, and among years, is also evidenced.

95 Both broodstocks spawned for longer, and an overall larger amount of eggs, from year 1 to year 2
96 (Table 1). The ratio of buoyant eggs increased from Year 1 to Year 2 in Broodstock 1, which also
97 spawned eggs with higher WW and DW. Broodstock 2 showed no differences in the ratio of buoyant

98 eggs, and the WW and DW of the spawned eggs decreased from Year 1 to Year 2 (Table 1). In both
99 broodstocks and years, the W% remained around 90 – 91%, and the fertilization rate of buoyant eggs
100 was close to 100% in all the monitored batches.

101 The average hatching rate decreased in both broodstocks (Table 2), as well as the minimum hatching
102 rate obtained by a batch. The number of batches with lower hatching rates, i.e. between 50 and 80%
103 or below 50%, increased from Year 1 to Year 2 in both broodstocks. Regarding larval performance
104 under starvation, as an indirect measure of the quality of the yolk reserves, it was worse from Year 1
105 to Year 2 for both broodstocks. The average mortality at 3 days post-hatch (dph) and at 5 dph
106 increased, the number of batches showing higher mortalities at these ages also increased, and the
107 lowest age of last survival of a batch decreased dramatically (i.e. minimal age of surviving starved
108 larva).

109 Considering these results, the different quality indexes lead to opposite conclusions. Based on the
110 total egg production, ratio of buoyant eggs and number of spawning days, there is an improvement
111 from Year 1 to Year 2 in both broodstocks. But this improvement did not correspond to the hatching
112 rate and the data obtained with larval performance such as mortality at 3 and 5 dph, and age of last
113 surviving larva; in this case, it can be concluded that the spawning quality decreased from Year 1 to
114 Year 2 in both broodstocks.

115 The actual age of each fish is unknown since they were captured from the wild, but all were from the
116 same geographical origin and kept captive for the same time period. Their weight average, the sex
117 ratio in the broodstock, their nutritional background and the management was equal for both
118 broodstocks. The differences between Year 1 and Year 2 could be due to the change in the food
119 regime, the age of the fish, but we hypothesize that the main reason would be the overall confinement.
120 Fish kept captive, despite the improvements in diet formulation, animal wellbeing and management
121 are reared under artificial conditions that can impair their physiological cycles and eventually, in the
122 case of broodstocks, lead to lower quality of spawnings.

123 **Conclusions**

124 Criteria used for assessing the spawning quality can lead to different conclusions. All criteria should
125 be used in order to obtain a reliable assessment, including criteria based on larval performance.

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130 rearing season.

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189 **Tables**

190 Table 1. Quality data based on production of floating eggs, spawning days and egg weight.

191 Superscripts denote significant differences ($P < 0.05$, Tukey's test).

192 Table 2. Quality data based on the hatching rate and the performance of hatched larvae. *:

193 prehatching.

194

195

196 Table 1. Quality data based on production of floating eggs, spawning days and egg weight. Superscripts denote significant differences ($P < 0.05$,
197 Tukey's test).

198

	Broodstock 1		Broodstock 2	
	Year 1	Year 2	Year 1	Year 2
Total buoyant eggs (mL)	3,520	14,150	4,140	8,925
Total spawned eggs (mL)	5,410	18,535	7,260	15,235
Ratio buoyant : sinking eggs	1.9	3.2	1.3	1.4
Total buoyant eggs (estimated number)	11.7×10^6	47.3×10^6	13.8×10^6	29.8×10^6
Total spawned eggs (estimated number)	18.1×10^6	62.0×10^6	24.3×10^6	51.0×10^6
Number of spawning days	27	58	32	61
Wet weight (μg per egg; average \pm SD)	511.6 ± 70.3	530.2 ± 65.5	543.9 ± 62.3^a	508.0 ± 32.8^b
Dry weight (μg per egg; average \pm SD)	44.2 ± 2.9^b	47.9 ± 5.5^a	48.2 ± 4.4^a	44.4 ± 2.4^b
Water percentage (%; average \pm SD)	91.2 ± 0.9	90.8 ± 1.7	91.0 ± 1.1	91.2 ± 0.9

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202

203 Table 2. Quality data based on the hatching rate and the performance of hatched larvae. *: prehatching. Superscripts denote significant differences (P
 204 < 0.05, Tukey's test).

205

	Broodstock 1		Broodstock 2	
	Year 1	Year 2	Year 1	Year 2
Hatching rate (% , average \pm SD)	92.6 \pm 9.2	78.5 \pm 17.7	85.3 \pm 18.2	65.7 \pm 32.0
Maximum hatching rate (%)	100	100	100	100
Minimum hatching rate (%)	57.3	2.1	24.2	0
Number of batches with hatching rate > 80%	27	31	22	31
Number of batches with hatching rate > 50%	0	22	8	14
Number of batches with hatching rate < 50%	0	5	2	16
Mortality at 3 dph (% , average \pm SD)	21.2 \pm 15.9 ^b	70.0 \pm 27.3 ^a	19.0 \pm 15.0 ^b	72.9 \pm 31.8 ^a
Number of batches with mortality at 3 dph < 50%	25	14	31	12
Number of batches with mortality at 3 dph < 80%	2	16	1	13
Number of batches with mortality at 3 dph > 80%	0	28	0	36
Mortality at 5 dph (% , average \pm SD)	29.6 \pm 20.8 ^b	74.3 \pm 24.3 ^a	26.0 \pm 17.6 ^b	78.2 \pm 27.0 ^a
Number of batches with mortality at 5 dph < 50%	23	7	29	11
Number of batches with mortality at 5 dph < 80%	3	19	3	10
Number of batches with mortality at 5 dph > 80%	1	32	0	40
Maximal age of surviving starved larvae	12	12	13	10
Minimal age of surviving starved larvae	9	1	8	-1*

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