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## **Influence of tank volume on vitellogenesis and spawning performances in sea bass *Dicentrarchus labrax* L.**

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### **Abstract:**

Sea bass, *Dicentrarchus labrax* (mean weight: 748±13 g), were maintained before and during vitellogenesis in 1, 3, 8, 16 and 32 m<sup>3</sup> tanks, and then they were transferred to 2 m<sup>3</sup> tanks, for the spawning season. During the first 2 months of the experiment, the growth rates were significantly lower in smaller tanks (1 m<sup>3</sup>). In August, the oocyte diameters were significantly lower in smaller tanks (1, 3 and 8 m<sup>3</sup>) than in larger (16–32 m<sup>3</sup>) tanks. At the end of the experiment, the fish mean weight in the 1 m<sup>3</sup> tanks was significantly lower than in the 3 m<sup>3</sup> tanks, but oocyte diameters and plasma oestradiol concentrations were not significantly different between the volumes. This shows a longer acclimation requirement in smaller volume rearing. Although all the females had not spawned, one spawn at least was collected in each volume. The variation in conditioning volume has not blocked the spawning process. The qualitative and quantitative characteristics of spawns were not significantly different between volumes. The conditioning volume of 3 m<sup>3</sup> seems to be a minimal volume required to obtain good reproduction of sea bass.

**Keywords:** Sea bass, Reproduction, Vitellogenesis, Aquaculture, Volume

## 29 Introduction

30 For temperate species fish such as the European sea bass, photoperiod and temperature are  
31 the main environmental factors controlling the process of sexual maturation and reproduction  
32 (Bromage 1995; Carrillo, Zanuy, Prat, Cerda, Ramos, Mananos & Bromage 1995; Mananos,  
33 Zanuy & Carrillo 1997; Bromage, Porter & Randall 2001; Rodriguez, Begtashi, Zanuy, Shaw &  
34 Carrillo 2001). The salinity, the oxygenation and the water quality play a minor role. Other  
35 factors related to the culture conditions, such as tank hydraulics, rearing density or farming  
36 manipulations, can also influence the reproduction of numerous species: common carp (Sehgal  
37 & Toor 1995), tilapia (Siddiqui, Al-harbi & Hafedh 1997; Ridha & Cruz 1999), *Pagrus auratus*  
38 (Cleary, Pankhurst & Battaglione 2000; Schreck, Contreras-sanchez & Fitzpatrick 2001). They  
39 can have a limiting or an activating role in the reproduction and they can influence the spawn  
40 quality (Bromage 1995). Although investment in rearing structures is an important part of the  
41 budget of a hatchery project, very few studies have been carried out on the influence of tank-  
42 rearing volume on spawning performance. Nevertheless, it has been reported that tank volume  
43 may influence the welfare of the fish. Hence, mortality associated with repeated handlings is  
44 lower for turbot (*Scophthalmus maximus* L.) held in small tank volumes ( $2\text{m}^3$ ) than those  
45 maintained in larger ones ( $16\text{m}^3$ ) (Mugnier, Fostier, Guezou, Gaignon & Quemener, 1998).  
46 Similarly, a temporary reduction (2 weeks) in the tank volume during the final stages of  
47 gametogenesis influences negatively the quality of gametes produced by brown (*Salmo trutta*)  
48 and rainbow trout (*Oncorhynchus mykiss*) (Campbell, Pottinger & Sumpter 1994). Fornies,  
49 Mananos, Carrillo, Rocha, Laureau, Mylonas, Zohar and Zanuy (2001) showed that the quality  
50 of the spawns of sea bass held individually in a  $2\text{m}^3$  tank is lower than that of fish held in  $15\text{m}^3$   
51 tanks. However, in his study, the fish densities and sex ratios were different. Thus, it is difficult  
52 to differentiate the respective effects of density and volume on the quality of gametes and the  
53 survival rates of the progeny.

54 Finally, very few studies have focused on only the effect of tank rearing volume on reproductive  
55 performances. Okumura, Okamoto, Oomori and Nakazono (2002) showed that water height and  
56 pond volume could limit the emergence of reproductive behaviour preceding spawning of  
57 *Epinephelus akaara*. Ambali and Little (1996) have shown that for the same condition of

58 biomass, the production of eggs of *Oreochromis niloticus* per m<sup>2</sup> is inversely proportional to the  
59 size of the reproduction ponds. The spawns of zebra fish (*Danio rerio*) maintained in volumes of  
60 200 or 100mL represent, respectively, 48% and 26% of the production obtained with breeders  
61 maintained in aquariums of 3.5 L (Goolish, Evans, Okutake & Max 1998). The objective of this  
62 experiment was to estimate the influence of tank volume during gametogenesis on individual  
63 spawning performances of the European sea bass, firstly, by follow-up of the effects on the  
64 gonadic development and on the plasmatic E2 profiles and, secondly, by comparing these  
65 observations with the quantitative and qualitative characteristics of the individual spawns  
66 observed during the reproductive season. This would allow showing a correlation between the  
67 reduction in the tank volume and the decrease in the spawn performances and also  
68 determination of the minimum volume that blocks the reproduction. The present study was  
69 conducted on sea bass *Dicentrarchus labrax* that spawns spontaneously in captivity and due to  
70 its economic importance for European aquaculture.

71

## 72 **Materials and Methods**

### 73 Fish and rearing conditions

74 The European sea bass *Dicentrarchus labrax* L. used in this experiment were hatched at the  
75 Ecloserie Marine de Gravelines (France) and reared in our experimental facilities for 3 years.  
76 During six months preceding the experiment, fishes were maintained in 6 tanks of 3 m<sup>3</sup> of  
77 volume. The experiment was divided into two phases: the first, "conditioning period", which was  
78 squared with the whole gametogenesis and the second, "spawning period", which starts at the  
79 end of vitellogenesis and covers the duration of the spawning period.

80 Sexually mature sea bass (mean weight: 748 +/- 13g) were randomly distributed in 1, 3, 8, 16  
81 and 32m<sup>3</sup> tanks (3 tanks for "1m<sup>3</sup> condition" and 1 tank for the other conditions), at the density  
82 of 3 fishes per m<sup>3</sup> and a sex ratio of 2 males: female during the conditioning period (from the  
83 end March to end December). Each tank of 1, 3, 8, 16 and 32 m<sup>3</sup> contained 3, 9, 24, 48 and 96  
84 fishes respectively. Under-skin magnetic tags implanted in gill cover individually identified the  
85 fish. Breeders were maintained under natural photoperiod and water temperature of the  
86 roadstead of Brest (latitude 48°21'N). At the end of the first period, the females were considered

87 ripe when mean diameter of oocyte reached 800-900 $\mu$ m. The males were selected based on  
88 their ripeness characters (spontaneous sperm release). Each selected female was transferred  
89 into spawning tank for an individual monitoring at same sex ratio than previously (2 males:  
90 female). 3 spawning tanks were brought into second phase for each conditioning volume (3  
91 replicates), according to the method used by Suquet, Normant, Gaignon, Quéméner and Fauvel  
92 (2005). Egg collectors allowed us to collect both floating and sinking eggs from each spawning  
93 device. Spawns occurred spontaneously.

94 The conditioning (1, 3, 8, 16 and 32 m<sup>3</sup>) and spawning (2m<sup>3</sup>) tanks were supplied with running  
95 seawater to a flow corresponding to 30% of the volume of each tank per hour. The oxygen level  
96 was maintained around 80% of the saturation. During the conditioning period, fish were fed "ad  
97 libitum", every day, with commercial feed (Neo Repro, Le Gouessant<sup>®</sup> - Lamballe - France,  
98 pellet diameter 9mm). During the spawning period, a small quantity of food was provided to  
99 avoid a weight loss and keep the tanks clean when the temperature exceeds 12 °C.

100

101 Studied parameters

102 Every month (from March to December), during the "conditioning period", all the fish of all  
103 experimental units were caught and anesthetized with ethylene glycol monophenylether  
104 (200ppm). Growth was estimated by individual weighing of all fish. From these, Specific growth  
105 rate (SGR, %day<sup>-1</sup>) was calculated as:  $100 \times (\ln w_f - \ln w_i) \text{ day}^{-1}$ , where  $w_i$  and  $w_f$  are the initial and  
106 final mean wet body weight respectively. Oocyte development was monitored by biopsy.  
107 Biopsies were carried out in all females of 1, 3 and 8 m<sup>3</sup> tanks and at 8 randomly chosen  
108 females of the other experimental units (16 and 32 m<sup>3</sup>). The diameter of the forty largest  
109 oocytes was recorded. Plasma levels of steroid hormones, estradiol (E2), were monitored  
110 during gametogenesis. E2 was quantified using a homologous enzyme-linked immunosorbent  
111 assay (ELISA) based on the procedure described by Nash, Davail-Cuisset, Bhattacharyya,  
112 Suter, Le Menn & Kime (2000). Measurements of steroid levels were performed in the same  
113 females as the determination of oocyte diameter.

114 During the spawning period, presence of spawn was checked every morning. The determination  
115 of eggs viability was made at the "4 cells stage", using 3 samples of 15 ml for each spawn. Eggs

116 and oocytes were sorted according 3 criteria: live and considered as fertilized eggs (floating  
117 eggs and visible cells), dead (sinking eggs) or not fertilized (floating eggs but invisible division of  
118 cells). 3 samples by spawn were placed in cylindro-conical incubators of 1liter in volume with a  
119 renewal rate of 300% per hour. The water temperature was the same as in the spawning tanks.  
120 Each day, the dead eggs were collected. At hatching, measurements on larvae were realized  
121 under weak anaesthesia with ethylene glycol monophenylether. The following parameters were  
122 evaluated:(1) Number of spawns by female, (2) fecundity; number of eggs by spawn and  
123 relative fecundity; total number of eggs / weight of female, (3) viability rate (%);  $100 \times (\text{number}$   
124  $\text{of live eggs}/\text{total number eggs})$ , (4) fertilization rate (%);  $100 \times (\text{number of fertilized eggs}/ \text{total}$   
125  $\text{number of eggs})$ , (5) egg diameter (mm); evaluated on 40 eggs fertilized without apparent  
126 abnormality in cells division. Values obtained for the spawns were means of 3 incubators. (6)  
127 Length of larvae (mm); mean total length of forty larvae alive and without skeletal  
128 malformations. (7) Hatching rate (%);  $100 \times (\text{number of larvae} / \text{number of eggs incubated})$ . (8)  
129 Malformation rate (%):  $100 \times (\text{malformed larvae}/\text{larvae})$  and non-lethal malformation rate (%):  
130  $100 \times (\text{malformed alive larvae} / \text{larvae})$ . A skeletal malformation is considered as non-lethal, if it  
131 allows the survival of larvae.

132

### 133 Statistical analysis

134 All results are expressed as mean  $\pm$  SE. Data were compared using one-way ANOVA using  
135 STATISTICA for Windows. Significant ANOVAs were followed by a post hoc multiple  
136 comparison test (Newman-Keuls). Differences were considered to be significant at  $P < 0.05$ .  
137 Before ANOVA analysis, data expressed in % were arc sinus square-root transformed.

138

139

### 140 **Results**

141 The minimal value of oxygen saturation observed in the 3m<sup>3</sup> tank during the conditioning period  
142 (75.2% air saturation) remains superior to the minimum required for the well-being of sea bass.  
143 This deficit (-13%) compared with the other conditions was measured for approximately 2

144 months. During the spawning period, the oxygen saturation rate remained above the minimum  
145 required for the well being of fish ( $> = 79\%$ ).

146

147 Conditioning period

148 Temperature ranged from 9.1°C (8th December) to 20.1°C (27th August) for all tanks. From  
149 March to August, the specific growth rate (SGR (%day<sup>-1</sup>) in the 1m<sup>3</sup> tank was significantly lower  
150 ( $0.07 \pm 0.02$ ,  $P < 0.05$ ) than in other tanks (3, 8, 16 and 32 m<sup>3</sup>) with a SGR equal to  $0.17 \pm 0.01$ ,  
151  $0.14 \pm 0.01$ ,  $0.17 \pm 0.01$ ,  $0.18 \pm 0.01$  respectively. Although after August this difference  
152 disappeared, the Specific Growth Rate of fishes from this condition remained significantly lower  
153 ( $0.11 \pm 0.01\%$  day<sup>-1</sup>) ( $P < 0.05$ ) than the fishes of other tanks,  $0.2 \pm 0.01$ ,  $0.16 \pm 0.01$ ,  $0.19 \pm$   
154  $0.01$ ,  $0.19 \pm 0.01$  % day<sup>-1</sup> for respectively 3, 8 16 and 32 m<sup>3</sup> tanks, for the whole conditioning  
155 period. This is illustrated by the evolution of weight of fishes in the different conditions during the  
156 conditioning period (Fig 1).

157 At the end of August, the mean oocytes diameter of females from 16 and 32 m<sup>3</sup> were  
158 significantly larger than for the females of 1, 3 and 8 m<sup>3</sup> tanks (Fig 2). This difference  
159 disappeared progressively and at the end of the conditioning period, no significant difference  
160 persisted (Table 1 and Fig 2).

161 Whatever the sampling date considered, the comparison of the mean concentration of E2,  
162 connected with volume, does not show any significant difference; for example the E2  
163 concentration at the end of conditioning period (ng.ml<sup>-1</sup>) was  $0.57 \pm 0.2$ ,  $1.79 \pm 0.14$ ,  $1.05 \pm$   
164  $0.33$ ,  $1.27 \pm 0.15$ ,  $1.06 \pm 0.18$  for respectively 1, 3, 8, 16 and 32 m<sup>3</sup>. The mean plasma  
165 concentration E2 of all females shows a significant peak in early October (Fig 3).

Figure 1 166

Figure 2 167

Figure 3 168

169

170 Spawning period

171 During this period, the temperatures ranged from 9.4 to 13.5°C. Fishes were transferred in the  
172 spawning units on 20th December. The first spawn occurred on 7th February and the last one

173 on 9th April. For the twice-spawning females, the spawning period lasted between 17 and 27  
174 days (Table I). The lowest fecundity per spawn was observed for the three females (n°2, 5 and  
175 15) conditioned in 1m<sup>3</sup>, which showed the lowest total fecundity with only a spawn. The best  
176 spawn fecundity and total fecundity were observed for two-spawning females (n° 11 and 14).  
177 After statistical analysis, no relation was identified between rank of the spawn and the fecundity,  
178 for the twice-spawning females.

179 The eggs viability rate was included between 50 and 98.2%, except for a spawn with a nil  
180 viability rate (female n° 8). 7 of 13 spawns showed a viability rate superior to 80%. No relation  
181 was identified between conditioning volume and viability rate and between rank of spawn and  
182 viability rate.

183 All fertilization rates were superior to 98% except for the first spawn of a female from 16 m<sup>3</sup>  
184 (86%). The mean eggs diameter of all spawns varied from 1,15 ± 0.005 mm (the first spawn of a  
185 female from 16 m<sup>3</sup>) to 1,24 ± 0.004 mm (the first spawn of a female from 32 m<sup>3</sup>). This spawn  
186 had a mean egg diameter that differed significantly (P< 0.05) from the other diameters of eggs.

187 There is no relation between the eggs diameter and fecundity rate or the weight of the female.  
188 The hatching rates varied from 22% to 92% without relation between this parameter and the  
189 volume of conditioning, spawn rank or viability rate.

190 Hatchings have started from 4 to 6 days after the beginning of the incubation and have  
191 happened during one or two days. No difference was showed between the spawns. The  
192 malformations rates have moved between 5.3% (female from 8 m<sup>3</sup>) and 30% (female from 32  
193 m<sup>3</sup>), except the first spawn of the second female from 8 m<sup>3</sup> with a malformations rate  
194 significantly different (62%). The non-lethal malformations represented 13 to 95% of the total  
195 malformations. There was no relation between malformation rate and the conditioning volume.

Table I

196  
197 The smallest larva measures 3.30 mm, the greatest: 4.7 mm. The smallest mean length is 3.66  
198 ± 0.03 mm (second spawn of female 3) and the greatest is 4.28 ± 0.03 mm (second spawn of  
199 female 14), without significant difference between spawns. At the end of the experiment, the  
200 ovarian samples of females showed atretic oocytes. The mean weight loss of the females  
201 during the spawning period has been upper to males (respectively 20,8 ± 1,56% et 14,6 ±

202 0,89%). Furthermore, the spawning females and those, which did not spawn, showed a weight  
203 loss of  $25.6 \pm 1,25\%$  and  $15,3 \pm 0,75\%$ , respectively.

204

## 205 **Discussion**

206 The growth observed for all experimental tanks is very weak during the first two months of the  
207 experiment, with some conditions showing a loss of weight (1 and 8 m<sup>3</sup>). In the second part of  
208 the conditioning period all tanks have similar growth rates.

209 In addition, the evolution of oocyte diameter shows a significant difference between the smallest  
210 volumes (1, 3 and 8 m<sup>3</sup>) and the largest, which disappears progressively; in November there is  
211 no more difference. The differences of growth or kinetic of maturation observed in the first part  
212 of the conditioning period are explained by a more difficult acclimatization phase for the fishes in  
213 smallest volumes (1, 3 and 8 m<sup>3</sup>), during which a permanent static behavior of the fishes,  
214 particularly, in 1 m<sup>3</sup> tanks was observed whereas the other fishes take up rapidly all the  
215 available space and show an active swimming behavior. A compensatory growth occurring after  
216 a growth depression as described by Arendt (1997) or by Ali, Nicieza and Wootton (2003) could  
217 explain this tightening.

218 The peak of plasma E2 concentrations observed in October corresponds with these obtained  
219 during previous studies held on sea bass. (Mananos *et al.* 1997; Prat, Zanuy, Bromage &  
220 Carrillo 1999; Asturiano, Sorbera, Ramos, Kime, Carrillo & Zanuy 2000). In the study of  
221 Asturiano *et al.* (2000), this rise appears when oocyte mean diameter reach 650-700  $\mu\text{m}$ ,  
222 whereas in our study the oocyte diameter reached 400  $\mu\text{m}$ . Moreover, previous study showed  
223 the appearance of the E2 peak one or two months before spawn (Mananos *et al.* 1997; Prat *et*  
224 *al.* 1999) or during the spawning period (Prat, Zanuy, Carrillo, De Mones & Fostier 1990); our  
225 study shows a peak earlier, four months before spawning period. These high levels of E2 are  
226 useful for the preservation of the oocytes viability until the environmental conditions allow the  
227 final maturation of oocytes. In our experimental conditions, the fall of the water temperature  
228 (under 12°C from November) could explain the delay in the beginning of the spawning period  
229 and the lapse of four months between the peak of E2 and the beginning of spawns.



230 Our study showed that it is possible to obtain spontaneous spawns in small volumes ( $2\text{m}^3$ ) in  
231 contradiction with Fornies et al. (2001). The results obtained for the relative fecundity (mean of  
232 232000 eggs  $\text{kg}_1$ ) are in agreement with Mananos et al. (1997), who calculated a value of  
233 293000 eggs  $\text{kg}_1$ . Because at least one female from each conditioning volume has  
234 spontaneously spawned, we can conclude that none of the volumes tested blocks the  
235 reproduction in the range studied. The low number of spawns obtained and the variability in  
236 spawn results does not allow to show a gradual effect of the conditioning volume. One female  
237 from the  $3\text{m}^3$  tank and two females from the  $8\text{m}^3$  tank had spawned twice and the total  
238 fecundities associated are among the four best ones. All the females conditioned in  $16\text{m}^3$  have  
239 spawned, but their fecundities are lesser. Only one female from the  $1\text{m}^3$  tank and one from the  
240  $32\text{m}^3$  tank had spawned and their fertilities were among the worst ones. The poor results  
241 observed for the females from the  $32\text{m}^3$  tank can be explained by the stress induced by the  
242 transfer from a large conditioning volume ( $32\text{m}^3$ ) to a small spawning volume ( $2\text{m}^3$ ) according to  
243 Mugnier, Fostier, Guezou, Gaignon and Quemener (1998) who showed that turbot (*S.*  
244 *maximus*) acclimatized in the smallest volume are more tolerant than those conditioned in a  
245 large volume. Thanks to the good environmental conditions (especially the water temperature)  
246 maintained during our study in contrast with the conditions of natural medium, the spawns  
247 started in the first days of February, earlier than observed in the natural environment by  
248 Boulineau-Coatanea (1969), who observed them during March-April. Bromage (1995) observed  
249 the first spawn when females measured 35 cm and weighed 1.5 kg, but the lack of spawn in  
250 some tanks of our study does not seem to be related to fish length, because certain fish used in  
251 our study spawned despite having dimensions lower than these limits. The variability observed  
252 in the qualitative and quantitative characteristics of spawns induces us to conclude on the effect  
253 of individual characteristics of the females that could conceal the possible effect of the volume.  
254 No volume in the range studied had a blocking effect on the spawns, but from the results,  
255 conditioning in the  $8\text{m}^3$  tank seems to be a minimal condition for the well-being and reproduction  
256 of the sea bass.  
257 This study will have to be supplemented by an analysis of the effect of the density, to seek for  
258 the best compromise between the maximal number of available breeders and the minimal

259 volume of stocking to use, in order to preserve the good performances of spawns and to  
260 improve the economic results of hatcheries.

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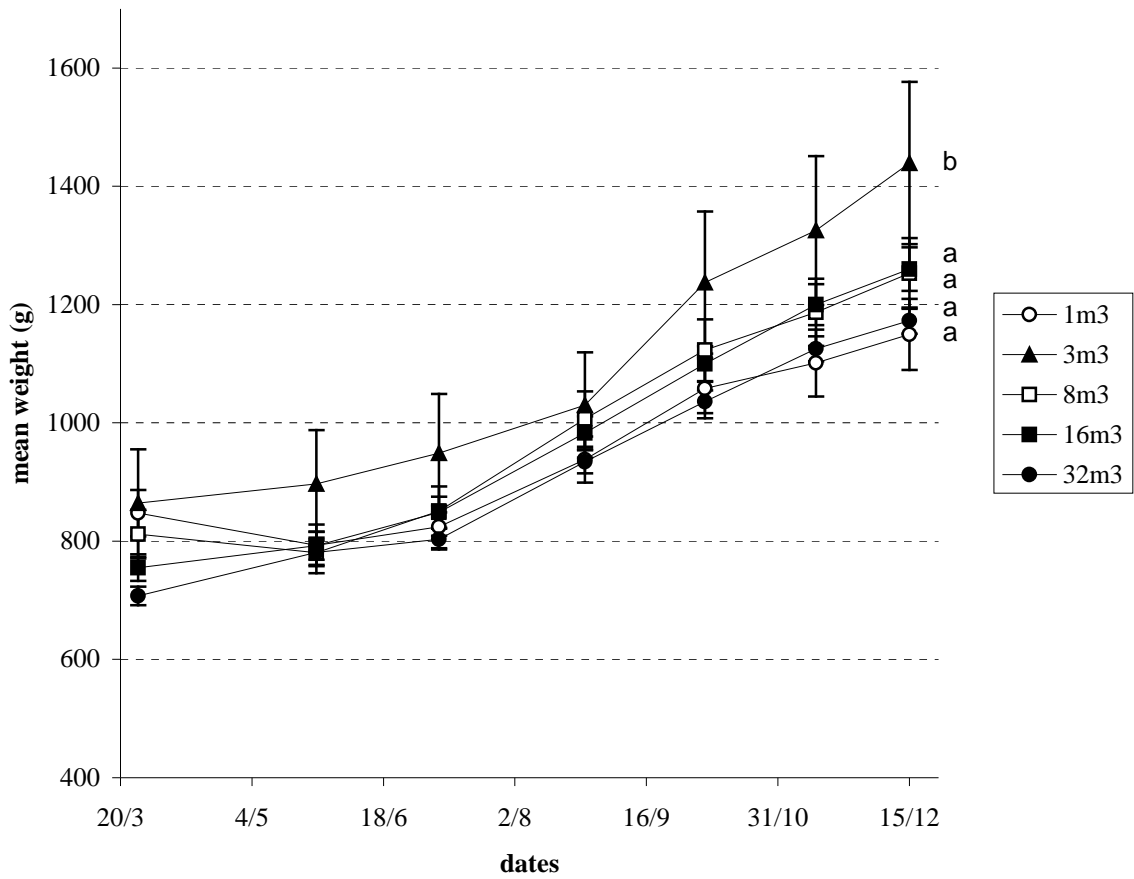
352 Suquet M., Normant Y., Gaignon J.L., Quéméner L. & Fauvel C. (2005) Effect of water  
353 temperature on individual reproductive activity of pollack (*Pollachius pollachius*). *Aquaculture*  
354 **243**, 113-120.

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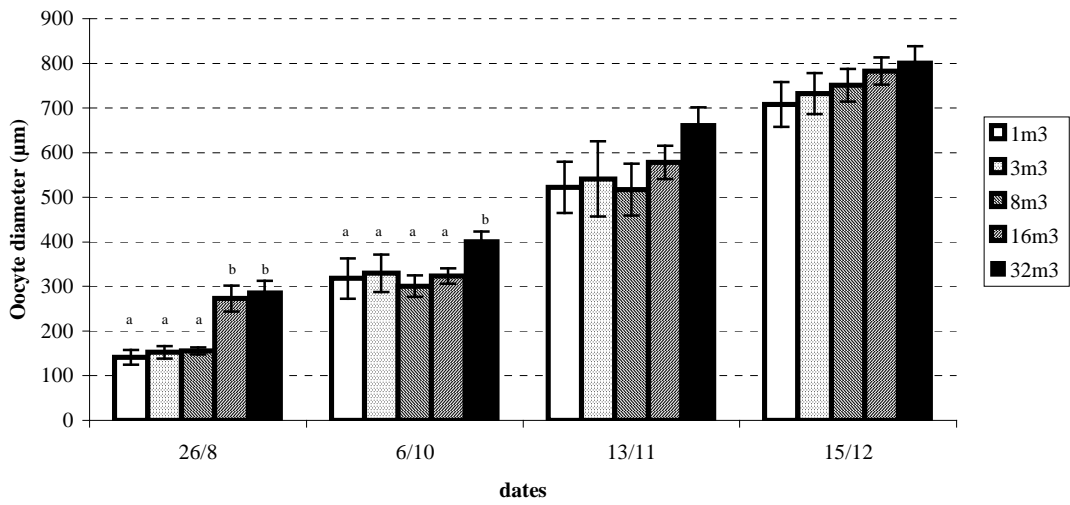
Table I. Individual females characteristics at the end of vitellogenesis and individual females spawning characteristics. The values for oocyte diameter correspond at mean±SE. Two numbers for the same female and the same parameter, corresponds to the values of two spawns. In a column, same letter denotes homogeneous groups (P>0.05).

♀	Conditioning volume (m <sup>3</sup> )	Data at the beginning of the spawning period (dec-15)				Day of spawn	Fecundity (eggs.spawn <sup>-1</sup> )	Relative fecundity (eggs.kg <sup>-1</sup> )	Viability rate (%)	Hatching rate (%)	Larval deformity rate (%)
		Weight (g)	SGR (% day <sup>-1</sup> )	Estradiol (ng ml <sup>-1</sup> )	Oocyte diameter (µm)						
1	8	1177	0.191	0.433	795.3±12.3	-	-	-	-	-	-
2	1	1256	0.108	0.958	770.7±12.6	-	-	-	-	-	-
3	16	1562	0.234	0.847	790.0±11.7	03/03	173 450	309 456	52.0	38.3	13.5a
						03/23	309 920		88.7	75.8	17.6a
4	3	1291	0.209	2.057	816.0±10.1	-	-	-	-	-	-
5	1	936	0.033	0.315	693.3±18.0	-	-	-	-	-	-
6	32	940	0.173	1.484	797.3±6.3	03/03	95 640	101 745	98.2	92.3	29.6a
7	16	1583	0.213	1.687	830.0±11.9	02/12	288 980	215 351	83.5	89.8	11.6a
						03/09	51 920		83.5	45.0	16.3a
8	16	1656	0.178	1.123	788.0±10.3	04/06	133 930	80 876	0.0	-	-
9	3	1674	0.158	1.599	714.7±9.3	-	-	-	-	-	-
10	32	919	0.315	0.477	825.3±9.7	-	-	-	-	-	-
11	8	1680	0.187	0.928	835.3±12.1	02/07	414 690	469 946	77.6	76.8	13.6a
						03/05	374 820		72.9	78.3	5.3a
12	32	1465	0.238	0.696	828.0±8.9	-	-	-	-	-	-
13	8	1400	0.223	0.467	848.0±12.4	03/23	207 610	276 257	64.7	31.3	62.0b
						04/09	179 150		80.1	51.6	19.7a
14	3	2237	0.182	1.715	666.0±8.1	03/03	387 260	379 865	88.9	68.3	8.3a
						03/30	462 500		94.5	70.7	19.7a
15	1	1469	0.140	0.450	660.0±9.3	03/07	35 240	23 989	63.6	21.9	18.2a

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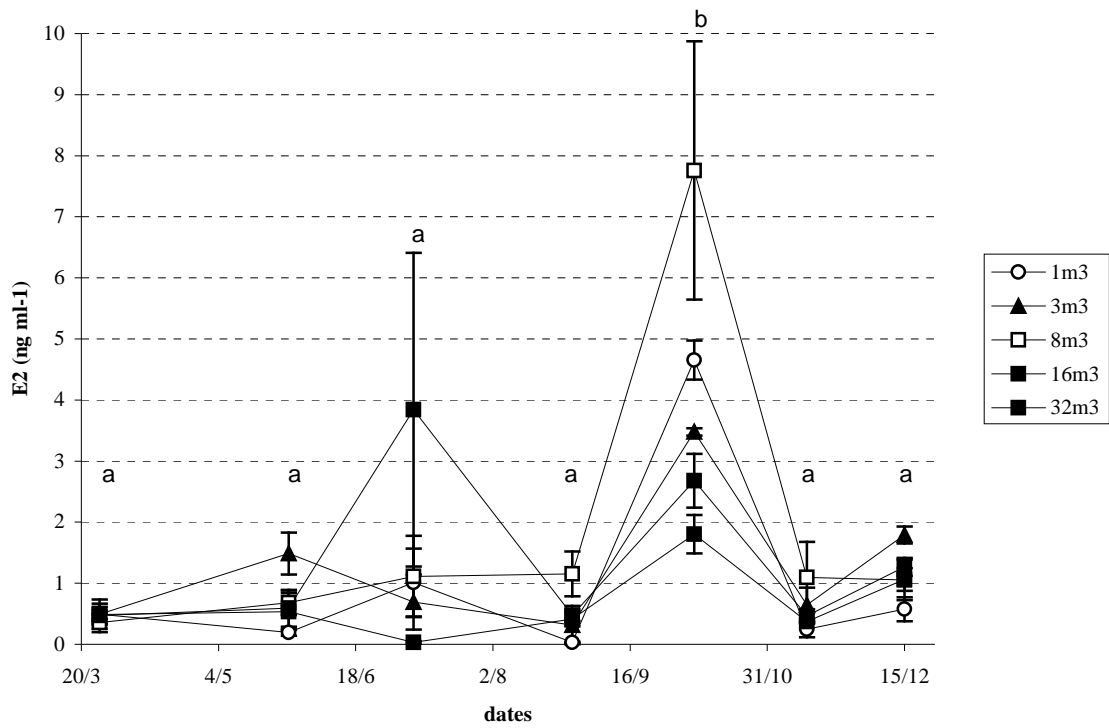


359 Figure 1: Change of mean weight over time in relation to the five conditioning volumes. Data are  
 360 given with SE (same letter denotes homogeneous groups ( $P > 0.05$ )). ( $n = 9$  for 1 and  $3\text{m}^3$ ,  $n = 24$ ,  
 361 48, 96 for respectively 8, 16 and  $32\text{m}^3$ )  
 362



362 Figure 2: Changes of mean oocytes diameters over time in relation to the conditioning volume.  
 363 Data are given with SE (same letter denotes homogeneous groups ( $P > 0.05$ )). ( $n = 3$  for 1 and  
 364  $3\text{m}^3$ ,  $n = 8$  for 8, 16 and  $32\text{m}^3$ )  
 365





365 Figure 3: Evolution of plasma E2 concentrations over time in relation to conditioning volume.  
 366 Values are given with SE (measurement days not sharing a common letter have an average  
 367 plasmatic oestradiol concentration, for the whole of observed females, significantly different  
 368 ( $P > 0.05$ )). (n= 3 for 1 and 3m<sup>3</sup>, n= 8 for 8, 16 and 32 m<sup>3</sup>)