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 m3 tanks, and then they were transferred to 2 m3 tanks, for the spawning season. During the first 2 months of the experiment, the growth rates were significantly lower in smaller tanks (1 m3). In August, the oocyte diameters were significantly lower in smaller tanks (1, 3 and 8 m3) than in larger (16–32 m3) tanks. At the end of the experiment, the fish mean weight in the 1 m3 tanks was significantly lower than in the 3 m3 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 m3 seems to be a minimal volume required to obtain good reproduction of sea bass.

Keywords: Sea bass, Reproduction, Vitellogenesis, Aquaculture, Volume
For temperate species fish such as the European sea bass, photoperiod and temperature are the main environmental factors controlling the process of sexual maturation and reproduction (Bromage 1995; Carrillo, Zanuy, Prat, Cerda, Ramos, Mananos & Bromage 1995; Mananos, Zanuy & Carrillo 1997; Bromage, Porter & Randall 2001; Rodriguez, Begtashi, Zanuy, Shaw & Carrillo 2001). The salinity, the oxygenation and the water quality play a minor role. Other factors related to the culture conditions, such as tank hydraulics, rearing density or farming manipulations, can also influence the reproduction of numerous species: common carp (Sehgal & Toor 1995), tilapia (Siddiqui, Al-harbi & Hafedh 1997; Ridha & Cruz 1999), *Pagrus auratus* (Cleary, Pankhurst & Battaglene 2000; Schreck, Contreras-sanchez & Fitzpatrick 2001). They can have a limiting or an activating role in the reproduction and they can influence the spawn quality (Bromage 1995). Although investment in rearing structures is an important part of the budget of a hatchery project, very few studies have been carried out on the influence of tank-rearing volume on spawning performance. Nevertheless, it has been reported that tank volume may influence the welfare of the fish. Hence, mortality associated with repeated handlings is lower for turbot (*Scophtalmus maximus* L.) held in small tank volumes (2m$^3$) than those maintained in larger ones (16m$^3$) (Mugnier, Fostier, Guezou, Gaignon & Quemener, 1998). Similarly, a temporary reduction (2 weeks) in the tank volume during the final stages of gametogenesis influences negatively the quality of gametes produced by brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Campbell, Pottinger & Sumpter 1994). Fornies, Mananos, Carrillo, Rocha, Laureau, Mylonas, Zohar and Zanuy (2001) showed that the quality of the spawns of sea bass held individually in a 2m$^3$ tank is lower than that of fish held in 15m$^3$ tanks. However, in his study, the fish densities and sex ratios were different. Thus, it is difficult to differentiate the respective effects of density and volume on the quality of gametes and the survival rates of the progeny. Finally, very few studies have focused on only the effect of tank rearing volume on reproductive performances. Okumura, Okamoto, Oomori and Nakazono (2002) showed that water height and pond volume could limit the emergence of reproductive behaviour preceding spawning of *Epinephelus akaara*. Ambali and Little (1996) have shown that for the same condition of
biomass, the production of eggs of *Oreochromis niloticus* per m² is inversely proportional to the size of the reproduction ponds. The spawns of zebra fish (*Danio rerio*) maintained in volumes of 200 or 100mL represent, respectively, 48% and 26% of the production obtained with breeders maintained in aquariums of 3.5 L (Goolish, Evans, Okutake & Max 1998). The objective of this experiment was to estimate the influence of tank volume during gametogenesis on individual spawning performances of the European sea bass, firstly, by follow-up of the effects on the gonadic development and on the plasmatic E2 profiles and, secondly, by comparing these observations with the quantitative and qualitative characteristics of the individual spawns observed during the reproductive season. This would allow showing a correlation between the reduction in the tank volume and the decrease in the spawn performances and also determination of the minimum volume that blocks the reproduction. The present study was conducted on sea bass *Dicentrarchus labrax* that spawns spontaneously in captivity and due to its economic importance for European aquaculture.

Materials and Methods

Fish and rearing conditions

The European sea bass *Dicentrarchus labrax* L. used in this experiment were hatched at the Ecloserie Marine de Gravelines (France) and reared in our experimental facilities for 3 years. During six months preceding the experiment, fishes were maintained in 6 tanks of 3 m³ of volume. The experiment was divided into two phases: the first, “conditioning period”, which was squared with the whole gametogenesis and the second, “spawning period”, which starts at the end of vitellogenesis and covers the duration of the spawning period. Sexually mature sea bass (mean weight: 748 +/- 13g) were randomly distributed in 1, 3, 8, 16 and 32m³ tanks (3 tanks for “1m³ condition” and 1 tank for the other conditions), at the density of 3 fishes per m³ and a sex ratio of 2 males: female during the conditioning period (from the end March to end December). Each tank of 1, 3, 8, 16 and 32 m³ contained 3, 9, 24, 48 and 96 fishes respectively. Under-skin magnetic tags implanted in gill cover individually identified the fish. Breeders were maintained under natural photoperiod and water temperature of the roadstead of Brest (latitude 48°21’N). At the end of the first period, the females were considered
ripe when mean diameter of oocyte reached 800-900µm. The males were selected based on their ripeness characters (spontaneous sperm release). Each selected female was transferred into spawning tank for an individual monitoring at same sex ratio than previously (2 males: female). 3 spawning tanks were brought into second phase for each conditioning volume (3 replicates), according to the method used by Suquet, Normant, Gaignon, Quéméner and Fauvel (2005). Egg collectors allowed us to collect both floating and sinking eggs from each spawning device. Spawns occurred spontaneously.

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

Studied parameters

Every month (from March to December), during the “conditioning period”, all the fish of all experimental units were caught and anesthetized with ethylene glycol monophenylether (200ppm). Growth was estimated by individual weighing of all fish. From these, Specific growth rate (SGR, %day$^{-1}$) was calculated as: 100 x (ln w_f – w_i) day$^{-1}$, where w_i and w_f are the initial and final mean wet body weight respectively. Oocyte development was monitored by biopsy. Biopsies were carried out in all females of 1, 3 and 8 m$^3$ tanks and at 8 randomly chosen females of the other experimental units (16 and 32 m$^3$). The diameter of the forty largest oocytes was recorded. Plasma levels of steroid hormones, estradiol (E2), were monitored during gametogenesis. E2 was quantified using a homologous enzyme-linked immunosorbent assay (ELISA) based on the procedure described by Nash, Davail-Cuisset, Bhattacharyya, Suter, Le Menn & Kime (2000). Measurements of steroid levels were performed in the same females as the determination of oocyte diameter. During the spawning period, presence of spawn was checked every morning. The determination of eggs viability was made at the “4 cells stage”, using 3 samples of 15 ml for each spawn. Eggs
and oocytes were sorted according 3 criteria: live and considered as fertilized eggs (floating eggs and visible cells), dead (sinking eggs) or not fertilized (floating eggs but invisible division of cells). 3 samples by spawn were placed in cylindro-conical incubators of 1 liter in volume with a renewal rate of 300% per hour. The water temperature was the same as in the spawning tanks. Each day, the dead eggs were collected. At hatching, measurements on larvae were realized under weak anaesthesia with ethylene glycol monophenylether. The following parameters were evaluated: (1) Number of spawns by female, (2) fecundity; number of eggs by spawn and relative fecundity; total number of eggs / weight of female, (3) viability rate (%); 100 x (number of live eggs / total number of eggs), (4) fertilization rate (%); 100 x (number of fertilized eggs / total number of eggs), (5) egg diameter (mm); evaluated on 40 eggs fertilized without apparent abnormality in cells division. Values obtained for the spawns were means of 3 incubators. (6) Length of larvae (mm); mean total length of forty larvae alive and without skeletal malformations. (7) Hatching rate (%); 100 x (number of larvae / number of eggs incubated). (8) Malformation rate (%); 100 x (malformed larvae / larvae) and non-lethal malformation rate (%): 100 x (malformed alive larvae / larvae). A skeletal malformation is considered as non-lethal, if it allows the survival of larvae.

Statistical analysis

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

Results

The minimal value of oxygen saturation observed in the 3 m³ tank during the conditioning period (75.2% air saturation) remains superior to the minimum required for the well-being of sea bass. This deficit (-13%) compared with the other conditions was measured for approximately 2
months. During the spawning period, the oxygen saturation rate remained above the minimum required for the well being of fish (≥ 79%).

Conditioning period

Temperature ranged from 9.1°C (8th December) to 20.1°C (27th August) for all tanks. From March to August, the specific growth rate (SGR (%day⁻¹)) in the 1m³ tank was significantly lower (0.07 ± 0.02, P<0.05) than in other tanks (3, 8, 16 and 32 m³) with a SGR equal to 0.17 ± 0.01, 0.14 ± 0.01, 0.17 ± 0.01, 0.18 ± 0.01 respectively. Although after August this difference disappeared, the Specific Growth Rate of fishes from this condition remained significantly lower (0.11 ± 0.01% day⁻¹) (P<0.05) than the fishes of other tanks, 0.2 ± 0.01, 0.16 ± 0.01, 0.19 ± 0.01, 0.19 ± 0.01 % day⁻¹ for respectively 3, 8, 16 and 32 m³ tanks, for the whole conditioning period. This is illustrated by the evolution of weight of fishes in the different conditions during the conditioning period (Fig 1).

At the end of August, the mean oocytes diameter of females from 16 and 32 m³ were significantly larger than for the females of 1, 3 and 8 m³ tanks (Fig 2). This difference disappeared progressively and at the end of the conditioning period, no significant difference persisted (Table 1 and Fig 2).

Whatever the sampling date considered, the comparison of the mean concentration of E2, connected with volume, does not show any significant difference; for example the E2 concentration at the end of conditioning period (ng.ml⁻¹) was 0.57 ± 0.2, 1.79 ± 0.14, 1.05 ± 0.33, 1.27 ± 0.15, 1.06 ± 0.18 for respectively 1, 3, 8, 16 and 32 m³. The mean plasma concentration E2 of all females shows a significant peak in early October (Fig 3).

Figure 1
Figure 2
Figure 3

Spawning period

During this period, the temperatures ranged from 9.4 to 13.5°C. Fishes were transferred in the spawning units on 20th December. The first spawn occurred on 7th February and the last one
on 9th April. For the twice-spawning females, the spawning period lasted between 17 and 27
days (Table I). The lowest fecundity per spawn was observed for the three females (n°2, 5 and
15) conditioned in 1m³, which showed the lowest total fecundity with only a spawn. The best
spawn fecundity and total fecundity were observed for two-spawning females (n° 11 and 14).
After statistical analysis, no relation was identified between rank of the spawn and the fecundity,
for the twice-spawning females.

The eggs viability rate was included between 50 and 98.2%, except for a spawn with a nil
viability rate (female n°8). 7 of 13 spawns showed a viability rate superior to 80%. No relation
was identified between conditioning volume and viability rate and between rank of spawn and
viability rate.
All fertilization rates were superior to 98% except for the first spawn of a female from 16 m³
(86%). The mean eggs diameter of all spawns varied from 1,15 ± 0.005 mm (the first spawn of a
female from 16 m³) to 1,24 ± 0.004 mm (the first spawn of a female from 32 m³). This spawn
had a mean egg diameter that differed significantly (P< 0.05) from the other diameters of eggs.
There is no relation between the eggs diameter and fecundity rate or the weight of the female.
The hatching rates varied from 22% to 92% without relation between this parameter and the
volume of conditioning, spawn rank or viability rate.
Hatchings have started from 4 to 6 days after the beginning of the incubation and have
happened during one or two days. No difference was showed between the spawns. The
malformations rates have moved between 5.3% (female from 8 m³) and 30% (female from 32
m³), except the first spawn of the second female from 8 m³ with a malformations rate
significantly different (62%). The non-lethal malformations represented 13 to 95% of the total
malformations. There was no relation between malformation rate and the conditioning volume.

Table I

The smallest larva measures 3.30 mm, the greatest: 4.7 mm. The smallest mean length is 3.66
± 0.03 mm (second spawn of female 3) and the greatest is 4.28 ± 0.03 mm (second spawn of
female 14), without significant difference between spawns. At the end of the experiment, the
ovarian samples of females showed atretic oocytes. The mean weight loss of the females
during the spawning period has been upper to males (respectively 20.8 ± 1.56% et 14.6 ±
Furthermore, the spawning females and those, which did not spawn, showed a weight loss of 25.6 ± 1.25% and 15.3 ± 0.75%, respectively.

**Discussion**

The growth observed for all experimental tanks is very weak during the first two months of the experiment, with some conditions showing a loss of weight (1 and 8 m³). In the second part of the conditioning period all tanks have similar growth rates.

In addition, the evolution of oocyte diameter shows a significant difference between the smallest volumes (1, 3 and 8 m³) and the largest, which disappears progressively; in November there is no more difference. The differences of growth or kinetic of maturation observed in the first part of the conditioning period are explained by a more difficult acclimatization phase for the fishes in smallest volumes (1, 3 and 8 m³), during which a permanent static behavior of the fishes, particularly, in 1 m³ tanks was observed whereas the other fishes take up rapidly all the available space and show an active swimming behavior. A compensatory growth occurring after a growth depression as described by Arendt (1997) or by Ali, Nicieza and Wootton (2003) could explain this tightening.

The peak of plasma E2 concentrations observed in October corresponds with these obtained during previous studies held on sea bass. (Mananos *et al.* 1997; Prat, Zanuy, Bromage & Carrillo 1999; Asturiano, Sorbera, Ramos, Kime, Carrillo & Zanuy 2000). In the study of Asturiano *et al.* (2000), this rise appears when oocyte mean diameter reach 650-700 µm, whereas in our study the oocyte diameter reached 400 µm. Moreover, previous study showed the appearance of the E2 peak one or two months before spawn (Mananos *et al.* 1997; Prat *et al.* 1999) or during the spawning period (Prat, Zanuy, Carrillo, De Mones & Fostier 1990); our study shows a peak earlier, four months before spawning period. These high levels of E2 are useful for the preservation of the oocytes viability until the environmental conditions allow the final maturation of oocytes. In our experimental conditions, the fall of the water temperature (under 12°C from November) could explain the delay in the beginning of the spawning period and the lapse of four months between the peak of E2 and the beginning of spawns.
Our study showed that it is possible to obtain spontaneous spawns in small volumes (2m$^3$) in contradiction with Fornies et al. (2001). The results obtained for the relative fecundity (mean of 232000 eggs kg$^{-1}$) are in agreement with Mananos et al. (1997), who calculated a value of 293000 eggs kg$^{-1}$. Because at least one female from each conditioning volume has spontaneously spawned, we can conclude that none of the volumes tested blocks the reproduction in the range studied. The low number of spawns obtained and the variability in spawn results does not allow to show a gradual effect of the conditioning volume. One female from the 3m$^3$ tank and two females from the 8m$^3$ tank had spawned twice and the total fecundities associated are among the four best ones. All the females conditioned in 16m$^3$ have spawned, but their fecundities are lesser. Only one female from the 1m$^3$ tank and one from the 32m$^3$ tank had spawned and their fertilities were among the worst ones. The poor results observed for the females from the 32m$^3$ tank can be explained by the stress induced by the transfer from a large conditioning volume (32m$^3$) to a small spawning volume (2m$^3$) according to Mugnier, Fostier, Guezou, Gaignon and Quemener (1998) who showed that turbotS. maximus) acclimatized in the smallest volume are more tolerant than those conditioned in a large volume. Thanks to the good environmental conditions (especially the water temperature) maintained during our study in contrast with the conditions of natural medium, the spawns started in the first days of February, earlier than observed in the natural environment by Boulineau-Coatanea (1969), who observed them during March-April. Bromage (1995) observed the first spawn when females measured 35 cm and weighed 1.5 kg, but the lack of spawn in some tanks of our study does not seem to be related to fish length, because certain fish used in our study spawned despite having dimensions lower than these limits. The variability observed in the qualitative and quantitative characteristics of spawns induces us to conclude on the effect of individual characteristics of the females that could conceal the possible effect of the volume. No volume in the range studied had a blocking effect on the spawns, but from the results, conditioning in the 8m$^3$ tank seems to be a minimal condition for the well-being and reproduction of the sea bass.

This study will have to be supplemented by an analysis of the effect of the density, to seek for the best compromise between the maximal number of available breeders and the minimal
volume of stocking to use, in order to preserve the good performances of spawns and to
improve the economic results of hatcheries.

References


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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).

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<th>Conditioning volume (m$^3$)</th>
<th>Data at the beginning of the spawning period (dec-15)</th>
<th>Day of spawn</th>
<th>Fecundity (eggs.spawn$^{-1}$)</th>
<th>Relative fecundity (eggs.kg$^{-1}$)</th>
<th>Viability rate (%)</th>
<th>Hatching rate (%)</th>
<th>Larval deformity rate (%)</th>
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Figure 1: Change of mean weight over time in relation to the five conditioning volumes. Data are given with SE (same letter denotes homogeneous groups (P>0.05)). (n= 9 for 1 and 3 m$^3$, n= 24, 48, 96 for respectively 8, 16 and 32 m$^3$)
Figure 2: Changes of mean oocytes diameters over time in relation to the conditioning volume. Data are given with SE (same letter denotes homogeneous groups (P>0.05)). (n= 3 for 1 and 3 m³, n= 8 for 8, 16 and 32 m³)
Figure 3: Evolution of plasma E2 concentrations over time in relation to conditioning volume. Values are given with SE (measurement days not sharing a common letter have an average plasmatic oestradiol concentration, for the whole of observed females, significantly different (P>0.05)). (n= 3 for 1 and 3 m$^3$, n= 8 for 8, 16 and 32 m$^3$)