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Production of meiotic gynogenetic and triploid sea bass, *Dicentrarchus labrax* L. 1. Performances, maturation and carcass quality

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Abstract

Meiotic gynogenetic and triploid sea bass were produced by pressure shocks according to a previously published protocol. Pressure-treated groups did not survive as well as controls during early development and larval rearing. Performances, sexual maturation and carcass quality were examined over a period of 34–45 months. At the age of 34 months, growth of the gynogenetic fish was comparable to that of the control but inferior in the triploid fish. A predominance of male fish was found within the triploid groups, while diploid and meiotic gynogenetic fish showed equal proportions of the sexes. Gonadal maturation in triploid fish was significantly impaired, particularly in the females that showed rudimentary ovaries. Triploid males exhibited primary maturation but proved to be gametically sterile. Pressure-induced triploids did not grow as well as diploids, but these results might be ascribed to specific on-growing conditions (communal rearing). The performance of gynogenetic sea bass was comparable to that of control. The superiority of diploid fish over their triploid counterparts was confirmed during the final growing period and more clearly so in females. Performances of triploids varied according to their maternal origin. Overall, striking qualitative differences between diploid and triploid fish were found at the age of

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34 and 45 months, although the results varied in a gender-specific manner. A strong maternal effect was also observed. The potential advantages of triploid sea bass for aquaculture purposes are discussed.

Keywords: Triploidy; Gynogenesis; Growth; Maturation; Sea bass; *Dicentrarchus labrax*

1. Introduction

The induction of polyploidy and gynogenesis as a tool for genome manipulation and sex control in fish generates considerable interest in aquacultural research. Much of the interest in induced polyploidy in commercially important fish is due to the assumption that growth rate of triploid fish would be somewhat higher than that of diploids and/or that their qualitative traits would be improved. Triploids are in fact assumed to be at least gametically sterile as they do not show proper reductional division during gametogenesis (Thorgaard and Allen, 1987) and therefore have the potential to outgrow diploids if the negative effects of treatment can be limited. The avoidance of reproduction would be of advantage in farmed fish species which become sexually mature before they reach marketable size as this would prevent the acquisition of commercially undesirable secondary sexual characteristics and potentially increase their somatic growth, carcass quality and survival. Since the original work on triploidy in the stickleback (*Gasterosteus aculeatus* L.) by Swarup (1959), triploidization has been induced with variable degree of success in various fish species as reviewed by Thorgaard and Allen (1987) and Purdom (1993). However, results on the association of growth improvement and induced triploidy remain conflicting (O'Keefe and Benfey, 1999) and seem to be species related and to depend on stock and rearing conditions. In particular, several workers have shown that triploids do not grow well when cultured in competition with their diploid counterparts as observed in salmonids (Quillet and Gaignon, 1990; Carter et al., 1994; Galbreath et al., 1994; McCarthy et al., 1996; Bonnet et al., 1999) and grass carp (Cassani and Caton, 1986).

Gynogenesis is a useful tool for the elucidation of genetic sex determination, the production of all-female progeny in species with homogametic (XX) females and the establishment of inbred lines. In several commercially important species, the artificial induction of gynogenetic diploids has been attempted, and techniques have been optimized, but information on growth and maturation in gynogenetically produced fish remains fragmentary.

In sea bass, previous studies described the methods to induce gynogenesis and triploidy by retention of the second polar body (Colombo et al., 1995; Gorshkova et al., 1995; Felip et al., 1997; Peruzzi and Chatain, 2000). Preliminary results on the performances of triploid and gynogenetic sea bass up to 3 years of age were reported by Colombo et al. (1996), Barbaro et al. (1996) and reviewed by Knibb et al. (1998). More recently, Felip et al. (1999a) described the growth and gonadal development in cold-shocked triploid sea bass during the first 2 years of age.

Besides the demonstration that triploidy might improve growth, the control of carcass yield and flesh quality (avoidance of decrease of fat, lipids and texture of flesh during sexual maturation) remain the main rationales for the application of triploidy in rainbow trout production for filleting and smoking big animals (3.5–4 kg) in France (Haffray et al., 1999). In sea bass, data on carcass and flesh quality of large-size animals (800–1200 g) are still lacking, even as diploid genotypes, and information on possible diversifications of production systems is required.

In the present study, we evaluate the zootechnical performances, sexual maturation, carcass and flesh quality in pressure-induced triploid and gynogenetic sea bass during almost 4 years of experimentation. The potential value and characterization of triploids for aquaculture purposes are also discussed.

2. Materials and methods

The experiment was performed over a 4-year period starting from April 1995 until February 1999, at the Station d'Aquaculture Expérimentale IFREMER of Palavas-les-Flots. The methods and treatment optima used for the induction of gynogenesis and triploidy were those described in Peruzzi and Chatain (2000).

The main handling periods operated during 4 years of experiment were as follows:

1. 0–6 months: Control, gynogenetic and triploid fish from two dams (A and B) were reared separately. Two additional tanks (dam A and B) were set up in order to verify the response of fish under mixed ploidy conditions.
2. 6–34 months: Fish were reared communally and tanks duplicated. Gynogenetic fish were culled at the end of the period.
3. 34–45 months: Diploid and triploid fish were reared separately in two duplicate tanks for each ploidy.

2.1. Broodstock and gamete collection

Sea bass broodstock obtained from wild stocks held at IFREMER Palavas was maintained in a closed recirculating system and spawned according to Peruzzi and Chatain (2000). Briefly, sperm was drawn from males and kept refrigerated before use. Sperm motility was checked under light microscopy according to an arbitrary scale (Billard et al., 1977) following activation with salt water. Ovulated oocytes were obtained by stripping the females at approximately 72 h following hormonal injection.

2.2. Sperm inactivation and artificial insemination

The DSD2 milt extender (Billard, 1984) was used following NaCl adjustment to 356 mOsm. Milt of sea bass males was diluted (1:20, milt/saline). Sperm motility was examined microscopically before and after dilution with saline as well as after irradiation

(total dose of 32,000 erg mm^{-2}) and compared to the motility of normal sperm. Batches of 50–70 ml of eggs were inseminated in 200-ml beakers with 4–6 ml of diluted irradiated or normal sperm, and after 5 s of mixing, water activation was initiated using a small volume of seawater. This stage was also considered as time zero in the development of the eggs. After further few seconds of gentle mixing, a larger volume of water was added and the eggs left undisturbed.

2.3. Pressure induction of triploidy and gynogenesis and determination of ploidy

Set values for pressure shocks were 8500 psi for 2 min applied at 6 min after fertilization (a.f.). Control eggs (unshocked samples) fertilized with either normal sperm (diploid control) or irradiated sperm (haploid control) were maintained in their beakers at 13 °C. Immediately after treatment, the eggs were gently rinsed and transferred into rearing tanks.

Twenty randomly selected hatched larvae per treatment were sampled at 72 h a.f., individually placed into disposable test tubes, deep frozen and prepared for propidium iodide (PI) flow-cytometric measurement as described by Peruzzi and Chatain (2000). At each time, haploid and diploid control groups were used as internal standards and their nuclear DNA content compared with those of erythrocytes of the parental types. For this analysis, whole blood was drawn from each fish by cardiac puncture, diluted in phosphate-buffered saline (PBS) and kept refrigerated until use. The ploidy analyses were repeated on all individuals in each experimental group at the age of 34 months (mean weight = 400 g) using blood samples.

2.4. Rearing method

2.4.1. Larval rearing (0–1.5 months)

Immediately after fertilization or application of the pressure shock, the eggs were transferred into 0.5-m^3 cylindroconical black gel-coated tanks with recirculated sand-filtered water (Biogrog, biological filter), disinfected under UV light. The volume of eggs varied according to the treatments they were submitted to in order to take into account the mortalities that the egg shock or the sperm irradiation will induce. It was, respectively, 50 and 70 ml for diploid (gynogenetic and normal) and triploid eggs and 25 ml/35 ml for the mixed diploid/triploid group. The total initial number of eggs introduced into each tank was, respectively, 44,300 (≈ 89 eggs/l) for diploids and 62,000 (≈ 125 eggs/l) for triploids. In the mixed group, 22,150 diploids were introduced together with 31,000 triploids (total ≈ 106 eggs/l)). These amounts correspond approximately to standard intensive rearing conditions (100 eggs/l). These numbers (N) were estimated from the formula: $N = 0.652\ V/vo$ (Chatain, 1994), where V is the total egg volume and vo the unitary egg volume (0.736 μl).

The larvae were kept in the dark until the age of 9 days. Starting from day 9, artificial incandescent lighting was used (photoperiod: 12:12 h L/D) avoiding all external light sources. Each tank was lit by a shaded cold-ray bulb (PAR 38, 120 W, GE) placed 1.2 m above the surface and producing 100 lx at the center of the tank and 40 lx at the edge.

The initial water temperature (14 °C) was maintained until hatching (3 days after fertilization), increased to 16 °C during the first 2 days post-hatching (DPH), and stabilized at this level until day 9. It was then increased to 20 °C in increments of 0.5 °C per day. Water turnover was 20% per hour. Initial seawater salinity (38‰) was decreased to 25‰ during the first 35 days of rearing then progressively readjusted to 38‰. Aeration rate ranged between 50 and 200 ml/min. Starting from day 5, the tanks were equipped with a cleaning system in order to remove the oily film developing on the surface and to facilitate swim bladder inflation (Chatain and Ounaïs-Guschemann, 1990).

Distribution of feed, consisting of live *Artemia salina*, was initiated on day 9, i.e., 4 days after mouth opening occurred. The prey density was adjusted twice or three times a day according to the larvae consumption. Freshly hatched nauplii were distributed from day 10 to day 20 followed by metanauplii enriched with a lipidic emulsion (Selco, Artemia system) for 12 h before distribution (one million *Artemia* per gram) and until day 48.

2.4.2. Nursery (1.5–3 months) and pregrowing (3–6 months)

At the age of 40 days, 650 larvae were sampled at random from each tank and transferred into other tanks similar to those of the larval rearing unit to be weaned and pregrown. Larvae without functional swim bladder were previously culled according to the sorting method described by Chatain and Corrao (1992). Seawater was treated as described above for the larval rearing phase, but the turnover was 100% per hour. Temperature was between 21 and 26 °C, with an average of 22 °C over the 5.5-month nursery and pregrowing period. Lighting was artificial and intensity set as during the larval rearing period. Larvae were fed commercial artificial pellet (Marine Start, Le Gouessant) between day 45 and 50, and then on Aqualim (Alevinage nos. 1, 2 and 3). Food distribution was ensured by an automatic feeder (food ratio ≈ 5–10% of the biomass) and until fish reached the adequate size (1 g, 3 months) to allow use of a self-feeder (food ad libitum). At the age of 3 and 6 months, a readjustment of the biomass was realized by reducing the number of fish per tank to 400 and 210 fish per tank, respectively.

2.4.3. On-growing (6–34 months)

At the age of 6 months, all fish were individually tagged (Section 2.7), mixed together and randomly transferred into an on-growing unit composed of two 4-m^3 cylindroconical black gel-coated tanks with recirculated water. They were then kept under communal rearing conditions until the age of 34 months. Throughout this period, the fish were fed exclusively on artificial pellet (Aqualim) provided ad libitum by a self-feeder. Light, photoperiod and temperature were natural varying from 13 °C in winter to 25 °C during summer periods.

2.4.4. On-growing (34–45 months)

Individually PIT-tagged fish (see Section 2.7) were reared for 1 more year (until age of 1384 days) in separate tanks according to their ploidy status (2*n* and 3*n*; 50 fish per tank) and in duplicates for security reasons only. At this stage, sex ratios were artificially equilibrated. Fish were fed as described above. Light, photoperiod and

temperature were natural, varying from 13 °C in winter to 25 °C during summer periods.

2.5. Rearing parameters, samples and measurements

Water quality was monitored by a daily check of temperature and salinity and a weekly check of nitrogen (NH_4 and NO_2) levels when the recirculating system was being used.

Fish performance and quality were estimated by recording the following parameters:

(1) total or standard length (L) on day 12 ($n = 10$), 21 ($n = 20$), 41 ($n = 40$) and on month 34 and 45 ($n = 50$);
(2) body weight (W) on months 3, 6, 9, 12, 15, 34, 45 ($n = 50$ per group);
(3) individual condition factor on months 34 and 45;
(4) total viscera, liver, gonad, perivisceral fat and digestive tract weight on 50 individuals per group and intra-muscular fat content on 10 individuals at 34 months of age (mean body weight = 400 g);
(5) all the above plus body width, fillet and bone weight at the age of 45 months on the entire population (mean body weight = 1 kg and approximately $n = 50$).
The yields for (3) and (4) were calculated relative to the total body weight.
(6) presence of urinary calculi in 21-day-old larvae, swim bladder inflation in 41-day-old larvae and skeletal (jaw and spine) deformities on 6-month-old fish ($n = 50$).

For measurements on larval samples, individuals were randomly captured, anaesthetized with MS 222 and observed by transparency on a Nikon V12 profile projector. Starting from the on-growing phase, all measurements and operations were carried out on fish anaesthetized with phenoxyethanol (300 ppm).

2.6. Measurement methods and precision

2.6.1. Survival

Fertilization rates were estimated by the ratio of the percentage of floating (living) eggs and sinking (dead) eggs (Chatain, 1994). Floating and sinking eggs were separated by simple decanting. Volumes were measured in a graduated syringe.

The number of surviving fish (N) at the end of the larval rearing (1.5 months), at the end of the weaning (2 months) and at 3 and 6 months of age was determined by individual counting. Operations were performed on anaesthetized fish.

2.6.2. Body weight, length and width

The fish weight was measured with a Mettler balance (precision to the nearest 0.001 g). The total length was used for measuring larvae and juveniles up to the age of 5 months, and the standard length for adult fish.

Body width was measured in 1 kg fish at the end of the experiment by use of an electronic digital caliper (precision of ± 0.03 mm between 0 and 200 mm, and of ± 0.04 mm beyond 200 mm).

2.6.3. Somatic and condition indices

The condition factor was calculated according to the formula K=[body weight/(body length)^3] × 100, the gonado-somatic index as GSI=(gonad weight/body weight) × 100 and the hepato-somatic index as HSI=(liver weight/body weight) × 100.

2.6.4. Skeletal deformities

The presence of spinal deformities (cyphosis, lordosis and vertebral fusion) was examined by soft X-rays (General Electrique 500 T; voltage 30 kV; automatic exposition cell at 60 mA per 0.03–0.05 s) on a Microvision monolayer film for mammography (Kronex-Dupont).

2.6.5. Urinary calculi

The presence of calculi in the kidney and near the urinary bladder is considered to be a pathological condition that might affect larval viability in hatchery-reared gilthead-sea bream, *Sparus aurata* (Modica et al., 1993) and sea bass (Chatain, 1994). For this purpose, 21-day-old larvae were observed by transparency on a profile projector and the incidence of calculosis recorded as described in Section 2.5.

2.6.6. Fillet quality

Flesh quality was estimated from the muscular fat content by classical lipid analysis at the age of 34 months (400 g) and by use of an electronic fat meter at the age of 45 months (1 kg). Electronic fat meter recordings failed at 34 months. The classical total lipid analysis was performed in triplicate on both ground-skinned fillets according the Soxlhet method (NF V4 402). For the fat meter (Torry Fish Fat Meter® Distell Industries, West Lothian, Scotland), the technique was based on microwaves and consisted of five measurements giving an estimation of the antero-posterior and dorso-ventral lipidic heterogeneity along the fish (Douirin et al., 1998). These measurements were calibrated with a chemical determination of sea bass muscular lipid content (S.E. 1.86%), the effectiveness of this method having been shown by Fauconneau et al. (1996). The fillet quality was estimated from the processing yield on 1 kg fish (45 months).

2.6.7. Viscera and gonads

Dissection of total viscera, liver and gonads was performed using standard methods after gutting the fish. Approximately 10 gonads/group were fixed in Bouin-Hollande, treated using conventional histological procedures and stained with toluidine blue and erythrosin orange G. Slides were observed using a photonic microscope (25 and 40 × magnification).

2.6.8. Motility test and fertilization trials

The sperm from control and triploid fish was drawn by abdominal pressure, and its motility was checked under a microscope after activation with seawater. When no sperm could be obtained, the activation test was performed on a testis smear. Fertilization trials were performed on egg samples held in individual 200-ml beakers placed in a thermo-regulated water bath. Percentages of developing eggs were recorded at 4 h a.f.

2.7. Fish tagging

At the age of 6 months (mean weight = 15–35 g) and before being mixed in a single rearing tank, the experimental groups were marked in the lower jaw or on the back using Visible Implant fluorescent elastomers (®Northwest Marine Technology, WA) of three different colors (blue, yellow and red). The operation was repeated several times during growth. The implants were injected just under the skin by use of a syringe equipped with a fine gauge needle. The size of the marks varied according to the size of the fish and, as a guidance, it started from a size of 2 mm. Detection of fluorescent marks was performed under UV illumination to increase the visibility in pigmented areas. Individual passively integrated transponder (PIT) tagging was performed at the age of 34 months on all fish in each group.

2.8. Statistical analysis

Statistical analyses were performed using STATISTICA (Statsoft, Maisons-Alfort, France) software. Swim bladder inflation, urinary calculi, survival and skeletal deformity rates and sex ratios were analyzed using χ^2 test (Elliot, 1977). Variance homogeneity was tested by Hartley test and homogeneous groups by Newman–Keuls (Dagnelie, 1975). All differences were accepted as significant when $P < 0.05$.

Different statistical approaches were used to analyze the data issuing from the various rearing periods (separate or communal):

2.8.1. Larval rearing (0–1.5 months), nursery (1.5–3 months) and pregrowing (3–6 months)

During these stages, the different groups of fish were reared in separate tanks (dam and "tank" factors being mixed and called " origin"). Therefore, weight differences were compared by a two-way analysis of variance (ANOVA type III sums of squares), the origin being considered as a random factor and the treatment (control, gynogenetic and triploid) as a fixed factor. The origin factor was nested within the treatment factor.

2.8.2. On-growing (6–34 months)

During this stage, the different groups of fish were tagged, gathered and reared in a communal tank (in security duplicates). The weight differences were then compared by a three-way ANOVA, where the origin and tank are random factors, whereas the treatment is still fixed. The origin factor is nested within the treatment factor and both are crossed with the tank factor.

2.8.3. On-growing (34–45 months)

Gynogenetic fish were discarded. Triploid and control groups were further separated and reared into different tanks. The employed ANOVA is therefore the one applied for the first rearing period (0–6 months). The same ANOVA was used to compare the gonado- and hepato-somatic indices, the perivisceral and intra-muscular fat ratios, the individual condition factor, the standard length, the fillet and the gutted yields.

3. Results

Pressure treatment of newly fertilized eggs of sea bass resulted in 100% triploidy in eggs from both dams at 72 h a.f. These results were confirmed by the ploidy analysis performed at the age of 34 months. Both gynogenetic groups resulted in 100% diploid individuals as confirmed by flow cytometry.

No survival was observed in any control haploid group beyond hatching stage (96 h a.f.) indicating the effectiveness of the UV treatment. Haploid state was further confirmed by flow-cytometric analysis on proportions of resulting embryos.

3.1. Larval, nursery and pregrowing period (0–6 months)

3.1.1. Survival

The overall survival of the diploid groups (13%) was higher during this period than in the gynogenetic (5%) and triploid groups (4%) and did not vary among dams except in their triploid form (Table 1).

The survival rates estimated 4 h a.f. ranged between 71% and 88% (Table 1). Gynogens and triploids were equivalent (mean = 79%; $\chi^2 = 1.26$; $df = 1$; $P = 0.2620$), but recorded a 9% decrease in fertilization rates compared to control groups (mean = 88%), ($\chi^2 = 9.89$; $df = 2$; $P = 0.0017$). Triploid fish originating from dam A (86%) performed better than those from dam B (71%).

Most of the mortality occurred during larval rearing. Survival rates estimated as percentages of fertilized eggs, were between 2% and 17% (Table 1). Again, triploids and gynogens were equivalent (mean = 9%; $\chi^2 = 0.0612$; $df = 1$; $P = 0.8046$), but recorded lower survival than controls (mean = 17%; $\chi^2 = 46.70$; $df = 2$; $P = 0.0001$). Unlike the performances recorded just after the shock, triploid fish obtained from dam B performed better (15%) than those from dam A (2%) at this stage of development.

Survival rates during the nursery period were similar to what is classically obtained during sea bass rearing (between 64% and 94%; Table 1). This time, gynogens performed as well as controls (mean = 90%; $\chi^2 = 3.1048$ $df = 1$; $P = 0.0781$). Only the triploids recorded low performances (70%; < 90% vs. other groups). Progenies obtained from dam B performed better than those from dam A, except in their triploid form.

Almost no mortality occurred during the pregrowing period: survival rates were between 87% and 99% (Table 1). All groups performed identically (mean = 98%) except for triploids issued from dam B (87%; $\chi^2 = 59.38$ $df = 1$; $P = 0.0001$).

Table 1
Early survival rates (%) of experimental fish originated from two dams (A and B)

Rearing period	CA	GA	TA	CB	GB	TB
Fertilisation (0–4 h a.f.)	88^{a}	84^{b}	85^{b}	87^{a}	78^{b}	71^{b}
Larval rearing (1–1.5 months)	16^{a}	9^{b}	2^{b}	17^{a}	6^{b}	15^{b}
Nursery (1.5–3 months)	88^{a}	83^{a}	75^{b}	94^{a}	94^{a}	64^{b}
Pregrowing (3–6 months)	97^{a}	99^{a}	99^{a}	97^{a}	99^{a}	87^{b}
Global over the 6-month period	12^{a}	6^{b}	1^{c}	14^{a}	4^{bc}	6^{b}

Control (C), gynogenetic (G), triploid (T); a.f.: after fertilisation.

3.1.2. Growth performances

Fish total length increased during the larval period from 5 to 20 mm (Table 2), and during the nursery and pregrowing period, total weight ranged between 14.5 and 25 g (Table 3). No length or weight differences were found between the control, triploid and gynogenetic groups during these first 6 months. A strong, significant "dam + tank" effect (called "origin" for convenience) was observed. This origin effect induced up to 5–7% differences in total length during the larval period (12 days: $F = 15.14$; $df = 3$ and 54; $P = 0.0001$; 41 days: $F = 24.15$ $df = 3$ and 234; $P = 0.0001$) and body weight differences of 10–30% during the nursery and pregrowing periods (3 months: $F = 19.68$; $df = 3$ and 294; $P = 0.0001$; 6 months: $F = 16.72$; $df = 3$ and 294; $P = 0.0001$). In any case, fish derived from origin A performed better than those derived from origin B, particularly in their triploid form.

3.1.3. Swim bladder inflation

Differences were observed within the experimental groups ($\chi^2 = 43.80$; $df = 5$; $P = 0.0001$) but could not be ascribed to differences between treated and control fish ($\chi^2 = 0.57$; $df = 1$; $P = 0.4491$). No special tendency related to the triploid or the gynogenetic state was found either. The lowest inflation percentage (45%) was observed in gynogenetic A, control A and triploid B ($\chi^2 = 0.86$; $df = 2$; $P = 0.6502$). Intermediate rates (71%) were found in control B and triploid A ($\chi^2 = 0.05$; $df = 1$; $P = 0.8256$), while the highest rate was observed in gynogenetic B (94%) ($\chi^2 = 8.58$; $df = 1$; $P = 0.0034$).

3.1.4. Urinary calculi and skeletal deformities

At 21 days a.f., only gynogenetic A, B and triploid A larvae showed urinary calculi in their bladder (5%). No malformation of the operculum was observed. Percentages of jaw and vertebral column deformities were similar (mean = 6%) in all groups (jaw: $\chi^2 = 1.06$; $df = 5$; $P = 0.9760$; vertebral column: $\chi^2 = 6.21$; $df = 5$; $P = 0.2859$).

3.1.5. Performances under competitive conditions (0–6 months)

No differences were found within the same ploidy group reared under communal or separate conditions. However, diploid fish (dam B) were 24% superior in weight ($F = 12.19$; $df = 1$; $P = 0.0006$) and 7% in length ($F = 14.7$; $df = 1$; $P = 0.0002$) to their triploid counterpart under both rearing conditions. Results from dam A were discarded as a drastic reduction in the percentage of triploid progenies under communal rearing was caused by a phenomenon of swim bladder hypertrophy.

Table 2
Growth in total length of experimental fish originated from two dams (A and B) during the larval period (0–1, 5 months)

Age (days)	CA	GA	TA	CB	GB	TB
12	6.11 ± 0.15^{a}	6.04 ± 0.15^{a}	6.61 ± 0.22^{a}	5.49 ± 0.22^{b}	5.88 ± 0.20^{b}	5.60 ± 0.15^{b}
21	9.08 ± 0.46^{a}	9.91 ± 0.33^{a}	8.64 ± 0.45^{a}	9.50 ± 0.36^{a}	9.48 ± 0.55^{a}	9.13 ± 0.30^{a}
41	16.44 ± 0.27^{a}	18.26 ± 0.36^{a}	19.21 ± 0.72^{a}	15.49 ± 0.50^{b}	19.09 ± 0.42^{b}	16.79 ± 0.30^{b}

Control (C), gynogenetic (G), triploid (T).

Table 3
Growth in weight of experimental fish originated from two dams (A and B) during the nursery, pregrowing and on-growing periods (3–45 months)

Age (months)	CA	GA	TA	CB	GB	TB
3	2.57 ± 0.28^{a}	2.55 ± 0.19^{a}	2.62 ± 0.21^{a}	2.17 ± 0.24^{b}	3.18 ± 0.28^{b}	1.58 ± 0.20^{b}
6	25.68 ± 2.08^{a}	25.64 ± 2.04^{a}	19.52 ± 1.50^{a}	19.92 ± 2.47^{b}	19.48 ± 2.10^{b}	14.41 ± 1.50^{b}
9	44.39 ± 4.43^{b}	46.81 ± 3.30^{a}	38.30 ± 3.35^{d}	39.18 ± 3.92^{c}	40.22 ± 3.70^{bc}	25.71 ± 3.45^{e}
12	49.50 ± 4.30^{a}	49.90 ± 4.20^{a}	41.30 ± 4.30^{b}	43.60 ± 4.80^{b}	43.40 ± 3.80^{b}	27.30 ± 3.80^{c}
34 male	374.83 ± 33.79^{b}	396.56 ± 30.73^{b}	353.91 ± 22.60^{bc}	440.14 ± 31.38^{a}	483.27 ± 25.76^{a}	316.68 ± 26.00^{c}
34 female	589.94 ± 45.31^{a}	546.25 ± 32.79^{a}	447.00 ± 38.87^{b}	571.68 ± 50.03^{a}	583.48 ± 26.29^{a}	423.14 ± 44.37^{b}
45 male	971.40 ± 61.48^{a}	–	877.90 ± 65.40^{b}	$974.\ 70 \pm 67.01^{a}$	–	808.80 ± 39.14^{b}
45 female	1251.10 ± 75.53^{a}	–	1082.40 ± 59.70^{b}	1298.10 ± 128.26^{a}	–	948.70 ± 111.40^{b}

Control (C), gynogenetic (G), triploid (T).

3.2. On-growing period (6–34 months)

3.2.1. Survival

The survival rates of experimental groups from the age of 12–34 months could not be estimated due to a technical failure of the individual tagging system. Firstly, partial or total loss of visible implant (fluorescent elastomer) occurred in approximately 30% of the population between 6 and 12 months of age. Some problems were also encountered with a new green elastomer provided by Northwest Marine Technology as replacement of the blue one. The fluorescence of this new elastomer approached, in fact, that of the yellow tag, misleading readers during detection under UV light.

3.2.2. Sex ratio and gonadal condition

The proportions of females in the diploid, gynogenetic and triploid progenies were different ($\chi^2 = 23.78$; $df = 11$; $P = 0.0137$) at the end of the experimentation (Fig. 1). This difference does not arise from duplicate rearing, the proportions of females between the two replicate tanks (mean = 52%) being not significantly different ($\chi^2 = 2.39$; $df = 1$; $P = 0.1219$). Differences in the proportions of females were ascribed to maternal origin ($\chi^2 = 9.71$; $df = 1$; $P = 0.0018$) with fewer females observed in the groups from dam B (43%

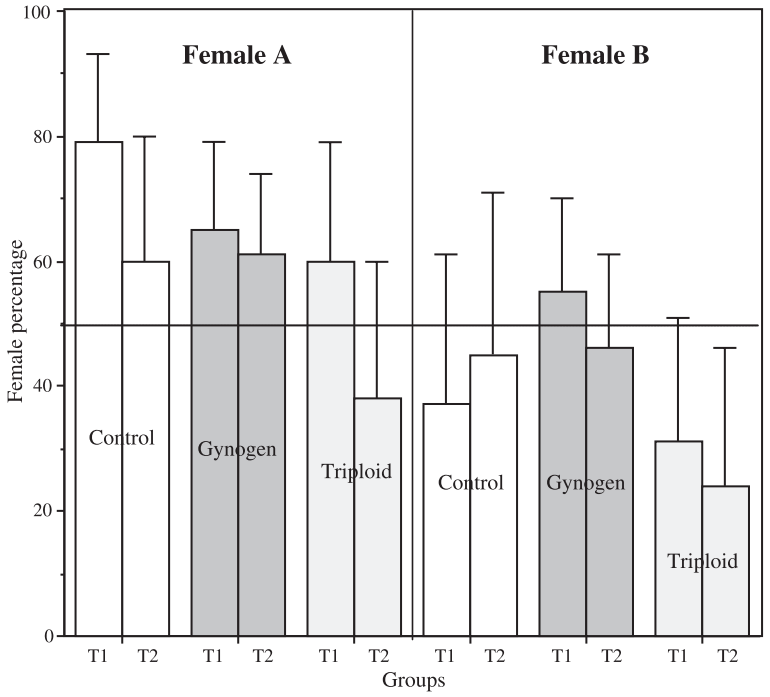

Fig. 1. Sex ratio of experimental groups of sea bass originated from two dams (A and B) and reared in two tanks (T1, T2). Control (□), gynogenetic (▩), triploid (▭).

vs. 60%), particularly among triploids. The triploid status affected the sex ratio significantly ($\chi^2 = 6.62$; $df = 2$; $P = 0.0365$). The proportion of females in triploid groups (mean = 40%) was lower than in the control and gynogenetic groups ($\chi^2 = 6.44$; $df = 1$; $P = 0.0011$). These last two groups showed similar, near 1:1 sex ratios (mean = 55%) ($\chi^2 = 0.19$; $df = 1$; $P = 0.6650$).

Macroscopic and histological analyses of ovaries and testes of 34- and 45-month-old triploid fish showed clear evidence of abnormal development compared to diploid fish. Triploid ovaries (Fig. 2a) were small or threadlike and contained very few oogonia and oocytes as compared to controls. Follicle development was impaired and retarded relative to diploids, but scattered vitellogenic oocytes could be observed in some triploid ovaries. Besides being larger, diploid ovaries were well developed and granular, showing secondary oocyte development and vitellogenesis. On the contrary, triploid males (Fig. 2b) had nearly developed whitish-gray testes but were never spontaneously fluent, and the gametes obtained from testis smears remained inactive after addition of seawater. Consequently, fertilization trials performed with gametes from squashed testes were all unsuccessful. The follicles of triploid males contained cell types ranging from spermatogonia to spermatids; no signs of spermiogenesis leading to spermatozoa were observed. Unlike triploids, all diploid males produced milt at stripping, and their gametes were fully active. Besides being larger, their well-developed white gonads contained numerous follicles filled with spermatozoa.

3.2.3. Growth performances

Measurements were performed every 3 months, but due to the problems encountered with the elastomer tags, only the weight measurements at 9, 12 (before the onset of the problem) and 34 months were statistically analyzed. At 34 month, the ploidy level was verified by flow cytometry, allowing distinguishing diploids from triploids. Gynogenetic fish were clearly identified by the only fluorescent implant that did not cause problem, the orange color. Fish weight ranged between 25–47 g at 9 months, 28–51 g at 12 months and 350–511 g at 34 months (Table 3).

As for the larval rearing period, the data analysis showed no clear correlation between triploid or gynogenetic state and growth. Again, a strong origin effect was pointed out

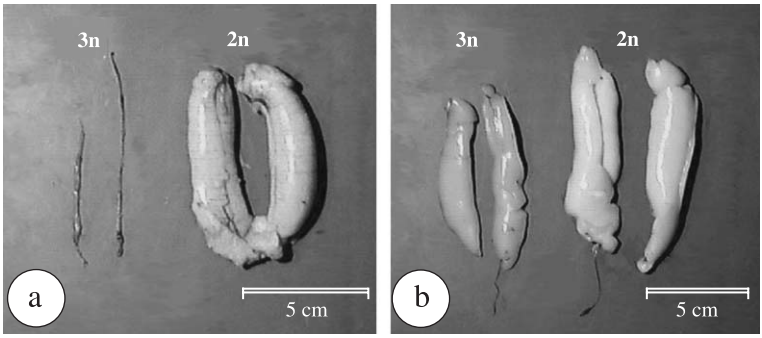

Fig. 2. Gonadal condition in triploid (3*n*) and diploid (2*n*) fish: (a) ovaries, (b) testes.

(9 months: $F=15.02$; $df=1$ and 593; $P=0.0001$; 12 months: $F=16.95$; $df=1$ and 593; $P=0.0001$; 34 months for females: $F=2.31$; $df=3$ and 173; $P=0.0078$; 34 months for males: $F=9.20$; $df=3$ and 161; $P=0.0001$). Only when fish reached the age of 34 months and could be sexed that the analysis of males and females highlighted a treatment effect, the weight of triploid females being 30% lower than the diploid ones ($F=7.77$; $df=2$ and 3; $P=0.0050$). For males, the same difference was recorded but was masked by an origin effect ($F=10.47$; $df=3$ and 188; $P<0.0001$).

This origin effect was not the same along the experiment. During the first year of life, the progenies of dam A were the best performing, but this tendency reversed between the second year of rearing and in 3-year-old fish when B progenies performed better than A ones. For each origin, gynogenetic or control fish showed similar performances, and these were always higher compared to their triploid counterpart.

The results stemming from the duplicated tanks showed differences for male and female fish: this was observed at 9 months (7%; $F=5.66$; $df=1$ and 593; $P=0.0018$) and 34 months of age (for females: $F=24.59$; $df=1$ and 173; $P<0.0001$). In this last case, 34-month-old males performed identically in the two environments unlike females that recorded an 11% weight difference.

3.2.4. Quality parameters

Condition factor (K factor) ranged between 1.87 and 2.14 in females and between 1.85 and 2.09 in males. Generally, triploids showed lower coefficients than control or gynogens, these two being equal; this difference was particularly evident in females (triploid < 10%; $F=8.56$; $df=3$ and 203; $P<0.0001$). In males, only the triploids B were different (< 5%) from their control or gynogenetic sibs ($F=23.86$; $df=3$ and 188; $P<0.0001$). In a general manner, progenies originated from dam A showed lower coefficients than those from dam B (Fig. 3).

Gonado-somatic index (GSI) ranged between 0.06 and 6.48 in females and between 2.08 and 4.00 in males. Triploids had extremely low GSI compared to gynogens or controls, especially in females: they were 28-fold smaller than the control, while gynogens were only 24% lower ($F=11.94$; $df=3$ and 160; $P<0.0001$). Male GSI differed by 69% between triploids and controls and by 13% between gynogens and controls ($F=25.10$; $df=2$ and 152; $P<0.0001$). Overall, progenies originated from dam B showed lower indexes than those from dam A.

Hepato-somatic Index (HSI) ranged between 2.05 and 3.08 in females and between 2.34 and 3.14 in males. As for the GSI, triploids showed lower indexes than gynogens, the latter being lower than controls; these differences were more important in females than males. The differences ranged between 40% and 31% for females and males, respectively (triploids vs. control), and 21% and 11% for gynogens vs. control (for females; $F=33.50$; $df=2$ and 3; $P=0.0080$; for males $F=100.1$; $df=2$ and 3; $P=0.0013$).

The proportion of fat surrounding the viscera ranged between 2.0% and 4.3% in females and between 2.5% and 3.2% in males. Perivisceral fat was highest in triploids and equal in gynogenetic or control fish. This result again appeared particularly true in females: their fat content was 110% higher compared to their control ($F=11.94$; $df=3$ and 160; $P<0.0001$). In males, only controls B were fatter (22%) than the other groups ($F=4.19$; $df=3$ and 152; $P=0.0070$).

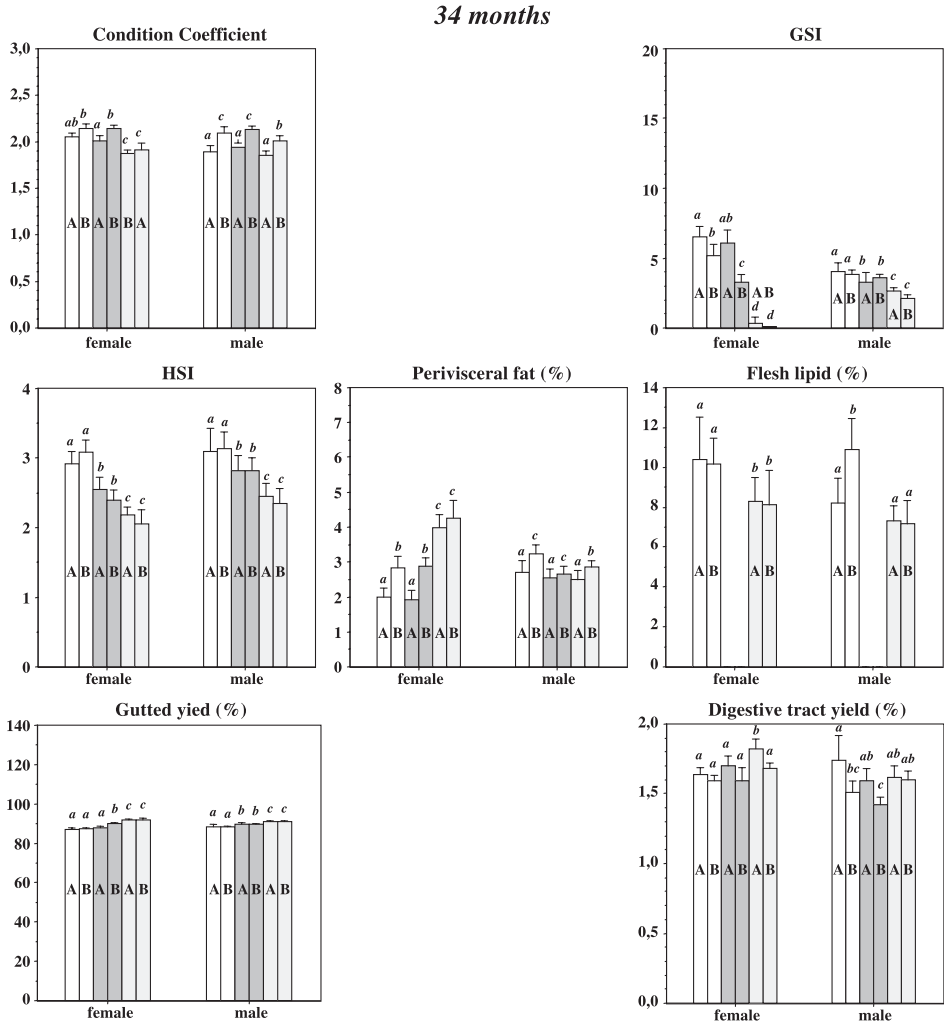

Fig. 3. Quality and condition of control (□), gynogenetic (▩) and triploid (▨) fish from females A and B at the age of 34 months. GSI: gonado-somatic Index; HSI: hepato-somatic index.

Flesh lipid content ranged between 8.1% and 10.4% for females and between 7.2% and 10.9% for males. Gynogens were not analyzed. Triploid fish contained less fat, particularly in females (25%; $F = 25.59$; $df = 1$ and 2; $P = 0.0860$). In males, only controls B were fatter (44%) than other groups ($F = 2.93$; $df = 3$ and 34; $P = 0.0668$).

Gutted yields ranged between 87% and 92% in females and between 88% and 91% in males. Triploids showed higher yields than gynogens, which were higher than controls, and these differences were more pronounced in females than in males. Differences ranged between 6% and 3% in females and males, respectively (triploid vs. control) and 2% and

1% for gynogens vs. control (for females; $F=11.02$; $df=2$ and 3; $P=0.0410$; for males $F=114.70$; $df=2$ and 3; $P=0.0012$).

Digestive tract yield ranged between 1.59% and 1.82% in females and between 1.42% and 1.74% in males. Little difference was recorded except in triploid A females, in which the yield was higher (11%) than in the other groups ($F=4.67$; $df=3$ and 160; $P=0.0037$).

3.3. On-growing period (34–45 months)

3.3.1. Survival

No mortality was recorded in the experimental groups during this period.

3.3.2. Growth performances

At the end of this period, weight ranged globally from 950 to 1298 g and from 828 to 975 g in females and males, respectively (Tables 3 and 4).

Triploid weight was lower than that of the control: 21% in females ($F=15.67$; $df=1$ and 2; $P=0.0524$); 17% in males ($F=14.38$; $df=1$ and 2; $P=0.0630$). SGR analysis confirmed this information. During the cold season, when growth rate was very slow (<0.1% body weight/day), all fish performed similarly. During the warm season, the lowest performance of triploids appeared clearly in females (44%; $F=28.32$; $df=2$ and 86; $P=0.0075$) and in males obtained from A dam (52%; $F=5.03$; $df=2$ and 102; $P=0.0082$).

3.3.3. Quality parameters

At the age of 45 months when fish reached approximately 1 kg of weight, they were sacrificed and analyzed as for the age of 34 months (Fig. 4).

Coefficient of condition ranged between 1.41 and 1.62 in females and between 1.41 and 1.50 in males. Triploids were characterized by lower coefficients. This was more evident in females (11%; $F=4.31$; $df=2$ and 86; $P=0.0164$) than in males. In males, indeed, only A triploid were found different from control B (6%; $F=3.34$; $df=2$ and 102; $P=0.0392$).

The thickness/standard length ratio ranged between 13.20% and 14.48% in females and between 13.23% and 13.75% in males. Only control females were thicker than triploids (7%; $F=3.59$; $df=2$ and 86; $P=0.0318$). Nevertheless, this difference was of the same range than the difference observed between controls (4%).

Gonado-somatic index ranged between 0.03 and 11.71 in females and between 2.46 and 4.00 in males. Triploids showed extremely low GSI compared to controls and particularly

Table 4
Specific growth rate (SGR) body weight per day of experimental fish originated from two dams (A and B) between 33 and 45 months

		CA	TA	CB	TB
SGR winter	male	0.011 ± 0.004^{a}	0.013 ± 0.055^{a}	-0.033 ± 0.004^{a}	-0.022 ± 0.023^{a}
	female	0.042 ± 0.025^{a}	0.092 ± 0.026^{a}	0.024 ± 0.045^{a}	0.033 ± 0.019^{a}
SGR summer	male	0.336 ± 0.038^{b}	0.219 ± 0.011^{a}	0.338 ± 0.023^{b}	0.325 ± 0.020^{b}
	female	0.366 ± 0.047^{a}	0.260 ± 0.055^{b}	0.395 ± 0.001^{a}	0.278 ± 0.050^{b}

Control (C), gynogenetic (G), triploid (T).

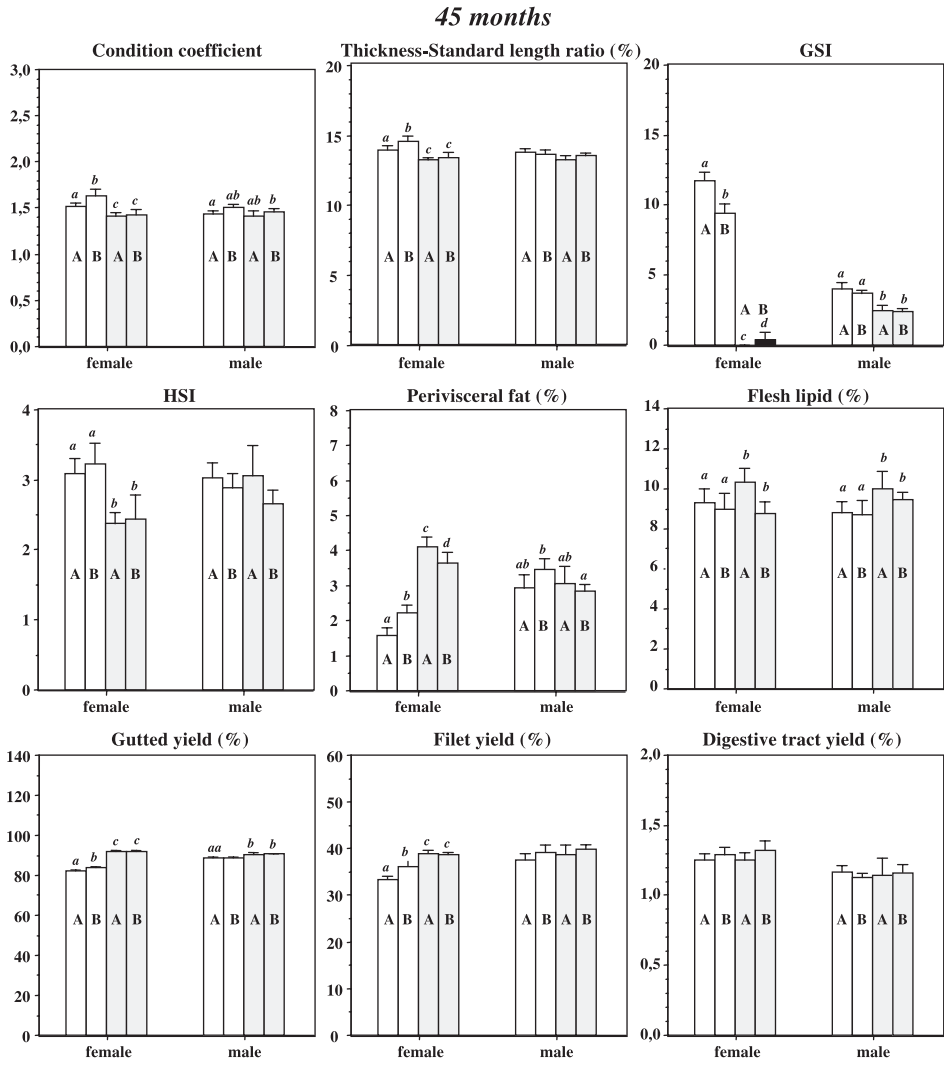

Fig. 4. Quality and condition of control (□) and triploid (▭) fish from females A and B at the age of 45 months. GSI: gonado-somatic index; HIS: hepato-somatic index.

in females, where differences reached 89-fold ($F = 65.70$; $df = 1$ and 2; $P = 0.0147$). In males, differences between triploid and diploids were around 60% ($F = 94.10$; $df = 1$ and 2; $P = 0.0104$).

Hepato-somatic index ranged between 2.38 and 3.23 in females and between 2.67 and 3.06 in males. Triploids showed lower indexes than controls, but these differences were only observed in females (32%; $F = 70.71$; $df = 1$ and 2; $P = 0.0065$).

The proportion of fat surrounding the viscera ranged between 1.57% and 4.10% in females and between 2.88% and 3.46% in males. Triploid females were much fatter than

controls: their fat content was 111% higher than in their diploid counterpart ($F=23.46$; $df=1$ and 2; $P=0.0390$). In males, indeed, only B triploids stored less perivisceral fat than their control (22%; $F=3.10$; $df=2$ and 1; $P=0.0494$).

Flesh lipid content ranged between 8.8% and 10.3% in diploid females and between 8.7% and 10% in males. Triploid flesh contained more lipids compared to diploids in both sexes: 9% in females ($F=2.92$; $df=2$ and 1; $P=0.0594$), 10% in males ($F=12.83$; $df=1$ and 2; $P=0.0697$).

Gutted yield ranged between 82% and 92% in diploid females and between 88% and 90% in males. Triploids had higher yields than controls, and these differences were fivefold more important in females than in males (11% in females; $F=151.96$; $df=1$ and 2; $P=0.060$; 2% in males $F=734.00$; $df=1$ and 2; $P=0.0012$).

Fillet yield ranged between 41% and 43% in females and between 42% and 44% in males. Triploids had higher yields than controls but only in females (11%; $F=11.21$; $df=2$ and 86; $P<0.0001$).

Digestive tract yield ranged between 1.25% and 1.32% in females and between 1.12% and 1.16% in males. There were no differences between the groups in both sexes.

4. Discussion

4.1. Survival

The first negative effects of pressure shocks on early development were recorded 4 h a.f.. At this time, fertilization rates of pressure-treated groups were 10% lower than controls. The same difference was recorded between dams at this stage of development. Negative effects of treatment (cold-shock) on fertilization rates in meiogenetic sea bass have been reported by Felip et al. (1999b). Smoker et al. (1995) clearly showed that the treatment rather than the ploidy status is responsible for lowered survival of cold-shocked gynogens in the pink salmon, *Oncorhynchus gorbuscha*.

Survival during larval rearing was also inferior in pressure-treated groups, the survival of control diploids being about twice that of triploid or gynogenetic fish. This negative effect of treatment on survival has been previously reported for sea bass larvae (Peruzzi and Chatain, 2000). Again, the most important differences were observed among dams (eightfold). Overall, the survival rates in the present work were low compared to what is generally obtained during this stage of the life cycle.

During the nursery and pregrowing phase (1.5–6 months), only the TB group displayed a lower survival (less than 20%), but this disadvantage with respect to the other groups was not confirmed during later periods (6–45 months). No differential mortality was observed within groups during the last phase of rearing (34–45 months of age).

A superior survival of diploid fish compared to triploids is generally observed under various conditions in salmonids (Bonnet et al., 1999; Galbreath and Thorgaard, 1995; Ojolick et al., 1995; Quillet and Gaignon, 1990; Solar et al., 1984) and cyprinids (Cherfas et al., 1994). Survival of triploid fish also varies according to the treatment and to the rearing conditions. In sea bass, no differences were reported in the survival of diploid and triploid sea bass reared separately between the age of 5 and 23 months (Felip et al., 1999a).

4.2. Sex ratios and gonadal condition

A predominance of male fish was found in this work within the triploid groups, while diploid and meiotic gynogenetic fish showed equal proportions of sexes. An excess of males was similarly observed among adults of triploid common carp (Cherfas et al., 1994), Thai silver barb, *Puntius gonionotus* (Koedprang and Na-Nakorn, 2000) and occasionally in rainbow trout (Solar et al., 1984). This was not reported for sea bass by Felip et al. (1999a) where a skewed sex ratio in favor of males was equally observed in diploid and triploid groups. Deviations from equal sex ratios in normal diploid offspring were often reported for cultured sea bass by other authors (Carillo et al., 1995; Colombo et al., 1996; Gorshkova et al., 1996). The present data and those previously obtained (Chatain et al., 2000) confirm that the mechanism of sex determination in this species does not correspond to a simple monofactorial system with female homogamety. A more complicated sex-determining mechanism with possible minor genetic or environmental factors interfering or overriding a major chromosomal mechanism have been suggested for this species by other workers (Colombo et al., 1996; Knibb et al., 1998).

Gonadal maturation in triploid fish was significantly impaired, particularly in females showing rudimentary ovaries. Triploid males exhibited primary maturation but proved to be gametically sterile during artificial breeding experiments. Similar results were reported by Felip et al. (1999a) and Colombo et al. (1996) on 2- and 3-year-old sea bass, respectively.

In this work, mean GSI of 34-month-old females was about 30 times lower than in control and about 90 times lower at 45 months of age. In males too, significantly, differences were observed for GSI between diploid and triploid fish, the values being nearly twice lower in 34 and 45-month-old triploid fish. Felip et al. (1999a) reported that the GSI of 2-year-old triploid females was reduced to 16% that of diploid females, whereas the GSI of triploid males was 40% that of their diploid siblings. Studies conducted on 3-year-old sea bass (Colombo et al., 1996) showed that the GSI of triploid fish was 172 times lower in females and 17 times lower in males compared to their diploid sibs just before the reproductive period.

4.3. Growth performances

No measurable growth differences between diploid and triploid sea bass were found in by Felip et al. (1999a) and Colombo et al. (1996) at the age of 2 and 3 years, respectively. In the present work, control and gynogenetic sea bass performed better than their triploid counterparts during the first 3 years of experimentation. The superiority of diploid fish over their triploid counterpart was confirmed during the final growing period and more clearly in females. Performances of triploids varied according to their maternal origin. No apparent negative effects resulted from rearing triploid groups in competition with diploids rather than in separate conditions during early stages (0–6 months). Moreover, a strong maternal effect was highlighted under separate rearing conditions. This finding, along with the previous on communal or separate conditions, indicates that the assumed "origin" effect could be ascribed to the maternal component only. Finally, results among dams were not consistent throughout the experiment. A significant influence of maternal strain effects on the relative growth of triploid progenies was also observed in rainbow trout by Guo et al.

(1990). These authors suggested that emphasis should be placed on the selection of a maternal strain exhibiting desiderable traits when producing triploids.

Studies conducted on other fish species have shown growth of triploids to be comparable to, or slightly lower than, that of diploids (Thorgaard, 1992; Galbreath et al., 1994; Hussain et al., 1995; Carter et al, 1994; O'Keefe and Benfey, 1999). The conclusions regarding the physiological competence of triploids affecting their behavior and performance remain nevertheless contradictory. While diploids and triploids in a number of species did not differ in terms of respiratory capacity (Sezaki et al., 1991), nutrient utilization (Oliva-Teles and Kaushik, 1990), or swimming capability (Parsons, 1993), some cases of stress tolerance (Ojolick et al., 1995) and reduced aerobic swimming ability (Virtanen et al., 1990) have been reported for triploid fish. In some cases, the altered ploidy level seems to affect behavior and to cause a genotype–environment interaction under particular conditions (Dunham, 1990). For instance, triploid channel catfish grown in tanks (with clear and calm water) were not as active as their diploid counterparts (Wolters et al., 1982). According to the same authors, lack of aggressive feeding behavior and general activity was more pronounced in less-favored environments such as ponds with turbid waters. Carter et al. (1994) has also observed a significantly higher incidence of dorsal fin damage due to aggressiveness of the diploids when reared communally with triploids in the Atlantic salmon, *Salmo salar*.

Unlike triploids, information on the performance of gynogenetic fish remains limited. Suzuki et al. (1985) reported that gynogenetic fish obtained by homologous and heterologous fertilization in the loach, *Misgurnus anguillicaudatus*, exhibited inferior growth compared to their diploid sibs. Comparative results were obtained on the carp, *Cyprinus carpio*, by Bieniarz et al. (1997). In the present study, the performance of gynogenetic sea bass resulted comparable to that of control up to the age of 33 months. In the same species, Colombo et al. (1996) reported that control fish grew faster than their gynogenetic counterpart until the age of 20 months.

4.4. Quality parameters

Striking differences between diploid and triploid fish were found in this work at the age of 34 and 45 months, which is not surprising as data were recorded right during the spawning season. Results also varied in a gender-specific manner. Most notably, triploid females exhibited lower *K* factor and HSI and higher gutted yield compared to diploid females. Fillet yield was also superior in one triploid progeny at the age of 45 months. Generally, fillet yield was higher in males than in females, independently from their ploidy status. Although obtained by different methods, differences in flesh lipids were also significant. Flesh lipids in triploids were inferior to controls at the age of 33 months, but finally increased. Comparable results were obtained for triploid males, although differences were less pronounced. Conversely, triploid females accumulated more fat bodies around the viscera and, during the final rearing, an increase in their flesh lipids was observed. A similar finding was made in females of rainbow trout (Lincoln and Scott, 1984), tilapia (Hussain et al., 1995) and silver barb (Koedprang and Na-Nakorn, 2000). This would suggest that lower energy diets might be required to decrease the buildup of lipid in triploid females as suggested for tilapia by Hussain et al. (1995). In the present

work, a reduction in perivisceral fat content was observed in triploid males (TB only) at the age of 34 and 45 months. This has been also observed in adult triploid rainbow trout by Chevassus (1994).

Finally, qualitative traits reported in the present work for diploid sea bass corresponded to what has been previously recorded in this species (Saillant et al., 2001). For example, these authors reported HIS values of 2.5–3.7% and muscle lipid contents of 9–12%.

Under our experimental conditions, pressure-induced triploids did not grow as well as diploids, but these results might be ascribed to specific on-growing conditions (communal environment), and their performance in on-farm environments should be evaluated. Furthermore, an exhaustive analysis of morphometric traits is currently underway to test the significance of parental or ploidy effect in our experimental groups. To date, we can conclude that the use of triploid fish in commercial production would avoid problems related to sexual maturation and potentially increase the market quality by providing a standard product throughout the year (unaffected by the physiological modifications linked to maturity). Indeed, during the spawning period, carcass quality and visual appeal diminish, making the diploids less appreciated for food. The marketing of triploid fish could solve these problems. Because of their sterility, farm-raised triploids would also respond to the ecological concern regarding the establishment of escaped fish or their interbreeding with native stocks, which were recently expressed by international organizations such as the FAO (Food and Agriculture Organization), ICES (International Council for Exploration of the Sea) and NASCO (North Atlantic Salmon Conservation Organization).

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