Genetic structure and genetic relatedness of a hatchery stock of Senegal sole (*Solea senegalensis*) inferred by microsatellites

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Abstract

*Solea senegalensis* is a fish species in an early stage of domestication. A commercial hatchery in southern Spain experienced a dramatic failure in spawning and embryo survival, after incorporation into the broodstock of fish reared in their hatchery. To assess the impact of management on the stock quality, a population genetics study was made of adult broodstock present at this hatchery in 2000. The broodstock was composed of a group of fish of wild origin and two more groups from F\textsubscript{1} progeny. A set of eight microsatellite loci was used to compare genetic variability among the three groups and to establish the relationships between pairs of individuals within each group.

Individuals from F\textsubscript{1} origin showed a substantial reduction in genetic variability when compared to those from the wild. There was a greater than 50% decrease in the number of alleles per locus corrected by sample size and 16% and 26% reductions in $H_e$ values in the two F\textsubscript{1} groups, respectively. The degree of genetic relationship between individuals, evaluated by the relatedness estimator, $r_{xy}$, and by graphical approaches, revealed a high proportion, almost 75%, of full-sibs and half-sibs in both F\textsubscript{1} groups, which might account for the reduction in overall variability. The high proportion of siblings also suggests a reproductive pattern in this stock in which few individuals breed successfully. This mating behaviour, along with factors inherent to selection and management systems, might be responsible for the negative effects on the reproductive capacity of the stock.

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1. Introduction

The sole, *Solea senegalensis*, is a promising species for European fish farming, owing to its high economic value, fast growth and need for market diversification. Advances in hatchery and rearing
techniques have been achieved for this species, although many problems must be solved before reproducible husbandry conditions can be established (Dinis et al., 1999). At this early stage, guidelines based on population genetics criteria are essential for founding and maintaining cultivated stocks and to avoid genetic erosion.

A domestication program for this species is underway in a commercial fish farm in southern Spain. The broodstock was founded in 1990 with 250 fish (50% males, 50% females approximately) from a wild population captured in a reservoir close to the hatchery facilities. Since then, the stock has been periodically supplemented with fish from the same natural source, except for the incorporation of fish from F1 hatchery progeny in the late 1990s. The performance in reproduction was very successful in the initial period with spawning rates of more than 15 million of eggs, but a progressive failure in the number and the quality of eggs occurred up to the point of null viability, being more noticeable in recent years. These changes in performance happened in the absence of any zootechnical modifications. According to records from the company, the broodstock was composed by three groups, one from wild origin and two others corresponding to selected F1 progeny, incorporated in 1998 and 1999, respectively. To assess the possible impact of management in the deteriorated performance of *S. senegalensis* in this hatchery, a population genetics study was planned on the 2000-year broodstock, consisting of an evaluation of genetic variability and genetic relationships between individuals.

A frequent difficulty when assessing relationships in cultured fish populations is the unavailability of complete pedigree records, or the disappearance of a large number of parents. However, appropriated estimators of parenthood in the absence of pedigree information have been developed. These estimators represent a measure of how many identical alleles are shared by one pair of individuals. Among them, the most common are from Li and Horvitz (1953), Queller and Goodnight (1989), Mathieu et al. (1990) and Lynch and Ritland (1999). The accuracy of these estimators depends on the number of unlinked loci used, the number of alleles per locus, the allele frequencies distributions (Lynch and Ritland, 1999) and the population composition (Van de Casteele et al., 2001).

Among the molecular markers available in population genetics, microsatellites emerged as those with finest resolution for labelling of individuals and populations, due to their high variability, abundance, neutrality, codominance and unambiguously scoring of alleles (Tautz, 1989; Weber and May, 1989). Microsatellite genotyping has proved to be an efficient tool for examining genetic structure (Wright and Bentzen, 1995) and pedigree tracing of hatchery populations, from various aquatic organisms (Goldstein and Schlottner, 1999). Recently, 15 microsatellites from Senegal sole were isolated (Porta and Alvarez, 2004), and 8 of them were selected for this study. These markers were used to compare genetic variability among the three component groups; one from wild origin and two others corresponding to selected F1 progeny, in order to establish genetic relatedness within each group in the absence of pedigree information.

2. Materials and methods

2.1. Fish samples and DNA extraction

The population screened represented the year 2000 broodstock (S00) of Senegal sole (*S. senegalensis*), belonging to a commercial fish farm in the southwest coast of Spain. The broodstock consisted on 250 tagged adults and was made up by three groups: (a) 63 individuals from the wild (W) captured from the same reservoir than founders, and located near the company. Every year sexually mature individuals (around 2 years of age) were captured from the reservoir and used as breeders; and (b) two sets of descendants produced in the own hatchery (F1), one represented by 152 mature individuals born in 1996 and incorporated in 1998 (F198), and the other one by 35 mature individuals born in 1997 and incorporated in 1999 (F199). Given that in hatchery conditions, the mean life span of the breeders is 5 years, it is possible that the parents of the F1 individuals were also present in the S00 stock.

DNA was extracted from blood samples using the saline precipitation method of Martinez et al. (1988). Genomic DNA was resuspended in 1× TE buffer and was kept at −20 °C prior to being processed.
The genotype of each individual was obtained after the amplification of 8 microsatellite markers, 7 of them selected from a set of 15 developed for Senegal sole: Sol5D, Sol9A, Sol13D, Sol19A, SolCA13, SolMII, SolA (Porta and Alvarez, 2004) and one microsatellite F1318/4/7, adapted from Solea solea (Iyengar et al., 2000). GenBank accession numbers are AF441388, AF441389, AF441385, AF441387, AF441390, AY426693, AY426692 and AF173849, respectively.

Polymerase chain reactions were performed in a 10 μl volume. The PCR reaction contained 100 ng (approximately) of genomic DNA, 1 x reaction buffer, 0.2 mM dNTPs, 0.5 μM of each primer, 0.2 U of Taq DNA polymerase and MgCl2 at different concentrations according to each locus. All PCR reactions were conducted in an Applied Biosystems 7200 thermal cycler. The conditions for amplification were: 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at the annealing temperature, variable according to each locus (Porta and Alvarez, 2004), and 45 s at 72 °C, with a final extension of 7 min at 72 °C. Denaturing buffer (95% deionized formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue) was added to each reaction in proportion 1:1. The mixture was heated for 10 min at 95 °C and was loaded in a 12% polyacrylamide–8 M urea gel. Samples were run on SE2001 manual sequencer for 2 h at 2000 V. The resulting gel was analyzed by the Silver staining method from Echt et al. (1996). Allele sizes were determined using the original clone, a 100 bp molecular weight marker and Poly-dA sonicated that produces a 1 bp ladder. The images were digitized using a scanner and the genotypes were determined by visual inspection.

2.3. Data analysis

Hardy–Weinberg (H–W) equilibrium and linkage disequilibrium were tested using of GENEPOP Ver. 2.3.3 (Raymond and Rousset, 1995). The exact P values were determined by a Markov Chain method when the allele number was higher than 4 (Guo and Thompson, 1992). Bonferroni adjustment of the P-values was used to correct multiple tests (Rice, 1989). Parameters used to obtain S.E. ≥ 0.01 were: dememorization number—1000, number of batches—400 and number of iterations per batch—2000. A complete enumeration method was performed when the number of alleles was 4 or less. The same software was also used to calculate $F_{IS}$ values (Weir and Cockerham, 1984).

Levels of genetic variation per group and for the whole stock were evaluated by the number of alleles per locus (A), as well as by observed ($H_o$) and expected ($H_e$) heterozygosities using FSTAT, Ver. 2.9.3 (Goudet, 2001; update from Goudet, 1995). As the (A) value is highly dependent on sample size, allelic richness ($A_n$), which is a measure of the number of alleles adjusted to the smallest common sample size, was also calculated. FSTAT software applies the rarefaction index from Hurlbert adapted to population genetics by Mousadik and Petit (1996).

Variability comparisons between the three samples (W, F1,98 and F1,99) were performed using different tests: (a) ANOVA for differences in the mean number of alleles per locus and allelic richness. (b) Kruskal–Wallis analysis of variance on ranks to analyze the differences in the mean values of observed and expected heterozygosities. For all these tests the SigmaStat Ver. 2.03 software was used.

The amount of genetic divergence among the three groups (W, F1,98 and F1,99) was calculated by the genetic variance statistic $F_{ST}$ (Wright, 1965). The mean $F_{ST}$ value for all loci was obtained in each group using the above version of GENEPOP.

The degree of genetic relationship between individuals (represented by unrelated, half-sibs and full-sibs assignments) was calculated by the $r_{xy}$ coefficient (Queller and Goodnight, 1989), using the Kinship program, Ver. 1.3.1 (Goodnight and Queller, 1999). This test implicitly weights loci according to their contribution to the estimator. It has been extensively applied to differentiate unrelated, half-sibs and full-sibs in several fish (McDonald et al., 2004; Sekino et al., 2004) and mammalian (Blouin et al., 1996) species.

To evaluate the bias of $r_{xy}$ values for unrelated, half-sibs and full-sibs categories, the observed allele frequencies of the W group were used to randomly simulate 2500 pairs of individuals in each relatedness category (Kinship, Ver. 1.3.1), assuming that the W population complies with both Hardy–Weinberg and linkage equilibrium. The mean $r_{xy}$ values from each distribution were compared by the Student’s t-statistic with those expected from $r_{xy}$ values of 0, 0.25 and 0.5, which correspond to unrelated, half-sibs and full-sibs, respectively. Among these distributions the
cutoff value for classification was determined as the midpoint between the means of the two contiguous distributions (Blouin et al., 1996). In this way, the probability that a dyad from one category could be misclassified as belonging to another category was determined.

To verify that this estimator could be applied to a real situation, a second set of \( r_{xy} \) pairwise distributions based on the genotypes from \( W \) individuals was obtained. For that, progeny genotypes were generated from all possible matings between \( W \) parents, using PROBMAG version 1.2 (Danzmann, 1997). These genotypes were classified according to their relatedness and then used to generate distributions for each category. The obtained \( r_{xy} \) distributions were compared with those obtained from allelic frequencies by the Mann–Whitney \( U \) statistical test.

Once the suitability of these markers was tested, the relationship between individuals was estimated. To calculate the relatedness coefficient (\( r_{xy} \)) between all dyads from the three groups, the allelic frequencies from the \( W \) group were adopted as representative of those in the base population, and so were used to weight the loci. This assumption is based on the fact that at the time the \( F_{198} \) and \( F_{199} \) were born, the broodstock was exclusively formed by fish from the wild and supposedly from the same natural population. Also, to test whether any individuals in \( W \) could be parents of individuals in the \( F_1 \) groups, exclusion methods were used by means of the PROBMAG software.

To discriminate further the family patterning within the three groups, two graphical methods were chosen. The UPGMA clustering was applied by using the Populations software, Ver. 1.2.28 (Olivier Langella, CNRS UPR9034). In this case, the distances matrix used corresponded to \( 1 - r_{xy} \) for all pairwise comparisons. As a complementary approach, the factorial correspondence analysis (AFC) was applied, using Genetix Ver. 4.04 (Belkhir et al., 2003).

3. Results

3.1. Genetic variability and genetic structure

The S00 stock showed 121 alleles for the 8 loci, ranging from 7 to 31 alleles per locus, with a mean value of 15 and a mean allelic richness, \( A_n \), of 9. The mean observed and expected heterozygosity values were 0.704 and 0.801, respectively. All loci showed departures from H–W equilibrium proportions of genotypes, and the \( F_{IS} \) values revealed deficits of heterozygosity for the \( Sol5D \) locus and an excess of heterozygosity for the \( F13I8/4/7 \) locus.

Genetic variability data from the three sets of the S00 stock (\( W \), \( F_{198} \), and \( F_{199} \)) are presented in Table 1. The \( W \) sample displayed the same number and identical range of alleles than S00, but its \( A_n \) mean value of 13 was significantly higher. The mean observed and expected heterozygosity values were 0.80 and 0.84, respectively. From the eight microsatellites, only \( SolA \) displayed significant departures from H–W proportions (\( P<0.01 \)) after Bonferroni adjustment. In spite of that, this locus was not discarded for subsequent analysis, since the H–W estimation was not so reliable due to the small size of the sample. Regarding \( F_{IS} \) values, only \( Sol5D \) and \( SolA \) showed deficit of heterozygotes, but no evidence of null alleles was observed after redesigning of the primers. No significant linkage disequilibrium was detected among all pairs of loci (\( P<0.01 \)). The results obtained from the \( W \) sample indicated that this set of microsatellites was suitable for subsequent analysis of relatedness.

The situation in the genetic structure of the \( F_1 \) groups was quite different, showing mean \( A_n \) values of 6 for both of them, which was half of that obtained for \( W \). The mean \( H_o \) values were of 0.7 for \( F_{198} \) and 0.62 for \( F_{199} \). Regarding the H–W equilibrium and the \( F_{IS} \) values, the \( F_{198} \) and \( F_{199} \) groups showed significant deviations, with several loci showing deficiency or excess of heterozygosity (Table 1). Concerning linkage equilibrium, all loci-pairs in \( F_{198} \) and 12 out of the total of 28 in \( F_{199} \) showed significant disequilibrium values.

The statistics tests revealed significant differences in the \( A_n \) and \( H_o \) values between \( W \) and both \( F_1 \) groups (\( P<0.01 \)), whereas differences were not significant between the two \( F_1 \) groups (\( P>0.01 \)). Concerning \( H_o \) values, no significant differences were revealed between the three groups.

The \( F_{ST} \) statistics was used to estimate genetic variance between groups. In this way, the highest \( F_{ST} \) value of 0.15 was obtained from \( F_{198} \) and \( F_{199} \) comparisons. Values of 0.086 and 0.07 were obtained after comparing \( W \) with \( F_{198} \) and \( F_{199} \), respectively.
Table 1
Genetic structure revealed by microsatellites from three groups (W, F198 and F199) comprising a commercial broodstock of *S. senegalensis*

<table>
<thead>
<tr>
<th>Locus</th>
<th>N. Ref.</th>
<th>Group</th>
<th>A</th>
<th>An</th>
<th>H_o/H_e</th>
<th>P</th>
<th>F_IS</th>
<th>A</th>
<th>An</th>
<th>H_o/H_e</th>
<th>P</th>
<th>F_IS</th>
<th>A</th>
<th>An</th>
<th>H_o/H_e</th>
<th>P</th>
<th>F_IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol5D</td>
<td>AF441388</td>
<td>Wild (n=63)</td>
<td>10</td>
<td>8.38</td>
<td>0.524/0.617</td>
<td>0.229</td>
<td>0.151</td>
<td>4</td>
<td>3.65</td>
<td>0.783/0.661</td>
<td>&lt;0.001</td>
<td>&lt;0.186</td>
<td>1</td>
<td>1</td>
<td>0.00/0.000</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>Sol9</td>
<td>AF441389</td>
<td>F1 98 (n=152)</td>
<td>17</td>
<td>14.50</td>
<td>0.825/0.875</td>
<td>0.013</td>
<td>0.057</td>
<td>10</td>
<td>6.96</td>
<td>0.730/0.696</td>
<td>&lt;0.001</td>
<td>–0.049</td>
<td>6</td>
<td>6</td>
<td>0.857/0.627</td>
<td>0.034</td>
<td>–0.375</td>
</tr>
<tr>
<td>Sol13D</td>
<td>AF441385</td>
<td>F1 99 (n=35)</td>
<td>16</td>
<td>13.94</td>
<td>0.873/0.902</td>
<td>0.231</td>
<td>0.032</td>
<td>10</td>
<td>6.80</td>
<td>0.724/0.758</td>
<td>&lt;0.001</td>
<td>0.046</td>
<td>10</td>
<td>10</td>
<td>1.000/0.842</td>
<td>0.010</td>
<td>–0.191</td>
</tr>
<tr>
<td>Sol19A</td>
<td>AF441387</td>
<td></td>
<td>7</td>
<td>6.93</td>
<td>0.810/0.769</td>
<td>0.870</td>
<td>–0.053</td>
<td>5</td>
<td>3.78</td>
<td>0.553/0.647</td>
<td>&lt;0.001</td>
<td>0.146</td>
<td>4</td>
<td>4</td>
<td>1.000/0.674</td>
<td>&lt;0.001</td>
<td>–0.494</td>
</tr>
<tr>
<td>Solca13</td>
<td>AF441390</td>
<td></td>
<td>12</td>
<td>10.56</td>
<td>0.810/0.866</td>
<td>0.074</td>
<td>0.066</td>
<td>11</td>
<td>6.65</td>
<td>0.803/0.652</td>
<td>&lt;0.001</td>
<td>–0.231</td>
<td>5</td>
<td>5</td>
<td>0.914/0.746</td>
<td>0.004</td>
<td>–0.23</td>
</tr>
<tr>
<td>SolMII</td>
<td>AV426693</td>
<td></td>
<td>10</td>
<td>9.13</td>
<td>0.825/0.842</td>
<td>0.861</td>
<td>0.019</td>
<td>7</td>
<td>5.19</td>
<td>0.849/0.736</td>
<td>&lt;0.001</td>
<td>–0.154</td>
<td>7</td>
<td>7</td>
<td>0.686/0.589</td>
<td>0.249</td>
<td>–0.167</td>
</tr>
<tr>
<td>SolA</td>
<td>AV426692</td>
<td></td>
<td>31</td>
<td>24.99</td>
<td>0.841/0.938</td>
<td>&lt;0.001</td>
<td>0.104</td>
<td>9</td>
<td>7.35</td>
<td>0.974/0.781</td>
<td>&lt;0.001</td>
<td>–0.248</td>
<td>9</td>
<td>9</td>
<td>0.886/0.771</td>
<td>0.109</td>
<td>–0.152</td>
</tr>
<tr>
<td>F1318/47</td>
<td>AF173849</td>
<td></td>
<td>18</td>
<td>15.51</td>
<td>0.905/0.889</td>
<td>0.041</td>
<td>–0.018</td>
<td>14</td>
<td>7.87</td>
<td>0.914/0.687</td>
<td>&lt;0.001</td>
<td>–0.332</td>
<td>6</td>
<td>6</td>
<td>0.886/0.748</td>
<td>0.013</td>
<td>–0.187</td>
</tr>
<tr>
<td>Mean values</td>
<td></td>
<td></td>
<td>15.13</td>
<td>13</td>
<td>0.802/0.837</td>
<td>0.043</td>
<td>8.75</td>
<td>6.031</td>
<td>0.79/0.70</td>
<td>–0.127</td>
<td>6</td>
<td>0.78/0.62</td>
<td>–0.251</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parameters calculated are: number of alleles (A), number of alleles adjusted to the smallest common sample size (An), observed (H_o) and expected (H_e) heterozygosities, H–W test P value (P) and fixation index (F_IS). Last row shows the mean values of each parameter.
3.2. Genetic relatedness

The $r_{xy}$ simulated distributions obtained from the W allelic frequencies and from mating genotypes of W individuals are presented in Fig. 1A. The mean of the $r_{xy}$ distributions (with their standard deviations), obtained from W allelic frequencies, were the following: 0.001 (0.147) for unrelated, 0.246 (0.155) for half-sibs and 0.496 (0.161) for full-sibs. These values show a clear agreement with the $r_{xy}$ expected values of 0.25 and 0.5, respectively ($P=0.991$).

The $r_{xy}$ distributions derived from potential progeny of W parents showed mean values (and standard deviations) of: 0.009 (0.153) for unrelated, 0.254 (0.151) for half-sibs and 0.506 (0.159) for full-sibs. These distributions were statistically similar to the corresponding ones obtained from W allele frequencies ($P=0.985$). The misclassification rates of $r_{xy}$ mean values from the two types of distributions are showed in Table 2.

![Fig. 1. (A) $r_{xy}$ distributions obtained from simulations using W alleles frequencies (solid lines with open symbols) or potential progeny of W parents (dashed lines with full symbols). Plots show $r_{xy}$ values for three relatedness categories: $\circ$ unrelated, $\triangle$ half-sibs and $\Box$ full-sibs. (B) Real data distributions of the $r_{xy}$ values from the three groups ($\bullet$ W, $\circ$ F198 and $\Box$ F199), representing a S. senegalensis broodstock.](image)

<table>
<thead>
<tr>
<th>True relationship</th>
<th>Misclassified as:</th>
<th>Simulations from allele frequencies</th>
<th>Simulations from parent matings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-sibs</td>
<td>Unrelated</td>
<td>1.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Full-sibs</td>
<td>Half-sibs</td>
<td>21.4</td>
<td>16.1</td>
</tr>
<tr>
<td>Half-sibs</td>
<td>Unrelated</td>
<td>22</td>
<td>20.2</td>
</tr>
<tr>
<td>Unrelated</td>
<td>Full-sibs</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Unrelated</td>
<td>Half-sibs</td>
<td>15</td>
<td>17.4</td>
</tr>
<tr>
<td>Half-sibs</td>
<td>Full-sibs</td>
<td>20</td>
<td>21.3</td>
</tr>
</tbody>
</table>

The values correspond to the fraction misclassified out of 2500 generated relatedness (%).

The $r_{xy}$ distribution patterns from the three groups (W, F198 and F199) are presented in Fig. 1B. The one corresponding to W showed a mean value of 0, which coincides with that of unrelated individuals category distribution. The F198 group showed a bimodal distribution, one with an $r_{xy}$ mean value of 0 which overlapped with the unrelated category distribution, and the other one with an $r_{xy}$ mean value of 0.5, which coincided with the full-sibs category distribution. The F199 group showed quite different pattern with a mean value of 0.45, which might correspond with half-sibs and full-sibs grouping.

The UPGMA phenogram obtained from the three groups (data not shown) was capable of individually discriminating the three of them as separated entities. A similar pattern of three well-differentiated groups, though not as precise as the UPGMA one, was documented by the AFC analysis (Fig. 2).

The UPGMA tree representing the F198 group only (Fig. 3) shows splits into two subgroups in the first branching: the F198A formed by 39 individuals and the F198B by 112 individuals. To test further whether the two subgroups corresponded to different matings, the $r_{xy}$ values were separately recalculated for each of the two F198 subgroups. The F198A showed a mean $r_{xy}$ value of 0.65, and the F198B a mean $r_{xy}$ value of 0.5. When individuals from two different subgroups were compared, $r_{xy}$ values close to 0 were observed.

When a similar analysis was performed on the F199 group, four subgroups were disclosed. The mean $r_{xy}$ values between individuals inside these subgroups were approximately 0.5, which corresponded to that of full-sibs. However, $r_{xy}$ values close to 0.25 were obtained when individuals from different subgroups were compared (Fig. 4).
To test whether any individuals in W could be parents of individuals in the F1 groups, the exclusion analysis did not allow a clear identification of any
parents. However, the putative disappearance of some of the parents might have reduced the screening ability of the method.

4. Discussion

In this study we have employed eight microsatellite loci to assess the impact of management on a farmed broodstock of *S. senegalensis*, composed by fish from wild (W) plus two more sets (F198 and F199) produced in the own hatchery. This stock, after a successful productive period, showed signs of progressive deterioration in the number of spawned and hatched eggs, following the introduction of the F1 groups. For investigating this issue, the genetic structure of the whole stock (S00) and the genetic relationship between individuals from each group were analyzed. The *F*<sub>ST</sub> values obtained indicated a high divergence between the two F1 groups, while the divergence between F198–W and F199–W was much lower.

The fact that the W sample contains all of the alleles observed in the S00 broodstock implies that the F1 groups did not contribute new alleles to the S00 stock. This observation, along with the *F*<sub>ST</sub> values obtained, supports the hypothesis that F1 individuals might have come from a population of similar genetic structure to W.

The comparison of genetic variability levels among the three groups (Table 1), revealed an important reduction in those of F1 origin relative to the W group. This reduction is seen in a 16% or 26% reduction of the *H*<sub>e</sub> values for F198 and F199, respectively, and in a greater than 50% decrease in the number of alleles per locus adjusted to the smallest common sample size (*A*<sub>n</sub>). In this case, the loss of variability not only affected alleles in lower frequencies, but also alleles in higher frequencies, thus suggesting that only few individuals have contributed to the F1 groups. These results support the idea that the parameter *A* is a more meaningful measure of genetic variation than *H*<sub>e</sub>, since the later is less sensitive to short bottlenecks that can occur in cultivated stocks (Hedgecock and Sly, 1990; Perez-Enriquez et al., 1999). The important loss of microsatellite alleles, which occurred in the breeding of W population to generate F1 groups, can also suggest a loss of alleles directly involved in the fitness of the cultivated stock. In that way, the incorporation of F1 individuals to the breeding stock might have produced genetic depression, which can have contributed along with other zootechnical factors, to the poor performance of the S00 broodstock. A similar case of loss in genetic variability has been largely reported in cultured stocks of fish and more precisely in the closely related species *S. solea*, detected by means of allozymes (Exadactylos et al., 1998).

To assess the causes of this genetic variability loss, the history of the broodstock management was tracked. The records from the company were incomplete and data on genetic relationships were absent. To circumvent this problem, the *rxy*-based genetic relatedness analysis was applied to pairs of individuals from the three groups. The pattern of the predicted distributions generated from W allelic frequencies (Fig. 1A) indicated that the loci used were able to discriminate: (i) unrelated from full-sibs with at least 95% accuracy, and (ii) pairs of unrelated and full-sibs from half-sibs with 78% accuracy. The *rxy* simulated distributions obtained from mating W genotypes, which is closer to a real situation (Fig. 1A), did not show bias when compared with the distributions simulated from W allelic frequencies. Conversely, left and right bias would increase or decrease, respectively, the two-type error (assignment of related dyads as unrelated), and consequently produce either an underestimation or overestimation of relatedness.

Once we tested the validity for relatedness analysis with these loci, we could infer the structure of each group from their distribution patterns and *rxy* mean values (Fig. 1B). In this way, the W group was composed by 77% of unrelated individuals and 23% of individuals with some degree of relatedness. The F198 group had a bimodal distribution of *rxy*, suggesting that it was formed by unrelated families of full-sibs. A re-examination of this group by UPGMA analysis (Fig. 3) revealed two clear groupings representing two unrelated families, named as F198A and F198B. Taking into account the genotypes of the individuals from F198A, we were able to infer the genotypes of their parents by Mendelian segregations, which allowed concluding that this subgroup was composed by 32 full-sibs and 7 half-sibs. Similarly, the F198B was found to be formed by 96 full-sibs and 16 individuals either half-sibs or unrelated. The F199...
group revealed a strong family structure, formed by full-sibs and half-sibs, that was resolved into four family groups with the UPGMA approach (Fig. 4), but contrary to the F1,98 group, no more precise relationships could be established.

The above data revealed that the S00 was composed by around 75% of siblings, which could partly account to the decrease in genetic variability in the whole stock, and consequently might have contributed along with other management factors to the poor performance shown after the incorporation of F1 individuals. The fact that a high proportion of individuals from F1 origin was closely related allowed speculating that in the breeding pattern in captivity of S. senegalensis, an unbalanced contribution of each parent had occurred. Actually, the participation of few parents might explain the loss in one generation, not only of rare, but also of more frequent alleles. This reproductive behaviour added to other factors, such as high fecundity (more than a million eggs per female in one season), differential selection pressure among families, management effects and the selection of breeders from the progeny, can represent a set of circumstances responsible for the drastic reduction in the genetic variability of the two studied F1 groups in just one generation.

In hatchery strains, it is generally recommended that unrelated fish from wild should be used as broodstock, though this might be difficult due to economical constraints. Instead a subset of hatchery-based fish is normally used to complete the broodstock size, with the advantage of being already adapted to domestication conditions. However, this practice might threaten the genetic variability of the broodstock when the mating behaviour of the species is unknown (Norris et al., 1999), and therefore is not possible to predict the pedigree structure of next generation. In those cases maximal retention of genetic variability from captive populations can be achieved through monitoring pedigree structure of descendants and suitably selecting reproducers among unrelated descendants (Norris et al., 2000). For knowledge of genetic structure and relatedness, microsatellites have proved in some cases to be excellent tools, as exemplified in this study.

The data generated in this study provide useful information on the population structure, on the effects of the management system used in this hatchery and on the reproductive pattern of this stock. This information can be applied to design suitable management guidelines for this stock or others from the same or related species.

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