# Mitochondrial Control Region and microsatellite data show low genetic variability in reared Senegalese sole, Solea senegalensis. Preliminary data.

P. Sánchez<sup>1,3</sup>, J. Viñas<sup>2</sup>, J.Alvarado-Bremer<sup>2</sup>, P.P. Ambrosio<sup>1,3</sup> and R. Flos<sup>1,3</sup>

<sup>1</sup> Departament d'Enginyeria Agroalimentària i Biotecnologia, Universitat Politècnica de Catalunya, Av. Canal Olímpic s/n, 08860 Castelldefels, Barcelona, Spain. E-mail: pablo.sanchez-fernandez@upc.edu

<sup>2</sup>Department of Marine Biology, Texas A&M University, 5007 Ave. U, Galveston, TX 77551, USA. <sup>3</sup>Centre de Referència de Recerca i Desenvolupament en Aqüicultura de la Generalitat de Catalunya.

## Introduction

Solea senegalensis is a flatfish of high commercial importance. Aquaculture of S. senegalensis has raised the interest of producers since mid 80's and has been in a developing stage for some years, though is still not optimised. One of the main hurdles to its definitive implementation is the difficulty in obtaining regular spawning and good quality fry, what leads to a juvenile scarcity for the ongrowing stage. In this framework, the need of a good broodstock management is essential. This work aimed to characterise the genetic variability level of a farmed Senegalese sole population and to compare it with a wild sample, as a first step for determining variation of this variability in culture and as a base for future studies related to breeding programs.

## Materials and methods

A total of 134 S. senegalensis were analysed. A control sample of 18 wild soles captured in the NE coast of Spain, and a sample of 116 individuals from a fish farm from the same location, who were the  $F_1$  progeny of wild broodstock captured in the SW coast of Spain, were obtained. For each individual the same protocol was used. Total DNA from muscular tissue was extracted, and subsequently used as template for the PCR reactions. Partial mitochondrial control region (CR) was amplified using the primers described at Alvarado-Bremer et al. (1995) and 8 specific microsatellite loci (Sol13B, Sol37, Sol19A, Sol1B, Sol12D, SolMII, SolCA13 and Sol14) were amplified using the primers described by Porta and Álvarez (2004).

The CR was sequenced using the ABI Prism<sup>®</sup> BigDye<sup>™</sup> Terminator Cycle Sequencing Ready Reaction Kit. Sequencing products and microsatellite PCR products were both read in an ABI Prism 310 Genetic Analyzer (Perkin Elmer). For the mitochondrial data values of haplotypic (h) and nucleotide ( $\pi$ ) diversity were computed with ARLEQUIN 2.0. Expected heterozygosity (He) for microsatellite data was estimated on Genepop 3.4. The genetic structure among both groups was also tested by analysis of the molecular variance (AMOVA) for both mtDNA and microsatellite data.

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### Results

Sequencing of a 414 bp CR of 66 farmed and 18 wild fish showed 23 variable positions which defined 15 haplotypes (hpl001 to hpl015). Six of them were found in the farmed fish, and 11 in the wild caught fish, and only two haplotypes (hpl011 and *hpl013*) were shared by both groups. Haplotype *hpl001* was exclusive from the farmed sample. It presented the highest frequency and was shared by 45 fish of 66 (68.2%). The estimated h values were significantly different (p < 0.001) being lower in farmed fish (0.493) than in wild fish (0.941). Similarly,  $\pi$  values were significantly (p < 0.05) lower in the harvested sample (0.007) than in the wild sample (0.014). Therefore both populations were genetically different (AMOVA,  $F_{st}$ of 0.414; p=0.000). Similar to the mitochondrial data, farmed fish presented lower levels of genetic variability when analyzing microsatellite data of the 134 individuals. The expected genetic diversity was 0.54 for farmed fish and 0.79 for wild caught fish, and normalized allele number (number of alleles/2N) was 0.06 in farmed fish and 0.35 in the wild sample. Consequently an AMOVA incorporating all loci was highly significant (p<0.0001) F<sub>st</sub>=0.067. Moreover, a locus by locus AMOVA also showed significant  $F_{ST}$  estimates in the 8 loci (p < 0.05).

#### Discussion

Genetic variability of farmed fish is significantly lower compared to the wild sample. As a consequence, this genetic variability reduction produces the clear differentiation observed between these two populations. The enormous loss of variability is probably related to two causes. First, a bottleneck produced by a founder effect during the establishment of this reproductive line. Second, taking into account the high h of the wild sample, it could be assumed that each haplotype found in the farmed fish represents a single mother. Considered the high presence of hpl001, a variable reproductive efficiency among individuals is inferred, resulting in an additional loss of variability. Our results indicate that there is a need of improving broodstock management and to increase the knowledge of sole reproductive biology in order to setting up successful commercial breeding programs.

### References

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