Title of the project
TOOLS FOR THE GENETIC IMPROVEMENT OF SEA BASS.
CONSTRUCTION AND PRELIMINARY APPLICATION OF A MEDIUM DENSITY LINKAGE
AND SYNTENY MAP

Acronym of the project
BASSMAP

<table>
<thead>
<tr>
<th>Type of contract</th>
<th>Total project cost</th>
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<tr>
<td><strong>Contract number</strong></td>
<td><strong>1,739,700 €</strong></td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td><strong>EU contribution</strong></td>
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<tr>
<td><strong>50 months</strong></td>
<td><strong>1,167,821 €</strong></td>
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Commencement date
01 November 2001

Period covered by the progress report
01 November 2004 – 31 December 2005

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Project Progress Report

Objectives:
The main objective is to prepare a chromosome map based on the linkage of genetic markers (linkage map) of the European sea bass. It incorporates both functional coding sequences (Expressed Sequence Tags, or ESTs) and non-functional sequences (DNA microsatellites and Amplified Fragment Length Polymorphisms, or AFLPs). The applicability of the linkage map is demonstrated in a commercial fish farm where Quantitative Trait Loci in sea bass are screened on the basis of these new genetic markers.

Results and Milestones:
A reference family (test panel) comprising two wild parents from the Adriatic Sea and 50 full sibs has been used for genetic mapping. Two hundred and eighty-nine microsatellites markers including 270 loci developed in view of this project, 226 AFLP markers and 29 EST loci have been genotyped in the reference family. Genotypes of all these loci have been deposited to a database (http://www.ResSpecies.org) to be further exploited for linkage mapping. 282 microsatellite, 164 AFLP and 29 EST markers are informative in the test panel. A first generation linkage map organised in 25 linkage groups was prepared based on 162 microsatellite DNA markers. A second generation linkage map provided a higher resolution by organising in total 369 polymorphic microsatellite and AFLP loci in 35 linkage groups. Of the 369 loci mapped, 29 represent type 1 (coding) markers amongst others located within the peptide Y (linkage group 1 or LG01), cytochrome P450 aromatase (LG06) and SOX (LG07) gene. The sex-averaged map spans 1506 centiMorgans (cM) of the sea bass genome. The sex-specific female map covers 1553 cM, whereas the male map only 1166 cM. The average recombination fraction was 0.124 in females and 0.084 in males, resulting in a female-to-male (F : M) recombination rate ratio of 1.48 : 1.
A Bacterial Artificial Chromosome (BAC) library with an average insert size of 164 kb and a final coverage of 7 times the full genome was constructed, gridded on filters and distributed between partners. The BAC clones have also been pooled to produce, together with the gridded filters, suitable material for the quick and effective screening for genetic markers such as ESTs and microsatellites. Screening the BAC library for gene-linked microsatellites and ESTs has started. Two types of complementary DNA (cDNA) libraries from larva/embryo and adult liver were constructed. A total of 848 cDNA clones have been isolated from these libraries and their 5'-ends were sequenced. Among them, about 300 clones have also been sequenced at the 3'-end. The ESTs developed have been partially
screened to produce gene-linked microsatellite markers. In total, 377 clones showed significant homology with around 100 unique and functionally relevant sequences. The defined cDNA clones will be selected to obtain finally about 100 interesting ESTs for the identification and characterization of candidate genes in Quantitative Trait Locus (QTL) mapping. A primary population of 2506 F1 progeny has been selected, phenotypically measured for nine commercial morphometric traits and fully genotyped with 13 microsatellites for parentage assignment. One female and eight males dominated the family structure. Heritabilities for these traits were estimated; for example body weight was shown to be rather low ($h^2 = 0.12 \pm 0.11$). Of these 2506 fish, 422 individuals were genotyped at 7 loci on linkage group 1 for a pilot QTL analysis. There is an area with a higher level of linkage at one end of the linkage group; a reliable interpretation requires further analysis.

Benefits and Beneficiaries:
In the first instance genetic management benefits the European sea bass aquaculture industry, where the lack of domesticated and selected stocks hampers economic and environmental sustainability. The sea bass industry becomes economically more stable and profitable, as its production relies on stocks tailored to farm conditions and customer demands. The large scale assignment of progeny to their parents proves the feasibility of batch rearing of families for selection. Our maps assist with the identification of loci particularly useful for selecting commercial traits such as sex, growth rate, disease resistance, stress response and sale characteristics. It will also benefit fisheries (stock management) and fundamental science.