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ABSTRACTS

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TWO ISOFORMS OF *vasa* MATERNAL FACTOR IN SENEGALESE SOLE: BIOTECHNOLOGICAL APLICATIONS.

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In the last decade v*asa* homologs have been cloned in several teleosts and their expression patterns during oogenesis, spermatogenesis and development have been analyzed. These previous studies show that the *vasa* gene is a germ cells molecular marker with a high biotechnological potential for identification and manipulation of germ cells in surrogate broodstocks. In teleosts different expression patterns were observed between males and females (Kobayashi et al., 2000) and during embryonic and larval development (Krøvel and Olsen, 2004) for *vasa* isoforms. In this study two isoforms of *vasa* gene in *S. senegalensis* were identified, characterized and the expression pattern was described. The Senegalese sole *vasa* cDNA 1 (*Ssvasa1*) was 2374bp while the Senegalese sole *vasa* cDNA 2 (*Ssvasa2*) was 1993bp. *Ssvasa* isoforms showed high homology with others *vasa* cDNAs when submitted at Blast analysis. Resulting amino acidic sequences of *Ssvasa* isoform were clustered in the VASA subfamily when compared with others DEAD box protein by neighbour-joining analysis. The RT-PCR and Q-PCR analysis performed in different tissues of adult fishes showed that *Ssvasa* gene expression was restricted to ovary and testis. Our results showed that the *Ssvasa1* RNA is maternally provided since expression was only detected until mid-blastula transition stage. Expression patterns of *Ssvasa1* and *Ssvasa2* were different during embryonic and larval development, similar with the observed in zebrafish (Krøvel and Olsen, 2004). Our results demonstrate that *Ssvasa1* RNA could be used as a molecular marker for testicular germ cells while *Ssvasa2* is more appropriate for the study of primordial germ cells during embryonic and larval development in Senegalese sole.

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Skeletal quality in Senegalese sole: characterization of fish resulting from environmental and nutritional trials

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The Senegalese sole (*Solea senegalensis*) is characteristic from southern Europe and Mediterranean. It has recently been adapted for aquaculture production since it is well accepted by consumers and reaches high commercial values. After the initial description of the ontogenic events of skeletogenesis, systematic evaluation of the incidence of skeletal malformations by several groups revealed high levels of skeletal deformities reaching up to 90% of the population. These high levels may impair development, growth and acceptability by the consumers, thus representing a constraint for commercial production of this species. A comparison on the intensiveness on rearing methodologies revealed a low incidence of skeletal deformities in early life stages captured in nature (0-23%) and a relatively higher number of deformed individuals when raised under extensive conditions in mesocosms (20-50%), as opposed to 60-80% of deformed fish obtained from intensive rearing. Data suggests that deformities occur naturally and have other causes than just those induced by rearing in captivity. Modulation of temperature during the autotrophic period caused both an increase of deformities and an increase in the anomaly charge paralleling the increase in incubation temperature.. Our results clearly demonstrate a significant effect of water temperature on skeletal development of Senegalese sole, indicating that temperature control is crucial for larval rearing. Nutritional imbalances during early larval stages have been shown to affect bone development and promote the occurrence of skeletal deformities. Vitamin K (VK) is a liposoluble vitamin classically involved in blood coagulation, and until recently largely disregarded in aquaculture nutrition. VK plays an important role in bone metabolism by acting over gamma carboxylation of matrix proteins. The effects of vitamin K in Solea senegalensis skeletogenesis were evaluated in larvae from three different dietary regimes supplemented with graded levels of phylloquinone (VK1) (0mg/kg, 50 mg/kg, 250 mg/kg) using enriched Artemia as carrier. A positive effect on bone quality was observed with VK1, with a reduction in both number of deformed fish and number of deformities per fish.
In order to characterize the alterations resulting from spine curvature vertebral deformities in Senegalese sole, we have analysed vertebral deformities in juvenile and adult fish using histological, histochemical and immunochemical approaches to verify the physiological response at tissue and cellular levels. We have observed an adaptive response to increased strain, with affected vertebrae presenting an increase in bone formation and in mineral as well as the development of chondroid at the articular surfaces and chondroid bone on the vertebral centra.
Even though relatively high numbers of deformities were observed under different environmental and nutritional conditions, the external anatomy was usually not severely affected, meaning that the aquaculture success of this species might not be constrained by skeletal deformities. Still, advances in nutritional and zootechnical conditions should be made to improve the overall skeletal quality of *Solea senegalensis*.

EFFECTS OF ENVIRONMENTAL CYCLES (LIGHT AND TEMPERATURE) ON SOLE LARVAE DEVELEOPMENT

Borja Blanco Vives

To be inserted