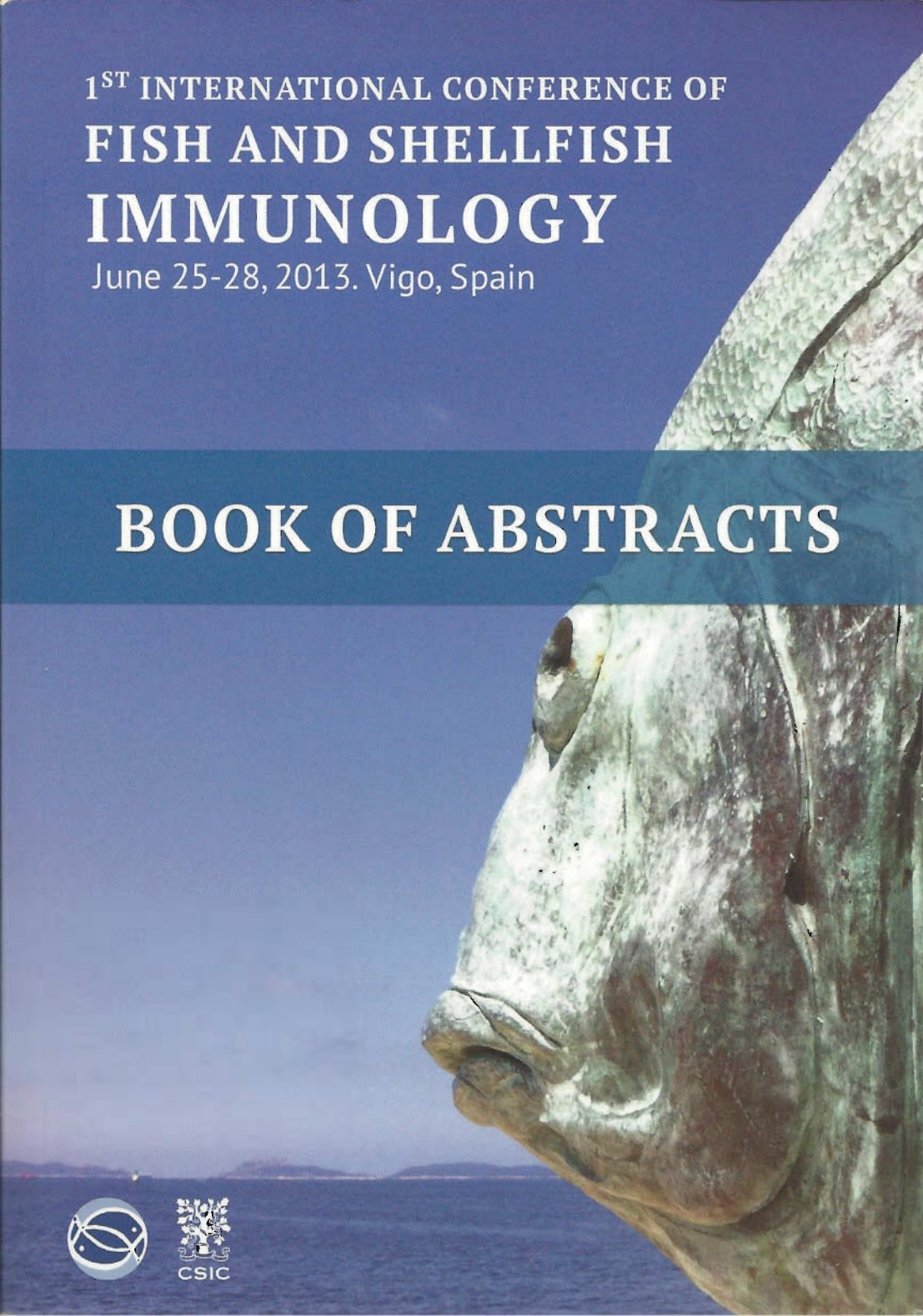
1ST INTERNATIONAL CONFERENCE OF FISH AND SHELLFISH IMMUNOLOGY

June 25-28, 2013. Vigo, Spain

BOOK OF ABSTRACTS



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CHARACTERIZATION OF SENEGALESE SOLE (*Solea senegalensis*) Mx PROMOTER

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Type I interferon (IFN) is a main component of the innate immune response against viral infections, creating an antiviral state in cells. Mx proteins are the best-studied IFN stimulated genes (ISGs) in fish. The antiviral activity against different viruses has been demonstrated for diverse fish Mx proteins, including Senegalese sole (*Solea senegalensis*) Mx protein (SsMx). To advance in the knowledge of the IFN pathway and the antiviral state in this species, is necessary to understand the regulatory mechanisms determining ISGs transcription. For this reason, the aim of the current study was the cloning and functional characterization of the SsMx promoter. To fulfill this objective, GenomeWalker™ Universal Kit was used to clone the SsMx promoter. A fragment of 1327 bp upstream of the transcriptional start site has been obtained. Sequence analysis showed a typical structure of an ISG promoter, including three ISREs (Interferon stimulated response element), a gamma activation sequence (GAS), a SP1 binding site, a STAT binding site and several GAAA/TTTC boxes. Then, the 1327-bp fragment obtained was cloned into a luciferase reporter vector, which was transfected into RTG-2 and CHSE-214 cells. The expression of luciferase was measured at different time points after stimulation of the IFN pathway with poly I:C. Interestingly, luciferase expression patterns differed depending of the cell line considered. In RTG-2 cells, the highest level of luciferase expression was observed at 24-48 h post-induction (p.i), decreasing afterwards, whereas in CHSE-214 cells a gradual increase of the luciferase expression up to 72 h p.i. was observed. Deletion and punctual mutation analyses have been performed to determine the contribution of each ISRE motif in the inducibility of the SsMx promoter. Results showed that ISRE1, sited closest to the transcriptional start site, is the main element contributing to the SsMx promoter response, while both, ISRE2 and ISRE3, have a minor additive effect on SsMx promoter induction.

This study has been funded by the project P09-CVI-4579, from Junta de Andalucía (Proyectos de Excelencia de la Junta de Andalucía).