# THE STIMULATION OF THE INNATE IMMUNE SYSTEM BY SJNNV PROTECTS JUVENILE EUROPEAN SEA BASS (*Dicentrarchus labrax*) AGAINTS SUPERINFECTION WITH RGNNV

**Carlos Carballo [1], Benjamín López-Jimena [2], Daniel Alvarez-Torres [3], Esther García-Rosado[1], Juan José Borrego[1], María del Carmen Alonso[1]**

[1]*Department of Microbiology, University of Malaga, Málaga, Spain*

[2]*IFAPA Centro El Toruño, Junta de Andalucía, El Puerto de Santa María, Cádiz, Spain*

[3]*Department of Genetics, Faculty of Sciences, University of Malaga, Málaga, Spain*

---

**Abstract**

European sea bass is highly susceptible to the infection by Viral Nervous Necrosis Virus (VNNV) whose genome is composed of two positive sense and single-stranded RNA segments: RNA1 (encoding the RNA dependent RNA polymerase, RdRp) and RNA2 (encoding the coat protein, CP). Betanodaviruses have been classified into four genotypes based on the sequence of the T4 region of the CP coding gene, although only the SJNNV and RGNNV genotypes have been detected in the Mediterranean area to date. Furthermore, the coexistence of these two genotypes in the same individual has been recorded by molecular techniques in a high percentage of wild and farmed fish species.

The interferon (IFN) system is the first defense against fish viral infections. Mx is the most studied IFN-induced protein; having being shown that some of them possess antiviral activity against fish viruses. The aim of the present study has been to determine the effect of the SJNNV-RGNNV coexistence on the pathogenesis of the RGNNV in European sea bass and to establish the role of the IFN-mediated immune system in the course of the RGNNV infection under coexistence.

In this study, viral replication and transcription of innate immunegenes have been determined by RT-real time-PCR (RT-qPCR) in the course of an experimental infection. Three different experimental conditions were considered: i) RGNNV-inoculated animals; ii) SJNNV-inoculated animals and iii) animals inoculated with SJNNV and superinfected with RGNNV. Superinfection was performed 24 h after the SJNNV inoculation. Control animals were mocked-injected with L-15 medium.

The RGNNV-infected group showed typical symptoms of the disease and displayed 76% cumulative mortality at the end of the experiment, whereas the mortality in the superinfected group was 4%, and no mortality was recorded in the SJNNV-inoculated group. The analysis of the Mx transcription by RT-qPCR showed a clearly differential induction of the sea bass innate immune system by RGNNV and SJNNV, since no transcription was recorded at any time tested (from 0 h to 48 h p.i.) after the RGNNV inoculation, whereas the injection of SJNNV resulted in an important increase of the Mx transcription from 24 h p.i. onwards. In the superinfected group the induction of the Mx gene transcription follows the same patterns that the ones described for the groups inoculated with SJNNV and RGNNV separately. These results suggest

that the induction of the IFN mediated system by the previous infection with SJNNV could be responsible for the decrease in the mortality recorded in the superinfected group, protecting sea bass of the posterior infection with RGNNV.

---

Keywords: European sea bass, SJNNV, RGNNV, Mx protein, IFN