

MyxfishControl: Epidemiology and control of an enteric myxosporosis of commercial Mediterranean fish (QLRT-2001-00722)

Timing: September 2002- December 2005

Participants: The consortium, coordinated by Prof. Pilar Alvarez Pellitero from the Instituto de Acuicultura de Torre de la Sal (C.S.I.C), is composed by six more partners: Universitat Autònoma de Barcelona, Dept. Biologia Animal, Biologia Vegetal i Ecologia, Facultat de Veterinaria (Spain); Universidad de Murcia, Dept. Biología Celular, Facultad de Biología (Spain); Université de Montpellier II Sciences et Techniques du Languedoc, Dept. Biologie, Evolution Environment (France); University of Thessalia, Dept. Health Sciences, School of Veterinary Medicine, (Greece); Istituto Zooprofilattico sperimentale dell'Umbria e delle Marche, Dept. Fish Pathology and Aquaculture (Italy) National Center for Mariculture, Oceanographic and Limnological Research, (Israel).

Background: *Enteromyxum leei* (Myxozoa) is an important pathogen of gilthead sea bream (*Sparus aurata*) and other sparids cultured in the Mediterranean area. The parasite produces acute enteritis followed by anorexia, cachexia and death of susceptible fish, and is the cause of high mortality both in netpens and in land-based mariculture systems. Different facilities along the Mediterranean coast and Red Sea coast of Israel are affected, and some ubiquitous wild fish species, other than sparids, are also susceptible to the disease. In spite of the current and potential importance of this disease, to date little was known about the mechanisms of infection, susceptibility of hosts, development and survival of the parasite outside the fish, and other aspects of the host-parasite relationships, including the immune response. A rapid and reliable diagnostic method was unavailable.

Objectives: The overall aims of the project were the evaluation of the status of the disease in Mediterranean sparid cultures and the development of prevention and control measures for this important parasite infection. The specific objectives included: 1) Validation of a PCR-based assay for the specific diagnosis of the parasite, its application in field epidemiological studies, and its use for the evaluation of the role of wild fish and invertebrates, inhabiting the neighbouring of enzootic culture facilities, as reservoirs of the disease; 2) Study of the transmission, pathogenesis and infectious process in sparid fish, in the course of experimental infections; and 3) Study of relevant aspects of the innate and adaptive immune responses to the disease, and use of this information for the application of immunoprophylactic measures.

Achieved milestones with molecular biology tools:

1. PCR-based assays (using oligonucleotide probes targeted to the 18S rRNA gene) have been validated for the specific diagnosis of the parasite, with excellent accuracy respect to gold standard (histology) diagnosis. PCR from

- non-lethal samples has a remarkable good performance: advisable for routine diagnosis and large scale studies. (Publication in press).
2. The PCR test has been applied in field epidemiological studies involving diverse gilthead sea bream and sharpsnout culture systems in all the participant countries. Sea cages (8 farms) and land based facilities (3 farms) have been surveyed. The highest prevalences were detected in land farms. (Manuscript in preparation).
 3. The PCR test has been applied to evaluate the role of wild fish and invertebrates, inhabiting the neighbouring of enzootic culture facilities, as reservoirs of the disease. More than 3600 specimens belonging to 34 families from five samplings sites in Mediterranean, Ionian and Red seas have been analysed. Positive fish belong to six families, mainly Mugilidae, Siganidae and Carangidae. In addition, 25 positive invertebrate samples out of 350 (Anthozoa, Polychaeta, Crustacea) have been detected. (Palenzuela et al. 2004. Applicability of PCR screening for the monitoring *Enteromyxum leei* (Myxozoa) infection in the Mediterranean aquaculture: an epidemiologic survey in sparid facilities. In: Biotecnologies for quality. S. Adams & J. A. Olafsen (Comp.). Aquaculture Europe, 2004, Barcelona, Spain, Oct. 20-23. Europ.Aquac.Soc. Spec. Publ. N° 34, pp.639-640. Other manuscripts on epidemiology are in preparation).
 4. The PCR assay has been used to manage infected fish stocks, in order to maintain a continuous source of the parasite and infected animals for the remaining workpackages.
 5. The transmission, pathogenesis and infectious process in sparid fish, have been studied in the course of experimental infections. The mechanisms involved in transmission and releasing of infective stages and in pathogenesis have been determined using field observations and histological, histochemical and DNA probes developed for detection of all the parasite life stages by *in situ* hybridisation protocols (Manuscript in preparation).
 6. Several factors of the innate and adaptive immune responses to the disease have been studied in sea bream and sharpsnout sea bream. The expression of pro-inflammatory cytokines in the head kidney (HK) of fish experimentally exposed to the disease were analysed. *IL-1 β* mRNA expression increased in HK of exposed, whereas *TNF α* gene expression decreased in exposed fish respect to control ones. (Cuesta et al. 2006. Gilthead sea bream (*Sparus aurata* L.) innate defence against the parasite *Enteromyxum leei* (Myxozoa). *Parasitology*, 132: 95-104; Cuesta et al. 2006. Cell-mediated cytotoxicity is the main innate immune mechanism involved in the cellular defence of gilthead sea bream (Teleostei: Sparidae) against *Enteromyxum leei* (Myxozoa). *Parasite Immunology* (in press). Other manuscripts in preparation).
 7. The possible use of levamisol as a preventive immunomodulator of the enteromyxosis was determined. No statistically significant effect on the expression of HK *IL-1 β* and *TNF α* was found in treated fish (Manuscript in preparation).