



The aetiology of systemic granulomatosis in meagre (*Argyrosomus regius*): The “*Nocardia*” hypothesis

M.I. Tsertou^{a,b,1}, M. Smyrli^{b,1}, C. Kokkari^b, E. Antonopoulou^a, P. Katharios^{b,*}

^a Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

^b Institute of Marine Biology, Biotechnology and Aquaculture, HCMR, Former American Base of Gournes, Heraklion 71003, Crete, Greece

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ABSTRACT

One of the most important bottlenecks of meagre (*Argyrosomus regius*) production is Systemic Granulomatosis (SG), a pathological condition affecting the majority of farmed populations. The aetiology of the disease, which is characterized by multiple granulomas in all soft tissues, is unknown; however, two hypotheses have been raised. The first is that it may be a metabolic disorder similar to systemic granulomas observed in other cultured fishes. The second hypothesis is that it is caused by bacterial pathogens, most likely *Nocardia* spp. (*i.e.* nocardiosis). The aim of this study was to test the bacterial pathogen hypothesis as the aetiological factor of SG. Meagre from various locations of Greece were sampled and examined using microbiological, molecular and histological techniques. The prevalence of granulomas in fish was 100% and was not correlated to an infectious agent, whereas nocardiosis was found in a single incidence (prevalence: 1.3%). The results suggest that nocardiosis is not the cause of SG, as it is most probably present in a confined geographical region in Greece. The metabolic disorder hypothesis for SG is more probable and more research has to be done to conclusively identify the aetiological factor of SG.

1. Introduction

Meagre (*Argyrosomus regius*) is an emerging species for the diversification of the European aquaculture due to its attractive and competitive biological attributes. These include a fast growth rate of ~1 kg per year in temperatures between 17–21 °C, low feed conversion ratio of 0.9–1.2, low fat content and excellent taste (Duncan et al., 2013; Monfort, 2010; Poli et al., 2003).

One of the requirements for the successful introduction of a new species in the aquaculture industry is the knowledge of the pathological problems that may arise. Recently, a review of the principal diseases affecting cultured meagre has been published, providing an overview of all the infectious and non-infectious diseases affecting the species (Soares et al., 2018). Vibriosis caused by various *Vibrio* species, nocardiosis and mycobacteriosis have been reported sporadically, while parasites seem to cause the most significant problems. For example, meagre has been reported to be parasitized by *Sciaenacotyle panceri* (Monogenea) in Sardinia and Corsica (Merella et al., 2009; Ternengo et al., 2010), *Benedenia sciaenae* (Monogenea) in Turkey (Toksen et al., 2007), *Diplectanum sciaenae* (Monogenea) in Spain (Andree et al., 2016), *Philometra* sp. (Nematoda) (Moravec et al., 2007) and

Amyloodinium ocellatum (Dinoflagellida) in Portugal (Soares et al., 2012). In farmed meagre, the monogeneans, *S. panceri* and *B. sciaenae* may become threats in cage aquaculture (Soares et al., 2018) and *A. ocellatum* in tanks or ponds (Soares et al., 2011).

One of the most important bottlenecks of meagre production is Systemic Granulomatosis (SG), a pathological condition affecting the majority of farmed populations. Systemic Granulomatosis is characterized by multiple granulomas in all soft tissues, which progressively may become calcified and necrotic (Ghittino et al., 2004; Katharios et al., 2011) (Fig. 1A, B, C and D). At the microscopic level, fresh squash preparations of affected tissues reveal the presence of granulomas encapsulated by several layers of fibrous tissue with an “onion-like” appearance (Fig. 1E). Histologically, the morphology of the granulomas consists of a central necrotic amorphous area surrounded by a multilamellar layer of epithelioid cells and fibrous tissue (Fig. 1F). The aetiology of this pathological condition is unknown, however two hypotheses have been raised. The first is that it is caused by bacterial pathogens, most likely *Nocardia* spp. (Elkesh et al., 2013), and the second that it may be a metabolic disorder (Ghittino et al., 2004; Katharios et al., 2011) similar to systemic granulomas observed in other cultured fish species (Balouet and Baoudin Laurencin, 1986; Baudin

* Corresponding author.

E-mail address: katharios@hcmr.gr (P. Katharios).

¹ These authors contributed equally to this work.

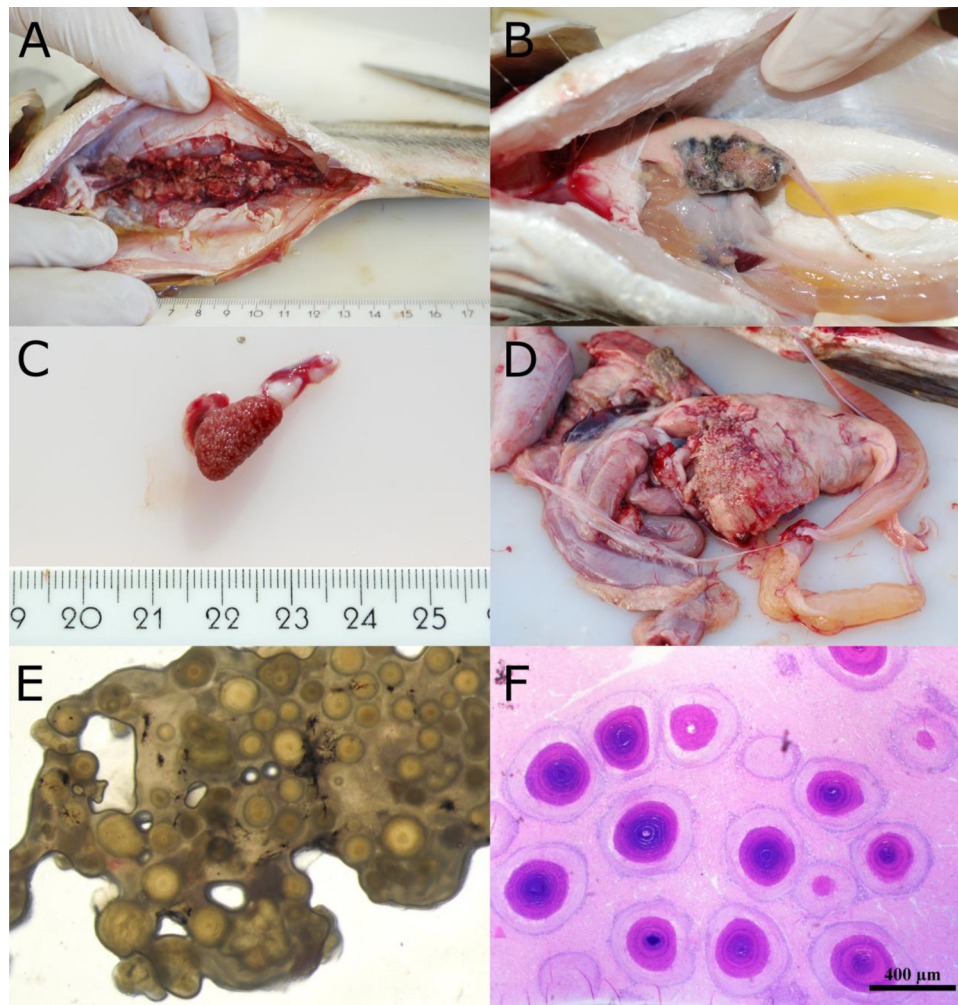


Fig. 1. A and B. Calcified kidney and liver from heavily affected meagre. C. Heart of meagre fully covered by granulomas. D. Multiple granulomas in the soft tissues of meagre. E. Fresh squash preparation from liver of meagre with granulomas (stereoscope x1). F. Histological section from meagre's heart with granulomas consisting of a central necrotic amorphous area surrounded by a multilamellar layer of epithelioid cells and fibrous tissue.

Laurencin and Messenger, 1991; Dunbar and Herman, 1971; Good et al., 2015; Paperna, 1987). Systemic Granulomatosis is not associated with high mortalities, however, it may lead to reduced growth and physiological performance during grow-out and, in addition, it affects the final product, making it unacceptable to the consumer (Fig. 1A–D). Granulomas in fish are a common immune response to a wide range of stimuli, including bacterial infections such as by *Nocardia* spp. and *Mycobacterium* spp., as well as to infections by the Mesomycetozoa *Ichthyophonus hoferi* (Roberts, 2012). *Nocardia* is a genus of actinobacteria related to severe epizootics in fish (Chen et al., 2000; Cornwell et al., 2011; Kudo et al., 1988; Vu-Khac et al., 2016). The clinical signs of nocardiosis include skin ulcers, small white to yellow nodules in the gills and the internal organs, while fish present anorexia and lethargy. Mortality is generally low in the range of 1–17% (Chen et al., 2000; Cornwell et al., 2011; Elkesh et al., 2013), although mass mortalities have been reported in the Japanese industry of cultured yellowtail kingfish (*Seriola lalandi*) and greater amberjack (*Seriola dumerili*) (Shimahara et al., 2008). Histopathology of *Nocardia* spp.-infected fish usually reveals chronic lesions in the form of granulomas. These granulomas are aggregations of macrophages differentiating into epithelioid cells that initially demarcate bacterial colonies and as inflammation progresses, the central area becomes necrotic.

The acid-fast bacteria of the genus *Mycobacterium* can also lead to granulomatous inflammation in fish (Colorni, 1992; Gauthier and Rhodes, 2009; Jacobs et al., 2009). Common symptoms of

mycobacteriosis are lethargy, anorexia, weakening, deformed body, abdominal swelling, dermal lesions and exophthalmos. The internal clinical signs of the infected fish include enlargement of soft tissues such as the spleen, kidney and liver, and grey or white nodules in internal organs (Avsever et al., 2014; Colorni, 1992; Gauthier and Rhodes, 2009; Jacobs et al., 2009; Jeronimo et al., 2013; Timur et al., 2015). Nocardiosis has been reported in farmed meagre in one incidence from Greece (Elkesh et al., 2013), while two cases of mycobacteriosis have been reported from Turkey (Avsever et al., 2014; Timur et al., 2015). These reports may suggest that SG is of an infectious aetiology. Thus, the aim of this study was to test the pathogen hypothesis as the aetiological factor of SG. To examine this hypothesis, we monitored a number of meagre populations farmed in various locations in Greece over a 3-year period in order to isolate and identify *Nocardia* spp., or other granuloma-associated pathogens and assess whether these bacteria represent an actual hazard for the species.

2. Materials and methods

2.1. Fish samples

From September 2013 to June 2016, a total of 150 meagre belonging to a range of developmental stages (from early juvenile to adults) and health status (apparently healthy, exhibiting disease signs and dead fish) were examined. Fish originated from different localities

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