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Enterospora nucleophila and *Cryptosporidium molnari* infection in farmed Gilthead Sea Bream (*Sparus aurata)*: preliminary results

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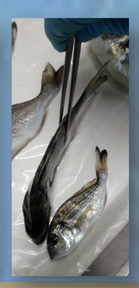
INTRODUCTION

Enteric parasites affecting farmed gilthead sea bream (GSB) (*Sparus aurata*) have become a serious threat for Mediterranean aquaculture in the last few years. Among these parasites *Enterospora nucleophila* and *Cryptosporidium molnari* are undoubtedly the most concerning ones, but available data on their occurrence in Mediterranean farms are still scarce.
Three species of Cryptosporidium found in fish hosts: *Cryptosporidium molnari*, *C. scophthalmi* and *C. huwi*, only *C. molnari* found in GSB. Considerable literature on detection tools, identification and molecular characterization of fish *Cryptosporidium* spp available, but scant data on their pathogenic power. *C. molnari* is likely to induce general clinical signs only when intensity of infection is high. Most common clinical signs: whitish faeces, abdominal swelling and ascites.

Even scarcer data on *E. nucleophila:* firstly described in 2014 and later seldom reported. Its presence in cultured GSB is associated with an emaciative syndrome, which is characterized by: anorexia, cachexia, pale internal organs, decreased growth indexes and significantly increased mortality rates. Pathogenic role of *E. nucleophila* still to be confirmed.
C. molnari and *E. nucleophila* reported from Spanish farms, but no available data on the epidemiology of these two parasites in Italian and Croatian facilities (except for some sporadic reports).
Aim of this work: improve the knowledge about these two parasites in Mediterranean aquaculture by carrying out an epidemiological survey in GSB farmed in Italy and Croatia. First year of investigation data are reported.

MATERIALS AND METHODS

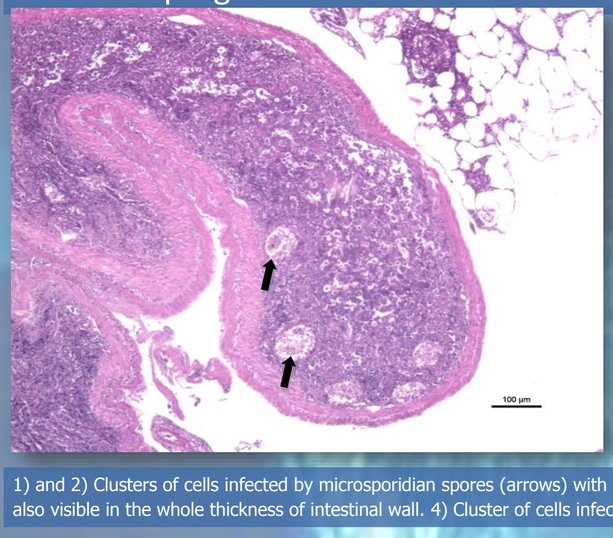
213 fish from Italy and 107 fish from Croatia examined**. Italy**: 173 samples from 2 cages facilities in the Tyrrhenian Sea and 40 from a hatchery; 53,8% of GSB collected from cages showing lethargy, pronounced weight loss up to cachexia and increased mortality rates**. Croatia**: 72 from a net-cages farm, 35 from a hatchery. Fish eggs, Nauplii of *Artemia salina* and rotifers (used as feed in the Croatian hatchery) also analyzed. *Artemia salina* nauplii, rotifers, fish eggs and larvae: whole sample processed for molecular analysis. Fries, fingerlings and adult fish: gastrointestinal tract extracted, mucosal surface deeply scraped for molecular analyses.
Molecular investigation. *Cryptosporidium* spp: Nested PCR amplifying 18S rDNA and gene sequencing. *E. nucleophila*: qPCR amplifying a very specific tract on 18S rDNA.
Histology performed on infected GSB.

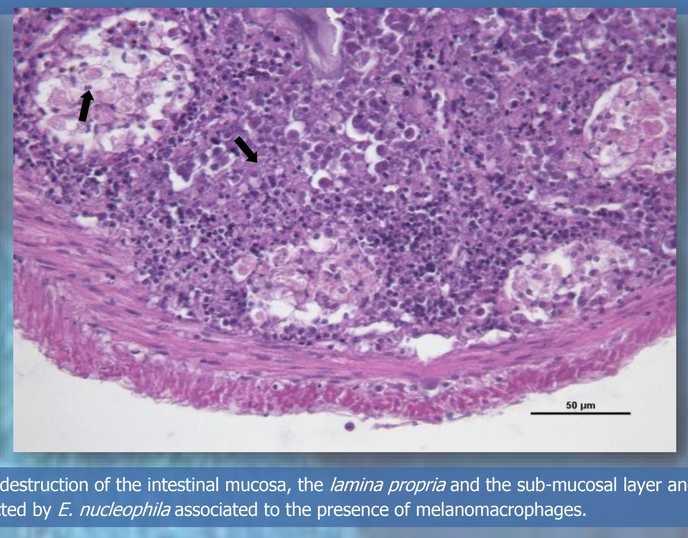


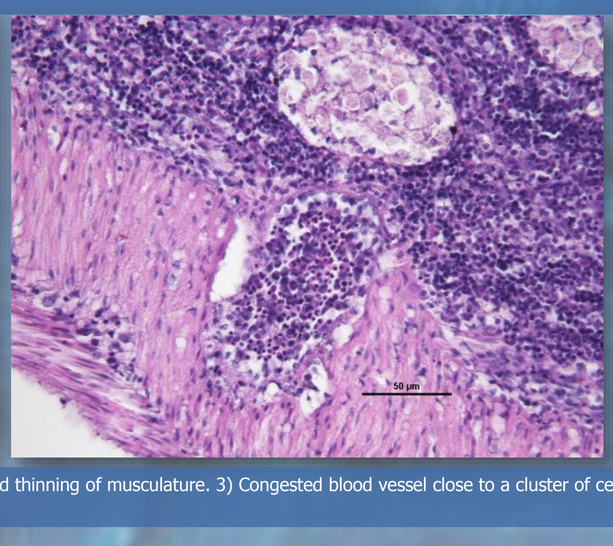
RESULTS

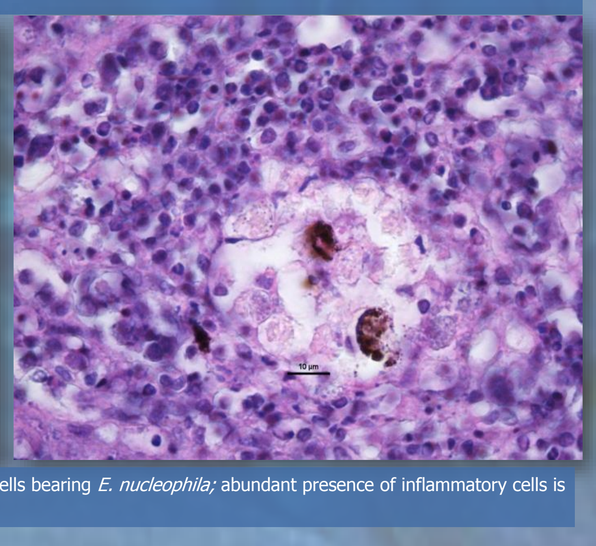
Italy. *E. nucleophila*: 63.6% of cage-farmed fish positive; 60.0% of fish from hatchery positive. *Cryptosporidium* spp: 4.5% of cage-farmed fish positive; 22.5% of fish from hatchery positive. **Croatia**. *E. nucleophila*: 50.0% of cage-farmed fish positive; 22.9% of fish from hatchery positive. *Cryptosporidium* spp: 5.6% of cage-farmed fish positive; none of the fish from the hatchery tested positive. *Artemia salina* nauplii, rotifers and fish eggs for both parasites. DNA sequence analysis led to the classification of *Cryptosporidium* spp as *C. molnari*.

Histology. *Enterospora nucleophila*. Massive infections: strong mucosal sloughing off and necrosis in association to inflammatory infiltration of lymphocytes and few mast cells. Eosinophilic granular cells and monocytes filled by spores also observed. Mild infections, intestinal mucosa structure preserved. Microsporidian spores detected both in gastric and intestinal mucosa, extending to the lamina propria and the sub-mucosal layer. ***Cryptosporidium molnari***. Evaluation of histological lesions are still in progress.









1) and 2) Clusters of cells infected by microsporidian spores (arrows) with destruction of the intestinal mucosa, the *lamina propria* and the sub-mucosal layer and thinning of musculature. 3) Congested blood vessel close to a cluster of cells bearing *E. nucleophila;* abundant presence of inflammatory cells is also visible in the whole thickness of intestinal wall. 4) Cluster of cells infected by *E. nucleophila* associated to the presence of melanomacrophages.

CONCLUSIONS

This preliminary study showed diffuse presence and high prevalences of *E. nucleophila* in Italian and Croatian farmed GSB. Prevalence high in both cages and hatchery, demonstrating that infection can occur at any time in the production cycle of GSB.
C. molnari also present with lower prevalences in both Italian and Croatian farms.
Further investigations are required to better comprehend epidemiology, transmission routes and pathogenic role in farmed GSB of both parasites. Additional studies will help investigate possible risk factors and establish critic control points along GSB production cycle in order to assess and manage the threats arousing from these emerging enteric parasites.