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FIRST REPORT OF *CERATOMYXA SPARUSAURATI* (PROTOZOA: MYXOSPOREA) AND THE OCCURRENCE OF EPITHELIOCYSTIS IN CULTURED SEA BREAM, *SPARUS AURATA* L. FROM MADEIRA.

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Abstract

The benign form of gill epitheliocystosis was observed in 3 out of 47 gilthead sea bream from a mariculture facility in Madeira, Portugal, causing limited epithelial hyperplasia on affected gill filaments. In 1 out of 47 fish massive infection of the gall-bladder and bile with the myxosporean *Ceratomyxa sparusaurati* was found. A disease monitoring program was established involving monthly sampling to access the possible threats of these infections on the sea bream culture of the island.

Introduction

Epitheliocystis has frequently been observed in the gills, skin and pseudobranch of several fish species (Hoffmann *et al.* 1969 in Turnball, 1993; Crespo *et al.* 1990), eliciting either a benign or proliferative reaction. It was already reported from North America, Europe, South east Asia and South Africa, leading to mortalities in the case of poor culture conditions (Paperna *et al.* 1981). In the sea bream, *Sparus aurata* L. epitheliocystis was already reported from the Mediterranean region and North east Spain (Paperna 1977; Padros & Crespo, 1995). Coelozoic myxosporeans of the genus *Ceratomyxa* have been reported from several marine fish species (Lom & Dyková, 1992) and in particular from sea bream, the myxosporean *Ceratomyxa sparusaurati* was recently described from cultured sea bream in Spain (Sitjá-Bobadilla *et al.* 1995; Palenzuela *et al.* 1997). The present paper reports the occurrence of the benign form of epitheliocystis in the gills of cultured sea bream and the infection with *C. sparusaurati* in the gall-bladder of the same fish host both from a mariculture in Madeira.

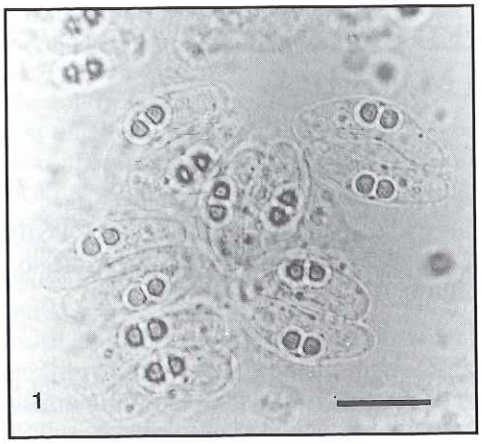
Material & Methods

Fourty-seven live sea bream collected from floating cages located at Caniçal, Madeira (southern coast), in May, June and July 1997, where examined for parasites. In the laboratory fish were anaesthetized with Benzocaine dissolved in 96% ethanol ((BDH Co.) and dissected to expose the internal organs. Squash preparations of gill filaments, stomach, intestine, liver, gall-bladder, spleen and kidneys were prepared and examined with a Zeiss Axioplan Microscope. Samples of kidney, liver and spleen were fixed primarily in Bouin's for 24h, and thereafter prepared for inclusion in paraffin, sectioned at 5 µm, and stained with Hematoxylin & Eosin (see Bancroft & Stevens 1990). Samples of gill filaments were fixed in 5% neutral formalin diluted in sea water, for 24h and further processed as mentioned above. Spore suspensions and pieces of gall-bladder were fixed in 2.5% glutaraldehyde for future electron microscopy study.

Results

1. *Ceratomyxa sparusaurati* Sitjá-Bobadilla, Palenzuela, Álvarez-Pellitero, 1995

In one out of the 47 fish examined, several spores and plasmodial stages of the coelozoic myxosporean *C. sparusaurati* were found filling the gall-bladder. Mature spores showed a somewhat crescent shaped structure. Mean thickness for 20 measured spores



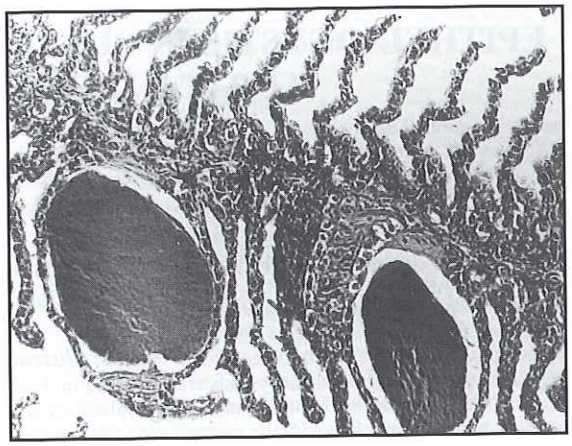


Figure 1: Mature spores of *Ceratomyxa sparusaurati* from the gall-bladder of cultured sea bream, *Sparus aurata*. (bar= 10 µm). **Figure 2**: Section of gill lamellae showing three epitheliocystis cysts (bar= 62.5 µm)

was 18.23±0.39 (S.E.), (range was 15 to 21.6 µm). Mean length was 4.69±0.11 (S.E.) (range 3.6 to 5.4 µm)(Fig. 1). Polar capsules were rounded about 2.4 µm in diameter. The number of turns of the polar filament were not discerned, as we used bright field objectives only. Aberrant spores with 3 polar caps and 3 valve projections were seen in the squashes. The developmental stages were either rounded, amoeboid or elongated plasmodia, some of them containing one or two spores.

2. Epitheliocystis

In 3 out of 47 fish examined, several white soft nodules were found on the gill filaments. Fresh nodules had a diameter of about 225 µm and were visible with the naked eye. Under the high power microscope strong basophilic cysts were observed in the gill filaments (Fig. 2). They were surrounded by hyperplastic gill epithelium with fusion of affected gill filaments. Other surrounding uninfected gill filaments were of normal appearance. It appeared to be a benign reaction as only localized epithelial hyperplasia was observed. No fish mortalities were registered in connection was this condition.

Discussion

Based on the morphology of developmental stages and spores of the *Ceratomyxa* species found in the gall-bladder of sea bream in the present work, there was no doubt to assign it to the newly described *Ceratomyxa sparusaurati* found in cultured sea bream from Spanish waters (Sitjá-Bobadilla *et al.* 1995; Palenzuela *et al.* 1997). The sea bream cultured in Madeira came from a nursery elsewhere, and were said to be exempt of parasites and diseases. To what extent this information is feasible is not known. Perhaps some fish were already infected with this myxosporean, prior to their settlement in Madeira. On the other hand, they could have acquired the myxosporean, from species of feral fishes around the floating cages. This is supported by the findings by Palenzuela *et al.* (1997) and Ching & Munday (1984, in Palenzuela *et al.* 1997) who reported the connection of the occurrence of myxosporean infections in cultured fish with the water supply. Diamant (1997) demonstrated experimentally, that the myxosporean *Myxidium leei* from the intestinal mucosa of sea bream, could be transmitted between fish, by ingestion of infected fish tissue or alternatively through water borne contami-

nation. In the present case if *C. sparusaurati* was transmitted to cultured uninfected sea bream either directly through the water supply, or through an intermediate host, this myxosporean should be found in feral fishes in Madeira. Observations on protozoan infections of several feral fishes in Madeira have already started, but are still at a very preliminary stage, which unables any decisive conclusion at the moment. Taking into account that from 47 fish examined during the summer, only one was infected with this myxosporean, it seems that for the moment there is no danger for the culture, although regular sampling should proceed. On the other hand, cultured sea bream in Spain, suffered prevalence up to 50%, a figure that was considered of concern by the authors (Palenzuela *et al.* 1997) as this myxosporean can lead to massive infections, causing severe pathological alterations to the epithelial cells of the gall-bladder. In respect to the occurrence of epitheliocystis only the benign form of this condition was observed in 3 of the fish examined. Epitheliocystis is said to be caused by a Chlamydial-agent (Paperna *et al.* 1981; Turnball, 1993). Electron microscopic observations on cysts collected from sea bream elsewhere, showed the presence of *Chlamidia*-like prokaryotic organisms (Paperna *et al.* 1981; Padros & Crespo, 1995). The condition in sea bream was considered a benign reaction, but Padros & Crespo (1995) observed the proliferative reaction in cultured sea bream from the North east of Spain. Epitheliocystis was reported from cultured sea bream from southern Portugal, although in this case the extent of damage to the gills was difficult to access, as co-infection with monogeneans was observed (Cruz e Silva *et al.* 1997). Although gills of sea bream in the present case, were only affected by the benign reaction of epitheliocystis, it is not to exclude the possible occurrence of a more dangerous proliferative form of this condition. No mortalities were registered associated with these infections, but a monitoring disease program is been carried out, involving monthly sampling. The present occurrence of this disease in sea bream in Madeira, is an alert for what could be a possible complication for the successful culture of this fish species.

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