Myxosporidians infections in Adriatic cage-reared fish

Ivona Mladineo

Institute of Oceanography and Fisheries, POB 500, 21000 Split, Croatia

Abstract
Myxosporidian parasite infestation was monitored in Adriatic cage-reared fish. Fish were collected once every three months from offshore netpens in the eastern Adriatic, over a nine month period. Fish samples comprised from 9 to 15 each of sea bass (Dicentrarchus labrax), sea bream (Sparus aurata), sharpsnout bream (Diplodus puntazzo) and black sea bream (Pagellus bogaraveo), all aged 1+. Only four myxosporean species were identified from these cage-reared fish in the Adriatic Sea. There were differing dynamics in their prevalence and intensity.

Introduction
The fast and progressive development of mariculture along the eastern Adriatic coast coupled with the trend of introduction of new fish species in intensive rearing systems, has lead to increasing incidence of a range of myxosporidian infections. These are becoming hard to eradicate and are often chronic problems. Although large scale mortalities caused by these pathogens are still rare, damage resulting in reduction of the immunocompetency of infected individuals may make the population susceptible to other pathogens and reduce the collective resistance of the population to disease such that occasionally very high mortalities may occur (Rigos et al., 1997). Slow growth rate and poor food conversion are other side effects that decrease final economical viability of the production. Additionally, there may be negative effect on fertility and reproductive success (Sitjà-Bobadilla and Alvarez-Pellitero, 1990; Alvarez-Pellitero and Sitjà-Bobadilla, 1993).

The subclinical and chronic impact of myxosporean infestation, mostly neglected in past and regarded as parasites of wild, has emerged as a complex, hard to eradicate and persistent problem in mariculture. With the continuous introduction of new species into rearing systems, myxosporean parasites are also introduced to the new environment and easily adapt to fish species already present which were completely allopathic for the parasite in the past (Diamant, 1997). Once kept under highly intensive rearing conditions with their host, myxosporean populations can expand rapidly causing occasional high mortalities and serious epizootic problems.

About 1100 myxosporean species have been described from fish, and they are recognized as the largest metazoan group infecting marine organisms (Kudo, 1966). In addition there are unrecognized synonyms of distinct species described under a single name and many have been described without species names (Lom and Arthur, 1989).

The aim of this study was to observe the myxosporidian fauna from divers facilities on Adriatic coast to determine information on their prevalence, abundance and their proliferation season. Such information is required to enable intervention at key times during the production cycle to help prevent or at least attenuate the potential damage to the stock.
Materials and Methods
Seven Adriatic fish facilities were monitored from June of 2001 to March 2002 for the presence of myxosporean parasites (figure 1). Fish were collected once every three months from off shore netpens, always from the same cage, so the same population of fish was followed over a nine-month period. Fish sample size comprised from 9 to 15 each of sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), sharpsnout bream (*Diplodus puntazzo*) and black sea bream (*Pagellus bogaraveo*), all aged 1+.

In total 654 individuals were examined; 296 sea bass (weighting 165.11 ± 59.61 g), 251 sea bream (255.69 ± 126.02 g), 72 sharpsnout bream (247.60 ± 35.97 g) and 35 black sea bream (198.66 ± 45.70 g). During fish sampling procedure, abiotic parameters (temperature, salinity, nutrient salts and oxygen) were measured.

Fish were put on ice and brought in laboratory between two to twelve hours after collection, depending on facility location. They were autopsied and biometrical measures were recorded.

Fresh smears were taken from gills, skin and fins, from three different parts of alimentary duct (pyloric area, middle intestine and rectal part), spleen, liver, gonads and kidney. If positive, smears were stained by May-Grünwald Giemsa. The number of myxozoans was counted semiquantitatively per microscope field at 400 x magnification: *
Myxosporean were measured and identified based upon the descriptions of Lom and Arthur, 1989.

In case of positive smears, tissue sections were prepared from paraffin-embedded blocks and stained by haematoxylin-eosin method.

For every fish species from a particular facility, prevalence and intensity were calculated according to Bush et al. (1997).

**Results**

Only four myxosporean species were found and identified from cage-reared fish in the Adriatic Sea during the nine-month survey period. Temperature and salinity are in table 1.

*Polysporoplasma sparis*

The spores were round, pumpkin-like, with the width marginally wider than the length. The length measured 13.25 µm ± 0.83, the width 13.5 µm ± 0.52, and the polar capsule length was 4.34 µm ± 0.3, width 3.41 µm ± 0.29. The polar capsules were round, of equal size and with 6 coils of the polar filament. In relation of polar capsule to spore length, the capsules were 3 times smaller than the spore. Anterior ends of polar capsule were set apart one from the other. The suture was prominent on the anterior end, with two thicker bulges on the posterior. In the spring time, very high intensity of pansporoblastic forms was detected from sharpsnout bream, with the presence of old granuloma in the glomerulo-tubular tissue, without MMC aggregation.

The parasite was found in the sea bream kidney only once during the spring period. It was found also in sharpsnout bream kept in cages neighbouring the infected sea bream, at low prevalence. The prevalence and intensity are in table 2.

As a histozoic and sporogenic, *P. sparis* in kidney was mostly surrounded by melanomacrophage centres (MMC). Spores were observed in disporoblast incorporated in glomerular tissue, sometimes masked by the high MMC agglomeration. Except for MMC, no significant histopathology was observed. Glomerular capillaries were not degenerated or infarciated by the spores, and the vascular lamina intima was continuous. Epithelial cells did not show hyperplasia or degeneration. In old spores, the fibrous capsule was feebly visible indicating the age of the process.

### Table 2: Prevalence (p %) and intensity (a) of *Polysporoplasma sparis* from 7 Adriatic facilities (I - VII); b - sea bream, sb - sharpsnout bream, bsb - black sea bream, bs - sea bass.

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<thead>
<tr>
<th>facility</th>
<th>July '01.</th>
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Sphaerospora dicentrarchi:
The length measured $4.2 \mu m \pm 0.26$, the width $4.4 \mu m \pm 0.56$, with the polar capsules length of $1.65 \mu m \pm 0.24$ and $1.25 \mu m \pm 0.26$ in width. Although the smallest myxosporidian identified in cage-reared fish, it is usually easily detected as large number of aggregated spores, in a cyst-like manner. No fibrous tissue formation or macrophages surrounding the aggregation could be observed.

Spores were small, spherical, and marginally wider than long. Polar capsules were also spherical, of equal size; set apart one from the other and 2.5 times smaller in length then the whole spore. The coils of the filament could not be counted under the light microscope precisely. Also no bulges or ornamentation could be observed.

The prevalence and the intensity increased during spring period, except for a facility where fish were submitted to high stress. Specifically, the cages were translocated from one bay to another, and caged fish transported for 4.5 miles. Prior the movement of cages no Sphaerospora sp. was isolated, however in the winter months it emerged in very high prevalence and moderate intensity; the pattern observed only in springtime on other facilities.

The usual site of infection of this histozoic parasite was the intestine (figure 2), but in high infections could be found also in kidney and gall bladder. Vacuolization and cell degeneration, with the lymphocytic infiltration was observed histologically, without any desquamation of the intestinal mucosae or haemorrhagic changes, indicating only ongoing inflammatory process in the intestine. In the gall bladder, similar changes were observed only in very intensive infection.

Strong host specificity was observed, the myxosporidian being isolated only from sea bass. The weight difference between infected

![Figure 2: Fresh smear of S. dicentrarchi in the intestine (400x).](image)
and uninfected fish was observed only on facilities with high intensity of infection, and no mortalities provoked by *S. dicentrarchi* occurred. The prevalence and intensity of *S. dicentrarchi* is in table 3.

*Ceratomyxa sparusaurati*

Spores were 4.93 µm ± 0.61 in length, 22.77 µm ± 7.13 in width, while the polar capsules were 2.71 µm ± 0.41 long and 2.82 µm ± 0.34 wide. Spores were arcuate with edges becoming thinner and longer in older spores. Young spores were triangular with open angle, with the anterior end convex and the posterior straight or slightly concave. Sutural line was straight, valves were thin and without ornamentation. Polar capsules were 1.8 x smaller than the spore length. They were drop-shaped, anteriorly set close to each other, with 5 coils of the polar filament, equal in shape.

The parasite was found in black sea bream and sea bream with a modest prevalence, but high intensity. Proliferation occurred in spring, with no geographic distribution limitation, thus the myxosporean was isolated from the north to the mid part of the Adriatic. *Ceratomyxa sp.* was not recovered from facilities in the southern Adriatic, but these also showed minimal prevalence of other parasite’ phyla (unpublished data).

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Table 4: Prevalence (p %) and intensity (a) of *Ceratomyxa sparusaurati* from 7 Adriatic facilities (I - VII); b - sea bream, sb - sharpsnout bream, bsb - black sea bream, bs - sea bass.

![Figure 3: Fresh smear of presporogenic developmental stage of *C. sparusaurati* (400x).](image3)

![Figure 4: Fresh smear of disporoblast of *C. sparusaurati* prior the detachment (400x).](image4)
During the winter period, high proliferation of sporogonal developmental stages was observed. The presporogenic replication was exhibit in tissue where the sporulation occurred i.e. the gall bladder epithelium (figure 3). After extrasporogenic vegetative replication, the plasmodium was released extracellularly and it matures coelozoically as a spore. The sporoblast contained two future spores (figure 4). The dimensions of the first stage where the polar capsules could be observed, were 5.8 µm ± 0.45 in width for the whole sporoblast, and 0.64 µm ± 0.50 for the polar capsules. In the next stage, where the spore with valves could be observed, the capsules measured 1.66 µm ± 0.26 (width) and 1.45 µm ± 0.12 (length), respectively, and the spore 8.85 µm ± 1.16 (width) and 2.95 µm ± 0.44 (length).

The only site of infection was the gall bladder. As the infections were mild in most cases, no histopathological changes were observed, except for hyperplasia and lymphocytic infiltration of the tissue surrounding the presporogenic stages. The prevalence and intensity are in table 4.

*Myxobolus* sp:
The spores were 9.78 µm ± 0.27 in length and 8.72 µm ± 0.51 in width, with drop-shape, lenticular in sutural and polar views. Shell valves were symmetrical and smooth. The suture line was not precisely in the median of spore, slightly inclined and prominent.

Myxobolus were found and identified only to the genus level from the intestine of sharpsnout sea bream from a north Adriatic facility and in one fish only (total N = 72). The prevalence was 10% with very low intensity, making this finding only of minor epizootical importance. The finding occurred in spring months.

**Discussion**

Myxosporidian studies date from last couple of decades (Krasin, 1985), however marine species have been poorly investigated comparing to freshwater ones, although the species and genera with the most primitively constructed spores and most primitive vegetative forms, occur only in the seas (Kovaliova and Schulman, 1985).

Previously, substantial research of myxosporidian from the Adriatic was conducted, but with emphasis on the parasites of wild fish species and limited only to the most southern part of the Adriatic (Parisi, 1912; Radujkovic and Raibaut, 1989). With the emerging of intensive mariculture and introduction of new fish species for production, marine myxosporidian as causes of pathology in cage-reared fish gained interest (Paperna and Baudin Laurencin, 1979, Alvarez-Pellitero et al., 1995).

In Adriatic finfish production, which is intensive but in limited quantities, serious myxosporosis never occurred, with the highest mortalities caused by bacterial pathogens and no data are available for the prevalence of the parasitofaunal population. Although this study was conducted over a relatively short period of nine months; the data may assist with better understanding of the patterns of emerging myxosporidiosis.

At first glance, the Adriatic myxosporean fauna in cage-reared fish is an impoverished one, but as no previous data exist for the marine area researched, no satisfactory comparison could be made. Only four different genera have previously been identified in farmed fish in this region, and those genera have been repeatedly recorded in larger studies con-
ducted in Greece, Israel, Italy, Turkey and Spain (Kent et al., 2001).

The most significant myxozoan in cultured sea bream in the Mediterranean, *Myxidium leei*, has been reported to induce mortalities and important losses in sparid fish (Diamant et al., 1994), however, it was not identified in the present study. In the most susceptible sparid, *Diplodus puntazzo*, losses can reach up to 80% (Athanassopoulou et al., 1999), but *Myxidium* sp. was not identified in this study perhaps because this fish species only represents a small proportion of Adriatic mariculture. From a personal communication, a *Myxidium* epizootic occurred two years ago in juvenile *D. puntazzo*, but this has not been recorded subsequently. This seems unusual as *Myxidium leei* is easily transmitted from fish to fish directly and by cohabitation (and other types of transmission cannot be ruled out), with an extremely wide range of susceptible fish species (Diamant, 1997).

*P. sparis* is one of the most recent myxosporeans isolated and identified as a pathogen in Mediterranean mariculture, previously described as *Sphaerospora* sp. (Sitjà-Bobadilla et al., 1992). However, the number of studies concerning *Polysporoplasma* is still limited (Sitjà-Bobadilla and Alvarez-Pellitero, 1995, Alvarez-Pellitero et al., 1995, Munoz et al., 1998). It provokes evident cellular changes in haemopoietic tissue and renal tubules, with hemorrhagic, leucocytic infiltration and MMC formation. Glomeruli become enlarged and destroyed by the parasite infiltration (Sitjà-Bobadilla et al., 1992). In Spanish semi-intensive cultured stock of sea bream, *Polysporoplasma* spores were isolated also from dermal ulcers in 20% fish, but this was not recorded in Adriatic facilities during the present study.

In the Adriatic, the main host harbouring *Polysporoplasma* was sea bream, with high intensity and different prevalence ranging from 7.14 to 100%. The summer and autumn months were the peak seasons of infection. A non-uniform prevalence of infection may be reflected by the diverse nutritional and zootechnical measures on different facilities, confirmed also by other authors (Sitjà-Bobadilla and Alvarez-Pellitero, 1995). The same authors found *Polysporoplasma* with a prevalence of 13.8% in sea bream held in semi-intensive systems and concluded that the culture conditions influence the infection dynamics. However, although the *Polysporoplasma* sp. prevalence differed to reflect zootechnical aspects of the facility, it was also isolated from facilities with very high level of optimal husbandry, nutritional and prophylaxis measures. This could be in connection with structure of the spores, having a great thickness of the valves that may contribute to high resistance even when prophylaxis/therapy is high.

Whilst the nine-month survey period is not a whole year, thus proper seasonality can not be discussed, research on a precise fish population showed summer and autumn to be the months of *Polysporoplasma*’ proliferation. However, other studies did not report a seasonal pattern (Sitjà-Bobadilla and Alvarez-Pallitero, 1995).

Most *Sphaerospora* species are hosted in the genito-urinary system (Dyková and Lom, 1982, Hermanns and Körting, 1985, Sitjà-Bobadilla and Alvarez-Pallitero, 1990, Baska, 1990), but *S. dicentrarchi* infects connective tissue at very high prevalence and causes massive mortalities in juvenile fish. Prevalence ranged from 7.14% to 100%, also with differ-
ent intensities, but with clear proliferation of disease in spring period. Positive statistical dependence between age and prevalence was reported in case of *Sphaerospora* (Sitjà-Bobadilla and Alvarez-Pelittero, 1992). The strong host specificity was demonstrated by absence of the parasite in fish species other than sea bass, although the prevalence in sea bass was high and the cages with different fish species were nearby.

For *Sphaerospora renicola* (Grossheider and Körting, 1993) and *S. truttae* (Özer and Wootten, 1999), the requirements of an actinosporean for the completion of the life cycle have been determined. In the case of *S. dicentrarchi*, experiments on transmission have not been successful. In *Sphaerospora* infestation, slight histological lesions in the gall bladder epithelium with inflammatory response in surrounding connective tissue are generally observed, with possible damage to pancreatic tissue (Alvarez-Pellitero and Sitjà-Bobadilla, 1993). Pathology is however, milder than the effect of freshwater *C. shasta*, with severely necrotic and occluded parts of descending intestine (Bartholomew et al., 1989).

*Ceratomyxa sparusaurati* is usually recorded in sea bream and sharpnout bream (Athanassopoulou et al., 1999), while the same genera (*Ceratomyxa arcuata*) was found in gadoid fish (Kalavati and MacKenzie, 1999) which are now being introduced to aquaculture (van der Meeren, 2002). It is interesting to note the finding of *Ceratomyxa* sp in common dentex, that revealed serious pathology only in fish with a very high prevalence of infection.

Total prevalence of this parasite in the Adriatic was not very high, but all fish examined in some periods, were parasitized (Alvarez-Pellitero et al., 1995). The mean prevalence was 18.63% with high intensity, but only in sparid species – sea bream, sharpnout bream and black sea bream. The highest prevalence and intensity were recorded during the spring, while the presporogenic phase peaked during the winter, which is more or less in accordance with previous work (Alvarez-Pellitero and Sitjà-Bobadilla, 1993). Bartholomew (1998) suggested that for *C. shasta*, fish may become infected at very low water temperature, but the progress of the disease is temperature dependent, which may also be applicable to marine species (Company et al., 1999).

Freshwater Myxobolidae occur also in actinosporean form, which lead to the discovery of a myxosporean life cycle (Wolf and Markiew, 1984). Since then, a range of studies demonstrated the invertebrate link in their cycle (Yokoyama et al., 1993) but few data are available about marine genera. Previously *Myxobolus* sp was isolated from the gall bladder of sea bass (Paperna and Baudin Laurencin, 1979) and bulbus arteriousus of brackish water tropic fish (Rajendran et al., 1998). Radujkovic and Raibaut (1992) had only one record of *Myxobolus* sp from the small intestine of annular sea bream (*Diplodus annularis*) with a very low prevalence of 3.8%. Thus, finding this myxozoan in an Adriatic cage-reared sharpnout bream, whilst interesting probably poses no current clear threat to the Adriatic mariculture industry.
References


