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The Effect of Seasonality on the Health and Growth of a Newly Recorded Myxobolus Species Infecting Cultured Sharp Snout Seabream (*Diplodus puntazzo* C.)

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Abstract: The prevalence, intensity, and pathology of a Myxobolus sp. affecting the kidneys of cultured *Diplodus puntazzo* C. during the period 2003-2005 are described. The study included data collected from the largest farm in southern Greece where a high rate of mortality due to myxosporean infection occurred. Sharp snout seabream reared in sea cages in southern Greece underwent microbiological, parasitological, and histological examinations, while measurement and identification of parasites was performed immediately after sampling. Samplings were made daily from winter 2003 to autumn 2005. Prevalence was estimated for each season (autumn, winter, spring, and summer) each year (2003, 2004, and 2005). The Myxobolus sp. was detected in high numbers and with a high prevalence of infection in the renal interstitial tissue, especially during the summer months. The growth rate of infected *D. puntazzo* was very slow during the study period in comparison to cultured *Sparus aurata* L. and uninfected *D. puntazzo*.

Key Words: *Diplodus puntazzo,* Myxobolus sp., Myxosprorida, pathology, seasonality

Introduction

Myxosporean infection occurs in a wide range of both marine and freshwater fish species. Some reviews have stressed the importance of its pathology in mariculture (1,2) and in freshwater farming (3). Recently, significant problems due to myxosporean infection in Mediterranean mariculture have emerged (4-7) *Enteromyxum leei* (formerly *Myxidium leei)* is often implicated in significant losses in cultured sharp snout seabream (*D. puntazzo*) and gilthead seabream (*S. aurata*) (8). Consistently high mortality rates have been associated with the absence of adequate treatment (6), despite the fact that recent studies have shown that salinomycin and amprolium treatment are successful in myxosporean infections, such as Myxobolus spp. in *D. puntazzo* (9,10), *Polysporoplasma sparis* in *S. aurata* (11,12), and *E. leei* in *D. puntazzo* (13). Other myxosporeans only rarely cause large scale mortality; however, damage resulting in reduced immunocompetency in infected individuals may render the population susceptible to other pathogens (14).

The aim of the present work was to study a Myxobolus sp. that is pathogenic in Greek mariculture, causing mortality and reductions in the growth rate of fish, which negatively affects the profitability of the industry.

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Materials and Methods

During 2003-2005, sharp snout seabream (2g-200g) reared in sea cages in southern Greece underwent microbiological, parasitological, and histological examinations. Samplings occurred from winter 2003 to autumn 2005. Each day, 2 fish from the same cage were sampled. Macroscopic examination was performed on the external surface of the gills, the entire body, and in all internal organs. Parasitological examination was also carried out (15). Measurement and identification of parasites was performed immediately after sampling, based on the keys of Lom and Arthur (16). Prevalence was estimated for each season (autumn, winter, spring, and summer) each year (2003, 2004, and 2005) and parasite intensity was estimated according to the intensity scale shown in Table 1.

Table 1. Intensity Scale.

No. of cysts per viewing field (× 10)	Intensity
1-2	+ (low)
3-4	++ (medium)
5-6	+++ (high)

Kidney and spleen samples were inoculated onto tryptone soy agar (TSA) for bacteriological examination, according to the method described by Roberts and Shepherd (17).

Gill, intestine, liver, spleen, stomach, swim bladder, and brain tissues were fixed in 10% buffered formalin, processed and stained with hematoxylin and eosin (H & E), Giemsa, and Von Kossa stains, according to the methods described by Drury and Wallington (18).

Results

Myxobolus sp. cysts containing mature spores (Figure 1) were observed in the renal interstitial tissue of cultured *D. puntazzo* at a high prevalence rate and intensity, most often in the summer months (Figures 2 and 3). The initial prevalence rate of the Myxobolus sp. infection in small fish (M. W. < 50g) was 5%, and was first observed in winter 2003. During 2003 the prevalence gradually increased, reaching the highest level (85%) during the summer, and then dropping to 65% during autumn.

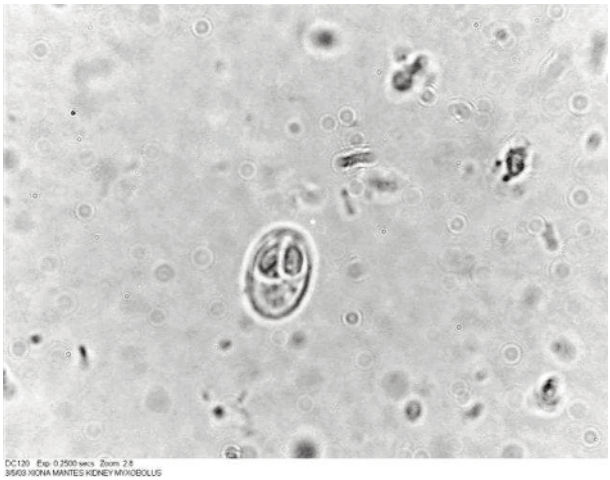

Figure 1. Myxobolus sp. spore from the renal interstitial tissue of cultured *D. puntazzo* 400. Myxobolus sp. spores were commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece.

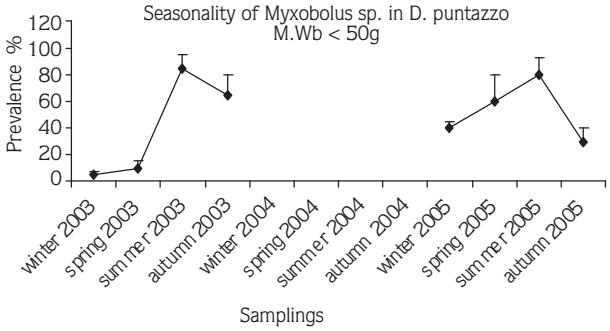

Figure 2. Seasonality of Myxobolus sp. in *D. puntazzo* M.W. < 50g. The Myxobolus sp. was commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece. Samples were collected from winter 2003 to autumn 2005. Prevalence (mean and standard deviation) is presented for each season (autumn, winter, spring and summer) each year (2003, 2004, and 2005).

Additionally, during 2005 Myxobolus sp. infection was first observed in the winter with a prevalence rate of 40%, which gradually increased to the highest level during the summer (80%), finally dropping to 30% during autumn. In large fish (M.W.> 50g) the initial prevalence rate of the Myxobolus sp. infection (33%) was first observed in winter 2003. During 2003 the prevalence gradually increased, reaching the highest level during the summer (85%), and then dropped to 40% during autumn. The same pattern was observed during

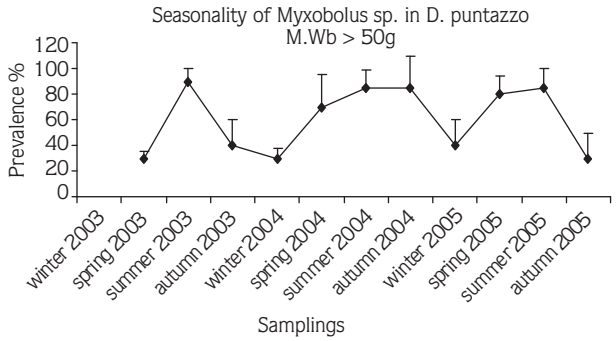

Figure 3. Seasonality of Myxobolus sp. in *D. puntazzo* M.W. > 50g. The Myxobolus sp. was commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece. Samples were collected from winter 2003 to autumn 2005. Prevalence (mean and standard deviation) is presented for each season (autumn, winter, spring and summer) each year (2003, 2004, and 2005).

2004 and 2005, whereas the cumulative mortality rate during the summer months of the 3 years reached 18.7%.

Early trophozoites were observed in the renal interstitial tissue, mainly during spring (Figure 4). Free spores were usually seen demarcated in melanomacrophage centers (MMC), whereas free crystallized material was observed often in the winter months in interstitial tissue. This material was calcified, as shown by Von Kossa staining, in histology sections. The parasite was first observed in spring 2003 (prevalence: 33%) and onwards in large fish (> 50g), and from winter 2003 (prevalence: 5%) in smaller fish (< 50g). Thereafter, the parasite was present in *D. puntazzo* aquaculture in increasing prevalence and intensity until today.

The spores are large, ellipsoidal in frontal view, and lemon-shaped in side view. They have a sutural edge emerging over the surface of the spore and a triangular appendix. Spore valves are symmetrical and smooth, with around 7-8 folds around the edge of the spore. The 2 ellipsoidal polar capsules taper slightly at the discharging canals of the polar filaments, which are equal in size. There is a sporoplasm with a large distinct iodinophilous vacuole. Spore, cyst, and trophozoite dimensions are shown in Table 2.

D. puntazzo growth during the survey is shown in Figure 5. In comparison, *Sparus aurata* data from the same farm is also shown, as well as data from normal average *D. puntazzo* growth obtained from 3 farms near the study area, in which myxosporean infection was not observed. During the period 2003-2004, the overall

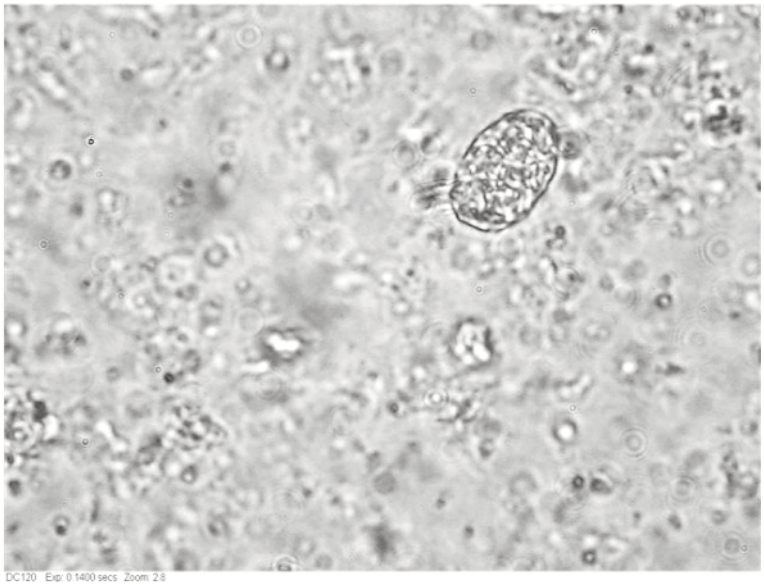

Figure 4. Myxobolus sp. trophozoite from the renal tissue of *D. puntazzo* 400 . Myxobolus sp. trophozoites were commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece.

growth of *D. puntazzo* was reduced by 37.5% of the sea bream normal growth and by 15% of the sharp snout sea bream normal growth. During the year 2005 the overall growth of *D. puntazzo* was reduced by 65% of the sea bream normal growth and by 20% of the sharp snout sea bream normal growth, when the Myxobolus sp. prevalence was also high (Figure 5).

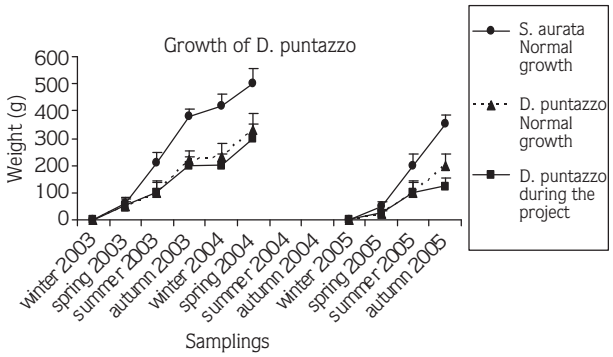

Figure 5. Growth of *D. puntazzo* during the period 2003-2005. The Myxobolus sp. was commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece. Samples were collected from winter 2003 to autumn 2005. Growth was estimated by daily measurement of weight and the mean and standard deviation is presented for each season (autumn, winter, spring and summer) each year (2003, 2004, and 2005).

Discussion

The epidemiology and pathology of myxosporean parasites other than *E. leei* in *D. puntazzo* are not well studied (5,6,14), making the identification of new species very difficult. An unidentified Myxobolus sp. has been reported from the intestine of *D. puntazzo* in Croatia (14), in low numbers and prevalence (10%). This was larger in spore size than the one observed in the kidneys of *D. puntazzo* in the present study. The latter histozoic parasite has also been observed in the kidney of cultured *S. aurata* from farms all over Greece, but with lower prevalence and intensity, mainly in the summer (Athanassopoulou, unpublished data).

From the above data it seems that the actual and potential pathogenic impact of myxosporean infection for Greek mariculture is a subject that requires more consideration. Myxosporeans are present in almost 40% of examined cases in Greek mariculture, sometimes causing significant loss of fish, or most commonly, reduced rate of growth (7,14). In the present study reduced growth rate of the infected fish was due to a possible organic disorder of the excretory system and the destruction of renal parenchyma. This has been reported in other myxosporean species, as well as in *D. puntazzo* during an experimental treatment in which the body weight (as an index of the general well being of the fish) showed statistically significant differences between uninfected, untreated infected fish, and fish treated with different innovative drugs (10). In the same study it was also shown that untreated (infected) control fish lost their phagocytic capacity as the prevalence of the infection increased. The reduction in the rate of growth observed in the present study becomes more important when the seasonal pattern of myxosporean infection is considered, in which the highest prevalence and intensity coincided with a period during which growth rate was maximum and very important to the profitability of the industry. During this critical period, if infection occurs with a low prevalence, salinomycin and amprolium treatment could be used, as it has been proven to be successful in myxosporean infections of Myxobolus spp. (9,10). Additionally, management methods that reduce the stress of infected fish and good quality feeding could improve the immune response of infected fish (19).

This preliminary report indicates the need for further research on the life cycle, transmission, and identification of myxosporean species affecting cultured Mediterranean fish. Additional study of the mechanisms of pathogenicity and the immunological aspects of this Myxobolus species is urgently needed.

Table 2. Dimensions (μm) of Myxobolus sp. spores and trophozoites from the renal tissue of *D. puntazzo*. Each value is the mean of 30 samples. Values in parentheses show the standard deviation, X ± (SD)/n = 30. The Myxobolus sp. was commonly observed in the renal interstitial tissue of cultured *D. puntazzo* reared in sea cages in southern Greece. Measurement and identification of parasites was performed immediately after sampling.

X ± (SD)/n = 30			
Mature spores		Trophozoites	
width	length	width	length
5-7 (0.72)	10-18 (0.8)	10-23 (8.9)	22-55 (9.6)

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