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Research Article

Diseases of White Sea Bream (*Diplodus sargus* L.) Reared in Experimental and Commercial Conditions in Greece

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Abstract: A bacteriological and parasitological study of the white sea bream, *Diplodus sargus* L., was conducted as this species is a potential candidate for aquaculture in Mediterranean countries. The study comprised fish reared in commercial cages (2 farms) located in different geographical localities, as well as fish reared experimentally in cages located in a natural enclosed lagoon. Fish were examined in the fresh state by means of smears from all internal and external organs and by histology. Microbiology was also performed at all sites. Mortality was low (12%) at farm 1 (Argolida), while at farm 2 (Korinth) and the lagoon it reached 32% and 42%, respectively. Microbial infections were low in prevalence and these concerned *Vibrio anguillarum*, *V. alginoliticus* and *Pseudomonas* sp. The parasitic fauna comprised external Monogenea, Digenea and internal Myxosporida. Monogenea were present throughout the year at all locations, but at low intensity. Three species of Myxosporida were recorded in this study: *Myxobolus* sp. in the kidneys, *Kudoa* sp. in the musculature and *Enteromyxum leei* in the intestine. Nephrocalcinosis was found in all sampled fish and this was suspected to be related to *Myxobolus* sp. infections. The study showed that *D. sargus* reared under captive conditions is prone to several parasitic and bacterial infections. Amongst them the most important in terms of pathology were the myxosporeans (*Myxobolus* sp. and *Kudoa* sp). These may play a role in the mortality observed, thus making this fish culture questionable in terms of cost effectiveness.

Key Words: *Diplodus sargus*, diseases, epidemiology, parasites

Introduction

The white sea bream (*Diplodus sargus*) has attracted great interest since the 1980s for Mediterranean aquaculture due to its high commercial value. To date only low-scale production has been achieved in Greece due to the slow growth rate of this species reared under captivity in shore cages (1). Despite this fact, scientific and economic efforts are still being made by different research and commercial groups in order to further develop the potential of new species (including the white sea bream) in aquaculture. Diversification is nowadays necessary because of overproduction in the industry and saturation of the market by sea bream and sea bass, affecting fish prices. However, the recent growth of the aquaculture industry in Greece along with the introduction of new fish species (*Diplodus puntazzo, Dentex dentex, Pagellus* spp. and *Diplodus sargus*) in intensive rearing systems has led to an increased occurrence of different pathogens; in addition, data concerning *D. sargus* biology, culture and pathology are very limited (1-6). Amongst these pathogens, Myxosporidia have caused serious problems in Sparidae

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during the last few years, limiting and constraining their further introduction in aquaculture (4,7,8). Microbial infections are currently under control in caged aquaculture due to efficient vaccination strategies, whereas Arthropoda infections are found in specific geographic areas of Greek waters (4,6,9). The most important pathogen myxosporean affecting Mediterranean fish is *Enteromyxum leei* (Diamant, Lom et Dykova 1994), often implicated in serious losses in cultured sharpsnout sea bream (*Diplodus puntazzo* C.) and sea bream (*Sparus aurata* L.) (8,10). The present work describes the main pathological conditions observed in *D. sargus* reared in both experimental and commercial cages in different Greek locations. The potential importance of these pathogens is assessed in terms of prevalence, abundance, intensity, pathology and mortality.

Materials and Methods

Sampling

During 2002-2003, *D. sargus* reared in commercial cages (2 farms) from 2 different geographical regions (Southern: Argolida and Korinth sites; and Western Greece: Corfu site) were examined. In total, 250 caged reared white sea bream (40-200 g in weight) were examined. The final stocking density of the caged fish in both farms was 10-12 kg/m^3 and fish were fed on commercial feeds. Five sampling rounds were conducted at each farm per year. Each sampling time randomly selected fish from *D. sargus* cages were taken. From these cages, 20-25 fish were collected and examined each time. Four experimental cages of 25 m^3 volume supplied with self- feeders were located in a natural enclosed lagoon in Western Greece stocked with 400 fish at a final density of 15 kg/m^3. In total, approximately 100 experimental fish were examined. Four samplings were performed per year and fish samples of 25 fish were collected from each cage. Salinity was 39‰ at all locations. Temperature profiles of the 3 locations in the study are shown in Figure 1.

Parasitological and bacteriological examinations

Fresh smears from all organs were examined microscopically by methods described by Athanassopoulou (11) and Roberts (12). Muscle tissue and gills were also examined under a stereoscope to detect myxosporan cysts and to count monogeneans. Identification of monogeneans was performed with the keys published by Euzet and Noisi (13). Parasites were recorded by means of prevalence and intensity. The intensity of the parasites was estimated according to the following keys:

No. of parasites per viewing field (x25)	Intensity keys
1-2	+
3-4	++
5-6	+++
7-8	++++
>8	+++++

Kidney and spleen samples were inoculated into different culture media with 2% salt as necessary (Tryptone Soy Agar, Citrate Bile Salt Agar, Brain Heart Infusion Agar) for 24-72 h at 23 °C according the methods described by Roberts (12). The identification of bacterial isolates was based on morphological and biochemical characteristics (API NE, 20NE, API Staph); in some cases, classical biochemical methods were also used (14,15). The biochemical characteristics of microbes isolated were also compared to those reported in the literature (15,16). Samples of fish showing bacterial and/or parasite infections from each location were processed histologically. Tissues of gills, intestine, liver, spleen, stomach, swim bladder and brain were fixed in 10% buffered formalin, and processed for routine histology. Sections for general histology were stained with Haematoxylin and Eosin (H&E), for the presence of bacteria by Giemsa and for calcium identification by Von Kossa stains, according to the methods described by Drury and Wallington (17).

Results

The growth of *D. sargus* was slightly better at Argolida (farm 1) (initial av. weight of 50 g), where, overall, temperatures were more constant throughout the study period (Figure 1). The lagoon from where the experimental fish originated was exposed to sudden increases and decreases in temperature that influenced

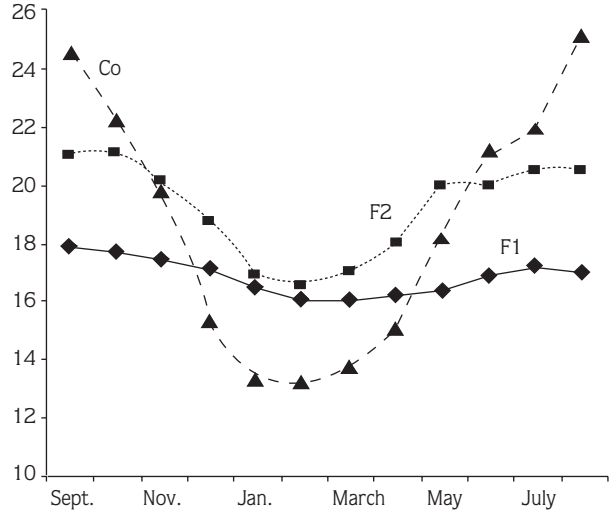

Figure 1. Average temperatures in: Corfu site (experimental site-Co), Argolida (F1) and Korinth (F2).

the fish growth rate initially. Fish at farm 2 (starting average weight of 100 g) also had good growth. However, at the end of the year the fish growth was almost equal at all locations (Figure 2).

The pathological changes in *D. sargus* and their prevalence from all locations studied are shown in Tables 1,2 and 3 and accumulative mortality is shown in Figure 3. At Argolida, mortality was low (12%), while at Korinth (farm 2) and in the lagoon it was higher (32% and 42%, respectively).

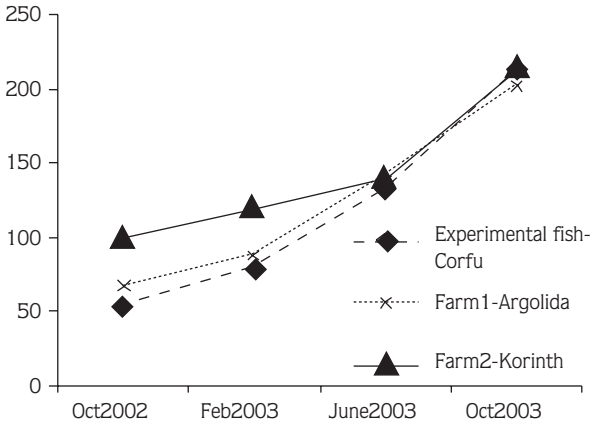

Figure 2. Growth (mean weight in grams) of *D. sargus* in different rearing facilities during 2002-2003.

Microbial infections had a low prevalence (<20%); these were *Vibrio anguillarum*, *V. alginoliticus* and *Pseudomonas* sp. infections (Table 1). *Vibrio alginoliticus* infections were not associated with a particular season. Mortality attributed to *V. alginolitycus* was low (<5%). Infected fish displayed abdominal oedema and external haemorrhages of the dorsal, anal and caudal fins. In necropsy, splenomegaly and some petechial haemorrhage were present in the intestine. In histology microcolonies in the spleen and a moderate inflammation of the proximal and final part of the gut were observed together with a few bacterial colonies.

Table 1. Prevalence of bacterial infections found in the different locations of the study Co: experimental, Corfu, F1: Farm 1, Argolida; F2: Corinth.

DATE	Location	*Pseudomonas* sp.	*Vibrio alginolyticus*	*Vibrio anguillarum*
10/2002	Co	10	0	0
	F1	0	15	0
	F2	0	12	0
12/2002	Co	20	0	0
	F1	0	20	0
	F2	0	20	0
2/2003	Co	20	0	0
	F1	0	0	0
	F2	0	0	0
6/2003	Co	0	10	60
	F1	0	0	0
	F2	0	0	0
10/2003	Co	0	20	0
	F1	0	0	40
	F2	0	0	0

Table 2. Prevalence of lesions and prevalence and intensity of nephrocalcinosis (NC) found in the different locations of the study. Co: experimental, Corfu, F1: Farm 1, Argolida; F2: Corinth; A = inflammation in brain; B = inflammation of intestine/stomach; C = inflammation in heart; D = Digenea.

DATE	Location	A	B	C	D	NC	
						Prevalence %	Intensity
10/2002	Co	0	0	20	80	0	
	F1	50	20	0	0	0	
	F2	50	20	0	0	0	
12/2002	Co	0	0	20	0	10	+
	F1	0	0	20	0	17.6 +	+
	F2	50	20	0	0	50	+
2/2003	Co	20	0	0	0	43	+
	F1	0	0	0	0	86	++
	F2	0	0	0	0	4.5	+
6/2003	Co	0	0	0	0	40	+
	F1	0	0	0	0	22.2	+
	F2	0	0	0	0	2.5	+

Table 3. Prevalence and intensity of parasites found in the different locations of the study. Co: experimental, Corfu, F1: Farm 1, Argolida; F2: Corinth.

DATE	Location	*Furnestinia* sp.		*Microcotyle* sp.		*Copepoda* spp.		*Enteromyxum leei*		*Kudoa* sp.		*Myxobolus* sp.	
		Prev. %	Intens.	Prev. %	Intens.	Prev. %	Intens.	Prev. %	Intens	Prev. %	Intensity	Prev. %	Intens.
10/2002	Co	80	++	40	+	20	+	0		60	++	40	++
	F1	80	++	40	+	0		0		0		80	++
	F2	60	++	0	+	0		20	+++ sporocysts	0		0	
12/2002	Co	0	++	18.9	+	0		0	0	20	++	10	++
	F1	75.6	++	5.9	+	0		17.6	+++	0		11.6	+
	F2	75		0				0	sporocysts	0		12.5	+
2/2003	Co	57	+	0		0		0	0	20	++	23.4	++
	F1	95	++	2.5	+	0		17.6	+++	0		11.6	++
	F2	90	++	0		0		0	sporocysts	0		12.6	++
6/2003	Co	25	+	0		0		0	0	0		90	+++
	F1	76.575	++	0		0		40	+++	0		90	+++
	F2		++	0		0		0	sporocysts	0		80	+++
10/2003	Co	0		18.9	+	0		0	0	20	++	10	++
	F1	75	+++	0		0		25	+	0		12.5	+
	F2	75	+++	0		0		0	spores	0		12.5	+

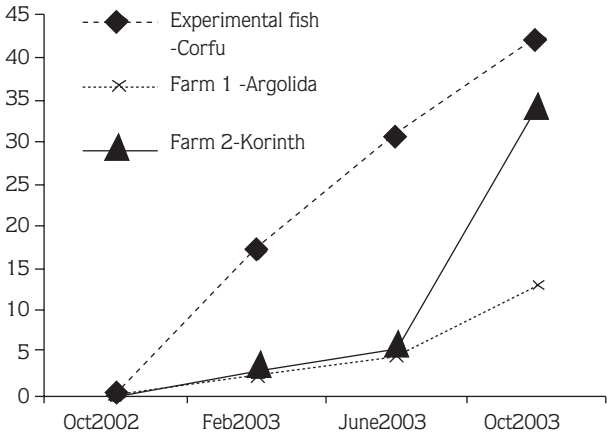

Figure 3. Accumulative mortality of *D. sargus* in different rearing facilities during 2002-2003.

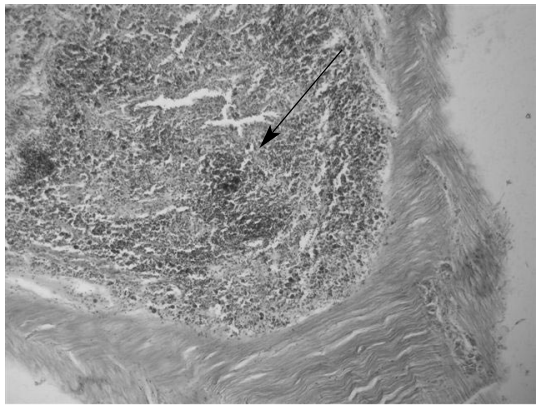

Figure 4. Inflammation in the stomach of *D. sargus* (arrow). H&E, X300.

Vibrio anguillarum infected fish had external haemorrhages but the pathology was more severe in the descending gastrointestinal tract, rectum and colon; enteric discharge associated with lumenal overgrowth and adherence of bacteria to the brush border was observed in the histology. Colonies were found also in the spleen and in the heart but no cardial myopathy was present. Mortality was higher in the spring (approx. 12%).

Pseudomonas sp. was present mainly in cold months, when mortality was about 10%. Moribund fish were discoloured, had haemorrhagic necrosis on the body and mouth, and a few exhibited exophthalmia and frayed fins. In necropsy, infected fish had an enlarged spleen and a haemorrhagic stomach and proximal gut. In the histological examination a moderate inflammation of the proximal part of the gut and in the mucosal layer of the stomach (Figure 4) was observed together with a few bacterial colonies. Inflammation associated with bacterial infection was also observed in the proximal intestine and in the brain. In the intestine and stomach this consisted of an intense aggregation of lymphocytes, whereas in the brain foci of bacterial colonies surrounded by inflammatory cells (mainly lymphocytes) were present.

The bacterial isolates were always present in histological sections.

Parasites comprised members of Monogenea (*Furnestinia* sp., *Microcotyle* sp), Digenea (metacercarea) and Myxosporida. Monogenea were found in the lateral part of gill filaments. The prevalence and intensity of *Furnestinia* sp. was high throughout the year, in all locations (Table 2). *Microcotyle* sp. infections were lower in prevalence and intensity in all locations. A mild hyperplasia of the epithelial cells was present in the histological sections of affected gills. Copepoda were only found in one sample (October 2002), at low prevalence and intensity, at the experimental site.

Digenea (metacercaria) were found only during one sampling in the experimental cages, having a high prevalence, encysted in the renal parenchyma. Histologically, large areas of demarcation by a double layer of connective tissue surrounding the parasites were observed in the interstitial renal tissue replacing the normal parenchyma. The centre of these granulomas was occupied by dense roundform material showing a few melanin spots and this was surrounded by a lighter stained material (Figure 5a, b). These parasites and histological lesions were not found in subsequent samplings.

Three species of Myxosporida were detected in this study (Table 2):

E. leei was isolated mainly from fish over 50 g at Argolida and Corinth from the gall bladder, the gut and less frequently the gills. The prevalence was low but the intensity of the sporocyst stage was high (farm 1). All affected fish appeared emaciated with enlarged gall bladder and distended abdomen. Pathology concerned the epithelial layer of the intestine, where degeneration, apoptosis and necrosis were observed. Haemorrhages in the mucosal layer were also present. An inflammatory

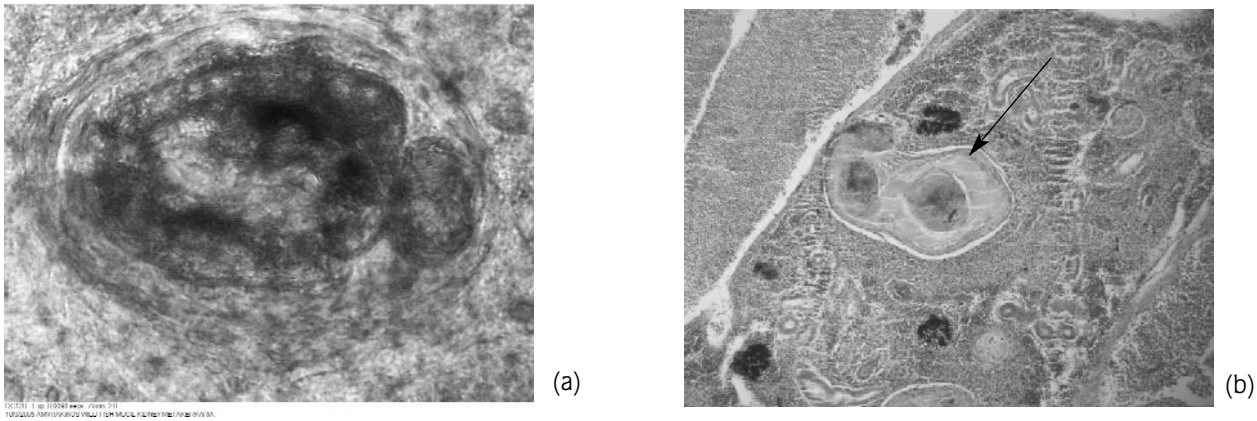

Figure 5. Metacercaria in the renal parenchyma of *D. sargus*. (a) Fresh preparation, X300, (b) granuloma (arrow) due to encysted metacercarea in the renal parenchyma. H&E, X200.

reaction was present near early trophozoites in the mucosal layer and this was retained until the parasite reached the sporoblast stage. Gall bladder epithelium was degenerated and all stages of parasite were present in the lumen.

Kudoa sp. (Figure 6) was found throughout the year only in fish from experimental cages in low prevalence and intensity. The myxosporean appeared in small, 1-5 mm long, elongated uncoloured external lesions located in the lateral body of muscles of the dorsal area. Affected muscles were placid in necropsy and in histology these appeared degenerated and necrotic due to sac-like groups of spores frequently surrounded by a fine fibrotic capsule that consisted of fibroblast-like cells.

Myxobolus sp. cysts were commonly found in the renal interstitial tissue of both experimental and cultured fish at a high prevalence and intensity in the summer months, while early trophozoites were observed in the renal interstitial tissue mainly during autumn. Free spores, similar in size and morphology to those previously reported, were demarcated by the tissue machrophages in melanomacrophage centres (MMC). In the winter months, free crystallised material was observed in the interstitial tissue. By Von Kossa staining of histology sections, a calcareous structure was proved.

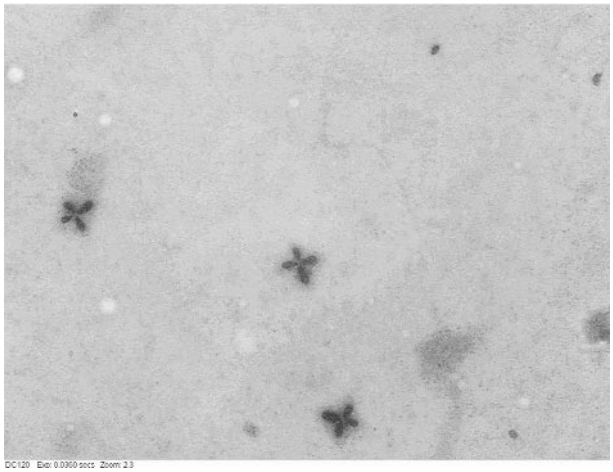

Figure 6. *Kudoa* sp. spores from the muscles of *D. sargus*. Giemsa X400.

Nephrocalcinosis was found almost throughout the year in the interstitial kidney tissue and inside the tubules and collecting ducts of both experimental and commercially reared fish (especially at Korinth) and was adversely associated with *Myxobolus* sp. cysts in the kidney. When the prevalence of *Myxobolus* sp. cysts was low, nephrocalcinosis lesions had a high prevalence. Histologically, tubules were enlarged, showing epithelial atrophy and degeneration. Free crystallised material was also present in the interstitial renal tissue without evidence of cellular changes in the surrounding haematopoietic tissue.

Discussion

The number of studies concerning *D. sargus* diseases is very limited, and the most commonly reported cases in fish under intensive culture are saddle-like syndrome (3) and nephrocalcinosis (2). Parasitic reports mostly concern data on parasites collected from wild fish (9,18), while there are no published data on microbial diseases of *D. sargus* other than a report of *V. anguillarum* infections (19). The present study showed that *D. sargus* reared

under captive conditions is prone to several parasitic and bacterial diseases that may play a role in its successful introduction in aquaculture, as they are able to induce mortality. Fish from experimental cages (Corfu), in particular, were prone to microbial and myxosporean infections. This may be due to the temperature profile of the area (higher temperature variation throughout the year) affecting the growth rate and inducing high mortalities. The small and shallow cages of the experimental fish may have favoured the increase in pathogens found in the study, while the temperature profile of the area could have contributed to the stress of the fish. Overall, the performance of *D. sargus* was best at Argolida (farm 1), also showing low mortality. This was probably related to the temperature profile of the region (constant throughout the year) and the low prevalence and intensity of myxosporean and microbial diseases observed in this location.

Monogeneans, although present throughout the year, did not pose a threat to the host, being present at low abundance and not eliciting any marked pathology.

Nephrocalcinosis, a chronic disease, has been reported from many cultured fish such as Salmonidae, halibut and sea bream (20,21). The disease has been reported in *D. sargus* reared under experimental conditions (2). The aetiology of the disease is not yet defined and it is generally thought that it is related to dietary factors and/or modifications of the chemical parameters of the water (20). In our study, as well as in previous studies (22), this condition was adversely associated with *Myxobolus* sp. infection in the kidney. In the present study, it was present in all rearing locations (cultured and experimental). Fish in our study were fed on different diets and thus it is unlikely that the condition was related to dietary factors. The fact that the condition was present in high prevalence when *Myxobolus* sp. infection was low may suggest that interstitial nephrocalcinosis represents a later stage of host reaction to this myxosporean. This process has been also observed in other Myxosporea such as *Myxidium rhodei*, affecting cyprinids (8). Calcinosis as a final stage of parasitosis is well reported in mammals and humans (23,24).

The *Myxobolus* sp. cysts found in *D. sargus* in our study were very similar in size and morphology to those observed in *D. puntazzo* (22). The latter histozoic parasite has been commonly found in the kidney of cultured *D. puntazzo* and *Sparus aurata* from farms all over Greece at a high prevalence and intensity in the summer and is currently under identification (24). Recently, the same parasite has been also found in the kidney of wild *D. annularis* and *D. vulgaris*, having a pathology similar to that of *D. puntazzo* (9). This is the first record of this parasite in reared *D. sargus*. Another unidentified *Myxobolus* species has been reported from the intestine of *D. puntazzo*, in Croatia (10) in low numbers and prevalence (10%). This latter species, however, was larger in spore size than the one found in the kidneys of *D. sargus*, *D. vulgaris, D. annularis* and *D. puntazzo* from Greece.

E. leei is considered very pathogenic to the sharpsnout sea bream, *Diplodus puntazzo*, making questionable the viability of its farming (5). The heavy and prolonged mortalities in some occasions, associated with the absence of an adequate treatment, seem to create a non-cost-effective operation. This myxosporean is a histozoic parasite that emerged during the 1990s, causing severe losses in the cultured gilthead sea bream (8). Subsequent works on other *Myxidium*-infected sparids such as sharpsnout sea bream (*D. puntazzo* C.), red porgy (*Pagrus major* L.) and common sea bream (*Pagrus pagrus* L.) revealed the potential threat of this myxosporean on cultured Sparidae species (5,8,25). In our study, however, *E. leei* was found in low prevalence and intensity that did not cause mortality, possibly suggesting that this host is more tolerant than other Sparidae such as *D. puntazzo*.

Histozoic myxosporea of the genus *Kudoa* infect many marine fish (26), parasitising mainly the body musculature of fish, and therefore are able to lower their market value, causing pseudocysts and soft texture (27). *Kudoa* sp. infection has been previously seen in different tissues of the gilthead sea bream and striped mullet (*Mugil cephalus* L.) (27). In Greek mariculture a case of kudoasis was previously reported in juvenile gilthead sea bream, associated with high mortalities (28). The present study describes the first report of this myxosporean infection in *D. sargus*. Although the pathogen heavily infected the muscular tissue of this species, mortalities were not induced, similar to findings reported by Rigos et al. (4) in sea bass. The potential impact of myxosporean infections for white sea bream requires and indeed necessities additional research on the mechanism of pathogenicity as this host is a recently introduced species for intensive aquaculture.

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