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PREVALENCE AND PATHOLOGY OF ECTOPARASITES OF MEDITERRANEAN SEA BREAM AND SEA BASS REARED UNDER DIFFERENT ENVIRONMENTAL AND AQUACULTURE CONDITIONS

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Abstract

Sea bream, *Sparus aurata* L., and sea bass, *Dicentrarchus labrax* L., were sampled from an off-shore cage farm in eastern Greece, an inshore cage fish farm in southern Greece, and a lagoon in northern Greece. The prevalence and intensity of Metazoan parasites and factors associated with the prevalence were investigated. In Farm 1 (eastern Greece), ectoparasite prevalence in sea bream and sea bass was 61.5% and 76.9% for Monogenea, none for Isopoda, and 0 and 23% for Copepoda. In Farm 2 the prevalence was 13.3% and 26.3% for Monogenea, 13.7% and 20% for Isopoda, and 0 and 13.6% for Copepoda. In the lagoon, the prevalence of ectoparasites was 100% and 21.9% for Monogenea and Copepoda, respectively. The lagoon had the highest prevalence of parasites among the studied ecosystems, however, the variety did not significantly affect the health of the infected fish. In Sparidae, gill lesions were due to monogeneans while skin and eye lesions were due to larvae of the isopod *Ceratothoa oestroides* that caused severe pathology. *Furnestinia echeneis* and *Diplectanum aequans* were host specific to sea bream and sea bass, respectively, and persisted in all aquaculture systems. *Lernanthropus kroyeri* was host specific and detected only in sea bass from the cage farms.

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Introduction

Fisheries and intensive fish farming are important financial resources in Mediterranean countries. The main fish species grown in the eastern Mediterranean are sea bass *(Dicentrarchus labrax,* family *Serranidae)* and gilthead sea bream *(Sparus auratus* Linnaeus 1758). Each accounts for about half of the total output. Intensive production started in Greece about 20 years ago. Greek production alone accounts for close to 60,000 tons of output, whereas hatcheries provide about 300 million fry to on-growers (FAO, 2002). Market demand and the increase in sea bass and bream output obviated the need to grow additional marine species with high market value and most new entrants in farming belong to the Sparidae (bream) family.

The most common parasites affecting farmed Mediterranean fish are the myxosporeans *Enteromyxum leei* (formerly *Myxidium leei*), often implicated in serious sparid losses, *Sphaerospora dicentrarchi* Sitja-Bobadilla and Alvarez-Peliterro 1992, and *S. testicularis* Sitja-Bobadilla et Alvarez-Pellitero 1990 (Sitja-Bobadilla and Alvarez-Pellitero, 1990, 1993ab; Alvarez-Pellitero and Sitja-Bobadilla, 1993).

The increase in prevalence of Isopoda is a serious problem (Papapanagiotou et al., 1999; Athanassopoulou et al., 2001a; Papapanagiotou and Trilles, 2001). Intensive fish farming in Mediterranean coastal waters provides close to the ideal environment for isopod parasites. Hence, isopod infestations comprise a frequent problem in farmed sea bream and, especially, sea bass, in the Mediterranean (Paperna, 1991). *Ceratothoa oestroides*, the most common isopod parasite, inflicts major damage (Varvarigos, 2004ab).

Other parasitic Metazoa have been studied because, under certain conditions, they can cause serious pathological problems and increased mortality, especially in young fish. Microcotylids, for example, feed on the host's blood and may cause severe anemia and even mortality (Roberts, 1989).

In the western Mediterranean, the gilthead sea bream is parasitized by two gill monogeneans: the *Furnestinia echeneis* (Wagener, 1857) Euzet and Audouin, 1959 (Monopisthocotylea: Diplectanidae) and the *Sparicotyle chrysophrii* (van Beneden and Hesse, 1863) Mamaev, 1984 (Polyopisthoco-tylea: Microcotylidae). These two monogeneans are globally frequent but moderate in intensity in wild populations. In contrast, they can be pathogenic to cultured fish (Reversat et al., 1992; Papoutsoglou et al., 1996). Two diplectanid monogeneans are commonly found on sea bass in the Mediterranean: *Diplectanum aequans* and *D. laubieri*. Both species, especially *D. aequans*, are potential pathogens (Gonzalez-Lanza et al., 1991).

Environmental upsets, such as weather extremes, excessively high temperature combined with poor dissolved oxygen, and, especially, temperature fluctuations in excess of 1.5°C predispose to parasitic disease by compromising fish defenses (Papoutsoglou et al., 1996). Infected caged fish may act as disease amplifiers, spreading vast amounts of pathogens. Wild fish may become infected from caged fish or *vice versa*. Wild fish shoals are important vectors of pathogens from farm to farm (Paperna, 1984).

The aim of this survey was to estimate the prevalence and intensity of Metazoan parasites in cultured sea bream and sea bass and study their pathology and factors influencing their prevalence in three geographic areas where approximately 40% of Greece's marine aquaculture is practiced. This is, to our knowledge, the first report concerning a large-scale investigation of ectoparasite impact on cultured sea bream and sea bass that takes into consideration different rearing, ecological, and temperature conditions.

Materials and Methods

Fish farms. Farm 1 is situated off the shore of Hios island in eastern Greece. The farm is fairly exposed, has a rocky bottom, and there are high currents in the area. The cage volume is 1500 m^3. Farm 1 is one of the oldest farms in the country and represents a mature ecosystem. Farm 2 is situated in Epidavros in southern Greece. It is a protected inshore farm with minimal currents. The bottom is muddy and the cage volume is 2000 m^3. Kavala is a bar-built lagoon in the delta of the Nestos River on the Macedonian coast of northern Greece. It covers an area of about. 0.785 km^2 and has a narrow connection to the sea (30 m wide, 1.5 m depth). The lagoon consists of four sections: the shallow basin is the main basin and is no deeper than 0.5 m; the southeastern basin consists of 13 artificially constructed channels up to 2 m deep; the northwestern basin is up to 4 m deep and used for over-wintering; and stocking basins. The tide fluctuates seasonally with a maximum of 45 cm. Salinity drops from winter to summer with an annual mean of 30 PSU. Average annual fish production is 120 kg/ha, based on natural and artificial stocking of fish fry with an on-growing facility, wintering basin, collecting basin, and permanent fish barrier (Ardizzone et al., 1988). These farming areas were selected because they represent three distinct geographic areas/ecosystems and a large proportion of Greek marine aquaculture is practiced there.

Measurement of ecological factors. Salinity, oxygen, and temperature were taken monthly by farm personnel as part of their routine management procedures. The two farms had similar and constant salinity and oxygen levels (40‰ and 2-8 mg/l) but the temperature at Farm 1 was lower and fluctuated more than at Farm 2 (Fig. 1). Both farms produced approximately 1500 tons of market-sized fish per year. The stocking density of the caged fish in both farms ranged 12-15 kg/m^3 and fish were fed commercial feeds. The temperature at Kavala lagoon ranged 15-30°C with one peak in summer. The salinity was lower (24-36‰) than in the farms (Theohari et al., 1997).

Fish samples. From Farm 1, 52 sea bream and 52 sea bass weighing 350-400 g were examined. From Farm 2, 225 sea bream and 205 sea bass weighing 50-300 g were examined. At each sampling, fish were randomly

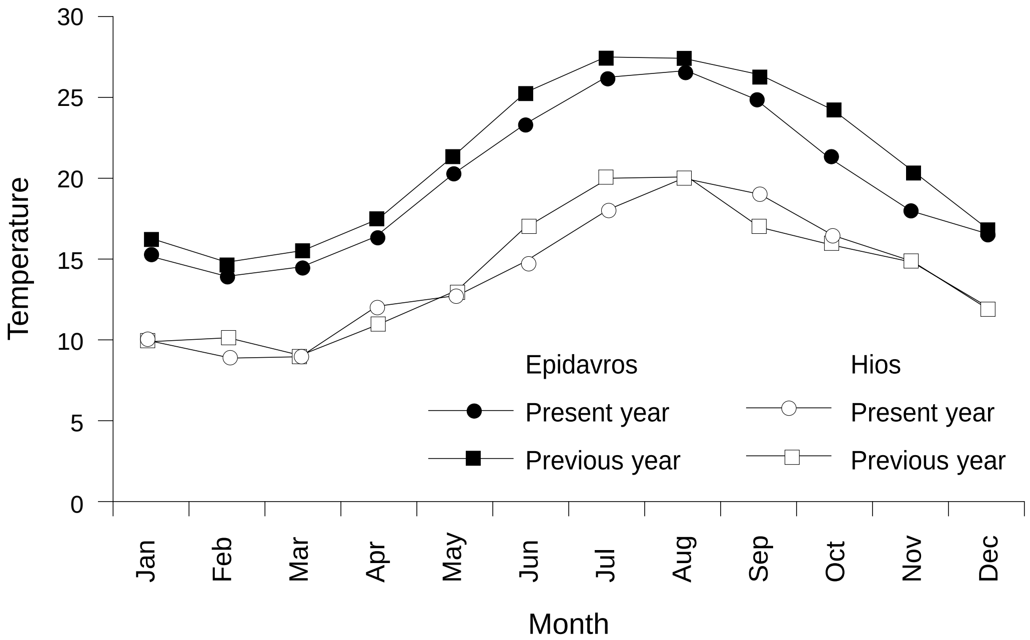

Fig. 1. Average temperature in Farm 1 off Hios Island and in Farm 2 in Epidavros.

selected from one cage of market-sized fish. Fish were sampled three times at Kavala lagoon (January, June, July 2001) Forty-five sea bream weighing 250-400 g were caught by gill nets or angling at randomly selected areas of the lagoon. Special care was taken to avoid parasite contamination between fish by careful manual separation of fish in individual plastic bags after capture.

Necropsy and parasitology. The external surfaces of the gills were examined macroscopically and total body weight was measured. Fresh skin and gill smears were examined under a stereoscope to detect parasites and count monogeneans. These were examined microscopically to identify smaller monogeneans by methods described by Papoutsoglou (1975) and Roberts (1989). Parasites were identified immediately after sampling using the keys of Yamaguti (1963), Papoutsoglou (1975), Euzet and Noisy (1978), and Euzet et al. (1993). Parasite intensity was described according to the key in Table 1.

Histology. Five percent of the randomly selected fish from each sampling were examined histologically. Tissues from the liver, spleen, kidney, heart, intestine, gills, and muscles were fixed in 10% buffered formalin. After decalcification, 5 µm histological sections were prepared and stained with hematoxylin-eosin, gram, and giemsa methods (Drury and Wallington, 1980).

Statistical analysis. The significance of associations between the parasite frequency and the farm, parasitic species, and season were evaluated for sea bream and sea bass separately, in multi-variable logistic regression models fitted in PROC LOGISTIC (SAS Vers. 8). In these models, the dependent variable was a binomial proportion with the number of affected fish in the numerator and the number of fish sampled in the denominator. Because of the contagious nature of parasitic infections and the positive correlation of results in fish from the same farm, there was an *a priori* concern for variability in the observed proportions of parasitized fish in excess of the binomial variance. Thus, the significant over-dispersion in the proportion of parasitized fish was accounted for by specifying the Williams adjustment in the model statement of all models. All considered factors were initially regressed on the proportion of parasitized fish in the multivariable logistic models (Zar, 1984).

These models were subsequently reduced in a stepwise approach (backward elimination of a variable followed by a test for forward selection of variables eliminated at previous steps) by employing a likelihood ratio test at each step until all variables in the model were significant at $p<0.05$. Two-factor interaction terms between the variables in the reduced models were then created and evaluated for significance. Selection of the final model was based on the examination of regression diagnostics, goodness-of-fit criteria, and biological plausibility.

Results

Post mortem examination. All fish infected by Monogenea had pale gills, increased mucus in gills and skin, skin lesions, and focal sloughing of scales. Fish (especially sea bass) infected

Table 1. Key to intensity of parasites.

No. parasites per viewing field	*Furnestinia sp. (x 40)*	*Microcotyle sp. (x 5)*	*Lernanthropus kroyeri (x 5)*	*Caligus minimus (x 10)*
1-2	+	+	+	+
3-4	++	++	++	++
5-6	+++	+++	+++	+++
7-8	++++	-	-	++++

by Isopoda and Copepoda had bleeding around the eyes, gills, and fins, sloughing of scales, and destruction or total lack of eye ball.

Parasitology. The parasite species and overall prevalence are shown in Table 2. Seasonality is shown in Figs. 2-6. In Farm 1, Monogenea (*F. echeneis* and *Microcotyle chrysophrii)* were a problem throughout the year, especially in autumn when mortality also occurred. The average 8-month mortality attributed to ectoparasites was 10%. In Farm 2, parasite prevalence was low and there were few mortalities. The average 8-month mortality due to ectoparasites was 2%. Isopoda (*C. oestroides*) were found only in Farm 2 and in high prevalence during the summer. Among Copepoda, only *Lernanthropus kroyeri* caused respiratory distress and mortality although the average 8-month mortality was less than 1%. Parasites in Kavala lagoon were diverse but no clinical signs or deaths were observed in the infected fish during the study period.

The intensity of infection is shown in Tables 3 and 4. Intensity was directly correlated to prevalence in Monogenea and Copepoda in both farms but not in the lagoon where intensity was very low for all parasites.

Prevalence of parasitism in sea bass. There were highly significant associations between the proportion of parasitized sea bass and the farm of origin (p<0.0001), parasite species (p = 0.01), and sampling season (p = 0.02). Fish in Farm 1 were 6.7 (95% CI 12.9-25) times more likely to be parasitized than those in Farm 2. Parasite intensity was also higher in Farm 1. The frequency of parasitism was 8 (95% CI 1.5-3.5) times higher in fish infected by Isopoda parasites and 2.7 (1.2-6.2) times higher in fish infected with Monogenea than in fish affected with Copepoda but there was no different between fish affected with Monogenea and Isopoda. Parasite frequency was highest in samples collected in autumn and did not differ in samples collected in spring or summer.

Prevalence of parasitism in sea bream. There were no significant two-way interactions in sea bream in any of the previously-mentioned factors. Neither type of parasite nor sampling season were associated with parasite frequency. Fish from Farm 2 had 6.7 (2.4-18.5) and 8.2 (2.4-28.6) times less likelihood of being parasitized than fish from Farms 1 and Kavala lagoon, respectively, while there was no difference between fish of Farm 1 and Kavala lagoon .

Histopathology of Monogenea. Generalized hyperplasia of epithelial and mucus cells of primary gill filaments was observed in fish infected with *F. echeneis,* especially when intensity was high (autumn). Fish infected with *Microcotyle chrysophrii* had focal inflammatory

Table 2. Prevalence of parasites in sea bream and sea bass from two farms and a lagoon.

Species	*Farm*	*Monogenea*[a]	*Isopoda*[b]	*Copepoda*[c]
Sea bream	1 (Hios)	32/52 (61.5%)	0	0
Sea bream	2 (Epidavros)	30/225 (13.3%)	31/225 (13.7%)	0
Sea bream	Lagoon	45/45 (100%)	0	13/45 (21.9%)
Sea bass	1 (Hios)	40/52 (76.9%)	0	12/52 (23.1%)
Sea bass	2 (Epidavros)	54/205 (26.3%)	41/205 (20%)	28/205(13.6%)

[a] *Furnestinia echeneis*, *Microcotyle chrysophrii*
[b] *Ceratothoa oestroides*
[c] *Caligus minimus*, *Lernanthropus kroyeri*, *Ergasilus* sp.

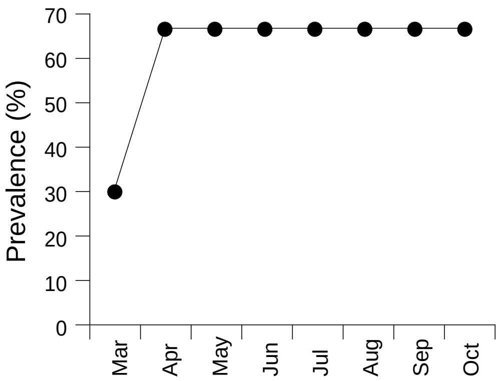

Fig. 2. Monogenea infections in sea bream from Farm 1.

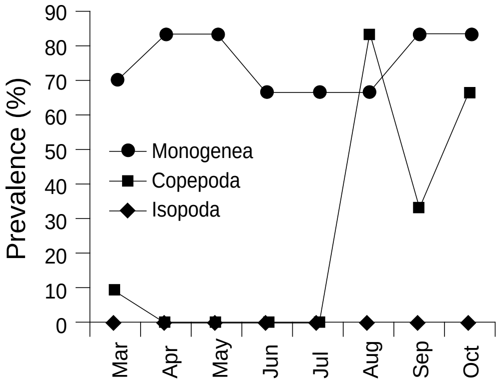

Fig. 3. Ectoparasite infections in sea bass from Farm 1.

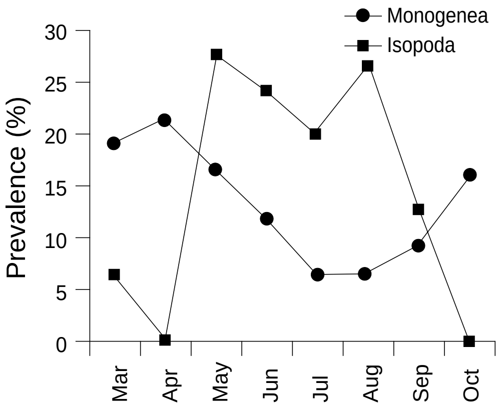

Fig. 4. Ectoparasites in sea bream from Farm 2.

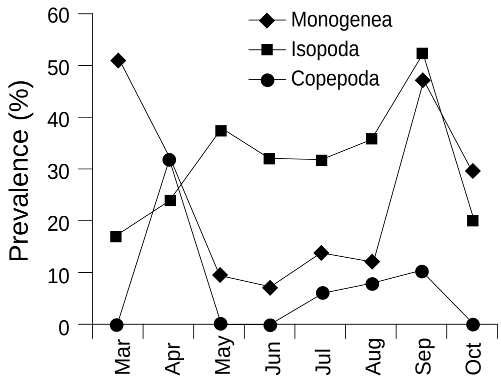

Fig. 5. Ectoparasites in sea bass from Farm 2.

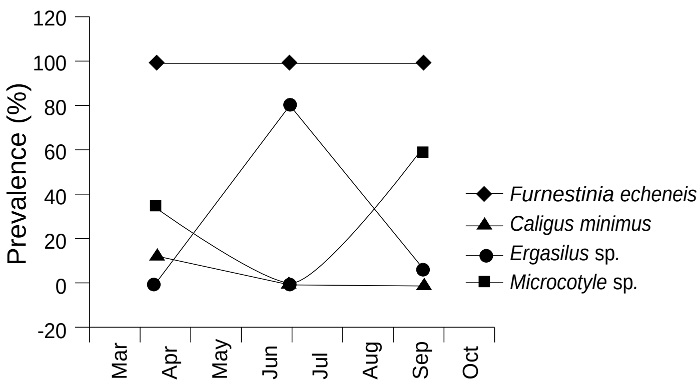

Fig. 6. Ectoparasites in sea bream from Kavala lagoon.

Table 3. Intensity of Monogenea parasites in different farming areas.

Month	*Farm 1*		*Farm 2*		*Lagoon*	
	Sea bream	*Sea bass*	*Sea bream*	*Sea bass*	*Sea bream*	
Mar	+	+	+	++		
Apr	++	++	+	+	Jan	+
May	+	++	+	+		
Jun	++	+	+	+		
Jul	++	+	+	+	June	+
Aug	+	+	+	+		
Sep	+++	++	++	++		
Oct	+++	+	++	+	Sep	+

Table 4. Intensity of Copepoda and Isopoda parasites in different farming areas.

Month	*Farm 1*		*Farm 2*		*Lagoon*	
	Sea bream	*Sea bass*	*Sea bream*	*Sea bass*	*Sea bream*	
Mar	0	+	++	+		
Apr	0	+	0	+	Jan	+
May	0	+	++	++		
Jun	0	+	+	++		
Jul	0	++	++	++	June	+
Aug	0	+	++	+++		
Sep	0	+	+	+++		
Oct	0	+	+	+	Sep	+

lesions around the attachment parts of the parasites. There was generalized hyperplasia, congestion of primary and secondary lamella, increased mucous cells, and thickening of filament edges when intensity of either monogenean was high.

Histopathology of Copepoda. Pathology was attributed only to *L. kroyeri*. Localized hyperplasia of epithelial and mucus cells of primary gill filaments as well as necrosis of epithelial cells leading to sloughing of cells was observed in most cases. Hemorrhage was present in only a few cases in the attachment areas of the parasites.

Histopathology of Isopoda. Pathology was attributed mainly to immature *C. oestroides* (larvae stages) in the skin and eyes of sea bass. The intensity of larvae was high during summer, especially in smaller sea bass where mortality was observed (5-20%). Small ulcers were observed in the skin where increased numbers of lymphocytes, eosinophils, neutrophils, and erythrocytes were present. Extensive granulomatous lesions were present in the eyes, with an increased number of inflammatory cells and hemorrhage. In some cases, there was a total loss of the eyeball. Parasites were present inside and around these lesions. Fish infected with mature parasites showed no serious pathology. Lesions were localized at the upper and lower jaws and the tongue and consisted of small granulomas.

Discussion

Parasites of euryaline fish have been reported mainly in wild populations (Radujkovic and Euzet, 1989). Isopod parasites have been reported in cultured sea bass in Corsica (Bragoni et al., 1984) and lagoons in Israel (Paperna, 1980) as well as Caligidae (Paperna, 1980). However, comparative studies of parasite loads of cultured sea bream and sea bass raised in different rearing systems do not exist.

According to our results, sea bass in Farm 1 were more likely to be parasitized by Monogenea than those in Farm 2, especially in autumn. This may be attributed to the lower overall temperatures and the specific wild fish fauna and climatic conditions of the site. Lower temperatures may affect the immune system, rendering fish more susceptible to infection. According to information provided by the industry, the area of Farm 1 is subject to greater rainfall than the area of Farm 2 (Bouboulis, pers. comm.), possibly influencing local water quality parameters. Although there are no data directly correlating the chemical quality of the water with the prevalence of Monogenea, Papoutsoglou et al. (1996) suggested that increased levels of suspended solids in cages could lead to increased parasitic loads. Earlier observations in Farm 1 showed that, after heavy rainfalls that are very common in autumn, an increased load of suspended solids and turbidity are present together with an increase in the prevalence and intensity of *D. aequans* in sea bass (Bouboulis, pers. comm.). In impoverished environments, monoxenous parasite species with simple single-host life cycles are likely to dominate (Diamant et al., 1999). Environmental changes and ecological disturbances are known to exert a marked influence on the emergence and proliferation of parasitic diseases. Further, rainfall intensity is a key determination of the transport of pathogenic microorganisms, including parasites (Patz et al., 2000). More studies and experimental data are needed to understand these observations in Mediterranean fish farming ecosystems.

In our study, Isopoda increased the susceptibility of sea bass (expressed as prevalence and intensity) to monogenean infections in Farm 2. This is also supported by our histopathological findings. In contrast, Copepoda infections did not have a similar effect on either fish.

The most common Isopoda infections in the Mediterranean are *Ceratothoa parallela* in sea bream (Papapanagiotou et al., 1999; Papapanagiotou and Trilles, 2001) and *C. oestroides* and *Nerocila orbignyi* in cage-cultured sea bass (Sarusic, 1999). *C. oestroides* attaches to the skin, gills, and mouth, causing mechanical injury and stress. They may transmit *Rickettsia*-like organisms (RLO; Athanassopoulou et al., 1999), whose prevalence is on the increase and relates to the geographic distribution of sea lice infections. In Farm 2, RLO were a constant problem (Athanassopoulou, 1997; Bouboulis, unpubl. data). Most damage is caused by pulli II larvae that attack small fish, causing blindness and severe ulcers, and feed on skin mucous. Adult stages, normally found in pairs in the buccal cavity, do not cause extensive damage but can cause anemia, slow growth rate, low reproduction rate, and secondary infections (gram-negative bacteria, lymphocystis, and *Rickettsia*-like infections; Woo, 1995). The severity of lesions produced by these parasites in sea bass in our study may explain why Isopod infections render fish so susceptible to other parasite infections. Although management measures can slightly reduce the prevalence, there is currently no effective treatment against these parasites and infection is an increasing problem in this country. Wild fish such as *Boops boops, Liza aurata*, *Boops* *salpa*, and striped bream (*Lithognatus mormyrus*) are thought to be carriers of this parasite, releasing stage II pulli that attack cultured fish (Bragoni et al., 1983). The lack of effective treatment and the diversity of wild fish may explain the severity of the ectoparasitic infections in this farm (Athanassopoulou, 1997; Athanassopoulou et al., 2001ab).

In our study, sea bream were more tolerant to parasitism and sampling season was not associated with parasite frequency. As in sea bass, sea bream from Farm 2 were less likely to develop parasites. This may be due to ecological parameters such as the more constant temperature that exists in the area of Farm 2.

The copepod *L. kroyeri* is another important parasite of cultured fish, often causing problems in sea bass. In our study, it was shown that a few parasites can cause mortality (albeit low) and clinical signs in fish, probably because of the size of the parasite and its method of feeding from the host. This is particularly prominent when mixed parasite infections occur. Parallel infection with Isopoda or Monogenea can increase mortality, especially in sea bass, while Copepoda, alone, does not render fish susceptible to other infections. This confirms earlier observations in sea bass in Kavala lagoon where mortality and pathology were attributed to multiple infections of these two parasite groups but not to Copepoda infections on their own (Athanassopoulou et al., 2001a, 2002). During our study, the lagoon had a high prevalence of parasites but their intensity and pathology was low. This may be due to the lower fish density in the lagoon that is generally observed in semi-intensive systems, the higher spreading of parasites in this environment, and the possibly less stress on the fish as normal in this environment.

Aquaculture development in Greece will inevitably disrupt natural marine ecosystems since rotation of aquaculture sites is not permitted. Therefore, improved surveillance and monitoring are needed to detect ecological changes that may lead to disease development as early as possible and to prevent spread. Further investigation and exploration are needed to gain better understanding and control of aquaculture in each ecosystem so as to prevent damage and contain the rising morbidity and mortality from parasitic disease.

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