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Protozoan and myxozoan infections in wild gilthead seabream (*Sparus aurata* L.) from North Lake of Tunis, Tunisia

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Abstract

A total of 150 gilthead seabream *Sparus aurata* L., from North Lake of Tunis, Tunisia, were studied for protozoan and myxozoan parasites. The parasitological survey revealed the presence of ectoparasites (*Amyloodinium ocellatum* Brown, 1931, *Trichodina lepsii* Lom, 1962 on the gills) and endoparasites (*Ceratomyxa sparusaurati* Sitjà-Bobadilla, Palenzuela et Alvarez-Pellitero, 1995 infecting the gallbladder, and *Eimeria sparis* Sitjà-Bobadilla, Palenzuela et Alvarez-Pellitero, 1996 parasitizing the intestine). This is the first record of *Amyloodinium ocellatum*, *Trichodina lepsii*, *Ceratomyxa sparusaurati*, and *Eimeria sparis* in *S. aurata* from Tunisian waters. Data on prevalence and intensity of infection are provided. A comparison of the present species with previously described species in cultured gilthead seabream from other Mediterranean countries is also presented. In this study *Trichodina lepsii* is identified for the first time in *Sparus aurata*. A taxonomic description of this species based on silver nitrate method is provided.

Keywords

Amyloodinium ocellatum, *Trichodina lepsii*, *Eimeria sparis*, *Ceratomyxa sparusaurati*, *Sparus aurata*, North Lake of Tunis, Tunisia, prevalence, intensity of infection

Introduction

Lake of Tunis situated in North-East of Tunisia is a natural eutrophic marine lagoon connected to the Mediterranean Sea by few canals and divided by a fairway into two sub-systems known as the South Lake (1100 ha) and North Lake (3100 ha) (Fig. 1)

The North Lake is characterized by an average depth of 1.4 m, a temperature ranging between 13°C in winter to 28°C in summer and high salinity (34 to 43 ppt). From 1985 to 1988 this Lake was subject of cleaning and bank restoration to eliminate the sources of pollution (Van Berk and Oostinga 1992, Ben Charrada 1995) which had led to an improvement of water quality and biological diversity. Several fish species live in this Lake, and among them gilthead seabream *Sparus aurata* L. is considered a commercially important fish. In Tunisia, the most important cultured marine species are basically the gilthead seabream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*). The total aquaculture production of *Sparus aurata* in Tunisia rose from 299 tonnes in 1996 to 1433 tonnes in 2009 (FAO fishery statistics, 2011). Except the parasitological study of Bahri *et al*. (1996) on the myxosporean of wild gilthead seabream from Gulf of Tunis, studies concerning protozoan and myxozoan parasites of *S. aurata* are scarce. Nevertheless, many studies have been carried on the metazoan of sparid fishes from Tunisian coasts (Ben Hassine *et al*. 1978, Anato *et al*. 1991, Benmansour and Ben Hassine 1998, Benmansour *et al*. 2001, Gargouri Ben Abdallah and Maamouri 2008, Gargouri Ben Abdallah *et al*. 2011).

With the increase of fish culture in marine cages in Tunisia and the risk of emergence of parasitic diseases responsible for economic losses, knowledge of parasite communities of *Sparus aurata* is an important tool for applying adequate prophylactic measures. Previous parasitological studies have reported diseases caused by parasites in cultured *S. aurata* from different Mediterranean countries (Paperna and Baudin-Laurencin 1979; Paperna 1984a; Alvarez-Pellitero and Sitjà-Bobadilla 1993; Diamant *et al*. 1994; Alvarez-Pellitero *et al*. 1995; Sitjà-Bobadilla *et al*. 1995, 1996; Sitjà-Bobadilla and Alvarez-Pellitero 1995, 2001; Sakiti *et al*. 1996; Rigos *et al*. 1999; Fioravanti *et al*. 2006a, b).

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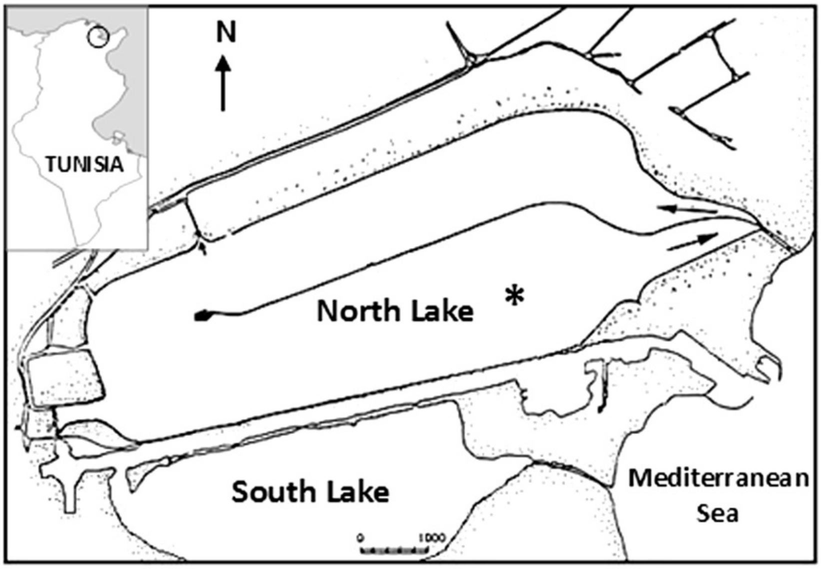


Fig. 1. Map showing location of study site (*) in North Tunisia

Therefore, the aim of the present work was to evaluate the risk of protozoan and myxozoan infections in wild gilthead seabream from North Lake of Tunis. Histological observations were also carried out in order to clarify the possible pathogenic effects of parasites on infected organs.

Materials and methods

From 2009 to 2010, 150 specimens of *Sparus aurata* ranging from 80–250 g in weight were collected from North Lake of Tunis. Fish were euthanized by lethal dose of MS 222 and transported to the laboratory for parasitological examination. Environmental variables were measured using a WTW multiparameter (Multi 340i) for water temperature (°C) and salinity (ppt) in each season (Table I).

Fresh smears of skin and portions of gills from the eight gill arches were examined for the presence of parasites. For the observation of the adhesive disc of trichodinids, smears from infected fish were air dried and impregnated for 10 min in 2% aqueous $AgNo_3$ solution (Klein 1958). Measurements (in µm) of trichodinids were made according to the recommendations proposed by Lom (1958). Details of denticles were presented according to Van As and Basson (1989).

Fresh spores of the myxosporean isolated from the gallbladder were measured according to the criteria described by Lom and Arthur (1989). The oocysts of the coccidian were measured from fresh smear under light microscope. Photographs of parasites were taken with a Nikon E600 microscope using differential interference contrast optics.

Prevalence of infection was defined as percentage of fish infected. Intensity of infection was semiquantitatively evaluated according to Alvarez-Pellitero *et al*. (1995) with the range: (+) 1–5; (++) 6–10; (+++) 11–25; (++++) 26–50; (+++++) 51–100; (++++++) > 100 parasites per fish.

For histological examination, fragments of infected gills, gallbladders and intestine were fixed in 10% neutral buffered formalin for 24 h. After fixation, the tissues were dehydrated through ethanol series, equilibrated in xylene and embedded in paraffin according to standard histological techniques. Sections of 5 µm were cut and stained with haematoxylin-eosin before examination under light microscope.

Results

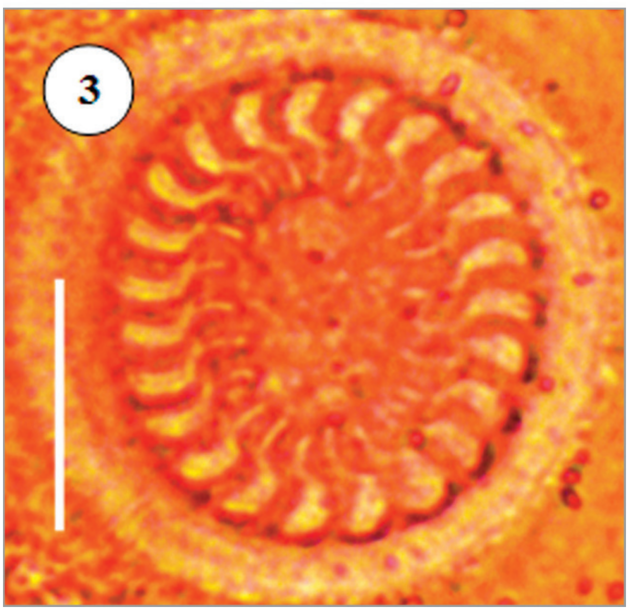
Specimens of *Sparus aurata* caught from North Lake of Tunis were found to be parasitised by two ectoparasites, *Amyloodinium ocellatum* and *Trichodina lepsii* and two endoparasites, the cœlozoic myxozoan *Ceratomyxa sparusaurati* and the apicomplexan *Eimeria sparis*. All species were previously reported to infect *S. aurata* from several Mediterranean aquaculture systems, except *Trichodina lepsii* which is identified for the first time in gilthead seabream.

Table I. Water parameters measured at the sampling period in North Lake of Tunis

Parameters	Autumn	Winter	Spring	Summer
Temperature (°C)	20.8	12	18.4	28
Salinity (ppt)	38.6	34.4	37.6	42.6

***Amyloodinium ocellatum* Brown, 1931**

The dinoflagellate *Amyloodinium ocellatum* identified on the gills of *Sparus aurata* during the sampling period presented trophonts measuring 80–90 µm in diameter. In heaviest infections, the entire gill was parasitized by numerous trophonts (30 trophonts per filament). The overall prevalence of this parasite was 32% with maximum prevalence in summer (73%) and minimum prevalence in autumn (15%). Prevalence and mean intensity values of *A. ocellatum* increased gradually from spring to summer and decreased in autumn (Table II). Infection by *A. ocellatum* was absent in winter when the water temperature was about 12°C. The examination of infected gills during highest infections showed histopathological changes such as haemorrhages and hyperplasia of epithelial cells (Fig. 10).



Figs 2–3. *Trichodina lepsii* infecting *Sparus aurata*. **2.** Giemsa stained *Trichodina lepsii*: detail of macronucleus. **3.** Silver nitrate impregnated adhesive disc of *T. lepsii*. Scale bar: **Fig. 2** = 20 µm; **Fig. 3** = 12 µm

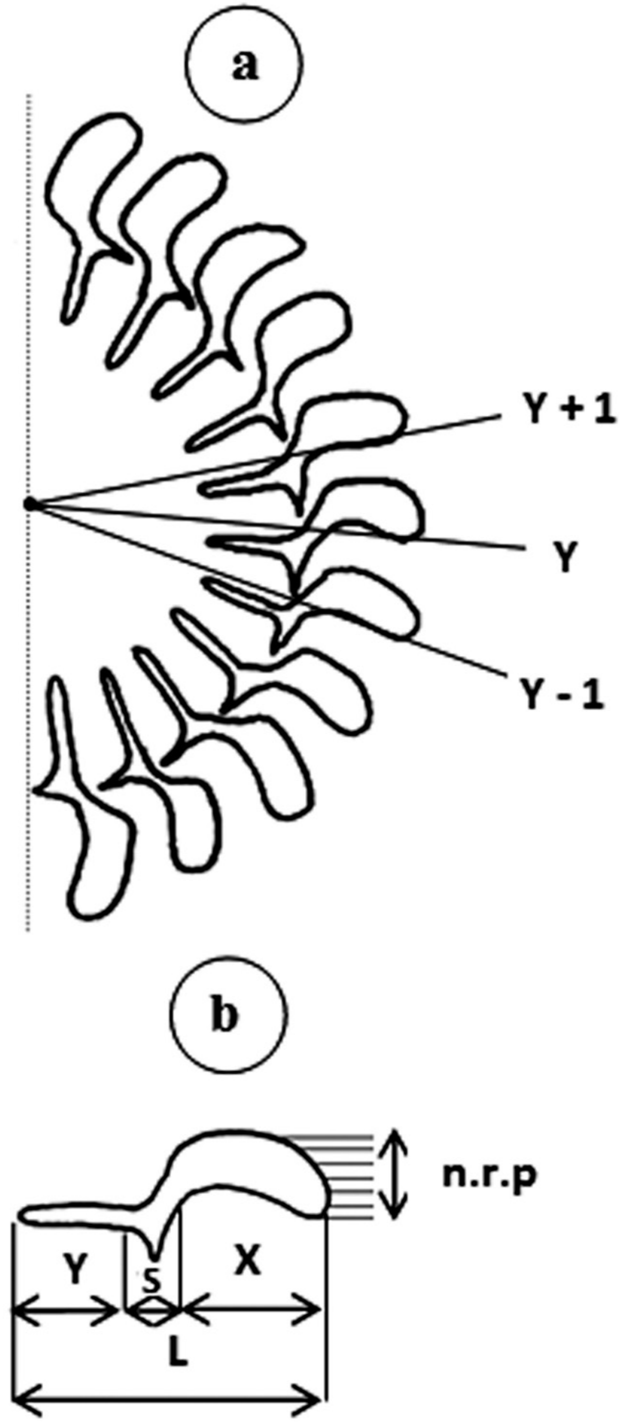
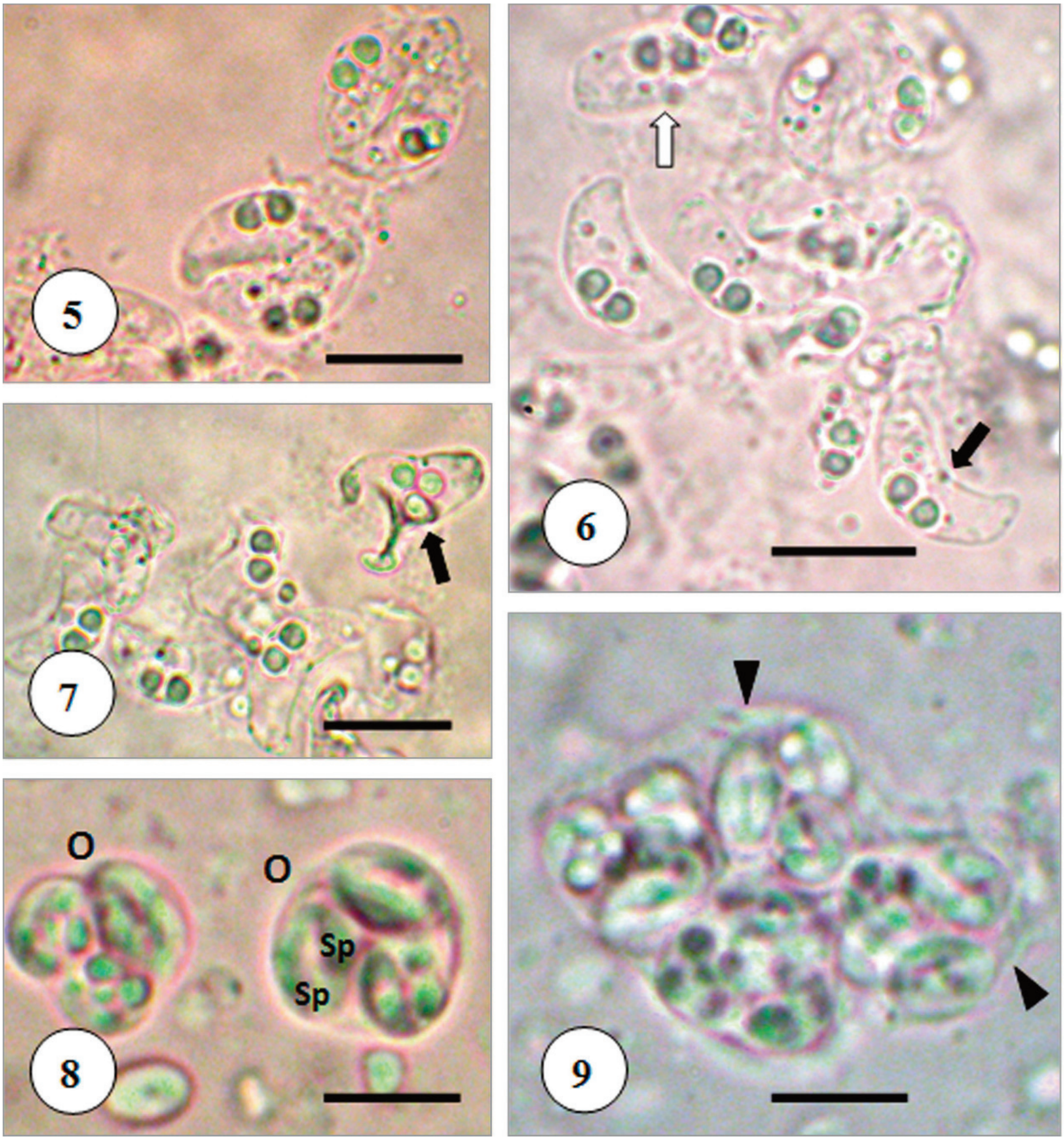


Fig. 4. Diagrammatic drawings of *T. lepsii*: (**a**) Part of adhesive disc to illustrate denticle structure and construction of X and Y axes as fixed references for the description of denticles. (**b**) Structure of the denticles, n.r.p – number of radial pins, L – denticle length, S – central part length, X – blade length, Y – ray length

Table II. Mean values of prevalence (P) and mean intensity (MI) of parasites of *Sparus aurata* in each season. EF: examined fish, PF: parasitised fish

	Amyloodinium ocellatum			***Trichodina lepsii***			***Ceratomyxa sparusaurati***			***Eimeria sparis***		
	PF/EF	(P%)	MI	PF/EF	(P%)	MI	PF/EF	(P%)	MI	PF/EF	(P%)	MI
Autumn	6/40	15	++	16/40	40	++++	3/40	7.5	+++	6/40	15	+++
Winter	0/40	0		20/40	50	+++++	2/40	5	++	3/40	7.5	+++
Spring	20/40	50	+++	30/40	75	++++++	8/40	20	++++	0/40	0	
Summer	22/30	73	++++	3/30	10	++	3/30	10	+++	0/30	0	



Figs 5–7. *Ceratomyxa sparusaurati* in fresh smears of bile: **5.** Disporous sporoblasts. **6.** Mature spores: flattened type (white arrow) and curved type (black arrow). **7.** Aberrant spore (arrow). Scale bar = 10 µm. **Figs 8** and **9.** Fresh oocysts of *Eimeria sparis* in the intestine of *Sparus aurata*: **8.** Two mature oocysts in the lumen of the intestine. **9**. Oocysts enclosed in a fine envelope (arrowhead). O – oocyst, Sp – sporozoite. Scale bars = 10 µm

Table III. Morphometric comparison of different populations of *Trichodina lepsii* Lom, 1962 with the present material (measurements in µm)

Host	*Liza aurata*	*Mugil cephalus*	*Mugil cephalus*	*Mugil cephalus*	*Perca fluvatilis*	*Sparus aurata*
Locality	Lake Tabacaria, Rumania	Bourgas Lake, Bulgaria	Black Sea, Turkey	Lake Qarun, Egypt	Blackman River, Tasmania	Lake of Tunis, Tunisia
Reference	Lom (1962)	Grupcheva (1975)	Özer and Öztürk (2004)	Al-Bassel *et al.* (2007)	Basson (2010)	Present study
Location	Gills	Gills	Gills	Gills	Gills, Skin	Gills
Body diameter	28–42 (35)	36–44	–	25–30 (28.4)	25.4–31.3 (27.7)	–
Adhesive disc diameter	19–27 (21)	24–27	19.2–27 (22.5)	22–27 (23)	20.5–25.6 (22.8)	23.6–28.8 (25.5)
Border membrane width	2	2.6–3.5	2.3–3.4 (2.8)	2–5 (3.1)	2.1–2.9 (2.5)	2.2–4.4 (3.8)
Denticle ring diameter	11–14 (12)	14–16	13.4–15 (14.1)	13–19 (15.5)	11–16.1 (13.4)	14–19 (16.5)
Denticle number	18–25	22–24	19–24 (22)	21–26 (22)	18–22 (20)	21–23 (22)
Number of radial pins/denticle	–	5	4–5	5–7 (6)	6–8 (7)	5–7 (6)
Denticle length	3	–	5.2–6 (5.6)	3–4.5 (3.5)	2.7–4.1 (3.3)	5.7–6 (5.7)
Blade length	2.5	2.6–3.5	2.8–3.4 (3.1)	3–4 (3.2)	2.5–3.6 (3)	2.8–3.2 (3.1)
Ray length	1.5	1.7–2.6	1.4–2 (1.7)	1–2 (1.3)	2.4–4.3 (3.1)	1.7–1.9 (1.8)
Central part width	1	0.9–1.3	0.6–1 (0.8)	2.5–3 (2.8)	0.6–1 (0.8)	1–1.2 (1.1)
Central circle diameter	–	–	9.4–10.9 (10.1)	–	–	7.6–11.5 (8.4)

***Trichodina lepsii* Lom, 1962** (Figs 2–4 and 11, Table III)

The ciliate *Trichodina lepsii* isolated from the gills was a small-sized trichodinid. From the Giemsa stained specimens the macronucleus presents horseshoe-shaped, elongated and with characteristic dilations at both ends (Fig. 2). Adhesive disc 25.5 (23.6–28.8) µm in diameter with large central circle 8.4 (7.6–11.5) µm (Fig. 3). Denticulate ring 16.5 (14–19) µm in diameter consists of 21–23 denticles with 5–7 radial pins per denticle (Fig. 4b). Blade 3 (2.8–3.2) µm in length, broad and occupies most area between Y axes with distal surface slightly rounded and parallel to border membrane (Figs 4a, b). Tangent point small and slightly rounded. Anterior blade margin almost parallel to Y axis, with no clear apex in most case (Fig. 4a). Posterior margin relatively curved. Blade apophysis not visible. Section above and below X axis similar in shape. Central part narrow, almost triangular in shape and extend the halfway towards Y-1 axis (Fig. 4a). Rays parallel to Y axes (Fig. 4a), relatively short with a thinner ray connection. We have observed slightly differences concerning the shape and the size of denticles for the present species which are considered as cases of intraspecific variation within the species.

Trichodina lepsii was present all over the year with marked seasonality (Table II). The overall prevalence was 46% and the maximum rate of infection was observed in spring (75%). Moreover, this ciliate was the most prevalent species in *Sparus aurata* from North Lake of Tunis.

Histological examination of infested gills revealed significant damages such as oedema, necrosis and sloughing off of epithelial cells (Fig. 11).

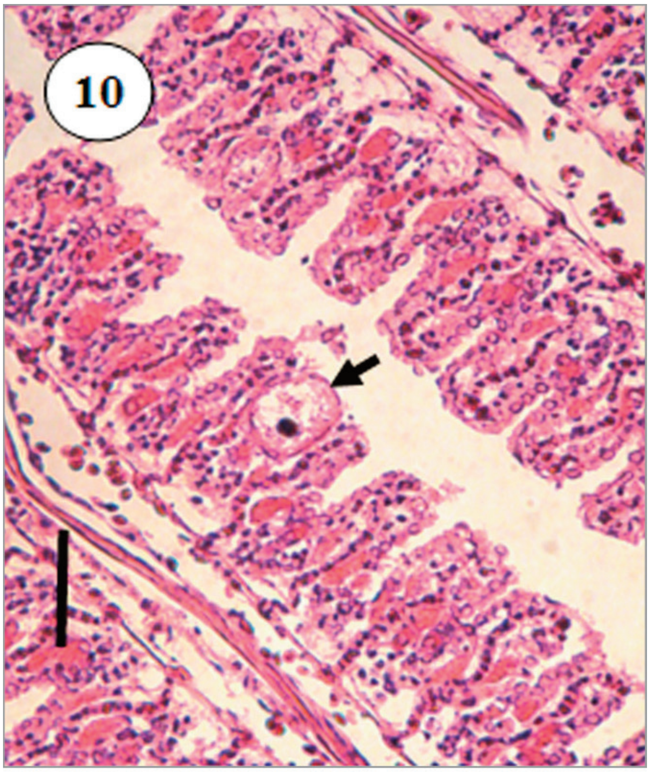
***Ceratomyxa sparusaurati* Sitjà-Bobadilla, Palenzuela et Alvarez-Pellitero, 1995** (Figs 5–7)

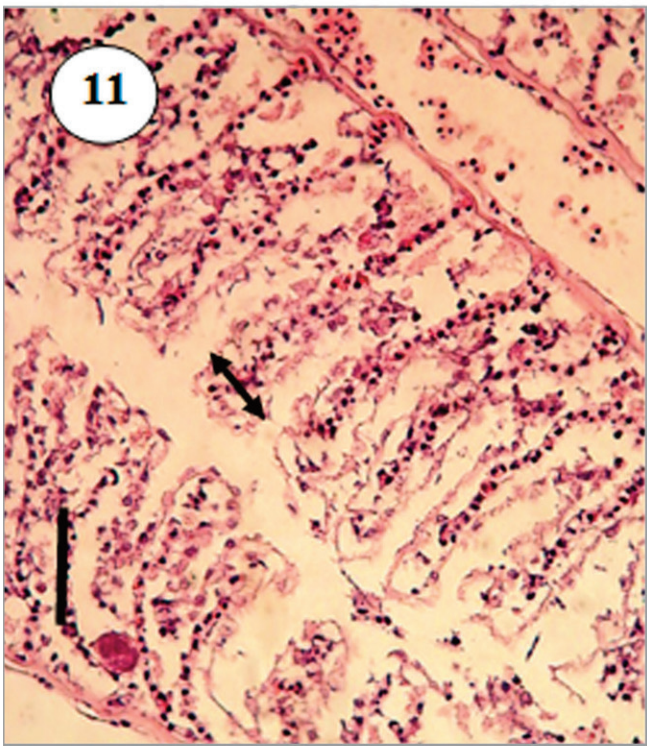
Trophozoites, disporous sporoblasts, and spores of *Ceratomyxa sparusaurati* were found in the gallbladder. Mature spores were crescent-shaped in the frontal view, with a convex anterior end and flat or slightly curved posterior one (Figs 5–6). Shell valves were smooth without mucous envelope and with rounded ends. Spores measured (n = 30) 4.2–7.2 (5.6 ± 0.6) µm in length and 14.0–18.0 (16.0 ± 1.7) µm in thickness. Polar capsules were subspherical measuring 2.2–3.4 (2.7 ± 0.3) µm in diameter. Sometimes, abnormal spores with three polar capsules and three shell valves were observed among the spores (Fig. 7).

Ceratomyxa sparusaurati was present all over the year with maximum prevalence in spring (20%) and minimum prevalence in winter (5%). The mean intensity values were variable throughout the season, the highest one was observed in spring (Table II).

***Eimeria sparis* Sitjà-Bobadilla, Palenzuela et Alvarez-Pellitero, 1996** (Figs 8–9)

Mature oocysts of *Eimeria sparis* (Figs 8–9) were spherical or subspherical in shape and measured (n = 30) 9.8–14.6





Figs 10 and **11.** Histological sections (H and E) of infected gills showing the pathological lesions of ectoparasites in *Sparus aurata*: **10.** Trophont of *Amyloodinium ocellatum* (arrow) attached to the gill filament causing hyperplasia. **11.** Gill infected by *Trichodina lepsii* showing oedema, necrosis and sloughing off (arrow) of epithelial cells. Scale bars = 50 µm

(12.2 ± 2.25) µm. The sporocysts were ellipsoid or oval in shape and measured (n = 50) 6–9 µm in length and 4–6 µm in width. Each sporocyst was composed by two sporozoites placed longitudinally (Fig. 8).

In this survey oocysts of *Eimeria sparis* was detected only in autumn 2009 and winter 2010 (Table II). The overall prevalence was 6%. In histological sections, no merogonial or gamogonial stages were observed only oocysts were present in the lumen of the intestine.

Discussion

In the present study 4 parasitic species were found in *Sparus aurata* from North Lake of Tunis, *Amyloodinium ocellatum* (32%), *Trichodina lepsii* (46%), *Ceratomyxa sparusaurati* (10.66%) and *Eimeria sparis* (6%). The prevalence and the intensity of infection of these parasites were variable throughout the season in relation to environmental factors.

The heaviest infections caused by *Amyloodinium ocellatum* were detected in summer when the water temperature reached 28°C and the salinity was about 42 ppt. However, when the temperature and the salinity of water decreased in winter and reached respectively 12°C and 38.6 ppt, the infection disappeared. According to Paperna (1984b), *A. ocellatum* cannot reproduce below 15°C which explain the absence of this protozoan from the examined fish in winter. The author has also reported that *A. ocellatum* has a wide tolerance to the temperature (18 to 30°C) and to the salinity (10 to 60 ppt). The infected gills of studied *S. aurata* showed inflammation, hyperplasia and haemorrhages. These histopathological changes caused by *A. ocellatum* have been previously reported by other authors (Paperna 1980, Kuperman and Matey 1999). Moreover, this dinoflagellate has caused mass mortalities of cultured fish at Eilat, Red Sea (Paperna 1980) and in the Philippines (Cruz-Lacierda *et al*. 2004).

The presence of *Trichodina* sp. has been previously reported in *S. aurata* from France (Paperna and Baudin Laurencin 1979); several culture systems in Spain (Alvarez-Pellitero *et al*. 1995); Adriatic Sea (Mladineo 2006) and different farming systems in Italy (Fioravanti *et al*. 2006a, b). However, the taxonomic identity of the *Trichodina* species was not established. In the present study, we report for the first time *Trichodina lepsii* from *S. aurata*. The trichodinid populations in *S. aurata* from North Lake of Tunis have uniformly shaped denticules and similar dimension with *T. lepsii* populations previously described in several fishes (Table III). Moreover, *T. lepsii* was recorded from several hosts, *Liza aurata* Risso, 1810 (Lom 1962); *Mugil cephalus* L., 1758 (Grupcheva 1975, Özer and Öztürk 2004, Al-Bassel *et al*. 2007); *Syngnathus typhle* L., 1758 (Grupcheva *et al*. 1989), *Pagrus caeruleostictus* Valenciennes, 1830 (Loubser *et al*. 1995) and *Perca fluviatilis* L., 1758 (Basson 2010). The presence of *T. lepsii* in *S. aurata* from Lake of Tunis is closely related to the environmental conditions, particularly to the temperature and the salinity. In this study we noted that the highest prevalence and mean intensities of *T. lepsii* were recorded during winter and spring, while the lowest infection rate occurred in summer when the temperature and the salinity of water were high, respectively 28°C and 42.6 ppt. Many authors have determined the relationship between seasonal dynamics of trichodinids and water quality and reported that the maximum rate of infection was in winter and/or in spring (Abu El-Wafa 1988; Hassan 1999; Özer and Erdem 1999; Özer 2000, 2003; Jerônimo *et al*. 2011). Previous studies have reported that the seasonal prevalence and intensity of trichodinids can be affected by organic pollution such as the level of nitrite, nitrate and phosphate (Ogut and Palm 2005) and the deposition of pig manure (Jerônimo *et al*. 2011). The fish examined in this study presented at higher infection, necrosis, oedema and hyperplasia of the gills. These pathological lesions have been previously observed associated with *Trichodina* spp. infection in several fish species by other authors (Hassan 1999, Afifi *et al*. 2000, Yemmen *et al*. 2011).

In the current study, the seasonal variations of *Ceratomyxa sparusaurati* prevalence are similar to those reported by Palenzuela *et al*. (1997) in intensive open system (i.e. higher in spring and summer and lower in autumn and winter). In general, many factors are involved in seasonal fluctuations of myxosporeans such as temperature (Alvarez-Pellitero and Sitjà-Bobadilla 1993, Alvarez-Pellitero *et al*. 1995), farming systems and size categories (Palenzuela *et al*. 1997, Fioravanti *et al*. 2006a, b). Fish heavily infected with *C. sparusaurati* present enlarged gallbladders and abdominal distension (Palenzuela *et al*. 1997). Nevertheless, we have not observed external sign of disease in the infected fish during the survey. Further myxosporeans have been reported in gilthead seabream, *Enteromyxum leei* Diamant, Lom et Dyková, 1994; *Polysporoplasma sparis* Sitjà-Bobadilla et Alvarez-Pellitero, 1995; *Henneguya* sp. Bahri, Ben Hassine et Marques, 1996 and *Leptotheca sparidarum* Sitjà-Bobadilla et Alvarez-Pellitero, 2001 which have not been observed in the sampled fish during our study.

Our observations on *Eimeria sparis* are limited to the oocysts and sporozoites. The different stages of merogony and gamogony were not observed. Comparatively, fish in the present study presented lower prevalence and infection intensities than those observed by Alvarez-Pellitero *et al*. (1995) in *S. aurata* from different cultured systems in Spain. On the contrary, Fioravanti *et al*. 2006b observed a very low prevalence (0.2%) of this parasite in cultured *S. aurata* from Italy.

In conclusion, prevalence and infection intensity of protozoan ectoparasites in wild *S. aurata* from North Lake of Tunis are influenced by environmental factors basically temperature and salinity. However, their pathogenicity was related to the intensity of parasites and to the host-inherent parameters. In the other hand, seasonal variations of the myxosporean *Ceratomyxa sparusaurati* and the apicomplexan *Eimeria sparis* seems to be mostly related to the presence of their intermediate hosts in the lake.

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