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Morphometric, molecular and ecological analyses of the parasites of the sharpsnout seabream *Diplodus puntazzo* Cetti (Sparidae) from the Spanish Mediterranean: implications for aquaculture

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Abstract

One of the fish species with the highest potential for aquaculture is the sharpsnout seabream, *Diplodus puntazzo* Cetti. Among other aspects, the development of new fish cultures requires studies of potential pathogens that may compromise survival of the fish in captivity. Moreover, both cultured and wild fish can act as sources or reservoirs of pathogens which may negatively affect other well-established cultures. We have studied the parasite fauna of the wild sharpsnout seabream, and monitored the survival of the parasites in culture conditions. The sharpsnout seabream was sampled from two different Spanish localities and examined for parasites. Additionally, 20 fish were maintained in captivity. Ten of them were examined for parasites after a period of 10 days and a further ten fish after another 10 days. All fish were parasitized with at least four species, with 19 parasite species being identified, seven of which were recorded for the first time in the sharpsnout seabream. These included *Microcotyle* sp., *Magnibursatus bartolii*, *Steringotrema pagelli*, *Galactosomum* sp., *Cardiocephaloides longicollis*, *Caligus ligusticus* and *Gnathia vorax*. We also report the first records of two parasite species in the wild sharpsnout seabream, the polyopisthocotylean monogeneans *Atrispinum seminalis* and *Sparicotyle chrysophrii*. Previously, these parasites had only been recorded in farmed sharpsnout seabream. Most parasites in the skin, gills and alimentary tract disappeared under the conditions of captivity, with the exception of the monogeneans of the genus *Lamellodiscus*. The information provided about the sharpsnout seabream parasite fauna will be useful to prevent possible problems in fish farms due to some parasite species. Many parasites of the sharpsnout seabream recorded in the present study are shared by the main fish species in Mediterranean aquaculture, the gilthead seabream, thus suggesting the possibility of cross-infections.

Introduction

Despite the recommendations of European governments and institutions to diversify fish cultures (Abellán & Basurco, 1999; Anonymous, 2012), Mediterranean aquaculture is still focused predominantly on two

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species, the gilthead seabream, *Sparus aurata* L., and the European seabass, *Dicentrarchus labrax* L. One of the species with higher potential for aquaculture is the sharpsnout seabream, *Diplodus puntazzo* Cetti (see Abellán & Basurco, 1999). Although catches of the sharpsnout seabream are low in Spanish fisheries (Food and Agriculture Organization,2010), this species has good aquaculture prospects because of its easy adaptation to conditions of captivity, high growth rate and food conversion efficiency (Favaloro *et al.*, 2002; Hernández *et al.*, 2003). The culture of this sparid is still under development, with a limited number of larvae produced in Italy, Greece and Portugal (Federation of European Aquaculture Producers, 2008; Vinagre *et al.*, 2010). In Spain this culture has been mostly experimental (Hernández *et al.*, 2001, 2002, 2003; Pajuelo *et al.*, 2008; Nogales Mérida *et al.*, 2010; Almaida-Pagán *et al.*, 2011). However, the introduction of the sharpsnout seabream into aquaculture has been compromised by the presence of many pathogens, often producing severe pathologies (Athanassopoulou *et al.*, 2005; Merella *et al.*, 2005; Katharios *et al.*, 2006; Montero *et al.*, 2007; Álvarez-Pellitero *et al.*, 2008; Golomazou *et al.*, 2009; Rigos & Katharios, 2010; Sánchez-García *et al.*, 2011).

Aquaculture conditions imply fish stress which favours pathogen transmission and, therefore, abnormally high infection levels (Ogawa, 1996). Fish parasite diseases are often associated with important economic losses in aquaculture because of fish mortalities, production decrease or increased costs of antiparasitic treatments (Murray & Peeler, 2005; Rhode, 2005). Often infections appear in farms because many of the pathogens causing diseases are also associated with sea cages. Considerable aggregations of wild fish are usually associated with sea cages in coastal areas (Sánchez-Jerez *et al.*, 2007), and parasite species infecting these neighbouring fish can be transmitted, resulting in wild fish acting as reservoirs of parasite infections in farmed fish (Kent, 2000; Mladineo *et al.*, 2009) or parasites in fish farms spreading to the surrounding environment (Rohde, 2005; Krkošek *et al.*, 2007). Thirdly, different fish species farmed in neighbouring installations can experience cross-infections (Di Cave *et al.*, 2002). These situations should be considered with special attention on new cultures with a short farming history, since their pathogens are unknown or poorly known.

This situation requires the development of effective control of cross-infections, based on considerable knowledge of pathogens living in farmed and wild fish and identification of potential harmful species (Hutson *et al.*, 2007; Rigos & Katharios, 2010). The sharpsnout seabream is, in fact, one of the fish species reported by Dempster *et al.* (2002) associated with sea cages in the Mediterranean and, therefore, wild populations of this species could be a source for pathogen transmission to farmed fish of the same or other species. Although a number of studies referring to parasites of sharpsnout seabream in the Mediterranean Sea exist (Athanassopoulou *et al.*, 1999, 2005; Di Cave *et al.*, 2002; Vagianou *et al.*, 2004; Merella *et al.*, 2005; Katharios *et al.*, 2006; Toksen, 2006; Mladineo & Maršić-Lučić, 2007; Montero *et al.*, 2007; Álvarez-Pellitero *et al.*, 2008), these are focused on pathogens of farmed fish and no data on the parasites of sharpsnout seabream in the wild are available. From an epizootiological point of view, the description of parasite communities of this species is indispensable to prevent economic and pathological impacts of certain parasitoses.

The aim of the present study was to identify the parasites infecting wild and farmed sharpsnout seabream. The parasite fauna of wild fish from two locations in the Spanish Mediterranean are described. Furthermore, we tested the effect of culture conditions on these parasites, in order to find those species that can survive and proliferate in farms.

Materials and methods

Collection and examination of fish

Seventy sharpsnout seabream aged 1 year or more were collected alive from two different locations in the Spanish Mediterranean in 2007: 50 fish with a total length (mean ± standard deviation) of 252.1 ± 11 mm, weight 300 ± 37.7 g were collected in Mar Menor off the coast of San Pedro del Pinatar, Region of Murcia (37°41′14″ N, 0°44′10″W); and 20 fish with a total length of 188.5 ± 18 mm, weight 111.95 ± 27.73 g were collected off the coast of Santa Pola, Valencian Community (38°11′23″N, 0°33′20″W). No more fish could be obtained since, as previously stated, the sharpsnout seabream is fished in low numbers in Spain. Thirty fish from Mar Menor and all the individuals from Santa Pola were killed by medullar section and immediately frozen. In order to study the effect of culture conditions on parasites, the other 20 specimens from Mar Menor were transported alive and reared in the aquaculture facilities of the Central Service for the Support to Experimental Research of the University of Valencia (SCSIE). Fish were maintained in marine water (salinity 37‰, temperature 20°C, 8:16 h light:dark photoperiod) and fed with commercial gilthead seabream pellets. After 10 days, ten fish were killed by medullar section; the remaining ten fish were killed after 20 days of captivity.

Fish were first examined for external parasites on the skin, fins and eyes, and following a post-mortem examination internal organs, such as digestive tract, gonads, liver, gills, kidney and brain, were examined in saline solution under a stereomicroscope (100× magnification). Parasites were removed and preserved in either 70% ethanol for morphological examination or in 100% ethanol for molecular study. Myxozoans were detected by examination of wet preparations of squeezed fresh organs, using a light microscope at magnifications of up to 1000× with differential interference contrast (DIC).

Morphometrics

Platyhelminths fixed in 70% ethanol were stained with iron acetocarmine (Georgiev *et al.*, 1986), dehydrated through a graded ethanol series, cleared in dimethyl phthalate, and examined as permanent mounts in Canada balsam under a light microscope. In the case of the diplectanid monogeneans *Lamellodiscus* spp., representative subsamples of 30 specimens from each fish were randomly selected to be identified and counted. For those species of *Lamellodiscus* with no more than 30 specimens,

all parasites were examined. Monogeneans were washed in distilled water and examined on semi-permanent preparations in glycerol–gelatine under a light microscope for their detailed morphological examination and identification. Crustaceans were examined under a light microscope in distilled water or in glycerine.

For some species, when the morphological identification was particularly confusing, detailed specific morphometric studies were performed. Fifteen mounted adult specimens of each of these species (i.e. *Lamellodiscus* spp. and *Peracreadium* sp.) from each location were selected for their detailed examination. Morphometric data of specimens of *Lamellodiscus* spp. and *Peracreadium* sp. were obtained by using a light microscope with a drawing tube. Haptoral parts of the diplectanids were measured according to Amine & Euzet (2005) and the resulting measurements were compared with the available published data. All measurements are given in micrometres.

Molecular analysis

Those species difficult to classify morphologically (*Lamellodiscus* spp. and *Peracreadium* sp.) were also studied through genetic comparisons. Three to four specimens of these species (see table 1), previously fixed in 100% ethanol, were transferred into 300 µl TNES urea (10 mM Tris–HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS), 4 M urea). Genomic DNA (gDNA) was extracted from single specimens using a phenol–chloroform standard procedure. The extracted DNA was resuspended in 20 µl of RNAse/DNAse-free water and left to dissolve overnight in the fridge. Polymerase chain reactions (PCRs) were performed with a programmable thermal cycler (Techne, TC-512, GMI, Ramsey, Minnesota, USA) in a final volume of 30 µl containing 0.3 U *Taq* DNA polymerase (BioLabs, Madrid, Spain) and the related 10 × Standard *Taq* Reaction Buffer with 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (dNTP), 10 mM of each PCR primer and 20–70 ng of template.

Partial 18S and entire internal transcribed spacer 1 (ITS1) of *Lamellodiscus* spp. were amplified and sequenced using the primers L7 (5′-TGA TTT GTC TGG TTT ATT CCG AT-3′ (Verneau *et al.*, 1997)) and IR8 (5′-GCT AGC TGC GTT CTT CAT CGA-3′ (Kaci-Chaouch *et al.*, 2008)) that anneal to the 18S and 5.8S rRNA genes, respectively. It has been shown that ITS1 is highly variable and not useful for inferring evolutionary relationships among *Lamellodiscus* spp., but it can be used to differentiate species (Desdevises *et al.*, 2000). Complete sequences of ITS1, 5.8S and ITS2 of *Peracreadium* sp. were amplified and sequenced using primers 18dF (5′-CAC ACC GCC CGT CGC TAC TAC CGA TTG-3′ (Hillis & Dixon, 1991)) and ITS2.2 (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′ (Anderson & Barker, 1993)). The thermocycling profile used for the amplification of sequences of *Lamellodiscus* spp. consisted of denaturation of DNA (95°C for 3 min); 38 cycles of amplification (94°C for 50 s, 50°C for 50 s and 72°C for 1 min 20 s); and 4 min extension hold at 72°C. The same profile was used for gene amplification of

Table 1. The range of monogenean and digenean species occurring in *Diplodus puntazzo* with accession numbers of the ITS and 18S rDNA sequences.

Species	Source	GenBank accession numbers
Lamellodiscus falcus s.l.	Present study	KC470292[a]
		KC470293[a]
		KC470294[a]
		KC470298[b]
		KC470299[b]
		KC470300[b]
Lamellodiscus ignoratus	Desdevises (2001)	AF294957[b]
	Kaci-Chaouch *et al.* (2008)	EU259026[a]
		EU259027[a]
		EU259029[a]
		EU259031[a]
Lamellodiscus ergensi	Desdevises *et al.* (2002)	AY038190[b]
	Kaci-Chaouch *et al.* (2008)	EU259056[a]
		EU259057[a]
		EU259058[a]
		EU259059[a]
Lamellodiscus theroni s.l.	Present study	KC470295[a]
		KC470296[a]
		KC470297[a]
		KC470301[b]
		KC470302[b]
		KC470303[b]
Peracreadium characis	Jousson *et al.* (1999)	AJ241796[a,b]
		KC470304[a,b]
		KC470305[a,b]
	Present study	KC470306[a,b]
		KC470307[a,b]

[a]ITS; [b]18S.

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Peracreadium sp. but with an annealing temperature of 55°C. After checking the presence of DNA in a 1% agarose gel in sodium acetate buffer and detection following GelRed™ Nucleic Acid Gel Stain staining and ultraviolet transillumination (VWR, Genoview, Barcelona, Spain), the PCR products were purified for sequencing using the GFX PCR DNA and Gel Band purification Kit (GE Healthcare UK Ltd, Pollards Wood, Bucks, UK). Cycle sequencing was conducted in a 48 capillary ABI 3730 sequencer (Applied Biosystems, Madrid, Spain) using the BIG Dye terminator v 3.1 Ready Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, using the same primers as those used for the PCR. The contiguous sequences were aligned using BioEdit v. 7.0.5. (Hall, 1999) and compared for similarities with sequences lodged in GenBank (detailed in table 1) using BLAST (Altschul *et al.*, 1990) and MEGA 4.1. (Tamura *et al.*, 2007).

In the case of the species of *Lamelodiscus*, not all sequences were available in GenBank. In particular, no information on *L. falcus* and *L. theroni* was found, and the sequences of *L. falcus s.l.* and *L. theroni s.l.* obtained in the present study were aligned and compared with the morphologically similar and phylogenetically closest species with available sequences, i.e. *L. ignoratus* Palombi, 1943 and *L. ergensi* Euzet et Oliver, 1966, respectively (Amine & Euzet, 2005).

Data analysis

For all parasite species, mean abundance and prevalence (P%) were calculated as defined by Bush *et al.* (1997), except for the myxozoans for which only prevalence was determined. Confidence intervals for the prevalence were also calculated. A comparison of prevalences and abundances between the samples of the two locations studied, and between wild and captive sharpsnout seabream, were calculated to identify significant differences. Statistical boostrap *t*-test for abundance and Fisher's exact test for prevalence were carried out with Quantitative Parasitology 3.0 (Rózsa *et al.*, 2000).

Results

All sharpsnout seabream analysed were infected by at least four parasite species. In total, 19 parasite species were found, 15 in the fish sampled in Mar Menor and 11 in the fish sampled off Santa Pola (table 2). Seven species of the parasites were shared by fish from the two localities. All parasites were identified to species level, except for the myxozoan *Ceratomyxa* sp., the digenean brain parasite *Galactosomum* sp. met. and the monogenean Microcotylidae gen. sp. The first two of these parasites were identified only up to genus level as their species identification requires further molecular or morphological analyses. Regarding Microcotylidae gen. sp., one single specimen was found and its morphological traits were not assignable to any known microcotylid species: (1) genital atrium armed with three types of spines (14 long peripheral, 9 small posterior and 4 long central); (2) vaginal pore dorsal and single, with narrow and sinuous conspicuous vaginal duct; and (3) 104 symmetrical clamps in the haptor.

Significant differences in the total mean abundance were found between wild fish from Mar Menor and Santa Pola (bootstrap *t*-test, $P < 0.01$), although the only parasites with significantly different mean abundance were the monogeneans *L. falcus* and *L. hilii* (bootstrap *t*-test, $P < 0.05$). Fish from both localities also showed significant differences in the prevalence of these monogenean species and of the digeneans *Cardiocephaloides longicollis* (Rudolphi, 1819) and *Peracreadium characis* (Stossich, 1886) (Fisher tests, $P < 0.05$). A greater variety of species was found in the fish from Mar Menor, which harboured eight parasite species not found in the fish from off Santa Pola. These include one myxozoan (*Ceratomyxa* sp.), two monogeneans (Microcotylidae gen. sp. and *Atrispinum seminalis* Euzet et Maillard, 1973), three digeneans (*Proctoeces maculatus* Looss, 1901; *Steringotrema pagelli* (van Beneden, 1871); and *Monorchis monorchis* Stossich, 1890) and two copepods (*Caligus ligusticus* Brian, 1906 and *Lernanthropus vorax* Richiardi, 1880). Fish from Santa Pola also harboured four parasite species not found in fish from Mar Menor, including two monogeneans (*Lamellodiscus bidens* Euzet, 1984 and *Sparicotyle chrysophrii* (van Beneden et Hesse, 1863)), one digenean (*Galactosomum* sp.) and one isopod (*Gnathia vorax* Lucas, 1849).

Parasite species richness and total abundance in the fish samples from Mar Menor gradually decreased in captivity conditions. Total parasite abundance was significantly lowered after 10 days of captivity (bootstrap *t*-test, $P < 0.01$), and reached even lower levels after 10 more days (bootstrap *t*-test, $P < 0.01$). In 10 days, many monogenean species (*A. seminalis*, *L. falcus s.l.*, *L. hilii* Euzet, 1984 and *L. theroni s.l.*) and the myxozoan *Ceratomyxa* sp. were still observed. In contrast, most digenean species had disappeared, and only two species were found: *P. maculatus* and the metacercariae of *C. longicollis*. The last 10 fish examined after 20 days in captivity were parasitized by the same three species of *Lamellodiscus*, the abundance of these species being higher (although not significantly different) than in the fish examined immediately after capture. Digeneans of the digestive tract were not found at this time, whereas parasites in strictly internal microhabitats (*C. longicollis* in the brain and *Ceratomyxa* sp. in the gall bladder) were still present.

Morphometrics

Table 3 summarizes the metrical data for the diplectanid monogeneans found in this study identified to the species level, compared with the original descriptions of *Lamellodiscus bidens* Euzet, 1984 and *L. hilii*. The identification of the other two morphotypes of '*Lamellodiscus*' was controversial (morphometric data in table 4). These morphotypes were similar to *L. falcus* or to *L. theroni* (further referred respectively to as *L. falcus sensu stricto* (*s.s.*) and *L. theroni sensu stricto*, for the original descriptions, and *L. falcus sensu lato* (*s.l.*) and *L. theroni sensu lato*, for the specimens of the present study). Both morphotypes in sharpsnout seabream had similar morphology and body length, but the haptor of *L. falcus s.l.* was, in general, narrower, with sclerotized structures thinner than those of *L. theroni s.l.* (fig. 1a). Moreover, the

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Table 2. The prevalence (%) and mean abundance (% MA) of myxozoan, heminth and crustacean parasites from *Diplodus puntazzo* in two localities of the Spanish Mediterranean, and from fish maintained in captivity for 10 and 20 days; *n* = number of fish examined, CI (95% confidence intervals) given in parentheses.

		Mar Menor (Murcia) *n* = 30		Santa Pola (Alicante) *n* = 20		Mar Menor (10 days in captivity) *n* = 10		Mar Menor (20 days in captivity) *n* = 10	
Taxon	Microhabitat	%	% MA	%	% MA	%	% MA	%	% MA
Cnidaria, Myxozoa									
Ceratomyxidae									
Ceratomyxa sp.[b]	Gall bladder	10.0 (0.3–44.5)	–	–	–	20.0 (7.1–38.5)	–	10.0 (0.3–44.5)	–
Platyhelminthes, Monogenea									
Capsalidae									
Encotyllabe vallei	Gills	3.3 (0.1–17.2)	0.0	40.0 (19.1–63.9)		–	–	–	–
Diplectanidae									
Lamellodiscus bidens	Gills	–	–	40.0 (19.1–63.9)	0.4	–	–	–	–
Lamellodiscus falcus	Gills	100 (88.4–100)	107.9	80.0 (56.3–94.7)	42.2	100 (69.1–100)	135.0	100 (69.1–100)	156.9
Lamellodiscus hilii	Gills	100 (88.4–100)	66.2	80.0 (56.3–94.7)	3.6	100 (69.1–100)	82.2	100 (69.1–100)	91.0
Lamellodiscus theroni	Gills	90.0 (73.5–97.9)	26.7	80.0 (56.3–94.7)	40.8	100 (69.1–100)	38.9	100 (69.1–100)	42.0
Microcotylidae									
Atrispinum seminalis	Gills	20.0 (7.1–38.5)	0.3	–	–	10.0 (0.3–44.5)	0.2	–	–
Microcotylidae gen. sp.[a]	Gills	3.3 (0.1–22.3)		–	–	–	–	–	–
Sparicotyle chrysophrii[b]	Gills	–	–	40.0 (19.1–63.9)	1.2	–	–	–	–
Platyhelminthes, Digenea									
Derogenidae									
Magnibursatus bartolii[a]	Gills/oesophagus	23.3 (9.9–42.2)	0.4	40.0 (19.1–63.9)		–	–	–	–
Fellodistomidae									
Proctoeces maculatus	Intestine/caeca	3.3 (0.1–17.2)	0.1	–	–	20.0 (7.1–38.5)	0.5	–	–
Steringotrema pagelli[a]	Intestine/caeca	6.7 (8.1–22.0)	0.1	–	–	–	–	–	–
Heterophyidae									
Galactosomum sp.[a]	Brain	–	–	20.0 (5.7–43.7)	0.2	–	–		
Monorchiidae									
Monorchis monorchis	Intestine/caeca	26.7 (12.2–45.8)	1.1	–	–	–	–	–	–
Opecoelidae									
Peracreadium characis	Intestine/caeca	80.0 (61.4–92.2)	4.5	40.0 (19.1–63.9)	3.6	–	–	–	–
Strigeidae									
Cardiocephaloides longicollis[a]	Brain	70.0 (50.6–85.2)	3.2	20.0 (5.7–43.7)	1.0	40.0 (12.1–73.8)	0.8	60.0 (26.2–87.8)	1.7
Crustacea, Copepoda				–					
Caligidae									
Caligus ligusticus[a]	Gills	6.7 (8.1–22.0)	0.1	–		30.0 (6.7–65.2)	0.6	–	–
Lernanthropidae									
Lernanthropus vorax	Gills	26.7 (14.7–49.4)	0.3	–	–	–	–	–	–
Crustacea, Isopoda									
Gnathidae									
Gnathia vorax[a]	Gills	–	–	100.0 (83.1–100.0)	13.4	–	–	–	–

[a] New records for *D. puntazzo*.
[b] New records for wild *D. puntazzo*.

Table 3. Comparative morphometrics (μm) of the monogenean species *Lamellodiscus hilii* and *L. bidens* infecting *Diplodus puntazzo*; n = number of specimens examined and range in size given in parentheses.

	L. hilii			*L. bidens*	
Morphometrics	Present study, Mar Menor, $n = 10$	Present study, Santa Pola, $n = 7$	Euzet (1984)	Present study, Santa Pola, $n = 2$	Euzet (1984)
Body length	833 ± 182 (640–1159)	790 ± 236 (500–1169)	800–1000	788 ± 243 (616–959)	700–800
Haptor width	201 ± 18 (181–225)	163 ± 75 (166–223)	250	201 ± 2 (200–203)	
Ventral bar (total length)	121 ± 7 (111–128)	114 ± 18 (104–147)	100–120	94 ± 10 (87–101)	95–105
Lateral bar (total length)	84 ± 6 (78–97)	81 ± 6 (76–90)	80–90	75 ± 2 (73–76)	65–80
Lamellodisc diameter	61 ± 9 (50–78)	61 ± 7 (51–68)	75	58 ± 10 (51–65)	60
Dorsal anchor (total length)	62 ± 4 (56–69)	60 ± 4 (54–65)	55–67	59 ± 4 (56–62)	52
Ventral anchor (total length)	68 ± 6 (61–78)	69 ± 6 (62–76)	70–80	63 ± 6 (59–68)	64
Male copulatory organ (total length)	77 ± 20 (52–108)	92 ± 16 (65–104)	100	66 ± 2 (65–68)	74

dorsal bar of *L. falcus s.l.* was undivided while that of *L. theroni s.l.* was divided in two pieces (fig. 1a, b). The copulatory organs of both species were both lyre-shaped, although the single piece of the copulatory organ of the specimens of *L. falcus s.l.* was observed to be markedly hooked (fig. 1c), and that of *L. theroni s.l.* was more straightened (fig. 1d).

The morphometric measurements of the specimens of the digenean *Peracreadium* sp. found in both localities are given in table 5.

Molecular analysis

For molecular markers, ITS1 and 18S, the sequences of *L. falcus s.l.* and *L. theroni s.l.* obtained here were aligned and compared with the available sequences in GenBank for *L. ignoratus* and *L. ergensi*, respectively (accession numbers in table 1). There was no variation in the length of the 18S sequences (525 bp). The 18S sequences for isolates of *L. falcus s.l.* were identical with the sequences of *L. ignoratus* retrieved from GenBank. The 18S sequences for the isolates of *L. theroni s.l.* were identical and exhibited 0.2% divergence from the sequence for *L. ergensi* retrieved from GenBank. In case of the ITS1 region, the sequences varied in length from 441 to 563 bp. ITS1 sequences obtained in the present study were also aligned and sequence divergences (% of p-distances and number of differences) computed are given in tables 6 and 7.

The ITS1 isolates of *L. falcus s.l.* were identical, but exhibited 1.2–26.0% divergence with the four sequences for *L. ignoratus* retrieved from GenBank (table 6). The divergences between the ITS1 sequences for isolates of *L. theroni s.l.* ranged between 0 and 0.2% whereas dissimilarities between them and the sequence for *L. ergensi* from GenBank ranged between 0.2 and 14.0% (table 7). The mean inter-individual uncorrected genetic distances for ITS1 sequences (± SD and range in parentheses) of '*L. ignoratus* complex' (i.e. the new sequences for *L. falcus s.l.* obtained in the present study together with the sequence for *L. ignoratus* from GenBank) was 10.5 ± 10.0 (0.0–26), and of '*L. ergensi* complex' (i.e. the new sequences for *L. theroni s.l.* obtained here together with the sequence for *L. ergensi* from GenBank) was 6.6 ± 6.9 (0.0–14.2). The mean intraspecific distances for each species separately were: *L. falcus s.l.*, 0; *L. theroni s.l.*, 0.1 ± 0.1 (0.0–0.2); *L. ignoratus*, 15.4 ± 8.2 (9.1–26.0); *L. ergensi*, 16.1 ± 5.7 (0.0–14.0).

Three specimens from Santa Pola and one from Mar Menor identified as *Peracreadium* sp. were sequenced (see GenBank accession numbers in table 1). The sequences obtained were aligned and compared with sequences of the partial 18S rDNA and complete ITS1–5.8S–ITS2 for *P. characis* from GenBank (AJ241896). The length of the 18S sequences was 120 bp in all sequences examined. The length of the complete ITS1–5.8S–ITS2 sequences was 1081 bp. The 18S sequences for isolates of *Peracreadium* sp. were identical with the GenBank sequence for *P. characis*. The ITS sequences for the isolates of *Peracreadium* sp. were identical, whereas the divergence between these and the sequence for *P. characis* in GenBank was 0.3% in all cases.

Discussion

We present here the first survey on the parasite fauna of the sharpsnout seabream in the wild, finding 19 different parasites species. The sharpsnout seabream harboured a diverse community of metazoan parasites, many of them previously reported in this species in the Mediterranean Sea (Radujkovic & Euzet, 1989; Sasal *et al.*, 1999; Bartoli *et al.*, 2005; Mladineo, 2006; Gargouri Ben Abdallah & Maamouri, 2008). The current study also provides new parasite records in the wild fish populations. The sharpsnout seabream is a new host record for seven species, including the polyopisthocotylean monogenean Microcotylidae gen. sp., the digeneans *Magnibursatus bartolii* Kostadinova, Power, Fernández, Balbuena, Raga et Gibson, 2003, *Steringotrema pagelli*, *Cardiocephaloides longicollis* and *Galactosomum* sp., the copepod *Caligus ligusticus* and the isopod *Gnathia vorax*. In addition, two parasite species, the polyopisthocotyleans, *Atrispinum seminalis* and *Sparicotyle chrysophrii*, which have already been reported in farmed fish, were found for the first time in wild populations of sharpsnout seabream. Most of these species were reported previously in other sparid fish which cohabit with sharpsnout seabream. Among these new records, ten parasite species were common in this fish, as they were noticeably frequent (prevalence ≥ 40%, in at least one locality, see table 2). The remaining

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Table 4. Comparative morphometrics (μm) of the monogenean species *Lamellodiscus ignoratus* and *L. ergensi* groups infecting *Diplodus puntazzo*; *n* = number of specimens examined and range in size given in parentheses.

	L. ignoratus group				*L. ergensi* group			
Morphometrics	*L. falcus s.l.* Present study, Mar Menor, $n = 15$	*L. falcus s.l.* Present study, Santa Pola, $n = 15$	*L. ignoratus* Amine *et al.* (2006)	*L. falcus* Amine *et al.* (2006), $n = 34$	*L. theroni s.l.* Present study, Mar Menor, $n = 15$	*L. theroni s.l.* Present study, Santa Pola, $n = 15$	*L. ergensi* Amine & Euzet (2005)	*L. theroni* Amine *et al.* (2007), $n = 22$
Body length	635 ± 103 (470–854)	511 ± 207 (465–715)	550 ± 61	394 ± 50	613 ± 71 (512–695)	597 ± 99 (413–773)	400–550	715 ± 77
Haptor width	115 ± 7 (106–129)	96 ± 37 (87–128)	173 ± 13		151 ± 14 (129–179)	138 ± 54 (111–193)		201 ± 29
Medial bar (total length)	53 ± 5 (45–58)	52 ± 9 (27–60)	71 ± 8	34 ± 1	85 ± 7 (78–99)	77 ± 4 (70–80)	90–95	94 ± 12
Lateral bar (total length)	43 ± 5 (38–51)	46 ± 8 (26–54)	51 ± 4	37 ± 1	43 ± 3 (38–47)	42 ± 4 (37–49)	46–52	54 ± 6
					44 ± 4 (40–52)	42 ± 6 (31–51)	46–51	55 ± 6
Lamellodiscus diameter	44 ± 6 (33–54)	42 ± 8 (21–49)	43 ± 4	29 ± 1	34 ± 3 (30–38)	33 ± 2 (30–37)	30–35	35 ± 3
Dorsal hook (total length)	34 ± 2 (31–37)	35 ± 1 (33–37)	34 ± 2	30 ± 1	56 ± 3 (48–58)	55 ± 3 (50–59)	51–57	70 ± 9
Ventral hook (total length)	38 ± 2 (33–40)	37 ± 2 (34–40)	41 ± 3	31 ± 1	68 ± 5 (62–72)	64 ± 4 (59–69)	63–69	82 ± 10
Male copulatory organ (total length)	40 ± 1 (41–52)	49 ± 1 (47–54)	46 ± 3	40 ± 1	45 ± 5 (35–50)	46 ± 3 (40–49)	44–47	53 ± 6

parasite species were infrequent (prevalence ≤ 20%) and, therefore, they could be more specific to other hosts in the area (mainly in other sparids, see Álvarez-Pellitero *et al.*, 1995; Golomazou *et al.*, 2006).

Most parasites were monoxenous (11 species, 8 monogeneans and 3 crustaceans). The heteroxenous species were mainly digeneans (7 species), together with the only species of myxozoan. Interestingly, no nematodes have been recorded in the current study or in previous studies of sharpsnout seabream (Gibson *et al.*, 2005). This fact is surprising, especially considering that the species of genera *Hysterothylacium* Ward et Magath, 1917 and *Contracaecum* Railliet et Henry, 1912 exhibit very low host specificity and have been reported frequently in many fish species in the Mediterranean (e.g. Petter & Maillard, 1987; Petter & Radujkovic, 1989; Fernández *et al.*, 2005; Pérez-del Olmo *et al.*, 2007).

Morphological and molecular identification

The identification of the species of *Lamellodiscus* is particularly complicated, as species taxonomy is often based on subtle morphological differences. *Lamellodiscus falcus* and *L. theroni* belong to two species groups ('*ignoratus*' and '*ergensi*' group, respectively) composed of species with very similar morphological traits; hooks, lateral bars and copulatory organs of the species within each group are highly similar (Amine *et al.*, 2007). In fact, *L. falcus* differs from *L. ignoratus* only in the smaller size of all sclerotized pieces and by slight differences in the morphology of the single piece of the male copulatory organ (referred as to 'impair piece' in Amine *et al.*, 2006). The material of *L. falcus s.l.* examined in the present study showed mixed morphological features of both *L. falcus* and *L. ignoratus* (table 4). The shape of the sclerotized structures was clearly similar to that described for *L. falcus* by Amine *et al.* (2006), especially in relation to the hooked shape of the single piece of the copulatory organ (see fig. 1). However, the range of total body lengths and the lengths of the medial and lateral haptor bars overlapped the ranges described for *L. falcus* and *L. ignoratus* (table 4). In contrast, the hooks in the Mediterranean material were larger than those described for *L. falcus*, and more similar to those of *L. ignoratus* (see Amine *et al.*, 2006). A similar situation occurred in the case of the specimens of *L. theroni s.l.* compared with its original description (Amine *et al.*, 2007). *Lamellodiscus theroni* differs from *L. ergensi* in the size of the body and the sclerotized structures, particularly those of the medial bar. In the case of the specimens of *L. theroni s.l.* found in the present study, the medial bar morphology was closer to that described for *L. theroni* and the total body length fell within the range described by Amine *et al.* (2007). However, the dimensions of the sclerotized haptor structures were smaller than those of *L. theroni*, and more similar to those of *L. ergensi* (table 4). In summary, these results show a distinct similarity between the morphology of the species of *Lamellodiscus* found in the present study and the descriptions of *L. falcus* and *L. theroni* but, in contrast, their dimensions were mostly similar to those of *L. ignoratus* and *L. ergensi*, respectively.

Finally, although the comparative sequence analysis showed intraspecific differences in ITS1, this variation was within the range of the intraspecific variation

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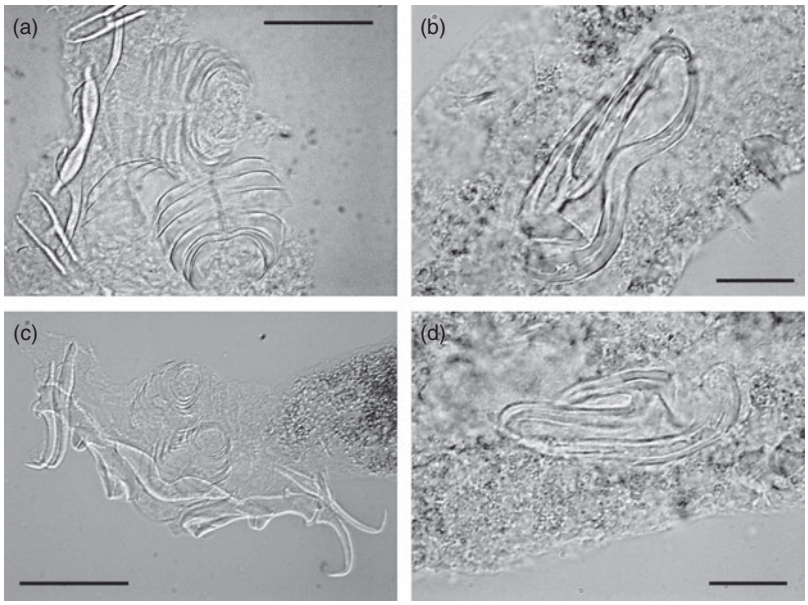

Fig. 1. The haptors of (a) *Lamellodiscus falcus s.l.* and (b) *L. theroni s.l.* and copulatory organs of (c) *L. falcus s.l.* and (d) *L. theroni s.l.* from *Diplodus puntazzo* from the Spanish Mediterranean. Scale bars: a, b = 500 μm; c, d = 100 μm.

provided by Kaci-Chaouch *et al.* (2008) for both *L. ergensi* and *L. ignoratus*. We agree with Poisot & Desdevises (2010) and Poisot *et al.* (2011) who suggested that this degree of molecular divergence in specimens of *Lamellodiscus* does not reinforce the separation of species despite their morphometric variations (*L. falcus* versus *L. ignoratus* and *L. theroni* versus *L. ergensi*). Recent studies emphasized that generalist *Lamellodiscus* species (i.e. members of *L. ergensi* and *L. ignoratus* groups) show higher morphometric variability on their different hosts than specialist species (e.g. *L. bidens* and *L. hilii*) (Poisot & Desdevises, 2010). This intraspecific variability induced by the host, combined with the possibility of morphometric differentiations caused by geographical variation, could explain many morphological variations. Studies incorporating morphological, morphometrical and genetic characterization are thus essential to understand the *Lamellodiscus* species classification.

With reference to digenean identification, specimens of *Peracreadium* sp. collected from both localities apparently belong to *P. characis* (see Bartoli *et al.*, 1989), since the general morphology mostly corresponded to this species but our individuals were somewhat smaller. The results obtained from the molecular analyses confirm that all

Table 5. Comparative morphometrics (μm) of the digenean species *Peracreadium characis* infecting *Diplodus puntazzo*; *n* = number of specimens examined and range in size given in parentheses.

Morphometrics	Present study, Mar Menor, *n* = 8	Present study, Santa Pola, *n* = 5	Bartoli *et al.* (1989), *n* = 10
Body length	1682 ± 276 (1470–2140)	1789 ± 150 (1615–2056)	2940 (2080–4250)
Body max. width	575 ± 189 (390–830)	532 ± 156 (364–765)	920 (785–1190)
Hind body max. length	901 ± 214 (976–1230)	895 ± 250 (715–1132)	1610 (910–2470)
Fore body length	518 ± 60 (410–590)	505 ± 55 (478–575)	900 (640–1230)
Oral sucker length	226 ± 26 (190–270)	232 ± 19 (215–255)	270 (210–350)
Oral sucker width	191 ± 33 (160–260)	189 ± 45 (143–248)	300 (230–390)
Pharynx length	174 ± 21 (150–220)	182 ± 34 (156–225)	210 (160–295)
Pharynx width	133 ± 34 (110–190)	112 ± 42 (88–172)	205 (140–300)
Ventral sucker length	263 ± 43 (230–340)	248 ± 39 (223–318)	400 (300–570)
Ventral sucker width	277 ± 26 (250–330)	289 ± 36 (238–314)	400 (310–520)
Anterior testis length	166 ± 42 (120–220)	175 ± 65 (112–258)	275 (220–370)
Anterior testis width	139 ± 54 (85–220)	102 ± 49 (73–192)	270 (210–340)
Posterior testis length	158 ± 50 (100–220)	150 ± 62 (92–235)	260 (190–375)
Posterior testis width	158 ± 65 (110–260)	139 ± 70 (95–247)	260 (210–320)
Ovary length	90 ± 14 (70–100)	86 ± 20 (68–110)	190 (150–290)
Ovary width	91 ± 21 (70 – 100)	94 ± 23 (69–108)	170 (100–250)
Egg length (mean)	71 ± 5 (63–78)	87 ± 7 (69–80)	66 (61–72)
Egg width (mean)	47 ± 5 (43–50)	42 ± 6 (37–50)	33 (30–39)

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Table 6. The range of nucleotides (above the diagonal) and genetic distances calculated as percentages (below the diagonal) in ITS1 sequences of *Lamellodiscus ignoratus* and *L. falcus s.l.* analysed in the present study (*L. ignoratus* complex).

	KC470292	KC470293	KC470294	EU259029	EU259027	EU259026	EU259031
L. falcus s.l. KC470292	–	0	0	35	5	3	105
L. falcus s.l. KC470293	0.0	–	0	35	5	3	105
L. falcus s.l. KC470294	0.0	0.0	–	35	5	3	105
L. ignoratus EU259029	8.4	8.4	8.4	–	38	35	103
L. ignoratus EU259027	1.2	1.2	1.2	9.1	–	8	109
L. ignoratus EU259026	7.0	7.0	7.0	8.4	1.9	–	106
L. ignoratus EU259031	25.1	25.1	25.1	24.6	26.0	25.3	–

isolates examined belonged to the same species, because the ITS sequences for isolates from Santa Pola and Mar Menor were identical. The low divergence (0.3%) between these sequences and the sequence for *P. characis* in GenBank fall well below the range for intraspecific sequence divergence given by Jousson *et al.* (1999) for the Opecoelidae, thus supporting the suggestion that specimens from the Mediterranean sharpsnout seabream also belong to *P. characis*. The present study therefore extends the range of the morphometric variation in this species.

Diversity of parasite fauna

The only myxozoan species found in the present study was *Ceratomyxa* sp. from the gall bladder. To date, two species of this genus have been reported in sharpsnout seabream: *C. diplodae* Lubat, Radujkovic, Marques et Bouix, 1989 and *C. puntazzi* Alama-Bermejo, Raga et Holzer, 2011 (Lubat *et al.*, 1989; Alama-Bermejo *et al.*, 2011). Both species parasitize the gall bladder and are very similar morphologically. Some myxozoan species are known to be highly damaging for aquaculture. In fact, one of the most pathogenic parasites for the culture of sharpsnout seabream has been the intestinal myxozoan, *Enteromyxum leei* Diamant, Lom et Dyková, 1994 (Montero *et al.*, 2007; Muñoz *et al.*, 2007; Álvarez-Pellitero *et al.*, 2008). Species of *Ceratomyxa* such as *C. sparusaurati* Sitjà-Bobadilla et Álvarez-Pellitero, 1995 in the gilthead seabream *Sparus aurata* L. (Palenzuela *et al.*, 1997) and *C. diplodae* in the sharpsnout seabream (Lubat *et al.*, 1989; Katharios *et al.*, 2007) have also been related to severe pathologies in Mediterranean sparids.

The most abundant group was the Monogenea, including eight species; all of them from the orobranchial cavity, usually on the gills (see table 2). Five of these species were monopisthocotyleans, four belonging to the genus *Lamellodiscus*. This genus is specific for the family Sparidae which can be parasitized by one or more species of *Lamellodiscus* (see Desdevises *et al.*, 2002). The fifth monopisthocotylean found in this study was the capsalid *Encotyllabe vallei* Monticelli, 1907, previously reported from the gills and pharynx of sharpsnout seabream and other sparids, including *S. aurata* (Radujkovic & Euzet, 1989). The remaining three monogenean species were microcotylid polyopisthocotyleans. Of these, *Atrispinum seminalis* has been recorded on the gills of five different species of *Diplodus* (see Gibson *et al.*, 2005), including the farmed sharpsnout seabream (Di Cave *et al.*, 2002; Athanassopoulou *et al.*, 2005). *Sparicotyle chrysophrii* is often recorded in wild and farmed *S. aurata* (e.g. Euzet & Audouin, 1959; Radujkovic & Euzet, 1989; Antonelli *et al.*, 2010a). Although this parasite has been found occasionally in farmed fish (Di Cave *et al.*, 2002), this study provides the first record in wild populations of sharpsnout seabream. The third microcotylid reported here, Microcotylidae gen. sp., has not been classified beyond family level since only one specimen was found, and its morphology does not correspond to any of the three genera previously reported in sharpsnout seabream, or to any other genus within the family (Mamaev & Parukhin, 1976; Maillard & Noisy, 1979; Mamaev, 1986; Radujkovic & Raibaut, 1989). Among the microcotylid species reported to date in sharpsnout seabream, *S. chrysophrii* appears most similar due to the presence of genital armature with three types of spines and a single vagina, and the lack of sclerotized plate. However, the number of the peripheral spines and the number of the haptoral clamps of *S. chrysophrii* are distinctly higher (Antonelli *et al.*, 2010a). More specimens of this morphotype should be examined in order to describe this possible new species.

Monopisthocotylean monogeneans represent a minor problem for the sparid cultures (Antonelli *et al.*, 2010b; Sánchez-García *et al.*, 2011). There are exceptional cases where some *Lamellodiscus* species have been related to

Table 7. The range of nucleotides (above the diagonal) and genetic distances calculated as percentages (below the diagonal) in ITS1 sequences of *Lamellodiscus ergensi* and *L. theroni s.l.* analysed in the present study (*L. ergensi* complex).

	KC470295	KC470296	KC470297	EU259056	EU259057	EU259058	EU259059
L. theroni s.l. KC470295	–	1	1	1	1	57	61
L. theroni s.l. KC470296	0.2	–	0	0	0	56	60
L. theroni s.l. KC470297	0.2	0.0	–	0	0	56	60
L. ergensi EU259056	0.2	0.0	0.0	–	0	56	60
L. ergensi EU259057	0.2	0.0	0.0	0.0	–	56	60
L. ergensi EU259058	13.3	13.3	13.3	13.1	13.1	–	8
L. ergensi EU259059	14.2	14.0	14.0	14.0	14.0	1.9	–

severe lesions and problems in some cultured sparids (Roubal, 1994), including the sharpsnout seabream (Katharios *et al.*, 2006). However, Sánchez-García *et al.* (2011) indicated that although the attachment mechanism of *Lamellodiscus* spp. seems quite traumatic (mostly due to the hooks piercing the gill epithelium) the damage provoked by these tiny parasites is mild and localized. In fact, despite the fact that some wild or captive sharpsnout seabream harboured more than 500 individuals of *Lamellodiscus* spp., fish were apparently healthy. In contrast, microcotylids, and *S. chrysophrii* in particular, represent a major parasitological problem in sparid cultures. This species is a well-known parasite of *S. aurata*, very often related to lethal epizootics in Mediterranean cultures (Faisal & Imam, 1990; Sanz, 1992; Sitjà-Bobadilla & Álvarez-Pellitero, 2009). *Sparicotyle chrysophrii* has also been reported to cause severe mortalities in sharpsnout seabream (Di Cave *et al.*, 2002). The finding of *S. chrysophrii* in wild sharpsnout seabream not only extends our knowledge on the occurrence of this emerging parasite, but also confirms that infected wild sharpsnout seabream can act as reservoirs of this monogenean for cultured gilthead seabream (Athanassopoulou *et al.*, 2005). No data exist on epizootics related to *A. seminalis*, but this parasite also seems potentially pathogenic, as its attachment and feeding strategies are similar to those of *S. chrysophrii*. Moreover, being monoxenous, it could also reach high loads in cultures.

With reference to digeneans, the derogenid *Magnibursatus bartolii* was found on gills or the oesophagus. As gills are not a usual infection site for digeneans, these parasites could have been regurgitated by fish; however, other hemiuroids, such as *Aponurus* spp. are frequently found on gills (Carreras-Aubets *et al.*, 2011). *Magnibursatus bartolii* has not been reported previously in sharpsnout seabream, but this parasite has been found in other sparids, such as *Boops boops* L. (the type-host) from the Spanish Mediterranean (Pérez-del-Olmo *et al.*, 2007) or *S. aurata* from Tunisia (Gargouri Ben Abdallah & Maamouri, 2008). More recently, a new species of *Magnibursatus*, *M. diplodii* Bayoumy & Abu-Taweel, 2012 was described in *Diplodus sargus* L. (Bayoumy & Abu-Taweel, 2012). The intestinal fellodistomids *Steringotrema pagelli* and *Proctoeces maculatus* were only found in fish from Mar Menor. This is the first record of *S. pagelli* in the sharpsnout seabream. *Proctoeces maculatus* has been cited previously in a survey of this fish off the Tunisian coast (Gargouri Ben Abdallah & Maamouri, 2008). This parasite has been recorded previously in other sparids (and interestingly also in one gobiid and pleuronectiforms, see Gibson *et al.*, 2005). The monorchid *Monorchis monorchis* and the opecoelid *Peracreadium characis* were previously reported in sharpsnout seabream, although only *P. characis* is strictly specific to this host (Bartoli *et al.*, 1989, 2005).

Metacercariae of *Cardiocephaloides longicollis* and *Galactosomum* sp. are reported for the first time from the brain of sharpsnout seabream. Although this is the first record of both parasites in sharpsnout seabream, it is not surprising since both exhibit low specificity towards second intermediate hosts (Naidenova & Mordvinova, 1997; Gibson *et al.*, 2005; Osset *et al.*, 2005, Culurgioni *et al.*, 2007). In heavy infections, parasites of the brain (including that of *C. longicollis*) have significant pathological effects as they can influence host behaviour in favour of parasite transmission to the final host (Chappel *et al.*, 1994; Lafferty, 2008; Fredensborg & Longoria, 2012). These species must be monitored, as they infect a vital organ, and could display synergic effects in heavy mixed infections.

The low number of crustacean parasites may be related to the loss of the individuals attached to skin during fish collection and processing (including handling or storage at −20°C). Although this is the first report of *Caligus ligusticus* in sharpsnout seabream, this species has been reported previously in sparid fish, including other *Diplodus* species, such as *D. sargus* (see Raibaut *et al.*, 1998 and references therein). Other caligids are known to parasitize sparid cultures (Ragias *et al.*, 2004; Mladineo, 2006) and they should be considered as potential pathogens for fish cultures as they have often been associated with important economic losses in farmed fish (Dawson, 1998; Lester & Hayward, 2006; Costello, 2009). *Lernanthropus vorax* has been often cited in wild sharpsnout seabream (Radujkovic & Raibaut, 1989; Raibaut *et al.*, 1998), and no pathological effects were reported. Nevertheless the presence of *L. vorax* should not be undervalued since other species of the same genus, such as *L. kroyeri* Van Beneden, 1851, have been frequently related to outbreaks and mortalities in sea bass culture (Yardimci & Pekmezci, 2012). The isopod *Gnathia vorax* was found for the first time on sharpsnout seabream. However, its presence is not surprising since gnathiid praniza larvae are non-specific, being found previously in other Mediterranean sparids (González *et al.*, 2004). The species of this family are considered as potential pathogens in culture conditions, due the anaemia provoked by haematophagus feeding (Marino *et al.*, 2004).

Parasite fauna and fish captivity

The current study provides useful information about the sharpsnout seabream parasites that can be transferred from the wild and survive in culture conditions. We observed that most parasites living in the external environment (i.e. ectoparasites and parasites of the digestive tract) disappeared under conditions of captivity, with the exception of *Lamellodiscus* spp. It is well known that most external parasites are highly susceptible to artificial culture conditions (Woo, 2006). Parasites on the skin and gills are affected by water quality and fish health, and parasites in the digestive tract are also affected by the food supplied in cultures. In contrast, parasites living in the blood and tissues can survive in such internal environments. The prevalence and abundance of metacercariae of *C. longicollis* did not vary significantly in captivity. Moreover, this was the only digenean found in fish captive for 20 days, probably because the metacercariae were protected within the brain tissues and are normally resistant encysted stages with little metabolic activity (Paperna & Dzikowski, 2006). A similar situation was observed for the myxozoan *Ceratomyxa* sp. protected within the gall bladder.

The most problematic parasites are usually the monoxenous species such as *Lamellodiscus* spp., which survive in cultures and are able to re-infect fish, reaching abnormally high parasite burdens. However, the importance of heteroxenous parasites should not be

underestimated, because in the surroundings of the sea cages or in the nets a substantial number of crustaceans and polychaetes that could act as intermediate/alternate hosts may be present.

The information provided in this study allows us to conclude which parasites could be a risk to sharpsnout seabream culture. There are three main groups (myxozoans, monogenean microcotylids and crustaceans) that should be taken into consideration for the culture of this fish. The myxozoan *Enteromyxum leei* was not found in the present study, although it has been reported in numerous cases, both in the wild and in culture (Le Breton & Marques, 1995; Merella *et al*., 2005; Álvarez-Pellitero *et al.*, 2008), even in cultures in the same location (San Pedro del Pinatar) in Mar Menor (Montero *et al.*, 2007). The microcotylid *S. chrysophrii* is an important finding. Its presence could cause respiratory dysfunction due to the epithelial damage and anaemia (Sitjà-Bobadilla & Álvarez-Pellitero, 2009). More crucial is the confirmation that wild sharpsnout seabream harbour one of the most damaging parasites in cultured *S. aurata* and therefore can act as reservoirs. The same effect could be expected in infections with other microcotylids such as *A. seminalis*. In fact, Merella *et al.* (2005) reported an epizootic induced by the high prevalence and intensity of the microcotylid *Atrispinum salpae* (Parona & Perugia, 1890) on cultured sharpsnout seabream. The third important group to take into consideration are the crustaceans. Although their numbers were quite low and they disappeared after 20 days of captivity, species of families such as caligids can provoke severe pathological problems. In fact, the main problem for salmon mariculture is the parasitosis caused by the caligid *Lepeophtheirus salmonis* (see Johnson & Fast, 2004). Moreover, in Mediterranean cultures crustaceans have been reported to provoke severe damage of different fish species (Costello, 2009; Yardimci & Pekmezci, 2012).

In view of the significant economic value of this fish, a detailed risk assessment study of sharpsnout seabream would be necessary to relaunch the culture of this species, minimizing possible future parasite problems. It should also be noted that most parasites of sharpsnout seabream recorded in present study are shared by the main fish species in Mediterranean aquaculture, such as the gilthead seabream. The proximity of cages containing this fish species could clearly result in cross-infections.

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Conflict of interest

None.

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