SCIENTIA MARINA 75(2)
June 2011, 369-378, Barcelona (Spain)
ISSN: 0214-8358
doi: 10.3989/scimar.2011.75n2369

Parasites and hystopathology of *Mullus barbatus* and *Citharus linguatula* (Pisces) from two sites in the NW Mediterranean with different degrees of pollution

MARTA CARRERAS-AUBETS, FRANCISCO-ESTEBAN MONTERO, FRANCESC PADRÓS, SILVIA CRESPO and MAITE CARRASSÓN

Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, E- 08193 Cerdanyola del Vallès, Barcelona, Spain. E-mail: Maite.Carrasson@uab.cat

SUMMARY: The usefulness of fish parasite communities as bioindicators of environmental stress was tested on two benthic fish species, the red mullet (*Mullus barbatus*) and the spotted flounder (*Citharus linguatula*), during the spring of 2006 at two sites of the Catalan coast (northwestern Mediterranean): an anthropogenic-impacted area located close to the city of Barcelona, and a less polluted area close to Blanes (Girona). Gonadosomatic and hepatosomatic indices and condition factor were determined for the fishes caught. Prevalence, mean intensity, mean abundance and species richness of the parasites found in the survey were calculated for both species and locations, and the main histological alterations were recorded. Cysts of unknown aetiology and intestinal coccidians were reported only in red mullets from the area close to Barcelona, which were highly parasitized by the digenean *Opecoeloides furcatus* and the nematode *Capillaria* sp. However, a higher prevalence of *Ichthyophonus* sp. was reported in the spotted flounder from Blanes. Cysts of unknown aetiology, some nematodes and *Ichthyophonus* sp. may be associated with pollution.

Keywords: bioindicators, Mediterranean, *Mullus barbatus*, *Citharus linguatula*, cysts of unknown etiology (CUEs), *Ichthyophonus* sp.

RESUMEN: PARÁSITOS E HISTOPATOLOGÍA DE *MULLUS BARBATUS* Y *CITHARUS LINGUATULA* DE DOS ZONAS SOMETIDAS A DIFERENTE GRADO DE CONTAMINACIÓN DEL MEDITERRÁNEO NOROCCIDENTAL. – Se ha comparado la utilidad de las comunidades parasíticas de peces como bioindicadores de estrés ambiental en dos especies bentónicas de peces , el salmonete de fango *Mullus barbatus* y la solleta *Citharus linguatula*, durante la primavera de 2006 en dos lugares de la costa catalana (Mediterráneo NO): un área fuertemente impactada cerca de la ciudad de Barcelona, y una menos contaminada cerca de Blanes (Girona). Se determinó el índice gonadosomático, el índice hepatosomático y el factor de condición de los peces capturados. Se calculó la prevalencia, intensidad media, abundancia media y riqueza específica de los diferentes parásitos encontrados por especie y localidad, y se analizaron las principales alteraciones histológicas. Se han encontrado quistes de etiología desconocida y coccidios intestinales tan sólo en los ejemplares de *M. barbatus* de Barcelona, los cuales también estaban altamente parasitados por el digeneo *Opecoeloides furcatus* y el nematodo *Capillaria* sp. Se ha detectado *Ichthyophonus* sp. tan sólo en los ejemplares de *C. linguatula*, presentando una mayor prevalencia en los ejemplares de Blanes. Los quistes de etiología desconocida, algunos nematodos e *Ichthyophonus* sp. podrían estar relacionados con la contaminación.

Palabras clave: bioindicadores, Mediterráneo, *Mullus barbatus*, *Citharus linguatula*, quistes de etiología desconocida, *Ichthyophonus* sp.

INTRODUCTION

The aquatic environment is a major sink for many potentially hazardous chemical pollutants, and the widespread presence of xenobiotics in sediment and biota is well-documented (Domingo and Bocio, 2007; Castells *et al.,* 2008). Levels of pollutants in sentinel organisms and the responses that these pollutants trigger in individuals have been successfully used as biomarkers of pollution (Broeg *et al.,* 1999; Handy *et* *al.,* 2002; Williams and Mackenzie, 2003; Au, 2004). Histopathology has been shown to be a useful tool for detecting sublethal and chronic damage of pollution in marine organisms (Stehr *et al.,* 1998; Bernet *et al.*, 1999; Korkea- aho *et al.*, 2006). Since fish parasites can reflect adverse effects of complex and variable environmental stresses (Lafferty, 1997; Landsberg *et al.*, 1998; Lafferty and Kuris, 1999; MacKenzie, 1999; Dzikowski *et al.*, 2003; Williams and Mackenzie, 2003), the study of fish parasite community structure has been proposed as a more sensitive indicator than the study of fishes themselves (Landsberg *et al*., 1998). However, although evidence supports the view of a relationship between parasitic load of fish and exposure to pollutants (Zander and Kesting, 1996; Schmidt *et al.*, 2003), the potential use of parasites as bioindicators for pollution biomonitoring is still controversial, since many natural factors also influence prevalence, infection intensity and biodiversity of parasites (Williams and Mackenzie, 2003) and both hosts and parasites can interact differently with each stressor (Lafferty and Kuris, 1999). This notwithstanding, from parasitological data obtained either from the field or following experimental treatment, Blanar *et al.* (2009) and Vidal-Martinez *et al.* (2010) point out the usefulness of parasites as bioindicators of environmental impacts.

Red mullet (*Mullus barbatus*, Linnaeus, 1758) and spotted flounder (*Citharus linguatula*, Linnaeus, 1758) are benthic fish species of commercial importance with a widespread occurrence in the Catalan Sea (northwestern Mediterranean). Their abundance and close association with the sediments where some toxicants accumulate make them suitable indicator species for the Mediterranean. *M. barbatus* has previously been used in several studies as a sentinel for pollution monitoring in Mediterranean waters (Porte *et al.*, 2002; Benedicto *et al.*, 2005; Zorita *et al.*, 2008; Insausti *et al.*, 2009). *C. linguatula* is a target species of the local trawling fleet and is vulnerable to fishing activities (De Juan *et al.*, 2007).The use of pleuronectiforms as bioindicators has been widely documented because of their highly susceptibility to suffer diseases (Johnson *et al.*, 1993: Khan and Hooper, 2007). Studies by Broeg *et al.* (1999) and Khan and Billiard (2007) have found changes in both histology and parasitism in fish exposed to a variety of pollutant types. However, information on the parasite fauna of *M. barbatus* and *C. linguatula* in the Catalan Sea is fragmentary. In the Tyrrhenian Sea, Adriatic Sea and other areas of the Mediterranean, the helminthofauna of *M. barbatus* has been studied by a number of authors (Vaccaro and Sivieri, 1969; Hristovski *et al.*, 1995; Bartoli and Prévót, 1996; Le Pommelet *et al.*, 1997; Martinez- Vicaria *et al.*, 2000), but to our knowledge the total parasite fauna of this species is unknown. The parasite fauna of *C. linguatula* was studied in detail in the eastern Atlantic (Marques *et al.*, 2006), but this information in the Mediterranean is limited and fragmentary (Cognetti-Varriale *et al.*, 1996; Verneau *et al.*, 1997; Cognetti-Varriale *et al.*, 1998). Similarly, although some data have previously been collected on both hepatic and splenic tissue alterations in *M. barbatus* (Pietrapiana *et al.*, 2002; Carrassón *et al.*, 2008), there is still a lack of information on the possible relationship of the parasites and pathology of these species to pollution.

The aim of this study was to compare the variation of parasites and histological alterations in two benthic fish species (*M. barbatus* and *C. linguatula*) from two differently polluted locations of the NW Mediterranean, and to discuss these variations in relation to pollution.

MATERIALS AND METHODS

Between March and April of 2006, 87 red mullet (*M. barbatus*) and 90 spotted flounder (*C. linguatula*) were collected from commercial trawlers at two different locations of the Catalan Sea (northwestern Mediterranean): the Blanes coast and the Barcelona coast. The Catalan coast has been divided into several water bodies according to anthropogenic pressure (mainly urban, industrial and agricultural wastes) and resulting environmental impacts; the water body corresponding to Barcelona coast has the worst ecological status of the Catalan Sea due to its closeness to a city of 1.7 million people from which it receives industrial waste and complex effluent (Agència Catalana de l'Aigua, 2005). The Blanes coast, close to a town of 39000 inhabitants, is considered a substantially less contaminated zone with a good ecological status (Agència Catalana de l'Aigua, 2005). Municipal sewage contributes greatly to the high values of organic carbon in the Barcelona area (Liquete *et al*., 2010). Heavy metal levels are high on the Barcelona shelf due to industrial development and a population increase during the 1920s and 1960s (Palanques *et al*., 1998, 2008; Sánchez-Cabeza *et al*., 1999), and organic pollutants (PCBs, DDT, etc.) show higher values in the sediment and organisms of the Barcelona area than in those of the Blanes area (Eljarrat *et al*., 2001; Porte *et al*., 2002). Fishes were captured at a depth of 52-130 m during the same period to avoid seasonal variations and minimize possible nutritional and reproductive changes.

Data of temperature and salinity for both sites were provided by the Agència Catalana de l'Aigua (ACA), the Government of Catalonia, Spain (Programa de Vigilància I Control de la Qualitat de les Aigües del Litoral Català, Water Framework Directive, 2000/60/EC).

Immediately upon capture, 50% of the individuals of each species from each location were individually introduced in a plastic bag (to prevent the possible loss of some parasites that could become detached) and frozen at -20ºC on normal ice to be used for parasitological analyses; the other 50% was fixed in buffered 10% formalin for histopathological analyses. Standard length (SL) was measured prior to dissection. Liver, gonads and eviscerated body were weighed.

Gonadosomatic index (GSI: gonad weight ×100/ eviscerated weight), hepatosomatic index (HSI: liver weight ×100/ eviscerated weight) and condition factor (CF: eviscerated weight ×100/(standard length)^3) were calculated to evaluate fish condition. GSI and HSI were calculated only for females. Differences were tested by means of Student's *t* test, after verifying a normal distribution.

For parasitological analysis, after thawing, the external surface of each fish and the plastic bag were inspected macroscopically and using binoculars. Different organs (oesophagus, stomach, intestine, liver, spleen, kidney, heart, gills, gonads, brain and muscle) were removed and carefully checked for parasites under the stereomicroscope and compound microscope. All parasites collected were counted and preserved in 70% ethanol. Digeneans and cestodes were stained in iron acetic carmine and mounted; nematodes were cleared in glycerine before identification. Parasites were identified to genus or species level when possible.

The parasitic community of two hosts was considered initially in terms of host size and locality. Fish from each location were grouped according to size of individuals. We considered two size groups for each species which generally correspond to an age of approximately 2 years (size 1) and 3 years (size 2): for *M. barbatus*, size 1 corresponds to a total length (TL)≤14.5 cm and size 2 with a TL≥14.5 cm (Kinacigïl *et al.*, 2001); for *C. linguatula*, size 1 corresponds to a TL≤16.1 cm, and size 2 to a TL≥16.1 cm (Vassilopoulou and Papacostantinou, 1994; García-Rodríguez and Esteban, 2000). Abundance of parasites was determined for each of the eight combinations of host species, size class and locality. The affinity of these eight groups was computed using a hierarchical analysis [unweighted pair group methods analysis (UPGMA), as the aggregation algorithm with Euclidean distance as a measure of similarity].

Parameters of prevalence (P), mean abundance (MA) and species richness (R) of parasites were calculated for both species and locations. Metazoan parasite diversity was calculated in terms of abundance of parasite item, using the Shannon Index (H'), one of the most commonly used parasite diversity indices (D'Amelio and Gerasi, 1997), and the Pielou evenness index (J'=H'/ log(total species)).

To compare the parasite prevalence and abundance between localities in the same host, a chi-square test and Student's *t* test were executed, respectively, after a normal distribution was found. Most of the data were not normally distributed (Kolmogorov-Smirnoff test), so they were normalized by logarithmic transformation.

The number of heteroxenous (H_{sp}) and monoxenous (M_{sp}) parasite species in each species host was obtained from a total count of heteroxenous *vs* monoxenous parasite species and the ratio H_{sp}/ M_{sp} in a given habitat was calculated according to D'Amelio and Gerasi (1997). Those species showing a prevalence value lower than 5% were defined as rare species (D'Amelio and Gerasi, 1997).

Samples of gills, intestine, liver, spleen, kidney, heart, gonads and musculature were obtained from fixed individuals and processed for routine histology. Sections (3-5 μm) were stained with haematoxylin-eosin and specific stains (Giemsa, Gram, Grocott, Ziehl-Neelsen and PAS) were used when necessary. Identification of the lesions and presence of the different types of parasites were performed according to their morphology in one section of each organ.

Protist parasites, Mesomycetozoea, Myxozoa and cysts of unknown aetiology (CUEs) due to their small size were detected only in histological slides. In this case, only prevalence of these parasites and lesions was calculated. Due to the high prevalence of *Ichthyophonus* sp. in many organs of *C. linguatula*, the infection level of *Ichthyophonus* sp. was evaluated in the organs with the highest infection levels (kidney, spleen heart and liver). Granulomas caused by the organism were counted: for each organ with granulomas, one tissue section was analysed at ×25 magnification and the number of granulomas was counted in 5 different fields of view, covering more than 80% of the total section.

RESULTS

Fish condition

Biometrical data of the fishes are shown in Table 1. Fish standard length ranged from 11.2 to 18 cm for *M. barbatus* and from 13 to 22.4 cm for *C. linguatula* with a homogenous range for both fish species analysed. Mean

TABLE 1. – Means and standard deviations of standard length (cm), eviscerated weight (g), gonadosomatic index (GSI), hepatosomatic index (HSI) and condition factor (CF) in *Mullus barbatus* and *Citharus linguatula* from both locations. Parenthesis values: number of specimens analysed; * significant difference between locations, ^{m} marginally significant values (*P*<0.10)

	Mullus barbatus		*Citharus linguatula*	
	Barcelona	Blanes	Barcelona	Blanes
Nº of specimens	45	42	48	42
Standard length (cm)	15.6 ± 1.43	15.1 ± 1.64	16.3 ± 1.7	16.4 ± 1.6
Eviscerated weight (g)	66.4 ± 16.1	56.2 ± 16.1	41.2 ± 13.9	47.5 ± 13.9
GSI	1.89 ± 0.75 (42)^m	2.21 ± 0.80 (30)^m	1.00 ± 0.26 (33)*	1.50 ± 0.34 (23)*
HSI	2.96 ± 0.82 (42)^m	2.26 ± 1.19 (29)^m	2.39 ± 0.63 (34)*	1.86 ± 0.34 (16)*
CF	1.75 ± 0.003*	1.16 ± 0.001*	0.97 ± 0.001*	1.07 ± 0.002*

Table 2. – Prevalence (P) and mean abundance (M.A.) of the parasites and pathologies found in *Mullus barbatus* and *Citharus linguatula* collected in Blanes and Barcelona. Msp: monoxenous lifecycle, Hsp: heteroxenous cycle, X: no available data, -: parasite species not present in the sample. Protists, Myxozoa, Mesomycetozoea and CUEs were identified by histological methods.

	Mullus barbatus				*Citharus linguatula*			
	Blanes		Barcelona		Blanes		Barcelona	
Parasite taxa (Lifecycle)	P	M.A.	P	M.A.	P	M.A.	P	M.A.
PROTISTS	20.00	X	30.44	X	60.00	–	56.00	–
Cryptocaryon sp. (*M*sp)	20.00	X	4.35	X	–	–	–	–
Trichodinidae (*M*sp)	–	–	–	–	60.00	X	56.00	X
Coccidians (*H*sp)	0.00	X	30.44	X	–	–	–	–
MYXOZOA (*H*sp)	75.00	X	56.52	X	10.00	X	21.7	X
MESOMYCETOZOEA	–	–	–	–	75.00	X	30.44	X
Ichthyophonus sp. (*M*sp)	–	–	–	–	75.00	X	30.44	X
DIGENEAN TREMATODES	50.00	3.00±6.74	90.91	5.23±6.31	22.73	0.27±0.55	16.00	0.16±0.37
Undetermined digeneans (*H*sp)	4.55	0.09±0.43	9.09	0.09±0.29	13.64	0.18±0.50	0.00	0.00
Opecoeloides furcatus (*H*sp)	45.46	2.73±6.75	86.36	4.77±6.03	–	–	–	–
Prosorhynchus sp. (*H*sp)	4.55	0.05±0.21	4.55	0.18±0.85	–	–	–	–
Aponurus sp. (*H*sp)	4.55	0.05±0.21	9.09	0.09±0.29	–	–	–	–
Derogenes latus (*H*sp)	4.55	0.09±0.43	4.55	0.05±0.21	–	–	–	–
Phyllidostomum sp. (*H*sp)	0.00	0.00	4.55	0.05±0.21	–	–	–	–
Lecitochirium sp. (*H*sp)	–	–	–	–	9.09	0.09±0.29	16.00	0.16±0.37
CESTODES	27.27	0.27± 0.46	18.18	0.18±0.40	68.18	0.86±0.77	64.00	0.80±0.76
O.Trypanorhyncha larval (*H*sp)	18.18	0.18±0.40	18.18	0.18±0.40	13.64	0.18±0.50	32.00	0.40±0.65
Botriocephalus sp. (*H*sp)	9.09	0.09±0.29	0.00	0.00	59.09	0.68±0.72	36.00	0.36±0.49
Undetermined larval (*H*sp)	–	–	–	–	0.00	0	4.00	0.04±0.20
NEMATODES	100.00	9.95±7.79	86.36	4.36±6.04	59.09	1.27±1.75	40.00	0.44±0.58
Undetermined larval (*H*sp)	5.00	0.05±0.22	4.35	0.04±0.21	25.00	0.25±0.45	8.70	0.09±0.29
Hysterothylacium fabri (*H*sp)	100.00	9.68±7.91	86.36	2.27±1.90	59.09	1.27±1.75	40.00	0.44±0.58
Capillaria sp. (*H*sp)	4.55	0.05±0.21	31.82	0.77±1.66	–	–	–	–
Ascarophis sp. (*H*sp)	0.00	0.00	13.64	1.09±4.68	–	–	–	–
Contracaecum sp. (*H*sp)	13.6	0.23±0.69	9.09	0.18±0.59	–	–	–	–
Cucullanus sp. (*H*sp)	0.00	0.00	4.55	0.05±0.21	–	–	–	–
CRUSTACEAN	9.09	0.09±0.29	0.00	0.00	–	–	–	–
Hastschekia mulli (*M*sp)	9.09	0.09±0.29	0.00	0.00	–	–	–	–
Cysts of Unknown Etiology (CUEs)	0.00	X	34.78	X	–	–	–	–

gonadosomatic index (GSI) of females showed higher values for fishes caught off Blanes, being significantly higher for *C. linguatula* (*t* test=6.242, *P*<0.001) and marginally significant for *M. barbatus* (*t* test=1.697, *P*=0.094). Mean hepatosomatic index (HSI) of females showed an opposite trend for the two species, being significantly higher in Barcelona samples than in those captured in Blanes for *C. linguatula* (*t* test=-3.828, *P*<0.001) and marginally significant for *M. barbatus* (*t* test=-1.782, *P*=0.079). Significant differences between localities were also found for condition factor values for both species (*t* test, *P*<0.05) (Table 1).

Parasitological and histopathological analysis

Five individuals of *M. barbatus* (3 from Blanes and 2 from Barcelona) and 11 of *C. linguatula* (9 from Blanes and 2 from Barcelona), out of a total of 87 specimens of *M. barbatus* and 90 of *C. linguatula* analysed, exhibited no parasites or tissue alterations. A total number of 18 parasite taxa were identified in *M. barbatus* samples, whereas only 10 were identified in *C. linguatula* samples (Table 2).

From the cluster analysis of the size and location combinations, we identified three groups depending on their parasite load (Fig. 1). Size 1 and size 2 from the 2 localities were clustered together in both species, indicating that size class is not a determinant of parasite community structure. The specimens of *M. barbatus* collected in Blanes (group A) were clearly separated from the other groups. *C. linguatula* individuals, either caught from Blanes or Barcelona (group C), were clustered together and clearly separated from the *M. barbatus* collected in Barcelona (group B) (Fig. 1). Based on these results, the samples were separated into three groups (A, B and C) for parasitological study:

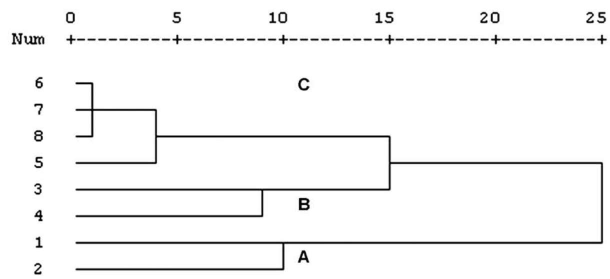

Fig. 1. – Dendrogram of dissimilarity between the parasite fauna of different groups (depending on location and size host) of *Mullus barbatus* and *Citharus linguatula*. 1, size 1 Blanes individuals of *Mullus barbatus*; 2, size 2 Blanes individuals of *Mullus barbatus*; 3, size 1 Barcelona individuals of *Mullus barbatus*; 4, size 2 Barcelona individuals of *Mullus barbatus*; 5, size 1 Blanes individuals of *Citharus linguatula*; 6, size 2 Blanes individuals of *Citharus linguatula*; 7, size 1 Barcelona individuals of *Citharus linguatula*; 8, size 2 Barcelona individuals of *Citharus linguatula*. Identified groups: A=1,2; B=3,4; C=5,6,7,8.

Group A: in the samples of *M. barbatus* from

Table 3. – Average number of granulomas (NG) of *Ichthyophonus* sp. per unit area counted in 5 fields of view from each section of kidney, spleen, heart and liver samples of *Citharus linguatula* from both sites. Prevalence of *Ichthyophonus* sp. in all the organs analysed of *Citharus linguatula* from the Blanes and Barcelona sites and chi-square test executed on prevalence. Parenthesis values: number of specimens analysed. * Significant differences ($P \leq 0.05$).

	Blanes NG.10^{-6}/ µm^2	Barcelona NG.10^{-6}/ µm^2	Blanes prevalence	Barcelona prevalence	χ^2	*P*
N	-	-	20	23		
Kidney	4.66±6.17 (11)	2.44±1.80 (5)	55	21.7	5.065	0.024*
Spleen	4.99±7.12 (9)	4.04±4.57 (4)	45	17.4	3.866	0.049*
Heart	2.28±2.38 (7)	5.19±4.84 (3)	35	13	2.890	0.089
Liver	0.90±1.30 (5)	1.21±0.87 (3)	25	13	1.010	0.315
Gill	-	-	25	4.3	3.800	0.051
Muscle	-	-	5	4.3	0.010	0.919
Intestine	-	-	20	8.7	1.139	0.286
Gonad	-	-	10	4.3	0.527	0.468

Blanes (n=42), the most prevalent and abundant group was the Nematoda, mainly represented by *Hysterothylacium fabri* (100% prevalence) (Table 2). This raphidascarid was mainly found as Larva 3 encysted in connective tissues of oesophagus and intestine but also in gonad, stomach, kidney and muscle. Myxozoan spores found in muscle and kidney also showed high values of prevalence (75%). The trematode *Opecoeloides furcatus*, with a prevalence close to 50%, was the second species in mean intensity (Table 2). Due to the methodology used in this work, we were unable to identify myxozoan spores and coccidians to species or even family level. No relevant histopathological alterations were found.

Group B: in 45 individuals of *M. barbatus* from Barcelona analysed, the Digenea and the Nematoda were the most prevalent groups (Table 2). *O. furcatus* and *H. fabri* were the most prevalent species. *O. furcatus* dominated the sample community in intensity. Myxozoans, *Capillaria* sp., coccidians and CUEs were present in more than 30% of the samples (Table 2). CUEs were located at the base of two adjacent gill lamellae. In most cases, the cysts, which showed an eosinophilic central core surrounded by basophilic material, elicited an inflammatory response and caused an alteration of the gill filament (Fig.2).

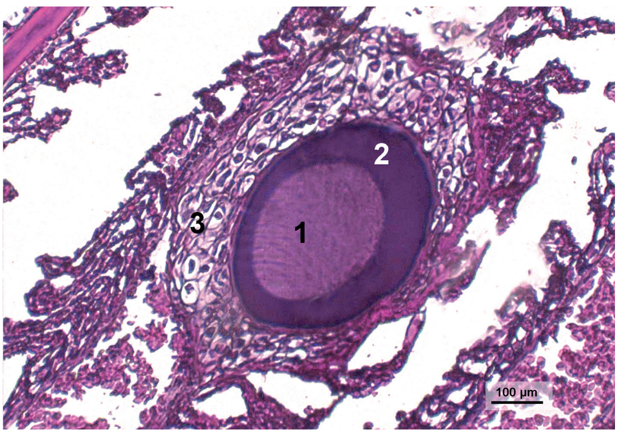

Fig. 2. – Cyst of unknown etiology in the gills of *Mullus barbatus*. 1, eosinophilic central core; 2, basophilic material; 3, inflammatory response.

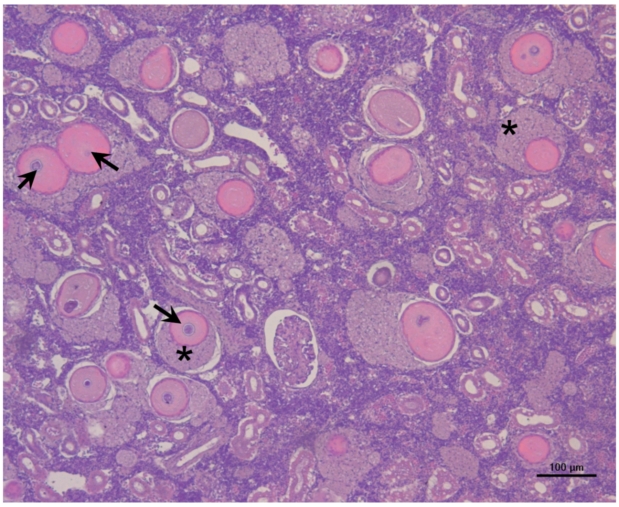

Fig. 3. – Ichthyophonosis in kidney of *Citharus linguatula*. Arrows, spores of *Ichthyophonus* sp.; asterisks, macrophage aggregates of response.

Group C: In Blanes samples of *C. linguatula* (n=42), the Nematoda was the first group in infection intensity and Mesomycetozoea the most prevalent (Table 2). Mesomycetozoea were exclusively represented by *Ichthyophonus* sp. A massive presence of spores was observed in all organs analysed for histopathology, some of them triggering a significant granulomatous response (Fig. 3). Kidney and spleen were the organs with the highest prevalence. The count of granulomas showed that the infection intensity was mainly strong in kidney, spleen, heart and liver (Table 3), with the highest intensity in spleen. Trichodinids, *H. fabri* L3 and *Bothriocephalus* sp. were present in approximately 60% of the hosts (Table 2).

In Barcelona samples of *C. linguatula* (n=48), the Cestoda and the protists were the most prevalent groups (Table 2). Cestodes were exclusively represented by one species of the genus *Bothriocephalus*, which was attached by the scolex to the intestine walls. *H. fabri* was the most prevalent and abundant parasite species. *Ichthyophonus* sp. was also observed in all organs analysed (Table 3).

TABLE 4. – Chi-square and *t* test executed in the prevalence and abundance of parasites and CUEs between the two sites in *Mullus barbatus* and *Citharus linguatula* samples. *t* test analysis was applied in variables normalized by logarithmic transformation except for *Hysterothylacium fabri* and Nematoda. * Significant values ($P \leq 0.05$), [m] marginally significant values ($0.05 < P < 0.10$)

	Mullus barbatus				*Citharus linguatula*			
	Prevalence		Abundance		Prevalence		Abundance	
	χ^2	*P*	T-test	*P*	χ^2	*P*	T-test	*P*
PROTISTS	0.612	0.434	-	-	0.053	0.818	-	-
Cryptocaryon sp.	2.550	0.110	-	-	-	-	-	-
Coccidians	7.271	0.007*	-	-	-	-	-	-
Trichodinidae	-	-	-	-	0.053	0.818	-	-
MYXOZOA	1.608	0.205	-	-	1.082	0.298	-	-
Ichthyophonus sp.	-	-	-	-	8.503	0.004*	-	-
DIGENEAN TREMATODES	8.844	0.003*	-2.617	0.012*	0.342	0.559	0.741	0.463
Opecoeloides furcatus	8.193	0.004*	-2.654	0.011*	-	-	-	-
Prosorhynchus sp.	0.000	1.000	-0.523	0.604	-	-	-	-
Aponurus sp.	0.358	0.550	-0.587	0.561	-	-	-	-
Derogenes latus	0.000	1.000	0.312	0.756	-	-	-	-
Phyllidostomum sp.	1.023	0.312	-1.000	0.329	-	-	-	-
Lecitochirium sp.	-	-	-	-	0.502	0.479	-0.697	0.490
CESTODES	0.518	0.472	0.707	0.483	0.091	0.763	0.283	0.778
Botriocephalus sp.	2.095	0.148	1.449	0.162	2.506	0.113	1.769	0.084[m]
NEMATODES	3.220	0.073[m]	2.662	0.011*	1.707	0.191	2.129	0.043*
Hysterothylacium fabri	3.220	0.073[m]	-4.270	0.000*	1.707	0.191	2.129	0.043*
Capillaria sp.	5.500	0.019*	-2.409	0.024*	-	-	-	-
Ascarophis sp.	3.220	0.073[m]	-1.407	0.174	-	-	-	-
Contracaecum sp.	0.226	0.635	0.259	0.797	-	-	-	-
Cucullanus sp.	1.023	0.312	-1.000	0.329	-	-	-	-
Hastschekia mulli	2.095	0.148	1.449	0.162	-	-	-	-
CUEs	8.547	0.003*	-	-	-	-	-	-

Site related differences in parasites and histological alterations

Mullus barbatus

Significantly statistical differences between groups A and B were found in relation to some parasites and pathologies described (Table 4). Coccidians, *Phyllodistomum* sp. digenean, *Ascarophis* sp. and *Cucullanus* sp. nematodes and CUEs were only found in the Barcelona site samples, coccidians ($P = 0.007$) and CUEs (P=0.003) showing significant differences with Blanes site (Table 4). The prevalence and abundance of *O. furcatus* and *Capillaria* sp. were significantly higher in Barcelona than in Blanes (respectives χ^2 and *t* test with P<0.05; Table 4), whereas the abundance of *H. fabri* L3 was significantly lower (*t* test=4.270, P=0.000; Table 4). Parasite diversity and evenness index showed higher significant values in Barcelona than at the Blanes site (*t* test= -2,290, P=0,027) and species richness was also slightly higher at this location (Table 5). The ratio of heteroxenous to monoxenous parasites was higher at the Barcelona site and the percentage of rare species was similar at both sites.

Citharus liguatula

Although cluster analysis grouped all individuals of *C. linguatula* in one group (C in Fig. 1), some differences between localities were found. Prevalence of *Ichthyophonus* sp. was significantly higher (χ^2=8.503, P=0.004; Table 4) at the Blanes site (due to significant differences in spleen and kidney; Table 3), but lower at the Barcelona site. *H. fabri* abundance also decreased significantly (T-test=2.129, P=0.043) in Barcelona area (Table 4). Parasite diversity and evenness index did not show significant differences between the two localities (*t* test=-0.545, P=0.589) and the species richness was the same in both sites (Table 5). Ratio between heteroxenous and monoxenous parasites was the same in both areas and there were no rare species.

TABLE 5. – Parasite diversity (H’), evenness index (J’), species richness (R) of parasites, distribution of monoxenous (M_{sp}) and heteroxenous (H_{sp}) parasite species, and percentage of rare species (P<5%) in *Mullus barbatus* and *Citharus linguatula* from each location. * Significant differences between locations.

	Mullus barbatus		*Citharus linguatula*	
	Barcelona	Blanes	Barcelona	Blanes
H’	2.19*	1.27*	2.05	1.74
J’	2.03	1.22	2.93	2.49
R	16	14	9	9
% M_{sp}	6.7	14.3	22.2	22.2
% H_{sp}	93.3	85.7	77.8	77.8
Ratio (H_{sp}/M_{sp})	13.9	6.0	3.5	3.5
% rare species	37.5	35.71	11.1	0

DISCUSSION

The present study provides some new data about the parasitofauna and pathology of two benthic Mediterranean species, *M. barbatus* and *C. linguatula*. Seventeen percent of the parasite taxa of *M. barbatus* (*Cryptocaryon* sp., *Prosorhynchus* sp., and *Phyllodistomum* sp.) and 30% of the parasite taxa of *C. linguatula* (*Ichthyophonus* sp., *Trichodina* sp. and *Lecitochirium* sp.) are cited for the first time (Gibson *et al.*, 2009).

Previous studies have indicated that the Barcelona continental shelf receives an important input of pollutants (municipal sewage, heavy metals, organic pollutants, etc.) that lead to high values compared to other areas of the Catalan coast (Eljarrat *et al*., 2001; Liquete *et al*., 2010; Palanques *et al*., 1998, 2008; Porte *et al*., 2002; Sánchez-Cabeza *et al*., 1999). Recent data from our laboratory on muscle samples of *M. barbatus* and *C. linguatula* from the Barcelona coast (unpublished data) confirm higher levels of organochlorine compounds than those from the Blanes coast, in agreement with the results of Porte *et al*. (2002) in *M. barbatus* from the same areas.

Values of the hepatosomatic index might reflect the pollution level of the aquatic milieu since HSI was higher in fishes caught from the most polluted area. This possible relationship between high HSI values and pollution has been observed previously (Vethaak *et al.*, 1992).

Results from the present study clearly indicate differences in the distribution and abundance of some of the parasites between the two study areas. *M. barbatus* showed higher diversity and richness values in Barcelona than in Blanes. Although pollution and stress are usually associated with a reduction in species richness and diversity of parasites (Schmidt *et al*., 2003), this is not a rule for all host species (Zander and Kesting, 1996; Landsberg *et al*., 1998). In fact, a wide range of positive and negative responses of parasite species to pollutants has been observed (Overstreet, 1997; Lafferty and Kuris, 1999; Dzikowski *et al*., 2003); since it is not always clear how diversity varies with particular impacts, because some species or parasite populations may increase while others decline (Poulin, 1992; Lafferty, 1997; Marcogliese, 2005), drawing conclusions based exclusively on a direct comparison of parasite indices is unreliable (Lang *et al.*, 1999). It is necessary to consider other factors (such as host specificity and abiotic factors and their interactions) when one is comparing results from different areas.

Among the parasite taxa, some studies suggest that nematodes would make a poor choice as indicator species (Lafferty, 1997; Blanar *et al.*, 2009). Nevertheless, we observed a significantly higher prevalence and abundance of *Capillaria* sp. in *M. barbatus* from the most polluted area, in agreement with data by Vidal-Martínez *et al*. (2010) indicating that nematodes have significant interactions with environmental impact. It is known that the response of nematodes to pollution varies according to the species and to the effects that different types of impact have on parasites (Lafferty, 1997). In our case, the response to pollution of *Capillaria* sp. in *M. barbatus* was positive, and this is in disagreement with the negative response normally indicated for heteroxenous parasites (Blanar *et al.*, 2009). On the other hand, infections caused by the nematode *H. fabri* in *M. barbatus* and *C. linguatula* were significantly lower in the impacted area near Barcelona. The potential influence of the different pollutants on the abundance of some ascaridoid nematodes is not clear. The Anisakidae nematode *Anisakis simplex* shows a wide tolerance to high levels of heavy metals accumulated in their tissues (Pascual and Abollo, 2005), although this species has demonstrated some sensitivity to a pesticide, carbofuran (Podolska *et al.*, 2008). In our case, the raphidascarid *H. fabri* could be affected by the variety of contaminants at the Barcelona site.

Digeneans are considered good indicators of environmental degradation, their number having been found to decrease with most types of pollution (Lafferty, 1997; Mackenzie, 1999, Blanar *et al.*, 2009). In our study, digenean increase with pollution in *M. barbatus*, and specifically that of *O. furcatus*, might indicate that environmental impact could affect the host response to the parasite but would not affect the survival and infectivity of the larvae, as pointed out by Morley *et al.* (2003). This result suggests that some larval parasite stages may not be as fragile as has been previously suggested.

Trichodinids are usually associated witn increase in organic matter (Yeomans *et al.*, 1997) and with a lowered immune response of the fish host due organochlorine compounds, heavy metals or petroleum hydrocarbons (Khan and Thulin, 1991, Broeg *et al*., 1999). In our study, however, we found similar levels of prevalence of trichodinids in *C. linguatula* in both areas in spite of the greater organic matter load (attributed to domestic sewage delivery, among other factors) present at the Barcelona site (up to 1.65%) (Liquete *et al.*, 2010) than at the Blanes site (annual mean sediment organic matter of 0.706%) (Pinedo *et al.*, 1997). This result could indicate that *C. lingutula* response to parasites might not be greatly altered by the environmental impact of the Barcelona site.

Infections caused by *Ichthyophonus* sp. in *C. linguatula* showed a significantly higher presence in Blanes, the less polluted area. *Ichthyophonus* sp. displays a perplexing physiological adaptability to a wide range of hosts and environmental conditions (Mellergaard and Spanggaard, 1997). Differences in susceptibility of the different fish host species to ichthyophoniasis due to temperature and salinity have been previously reported (see Franco- Sierra *et al.*, 1997), but considering that the two areas in our study exhibit very similar temperature and salinity values, these factors do not seem to be related to the differences observed. Oral transmission via an intermediate fish host carrying an infective stage of the parasite was demonstrated by Kocan *et al.* (1999). However, the presence of *Ichthyophonus* sp. in planktivorous fishes has led some authors (Hershberger *et al.*, 2008; Kocan *et al.*, 2010) to propose that a free-living stage of the parasite is also a possible route of infection. The inability of the free-living stage of *Ichthyophonus* sp. to survive in a highly polluted environment would explain the lower impact of this disease in the most polluted area.

No relevant histopathological alterations were described in any of the organs analysed, with the exception of CUEs, which were found at the base of the gill lamellae. These structures are reported for the first time in Mediterranean waters and also in the Mullidae family. CUEs have been previously identified in other fish groups and in other geographic locations (MacLean *et al.*, 1987; Munday and Brand, 1992; Nowak *et al.*, 2004). In our study, these cysts appeared only significantly in the Barcelona area, prevalence values being similar to those obtained in some impacted areas in Tasmania (Munday and Brand, 1992; Nowak *et al*., 2004) and Canada (Munday and Brand, 1992), and higher than those found in Sydney waters (Nowak, 1996). Moreover, studies on fish caught from the Barcelona area carried out in our laboratory in 2007-2010 (unpublished data) demonstrated the presence of CUEs in 13 of a total of 18 species analysed. Although some studies found no direct relationship between CUEs and pollution (Nowak, 1996), Munday and Brand (1992) suggested a link between pollution and presence of these cysts. Our results lead us to suggest that this histological alteration of the gills might be an interesting bioindicator, since CUEs are structures which can be rapidly and easily identified in the gill tissue. However, further experimental studies should thoroughly confirm the possible relationship between contaminant agents and CUEs.

From the present study, information on parasites found in *M. barbatus* leads us to point out, as previously suggested by other authors (Porte *et al.*, 2002; Zorita *et al.*, 2008; Insausti *et al.*, 2009), that *M. barbatus* is a suitable species for bioindicator analysis. The usefulness of the determination of *H. fabri*, *Capillaria* sp. and *O. furcatus* as biomarkers for pollution biomonitoring in *M. barbatus* from the Catalan sea seems to be promising. In addition to the fact that these parasites show significant differences between localities in relation to pollution, they can allow quick and easy identifications following the main criteria used in the bibliography (Broeg *et al*., 1999; Handy *et al*., 2002). However, it is necessary to estimate the threshold at which parasites respond to environmental insults to confirm the relationship between parasites and pollution, and this must be obtained by means of experimental data (Vidal-Martínez *et al.*, 2010).

Only *Ichthyophonus* sp. could be considered for biological monitoring in *C. linguatula*. The most suitable organ for this purpose would be the spleen due to the ease of its ablation, the possibility of obtaining complete radial sections and the significant differences we found between sites. However, the parasite communities of *C. linguatula* are too similar between locations. Mackenzie (1999) discusses the importance in the selection of suitable host-parasite systems for monitoring. The choice of host species to monitor must be made judiciously. From our work it is apparent that, whereas *M. barbatus* is a suitable species, *C. linguatula* should not be considered as a target species in Mediterranean biomonitoring.

ACKNOWLEDGEMENTS

The authors thank the Agència Catalana de l'Aigua (ACA) of the Generalitat de Catalunya for providing data of some water abiotic factors. They also thank the Departament d'Agricultura, Alimentació i Acció Rural of the Generalitat de Catalunya for providing data of captures in the Mediterranean Sea.

Funding for this work was obtained from the Spanish Ministerio de Educación y Ciencia through the research project Biomare CTM2006- 13508- C02-01MAR and the Complementary Action CTM2006-28145- E/MAR, and "Biomarcadors en la dinàmica d'ecosistemes marins sotmesos a impacte antropogènic" (PNL2005-35, noves línies recerca, UAB 2005) . M. Carreras Aubets benefits from a FPU grant from the Ministerio de Educación y Ciencia. The authors would finally like to thank the two anonymous referees for their valuable comments and suggestions for improving this work.

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Scient. ed.: C. Porte.
Received June 7, 2010. Accpeted October 22, 2010.
Published online March 14, 2011.