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Coastal fish farming does not affect the total parasite communities of wild fish in SW Mediterranean

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ABSTRACT

The influence of sea-cage salmon farms in increasing the parasite loads of wild salmonids has received considerable attention due to the potential negative consequences for both natural populations and cultivated stock. However, studies dealing with the parasitological loads of reared fish of other species and their relation with farm-associated wild fish are scarce. In this work, cultured and aggregated, wild fish from 3 different fish farms and 2 control locations were compared by hook and line and spear-fishing. It was found that reared sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) did not share macroparasites with farm-associated wild fish (bogue *Boops boops* and Mediterranean horse mackerel *Trachurus mediterraneus*). Similarly, no effect of farms on the total parasite community was detected when it was compared farm-associated and not farm-associated wild bogue and mackerel neither a host-range enlargement that has been detected in some other works. Reduced numbers of cestodes, nematodes and the digenean *Lecithocladium excisum* (which is first recorded parasitizing *T. mediterraneus*) occurred in farm-associated wild fish compared to not farm-associated fish. In contrast, the digenean parasites *Bacciger israelensis* and *Prodistomum polonii* were favoured by the farm effect. Influence of farms on wild fish, such as diet modification, may be detrimental for some parasite species, while these same conditions could enhance others.

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1. Introduction

Mediterranean sea-cage fish farming influences wild fish communities through effects on abundance, biomass (Dempster et al., 2002), feeding habits and physiology (Fernandez-Jover et al., 2007). Due to the sustained increase in production of marine aquaculture (FAO Newsroom, 2006, Federation of European Aquaculture Producers www.feap.info/feap), a potentially serious environmental effect for both farmed and wild stocks is increased abundance and prevalence of fish pathogens and the potential transfer between cultured and wild fish communities. Typical of fish reared in sea-cage aquaculture, fish are held at high density, therefore it may favour transmission of pathogens and direct life cycle parasites. Parasitism in cultivated fish can cause high mortalities and economic loss, like the cymothoid *Ceratothoa parallela* (Papapanagiotou and Trilles, 2001) or the monogeneans *Furnestinia echeneis* in sea bream and *Diplectanum aequans* and *Diplectanum laubieri* in sea bass (Zarza and Aizpurua, 2001). Management actions include the application of

formalin, ethanol and the use of immunostimulants. However, infections are very difficult to manage once the parasite has spread out, therefore, prevention and prophylaxis still form the main management actions (Zarza and Aizpurua, 2001).

Interactions between wild and cultured fish involving transmission of pathogens have been demonstrated for salmonids (Sepúlveda et al., 2004; Morton et al., 2005; Krkošek et al., 2005) and tuna, with the transmission of pathogens through imported frozen fish to farmed fish (Gaughan, 2002). However, it is generally difficult to detect the effects of farm-originated pathogens in local wild fish populations. Strong evidence of such an event concerning sea bass and sea bream has been reported for the bacterial infection by *Mycobacterium marinum* (Diamant et al., 2000; Ucko et al., 2002), but investigations on macroparasites, such as monogeneans or digeneans, are scarce (Raynard et al., 2007). Sea-cage farming may act as a 'pathogen amplifier of the infection', elevating the prevalence and abundance at levels unseen in the wild (Zlotkin et al., 1998; Horton and Okamura, 2001; Papapanagiotou and Trilles, 2001), favouring infection with unusual parasites (Papapanagiotou et al., 1999; Kent, 2000) or enlarging the host range of parasites, such as the myxosporean *Kudoa iwata* (Diamant et al., 2005). Although macroparasites such as monogeneans are well known to be highly host specific (Sasal et al., 2004), transmission of such parasites between different species of reared fishes at the same facilities may be

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also possible (Mladineo and Maršić-Lučić, 2007), indicating the potential enlargement of parasite host range under farming conditions.

To our knowledge, no study in the Mediterranean has demonstrated the transfer of parasites from farms to wild populations. The specific characteristics of the Mediterranean basin (higher temperatures, high biodiversity and different cultured species) imply that farmed and wild fish pathogen interactions may differ from elsewhere. The objectives of the present study were to describe the parasitic assemblage of reared sea bass (*Sparus aurata*) and sea bream (*Dicentrarchus labrax*) as well as farm-aggregated species and their non-associated wild counterparts: bogie (*Boops boops*) and Mediterranean horse mackerel (*Trachurus mediterraneus*). This work is focused on macroparasites, including copepods, isopods, cestodes, monogeneans, digeneans, acantocephalans and nematodes, in order to detect i) shared parasites species between cultivated and wild fish, and ii) variations in the parasite communities between farm-associated and not-associated wild fish. Further, any possible change in the host specificity of parasite species due to the presence of fish farms was assessed.

2. Materials and methods

Three fish farms located in the south-east of Spain were studied. Specific characteristics for each location are given in Table 1. All farms were grow out sites and reared sea-bass (*D. labrax*) and sea-bream (*S. aurata*), with no existing fallowing periods. In all farms, fish were grown from 10 to 30 g and are slaughtered after approximately 10 months, with weights ranging from 450–600 g to 450–900 g for sea bass and sea bream respectively. No treatment against pathogens was applied to the reared fish prior or during the study period. Farmed fish were captured on hook and line at the three fish farms to compare parasite communities. The main wild species, *T. mediterraneus* and *B. boops*, aggregated around the three fish farms were spear-fished, at depths between 15 and 25 m.

Individuals from wild, not farm-associated populations (30 non-associated *T. mediterraneus* and 30 *B. boops*) were obtained from commercial, bottom-trawling fisheries at depths between 50 and 80 m at two locations at least 10 km away from the nearest fish farm. Non-associated fish were obtained at six different days during the sampling period, choosing five fish of every species each day by means of a computer-generated, random numbers table. All fish were captured between May and June 2006 to avoid seasonal variation in parasitic infections (Mackenzie and Gibson, 1970) and were chosen to be of similar size to avoid size effects on the parasitic communities.

Immediately after capture, fish were checked for external parasites, labelled, individually packed and stored in ice for about 4 h. At the laboratory, fish were measured, weighted and examined for both ecto- and endoparasites. The bucal cavity, skin, fins and total branchial

arches were carefully checked under a dissecting microscope at 45× magnification. Fish were gutted and the carcass was checked. Gut washes were done in order to find parasites in the digestive tract. To do so, fish intestines were extracted, opened with dissection scissors, dropped in 500 mL jars with 10% salt-water and vigorously agitated to allow parasites to fall by density. This method was repeated 3–4 times until total separation of parasites, intestines and stomach contents. In addition, intestines were carefully checked for remaining attached parasites under a dissecting microscope. Parasites were preserved in 70% ethanol and stained with carmine. Prevalence, mean intensity, and mean abundance were determined for each parasite species, according to Bush et al. (1997); prevalence is the number of infected fish with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species (commonly expressed as a percentage); mean abundance of infection, i.e. the total number of parasites of a particular species found in a sample divided by the total number of hosts examined for that parasite species; and mean intensity of infection is the average intensity, i.e. the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. Mann–Whitney tests were used to find significant differences between farm-associated and not-associated fish indexes. Following Diamant et al. (1999) and Pérez-del Olmo et al. (2007), species richness and intensity of the total parasite community as well as richness and intensity of total monoxenous (parasites with a direct life cycle) and heteroxenous species (parasites with more one or more intermediate host and one final host) was chosen to describe the parasite communities. High stocking density may result in higher infections of several pathogenic organisms, principally those directly transmitted such as monogeneans. Therefore, it was also used monoxenous/heteroxenous species richness and intensity ratios (S_m/S_h and I_m/I_h respectively) to detect if populations of monoxenous parasites were enhanced due to the presence of farms. Analyses were done using SPSS12.0 (SPSS Inc.) and the program Quantitative Parasitology (QP3.0, Rózsa et al., 2000), which employs Fisher's test for prevalence and a bootstrap test for mean abundance and mean intensity. When p -value was lower than 0.05 but confidence intervals overlapped, the result was considered as statistically non-significant.

In order to detect differences in the total pool of different variables (species of parasites) analyzed, several multivariate analysis were used. All were performed using the PRIMER statistical package (Plymouth Marine Laboratory, England). In order to obtain triangular similarity matrices to represent in 2 dimensions the differences in the complete parasite profile of the different groups of fish (farm-associated and non-associated), Bray–Curtis similarity coefficient was calculated (Clarke and Warwick, 1994). Non-metric multidimensional scaling (nMDS) based on the same Bray–Curtis similarities was used as the ordination

Table 1
Production characteristics of the 3 Mediterranean farms growing *Sparus aurata* and *Dicentrarchus labrax* (localities of Altea, Campello and Guardamar) and the 2 locations where not-farm associated fish were captured (Santa Pola and Villajoyosa). In the bottom section of the table, 'n' indicates number of individuals (replicates) used for experiments from every locality.

Location	Position	Distance from shore (km)	Years in operation before Spring 2006	Depth (m)	Number of cages	Production (tonnes/year)	
Farm Altea	38°34,271'N	0°02,068'W	2.7	5.2	34	12	500
Farm Campello	38°25,234'N	0°12,050'W	3.2	9.1	30	12	350
Farm Guardamar	38°05,743'N	0°36,341'W	3.7	5.9	24	24	1000
Santa Pola	38°10,43.01'N	0°32,19.98'W	–	–	–	–	–
Villajoyosa	38°30,17.39'N	0°13,23.52'W	–	–	–	–	–
Location	<i>B. boops</i> (n)		<i>T. mediterraneus</i> (n)		<i>D. labrax</i> (n)		<i>S. aurata</i> (n)
Farm Altea	9		7		11		10
Farm Campello	11		10		10		10
Farm Guardamar	9		8		10		23
Santa Pola	15		15		–		–
Villajoyosa	15		15		–		–

method with the objective of visually detecting differences between farm-associated and not-associated groups of fish. This technique constructs a “map” or a configuration of the samples, in a specified number of dimensions, which attempts to satisfy all the conditions imposed by the rank similarity matrix (Clarke and Warwick, 1994). Data was square root transformed to diminish the weight of the dominant parasite species. In addition, in order to detect the degree of similarity within farm-associated and not-associated wild fish as well as the main parasite species contributing to differences between these groups of fish, a SIMPER analysis was applied. Analysis of Similarities (ANOSIM) was used to test if any differences occurred between associated and not-associated fish based on their total parasitic community (Clarke and Green, 1988). ANOSIM procedure calculates the *R*-statistic which indicates the magnitude of the difference between populations and a significance level. The *R*-statistic ranges from 0 to 1; *R*>0.75 indicates high differences in overall community structure whereas values for *R*<0.25 indicate little separation; intermediate *R* values reflect varying degrees of overlap but generally different community structure.

3. Results

3.1. Biometrical parameters of farm-aggregated species and non associated counterparts

Even though of similar standard length, farm-associated fish were heavier than not-associated individuals ($p<0.05$). Weights were 155.8 ± 20.7 g for not-associated *B. boops* while farm-associated ones were 354.6 ± 119.4 g. Standard lengths were 23.2 ± 2.4 cm and 26.2 ± 3 cm for not-associated and farm-associated *B. boops* respectively. Not-associated *T. mediterraneus* weighted 258.1 ± 125.7 g and measured 20.2 ± 7 cm, while farm-associated fish were 383.2 ± 147.3 g and 27.9 ± 4.6 cm (Table 2).

3.2. Parasitic assemblage of farm-aggregated species and their non-associated wild counterparts

3.2.1. *B. boops*

Ten different species plus unidentified nematodes and cestode larvae parasitized *B. boops* (Table 3). There were not significant differences for the total parasite richness or for the mean number of parasites between associated and non-associated *B. boops*. Results for monoxenous species were very similar for associated and not farm-associated *B. boops*. In the same way, no differences were found for the mean number of monoxenous individuals per fish and for the I_m/I_h ratio (intensity of monoxenous/intensity of heteroxenous parasites). Finally, the S_m/S_h index gave similar results for both groups of fish (Table 2).

Digeneans *Bacciger israelensis* (Monorchiiidae) and *Aphanurus stossichii* (Hemiuridae), as well as the monogenean *Microcotyle erythrini* (Microcotylidae) were found to be responsible for the highest prevalences in both associated and not farm-associated *B. boops* (86.2%, 82.8%, and 79.3% respectively). In addition, the monorchiid *B. israelensis* was dominant in farm-associated *B. boops* with the highest mean abundance and mean intensity (49.6% and 59.6% respectively; Table 3). Some parasite taxa were only found in not-associated *B. boops* (*Proisorhynchus crucibulum*), whereas others were only found in farm-associated fish (the digenean *Arnola microcirrus* and the monogenean *Cyclocotyla bellones*), however these taxa were always found in low numbers (Table 3).

Significant differences between associated and not-associated fish were found for the prevalence of nematodes ($p=0.01$) since farm-associated *B. boops* had a lower infection level than not-associated fish. In addition, statistically significant differences were found for the prevalence of the monogenean *Pseudaxine trachuri*. Not-associated *B. boops* were more infected ($P\%=30$) than their farm-associated counterparts ($P\%=3.4$). Finally, significant differences were also found for the mean abundance and/or mean intensity of the monorchiid *B. israelensis* ($p=0.05$ for mean abundance and intensity) due to the higher values detected in farm-associated *B. boops*.

Multidimensional Scaling (MDS) analysis did not clearly differentiate associated and not farm-associated *B. boops* (Fig. 1). Bray–Curtis similarity between not-associated and associated fish was not clearly marked (Table 6). The main parasite taxa contributing to this difference were *B. israelensis*, *Lecithocladium excisum* and *A. stossichii*, showing a mean variability of 35.5%, 14.3% and 13.7% respectively between not-associated and farm-associated fish. Finally, no significant difference between farm-associated and not-associated fish for the total parasite community was detected by ANOSIM (*R*-value 0.101; $p=0.02$).

3.2.2. *T. mediterraneus*

Nine different species plus unidentified nematodes, cestode larvae and acanthocephalans were detected for *T. mediterraneus* (Table 4). No differences were detected between associated and not-associated *T. mediterraneus* for parasitic richness, mean number of parasites individuals per fish, total monoxenous and total heteroxenous species (Table 2). Similarly, ratios of I_m/I_h (intensity of monoxenous/intensity of heteroxenous parasites) and S_m/S_h gave similar results for both groups of fish.

For *T. mediterraneus* captured in association with farms, the hemiurid *L. excisum* (56.5%), cestode larvae (47.8%) and the leprocreadiid *Prodistomum polonii* (43.5%) were recorded in highest prevalence. Cestode larva also showed a high mean intensity of which reached 36.3. In the case of not-associated *T. mediterraneus*, highest prevalences were detected for *L. excisum* (89.3%), cestode larvae

Table 2

Biometrical parameters and parasite community descriptors for the four studied fish species. *Boops boops* and *Trachurus mediterraneus* are wild fish, *Sparus aurata* and *Dicentrarchus labrax* are reared fish. Data are mean \pm standard deviation. * indicates significant differences at 95% confidence. S_m : Species richness of monoxenous parasites. S_h : Richness of heteroxenous parasites. I_m : Intensity of monogenean parasites. I_h : Intensity of heteroxenous parasites.

	<i>B. boops</i>		<i>T. mediterraneus</i>		<i>S. aurata</i>	<i>D. labrax</i>
	Farm	Not-associated	Farm	Not-associated	Reared	Reared
Sample size	29	30	25	30	43	31
Tot weight (g)	354.6 ± 119.4	$155.8 \pm 20.7^*$	383.2 ± 147.3	$258.1 \pm 125.7^*$	325.8 ± 248.1	443.3 ± 142.3
Standard length (cm)	26.2 ± 3	23.2 ± 2.4	27.9 ± 4.6	25.7 ± 3.4	20.2 ± 7	29.8 ± 3.5
Total no. of taxa	11	10	12	9	3	1
Mean no. of parasite species/fish	3.7 ± 1.5	4 ± 1.7	3 ± 1.9	3.2 ± 1.5	2 ± 0.1	0.5 ± 0.5
Mean no. of parasite individuals/fish	15.1 ± 21.1	5.1 ± 3	14.6 ± 25.3	10.4 ± 14	13.1 ± 29.1	18.4 ± 23.6
Mean no. of monoxenous species/fish	0.9 ± 0.5	0.8 ± 0.6	0.3 ± 0.6	0.1 ± 0.4	1.9 ± 0.6	0.5 ± 0.5
Mean no. of monoxenous individuals/fish	2.7 ± 1.5	4.3 ± 3.5	2.3 ± 0.9	2.2 ± 1.6	19.3 ± 35.8	18.4 ± 23.6
Mean no. of heteroxenous species/fish	2.8 ± 1.4	3.3 ± 1.6	2.6 ± 1.5	3.1 ± 1.3	–	–
Mean no. of heteroxenous individuals/fish	18.6 ± 26	5.1 ± 3.6	14.9 ± 25.3	10.5 ± 13.9	–	–
S_m/S_h	0.3 ± 0.2	0.3 ± 0.3	0.1 ± 0.2	0.1 ± 0.1	–	–
I_m/I_h	0.5 ± 0.4	1.2 ± 1.2	0.1 ± 0.3	0.1 ± 0.4	–	–

Table 3 Prevalence (P%), mean abundance (MA) ± Standard Deviation and mean intensity (MI) ± Standard Deviation for *Boops boops* parasites taxa. *p*-values represent level of significance of comparisons for prevalence, mean abundance and mean intensity between farm-associated and not-associated *B. boops*. Bold letters indicate significant differences. Prevalence was tested with Fisher's test, mean abundance and mean intensity with bootstrap test.

<i>B. boops</i>	Farm			Not-associated			<i>p</i> -value			
	P%	MA	MI	P%	MA	MI	P	MA	MI	
Cestode larvae										
Tetraphylidae	55.2	1.9 ± 3.38	3.44 ± 3.97	55	1.9 ± 2.55	3.45 ± 2.54	1	0.99	0.99	
Digeneans										
Accacoeliidae										
<i>Tetrochetus coryphaenae</i>	10.3	0.14 ± 0.44	1.33 ± 0.58	20	0.2 ± 0.41	1 ± 0	0.59	0.7	0.34	
Bucephalidae										
<i>Prosorhynchus crucibulum</i>	0			2	0.15 ± 0.49	1.5 ± 0.71	0.06	0.26	1	
Derogenidae										
<i>Arnola microcirrus</i>	1	0.03 ± 0.19	1 ± 0	0			1	0.44	1	
Hemiuridae										
<i>Aphanurus stossichii</i>	82.8	2.86 ± 2.66	3.46 ± 2.54	70	4.05 ± 3.94	5.79 ± 3.45	0.32	0.21	0.03	
<i>Hemiurus communis</i>	3.4	0.03 ± 0.19	1 ± 0	10	0.1 ± 0.31	1 ± 0	0.29	0.29	1	
<i>Lecithocladium excisum</i>	37.9	1.69 ± 2.48	4.45 ± 1.92	45	5.15 ± 9.73	11.44 ± 11.99	0.77	0.12	0.07	
Monorchiidae										
<i>Bacciger israelensis</i>	86.2	49.62 ± 85.8	57.56 ± 90.07	70	5.55 ± 7.89	7.4 ± 8.36	0.34	0.05	0.05	
Monogeneans										
Diclidophoridae										
<i>Cyclocotyla bellones</i>	3.4	0.03 ± 0.19	1 ± 0	0			1	0.45	1	
Gastrocotylidae										
<i>Pseudaxine trachuri</i>	3.4	0.03 ± 0.19	1 ± 0	30	0.25 ± 0.72	1.67 ± 1.15	0.05	0.24	1	
Microcotylidae										
<i>Microcotyle erythrini</i>	79.3	2.24 ± 1.81	2.83 ± 1.56	60	3.05 ± 3.76	5.08 ± 3.63	0.2	0.37	0.06	
Nematodes										
Not identified	3.4	0.03 ± 0.19	1 ± 0	40	0.5 ± 0.69	1.25 ± 0.46	0.01	0.02	1	

(85.7%, also showing the highest mean abundance and intensity, 16.8 and 19.6 respectively), the hemiurid *Ectenurus lepidus* (57.1%), and nematodes (46%) (Table 4).

Some parasite taxa were only found in farm-associated *T. mediterraneus*. This is the case for Acantocephalans, the copepod *Lernanthropus* sp. and the digenean monorchiid *Lasiotocus typicus*, however, they were always found in low numbers (Table 4). Significant differences between associated and non associated *T. mediterraneus* were found for the prevalence of the leprocreadiid digenean *P. polonii* (*p*=0.03) and cestode larvae (*p*=0.01); the former showing higher prevalence in farm-associated fish, whereas the last showed higher prevalence in not-associated fish. In addition, significant differences between associated and not farm-associated fish were also found for the prevalence of nematodes (*p*=0.01), and the hemiurid *L. excisum* (*p*=0.01); both nematodes and *L. excisum* showing higher values in not farm-associated *T. mediterraneus* (Table 4).

Finally, the hemiurid *L. excisum* is first described in this work parasitizing *T. mediterraneus*. High prevalences were found for this parasite in both associated (56.5%) and not farm-associated (89.3%) and reached mean intensities of 4.5 and 7.8 in farm-associated and not associated fish respectively (Table 4).

Although MDS analysis did not clearly differentiate associated and not-associated *T. mediterraneus*, farm-associated fish were more highly dispersed on the plot compared to that of not-associated fish (Fig. 2), indicating greater variability in parasite assemblages at farms (Table 5). This was confirmed by the percentage of similarity within

not-associated and farm-associated fish shown by SIMPER analysis: not-associated *T. mediterraneus* showed a similarity level two times higher than that obtained for farm-associated fish (Table 6). The tetraphylidae cestoda, *L. excisum* and *P. polonii*, explained 60% of the total variability between not-associated and farm-associated *T. mediterraneus* (Table 7). A lack of significant differences for the total parasitic community was found since the ANOSIM test showed an *R* value of 0.182 (*p*=0.001).

3.3. Parasitic assemblage of reared *S. aurata* and *D. labrax*

Three species parasitized the cultivated species *S. aurata* and only one species was found in *D. labrax* (Table 5), all of which were gill monogeneans. No intestinal parasites were detected. Differences among localities were found for the prevalence of *Encotyllabe vallei* (*p*=0.003) and the mean intensity of *Furnestinia echeneis* (*p*=0.001) in *S. aurata*. In addition, significant differences were found for the prevalence (*p*=0.001) of *Diplectanum aequans* in *D. labrax*. No skin copepods or isopods were found on any of the two reared species. No parasite potentially coming from farm-aggregated wild fish were found infecting either *S. aurata* or *D. labrax*.

Reproductive maturity for sea-bass and sea-bream is reached at around 1–2 and 2–4 years respectively. As a protogynic species, 100% of *B. boops* captured were female individuals. Therefore, no sex or reproductive stage effects were detected for these species. For *T. mediterraneus*, 70% of individuals were females and they were all captured prior to the spawning season (summer). No significant differences between sexes were found for any parasite species or parasite index for *T. mediterraneus* (Bauchot and Hureau, 1986).

4. Discussion

In SW Mediterranean, it has been found that reared sea bass and sea bream did not share macroparasites with farm-associated bogue and Mediterranean horse mackerel and that there was no effect on the total parasite community when associated wild fish were compared to their non-aggregated counterparts. However, a farm effect on parasite species was detected with modified abundances and prevalences while parasitizing farm-associated *B. boops* and *T. mediterraneus*.

4.1. Potential influence of coastal sea-cage aquaculture on parasitic communities of farm-aggregated wild *B. boops* and *T. mediterraneus*

No obvious differences in the total parasite community between farm-associated and not-associated fish were detected for both wild *B. boops* and *T. mediterraneus*. Similarly, there were no differences in

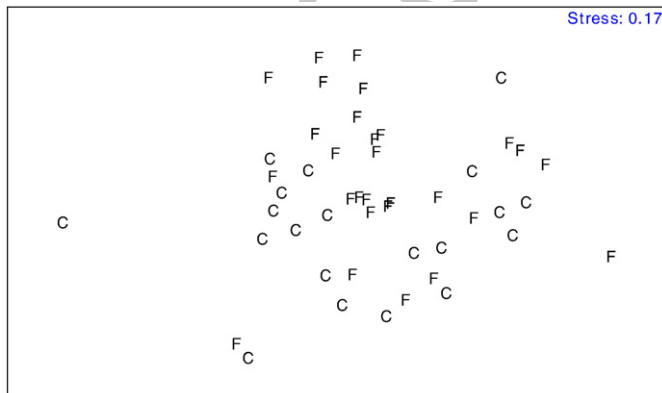


Fig. 1. MDS distribution based on Bray–Curtis similarities of farm-associated (F) and not farm-associated (C) *Boops boops*.

t4.1 **Table 4**

Prevalence (P%), mean abundance (MA) ± Standard Deviation and mean intensity (MI) ± Standard Deviation for *Trachurus mediterraneus* parasite taxa. *p*-values represent level of significance of comparisons for prevalence, mean abundance and mean intensity between farm-associated and not-associated *T. mediterraneus*. Bold letters indicate significant differences. Prevalence was tested with Fisher's test, mean abundance and mean intensity with bootstrap test.

<i>T. mediterraneus</i>		Farm			Not-associated			<i>p</i> -value			
		P%	MA	MI	P%	MA	MI	P	MA	MI	
t4.5	Acanthocephalans	4.3	0.04 ± 0.21	1 ± 0	0			0.45	0.43	>1	
t4.6	Cestode larvae	47.8	17.35 ± 36.4	36.27 ± 46.48	85.7	16.79 ± 26.32	19.58 ± 27.5	0.01	0.94	0.3	
t4.7	Copepods	4.3	0.04 ± 0.21	1 ± 0	0			0.45	0.44	1	
t4.8	Digeneans										
t4.9	Fellodistomidae										
t4.10		<i>Monascus filiformis</i>	21.7	1.09 ± 2.43	5 ± 2.83	7.1	0.14 ± 0.59	2 ± 1.41	0.22	0.15	0.12
t4.11		<i>Tergestia laticollis</i>	30.4	2.22 ± 6.35	7.29 ± 10.23	10.7	0.29 ± 0.94	2.67 ± 1.53	0.15	0.24	0.31
t4.12	Hemiuridae	<i>Ectenurus lepidus</i>	32.1	0.91 ± 1.83	2.33 ± 2.35	57.1	1.25 ± 1.43	2.19 ± 1.22	0.11	0.25	0.87
t4.13		<i>Lecithocladium excisum</i>	56.5	2.52 ± 3.13	4.46 ± 2.93	89.3	6.93 ± 5.73	7.76 ± 5.5	0.01	0.001	0.02
t4.14	Lepocreadiidae	<i>Prodistomum polonii</i>	43.5	6.13 ± 12.96	14.1 ± 16.9	14.3	0.21 ± 0.57	1.5 ± 0.58	0.03	0.1	0.08
t4.15	Monorchiidae	<i>Lasiotocus typicus</i>	4.3	0.22 ± 1.04	5 ± 0	0			0.45	0.43	1
t4.16	Monogeneans										
t4.17	Gastrocotylidae	<i>Gastrocotyle trachuri</i>	4.3	0.04 ± 0.21	1 ± 0	3.6	0.04 ± 0.19	1 ± 0	1	0.81	1
t4.18		<i>Pseudaxine trachuri</i>	26.1	0.7 ± 1.36	2.67 ± 1.37	10.7	0.25 ± 0.84	2.33 ± 1.53	0.27	0.2	0.76
	Nematodes	Not identified	12	0.22 ± 0.67	1.67 ± 1.15	46	1.5 ± 2.22	3.7 ± 2.11	0.01	0.01	0.06

333 the S_m/S_h and I_m/I_h ratios. Diamant et al. (1999) proposed that total
 334 heteroxenous/monoxenous parasites and S_h/S_m ratios are useful tools
 335 to compare two distinctly different habitats; low values of these ratios
 336 indicating anthropogenically impacted environments and high values
 337 indicating ecologically stable habitats. Our results suggest that these
 338 ratios do not appear to be good descriptors of fish farming impact on
 339 the environment.

340 4.2. Parasite communities of reared fish, *S. aurata* and *D. labrax*

341 In the present study, no result was found pointing at a potential
 342 host enlargement or cross infection of macroparasites between
 343 cultivated and wild fish. Indeed, no shared macroparasites were
 344 found to infect reared and wild fish at the same time, indicating that
 345 these parasites always present high levels of host-specificity. This
 346 result contrasts with the findings of Mladineo and Maršić-Lučić
 347 (2007), who suspected host switching of *Lamellodiscus elegans* from
 348 wild sparids such as *Diplodus annularis*, *D. vulgaris*, and *D. puntazzo*, to
 349 reared sea bream, all of them sparid species. The carangid *T.*
 350 *mediterraneus*, is not in the same family as either *D. labrax* or *S.*
 351 *aurata*. Greater transfer between species of the same family should be
 352 expected. However, signs of shared parasites between the sparids *S.*
 353 *aurata* (cultivated fish) and *B. boops* (wild) were not detected.
 354 Similarly, Mladineo et al. (2009) have recently state that there is no
 355 sign of a transmission of the monogenean *Sparicotyle chrysoiphrii* and

the isopod *Ceratothoa oestroides* between wild *B. boops* and cultivated
S. aurata and *D. labrax*.

Endoparasites were largely absent from cultivated fish, which is
 likely to be due to a diet composed almost entirely of commercial food
 pellets which prevents consumption of intermediate hosts for the
 different parasite species. Respect to monogenean parasites, with the
 exception of *Encotyllabe valley*, the same species here documented
 were detected by Papoutsoglou et al. (1996) on sea bass and sea
 bream at two farms in Greece. However, these authors also detected
 low numbers of the copepod *Caligus minimus*, a species which was
 completely absent from our samples. Finally, Mladineo (2005) found a
 similar prevalence of *D. aequans* parasitizing reared sea bass in the
 Adriatic Sea, though in lower mean abundance compared to this
 study. Further, *S. chrysoiphrii* in sea bream was found with a lower
 prevalence and mean abundance than in this study. Differences in
 parasite numbers between studies may be due to the different
 sampling times or locational differences.

4.3. Heteroxenous parasites

Surprisingly, results showed that, in the case of *B. boops*, the mean
 number of heteroxenous parasites individuals was three times higher
 in farm-associated than in not-associated fish, as seen for the
 monorchiid *B. israelensis*, which was found to be more abundant in
 farm-associated than in not-associated *B. boops* and was the main
 contributor to the high levels of heteroxenous individuals found. Fish
 farms could favour the aggregation of ctenophores and amphipods
 which are known intermediate hosts for *B. israelensis* (Pérez-del Olmo
 et al., 2008). Thus, a study on densities of potential intermediate hosts
 in the life history of these parasites, close to and distant from fish
 farms is needed to test this hypothesis.

In contrast to the results presented above, epidemiological
 parameters were higher in not-associated than in farm-associated
 fish for *B. boops* and *T. mediterraneus*. For example, prevalences of taxa
 such as nematodes in *B. boops*, and cestodes, nematodes and the
 digenean *L. excisum* in *T. mediterraneus* were greater in not-associated
 fish. This result suggests that fish farming activity may also negatively
 affect the epidemiology of some parasites. Artificial food pellets used
 in fish farms are known to be enriched with antioxidants, metals or
 immunostimulants (Bricknell and Dalmo, 2005). In some cases, food
 may include therapeutants when the reared fish are affected by
 diseases (Wu, 1995), which may also negatively influence some
 parasite species in wild fish that consume this food.

Despite the fact that wild fish may decrease or stop the
 consumption of intermediate hosts during residence around fish
 farms (Fernandez-Jover et al., 2007, 2008), which is a disadvantage for

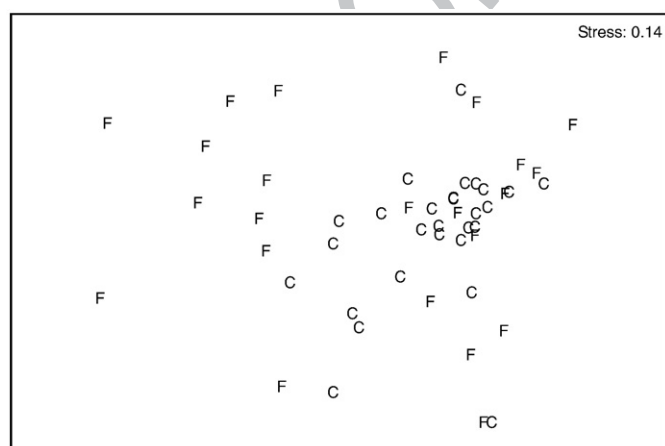


Fig. 2. MDS distribution based on Bray-Curtis similarities of farm-associated (F) and not-associated (C) *T. mediterraneus*.

Table 5 Prevalence (P%), mean abundance (MA) ± Standard Deviation and mean intensity (MI) ± Standard Deviation of reared *Sparus aurata* and *Dicentrarchus labrax* parasites and respective p-values. Table shows total cultivated individuals (TOTAL) and values per farm (Altea, Campello and Guardamar). Bold letters indicate significant differences. Prevalence was tested with Fisher's test, mean abundance and mean intensity with bootstrap test.

		TOT			ALTEA			CAMPELLO			GUARDAMAR			P	MI	
		P%	MA	MI	P%	MA	MI	P%	MA	MI	P%	MA	MI			
	<i>S. aurata</i>															
	Monogeneans															
	Capsalidae	<i>Encotyllabe vallei</i>	9.3	0.2 ± 0.6	2 ± 0	0		40	0.8 ± 1	2 ± 0	0			0.003	1	
	Diplectanidae	<i>Furnestinia echeneis</i>	88	15 ± 31.2	16.9 ± 32.7	80	3.4 ± 3.5	4.3 ± 3.4	100	50.6 ± 51.3	50.6 ± 51.3	87	4.5 ± 5.1	5.2 ± 5.2	0.4	0.001
	Microcotylidae	<i>Sparicotyle chrysophrii</i>	88	23.4 ± 45.2	26.5 ± 47.2	90	6.9 ± 7.9	7.7 ± 7.9	90	56.4 ± 85.4	62.7 ± 88.1	91	16.2 ± 14.5	18.7 ± 13.9	1	0.3
	<i>D. labrax</i>															
	Monogeneans															
	Diplectanidae	<i>Diplectanum aequans</i>	55	18.3 ± 23.6	33.5 ± 22.5	100	27.7 ± 20.8	27.7 ± 20.8	0			60	26.4 ± 28.6	44 ± 23.5	0.001	0.05

heteroxenous parasites, they undertake seasonal migrations away from farms (Fernandez-Jover et al., 2008). Consequently, wild fish resume consuming their natural diet which may prevent the total disappearance of such heteroxenous parasites and explain the remaining low levels of some parasites observed in farm-associated fish.

Finally, in this work, it is reported for the first time the presence of the hemiurid *L. excisum* on *T. mediterraneus* (in both farm-associated and not-associated fish), while this parasite is known to be commonly found in *B. boops* in the Mediterranean Sea. In addition, as the values of epidemiological parameters for this parasite were high, it is possible to conclude that *L. excisum* is a common inhabitant of this fish species in the studied area. However, it is unlikely that this constitutes an enlargement of the parasites host range as *L. excisum* (i) has been previously described as a generalist by Pérez-Del Olmo et al. (2008), and (ii) has been reported parasitizing the related fish species *T. trachurus* in the Atlantic Ocean (MacKenzie et al., 2004, 2008).

4.4. Monoxenous parasites

In the case of monoxenous parasites, no obvious differences between farm-associated and not-associated fish were found for both *T. mediterraneus* and *B. boops*. In addition, copepod or isopod species usually reported in the literature parasitizing *B. boops* were not detected, even though during sampling low numbers of isopods on associated wild sparids (*B. boops* or *Oblada melanura*), and copepods on the carangid *Trachinotus ovatus* were visually detected (pers. obs.).

These results largely contradict the idea that sea-cage fish farming enlarges the parasite host range of associated wild fish and enhance populations of parasite species that are directly transmitted among fish. This mechanism was thought to act through high densities of wild fish around sea cages leading to supposedly increased contacts between fish and infective stages of parasites (Dempster and Sanchez-Jerez, 2008). Indeed, due to the particular characteristics of aquaculture, which involves high stock densities, increased levels of diseases and of parasite populations have been documented, especially those directly transmitted among fish such as monogeneans (Ogawa, 1996; Zarza and Aizpurua, 2001). However, our results suggest that this

Table 6 Bray–Curtis average similarity (%) within wild farm-associated and not-associated *Boops boops* and *Trachurus mediterraneus*.

	NOT-ASSOCIATED	FARM
%Similarity		
<i>B. boops</i>	39.1	46.2
<i>T. mediterraneus</i>	52.2	24.1

mechanism does not occur for any of the species investigated during the time frame of this work.

Pollution also affects parasite communities of fish. Monoxenous parasite species, which are opportunistic, may be enhanced when environmental quality decreases (Diamant et al., 1999). Pérez-del Olmo et al. (2007) showed that, in NW Spain, monogeneans parasites of *B. boops* were favoured after a crude-oil spill. In the Mediterranean, Dzikowski et al. (2003) found higher species richness and S_h/S_m ratio in low-polluted mugilids compared to fish captured at polluted sites. However, monoxenous parasite community of wild associated *T. mediterraneus* and *B. boops* do not appear to be substantially modified by pollution from aquaculture. This is probably due to the high differences in nature and origin of the two kinds of pollution. However, other monoxenous parasites groups that were not investigated in this work, such as mastigophorans or ciliophorans, should be taken into account in future research since their population dynamics may be modified under fish farming conditions.

5. Conclusion

Wild fish parasitic communities were not severely affected by the influence of coastal sea-cage aquaculture. However, our data suggest that some parasite and fish species may be differently affected when resident around farms. Therefore, without a full investigation of the numerous wild fish species that reside around farms and their associated parasite communities, it is not possible to conclude that farms have no detrimental effects on wild fish parasite loads. The fish community associated with cages may be formed by more than 30 different fish species in the SW Mediterranean (Dempster et al., 2002), including those that do not feed on the lost pellets but on their usual fish prey (Sanchez-Jerez et al., 2008) and may reach abundances 2900 times higher than those found at pelagic control locations (Dempster et al., 2004). Due to the continuing growth of the aquaculture industry (FAO, 2007) and the introduction of new species to culture (Duarte et al., 2007), interactions between wild and

Table 7 Bray–Curtis dissimilarity (%) between (a) wild associated and not farm-associated *Boops boops* and *Trachurus mediterraneus*, and (b) main parasite taxa responsible for these differences.

<i>B. boops</i>		<i>T. mediterraneus</i>		
a	b	a	b	
60.1	<i>B. israelensis</i>	35.5	67.3	Cestoda tetraphylidae
	<i>L. excisum</i>	14.3		<i>L. excisum</i>
	<i>A. stossichii</i>	13.7		<i>P. polonii</i>
				12

469 cultured fish will inevitably increase and continued research will be
470 necessary.

Q2471 6. Uncited references

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473 Nowak, 2007
474 Öktener and Trilles, 2004

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