

Delta de l'Ebre is a natural bay model for *Marteilia* spp. (Paramyxea) dynamics and life-cycle studies

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ABSTRACT: *Marteilia* spp. are paramyxean parasites that affect several bivalve species of economic interest, such as *Ostrea edulis* and *Mytilus galloprovincialis*. Certain aspects of *Marteilia* spp., such as their life cycle and host affinity and infection dynamics, still remain unknown. The 'Delta de l'Ebre' constitutes a natural model for the study of the life cycle of the parasite *Marteilia*, since uninfected mussels and flat oysters immersed in the bays can become infected. This, along with the geographical and ecological characteristics of the bays, make it a very interesting location to study the *Marteilia* life cycle. Preliminary results concerning marteiliosis, mainly in mussels, such as prevalence dynamics, infectious periods, host affinity and host intermediate candidates are reported in the present paper. This information will be required for further, more exhaustive, studies in the bays of the Ebre delta.

KEY WORDS: *Marteilia* · Paramyxea · *Mytilus galloprovincialis* · Intermediate host · Host specificity · Ebre delta bays

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INTRODUCTION

Paramyxean parasites of the genus *Marteilia* affect some marine bivalve molluscs of economical interest such as the European flat oyster *Ostrea edulis*, and the blue mussel *Mytilus galloprovincialis* and *M. edulis*, which are important aquaculture species in Europe.

Historically, *Marteilia* species have been responsible for high mortalities in mollusc cultures in European countries. More particularly, *M. refringens* affects flat oysters in estuaries, bays and lagoons from a northern limit in Brittany on the French Atlantic coast to a southern limit identified as being the Mediterranean Sea (Comps 1979). Due to the detrimental impact of this parasite on flat oyster cultures since the 1970s, infection with *M. refringens* has been listed as a disease notifiable to the OIE (the World Organisation for Ani-

mal Health). The parasite has been a subject for study and scientific investigations since its first description in 1968 in the Aber Wrach, Brittany, France (Comps 1970). More than 3 decades of research have clarified many taxonomical, epidemiological and pathological aspects of the disease (Herrbach 1971, Grizel et al. 1974, Perkins 1976, Balouet 1977, Berthe et al. 1998, 2000, 2004, Le Roux et al. 2001, Longshaw et al. 2001, Audemard et al. 2002, Lopez-Flores et al. 2004, Novoa et al. 2005). However, this organism still presents questions regarding its host affinity, infection dynamics and life cycle.

In Europe, 2 species or types of *Marteilia*, *M. refringens* and *M. maurini*, are responsible for marteiliosis of oysters and mussels, respectively (Le Roux et al. 2001). Recent studies, based on molecular data, suggest that both parasites, *M. refringens* and *M. maurini*, are con-

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specific and correspond to 2 types of the same genomic species (Lopez-Flores et al. 2004), most probably undergoing an allopatric speciation process. However, these molecular results have to be confirmed based on robust epidemiological and ecological datasets.

More information is required to control disease emergence, to monitor infested cultures and to clarify the ecological behaviour of the 2 different *Marteilia* types infecting oysters and mussels in Europe.

Host affinity studies have investigated the relative host specificity of the 2 types of *Marteilia* with respect to their molluscan hosts. Results showed some cases of co-infection (both types in the same individual) and a lack of a strict host specificity (Le Roux et al. 2001, Lopez-Flores et al. 2004, Novoa et al. 2005). This has important consequences for further understanding of the transmissibility of the disease and to establish monitoring strategies.

Comparative studies of *Marteilia* types and their ecology also appear important in a polyphasic approach to their relative taxonomic relations. Indeed, the hypothesis of co-specificity might be confirmed if both *Marteilia* types were to present similar behaviour and dynamics in mussels as well as in flat oysters. Marteiliosis evolution and distribution have been well documented for *Ostrea edulis*. Many studies have been conducted on *M. refringens* in flat oysters, with particular emphasis on the transmission period, the annual dynamics of the parasite, and its life-stage distribution over time and within tissues. In mussels, studies describing annual patterns of infection intensity, spatial distribution and pathogenesis have been published (e.g. Villalba et al. 1993). However, more information is necessary to compare the disease profiles in flat oysters and mussels.

Since 1968, several experiments to transmit the disease to non-infected flat oysters have been attempted without success (Balouet 1977, Comps & Joly 1980, Berthe et al. 1998). Results of these experiments supported the hypothesis of a heterogenic life cycle including an intermediate host. To investigate this hypothesis, an interesting experimental model was designed based on natural ponds with low biodiversity (Audemard et al. 2001). These ponds, also known as 'claires', reduce the high number of potential hosts present in the estuarine areas. Claires are shallow ponds traditionally used for fattening and greening oysters and where the *Marteilia refringens* life cycle was demonstrated to be completed. This model has facilitated the initial screening step of research for an intermediate host by decreasing the biodiversity 10-fold compared to that in estuaries. Audemard et al. (2001) demonstrated that *Paracartia* [*Acartia*] *grani* (Copepoda) is an alternate host of *M. refringens* and could be an intermediate host in the parasite life cycle

(Audemard et al. 2002). The use of molecular tools (Le Roux et al. 1999) facilitated research in this field by screening for *M. refringens* DNA in the different species living in the claires.

The experimental transmission of the parasite to mussels did not provide conclusive results, except for a unique equivocal report of transmission from oysters to mussels (Comps & Joly 1980). Thus, again, the hypothesis of a heteroxenic life cycle and an intermediate host was postulated (Balouet 1977, Berthe et al. 1998). However, no potential intermediate host has been proposed for the *Marteilia* infection in mussels.

The claire pond model for marteiliosis provided important preliminary data to design a more natural and comprehensive study model in order to answer unresolved questions, especially concerning *Marteilia* in mussels.

In the estuarine bays of the Ebre River (NW Mediterranean coast of Spain), *Marteilia* has been recorded in both oysters and mussels (Riera et al. 1993, Durfort et al. 1996, Carrasco et al. 2001). The dimensions of the bays, their hydrodynamics, biodiversity and shellfish aquaculture make them an interesting choice as a natural model to further our knowledge of the *Marteilia* spp. cycle.

The current paper presents the dynamics of the parasite in 2 bays of the Ebre delta, and the relationship with key environmental factors. Preliminary comparative studies on host affinity and determination of the main zooplankton species present in the bays were carried out. These preliminary data are indispensable for further studies using this model.

MATERIALS AND METHODS

Study site. The Ebre delta is located in the north-western Mediterranean (Fig. 1). Sediments of the Ebre River form this delta, which encompasses 2 semi-enclosed bays: the northern Fangar Bay and the southern Alfacs Bay.

The delta is a complex water system. River water is diverted into a channel system 50 km upstream of the river outlet to be used in rice and horticulture production. After agricultural use it drains from the fields into Fangar Bay (with a capacity of $10 \text{ m}^3 \text{ s}^{-1}$) to the north and Alfacs Bay (with a capacity of $13 \text{ m}^3 \text{ s}^{-1}$) to the south (Camp & Delgado 1987). Freshwater supply exceeds evaporative loss in both Fangar and Alfacs Bay (Camp & Delgado 1987). Both bays receive approximately the same quantities of freshwater, whereas the residence times in the bays are different. The annual average salinity in the bays is 35‰ (slightly below the salinity of the open sea, 37 to 38‰), but salinity variation is very pronounced. Temperatures during the year range from 8 to 28°C, with some exceptions in particu-

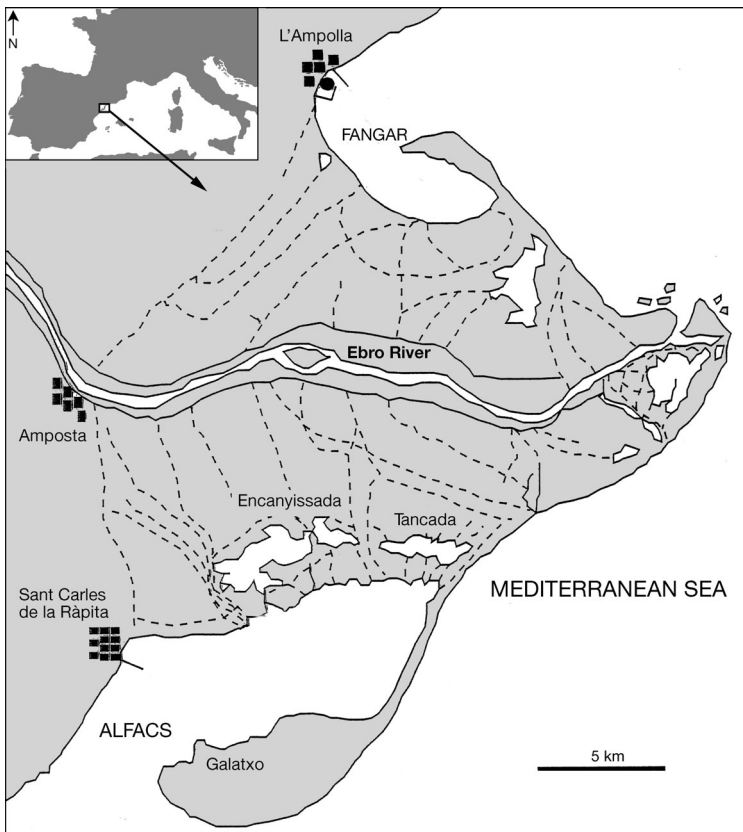


Fig. 1. Study site: Delta de l'Ebre, Catalanian coast, north eastern Spain

lar cases. The studied bays have a maximum depth of 6 m. The tides have a maximum amplitude of 20 cm, and the alternation between a stratified and mixed water column in the annual cycle is determined by seasonal winds (Delgado & Camp 1987).

Fangar Bay has an extension of 10 km², a maximum depth of 4 m, a capacity of 16 × 10⁶ m³ and is open to the sea across a 1 km mouth. Freshwater retention time is an average of 1 d, which produces short fluctuations in temperature and salinity (1 to 2 d). The annual average chlorophyll *a* concentration in this bay is 3.44 mg m⁻³ (Delgado 1987).

Alfacs Bay has an extension of 50 km², a maximum depth of 6 m, a capacity of 191 × 10⁶ m³ and is open to the sea across 3 km. Freshwater retention time is 10 d and, therefore, fluctuations of temperature and salinity are longer (10 to 20 d). The annual average chlorophyll *a* concentration in this bay is 3.20 mg m⁻³ (Delgado 1987). Different species of *Acartia* spp. (Copepoda) have been reported to form part of the zooplankton community in this bay (M. Alcaraz pers. comm.).

Both bays are exploited for mollusc aquaculture and fisheries. For the purpose of the present study, temperature and salinity were measured weekly at 2 m depth using a manual conductivity meter (WTW) during the

experimental 2003 culture period, from February to December 2003. For the second experimental period (October 2003 to September 2004), temperature and salinity were recorded every hour using a SBE 16plus moored at 2 m depth.

Mollusc experimental cultures. Blue mussels *Mytilus galloprovincialis*, European flat oyster *Ostrea edulis* and Pacific oysters *Crassostrea gigas* were used as experimental animals. They were cultivated on ropes prepared according to local farming practices. Ropes were deployed in both bays as described below.

2003 experiments: Mussel ropes were set up with spat (30 mm average size) naturally collected within the Alfacs Bay and on the Maresme coast (north of Barcelona) in February 2003. Flat oyster ropes were prepared in March of 2003 with oysters (1 yr old, 65 mm average size) originating in French Mediterranean and Italian Adriatic waters. Pacific oyster ropes were prepared in February of 2003 with spat (4 mm average size) originating from Marennes-Oleron, France. All cultures were placed in both bays in duplicate.

2003/2004 experiments: Mussel ropes were prepared in October 2003 with spat (32 mm average size) originating in the northern Italian Adriatic and the French Mediterranean. Flat oyster ropes were prepared in May of 2004 with 1 yr old (60 mm average size) oysters originating from Galicia, Spain. Duplicates of these ropes were placed in both bays. Pacific oyster ropes used in 2004 were the same as in 2003.

Sampling strategy. Epidemiological study in the Ebre Delta: A total of 60 individuals in 2003 and 150 individuals in 2003/2004 were collected from each species in both bays every 3 mo over the 2 yr period in order to be able to detect 5 and 2% of prevalence, respectively (Ossiander & Wedemeyer 1973). Additionally, 150 mussels deployed in a site of Fangar Bay were sampled monthly during late spring and summer in 2004, to provide a more detailed description of parasite dynamics during this period.

Zooplankton sampling: An initial sampling of zooplankton was performed in October 2003, as a first preliminary analysis, and then every 2 wk from June to August 2004. Horizontal net hauls using 100 µm mesh were performed for zooplankton sampling. Zooplankton was fixed in 10% formaldehyde in filtered seawater and observed using a stereomicroscope (Nikon SMZ800) for identification. Furthermore, qualitative abundance was noted for each taxon.

Histology. Specimens were measured and analysed by histology to establish their status regarding marteiliosis. Other parasites and proliferative diseases observed were also recorded. Samples were placed in 10% formalin in sterile seawater, and, after dehydration and paraffin embedding, tissues were cut 3 µm thick and stained with haematoxylin and eosin (H&E). Slides were examined under light microscopy (Carl Zeiss, Axiostar 34241).

Molecular biology. To study the types of *Marteilia* present in the Ebre delta, 6 flat oysters and 3 mussels that were established to be positive by histology during the epidemiological 2003 study were used for molecular studies.

DNA extraction: Soft tissues, which had been stored at –20°C, of the positive individuals were used for DNA extraction. After grinding, samples were suspended in 10 vol of extraction buffer (NaCl 100 mM, 10 mM Tris [pH 8], 2.5 mM EDTA [pH 8], SDS 0.5%) containing Proteinase K (100 µg ml⁻¹). Following an overnight incubation at 55°C, DNA was extracted using a standard protocol with phenol/chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989).

DNA amplification and cloning: Polymerase chain reaction (PCR) analysis was performed, as described by Le Roux et al. (1999), with 2 different pairs of primers. A pair of universal primers (CS1/CAS1—eukaryotic species, including *Marteilia* spp.) was used as an internal control of DNA quality. A second pair, Pr4/5, was specific for *Marteilia* species ITS1 (Le Roux et al. 2001). PCR reactions were carried out according to the standard conditions for Silver-star *Taq* polymerase (Eurogenetec). After a denaturation step at 94°C for 5 min, 30 cycles were run with a PTC-100™ thermocycler (MJ Research) as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min and elongation at 72 °C for 1 min. A final elongation step at 72°C for 10 min was performed. Amplified products

Table 1. Prevalence of *Marteilia* spp. in bivalve cultures (*Mytilus galloprovincialis*, *Ostrea edulis*, *Crassostrea gigas*) representative of commercial production in Alfacs Bay in 2003. Table also shows the spat origin. A: Alfacs; I: Italy; Fr: France; -: not sampled

Sampling date (2003)	Mussel A (%)	Flat oyster I (%)	Flat oyster Fr + I (%)	Pacific oyster Fr (%)
Feb	–	–	–	–
Mar	–	0	–	–
May	1.67	0	1.67	0
Jul	0	5	5	0
Nov	–	3.34	–	–

Table 2. Prevalence of *Marteilia* spp. in bivalve cultures (*Mytilus galloprovincialis*, *Ostrea edulis*, *Crassostrea gigas*) representative of commercial production in Fangar Bay in 2003. Table also shows the spat origin. A: Alfacs; F: Fangar; M: Maresme; I: Italy; Fr: France; -: not sampled

Sampling date (2003)	Mussel F+A (%)	Mussel M (%)	Flat oyster I (%)	Flat oyster Fr + I (%)	Pacific oyster Fr (%)
Feb	0	3.34	–	–	–
Mar	–	–	0	–	–
May	0	5	0	–	–
Aug	0	5	1.67	1.67	0
Nov	–	–	16.67	–	–

were analysed electrophoretically on 1% agarose gels and cloned using the pCR 2.1 Vector System (Invitrogen). Minipreps of recombinant plasmids were prepared according to standard alkaline lysis protocols with an additional phenol/chloroform extraction and ethanol precipitation (Sambrook et al. 1989).

Restriction-fragment length polymorphisms (RFLP): PCR was performed with Primer Pair 4/5, and, as template, DNA was extracted from recombinant plasmids (10 to 15 cloned amplicons for each *Marteilia* spp. infected animal). Polymorphisms among the PCR products were identified by *Hha*I enzymatic digestion (Promega) as described in Le Roux et al. (2001). The resulting restriction fragments were analysed electrophoretically on 2% agarose gels.

RESULTS

Epidemiological studies

2003 study

Detection of *Marteilia* spp. was sporadic in flat oysters *Ostrea edulis* and mussels *Mytilus galloprovincialis* from both bays, whereas no Pacific cupped oysters *Crassostrea gigas* were found to be infected. Recorded prevalence values mostly ranged between 1 and 3 out of 60 individuals (1.67 to 5%) (Tables 1 & 2). Uninfected flat oysters from the Italian Adriatic coast became infected in both bays in July/August. Important differences were observed in November, when 10 out of 60 flat oysters (16.67%) were parasitized in Fangar Bay, whereas, in Alfacs Bay, 2 out of 60 individuals (3.34%) were infected (Fig. 2). Other parasites such as the ciliates *Ancistrocoma* spp. (in 1 individual in the Alfacs November sample), the copepods *Myticola* spp. (in 1 out of 60 individuals in the Fangar November sample) and the trematodes *Bucephalus haimeanus* (between 2 and 10 out of 60 parasitized individuals in the Alfacs and in 1 out of 60 individuals in the Fangar

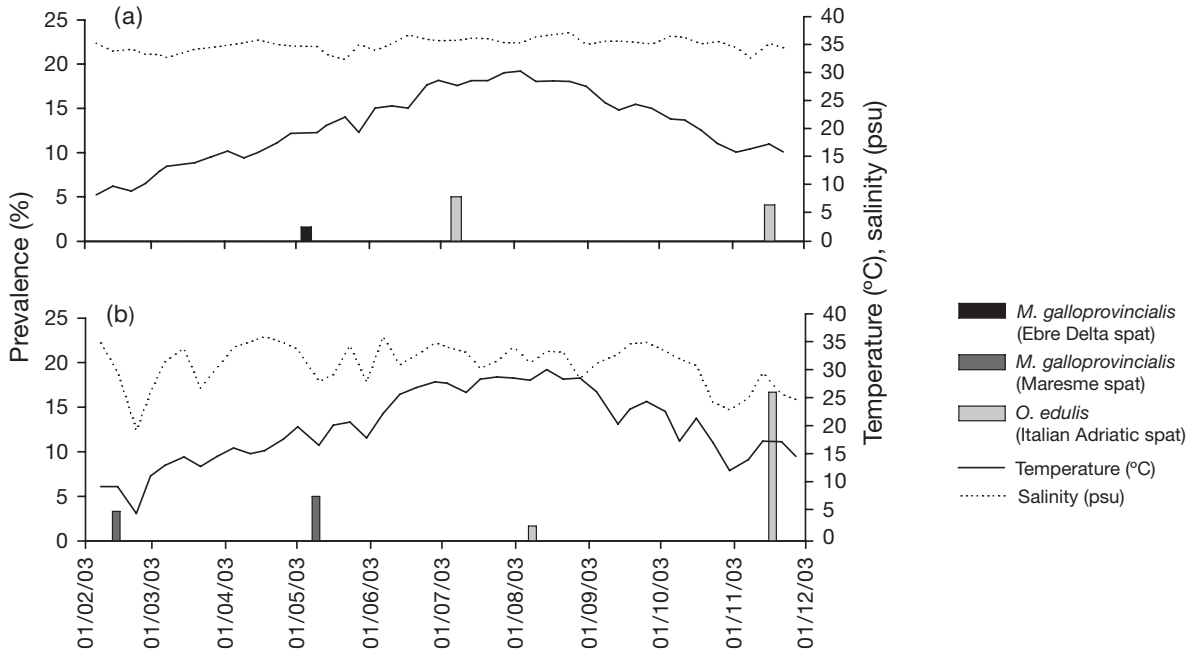


Fig. 2. *Marteilia* spp. prevalence (%) in the cultivated bivalves (*Mytilus galloprovincialis*, *Ostrea edulis*, *Crassostrea gigas*) from the Ebre River delta in 2003: (a) in Alfacs Bay and (b) in Fangar Bay. Complementary environmental data: temperature (°C) and salinity (psu). Dates given as d/mo/yr

sample) were also detected in *Ostrea edulis* samples. These parasites were not present in mussel or Pacific oyster samples during the same period. Due to unusually high temperatures (>28°C), mussels showed 100% mortality in mid-June in Alfacs Bay and in mid-August in Fangar Bay. New mussel spat, of different origins, were introduced for producers using the bays of the Ebre River delta, to yield a subsequent culture crop.

2003/2004 study

In October 2003, before the experimental ropes were submerged in the bays, a preliminary screening was carried out to assess the status of the different stocks. No infected individuals were detected for Adriatic Ital-

Table 3. Prevalence of *Marteilia* spp. in bivalve cultures (*Mytilus galloprovincialis*, *Ostrea edulis*) representative of commercial production in Alfacs Bay in 2003/2004. Table also shows the spat origin. I: Italy; Fr: France; G: Galicia, Spain; -: not sampled

Sampling date	Mussel		Flat oyster G (%)
	I (%)	Fr (%)	
Oct 2003	0	6.67	-
Feb 2004	0.60	18.49	-
Mar 2004	-	-	0
May 2004	0.60	20.60	0
Jul 2004	3.30	15.21	-

ian mussels, whereas 6.67% of the French mussels were infected (Tables 3 & 4). Prevalence data for both the *Mytilus galloprovincialis* samples (French and Italian origin) are shown in Fig. 3 for both bays (a and b/c). Additional samples were taken in Fangar Bay between May and September 2004 for the mussel of French origin, to assess the infection period more accurately (Fig. 3c). Only 1 out of 150 (0.60%) Adriatic Italian mussels cultured in Alfacs Bay was parasitized in February and May. This prevalence increased to 3.34% in July. In Fangar Bay, no infected individuals could be detected in October, February or May, whereas 13.34% of the individuals were found parasitized in August.

Table 4. Prevalence of *Marteilia* spp. in bivalve cultures (*Mytilus galloprovincialis*, *Ostrea edulis*) representative of commercial production in Fangar Bay in 2003/2004. Table also shows the spat origin. I: Italy; Fr: France; G: Galicia, Spain; -: not sampled

Sampling date	Mussel		Flat oyster G (%)
	I (%)	Fr (%)	
Oct 2003	0	6.67	-
Feb 2004	0	14	-
Mar 2004	-	-	0
May 2004	0	9.30	0
Jun 2004	-	18.67	0
Jul 2004	-	12	-
Aug 2004	13.34	18	-

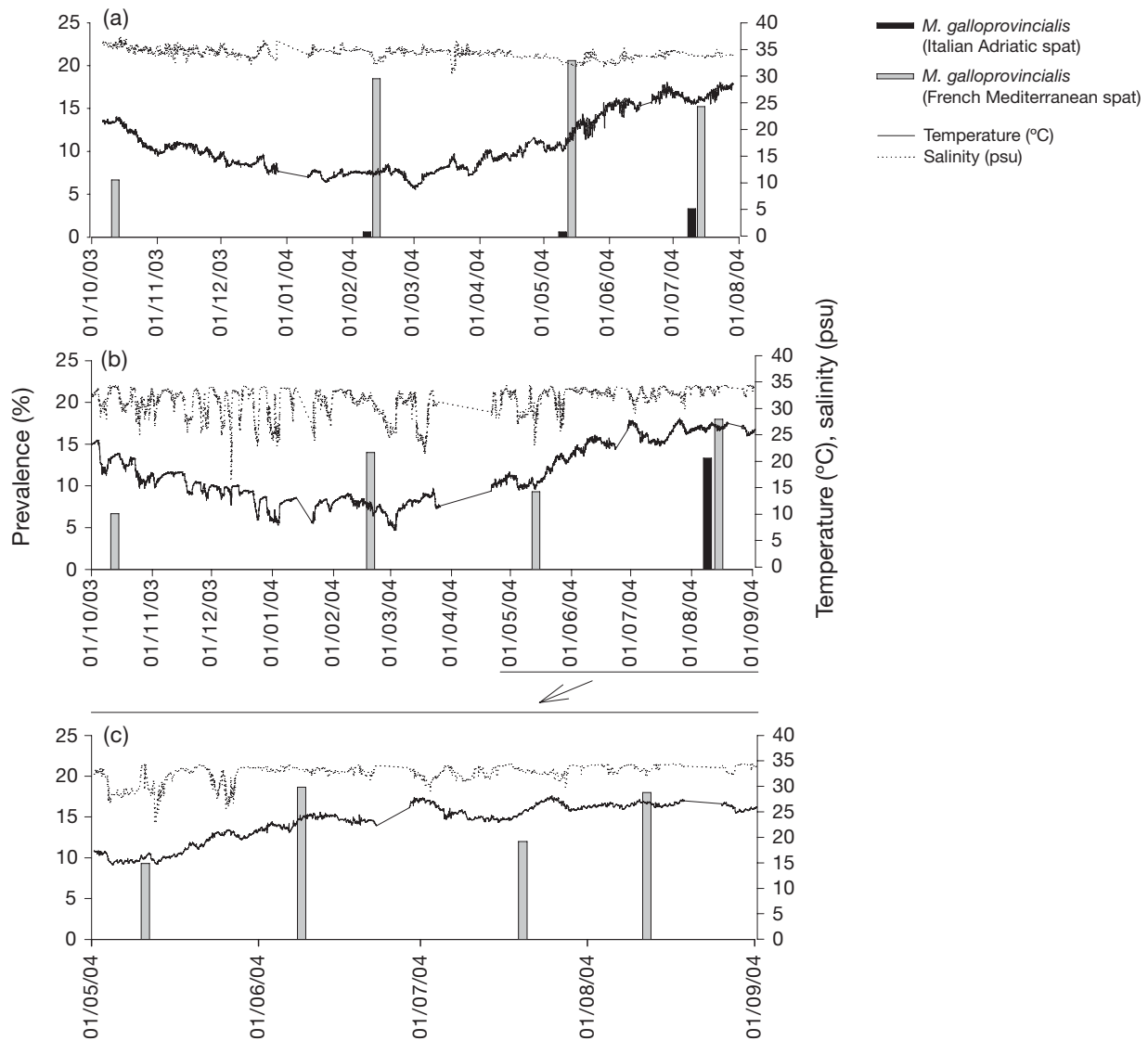


Fig. 3. *Marteilia* spp. prevalence (%) in the cultivated bivalves (*Mytilus galloprovincialis*, *Ostrea edulis*, *Crassostrea gigas*) from the Ebre River delta in 2003/2004 period: (a) in Alfacs Bay, (b) in Fangar Bay and (c) in Fangar Bay for French spat mussel during late spring and summer. Complementary environmental data: temperature (°C) and salinity (psu). Dates are d/mo/yr

In June, massive mortalities (100%) of *Ostrea edulis* of unknown cause occurred in both bays. Therefore, prevalence data were not available for the entire duration of the experiment. However, data collected in May and June did not show the presence of *Marteilia* spp. in flat oysters. None of the *Crassostrea gigas* samples were analysed after the 2003 negative results. Other parasites such as the ciliates *Ancistrocoma* spp., the trematodes *Protoeces* spp. and rickettsias were also detected in mussels. For *Mytilus galloprovincialis* of French origin, parasite prevalence was 2.67%, for both trematodes and ciliates, in the October sample, before the mussels were immersed in the bays. Throughout the year, the 3 types of parasites

(ciliates, trematodes and rickettsias) were sporadically detected, with prevalence ranging between 0.60 and 2.67%. In addition, cases of proliferative disease recorded as neoplasia were noted in 3.34% of the individuals of the Alfacs May sample. For the mussels of Italian origin, no parasites were noted in October pre-immersion samples. However, ciliates *Ancistrocoma* spp. and trematodes *Protoeces* spp. were detected, with a prevalence of 2% for both parasites in the Fangar February sample, and rickettsias were detected, with a 0.60% of prevalence in the Alfacs May sample. Neoplasia was also detected in 1 (out of 150 individuals) mussel in the Fangar June sample. For flat oysters, parasites such as ciliates *Ancistro-*

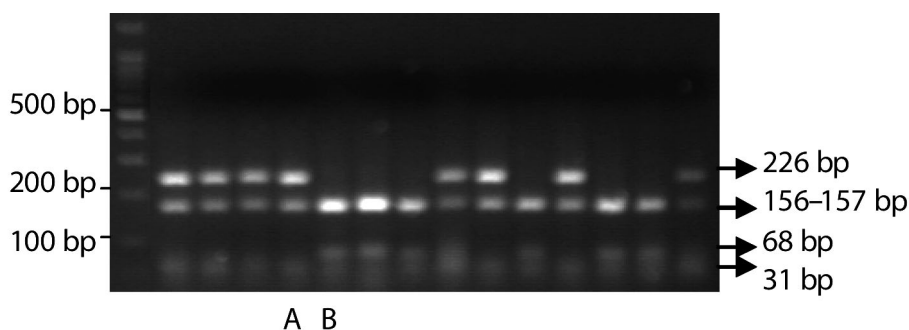


Fig. 4. *Marteilia* spp. Co-infected flat oyster *Ostrea edulis*. Electrophoresis gel of *HhaI* PCR-RFLP on partial ITS *Marteilia* spp. Lane A corresponds to the Type 'O' profile (3 restriction fragments: 226, 156 and 31 base pairs) and Lane B corresponds to the Type 'M' profile (4 restriction fragments: 156, 157, 68 and 31 base pairs)

coma spp., copepods *Myticola* spp. and rickettsias were also detected in the Fangar June samples, with higher prevalence rates (9.33, 5.33 and 6.66%, respectively). In Alfacs Bay, these parasites were not detected in *O. edulis* during the same period.

Marteilia spp. type study

All the amplicons (14 to 15 amplicons ind.⁻¹) analysed by PCR-RFLP from 3 of the *Marteilia*-infected mussels in 2003, showed a *M. maurini* profile according to the definition of Le Roux et al. (2001). However, from the 6 flat oysters analysed by PCR-RFLP, 1 oyster from Alfacs Bay led to the detection of both types, with 8 amplicons of *M. maurini* profile and 6 amplicons of *M. refringens* profile (Fig. 4).

Zooplankton study

In the summer of 2004, both copepod abundance and species diversity in the bays were low. However, in October 2003 samples, the number of species and

number of individuals were higher, with major groups of copepods (Calanoida, Cyclopoida and Harpacticoida) being represented. The study of calanoid copepods revealed the presence of 2 main *Acartia* species in these samples; the most abundant one was *A. latisetosa*, and, in lower abundance, *A. grani* (*Paracartia grani*) was also present. Some representatives of *A. clausi* were also observed (Table 5). In relation to other copepod groups, Cyclopoida were mainly represented by the genus *Oithona*, and this taxon was also very abundant. Harpatocoids were less abundant. Regarding the summer 2004 samples, only a few samples, from Alfacs Bay, contained copepod individuals. In these samples, *A. latisetosa* was the most abundant copepod, with some *A. grani* being detected. A number of unidentified copepodite stages, crustacean larvae, bivalve larvae and gastropod larvae were also well represented in some of the samples during the same periods.

DISCUSSION

The Ebre delta is the first Spanish shellfish production site on the Mediterranean coast. The production of bivalves is estimated at 2657 metric tons per year (2004, www.gencat.net/darp). Major cultivated species are the mussels *Mytilus galloprovincialis* (1700 t yr⁻¹), Pacific oysters *Crassostrea gigas* (854 t yr⁻¹) and Japanese carpet clams *Ruditapes philippinarum* (94 t yr⁻¹). In the past, European flat oyster *Ostrea edulis* culture was also carried out in the bays of the Ebre River delta, but several mortality outbreaks in the 1990s led farmers to replace this species with *C. gigas*. Oyster and mussel culture is traditionally achieved on ropes tied to wooden frames, also called 'bateas'.

From the preliminary data we obtained during our study, the bays of the Ebre Rive delta appear to be provide an interesting natural environmental model to

Table 5. Qualitative abundance of different copepod taxa in the bays of the Ebre River delta in autumn (Au) 2003 and summer (Su) 2004. +++: very abundant; ++: common; +: rare

Taxon	Abundance	Season
Calanoida		
<i>Acartia latisetosa</i>	+++	Au, Su
<i>Acartia grani</i>	+	Au, Su
<i>Acartia clausi</i>	+	Au
Cyclopoida		
<i>Oithona</i> sp.	+++	Au
Harpacticoida		
Indeterminate species	++	Au

study the dynamics of *Marteilia* species. The cycle of the parasite was completed in the bays, as demonstrated by the infection of uninfected mussels and flat oysters. From this point of view, our results are similar to those previously published by Audemard et al. (2004), with parasite transmission occurring between June and August, when water temperatures are $>17^{\circ}\text{C}$.

Marteilia species were detected in both *Mytilus galloprovincialis* and *Ostrea edulis* from Alfacs and Fangar Bays. However, they were not reported in *Crasostrea gigas*. The presence of *M. refringens* in Pacific oyster *C. gigas* has previously been reported, but is currently considered as a peculiar and unusual location of the parasite in filter-feeding organisms (Berthe et al. 2004).

Marteilia was detected in mussels all year round. However, the infection of uninfected mussels shows a clear seasonal window, for 2004, between May and July/August, when temperatures are $>17^{\circ}\text{C}$, as occurs in flat oysters (Balouet 1977, Audemard et al. 2001). New infections probably began to occur in late May and June, just after temperatures rose to $>17^{\circ}\text{C}$ (Grizel 1985). However, the high prevalence rates recorded in February, when temperatures are minimal, is not well understood. This phenomenon has also been observed by other investigators (Villalba et al. 1993). Significant and sudden changes in temperatures (like thermal shocks) may trigger some erratic or sporadic event of parasite activation in winter. This is probably true when considering the temperature variations recorded in our model (see Fig. 3).

This may lead to new considerations on parasite transmission, such as a possible mechanism of parasite multiplication, with limited dispersion (no new ropes becoming infected) being activated by changes in cold temperature regimes. Or it may be that transmission exists throughout the year, because mature sporangia occur in all seasons, but different types of intermediate hosts are involved. Another possibility would also be that direct, short-distance transmission occurs (for example, through faeces that may be shed in the close vicinity of individuals belonging to the same rope) in winter time and indirect, long-distance transmission in summer time. By monitoring the dynamics of infection on a monthly basis throughout the year and more frequently during the critical periods, new information on the dynamics of *Marteilia* can be gained.

Results of our *Marteilia* type study indicate that, generally, *M. maurini* would be found in mussels, while *M. refringens* would be found in oysters, but lack of strict host affinity is evident in our data and has also been shown in previous studies (Le Roux et al. 2001, Lopez-Flores et al. 2004, Novoa et al. 2005). It may be hypoth-

esised that in specific situations of high parasitic loads in the environment, a predominant type could infect both flat oysters and mussels. That is what could have happened in the co-infected oysters in the present study. Alfacs Bay supports a higher mussel than flat oyster density, and the possibility of cross-infection occurring (from mussels to flat oysters) is probably high. It is not clear if the types of *Marteilia* are conspecific or not, and controversial opinions circulate among authors regarding the co-infection. On the other hand, the frequency of detection may vary with the period of the year, and more attention should be given to temporal scaling of the phenomenon.

Strictly speaking, what is usually interpreted as co-infection is rather co-detection, as PCR detection gives no data are available on the actual infection status. Viability and development studies of the *Marteilia refringens* type in mussels and the *M. maurini* type in flat oysters would be interesting. Unfortunately, the typing techniques currently available do not permit a topographical analysis equivalent to *in situ* hybridisation or immunohistochemistry. This would be of utmost interest in relation to the risks of transfers of infected animals between geographical regions. Knowledge of the complete cycle of *Marteilia* spp. would also facilitate experimental cross-infections in the laboratory, to acquire further information for new legislation designed to prevent transfers of the parasite.

Paracartia grani is considered a potential intermediate host of *Marteilia refringens* (Audemard et al. 2002), and is present in the both bays. However, this species is not one of the most abundant calanoid copepods in the bays, in contrast with its role in the claire pond model (Audemard et al. 2001). Other species, including *Acartia latisetosa*, are more abundant during spring, summer and fall, and could constitute better candidates for intermediate hosts in *Marteilia* cycle. Further investigations of these 2 species and other potential intermediate/alternate hosts will be required to analyse the presence of *Marteilia* in the zooplankton. Furthermore, experimental comparative infection of *P. grani* by *Marteilia* spp. from mussels and from oysters could provide interesting information regarding the potential involvement of different copepod species in the cycle of the parasite according to the bivalve host species and *Marteilia* type.

The Ebre River delta presents perfect qualities to study the dynamics of *Marteilia* species in a natural environment. The characteristics are similar to those of the claire pond model, at a higher scale of biodiversity and hydrodynamics. This should allow us to study the infection dynamics of the parasite in mussels, as well as in intermediate hosts, in order to further clarify aspects of the life cycle of *Marteilia* and eventually resolve the remaining enigmas of this parasite.

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