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Infection dynamics of *Marteilia refringens* in flat oyster *Ostrea edulis* and copepod *Paracartia grani* in a claire pond of Marennes-Oléron Bay

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ABSTRACT: The protozoan parasite *Marteilia refringens* has been partly responsible for the severe decrease in the production of the European flat oyster *Ostrea edulis* Linnaeus in France since the 1970s. The calanoid copepod *Paracartia grani* Sars was recently found to be a host for *M. refringens* in French shallow-water oyster ponds ('claires'). This study reconsidered *M. refringens* transmission dynamics in the light of this finding, taking into account not only oyster infection dynamics and environmental factors but also data concerning the copepod host. *P. grani* population dynamics in the claire under study revealed that this species is the dominant planktonic copepod in this confined ecosystem. During winter, *M. refringens* overwintered in *O. edulis*, with *P. grani* existing only as resting eggs in the sediment. The increase in temperature in spring controlled and synchronized both the release of *M. refringens* sporangia in the oyster feces, and the hatching of the benthic resting eggs of the copepod. Infection of oysters by *M. refringens* was limited to June, July and August, coinciding with (1) the highest temperature recorded in the claire, and (2) the highest abundance of *P. grani*. PCR detection of *M. refringens* in *P. grani* during the summer period was linked to the release of parasite sporangia by the oyster. Our results are supported by previous results on the effective transmission of this parasite from the oyster to the copepod.

KEY WORDS: *Marteilia refringens* · *Ostrea edulis* · *Paracartia grani* · Infection dynamics · Claire pond

INTRODUCTION

Marteilia refringens (Grizel et al. 1974) is a major pathogen of the European flat oyster *Ostrea edulis* Linnaeus. Since the early 1970s, it has been responsible for important oyster mortalities and economic losses in the European oyster industry (Alderman 1979, Grizel 1985, Figueras & Montes 1988). Recently, Audemard et al. (2002) demonstrated that the planktonic copepod *Paracartia grani* Sars is a host of *M. refringens*. This result was obtained by overcoming 2 major impediments in studying the *M. refringens* life cycle (Balouet et al. 1979, Grizel 1985): (1) the considerable number of potential host species present in enzootic zones, and (2) the lack of appropriate tools for detecting the parasite regardless of host species and developmental stage. The problem of species richness was circumvented by choosing shallow-water oyster ponds ('claires') in the Marennes-Oléron Bay (SW France). *M. refringens* transmission to oysters in claire ponds

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was previously demonstrated by Berthe et al. (1998). Claire ponds were also shown to contain fewer than 100 recognizable species, including macrofauna, meiofauna, and zooplankton (Reymond 1991, Audemard et al. 2001), compared to more than 1000 on the open shore and coast Marennes-Oléron Bay (Bodin 1972, Sautour 1991, de Montaudouin & Sauriau 2000). DNA-based techniques are the most appropriate tools for detecting the parasite among potential hosts because they are independent of both developmental stage of the parasite and its host-species location (Mialhe et al. 1995). Based on the sequence of the small subunit ribosomal DNA (SSU rDNA) of *M. refringens*, specific polymerase chain reaction (PCR) and *in situ* hybridization assays were developed (Le Roux et al. 1999, Berthe et al. 2000).

In *Ostrea edulis*, *Marteilia refringens* infects the digestive gland (Grizel et al. 1974), and in *Paracartia grani* it infects the ovarian tissue (Audemard et al. 2002). Transmission experiments from *O. edulis* to *P. grani* under laboratory conditions were successful, demonstrating that the 2 species are contiguous in the life cycle of the parasite (Audemard et al. 2002). *P. grani* inhabits coastal, semi-confined ecosystems along the tropical and temperate Atlantic coast. As firstly noted by Sars (1904), this copepod is often observed near oyster beds and has been reported during spring/summer seasons in estuaries and bays that are within the geographical range of the disease (Lubet 1953, Paulmier 1965, Le Fèvre-Lehoërff 1971, Gallo 1981, Alcaraz 1983, d'Elbée & Castel 1991, 1995, Siokou-Frangou et al. 1998, C. Razouls & F. de Bovée 1999: www.obs-banyuls.fr/Copepodes/RAZOULS1.htm)).

Marteilia refringens transmission and infection of oysters has been documented relative to oyster host and environmental parameters (Balouet 1979, Grizel 1985). The aim of the present study was to reconsider *M. refringens* transmission and seasonality in the light of the new data concerning its life cycle. Based on a 2 yr study within a claire, the parasite dynamics were documented by measurement of parasite intensity and infection period for the flat oyster, and by detection of the parasite in the copepod. The zooplankton community of the claire was studied in terms of species richness and abundance, with particular emphasis on *Paracartia grani* and other Acartidae present in this semi-enclosed ecosystem.

MATERIALS AND METHODS

Sampling strategies within the claire. This study was carried out in 1998 and 1999 in a claire pond from the CREAA (Centre régional d'expérimentation et d'application aquacole) aquaculture facilities located on the eastern coast of the Oléron island, Marennes-Oléron Bay, SW Atlantic coast of France (see Audemard et al. 2001 for details). Claires are shallow-water ponds traditionally used for oyster culture and final conditioning in this area (Goulletquer & Héral 1997). They communicate with the open bay through channels, and function as an open system, with exchange of water occuring for a few days at spring tide. During neap tides, they function as a closed system (see Fig. 1). Claires were originally chosen as a study model for the life cycle parasite *Marteilia refringens* because in these ponds (1) the parasite is enzootic and its transmission effective (Berthe et al. 1998), and (2) species richness is low (Audemard et al. 2001).

During both study years, temperature was recorded in the claire and the presence of *Marteilia refringens* in *Paracartia grani* was documented using PCR. Water temperature was measured automatically every 15 min with an ONSET probe located 10 cm above the bottom, and the mean temperature was calculated for each day. In 1998, the pond was drained completely once a month to sample benthic species which were analyzed for *M. refringens* presence (Audemard et al. 2001). After sampling, the claire was refilled by bay-water inflow through the channels. The dates of draining were 24 February, 31 March, 29 April, 28 May, 26 June, 27 July, 26 August, 24 September, 2 November and 3 December (see Fig. 1a). During 1998, we studied the intensity of parasitism in oysters and zooplankton population dynamics. In 1999, we documented the infection period of the oysters.

Dynamics of *Marteilia refringens* in *Ostrea edulis*. *Parasite intensity:* From February to November 1998, *Marteilia refringens* intensity was determined in the claire in 2 batches of flat oysters (Batches 97 and 98). Batch 97 consisted of naïve individuals spawned in 1996 and held in plastic mesh bags directly on the bottom of the claire from July 1997 onwards. During summer 1997 they became naturally infected in the claire, although no infected flat oysters were present, as demonstrated previously (Trial 10 in Berthe et al. 1998). Batch 98 consisted of naive oysters spawned in 1997 and placed in the claire at the beginning of the experiment in February 1998. They were held in plastic mesh bags randomly placed directly on the bottom of the claire.

We sampled 30 oysters each from Batches 97 and 98 every 3 wk from February to November 1998 by randomly selecting individuals from the plastic mesh bags. The soft tissues of the oysters were cut along a sagittal plane and placed in Davidson's fixative AFA (10% glycerin, 20% formalin, 30% 95 ethanol, 30% dH_2O, 10% glacial acetic acid). The samples were subsequently treated by conventional histological procedures. Cut sections were 2 µm thick (standard procedure at the IFREMER laboratory, La Tremblade), and

were stained with hematoxylin and eosin. On each sampling date, the intensity of infection of each sampled oyster was estimated by counting the number of parasites under a light microscope in 5 randomly chosen microscopic fields (magnification 1000×). This allowed statistical comparisons of the different levels of variation in parasite intensity (between dates, between oysters of a given date, and within an oyster) through the use of a mixed-model nested ANOVA (Sokal & Rohlf 1981). The design, which included date as a fixed factor and oyster as a random factor with 5 replicated measurements per oyster, was unavoidably unbalanced (unequal number of infested oysters per date). Since there are no exact tests of significance in the case of an unbalanced nested ANOVA (see Sokal & Rohlf 1981), the analysis was restricted to a subset of dates with a common number of infected oysters. We used 7 out of 10 available dates with at least 15 infected oysters; in the case of higher sample sizes, the 15 individuals were selected at random.

A 1-way analysis of variance with replication was also used to test the significance ($\alpha = 0.05$) of date-to-date variation in the whole data set (10 and 8 dates for Batches 97 and 98, respectively). The parameter tested was the mean parasite intensity per infected oyster. The balanced ANOVA procedure of the MINITAB (Release 10.2) package was used, and prior to each analysis, data on the number of parasites was square-root-transformed to insure homogeneity of variances (Sokal & Rohlf 1981).

Infection period in flat oysters: We determined the infection period from March 1999 to January 2000 (Table 1). Dual sets of experiments were designed based on the use of the claire as an enzootic zone, and the laboratory as a *Marteilia refringens*-free environment. Independent batches of 30 *M. refringens*-free oysters spawned in 1998 were successively held in the claire for 1 mo from March to September, and then replaced in *M. refringens*-free conditions in the laboratory in separate tanks. An extra batch of 30 *M. refringens*-free flat oysters was held in the claire from mid-October 1999 to the end of January 2000 to assess the absence of infection during the winter period, as previously reparted by Grizel (1985).

Table 1. Determination of *Marteilia refringens* (*M.r.*) infection period in *Ostrea edulis* performed from March 1999 to January 2000. Analysis of infection in oysters deployed monthly in the pond and number of days/nights (D/N) when minimum temperature was higher than 17°C. nd: no data

Trial No.	Month(s)	*M.r.* in oysters	D/N >17°C
1	Mar 1999	No	0
2	Apr 1999	No	1
3	May 1999	No	19
4	Jun 1999	Yes	28
5	Jul 1999	Yes	31
6	Aug 1999	Yes	31
7	Sep 1999	No	24
8	Oct 1999 to Jan 2000	No	nd

We held 1 batch of 30 *Marteilia refringens*-free flat oysters in the claire from March to the end of the experiment as a positive control, and a last batch was held in the laboratory in a separate tank to control for the absence of parasite transmission under laboratory conditions (negative control). Water temperature in the claire as well as in laboratory tanks was continually recorded.

In October, the first 7 batches reared in the laboratory as well as the controls were screened for *Marteilia refringens* presence using both digestive gland imprints and histology. The final batch exposed in the claire in October 1999 was analyzed in February 2000.

To prepare digestive gland imprints, pieces of tissue (3 mm in section) were cut from the digestive gland and excess water was removed on blotting paper. The sections were dabbed onto glass slides. The slides were dried in air and fixed in methanol for 2 to 3 min. The tissue imprints were stained using a commercially available staining kit (Hemacolor, Merck) according to the manufacturer's instructions. After staining, the slides were rinsed under tap water and dried completely before mounting in a synthetic resin. An observation time of 5 mn per slide was used with high magnification.

Richness and abundance of zooplankton species in the claire. In 1998, quantitative sampling of the zooplankton was performed weekly by filtering 400 l of claire pond water through a 100 µm mesh. When the complete draining of the claire fell on the same date, the zooplankton was sampled before draining the pond. Animals retained were preserved in absolute ethanol and later identified to species level, sorted and counted.

Presence of *Marteilia refringens* in *Paracartia grani*. In 1998 and 1999, the presence or absence of *Marteilia refringens* in pools of >30 *Paracartia grani* was documented using PCR with primers specific for *M. refringens*. In 1998, sampling was performed from mid-May to the beginning of September and resulted in 8 samples containing *P. grani*. In 1999, sampling was performed from mid-June to the end of October and resulted in a total of 12 samples with *P. grani* (see Fig. 1). Protocols have been described in detail by Le Roux et al. (1999). In short, tissue from >30 *Paracartia grani* was pooled and suspended in 10 vol of extraction buffer (NaCl 100 mM, EDTA 25 mM, pH 8, sodium dodecyl sulfate 0.5%) containing Proteinase K (100 µg ml). Following overnight incubation at 50°C, DNA was extracted with phenol/chloroform, and precipitated

with ethanol. PCR was performed as described by Le Roux et al. (1999) with 2 different primer sets: (1) CS1/CAS1, had sequences shared by 50 eukaryotic species including *Marteilia refringens* and served as a 'universal' primer providing an internal control of amplification experiments; (2) SS2/SAS1 was specific for *M. refringens* 18S rDNA (Le Roux et al. 1999, Berthe et al. 2000). The volume of the PCR reaction was 50 µl comprising approximately 10 ng of purified DNA mixed with 5 µl of 10× PCR buffer, 5 µl of 25 mM $MgCl_2$, 5 µl of dNTP at 2 mM, 0.5 µl of each primer at 100 µM, and 0.25 µl (1 U) of *Taq* DNA polymerase (Promega). Samples were overlaid with mineral oil, denatured for 5 min at 94°C and amplified for 30 cycles: 1 min at 94°C for denaturation, 1 min at 55°C for primer annealing, and 1 min at 72°C for elongation in a thermal cycler apparatus (Appligene). Polymerization at 72°C was then extended for 10 min to ensure completion of the amplified products. Products were electrophoresed on 1% agarose (in 1× tris borate EDTA) gels, stained with ethidium bromide and then visualized using UV light.

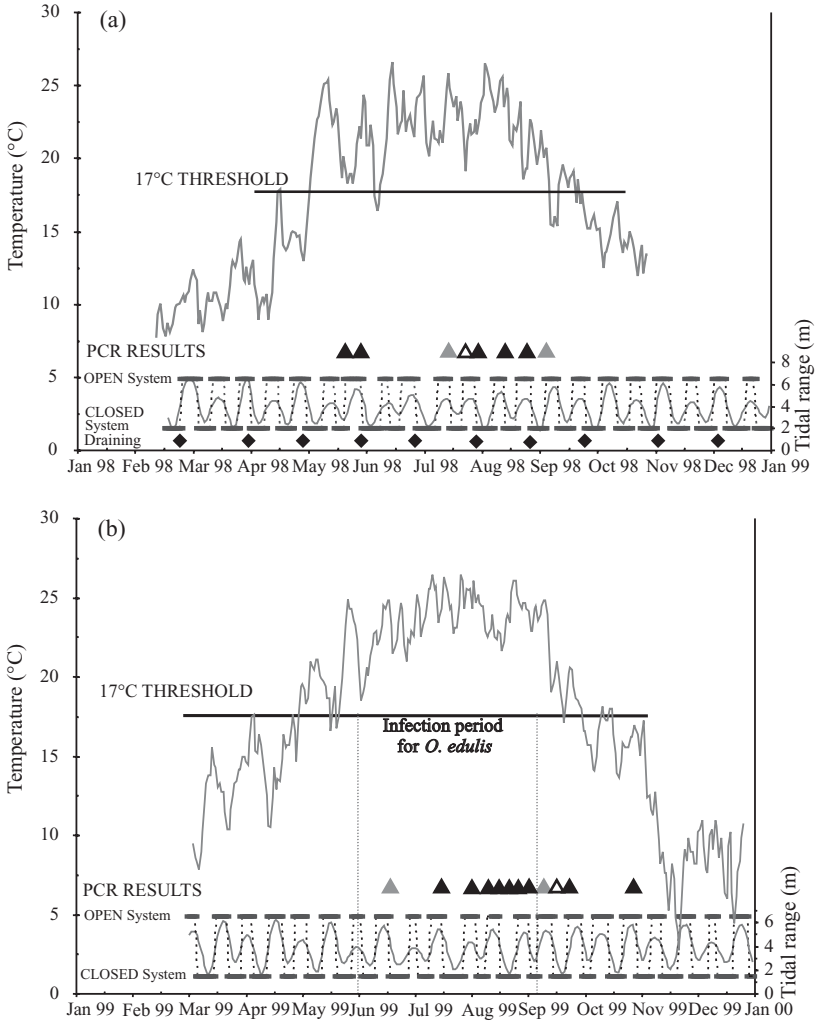

Fig. 1. Environmental parameters in claire pond in (a) 1998 and (b) 1999, showing day-to-day changes in mean temperature, tidal range and water management (system open/closed to fresh marine-water inputs, draining dates). Results of PCR for *Paracartia grani* are also given: (Δ) no PCR amplification obtained using conserved primers; (▲) PCR amplification obtained but no *Marteilia refringens* detected; (▲) *M. refringens'* DNA detected

RESULTS

Environmental parameters in claire and experimental tanks

In 1998, water temperatures ranged from 8°C in February to 29°C early July (Fig. 1a). Mean values were lower than 15°C until mid-April, and higher than 17°C from May to late-September.

In 1999 in the claire, water temperature ranged from 8 to 10°C in March to 29°C at the end of July (Fig. 1b). Temperature exceeded 17°C from the end of March to late-October. Temperature lower than 3°C was recorded in mid-November. Temperature exceeding 17°C in experimental tanks, was re-corded from mid-May to late-September.

Dynamics of *Marteilia refringens* in *Ostrea edulis*

Parasite intensity

The bulk of the variation in parasitism intensity comprized variations among individuals, i.e. 50 and 60% for Batches 97 and 98, respectively. The contribution to total variation among the 5 replicate measurements within 1 oyster represented ca. 33% for both batches. Despite these significant levels of variability within and between individuals (Fig. 2), seasonal variability was highly significant ($p < 0.001$ for both batches). Intensity of infection increased 5-fold in Batch 97 from 24 February to 29 April, then remained approximately stable until the summer due to a large variability in parasite

intensity among individuals, as demonstrated by the standard deviation (Fig. 2). *Marteilia refringens*-free flat oysters (Batch 98) remained uninfected until mid-June 1998. During summer, a significant increase in parasite intensity was observed in both Batches 97 and 98. The remaining oysters from Batch 97 died after 22 July 1998, when the intensity of infection was 7 times higher than in February. This was confirmed by an *a posteriori* Newman-Keuls test, indicating that the lowest mean values of parasitism intensity were recorded in February and March (Batch 97), and the highest mean values in April (Batch 97) and July (both batches).

Infection period in flat oysters

All positive-control oysters held in the claire from March to October 1999 became infected, yet no negative-control oysters held in the laboratory conditions did so. In experimental samples, *Marteilia refringens* was only detected in June, July and August (Table 1). Minimum temperatures exceeded 17°C on more than 28 d during each of these months (Table 1, Fig. 1b).

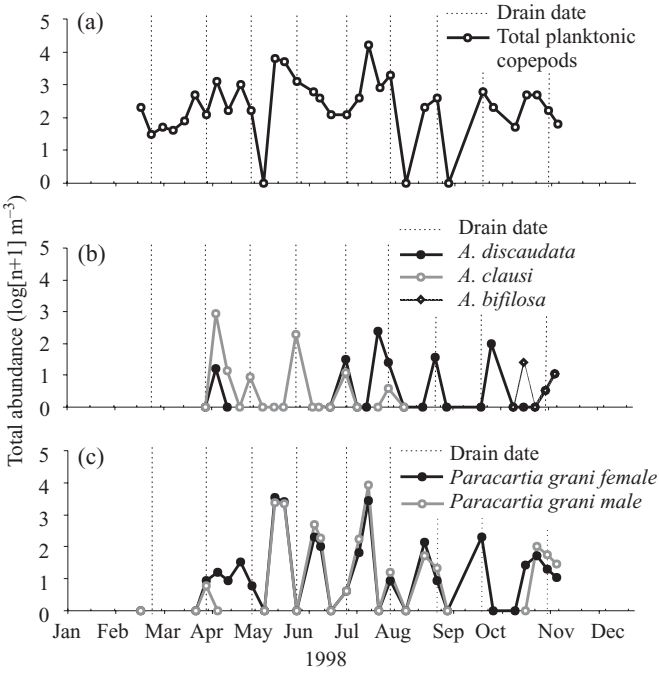

Fig. 3. Zooplankton dynamics in claire pond, showing abundance of (a) total planktonic copepods, (b) Acartidae, (c) male and female of *Paracartia grani*. Dotted vertical lines: dates when pond was drained

Zooplankton in the claire

The planktonic copepod community of the claire pond comprized the calanoid copepods *Acartia bifilosa* (Giesbrecht), *A. clausi* Giesbrecht, *A. discaudata* (Giesbrecht), *Centropages hamatus* (Lilljeborg), *Eurytemora pacifica* Sato, *Paracartia grani* GO. Sars and *Temora longicornis* (Müller). *E. pacifica* is an alien species, first recorded for European waters in the Marennes-Oléron Bay in 1985 (Goulletquer et al. 2002). The harpacticoid *Euterpina acutifrons* (Dana) was also identified and, in addition, there was a group of cyclopoid copepods not identified in the course of this study (see Audemard et al. 2001 of detail on other species).

No copepods were observed in the samples collected on 7 May, 6 August and 2 September (Fig. 3a). Abundance maxima were on 14 and 20 May (6050 and 4900 ind. m^{-3}, respectively) and on 13 July (17 350 ind. m^{-3}).

Acartia clausi entered the pond from oceanic waters in spring and early summer (Fig. 3b). Its abundance significantly decreased from April (855 ind. m^{-3} on 7 April, and 200 ind. m^{-3} on 28 May) to July, when no individuals were observed.

Acartia discaudata on the other hand, was abundant during the summer, with the highest value recorded on 20 July (250 ind. m^{-3}).

Acartia bifilosa was found only once in autumn (25 ind. m^{-3} on 22 October) when other *Acartia* species were less abundant.

Paracartia grani was the most abundant acartid species in the claire pond, especially during summer. On 20 May, 8 June and 18 August, *P. grani* was the only zooplanktonic copepod species

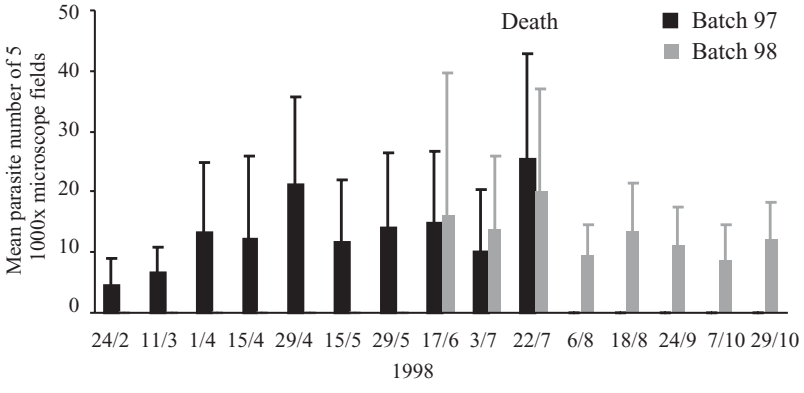

Fig. 2. *Marteilia refringens* infecting *Ostrea edulis*. Mean intensity in 2 batches of flat oysters (Batch 97 previously infected, Batch 98 uninfected) in 1998 with standard deviations. Dates are d/mo

recorded. It appeared on 31 March and was present until the end of sampling in November (Fig. 3c). *P. grani* was most abundant on 14 and 20 May (5800 and 4900 ind. m^{-3}, respectively) and 13 July (11 500 ind. m^{-3}). No males were observed from April until 14 May, when 2300 males m^{-3} were observed. Males were similarly absent from 2 September until 29 October, when 100 males m^{-3} were counted compared to 50 females m^{-3} (Fig. 3c). Female abundance was highest on 14 and 20 May (3575 and 2650 ind. m^{-3}, respectively). Male abundance was highest on 13 July (8750 ind. m^{-3} against 2750 ind. m^{-3} for females).

Marteilia refringens dynamics in *Paracartia grani*

The quality of DNA extracted from *Paracartia grani* was assessed with conserved primers CS1/CAS1 before amplification with *Marteilia refringens*-specific primers (SS2/SAS1). Extractions of samples from 20 July 1998 and 15 September 1999 yielded no amplifiable DNA.

In 1998, *Marteilia refringens* was detected on 20 and 28 May, 27 July, and 12 and 26 August, but not on 13 July or 2 September (Fig. 1a). In 1999, *M. refringens* DNA was detected on 14 and 30 July, 5, 13, 17, 25 and 31 August, 23 September and 29 October (Fig. 1b), but was not detected on 16 June or 7 September.

DISCUSSION

Claire ponds were chosen as a model for studying the life cycle of *Marteilia refringens* because the transmission of the parasite to flat oysters is effective in this ecosystem (Berthe et al. 1998) and the species richness of potential hosts is a tenth of that in intertidal areas of the Marennes-Oléron Bay (de Montaudouin & Sauriau 2000, Audemard et al. 2001). Using the claire pond model, the copepod *Paracartia grani* was revealed to be a host of *M. refringens* (Audemard et al. 2002). In the present study, we have described the dynamics of *M. refringens* transmission by considering its known hosts (*Ostrea edulis* and *P. grani*) and the environmental parameters of the claire pond.

Population dynamics of *Paracartia grani* in the claire

Among the copepod species identified during this study, *Paracartia grani* was sometimes the only species sampled. The abundance of *P. grani* in the pond was greater or equal to values recorded in other environments in bays and estuaries. The peak abundance of 11 500 ind. m^{-3} in July was comparable to values observed in Arcachon Bay (1000 to 10 000 ind. m^{-3}; Castel & Courties 1982), but was higher than that observed in the Mundaka estuary (100 to 1000 ind. m^{-3}; Villate 1991) and in Marennes-Oléron Bay (<100 ind. m^{-3}; Sautour 1991). Of the Acartidae present in the claire pond, only *P. grani* appears to be particularly adapted to this shallow-water environment. Previous studies have shown that *P. grani* is abundant in confined ecosystems characterized by variability in both physical (temperature and salinity) and biological (quality and quantity of available food) parameters (Rodríguez & Jimenez 1990, Rodríguez et al. 1995, Guerrero & Rodríguez 1998). The abundance of this species was influenced in part by the draining period of the experimental claire, for instance in early May and mid-September. The high reproductive rate of *P. grani* (Rodríguez et al. 1995) together with its physiological capabilities to react to fluctuations in food supply (Calbet & Alcaraz 1996, 1997), however, can explain the high abundance observed between draining periods <1 mo of the pond.

Claire ponds are considered to be a selective environment (i.e. an environment suitable for only certain species) because of the conditions they provide in terms of temperature range, sediment grain sizes and water-renewal cycles (de Montaudouin & Sauriau 2000, Audemard et al. 2001). Moreover, because claires are shallow ponds when closed, the environmental conditions can become more selective (less species can survive) than when bay water can enter. During the present study, the monthly draining of the pond in 1998, added another environmental disturbance. Despite the environmental singularity of these ponds, the temporal succession of the *Acartia* complex (*Paracartia grani*, *A. clausi*, *A. discaudata* and *A. bifilosa*) followed the general trends described previously for more open environments (Collins & Williams 1981, Castel & Courties 1982, Alcaraz 1983, Villate 1991, Sautour & Castel 1993). *A. clausi* is a neritic species that was observed only in spring and autumn when the claire was open, and thus when the environmental parameters were not too selective. *A. discaudata* is considered an 'intermediate' species between *P. grani*, characteristic of confined environments, and *A. clausi*, observed in neritic environments (Alcaraz 1983). *A. discaudata* was observed more frequently in the claire from spring to autumn, when the claire was open. Its abundance never exceeded 250 ind. m^{-3}, however, much lower than the 10 000 ind. m^{-3} attained by *P. grani*. The estuarine species *A. bifilosa*, observed in the Marennes-Oléron Bay in autumn (Sautour & Castel 1993), entered the claire during this season but disappeared when the claire was closed.

A previous study revealed that *Marteilia refringens* infects the ovarian tissue of *Paracartia grani* (Audemard

et al. 2002). The ovarian tissue was frequently overrun by large numbers of small *M. refringens* cells within the ovocytes. This infection may have consequences on the population dynamics of the copepod, as it could impact the reproductive processes of infected females. However, the low prevalence of the parasite (26%) in the claire (Audemard et al. 2002), the high abundance of *P. grani* documented in the present study, and the high egg production of *P. grani* (Rodríguez et al. 1995) may limit the parasite's impact on copepod population dynamics. The predominance of males over females in the claire during summer has also been observed in Malaga harbor (Rodríguez & Jímenez 1990), where *M. refringens* is not known to occur. This suggest that infection of females by *M. refringens* does not necessarily affect female abundance.

The appearance of *Paracartia grani* is controlled by temperature. In winter this species is present as benthic resting eggs, which hatch in response to rising temperature in spring (Rodríguez et al. 1995). In this study, *P. grani* appeared in the claire at the end of March, when the temperature was 15 to 17°C. This timing matches the release of *Marteilia refringens* sporangia by the infected oysters, which normally begins when temperatures reach 12°C and which was observed until early April (Audemard et al. 2001). Synchronous release of parasite sporangia and the emergence of copepods at temperatures from 12 to 17°C may facilitate the transmission of *M. refringens* from *Ostrea edulis* to *P. grani*.

Dynamics of *Marteilia refringens* in *Ostrea edulis*

Ostrea edulis in the claire in 1999 became infected during a 3 mo period, from June to August. The time of exposure required by oysters to become infected was confirmed to be <1 mo. In these months, temperatures remained above 17°C, which is commonly regarded as the thermal threshold required for *Marteilia refringens* transmission to flat oysters (Grizel 1985). Temperatures in the claire exceeded this thermal threshold from May to September in both 1998 and 1999, which implies a similar infection period in both years.

Increasing parasitism intensity was noticed in Batch 97 from March 1998. The presence and development of the parasite in this batch (consisting of infected oysters) could not be explained by new infections, as *Marteilia refringens*-free flat oysters remained uninfected until mid-June 1998. The increase in parasitism intensity in previously infected oysters might have resulted from internal multiplication of the parasite linked to the spring increase temperature from 10°C in March to at least 15°C in April. This result is consistent with the thermal threshold of 12°C considered to allow internal parasite multiplication (Grizel 1985).

During summer, the increase of parasite intensity observed in both Batches 97 and 98 can be explained by both new infection and internal multiplication of the parasite. All the oysters initially infected (Batch 97) died after the 22 July 1998, when the intensity of infection was 7 times higher than in February.

Dynamics of *Marteilia refringens* transmission between oyster and copepod

It has been experimentally demonstrated that *Paracartia grani* can be infected by *Marteilia refringens* through exposure to sporangia released from infected oysters (Audemard et al. 2002). During summer, the high prevalence of the parasite in oyster populations (up to 96%; Audemard et al. 2001), and the increase in parasite intensity observed in the oysters, resulted in the release of a high number of sporangia to the environment, and consequently a higher infection rates of *P. grani*. This phenomenon can explain the recurrent *M. refringens* DNA detection by PCR in *P. grani* in May and August in 1998, and from mid-July to the beginning of September in 1999.

First attempts of experimental transmission of *Marteilia refringens* from *Paracartia grani* to *Ostrea edulis* failed. However, this may have been due to non-optimal experimental conditions and does not rule out the hypothesis of a possible transmission of the parasite from the copepod to the oyster (Audemard et al. 2002). The PCR diagnosis of *M. refringens* in *P. grani* in 1999 revealed that the parasite was still detectable after the infection period of the oysters. On the other hand, *M. refringens* was not detected in *P. grani* at the beginning of the oyster infection period, in June. The analysis of only 1 date in June, however, is not sufficient to conclude that *M. refringens* was not present in *P. grani*. The detection of the parasite in *P. grani* after the infection period for oysters is more problematic. The detection of *M. refringens* in *P. grani* by PCR, however, only indicates the presence of *M. refringens* DNA. This can be attributable either to an infection, or to the presence of the DNA on the body surface or within the digestive tract of the copepod. Absence of infection of the oysters even in the presence of copepods presenting parasitism could also be explained by the absence of development in the copepod of the stage infective for the oyster. This could be due to physiological changes experienced in fall by *P. grani*. In this season, its ovarian tissue produces benthic resting eggs instead of the subitaneous eggs produced in spring and summer (Guerrero & Rodríguez 1998). This change could affect or prevent the development of *M. refringens* within the copepod ovarian tissue, and as a consequence the stage infective for the oyster would not be produced, preventing oyster infection.

Finally, as the direct transmission of the parasite from copepod to oyster has not been demonstrated, the involvement of another host between the copepod and the oyster, that was absent from the claire or not susceptible to parasite infection until September, cannot be excluded. The life cycle of *Marteilia refringens* still requires complete resolution. However, high *M. refringens* prevalence in the oyster (up to 96% reported in Audemard et al. 2001) and high abundance of *Paracartia grani* in the claire have been demonstrated by this study. Transmission efficiency of the parasite was probably enhanced in the claire (a confined environment to which *P. grani* is particularly adapted), compared to more open environments such as bays and estuaries, where marteiliosis has also been reported. Future studies will need to investigate further *M. refringens* transmission dynamics in more open and complex environments.

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LITERATURE CITED

Alcaraz M (1983) Coexistence and segregation of congeneric pelagic copepods: spatial distribution of the *Acartia* complex in the ría of Vigo (NW of Spain). J Plankton Res 5:891–900

Alderman DJ (1979) Epizootiology of *Marteilia refringens* in Europe. Mar Fish Rev 41:67–69

Audemard C, Barnaud A, Collins CM, Le Roux F, Sauriau PG, Coustau C, Blachier P, Berthe FCJ (2001) Claire ponds as an experimental model for *Marteilia refringens* life-cycle studies: new perspectives. J Exp Mar Biol Ecol 257:87–108

Audemard C, Le Roux F, Barnaud A, Collins CM and 6 others (2002) Needle in a haystack: involvement of the copepod *Paracartia grani* in the life-cycle of the oyster pathogen *Marteilia refringens*. Parasitology 124:315–323

Balouet G (1979) *Marteilia refringens* — considerations of the life cycle and development of Aber disease in *Ostrea edulis*. Mar Fish Rev 41:64–66

Balouet G, Chastel C, Cahour A, Quillard A, Poder M (1979) Etude épidémiologique et pathologique de la maladie de l'huître plate en Bretagne. Sci Pêches 289:13–23

Berthe FCJ, Pernas M, Zerabib M, Haffner P, Thébault A, Figueras AJ (1998) Experimental transmission of *Marteilia refringens* with special consideration of its life cycle. Dis Aquat Org 34:135–144

Berthe FCJ, Le Roux F, Peyretaillade E, Peyret P, Rodriguez D, Gouy M, Vivarès CP (2000) Phylogenetic analysis of the small subunit ribosomal RNA of *Marteilia refringens* validates the existence of the phylum Paramyxea (Desportes and Perkins, 1990). J Eukaryot Microbiol 47:288–293

Bodin P (1972) Copépodes harpacticoides marins des environs de La Rochelle. IV Espèces de la zone intertidale des Nauteries. Téthys 4:651–682

Calbet A, Alcaraz M (1996) Effects of constant and fluctuating food supply on egg production rates of *Acartia grani* (Copepoda: Calanoida). Mar Ecol Prog Ser 140:33–39

Calbet A, Alcaraz M (1997) Growth and survival rates of early developmental stages of *Acartia grani* (Copepoda: Calanoida) in relation to food concentration and fluctuations in food supply. Mar Ecol Prog Ser 147:181–186

Castel J, Courties C (1982) Composition and differential distribution of zooplankton in Arcachon Bay. J Plankton Res 4:417–433

Collins NR, Williams R (1981) Zooplankton of the Bristol Channel and Severn Estuary. The distribution of four copepods in relation to salinity. Mar Biol 64:273–283

d'Elbée J, Castel J (1991) Zooplankton from the continental shelf of the southern bay of Biscay exchange with Arcachon basin, France. Ann Inst Océanogr 67:35–48

d'Elbée J, Castel J (1995) Evolution spatio-temporelle du zooplancton dans le bassin d'Arcachon: le rôle des variables de milieu. Cah Biol Mar 36:33–45

de Montaudouin X, Sauriau PG (2000) Contribution to a synopsis of marine species richness in the Pertuis Charentais Sea with new insights in soft-bottom macrofauna of the Marennes-Oleron Bay. Cah Biol Mar 41:181–222

Figueras AJ, Montes J (1988) Aber disease of edible oysters caused by *Marteilia refringens*. Am Fish Soc Spec Publ 18: 38–46

Gallo JM (1981) Les copépodes pélagiques néritiques de La Rochelle. Ann Soc Sci Nat Charente-Marit 6:847–850

Goulletquer P, Héral M (1997) Marine molluscan production trends in France: from fisheries to aquaculture. NOAA Tech Rep NMFS 129:137–164

Goulletquer P, Bachelet G, Sauriau PG, Noël P (2002) Open atlantic coast of Europe — a century of introduced species into French waters. In: Leppäkoski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe, distribution, impacts and management. Kluwer Academic Publishers, Dordrecht, p 276–290

Grizel H (1985) Etude des récentes épizooties de l'huître plate *Ostrea edulis* Linné et de leur impact sur l'ostréiculture bretonne. Thèse Doctorat d'Etat es Sciences, Université des Sciences et Techniques du Languedoc, Montpellier

Grizel H, Comps M, Bonami JR, Cousserans F, Duthoit JL, Le Pennec MA (1974) Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linné. Sci Pêches 240: 7–30

Guerrero F, Rodríguez V (1998) Existence and significance of *Acartia grani* resting eggs (Copepoda: Calanoida) in sediments of a coastal station in the Alboran Sea (SE Spain). J Plankton Res 20:305–314

Le Fèvre-Lehoërff G (1971) Etude d'un cycle nycthéméral dans l'estuaire de la rivière de Morlaix. Hydrologie et zooplancton. Rev Trav Inst Pêches Marit 35:347–366

Le Roux F, Audemard C, Barnaud A, Berthe F (1999) DNA probes as potential tools for the detection of *Marteilia refringens*. Mar Biotechnol 1:588–597

Lubet P (1953) Variations saisonnières du zooplancton du bassin d'Arcachon. Bull Soc Zool Fr 78:204–216

Mialhe E, Bachere E, Boulo V, Cadoret JP and 6 others (1995) Future of biotechnology-based control of disease in marine invertebrates. Mol Mar Biol Biotechnol 4:275–283

Paulmier G (1965) Le microplancton de la rivière d'Auray. Rev Trav Inst Pêches Marit 29:211–224

Reymond H (1991) Dynamique de la chaîne hétérotrophe benthique des marais maritimes en période estivale et son impact sur les productions aquacoles de carnivores: *Penaeus japonicus*, un modèle d'étude. Thèse de Doctorat, Université Pierre et Marie Curie, Paris VI

Rodríguez V, Jiménez F (1990) Co-existence within a group of congeneric species of *Acartia* (Copepoda Calanoida): sexual dimorphism and ecological niche in *Acartia grani*. J Plankton Res 12:497–511

Rodríguez V, Guerrero F, Bautista B (1995) Egg production of individual copepods of *Acartia grani* Sars from coastal waters: seasonal and diel variability. J Plankton Res 17:2233–2250

Sars GO (1904) Description of *Paracartia Grani*, GO. Sars, a peculiar calanoid occuring in some of the oyster-beds of western Norway. Bergens Mus Årb 1904(4):1–16

Sautour B (1991) Populations zooplanctoniques dans le bassin de Marennes-Oléron; dynamique de population, nutrition et production des copépodes dominants. Thèse de Doctorat, Université Bordeaux I

Sautour B, Castel J (1993) Distribution of zooplankton populations in Marennes-Oléron Bay (France), structure and grazing impact of copepod communities. Oceanol Acta 16: 279–290

Siokou-Frangou I, Papathanassiou E, Lepretre A, Frontier S (1998) Zooplankton assemblages and influence of environmental parameters on them in a Mediterranean coastal area. J Plankton Res 20:847–870

Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research, 2nd edn. WH Freeman & Co, New York

Villate F (1991) Zooplankton assemblages in the shallow tidal estuary of Mundaka (Bay of Biscay). Cah Biol Mar 32: 105–119

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