

Spatio-temporal patterns of perkinsosis in the Manila clam *Ruditapes philippinarum* from Arcachon Bay (SW France)

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ABSTRACT: Pathogens belonging to the genus *Perkinsus* infect many bivalve molluscan species around the world, including the Manila clam *Ruditapes philippinarum*. We investigated the spatial distribution of this parasite at 34 stations throughout Arcachon Bay (SW France). Prevalence of perkinsosis was 93% and mean infection abundance was 96×10^3 cells g^{-1} wet gill. Lowest mean abundances were found close to the Leyre River mouth and a significant negative correlation was observed between mean abundance and salinity. Perkinsosis was rare at the oceanic site where salinities and other environmental parameters were stable. A second aim of this study was to survey perkinsosis during annual cycles at 4 sites within Arcachon Bay. Prevalence and intensities (\pm SE) of the disease were high, on average between 70 and 100%, and $130 \times 10^3 \pm 6.7 \times 10^3$ cells g^{-1} wet gill. No seasonal cycle was evident. Clams were infected at 9 mm shell length and infection increased with clam size. The third objective was to determine the disinfection and infection kinetics through a 21 mo reciprocal transplantation between a nearly *Perkinsus* sp.-free area and a highly affected site. Disinfection appeared to be a very slow process and was similar at the site with favorable conditions for *Perkinsus* sp. as at the site with unfavorable conditions. Conversely, infection acquisition appeared to be episodic with spatially defined areas. Consequently, the overall lack of a clear seasonal infection pattern is interpreted as the combination of episodic infection events and slow disinfection kinetics.

KEY WORDS: *Ruditapes philippinarum* · *Perkinsus* sp. · Arcachon Bay · Clam · Spatio-temporal variations · Disinfection · Infection

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INTRODUCTION

Pathogens belonging to the protozoan genus *Perkinsus* occur around the world and infect numerous species of oysters, clams and abalones. They have been associated with massive mortalities of commercially important mollusks including *Crassostrea virginica* in the USA (Andrews & Hewatt 1957) and *Ruditapes decussatus* in Spain and Portugal (Ruano & Cachola 1986, Azevedo 1989). The *Perkinsus* genus includes several species of molluscan parasites, including *P. marinus* from *C. virginica* (Mackin et al. 1950, Burrenson et al. 1994), *P. olseni* from the blacklip abalone *Haliotis ru-*

ber (Lester & Davis 1981), and clams *R. decussatus* and *R. philippinarum* (Azevedo 1989, Navas et al. 1992), *P. qugwadi* from Japanese scallops *Patinopecten yessoensis* (Blackbourn et al. 1998), *P. chesapeakei* from the clams *Mya arenaria* and *Macoma balthica* (McLaughlin & Faisal 1999, Coss et al. 2001, Dungan et al. 2002), *P. mediterraneus* from the European flat oyster *Ostrea edulis* (Casas et al. 2004), *P. honshuensis* from *R. philippinarum* (Dungan & Reece 2006) and *P. beihaiensis* from *C. ariakensis* (Moss et al. 2008).

The Manila clam *Ruditapes philippinarum* is from the Indo-Pacific but is now widely distributed all over Europe. It was introduced in France in 1972, rapidly

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became naturalized, and is now a common bivalve of tidal flats along the Atlantic coast. The Manila clam has important commercial value. French production was 3000 t in 2005. Arcachon Bay (a southwestern French Atlantic lagoon) ranks first in terms of national production, with ~1000 t being produced in the bay in 2008.

In Asia, *Perkinsus* sp. has been identified in Manila clams *Ruditapes philippinarum* in Korea (Choi & Park 1997, Park et al. 1999, Lee et al. 2001, Park & Choi 2001), Japan (Hamaguchi et al. 1998, Choi et al. 2002), and China (Liang et al. 2001). *Perkinsus olseni* is considered a severe pathogen with a highly destructive potential in clams belonging to the genus *Ruditapes* in Spain, Portugal and Italy (Azevedo 1989, Figueras et al. 1992, Sagristá et al. 1995, Elandalloussi et al. 2008). In Europe, *Perkinsus* sp. was found in *R. philippinarum* in Spain (Navas et al. 1992), Italy (Da Ros & Canzonier 1985, Da Ros et al. 1998) and France (Lassalle et al. 2007).

Perkinsus spp. pose major threats to molluscan aquaculture around the world. Thus, it is important to determine the infection level and the dynamics of the *Perkinsus* sp. parasite. The present study makes a contribution by investigating perkinsosis in *Ruditapes philippinarum* from Arcachon Bay during an annual cycle. Previous studies gave an instantaneous picture of *Perkinsus* sp. infection in Arcachon Bay (Fouche et al. 1997, Lassalle et al. 2007). The aims of this study were to (1) map the infection distribution in 34 stations throughout Arcachon Bay; (2) survey *Perkinsus* sp. infection during annual cycles in 4 sites; and (3) examine perkinsosis abundance dynamics through a cross-transplantation experiment between a site with very low and a site with high perkinsosis intensity.

MATERIALS AND METHODS

Study area. Arcachon Bay (44° 40' N, 1° 10' W) is a 180 km² semi-sheltered lagoon on the southwest coast of France (Fig. 1). Intertidal mudflats occupy 110 km² of the bay and these are mostly covered by seagrass (*Zostera noltii*) beds. The Manila clam *Ruditapes philippinarum* is generally located in the mid intertidal zone but can also be found in areas from 2.75 m above chart datum to shallow channels (Blanchet et al. 2005, Cottet et al. 2007). In terms of biomass, it is the dominant species in these mudflats. Arcachon Bay receives seawater inputs from the Atlantic Ocean and freshwater inputs mostly from the Leyre River but also from several small streams located around the bay. The mixing of these fresh and oceanic waters as well as the slow renewal by tides induce temperature and salinity gradients within the bay (Plus et al. 2006). Water salinity and sediment temperature in Manila clam habitats

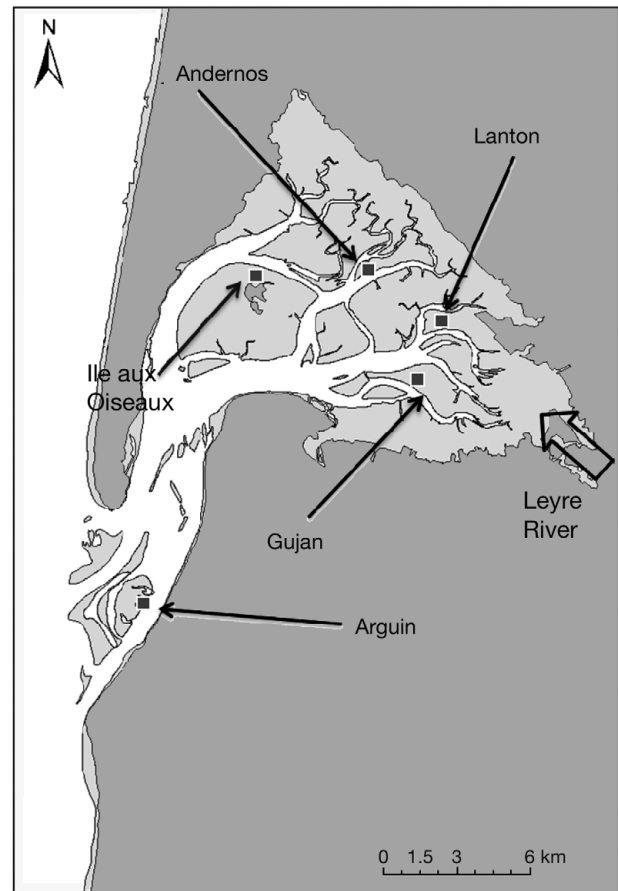


Fig. 1. Arcachon Bay showing the 4 sampling stations selected for the temporal survey (Ile aux Oiseaux, Andernos, Lanton and Gujan) and the station where the cross-transplantation experiment was done (Arguin)

vary from 4 to 35 and from -2 to 44°C, respectively (Dang et al. 2008). Five sites scattered within the bay were investigated in the present study: Andernos, Ile aux Oiseaux, Gujan, Lanton, and the oceanic site of Arguin (Fig. 1).

The environmental characteristics of each site — sediment granulometry, sediment temperature, tidal level and immersion time — were determined in a previous study (Dang et al. 2010) and are reported in Table 1. Water salinity for each site was provided by Auby et al. (1999). These environmental factors allowed the grouping of the sites into 3: the oceanic site of Arguin, which is characterized by higher sediment grain size and the relative stability of environmental variables such as sediment temperature and water salinity (Table 1); the inner sites Andernos and Ile aux Oiseaux, which display a lower sediment grain size, higher percentage of silt because of their muddy intertidal flat location, and higher salinities; and the inner sites Gujan and Lanton, which share the same charac-

Table 1. Environmental variables characterizing the sampling sites: sediment grain size (median, μm), organic matter of sediment dry weight (%), sediment silt (%), sediment temperature ($^{\circ}\text{C}$), water salinity, tidal level (m) and immersion time (%)

Site	Sediment grain size	Sediment organic matter	Sediment silt	Sediment temperature			Water salinity			Tidal level	Immersion time
				Min.	Max.	Mean	Min.	Max.	Mean		
Andernos	163	3.3	14.5	-1.0	35.4	15.8	18.5	34.5	30.0	2.11	60.42
Lanton	78	10.1	41.0	-1.7	37.8	16.0	4.8	34.4	26.7	1.89	70.83
Ile aux Oiseaux	97	13.0	42.4	0.2	37.9	16.1	12.1	34.8	29.6	2.63	45.14
Gujan	69	5.6	47.2	-0.2	43.7	16.1	4.8	34.4	26.7	1.46	75.00
Arguin	360	1.0	3.5	-0.2	30.0	15.1	31.2	35.4	34.2	2.07	64.58

teristics as Andernos and Ile aux Oiseaux except for their lower salinities (Table 1). Ile aux Oiseaux has the highest tidal level (2.63 m) and Gujan has the lowest (1.46 m) (Table 1).

Spatial distribution. Manila clams were sampled at 34 geographically referenced stations within Arcachon Bay between mid-May and mid-June 2006 (see Fig. 2). Stations were randomly allocated within the $\sim 70 \text{ km}^2$ distribution area of the species, as described by stock assessment studies (Caill-Milly et al. 2006). The clam density for each sampled station was given by Caill-Milly et al. (2006). Tidal elevation (m) of each station was provided by both SHOM (Service Hydrographique de la Marine, Brest) and DDE (Direction Départementale de l'Équipement de la Gironde, Bordeaux). Distances between each station and the ocean were calculated using the Arcview 3.2 GIS software. Temperature was provided by Auby et al. (1999). The number of days per year when salinity was < 30 was estimated for each station by M. Plus (unpubl. data).

Ten adult Manila clams (30 to 40 mm) were randomly collected by hand at each of the 34 stations and *Perkinsus* sp. was subsequently detected using the following protocol. Clams were opened, and gills were excised and processed as described by the quantitative method of Ray (1952) as modified by Choi et al. (1989). Gills were incubated in 10 ml of fluid thioglycolate medium (FTM) supplemented with 100 μl of antibiotics (streptomycin and penicillin G) and 500 μl of antifungal (Nystatin). Gills were incubated for at least 5 d in the dark at room temperature. After incubation, the FTM was discarded following centrifugation and remaining gill tissues were digested twice with 2N NaOH at 60°C for 1 h. The resulting solution was centrifuged and the supernatant discarded. Finally, the pellet was washed twice with a sterile solution of phosphate-buffered saline (PBS 1 \times) and resuspended in 1 ml of the same solution. The number of *Perkinsus* sp. hypnospores was estimated using a Malassez chamber under light microscopy. The prevalence of perkinsosis was defined as the percentage of individuals having gills that were infected by *Perkinsus* sp. The mean

abundance was defined as the number of protozoan cells per gram of wet analyzed gills (cells g^{-1} wet gill); in this case, all analyzed clams were considered. The mean intensity of the disease was defined as the number of parasite cells per gram of wet infected gill (cells g^{-1} wet gill); only the infected clams were considered in the calculation of intensity.

Clam shell length and infection abundance. To assess size-specific infection abundance, clams of 7 to 42 mm shell length were randomly collected by hand, but only at a single station (Lanton). A minimum of 4 ind. for each 2 mm shell length class was considered for perkinsosis analysis.

Temporal survey on 4 sites. To follow infection prevalence and intensity in adult clams in relation to time and space, 4 populations were surveyed during 1 yr (February 2006 to February 2007). However, these surveys were extended to a larger time scale where sampling was performed for other purposes. Every month, 30 adult clams (30 to 40 mm) were randomly collected by hand at each of the 4 sites: Ile aux Oiseaux (from December 2005 to February 2007), Andernos (from November 2005 to September 2007), Gujan (from February 2006 to February 2007) and Lanton (from January 2006 to November 2007).

Disinfection kinetics. In Arcachon Bay, clams from Arguin were almost free of perkinsosis, whereas clams located inside the bay were highly infected with *Perkinsus* sp. At Arguin, the mean *Perkinsus* sp. abundance ($\pm \text{SE}$) was $2.1 \times 10^2 \pm 2.3 \times 10^2$ cells g^{-1} wet gill, whereas it was $1.2 \times 10^5 \pm 1.9 \times 10^5$ cells g^{-1} wet gill at Lanton. Moreover, the mean prevalence was 7% at Arguin against 88% at Lanton.

To assess perkinsosis disinfection kinetics, infected clams from Lanton were transplanted into enclosures at a site of much lower *Perkinsus* sp. abundance (Arguin) in November 2006. Four hundred specimens (average shell length $\pm \text{SD} = 31.5 \pm 1.1$ mm) were placed in 4 topless 0.25 m^2 enclosures. Every month, 5 clams per enclosure were randomly sampled (total = 20 clams) for perkinsosis diagnosis, and compared with 20 clams from the native site (Lanton). At the studied

shell length, clams did not significantly grow (Dang et al. 2010), discounting any dilution effect of the number of *Perkinsus* cells per gram of gill.

Infection kinetics. To assess infection kinetics, reciprocal transplantation of 400 adult clams (average shell length \pm SD = 35.3 ± 3.2 mm) from Arguin (low *Perkinsus* sp. abundance) to Lanton (high *Perkinsus* sp.) was conducted between November 2006 and May 2007 following the same protocol as previously described for Arguin in the previous section. Clams native to Arguin were also monitored as a control for this experiment.

Due to the low incidence of infection at Lanton during our experiment, other stations within inner Arcachon Bay were also investigated. Indeed, clams of different origins were transplanted at different locations inside the bay for a growth experiment (Dang et al. 2010). In April 2006, clams from Arguin (20 to 44 mm) were introduced to Ile aux Oiseaux (number of analyzed clams for perkinsosis diagnosis, $n = 6$), in June 2006 to Andernos ($n = 10$), in April 2007 to Lanton ($n = 19$) and in May 2007 to Gujan ($n = 9$). These clams (lightly infected) were maintained very close (centimeters away) to clams from the inner bay stations (highly infected) for 2 yr. Transplanted clams were held inside an enclosure at a density of 320 ind. m^{-2} . They were collected by hand in October 2007 and their *Perkinsus* sp. infection quantified to investigate whether infection at Lanton was representative of infection in other parts of the bay (and whether clams from Arguin were resistant to perkinsosis).

Statistical analyses. In the spatial analysis, regressions were performed between *Perkinsus* sp. mean infection abundance and the following 5 variables: tidal level, distance from the ocean, maximum temperature, period of salinity <30 , and clam density.

To determine correlation between clam shell length and perkinsosis mean abundance, a regression was performed.

In temporal surveys, differences in prevalence between sites were tested using ANOVA after arcsin \sqrt{P} data transformation ($P = \text{prevalence}/100$) (Zar 1984). Differences in infection intensities between sites over time were tested using a 2-way ANOVA (factors: site, time) after logarithmic data transformation. Maximum type I error rates were set at $\alpha = 0.05$. Prior to ANOVA, homogeneity of variance was confirmed using Cochran's test.

For the disinfection and infection experiments, regressions were performed between time and *Perkinsus* sp. mean log abundance. To evaluate whether disinfection during the course of the experiment was significant and to determine the influence of site, a 2-way ANOVA was performed (factors: site, time). Statistical analysis was performed using Statistica 7.1 software. All reported means are accompanied by SEs.

RESULTS

Spatial distribution

The prevalence of *Perkinsus* sp. in clams was relatively high (between 60 and 100%). Mean perkinsosis prevalence within the bay was 93%. The disease was present in all parts of the bay except at Arguin, where the protozoan was rare. The mean prevalence at Arguin was 5% and the mean abundance of infection was very low (213 cells g^{-1}). In contrast, mean abundances of infection in the inner bay stations were high and ranged between 1.1×10^3 and $230 \times 10^3 \text{ cells g}^{-1}$ wet gill (average \pm SE: $96 \times 10^3 \pm 9.9 \times 10^3 \text{ cells g}^{-1}$ wet gill; Fig. 2). *Perkinsus* sp. distribution within the bay seemed heterogeneous. However, with the exception of Arguin, the lowest values of mean infection abundance were found at the Leyre River mouth. Analysis of data from the 34 stations within the bay showed significant negative correlations only between mean *Perkinsus* sp. abundance and distance to the ocean ($r = -0.39$, $n = 34$, $p = 0.022$) and between mean *Perkinsus* sp. abundance and number of days when salinities were <30 ($r = -0.37$, $n = 34$, $p = 0.033$). No significant relation was observed between *Perkinsus* sp. infection and clam density for densities ranging from 10 to 160 ind. m^{-2} .

Clam shell length and infection abundance

The minimum shell length range of infected clams at Lanton was 9 to 10 mm (Fig. 3). *Perkinsus* sp. infection abundance significantly increased with shell length (regression, $r = 0.23$, $n = 97$, $p = 0.024$) (Fig. 3).

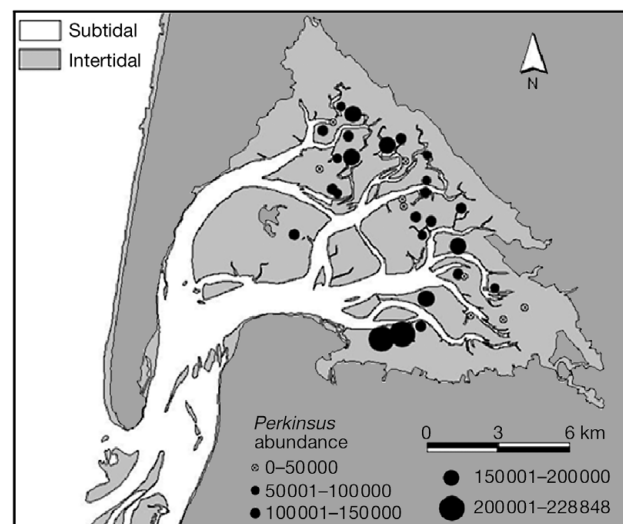


Fig. 2. *Perkinsus* sp. Mean abundance (cells g^{-1} wet gill) at 34 stations in Arcachon Bay that were sampled in May–June 2006

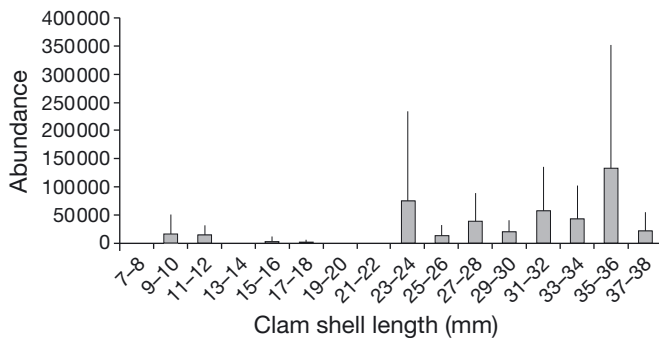


Fig. 3. *Perkinsus* sp. Size-specific mean abundance (\pm SE; cells g^{-1} wet gill) (2 mm clam shell length categories) at Lanton (n = 4)

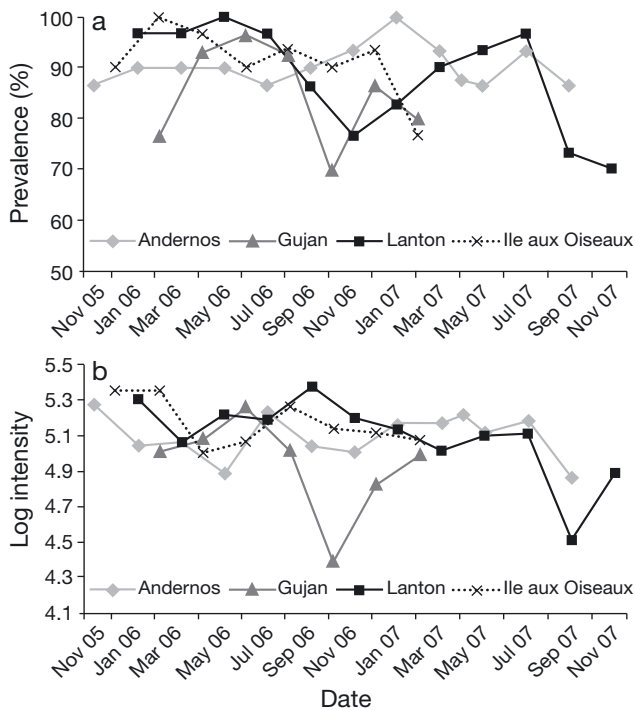


Fig. 4. (a) Prevalence and (b) mean log intensity (cells g^{-1} wet gill) of perkinsosis in the 4 inner sites of Ile aux Oiseaux, Andernos, Lanton and Gujan from November 2005–November 2007

Temporal survey of 4 sites

Prevalence at the 4 inner sites was high (between 70 and 100%; Fig. 4a). Average yearly prevalences were not different between sites (1-way ANOVA, $p = 0.56$).

Mean intensities of infection were high (between 4 and 5 on a logarithmic scale). No significant difference was found between sites ($F = 2.2$, $df = 3$, $p = 0.08$) (Fig. 4b) and with time ($F = 0.74$, $df = 6$, $p = 0.61$), and interaction was also insignificant ($F = 1.2$, $df = 18$, $p = 0.26$). The average intensity (over time and across sites) was $130 \times 10^3 \pm 6.7 \times 10^3$ cells g^{-1} wet gill.

Disinfection dynamics

To obtain information on the disinfection dynamics, clams originating from Lanton were held at Arguin for 1.5 yr. Mean infection abundance on a log scale showed a significant decrease (regression: $r = -0.56$, $df = 15$, $p = 0.024$) (Fig. 5), from $120 \times 10^3 \pm 32 \times 10^3$ cells g^{-1} wet gill at the start to $33 \times 10^3 \pm 10 \times 10^3$ cells g^{-1} wet gill at completion. Nevertheless, mean infection abundance remained high. A significant decrease in mean infection abundance was also observed in the survey of control clams at Lanton (regression: $r = -0.79$, $df = 7$, $p = 0.019$) (Fig. 5). Disinfection did not depend on site (2-way ANOVA: $F = 0.04$, $df = 1$, $p = 0.84$) but was dependent on time (2-way ANOVA: $F = 2.68$, $df = 7$, $p = 0.01$). These 2 factors did not interact (2-way ANOVA: $F = 0.66$, $df = 7$, $p = 0.7$).

Infection dynamics

To understand the *Perkinsus* sp. infection dynamics, a reciprocal transplantation experiment from Arguin to Lanton was conducted. The infection variability with time (Fig. 6) is due to the logarithmic representation. Moreover, SEs are very important in this figure. The infection abundance was considered stable during the experiment, with $0.76 \times 10^3 \pm 0.76 \times 10^3$ cells g^{-1} wet gill at the start and $1.3 \times 10^3 \pm 0.4 \times 10^3$ cells g^{-1} wet gill at completion (regression: $r = 0.29$, $df = 16$, $p = 0.262$) (Fig. 6).

In contrast, clams transplanted from Arguin to Andernos in June 2006 (t -test: $p < 0.001$), to Gujan in May 2007 (t -test: $p < 0.05$) and to Ile aux Oiseaux in April 2006 (t -test: $p < 0.001$) developed a significantly increased *Perkinsus* sp. burden. Initial mean *Perkinsus* sp. abundance was similar ($0.76 \times 10^3 \pm 0.76 \times 10^3$ cells

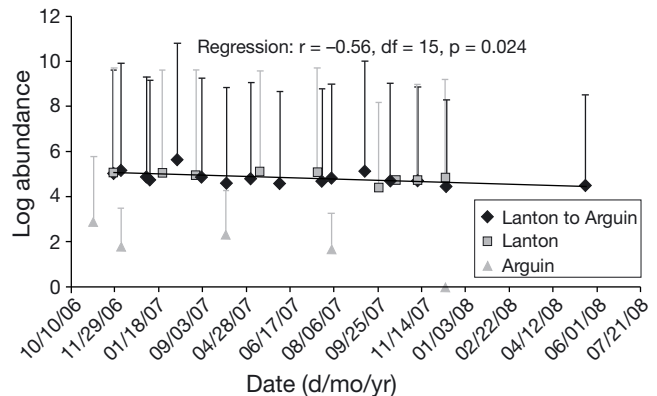


Fig. 5. Mean log infection abundance (\pm SE; cells g^{-1} wet gill) of perkinsosis in clams that were transplanted from Lanton to Arguin and in control clams at lanton and Arguin, respectively. (—) Regression of mean infection abundance of transplanted clams over time

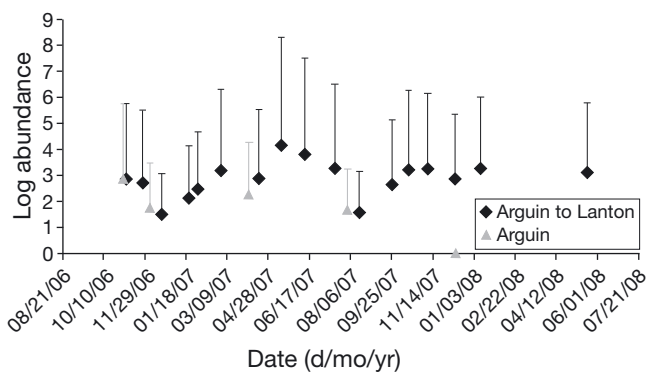


Fig. 6. Mean log infection abundance (\pm SE; cells g^{-1} wet gill) of perkinsosis in clams that were transplanted from Arguin to Lanton and in control clams at Arguin

g^{-1} wet gill) at all 3 additional sites (Andernos, Ile aux Oiseaux and Gujan). The highest increase was at Andernos, where the mean abundance burden reached $160 \times 10^3 \pm 43 \times 10^3$ cells g^{-1} wet gill at experiment completion. At Ile aux Oiseaux, mean *Perkinsus* sp. abundance reached $35 \times 10^3 \pm 17 \times 10^3$ cells g^{-1} wet gill, whereas it reached $2 \times 10^3 \pm 1 \times 10^3$ cells g^{-1} wet gill at experiment completion in Gujan.

DISCUSSION

No seasonality of perkinsosis was apparent in Arcachon Bay during this 2 yr survey. The stability of *Perkinsus* sp. infection could be explained by 2 hypotheses: (1) infection and disinfection did not occur or were negligible during the experimental period; or (2) the stability of infection resulted from the same quantity of parasites being acquired by and purged from the host.

The trend of decrease after peaks of infection intensity generally coincides with the death of the most heavily infected clams or with the regression of infection (Villalba et al. 2005). Considering that disinfection is a very slow process, decrease in infection intensities thus corresponded either with mortality or with the appearance of a low-infected cohort. Based on the low growth rate in Arcachon Bay (Dang et al. 2010), the latter hypothesis is doubtful. No changes in the physical environment (e.g. a low salinity event) could be related to the decrease of infection at Gujan from August 2006 to October 2006.

Salinity is an important abiotic factor structuring the abundance and prevalence of *Perkinsus* sp. The decrease in salinity induced by episodic events such as heavy rains could affect the local development of the disease (Leite et al. 2004). When *P. marinus*-infected oysters are transferred to a low salinity site, disease

progression and mortality are delayed, suggesting a physiological effect of salinity on the parasite (Ray & Mackin 1954, Andrews & Hewatt 1957). For *P. olseni*, the optimal salinity range is 25 to 35 (Auzoux-Bordeneuve et al. 1995). High infection intensity and prevalence of *P. olseni* generally correspond to high salinities (Ray & Mackin 1954, Andrews & Hewatt 1957, Burreson & Ragone Calvo 1996, Cigarría et al. 1997, Park & Choi 2001). In the present study, salinity could partly explain *Perkinsus* sp. distribution throughout the bay, with a negative correlation being obtained between the number of days when salinities were <30 and mean perkinsosis abundance. Moreover, the lowest mean abundances were found at the Leyre River mouth. However, Park & Choi (2001) showed that perkinsosis was absent from sites where salinity remained constant all year long. This could partly explain why perkinsosis was almost absent at Arguin (with stable high salinity year-round). Moreover, the probability of a clam meeting an infective particle is certainly lower in Arguin than in the inner sites, Arguin being the most oceanic area of the bay and characterized by high circulation dynamics that favor the washing of infective particles. Furthermore, Choi et al. (2002) reported that *Perkinsus* infection levels relate significantly to sediment type: clams living on muddy flats tend to have higher levels of infection than those on sandy tidal flats.

In contrast to the present study, a seasonal cycle of infection intensity of *Perkinsus* sp. was observed during a 5 yr survey of *Ruditapes decussatus* in Galicia, Spain (Villalba et al. 2005). Highest values of *Perkinsus* sp. infection were found from spring to early autumn, which was associated with increasing temperatures ($>15^{\circ}C$). Infection regression occurred in winter due to lower temperatures (annual minimum: 9 to $10^{\circ}C$) (Villalba et al. 2005). Similarly, La Peyre et al. (2002) showed that *P. olseni* exhibited highest metabolic activity at $15^{\circ}C$. The increasing north-south latitudinal gradient of *Perkinsus* sp. abundance reported by Lassalle et al. (2007) confirmed that this protozoan prefers higher temperatures. Chu et al. (1996) and Park & Choi (2001) reported that high water temperatures enhance infection intensity and prevalence of *P. marinus*. However, no relation between spatio-temporal patterns of perkinsosis infection and temperature could be deduced from the present study.

The mean infection abundance at the end of the disinfection experiment was 3.6 \times lower than that at the beginning (14 mo after). However, even if environmental conditions at Arguin were not favorable for *Perkinsus* sp. development, disinfection here was similar to that in the inner bay, where conditions were favorable for *Perkinsus* sp. development. In our studied sites, disinfection appeared to be a slow pheno-

menon. A similar result (2.6×10^3 *Perkinsus* cells g^{-1} wet gill) was found in infected Manila clams from the Gulf of Morbihan (France) that were transplanted to Landéda (France)—a site that was free of *Perkinsus* sp. (Lassalle et al. 2007), and in which 100% of initial infection was measured 2 yr later at the same season (unpubl. results).

Moreover, it seems that low salinity events have little impact on *Perkinsus* sp. disinfection rate within the bay. Indeed, the Leyre River discharge was strongly higher in March 2006, March 2007 and June 2007 relative to the same months of the past 20 yr (Météo France data). Precipitation was substantially higher in March 2006, February 2007, March 2007 and May 2007 relative to that in the past 20 yr (Météo France data). These outputs of freshwater did not induce a decrease of *Perkinsus* sp. infection at studied sites, showing that the *Perkinsus* sp. at Arcachon is little affected by low-salinity events.

In the reciprocal transplantation experiment, mean *Perkinsus* sp. abundance evolution depended on the destination site. No infection of the transplanted clams occurred at Lanton between November 2006 and May 2008, although it is reputed to be a highly infected site. However, infection occurred at Andernos between June 2006 and October 2007, at Ile aux Oiseaux between April 2006 and October 2007, and to a lesser extent at Gujan between May and October 2007. The greatest infection acquisition occurred at Andernos. These 4 inner sites are separated by a few kilometers. In the case of Lanton, no infection was observed for 2 yr, suggesting that the *Perkinsus* sp. load in clams was acquired at least 2 yr before and hardly decreased. This dynamics (episodic high infection and high infection stability) explains why no seasonal pattern was observed. Consequently, the hypothesis that the *Perkinsus* sp. load remained stable because of an equilibrium between infection and disinfection is not supported.

In conclusion, the infection phenomenon at Lanton appeared to be an episodic event in space and time. The high infection load that was observed in Lanton was the result of a few multiple infection events. This could suggest that large clams that have endured several infection events during their life spans were more infected than small clams. The present study showed that clams of 9 to 10 mm shell length at Lanton were infected by *Perkinsus* sp. and that infection significantly increased with clam shell length. A similar association between infection intensity and clam size was observed in *Ruditapes decussatus* from Galicia (Villalba et al. 2005) and in *R. philippinarum* from Korea (Park et al. 1999, Park & Choi 2001). Infection was never found in carpet-shell clams that were <20 mm in shell length in Spain (Villalba et al. 2005), and the

threshold in Manila clams from Korea was 15 mm shell length (Choi & Park 1997).

In the transplantation experiment, clams from Arguin were placed in enclosures that were close to clams coming from the inner bay, i.e. infected by perkinsosis (Andernos, Lanton, Gujan and Ile aux Oiseaux). As no infection occurred at Lanton, contamination between proximate live individuals did not happen. This indicated that the *Perkinsus* sp. present at Arcachon did not have a significant direct transmission from live host to live host. Transmission of *Perkinsus* sp. between clams apparently differs from that of *P. marinus* in oysters, the latter occurring with host spawning, excretory activities, alternate host or vector activities, heterotrophic parasite proliferation, or periodic resuspension of parasite cells present in the sediment (Bushek et al. 2002, Ragone Calvo et al. 2003). The principal mode of *P. marinus* transmission, however, is via the direct dissemination of parasite cells from dead oysters (Bushek et al. 2002, Ragone Calvo et al. 2003). Significant clam mortality might also be required for substantial transmission of *Perkinsus* sp. at Arcachon. Another study during the same period at Lanton showed that clam mortality, Z , was not particularly high ($Z = 0.52 \text{ yr}^{-1}$) (Dang et al. 2010).

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LITERATURE CITED

- Andrews JD, Hewatt WG (1957) Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* on oysters of Chesapeake Bay. *Ecol Monogr* 27:1–25
- Auby I, Trut G, d'Amico F, Beliaeff B (1999) Réseau hydrologique du Bassin d'Arcachon—Synthèse des résultats 1988–1997. Report No. R.INT.DEL/AR/99-09, Ifremer, Paris, p 1–60
- Auzoux-Bordenave S, Vigario AM, Ruano F, Domart-Coulon I, Doumenc D (1995) *In vitro* sporulation of the clam pathogen *Perkinsus atlanticus* (Apicomplexa, Perkinsea) under various environmental condition. *J Shellfish Res* 14:469–475
- Azevedo C (1989) Fine structure of *Perkinsus atlanticus* n. sp. (Apicomplexa, Perkinsea) parasite of the clam *Ruditapes decussatus* from Portugal. *J Parasitol* 75:627–635
- Blackbourn J, Bower SM, Meyer JR (1998) *Perkinsus qugwadi* sp. nov. (incertae sedis), a pathogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Can J Zool* 76:942–953
- Blanchet H, de Montaudouin X, Chardy P, Bachelet G (2005) Structuring factors and recent changes in subtidal macro-

- zoobenthic communities of a coastal lagoon, Arcachon Bay (France). *Estuar Coast Shelf Sci* 64:561–576
- Burreson EM, Ragone Calvo LM (1996) Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *J Shellfish Res* 15:17–34
- Burreson EM, Alvarez RS, Martinez VV, Macedo LA (1994) *Perkinsus marinus* (Apicomplexa) as a potential source of oyster *Crassostrea virginica* mortality in coastal lagoons of Tabasco, Mexico. *Dis Aquat Org* 20:77–82
- Bushek D, Ford SE, Chintala MM (2002) Comparison of *in vitro*-cultured and wild-type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. *Dis Aquat Org* 51: 217–225
- Caill-Milly N, Duclercq B, Morandeau G (2006) Campagne d'évaluation du stock de palourdes du bassin d'Arcachon. Rapport Ifremer, available at <http://archimer.ifremer.fr/doc/2006/rapport-2218.pdf>
- Casas SM, Grau A, Reece KS, Apakupakul K, Azevedo C, Villalba A (2004) *Perkinsus mediterraneus* n. sp., a protistan parasite of the European flat oyster *Ostrea edulis* from the Balearic Islands, Mediterranean Sea. *Dis Aquat Org* 58: 231–244
- Choi KS, Park KI (1997) Report on the occurrence of *Perkinsus* sp. in the Manila clams, *Ruditapes philippinarum* in Korea. *J Aquac* 10:227–237
- Choi KS, Wilson EA, Lewis DH, Powell EN, Ray SM (1989) The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. *J Shellfish Res* 8:125–131
- Choi KS, Park KI, Lee KW, Matsuoka K (2002) Infection intensity, prevalence, and histopathology of *Perkinsus* sp. in the Manila clam, *Ruditapes philippinarum*, in Isahaya Bay, Japan. *J Shellfish Res* 21:119–125
- Chu FLE, Volety AK, Constant G (1996) A comparison of *Crassostrea gigas* and *Crassostrea virginica*: effects of temperature and salinity on susceptibility to the protozoan parasite, *Perkinsus marinus*. *J Shellfish Res* 15:375–380
- Cigarría J, Rodríguez C, Fernández JM (1997) Impact of *Perkinsus* sp. on Manila clam *Ruditapes philippinarum* beds. *Dis Aquat Org* 29:117–120
- Coss CA, Robledo JAF, Ruiz JM, Vasta GR (2001) Description of *Perkinsus andrewsi* n. sp. isolated from the Baltic clam (*Macoma balthica*) by characterization of the ribosomal RNA locus, and development of a species-specific PCR-based diagnostic assay. *J Eukaryot Microbiol* 48: 52–61
- Cottet M, de Montaudouin X, Blanchet H, Lebleu P (2007) *Spartina anglica* eradication experiment and *in situ* monitoring assess structuring strength of habitat complexity on marine macrofauna at high tidal level. *Estuar Coast Shelf Sci* 71:629–640
- Da Ros L, Canzonier WJ (1985) *Perkinsus*, a protistan threat to bivalve culture in the Mediterranean basin. *Bull Eur Assoc Fish Pathol* 5:23–25
- Da Ros L, Marin MG, Nesto N, Ford SE (1998) Preliminary results of a field study on some stress-related parameters in *Tapes philippinarum* naturally infected by the protozoan *Perkinsus* sp. *Mar Environ Res* 46:249–252
- Dang C, de Montaudouin X, Gonzalez P, Mesmer-Dudons N, Caill-Milly N (2008) Brown muscle disease (BMD), an emergent pathology affecting Manila clam *Ruditapes philippinarum* in Arcachon Bay (SW France). *Dis Aquat Org* 80:219–228
- Dang C, De Montaudouin X, Gam M, Bru N, Paroissin C, Caill-Milly N (2010) The Manila clam population in Arcachon Bay (SW France): Can it be kept sustainable? *J Sea Res* 63:108–118
- Dungan CF, Reece KS (2006) *In vitro* propagation of two *Perkinsus* spp. parasites from Japanese Manila clams *Venerupis philippinarum* and description of *Perkinsus honshuensis* n. sp. *J Eukaryot Microbiol* 53:316–326
- Dungan CF, Hamilton RM, Hudson KL, McCollough CB, Reece KS (2002) Two epizootic diseases in Chesapeake Bay commercial clams, *Mya arenaria* and *Tagelus plebeius*. *Dis Aquat Org* 50:67–78
- Elandalloussi LM, Leite RB, Rodrigues PM, Afonso R, Cancela ML (2008) Effect of the herbicide Roundup® on *Perkinsus olseni* *in vitro* proliferation and *in vivo* survival when infecting a permissive host, the clam *Ruditapes decussatus*. *Bull Environ Contam Toxicol* 80:512–515
- Figueras A, Robledo JAF, Novoa B (1992) Occurrence of haplosporidian and *Perkinsus*-like infections in carpet-shell clams, *Ruditapes decussatus*, of the Ria de Vigo (Galicia NW Spain). *J Shellfish Res* 11:377–382
- Fouche D, Renault T, Le Deuff RM, Grizel H (1997) Etat des connaissances sur la pathologie chez les mollusques bivalves. Rapport Ifremer R.INT.DEL/97.01/La Tremblade, available at <http://archimer.ifremer.fr/doc/1997/rapport-1923.pdf>
- Hamaguchi M, Suzuki N, Usuki H, Ishioka H (1998) *Perkinsus* protozoan infection in short-necked clam *Tapes (=Ruditapes) philippinarum* in Japan. *Fish Pathol* 33:473–480
- La Peyre JF, Nickens AD, Casas SM, Villalba A (2002) Viability and growth of *Perkinsus marinus* and *Perkinsus atlanticus* at three temperatures. *J Shellfish Res* 21:374
- Lassalle G, de Montaudouin X, Soudant P, Paillard C (2007) Parasite co-infection of two sympatric bivalves, the Manila clam (*Ruditapes philippinarum*) and the cockle (*Cerastoderma edule*) along a latitudinal gradient. *Aquat Living Resour* 20:33–42
- Lee MK, Cho BY, Lee SJ, Kang JY, Jeong HD, Huh SH, Huh MD (2001) Histopathological lesions of Manila clam, *Tapes philippinarum*, from Hadong and Namhae coastal areas of Korea. *Aquaculture* 201:199–209
- Leite RB, Afonso R, Cancela ML (2004) *Perkinsus* sp. infestation in carpet-shell clams, *Ruditapes decussatus* (L.), along the Portuguese coast. Results from a 2-year survey. *Aquaculture* 240:39–53
- Lester RJG, Davis GHG (1981) A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. *J Invertebr Pathol* 37:181–187
- Liang YB, Zhang XC, Wang LJ, Yang B, Zhang Y, Cai CL (2001) Prevalence of *Perkinsus* sp. in the Manila clam, *Ruditapes philippinarum*, along the northern coast of the Yellow Sea in China. *Oceanol Limnol Sin* 32:502–511 (in Chinese with English abstract)
- Mackin JG, Owen HM, Collier A (1950) Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science* 111:328–329
- McLaughlin SM, Faisal M (1999) A comparison of diagnostic assays for detection of *Perkinsus* spp. in the softshell clam *Mya arenaria*. *Aquaculture* 172:197–204
- Moss JA, Xiao J, Dungan CF, Reece KS (2008) Description of *Perkinsus beihaiensis* n. sp., a new *Perkinsus* sp. parasite in oysters of southern China. *J Eukaryot Microbiol* 55: 117–130
- Navas JI, Castilho MC, Vera P, Ruiz-Rico M (1992) Principal parasites observed in clams, *Ruditapes decussatus* (L.), *Ruditapes philippinarum* (Adams and Reeve), *Venerupis pullastra* (Montagu) and *Venerupis aureus* (Gmelin), from the Huelva coast (S.W. Spain). *Aquaculture* 107: 193–199
- Park KI, Choi KS (2001) Spatial distribution of the protozoan

- parasite *Perkinsus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. *Aquaculture* 203:9–22
- Park KI, Choi KS, Choi JW (1999) Epizootiology of *Perkinsus* sp. found in the manila clam *Ruditapes philippinarum* in Komsoe Bay, Korea. *J Korean Fish Soc* 32:303–309
- Plus M, Maurer D, Stanisière JY, Dumas F (2006) Caractérisation des composants hydrodynamiques d'une lagune mésotidale, le bassin d'Arcachon. Rapport Ifremer R.INT.DIR/LAB/AR/06.007, available at <http://archimer.ifremer.fr/doc/2006/rapport-2352.pdf>
- Ragone Calvo LM, Dungan CF, Roberson BS, Burreson EM (2003) Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Dis Aquat Org* 56:75–86
- Ray SM (1952) A culture technique for the diagnosis of infections with *Dermocystidium marinum*. *Science* 116:360–361
- Ray SM, Mackin JG (1954) Studies on the transmission and pathogenicity of *Dermocystidium marinum*. Texas A&M Research Foundation, Project 23, Tech Rep No 11
- Ruano F, Cachola R (1986) Outbreak of a severe epizootic of *Perkinsus marinus* (Levin-78) at Ria de Faro clam's culture beds. *Proc 2nd Int Colloq Pathol Mar Aquac (PAMAQ II)*, Oporto, Portugal, p 41–42
- Sagristá E, Durfort M, Azevedo C (1995) *Perkinsus* sp. (Phylum Apicomplexa) in Mediterranean clam *Ruditapes semidecussatus*: ultrastructural observations of the cellular response of the host. *Aquaculture* 132:153–160
- Villalba A, Casas SM, Lopez C, Carballal MJ (2005) Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). II. Temporal pattern of disease dynamics and association with clam mortality. *Dis Aquat Org* 65:257–267
- Zar JH 1984. *Biostatistical analysis*. Prentice Hall, Englewood Cliffs, NJ

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