

ORIGINAL ARTICLE

Environmental factors contributing to the development of brown muscle disease and perkinsosis in Manila clams (*Ruditapes philippinarum*) and trematodiasis in cockles (*Cerastoderma edule*) of Arcachon Bay

Cindy Binias¹, Van Tu Do¹, Florence Jude-Lemeilleur¹, Martin Plus², Jean-Marie Froidefond¹ & Xavier de Montaudouin¹

¹ University Bordeaux 1, CNRS, UMR EPOC 5805, Arcachon, France

² IFREMER, LER/AR, Arcachon, France

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Correspondence

Xavier de Montaudouin: University Bordeaux 1, CNRS, UMR EPOC 5805, Marine Station-2, rue du Pr Jolyet, 33120 Arcachon, France.

E-mail: x.de-montaudouin@epoc.

u-bordeaux1.fr

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Abstract

The aim of the present study was to identify environmental factors that could explain the distribution of different pathologies of commercially exploited intertidal bivalves in an Atlantic lagoon, Arcachon Bay. In particular, the role of the salinity gradient as a driver was explored. The Manila clam *Ruditapes philippinarum* experienced two severe pathologies, perkinsosis, which is due to a protozoan parasite, and brown muscle disease (BMD), the etiological agent of which remains unknown. *Perkinsus olseni* infection was very low in a small low-salinity area but, at the scale of the entire lagoon, was more influenced by organic matter content in the sediment and by emersion time. BMD prevalence was also 2.6 times higher in the higher organic content area but was also negatively correlated with salinity. The sympatric cockle (*Cerastoderma edule*) was affected by eight trematode species. These parasites have a complex life cycle that generally involves three free-living host species. The distribution of the different trematode communities was rather patchy within Arcachon Bay with no clear relationship with measured environmental factors. The dominance of particular trematode species could be due to the presence of other hosts involved in their life cycle, making it more difficult to detect a major environmental driver. This survey demonstrated that salinity is not a major factor explaining disease distribution in this temperate lagoon. This result has consequences for the research of 'refuge areas' (free of diseases) or 'hot spots' (heavy infection) for species of high economic value.

Introduction

In coastal ecosystems, salinity is generally a strong driver of community structure (Blanchet *et al.* 2005). This is particularly true in systems such as estuaries where the gradient is obvious (Wolff 1973; Ysebaert & Herman 2002; Rybarczyk & Elkaim 2003). Typically, three types of faunal communities are encountered, associated with euhaline, polyhaline, mesohaline and oligohaline waters, respectively. All of these have both pelagic (Herman *et al.*

1968; Orsi & Mecum 1986; Baretta & Malschaert 1988; David *et al.* 2006) and benthic components (Attrill & Rundle 2002; Ysebaert & Herman 2002). Parasite species may also display strong relationships to salinity gradients when their life cycles involve free-living stages sensitive to water mass characteristics. This has been described in protozoans such as *Perkinsus* spp. that parasitize numerous mollusc species (Ahn & Kim 2001; La Peyre *et al.* 2006). When the host dies, the parasite transforms into a stage, the hypnospore, that spends some time in the

water before being inhaled by a new host (Choi & Park 2010). The various species of the genus *Perkinsus* are known to be limited by low salinity (Leite *et al.* 2004). Most trematode species also display free-swimming larval stages in their life cycles, usually including two such stages. The parasite reproduces sexually in the definitive host. Eggs are emitted into the water with faeces and develop into miracidium larvae which infect the first intermediate host, always a mollusc. Asexual reproduction in this host leads to the formation of a new type of larvae, cercariae, which are shed into the water and swim or drift before infection of the second intermediate host. There, larvae remain in a latent stage, the metacercariae, and wait for their host to be preyed upon by the final host in order to complete their life cycle. Many studies have demonstrated that these swimming larvae are sensitive to salinity (Mouritsen 2002; Koprivnikar *et al.* 2010), suggesting that this factor could contribute to explaining the structure of parasite communities.

However, in lagoons, salinity gradients are not always so pronounced and other structuring factors may interfere with the process. These include emersion rate, seagrass occurrence, and sediment grain size (Bachelet *et al.* 1996; Marzano *et al.* 2003; Blanchet *et al.* 2004). This results in a mosaic of communities where the major structuring factors are not always easy to detect. In relation to parasites, there is little knowledge on the factors that drive their distribution in these sheltered areas. For fishermen, such knowledge could contribute to identifying 'refuge areas' that are more or less exempt from infectious diseases (Hoffmann *et al.* 2009).

Arcachon Bay is a good system to illustrate and study these questions. It is a typical tidal lagoon with a cape sheltering most of the system and with freshwater inputs. Previous studies performed on free-living intertidal fauna demonstrated that the salinity gradient was not the only driver of the distribution of pelagic (Vincent 2002) and benthic communities (Bachelet & Dauvin 1993; Blanchet *et al.* 2004). Arcachon Bay also supports the highest production of Manila clam *Ruditapes philippinarum* in France and periodically sustains a small cockle (*Cerastoderma edule*) fishery also.

Both bivalves are susceptible to pathological conditions. Perkinsosis (*Perkinsus olseni*) is very prevalent in Manila clams in the whole bay, with no detected gradient (Dang *et al.* 2010). In 2005, a new disease of the bivalve, brown muscle disease (BMD), was described for the first time (Dang *et al.* 2008). The infectious agent is still unknown, although a virus is suspected (Dang *et al.* 2009). Finally, trematodes are abundant, mostly in cockles. An assemblage of 13 trematode species was identified in previous studies (Desclaux *et al.* 2002; de Montaudouin *et al.* 2009) but the distribution of these parasites at

the lagoon scale remains unknown. Trematodes induce a less severe impact on their cockle hosts than that observed in Manila clams infected with perkinsosis and BMD, except when the intensity (number of parasite individuals per infected host (Bush *et al.* 1997)) becomes high in cockles infected as second intermediate hosts (Desclaux *et al.* 2004) or when the cockle is the first intermediate host (Jonsson & André 1992; Thielgtes 2006).

Our specific aims were: (i) to describe a series of biotic and abiotic factors in Arcachon Bay in order to identify homogeneous entities, i.e. groups of stations that were defined by similar environmental characteristics with (salinity, temperature, emersion rate, grain-size median, seagrass biomass); (ii) to assess levels of infection in cockles and clams; and (iii) to identify environmental factors correlated with the various diseases. The general aim was to explore whether it is possible to identify 'hot spots' (accumulation of infections) or 'refuge areas' (sites free of pathogens) by measuring biotic and/or abiotic factors.

Study Site

Arcachon Bay (44°40' N, 1°10' W) is a macrotidal (tidal range = 0.9–4.9) coastal lagoon situated on the southwestern coast of France. This 156-km² ecosystem is connected to the Atlantic Ocean by a 2- to 3-km-wide and 12-km-long channel. Channels represent 41 km² of the lagoon surface and penetrate between large intertidal areas (115 km²). A significant proportion of these tidal flats (46 km²) is covered by *Zostera noltii* seagrass beds (Plus *et al.* 2010). Arcachon Bay receives freshwater inputs from the northeast and south but mainly from a river (Leyre) located in the southeastern end of the lagoon. The balance between marine and freshwater inputs and the slow renewal of water by tides induce salinity and temperature gradients (Robert *et al.* 1987).

Material and Methods

Sampling procedure

In October and November 2009, a total of 39 stations were sampled along two axes (i.e. two sub-areas) drawn between the most seaward part of the lagoon and the most landward, within the Manila clam and cockle habitats (Fig. 1). Stations were sampled at low tide. Six 0.25-m² quadrats were sampled by hand to collect clams and cockles. When the number of collected individuals was insufficient, additional individuals were collected haphazardly in the immediately surrounding area. After all stations had been sampled, we selected individuals

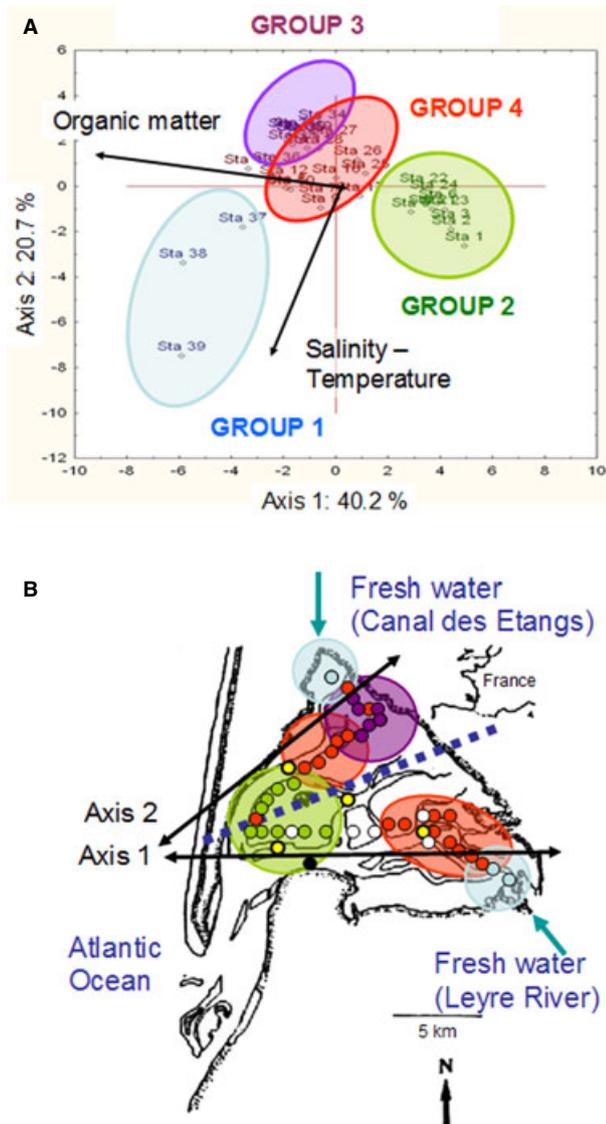


Fig. 1. Principal component analysis (PCA) based on 21 environmental factors (A) from 33 stations (B). Four groups can be separated. Organic matter content in the sediment contributed to Axis 1, and salinity and temperature contributed to Axis 2. Group 1: blue; Group 2: green; Group 3: violet; Group 4: red. White: no values. Dots with the above-mentioned colours represent stations where bivalves were collected. Yellow dots represent stations that were surveyed within the ARCHYD network to obtain ground-truth values of salinity and temperature for model validation. Black dot shows the Eyraç tide gauge to calibrate sea surface height with the model and to deduce emersion time.

with a shell length of 28–36 mm, a length range that was common to all stations, for further analysis. Sediment was sampled for analysis of organic matter and median grain size. Pictures were taken for analysis of seagrass biomass (see below) using a Fujifilm FinePix S9500 camera at a resolution of 1600 × 1200 pixels.

Environmental factors

Grain size and organic matter

At each station, the upper 3 cm of sediment was collected and subsequently analysed for grain-size distribution (wet sieving) and organic matter (loss of weight of dry sediment at 450 °C for 4 h).

Biomass of seagrass foliage

A rapid but reliable method of assessing the biomass of *Zostera noltii* leaves was developed for this study, during a preliminary sampling trip. Fifteen 15 × 15 cm quadrats were carefully laid over the sediment surface at low tide. These quadrats were visually selected to represent a large range of vegetation cover, from 0% (bare sediment) to 100%. For each quadrat, a numerically identified photograph was taken from directly overhead, 1 m above the surface.

All leaves in the quadrat were cut at their base with scissors. Back at the lab, each sample was washed, weighed (fresh weight) and dried at 60 °C for 48 h to obtain a dry weight.

On each photograph, polygons corresponding to bare sediment were drawn using image analysis software (ENVI 4.2, ITT Corp., White Plains, NY, USA). The surface of these polygons was automatically calculated and the seagrass cover was deduced from the quadrat surface. The leaf dry weights and the surface covers were correlated following logarithmic transformation of both variables (and after removing one picture without grass). The relationship was described by the following equation:

$$\ln(DW) = 1.450 \times \ln(S) - 1.733, \text{ with } R = 0.98 \text{ (n} = 14 \text{ pictures)}$$

where $\ln(S) = 0.690 \times \ln(DW) + 1.195$

DW is the dry weight in $\text{g}\cdot\text{m}^{-2}$ of *Z. noltii* leaves and S is the percentage of sediment covered by *Z. noltii*. The biomass in fresh weight (FW) could also be obtained:

$$FW = 13.2 \times DW, R^2 = 0.95 \text{ (n} = 15)$$

Therefore, from a numerically identified picture, it was possible to obtain a biomass with reliable precision. This method was more precise for lower seagrass covers; as vegetation cover approaches 100%, the method becomes less precise because 100% cover may correspond to many biomass values, depending on seagrass bed thickness.

The drawing of polygons allowed us to obtain the relationship between leaf biomass and seagrass cover but was highly time-consuming for routine survey. Consequently, we used a line-drawing method to estimate foliage biomass more rapidly. This method consisted of drawing three equidistant lines across each numerical image and

counting the intersections between lines and leaves. The 15×15 cm frame was placed with leaves perpendicular to one of the square sides and lines were drawn at right angles to leaves. The best correlations were found after logarithmic transformation of both variables (intersection and biomass) and described as follows:

$$\ln(\text{DW}) = 1.514 \times \ln(\text{mean number of intercepts per line}) - 1.9113, \text{ with } R = 0.98 \text{ (n = 14)}$$

This method was utilized to determine aerial biomass in the 39 investigated stations (10 frames per station). Less than 10 min per photograph, including line drawing, were necessary to estimate aerial biomass.

Temperature, salinity and emersion time

Temperature, salinity and emersion rates for each sampling site were obtained by means of a mathematical model (MARS; Lazure & Dumas 2008) that had been previously applied and validated on Arcachon Bay (Plus *et al.* 2009). This three-dimensional hydrodynamic model calculates free surface height variations, current speed and direction, water temperature and salinity, at a 235-m resolution on the horizontal plane (10 meshes on the vertical) and at time steps ranging from 10 to 60 s. The model was used during a 3-year period (November 2006 to November 2009), and the following parameters were recorded for each site:

- 1 Temperature and salinity minima, maxima and means.
- 2 Percentage of time spent in emersion.
- 3 Frequency histograms for temperature (percentage of time spent at temperatures below the following thresholds: 8, 12, 16 and 20 °C). These threshold values cover the range of values found in this area and allow detection of particularly low and high temperatures from 'rare events'.
- 4 Frequency histograms for salinity (percentage of time spent at salinities below the following thresholds: 13, 28 and 34). These threshold values cover the range of values found in this area and allow detection of particularly low and high salinities from 'rare events'.

Boundary conditions were provided by the global tidal solution FES99 (Lefèvre *et al.* 2002) and the atmospheric forcing parameters – air temperature, atmospheric pressure, nebulosity, relative humidity and surface wind stress – were provided by the ARPEGE model (Centre National de Recherche Météorologique, Toulouse, France).

Comparisons between available ground-truth values and model simulations were performed to validate the mathematical model for the study period (from November 2006 to November 2009). Empirical temperature and salinity data were taken from the ARCHYD database (Ifremer), selecting four stations located along the clam sampling axes (Fig. 1). Sea surface height (SSH) observations at the Eyrac tide gauge (Fig. 1) were provided by

the REFMAR website (refmar.shom.fr) and remain the property of SHOM (Naval Hydrographic and Oceanographic Service) and the Gironde DDTM (Sea and Territory Departmental Directorate). Model evaluation was performed following Piñeiro *et al.* (2008), regressing observed *versus* predicted values and testing the significance of slope = 1 and intercept = 0. This analysis was complemented by RMSD (root-mean squared deviation) and EFF (model efficiency) calculations:

$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^n (Y_{\text{imod}} - Y_{\text{iobs}})^2}{n}}$$

$$\text{EFF} = 1 - \frac{\sum_{i=1}^n (Y_{\text{imod}} - Y_{\text{iobs}})^2}{\sum_{i=1}^n (Y_{\text{imod}} - Y_{\text{iobs}})^2}$$

where Y_{imod} and Y_{iobs} are respectively the predicted and observed values and n is the total number of values.

Table 1 summarizes the results of the model validation. Observed *versus* predicted values regressions showed that the model behaved satisfactorily. Best model performances were obtained for sea surface elevation and temperature, with a very high percentage of variance in observed values explained by the model (respectively 97% and 98%). The worst model performance was obtained for salinity but the coefficient of determination still remained high (85%). All tests for slope = 1 and intercept = 0 were passed and model efficiency was near 1.

Table 1. Regression parameters (slope a and bias b , for the $Y_{\text{obs}} = aY_{\text{mod}} + b$ equation), coefficient of determination (r^2), Theil's partial inequality coefficients (U_{bias} , U_{slope} and U_{error} , are the proportions of observed variance not explained by the predicted values but due to, respectively, mean differences between observed and predicted values, slope error and unexplained variance), root mean-squared deviation (RMSD, expressed in the same units as the variables) and model efficiency (Eff; the closer Eff is to 1, the better the model), for observed *versus* predicted variables (temperature – TEMP, salinity – SAL, sea surface height SSH).

| | SSH | Temperature | Salinity |
|------------------------------|-------|-------------|----------|
| a | 1.008 | 0.958 | 0.910 |
| Significance of test $a = 1$ | 0.20 | 0.09 | 0.18 |
| b | 0.016 | 0.376 | 3.086 |
| Significance of test $b = 0$ | 0.33 | 0.35 | 0.16 |
| Degree of freedom | 915 | 34 | 34 |
| r^2 | 0.97 | 0.98 | 0.85 |
| U_{bias} (%) | 0.034 | 0.125 | 0.039 |
| U_{slope} (%) | 0.002 | 0.071 | 0.049 |
| U_{error} (%) | 0.964 | 0.804 | 0.912 |
| RMSD | 0.19 | 0.78 | 0.79 |
| EFF | 0.96 | 0.98 | 0.84 |

Theil's partial coefficients show that most of the errors in model predictions were due to unexplained variance and not to bias or to misdirection. Temperature, salinity and SSH root-mean squared deviations were 0.78 °C, 0.79 and 19 cm, respectively.

Bivalve models and associated pathology

Manila clam and Perkinsus

All collected Manila clams (*Ruditapes philippinarum*) belonging to shell length class 28–36 mm were opened (34 stations of 39 investigated stations harboured Manila clams with adequate shell length) and gill samples were excised. Five gills from clams with identical lengths were pooled and weighed for analyses of *Perkinsus* infection levels as determined by the FTM (fluid thioglycolate medium) assay (Ray 1966). Depending on clam availability, between two and six pools (except one pool in a single station, no. 21) were obtained. For induction of prezoosporangia (hypnospores), gill samples were placed in separate 15-ml tubes containing 9.5 ml FTM supplemented with 66 µg·ml⁻¹ streptomycin, 32 µg·ml⁻¹ penicillin G and 40 µg·ml⁻¹ nystatin (final concentrations), to prevent bacterial and fungal activity. The tubes were incubated at room temperature for 7 days, in the dark. After incubation, the samples were stored at 4 °C until hypnospore enumeration. To lyse tissues, samples were centrifuged at 664 g for 10 min. Pellets were added with 5 ml 2 N NaOH, and incubated at 60 °C for at least 1 h. This step was repeated before pellets were rinsed twice with 10 ml 0.1 M phosphate-buffered saline (PBS). Final pellets were resuspended in 1 ml PBS and hypnospores were counted twice using a Malassez counting chamber.

The concentration of *Perkinsus* was correlated to the different variables of the environment (Pearson correlation, after verifying normality of residuals), and was compared among the four spatial groups (Kruskal–Wallis test due to heteroscedasticity) and between the two axes, i.e. between the two sub-areas of the lagoon (Student's *t*-test) (STATISTICA 7 software, Statsoft Inc., Tulsa, Oklahoma, USA).

Manila clam and BMD

All collected clams belonging to the 28- to 36-mm shell length class were opened and an index of the pathology (muscle print index, MPI) estimated. On the posterior adductor muscle (the only affected one), the MPI was used to designate the surface colonized by the brown muscle print on a scale of 0–4 as follows: 0 (healthy), 1 (0–25%), 2 (25–50%), 3 (50–75%) and 4 (75–100%). When both valves displayed different pathology indices, the highest category was selected to characterize the stage of BMD. Prevalence was defined as the percentage of infected hosts (Bush *et al.* 1997).

Cockles and trematodes

When possible, five cockles between 13- and 29-mm shell length per station were opened. The flesh was separated and squashed between two large glass slides. Trematodes were identified and counted under a stereomicroscope (de Montaudouin *et al.* 2009). Trematode abundance was defined as the mean number of metacercariae per individual host, and prevalence as the percentage of infected hosts (Bush *et al.* 1997). A correspondence analysis was performed on data which consisted of a '35 stations × eight trematode species' matrix when each species occurred. Data (averaged metacercariae abundance) were log₁₀(x + 1)-transformed. In the case of *Bucephalus minimus*, it was not possible to separate and count sporocysts and a value of 1 was arbitrarily imposed in the matrix. Particular attention was devoted to the identification of the 'contributive' taxa. A taxon was considered 'contributive' when its contribution to the dimension's inertia was at least twice the mean theoretical contribution of a taxon. Considering that the eight taxa of the matrix contributed to 100% inertia, a 'contributive' taxon inertia should arbitrarily be over (100/8) × 2 = 25%.

Results

Environmental factors

The principal component analysis separated four spatial groups (Fig. 1A). Group 1 isolated a small number of stations at the mouth of the two freshwater inputs (Canal des Etangs and Leyre) (Fig. 1B). This group was characterized by low mean salinity (22.6), high frequency of T < 16 °C (50%), high organic matter and silt and clay contents in the sediment (7.8% and 38.5%, respectively), null emersion time, and low seagrass coverage (7%) (Table 2). Group 2 gathered stations in oceanic position with high median grain size (191 µm), high mean salinity (32.8), mean temperature similar to elsewhere in the lagoon (17 °C) but with low occurrence of cold events, i.e. low frequency of T < 8 °C (4.2%), low organic matter and silt and clay content in the sediment (3.2% and 16.3%, respectively) (Fig. 1, Table 2). Groups 3 and 4 displayed medium mean salinity (30.1), medium frequency of T < 8 °C (7.7%) (Table 2). Axis 2 displayed a higher percentage of silt and clay, organic matter in the sediment and emersion time and more extreme water temperatures than Axis 1 (Table 3).

Manila clam and perkinsosis

The mean concentration of *Perkinsus* in the bay was 62 000 cells g⁻¹ (gill fresh weight, FW) and could reach 209 000 cells g⁻¹ (gill FW) (station 33, Axis 2, Fig. 2). It

Table 2. Different characteristics of the environment averaged for each of the four groups that were defined by the principal component analysis (Fig. 2). ANOVA (F-value) or Kruskal–Wallis tests (H) were performed to compare values among groups.

| | Group 1 | Group 2 | Group 3 | Group 4 | F | H | P |
|--------------------------------------|------------------------------------|-------------------------------------|----------------------------------|----------------------------------|-------|-------|------------------|
| Sediment | | | | | | | |
| Median (μm) | 103 ^b \pm 52.8 | 191.2^a \pm 85.3 | 96.5 ^b \pm 37.8 | 108.3 ^b \pm 22.5 | | 13.23 | 0.004 |
| Silt (%) | 38.5 ^a \pm 11.3 | 16.3^b \pm 16.3 | 38.8 ^a \pm 3.2 | 29.5 ^a \pm 8.3 | 8.32 | | <0.001 |
| Organic matter (%) | 7.8 ^{b,c} \pm 2.6 | 3.2^a \pm 2.5 | 9.1^c \pm 1.3 | 6.2^b \pm 2.5 | 7.91 | | 0.000 |
| Seagrass cover (%) | 6.2 ^a \pm 10.8 | 72.2 ^b \pm 22.7 | 40.1 ^{a,b} \pm 23.4 | 44.9 ^{a,b} \pm 26.7 | 6.24 | | 0.002 |
| Salinity | | | | | | | |
| Mean | 22.5^c \pm 3.7 | 32.8^b \pm 0.3 | 30.3 ^a \pm 0.3 | 29.9 ^a \pm 1.4 | | 23.16 | <0.001 |
| F < 13 | 16.4 ^a \pm 8.7 | 0.0 ^b \pm 0 | 0.1 ^{a,b} \pm 0.3 | 0.3 ^{a,b} \pm 0.9 | | 14.64 | 0.002 |
| F < 28 | 66.6^a \pm 19.0 | 1.8 ^b \pm 0.7 | 20.1 ^b \pm 3.9 | 22.7 ^b \pm 13.1 | | 22.87 | <0.001 |
| F < 34 | 99.9 ^a \pm 0.0 | 75.1^b \pm 7.8 | 99.8 ^a \pm 0.1 | 98.8 ^a \pm 1.5 | | 21.92 | <0.001 |
| Temperature | | | | | | | |
| Mean | 16.7 ^a \pm 0.2 | 16.7 ^a \pm 0.1 | 17.0 ^b \pm 0.1 | 17.0 ^b \pm 0.1 | 31.80 | | <0.001 |
| Minimal | 1.2 ^{b,c} \pm 0.4 | 3.1^a \pm 0.9 | 0.8 ^c \pm 0.5 | 1.9 ^b \pm 0.9 | 9.55 | | <0.001 |
| Maximal | 34.4 \pm 0.5 | 33.1 \pm 2.2 | 34.1 \pm 2.1 | 32.2 \pm 2.6 | 1.36 | | 0.274 |
| F < 8 °C | 9.5 ^a \pm 0.3 | 4.2^b \pm 1.1 | 7.9 ^a \pm 0.2 | 7.5 ^a \pm 0.7 | | 23.74 | <0.001 |
| F < 12 °C | 31.6 ^a \pm 0.7 | 27.1^b \pm 0.2 | 28.0 ^a \pm 0.2 | 28.1 ^a \pm 0.5 | | 23.11 | <0.001 |
| F < 16 °C | 50.4 ^a \pm 0.7 | 48.4 ^{a,b} \pm 0.4 | 48.0 ^b \pm 0.2 | 48.3 ^{a,b} \pm 0.3 | | 12.13 | 0.006 |
| F < 20 °C | 62.7 ^{a,c} \pm 1.3 | 63.1 ^a \pm 1.4 | 59.5 ^b \pm 0.3 | 59.9 ^{c,b} \pm 0.7 | | 23.07 | <0.001 |
| Current F < 0.25 m s ⁻¹ | 93.3 \pm 6.9 | 95.6 \pm 4.5 | 91.3 \pm 10.4 | 82.2 \pm 15.7 | | 4.97 | 0.174 |
| % Emersion time | 0.0 ^a \pm 0 | 30.9 ^b \pm 16.9 | 32.9 ^b \pm 11.4 | 13.0 ^a \pm 15.1 | 6.26 | | 0.002 |
| Clam density (ind. m ⁻²) | 16.2 ^{a,b} \pm 28.1 | 6.3 ^a \pm 7.9 | 51.4 ^b \pm 41.7 | 23.9 ^{a,b} \pm 18.6 | | 10.84 | 0.012 |
| Distance (km) | | | | | | | |
| Leyre | 5.9 ^{a,b} \pm 8.1 | 13.8 ^a \pm 1.1 | 12.4 ^{a,b} \pm 1.3 | 8.2 ^b \pm 4.3 | | 10.66 | 0.013 |
| Canal des Etangs | 9.8 ^{a,b} \pm 8.1 | 8.2 ^{a,b} \pm 0.9 | 3.3 ^a \pm 1.3 | 8.2 ^b \pm 4.1 | | 9.07 | 0.028 |
| Atlantic Ocean | 12.7 ^a \pm 1.9 | 3.0^b \pm 1.0 | 9.6 ^a \pm 0.4 | 9.5 ^a \pm 1.9 | | 22.06 | <0.001 |

Superscript letters gather groups that are similar for a given parameter, values that are different from values of the three other groups ($P < 0.05$) are in bold and/or italics.

was positively correlated to organic matter concentration in the sediment, percentage of emersion, and distance to Leyre River and was negatively correlated to frequency of $T < 16$ °C and distance to Canal des Etangs (Table 4). There was a significant difference of *Perkinsus* concentration among spatial groups (Kruskal–Wallis, d.f. = 3, $H = 22.58$, $P < 0.001$), Group 1 (7400 cells g⁻¹ gill FW), being different from Groups 2, 3 and 4, which displayed similar infection (70 193 cells g⁻¹ gill FW). *Perkinsus* abundance was three times higher in Axis 2 (mean = 95 701 cells g⁻¹ gill FW) than in Axis 1 (mean = 28 917 cells g⁻¹ gill fresh weight) (Student's t -test, $df = 33$, $t = -4.62$, $P < 0.001$).

Manila clam and brown muscle disease

BMD prevalence per station in Manila clams throughout the bay was 11% (Fig. 3). BMD prevalence and MPI were positively correlated with silt and organic matter contents in the sediment and with salinity frequency $S < 34$. They were negatively correlated with minimal temperatures. In addition, MPI was positively correlated with mean water temperature and with Manila clam density (Table 4). However, the influence of freshwater input was not

similar between Canal des Etangs and Leyre. There was no difference of prevalence between spatial groups (one-way ANOVA, $F_{3,28} = 2.03$, $P = 0.13$). However, muscle print index increased from the most oceanic spatial group (G2) (MPI_{mean} = 0.8) to the more continental ones (G3 and G4) (MPI_{mean} = 2.4) groups (one-way ANOVA, $F_{3,28} = 11.74$, $P < 0.001$). Prevalence was 2.6 times higher along Axis 2 (16.1%) (see Fig. 1 for localization of both axes) than along Axis 1 (4.5%) (Mann–Whitney U -test, $Z = -2.70$, $P = 0.007$) (Fig. 3). MPI was similar in both axes (1.84) (Mann–Whitney U -test, $Z = -1.55$, $P = 0.12$).

Cockles and trematodes

Cockles were present in 35 of the 39 stations investigated. Eight trematode taxa were found. Seven species utilize the cockle as the second intermediate host. One of them belongs to the genus *Himasthla* and could be a complex of two species, *Himasthla quissetensis* and *Himasthla continua*. The 8th species, *Bucephalus minimus*, utilizes the cockle as the first intermediate host with a global prevalence of 13% ($n = 144$ cockles). Dimension 1 of the correspondence analysis (44.6% of inertia) discriminated *Himasthla interrupta*, whereas dimension 2 (23.6%

Table 3. Different characteristics of the environment in both axes (Fig. 1). Student's *t*-test (*t*-value) was performed to compare values between axes.

| | Axis 1 | Axis 2 | <i>t</i> | P |
|--------------------------------------|--------|--------|--------------|------------------|
| Sediment | | | | |
| Median (μm) | 131.9 | 127.4 | 0.20 | 0.838 |
| Silt (%) | 23.5 | 32.1 | -2.12 | 0.041 |
| Organic matter (%) | 4.3 | 7.3 | -3.11 | 0.004 |
| Seagrass cover (%) | 44.6 | 51 | -0.60 | 0.550 |
| Salinity | | | | |
| Mean | 29.5 | 30.9 | -1.38 | 0.178 |
| F < 13 | 2.6 | 0.8 | 1.02 | 0.315 |
| F < 28 | 25.2 | 15.2 | 1.49 | 0.144 |
| F < 34 | 91.2 | 92.3 | -0.27 | 0.790 |
| Temperature | | | | |
| Mean | 16.9 | 16.9 | 0.30 | 0.765 |
| Minimal | 2.5 | 1.7 | 2.28 | 0.029 |
| Maximal | 32.1 | 33.7 | -2.05 | 0.048 |
| F < 8 °C | 6.6 | 6.9 | -0.39 | 0.700 |
| F < 12 °C | 28.2 | 27.9 | 0.69 | 0.489 |
| F < 16 °C | 48.6 | 48.3 | 1.33 | 0.191 |
| F < 20 °C | 61.3 | 60.9 | 0.57 | 0.573 |
| Current F < 0.25 m·s ⁻¹ | 85.3 | 91.9 | -1.53 | 0.135 |
| % Emersion time | 11.4 | 28.3 | -3.16 | 0.003 |
| Clam density (ind. m ⁻²) | 17.6 | 27.7 | -1.11 | 0.273 |
| Distance (km) | | | | |
| Leyre | 7.2 | 13.3 | -5.10 | <0.001 |
| Canal des Etangs | 10.9 | 4.5 | 7.83 | <0.001 |
| Atlantic Ocean | 8.4 | 7.4 | 0.83 | 0.414 |

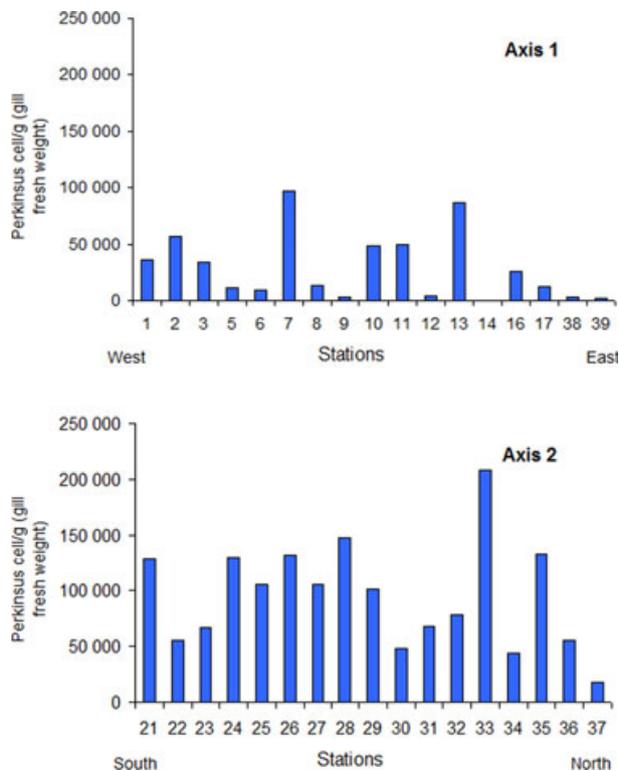
Values that are different ($P < 0.05$) are in bold.

inertia) discriminated *Curtuteria arguinae* and *Himasthla* spp. (Fig. 4A).

Three trematode communities could be identified in the bay (Fig. 4B). *Himasthla interrupta* was present in the northern part of the bay only (mean abundance per station comprised between 3 and 22 metacercariae per cockle) and was accompanied by a high *B. minimus* prevalence (24% with $n = 25$ cockles), *Curtuteria arguinae* was the dominant trematode in the southwest end of the bay, i.e. the most oceanic part (mean abundance per station was 9–43 metacercariae per cockle, $n = 34$ cockles), often accompanied by *Diptherostomum brusinae*. The southeastern end of the bay was characterized by a higher abundance in *Himasthla* spp. (mean abundance per station was 9–43 metacercariae per cockle, $n = 34$ cockles) which represented the only trematode taxon present there.

Discussion

The aim of the study was to correlate the distribution of three types of bivalve diseases within a lagoon (Arcachon Bay) in order to identify the main drivers. Salinity is often cited as a strong contributing factor (Ahn & Kim

**Fig. 2.** Abundance of *Perkinsus olsenii* (cells g^{-1} gill fresh weight) per station in Arcachon Bay.

2001; Elandalloussi *et al.* 2008) but many confounding factors may intervene along the gradient. The unusual feature of Arcachon Bay was the presence of two major freshwater inputs that induce a bi-directional salinity–temperature gradient, east–west and north–south. We demonstrated that the effect of salinity was restricted to a very narrow area around the mouth of the river, for perkinsosis only. Indeed, *Perkinsus* concentration was significantly lower than elsewhere only in Group 1, at three stations near river mouths. The distribution of all diseases was explained less in the ‘oceanic versus continental’ axis than between either northwest or southwest sub-areas of the lagoon. This suggests that factors other than salinity are contributory.

The principal component analysis discriminated groups of stations in accordance with what could be expected in such an ecosystem: an oceanic-influenced group (Group 2), a continent-influenced group (Group 1) and two intermediate groups (Groups 3 and 4) (Bouchet 1993).

The mean abundance of perkinsosis infection was high compared to previous studies (Lassalle *et al.* 2007) but was lower than that assessed in Arcachon Bay in 2006 [96,000 cells g^{-1} (gill FW) (Dang *et al.* 2010)]. This difference can be explained by the distribution of sampling stations, which in the present study took covered a

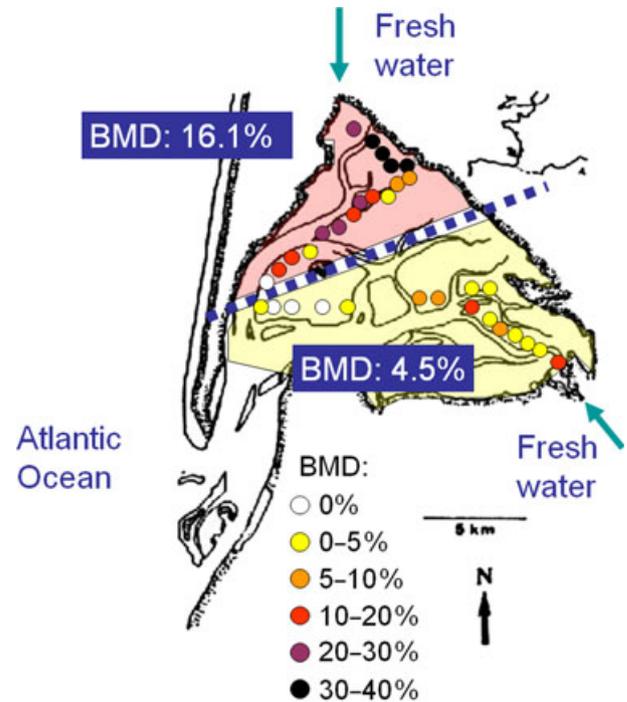
Table 4. Correlation between (1) *Perkinsus* concentration, brown muscle disease (BMD) prevalence, BMD's muscle print index (MPI) and (2) different parameters of the environment (n = 34).

| | <i>Perkinsus</i> concentration (cells g ⁻¹ of gills) | | BMD | | | |
|--------------------------------------|---|--------------|----------------|------------------|--------------|------------------|
| | | | Prevalence (%) | | MPI | |
| | R | P | R | P | R | P |
| Sediment | | | | | | |
| Median (µm) | -0.04 | 0.829 | -0.27 | 0.141 | -0.28 | 0.119 |
| Silt (%) | 0.25 | 0.149 | 0.45 | 0.010 | 0.39 | 0.025 |
| Organic matter (%) | 0.35 | 0.040 | 0.52 | 0.003 | 0.42 | 0.015 |
| Seagrass cover (%) | 0.09 | 0.594 | 0.07 | 0.713 | -0.35 | 0.051 |
| Salinity | | | | | | |
| Mean | 0.29 | 0.101 | -0.10 | 0.580 | -0.26 | 0.145 |
| F < 13 | -0.31 | 0.073 | 0.08 | 0.672 | -0.03 | 0.865 |
| F < 28 | -0.27 | 0.127 | 0.07 | 0.681 | 0.29 | 0.098 |
| F < 34 | 0.03 | 0.875 | 0.37 | 0.039 | 0.72 | <0.001 |
| Temperature | | | | | | |
| Minimal | -0.29 | 0.093 | -0.37 | 0.034 | -0.46 | 0.008 |
| Maximal | 0.29 | 0.090 | 0.12 | 0.514 | -0.02 | 0.900 |
| Mean | 0.09 | 0.614 | 0.12 | 0.6644 | 0.66 | <0.001 |
| F < 8 °C | 0.00 | 0.990 | 0.32 | 0.071 | 0.65 | <0.001 |
| F < 12 °C | -0.26 | 0.131 | 0.18 | 0.330 | 0.21 | 0.245 |
| F < 16 °C | -0.35 | 0.043 | -0.00 | 0.987 | -0.24 | 0.176 |
| F < 20 °C | -0.17 | 0.344 | -0.23 | 0.213 | -0.73 | <0.001 |
| Current | | | | | | |
| F < 0.25 m·s ⁻¹ | 0.09 | 0.626 | -0.16 | 0.390 | -0.20 | 0.270 |
| % Emersion time | 0.34 | 0.048 | 0.13 | 0.491 | 0.03 | 0.875 |
| Clam density (ind. m ⁻²) | 0.10 | 0.569 | -0.12 | 0.502 | 0.37 | 0.037 |
| Distance (km) | | | | | | |
| Leyre | 0.43 | 0.011 | 0.28 | 0.114 | -0.28 | 0.113 |
| Canal des Etangs | -0.51 | 0.002 | -0.60 | <0.001 | -0.20 | 0.260 |
| Atlantic Ocean | -0.14 | 0.422 | 0.20 | 0.264 | 0.62 | <0.001 |

R, Pearson correlation coefficient.

Significant correlations (P < 0.05) are in bold.

broader area and included stations with low infection. Salinity is an important factor structuring the abundance of *Perkinsus* spp. in some locations (Leite *et al.* 2004). For *Perkinsus olseni*, the optimal salinity range is 25–35 (Auzoux-Bordenave *et al.* 1995) and high infection generally corresponds to high salinity (Burrison & Ragone Calvo 1996; Cigarriá *et al.* 1997; Park & Choi 2001). In Arcachon Bay a similar gradient was obtained previously (Dang *et al.* 2010) but was mainly due to stations sampled near freshwater inputs. In the present study, the refuge function of low-salinity areas was evident (Group 1) but concerned <5% of the axis lengths. It should be

**Fig. 3.** Brown muscle disease (BMD) prevalence per station in Arcachon Bay. Mean BMD prevalence per axis (i.e. sub-area) is noted.

determined whether these low salinity areas are effective for commercially relevant growth rates in the shellfish. The salinity gradient of these areas was not high enough to induce a perkinsosis infection gradient. Unexpectedly, the highest difference in perkinsosis infection was observed between Axes 1 and 2. The environmental characteristics that were significantly different between axes involved sediment and emersion. The highest content in silt and clay, and organic matter in the sediment coincided with the highest emersion rate, the highest extreme temperatures and eventually the highest perkinsosis infection (Axis 2). Higher infection in muddy sediment than in sands has already been reported (Choi *et al.* 2002). Our hypothesis is also that the highest emersion rate (higher average hypsometric level) is due to (and/or is the consequence of) a smaller input of oceanic water during flood-tides, a lower water turnover and a higher sedimentation of fine particles. This lower turnover is consistent with an unpublished report that one-third of the water mass transits through Axis 2 against 2/3, through Axis 1 (SOGREAH 2001). That would increase the retention of *Perkinsus* hypnospores and facilitate infection between clams.

A similar observation was made with brown muscle disease (BMD). Interpretation is more difficult due to the lack of knowledge concerning the infectious agent of this disease, which might be a virus (Dang *et al.* 2009). Here

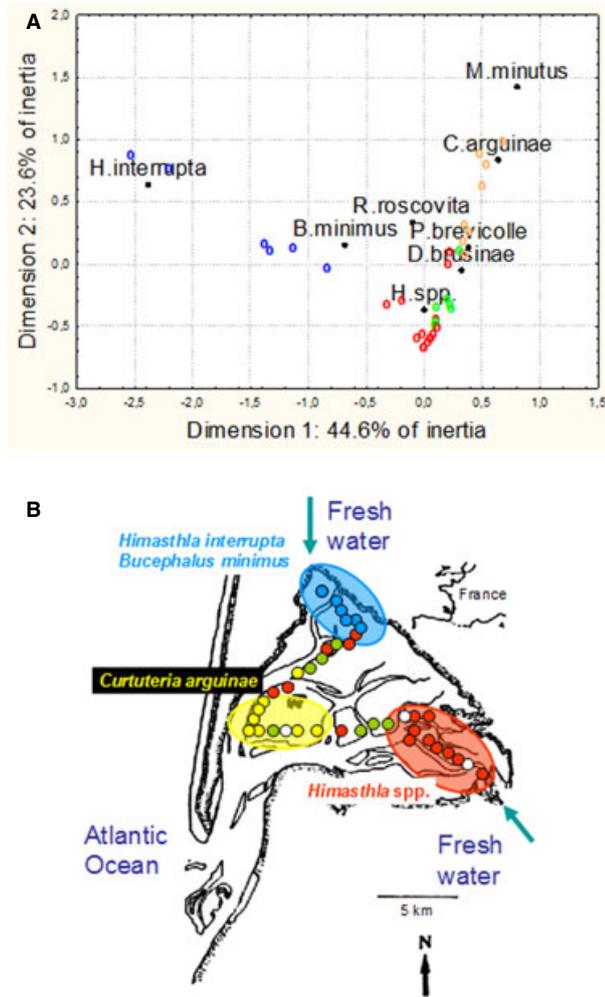


Fig. 4. Correspondence analysis discriminating the 34 stations harbouring cockles in relation to trematode species in cockles (A) and different assemblages of trematodes in cockles from this correspondence analysis (B). Dominant species are indicated and discriminated by different colours. Stations with no characteristic trematode assemblage are in green.

again, Axis 2 displayed higher prevalence in relation to sediment characteristics (but similar muscle print index MPI). This axis was also characterized by higher clam densities (+60%, although $P > 0.05$), which could facilitate disease transmission.

The situation of trematodes was different because many species were involved and the distribution corresponded to a community analysis pattern. At the lagoon scale, three communities were discriminated by one or two dominant trematode species, and these were related to the presence of their other hosts (Sapp & Esch 1994; Hechinger & Lafferty 2005; Byers *et al.* 2008), the first intermediate and definitive hosts, which are sensitive to environment characteristics. Trematode communities

were also different between the two areas of freshwater input (Leyre River and Canal des Etangs).

Our analyses were all correlative. Relationships between causes and consequences were not demonstrated but the identification of two different sub-ecosystems arose, independently of salinity gradient. For Manila clams, the notion of 'refuge' concerns a very small area and is not relevant for BMD. Hence, this is not interesting in terms of fishery management. However, the difference in infection between the two axes is important and suggests that fisheries models should be developed by sub-areas and not at the whole ecosystem scale (Bald *et al.* 2009). Trematode communities in cockles have a patchy distribution but the level of infection remained low compared with known pathological thresholds (Desclaux *et al.* 2004; Gam *et al.* 2009) and should, therefore, have a low impact in this system.

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