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BROWN MUSCLE DISEASE AND MANILA CLAM *RUDITAPES PHILIPPINARUM* DYNAMICS IN ARCACHON BAY, FRANCE

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ABSTRACT Brown Muscle Disease (BMD) affects Manila clam *Ruditapes philippinarum*. It was described for the first time in 2005 in Arcachon Bay, France. The pathology consists in a progressive necrosis of the posterior adductor muscle, valve gaping, clam migration to the sediment surface, and death. This study aims to quantify the prevalence of BMD in the bay and to evaluate the effect of BMD on Manila clam dynamics. The prevalence was assessed on 50 stations spread within Arcachon Bay. About 62% of Manila clam habitat surface was infected by BMD. A survey of buried and surface clams was conducted from November 2006 to March 2008 in Lanton, a site infected by BMD. Modal progression analysis separated confidently cohorts from 2003 to 2005 recruitments. This pathology only affected adult clams (>25 mm, >2 years). For both buried and surface individuals, shell length was significantly correlated with BMD infection. Surface clams had prevalence (67%) higher than buried clams (23%) and showed greater mortality rate after 15 d in running water: 82% against 12% for buried individuals. The final disease index (FDI) and the condition index (CI) were monthly evaluated on 50 clams located at each position in the sediment. CI displayed a significant decrease after BMD's infection from light to severe disease stages. Length data analysis through Bhattacharya's method (FISAT II software) allowed identifying four cohorts. The 2003s cohort enabled to calculate mortality rate that was 39% for 5 months and to estimate that BMD was responsible of 95% of that mortality. The temperature was certainly an important factor in BMD transmission, because cohort dynamics results argued that BMD developed during spring and summer.

KEY WORDS: Manila clam, disease, dynamic, brown muscle disease, mortality, *Ruditapes philippinarum*

INTRODUCTION

Brown Muscle Disease (BMD) was described for the first time in 2005 in Arcachon Bay (Dang et al. 2008). This pathology affects the Manila clam *Ruditapes philippinarum* (Adams & Reeve 1850), one of the most exploited bivalve molluscs in the world. This species has a high commercial value in France. Arcachon Bay (SW France) harbors the most important national stock and production (Caill-Milly et al. 2006). BMD induces a transformation of the posterior adductor muscle, which becomes infused by conchiolin and calcified (Dang et al. 2008). The disease affects both types of muscular tissue, with striated muscle becoming impacted to a higher degree than smooth muscle. Histological observations revealed an important inflammatory response with a large invasion of hemocytes into tissues and a heavy necrosis of muscular fibers. The causal agent of the disease is not identified. Histological and molecular assays discarded bacteria and protozoans, and macroscopic survey under binocular excluded digenean trematodes (Dang et al. 2008, Dang et al. Submitted). More recently, ultrastructural observations by transmission electron microscopy revealed the presence of virus-like particles (Dang et al. In press). Preliminary results from a one-year monthly survey conducted on adult clams in four sites of Arcachon Bay showed that mean prevalence could reach 30%. In some places, peaks of prevalence occurred during the cold season (Dang et al. 2008). At one occasion, adult clams were also collected at the surface of the sediment (i.e., an abnormal position for this endogenous bivalve) and exhibited a three times higher prevalence than buried clams (78% *vs* 27%). These studies revealed the deleterious effect of the disease at the individual scale but did not allow to assess neither its impact at the population scale nor a clear phenology of the disease/clam system. Previous diseases have already seriously affected clam populations like Brown Ring Disease (BRD) (Allam et al. 2002) or perkinsosis (Ngo & Choi 2004, Villalba et al. 2004, Villalba et al. 2005), but also caused by virus-like particles (Novoa & Figueras 2000). The present paper proposes to give an insight on the effect of BMD on clam populations and to clarify some aspects of the disease transmission. The first step consisted in verifying that BMD was not a local pathology through a large scale sampling campaign performed within Arcachon Bay. Then, an attempt was made to describe clam dynamics and link it to BMD occurrence, prevalence and intensity. Finally, the impact of BMD on clam condition and mortality was assessed.

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MATERIAL AND METHOD

Study Area

Arcachon Bay (44°40′N, 1°10′W) is a 156-km^2 semi-sheltered lagoon in the southwest of France (Fig. 1). Tidal flats represent 110 km^2, partly covered by *Zostera noltii* seagrass beds and colonized by Manila clams (Blanchet et al. 2004, Caill-Milly et al. 2006). Tidal range varies between 0.9 and 4.9 m depending on site and tide coefficient. Clams are generally situated in the mid intertidal zone but can be found from 2.75 m above the 0 of low tide (Cottet et al. 2007) to the tidal channel (Blanchet et al. 2005). Most of the present study was conducted at Lanton, a site where the Brown Muscle Disease was most prevalent (Dang et al. 2008). Lanton is in the inner part of the Bay with an influence of the Leyre River (Fig. 1). The sampling site was situated at 1.9 m above the level of low tide. Sediments were fine muddy sands (median grain size = 78.5 μm; 41% silt and clay), sediment temperature fluctuated between –1.7 and 37.8°C and salinity between 4.8 and 26.7 psu (Dang et al. 2008).

Spatial Survey

In May and June 2006, 480 stations were sampled in Arcachon Bay following a stratified strategy to evaluate the

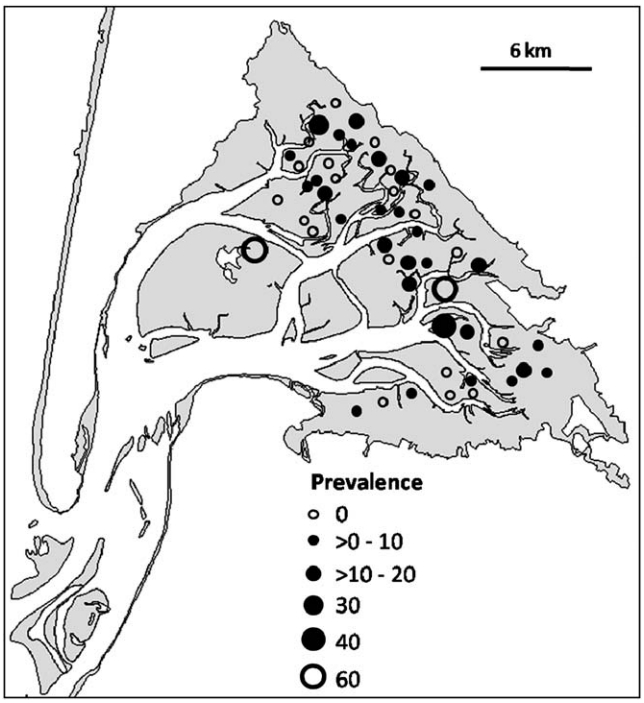

Figure 1. Prevalence of brown muscle disease in buried Manila clams *Ruditapes philippinarum* sampled in 50 stations within Arcachon Bay.

Manila clam standing stock (Caill-Milly et al. 2006). To obtain a sufficient number of clams for BMD analysis, these stations were pooled in 50 stations (Fig. 1). Ten adult clams (from 30–40 mm) were collected from each pooled station. Shells were observed with the naked eye and under a binocular microscope to determine the BMD prevalence.

Manila Clam Dynamics

From November 2006 to March 2008, clams were collected monthly at Lanton. During that period, sediment temperature was recorded every hour with an automatic probe (BoxCar Pro, Version 3.51). The sampling strategy had to integrate the fact that clam density was low, the quantity of juveniles negligible (Caill-Milly et al. 2006), that it was impossible to sieve samples *in situ* because of the absence of proximal water at low tide, and it was difficult to walk in these very fine sediments. It should also give estimation of the ratio of surface clams (sometimes very scarce). Consequently, it was decided to collect clams by hand following three radials of 50-cm width. Buried clams were sampled along five meters (total monthly sample area: three 2.5-m^2 radials) and live surface clams were collected along 20 meters (total monthly sample area: three 10-m^2 radials). All clams were measured at the nearest 0.1 mm. Modal progression analysis (Bhattacharya 1967) was performed using FISAT software (FISAT II, version 1.2.2, FAO-ICLARM) to separate the different cohorts (Gayanilo et al. 2005). This analysis was performed with respect of Manila clam individual growth and recruitment parameters previously determined in this site (Dang et al. Submitted): it was assumed that recruitment occurred in October and that growth followed Von Bertalanffy growth function:

$$L_t = 42.10\,(1 - e^{-0.51t})$$

with L_t = shell length (mm) and t = time (year).

At each occasion, 50 buried clams and 50 surface clams were dissected to measure condition index (CI) and Brown Muscle Disease. CI was defined as the ratio of dry flesh weight (mg) to shell weight (g). Finally, 30 clams monthly sampled in depth and the same quantity collected in surface were maintained separately in the laboratory, in running seawater for 15 days at ambient temperature. This experiment ran for one year, from August 2007 to July 2008. During this period, all dead clams were counted and dissected. At the end, the surviving individuals were all dissected.

Brown Muscle Disease and Pathology-Dependent Mortality.

At each occasion, 50 clams from each position (buried and surface) were opened and an index of the pathology estimated. On the posterior muscle (the only affected one), two Final Disease Indices (FDI) were estimated, one for striated muscle and one for smooth muscle. The value increases from 1–16 with pathology progression (Dang et al. 2008). The mean of both values (mFDI) was calculated for each clam and four stages were defined, based on mFDI values: Stage a (1–4), b (5–8), c (9–12), and d (13–16). In a group (or a cohort) of clams, the mean intensity of BMD was defined as the mean of mFDI of each clam, including zero values corresponding to healthy individuals.

Pathology dependent mortality was estimated for 2003s clam cohort. The assumption was that clams do not recover from BMD and was based on our observation (see discussion). Therefore, when following the fate of a cohort, the decrease of the mean BMD intensity can be interpreted as the death of the most affected clams. The number of clams that should have disappeared was calculated between a peak of BMD and a minimum. In November 2006, when BMD intensity was the highest, a matrix was constructed with the mFDI of each clam considered in the mean BMD intensity. Then, clam with the highest mFDI was taken off the matrix, simulating the death of this clam and thus obtaining a new, lower mean intensity. This was sequentially reiterated until the BMD intensity reached the lowest value corresponding to the following March.

RESULTS

Spread of BMD in Arcachon Bay

A total of 31 stations out of 50 harbored BMD-affected clams (Fig. 1). Considering the sampling strategy (Caill-Milly et al. 2006), it can be considered that 62% of the clam habitat was affected by BMD. However, prevalence was generally <20% (27 stations) and was over 30% at only three occasions.

Manila Clam Abundance

Between November 2006 and March 2008, the mean clam abundance collected by hand was 40 ind/m^2 with 89% being buried (Fig. 2). Clams' shell length was comprised between 9 and 42 mm, but hand sampling was considered as fully efficient over 20 mm. The highest proportions of surface clams (>15% of total abundance) were found when the averaged temperature of the last 24 h was under 12°C. Conversely, the minimum

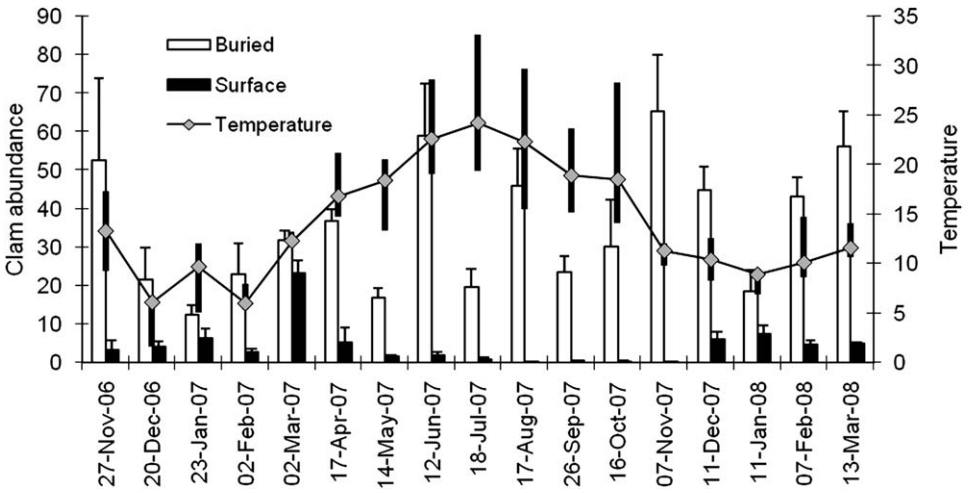

Figure 2. Abundance of Manila clams *Ruditapes philippinarum* (ind/m^2, +1 standard error) found in buried position or at the sediment surface and mean temperature during the 24 previous hours (°C, bars for minimum and maximum).

proportion of surface clam was generally found when temperature was over 18°C (Fig. 2). In November 2007, however, the proportion of surface clam was very low (1%) despite a temperature 11°C.

In pooling all clams (3,125 individuals), a correlation was demonstrated between shell length and BMD infection. Infection in buried individuals was null between 9 and 26 mm, and started at 27 mm (Fig. 3a). Assuming October recruitment and the above-cited Von Bertalanffy growth (also assumed independent of pathology) function, 27-mm shell length corresponded to a 2-y individual. At that date BMD prevalence reached 24%. Then, prevalence slowly and regularly increased

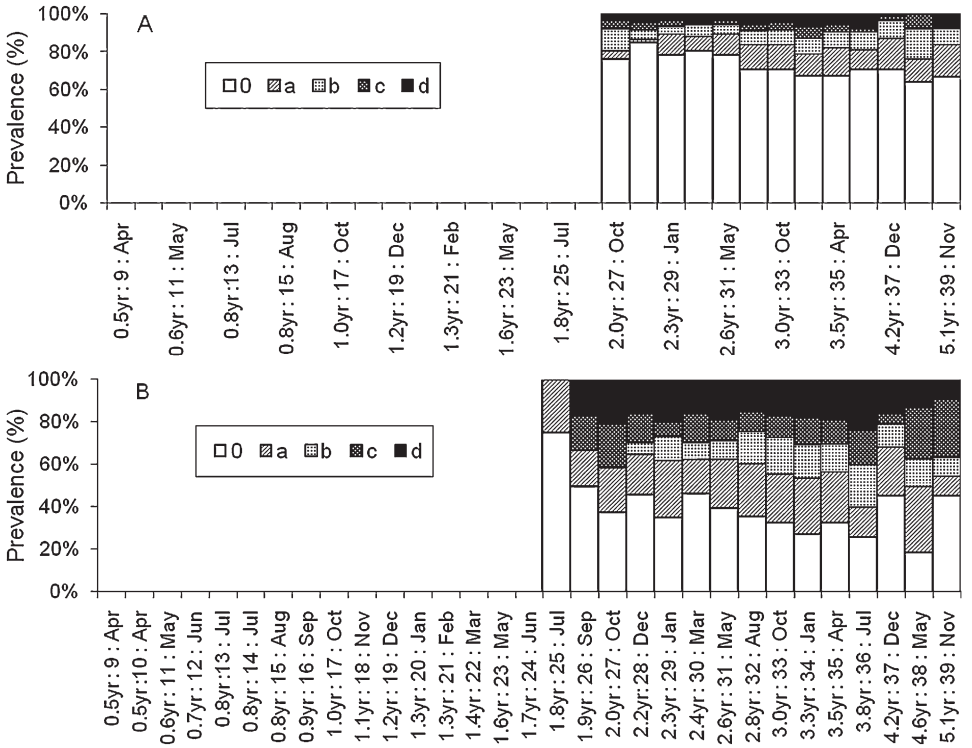

Figure 3. Prevalence of brown muscle disease in Manila clams *Ruditapes philippinarum*, including the different stages of the pathology (see text). X-axis: for example "0.5 y: 9: Apr" means that the predicted age of the clam is 0.5 y, corresponding to a shell length of 9 mm, expected to occur in the field in April. A: buried clams; B: surface clams.

(linear correlation, $R^2 = 0.72$, $P < 0.05$) to reach 33% at a length of 39 mm (5-y individuals). Infection in surface individuals started at 25 mm (Fig. 3b) that also corresponded to the smallest clams found at the sediment surface. This length corresponded to a 1.8-y individual infected in July. At that date BMD prevalence reached 25%. Then, prevalence slowly and regularly increased (linear correlation, $R^2 = 0.35$, $P < 0.05$) to reach 80% at a length of 38 mm (4.6-y individuals). The average percentages within diseased clams were 36, 20, 19 and 24% for stages a, b, c and d, respectively. Each stage was however considered as equally represented (χ^2, $P > 0.05$).

Health of Surface Clams

During the 15-d period after sampling, surface clams died throughout the year in greater proportion (Kolmogorov-Smirnov test, $P < 0.01$) than buried clams (Fig. 4). Mean mortality was 12.4% for surface clam with a maximum of 82% (July). Between May and October, the mortality was always over 20%. Conversely, mean mortality was only 0.5% for buried clams with a summer maximum at 7%.

The monthly survey showed that surface clams were more heavily affected by BMD (Fig. 5). Indeed, the mean prevalence was 67% (minimum: 44%, maximum: 96%) against 26% for buried clams (minimum: 14%, maximum: 36%). There was no clear seasonal pattern.

Clam condition index (CI) was affected by BMD (ANOVA, $P < 0.05$) (Fig. 6). In healthy clams, CI fluctuated between 37.8 and 69.8‰, following the reproductive cycle, with an annual mean of 43.8‰. CI index decreased with the severity of the disease, with annual means of 40.7, 38.8, 36.9 and 35.2‰ for stages a, b, c, and d, respectively. The mean decrease of the CI in stage-d clams was 20% of healthy clam's CI but could occasionally reach 30% (January).

BMD-Dependent Mortality and BMD Transmission Period

Pooled data did not authorize neither to detect any seasonality in BMD transmission nor to estimate the part of mortality because BMD. To reach this aim, it was necessary to work at cohort scale. Modal progression analysis (Bhattacharya's method) separated confidently (separation index >2) four cohorts, from 2003 to 2005. However, because of hand sampling, 2003 was the only cohort that was exhaustively collected, with mean shell length = 30.9 mm at the beginning of the study (November 2006) and 35.9 mm at the cohort disappearance (July 2007) (Fig. 7). At start, the clam density was 43 ind/m^2 (i.e., 75% of the total population abundance)(Fig. 2), and mFDI of the cohort was 6.1 (Fig. 8). Most individuals were buried (more than 80% except in January 2007 with only 64%). After five months (in March 2007), the density dropped to 16.7 ind/m^2, corresponding to a mortality rate of 39%. At that time, mFDI had decreased (ANOVA, $P < 0.05$) and reached the lowest value with 1.7. To obtain this mFDI, it was necessary to "kill" 32% of the individuals (the most affected by BMD, see Materials and Methods). Consequently, it was assumed that 95% of the total mortality was caused by BMD. mFDI remained even until April 2007 and progressively increased afterwards with sediment temperature, especially in July 2007.

DISCUSSION

Brown Muscle Disease (BMD) appeared as a prevalent pathology for Manila clams in Arcachon Bay where it can be considered as the second disease in term of prevalence, behind perkinsosis (Lassalle et al. 2007), but before helminthosis (Dang et al. Submitted) or brown ring disease (Lassalle et al. 2007). BMD affected adult Manila clams only (>25 mm or >2 y). Three non mutually exclusive hypotheses could be evoked to explain the lack of young BMD-affected clams. (1) BMD has not affected clams for two years, and consequently did not occur in the youngest cohorts. However, the regular increase of prevalence with shell length (and clam age) made this option doubtful. (2) Young clams rapidly died when contracting BMD and were never sampled at intermediate stages. The fact that diseased clams were retrieved at the surface of the sediment before those in buried situation (25 mm *vs.* 27 mm, corresponding to 3-month elapsed time) partly argued for this hypothesis. Indeed, it could be assumed that at these shell lengths diseased clams could survive but were still not able to maintain themselves in buried position. However, out of the 1,084 clams collected at the surface of sediment, none had a shell length <25 mm. 3). The pathology necessitates adult hosts. Size-dependent pathology was well-described for trematodes parasites that use

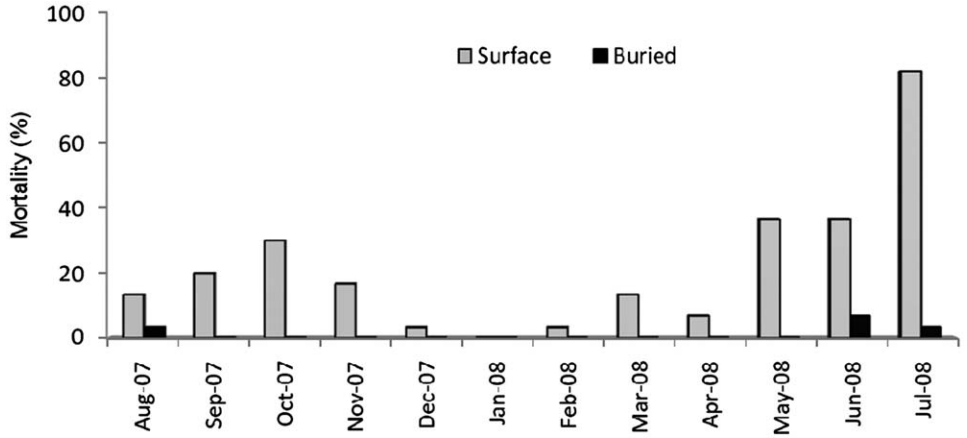

Figure 4. Mortality rates of Manila clams *Ruditapes philippinarum* collected in the field at the sediment surface and in buried position after 15 days in running water in the laboratory.

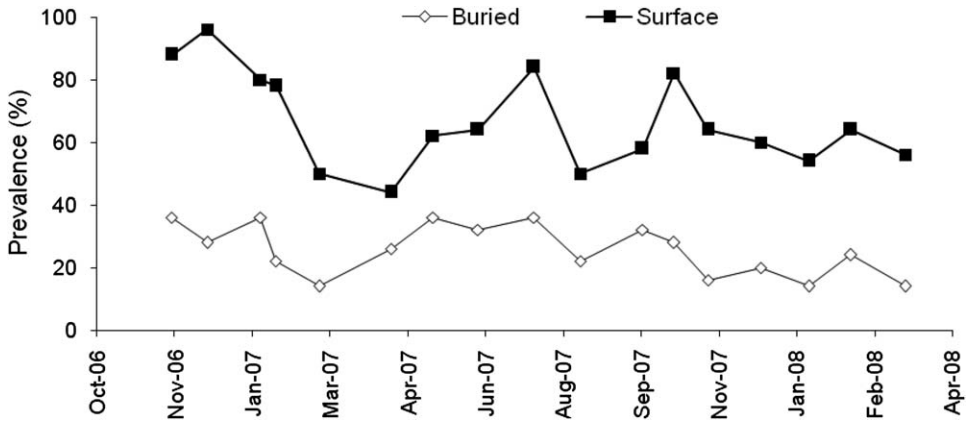

Figure 5. Prevalence of brown muscle disease in Manila clams *Ruditapes philippinarum* collected in the field at the sediment surface and in buried position.

host's gonad to develop, in their mollusc first intermediate hosts (Kube et al. 2006, Lajtner et al. 2008) or for protozoans like Perkinsus genus, which could infest all tissues of molluscs. This parasite can infect clams and the infection intensity has been correlated to the clam size (Choi & Park 1997, Park et al. 1999, Park & Choi 2001, Villalba et al. 2005). In *Ruditapes decussatus* from Spain, no infection was observed in clams <20 mm (Villalba et al. 2005) whereas no infection was found in clams <15 mm in *Ruditapes philippinarum* from Korea (Choi & Park 1997). Other disease like brown ring disease affecting *R. philippinarum* was not size dependent and could attain both adults and juveniles (Paillard 2004, Paillard et al. 2006).

Results from 2003s clam cohort as well as from correlation between shell length and BMD infection suggested that BMD developed during spring and summer months when mean sediment temperature was >13°C, with a peak in July (T = 21°C). This was also the period when the most important proportion of clams laid at the sediment surface with the lowest expectation of life. Dang et al. (2008) had rather pointed out winter peaks of BMD infection but from a mixture of clam cohorts that did not allow correct estimations. Temperature is considered as a major key factor in disease transmission but with contrasting correlations following species. Brown ring disease occurs within the lower values of temperature range (Paillard 2004) when perkinsosis is a rather "warm disease" (Villalba et al. 2005). In the same way, temperature could also be an important factor in some viral diseases. For instance, the ayoka-virus affecting the Japanese pearl oyster *Pinctada fucata martensii* induces high mortalities and symptoms tends to occur during summer when the temperature is up to 25°C (Miyazaki et al. 1999). Furthermore, the lymphocystis disease virus, which infects the Japanese flounders *Paralichthys olivaceus* only replicated in its host when water temperature was around 20°C (Kitamura et al. 2007).

The similar proportion of individuals at each BMD stage suggested that the disease progresses regularly. The muscle was not the only affected tissue, because the condition index (CI) concomitantly decreased with a mean loss of 20%. The mean CI of clams at stage d (35‰) was lower than the lowest CI of healthy clams along their sexual cycle (38‰). The decrease of Manila clams CI was certainly caused by a loss of energy and particularly glycogen reserves to struggle against BMD progression through immune responses. This was supported by the important hemocytic infiltration observed

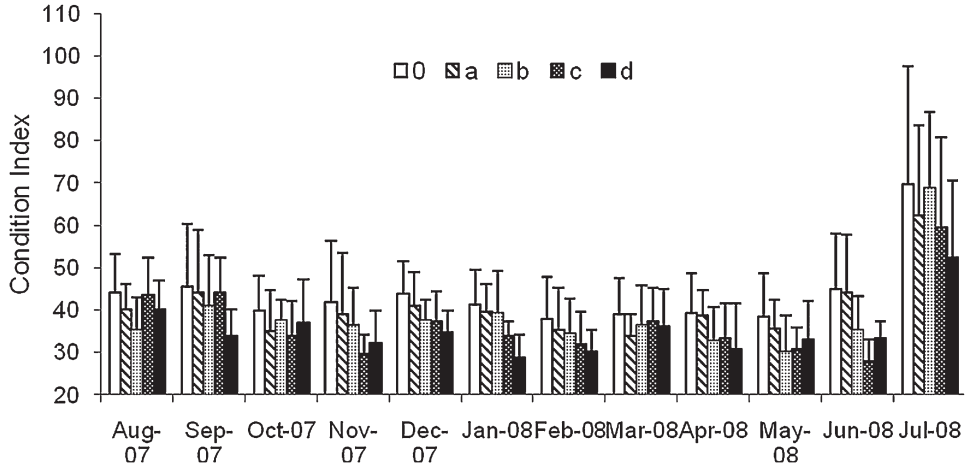

Figure 6. Condition index (+1 standard deviation) of Manila clams *Ruditapes philippinarum* in healthy clams (0) and brown muscle diseased clams (stages a to d, see text).

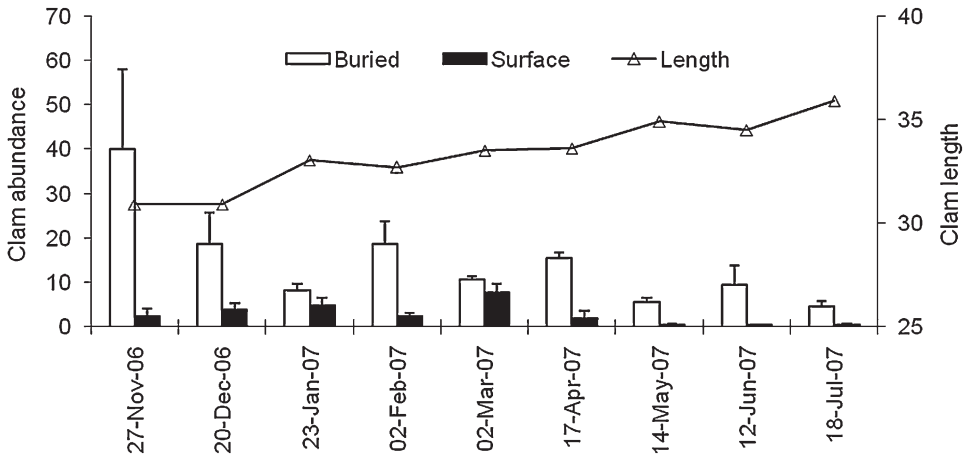

Figure 7. Abundance (ind/m^2, +1 standard error) of Manila clams *Ruditapes philippinarum* from 2003s cohort, found in buried position or at the sediment surface and mean shell length (mm).

within the muscle (Dang et al. 2008, Dang et al. In press). However, CI of diseased clams also followed seasonal rhythm with a peak in July, suggesting that they were still able to reproduce. Some clam diseases like perkinsosis or brown ring disease (BRD) could reduce the clam CI (Casas 2002, Leite et al. 2004, Flye-Sainte-Marie et al. 2007). *Perkinsus olseni* led to a decrease of CI in infected clams *R. decussatus* in Portugal (Leite et al. 2004) and *R. philippinarum* in Korea (Park et al. 1999). Some authors observed a significant reduction of the CI in infected clams only during gametogenesis and not during the postspawning period (Casas 2002). Clams with high infection of BRD exhibited a reduction of 27% to 35% of their CI, indicating a significant disease-associated weight loss (Flye-Sainte-Marie et al. 2007).

Assessment of mortality caused by BMD was a complicated task because BMD occurred in adults whose age was difficult to assess. However, we could separate 2003s cohort during part of its lifespan. This was a good cohort to analyze because we were sure to collect all individuals (shell length at start >30 mm) and the length was still under exploited threshold (36 mm against 40 mm for legal catch size in 2007). Mortality could consequently be considered as natural. Mortality rate caused by BMD was assessed using a theory developed for digenean trematodes. The main hypothesis was that, in a given cohort, the decrease of the pathology intensity was caused by the death of the most affected individuals (Anderson & Gordon 1982, Kennedy 1984, Lester 1984, de Montaudouin et al. 2003, Desclaux et al. 2004). However, the decrease of a given pathogenicity index (as mFDI, parasite intensity,…) may result from different phenomenon: (1) emigration of parasitized animal, or recruitment, or immigration of unparasitized animals. With such a sedentary species and working at the cohort level, this hypothesis could be excluded; (2) recovery processes of diseased clams as described for the brown ring disease (Paillard 2004). None of our observations suggested the least sign of recovery. However this possibility cannot be completely eliminated: (3) death of the most affected clams. This is for the moment the most probable issue. The mortality of 2003s cohort clams during the five studied months was 39%. For such a class-size (>30 mm), mortality rates ranged between 3.1 and 3.7% per month (i.e., less than 19% in

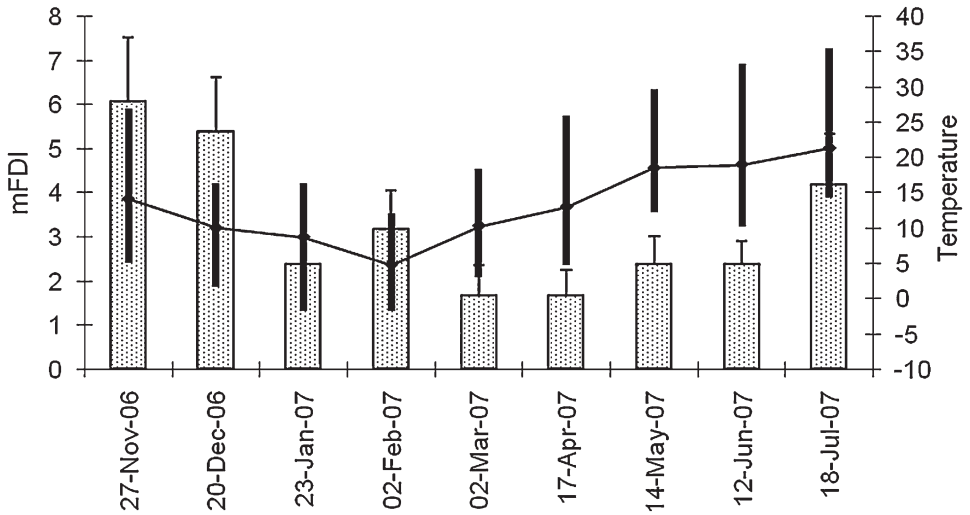

Figure 8. Mean FDI of Manila clams *Ruditapes philippinarum* from 2003s cohort and mean sediment temperature of the 24 previous hours (°C, bars for minimum and maximum).

5 months) in this precise site for BMD-free clams (Dang et al. Submitted-b). This rate was considered as high but was recorded in enclosures that may attract predators as crabs or rough tingles (*Ocenebra*). The herein clam mortality values (39% in 5 months) could consequently be considered as much higher than expected. The calculated part of BMD responsibility in the clam mortality was 32%, which gives to this factor a major importance (95%) in explaining natural adult mortality.

The present study evidenced the important impact (high prevalence and high mortality rates) of an emergent pathology on Manila clam population in Arcachon Bay. Even if the causal agent is not actually identified, BMD transmission and/or development period has been assessed. Further studies will focus on BMD etiology. As a viral etiology is highly suspected, the purification of viral particles will be attempted and an experimental infection will be realized. Thus, disease transmission parameters (i.e., environmental factors [temperature, salinity]) or minimum size of infection will be confirmed.

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LITERATURE CITED

Allam, B., C. Paillard & S. E. Ford. 2002. Pathogenicity of *Vibrio tapetis*, the etiological agent of brown ring disease in clams. *Dis. Aquat. Organ*. 48:221–231.

Anderson, R. M. & D. M. Gordon. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* 85:373–398.

Bhattacharya, C. G. 1967. A simple method of resolution of a distribution into gaussian components. *Biometrics* 23:115–135.

Blanchet, H., X. de Montaudouin, P. Chardy & G. Bachelet. 2005. Structuring factors and recent changes in subtidal macrozoobenthic communities of a coastal lagoon, Arcachon Bay (France). *Estuar. Coast. Shelf Sci*. 64:561–576.

Blanchet, H., X. de Montaudouin, A. Lucas & P. Chardy. 2004. Heterogeneity of macrozoobenthic assemblages within a *Zostera noltii* seagrass bed: diversity, abundance, biomass and structuring factors. *Estuar. Coast. Shelf Sci*. 61:111–123.

Caill-Milly, N., B. Duclercq & G. Morandeau. 2006. Campagne d'évaluation du stock de palourdes du bassin d'Arcachon. IFREMER report. pp. 1–51.

Casas, S.M. 2002. Estudio de la perkinsosis en la almeja fina, *Tapes decussatus* (Linnaeus 1758), de Galicia. PhD Thesis. In: University of Santiago de Compostela, Spain.

Choi, K. S. & K. I. Park. 1997. Report on the occurrence of *Perkinsus* sp. in the Manila clams, *Ruditapes philippinarum* in Korea. *J Aquac*. 10:227–237.

Cottet, M., X. de Montaudouin, H. Blanchet & P. Lebleu. 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.

Dang, C., X. De Montaudouin, J. Bald, L. Lanceleur, I. Paul-Pont & N. Caill-Milly. Trematode parasites in the non indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia* (Submitted-a).

Dang, C., X. De Montaudouin, M. Gam, C. Paroissin & N. Caill-Milly. The Manila clam population dynamics in Arcachon Bay (France): growth, mortality, reproduction, production and productivity. *J. Sea Res*. (Submitted).

Dang, C., X. de Montaudouin, P. Gonzalez, N. Mesmer-Dudons & N. Caill-Milly. 2008. Brown muscle disease (BMD), an emergent pathology affecting Manila clam *Ruditapes philippinarum* in Arcachon Bay (SW France). *Dis. Aquat. Organ*. 80:219–228.

Dang, C., P. Gonzalez, N. Mesmer-Dudons, N. Caill-Milly, J. R. Bonami & X. De Montaudouin. Virus-like particles associated with brown muscle disease in Manila clam (*Ruditapes philippinarum*) in Arcachon Bay (France). *J. Fish Dis*. (In press).

de Montaudouin, X., H. Blanchet, I. Kisielewski, C. Desclaux & G. Bachelet. 2003. Digenean trematodes moderately alter *Hydrobia ulvae* population size structure. *J. Mar. Biol. Ass. UK* 83: 297–305.

Desclaux, C., X. de Montaudouin & G. Bachelet. 2004. Cockle (*Cerastoderma edule*) population mortality: the role of the digenean parasite *Himasthla quissetensis*. *Mar. Ecol. Prog. Ser*. 279:141–150.

Flye-Sainte-Marie, J., S. Pouvreau, C. Paillard & F. Jean. 2007. Impact of Brown Ring Disease on the energy budget of the Manila clam Ruditapes philippinarum. *J. Exp. Mar. Biol. Ecol*. 349:378–389.

Gayanilo, F. C., P. Sparre & D. Pauly. 2005. FAO-ICLARM stock assessment tools II, Revised version, Rome, FAO .

Kennedy, C. R. 1984. The use of frequency distribution in an attempt to detect host mortality induced by infections of diplostomatid metacercariae. *Parasitology* 89:209–220.

Kitamura, S. I., J. Y. Ko, W. L. Lee, S. R. Kim, J. Y. Song, D. K. Kim, S. J. Jung & M. J. Oh. 2007. Seasonal prevalence of lymphocystis disease virus and aquabirnavirus in Japanese flounder, *Paralichthys olivaceus* and blue mussel, *Mytilus galloprovincialis*. *Aquaculture* 266:26–31.

Kube, S., J. Kube & A. Bick. 2006. A loss of fecundity in a population of mudsnails *Hydrobia ventrosa* caused by larval trematodes does not measurably affect host population equilibrium level. *Parasitology* 132:725–732.

Lajtner, J., A. Luciæ, M. Marušiæ & R. Erben. 2008. The effects of trematode *Bucephalus polymorphus* on the reproductive cycle of the zebra mussel *Dreissena polymorpha* in the Drava River. *Acta Parasitol*. 53:85–92.

Lassalle, G., X. de Montaudouin, P. Soudant & C. Paillard. 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.

Leite, R. B., R. Afonso & M. L. Cancela. 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, R. J. G. 1984. A review of methods for estimating mortality due to parasites in wild fish populations. *Helgol. Meersunters*. 37:53–64.

Miyazaki, T., K. Goto, T. Kobayashi, T. Kageyama & M. Miyata. 1999. Mass mortalities associated with a virus disease in Japanese pearl oyster *Pinctada fucata martensii*. *Dis. Aquat. Organ*. 37:1–12.

Ngo, T. T. T. & K.-S. Choi. 2004. Seasonal changes of Perkinsus and Cercaria infections in the Manila clam *Ruditapes philippinarum* from Jeju, Korea. *Aquaculture* 239:57–68.

Novoa, B. & A. Figueras. 2000. Virus-like particles associated with mortalities of the carpet-shell clam *Ruditapes decussatus*. *Dis. Aquat. Organ*. 39:147–149.

Paillard, C. 2004. A short review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. *Aquat. Living Resour*. 17:467–475.

Paillard, C., S. Gausson, J. L. Nicolas, J. P. le Pennec & D. Haras. 2006. Molecular identification of *Vibrio tapetis*, the causative agent of the brown ring disease of *Ruditapes philippinarum*. *Aquaculture* 253:25–38.

Park, K. I. & K. S. Choi. 2001. Spatial distribution of the protozoan parasite *Perkinsus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. *Aquaculture* 203:9–22.

Park, K. I., K. S. Choi & J. W. Choi. 1999. Epizootiology of *Perkinsus* sp. found in the manila clam *Ruditapes philippinarum* in Komsoe Bay, Korea. *J. Korean Fish. Soc*. 32:303–309.

Villalba, A., S. M. Casas, C. Lopez & M. J. Carballal. 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.

Villalba, A., K. S. Reece, M. Camino Ordas, S. M. Casas & A. Figueras. 2004. Perkinsosis in molluscs: a review. *Aquat. Living Resour*. 17:411–432.