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First assessment of Perkinsosis and brown ring disease co-infection in *Ruditapes decussatus* in the North Lake of Tunis (southern Mediterranean Sea)

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Carpet shell clam populations on the Tunisian coastline are susceptible to several microbial pathogen challenges. In this study we report the results of five years' surveillance, conducted from January 2004 till June 2009, for detection of Perkinsosis and brown ring disease (BRD). The survey covered three sites of natural populations of Ruditapes decussatus *in a Tunisian lagoon, the North Lake of Tunis. Perkinsosis was detected preferentially in winter periods from the external and marine site, BRD was detected more frequently in the summer periods in a more proximal collection site (in the lake) and was positively correlated with concentrations of heterotrophic* Vibrio *sp. Our results suggest that several factors other than temperature and salinity might explain spatial distribution variability and natural intensities for these infections in carpet shell clam populations.*

Keywords: *Ruditapes decussatus*, Perkinsosis, brown ring disease, prevalence, environmental factors

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INTRODUCTION

Parasitism in the form of the diseases Perkinsosis and brown ring disease (BRD) represent the main potential biotic factors affecting uncultured *Ruditapes philippinarum* and *Ruditapes decussatus* bivalve populations (Park & Choi, 2001; Leite *et al.*, 2004; Paillard *et al.*, 2004; Villalba *et al.*, 2005). Perkinsosis results from infection by *Perkinsus* spp., a genus of protozoan parasites that colonize the animal flesh whereas BRD is caused by marine bacteria such as *Vibrio tapetis*, by disrupting the inner shell. Both pathogens are known to interfere with the host energy balance and have been responsible for mass mortalities of clams. The presence of BRD in clam populations is influenced by environmental parameters, mainly temperature; elevated temperatures are known to adversely affect the replication of *V. tapetis* (Leite *et al.*, 2004; Paillard *et al.*, 1994; Borrego *et al.*, 1996; Castro *et al.*, 2002; Ngo & Choi, 2004; Villalba *et al.*, 2004, 2005; Park *et al.*, 2006a; Flye-Sainte-Marie *et al.*, 2007).

Ruditapes decussatus is more resistant to infections than *R. philippinarum*, however, severe infections have been reported on northern parts of the Mediterranean and Adriatic coasts, in contrast to less severe infections associated with southern coasts of the Mediterranean (Park & Choi, 2001; Leite *et al.*, 2004; Ngo *et al.*, 2004; Ben Salah, 2005; Allam *et al.*, 2006; Lassalle *et al.*, 2007; El Bour *et al.*, 2008). Most studies have only considered a single parasite or infectious source for one clam host and there are only few reports available documenting the co-occurrence of several microparasites in *Ruditapes decussaus* (Ngo *et al.*, 2004; Lassalle *et al.*, 2007).

From 1999 to 2002, mass mortalities were observed locally in natural populations of carpet clams in the North Lake of Tunis (Attia—El Hili *et al.*, 2007). This lake is mainly populated by carpet shell clams and constitutes the main production area of this economically significant species for Tunisia. During this period, *Perkinsus* parasites and *Vibrio* spp. were detected suggesting either one or both were responsible for the observed deaths. In this study, we report the results of five years of seasonal surveillance, beginning from January 2004 to June 2008, of both Perkinsosis and BRD infections in natural populations of *R. decussatus* collected from three surveillance sites in North Lake.

MATERIALS AND METHODS

Study site

The North Lake of Tunis lagoon (a coastal sub-marine environment) is located in the western part of the Gulf of Tunis (southern Mediterranean Sea), between 36°45′ and

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36°52′ north, 10°10′ and 10°20′ east, with a surface area of 2400 ha, (Figure 1).

Prior to the 1980s sewage effluent and runoff from Tunis City drained directly into North Lake, causing dystrophic crisis resulting in mortalities of fish and shellfish (Belkhir *et al.*, 1985). The construction of a separation dam and a system of inlet and outlet gates improved the water circulation within North Lake, resulting in shorter residence time of water in the lake (from 17 to 24 days) (Ben Charrada, 1992). Because of the economic and ecological importance of fisheries in the North Lake, several environmental monitoring stations, recording a variety of pollutants, mainly chemical and potentially toxic phytoplankton species, have been established (Armi *et al.*, 2009).

Surveillance stations

A dike-like structure in North Lake divides the lagoon along an east–west line in order to improve water circulation. This is mainly achieved by the Kheirredine channel or gate in the east part of the lake. Consequently three surveillance stations were established in this area. Stations were located and maintained using a Global Positioning System (GPS; Garmin GPS 45). For surveillance, two internal (within the lake) stations were chosen: (i) Chikly (mean depth: 2.3 m) located centrally; and (ii) STEG (mean depth 2.5 m) in the south-eastern region, very close to a warm water outfall from the Thermal Electricity Central plant. A third surveillance station, the Channel station, was selected near the Gulf of Tunis. Channel station is a marine environment and the deepest area under surveillance (mean depth of 5 m).

For all sites, temperature (°C), salinity (psu) and dissolved oxygen (mg l^{-1}) were measured *in situ* using a multi-parameter probe (type WTW).

Sample collection

More than 150 clams were randomly sampled at all sites twice a year (in January and June) from 2004 to 2008, as recommended by the guidelines of the International Aquatic Animal Health Code (OIE, 2001). Animal shell length was measured along its longest axis using Vernier callipers, to the nearest 0.5 mm. Live clams were processed as soon as received in the laboratory using the diagnostic algorithms for Perkinsosis, BRD and total bacteriological analyses. Dead or moribund clams were counted and removed prior to analysis.

Diagnostic algorithm for Perkinsosis

Clam samples were depurated in individual seawater tanks overnight (one sample per tank) to remove sediments in the guts and tissues before recording animal weight. For histological preparation, a transverse section was cut from the middle of the animal body and fixed with Davidson's solution. All sections were dehydrated in ethanol, embedded in paraffin sliced to 5 mm and stained with Harris' haematoxylin–eosin before examination under a light microscope. The prevalence (percentage of clams infected) and infection intensity of *Perkinsus* spp. were determined using the histological slides. From each sampling group a minimum of 35 individuals were analysed.

Diagnostic algorithm for BRD

Samples of 100 live clams from each station were collected and examined to detect external and internal BRD symptoms on their shells. Animal diagnosis was based on the external observation of brownish organic (conchiolin) deposits adhering to the internal surface of the valves according to the criteria of Paillard *et al.* (2004). Conchiolin deposits stages (CDS) range from microscopically observable brown spots on inner face of shell in earliest stage (CDS1), to a macroscopic thick brown deposit covering most of inner shell in the most advanced stage (CDS7). Prevalence of BRD in surveillance sites was determined for all clam shells collected. Scanning electronic microscopy (ESEM Type JEOL JSM-5400) was used to examine different shell fragments in the presence or absence of brown spots.

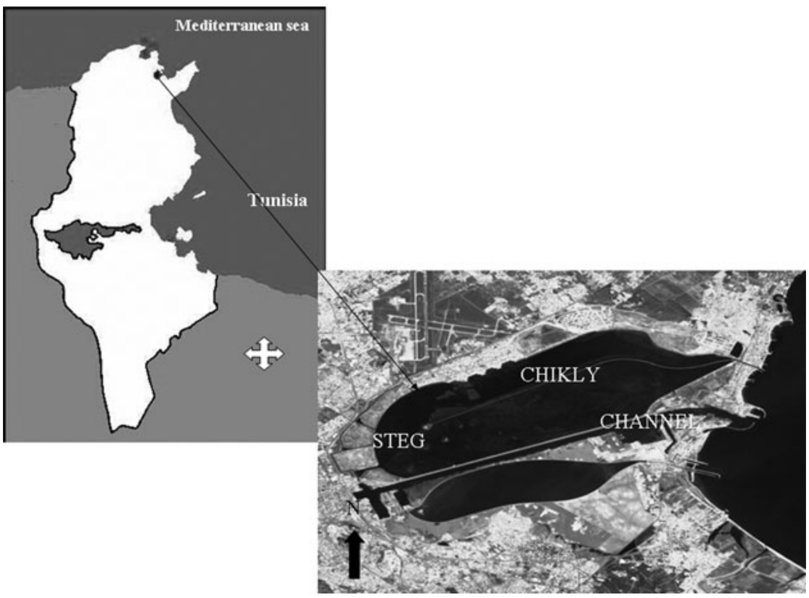

Fig. 1. Sampling sites location in the lagoon of North Lake of Tunis.

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Bacterial enumeration

Total heterotrophic mesophyllic bacteria and Vibrionaceae colonies counts were determined by the plate counting agar method (PCA), using either Zobell agar (ZA) or Zobell mannitol agar (ZMA), respectively. Bacteria from the extrapallial fluid and flesh of clams were enumerated. The replicate plating method of Paillard *et al.* (2004) was used to isolate *V. tapetis*.

Statistical tests

A Pearson test was performed using the program XL-Stat to determine correlations between Perkinsosis and BRD prevalence in clams and the association of bacteriological or environmental variables.

RESULTS

Environmental conditions and sampling efforts

Due to the shallow depths in the lake (<3 m), water temperatures varied widely between the winter and summer periods. Temperature averages ranged between 10°C and 13°C in winter periods and 24°C in summer. Recorded salinities ranged from 39‰ to 40‰ for summer and were lower in winter (35‰). Oxygen averages fluctuated between 6 mg/l to 8 mg/l during winter periods and 8.4 mg/l to 14 mg/l during summer periods. Adult clams surveyed ranged from 228 mm to 340 mm for all sites.

Prevalence and infection intensity of *Perkinsus* spp.

Prevalence and infection intensity of *Perkinsus olseni* (Apicomplexa protozoan) was determined from histological slides according to the scale developed by Ngo *et al.* (2004). Parasites were found and counted in all types of tissues; the mantle, gill filaments, digestive tubules and gonads (Figure 2A). In addition to *Perkinsus* spp., other parasites (metacercariae parasites) were observed, but in the mantle only, in animals collected from the three sites. No significant temporal differences among all sites were observed (Figure 2B).

In winter periods, Perkinsosis infection was detected mainly in the external site (Channel station). Prevalence varied from 2% to 60% with the highest value found in the

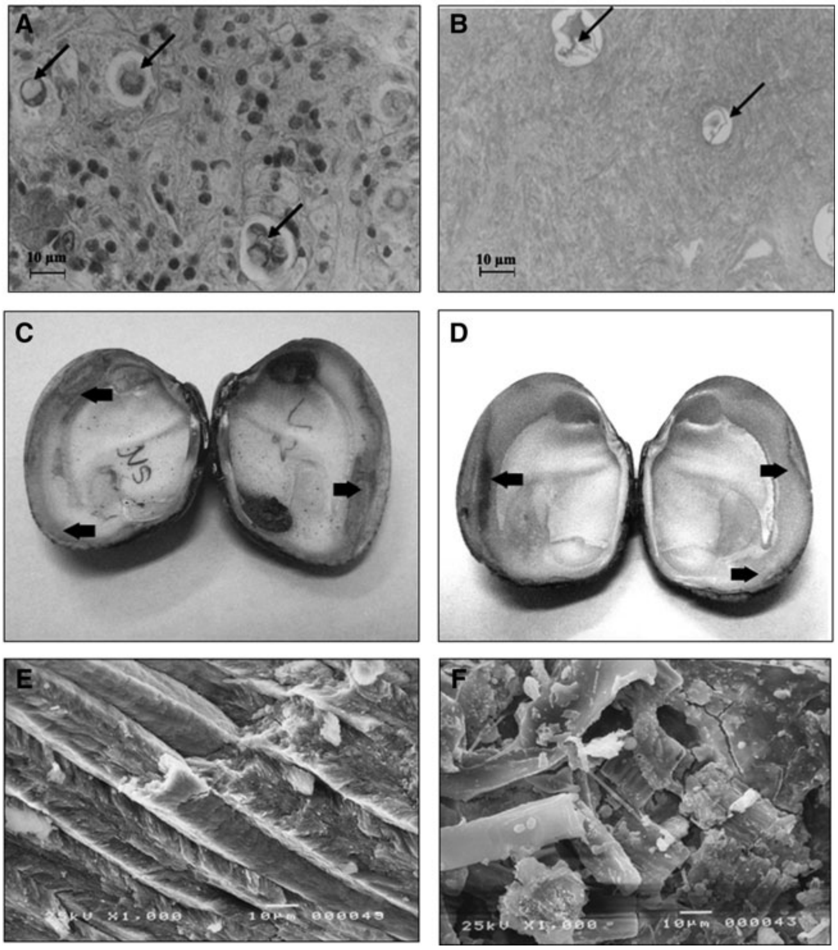

Fig. 2. (A, B) Histological sections of the mantle tissue of *Ruditapes decussatus* infected by *Perkinsus olseni* (A) and cysts of metacercaria (B) (scale bar = 10 µm); (C, D) view of inner side of shells, featuring brown ring disease symptoms and conchiolin deposits in the outer edge of shells; (E) scanning electon microscopy (SEM) observation of healthy shell compact and normal inner shell organization (normal periostracum lamina); (F) SEM of infested shells revealing disorganized periostracum in the presence of numerous bacteria.

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central zone of Chikly (Figure 3A). In summer periods, the frequency of Perkinsosis was lower and only detected at internal sites of North Lake (Chikly and STEG), with rates ranging from 2% to 8%.

Prevalence and BRD intensity

Brown ring disease symptoms were observed at least once during the surveillance period in all three sites as evidenced by the brownish organic conchiolin deposits present in the outer edges of shells of infested clams. Intensity of infection ranged from CDS4 to CDS6 of the Paillard classification scheme, indicating well-developed BRD (Figure 2C, D). SEM examination revealed disorganized periostracal lamina with numerous adherent bacteria compared to a healthy compact shell with no damages (Figure 2E, F).

Brown ring disease was detected more often in summer than winter sampling seasons although less frequently than Perkinsosis (detection varied from 40% to 60% of samples) resulting in lower disease prevalence (from 1% in Channel station to 10% in Chikly station; Figure 3B). Heterotrophic bacteria and Vibrionaceae counts indicated that there was little increase in viable bacterial numbers in summer periods during the surveillance period. Vibrionaceae were recovered consistently at higher numbers than heterotrophic bacteria, but both types of bacteria were positively correlated with disease (Pearson correlation coefficient = 0.453, $P < 0.05$). Clams feed primarily on sediments and can concentrate 10^4 to 10^6 colony forming units of bacteria, mainly *Vibrio* species which increase in summer periods (Figure 3C, D). This concentration reflects the negative impact of effluent discharge, an effect that is enhanced by poor water circulation, resulting in bacterial enrichment in clams in all lake zones. The geographical distribution of microbial disturbances showed preferential occurrence of BRD in summer mainly in the central zone contrary to Perkinsosis affecting clams in the external surveillance station within the channel. The most infested station during the period of time of the surveillance was the central area; this area was characterized by poor water circulation which likely contributed to increased

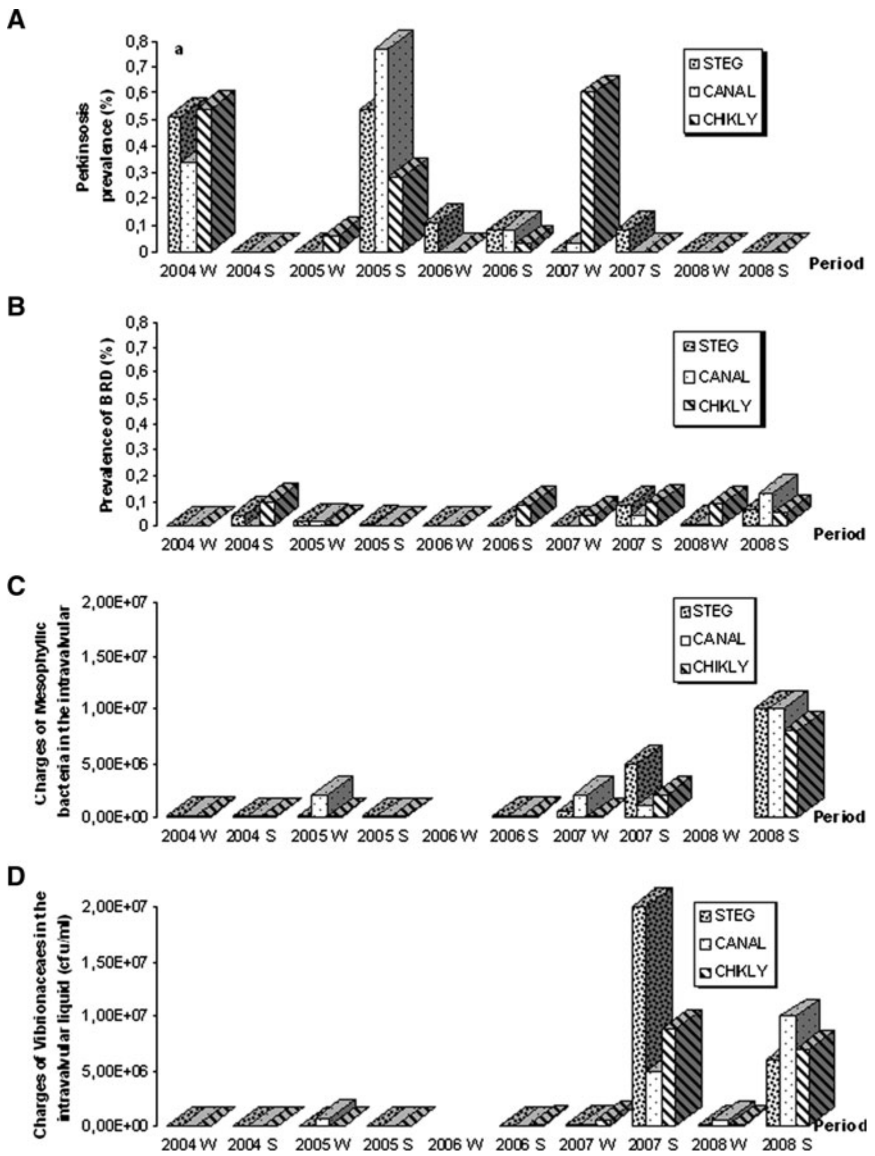

Fig. 3. Distribution of both prevalence of perkinsosis (A), brown ring disease (BRD) (B) and different bacterial charges (total mesophyllic (C) and Vibrionaceae (D)) quantified for all sites during the surveillance period. S, summer; W, winter.

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bacterial infection. A negative correlation was observed between BRD and Perkinsosis (Pearson correlation coefficient = −0.360, $P < 0.05$), and a positive correlation was shown between BRD and total mesophyllic bacteria or Vibrionaceae counts (Pearson correlation coefficient = 0.251, $P < 0.05$).

DISCUSSION

Due to the increasing importance of the carpet shell clam to the Tunisian economy, zoosanitary surveys were conducted in a coastal area of Tunisia. To our knowledge, the work described here is the first systematic surveillance of microbiological conditions affecting the carpet shell clam, and leading to Perkinsosis and BRD diseases in the North Lake of Tunis.

Brown ring disease, a bacterial disease affecting clam shells, and Perkinsosis, caused by protozoan parasites, were documented in the three surveillance sites. Prevalence ranged from 2% to 60% for Perkinsosis and from 2% to 13% for BRD. Although these diseases appear to be mutually exclusive (negatively correlated), both diseases are capable of causing mass mortalities (Paillard *et al.*, 2004; Villalba *et al.*, 2004, 2005). Both Lassalle *et al.* (2007) and Fly-Sainte-Marie *et al.* (2009) highlighted lethal effects of diseases affecting haemocyte parameters, growth rate, condition index and reduction in reproductive output for both *R. decussatus* and *R. philippinarum*. Comparatively, Soudant *et al.* (2008) demonstrated that the combined effects of parasites and harmful algal blooms modify clam haemocytic characteristics more than each individual infection.

Our present data identified *Perkinsus olseni* infection in carpet shell clams capable of spreading to all types of tissues. Similar results were previously presented by El Hili *et al.* (2007) for naturally occurring populations of carpet shell clams from another northern Tunisian location (Lagoon of Bizerte) while others have shown this for other northern Mediterranean Sea coasts (Leite *et al.*, 2004; Ngo & Choi, 2004).

Advanced BRD stages (CDS > 4) were observed and there was a positive correlation between BRD prevalence and heterotrophic Vibrionaceae counts. This confirmed previous results obtained for BRD occurring in both Manila and carpet shell clams in European areas (Paillard *et al.*, 2006; Nolwenn-Trinkler *et al.*, 2010) and 'BRD like diseases' described for several local bivalves species in Tunisia by El Bour *et al.* (2008). Due to a high level of bacteria (mainly *Vibrio* species which are naturally resistant to haemolymph bactericidal activities) changes in haemocyte counts and metabolic activities under environmental conditions favourable for microbial parasitism often lead to an infectious state in the bivalve (Tuntiwaranuruk *et al.*, 2004; Allam *et al.*, 2006; Ciacci *et al.*, 2009). The prevailing conditions found at the North Lake of Tunis (warm temperatures and high salinity) are predicted to be favourable for parasite infestation in clam populations. Salinity has previously been shown to be a prime environmental factor contributing to the spatial and temporal distribution of *Perkinsus* spp. (Park *et al.*, 2006b).

Brown ring disease occurs at lower temperatures (Paillard *et al.*, 2004; Villalba *et al.*, 2005; Dang & Montaudoin, 2009) unlike Perkinsosis, which is a 'warm disease' and its occurrence should be more frequent in the local conditions of the lagoon. Present data indicate greater prevalence of Perkinsosis in winter periods at the external surveillance site (exposed to traffic and water dynamics) and BRD in summer periods within the more internal part of the lake. In order to confirm this hypothesis, monthly surveys for parasites should be continued.

Other factors must also be considered to explain variation of spatial distribution and intensity of both diseases. Leite *et al.* (2004) signalled that the density of clam populations, the transfer and presence of various species in the same location are factors influencing the spread of Perkinsosis. In a recent study, Fly-Sainte-Marie *et al.* (2008) noted the influence of sediment grains in natural habitats with respect to the control of BRD spread and infection intensity. Alternatively, Labreuche *et al.* (2006) and Samarin *et al.* (2007) highlighted that genetic internal molecular features and immunological resistance to pathogens should be considered as much more influencing factors in pathogen spreading within bivalve populations. The factors which play a significant role in Tunisia have yet to be fully determined.

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REFERENCES

Allam B., Paillard C., Auffret M. and Ford S.E. (2006) Effect of the pathogenic *Vibrio tapetis* on defence factors of susceptible and non-susceptible bivalve species: II. Cellular and biochemical changes following *in vivo* challenge. *Fish and Shellfish Immunology* 20, 384–397.

Armi Z., Turki S., Trabelsi E. and Ben Maiz N. (2009) Nutrient loading and occurrence of potentially harmful phytoplankton species in North Lake of Tunis (Tunisia). *Cahiers de Biologie Marine* 49, 311–321.

Belkhir M., Gharbi H., Dridi K., Amara H., Ksouri A. and Hadj Ali Salem M. (1985) Le lac de Bizerte: étude physico-chimique et microbiologique de l'écosystème lagunaire. *Rapports et Documents* 3, 1–22

Ben Charrada R. (1992) Le lac de Tunis après les aménagements: paramètres physico-chimiques de l'eau et relation avec la croissance des algues. *Marine Life* 1, 29–44.

Ben Salah C. (2005) *Etude parasitaire et lésionnelle chez la palourde (R. decussatus) dans le lac de Bizerte.* Thèse de Médecine Vétérinaire. Université de Tunis El Manar, 58 pp.

Borrego J.J., Luque A., Castro D., Santamaria J.A. and Martinez-Manzares E. (1996) Virulence factors of the *Vibrio* PI, the causative agent of brown ring disease in the Manila clam, *Ruditapes philippinarum: Aquatic Living Resources* 9, 125–136.

Castro D., Pujalte M.J., Lopez-Cortes L., Garay E. and Borrego J.J. (2002) Vibrios isolated from the cultured Manila clam (*R. philippinarum*): numerical taxonomy and antibacterial activities. *Journal of Applied Microbiology* 93, 438–447.

Ciacci C., Citterio B., Betti M., Canonico B., Roch P. and Canesi L. (2009) Functional differential immune responses of *M. galloprovincialis* to bacterial challenge. *Comparative Biochemistry and Physiology. Series B. Biochemistry and Molecular Biology* 153, 365–371.

Da Dang C. and Montadouin X. (2009) Brown muscle disease and Manila clam *Ruditapes philipinarum* dynamics in Arcachon Bay, France. *Journal of Shellfish Research* 28, 355–362.

El Bour M., Jaafar-kéfi F., Hamrouni M., Mahmoud N., Dellali M., Trigui-El-Menif N. and Aloui-Bejaoui N. (2008) Etude de dépistage de la maladie de l'anneau brun chez des espèces de bivalves des côtes tunisiennes. *Bulletin de la Société Zoologique de France* 133 107–115.

El Hili H., Ben Salah C., Ayari W. and Ben Amor N. (2007) Etude descriptive de l'infestation de la palourde (*R. decussatus*) *à Perkinsus olseni* dans le lac de Bizerte en Tunisie. *Rapport de la Commission International de la Mar Méditerranée* 38, 421.

Flye-Sainte-Marie J., Pouvreau S., Paillard C. and Jean F. (2007) Impact of brown ring disease on the energy budget of the Manila clams *Ruditapes philippinarum. Journal of Experimental Marine Biology and Ecology* 349 378–389.

Flye-Sainte-Marie J., Jean F., Ford S.E. and Paillard C. (2008) Effect of grain-size on development of brown ring disease in Manila clam *Ruditapes philippinarum. Aquaculture* 278 184–187.

Fly-Sainte-Marie J., Soudant P., Lambert C., Le Goïc N., Goncalvez M., Travers A. and Paillard C. (2009) Variability of the hemocyte parameters of *R. philippinarum* in the field during an annual cycle. *Journal of Experimental Marine Biology and Ecology* 377, 1–11.

Labreuche Y., Soudant P., Goncalves M., Lambert C. and Nicolas J.L. (2006) Effect of extra-cellular products from pathogenic *Vibrio aestuarinus* strain O1/O32 on lethality and cellular immune responses of the oyster *Crassostrea gigas. Developmental and Comparative Immunology* 30, 367–379.

Lassalle G., Montaudouin X., Soudant P. and Paillard C. (2007) Parasite co-infection of two sympatric bivalves, the Manila clam (*R. philippinarum*) and the cockle (*Cerastoderma edule*) along a latitudinal gradient. *Aquatic Living Resources* 20, 33–42.

Leite R.B., Alfonso R. and Cancela M.L. (2004) *Perkinsus* sp infestation in carpet-shell clam (*R. decussatus*), along the Portuguese coast. Results from a 2-year survey. *Aquaculture* 240, 39–53.

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

Nolwenn-Trinkler T., Sinquin G., Querne J. and Paillard C. (2010) Resistance to brown ring disease in the Manila clam *Ruditapes philippinarum*: a study of selected stocks showing a recovery process by shell repair. *Journal of Invertebrate Pathology* 104, 8–16.

OIE (2001) *Diagnostic manual for Aquatic diseases.* 3rd edition. Paris: Office International des Epizooties, 273 pp.

Paillard C., Maes P. and Oubella R. (1994) Brown ring disease in clams. *Annual Review of Fish Diseases* 4, 219–240.

Paillard C., Maes P. and Oubella R. (2004) Effect of temperature on defence parameters in Manila clams *Ruditapes philippinarum* challenged with *Vibrio tapetis. Diseases of Aquatic Organisms* 59, 249–262.

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

Park K.I. and Choi K.S. (2001) Spatial distribution of the protozoom parasite *Perkinus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. *Aquaculture* 203, 9–22.

Park K.L., Paillard C., Le Chevalier P. and Choi K.S. (2006a) Report on the occurrence of brown ring disease (BRD) in Manila clam, *Ruditapes philippinarum*, on the west coast of Korea. *Aquaculture* 255, 610–613.

Park K.I. Figuers A. and Choi K.S. (2006b) Application of enzyme-linked immunosorbent assay (ELISA) for the study of reproduction in the Manila clam *Ruditapes philippinarum (Mollusca: Bivalvia). II. Impacts of Perkinsus olseni* on clam reproduction. *Aquaculture* 251, 182–191.

Samain J.F., Dégremont L., Soletchnik P., Hauré J., Bédier E., Ropert M., Moal J., Huvet A., Bacca H., Van Wormhoudt A., Délaporte M., Costil K., Pouvreau S., Lambert C., Boulo V., Soudant P., Nicolas J.L., Le Roux F., Renault T., Gagnaire B., Géret F., Boutet I. and Boudry P. (2007) Genetically based resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*) and its relationship with physiological, immunological characteristics and infection process. *Aquaculture* 268, 227–243.

Soudant P., Leite R., Chu F.L.E., Villaba A. and Cancela L. (2008) Bivalves–*Perkinsus* sp. interactions. In Villaba A. (ed) *Workshop for the analysis of the impact of Perkinsosis to the European shellfish industry*. Centro de Investigacións Mariñas, Consellería de Pesca e Asuntos Marítimos da Xunta de Galicia, Vilanova de Arousa, Spain and Centro Tecnológico del Mar—Fundación CETMAR, Vigo, Spain. 2008, pp. 77–109.

Tuntiwaranuruk C., Chalermwat K., Upathum E.S., Kruatrachue M. and Azevedo C. (2004) Investigation of *Nematopsis* sp. oocysts in 7 species of bivalves from Chonburi Province, Gulf of Thailand. *Diseases of Aquatic Organisms* 58, 47–53.

Villalba A., Reece K.S., Ordas M.C., Casas S.M. and Figueras A. (2004) Perkinsosis in molluscs: a review. *Aquatic Living Resources* 17, 411–432.

and

Villalba A., Casas S.M., Lopez C. and Carballal M.J. (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. *Diseases of Aquatic Organisms* 65, 257–267.

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