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# Brown muscle disease: Impact on Manila clam *Venerupis* (=*Ruditapes*) *philippinarum* biology

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ABSTRACT

This study assessed the effect of Brown Muscle Disease (BMD) on Manila clam *Venerupis philippinarum* fitness. BMD was discovered in 2005. It affects the posterior adductor muscle and leads to clam gaping and eventually death. Three statuses of clams were compared: buried individuals with no signs of BMD (BUR); clams at the surface of the sediment with no signs of BMD (SURF) and clams at the surface of the sediment exhibiting signs of brown muscle disease (BMD). Physiological (condition index), immune (hemocyte parameters) and molecular (gene expressions) parameters collected seasonally were analyzed and compared.

Results demonstrated a seasonal pattern in condition index (CI) with peaks in spring/summer and decreases in autumn/winter. At each season, the highest CI was observed in BUR and the lowest CI was observed in BMD.

In terms of immune response, phagocytosis rate and capacity were higher in clams with BMD whereas the health status of the clams did not influence the total hemocyte count. Genes involved in the immune system (*comp*, *tnf*, *inter*) were upregulated in clams with BMD. The molecular analysis of gill and posterior muscle showed higher mitochondrial metabolism (*cox-1*, 16S) in cells of infected clams, suggesting a stronger energetic demand by these cells. Finally, genes involved in oxidative stress response (*cat*, *sod*), detoxification (*mt*) and DNA repair (*gadd45*) were also overexpressed due to reactive oxygen species production.

Most of the studied parameters underlined a cause–effect correlation between Manila clam health status (BUR, SUR, BMD) and physiological parameters. An important stress response was observed in BMD-infected clams at different scales, *i.e.* condition index, immune parameters and stress-related gene expression.



## 1. Introduction

Capture fisheries and aquaculture supplied the world with about 148 million tons (Mt) of product in 2010 with a total value of 217.5 US$ billion [1]. Freshwater and marine bivalves represented 14.4 Mt of this production, with strong domination by Ostreidae and Veneridae. However, the emergence of infectious diseases represents a limiting factor for aquaculture and fisheries and has led to serious economic losses [2]. There are several examples throughout the world where the development and spread of infectious diseases acted as a precursor to production collapse. In most cases, protozoans, bacteria and virus pathogens were involved. Concerning oysters, the “winter mortality” that occurred in oyster *Saccostrea glomerata* in 1924 (Australia) was perhaps the earliest epizootic of bivalves associated with a parasitic etiological agent, the protozoan *Bonamia roughleyi* [3]. From then on, many other host/parasite interactions associated with massive mortalities have been identified, *e.g. S. glomerata*/*Marteilia sydneyi* (QX disease) in Australia [4,5], *Ostrea edulis*/*Bonamia ostreae* and *Marteilia refringens* in Europe [6], *Crassostrea angulata*/irido-like virus in France [7], *Crassostrea gigas*/herpes-like virus around the world [8].

After *Crassostrea* spp., the Manila clam, *Venerupis* (=*Ruditapes*) *philippinarum*, is the most exploited bivalve in the world with 3.24 Mt and 0.65 Mt produced from aquaculture and capture fisheries respectively [1]. Manila clam stocks also suffer from many diseases,

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mainly due to bacteria *Vibrio tapetis* (Brown Ring Disease) [9] and protozoans *Perkinsus* spp. In 2005, a new disease named Brown Muscle Disease (BMD) was discovered, in Arcachon Bay [10] where the Manila clam fishery is of major importance [11]. BMD was rapidly involved in high mortalities in adult Manila clams in the area [10]. The disease was present in 62% of the Manila clam habitat surface [12] with a prevalence reaching in some areas 30% of adult specimen [10]. Dang & de Montaudouin [12] focused on a high prevalence area of Arcachon Bay and showed that 95% of the mortality of the 4-yr old cohort was due to BMD. At the individual scale, condition index of Manila clams affected by BMD was reduced by 37% [12]. The disease affects the posterior adductor muscle which becomes infused with conchiolin and becomes progressively calcified. Diseased clams are often associated with the presence of clams at the surface of sediment, which is an abnormal position for this infaunal bivalve. An analysis of "surfaced clams" revealed an infection rate three times higher than buried (*i.e.* normal position) sympatric clams [10]. In laboratory trials, surfaced clams displayed greater mortality rates after 15 days than buried individuals (82% against 12%). Ultrastructural observation by transmission electron microscopy revealed the presence of virus-like particles [13]. At sub-lethal level, the effects of BMD on the physiology of clams, *e.g.* its condition index, immune response or interest gene expressions, are unknown and constitute the basis of this study. However, BMD in clams in this area cannot be dissociated from perkinsosis which, in our ecosystem, is very prevalent and co-occurs with BMD [14]. Perkinsosis was discovered in 1950 by Mackin et al. [15] in *Crassostrea virginica*, and since then an abundant literature has focused on this disease and its etiological agent, their distributions, hosts and epizoology [16]. Recently, *Perkinsus* sp. mean abundance and prevalence in Arcachon bay was assessed at around $10^5$ cells $g^{-1}$ of gill and 100%, respectively [17,18]. At this level of infection, only sub-lethal effects on physiology, reproduction and growth have been observed (Casas 2002 in Ref. [16]). Besides their direct pathological consequences, infection by *Perkinsus* sp. may render hosts more susceptible to other diseases [19–21].

The use of energetic budgets for defense against pathogens may slow growth and reduce condition of market sized individuals, and may lead to deficiency of energy reserves required for gametogenesis [16]. In general terms, infection causes host weakening so that it becomes increasingly difficult for affected individuals to overcome any other adverse conditions [16].

The ability to activate or induce defense reactions in response to microbial challenge, inflammatory conditions or environmental stress is a fundamental property of organisms [22]. Bivalves possess natural innate defenses, including an open circulatory system in which hemocytes are the primary effectors of cellular immunity. These cells are able to detect foreign material and induce an efficient immune response [23,24]. The health status of marine bivalves can be inferred from immune parameters, such as hemocyte concentration and phagocytosis [25]. However, factors other than diseases, like temperature, salinity, nutritional stress or air exposure, can modulate immune reaction [25–28]. Consequently, measurement of other parameters, such as condition index or expression modulation of genes involved in defence against pathogens, is essential to assess the influence of infection on the global immune response [24]. During the last decade, development of transcriptomic approaches has led to the description of the impacts of a number of xenobiotics and parasites on host cell response. However, concerning bivalves, only a few studies have determined the modulation of gene expression patterns during the infection process. Recent analysis revealed that perkinsosis was able to increase the transcription level of genes involved in the oxidative stress defence and apoptotic mechanisms in the eastern oyster *C virginica* [29]. In the same way, bonamiosis due to the parasite *B. ostreae* has been associated with differential expression of various genes notably those involved in immune response such as cytokines, stress proteins, eicosanoids or genes involved in phagocytosis [30,31]. However, for *V. philippinarum* such molecular reports remained scarce. *Perkinsus* sp. was assumed to increase the expression of different sets of lectins [32] and a modified-expression of metallothioneins had been observed during exposure to the bacteria *V. tapetis*, the trematode parasite *Himasthla elongata* and cadmium used alone or mixed in controlled conditions [33]. The first partial transcriptome analysis by high throughput sequencing of Manila clams has been recently achieved, making numerous gene sequences available [34]. In this context it appeared particularly relevant to determine the molecular impact of BMD in *V. philippinarum* to contribute to the limited knowledge currently available on the cellular effects of this disease.

Our previous field observations showed that adult Manila clams in Arcachon Bay may be divided into three "statuses": 1) individuals that are buried in the sediment. Most of them are BMD-free but are already infected by the parasite *Perkinsus* sp.; 2) individuals that are at the surface of the sediment but do not exhibit BMD signs; 3) individuals that are at the surface of the sediment and exhibit BMD signs. Our aim was to compare the adaptive response of bivalves in these three statuses at three organization biological levels: 1) Physiological with the measurement of condition index; 2) Cellular with evaluation of hemocyte parameters and 3) Molecular with expression levels of genes involved in important cellular functions (mitochondrial metabolism oxidative stress response, detoxification, DNA repair and immune response). *Perkinsus* sp. abundance was also measured. This correlative approach, including seasonal variations, will provide a better understanding of Manila clams response to the BMD.

## 2. Materials and methods

### 2.1. Sampling site

Arcachon Bay is a 156 $km^2$ semi-sheltered lagoon along the Atlantic coast of France (Fig. 1).

It is subject to both oceanic and continental influence. The Manila clam is, in terms of biomass, the dominant species of

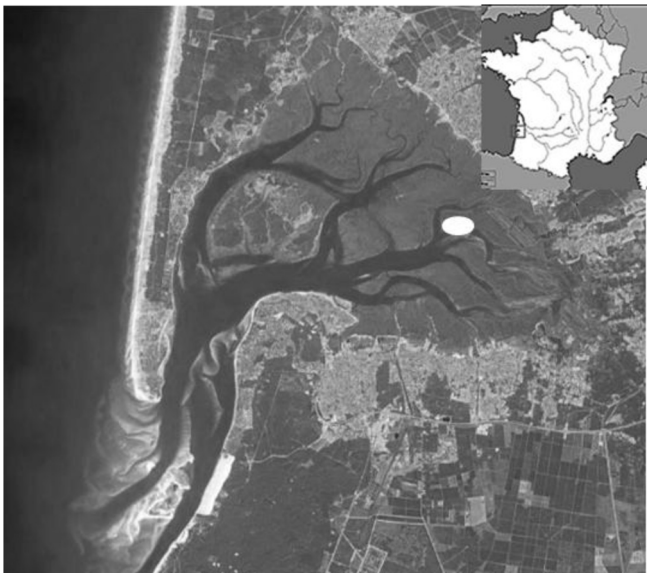

**Fig. 1.** Location of sampling site (white ring).