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Short communication

Effect of sediment grain-size on development of brown ring disease in the Manila clam *Ruditapes philippinarum*

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ABSTRACT

Brown ring disease (BRD) in the Manila clam is induced by the bacterium *Vibrio tapetis*. During the infection process, the pathogen enters the extrapallial compartment of the Manila clam and induces the formation of a characteristic brown deposit that gives the disease its name. Although post-infection processes have been widely described for this disease, the mechanisms of entry of the bacteria into the extrapallial compartment remains unclear. From relationships between clam size and BRD prevalence, and between grain-size distribution in natural habitats and prevalence, we propose a simple explanation for this step: *V. tapetis* benefits from mechanical disruptions of the periostracal lamina or valve margins to colonize the extrapallial compartment. Such disruptions may be induced by the presence of large sediment grains in natural habitats, which become lodged in the shell opening. This hypothesis suggests that limiting handling of clams may help to limit development of BRD in cultured clam beds.

1. Introduction

The Manila clam, *Ruditapes philippinarum*, was introduced in France for aquaculture purposes between 1972 and 1975 (Flassch and Leborgne, 1992). In France, this venerid culture became increasingly widespread, and since 1988 natural populations have colonized most embayments along the French Atlantic coast, resulting in important fisheries.

Brown ring disease (BRD) in this species is a bacterial disease induced by the pathogen *Vibrio tapetis*. It was first observed in 1987 in northern Brittany (France) and has rapidly been reported along the French Atlantic coast (Paillard, 1992). The disease is now observed along the entire European Atlantic coast, from Norway to southern Spain, and in Italy (Paillard, 2004b; Paillard, unpublished data). The disease, which causes mass mortalities in cultured clam beds, has severely affected venerid culture in northern Brittany but has a lower impact in natural beds, where maximum prevalence reaches only 30% (Paillard, 2004b).

During the infection process, the pathogen proliferates within the extrapallial compartment and disrupts the normal production of periostracal lamina, inducing the formation of a brown conchiolin deposit on the inner shell; this characteristic clinical sign gave the disease its name (Paillard, 1992). BRD progression has been described in depth in Paillard et al. (1994) and Paillard (2004b). The following steps for the disease progression have been proposed by Paillard (2004b): (1) adherence of the pathogen, *V. tapetis*, to the periostracal lamina; (2) penetration and colonization of the extrapallial compartment; and (3) formation of the anomalous brown conchiolin deposit, in which the pathogen becomes embedded. Although post-infection processes (i.e. after penetration into extrapallial compartment) have been widely described (see Paillard, 2004b, for a review), mechanisms of entry of *V. tapetis* into the extrapallial fluids remain poorly understood. Paillard (2004b) stated that "in favourable conditions for the pathogen, *V. tapetis* colonization provokes some alteration and rupture of the periostracal lamina which allows the penetration of the bacteria into the extrapallial fluids". By revisiting three unpublished data sets, we propose a simple hypothesis to explain the entry of *V. tapetis* into the extrapallial compartment.

2. Methods

All three data sets were collected from intertidal natural populations in the Gulf of Morbihan (southern Brittany, France) between 1999 and 2006 (Fig. 1). For all data sets, BRD signs in the clams were monitored on the inner surface of the clam shells according to the

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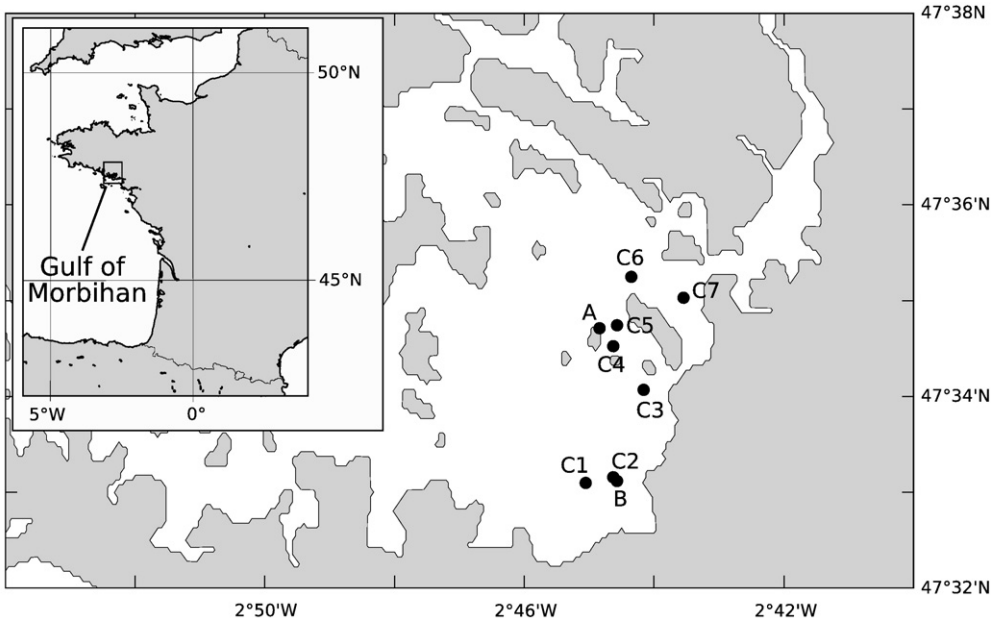

Fig. 1. Map of the sampling sites of the data sets used for this study. A: Ile de Bailleron; B: Ile aux Oiseaux. C1–C7: Sampling sites of the third data set.

criteria of Paillard and Maes (1994) in which conchiolin deposits stages (CDS) range from microscopic brown spots on the inner face of the shell in the earliest stage (CDS 1), to a thick brown deposit covering most of the inner shell in the most advanced stage (CDS 7).

The first data set came from a monthly monitoring of haemocyte parameters of Manila clams at Ile de Bailleron (Fig. 1, site A) (Flye-Sainte-Marie et al., unpublished data). During this survey, a total of 17 samples of 60 clams each was collected between the 6th of July 2004 and the 20th of September 2005 (total: 1020 individuals). This survey included individuals from a wide size range: from 26 mm to 55 mm (mean=37.80 mm, SD=4.46) along the maximum length axis. The prevalence of BRD was low (mean=9.7%, SD=3.5%) and showed no significant variation during the sampling period ($\chi^2=12.96$, $df=16$, $p=0.675$) allowing pooling of the whole data set in order to extract a size–prevalence relationship. In this data set, disease intensity was also low: 80% of the affected clams had a CDS of 3 or less.

The second data set was collected on the 29th of March 2006 in Ile aux Oiseaux (Fig. 1, site B). A total of 530 individuals was collected and the size ranged from 11 mm to 42 mm (mean=28.01 mm, SD=6.49). In this data set, disease prevalence and intensity were also low: average prevalence was 4.1% and 80% of the affected clams presented a CDS of 3 or less.

For these two data sets, individuals were distributed into 2-mm size classes; prevalence (percentage of affected individuals) was calculated for each size class. Size classes with fewer than 40 individuals (left and right tails of the size distributions) were excluded from computations. Relationships between clam size and prevalence were analysed using linear models, the *p*-value corresponds to the significance of the linear model (i.e. probability of the difference between the value of the slope and 0).

The third data set came from a survey of Manila clam density, BRD prevalence and *V. tapetis* abundance in the sediment, as estimated by an ELISA test (Allam et al., 2002) in the Gulf of Morbihan during 1999 (Paillard, unpublished data). During this study, sediment cores were collected at some of the sampling stations. Grain-size distribution in sediment cores was measured following Weiss and Frock, (1976). For seven stations (Fig. 1, sites C1–C7) both BRD prevalence and grain-size distribution were available, which allowed analysis of the relationship

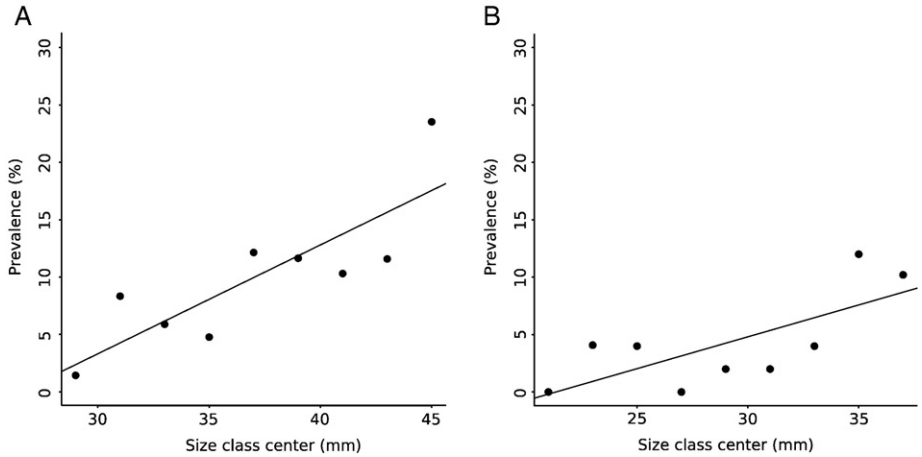

Fig. 2. Relationship between center of the size classes and prevalence in the data set from (A) Ile de Bailleron ($y=0.94x-0.25$; $r^2=0.52$; $p=0.005$) and the data set from (B) Ile aux Oiseaux ($y=0.55x-0.12$; $r^2=0.66$; $p=0.028$).