Impact of Brown Ring Disease on the energy budget of the Manila clam Ruditapes philippinarum

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Abstract

Brown Ring Disease (BRD) is a bacterial disease caused by the pathogen, *Vibrio tapetis*. The disease induces formation of a brown deposit on inner shell of the Manila clam, *Ruditapes philippinarum*. Development of this disease is correlated with a decrease in the condition index of infected clams. Experiments were conduced in order to assess the effect of the development of BRD on two parameters affecting the energy balance of the clams: the clearance and the respiration rates. Experiments were performed in a physiological measurement system that allowed simultaneous measures of clearance and respiration rates. During both acclimation and measurements clams were fed with cultured *T-iso* and temperature was close to seasonal field temperature (10°C). Our results showed that severely diseased clams (conchiolin deposit stage, CDS $\geq$ 4) are subject to weight loss in comparison to uninfected ones, indicating that BRD induces a desequilibrium in the energy balance. We demonstrated a reduction of the clearance rate of severely diseased clams which led to a decrease in energy acquisition. Respiration rate showed asignificant decrease with BRD symptoms, but evidence in the literature allowed us to hypothesize that energy mobilised for an immune response and lesion repair increases overall organism maintenance costs. Both factors should thus contribute to the degradation of the energy balance of diseased clams. Because effects of BRD on naturally infected clams only appears significant for CDS $\geq$ 4, when brown ring assumes a significant place on the inner shell, we consider that the Manila clam is tolerant of low disease levels.

Key words: clearance rate, filtration, respiration rate, Brown Ring Disease, energy budget, *Ruditapes philippinarum*

1 Introduction

Brown Ring Disease (BRD) in the Manila clam, *Ruditapes philippinarum*, was first observed in North Finistère (France) in 1987 (Paillard et al., 1989), and can be responsible for mass

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mortalities (see e.g. Paillard et al., 1989; Castro et al., 1992; Paillard, 1992, 2004b). This disease was shown to be caused by a *Vibrio* sp. (Paillard and Maes, 1990), which was named *Vibrio tapetis* (Borrego et al., 1996b). The infection disrupts the production of periostracal lamina and causes an anomalous deposition of periostracum on the inner shell of infected clams (Paillard and Maes, 1995a,b). Infected clams thus exhibit a characteristic brown deposit on the inner surface of the valves (Paillard et al., 1989) that gave the disease its name. Disease progression is estimated by the extent of the symptomatic deposit decribed by Paillard and Maes (1994). Infected clams show depressed defence-associated activities (Allam et al., 2000; Paillard et al., 2004). The effects of BRD on Manila clams have been reviewed recently by Paillard (2004b).

Different studies of marine bivalve infections by pathogens and/or parasites have shown reduction in reproduction efficiency, condition and growth. The protozoan parasite *Perkinsus marinus* significantly reduces growth rate, condition index and gametogenesis of its host, *Crassostrea virginica* (Kennedy et al., 1995; Paytner, 1996; Dittman et al., 2001). A similar pattern has also been shown for *Perkinsus olseni* in the clam *Tapes decussatus* (see review in Villalba et al., 2004). The latter parasite also reduces the reproductive output of *R. philippinarum* (Ngo and Choi, 2004; Park et al., 2004). The ascetosporan parasite *Haplosporidium nelsoni* also inhibits gametogenesis and reduces condition and glycogen reserves of its host, the oyster *C. virginica* (Barber et al., 1988a,b; Ford and Figueras, 1988). These results allowed the authors to conclude that infection induces an alteration of the host's energy budget. Nevertheless, few studies have been performed to document the influence of pathogens and/or parasites on components of the energy budget of bivalves such as food consumption and metabolism. The influence of the parasitism by the gastropod *Boonea impressa* on these parameters in the oyster *C. virginica* was documented by Ward and Langdon (1986) and Gale et al. (1991). The effects of *P. olseni* on food consumption and metabolism of *Tapes decussatus* were documented in Casas (2002). Newell (1985) showed that the infection by the parasite *Haplosporidium nelsoni* reduced clearance rate but does not affect oxygen comsumption rate of the host *C. virginica*.

Experimental infections of Manila clams by *Vibrio tapetis* induced development of BRD, weight loss and depletion of glycogen reserves, suggesting an energetic cost of the disease (Plana, 1995; Plana et al., 1996). In the field, Goulletquer (1989) also showed that winter mass mortalities of Manila clams were associated with low condition index and glycogen reserves. These mortalities were subsequently associated with BRD and Paillard (1992) demonstrated that BRD infected Manila clams exhibited low condition index. All these results lead to the conclusion that the development of BRD affects the energy balance of the Manila clam. The aim of this physiological study was to document the influence of the development of BRD on two components of the energy budget of naturally infected Manila clams: the clearance rate, a parameter involved in energy acquisition, and the respiration rate, reflecting the overall metabolism of the Manila clam.

2 Materials and methods

2.1 Biological material and acclimation procedure

Manila clams, *R. philippinarum*, were provided by the SATMAR hatchery and were grown in the Chausey Islands (Manche, France). Clams ranging from 37 mm to 50 mm were then transferred to Landéda (North Finistère, France) during autumn 2005. By early January 2006, BRD prevalence was 50%.

Samples were collected at low tide and transferred to IFREMER Argenton Shellfish Laboratory (North Finistère, France) on 13 January, and 2 and 28 February, 2006. As gametogenesis is initiated at 12°C in this species (Laruelle et al., 1994), clams from the three samples had empty gonads. Once in the laboratory, clams (37 to 50 mm length) were held in flow-through tanks in sieves containing field sediment. Tanks were supplied with thermoregulated, filtered (1 μm) seawater enriched in cultured microalgae *Isochrysis aff. galbana (T.iso)*. Salinity (35 ‰) and temperature (10°C) were kept constant during all experiments and were near to seasonal field conditions (field average temperature measured at Landéda during the experimental period was 9°C). Minimal acclimation time of one week before measurements was respected in order to limit influence of the stress due transfer from field to laboratory on our measurements. We limited acclimation time to a maximum of 4 weeks to avoid reparation processes or further development of BRD symptoms in laboratory conditions.

2.2 Physiological measurements

2.2.1 Physiological measurement system

Ecophysiological measurements were performed in the IFREMER Argenton Shellfish Laboratory, which is equipped with an experimental apparatus allowing the simultaneous monitoring of clearance rate (CR, $L\,h^{-1}$) and respiration rate (RR, $mgO_2\,h^{-1}$) in individual flow-through chambers, for seven individuals at a time (Savina and Pouvreau, 2004).

The apparatus consists of eight flow-through transparent chambers each having a volume of 1.2 L, (Fig.1 B) filled with 370 g of sand. Sand was sieved on 1 mm mesh to avoid recirculation of small particles in the system and baked 4 h at 450°C to limit bacterial metabolism in the chambers. The first chamber (C0) was used as a control, the seven others (C1-C7) contained animals. Upstream water was thermoregulated, filtered (1 μm), and enriched with cultivated algae at a controlled concentration by means of a peristaltic pump. The flow rate through the chambers was controlled using manual flow meters (FM0 to FM7). Temperature, oxygen concentration and fluorescence were monitored alternately in the outflowing water of each chamber using an oxygen–meter (O_2; WTW sensor) and a fluorometer (F; Seapoint sensor). Data mesured by these sensors were collected by the controller and sent to a computer via a local area network. The controller controlled the valves that shunted water to either the measurement probes or to

waste. The system allowed sampling of out-flowing water for particle counts.

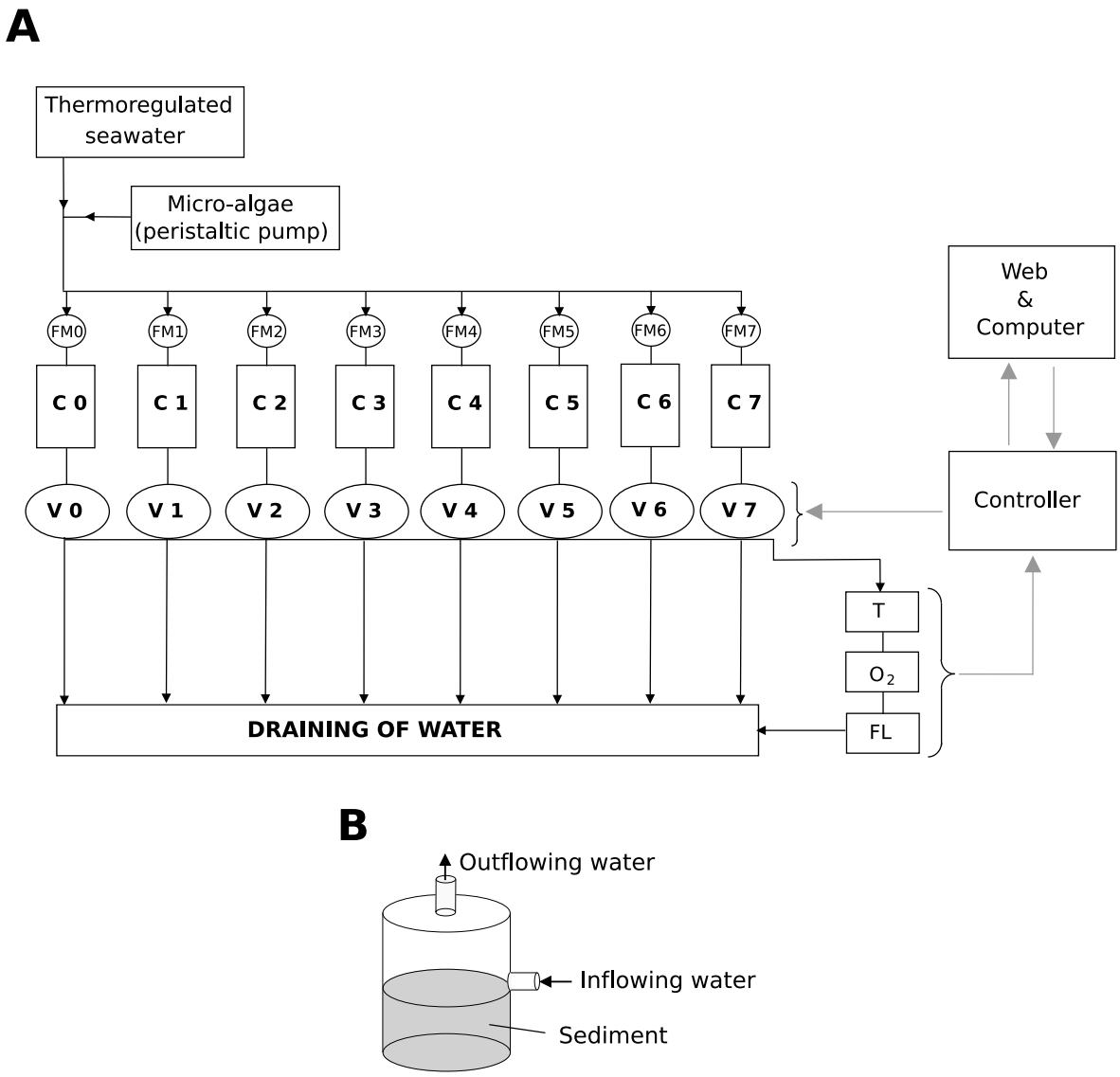

Fig. 1. The ecophysiological measurement system. A: General view (C: chamber, FM: manual flow meter, V: valve, T: temperature sensor, O_2: oxygen sensor, FL: fluorometer); B: individual chamber.

2.2.2 Experimental conditions

A total of 15 experiments was performed between 15 January and 28 March 2006. For all experiments, conditions were similar. Temperature varied between 9.6 and 10.4°C and cultured algae concentration (*Isochrysis aff. galbana; T–iso*) in inflowing water was set to 50 $cells\,\mu L^{-1}$. Preliminary experiments (unpublished data) showed that this alga concentration was below the pseudofaeces production threshold for *R. philippinarum*. No pseudofaeces production was observed in any experiment thus ingestion rate was equivalent to filtration rate.

2.2.3 Measurement procedure

For each experiment, individuals were placed in one of the eight individual chambers. A time interval of at least $2\,h$ between the transfer of clams to the chambers and the beginning of any measurements allowed the clams to return to a normal activity. This time was chosen after preliminary experiments which showed that clams were consistently active $2\,h$ after being transferred to the chambers. The first chamber was used as a control and had sediment but no clams. Flow rate in the chambers was constant and equal to $6.7\,L\,h^{-1}$. This value was chosen after preliminary tests and allowed measurement of the respiration rate, while preventing the clam from removing too much algae. Experimental chambers were successively measured continuously for $5\,min$. Prior to each experimental chamber measurement, the control chamber was continuously measured for $3\,min$. Between each measurement a 5–min interval with no measurement allowed water from the next chamber to reach the sensors. This resulted in a record every $2\,h$ and $20\,min$ for each animal.

Every day, a new alga culture was used. Because the concentration of the culture varied, the flow rate of the peristaltic pump was adjusted to provide an algal concentration of $50\,cells\,\mu L^{-1}$. Every day, excurrent water samples were collected for cell counting (Coulter counter, multisizer), which allowed an intercalibration between fluorescence and cell concentration.

Characterization and classification of the BRD symptom requires observation of the inner surface of the clam shell (Paillard and Maes, 1994). Although this can be done in living clams, it is stressful (Ford and Paillard, 2006). As a consequence, diagnosis of BRD had to be done after the physiological measurements. Rhythmic variations in activity are well documented in bivalves (e.g. Bougrier et al., 1998; Kim et al., 2003; Ortmann and Greishaber, 2003; Rodland et al., 2006), including the Manila clam (Kim et al., 1999, 2001, 2004). They must be taken into account in order to obtain reliable individual measurements of clearance rate and respiration rate. A preliminary experiment was performed to determine the minimum experimental time needed to integrate these variations of activity. It was performed over 5 days under the experimental conditions cited above. After each respiration rate and clearance rate measurement, the average values were calculated from the beginning of the experiment. For both respiration and clearance rate, in all measured individuals, the average stabilized after $48\,h$. Thus $48\,h$ was assumed to be the minimum experimental time needed to obtain measures that would reliably integrate variations in the activity of Manila clams.

2.3 Ecophysiological data processing

2.3.1 Average clearance rate

For each record, average values of the fluorescence of the effluent water from the control and from the experimental chamber were calculated. For each new cell culture, water-sample cell concentrations were measured using the Coulter Counter Multisizer 3 to allow the calculation of a regression equation between fluorescence and cell concentration. Using this equation, average fluorescence values were converted to cell concentration ($cells\,L^{-1}$). Then, for each record, the

clearance rate (CR, $L\,h^{-1}$) was calculated following the equation (Hildreth and Crisp, 1976):

$$CR = \frac{C_c - C_a}{C_a} \times FR \tag{1}$$

where C_c is the cell concentration ($cells\,L^{-1}$) in the effluent water from the control chamber, C_a is the cell concentration ($cells\,L^{-1}$) in the effluent water from the measured animal chamber and FR is the flow rate ($L\,h^{-1}$) through the chambers. The average of all records (c.a. 21 records per animal) was then calculated for each animal.

2.3.2 Average respiration rate

For each record, average values of the oxygen concentration of the effluent water from the control and from the experimental chamber were calculated. Then, the oxygen consumption rate (RR, $mg\,O_2\,h^{-1}$) was calculated following the equation:

$$RR = (O_c - O_a) \times FR \tag{2}$$

where O_c is the oxygen ($mg\,O_2\,L^{-1}$) in the effluent water from the control chamber, O_a is the oxygen ($mg\,O_2\,L^{-1}$) in the effluent water from the measured animal chamber and FR is the flow rate ($L\,h^{-1}$) through the chamber. Then the average of all records (c.a. 21 records per animal) was then calculated for each animal.

2.3.3 Maximum clearance rate

Our measurement procedure allowed us to examine instantaneous measurements of clearance rate (e.g. each of the 21 records). Maximum clearance rate was defined as the average of the three highest values obtained in the 21 records. Maximum clearance rate provides information about the filtration capacity of the clams, and permits discrimination between the impact of BRD on behaviour and that on physiological capacities.

2.3.4 Filtration and respiration–time activity

Filtration–time activity (FTA) and respiration–time activity (RTA) are defined as the proportion of time spent in the activity of filtration and respiration respectively (Bougrier et al., 1998; Huvet, 2000). Following Savina and Pouvreau (2004), FTA and RTA were calculated for each individual as the ratio between the number of records showing a measurable filtration or respiration activity and the total number of records. These values provide information about the behaviour of the clams.

2.3.5 Standardisation of clearance and respiration rates

In order to compare values obtained for different size individuals, clearance and respiration rates were corrected for weight differences between individuals following the formula of Bayne et al. (1987):

$$Y_s = (\frac{W_s}{W_m})^b \times Y_m \tag{3}$$

Were Y_s is the physiological rate for an individual of standard shell dry weight W_s , Y_m is the measured physiological rate for an individual of shell dry weight W_m and b the weight exponent for the physiological rate function. Clearance and respiration rates were standardised for a clam with a shell dry weight of $11\,g$ (corresponding to a length of $43.9\,mm$). Considering that filtration and respiration processes scale with somatic tissue weight and that BRD may induce a loss of reserves rather than somatic tissue, shell dry weight was used as a proxy of somatic weight for correction of physiological rates for size. Reviews concerning weight exponents calculated for several bivalves (Pouvreau et al., 1999; Savina and Pouvreau, 2004, for clearance and respiration, respectively) showed that average weight exponents are generally around 2/3 for clearance and 3/4 for respiration. These values were thus chosen for standardisation of clearance and respiration rates respectively.

2.4 Biological sample treatment

2.4.1 Condition index

After each experiment, clams were individually numbered, flesh was removed from the shell, freeze-dried to constant mass (48h) and weighed. Shells were measured along the maximum length axis, dried and weighed. Condition index (CI) was calculated following

$$CI = \frac{Flesh\ Dry\ Weight}{Shell\ Dry\ Weight} \times 100 \tag{4}$$

2.4.2 Characterisation and classification of BRD syndrome

Disease stage was classified according to the description of Paillard and Maes (1994). According to these authors, conchiolin deposit stage (CDS) range from microscopic brown spots on the inner face of the shell in the earliest stage (CDS 1) to a complete thick brown ring in the most advanced stage (CDS 7).

2.4.3 Statistical analysis

Statistical analysis were performed using the R software (R Development Core Team, 2006). ANOVA was used to test the effect of BRD development stage (CDS) on measured physiological rates after checking homoscedasticity (Barttlet test). Since FTA and RTA are ratios, data were arcsin–transformed and the same procedure used.

3 Results

3.1 Effect of BRD stage on condition index

Development of BRD symptoms was associated with a weight loss of the clams. Condition index decreased as the severity of BRD symptoms increased (Fig. 2). There were significant differences of condition index among CDS levels (ANOVA, *F*-value=12.89, *P*-value= $1.7\,10^{-11}$). In clams with CDS > 4, condition index was 27 to 35 % less than that of asymptomatic (CDS = 0) clams (HSD Tukey, *P*-value < 0.05, see Fig. 2); however there were no significant differences between clams in CDS=0 and in those in CDS=1–4.

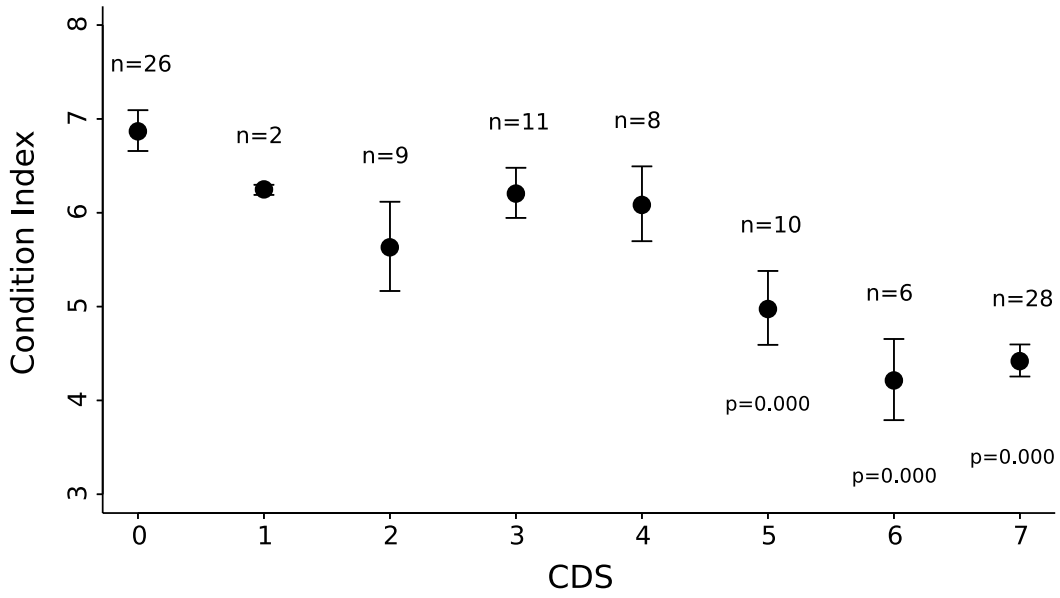

Fig. 2. Relationship of mean condition index (mean $\pm$ SE) with conchiolin deposit stage (CDS). p is the HSD Tukey *P*-value for the comparison with CDS=0. n = number of individuals in each CDS class.

3.2 Effect of BRD stage on respiration rate

Respiration rate decreased with the extent of BRD development (Fig. 3) and was significantly lower for clams with CDS $\geq$ 5 in comparison to asymptomatic (CDS=0) ones (ANOVA, *F*-

value=4.86, *P*-value= $1.1\,10^{-4}$; HSD Tukey, *P*-value < 0.05, see Fig. 3). No clear effect of BRD was found for respiration–time activity.

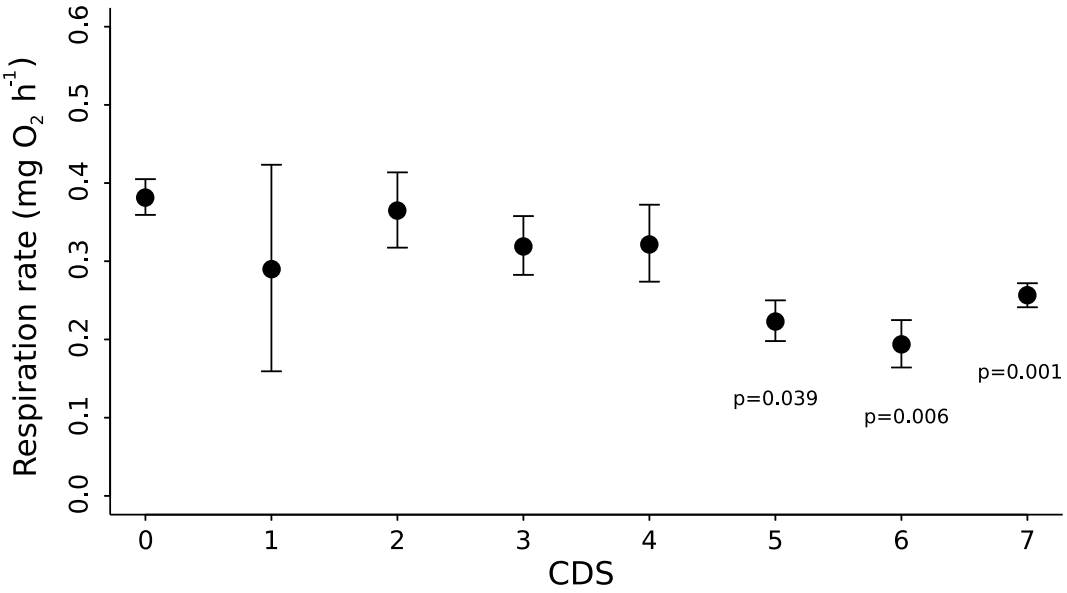

Fig. 3. Relationship of mean respiration rate (mean ± SE) with conchiolin deposit stage (CDS). p is the HSD Tukey *P*-value for the comparison with CDS=0. The number of individuals in each CDS class is indicated in Fig. 2.

3.3 Effect of BRD stage on clearance rate

Average clearance rate was negatively affected by the development of the brown ring symptom (Fig. 4). The clearance rate of clams with CDS $\geq$ 4 was significantly lower than that of asymptomatic (CDS=0) clams (ANOVA, *F*-value=9.01, *P*-value= $2.0\,10^{-8}$; HSD Tukey, *P*-value < 0.05, see Fig. 4). The clearance rate of the former was 45% to 62% lower compared to latter.

Maximum clearance decreased with the development of BRD symptoms (Fig. 5), and was significantly lower in clams with CDS $\geq$ 4 compared to asymptomatic ones (CDS = 0) (ANOVA, *F*-value=8.35, *P*-value= $7.4\,10^{-8}$; HSD Tukey, *P*-value < 0.05, see Fig. 5). Maximum clearance rate in the former decreased between 41% and 56% in comparison to latter, thus suggesting that filtration capacity is affected by the development of BRD.

Filtration–time activity was also negatively affected by the development of BRD (Fig. 6). In individuals with CDS $\geq$ 4, FTA was significantly lower than for asymptomatic individuals (ANOVA, *F*-value=6.40, *P*-value= $3.8\,10^{-6}$; HSD Tukey, *P*-value < 0.05, see. Fig. 6) except for individuals with CDS = 6 (HSD Tukey, *P*-value > 0.05, see. Fig. 6) presumably because of the low number of individuals (n=6). On average, individuals with CDS < 4 spent between 80 and 95% in filtration activity, whereas severely diseased ones (CDS $\geq$ 4) spent between 67 and 59%.

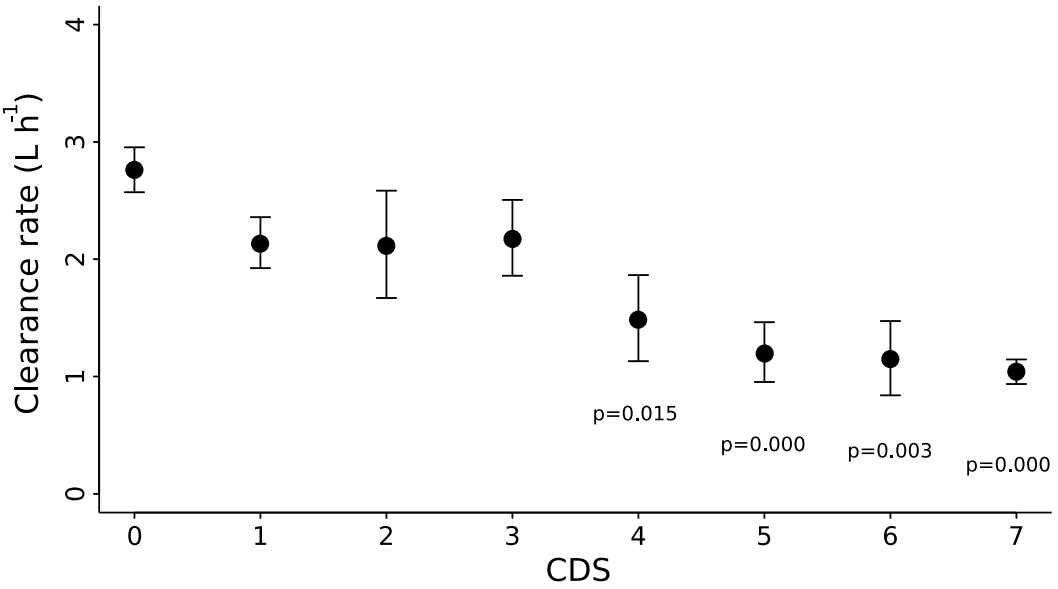

Fig. 4. Relationship of mean clearance rate (mean ± SE) with conchiolin deposit stage (CDS). p is the HSD Tukey *P*-value for the comparison with CDS=0. The number of individuals in each CDS class is indicated in Fig. 2.

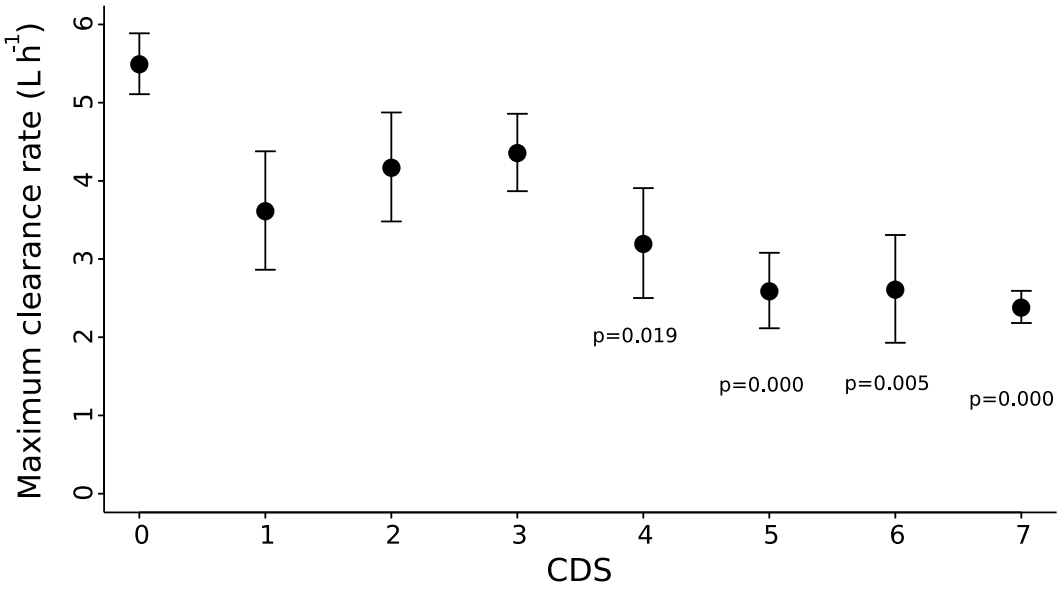

Fig. 5. Relationship of mean maximum clearance rate (mean ± SE) with conchiolin deposit stage (CDS). p is the HSD Tukey *P*-value for the comparison with CDS=0. The number of individuals in each CDS class is indicated in Fig. 2.

4 Discussion

Effect of BRD development on the Manila clam energy budget Clams with high CDS (> 4) exhibited a decrease of 27% to 35% in their condition index, indicating significant weight

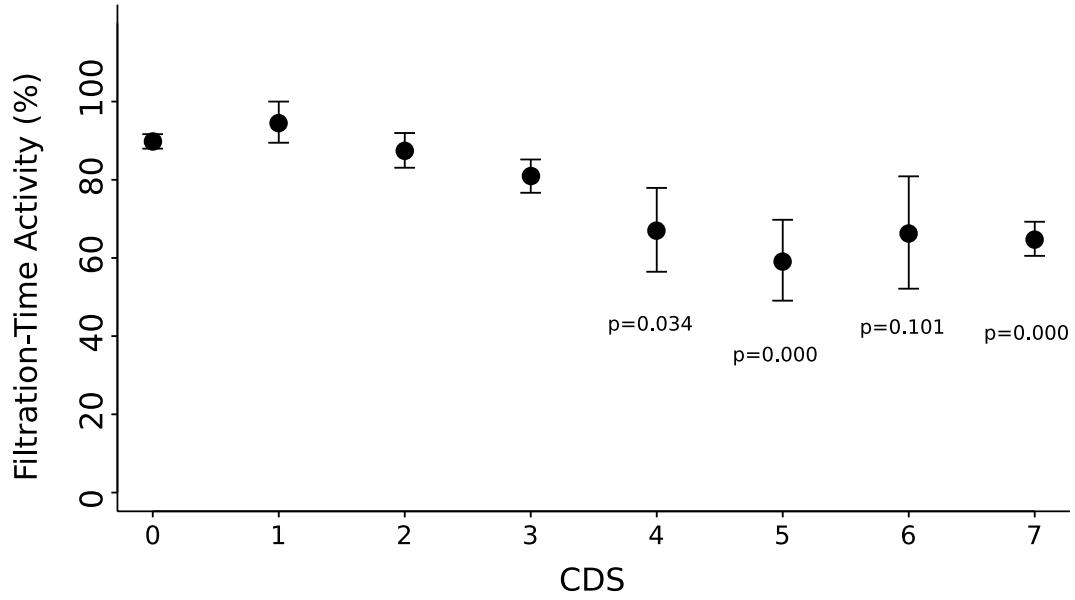

Fig. 6. Relationship of filtration–time activity (FTA; mean ± SE) with conchiolin deposit stage (CDS). p is the HSD Tukey *P*-value for the comparison with CDS=0. The number of individuals in each CDS class is indicated in Fig. 2.

loss associated with the disease. These results are in accordance with Goulletquer (1989), who showed that winter mortalities in Manila clams along the French Atlantic coast in the 1980s were associated with a decrease in the condition index and in the glycogen reserves, wich constitute the main energy reserves for bivalves (see reviews by Gabbot, 1976, 1983; Lucas, 1993). These winter mortalities were subsequently attributed to BRD (Paillard, 1992). Our results also confirm the observations of Plana (1995) and Plana et al. (1996), who showed that Manila clams experimentally infected by *Vibrio tapetis* showed a significant decrease in dry weight and glycogen reserves in comparison to uninfected individuals. In marine bivalves, weight loss associated with microparasite infection is a general pattern. *Haplosporidium nelsoni* and *Perkinus marinus* infections have been shown to reduce condition of the Eastern oyster *Crassostrea virginica* (see e.g. Barber et al., 1988a; Paytner, 1996). *P. olseni* was also shown to reduce condition in the clams *Tapes decussatus* (see. review in Villalba et al., 2004) and *Ruditapes philippinarum* (Park et al., 1999). Our results show that BRD, as is the case with other pathological conditions, affects the energy budget of the Manila clam.

Effects of BRD development on filtration activity A first source of alteration of the energy budget of infected Manila clams is the decrease of the average clearance rate. Clams exhibiting elevated CDS (≥ 4) showed a 45% to 62% decrease in their clearance rate, thus decreasing the energy input. Maximum clearance rates are significantly affected by the development of BRD also, which tends to show that filtration capacity is affected by BRD. Different explanations can be hypothesized to explain this decrease in the filtration capacity.

(1) *Vibrio tapetis*, the etiologic agent of BRD, could induce gills lesions that would affect filtration ability. Such gills lesion were found in Manila clams populations experiencing

winter mortalities in British Columbia by (Bower, 1992), but these mortalities were not linked to BRD. Moreover gills lesions have never been associated with the development of this disease (Paillard, unpublished observations).

(2) Another explanation is that gill activity could be inhibited by a factor secreted by *Vibrio tapetis*. McHenery and Birkbeck (1986) showed that suspensions of marine vibrios (*V. anguillarum* and *V. fisheri*) inhibited filtration by adult *Mytilus edulis*. These authors hypothesized the involvement of an inhibitory surface or secreted factor, which inhibited gill ciliary activity, to explain this phenomenon. Secretion of toxins has been shown for different pathogenic *Vibrio* species. Labreuche et al. (2006) showed that *Vibrio aesturianus* extracellular products (ECP) induced an inhibition in phagocytic and adhesive capabilities of *Crassostrea gigas* haemocytes. These authors concluded that an important part of the pathogenicity of this bacteria is attributable to *Vibrio aesturianus* extracellular products. *Vibrio tapetis* ECP have been shown to induce vacuolization, rounding, shrinking, detaching, and finally, destruction of fish cultured cells (Borrego et al., 1996a) and to inhibit adhesion properties of *R. philippinarum* haemocytes (Choquet, 2004). The existence of deleterious ECP effects by *V. tapetis* supports the hypothesis that filtration activity could be inhibited by one or more factors produced by this bacterium.

(3) Goulletquer et al. (1989) showed that the siphons of clams with heavy brown ring deposit tend to remain retracted and to maintain them at a 45° angle rather than vertically. This interference could thus decrease the pumping efficiency. Furthermore, these authors showed that thickness of the brown deposit induces a lack of tightness of pallial cavity, which could contribute to the degradation of the filter–pump efficiency. Thus a mechanical interference could be hypothesized to explain decreased filtration capacity.

Considering that the effects of BRD on filtration become significant only at CDS $\geq$ 4, when the brown deposit assumes a significant place on the inner shell surface (Paillard and Maes, 1994) and that the relationship between *V. tapetis* densities and CDS is higly variable, as high *V. tapetis* concentrations can be found in clams with low CDS (Paillard, unpublished data) the latter hypothesis appears most consistent with available data. In the field, Manila clams live mainly in the intertidal zone and filtration is not possible during low tide. Moreover, food concentration may vary with the tidal cycle and over short time scales (Smaal and Haas, 1997; Smaal and Zurburg, 1997). Consequently, for such an intertidal bivalve, food is available during a limited period only; thus, decreased filtration capacity should lead to a pronounced loss of efficiency in the exploitation of available trophic resources.

Our results also show that filtration behaviour is affected by the development of BRD because filtration–time activity is reduced in clams with CDS $\geq$ 4. Ward and Langdon (1986) showed that physical irritation of the mantle by the parasitic gastropod *Boonea impressa* induces frequent valve adductions in *Crassostrea virginica* resulting in a decreased clearance rate. The presence of a thick conchiolin deposit along the shell margin may similarly cause an irritation that reduces time spent in filtration activity. Finally, burrowing activity has been shown to be affected in strongly diseased clams (Goulletquer et al., 1989; Paillard, 1992). We can thus hypothesize that the development of BRD induces an inhibition of the overall activity of diseased clams.

This study shows that decrease in the average clearance rate of Manila clams with CDS ≥ 4 can be attributed both to a decrease in the filtration capacity and a change in the filtration behaviour. Plana and Le Pennec (1991) and Plana (1995) showed that BRD induces a degeneration of the digestive diverticula and concluded that digestion efficiency may be negatively affected by development of BRD. Such a decrease in the average clearance rate combined with a possible loss in digestion efficiency translate into reduced energy input that explains part of the observed weight loss linked to BRD development.

Effects of BRD development on overall metabolism Respiration rate decreased with the development of BRD symptoms. Highly diseased clams (CDS ≥ 5) had lower respiration rates than asymptomatic ones. As feeeding rate is depressed at the same CDS levels, this decrease in oxygen consumption could be interpreted as a compensatory reduction of metabolic rate. Nevertheless, feeding processes may be associated with an important energy expenditure (Bayne, 2004) because digestion and absorption may amount to 15-20% of the total energy expenditure (Widdows and Hawkins, 1989); moreover feeding in bivalves is dependent on the secretion of mucus (Beninger and Venoit, 1999). Thus, part of the respiration measured during our experiments may be attributable to the feeding processes. This may explain this decrease of respiration rate at CDS$\geq$5, when filtration was reduced.

Furthermore, Plana et al. (1996) and Plana (1995) presented evidence suggesting that starved Manila clams experimentally infected by *V. tapetis* lost weight at a greater rate than did uninfected control clams. This differential weight loss and energy depletion could not be explained by a difference in energy acquisition since both groups were starved. Rather, these results suggest an extra energy expenditure linked to the infection and thus other explanations for the effect of BRD on the clam's energy budget should be considered:

(1) Energy is required for the growth of the *Vibrio tapetis* population which is normally restricted to the extrapallial fluids. This bacteria, was shown to have a high growth rate (0.84 h^{-1}) when cultured in Manila clam extrapallial fluids (Haberkorn, 2005). Taking into account this growth rate, the average bacterial burden in extrapallial fluids at CDS=7 ($2.25\,10^5\ cells\,ml^{-1}$; measured by ELISA test; Paillard, unpublished data) and a generic value of yield efficiency for microrganisms (0.024 $g\,DW$ produced per $K\,J$ consumed; calculated from Prochazka et al., 1970), the energy consumption of the *V. tapetis* population for its growth was estimated as less than 1% of the metabolised energy (respiration) of the clam. This value may be overestimated because growth rate value was obtained at 18°C, temperature which is near to the thermal growth optimum of *V. tapetis* (Haberkorn, 2005). This rough estimate allows us to conclude that the host energy loss due to the *V. tapetis* population growth is very low and can not explain the weight loss and reserve depletion observed by Plana et al. (1996) and Plana (1995). Choi et al. (1989) showed that, for heavily infected oysters *Crassotrea virginica*, the protozoan parasite *P. marinus* consumes more energy than the oyster has available after meeting its own metabolic needs. This difference can be explained by the fact that, in the case of BRD, the parasite's biomass relative to the host's biomass is much lower.

(2) Lesions have been observed in the digestive gland and the mantle of highly infected clams

(Paillard, 1992; Plana and Le Pennec, 1991; Plana, 1995; Paillard, 2004b); thus, an energy demand could be associated with cell repair functions and clearance of damaged tissues. However, such processes are difficult to assess, although there is evidence for their energetic cost (Freitak et al., 2003; Romanyukha et al., 2006). An energetic cost may also be associated with production of the symptomatic conchiolin deposit. Conchiolin being mainly composed of proteins (Goulletquer et al., 1989), its production may also be energetically costly. Nevertheless, the dynamic of this production remains poorly known and can't be precisely estimated.

(3) The energy mobilised for the development of immunological response to the pathogen (see reviews in Paillard, 2004b,a) may deplete reserves. The metabolic cost of immunity is difficult to assess during an immune challenge (Schmid-Hempel, 2003) and very few studies exist that show evidence of the energetic trade-off among immunity and other competing physiological and behavioral functions in molluscs. Activation cost of the response of immune system has been convincingly demonstrated in a wide range of animals and uses up a tangible part of an organism's energy budget (see review by Schmid-Hempel, 2003). In mammals (Lochmiller and Deerenberg, 2000) the metabolic costs of mounting an immune response range from about 10% up to 30% of the resting metabolic rate; in birds, an increase in basal metabolic activity of 9% has been measured, correlated with a 3% weight loss (Kerimov et al., 2001). Butterfly pupae raised their standard metabolic rate 8% when both humoral and cellular immune responses leading to melanization are induced (Freitak et al., 2003). Development of BRD may induce an energetically costly response of the clam's immune system in the context of the trade-off concept, coupled to finite energy inputs and reserves that must be allocated to a wide variety of competing biological functions. Furthermore, shell reparation by calcification of the conchiolin deposit has been documented (Paillard and Maes, 1994). An energy demand could thus be associated with shell repair functions. Palmer (1992) emphasized that the main energetic cost of production of shell in marine molluscs can be attributed to shell organic matrix. Goulletquer and Wolowicz (1989) showed that the shell matrix accounts for 2–3% of the total shell weight in the Manila clam. Consequently, the energetic cost of shell repair is probably not very high.

All these energetic costs are included in the organism's overall maintenance costs and should lead to variations in the basal metabolism. Further measurements of respiration rate on starving animals are needed to document the influence of BRD development on the basal metabolism of *R. philippinarum*.

Conclusions In conclusion, the present study shows that in naturally infected clams, the observed effects of BRD become significant only for CDS ≥ 4, when the conchiolin deposit assumes a significant place on the inner shell surface. This emphasize that the energetic cost of BRD is dependent on the intensity of the symptoms. Alteration of the energy balance of the Manila clams by the presence of *V. tapetis* can be summarized by the scheme in Fig. 7. One primary way in which the energy balance is affected is the decrease in the energy inputs through a degradation of the filtration capacity and a modification of the filtration behaviour. A second way may be an increase in maintenance costs, due to the defence processes induced against the

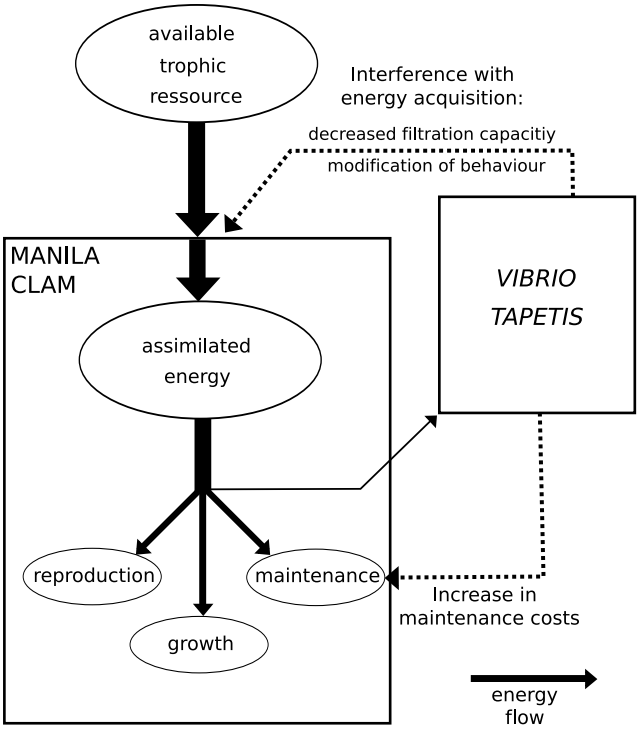

Fig. 7. Energetics of Manila clam / *Vibrio tapetis* interactions. Althought a small amount of energy is required for *V. tapetis* population growth, the energy balance of the Manila clams is alterated by the development of BRD through two main ways: (1) interference with energy acquisition by a decrease in the filtration capacity and a modification of the filtration behaviour and (2) increased maintenance costs.

pathogen. Further investigations are needed to determine the relative contribution of these two factors on the overall degradation of the host energy budget.

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