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A quantitative estimation of the energetic cost of brown ring disease in the Manila clam using Dynamic Energy Budget theory

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Abstract:

Brown ring disease (BRD) in the Manila clam, Ruditapes philippinarum, is a bacterial disease caused by the pathogen Vibrio tapetis. This disease induces the formation of a characteristic brown conchiolin deposit on the inner shell and is associated with a decrease in condition index indicating that the development of the disease affects the energy balance of the clam. A previous study showed that the energy budget of the host was affected by a decrease in filtration activity, and hypothesized that a second way to degrade the energy balance was the increase in maintenance costs associated to the cost of immune response and lesion repair. This paper focusses on this second way of degradation of the energy balance.
A starvation experiment confirmed that the energy balance was affected by BRD, independently of the effects on filtration activity, indicating an increase in the maintenance costs. An energy budget model of the Manila clam, based on DEB theory, was developed and allowed to properly predict weight loss during starvation.Vibrio development and its effects on the energy budget of the host was theoretically introduced in the model. Coupling modelling and experimental observations allowed to provide a quantitative and dynamic estimation of the increase in maintenance costs associated with the development of BRD. The estimation which is given here, indicates that during an infection the maintenance cost can almost double compared to the uninfected situation.
Further development of the model, especially focussed on Vibrio dynamics and its effects on filtration activity is needed to provide a more extensive description of the energetic cost of BRD in the Manila clam.

Keywords: Manila Clam; Brown Ring Disease; Energy Budget; Energetic Cost; Dynamic Energy Budget Theory; Maintenance Cost

1 Introduction

Brown ring disease (BRD) in the Manila clam, Ruditapes philippinarum, is a bacterial disease caused by the pathogen Vibrio tapetis (Paillard and Maes, 1990; Borrego et al., 1996). This clam species was introduced in western Europe at the beginning of the 70s (Flassch and Leborgne, 1992) for aquaculture purpose; venerid culture became subsequently increasingly important along the French Atlantic coast. BRD appeared in cultured clam beds of the Northwest cost of France in 1987 and caused mass clam mortalities (Paillard, 1992; Paillard et al., 1994). Disease progression has been recently reviewed in Paillard (2004). The pathogenic agent, V. tapetis, penetrates and develops within the peripheral extrapallial compartment (i.e.

peripheral space between mantle and shell). The infection disrupts the normal production of periostracal lamina and causes an abnormal deposit of periostracum on the inner shell (Paillard and Maes, 1995a,b). Therefore, diseased clams exhibit a characteristic brown deposit on the peripheral inner shell surface (Paillard et al., 1989) that gave the disease its name. Monitoring disease progression is based on the extent of the symptoms following a classification scale described by Paillard and Maes (1994). Defence processes against the bacterium occur in the extrapallial fluids in part at least through phagocytosis by hemocytes (Allam and Paillard, 1998; Allam et al., 2000, 2001).

Infection by pathogens and/or parasites in bivalves has often been associated with evidences of modification of the energy budget of the host: reduction in reproduction efficiency, condition and growth. Several studies showed that infections by protozoan parasites of the genera *Perkinsus* reduce gametogenesis or reproductive output, condition index and growth rate in various host species: the oyster, *Crassostrea virginica* (Kennedy et al., 1995; Paytner, 1996; Dittman et al., 2001), the European clam, *Tapes decussatus* (see review in Villalba et al., 2004) and the Manila clam, *R. philippinarum* (Ngo and Choi, 2004; Park et al., 2006). A similar pattern has also been shown for the infection by the ascetosporan *Haplosporidium nelsoni* which reduces gametogenesis, condition and glycogen reserves of the oyster *C. virginica* (Barber et al., 1988a,b; Ford and Figueras, 1988). BRD has also been associated with a decrease in flesh weight, condition and glycogen reserves (Paillard, 1992; Plana, 1995; Plana et al., 1996; Flye-Sainte-Marie et al., 2007b). All these observations indicate that infections affect the energy balance of the host; nevertheless, no studies provided a quantitative approach for these processes.

An experimental approach based on measurements of clearance and respiration rates detailed the impact of BRD on the energy budget of the Manila clam (Flye-Sainte-Marie et al., 2007b). This study showed that clams presenting heavy symptoms presented a significant weight loss, indicating that BRD can be associated with a modification of the energy budget of the host. This study emphasized that one way of alteration of the energy balance is an inhibition of the filtration activity leading to a decrease in the energy input. Surprisingly, clams presenting heavy symptoms had also significantly lower respiration rates (Flye-Sainte-Marie et al., 2007b). Respiration results from various processes in the organism (assimilation, maintenance, growth... see e.g. Kooijman, 2000), thus (Flye-Sainte-Marie et al., 2007b) hypothesized that the decreased respiration rate can be explained by the decrease in filtration activity rather than a decrease in the organism maintenance costs. This hypothesis is supported by the observations of Plana (1995) and Plana et al. (1996) that indicated that starved Manila clams infected by *V. tapetis* presented a higher weight loss than uninfected ones. This result suggests that the energy budget of infected clams is also affected independently of feeding activity. Flye-Sainte-Marie et al. (2007b) thus hypothesised that BRD development was associated with an increase in maintenance costs due to immune response and lesions repair.

Dynamic Energy Budget (DEB) theory (Kooijman, 1986, 2000) provides a mechanistic framework for the study of mass and energy budgets in living systems. This theory describes quantitatively energy flow through living organisms and its allocation to growth, development, reproduction and maintenance. This theoretical approach have been applied to model growth and reproduction under influence of environmental factors in various bivalve species (Ross and Nisbet, 1990; van Haren and Kooijman, 1993; Ren and Ross, 2001, 2005; Bacher and Gangnery, 2006; Pouvreau et al., 2006; Cardoso, 2007). The explicit rules of allocation of energy to maintenance provide a powerful tool to study the impact of disease on the energy budget of the host.

By coupling experimental starvation data of infected and uninfected clams and a modelling approach, the aim of present study is to propose an approach allowing a quantitative estimation of the energetic cost of BRD.

2 Methods

2.1 Experimental data : starvation experiment

2.1.1 Biological material and infection procedure

Manila clams, *R. philippinarum*, from a natural population, ranging from 33 mm to 42 mm length (following the maximum length axis; average : 36.4 mm $\pm$SD 1.7) were collected the 11 May 2007 at low tide in Baie de Lanveur, Bay of Brest (Finistère, France). Clams were transferred to the Laboratoire des Sciences de l'Environnement Marin (LEMAR, IUEM, Plouzané, North Finistère, France). Clams were held in 90 L tank equipped with a pump and an airing system allowing homogenisation and oxygenation of the water. Clams were acclimated to experimental conditions (15.7 °C $\pm$ 0.3; 0.5 μm filtered seawater; salinity 33.5 ‰) for 13 days before beginning the experiments.

Experimental infections were performed at 24 May 2007. Brown Ring Disease was experimentally induced in individual clams by injection of $5\,10^7$ cells of *Vibrio tapetis* (strain CECT 4600) into the pallial cavity as described by Paillard and Maes (1990). Prior to injection, virulence of the bacterial strain was checked following the Choquet et al. (2003) protocol. Individuals were separated in four groups: a control group (170 individuals) injected with sterile sea–water, two groups of 170 individuals each injected with *V. tapetis*, and a group of 40 individuals dissected for estimation of initial weight.

2.1.2 Starvation experiment

Each group (control + 2 infected groups) were held in a 90 L tank equipped with a pump and an aeration system allowing homogeneisation and aeration of the water. Goulletquer (1989) showed that the retention efficiency was null for 1 μm particles in *R. philipinarum*, thus tanks were filled with 0.5 μm filtered seawater (salinity 33.5 ‰) to avoid any available potential trophic particle in the water. The tanks were held in a thermoregulated room (13°C) and water in the tanks was heated to 15.7°C, temperature was controlled using a thermostat. An autonomous temperature data logger (EBI-125A, Ebro, Germany) recorded the water temperature in the tanks every 20 min to control thermoregulation. The average measured temperature in the tanks was 15.7 ± 0.3 °C. Half of the water was renewed three times a week.

The experiment lasted for 3 month. Every month (2007-June-25, 2007-July-20 and 2007-August-21), 30 clams from each tank were dissected. Wet weight was measured, flesh was freeze-dried to constant mass (48h) and dry weight was measured. Shells were sized following the maximum length axis. Disease progression was monitored on the shells. 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).

In order to avoid variability of weight only due to size differences and because no growth was observed during the experiment all dry flesh weights were corrected for size differences and standardized for a shell length of 37 mm (round up of the average shell length). This correction was based on the assumption that average flesh weight is proportional to cubed length (L^3).

To present the results of this experiment, individuals were classified into three groups: asymptomatic individuals, individuals presenting light symptoms ($1 \leq \text{CDS} \leq 3$) and clams presenting heavy symptoms ($\text{CDS} \geq 4$). In each group, mean and confidence intervals of standardized dry flesh weight were estimated using ordinary bootstrap (Efron and Tibshirani, 1993; Davison and Hinkley, 1997) with 999 replicates; 95% percentiles were used to determine width of the confidence intervals. Differences in mean lengths were tested using a classical student t test, after a Fisher test of homoscedasticity. All statistical analysis were performed using the R statistical software (R Development Core Team, 2006).

At the beginning of the experiment mean flesh dry weight was 0.463 g (± 0.017; standardized for a 37 mm–individual).

2.2 Model formulation

2.2.1 Structure of the model and basic concepts

The structure of the model is illustrated in Fig. 1, the host module is based on DEB theory (Kooijman, 2000), the interaction with the pathogen is adapted from Flye-Sainte-Marie et al. (2007b).

According to Kooijman (2000), the energy budget of an organism can be fully described by the dynamics of three state variables: (1) the mass of structure (M_V), which corresponds to the somatic tissue excluding reserves; (2) the mass of reserves (M_E) and (3) the mass of the "reproduction buffer" (M_R), which corresponds to reserves allocated to reproduction. The flux of energy from food goes into reserves. Flux of energy from reserves is allocated to somatic maintenance plus growth and to maturity (maturation, maturity maintenance and reproduction) with a constant ratio according to the "kappa–rule". DEB theory gives priority to maintenance, which means that somatic and maturity maintenance costs are first "paid" from the flux of energy from reserves, the remaining energy is then allocated to growth and maturity (or reproduction buffer). If maintenance costs cannot be "paid" from the flux of energy coming from reserves the maintenance is primarily paid from reproduction buffer and if the reproduction buffer is empty maintenances costs are "paid" from structural volume.

Interaction between the energy budget of the Manila clam and *V. tapetis* are derived from Flye-Sainte-Marie et al. (2007b). Since experimental data about *V. tapetis* dynamics within the extrapallial fluids are not available this part of the model is essentially theoretical. The justification for this theoretical approach is that the aim of this study is not to provide realistic dynamics of *Vibrio* within an infected clam, but to provide realistic dynamical modifications of the energy budget of the infected host. We suppose that after infection, *V. tapetis* grows within the extrapallial fluids and secretes a product. According to DEB theory, immunity work is taken into account in maturity maintenance costs. Thus the product induces an increase in maintenance costs (which corresponds to the activation of the immune response) and this increase in maturity maintenance costs increases the death rate of the *Vibrio*. The product has also a toxicity effect which increases somatic maintenance costs (lesions). The effect on filtration activity described in Flye-Sainte-Marie et al. (2007b) is not taken into account in this study since experimental data comes from a starvation experiment.

2.2.2 Dynamics of the Manila clam budget

Notation of the symbols for the energy budget of the Manila clam is from Kooijman (2000) and Kooijman et al. (2008). According to Kooijman (2000) biomass can be partitioned into structure (M_V), reserve (M_E) and reproduction buffer (M_R). The

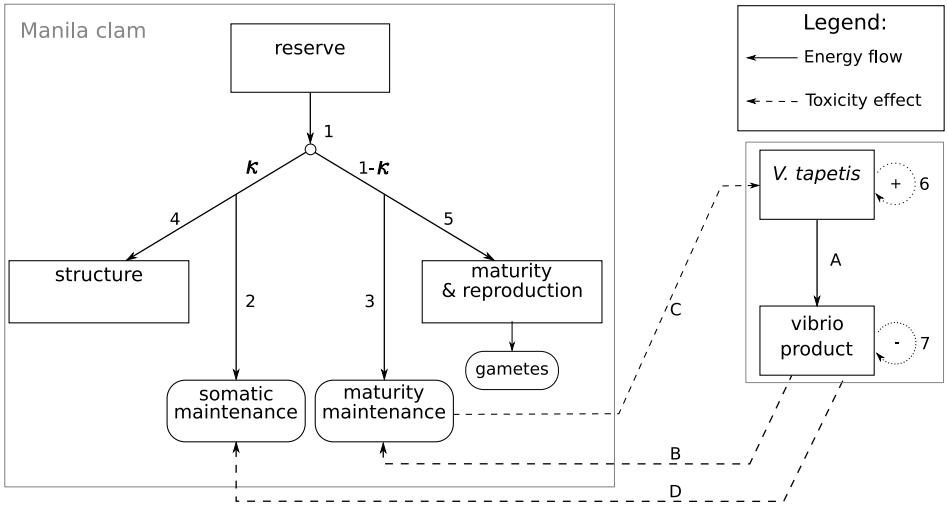

Fig. 1. Conceptual scheme of the Manila clam/*V. tapetis* interactions during starvation (energy acquisition processes are not modelled in this study). Manila clam energy budget under starvation: 1: use of reserves, 2: Somatic maintenance, 3: maturity maintenance, 4: somatic growth, 5: maturity and reproduction, 6: growth of *Vibrio* population, 7: decay of *Vibrio* product. *V. tapetis* and interaction with the host: A: secretion of *Vibrio* product, B: effect (toxicity) of *Vibrio* product on maturity maintenance (immune response), C: effect of maturity maintenance on *Vibrio* death rate, D: effect (toxicity) of *Vibrio* product on somatic maintenance (lesions).

DEB model presented in this study is an application of the formulation extensively described in Kooijman et al. (2008). This formulation includes maturity (M_H) as a state variable and is quantified as the cumulative investment of reserve in maturity. Birth, defined in the DEB theory concept as the first feeding of the organism, occurs at the amount of maturity M_H^b (maturity at birth); and puberty, defined as the first investment of reserve in reproduction buffer, occurs at the amount of maturity M_H^p (maturity at puberty). The choice of this formulation has been motivated by the fact that infection before puberty (before reaching a particular amount of maturity : M_H^p, maturity at puberty) may affect the maturation rate. Maturity itself represents information and has no mass or energy. Equations of the standard DEB model described by Kooijman (2000) were rewritten to work with the following state variables (Kooijman et al., 2008):

- volumetric length L (cm) that can be converted to observed length (L^{Obs}) with the relation $L = L^{Obs}/\delta_{\mathcal{M}}$, with $\delta_{\mathcal{M}}$ the shape coefficient (dimensionless).

and scaled state variables, corresponding to the variables divided by the maximum surface specific assimilation rate ($\{\dot{J}_{EAm}\}$, mol d^{-1} cm^{-2}) :

- scaled reserve U_E (d cm^2).
- scaled maturity U_H (d cm^2)
- scaled reproduction buffer U_R (d cm^2)

Table 1
Variables, scaled variables, parameters, compound parameters and quantities for the Manila clam.

symbol	unit	description
Variables for the Manila clam		
X	mol cm^{-3}	food density
M_V, M_E and M_R	mol	structural, reserve and reproduction buffer mass respectively
M_H	mol	cumulative investment in maturity
$\dot{J}_X$	mol d^{-1}	Ingestion rate
$\dot{r}_B$	d^{-1}	von Bertalanffy growth rate
$\dot{J}_{EM}$	mol d^{-1}	somatic maintenance mass flux
$\dot{J}_{EJ}$	mol d^{-1}	maturity maintenance mass flux
Scaled variables for the Manila clam		
L	cm	volumetric structural length
$U_H = M_H/\{\dot{J}_{EAm}\}$	d cm^2	scaled maturity
$U_R = M_R/\{\dot{J}_{EAm}\}$	d cm^2	scaled reproduction mass
$U_E = M_E/\{\dot{J}_{EAm}\}$	d cm^2	scaled reserve mass
$f = X/(X_K + X)$	–	scaled functional response
$e = \dot{v}U_E L^{-3}$	–	scaled reserve density
Parameters for the Manila clam		
$\delta_{\mathcal{M}}$	–	shape coefficient
$\{\dot{J}_{EAm}\}$	mol d^{-1}cm^{-2}	surface area-specific maximum assimilation rate
y_{VE}	mol mol^{-1}	yield of structure on reserve
$\dot{v}$	cm d^{-1}	energy conductance
$\dot{k}_J$	d^{-1}	specific maturity maintenance
κ	-	allocation fraction
κ_R	-	reproduction efficiency
U_H^b and U_H^p	d cm^2	scaled maturity at birth and at puberty respectively
T_A, T_H, T_{AH}, T_L, T_{AL}	K	Arrhenius temperatures
Compound parameters for the Manila clam		
X_K	mol cm^{-3}	half-saturation constant
$\{\dot{J}_{XAm}\}$	mol d^{-1}cm^{-2}	maximum specific ingestion rate
$L_m = \frac{\dot{v}}{\dot{k}_M g}$	cm	maximum structural length
$\dot{k}_M$	d^{-1}	somatic maintenance rate coefficient
g	–	energy investment ratio

Thus scaled reserve and scaled reproduction buffer can be converted to mass (g) by multiplying by $\{\dot{J}_{EAm}\}\,\bar{\mu}_E$ ($= \{\dot{p}_{Am}\}\,\bar{\mu}_E/w_E$) with $\bar{\mu}_E$, the chemical potential of reserves (g mol^{-1}), $\{\dot{p}_{Am}\}$, the maximum surface specific assimilation rate (J d^{-1}cm-2) and $\bar{\mu}_E/w_E$, the energy content of reserve (J g^{-1}). Variables, scaled

variables, parameters and compound parameters used in this study are summarized in Tab. 1.

Food uptake is not taken into account since clam were starving during the experiment. Change of state variables in time of this formulation for the DEB model were extensively described by Kooijman et al. (2008). According to these authors, length change can be written as:

$$\frac{d}{dt}L = \dot{r}_B(eL_m - L) \tag{1}$$
$$\text{with } \dot{r}_B = \frac{\dot{k}_M g}{3(e+g)};\ L_m = \frac{\dot{v}}{\dot{k}_M g};\ g = \frac{\dot{v}[M_V]}{\kappa\{\dot{J}_{EAm}\}y_{VE}} \text{ and } e = \frac{\dot{v}U_E}{L^3}$$

where $\dot{r}_B$ (d^{-1}) is the von Bertalanffy growth rate, L_m (cm) is the maximum length of the species, g (dimensionless) the energy investment ratio and e (dimensionless) is the scaled reserve density.

Since before birth the organism does not feed, and birth occurs at a particular amount of maturity (M_H^b) emphi. e. at a particular amount of scaled maturity (U_H^b), change in scaled reserve can be written as:

$$\frac{d}{dt}U_E = fL^2 - S_C \quad \text{for } U_H > U_H^b \quad \text{else} \quad \frac{d}{dt}U_E = -S_C \tag{2}$$
$$\text{with } S_C = L^2\frac{ge}{g+e}\left(1 + \frac{L}{gL_m}\right)$$

where S_C (cm^2) is the "flux" of utilisation of scaled reserves.

According to Kooijman et al. (2008) maturity is quantified as the cumulative investment of reserves into maturity. Investment of reserves into maturity stops at puberty, when a particular amount of maturity (M_H^p) or a particular amount of scaled maturity (U_H^p) is reached. After puberty energy from reserves is allocated to reproduction buffer rather than maturity (Kooijman, 2000), thus maturity is constant after puberty and equals to M_H^p. Thus change in scaled maturity and scaled reproduction buffer over time can be written as:

$$\frac{d}{dt}U_H = (1-\kappa)S_C - \dot{k}_J U_H \quad \text{for } U_H < U_H^p \tag{3}$$
$$\frac{d}{dt}U_R = (1-\kappa)S_C - \dot{k}_J U_H^p \quad \text{for } U_H = U_H^p \quad \text{else } \frac{d}{dt}U_R = 0 \tag{4}$$

Factors triggering spawning events in the Manila clam are not well known (see e.g. Flye-Sainte-Marie et al., 2007a), and not spawning events were observed during

the starvation experiment, spawning events were subsequently not modelled in this study.

Somatic and maturity maintenance ($\dot{J}_{EM}$ and $\dot{J}_{EJ}$ respectively, mol d^{-1}) costs can be quantified as:

$$\dot{J}_{EM} = \kappa \frac{L^3}{L_m} \{\dot{J}_{EAm}\}$$
$$\dot{J}_{EJ} = \dot{k}_J \, U_H \, \{\dot{J}_{EAm}\}$$

DEB theory gives priority to maintenance (Kooijman, 2000). Thus, according to Pouvreau et al. (2006), if maintenance costs cannot be paid from the flux coming from reserves it is primarily supplemented from the reproduction buffer with the assumption that conversion efficiency equals to the conversion efficiency of producing gametes from reserves (κ_R). If this is also not sufficient, it is further supplemented from structure. In this case we assume that the overhead cost in paying maintenance from structure rather than reserve equals to the overhead cost of producing structure from reserve. In this case the change in structural length over time ($\frac{d}{dt}L$) becomes negative.

2.2.3 Dynamics of Vibrio tapetis

Since data are not available for the dynamics of *V. tapetis* in an infected clam, this part is essentially theoretical, the objective of this part of the model is to simulate a dynamic perturbation of the host's energy budget.

Suppose that at time $t = t_B$ an individual *Vibrio* successfully enters in the extrapallial compartment of a clam. The mass of *Vibrio* inside the host is denoted by M_B, so $M_B(t_B) = M_{B0}$ and the *Vibrio* density in the extrapallial fluids $[B]$ (mass of bacteria by volume of extrapallial fluids, so to structural volume). Based on the assumption that *Vibrio* growth is limited by the volume of the extrapallial compartment (or the nutrients present in that volume), a logistic growth equation is applied for the *Vibrio* population growth. As discussed in Flye-Sainte-Marie et al. (2007b), we assume that the energy needed for the *Vibrio* population growth are negligibly small. This assumption is supported by the fact that the *Vibrio* concentration in the extrapallial fluids remain small (typically $< 10^5$ cells ml^{-1} Paillard, unpublished data; i.e. $< 10^{-}7$ gDW ml^{-1} using conversion coefficients given in Bratbak and Dundas, 1984 and in Ohman and Snyder, 1991). The presence of the *Vibrio* induces an immune response : a mobilisation of hemocytes to the extrapallial compartement and phagocytosis of the pathogenic bacteria (see Paillard, 2004, for a review). DEB theory assumes that cost of immunity is included in maturity maintenance costs (Kooijman, 2000). Thus maturity maintenance might be affected by the presence of the *Vibrio*. We assumed that immune response is proportional to the increase in maturity maintenance costs induced by the pathogen and that it follows a Holling type II functional response. The choice of the Holling type II functional

response have been motivated by the fact that phagocytosis of bacteria by hemocyte is a process similar to a feeding process. Subsequently, the change in the *Vibrio* concentration can be written as:

$$\frac{d}{dt}[B] = \left(\dot{r}_m^B \left(1 - \frac{[B]}{[B_m]}\right) - \dot{r}\right)[B] - f_B \left(\dot{k}_J(0) - \dot{k}_J(Q)\right) U_H\{\dot{J}_{BAm}\} \quad (5)$$
$$\text{with } f_B = \frac{[B]}{[B_K] + [B]}$$

where $\dot{r}_m^B$ (d^{-1}) is the maximum growth rate of the *Vibrio* population, $[B_m]$ (mol cm^{-3}) the maximum *Vibrio* concentration, $\dot{r} = \frac{d}{dt} \ln L^3$ (d^{-1}) is the dilution by growth rate, $\dot{k}_J(0)$ (d^{-1}) the maturity maintenance rate coefficient without *Vibrio*, $\dot{k}_J(Q)$ (d^{-1}) the maturity maintenance rate coefficient modified by *Vibrio* presence (Q denote the *Vibrio* product, see below for more explanation, see section "Effect of *Vibrio* on the host" for the effect of *Vibrio* on host maintenance), f_B (dimensionless) the functional response of immune response on *Vibrio*, and $\{\dot{J}_{BAm}\}$ (mol cm^{-2} d^{-1}) the maximum surface specific *Vibrio* elimination rate.

Suppose further that *Vibrio* excretes a compound Q that has the potential to change one or more parameters values of the host, following the DEBtox rules for effects of chemical compounds on the budget of organisms (Kooijman and Bedaux, 1996). Note that this product is theoretical and aims at linking the effect of the *Vibrio* on the host to the *Vibrio* population. The specific production rate is taken to be constant, as well as the specific decay rate. So the change in the *Vibrio* product concentration $[Q]$ (mol cm^{-3}) is:

$$\frac{d}{dt}[Q] = j_Q[B] - (\dot{k}_Q + \dot{r})[Q] \quad (6)$$

where j_Q is the mass-specific production rate (moles of product per mole of *Vibrio* per time) and $\dot{k}_Q$ the specific decay rate of the *Vibrio* product.

We assume that all interactions between the *Vibrio* and the Manila clam energy balance are linked to the vibrio product.

Variables and parameters for the *Vibrio* dynamics and its effects are summarized in Tab. 2.

2.2.4 Effects of Vibrio *on the Manila clam energy balance*

Effects of the development of BRD on the energy balance on the Manila clam were studied in Flye-Sainte-Marie et al. (2007b). This study emphasized that the energy balance of the Manila clam was affected in two main ways: a reduction of the filtration activity and an increase in the maintenance costs, presumably associated

Table 2
Variables and parameters for *Vibrio* and its interaction with the Manila clam energy budget. Where similar units occur in the specification of a parameter, they relate to different categories (such as *Vibrio versus Vibrio* products), and can, therefore, not be reduced.

symbol	unit	description
Variables		
$[B]$	$\mathrm{mol.cm^{-3}}$	*Vibrio* density
$[Q]$	$\mathrm{mol\,cm^{-1}}$	concentration of *Vibrio* product
f_B	-	functional response for haemocytes
Parameters		
$[B_K]$	$\mathrm{mol.cm^{-3}}$	half saturation constant of immune response
$[B_m]$	$\mathrm{mol.cm^{-3}}$	maximum *Vibrio* density
$\dot{r}_m^B$	$\mathrm{d^{-1}}$	specific growth rate of *Vibrio*
j_Q	$\mathrm{mol\,mol^{-1}\,d^{-1}}$	specific production rate of *Vibrio* product
$\dot{k}_Q$	$\mathrm{d^{-1}}$	specific decay rate of *Vibrio* product
$\{\dot{J}_{BAm}\}$	$\mathrm{mol\,cm^{-2}\,d^{-1}}$	maximum surf. spec. *Vibrio* elimination rate
$[Q_M^0]$, $[Q_M]$	$\mathrm{mol\,cm^{-3}}$	toxicity parameters for somatic maintenance
$[Q_J^0]$, $[Q_J]$	$\mathrm{mol\,cm^{-3}}$	toxicity parameters for maturity maintenance

to immune response and lesion repair.

Since clams were under starvation during the experiment and food uptake was not modelled in this study the effect of *Vibrio* on filtration activity was also not taken into account. The effect of the *Vibrio* on the immune system has been modelled has an increase of the maturity maintenance rate coefficient ($\dot{k}_J$). *Vibrio* infection also induces lesions (see Paillard, 2004). Since lesions are perturbations or destruction of structural body tissue, their repair should be attributed to the structural maintenance cost. Thus costs for lesions repair were modelled as an increase in the somatic maintenance rate coefficient ($\dot{k}_M$). These effects were assumed to be linked to the *Vibrio* product according to the DEBtox rules for effects of chemical compounds on the budget of organisms (Kooijman and Bedaux, 1996). These effects were thus quantified as:

$$\dot{k}_M(Q) = \dot{k}_M(0)(1 + \max(0, [Q] - [Q_M^0])/[Q_M])$$
$$\dot{k}_J(Q) = \dot{k}_J(0)(1 + \max(0, [Q] - [Q_J^0])/[Q_J])$$

where $[Q_M^0]$ and $[Q_J^0]$ are the no-effect concentrations, when $[Q]$ is under these concentration the product has no effect. $[Q_M]$ and $[Q_J]$ are tolerance concentrations; the larger their values, the smaller the effects. The values $\dot{k}_M(0)$ and $\dot{k}_J(0)$ correspond to the uninfected situation. The motivation of these linear relationships is that the real functions might be more complicated, but we use a linear approximation for small effects; small changes in parameter values, however, not necessarily translate into small effects on the budget.

2.2.5 Effect of temperature on physiological rates

Physiological rates depends on body temperature. This dependency can be described by the Arrhenius relation within a species-specific tolerance range of temperatures. According to Kooijman (2000) this dependence can be described by the following relation:

$$\dot{k}(T) = \dot{k}_1 \exp\left(\frac{T_A}{T_1} - \frac{T_A}{T}\right)\left(1 + \exp\left\{\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right\} + \exp\left\{\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right\}\right)$$

where $\dot{k}(T)$ is the value of the physiological rate at temperature T, $\dot{k}_1$ is the physiological rate at the reference temperature T_1 (arbitrary chosen), T_A is the Arrhenius temperature, T_H and T_L are the upper and lower boundaries of the tolerance range, and T_{AH} and T_{AL} are the Arrhenius temperature for the rate of decrease at both boundaries. All temperatures are expressed in Kelvin (K).

All physiological rates of the budget of the Manila clam were corrected using this relation. Growth rate of the *Vibrio* population was corrected by taking into account only its Arrhenius temperature (T_A^B) because available data did not allowed to estimate additional temperature–related parameters.

2.2.6 Parameter estimates

Parameters values of the host module of the model were taken from Flye-Sainte-Marie (2008)

The Arrhenius temperature for the *Vibrio* (T_A^B) was estimated from experimental growth experiments from Haberkorn (2005) using the procedure described in Flye-Sainte-Marie (2008). Since experimental data on *V. tapetis* dynamics during the infection process were not available, most of the parameters related to *Vibrio* dynamics and its interaction with the budget of the Manila clam were manually fitted until meeting the following constraints: (1) to provide a realistic maximum bacteria density, and (2) to adjust the total weight loss to the observations. As explained previously, the *Vibrio* product is here used as a theoretical way to quantify the effects of the *Vibrio* population on the host. Thus the parameters concerning *Vibrio* products and their effects given here are arbitrary and should not be compared to real effects of *Vibrio* extracellular products.

The parameter values that we used in this study are listed in Table 3.

Table 3
Parameter estimates for the Manila clam, the *Vibrio* and the interaction with the Manila clam budget used in the application presented in this study. All rates are given for the arbitrary reference temperature T_1=288 K (15°C). The meaning of the symbols is explained in Tables 1 and 2.

Symbol	Value	Unit	Source
Parameters for the Manila clam			
κ	0.89	–	Flye-Sainte-Marie (2008)
κ_R	0.95	–	Kooijman et al. (2008)
g	1.384	–	Flye-Sainte-Marie (2008)
$\dot{v}$	0.0292	cm d^{-1}	Flye-Sainte-Marie (2008)
$\dot{k}_J$	0.0091	d^{-1}	Flye-Sainte-Marie (2008)
$\dot{k}_M$	0.0091	d^{-1}	Flye-Sainte-Marie (2008)
U_H^b	4.76 10^{-7}	d cm^2	Flye-Sainte-Marie (2008)
U_H^p	0.1274	d cm^2	Flye-Sainte-Marie (2008)
$\delta_{\mathcal{M}}$	0.29	–	Flye-Sainte-Marie (2008)
T_1	288	K	Arbitrary
T_A	6071	K	Flye-Sainte-Marie (2008)
T_H	300	K	Flye-Sainte-Marie (2008)
T_L	275	K	Flye-Sainte-Marie (2008)
T_{AH}	30424	K	Flye-Sainte-Marie (2008)
T_{AL}	299859	K	Flye-Sainte-Marie (2008)
Parameters for the Vibrio and Vibrio products			
T_A^B	6843	K	this study, data from Haberkorn (2005)
$\dot{r}_m^B$	4.65	d$^-$1	this study
$[B_m]$	1 10^{-5}	C-mol cm^{-2}	this study
j_Q	0.11	d^{-1}	this study
$\dot{k}_Q$	0.066	d^{-1}	this study
$[B_K]$	1.10$^-$7	C-mol cm^{-3}	this study
$\{\dot{J}_{BAm}\}$	4.7 10^{-4}	C-mol cm^{-2} d^{-1}	this study
$[Q_M^0]$	1 10^{-6}	C-mol cm^{-3}	this study
$[Q_M]$	1 10^{-5}	C-mol cm^{-3}	this study
$[Q_J^0]$	0	C-mol cm^{-3}	this study
$[Q_J]$	3.7 10^{-7}	C-mol cm^{-3}	this study

3 Results

3.1 Starvation experiment

At each date, there were no significant differences in the average dry weight between uninfected clams from the control and asymptomatic clams form the infected

batches (*t* test; all p-values > 0.05). This allowed to classify individuals into three groups: asymptomatic individuals (including control), individuals presenting light symptoms ($1 \leq CDS \leq 3$) and clams presenting heavy symptoms ($CDS \geq 4$). There was no significant difference in the number of individuals in each group between the three sampling dates ($\chi^2 = 1.88$, df = 4, *p*-value = 0.75) and mortality was low (< 10%) during the experiment. This allowed to hypothesize that there was negligible transfer of individuals between groups during the experiment.

At each sampling date there were no significant differences in total flesh dry weight between asymptomatic clams and clams presenting light symptoms. Fig. 2 shows the evolution of total flesh dry weight in asymptomatic clams and clams presenting heavy symptoms during the experiment. In asymptomatic clams weight loss was almost linear. Dry weights in individuals presenting heavy symptoms were significantly lower than in asymptomatic one two and three month after the beginning of the experiment (randomized-*t*, tests *p*–values < 0.05). No difference between mean standardized dry flesh weight of asymptomatic and clams presenting light symptoms could be detected (randomized-*t* tests; p-values > 0.05) at any time.

Growth during the experiment was negligible; no difference in mean length could be detected between beginning and end of the starvation experiment, for any group of clams (*t* test; all p-values > 0.05)

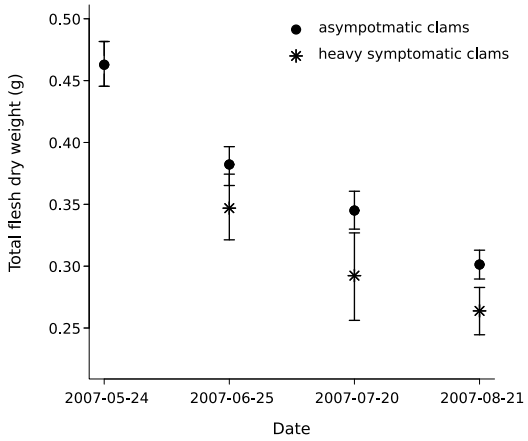

Fig. 2. Evolution of total flesh dry weight (mean $\pm$ 95% bootstrap percentile interval) during the starvation experiment in asymptomatic clams (●) and in clams presenting heavy symptoms ($*$, $CDS \geqslant 4$)

3.2 Model simulations : impact of BRD development on maintenance cost

The interaction model between the energy budget of the Manila clam and the *Vibrio* was used to estimate the energetic costs of disease development in the highly diseased clams of the starvation experiment. For this purpose, two simulations were performed. The first simulation only represents the energy budget of an uninfected

clam under starvation conditions. For the second simulation, the *Vibrio* infection and its effects are introduced. This simulation is supposed to reproduce the energy budget of a Manila clam presenting heavy symptoms during starvation.

Initial conditions (*i.e.* state variables at the beginning of the simulation) were estimated from the initial mean length and initial mean total flesh dry mass. Initial structural length (L_{t0}) was estimated as $L_{t0} = \delta_{\mathcal{M}} L_{t0}^{Obs}$ and allowed to estimate initial structural weight (with d_V = 0.216 g cm^3 the structural volume to dry weight coefficient, from Flye-Sainte-Marie, 2008). The difference between the structural weight and the mean observed weight allows to estimate the initial weight of reserve plus reproduction buffer. Since at the sampling period (April) the clams are in the main growing period of the year on French Atlantic coasts (see e.g. Flye-Sainte-Marie et al., 2007a) the weight of reserves were assumed to be 60% of the maximum energy density ($[Em]$ = 2200 J cm^{-3} from Flye-Sainte-Marie, 2008). This allowed to estimate the initial weight of the reproduction buffer. Initial weight of reserves and reproduction buffer were then scaled (with $\bar{\mu}_E/w_E$=15.5 10^3 J g^{-1} and $\{\dot{p}_{Am}\}$=64.2 J cm^{-2} d^{-1} from Flye-Sainte-Marie, 2008). For both simulations, since Manila clam were in condition of starvation, the scaled functional response (f) was set to 0. Temperature for the simulation was constant and equal to 15.7°C (288.7 K). Initial *Vibrio* product concentration $[Q^{t0}]$ was set to 0. The simulation was performed over 100 days. For the infected clams, the infection was induced at the beginning of the simulation and the initial concentration of *Vibrio* was supposed to be $[B_0] = 3\,10^{-9}$ C-mole cm^{-3}; this value was computed from average *Vibrio* concentration observed in extrapallial fluids of clams with low symptom development (CDS=1) and recalculated in C-moles cm^{-3} using data on carbon content of bacteria from Ohman and Snyder (1991). Since 37 mm-individuals typically reproduce, the initial scaled maturity was taken to be equal to scaled maturity at puberty. Initial conditions are summarized in Tab. 4.

Table 4
Initial values of the state variables for the simulation of the impact of the disease development during starvation condition.

symbol	value	unit	description
L_{t0}	1.073	cm	Initial volumetric length
U_E^{T0}	20	d cm^2	Initial scaled reserves
U_R^{T0}	32	d cm^2	Initial scaled reproduction buffer
$U_H^{T0} = U_H^p$	0.1274	d cm^2	Initial scaled maturity
$[Q^{t0}]$	0	C-mol cm^3	Initial *Vibrio* product concentration

Evolution of the state variables of the model during the simulations for the uninfected and the infected clam are shown in Fig. 3.

In the uninfected clam (continuous curve in Fig. 3) structural length remains con-

stant over the whole simulation period (Fig. 3 A), indicating that there is no (or very low) allocation to somatic growth. Scaled reserve exponentially decrease during the simulation and are almost null after 100 days (Fig. 3 B). Scaled reproduction buffer is almost constant for $\approx$ 10 days, indicating that allocation of reserve to reproduction balance the maintenance costs; but after this period, maintenance costs cannot be balanced by the flux coming from reserves, and maintenance costs are paid from reproduction buffer which is almost empty after 100 days (Fig. 3 C).

In the infected clam (doted curves in Fig. 3), simulated *Vibrio* concentration rapidly increase after the infection and reaches its maximum after few days (Fig. 3 D). Increase in *Vibrio* product concentration (Fig. 3 E) increases maturity maintenance cost (immune response) and the *Vibrio* is eliminated after $\approx$ 35 days. In starvation conditions the *Vibrio* infection has small effects on the use of reserves (Fig. 3 B). The increased maintenance costs are mainly paid from the scaled reproduction buffer after a few days; the reproduction buffer is empty after $\approx$ 80 days. (Fig. 3 B) and maintenance is then paid from structure, this explains why simulated length decreases after $\approx$ 80 days (Fig. 3 A).

From the simulated evolution of length, scaled reserves and scaled reproduction buffer it was possible to estimate the evolution of total dry mass using the coefficients cited above. The evolution for the uninfected and infected clams is shown in Fig. 4. In uninfected clams the model simulates a linear decrease of total flesh dry weight during starvation and simulation is close to the observed points and stays within the bootstrap percentile intervals of the observations. Parameters for the *Vibrio* and the interaction with the Manila clam budget were estimated in order to provide a good representation of the weight loss during starvation until day 60. Nevertheless, after $\approx$ 80 days, when maintenance is paid from structure, the model simulates an acceleration of the total flesh weight loss, whereas data suggest a deceleration of the weight loss.

From the simulations it was also possible to estimate the total energy allocated to maintenance. The aim of the work was to estimate the increase of maintenance costs associated with BRD development. Thus the results are here presented as the total maintenance costs which correspond to the sum of the energy flow invested in somatic and maturity maintenance (Fig. 5). For the uninfected clam the total flow invested in maintenance remains constant all along the simulation (Fig. 5). In the infected clam the maintenance cost rapidly increases after the infection due to the increase in vibrio product concentrations (see. Fig. 3 E). The total maintenance reaches a maximum value of $\approx$ 57 J d^{-1} after $\approx$ 25 days. The decrease simulated after $\approx$ 80 days is associated with the decrease in somatic maintenance due to reduction of structural length (shrinking). The maximum value of the energy flow to maintenance associated to BRD development ($\approx$ 57 J d^{-1}) is comparable to the maximum assimilation flux can be estimated to $\dot{p}_A$ = 77.8 J d^{-1} for an individual of this size and at the temperature of the experiment (value estimated from $\{\dot{p}_{Am}\}$ and T$_A$ values given in Flye-Sainte-Marie, 2008) .

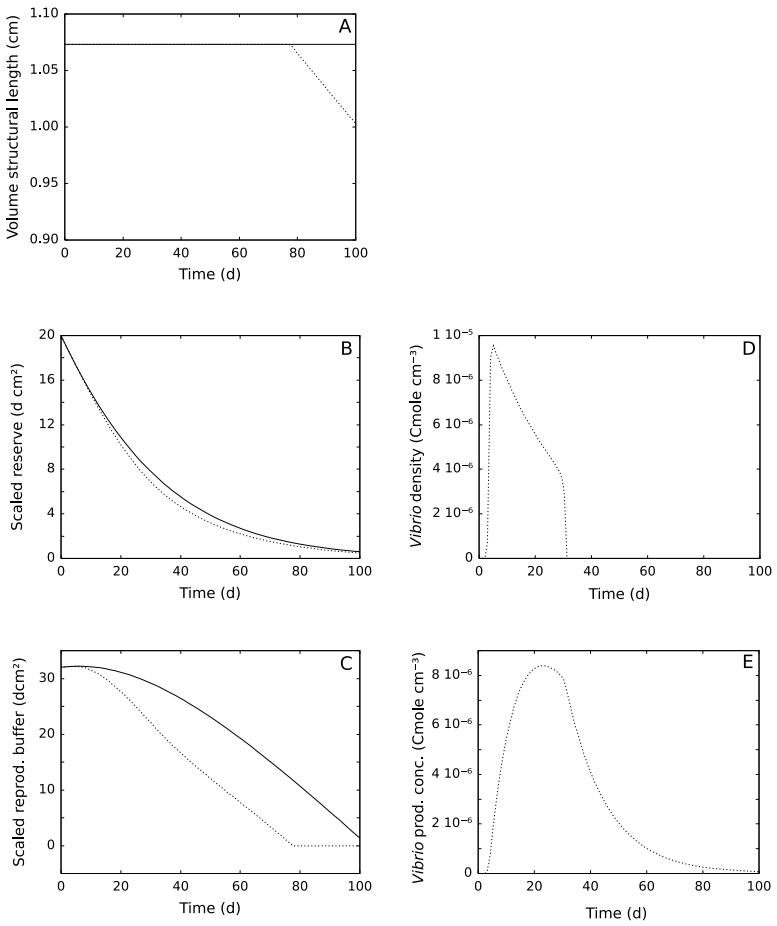

Fig. 3. Evolution of state variables during the simulation for uninfected (continuous curve) and infected (doted curve) clams in starvation conditions (f=0). A: evolution of structural length, B: evolution of scaled reserves, C: evolution of scaled reproduction buffer, D: evolution of *Vibrio* concentration, E: evolution of *Vibrio* product concentration.

4 Discussion

Starvation experiment

The starvation experimental data indicated that individuals presenting a high development of symptoms had a significant higher weight loss than asymptomatic ones after two months of starvation. This observation confirms the observations of Plana (1995) and Plana et al. (1996), and indicates that an energy loss can be associated with the development of BRD independently of the decrease in filtration activity shown by Flye-Sainte-Marie et al. (2007b). As discussed in this article this energy loss might be associated to an increase in the maintenance cost due to the energy needed for the immune response and for lesion repair. The energy needed for *Vibrio* population growth might be negligible (Flye-Sainte-Marie et al., 2007b).

No significant difference in weight could be found between the group of asymp-

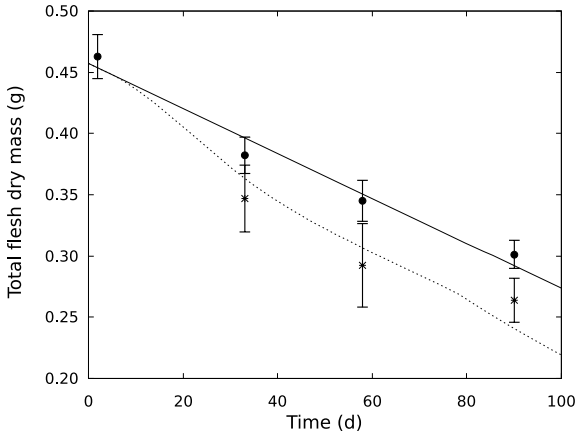

Fig. 4. Simulation of the evolution of total flesh dry mass for uninfected (continuous curve) and infected (doted curve) clams in starvation conditions (f=0). Experimental data: average total flesh dry weight (mean $\pm$ 95% confidence interval) during the starvation experiment in asymptomatic clams (●) and in clams presenting heavy symptoms (⁎, CDS$\geqslant$4)

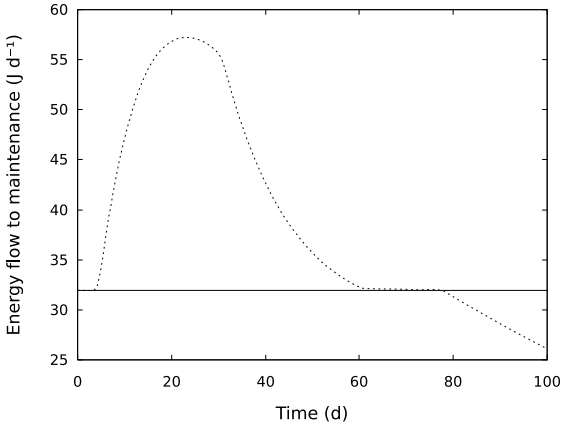

Fig. 5. Total energy allocated to maintenance (somatic and maturity maintenance for uninfected (continuous curve) and infected (doted curve) clams.

tomatic clams and the group of clams presenting moderate symptoms development: energy loss associated with low BRD development levels is negligible. This suggests that BRD development has poor effects on physiology at low disease intensity. This observation is consistent with most previous observations. The low disease intensity observed during a one–year survey in the Gulf of Morbihan could not be associated with significant variations in any measured hemocyte parameter or in the condition index (Flye-Sainte-Marie, 2008). It was also not possible to show any significant difference in clearance rate, respiration rate or condition index in clams presenting low symptom development (CDS < 4, Flye-Sainte-Marie et al., 2007b). Thus the stage CDS = 4 can be considered as a critical stage above which physiology is strongly modified but under which effects of disease are negligible.

The proportion of clams in each group (asymptomatic, low symptoms and heavy symptoms) did not significantly varied after 30 days and low mortality was observed during the experiment. This tends to indicate that there was negligible trans-

fer of individuals between the three groups during the experiment. This can support the hypothesis that each group corresponds to a "batch" of clams that can be followed over time on the base of symptoms intensity. A recent study showed that rough handling significantly increased the infection probability (Jean et al., submitted) presumably through disruptions of the periostracal lamina that would allow the pathogen to colonize the extrapallial compartment (Flye-Sainte-Marie et al., 2008). Thus, in such an experiment the success of infection, and the intensity of the development of the disease may be related to the potential lesions during the initial handling (transport before and handling before the beginning experiment). This may explain the fact there was negligible transfer of individuals between the three groups during the experiment.

Budget of the uninfected Manila clam

The prediction of weight loss during starvation was close to the observations. For the simulated uninfected clam, the length is constant over the whole simulation period, which is consistent with the observations since no growth was observed during the experiment. This indicates that parameter estimates for the energy budget of Manila clam are reliable and emphasize that the prediction of the energy budget of Manila clam at constant environmental conditions by the DEB model developed in this study are reliable.

These parameters values model should also be validated with varying food density and temperature, but this implicates the estimation of food acquisition related parameters which was not necessary for the application presented in this study.

Impact of disease on the maintenance cost

The main objective of present study was to propose an approach to estimate quantitatively the impact of the development of the disease on the maintenance cost of the Manila clam by coupling simple observation (weight) and modelling. The strategy was to quantify the increase in total maintenance cost that can explain the observed weight loss in infected individuals. Since bacterial infections are dynamic processes, the associated increase in maintenance costs had to be treated dynamically. For this purpose, it was necessary to be able to follow a batch of infected clams and a batch of uninfected clams over time in the starvation experiment, this was possible on the basis of the observation of symptoms. The batch of asymptomatic clams is supposed to represent the uninfected case, as the batch of clams with heavy symptoms is supposed to represent the infected case.

The dynamics of the *Vibrio* within the clam were treated here theoretically since no experimental data were available, *Vibrio* dynamics only aim to modify dynamically the maintenance cost of the Manila clam. This justifies that the parameters were only estimated in order to represent the observed weight loss. Thus parameter

values have to be considered carefully and simulated dynamics of the *Vibrio* may not be realistic. The *Vibrio* population is here eliminated by the host after $\approx$ 30 days, which is difficult to check since accurate detection and quantification of the *V. tapetis* burden in clams is difficult (Drummond et al., 2006). Further development in the *V. tapetis* detection techniques and experiments is needed to obtain data allowing a more realistic modelling of the *Vibrio* dynamics.

In spite of this difficulty it was possible to provide a realistic dynamic increase in the maintenance costs since the simulated trajectory of dry weight in the infected case was near to the observed weight of the clams with heavy symptoms. The simulation only deviated from the observation at the end of the simulation when maintenance was paid from structure. In this case, for simplification purposes, the assumption that the overhead costs in paying maintenance from structure was equal to the overhead costs in making structure from reserve was done. It is possible that this assumption is wrong and that the overhead costs in paying maintenance from structure are lower. Data from Whyte et al. (1990) during a long-term starvation experiment performed over 405 days in the oyster *Crassostrea gigas* indicated that the rate of total weight decrease was higher between 0 and 30 days than between 60 to 405 days. These data could suggest a regulation of the maintenance cost in conditions of extreme starvation, such a regulations is not taken into account in the model. Further investigations are needed to detail this particular extreme situation within the context of DEB theory.

The dynamic quantification of energy flow to total (maturity and somatic) maintenance indicated that the cost of development of the disease was highest 30 days after infection. The maximum reaches the value of 57 $\mathrm{J\,d^{-1}}$, which is almost two times the total energy flow to maintenance cost in uninfected clams (32 $\mathrm{J\,d^{-1}}$). Thus BRD induces an important modification of the energy budget in the Manila clam. The maximum value can be compared to the maximum assimilation flux for an individual of this size and at the temperature of the experiment ($\dot{p}_A$ = 77.8 $\mathrm{J\,d^{-1}}$). Theoretically, only a well fed individual could compensate the maximum maintenance from assimilation. This increased maintenance cost, coupled with the decrease of filtration activity may explain the overall cost of BRD development.

5 Conclusions

Experimental data presented in this study indicate that an energetic cost can be associated to high intensity of BRD development, independently of the decrease in filtration activity. This observation confirms the hypothesis presented in Flye-Sainte-Marie et al. (2007b) suggesting that the energy budget of the Manila clam is both affected by a decrease in the filtration activity and an increase in the maintenance costs.

The DEB model presented in this study allows to predict properly both length growth and weight loss at constant environmental condition. In spite of the lack of data about *Vibrio* dynamics this study shows that on the basis of simple measurements of weight loss during starvation it is possible, by coupling modelling and observation, to provide a quantitative and dynamic estimation of the increase in maintenance associated with the development of BRD. The estimation given here indicated that during an infection the maintenance cost can almost double compared to the uninfected situation.

Further development of the model, especially focussed on the *Vibrio* dynamics and its effects on filtration activity is needed to provide a more extensive description of the energetic cost of BRD in the Manila clam.

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