Application of a dynamic energy budget model to the Pacific oyster, Crassostrea gigas, reared under various environmental conditions

Stéphane Pouvreau[a], , Yves Bourles[a, b], Sébastien Lefebvre[c], Aline Gangnery[d] and Marianne Alunno-Bruscia[b]

[a]UMR 100 (Physiologie et Écophysiologie des Mollusques Marins), Station IFREMER d'Argenton, Presqu'île du Vivier, 29840 Argenton, France

[b]CREMA (Centre de Recherche sur les Ecosystèmes Marins et Aquacoles), UMR 010 CNRS-IFREMER, Place du Séminaire, B.P. 5, 17137 L'Houmeau, France

[c]UMR 100 (Physiologie et Écophysiologie des Mollusques Marins), Laboratoire de Biologie et Biotechnologies Marines, Université Caen, Boulevard Maréchal Juin, 14032 Caen cedex, France

[d]Laboratoire Environnement Ressources de Normandie, Station IFREMER, Avenue du Général de Gaulle, BP 32, 14520 Port en Bessin, France

*: Corresponding author : spouvreau@ifremer.fr

Abstract: The Dynamic Energy Budget (DEB) model (Kooijman, S.A.L.M., 1986. Energy budgets can explain body size relations. J. Theor. Biol. 121, 269–282; Kooijman, S.A.L.M., 2000. Dynamic Energy and Mass Budgets in Biological Systems. Cambridge University Press, Cambridge, 424 pp.) has been adapted to describe the dynamics of growth and reproduction of the Pacific oyster (Crassostrea gigas) reared in different areas under conditions ranging from controlled to natural. The values of the model parameters were estimated from available physiological data and from published information. The sets of data used to validate the model came from three long-term growth experiments (> 5 months) performed on Pacific oysters reared under different conditions of food and environment. The forcing variables were temperature and phytoplankton densities, the latter being assessed from in vivo fluorescence and chlorophyll-a concentration measurement. The successful validation of the model on the three data sets demonstrated its ability to capture the dynamics of the energy budget in the Pacific oyster in various environments with the same set of parameters. The only parameter that varied between simulations was the half-saturation coefficient (XK), because of a different diet composition between the three environments under test. The model successfully reproduced quantitatively the growth and reproduction and the timing of spawning. These first simulation data led us to propose several promising perspectives of application for this model in shellfish ecosystems.

Keywords: Crassostrea gigas; Dynamic energy budget; Ecophysiology; Growth; Reproduction; Reproductive effort

1. Introduction

Marine bivalves, and especially the Pacific oyster *Crassostrea gigas*, are economically important in French aquaculture. Exceeding 109 million kg in 2001 (Girard et al., 2005), the production of this species has become a major industry on the French coasts. As a consequence, oysters are often the dominant organism in terms of their biomass in several shellfish areas and form an integral part of the ecosystem in which they occur. These constraints have prompted the necessity for tools to understand ecological processes in shellfish ecosystems and to help management of the oyster farming. Understanding of the interaction between this species and its environment, could be facilitated through the development of ecological models (e.g. Héral, 1991).

The last fifteen years have seen the development of numerous energetic models that explain the growth of molluscs according to their environment, i.e. temperature and food supply. Most of them are net-production models based on the Scope for Growth concept (Bayne, 1976). They use a detailed empirical sequence of steps for nutrition and resource allocation, based on allometric relationships, and assume that assimilated energy is immediately available for maintenance, the rest is used for growth or stored as reserves (e.g. Bacher et al., 1991; Powell et al., 1992; Schneider, 1992; Raillard et al., 1993; Barillé et al., 1997; Grant and Bacher, 1998; Scholten and Smaal, 1998; Ren and Ross, 2001). Some of the models developed for bivalves are more mechanistic (Ross and Nisbet, 1990; Van Haren and Kooijman, 1993; Cardoso et al., 2001; Ren and Ross, 2006). They are based on the dynamic energy budget or DEB theory proposed by Kooijman (1986) and extensively discussed in Kooijman (2000). DEB models differ from net-production models in several aspects. They assume that assimilated energy is first stored in reserves, and reserves are utilised to fuel other metabolic processes (maintenance, growth, development and reproduction). DEB models do not use empirical allometric relationships, but simply state that feeding is proportional to surface area whereas maintenance scales to structural body volume. More generally, DEB theory aims for a generic theory of energy budgets that assumes common physiological processes across species and life stages via a set of common DEB parameters, the only difference between species lying in the value of these parameters. These promising considerations led us to conduct investigations to apply the DEB model proposed by Kooijman (2000) to catch and simulate the life history (namely growth and reproduction) of *C. gigas* under different environmental conditions from controlled to natural ones.

The first problem in building such a model lies in the estimation of the DEB parameters. Most of them were previously assessed for the Pacific oyster by Van der Veer et al. (2006) independent of this study. Our contribution was aimed at checking the generic property of these parameters to determine whether their assessment of DEB parameters was sufficient to model oyster growth in various environments and for different populations. We also wondered whether the intra-specific differences are so great that the DEB parameters need to be recalculated for each new population and/or new environment.

A second problem lies in the diversity of the sources of food (phytoplankton, protozoa, micro-zooplankton, bacterial aggregates, detrital organic matter etc.) as well as its dilution in seston by mineral particles. The exact determination of ingestion fluxes is, thus, very hazardous because of the difficulty to monitor food and ascertain its origin. Energy allocation can be prejudicially affected by this lack of information. To overcome these problems and develop a generic model for *C. gigas*, we have designed a DEB model testing it on several populations of oysters reared under conditions clearly identified through daily or weekly measurements of water temperature, salinity, oxygen concentration as well as food quality

and phytoplankton concentration. This paper is focused on oysters after metamorphosis, *i.e.* juvenile and adult stages; embryo and larval stages are not considered.

2. Material and methods

2.1. DEB Model

The dynamic energy budget model used in this study is developed on the basis of DEB theory (Kooijman, 1986, 2000) and is depicted in Fig. 1. It includes some specific extra parameters needed to model the bio-energetics of the Pacific oyster. According to Kooijman (2000), the dynamics of growth and reproduction can be fully described by three differential equations: the first one specifies the growth of the structural body volume, V, the second one describes the dynamics of the energy reserves, E, and the third one deals with the storage and use of the energy allocated to development and reproduction, E_R.

Food uptake is assumed to: (i) follow a type-II Holling function response depending on food density X (expressed in µg chl-a l^{-1} in this study) and (ii) be proportional to the surface area of the structural body volume (V, cm^3); thus, the ingestion rate $\dot{J}_X$ (mg d^{-1}) can be written as:

$$\dot{J}_X = \left\{ \dot{J}_{Xm} \right\} f \cdot V^{2/3} \quad with \ f = \left(\frac{X}{X + X_k} \right) \tag{1}$$

where f is the scaled functional response (dimensionless), X_K is the saturation coefficient or Michaelis-Menten constant (µg chl-a l^{-1}) and $\left\{ \dot{J}_{Xm} \right\}$ the area-specific maximum ingestion rate, expressed in mg d^{-1} cm^{-2}. As a first approximation, the absorption efficiency (*ae*, dimensionless) and the food-energy conversion μ_X (J mg^{-1}) are assumed to be constant, and consequently the assimilation energy rate, $\dot{p}_A$, i.e. the total energy input per individual, becomes:

$$\dot{p}_A = ae \cdot \mu_X \cdot \dot{J}_X = ae \cdot \mu_X \cdot \left\{ \dot{J}_{Xm} \right\} f \cdot V^{2/3} = \left\{ \dot{p}_{Am} \right\} f \cdot V^{2/3} \tag{2}$$

where $\left\{ \dot{p}_{Am} \right\}$ is defined as the area-specific maximum assimilation rate and is expressed in J d^{-1} cm^{-2}.

Because of the energy conservation law, the dynamics of the reserve can be written as:

$$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C \tag{3}$$

where $\dot{p}_C$ denotes the energy consumed (fixed and dissipated) by the body tissues; it is called the catabolic power or utilisation rate. Kooijman (2000, chapter 3.4) showed that $\dot{p}_C$ can be written as :

$$\dot{p}_C = \frac{[E]}{[E_G] + \kappa \cdot [E]} \left(\frac{[E_G] \cdot \left\{ \dot{p}_{Am} \right\} V^{2/3}}{[E_m]} + \left[\dot{p}_M \right] V \right) \tag{4}$$

When combined with Eqs. [2] and [4], Eq. [3] fully describes the dynamics of the reserve, E, necessary to establish this model. In formula (4), $[E]$ corresponds to the energy density in an organism. It is defined as E/V and expressed in J cm^{-3}; according to the food density in the environment it may vary between 0 J cm^{-3} and the maximum energy density $[E_m]$. The parameter κ corresponds to a fixed fraction of the utilisation rate, $\dot{p}_C$, which is spent on growth *plus* maintenance, with maintenance having priority (the rest, *1-κ*, is allocated to development (maturity) and reproduction (gametes) *plus* maturity maintenance). $[E_G]$ denotes the volume-specific cost for growth (J cm^{-3}), a constant for structural mass including all types

of overheads, and not only the costs of biosynthesis. $\left[\dot{p}_M\right]$ denotes the volume-specific maintenance rate (J cm^{-3} d^{-1}).

Maintenance $\dot{p}_M$ (J d^{-1}) stands for a collection of processes necessary to 'stay alive'. For example, in the case of the Pacific oyster, it includes the maintenance of concentration gradients across membranes, the osmo-regulation, the turnover of structural body proteins, a certain level of muscle tension and movement for shell closure, a continuous production of shell and mucus. Maintenance cost is mainly scaled with volume: $\dot{p}_M=\left[\dot{p}_M\right]V$. Then, the dynamics for the structural body volume V is deduced from $\dot{p}_C$ and $\dot{p}_M$ according to the κ rule:

$$\frac{dV}{dt}=\frac{\dot{p}_G}{[E_G]}=\frac{\kappa\cdot\dot{p}_C-\dot{p}_M}{[E_G]} \qquad [5]$$

Under prolonged starvation and when reserve density drops below the non-growth barrier ($\kappa\,\dot{p}_C<\dot{p}_M$), the maintenance costs are first paid by using energy stored in the reproduction buffer E_R (see below). When E_R is totally emptied, maintenance is then paid by direct shrinking of structural volume.

Growth and development are parallel processes in the DEB model. So, similarly to somatic allocation, the energy allocated to development *plus* reproduction is equal to $(1-\kappa).\dot{p}_C$ and is also split into two sub-fluxes denoted $\dot{p}_R$ and $\dot{p}_J$; the former is scaled with dV/dt whereas the latter does it with $\dot{p}_M$ and corresponds to maturity maintenance costs (at a given level of complexity). Kooijman (2000, chapter 3) demonstrated that $\dot{p}_J$ can be defined as:

$$\dot{p}_J=\left(\frac{1-k}{k}\right)Min(V,V_P)\cdot\left[\dot{p}_M\right] \qquad [6]$$

where V_P corresponds to the volume at sexual maturity. Once an individual has reached V_P, the dynamics for energy allocated to reproduction buffer E_R is then:

$$\frac{dE_R}{dt}=(1-\kappa)\cdot\dot{p}_C-\dot{p}_J \qquad [7]$$

In autumn and winter, the Pacific oyster stores glycogen in specialised cells named vesicular cells (e.g. Berthelin et al., 2000; Li et al., 2000). In the model, we assume that this storage period corresponds to an increase in E_R. In spring and summer, the previously stored energy and new inputs of energy in E_R are converted into gametes (ovocyte or spermatozoa). Similarly to the cost for structure ($[E_G]$), the energy allocated to reproduction buffer E_R is fixed into the cells (vesicular cells, spermatozoa and ovocyte) with some efficiency denoted κ_R; the rest $1-\kappa_R$ is dissipated as overhead in structure building. This conversion constitutes a significant loss of energy whose value should be very similar to the loss of the energy dissipated for structural volume synthesis.

Once enough energy has been accumulated in the reproduction buffer, i.e. once a certain gonado-somatic index (GI, %) has been reached, and if external temperature is above 20°C, the buffer is completely emptied and further accumulation becomes possible. The number of gametes (spermatozoid or oocyte) $\dot{R}$ produced per individual during one spawning is :

$$\dot{R}=\frac{GDW}{w_\gamma} \qquad [8]$$

where w_γ is the dry mass of a spermatozoid or an oocyte of *C. gigas*.

Physiological rates (in the model, assimilation and maintenance) depend on the body temperature. This dependency is usually well-described by the Arrhenius relation within a species-specific tolerance range of temperatures. The lower and upper boundaries of the

tolerance range are named T_L and T_H, respectively. Within this range, all the physiological rates follow the relation: $\dot{k}(T)=\dot{k_1}\exp\left\{\dfrac{T_A}{T_1}-\dfrac{T_A}{T}\right\}$ where $\dot{k}(T)$ is the value of the physiological rate at ambient temperature T (in K), $\dot{k_1}$ is the value of the physiological rate at a chosen reference temperature T_1, and T_A is the so-called Arrhenius temperature (in K).

At temperatures lower than T_L, the true rate is generally lower than expected from the standard Arrhenius formula, because organisms are in a condition similar to a resting phase. At temperatures above T_H, the rate can also be lower than expected because organisms approach a condition of rapid death. To take into account both boundaries, the basic formula described above becomes:

$$\dot{k}(T)=\dot{k_1}\exp\left\{\frac{T_A}{T_1}-\frac{T_A}{T}\right\}\left(1+\exp\left\{\frac{T_{AL}}{T}-\frac{T_{AL}}{T_L}\right\}+\exp\left\{\frac{T_{AH}}{T_H}-\frac{T_{AH}}{T}\right\}\right)^{-1} \qquad [9]$$

where T_{AL} and T_{AH} are the Arrhenius temperatures (in K) for the rate of decrease at both boundaries.

2.2. Experimental data

The data sets used to test the model came from three independent and long-term growth experiments (>5 months) performed on Pacific oysters under different conditions of food and environment: (i) in experiment A, the oysters were placed in experimental facilities and reared at two contrasted food density levels; (ii) in experiment B, they were placed in an oyster pond and reared at a fluctuating food density, and (iii) in experiment C they were reared in a natural environment (Thau lagoon) over a complete annual cycle.

Experiment A was conducted over a 130-d period extending from April to August 2004 (see Bourles, 2004, for details). The oysters in this experiment were collected as spat in the bay of Arcachon (south-western France) in August 2003, and in March 2004 transferred to holding facilities within the IFREMER shellfish laboratory located at Argenton (Brittany, France). After an acclimation period, the bivalves were randomly split into two groups. The first group was fed ad libitum ('fed group') with a mixture of three cultivated micro-algae (25% *Chaetoceros calcitrans*, 25% *Skeletonema costatum* and 50% *Isochrysis galbana* named T-iso) distributed at a daily ration per oyster of 12% (dry mass algae/dry mass oyster); phytoplankton density around the fed oysters generally exceeded 15 µg chl-a l^{-1}. The second group was fed with only a 2% daily ration ('lean group') of the same mixture of algae, and phytoplankton density was always below 5 µg chl-a l^{-1}. Throughout experiment A, the oysters were stacked in 300-L raceways supplied with 5-µm-filtered running seawater at a mean salinity of 34.5. The water temperature was regulated every day to mimic the mean seasonal cycle of seawater temperature within the Marennes-Oléron Bay considered as the reference site (e.g. Deslous-Paoli and Héral, 1988). The tanks were periodically washed. Temperature, phytoplankton density, but also ingestion and oxygen consumption rates were monitored daily.

Experiment B lasted from June to October 2002. Oysters were cultivated in a 600-m^3 pond as part of an integrated mariculture system located on Oléron island (described extensively in Lefebvre et al., 2004). They were fed with pure phytoplankton (mainly diatoms). Biometry (flesh and shell mass) was conducted at the start and end of the experiment. In situ oxygen, temperature and in vivo chlorophyll-a were monitored daily. Three batches of oysters from different origins were placed in the pond. Batch 1 was composed of about 30-mo-old oysters from Brittany (France) transferred to the pond in June 2002. Batch 2 consisted of smaller oysters from the same origin, but transported to the pond

one month later, presumably after spawning. Batch 3 was composed of oysters from the Marennes-Oléron Bay and transferred to the pond after spawning.

Experiment C consisted of an annual growth survey conducted in the Thau lagoon (southern France). This study was carried out between September 2000 and October 2001 as described in Gangnery et al. (2003). Briefly, oysters were installed on ropes in late September 2000 at one site located in the north-western part of the lagoon. In compliance with the culture methods in use in this lagoon, 27 groups of 3 oysters were glued on 3-m-long ropes with cement, and density on the ropes was adjusted to 34 individuals per metre of rope; water depth was 4 m at the study site. The potential food consisted of natural phytoplankton and growth was followed monthly over a year. Temperature, salinity and chlorophyll-a were determined once a week during the growth period and every month outside this period.

The methods used to assess the growth of oysters over these three experiments were similar. Under each condition, oysters (n >12) were randomly collected twice a month in experiments A and C, and at the start and end of experiment B. They were cleaned and weighed after draining. Individual total mass (*TW*, g) was recorded. Then, the oysters were opened, and their flesh was removed and drained prior to weighing. The wet mass of total soft tissues per oyster was termed *WW*. In some cases, the dry mass of soft tissues was determined after freeze-drying and termed *DW*.

2.3. Model simulations

The model was implemented in STELLA 8.0 software (High Performance Systems, Hannover, NH, USA). Most of the model parameters used in this study came from Van der Veer et al. (2006). Otherwise, they were estimated according to the literature or unpublished IFREMER data (see Table 1). The state variables and processes expressed in energy were converted into the appropriate units by using: (i) for structural volume: density = 1 g cm^{-3} and 1 g wet mass = 0.15 g dry mass = 0.17 g ash-free dry mass (Whyte et al., 1990); (ii) for energy reserve (mainly glycogen): 1 mg ash free dry mass (AFDM) = 17 J (e.g. Brody, 1945); (iii) for respiration rate (proportional to $\dot{p}_C$): 1 mg O$_2$ = 13.8 J (Bayne and Newell, 1983), (iv) for gametes production: 1 spermotozoid=161 picogDW and 1 oocyte= 93000 picogDW (Ernande et al., 2004).

Table 2 lists the initial value of the state variables for each experiment. Structural volume was calculated with regard to the length by using δ, the shape correction: $V = (\delta L)^3$. For each simulation, the initial values for energy storage E and for the reproduction buffer E_R were then deduced to obtain not only the correct and initial total dry mass (*DM*) but also realistic initial values for GI index and energy density [E].

For each of the three experiments (A, B and C), the forcing variables used to run the model were the temperature and food density relative to the experiment concerned. They are shown in Fig. 2.

The model outputs, e.g. not only the total dry mass and potential spawning, but also the absorption and respiration rates, were compared to the growth and ecophysiology data obtained in these three experiments, respectively. The goodness-of-fit between prediction (Y) and observation (X) was tested according to the R² value of the regression Y=X. The only parameter that had a different value between the various simulations was X_K, the half-saturation coefficient because of differences in diet composition between experiments (see Kooijman, 2006).

3. Results

3.1. Model simulations

Experiment A. $X_{K} = 17\,\mu g$ chl-a l^{-1} produced the best fit between observation and simulation by giving a very good agreement ($R^2 = 0.98$, n = 14, p < 0.0001) between the model-predicted growth and the experimental one from the start to the end of the experiment (April to August) whatever the food density (Fig. 3a). A more thorough analysis not only confirmed the very good simulation of somatic growth (reserve and structure) under both ('lean' and 'fed') conditions, but also highlighted, with a rather high reliability, the reproduction of a spawning event in the latter. On the other hand, under the low-food density, as predicted by the model, no spawning was observed. The model also calculated the assimilation and oxygen consumption rates, which had both been continuously measured throughout the experiment. Fig. 3b and c illustrate the comparison of model-simulated and recorded assimilation and respiration rates under both feeding conditions. Apart from some discrepancies in July (around the spawning period), the model showed a high correlation between simulations and observations for assimilation and respiration ($R^2 = 0.77$, n = 198, p < 0.0001 and $R^2 = 0.96$, n = 192, p < 0.0001, respectively) after pooling both feeding conditions.

Experiment B. The aim of experiment 2 was to test the model in a more fluctuating environment and on several populations of oysters from various origins. For this environment, the X_K value was set equal to 8 μg chl-a l^{-1}. Comparison of the model-predicted growth with observed growth during experiment B (Fig. 4a) showed a good fit for the three populations of oysters. After pooling of all the data, the overall correlation coefficient between observation and simulation was $R^2 = 0.81$ (n = 8, p < 0.002). Final masses were very well predicted by the model in the three populations (Fig. 4a). In 'batch 1', oysters were transferred to the pond early enough in the season to allow continuation and completion of their gametogenesis in summer and the timing and magnitude of the spawning indicated by the mass loss observed in mid-September were clearly predicted by the model. The other two batches of oysters showed no sign of such an event.

Experiment C. In experiment C the model was applied under typical field conditions and over a complete annual cycle. The best fit between observation and simulation was obtained with $X_K = 3.5$ μg chl-a l^{-1} (Fig. 4b). An overall analysis of the prediction against observation indicated a goodness-of-fit: R^2 of 0.92 (n = 24, p < 0.0001). The model simulated not only oyster growth over a complete annual cycle, but also the gametogenesis and spawning periods in the Thau lagoon. In agreement with experimental data on dry flesh mass, the model showed two sharp drops: at the end of June and August. It anticipated slightly the first period of spawning, whereas the second one was predicted at the correct time. The quantities of gametes released during the first and second spawning were 0.2 and 0.5 g DW, respectively, in agreement with observations. However, a more thorough analysis showed some discrepancies between the model and data with a slight underestimation of growth in spring and a small overestimation in summer.

3.2. Analysis of the model internal functioning

The simulations in the Thau lagoon (experiment C) were used to obtain clues as to how the model treats physiological responses.

One of the internal properties of the DEB model is that the energy utilisation rate $\dot{p}_C$ varied so that the scaled energy density (e = $[E] / [E_m]$) tends to reach the value of the scaled

functional response f. Fig. 5a clearly illustrates this internal property in the Thau lagoon conditions: f varied according to food densities, and after a short delay the scaled energy density e follows exactly the same trend, but with a smoothing due to the storage compartment.

According to the assimilation values, food was limiting in winter, and the assimilation rate was just sufficient to meet the maintenance costs (Fig. 5b): in December, the assimilation rate of a 6 to 8-mo-old oyster was about 27 J d^{-1}, and maintenance costs reached the same value (27 J d^{-1}). At the other extreme, a 1-y-old oyster assimilated more than 700 J d^{-1} in summer with maintenance rates accounting for 202 J d^{-1}; the resulting positive energy budget allows rapid growth and active gametogenesis.

Finally, the model was run over a 10-y period by setting the temperature and food density measured in Thau lagoon as forcing variables. At the end of the simulation, the dry mass (outside the spawning period) had reached about 8 g corresponding to a maximum length of about 18 cm.

4. Discussion

Over the last decade, modelling approaches have gained popularity in studies on shellfish energetics (e.g. Bacher et al.*,* 1991; Powell et al., 1992; Schneider, 1992; Raillard et al., 1993; Van Haren and Kooijman, 1993; Barillé et al.*,* 1997; Grant and Bacher, 1998; Scholten and Smaal, 1998; Ren and Ross, 2001, 2006) especially in coastal management where carrying capacity and environmental changes are essential. Concerning the Pacific oyster, the model presented in Barillé et al. (1997) uses the widely known 'scope for growth' concept. It simulates the temporal evolution of the somatic and storage-gonad compartments according to several environmental variables (total particulate matter, particulate organic matter, particulate inorganic matter, chlorophyll-a, phaeopigments, proteins, lipids and carbohydrates). This highly parameterised model successfully reproduced the growth and spawning of oysters reared in the Bay of Marennes Oléron, but showed its limits when applied to other conditions. Ren and Ross (2001) also developed a bioenergetic model for the Pacific oyster. Though their model uses some aspects of the theory developed by Kooijman (2000) by distinguishing structure from reserve, it relies less on mechanics and, thus, differs from DEBs in two fundamentals aspects: (i) the use of allometric functions relying on an empirical basis (Kooijman, 2000, chapters 1 and 2), and (ii) the direct subtraction of respiration from assimilation before energy allocation. Our study is the first application of the generic DEB model developed by Kooijman (2000) to the Pacific oyster. It highlights the good agreement between model-based simulations and real observations, even though it constitutes only the first step of our research program. Such a concordance indicates that the DEB theory, developed by Kooijman (1986) for all kinds of animals, is an efficient tool for capturing the bioenergetics and physiology of molluscs, especially the Pacific oyster. It also demonstrates that: (i) the accuracy on the measurements of the estimates used here for the forcing variables and DEB parameters was sufficient, (ii) the food sources selected in this study were highly decisive for growth and reproduction, and (iii) the reproductive processes had been clearly formulated at this first stage.

Most of the estimates used for the DEB parameters in this study were taken from Van der Veer et al. (2006). However, the very small number of data used to determine some of the estimates together with their calculation from relationships with other parameters had caused these authors to question the quality of their estimates. Even the estimation procedure itself is a source of uncertainty: for example, Van der Meer (2006) illustrated clearly that

simultaneous regressions on combined sets of data on the blue mussel, *Mytilus edulis* (i.e. weighted non-linear least squares regression) gave more reliable estimates than simple regressions on isolated data sets as done in Van Haren and Kooijman (1993). Nevertheless, the proven agreement between the simulation results produced by the model and observations indicates that, despite the lack of calibration, most of the estimates can be considered fairly reliable. The quality of a parameter value on the global output of the model can be tested by performing a sensitivity analysis. Such an analysis was performed on our model by Bacher and Gangnery (2006). They showed that parameters governing food consumption, i.e. $\{\dot{p}_{Am}\}$ and X_k, had most effect on growth: for example a 10% increase in the parameter X_k induced a variation of ca. 10% in the tissue mass. This result indicates that more precise laboratory and field experiments are required to gain more insight into especially these two parameters.

As mentioned, in the three experiments analysed in this study, the great concordance between observation and simulation data suggested that growth and reproduction were both largely determined by phytoplankton dynamics and seasonal cycle of temperatures in the environments tested here. Indeed, each experiment relied on a good knowledge of the quantity (daily in vivo fluorescence or weekly chlorophyll-a measurements) and quality (cultured phytoplankton, mono-specific blooms or natural suspended matter rich in phytoplankton) of potential food sources. Under such ideal conditions, the simple type-II feeding function seems to be sufficient to quantitatively reproduce ingestion rate by using chlorophyll-a as a quantifier for food. Under more complex field conditions with many sources of food often diluted with a high mineral seston load, this simplistic form of the feeding function would probably require further improvements (e.g. Kooijman, 2006). The effect of diet composition on ingestion rate is poorly known in the Pacific oyster (e.g. Barillé et al., 1997). Furthermore, many species are known to modify their diet in the course of development or reproductive cycle, but to our knowledge the literature presents no information on such behaviour in *Crassostrea gigas*.

Concerning ingestion rate assessment, another problem is the choice of the correct quantifier for food. As mentioned, in this study we used chlorophyll-a for two reasons: (i) the food supplied to the oysters consisted mainly, but not exclusively, of phytoplankton, and (ii) chlorophyll-a is often used as a proxy for phytoplankton biomass. The simulations indicated that this quantifier was quite good in the three experiments, but the half-saturation coefficient (X_K) expressed in µg chl-a l^{-1} showed variations according to experiment. Under cultured algae conditions, X_K was higher than under natural conditions (17 vs 3 µg chl-a l^{-1} in experiments A and C, respectively). We therefore wonder whether this variation results from a bad quantification of phytoplankton abundance by chlorophyll-a and whether the use of another quantifier for food (total volume of phytoplankton or total amount of carbon for example) would reduce it. The former assumption is supported by Zonneveld (1998), who clearly demonstrated huge variations in phytoplankton Chl-a-to-carbon ratio according to environmental conditions. Further investigations are, therefore, needed to find a suitable generic quantifier for food, likely POC, and to develop a more elaborated equation to describe the effect of food composition and quality on the half-saturation coefficient (see Kooijman, 2006). Differences between sites in X_K but also in $\{\dot{p}_{Am}\}$ values may also be due to phenotypic adaptation in the clearance and selection capacities of oysters. It has been shown that these capacities depend on the size of gills and palps (e.g. Barillé et al., 2000; Honkoop et al., 2003) and individuals can adapt these organs according to local food conditions (e.g. Essink et al., 1989). Consequently, we assume that potential plasticity in ingestion capacity could partly explain the differences we found in X_K values among experiments. And more generally, adaptive mechanisms within a species could potentially increase the variability in some DEB parameters.

If food sources and selection efficiency are often the origin of discrepancies in the modelling of mollusc growth, reproductive processes (gametogenesis and spawning) are also complex processes that need special attention to be properly implemented into a model. The amount of energy invested in reproduction usually accounts for a significant part of the energy budget in bivalves, especially in the Pacific oyster (e.g. Deslous-Paoli and Héral, 1988) as demonstrated in the present study by the low κ-value (0.45) for this species. Furthermore, the sensitivity analyses performed by Bacher and Gangnery (2006) on the present model showed that parameter κ also had a significant effect on the growth simulation. Therefore, particular attention was paid to the formulation of reproductive processes in our model. Most of this formulation has already been described by Kooijman (2000) but we needed to add some species-specific aspects. The reproductive cycle of a bivalve can be viewed as a two-phase process: the first one is devoted to storage accumulation (generally glycogen in bivalves), and the second one, which is fuelled by accumulated stores (e.g. Li et al., 2000) and/or directly available food (Enriquez-Diaz, 2004), is dedicated to gametogenesis. This suggests that the energy used for gametogenesis includes both recently assimilated and previously stored reserves. Our model directly reproduces this connection between storage accumulation and gamete synthesis, since the state variable, E_R, considers storage and gametes to be in the same compartment.

In autumn and winter, the Pacific oyster stores glycogen in specialised cells, called vesicular cells (e.g. Berthelin et al., 2000; Li et al., 2000). In spring and summer, the previously stored energy and the new energy input are converted into gametes (oocytes or spermatozoa). Consequently, in our model, throughout the year, the energy allocated to the reproduction buffer, E_R, is fixed (within vesicular cells, spermatozoa and oocyte) with a certain efficiency called κ_R, the rest ($1-\kappa_R$) is dissipated as overheads for structure building. This conversion constitutes a significant loss of energy corresponding to the cost of gametogenesis. This cost should be almost equal to the loss of energy dissipated over structural volume synthesis (integrated in $[E_G]$); this is why κ_R was set equal to 0.7. This cost should also be expected to differ with sex (male or female). As oysters can, however, change sex during their life cycle with no quantitative change in their growth or gonad mass, we first assumed that κ_R was exactly the same for males and females, but these assumptions have to be confirmed or refuted by further studies. Moreover, Ren and Ross (2001) have already stressed the strong need for quantitative investigation on the energetics associated with reproduction.

The spawning process was formulated in a simplistic way in the model by assuming that, once enough energy has been accumulated in the reproduction buffer, i.e. once a certain gonado-somatic index (*GI*, %) has been reached, provided that external temperature is above 20°C, spawning can occur, and then the buffer is completely emptied. This kind of formulation for a spawning trigger in bivalves has been used in previous models (e.g. Pouvreau et al., 2000). For the moment, it seems to constitute a suitable way to formulate this process: for example the model predicted two spawning periods in Thau lagoon. Both predictions were validated in quantity and timing by the observation of significant losses in total dry mass, but also by histological observations performed on this population of oysters (Pouvreau, pers. comm., 2002). Further field testing of the model in various environments should indicate whether this simplistic formulation is sufficient or not.

We extended the basic model to include the possibility of using the energy stored in E_R to sustain maintenance under starvation conditions; knowledge on this issue is very limited. The observation in the field, in autumn and winter, of decrease in soft tissue mass (Deslous-Paoli and Héral, 1988) might indicate that this shrinking results mainly from a reduction of the energy reserve, E, and to a lesser extent from a decrease in the energy stored in the reproduction buffer, E_R. In autumn E_R can be either totally emptied (after a total spawning) or

filled partially with a new storage of glycogen in vesicular cells for the next reproduction cycle or filled partially with un-spawned gametes. Unfortunately, few studies have dealt with the latter case, i.e. the use of E_R to sustain maintenance under condition of starvation, a process known as gametic degeneration, resorption or atresia. The process of resorption in *C. gigas* has often been reported (e.g. Steele and Mulcahy, 1999; Enriquez-Diaz, 2004); it is considered to be a 'self-cleaning' stage that prepares the gonads for a new sexual cycle and is accompanied by a progressive autumnal reduction in gonad mass. The formulation used in our model to take into account the resorption process is presumably too simple and probably requires further refinements.

In conclusion, by demonstrating its rather good ability to mimic growth and reproduction under various controlled conditions, this DEB model appears to be generally suitable for simulation of bioenergetics of oysters in the field. Nevertheless, for use in a generic approach, the sources of food and selection efficiency are likely to need further refinements as well as the formulation of reproductive physiology. For our objective -the development of a species-specific model- addition of some extra parameters to the original model of Kooijman (2000) may be required. However, it is a delicate step that needs proper justification (Kooijman, 2000), and for the moment does not seem to be required. By being appropriate for general questions and inter-specific comparisons, the DEB model developed by Kooijman (2000) provides a satisfactory fit without needing any modification. The next step will be to investigate the suitability of this model to simulate growth and reproduction in the field under environmental conditions more complex than those handled in this study.

Acknowledgements

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Table 1. List of the core-parameters (+ additional ones) implemented in the DEB model of oyster growth assessed in this study.

Parameters	Symbol	Units	Value	References
Primary parameters:				
Arrhenius temperature	T_A	K	5800	Van der Veer et al. (2006)
Half saturation coefficient	X_K	µg chl-a l^{-1}	3.5 – 8-17	Free-fitting (see Kooijman, 2006)
Max. surf. area-specific ingestion rate	$\{p_{Xm}\}$	J cm^{-2} d^{-1}	560	Van der Veer et al. (2006)
Assimilation efficiency	*ae*	-	0.75	Van der Veer et al. (2006)
Volume-specific maintenance costs	$[p_m]$	J cm^{-3} d^{-1}	24	Van der Veer et al. (2006)
Maximum storage density	$[E_M]$	J cm^{-3}	2295	Van der Veer et al. (2006)
Volume-specific costs for structure	$[E_G]$	J cm^{-3}	1900	Van der Veer et al. (2006)
Structural volume at sexual maturity	V_p	cm^{-3}	0.4	Pers. unpubl. data
Fraction of p_C spent on maintenance plus growth	κ	-	0.45	Van der Veer et al. (2006)
Fraction of reproduction energy fixed in eggs	κ_R	-	0.7	This study (see explanation in text)
Shape coefficient	δ_M	-	0.175	Van der Veer et al. (2006)
Additional parameters:				
Lower boundary of tolerance range	T_L	K	281	Van der Veer et al. (2006)
Upper boundary of tolerance range	T_H	K	305	Van der Veer et al. (2006)
Rate of decrease at lower boundary	T_{AL}	K	75000	Van der Veer et al. (2006)
Rate of decrease at upper boundary	T_{AH}	K	30000	Van der Veer et al. (2006)
Energy content of reserves (in ash-free dry mass)	μ_E	J mg^{-1}	17.5	Deslous-Paoli and Héral (1988)
Gonado-somatic index triggering spawning	GI	%	35	Pers. unpubl. data
Temperature threshold triggering spawning	T_S	°C	20	Pers. unpubl. data

Table 2. Initial values of the state variables: structure (V), storage (E) and reproduction buffer (E_R) for different conditions in experiments A, B and C. * calculated with a shape coefficient $\delta = 0.2$ specific to suspended culture in the Thau lagoon. ** the low value of initial dry mass according to length and season (summer) indicates a possible spawning of oysters ($[E]$ low and E_R set to zero) prior to the experiment.

Experiment	Length L (cm)	Structure V (cm^3)	Storage E (J)	Reproduction buffer E_R (J)	Total dry mass (g)
Experiment A	4	0.35	200	0	0.08
Experiment B					
Batch 1	7.6	2.3	2000	4000	0.70
Batch 2	7.9	2.6	500	0	0.55**
Batch 3	8.3	3.1	3500	8500	1.15
Experiment C	4.7	1*	500	500	0.25

Fig. 1.　Scheme of the DEB growth model for the Pacific oyster (*Crassostrea gigas*). State variables are grey and forcing variables are circled. Overheads consist of mineral, products and heat fluxes. They include for example carbon dioxide production and oxygen consumption.

Fig. 2.　Temporal variations of the forcing variables, i.e. phytoplankton concentration and temperature, in experiments A (top panel); B (middle panel) and C (lower panel).

Fig. 3.　Comparison of observations ± SD (dots) and simulations (line) of the Pacific oyster in Experiment A under two food conditions. 'Fed oysters': fed ad libitum with an environmental phytoplankton density above 15 µg chl-a l^{-1}. 'Lean oysters': fed with the same mixture of algae, but at a phytoplankton density of less than 5 µg chl-a l^{-1}. Arrows indicate the spawning event under the high-food condition.
a: Growth of flesh dry mass (g)
b: Assimilation rate (J d^{-1} ind^{-1})
c: Respiration rate (mg O_2 ind^{-1})

Fig. 4.　Comparison of observations ± SD (dots) and simulations (line) of body dry mass in the Pacific oyster in
a: Experiment B for each stock
b: Experiment C (Thau lagoon).

Fig. 5.　Analysis of internal functioning of the model for one-year-old Pacific oysters by taking the environmental conditions in the Thau lagoon as reference:
a: Simulated functional response and scaled energy density;
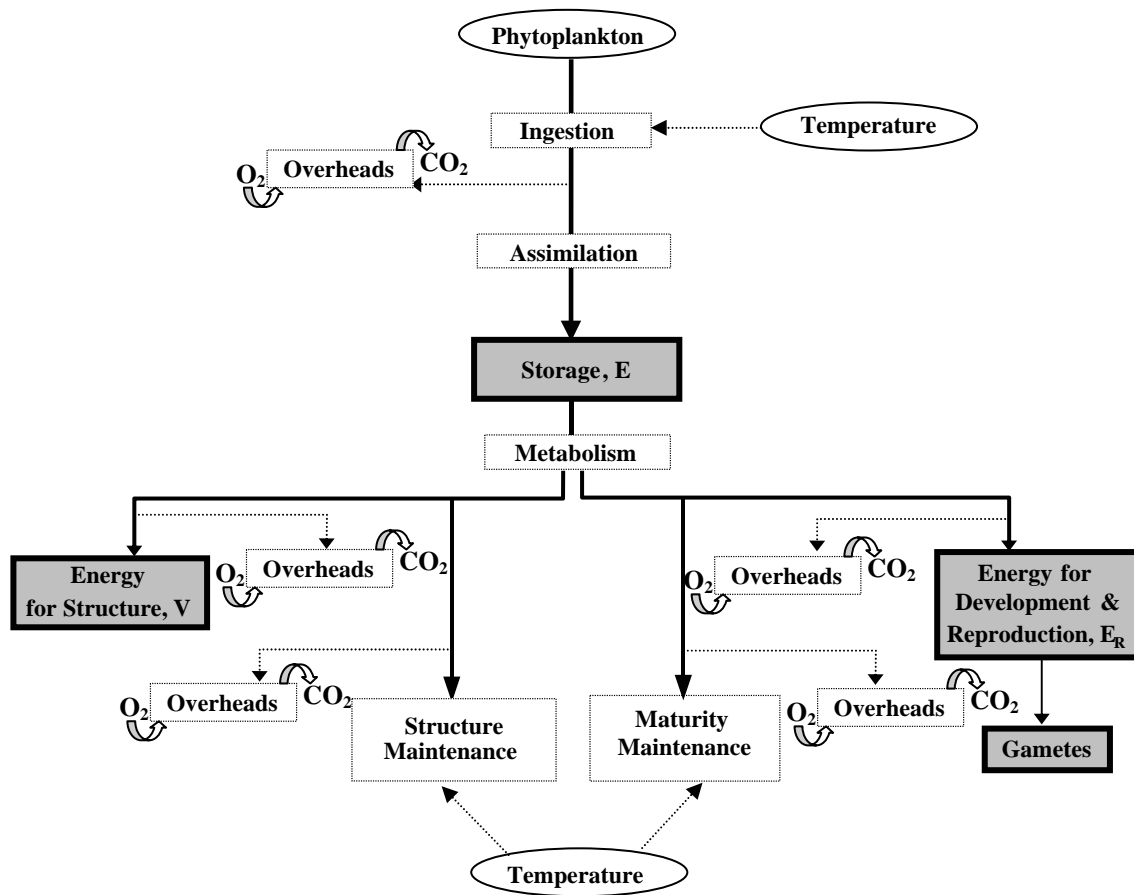b: Simulated energy budget (assimilated energy and maintenance).

Figure 1. Scheme of the conceptual DEB growth model for the Pacific oyster (*C. gigas*). State variables are grey-coloured and forcing variables are circled. Overheads consist of mineral, products and heat fluxes. They include for example Carbon dioxide production and Oxygen consumption.
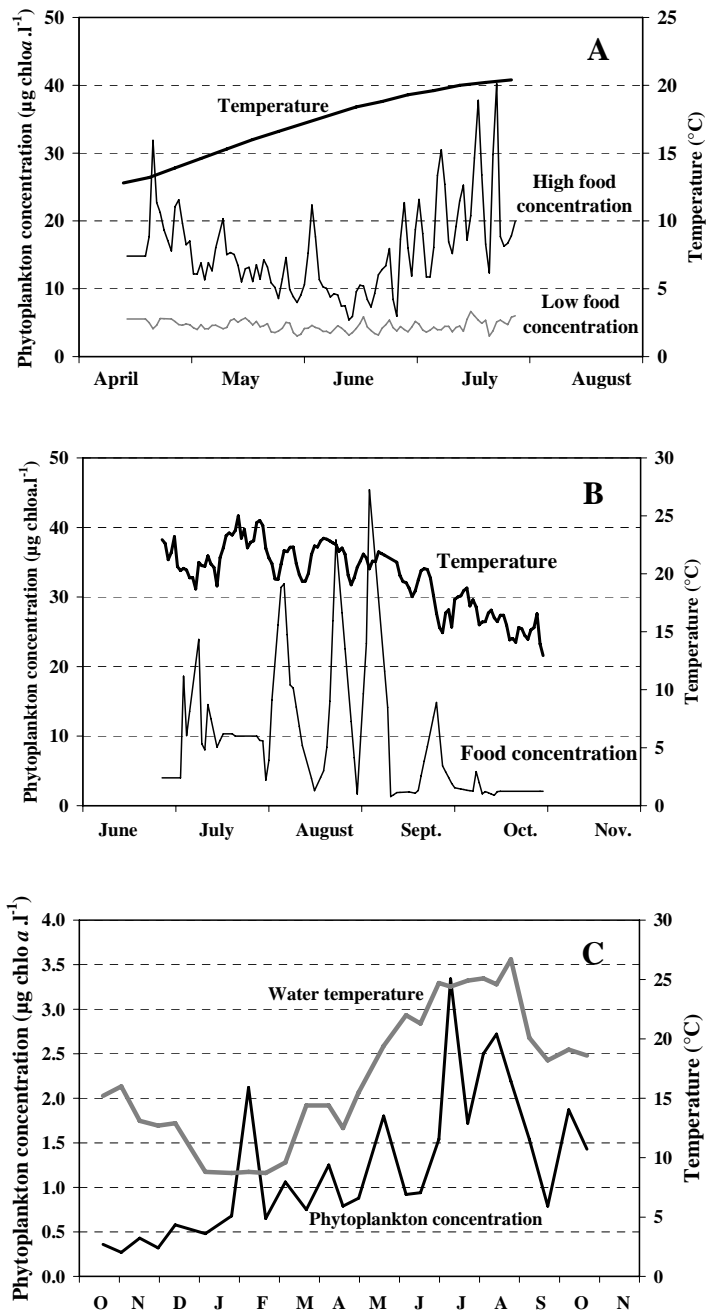
Figure 2. Temporal variations of the forcing variables, *i.e.* phytoplankton concentration and temperature in experiments A, B and C.
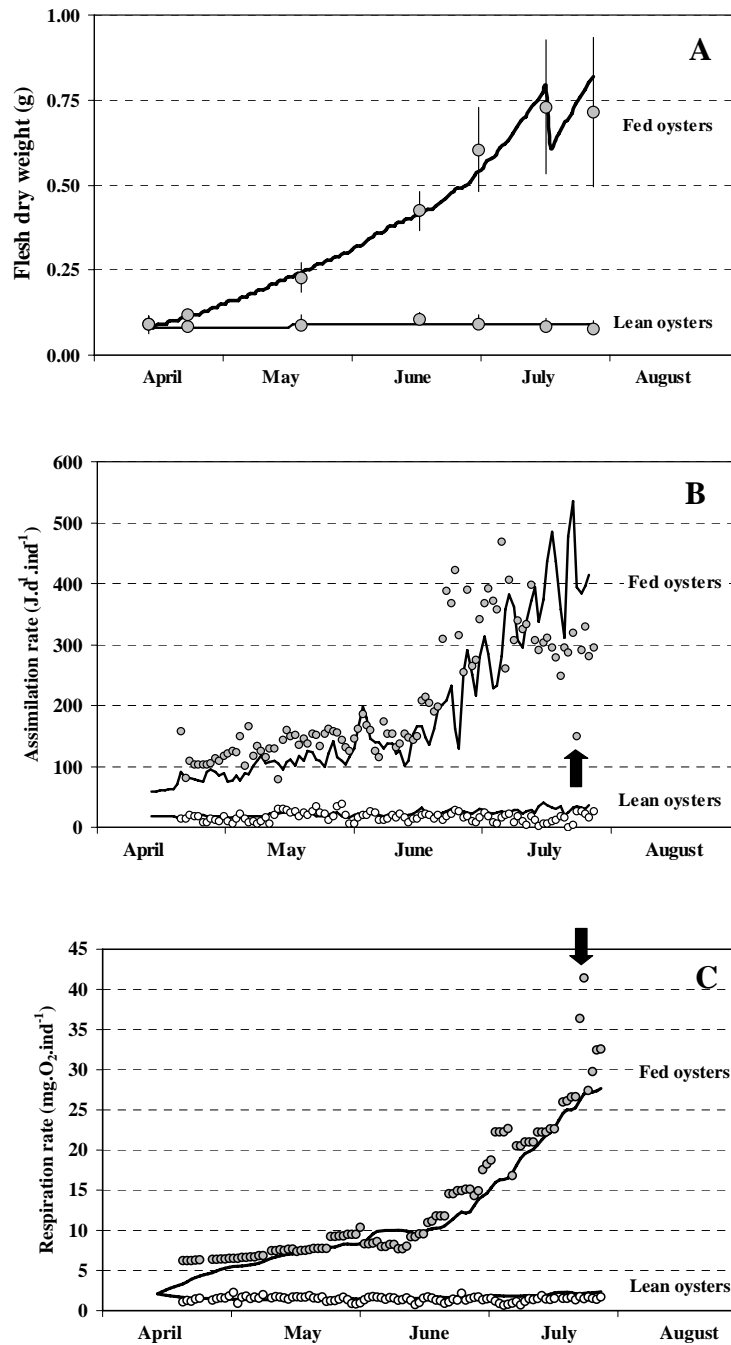
Figure 3: Comparison of observations and simulations in Experiment A. Figure 3A: growth simulation (line) of flesh dry mass of the Pacific oyster under two food conditions. 'Fed oysters' were fed *ad libitum* with an environmental phytoplankton density above 15 µg chloa. l[-1]. At the opposite, 'Lean oysters' were poorly fed with the same mixture of algae, but at a phytoplankton density less than 5 µg chloa. l[-1]. Dots (symbols) are observed data (± SD). In figure 3B and 3C: Assimilation and respiration simulation (line) of the Pacific oyster under two food conditions. Dots (symbols) are observed mean daily data. Under the high-food condition, arrows indicate the spawning event leading to an occasional, though sharp, decrease in assimilation and a sharp increase in respiration. These variations are not predicted by the model, because of the missing integration of such processes in the model.
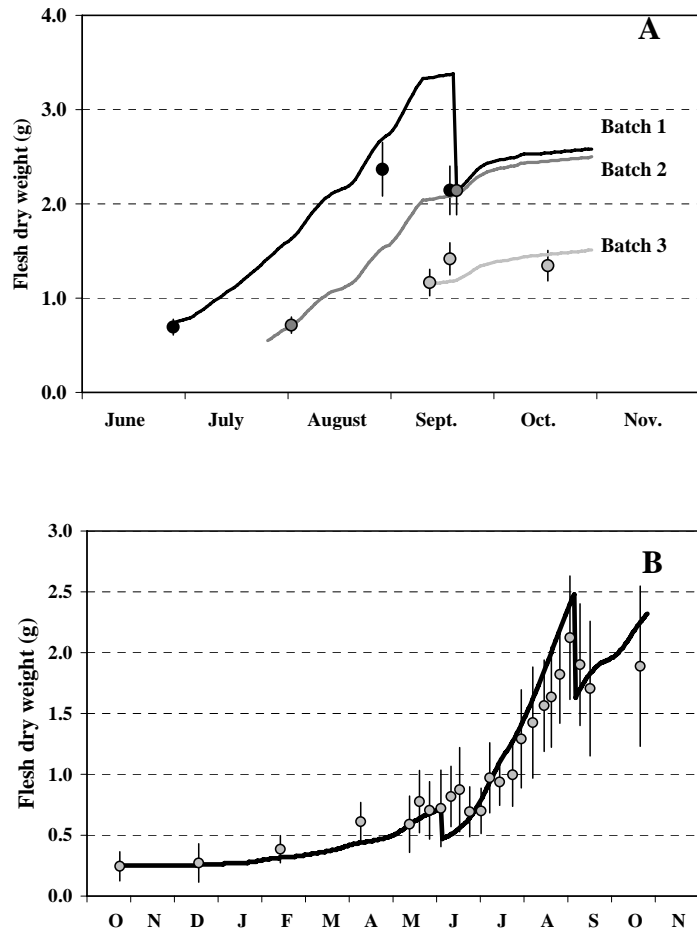
Figure 4. Growth simulations (lines) of flesh dry mass of the Pacific oyster in experiment B for each stock of oysters (fig. 4A) and in experiment C (Thau lagoon, fig. 4B). Dots (symbols) are observed data (± SD).
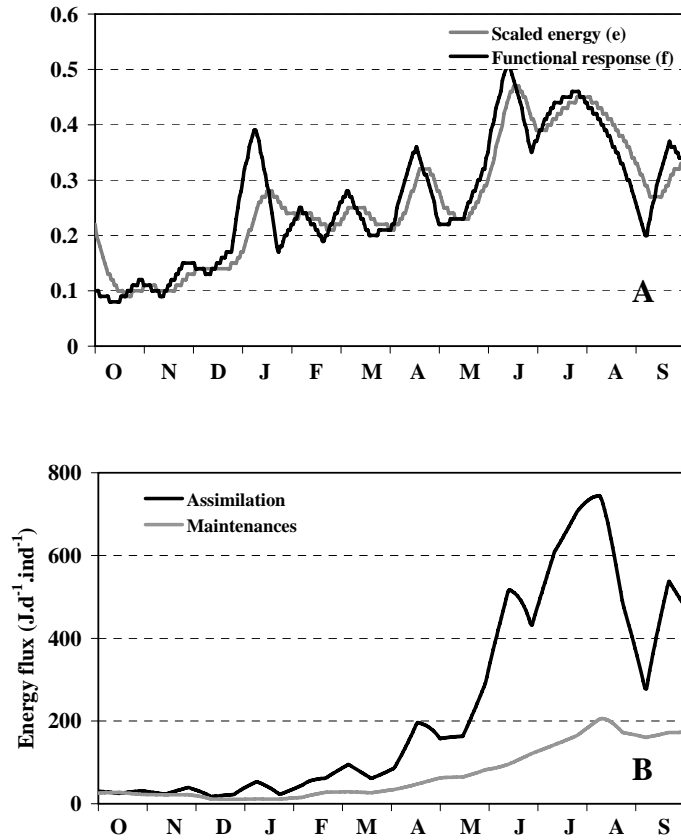
Figure 5: Analysis of internal functioning of the model for one-year-old Pacific oysters by taking the environmental conditions in the Thau lagoon as reference: A: Simulated functional response and scaled energy density; B: Simulated energy budget (assimilated energy and maintenance).