FIRST PART OF THE ESSAY:
"A general paragraph about the items in the subprogram that you thought were most inspiring, or difficult, or in need for further research."

Structural Homeostasis, Weak Homeostasis and Reserve Dynamics

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The Homeostasis concept is one of the cornerstone of the DEB theory. In Chapter 7, there is a very interesting part concerning homeostasis, more precisely structural homeostasis. For me, the concept of homeostasis sound nice in physiology: cells achieve full controls over all transformations with the help of enzymes. The properties of enzymes depend on their micro-environment. So a constant chemical composition, *i.e.* homeostasis, appears to be essential for full control.
Structural mass (V,M_V) and reserve (E,M_E) do not change in composition: this is **the strong homeostasis assumption**. But, the amount of reserves can change relative to the amount of structure, depending on food densities (f). When food density does not change during the life cycle, the individual is in a state equilibrium, reserves and structure are in constant proportion: this is **the weak homeostasis assumption**. **The essential point of this assumption is that, under constant environmental conditions, the individual grow in such a way the reserve density [E] does not change (see figure 1).** So, this assumption is crucial since it helps to determine the reserves dynamics, and the formula $\frac{d[E]}{dt} = [\dot{p}_A] - \frac{\dot{v}[E]}{V^{1/3}}$.
A first demonstration of this formula is given at {83-85} and other one at {247-249}. The aim of my essay, with the help of my colleagues, is to go back on these two demonstrations.

First demonstration {83-85}:

Since energy capacity of the blood is small, the variation of energy in blood is assume to be closed to 0, which means that the dynamic of energy reserves can be written as (conservation law):

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

The dynamics of the reserves follows from three important requirements:
1. The reserve dynamics should be partitionable;
2. The reserve density ([E]=E/V) at steady state (food density and temperature are constant) should not depend on structural body mass (V). This is the weak homeostasis assumption;
3. The use of reserves should not directly relate to food availability.

These three requirements are very important for the axiomatic approach concerning the reserve density dynamics. The dynamics for the reserve density has to be set up first, in a general form, as following:

$\frac{d[E]}{dt} = [\dot{p}_A] - F([E],V)$ but why don't you consider a function F(E,V), E and V being the state variables of the model ?

[E] varies according to an assimilation rate and an utilisation rate (here, the F function) that depends on V, but also on [E].

Weak homeostasis says that, at equilibrium (f=cst, d[E]/dt=0), [E] is independent of V. But [pA] is a function of $V^{2/3}/V=V^{-1/3}$. So at equilibrium ([E]*), when weak homeostasis can be apply, it is necessary to have : $F([E]^*,V)=H([E]^*/\theta) \cdot V^{-1/3}$, to obtain [E] independent of V.

For non-equilibrium conditions, we must have a function, called G that depends on V but that must disappear at steady state, so we need to multiply it by ([E]*-[E]). The general form for the reserve density is therefore:

$$\frac{d[E]}{dt} = [\dot{p}_A] - \frac{H([E],\theta)}{V^{1/3}} + ([E]^* - [E])G([E],V)$$

[pA] and [E]* depend on food density. But the third requirement implies that d[E]/dt is not directly linked to food density, so G must be set to zero. We are not sure to have fully understood this last step ?

If this last step is correct, the axiomatic approach gives :

$$\frac{d[E]}{dt} = [\dot{p}_A] - \frac{H([E],\theta)}{V^{1/3}}$$

By using the first requirement (partitionability of reserve dynamics) , it is relatively clear to me that H ([E],θ) is in fact a first-degree homogeneous function (f(x)=ax) since it follows $k_A H([E],\theta) = H(k_A[E], \theta)$ so $H([E]) = \dot{v}[E]$. Consequently, we obtain the famous first order equation :

$$\frac{d[E]}{dt} = [\dot{p}_A] - \frac{\dot{v}[E]}{V^{1/3}}$$ (equation 7.23 equivalent to equation 3.10)

The major problem for me is that this equation is a simplification of the general mathematical equation (in which I'm completely agree) :

$$\frac{d[E]}{dt} = \frac{1}{V}\frac{dE}{dt} - \frac{[E]}{V}\frac{dV}{dt} = [\dot{p}_A] - [\dot{p}_C] - [E]\frac{d\ln V}{dt}$$ (equation 3.7)

Are these two equations (7.23 and 3.10) identical ? For me, they are not identical (see figure in annexe). And on page 249, we can see that in fact equation 7.23 is a simplification of equation 7.21 (see below). This simplification seems to be correct only if g>>1, in other word only if [Eg]>>[Em] (since k is close to 1).

The volume specific catabolic flux (3.9) is obtained by saying that:

equation 7.23=equation 3.10

So, for me, this equality is an approximation that is only possible when [Eg] >> [Em]. If not, this equality is violated, and [pc] cannot be approximated by (3.9).

<u>Second demonstration {247-249}:</u>

In chapter 7.6, there is another approach (a structural approach) to demonstrate, **at a cellular level**, the first order process for dynamic reserve density. This other approach used the structural homeostasis assumption, that is like a structural mechanism behind the weak homeostasis assumption.

In order to have a reserve precursor density constant in the cytosol (for optimal enzyme kinetics), we need to have a constant carriers (enzyme+molecule) density on the cell + vesicle membranes. The density of carriers on membrane is proportional to the surface area of membrane {Mc}. At substrate constant, density of carriers is constant if $\{Mc\}=Mc/V^{2/3}$ is constant (structural homeostasis): $Mc=\alpha V^{2/3}$.

In other word, the structural homeostasis implies that there is a direct coupling between the linear dimensions of the n vesicles (l_i) and the linear dimension of the cell (L).

Structural homeostasis implies $l_i/L=cste \Leftrightarrow L=\alpha l_i$

The total amount of membranes (external membrane + vesicle membrane) in a cell is named Mc. Mc is proportional to $(l_i+L).^2$ so to L^2 because of structural homeostasis. So we have:

$Mc=\alpha L^2$ (equation A)

Weak homeostasis implies [E]=cste ⇔ E=αV (equation B)

Equation A + B gives that both homeostasis implies Mc/E=αL²/V ⇔ Mc=αE.V$^{-1/3}$ (Equation in figure 7.21)

A cell contains n vesicles. We named Ei and Mi, the energy reserve and the amount of membrane for vesicle i. If a cell contains n vesicles, we have : E=nEi, E=nV[Ei], [E]=n[Ei] and Mc=n{Mci}. So: Mc=n{Mci}V$^{2/3}$=[E]/[Ei] {Mci}V $^{2/3}$. And consequently dynamics for Mc follows equation (7.18).

The dynamics of the amount of membranes M_C can also be obtain with the DEB theory (equation 7.13, below):

$\frac{dM_C}{dt} = \dot{p}_C \eta_{CC} - M_C \dot{k}_C$, where η_{CC} and $\dot{k}_C$ denotes respectively the conversion efficiency of catabolic power into M_C and k_C a decay rate of destruction of membranes.

Similarly, for structural volume, we can write that:

$\frac{dM_V}{dt} = [M_V]\frac{dV}{dt} = \dot{p}_C \eta_{VC} - M_V \dot{k}_M$ where $\dot{k}_M$ is the maintenance rate coefficient $\dot{k}_M = \frac{[\dot{p}_M]}{[E_G]}$

This equation, Equation 7.14, can also be obtained on the basis of :

$$\kappa \dot{p}_C = [E_G]\frac{dV}{dt} + \dot{p}_M = \frac{[E_G]}{[M_V]}\frac{dM_V}{dt} + \dot{p}_M = \mu_{GV}\frac{dM_V}{dt} + \dot{p}_M$$

We need to write : $\eta_{VC} = \kappa \eta_{VG}$ that I don't understand ?

We have:

$$\frac{d[E]}{dt} = \frac{1}{V}\frac{dE}{dt} - \frac{[E]}{V}\frac{dV}{dt} = [\dot{p}_A] - [\dot{p}_C] - \frac{[E]}{V}\frac{dV}{dt}$$ Equation 3.7

With (7.14), we can deduce dV/dt and replace it in 3.7. That's give the catabolic flux equation in (7.16). Using (7.16), (7.13) and (7.18), **we can deduce the complete formulas for d[E]/dt (equation 7.21) at a cellular level.**

This equation is strongly different than equation than equation 3.10, and need to be simplified in order to obtain the equation 7.23. There is several simplification steps, than can be summarised as follows :

1. Membrane kinetics is very fast with respect to reserve kinetics
2. [Eg]>>[Em]

SECOND PART OF THE ESSAY:

"A specific paragraph about how you plan to apply the theory in your own research."

S. Pouvreau:

Physiological models explaining growth and reproduction of molluscs in their environment in relation to food supplies have already been achieved on numerous bivalves species. Generally, these models are based on the widely used scope for growth concept. During my PhD, I have developed such a model (Pouvreau et al., 2000) for the pearl oyster, Pinctada margaritifera. But I found in the SFG theory many inconsistencies, at several levels.

Firstly, I was really annoyed with the allometric equation ($Y=aW^b$)and especially the allometric exponent (b). At this period, it was clear to me that the weight (W) should be a somatic weight and b a constant value within a specie and between species. And that this value could only differ according to the process (anabolism/catabolism). For pearl oyster, I try to demonstrate this with filtration rate measurement (Pouvreau et al., 1999).
Secondly, I was really annoyed with the SFG concept itself where respiration rate appears as a 'strange black box', hastily subtracted to assimilation. During this period, I had not enough time to try to apply another approach. I tried to read the first edition of the Kooijman book, and I found it too'hard' and too complicate. So I take a more 'easy road' : the scope for growth.

After the DEB course, it appears clearly for me that this 'easy road' is not the good one and it is probably a blind alley. I was really attracted by the first chapter of the DEB book: (1) the radical rejection of the standard application of allometric equations, that restrict the usefulness of almost all existing theories on energetics, (2) the axiomatic approach like physician of the theory, and (3) the major role playing by a necessary storage compartment-buffer since it is true that individuals react slowly to changes in their feeding conditions. Concerning this point of view, the SFG concept is too 'reactive'.

Now, I work on the ecophysiology of growth and reproduction of commercial bivalves (mainly oysters). My scientific aim is to clearly understand how a bivalve modifies its growth and reproduction according to its environment (physics parameter and food supply). There is a lot of practical applications for this scientific field: zootechnical optimization in hatchery, carrying capacity of ecosystem, impact studies in case of environmental changes…. To reach this aim, I and other colleagues (M. alunno-bruscia, and our phD Y. Bourles) are trying to build mechanistic models for growth (adult and larvae) and reproduction for several bivalves and filter-feeders (C. gigas, P. margaritifera, C. fornicata…). In my opinion, it can be helpful to use the DEB concept in our modelling approach. We have translate the code given in the paper of van der Veer et al. (2001) to adapt it to our species under STELLA software. We have a phD student (Y. Bourles) that is working on it to evaluate its robustness. Nevertheless, I have still some questions and doubt concerning dynamics of reserves, see our essays.

The major bottlenecks would be, in my opinion, the acquisition of some parameters. We have experimental facilities (marine laboratory with algae culture room adapted to grow filter-feeders) and several field-tool (ecophysiological systems). But, I'm still not sure to really know how to use them to obtain DEB parameters: [Eg] and kappa for example. Perhaps, also that some DEB concept concerning the way that gametes production is treated would perhaps do not work with our species. The emersion time for our intertidal specie appears also to me as a problem, concerning the DEB theory. I hope that the several steps of validation will help us to solve this problem.

L. Pecquerie:
During spring surveys, we observe a high individual variability of lengths among the one-year-old anchovy cohort. We hypothesize that this variability is mainly due to differences in the hatching dates, the life histories and/or the genotypes. As almost all the one-year-old cohort is able to spawn in spring, we would like to study the impact of this variability on both spatial and temporal spawning distribution under different environmental conditions (temperature and food). For this purpose, we need to model the growth and reproduction of the anchovy population according to the environment.

The food and temperature variables are given by a 3D hydrodynamic model coupled to a primary production model. We choose to model the growth of a mean individual according to the DEB theory. We will have to

specify the way the reproduction buffer will be handled, as the batch fecundity of the individuals is indeterminate (environmental determinism). The variability of growth will be introduced by different hatching dates and trajectories of individuals over the continental shelf of the Bay of Biscay. The parameter values of the DEB model will first be the same for each individual. If it does not fit the variability observed in the data, different set of parameters

X. Bodiguel:

Polychlorinated Biphenyls (PCBs), are characterized by a high persistence in the environment, a bioaccumulation by the marine organisms and a potentially toxic character. The bioaccumulation depends on the physical and chemical properties of the compounds and biological factors like feeding, growth and reproduction. With a high trophic level, the Mediterranean hake is potentially exposed to these contaminants and it is significant to evaluate its contamination level and its contamination mechanisms.

We choose to model the bioaccumulation phenomenon according to the DEB theory. First, we will have to model the growth of male and female hakes, and then, to simulate the contaminant bioaccumulation (especially organic contaminants) during their life. We will have to specify the contaminant kinetics according to their chemical properties: lipid associated for the organic contaminants (Log Kow>6), and protein associated for the metallic ones. Finally, we will have to extend the individual model to the whole population and eventually to the trophic web.

Y. Bourles:

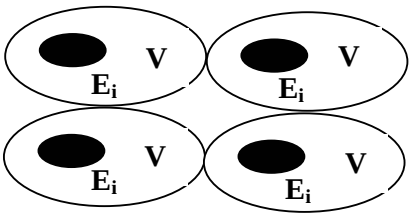
Several physiological and bioenergetic models of the growth and the reproduction of the Pacific oyster (*Crassostrea gigas*) are already available in the literature. But most of them exhibit two recurrent limits: (1) they do not allow to simulate properly the growth and reproduction of *C. gigas* over time (*i.e.* over a year), especially during the summer at the end of their gametogenesis; (2) furthermore, they cannot be applied to other shellfish culture areas or ecosystems than where they are developed. Thus, it appears as an evidence that a generic model which can answers these two limits is lacking. Such a model will be very useful to simulate and compare the ecophysiological behaviour of *C. gigas* in different ecosystems all along the year. And perhaps it would become a strong tool to understand the huge intra-specific variability and the wide extent of this species. In a more concrete application, the accurate simulation of such a model of the growth of *C. gigas* in a given site could be used to estimate the trophic capacity of this site (or at the opposite, the ultimate density of oysters the site can support for a known food density and temperature through the year).
A preliminary attempt of a DEB model based on a work of van der Veer *et al*. (2001) on flatfish was applied to *C. gigas* (experimental conditions, with cultivated phytoplankton inflow). In this preliminary step, this model fitted pretty well to the observed growth. As we feel that the DEB theory can be a new approach stronger and wider than the generalized Scope For Growth approach for instance, we want to test it to build our generic model of the ecophysiology of the Pacific oyster. First, we want to check the DEB parameters in various experiments and then we would like to confront the model in development to data sets from different natural sites, to compare and to verify the simulations are faithful with the reported data. We hope to improve our generic model step by step, making it more complex (as it would be necessary) in respect to the various specificities of the different areas.

The problem concerning the dynamic of reserves : what is the dilution by growth ?

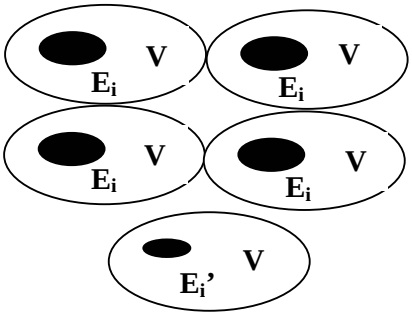
Case N°1 :
NON EQUILIBRIUM

f variable

p_A



Somatic growth



Although f is constant, [E] varies = dilution by growth (a new cell has less energy reserve than the mother cells), that gives :

$$\frac{d[E]}{dt} = [\dot{p}_A] - [\dot{p}_C] - [E]\frac{d\ln V}{dt}$$

= DEB theory out of equilibrium. General case for growth ?

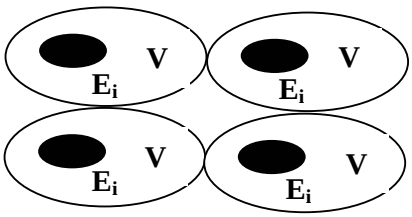
V=ΣVi & E=ΣEi

[E]=E/V=Ei/Vi

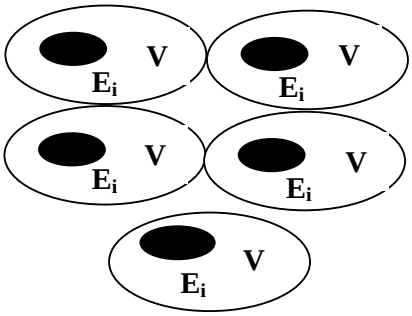
Case N°2 :
AT EQUILIBRIUM

f constant

p_A



Somatic growth

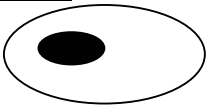


When f is constant, [E] does NOT vary = no dilution by growth (a new cell has the same energy reserve than the mother cells), that gives :

$$\frac{d[E]}{dt} = [\dot{p}_A] - \frac{\dot{v}[E]}{V^{1/3}}$$

= DEB theory at equilibrium, weak homeostatis, no dilution by growth ?

Legend :



= It is a theoretical cell of volume Vi and reserve Ei (or E'i)