PhD position at Ifremer (Call 2008-2009)

Title of the PhD project:

Explaining growth variability of the Pacific oyster in different coastal ecosystems: characterisation of the trophic and abiotic environment of *Crassostrea gigas* by the coupling of isotopic analysis and DEB modelling.

(Acronym: **IsoDEB**)

- Scientific adviser:
Marianne Alunno-Bruscia (Ifremer/PFOM/PI & UMR M 100 Ifremer-UCBN PE²M)

- Supervisor :
Sébastien Lefebvre (Université de Caen & UMR M 100 Ifremer-UCBN PE²M)

- Duration : 3 years (Nov. 2008-Nov. 2011)

- Laboratories/locations:
UMR M 100 Ifremer-UCBN PE²M (Caen http://www.unicaen.fr/ufr/ibfa/lbbm/ & Argenton, http://www.ifremer.fr/argenton/)

- Graduate school/University :
Ecole doctorale de chimie biologie Université de Caen Basse-Normandie

- Conditions/modalities : please look at the following website for deadlines and conditions of eligibility:

http://www.ifremer.fr/ds/animation_scientifique/bourses/doctorales/appel/index.html

- **Abstract & keywords** *(for posting on international sites)*

Abstract. Growth performances of the Pacific oyster (*Crassostrea gigas*) mainly rely on environmental factors, *e.g.* water temperature and food resources. However, the shellfish farming industry does not presently have a reliable tool to help understand and characterize the trophic functioning of marine ecosystems. Nor does it have relevant indicators to quantify and explain the variability in growth performances of oysters among culture sites on a national scale, with which to address future management questions. The main aim of the present PhD project is to develop and validate an operational tool for trophic characterization of shellfish farming areas, combining stable isotopes and modelling, which will explain differences in the growth of *C. gigas* between years and sites. More specifically, the PhD project will use a monitoring network on growth and survival of *C. gigas* (Ostreos project), and different types of biomarkers for the origin and fate of organic matter in the environment, (stable isotopes and fatty acids) coupled with bioclimatic conditions. These data will be integrated into a dynamic energy budget (DEB) model of oyster growth to obtain ecophysiological indicators (nutrition level, availability of reserves, maintenance and growth rate) of growth in *C. gigas* depending on spatio-temporal environment fluctuations throughout the rearing cycle.

Keywords: growth variability, Pacific oyster, trophic resources, isotopes, Dynamic Energy Budget (DEB) model, shellfish ecosystems.

Academic training and experience required
Qualifications: M.Sc. or equivalent degree in biology or ecology (*e.g.* Master II, “Diplôme d’Ingénieur en Agriculture”).
Specific knowledge: a strong background in marine ecology and/or ecophysiology, experience with trophic markers is required as well as knowledges/experience in mathematical modelling. An aptitude for both laboratory and field work will be appreciated.
Personal skills: a capacity for team work and a desire to travel and work in different places. Adequate spoken and written English is also required.

Study context and state of the art

Coastal zones form a productive but complex interface between marine and terrestrial environments, (Yokohama *et al.*, 2005). The broad diversity in their mode of ecological function, both on both spatial and temporal scales, is mainly due to the "bottom-up" control on primary production (*e.g*. hydrodynamics, inputs from catchments, local climatology) at the base of their trophic networks (Bergamino *et al.*, 2007). Sites used for oyster farming thus show much variation in potential (differences in yield, duration of culture etc.), both at regional and national levels, and such differences need to be better understood for the sustainability of this industry.

The characterization of French shellfish farming areas, and in particular the biological performance of Pacific oyster (*Crassostrea gigas*) in these coastal ecosystems, has been performed routinely since 1993 by the Remora network ("mollusc aquacultural yield network"), which monitors oysters on the basis of just the biometric parameters of growth, mortality and quality (Bedier *et al.* 2007). Robust and simple, this network is based on monitoring oyster batches of common origin positioned on 45 sites in the principal French production areas. The Remora network makes it possible to assess the productivity of the different basins where oysters are farmed, by comparing rearing performances (*e.g.* Soletchnick *et al.* 2007), but does not offer any tools for a functional understanding of their ecology or their evolution in the context of global change.

Oyster biological performances (growth and reproduction) in systems with such low human influence depend primarily on the quantity and quality of food, on temperature and on the metabolism of the organisms themselves. These factors can be integrated into bio-energetic growth models based on DEB ("dynamic energy bugdet", Kooijman 2000, Nisbet *et al.* 2000) theory, which have been validated in controlled systems or ecosystems with no tides or strong turbidity (Pouvreau *et al.* 2006), and that are presently being tested for more complex ecosystems (Y. Bourlès, PhD in progress). However, the generalized application of this type of model to complex intertidal systems still requires a certain number of questions to be answered (Pouvreau *et al.* 2007, Bourlès *et al.* 2007). In particular, it is not easy to identify the different food sources or their spatio-temporal variation in order to characterise food availability for the bivalve. From regularly-made growth measurements on oyster stocks, together with temperature records, it is nevertheless possible to trace the quantitative trophic history of these organisms by inverse analysis (*e.g.* Casas 2005, Marin-Leal *et al.*, 2008). This history can then be compared with quantitative and qualitative hydrobiological variables (salinity, concentration of nutrient salts, seston, chlorophyll *a,* and flora) measured in the coastal ecosystems in order to characterize shellfish farming ecosystems (Harma *et al.*, in prep).

Bivalves in general, and the Pacific oyster in particular, use different food sources of marine or terrigenous origin (planktonic and benthic microalgae, bacteria, protozoa, detritus) present in their environment to satisfy their energy requirements (Riera and Richard, 1996). The trophic niche of the species is broad (Rossi *et al.* 2004) and relatively plastic: depending on food source availability, the ecosystem in question and the season (Leal-Marin *et al.* 2008). Phytoplankton is often the most common source of food in these ecosystems with marine types dominant, but microphytobenthos and refuse of macroalgal or terrigenous origin are frequently consumed in estuarine and/or intertidal zones (*e.g.* Riera and Richard, 1996; Marin-Leal *et al.* 2008).

The trophic environment of oysters can be characterised using different markers of the origin and fate of organic matter in a complementary way. Natural stable isotopes are one kind of marker that can be used, among other purposes, to quantify trophic interactions (West *et al.* 2006). Fatty acid profiles of organisms allow the identification of different trophic groups, like bacteria, and different types of micro or macro-algae to be distinguished. Fatty acids can therefore be used to confirm the results of an isotopic approach (Alfaro *et al.* 2006). The use of the isotopic technique must satisfy two conditions: *i)* the isotopic value of the food sources must be distinct (meaning that several elements need to be studied simultaneously: C, N and S) and known in time and space, and *ii)* the enrichment between a predator and its prey (*i.e.* fractionation) must be known perfectly. Even if the orders of magnitude in these relationships are known and repeatable (Maksymowska *et al.* 2000), isotopic values of food sources can vary significantly in time and space and their extrapolation between ecosystems or seasons requires verification (Marin-Leal *et al.* 2008). In addition, isotopic fractionation is influenced by many parameters (metabolism, age, source type, physiological state), and the values proposed thus far for *C. gigas* are limited to the experimental conditions tested (Dubois *et al.* 2007, Yokohama *et al.* 2008). Some research to define fractionation mechanistically has been done (*e.g.* Olive *et al.* 2003), and such approaches should also now be envisaged using precise bioenergetic modelling tools such as DEB.

General objectives & value of the project

The general objective of this doctoral project is to develop and validate a working tool for trophic characterization of shellfish farming areas, combining an isotopic approach with one of modelling and using the national project Ostreos as an experimental basis. This tool will offer an improved means of explaining the growth variability observed in *C. gigas* between sites or years. It will also provide key management information for the industry via the characterisation of the different oyster-growing areas, and thus contribute to the sustainability and development of shellfish farming.

In addition to the obvious operational character of the tool to be developed, the major innovation of this PhD project is the coupling of DEB theory with isotopic markers (*i.e.* the transformation of the existing energy model into a model based on elements), which will allow significant advances in isotopic ecology research though the explicit examination of fractionation.

Methods

The PhD project will consist of the following methodological approaches:

Action 1/ To create an isotopic DEB model (a model based on elements to complement the existing one based on energy; Pouvreau *et al.* 2006) which will *i)* take into account a formalization of the fractionation and *ii)* establish the assessments of energy and mass for carbon and nitrogen in a mechanistic way and allow discrimination between light and heavy isotopes. This model will be tested using data from oyster growth monitoring in controlled environments (Argenton experimental site) in which chosen factors can be manipulated (trophic level and sources, temperature). Simultaneous isotopic analysis is envisaged on whole individual oysters and on separate organs, as the renewal rates and signature acquisition times are known to be different between tissues (*e.g.* low renewal for the muscle, high for digestive gland; Paulet *et al.* 2006). Multi-organ analysis will thus provide a more precise image of intra-annual variability (Lorrain *et al.* 2002). The work of building the isotopic DEB model will be facilitated by the student's participation in the "DEB course" in April 2009 (http://www.bio.vu.nl/thb/deb/course/) and in the two annual meetings of European Research Group AquaDEB (http://www.ifremer.fr/aquadeb/).

Action 2/ To determine the trophic mode of oysters at several study sites in three principal shellfish farming basins: Normandy, Rade de Brest, Marennes-Oléron, (link with the Ostreos project). Initially, this will involve analyzing the isotopic value of different tissues of *C. gigas* and its potential food sources, and measuring hydrobiological variables (qualitatively and quantitatively) to characterise its environment. As a second step, these data (growth and environmental) will be analyzed with the isotopic DEB model. (using inverse analysis, Marin-Leal *et al.* 2008). Action 2 will be based on a large-scale field transplant experiment (*i.e.* across several sites) using identical batches of young oysters, in collaboration with the Ifremer regional environmental resource laboratories (LER) or their partners (Remora network and Ostreos project; Remonor and Hydronor SMEL CG50 networks). The growth monitoring of these oysters (mass, size, etc.) and the analysis of the isotopic values of their tissues will be carried out frequently for the selected sites: at 15 days to one month intervals for periods of growth and at 2 months intervals outside of the periods of high growth. The inputs of potential food sources will then be estimated from the isotopic values of these sources and the isotopic DEB model. The trends observed will be verified by qualitative analysis of the fatty acids in the oyster tissues. Lastly, the inputs thus obtained will be compared with hydrobiological data (chlorophyll *a*, flora, nutrient salts, temperature, seston and salinity) collected by coastal environment monitoring networks (Rephy, Hydronor) using multivariate data analysis (PCA and canonical analysis).

Schedule: 3-year work plan

The PhD project will last three years, and involve working in two laboratories of the UMR M 100 Ifremer-UCBN EP ² M (Ifremer Argenton & Université de Caen Basse-Normandie), and collaboration with Ifremer (Normandy, Morbihan/Pays de la Loire) and their partners (*e.g.* SMEL CG 50).

The first year (Nov. 2008-Nov. 2009) will consist of reviewing the state of the art and making preliminary analysis of historical time series of Remora and Remonor monitoring data in partnership with the LER/NR and/or the SMEL. The setting up of the field experiment for growth monitoring (action 2) will take place from March 2009 (link with the Ostreos project). The student will also work on the adaptation of the DEB theory to the project, while following the internet "DEB course". This year will be spent at the Université de Caen Basse-Normandie.

The second year (Dec. 2009-Nov. 2010) will be devoted to setting up and monitoring isotopic fractionation experiments in controlled environments (Argenton experimental site; action 1). A stay of a few weeks is envisaged with B. Kooijman at Vrije Universiteit Amsterdam to finalize the development of the isotopic DEB model (paper n°1). The analyses of the first year of growth monitoring in the study sites will be also undertaken. If time and the progress of the project allow, a second year of monitoring will be set up at the study sites in order to determine inter-annual variability. During this second year, the student will work with the LPI/PFOM at Ifremer Argenton.

The third year (Dec. 2010-Nov. 2011) will be devoted to the validation of the DEB model by fractionation experiments carried out in controlled conditions (paper n°2). This model will be then used to determine the trophic mode of oysters at the study sites (paper n°3). The thesis also will be written during this final year, based on the scientific manuscripts produced from the second year onwards. During this final year the student will be based at the Université de Caen Basse-Normandie.

Expected results & applications

Examining food sources and abiotic parameters, like temperature, using DEB modelling should enable us to explain a large part of production variability between French shellfish farming areas. This project will seek to produce a simple and robust methodology, and to find biological indicators that can easily be transferred to other research activities (Ifremer Ostreos project, SMEL Remonor network) and management end-users (SRC, CNC, and planning departments). The results of this project should also justify monitoring for other potential sources of food than just phytoplankton (microphytobenthos, refuse, etc.). Lastly, the methodology could be applied to other cultivated or exploited species (mussel, cockle etc.), or even to invasive species, so as to improve current knowledge on the mechanisms of trophic competition.

Planned collaborations & links with other projects

- Ifremer Projects: Ostreos (Edouard Bédier), OGIVE (Aline Gangnery)
- Regional project UMR PE²M/LBBM (Salssa: Spatial Analysis at Large Scale for Sustainable Aquaculture, with SMEL, DRAM, AESN)
- Bas Kooijman (Vrije Universiteit Amsterdam): Possibility of PhD co-supervision (depending on the student's wishes)
- European Research Group: AquaDEB (Stéphane Pouvreau, Cédric Bacher, Bas Kooijman)
- UMR CNRS Université de La Rochelle (Robert Galois)
- Ifremer Dyneco department (Stanislas Dubois)

Some of the collaborators mentioned above will also be on the doctoral committee, which will meet once a year during the PhD project.

References

Alfaro A. C., Thomas F., Sergent L., Duxbury M. (2006). Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coastal Shelf Sci. 70: 271-286.

Bédier E., S. Claude, C. Simonne, F. d'Amico, H. Palvadeau, P. Guilpain, P. Le Gall, S. Pien (2007). Réseau national de suivi des performances de l'huître creuse (Crassostrea gigas) Remora. Synthèse des résultats des stations nationales. Campagne 2006. Rapport IFREMER RST/LER/MPL/2007-11, 76 p.

Bergamino N., Loiselle S.A., Cozar A., Dattilo A.M., Bracchini L., Rossi C. (2007). Examining the dynamics of phytoplankton biomass in Lake Tanganyika using empirical orthogonal functions. Ecol. Model. 204 : 156-162.

Bourlès Y., Pouvreau S., Alunno-Bruscia M., Leguay D., Arnaud C., Goulletquer P., Kooijman S.A.L.M. (2007). Modelling growth and reproduction in the Pacific oyster (*Crassostrea gigas*) : what's new with the oyster DEB-model? International Conference of Shellfish Restoration, Vlissingen, 12-16 Nov. 2007. http://documents.plant.wur.nl/imares/abstracts-icsr.pdf

Casas, S. 2005. Modélisation de la bioaccumulation de métaux traces (Hg, Cd, Pb, Cu et Zn) chez la moule, *Mytilus galloprovincialis*, en milieu méditerranéen. Thèse de doctorat : Océanologie biologique, environnement marin : Toulon, 314 p.

Dubois S., Blin J. L., Bouchaud B., Lefebvre S. (2007). Isotope trophic step fractionation of suspension-feeding species: implications for food partitioning in coastal ecosystems. J. Exp. Mar. Biol. Ecol. 351: 121-128.

Harma C., Blin J-L., Lefebvre S. (en préparation) An opportunistic suspension feeder as a spatial bio-indicator of trophic web functioning. A soumettre à Journal of Applied Ecology.

Kooijman S.A.L.M. 2000. Dynamic energy and mass budgets in biological systems. Cambridge University Press, Cambridge.

Lorrain A., Paulet YM, Chauvaud L., Savoye N., Donval A., Saout C. 2002. Differential delta C-13 and delta N-15 signatures among scallop tissues: implications for ecology and physiology. J. Exp. Mar. Biol. Ecol. 275 : 47-61.

Maksymowska D., Richard P., Piekarek-Jankowska H., Riera P. (2000). Chemical and isotopic composition of the organic matter sources in the gulf of Gdansk (Southern Baltic Sea). Estuar. Coastal Shelf Sci. 51 : 585-598.

Marin-Leal J.C., Dubois S., Orvain F., Galois R., Blin J.L., Ropert M., Bataillé M.P., Ourry A., Lefebvre S. (2008) Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and modelling as tool to estimate the trophic ecology of cultivated oysters in two contrasting environments. Mar. Biol. 153 (4) : 673-688

Nisbet R.M., Muller, E.B., Lika, K., Kooijman, S.A.L.M. (2000). From molecules to ecosystems through dynamic energy budget models. J. Anim. Ecol. 69 : 913-926.

Olive P.J.W., Pinnegar J.K., Polunin N.Y.C., Richards G., Welch R. 2003. Isotope trophic-step fractionation: a dynamic equilibrium model. J. Anim. Ecol. 72 : 608-617.

Paulet Y.M., Lorrain A., Richard J., Pouvreau S., (2006). Experimental shift in diet delta C-13: A potential tool for ecophysiological studies in marine bivalves. Organic Geochem. 37 : 1359-1370.

Pouvreau S., Bourlès Y., Lefebvre S., Gangnery A., Alunno-Bruscia M. 2006. Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. J. Sea Res. 56 : 156-167.

Pouvreau S., Maurer D., Bernard I., Bourlès Y., Néaud-Masson M., Rumèbe M., Alunno-Bruscia M.. (2007). Modelling growth and reproduction in the Pacific oyster (*Crassostrea gigas*) : application of the 'Oyster-DEB model' in Arcachon Bay (France). International Conference of Shellfish Restoration, Vlissingen, 12-16 Nov. 2007. http://documents.plant.wur.nl/imares/abstracts-icsr.pdf

Riera P., Richard P. 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. Estuar. Coastal Shelf Sci. 42: 347-360.

Rossi F., Herman P., Middelburg J. 2004. Interspecific and intraspecific variation of $\delta^{13}C$ and $\delta^{15}N$ in deposit- and suspension- feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. Limnol. Oceanogr. 49: 408-414.

Soletchnik P., M. Ropert, J. Mazurié, P.-G. Fleury, F. Le Coz. 2007. Relationships between oyster mortality patterns and environmental data from monitoring databases along the coasts of France. Aquaculture 271, 384-400.

West J., Bowen G., Cerling T., Ehleringer J. 2006. Stable isotopes as one of nature's ecological recorders. Trens Ecol. Evol. : 21: 408-414.

Yokoyama H., Tamaki A., Koyama K., Ishihi Y., Shimoda K., Harada K. 2005. Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. Mar. Ecol. Prog. Ser. 304: 101-116.

Yokoyama H., Ishihi Y., Yamamoto S., (2008). Diet-tissue fractionation of the Pacific oyster *Crassostrea gigas*. Mar. Ecol. Prog. Ser. (on line first) DOI 10.3354/meps07306.