- Title of PhD project : **Impact of toxic blooms on the reproduction of oysters of economic interest in the bay of Brest**

- Supervisor: Philippe Soudant (CNRS / LEMAR)
- Scientific director: Caroline Fabioux (UBO / LEMAR)
- Researchers implicated: Hélène Hégaret (CNRS / LEMAR), Marc Suquet (IFREMER / LEMAR), Stéphane Pouvreau (IFREMER / LEMAR), Arnaud Huvet (IFREMER / LEMAR)
- Laboratory: LEMAR / IUEM / UBO teams 1 and 2
- Doctoral School of attachment: Doctoral School of Marine Sciences _UBO

Scientific background of the thesis:

Toxic microalgae blooms are becoming more frequent and intense and geographic extent is increasing (Anderson, 2014). In the bay of Brest, the blooms of the dinoflagellate *Alexandrium minutum*, producer of paralytic toxins (PST), are recurrent since the summer of 2012. In July 2012, the concentration of *A. minutum* reached record concentrations with more than 40 million cells / L of water (REPHY Bulletin, 2012- Week 29, Concarneau Ifremer), causing the closure of shellfish sales for several weeks, because of the concentration of toxins accumulated by these filter feeders. If toxic microalgae blooms are essentially known and studied for their danger to human health associated with the consumption of contaminated shellfish or fish, they impact directly marine organisms. Thus, microalgae producing PST cause many physiological changes in marine bivalves. Microalgae change valval behavior and filtration activity of oysters (Tran et al., 2010, and Haberkorn al., 2011, Tran et al., 2015), the degradation of many tissues including the gonadal tissue (Galimany et al. 2008, Hégaret et al. 2009, Haberkorn et al. 2010a), cellular reactions such as infiltration or diapedesis of the hemocytes (Hégaret et al. 2009, Haberkorn et al. 2010a, Lassudrie 2014) or changes of immune responses (Hégaret et al. 2005a and b, 2007a, b, 2009). These toxic blooms appear more generally during the season of the reproduction of most bivalves and could impact on the development of gametes but also spawning, larval development or recruitment of juveniles.
Several recruitment defects were observed in the natural environment when toxic microalgae bloom, and at the present time, even if it is not possible to prove that these blooms are the cause of these defects, this hypothesis has been proposed several times (Erard-Le-Denn et al., 1990; Summerson and Peterson, 1991). Other studies also show an effect of the PST-producers microalgae on embryonic and larval development of several species of bivalves (Yan et al., 2003; Springer et al 2002; Leverone et al 2006. Padilla et al. 2006; Shumway et al 2006). For example, Yan et al. (2001) observed that the microalgae producer of PSTs, significantly decreased the egg hatching rate, fertilization rate and larval survival of the scallop, *Chlamys farreri*. Basti et al. (2013) have shown, in the pearl oyster *Pinctada fucata* exposed *in vitro* to the dinoflagellate *Heterocapsa circularisquama*, a significant reduction of the mobility and speed of sperm, the egg viability, fertility and embryo development rates. Recent studies made in LEMAR laboratory highlight that the exposure of the gametes of the oyster *Crassostrea gigas* to the dinoflagellate *A. minutum* caused a reduction in mobility and ATP content and a modification of the DNA content of sperm (Haberkorn et al, 2010b, Le Goic et al., 2013) and an increase of the production of reactive oxygen species in the oocytes (Le Goic et al., 2014). Thus, the blooms of *A. minutum* observed over the last three years in the bay of Brest could impact on the reproduction of the oyster *C. gigas*, a species of primary importance to the economy of Britain.

In this context, the thesis will aim to determine the impact of the blooms of toxic *A. minutum* on each stage of the reproduction of *C. gigas* in the laboratory (axis 1), but also on the field during spring blooms of *A. minutum* (axis 2).

Axis 1: Experiments of exposure of *A. minutum* will be conducted in the lab on adult male and female oysters during gametogenesis and on each stage of development (gametes, embryos and larvae) based on the expertise of the laboratory in *in vitro* reproduction of *C. gigas* and toxic microalgae culture. This experimental part will allow:
- To measure the accumulation of toxins in the gonadal tissue, gametes and developmental stages.
- Finely, to decrypt the effect of toxic algae on the kinetics of gametogenesis and reproductive investment, quality of gametes and embryo development with tools previously developed by our team for the study of reproduction of marine bivalves (histology, image analysis, flow cytometry, proteomics, transcriptomics) (Fabioux et al., 2005; Dheilly et al, 2012;. Corporeau et al, 2012;. The Goic et al, 2013 and 2014.; Sousa et al, 2014;. Boulais et al, 2015)..

Axis 2: Data on the VELYGER network (http://wwz.ifremer.fr/velyger) highlighted delays in the spawning date or less abundant recruitment than expected for *C. gigas* in recent years and the effect of the toxic blooms of *A. minutum* in these phenomena will be studied *in situ*. Oysters will be sampled regularly in the bay of Brest during the breeding season. Gametogenesis and gamete quality will be analyzed with the same tools used for laboratory experiments. These data will be correlated with physico-chemical data, and monitoring of toxic phytoplankton species thanks to the different monitoring networks existing in the bay of Brest, including the marine observatory of the IUEM (Observatory of Sciences of the Universe of the National Institute for Earth Science and the Environment) and REPHY network (Ifremer). In parallel, the potential existence of a link between egg-laying defects and / or poor recruitment of *C. gigas* and blooms of A*. minutum* will be analyzed i) through the time series acquired over the past seven years in the bay of Brest in VELYGER network (Ifremer, reproduction of *C. gigas*) and REPHY (Ifremer, monitoring of toxic phytoplankton) and ii) these same monitoring carried out in 3 years and on monitoring the valve activity of oysters equipped with valvometers providing information on both exposure to *A. minutum* and spawning activity of female oysters (collab. EPOC Laboratory, Arcachon).

Axis 3: In parallel to the study on *C. gigas* (axes 1 and 2), the last line of the thesis is a preliminary study of the effect of *A. minutum* blooms on the reproduction of the flat oyster *Ostrea edulis*. This species, endemic to our shores, is also exposed to *A. minutum* in the bay of Brest. The reproduction of *O. edulis* is less understood than *C. gigas*, but *O. edulis* is an interesting model because it incubates larvae before releasing them into the sea water. Exposures of flat oysters in gametogenesis and incubanting to *A. minutum* will be made, in the lab, to measure some indicators of reproduction such as reproductive investment and larval survival and its results will be compared with those obtained on *C. gigas*.

Positioning of the subject and the laboratory:

LEMAR has for several years assessed the impact of toxic algae on the biology of bivalves and the consequences on ecosystem functioning. This work has already been completed by more than twenty recent publications. This research activity within the LEMAR concerns to date several researchers, postdocs, graduate students and technicians. Our team aims to, through various national and international projects and collaborations i) further characterize the effect of toxic phytoplankton in filter-feeding shellfish to individual and population levels, and ii) to better understand and characterize the physiological responses of bivalves, to better understand the accumulation and depuration of toxins, due to exposure to toxic phytoplanktonic blooms.
This thesis, positioned in the bay of Brest, participate in cross-axes "ecology and physiology of photosynthetic organisms" and "Rade de Brest" of LEMAR. In a broader context, it fits perfectly within the “Zone atelier Brest Iroise” (Zabri) and the axis 6 of Labex "Sea Evolution of marine habitats and adaptation of populations."

The functioning of the thesis will build on the ANR project ACCUTOX funded by ANR CESA (2013-2017) and new projects submitted in the course of the year.

International collaborations:
- Aswani Volety _ University of North Carolina Wilmington, USA
- Sandra Shmway_ University of Connecticut Department of Marine Sciences, USA
- Monica Bricelj_ Institute of Marine and Coastal Sciences, The state University of New Jersey, USA
- Leila Basti_ National Research Institute of Fisheries Science, Yokohama, Japan