Acronym and tittle of the thesis : HARn'Bi

Tittle	Consequences of toxic microalgal blooms on reproduction and development of bivalve molluscs of commercial interest
Acronym	**HARn'Bi**
Keywords	HAB, bivalves, reproduction, transcriptomics, epigenetic

Research Unit and host team

Resarch Unit	Laboratoire des Sciences de l'Environnement Marin
	UMR 6539 LEMAR
Research Unit manager	Sarthou Géraldine
Research team	PANORAMA : Physiologie intégrative et adaptation des organismes marins : du gène à la population
Research team manager	Hélène Hégaret – Caroline Fabioux – David Mazurais
Resarch team activity	L'équipe PANORAMA s'appuie sur des compétences pluridisciplinaires, de l'observation in situ et de l'expérimentation pour comprendre et prédire les impacts des forçages environnementaux sur les organismes, aux échelles d'organisation biologique allant du gène à l'individu.

Thesis supervisors

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Project Description

Project summary :
Harmful Algal Blooms (HAB) are now considered as a major concern for marine ecosystems sustainability worldwide (Accords de Paris 2017, IOC-UNESCO). HAB have been mainly studied for food safety regarding their toxins affecting human health through the consumption of contaminated shellfish and associated significant economic losses due to prohibition of fishing and sale of shellfish during HAB events. However, some HAB species appear of great concern to marine organism health, including recognized ichthyotoxic species regularly blooming on French coasts, as well as "emerging" species recently observed along European coasts. Filter-feeding bivalve mollusks, as primary consumers, are directly affected by HAB. Bivalve stock renewal for aquaculture and fisheries which relies on successful reproduction could be affected by HAB. In this context, the objective of this project is to decipher the direct and trans-generational consequences of exposure of bivalves to toxic microalgae. The toxicity of several species of microalgae regularly blooming in France will be evaluated on the main life traits related to reproduction and development of bivalve species of commercial interest (oyster, mussels, scallops). Transcriptomic,

epigenetic and genetic approaches will be developed on the most problematic bivalve/microalgae couples, to understand the modes of action of toxins on molecular signaling pathways of bivalves, how exposure of shellfish during the reproductive period can impact the following generations and if HAB could represent a selection pressure for natural and exploited populations.

Hypothesis, approaches, techniques :

Anthropization of coastal areas and global warming promote more frequent HAB events worldwide. In France, HAB often occur during bivalve breeding season, with potentially important, but unestimated, consequences on natural and cultured populations. In the USA, HAB of the genus *Karenia*, locally induced the breakdown of scallop fishery, due to a reproductive failure, generating a loss of 3 million dollars. Our recent experimental studies show that the dinoflagellate *Alexandrium minutum* severely affects gametes, development and settlement of oyster larvae (Le Goïc et al., 2013 & 2014; Castrec et al., 2019 & 2020).
To date, HAB have been primarily studied for their symptomatically-inducing toxins in humans (paralytic, amnesiac, diarrheal toxins), but they can also produce other chemically unknown bioactive compounds (Long et al., 2021) that are far more toxic to marine species. In addition, emerging and ichthyotoxic microalgae species have been observed along the French or European coasts (Nézan et al., 2014). Their effects on marine organisms are unknown. All these data point out the urgent need to characterize the toxicity of HAB proliferating along our coasts on bivalves of commercial interest and to analyze consequences on these populations and, in particular their impact on reproduction and larval recruitment. This is what we propose with this thesis project, which is part of the ANR HABIS project "Harmful Algal Blooms: a threat for sustainability of exploited BIvalveS" (2023-2027) led by LEMAR.

The thesis will be divided into three interconnected axes
1) To decipher mechanisms underlying the toxicity of *A. minutum* bioactive extracellular compounds (BEC) on *Crassostrea gigas* oyster gametes and their offspring, a model for which we already have data. We will conduct i) molecular transcriptomic (RNAseq) approaches to highlight impacted molecular networks, ii) cellular approaches by flow cytometry and electron microscopy to visualize the possible cytolytic effects of BEC that we assume and iii) epigenetic approaches to test the involvement of methyl marks in the transgenerational transmission of negative effects of BEC.
2) The question of ubiquitous or specific interactions between toxic microalgae and bivalves will be addressed. Two or 3 bivalves/microalgae couples will be selected upstream in the HABIS project thanks to a screening of toxic bivalves/microalgae couples in biotests on bivalves gametes and larvae. On these couples, we will carry out systematic exposures of adults in gametogenesis, embryos and larvae and analyze physiological (reproductive investment), cellular (gamete quality by cytometry and *in vitro* fertilization test) and molecular (expression of candidate genes by quantitative PCR) mechanisms affected by HAB exposure.
3) Genomic analyses by RADseq will be performed on embryos before and after exposure to a toxic microalgae to determine if HAB can constitute a differential selection pressure during the early life stages. This work will be carried out on the pair considered as the most relevant from axes 1 and 2.

This project requires mastery of controlled reproduction and embryo-larval rearing of bivalves. LEMAR has all the necessary expertise and technical devices, except for pectinids (scallops) for which the experiments will be conducted at the Tinduff hatchery. Similarly, the culture of toxic microalgae necessary for the exposure of bivalves in the laboratory is mastered by the different partners of the HABIS project.

Scientific environment, positioning in regional/national/international context:

The PhD student will benefit from a rich scientific environment. The thesis will be carried out at LEMAR (IUEM, UBO) in collaboration with the BOREA laboratory (UniCaen). The subject is in perfect adequacy with the axis 2, “Phenotypic plasticity in response to anthropogenic constraints and toxic microalgae: ecotoxicological approach”, of the PANORAMA team of LEMAR. The ecotoxicological approach that will be carried out will feed the LEMAR transversal axes "ETIC: Exposure, transfer and ecotoxicological

impacts of contaminants in the marine environment" and "PEPS: physiology and ecology of photosynthetic organisms" through the work that will be carried out on toxic microalgal species.
Locally, this subject is in line with the EUR ISblue, themes 4 "The living ocean and ecosystem services", and 3 "Sustainability of coastal systems" in relation with its strong socio-economic component. The PhD student will thus benefit from the ISblue dynamics for the international opening and the link between research and training. The thesis that will focus in the coastal zone, particularly in the bay of Brest, will contribute to the dynamics of theme 3 "Sustainability between conservation, restoration, exploitation and uses" of the ZaBrI (zone Atelier Brest–Iroise).
On a regional scale, fishing and shellfish farming are major socio-economic and historical activities and their sustainable management is a priority for Brittany. However, the results of this project will be applicable to the global scale. Indeed, the oceans provide most of the protein consumed by humans and this part will increase with population growth. However, HAB could challenge the sustainability of these coastal ecosystems and the related socio-ecosystems. In accordance with the Sustainable Development Goals 2 "Zero Hunger" and 14 "Aquatic Life" of UNESCO, this thesis will contribute to the acquisition of scientific knowledge on the vulnerability of these resources.

Scientific collaborations (nature/partnership/country) and envisaged socio-economic partnership:

The thesis will be carried out mainly at the LEMAR lab of IUEM. It includes an important collaboration (co-supervising) with the BOREA laboratory of UniCaen where the PhD student will work for a few months on epigenetic approaches, with Guillaume Rivière, an expert in epigenetics of marine bivalves, and in connection with the bioinformatics platform of Ifremer (Datarmor - SeBiMer). The thesis is fully in line with the axis 4 of the ANR HABIS project including several scientific partners all experts in toxic microalgae and their toxins or in the physiology and reproduction of bivalve molluscs (M. Lassudrie, LER-BO Ifremer Concarneau ; D. Réveillon, PHYTOX ifremer Nantes), as well as local collaborations with the Tinduff hatchery, the only hatchery in Europe specialized in the reproduction of scallops. We will also have the possibility to interact with the flat oyster hatchery of Porscav.
The problematic of HAB and their impacts on marine resources being a global issue, we have been working for years in collaboration with researchers from several countries, through international projects or outgoing/incoming students or visiting researchers as with Brazil (Mirella DaSilva), the United States (Gary Wikfors), Spain (Juan Blanco) for example. This project is also part of the collaborative project Catalyst seedings (2022-2024), led by Anne Rolton Vignier between the Cawthrow Institute (New Zealand), CNRS-LEMAR (H. Hégaret) and Ifremer LER-BO (M. Lassudrie) on the effect of HAB on the reproduction of *C. gigas* oysters, which could potentially include a research stay in New Zealand for the PhD student.

Candidate profile

Profil souhaité du candidat (spécialité/discipline principale, compétences scientifiques et techniques requises) :

- Biologist, master degree or equivalent
- At least theoricall skills, if possible practical, in physiology, molecular and cellular biology, developmental biology, microalgal cultures
- Good level of English, writing skills, teamwork skills