CHINA SCHOLARSHIP COUNCIL

Appel à projets
Campagne 2022
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Title of the research project :

Thesis supervisor (HDR) :

Name :   Surname :

Title :

email :

Professional adress :
(site, dresse, bulding, office…)

Research Unit

Name :

Code *(ex. UMR xxxx)* :

Doctorate School

Thesis supervisor's doctorate school (candidate's futur
doctoral school) :

PhD student currently supervised by the thesis supervisor (number, year of the first inscription) :

PhD Project : REPROSTREA : Gametogenesis and development of the flat oyster : genomic approach for an improvement of the reproduction

A- Scientific background :

Historically, French oyster farming has gone through a succession of development phases, stock exploitation or disease outbreaks mainly due to Bonamia and Martelia parasites. The latter have particularly disrupted production and are still today in many shellfish growing areas a major obstacle to oyster farming, particularly the flat oyster *Ostrea edulis*. The *O. edulis* oyster has an original reproductive cycle and, to date, much of our current knowledge is based on the work of Walne (1966) and has changed very little since then, particularly with regard to the molecular aspect of the different phases of gametogenesis. The lack of molecular and proteomic data on the reproduction of *O. edulis* constitutes a real obstacle to the development of its production in hatcheries, even though this control of the reproductive cycle is the basis of the production system of all aquaculture species. Moreover, the conditioning of reproducers is one of the key phases in the success of larval production, so prior knowledge of the sexual cycle is essential to control this stage. *O. edulis* does not have sex chromosomes but presents an asynchronous hermaphrodism, a rhythmic consecutive sexuality as well as the possibility that several sexual inversions may occur during the same breeding season. This behaviour poses a major problem in the operation of hatcheries and particularly in the context of producing families on a bi-parental basis for application in genetic selection. Control of the reproduction cycle, the possibility of synchronising gametogenesis and even orienting it are therefore necessary to optimise the production of these families and, more generally, the production of Ostrea larvae. This project is part of a context of improving the reproduction of *O. edulis* and proposes to develop a new study method based on the possibility of triggering gametogenesis using specific inducers coupled with control of the conditioning of the genitors from a nutritional (selection of phytoplanktonic strains) and thermal point of view. The influence of these parameters on reproductive success and larval quality will also be taken into account.

This project is based in particular on the use of several molecular and proteomic methodologies, using previously obtained *O. edulis* genomic results (genome sequenced at a chromosomal level) and on the coupling of experimental and natural environment approaches.

B - Details of the proposal

B-1 Objectives:

The final goal of this project is to provide gametogenesis inducers in order to better synchronize gonad development in *O. edulis* in an oyster hatchery context in order to improve spat production and two main objectives have been defined.

The first objective concerns the acquisition of fundamental data on the reproductive cycle and larval development of *O. edulis* by transcriptomic (RNAseq and quantitative PCR), proteomic (via the monitoring of concentration kinetics of a number of proteins) and metabolic (gametogenesis quality indices, carbohydrate and lipid indices) approaches. All the data acquired will be integrated in a comparative approach with other mollusc models for which

gametogenesis has already been described, the originality of *O. edulis* being based on the existence of a complex hermophrodism system and a larval incubation phase. The influence of parameters such as diet composition and temperature will be studied in order to better understand the physiology of reproduction. This axis will be mainly carried out through laboratory experiments and field monitoring. A follow-up of gametogenesis will also be undertaken at the level of two field-experimental sites for which we will have data on environmental parameters.

The second objective concerns the research, production and testing of the effects of molecules/effectors capable of triggering gonadal maturation in *O. edulis*. A first list of potential effectors (whose sequences have been identified in the genome) has already been drawn up and may be completed on the basis of the results obtained in the first objective. These effectors will be tested in order to determine whether it is possible to influence the relative development of one or other of the gonads and/or to synchronize gametogenesis in order to limit the strong heterogeneity observed at present. In this project, several molecules (proteins and peptides) will be tested separatly and in simultaneously in order to optimize the chances of obtaining the expected result. This part of the project will be spread over its entire duration and will be continued even if a first molecule is successful, as the objective is to develop several inducers if possible capable of differentially influencing both sexes.

B-2 Methodology:

The samples will be partly produced at the hatchery level in order to perfectly master the temporal kinetics of the development of the gonads but also of the larvae. Several successive cycles of gametogenesis are planned each year in order to have a large number of replicates but also to be able to test several experimental conditions in terms of nutrition and thermal diet. Sampling in natural populations will also be carried out to compare the data acquired in a controlled environment with the data from the natural environment. In order to do so, an aid of the profession will be set up. These samplings will also be based on the existing sets of families produced within the framework of a previous european project based on genetic selection. Based on both the data available in the literature and the presence or not of those molecules in the flat oyster genome, we propose to develop the production of several potential inducers by recombinant protein approach in order to test their potential to trigger gametogenesis. The study of their effect on the gametogenesis will be tested in experimental populations. The different stages of gametogenesis will be determined by histological analyses to which we will couple biochemical analyses and will allow us to obtain fine kinetics on the development of the gonads allowing also to correlate for transcriptomic analysis. The whole molecular description of the reproductive cycle, larval development, the effect of different conditions/effectors on gametogenesis will be carried out by coupling between a global RNAseq approach and quantitative PCR (on target genes). RNAseq libraries will be realized in the laboratory and sequenced on external platforms. The transcriptomic data obtained will be compared with those obtained on other mollusc models in order to highlight similarities and/or differences between the modes of reproduction in these organisms. An immuno-chemical analysis will be perfomed using specific antibody directed against targeted protein (by ELISA and histo-immuno labelling). All bio-informatic analyses will be conducted in the laboratory.

B-3 Scientific integration of the project in oyster research field:

This thesis project is including as part of a European FEAMP program called ARCHE which aims to better understand the functioning of hermaphroditism in marine bivalves. This project, which combines both a basic and an applied research approach in the field of biological sciences, aims to offer a multi-disciplinary training to a motivated student. Depending on the progress of the work, new research axes will be explored such as the study of the influence of DNA methylation and the role of parasitism on the reproductive capacities of the flat oyster. All the experimental designs and the access to field samples will allow to adress those questions.

The phD project will be conducted in a laboratory strongly involved in the field of genomics of marine organisms and which is involved in several international consortium in genomics fields. All technical and analytical skills are already available and will be taught to the student by the thesis supervisor. Moreover, the student will benefit from the various services available at the Roscoff biological station (bioinformatics analysis platform, experimental structure) and from the international collaborations developed by the thesis supervisor within the framework of the European project ARCHE. The student will also have the opportunity to develop new techniques within the laboratory according to the evolution of the acquired results.

C- References

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Boutet et al., (2021) : Chromosomal assembly of the flat oyster *Ostrea edulis* genome as a new genetic ressources for aquaculture (in prep)

4°) Profile of the Applicant (skills/diploma…)

Master degree with an interest for mollusk physiology, competences in bio-informatics for transcriptomic and genomic analyses, experience in molecular biology and laboratory work, ability to work in team and competence in English writing.