

PROPOSITION DE SUJET DE THESE

Formulaire demande de financement : ARED - ISblue – Etablissement(s) - ...

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Acronyme : EXCLAM

Présentation de l'établissement porteur (bénéficiaire de l'aide régionale)

Établissement porteur du projet : UBO UBS Institut Agro Rennes

IMTA ENSTA ENIB

Ecole Doctorale : EDSML

SPI BZH SPIN MATHSTIC Bretagne Océane pour les projets ISblue

Identification du projet

Intitulé du projet	Multiexposure of <i>Ruditapes philippinarum</i> clam hemocytes <i>in vitro</i> in a new cell culture model
Nom	MADEC
Prénom	Stéphanie

Demande d'ARED

Se reporter à la notice ARED Région Bretagne et préciser :

Priorité régionale	Atténuation et/ou adaptation au changement climatique
DIS	Economie maritime pour une croissance bleue
Levier thématique	Les bioressources et biotechnologies marines
DIS secondaire	Economie de la santé et du bien-être pour une meilleure qualité de vie
Levier thématique secondaire	Prévention – nutrition - environnement - travail
Axe transversal	Les transitions environnementales et citoyennes

Organisme de tutelle : encadrement et unité de recherche

Porteur du projet HDR

Date obtention de l'HDR	06 décembre 2022
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Nom	MADEC
Prénom	Stéphanie
Adresse électronique	Stephanie.madec@univ-brest.fr
Tel	0290915565
Expérience d'encadrement	Co-encadrement de 4 thèses

Unité de recherche

Nom de l'unité	Laboratoire des Sciences de l'Environnement Marin
Acronyme de l'Unité (umr xx, ...)	UMR6539
Nom et prénom du responsable	Sarthou Géraldine
Le cas échéant, nom de l'équipe de recherche	Equipe PANORAMA
Le cas échéant, nom du responsable de l'équipe de recherche	C. Fabioux/H. Hegaret/D. Mazurais

Co-directeur de thèse – si nécessaire

Nom	MILAN
Prénom	Massimo
Unité de recherche	Department of Comparative Biomedicine and Food Science
Etablissement de tutelle	Université de Padoue (Italie)
Expérience d'encadrement	Co-encadrement de 2 thèses

Co-encadrant (s) de thèse – si nécessaire

Nom	EVEN
Prénom	Yasmine
Unité de recherche	Laboratoire des Sciences de l'Environnement Marin
Etablissement de tutelle	Université de Bretagne Occidentale
Expérience d'encadrement	Co-encadrement d'une thèse

Description du projet : complément

Lieu principal de déroulement du projet en Bretagne : Laboratoire des Sciences de l'Environnement Marin

Lieu principal de déroulement du projet si hors Bretagne : Laboratoire BCA (Comparative Biomedicine and Food Science), Université de Padoue, Italie

Libellé (attention veiller à respecter le nombre de caractères imposés par le serveur de la Région)

Résumé synthétique du projet
(2 000 caractères maximum)

The coastal environment is heavily impacted by human activities. Some estuaries are degraded due to urban, agricultural or aquaculture discharges and are loaded with faecal bacteria, biocides, and heavy metals known to induce cross-resistance to antibiotics. These phenomena are likely to increase in the coming years due to heavy rainfall associated with climate change, warming seawater associated with emerging infectious diseases and the expected continued growth of aquaculture and concomitant antibiotic use. Recent findings indicate that bivalves possess an immune memory capacity that could be used to enhance their immune response to infections via immune priming, a recent and potentially powerful tool for limiting antibiotic use in aquaculture.

Bivalves are known to bio-accumulate contaminants and sometimes host bacteria resistant to pathogenic antibiotics. There is considerable work in the literature on the impact of pollutants on the bivalve immune system at the organismal level, but due to the lack of a cell culture model, little data exists at the cellular level to assess the effect of the environment on bivalve haemocytes in the medium term. There is also little data on the relationship between the bivalve immune system and its microbiota.

Thanks to recent technological developments in our laboratory on the cell culture of bivalves, we plan to expose clam hemocytes to :

- pollutants, with the aim of identifying their effects on the state of health of the cells by considering the pollutants alone or in a mixture, at different culture times (acute/sub-chronic) and to determine the cellular mechanisms impacted.

- priming agents (bacteria, bacterial extracellular products) to identify their effects on immunity, their mechanisms of action and to study at the molecular level the hemocytes-microbiota interactions.

The second part of the thesis will aim to reproduce these experiments under conditions of climate change to find out to what extent these effects can be modified.

Hypothèses, questions posées, points de blocage, approche méthodologique, technique
(4 000 caractères maximum)

This thesis project is part of the ISblue Maresistome Flagship project (2022-2026) on the emergence and dissemination of antibiotic resistance in polluted coastal ecosystems. This project requires a global approach to human, animal and environmental health called "One Health", as advocated by the WHO. One of the objectives of the project is to decipher the mechanisms of action involved in an innovative strategy (immune priming) by improving the immune response of bivalves against pathogens through interaction with bacteria. This will limit the use of antibiotics for sustainable aquaculture. The effect of pollutants (biocides, heavy metals) will also be studied *in vivo* and *in vitro*.

Objective 1: What is the impact of pollutants on clam immunity at the cellular level?

In vivo experiments will compare the immunity of clams between a polluted site and a protected site. Their haemolymph will be collected to assess haemocyte viability, proliferation and enzymatic activities (oxidative stress, degradation and inflammation enzymes), and to associate them with clam immune parameters (phagocytosis). Eukaryotic gene expression (RNAseq) related to clam immunity will be analysed in haemolymph and digestive glands (coll. Department of Comparative Biomedicine and Food Science (BCA), Univ Padova, Italy).

The toxicity and intracellular mechanisms of action of the pollutants will also be studied *in vitro*. These will be selected on the basis of environmental assays (Maresistome project). Haemolymph will be

collected from healthy clams, and haemocytes will be exposed to these pollutants in order to assess their impact on their viability, proliferation, phagocytic capacity, enzymatic activities and gene expression (qPCR). The dose-response effects of these pollutants will also be studied at different culture times in order to evaluate for the first time the short- and medium-term cellular effects thanks to a technique recently developed in collaboration with the ecotoxicology laboratory in Padua. The effects of pollutants in mixtures, closer to environmental conditions, will make it possible to identify potential additive, antagonistic or synergistic actions at sub-lethal concentrations.

Objective 2: What are the intracellular mechanisms of action of priming agents?

In vivo experiments currently underway (post-doc BIENVENUE) aim to stimulate the resistance of clams to infection by testing different priming agents by pre-exposing clams with bacteria (non-virulent and inactivated *V. tapetis*) or their extracellular products, and then exposing the animals to a pathogenic bacteria (virulent *V. tapetis*). The most effective priming agent will be tested on haemocyte cell cultures to study its mechanism of action (RNAseq).

Risk and solution: In case of lack of effect of priming agents applied by clam dipping, these agents will be directly injected into the animals. Animals will be pooled in case of insufficient biological material. LEMAR has strong expertise in the inactivation of *V. tapetis* and has non-pathogenic strains.

Objective 3: What is the molecular dialogue between haemocytes and clam microbiota?

In order to identify the molecular mechanisms underlying the communication between haemocytes and the bacteria of the microbiota, *in vitro* experiments (co-cultures in microplates) will allow the analysis of the secretome and transcriptome of haemocytes (collaboration with the BCA laboratory, Padua), and to evaluate the impact of contaminants on the cellular dialogue between haemocytes and microbiota.

Objective 4: Does climate change impact the effects of pollutants, priming effects and interactions between host cells and their microbiota?

In vitro exposures of haemocytes to pollutants, priming agents and bacteria from the microbiota will be repeated under conditions of climate change (increased temperature, decreased pH).

Risk and solution: If the change in temperature and pH parameters affects cell viability in the control condition, it will be possible to collect haemocytes from clams previously acclimatised to the climate change conditions and carry out multi-exposures of haemocytes in the acute condition.

Environnement scientifique, positionnement dans contexte régional/national/international (2 000 caractères maximum)

At the regional and national level: This project will make it possible to establish links with the LABOCEA laboratory, which has been commissioned to carry out analyses of pollutants on the environmental samples of the Maresistome project, which is the subject of the thesis application.

We will study the effect of pollution on the immunity of clams and test their capacity to resist infection via immune priming with the collaboration of the SATMAR company, which will supply us with healthy animals.

The thesis project will allow the integration of the GDR in Aquatic Ecotoxicology, a place of animation and scientific construction to better diagnose and predict the impact of pollutants in aquatic animals. It aims to promote interdisciplinarity and the emergence of integrative approaches to move towards a predictive ecotoxicology.

At the international level: This project will reinforce LEMAR's research on marine bacteria and bivalves by new studies on the effect of marine pollution on bivalve immunity at the cellular level, and the link with antibiotic resistance, which are considered emerging research themes at the international level. The project will maintain the collaboration with the BCA laboratory of the University of Padua (Italy) and

create a new collaboration with the Ecotoxicology laboratory of the Biology Department of the University of Padua. It will reinforce the CNRS ClimClam international research project (IRP), coordinated by C. Paillard (LEMAR) and L. Bargelloni (BCA), which aims to study the complex Environment-Clam-Pathogens-Microbiota system in a context of climate change. In addition, a global research network on clam health will be launched during the project and several partners are already envisaged: Italy (L Bargelloni), Korea (MC Chu), Japan (T Matsuyama), USA (B Allam), Portugal (C Azevedo), Spain (A Figueras). Furthermore, the project is part of the ZABrI, included in the national network Réseau des Zones Ateliers (RZA) and in the European and international networks Long Term Ecosystem Research (LTER), which will greatly facilitate networking for the development of new projects and the dissemination of results through publications and presentations at national and international conferences.

Collaborations scientifiques (nature/partenariat/pays) et partenariat socio-économique envisagé

The proposed thesis will be in collaboration with two laboratories of the University of Padua (Italy):
-the BCA laboratory (Department of Comparative Biomedicine and Food Science) which will be responsible for the transcriptomic analyses
-the Ecotoxicology laboratory of the Department of Biology, which will participate in the choice of pollutant doses and cellular tests to assess toxicity.

Pour les demandes Région Bretagne

Adéquation du projet avec le DIS de Rattachement
Pour les demandes Région Bretagne (3 000 caractères maximum)

The project is mainly linked to the strategic innovation area **D1 Maritime economy for blue growth**.
The thematic levers identified concern:

- **marine bioresources and biotechnologies**

The project proposes innovative solutions to reduce antibiotic resistance in the environment by limiting the use of antibiotics in aquaculture. One of the strategies used in the thesis project is to stimulate the immune system of clams with different bacteria (immune priming) against their pathogen *Vibrio tapetis*.

The project also relies on *in vitro* experiments to evaluate the effect of pollutants or priming agents on a new cell culture model in order to identify new markers of the health status of clams. This new cell model could eventually be used for high-throughput analyses of the effect of the environment, such as various pollutants, climate change, and molecules of biotechnological interest such as new, more environmentally friendly antifouling agents, on marine bivalves.

- **environment, ocean health and coastal management**

This project aims to improve knowledge of the coastline, both regionally and globally, by comparing our results with a contrasting environment such as the Bay of Venice. It aims to better understand present events and help society to anticipate future social and environmental challenges. The results may provide decision-making tools for the implementation of public policies for risk prevention and management of coastal and littoral areas.

The project is also linked to the strategic innovation area **D4 Economy of health and well-being for a better quality of life**, and in particular the thematic lever "prevention - nutrition - environment -

work". We will indeed contribute to responding to various issues supported by the Brittany Region. On a fundamental level, it will provide better knowledge of the effect of pollution on the health and immunity of marine bivalves, an essential economic source for the region, and the identification of new environmental health markers. The role between immunity and the microbiota of clams will be particularly studied. The thesis project is part of a larger project on the effect of different pollutants from the catchment area and present on the coastline in the emergence and dissemination of antibiotic resistance in the coastal environment and in particular in the microbiota of bivalves. This project will provide more knowledge on the land-sea continuum, on the possible dissemination of antibiotic resistance in the marine environment and within the trophic chain and is fully in line with the One Health theme.

The results expected from the thesis will help to make Brittany an international centre of excellence in the fight against environmental risks, by investigating the new challenges linked to climate change.

Si priorité régionale, préciser (200 caractères maximum)

-Adaptation to climate change: measuring the effect of temperature and pH on clam exposure to pollutants/priming agents

-One Health: pollution-immunity-clam resistance link

Demande de (co)financement ISblue

Vous sollicitez un financement ISblue,

Précisez le lien du sujet avec les thèmes ISblue

Thème ISblue	Thème principal	Thème secondaire (si nécessaire)	Autre (si nécessaire)
la régulation du climat par l'océan			
les interactions entre la Terre et l'océan		X	
la durabilité des systèmes côtiers			
l'océan vivant et les services écosystémiques	X		
les systèmes d'observation à long terme			

Expliquez/précisez en quelques lignes dans quelle mesure votre demande correspond à l'un ou plusieurs des critères ISblue ci-dessous :

1- Originalité, impact potentiel du projet (4 lignes maxi)

Our project aims to study the effect of pollutants on the immunity of clams in relation to their resistome, involving an original integrative approach in marine ecotoxicology. The new cell culture model aims to identify new markers of marine ecosystem health, effective priming agents and to better understand the effect of pollutant mixtures.

2- Positionnement international du sujet, cotutelle ou co-encadrement international (4 lignes maxi)

The proposed thesis will be in international co-supervision with Dr. M. Milan, expert in genomics at the BCA laboratory of the Univ. of Padova, who will supervise the *in vivo* and *in vitro* transcriptomic analyses of the thesis. The Ecotoxicology laboratory of the University of Padua, directed by V. Matozzo, will

participate in the selection of enzymatic activities on haemocytes.

3- Effet intégrateur entre unités de recherche et / ou interdisciplinarités (4 lignes maxi)

Apart from the development of international collaboration with the two Italian laboratories, the thesis is integrated into the interdisciplinary Maresistome project, which brings together a consortium of 4 ISblue units, Ifremer, ANSES, and local players such as EPAGA. The PhD student will therefore be able to benefit from this diversified scientific environment, especially during the project's review meetings.

4- Potentiel d'insertion à un haut niveau dans la communauté académique ou non académique du docteur (4 lignes maxi)

The doctoral student will benefit from a network of national and international scope. Thanks to these collaborations and to the opportunity to teach at the UBO, and possibly at the University of Padua, the graduate will be able to respond to a variety of job offers within research organisations and universities or in private laboratories specialising in ecotoxicological analyses.

Financement du projet de thèse

En cas de financement à 50 %, le cofinancement est-il déjà identifié (oui/non) : non

Si oui, préciser la nature du cofinancement (ANR, partenaire privé, Ademe, etc.) :

Si le cofinancement n'est pas encore confirmé, date prévue de réponse du cofinancier :

En cas de non-obtention du cofinancement demandé, une autre source de cofinancement est-elle identifiée (oui/non) :

Si oui, laquelle :

Sollicitez-vous un co-financement Is-Blue (oui/non) ? oui

Important : Veillez à bien compléter les différents co financements sollicités sur le serveur Thèses en Bretagne Loire lors du dépôt de votre dossier.

Projet de thèse en cotutelle internationale

S'agit-il d'un projet de thèse en cotutelle internationale dans le cadre d'une convention (oui/non) : non, il s'agit d'un projet de thèse en co-direction internationale

Si oui, préciser l'établissement pressenti (et le pays de rattachement) : Université de Padoue (Italie)

Ce projet de thèse fera-t-il l'objet d'un cofinancement international (oui/non) : non

(Rémunération du doctorant par l'établissement implanté sur le territoire régional (18 mois sur 36 mois), et l'établissement étranger, qui s'engage également à rémunérer le doctorant dans le cadre de son séjour à l'étranger, soit durant 18 mois -a minima-)

En cas de cofinancement international, préciser -si vous en avez connaissance- l'organisation du calendrier des périodes de séjour :

Préciser quel est le stade du projet international (joindre une lettre d'engagement du partenaire)

Vous sollicitez un financement UBO EDSML qui sera porté à la décision du Conseil de l'ED

Indiquez le ici, oui non **et sur le serveur TEBL (indispensable)**

Le candidat

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

The successful candidate will have a particular interest in marine sciences, cell biology, toxicology, microbiology, and omics data analysis. The successful candidate will be self-motivated, curious and able to work as part of a research team. English language skills are required. Skills in biochemical analysis (cell functionality), cytometry, molecular biology, bioinformatics and biostatistics (use of R software) would be a plus.

ATTENTION : Tout dossier non déposé sur le serveur dans les délais indiqués, ne pourra être pris en compte notamment par les instances ISblue, conseil de l'EDSML.

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