Project information

Keywords
In vitro. Scallops.

Project title
Single and mixture exposure of key Arctic contaminants (POPs, heavy metals and PAHs) in Icelandic scallops (Chlamys islandica) using in vitro approach.

Year
2016

Project leader
Perrine Geraudie

Geographical localization of the research project in decimal degrees (max 5 per project, ex. 70,662°N and 23,707°E)
69°40′58″N 18°56′34″E; 41°23′N 2°11′E; 46°09′33″N 1°09′06″W

Participants

Pr. Cinta Porte, IDAEA–CSIC, Barcelona, Spain

Dr. Nicholas Warner, NILU.

Dr. Helene Guyon-Thomas, LIENSs, La Rochelle, France

Flagship
Hazardous Substances

Summary of Results

In this project, we are developing new *in vitro* (microsomal subcellular fractioning) and *ex vivo* (tissue explant culture) tools to evaluate toxicity and subcellular effects of different contaminants. For that, we have used the model species *C. Islandica* which is a key species of the Barents Sea. We have also used the common and worldwide bivalve species, *Mytilus edulis*, to do preliminary test and pre validation of the method, as the blue mussels is the most used bivalve species in Ecotoxicology and can be find everywhere. This has been done in collaboration with CSIC and in the context of the PhD study conducted there. The characterization of the lipid profile on the subcellular microsomal fraction of the cold-water scallop, have been done, following the method used and previously developed on blue mussels. The analytical method allowed the detection of a minimum of 166 different class of lipid. In particular, the main lipids were identified and their involvement during key physiological and fitness processes were studied. Phospholipids, main constituents of cell membranes: e.g. phosphadylcholines (PCs). Neutral lipids, used as energy storage: e.g. diacylglycerols (DAGs) and triacylglycerols (TAGs). The highest accumulation of lipids in the digestive gland was observed in summer compared to winter and spring seasons. Strong seasonal variation of TAGs was observed, whereas membrane lipids (PCs, PCs-Plasm, lyso-PCs) remain stable.

Digestive gland lipids showed high degree of unsaturation (e.g. 38:6, number of carbons: number of double bonds).

The characterization of two key neurotransmitters (Dopamin and Serotonin) in Scallops has also been done. The method has been optimized according to published studies on similar work, and the extraction and pre cleaning process. However, no study reported data on neurotransmitter characterization using analytical methods in bivalves. The results show a really good recovery

percentage for the Serotonin (reaching 84% during the best conditions), while the results for Dopamine ranged from 38 to 57%. The project is on the final stage of improving the analytical methodology in order to obtain satisfactory recovering and cleaning results.

The microsomal subcellular fractioning has been successfully validated in scallops as it has been used for the investigation of lipid metabolism. The optimization *ex vitro* tools using microsomal fractioning and digestive gland explant of scallops, is still under process and will provide an innovative and cost effective tools for studying the toxicity of contaminants and the subcellular effects of complex mixture. It will also provide an alternative to study MOA of chemicals, providing a 3D experimental system to evaluate endocrine disrupting potency of single and mixture exposure.

Master and PhD-students involved in the project

Master student: Marine Barbarin, LIENSs, La Rochelle.

PhD students: Elisabeth Alejandra Gilabert, Elisabeth Perez-Albaladejo, CSIC, Barcelona; Marine Breitweiser, LIENSs La Rochelle.

For the Management

The results obtained on the lipid metabolism, and lipid class characterization could be used for fishery and increase the knowledge on scallop physiology and energy metabolism. This is also crucial for ecosystemic study and population dynamic as lipid metabolism are crucial for Arctic species.

Published Results/Planned Publications

An article has been submitted to Comparative physiology and endocrinology entitled: "Characterization of the lipid profile of the cold-water scallop, *Chlamys islandica*, a model organism of the Arctic and Subarctic seas".

Two publications are planned to be written at the end of the project: 1) Neurotransmitters as biomarkers as indicators for reproductive toxicity in bivalves from xenobiotic exposure. 2) New in vitro and ex vivo tools for evaluating chemical toxicity as well as mechanisms of actions of key contaminants.

Communicated Results

The results were presented to the conference SETAC young in Gainesville, USA (28 february-2 March). Title of the oral presentation was: "The use of bivalve species for ecotoxicological studies in Arctic".

Two presentations in relation to the project were done at the ESCPB in Barcelona,

which was organized from the 4th to the 7th of September 2016.

The first presentation was entitled: "Subcellular effects of key Arctic contaminants in bivalves using *in vitro* approach: how synergetic behavior drives biomarker responses from single to mixture exposure".

The second presentation was entitled: "Characterization of the lipid profile of the cold-water scallop, *Chlamys islandica*, a model organism of the Arctic and Subarctic seas".

The project has been presented locally during the national outreach program Forskningdagene (24-27th September 2016) and in particular through the specific outreach program arranged by the Framsenteret, "Forkserne kommer". The project has also been connected to the science outreach program called "Kul forskning for Barn" which has been conducted at Polaria as well as in a local kindergarden. In addition to small and basic experiments, an introduction to the bivalve anatomy has been done to young kids aged from 4 to 6 year old.

Interdisciplinary Cooperation

The project was based on multidisciplinary approach including analytical method to measure neuropeptides, *in vitro* and *ex vivo* tools, biochemical methods and molecular biology. A strong cooperation with ecotoxicological experts, worldwide known, was extremely beneficial to the project, providing strong experience which could be used to deliver relevant data in a short time. The Spanish partner, the CSIC, provided analytical tools to assess subcellular lipidomic metabolism. The French laboratory, LIENSs, had successfully conducted the molecular characterization of key genes involved in the reproductive function of Scallops. Finally, national collaboration, with NILU, provided crucial local cooperation needed to coordinate the management of the project and in particular to establish the new analytical methodology which requires close communication to combine fundamental biological and neurophysiological knowledge (provides by APN), with chemistry and analytical skills.

Budget in accordance to results

The total budget was cut from the original plan of 50 000 Nok. The major consequences were observed on NILU methodological development of neurotransmitter measurement to be used as biomarkers in gonadal tissues.

The Framsenteret funding allowed the project to reach the main objectives "developing new in vitro and ex vivo methodologies in scallops". The funding helped covering the costs of the lipid characterization. The project was connected to ongoing projects conducted by the different partners, as well involving PhD and master students. Thus the Framsenteret funding was mostly allocated to direct costs for analysis resources

while covering salary costs for APN, and NILU employees.

Could results from the project be subject for any commercial utilization

Yes

If Yes

Considering the fact that Scallops have an economical importance in Norway and in the world as food delicatessen, and that the neurotransmitters Serotonin and Dopamin are of great interest for fishery to control the spawning but also to increase the value of food item, as serotonin or other neuropeptides such as tryptophan, have been recognized to improve health condition and reduce heart attack risk. To be able to quantify neurotransmitters and to study their involvement in the scallop physiology is of great interest and could increase commercial interest for the scallops. Moreover, knowledge on neuropeptides and neurophysiology is crucial for animal biology and could be used in other organisms from higher trophic levels, including mammals.

Conclusions

Future research activities and perspectives : The final part of the project is still under progress and will be achieved by the end of the year. The Scallop gonad and digestive gland explants (pieces of about 3 mm3 (<20 mg) will be prepared under sterile conditions according to the method developed by Gerbron et al., 2010. Then the explants will be incubated with single as well as different cocktails of contaminant for up to 96h (24h pre-exposure and 72h exposure to contaminants) before to measure cellular toxicity and biomarker responses in gonad and digestive gland tissues.

Cellular toxicity and molecular tools which can be associated to short-term subcellular changes will be used for the characterization of in vitro effects of cocktail contaminant exposure in Icelandic scallops. We plan to use the new *in vitro* and *ex vivo* tools will be used in bivalve species to study the effects of contaminants in single and mixture exposure on physiology. In particular interest, the impacts of obesogens will be study on subcellular fraction of digestive gland, where the lipid metabolism has been shown to take place. This work will be investigated together with the CSIC, in Barcelona, Spain, in close collaboration with the PhD study which is currently investigating:" NEW TOOLS FOR THE ASSESSMENT OF LIPID DISRUPTORS AND ENVIRONMENTAL OBESOGENS", and the explant culture which is under optimization using scallop digestive gland will be transfer to their model species, the blue mussels.

Moreover, the impacts of Fluoxetine, an antidepressor drug will be investigated in Icelandic scallops both using *in vivo* and *ex vivo* methods, if the project submitted to "Hazardous substances" program of the Framsenteret in November 2016 is funded. The methods, will also be transferred to a PhD and a master students from the LIENSs, during a workshop organized in December at the University of La Rochelle.

A project using the same model species, Icelandic scallops, has been submitted to the Framsenteret Flagship Polavhet, to investigate the effects of marine diesel and climate change on the physiology and fitness of bivalves. The findings of the ongoing study will constitute a relevant knowledge on scallop physiology, especially lipid metabolism, reproduction, and neurotransmitter activities which can be the base to study the impacts of environmental stressors on bivalve fitness. Moreover, subcellular effects, and mechanisms of actions will be investigated using both *in vitro* and *ex vivo* recently developed tools.

Finally, the *in vitro* methods (e.g. microsomal subcellular fractioning) will be used in 2 other higher trophic species, such as fish and seals from Arctic areas. First, on Arctic charr, in collaboration with the University of Tromsø. As well as in collaboration with the Norsk Polar Institut, where liver samples from a previous study will be used to assess the impact of key Arctic contaminants, and results will be compared with fieldwork data where different biomarkers were investigated in correlation to contaminant levels.