

# Protim Proteomic identification of ovarian fluid components which are able to extend fish egg viability

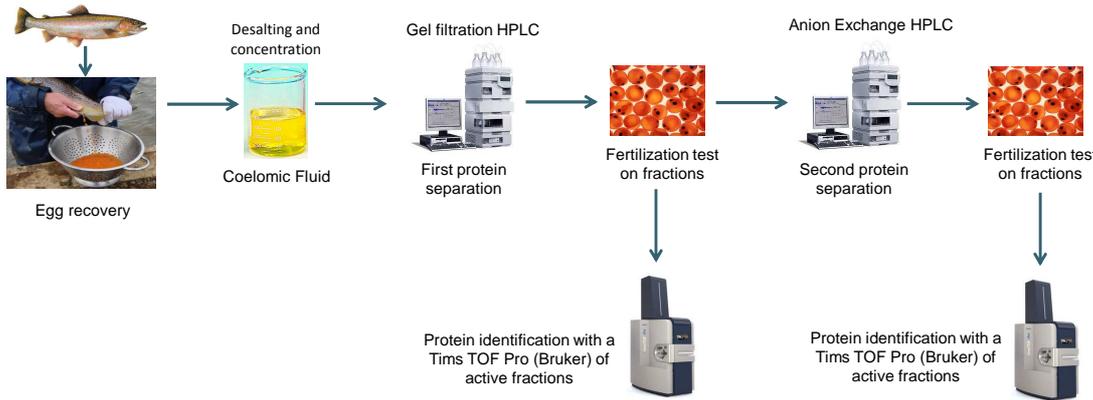
Guével Blandine<sup>1</sup>, Guého Aurélie<sup>2</sup>, Rime Hélène<sup>2</sup>, Com Emmanuelle<sup>1</sup>, Lavigne Régis<sup>1</sup>, Montfort Jérôme<sup>2</sup>, Labbé Catherine<sup>2</sup>, Bobe Julien<sup>2</sup>, Pineau Charles<sup>1</sup>

<sup>1</sup>PROTIM - REMEDE, Inserm U1085 - Irset, Campus de Beaulieu, Rennes, France  
<sup>2</sup>LPGP, UR1037 Fish Physiology and Genomics, INRA, Rennes, France

## Introduction

The control of egg quality is a major issue for the development of a sustainable aquaculture in the context of an increasing demand for aquaculture food products. In most fish species, the egg ability to be fertilized exhibits a rapid and dramatic decrease after ovulation. In contrast, salmonid fishes can hold their eggs after ovulation for several days without any significant decrease in egg viability. The EggPreserve project aims at identifying proteins in ovarian fluid that are responsible for preserving egg viability and ability to be fertilized after ovulation in salmonids.

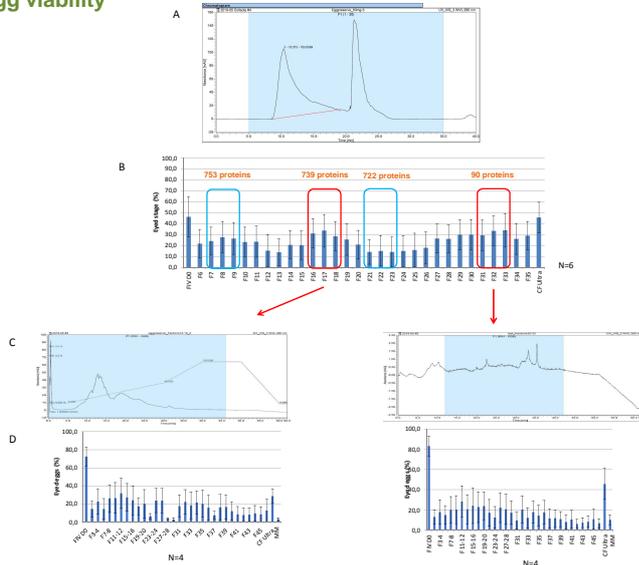
## Material & Method



**Figure 1 :** Coelomic fluids were desalted and concentrated by dialfiltration and then fractionated by UHPLC using sequential chromatography (Gel Filtration and Anion Exchange). The different fractions were systematically assessed for egg quality preservation with a biological test and proteins in fractions of interest were identified by mass spectrometry (Tims TOF Pro; Bruker) using classical bottom-up approach. In parallel, several ovarian fluid proteomes of salmonid and non-salmonid species were characterized by MS and compared with OrthoDB to identified specific proteins of salmonids.

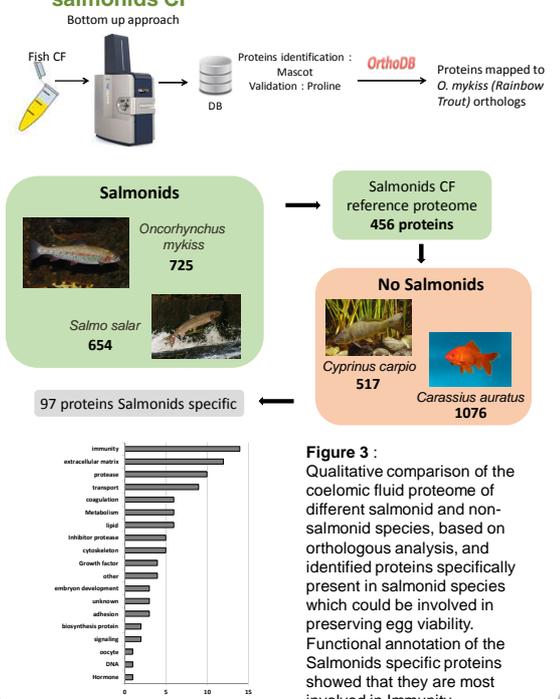
## Results

### 1. Characterisation of Rainbow Trout coelomic fluid proteins involved in egg viability



**Figure 2 :** The 30 Gel Filtration fractions of ovarian fluids were assessed by incubation with ovulated eggs before ovulation (A). We identified 3 groups of fractions allowing the conservation of the eggs, and a group of fractions having the opposite effect (B). A second fractionation using Anion Exchange was performed for 2 of the previous groups (in red) of Gel Filtration fractions preserving eggs viability (C). These fractions were also tested on ovulated eggs (D). In parallel, mass spectrometry analysis on all active fractions resulting from both chromatographic runs allowed the identification of proteins of interest, probably involved in egg quality (numbers above the selected fractions in B).

### 2. Proteomic comparison of salmonids CF to non salmonids CF



**Figure 3 :** Qualitative comparison of the coelomic fluid proteome of different salmonid and non-salmonid species, based on orthologous analysis, and identified proteins specifically present in salmonid species which could be involved in preserving egg viability. Functional annotation of the Salmonids specific proteins showed that they are most involved in Immunity.

## Conclusion & Perspectives

We have initiated an extensive mass spectrometry analysis of ovarian fluid proteomes from salmonid and non-salmonid species in order to increase our understanding of ovarian fluid protein composition. We have identified proteins that could probably be involved in the enhancement of egg viability in salmonid species compared to non-salmonid species. Our work will help deciphering of the biological mechanisms of egg quality control in fish.

This project can have a direct, and possibly rapid, impact for the aquaculture sector with the marketing of a commercial synthetic fluid that mimics ovarian fluid.

### References :

Bonnet E, Jalabert B, Bobe J: A 3-day in vitro storage of rainbow trout (*Oncorhynchus mykiss*) unfertilized eggs in coelomic fluid at 12 degrees C does not affect developmental success. *Cybulum* 2003, 27:47-51.  
 Rime H, Guillon N, Pineau C, Bonnet E, Bobe J, Jalabert B: Post-ovulatory ageing and egg quality: a proteomic analysis of rainbow trout coelomic fluid. *Reprod Biol Endocrinol.* 2004:26-.