# Innovative metabolomic workflows to study growth kinetics

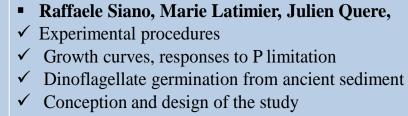
## dinoflagellate cysts revived from modern and ancient sediment.

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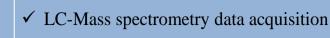
3 Teams: Biology, Chemistry & Metabolomics







Manoella Sibat, Philipp Hess





**Florence Souard; Yann Guitton** Data analysis workflows W4M 3.0 Galaxy online

Nicolas Elie Molecular Network (MetGem) Pierre-Marie Allard ; Jean luc Wolfender Molecular Network (GNPS) & MetaboMaps Pipeline

Florence Mondeguer. Experimental design – LC-MS/MS data analysis Biostatistics (MPP) - Molecular Network Data interpretations

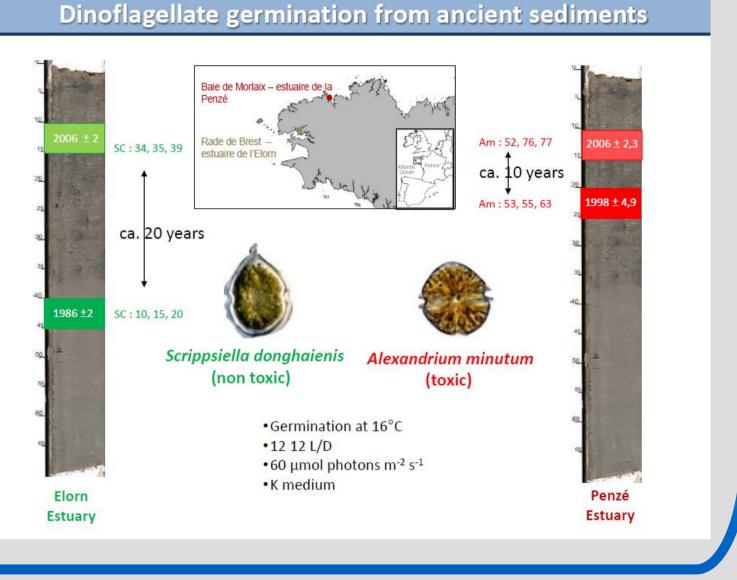
Keywords: Paleoecology; Dinoflagellates cyst; Harmful Algal Blooms (HAB); LC-QToF data processing, Metabolomics workflows; Statistics analysis; Network

**Introduction** : Aquatic ecosystems worldwide have been negatively affected by eutrophication, many of them driven by increasing nutrient inputs from untreated domestic sewage and industrial and agricultural wastewater. During their life cycle, Alexandrium minutum and Scrippsiella donghaienis can produce resistant and revivable cysts that can accumulate in the sediments of the coastal marine environment for hundreds of years. As a working hypothesis, we presumed that these two genera have preserved their adaptation to such ecosystem changes in the form of cysts in the old sediments. From revivified cysts, some cultures of toxic and non-toxic dinoflagellates (A. *minutum* and *S. donghaienis*) were analyzed by a untargeted metabolomics approach by LC-HRMS to study the possible

### Hypothesis Metabolomic Approach

#### **Phenotype untargeted approach**

Q1: Can metabolomics profiles show the inter- and



adaptive responses of phytoplankton to these modifications of coastal ecosystems.

Strains of S. donghaienis were obtained from sediments isotopically dated to 1986 (ancient) and 2006 (modern) while those of A. minutum dated from 1996 (ancient) and 2006 (modern). In total 84 samples were obtained, with each experimental condition (species, age, growth phase) being cultured in triplicate in phosphate depleted conditions. More specifically, two workflows have been used for univariate and multivariate modelling on:

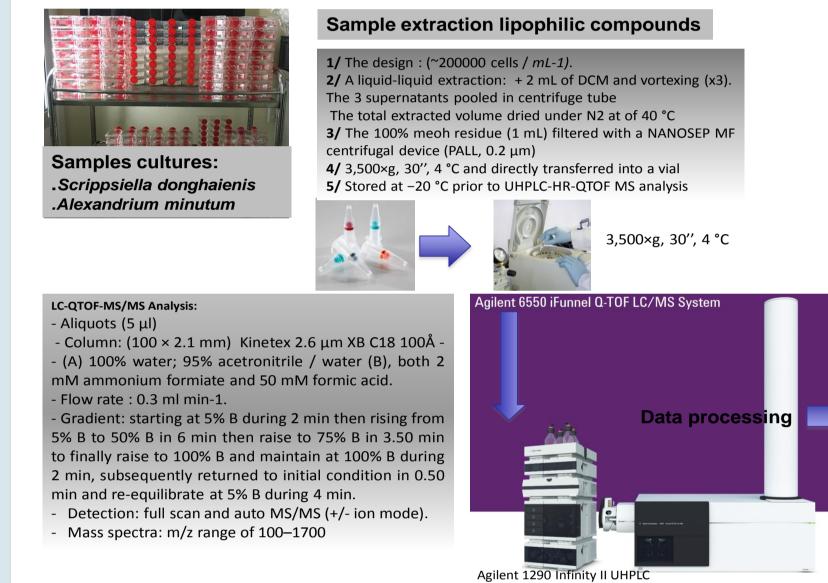
- chemometrics platform, Agilent Mass Profiler Professional (MPP)
- collaborative portal dedicated to metabolomic data processing, analysis and annotation

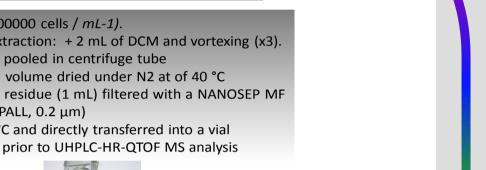
"Workflow4Metabolomics"

- molecular network

intra-specific diversity of dinoflagellate in a same culture conditions?

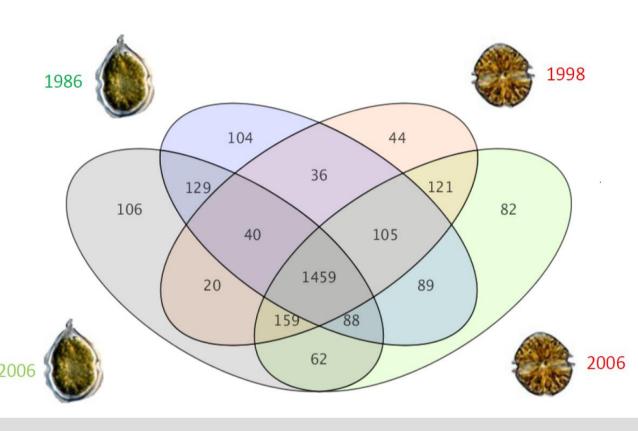
Q2: Can metabolomics profiles of different ages inform about the potential adaptation of a species to the environmental changes occurred across the last century ?





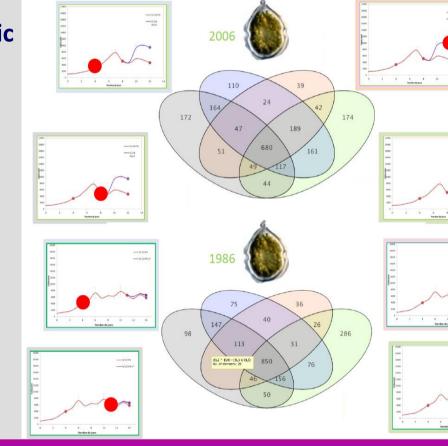
#### 2 Metabolomics Workflows tested

1st step from: Agilent Mass Profiler Professional (MPP) soft a chemometrics platform



A Q-TOF LC/MS metabolomic fingerprinting approach to investigate differential metabolites of Scripsiella **Donghaienis** versus Alexandrium Minutum cultures. **Specific metabolites can be** identified between Scrippsiella Donghaienis (green) and Alexandrium

Minutum (red) cultures.



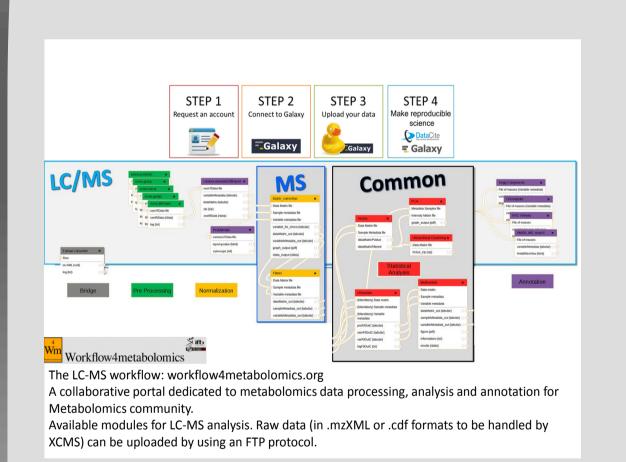
Different lipophilic metabolites produced during S. Donghaienis

growth.

### Pacman? or Start exploring the network with a desktop software for live Molecular Networking

#### 2 Metabolomics Workflows tested

2nd step from: Collaborative portal Galaxy-W4M the Workflow4Metabolomics 3.0 Data Processing



OXNET

ALEI

RED ATI

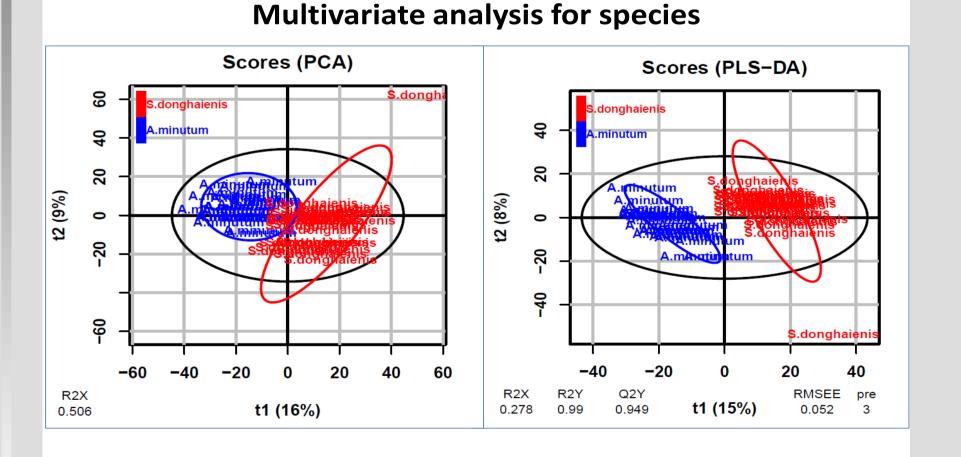
Atlantic Area European Regional Development Fund

Chemistry

DE ALERTA Más segur

The 2 workflows (MPP 1 & W4M) give the same trends but different and complementary biological relevant metabolite information. The two strains do not the same have distribution:

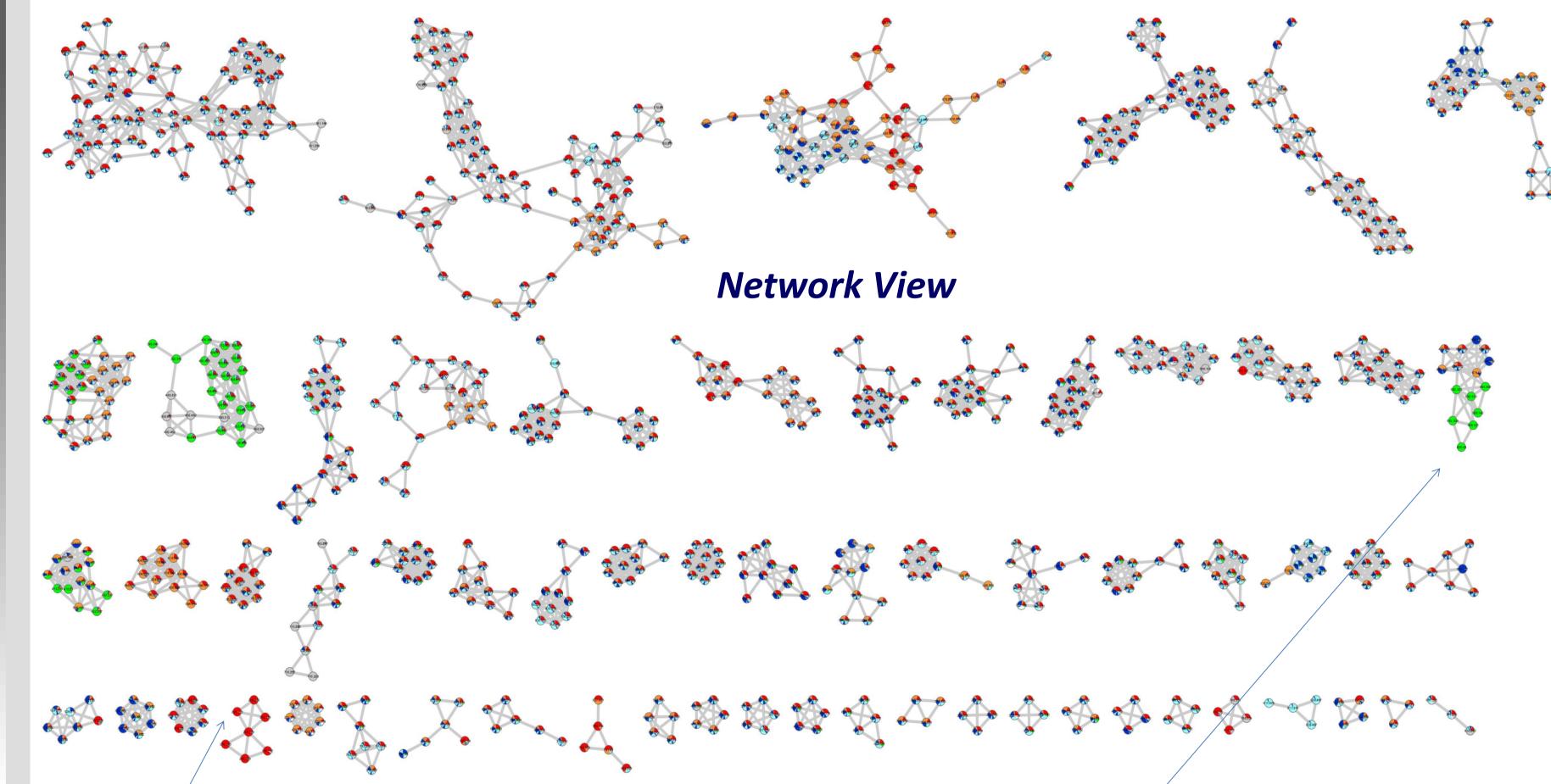
- Neither in the difference between "ancient" and "modern"
- Neither according to the different phases of growth

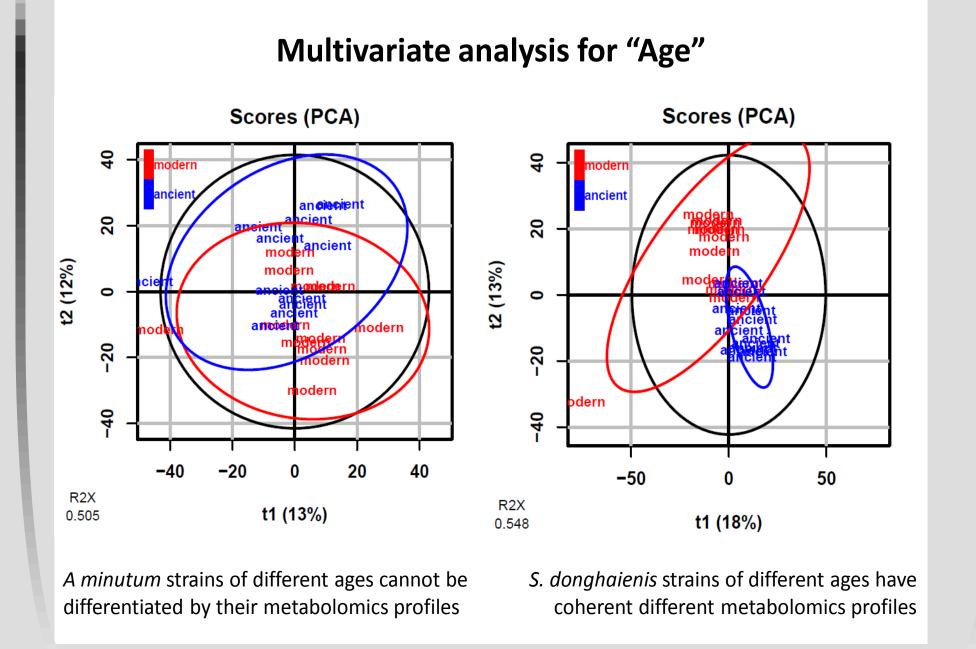


Scrippsiella Donghaienis vs Alexandrium Minutum

The MetGEM<sup>1</sup> new software provides two ways of overcoming GNPS weaknesses. With MetGem, you have a userfriendly, local software for MS/MS comparison and spectral network generation, without the requirement of several different software (FTP client, browser and molecular interaction networks visualizer).

<sup>1</sup>Elie, N., Olivon, F., Grelier, G., Litaudon, M. and Touboul, D. (2018). HDiSpeC: A new desktop software for live Molecular Networking and t-SNE projections of MS/MS spectra. 11èmes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique (RM. 23-25 mai 2018, Liège, Belgique.

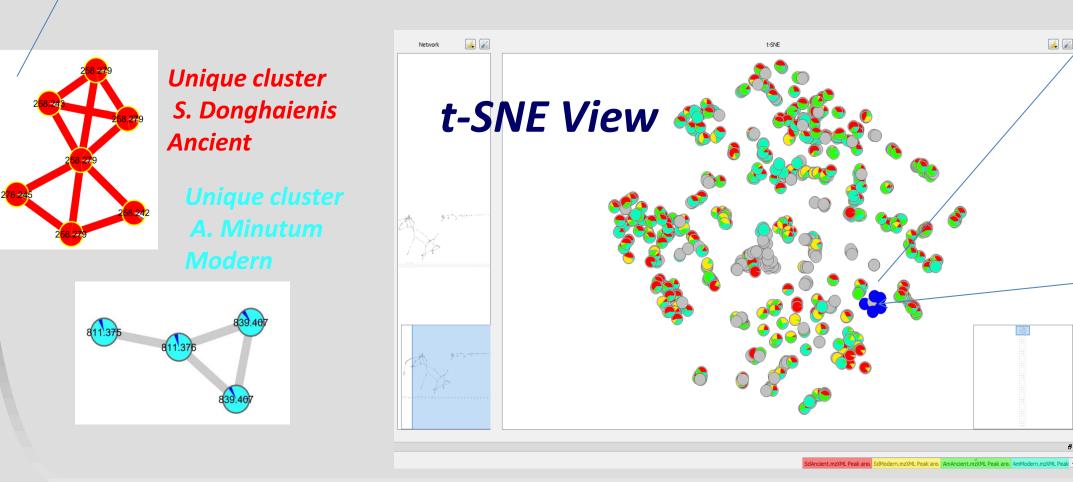




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A representation of high-dimensional dataset MS/MS spectra, life cycle of Alexandrium minutum and Scrippsiella donghaienis.

The color scheme: Scrippsiella Donghaienis (Ancient; ) and Alexandrium Minutum (Ancient ; I ) shows a great similarity between the two species. In blue the concept proof with **10 external standards**.



*Recent technique known as t-distributed stochastic* neighbor embedding (t-SNE) can provide a representation

of high-dimensional datasets like MS/MS spectra

Compound Name	Formula	Mass	(M+H)+
GYM-A	C32H45NO4	507,33486	508,3421
13-desMe-SPX C	C42H61NO7	691,4448	692,4521
PnTX-G	C42H63NO7	693,46045	694,4677
DTX-2- H2O	C44H68O13	804,46599	787,4623
OA	C44H68O13	804,46599	805,4745
AZA-3	C46H69NO12	827,48198	828,4893
DTX-1	C45H70O13	818,48164	819,4889
AZA-1	C47H71NO12	841,49763	842,5049
AZA-2	C48H73NO12	855,51328	856,5206
PTX2 NH4+	C47H70O14	858,47656	876,5104