

First insight on metabolomics workflows and differences between dinoflagellate: characterization and discrimination of modern and ancient strains and their algal growth.

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Keywords: Non targeted analysis; paleoecology; dinoflagellates cyst; Harmful Algal Blooms (HAB); LC-QToF; data processing, metabolomics workflows; statistics analysis

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 donghaiensis* 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 revived cysts, some cultures of toxic and non-toxic dinoflagellates (*A. minutum* and *S. donghaiensis*) were analyzed by an untargeted metabolomics approach by LC-HRMS to study the possible adaptive responses of phytoplankton to these modifications of coastal ecosystems.

Strains of *S. donghaiensis* 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.

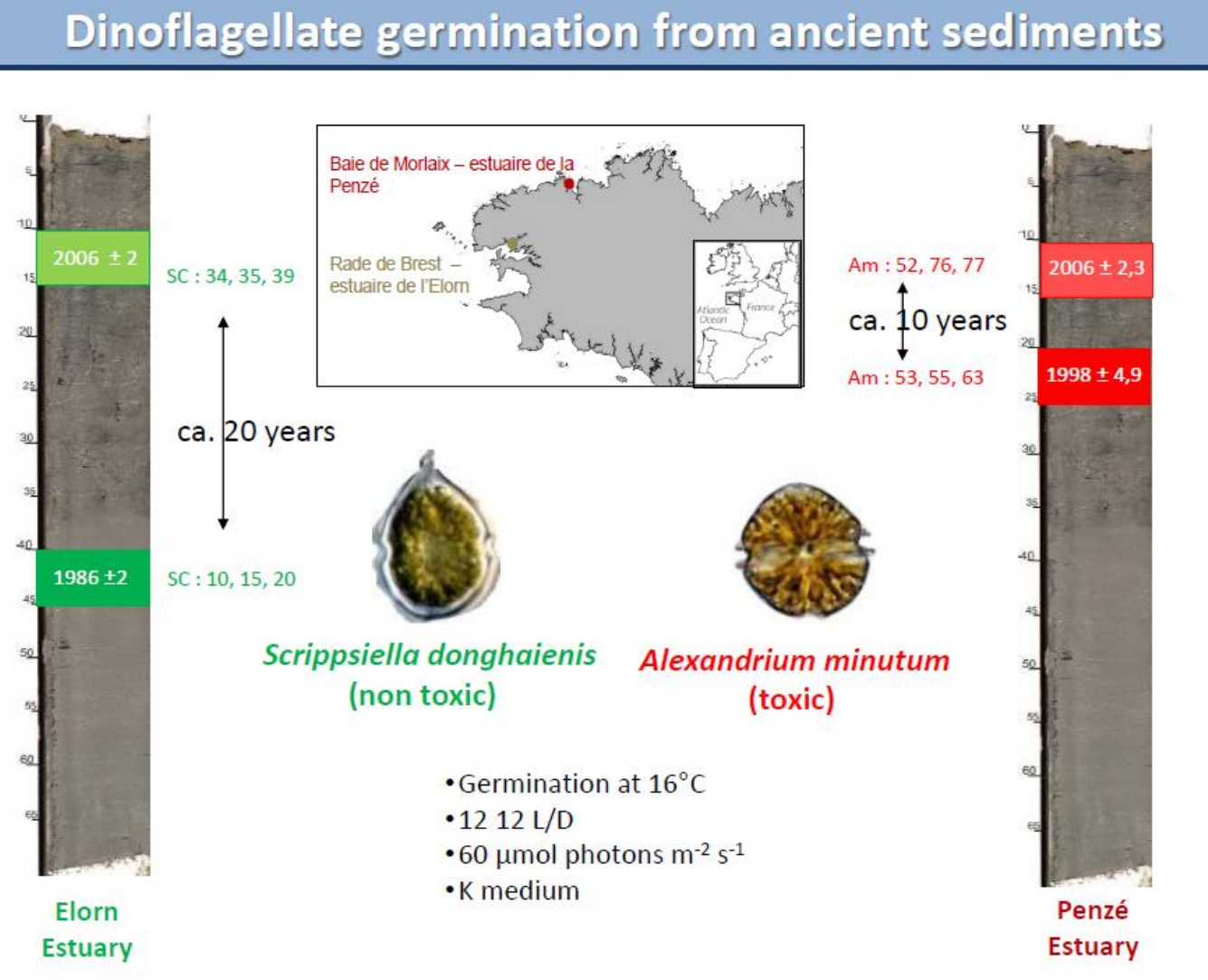
More specifically, two workflows have been used for univariate and multivariate modelling on:

- a chemometrics platform, Agilent Mass Profiler Professional (MPP)
- a collaborative portal dedicated to metabolomic data processing, analysis and annotation "Workflow4Metabolomics"

3 Teams - 3 subjects: Biology – Chemistry - Metabolomics

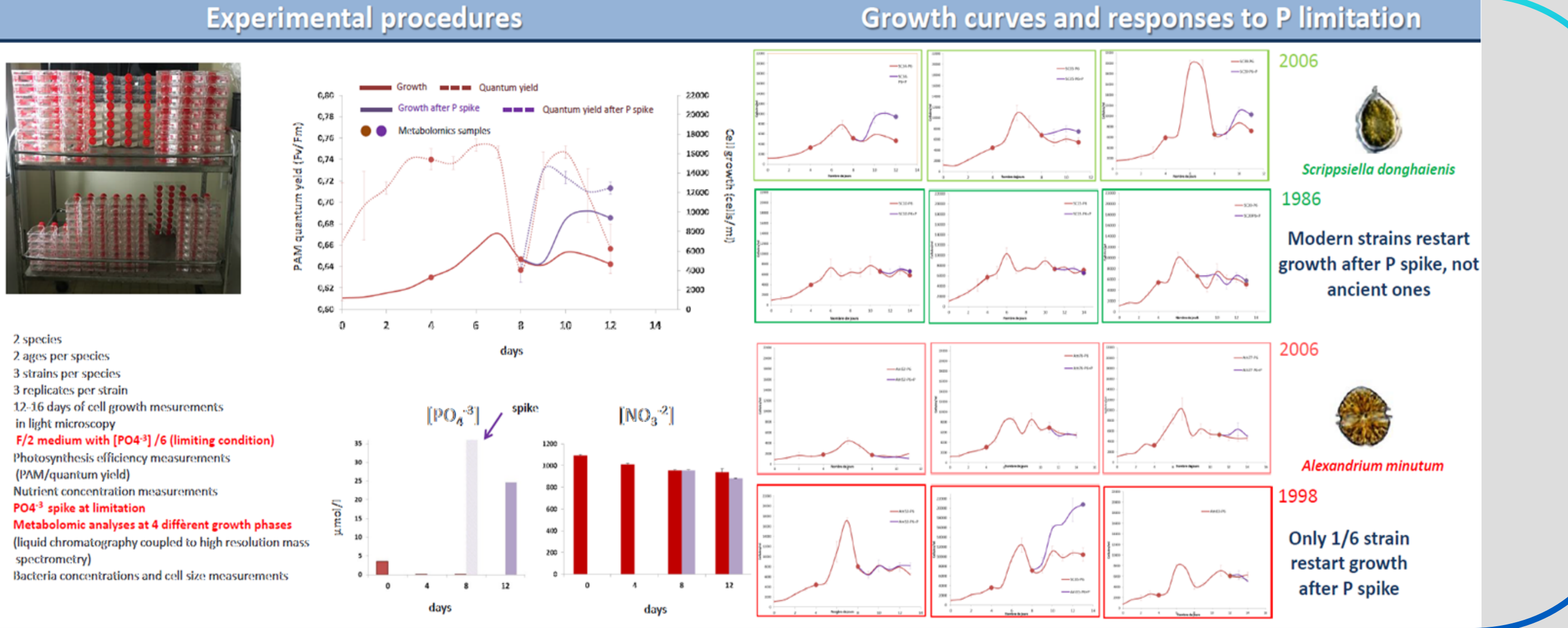
	<ul style="list-style-type: none"> Marie Latimier, Julien Quere Experimental procedures growth curves, responses to P limitation 	
	<ul style="list-style-type: none"> Raffaele Siano Dinoflagellate germination from ancient sediment Conception and design of the study 	
	<ul style="list-style-type: none"> Manoella Sibat LC-Mass spectrometry data acquisition Philipp Hess Conception and design of the study 	
	<ul style="list-style-type: none"> Florence Souard Data analysis workflows W4M 3.0 Galaxy online Yann Guitton Data analysis workflow W4M 3.0 Galaxy online & MPP Florence Mondeguer Experimental design - Mass Spectrometry & data analysis Mass Profiler Pro (MPP) chemometrics platform Agilent Data interpretation 	

HYPOTHESIS Phenotype Untargeted approach

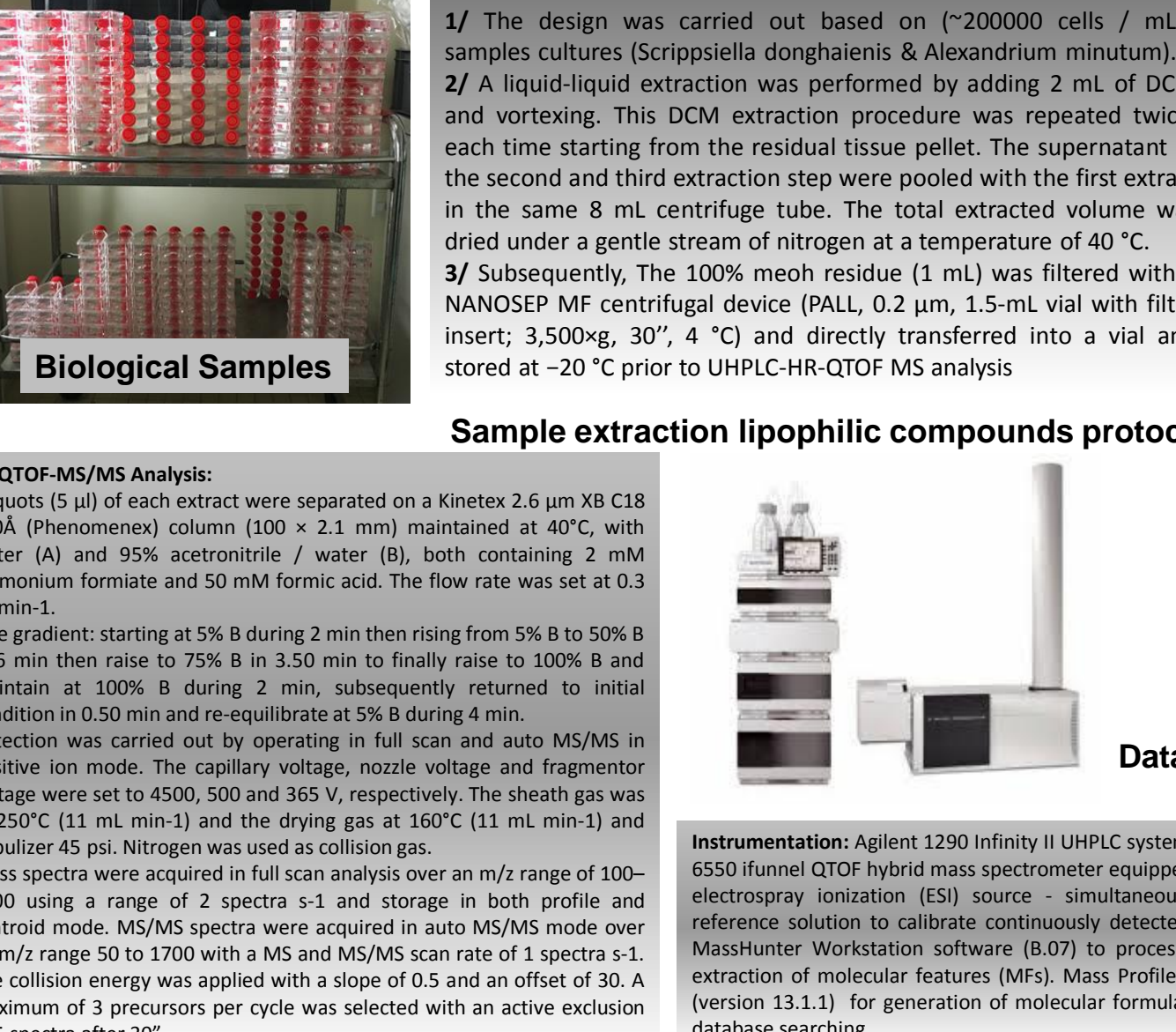


Q1: Can metabolomics profiles show the inter- and intra-specific diversity of dinoflagellate in a same culture conditions?
 Q2: Can metabolomics profiles of different ages inform about the potential adaptation of a species to the environmental changes occurred across the last century?

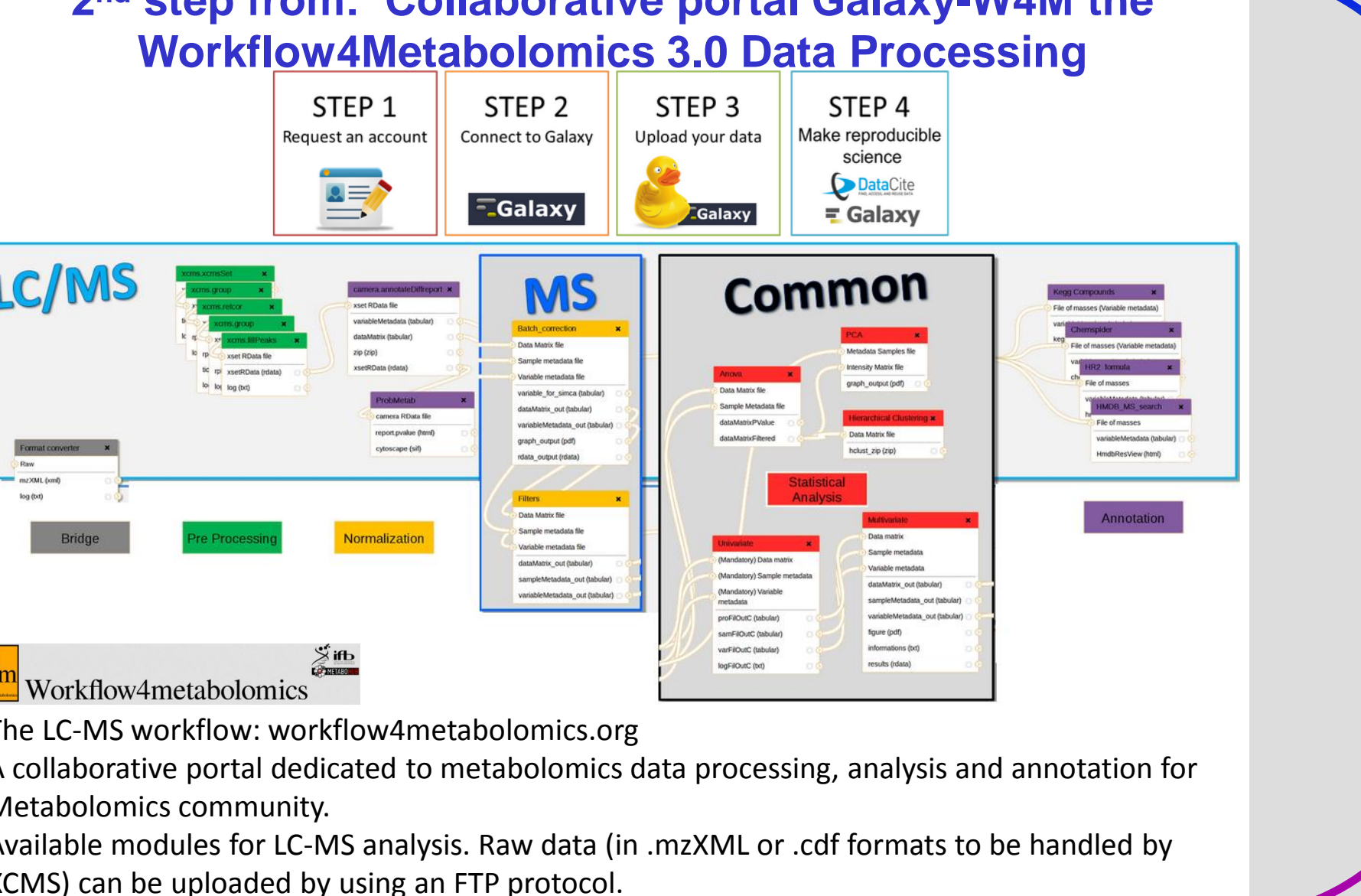
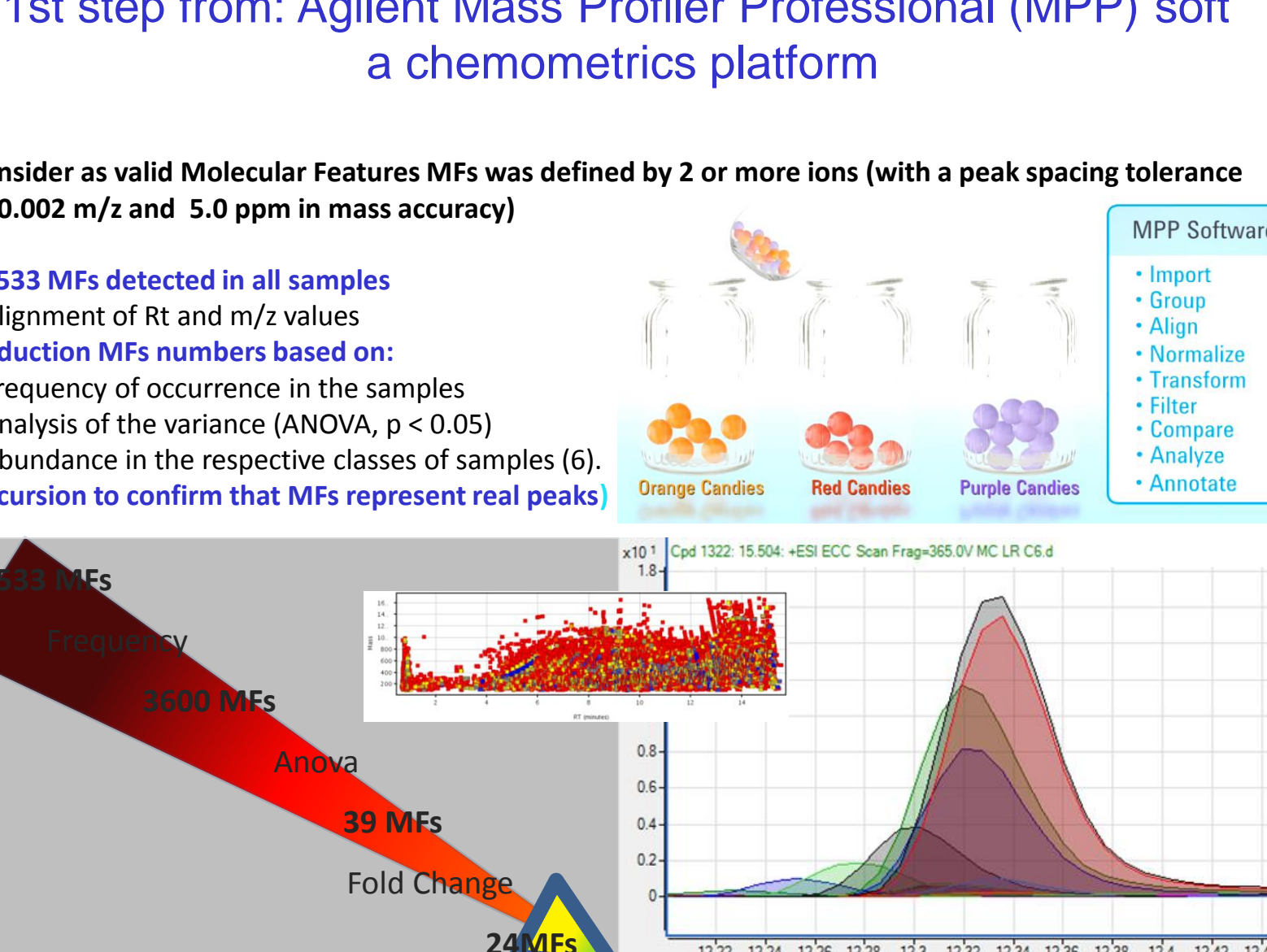
Experimental Design



Chemistry



2 Metabolomics Workflows tested

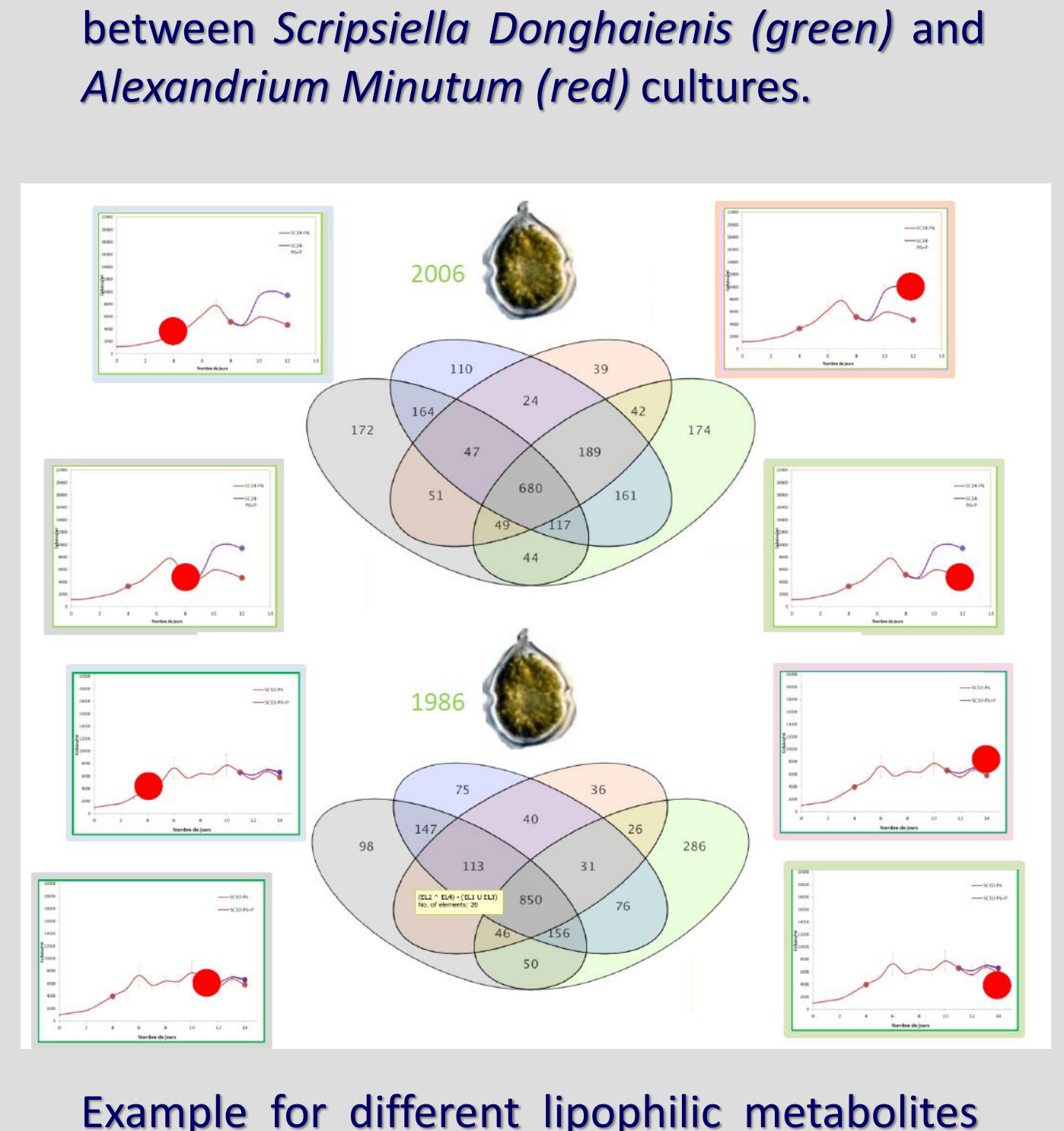
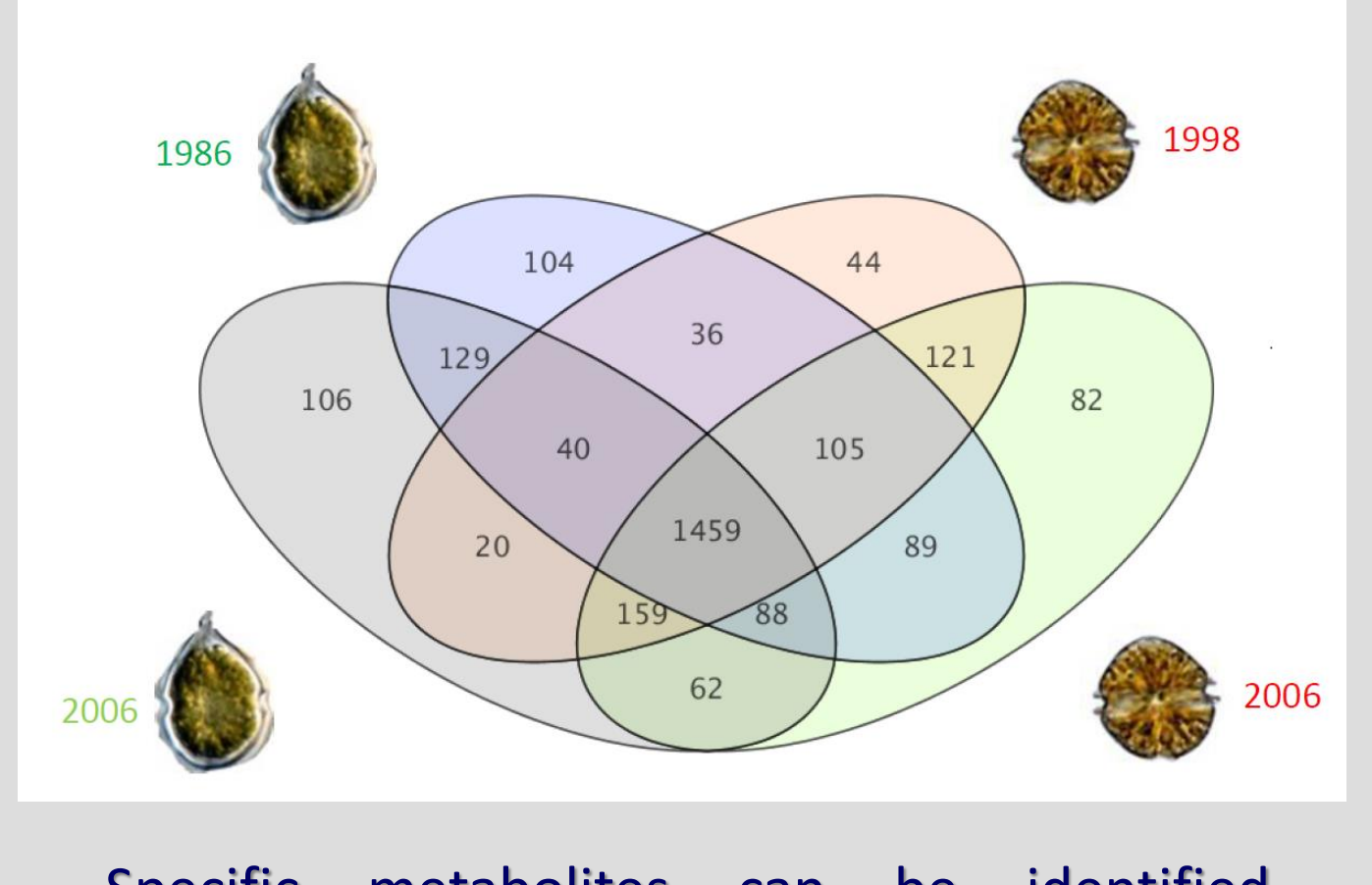
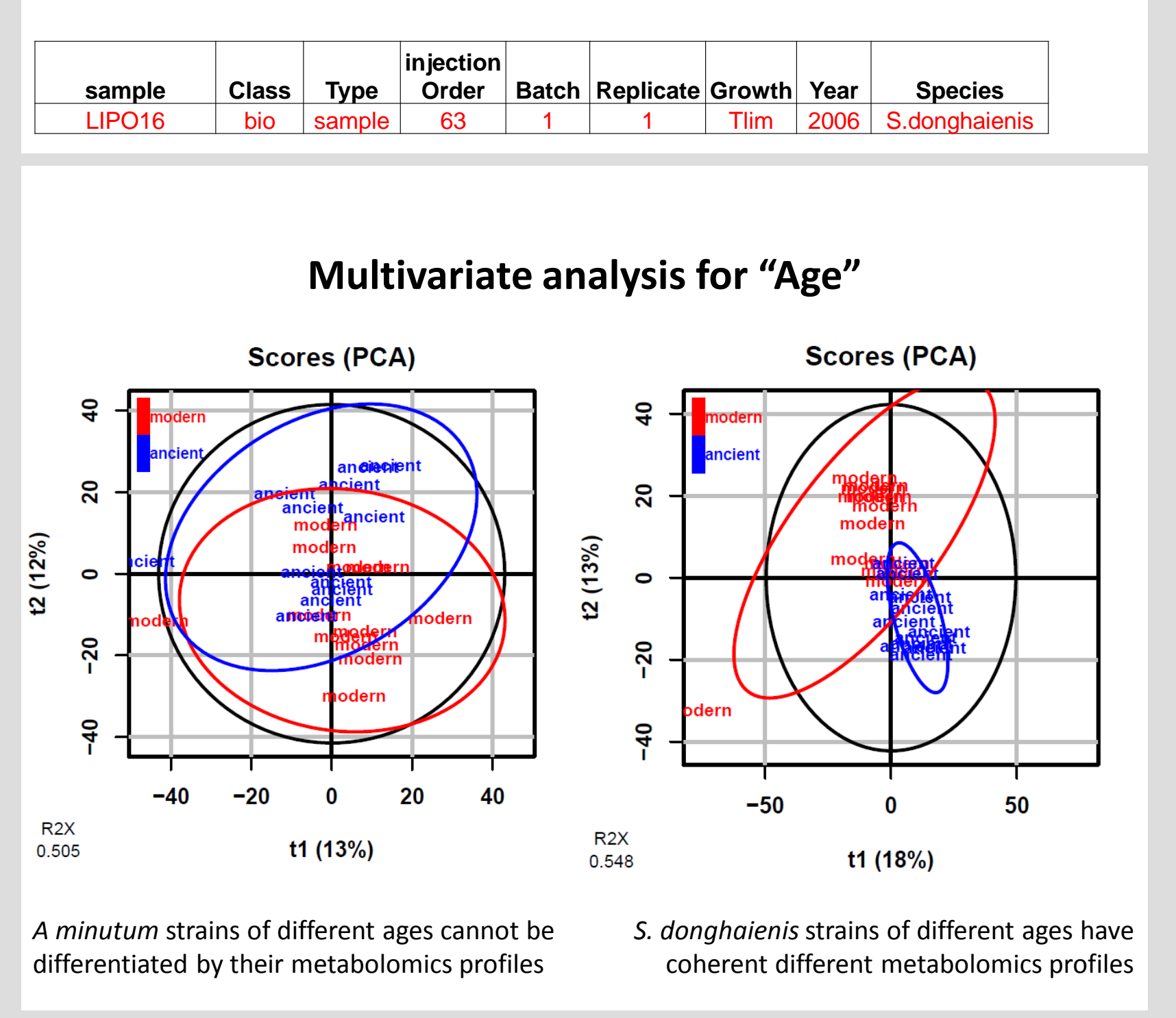
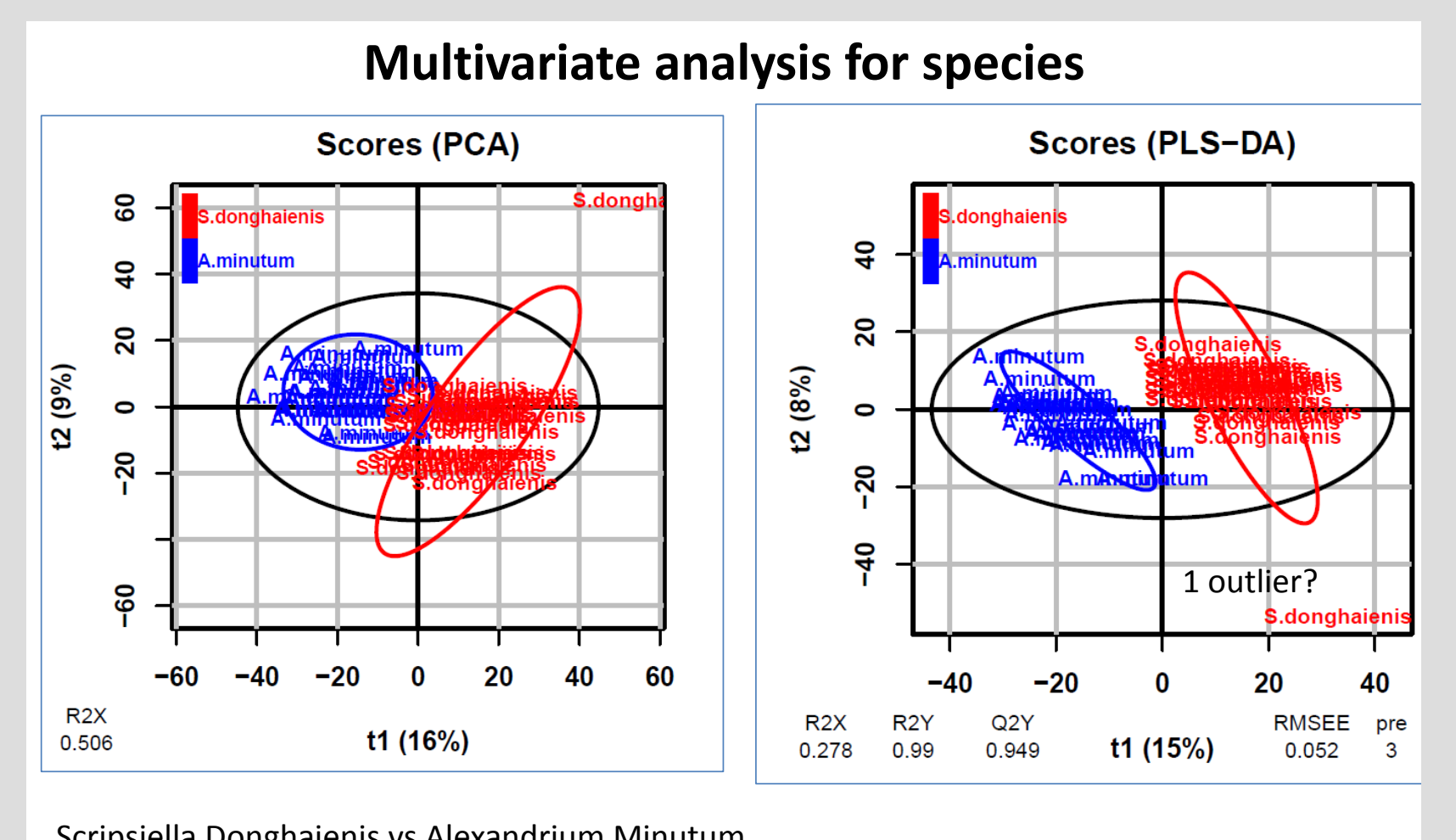


Statistical Best Results

2 workflows : From Mass Profiler Professional (MPP) / From Collaborative portal Galaxy-W4M / Specific Results (MPP)

1 sample Meta data

sample	Class	Type	Injection Order	Batch	Replicate	Growth	Year	Species
LIP001	bio	sample	17	1	1	Temp	1986	S.donghaiensis
LIP002	bio	sample	18	1	1	Temp	1986	S.donghaiensis
LIP003	bio	sample	27	1	3	Temp	1986	S.donghaiensis
LIP004	bio	sample	50	1	1	Tim	1986	S.donghaiensis
LIP005	bio	sample	35	2	1	Tim	1986	S.donghaiensis
LIP006	bio	sample	66	1	3	Tim	1986	S.donghaiensis
LIP007	bio	sample	45	1	1	Tim	1986	S.donghaiensis
LIP008	bio	sample	54	1	2	Tim	1986	S.donghaiensis
LIP009	bio	sample	26	1	3	Tim	1986	S.donghaiensis
LIP010	bio	sample	52	1	3	Ajout P	1986	S.donghaiensis
LIP011	bio	sample	34	1	2	Ajout P	1986	S.donghaiensis
LIP012	bio	sample	65	1	3	Ajout P	1986	S.donghaiensis
LIP013	bio	sample	44	1	1	Temp	2006	S.donghaiensis
LIP014	bio	sample	16	1	2	Temp	2006	S.donghaiensis
LIP015	bio	sample	23	1	3	Temp	2006	S.donghaiensis
LIP016	bio	sample	63	1	1	Tim	2006	S.donghaiensis
LIP017	bio	sample	33	1	2	Tim	2006	S.donghaiensis
LIP018	bio	sample	64	1	3	Tim	2006	S.donghaiensis
LIP019	bio	sample	42	1	1	Tim	2006	S.donghaiensis
LIP020	bio	sample	15	1	2	Tim	2006	S.donghaiensis
LIP021	bio	sample	53	1	3	Tim	2006	S.donghaiensis
LIP022	bio	sample	62	1	1	Ajout P	2006	S.donghaiensis
LIP023	bio	sample	30	1	2	Ajout P	2006	S.donghaiensis
LIP024	bio	sample	69	1	3	Ajout P	2006	S.donghaiensis
LIP025	bio	sample	41	1	1	Temp	1996	A.minutum
LIP026	bio	sample	14	1	2	Temp	1996	A.minutum
LIP027	bio	sample	49	1	3	Temp	1996	A.minutum
LIP028	bio	sample	22	1	1	Tim	1996	A.minutum
LIP029	bio	sample	43	1	2	Temp	1996	A.minutum
LIP030	bio	sample	32	1	3	Temp	1996	A.minutum
LIP031	bio	sample	68	1	3	Tim	1996	A.minutum
LIP032	bio	sample	20	1	1	Ajout P	1996	A.minutum
LIP033	bio	sample	20	1	2	Temp	2006	A.minutum
LIP034	bio	sample	51	1	2	Temp	2006	A.minutum
LIP035	bio	sample	29	1	3	Temp	2006	A.minutum
LIP036	bio	sample	59	1	1	Tim	2006	A.minutum
LIP037	bio	sample	67	1	2	Tim	2006	A.minutum
LIP038	bio	sample	39	1	3	Tim	2006	A.minutum
LIP039	bio	sample	19	1	1	Tim	1996	A.minutum
LIP040	bio	sample	57	1	2	Tim	1996	A.minutum
LIP041	bio	sample	29	1	1	Ajout P	1996	A.minutum
LIP042	bio	sample	58	1	2	Ajout P	1996	A.minutum
LIP043	bio	sample	31	1	3	Tim	2006	A.minutum
LIP044	bio	sample	38	1	2	Tim	2006	A.minutum
LIP045	bio	sample	18	1	3	Tim	2006	A.minutum
LIP046	bio	sample	47	1	1	Ajout P	2006	A.minutum
LIP047	bio	sample	28	1	2	Ajout P	2006	A.minutum
LIP048	bio	sample	27	1	3	Ajout P	2006	A.minutum
blanc1_01	blank	blank	7	1	1	ras	ras	ras
blanc1_02	blank	blank	8	1	1	ras	ras	ras
blanc1_03	blank	blank	9	1	3	ras	ras	ras
blanc2_01	blank	blank	10	1	4	ras	ras	ras
blanc2_02	blank	blank	11	1	5	ras	ras	ras
blanc2_03	blank	blank	12	1	6	ras	ras	ras
QC5_01	bio	pool	13	1	1	ras	ras	ras
QC5_02	bio	pool	48	1	2	ras	ras	ras
QC5_03	bio	pool	71	1	3	ras	ras	ras



Here we present a Q-TOF LC/MS metabolomic fingerprinting approach
 • to investigate differential metabolites of *Scripsiella Donghaiensis* versus *Alexandrium Minutum* cultures.
 • to focus on the specific metabolites of the microalga, specie, age or growing
 The 2 workflows (MPP 1 & W4M) give the same trend but different and complementary biological relevant metabolite information. The two strains do not have the same distribution:
 • Neither in the difference between "ancient" and "modern"
 • Neither according to the different phases of growth