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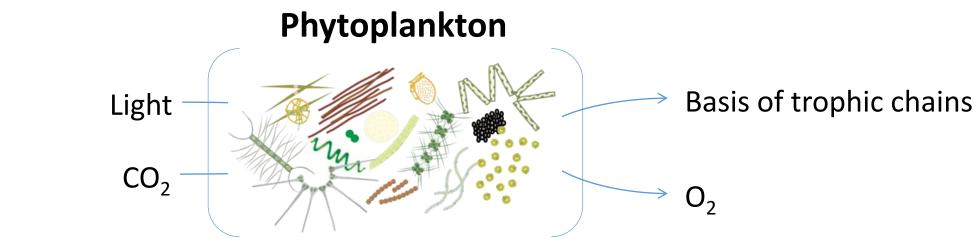
# Using metabolomics to highlight biomarkers of (non)-exposure of mussels and oysters to phycotoxins

## Valentin Derrien<sup>1,2</sup>, Justine Massias<sup>2</sup>, Axel Raux<sup>2</sup>, Amélie Derrien<sup>3</sup>, Killian Guyomard<sup>4</sup>, Lionel Dégremont<sup>5</sup>, Virginie Le Razavet<sup>4</sup>, Florence Mondeguer<sup>1</sup>, Yann Guitton<sup>2</sup>, Damien Réveillon<sup>1\*</sup>

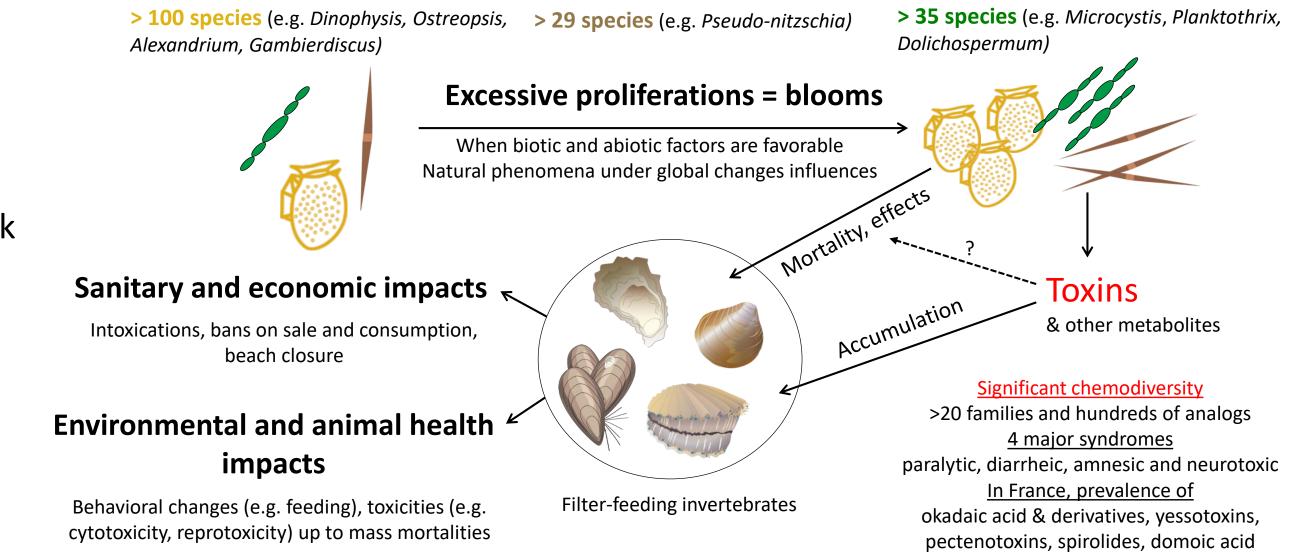
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### Context

- Some micro-algae species produce toxins that can cause food poisoning in humans through the consumption of contaminated shellfish
- Monitoring programmes have been implemented to protect the consumers  $\rightarrow$  quantification by targeted analyses using LC-MS/MS
- Alternatively, we could try to consider the consequences of a contamination by phycotoxins on the global metabolite expression of bivalves to find biomarkers of exposition or non-exposition  $\rightarrow$  metabolomics?



Huge biodiversity including harmful dinoflagellates/diatoms/cyanobacteria



<u>Objectives</u>: Innovate for the assessment of shellfish safety via a double approach: metabolomics + molecular network Highlight **biomarkers** characteristic of a (non-)exposure of bivalves to phycotoxins

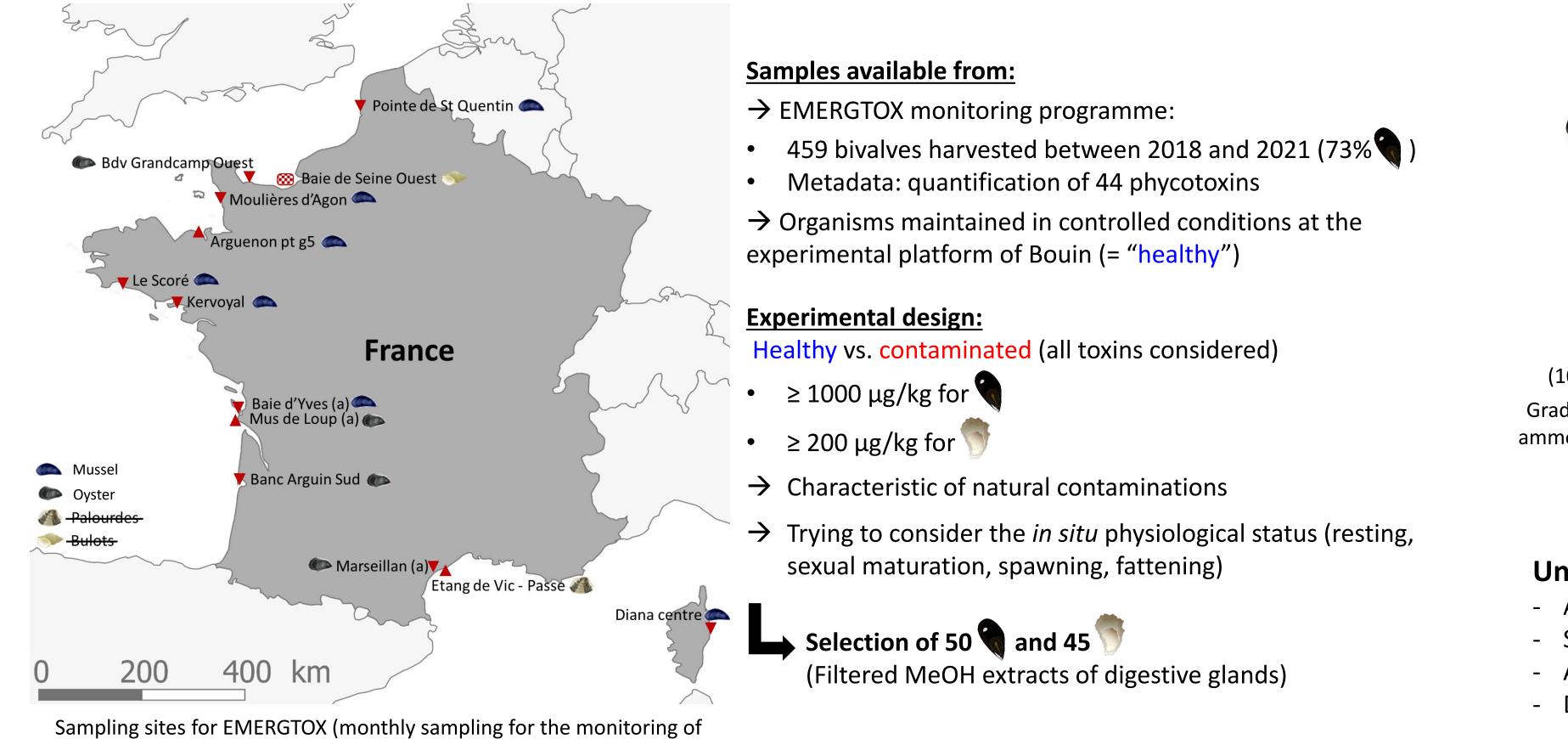
<u>Strategy</u>: Use the latest generation of high resolution mass spectrometers and bioinformatic tools, in collaboration with the metabolomic plateform of a leading partner in food chemical safety (LABERCA).

<u>Background</u>: Based on preliminary results suggesting metabolites characteristic of non-exposure in mussel (Mondeguer et al., 2012, Arrement - LABERCA collaboration) and on the implementation of a monitoring method based on biomarkers obtained from untargeted metabolomics by LABERCA

Overview of the problems related to harmful algal blooms (HABs)

### Metabolomic approach

## 1) Samples and experimental design





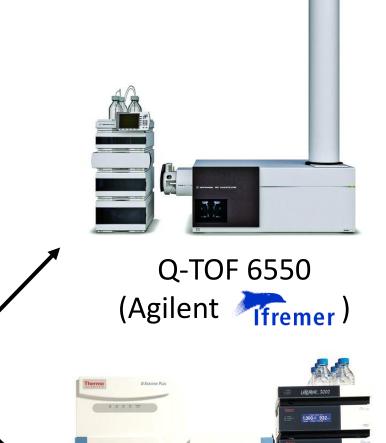
Comparison/complementarity

Q-TOF & Q-Exactive

High Resolution

Mass spectrometry

Electrospray (+ and -MS)



DDA (5 precursors/scan, 3 (100 x 2,1 mm, 1,7 μm) collision energies) Gradient: ACN/water/2 mM ammonium formate + 50 mM formic acid



Q-Exactive (Thermo, LABERCA)

### Untargeted metabolomics (following the guidelines of Broadhurst et al., (2018)

- Analytical and extraction process blanks, quality controls
- Standard reference materials (4 IS spiked in all samples + mix of 10 phycotoxins)
- Appropriate order of analysis (randomized for samples)
- Data-dependant analysis

UPLC

Kinetex<sup>®</sup> C18

### 4) Statistical analyses and selection of 'biomarkers'

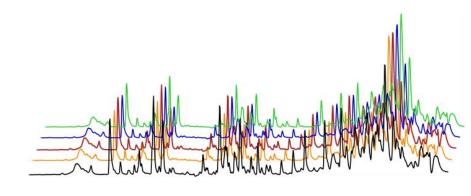
-MS

Supervised orthogonal partial least squares – discriminant analyses (OPLS-DA) were constructed (and validated by permutation tests) using the classes healthy and contaminated.

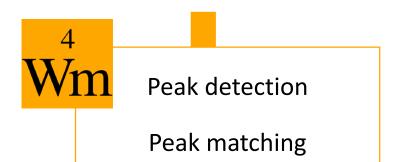
+MS

### 3) Data processing

regulated and emerging toxins in mollusks)



From chromatograms...



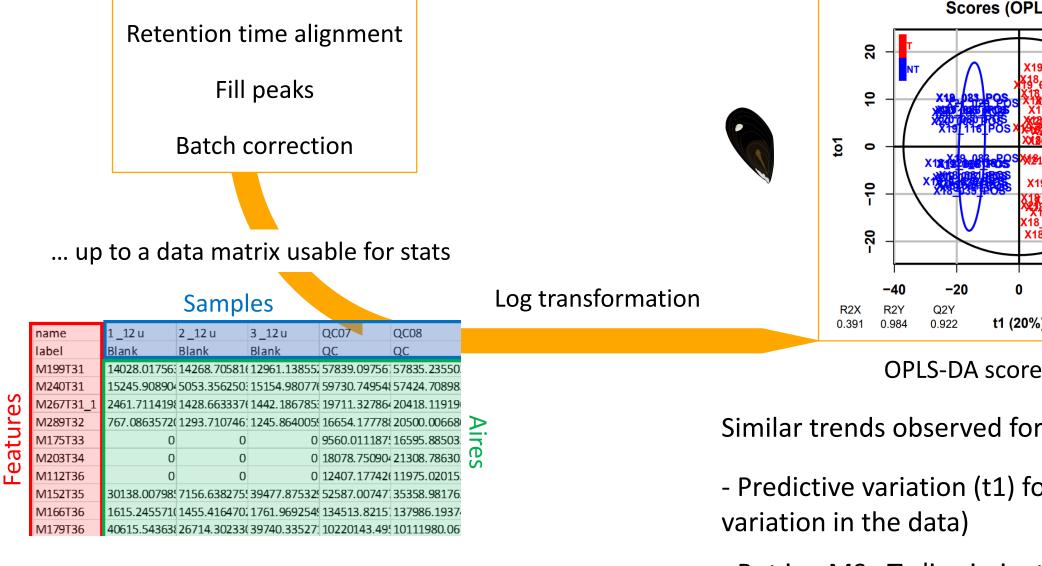
# Scores (OPLS-DA) Scores (OPLS-DA) -MS +MS

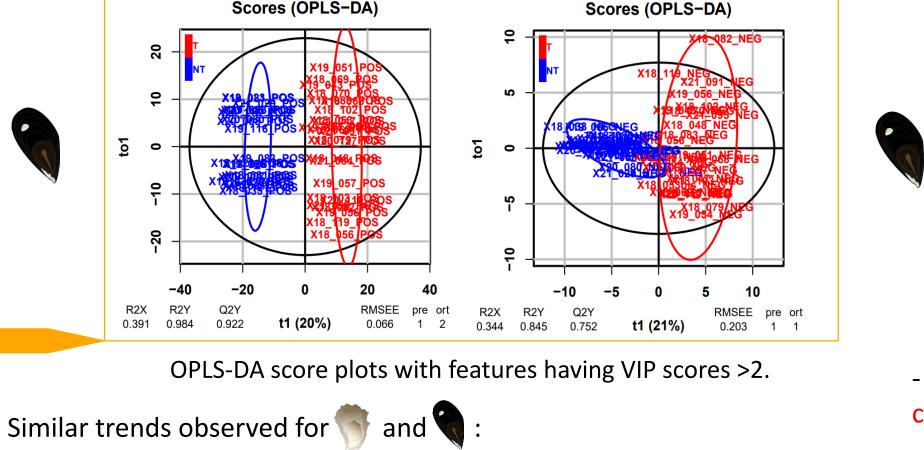
+MS				
Feature	m/z	rt (s)	mean area in <mark>T</mark>	mean area in NT
M387T340	387.2961	339.9139	1.74E+05	4.98E+01
M276T137	276.1262	137.4513	1.31E+06	4.98E+04
M476T251	475.9208	251.2862	3.26E+04	1.46E+01
M325T324	325.2590	323.6006	7.08E+04	1.02E+04
M475T167	474.9731	167.4614	3.61E+04	1.01E+02
-MS				
Feature	m/z	rt (s)	mean area in <mark>T</mark>	mean area in <mark>NT</mark>
M695T624	694.9652	623.5121	2.20E+04	0.00E+00
M661T666	661.1458	666.4104	5.09E+04	0.00E+00
M583T732	583.1859	732.0558	1.66E+05	1.87E+03
M695T760	695.1200	760.2427	1.39E+04	0.00E+00
M625T390	625.0181	390.0057	1.46E+04	5.40E+01

Relative abundances of some significant features in contaminated (T) and healthy (NT) organisms

# **Results and perspectives**

- The untargeted metabolomic workflow allowed for the selection of several putative biomarkers of (non)-exposure to phycotoxins
  - mainly for contaminated bivalves
  - but some could be biomarkers of health status
- Data from the Q-Exactive are being processed





- Predictive variation (t1) for -MS > +MS (21-27% vs. 16-20% of all

- But in +MS: 7 discrimination healty - contaminated (R2Y 0.98-0.99 vs. 0.77-0.84) and *¬* predictive performance (Q2Y 0.72-0.92 vs. 0.58-0.75)

 $\rightarrow$  features discriminating the two groups can be highlighted = putative biomarkers?

+MS Feature *m/z* rt (s) mean area in T mean area in N M566T313 566.1042 312.6718 1.40E+05 3.55E+02 M191T39 191.0741 38.6389 1.79E+05 4.68E+02 M301T56 301.1391 55.8024 5.86E+05 4.94E+03 1440T338 440.2787 337.6721 9.24E+04 6.01E+02 M323T345 323.0939 344.8371 0.00E+00 1.54E+04 Feature *m/z* rt (s) mean area in T mean area in N M585T733 585.1837 733.4698 9.41E+04 2.42E+01 M152T36 152.0392 35.7016 2.52E+04 2.64E+03 M534T675 534.2579 674.5250 3.07E+03 3.49E+04 695T763 695.1206 763.3494 7.35E+04 2.55E+01 M623T676 623.2244 675.7263 3.41E+03 2.07E+04

- Most significant features characteristic of contaminated organims

- Some contrary examples: M323T345 for 🥎 M534T675 for 📢

- One feature possibly common between the two bivalves (M695T760-763)
- M440T338 may correspond to lysoPC (12:0) (SGNPS annotation)

 $\rightarrow$  Complementarity between the two HRMS will be assessed

- Sensitivity and selectivity should be tested
- $\rightarrow$  using less contaminated samples
- Annotation has to be improved
  - only few biomarkers present in molecular networks
  - should consider targeted-MS/MS
  - try other annotation tools (e.g. MS/R/US)
- Promising results towards innovating for the assessment of shellfish safety

 $\rightarrow$  but the list of candidates biomarkers will ultimately have to pass challenge tests

References : Mondeguer, F., Antignac, J.-P., Guitton, Y., Monteau, F., Borgne, S.L., Hess, P. (2012). Nouvelle stratégie de caractérisation non ciblée de type métabolomique au service de l'identification de composés bioactifs accumulés dans les mollusques bivalves. Spectra analyse 284 ; Broadhurst, D., Goodacre, R., Reinke, S.N., Kuligowski, J., Wilson, I.D., Lewis, M.R., Dunn, W.B. (2018). Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabolomics 14 (6):72.

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