Journée Cyanobactéries FIRE

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Transfer of cyanobacteria and cyanotoxins from fresh to marine waters

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anses

The Bieautox project (Brittany, France)

Evaluation of cyanobacteria and intracellular/extracellular cyanotoxin transfer from fresh to estuarine waters along a salinity gradient in an estuarine continuum

Upstream → Downstream

First freshwater site F1 Reservoir water production

2nd freshwater site F2 St Eloi River

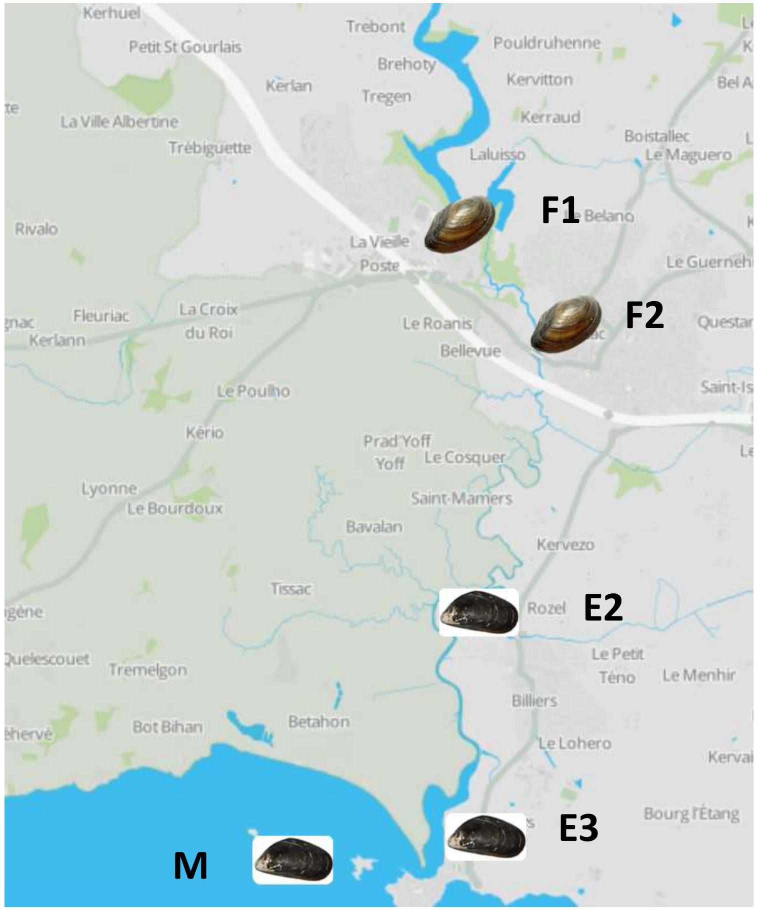
First estuarine site E1 St Eloi river

2nd estuarine site E2 Port

Entrance of marine section, M

2-year survey, monthly or bi-weekly, sampling of phytoplanktonic populations, quantification of cyanotoxins microcystins in phytoplankton, in water and in bivalves (LC-MS/MS)

Using sentinel species as bioindicators of cyanotoxins



Phytoplankton may not represent water contamination levels

Spatio-temporal variations in localisation/abundance of cyanobacteria
Dilution effect in the estuary
Sporadic events of cyanotoxin transfer

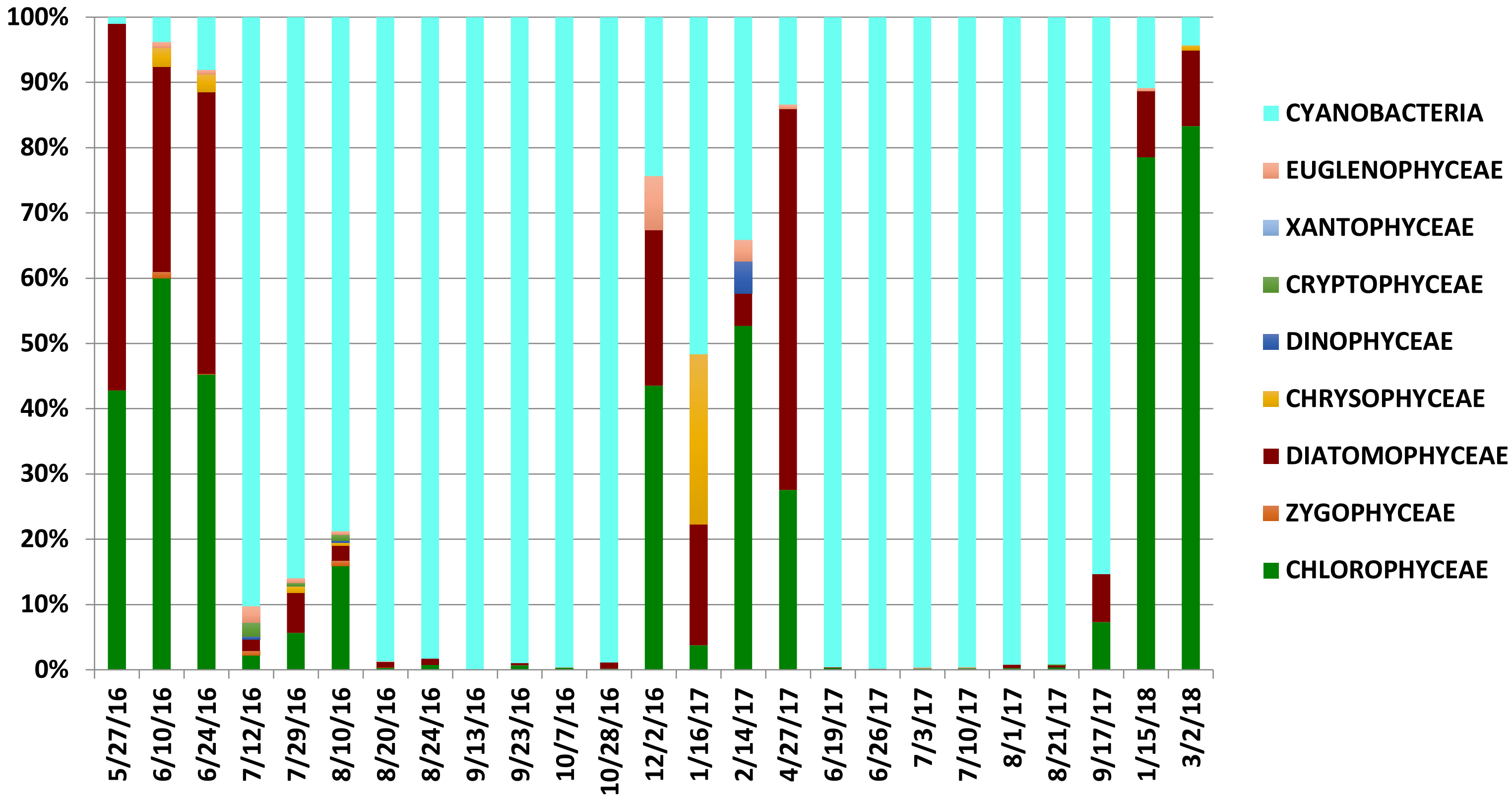
Sentinel species to address changes in cyanotoxin concentrations : filtering bivalves as temporal integrator of cyanotoxin bioavailability

Anodonta anatina sp, (20-30 cm)
Laboratory and multi-site validation

PhD A. Lepoutre 2015-19

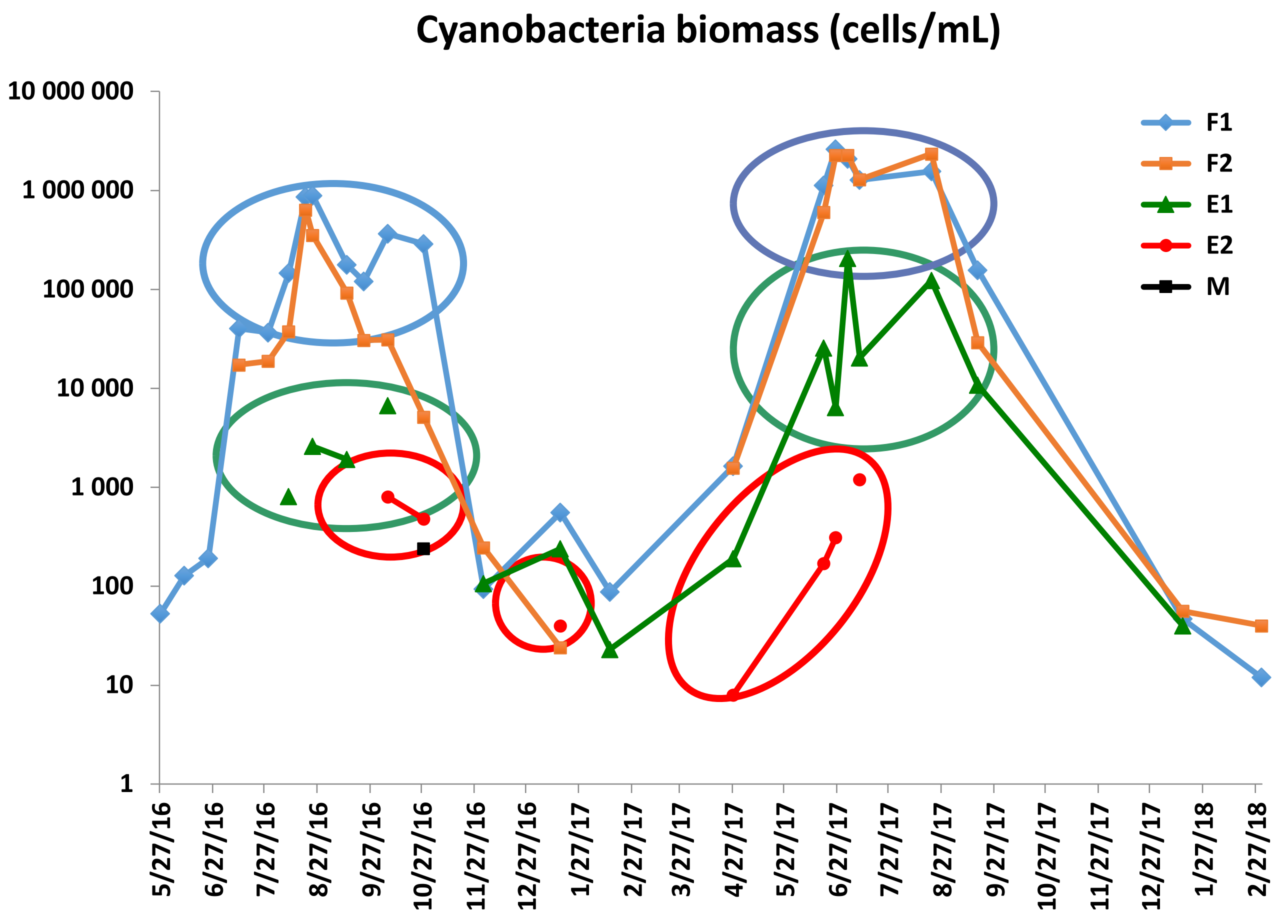
Mytilus edulis (4-6 cm)

Phytoplankton community structure at site F1



- **Cyanobacteria dominance in summer** from July to October 2016 and from June to September 2017

Cyanobacteria population dynamics



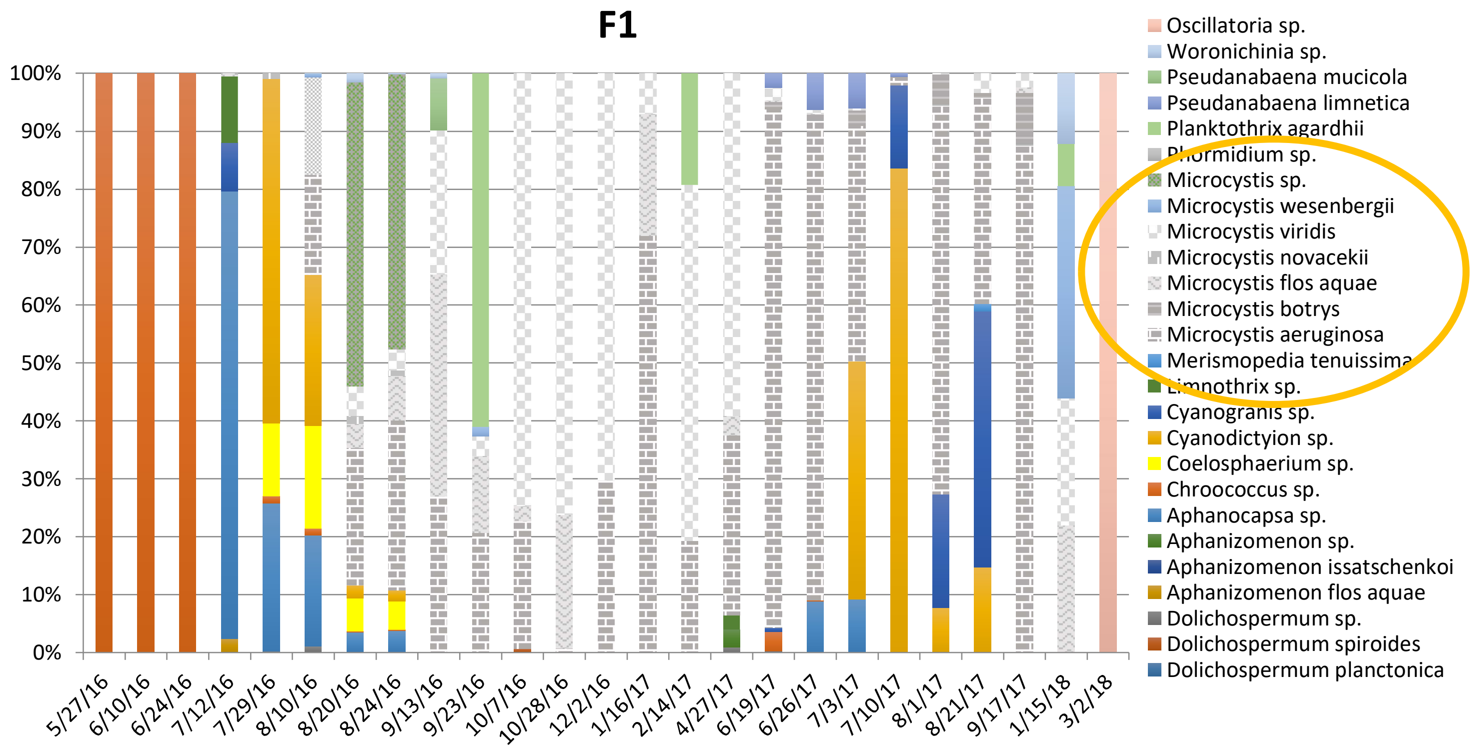
Recurrent summer proliferation at the freshwater sites in 2016 and 2017 with maximum of **2 millions cells/mL**

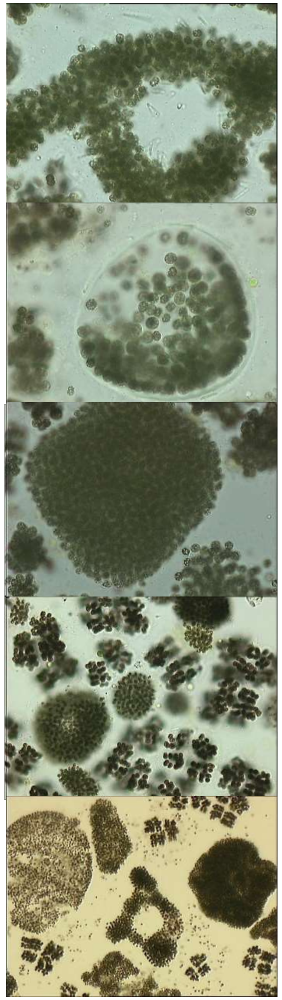
Transfer of cyanobacteria to first estuarine site E1 (over 100 000 cells/mL in July/Aug 2017).
Progressive dilution

Transfer of cyanobacteria to more saline second estuarine site E2, maximum 1000 cells/mL in Sept-Oct 2016 and June-July 2017
Dilution

➢ **Downstream cell densities related to upstream ones : transfer and not *in situ* growth**

Diversity of cyanobacteria





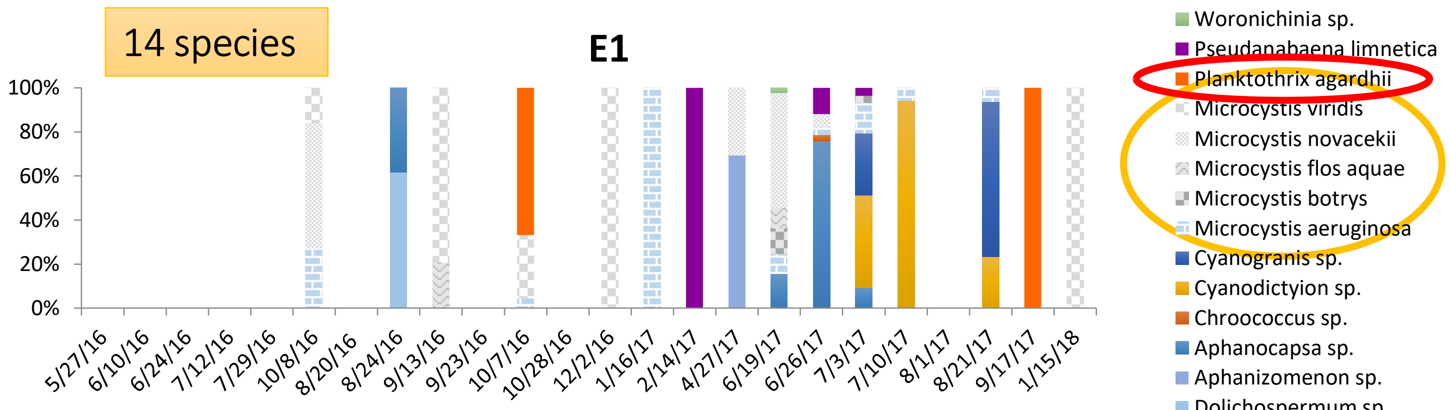
26 species of cyanobacteria at the freshwater site F1

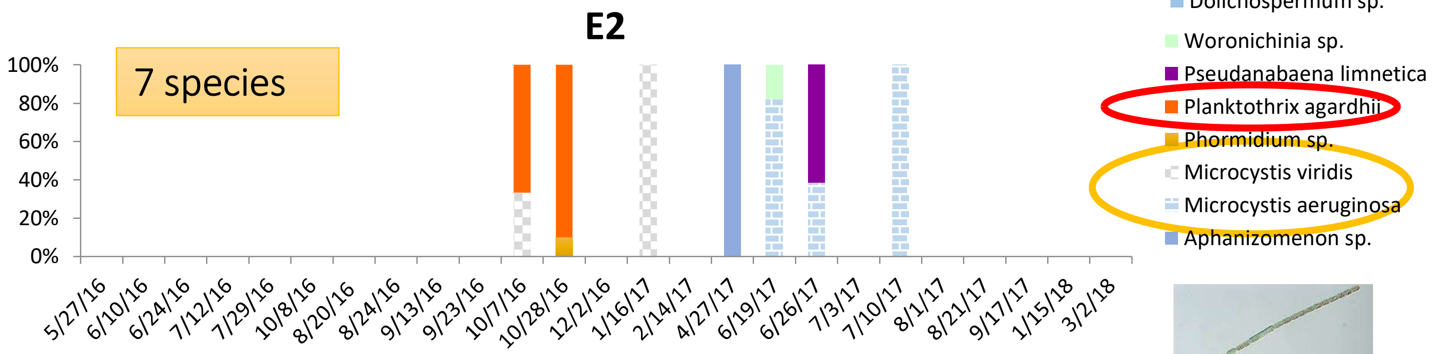
Dominance of *Microcystis sp* (7 species with 3 coexisting)

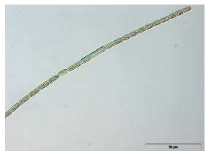
Some of these cyanobacterial species transfered downstream, with a selection

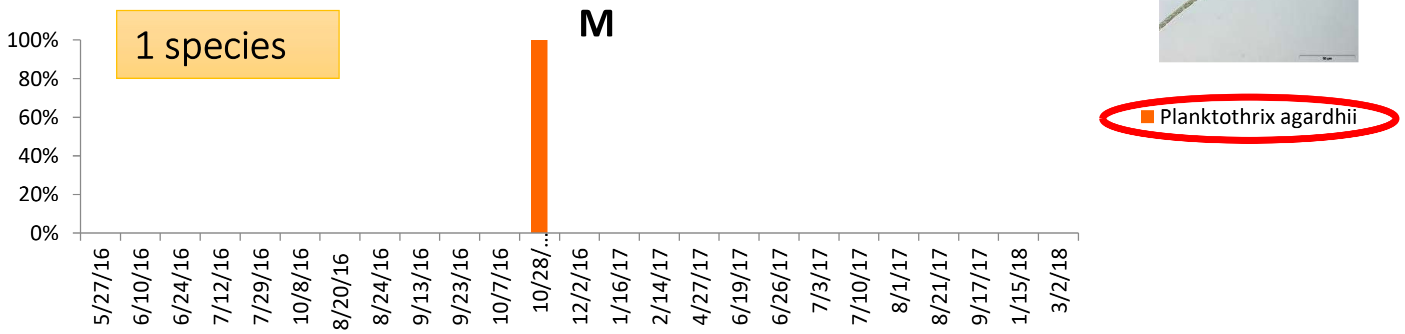
Bormans, M., Zouher, A., Mineaud, E., Brient, L., Savar, V., Robert, E., **Lance, E.**, 2019. Demonstrated transfer of cyanobacteria and cyanotoxins along a freshwater-marine continuum in France. **Harmful Algae**

Diversity of cyanobacteria









Microcystis aeruginosa survive despite the fragility of its colonies

Only *P. Agardhii* observed once in the marine section

Progressive species selection
Possible species resistance to the longitudinal salinity gradient

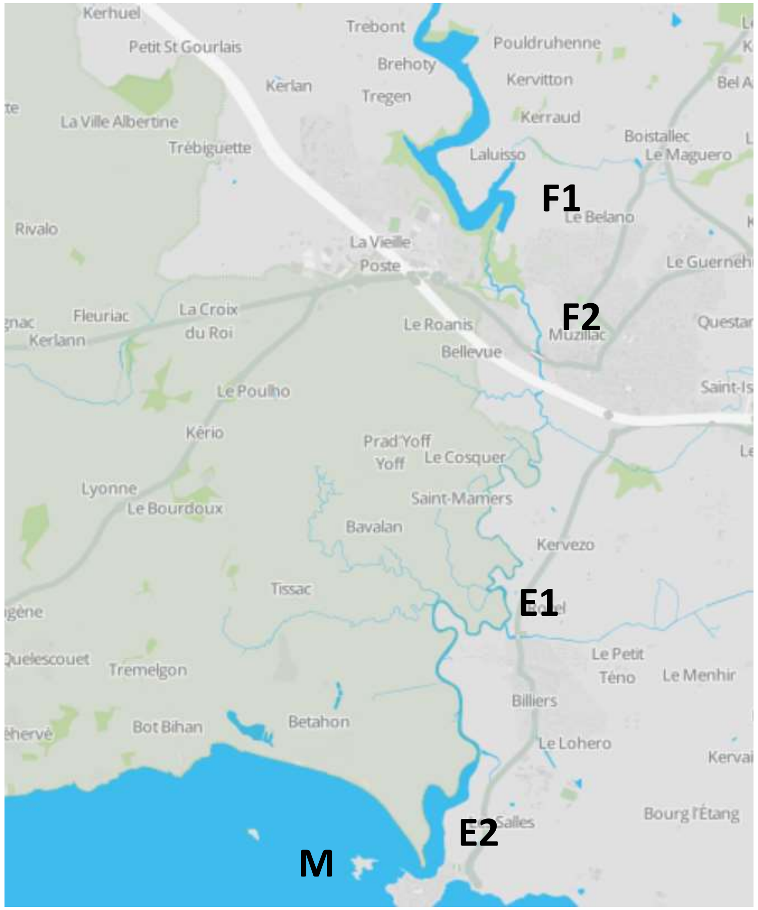
Physiological/morphological parameters controlling species selection currently investigated
PhD thesis MG Des Aulnois, Ifremer

Intracellular microcystins

Frequency of occurrence of intracellular MCs in phytoplanctonic biomass

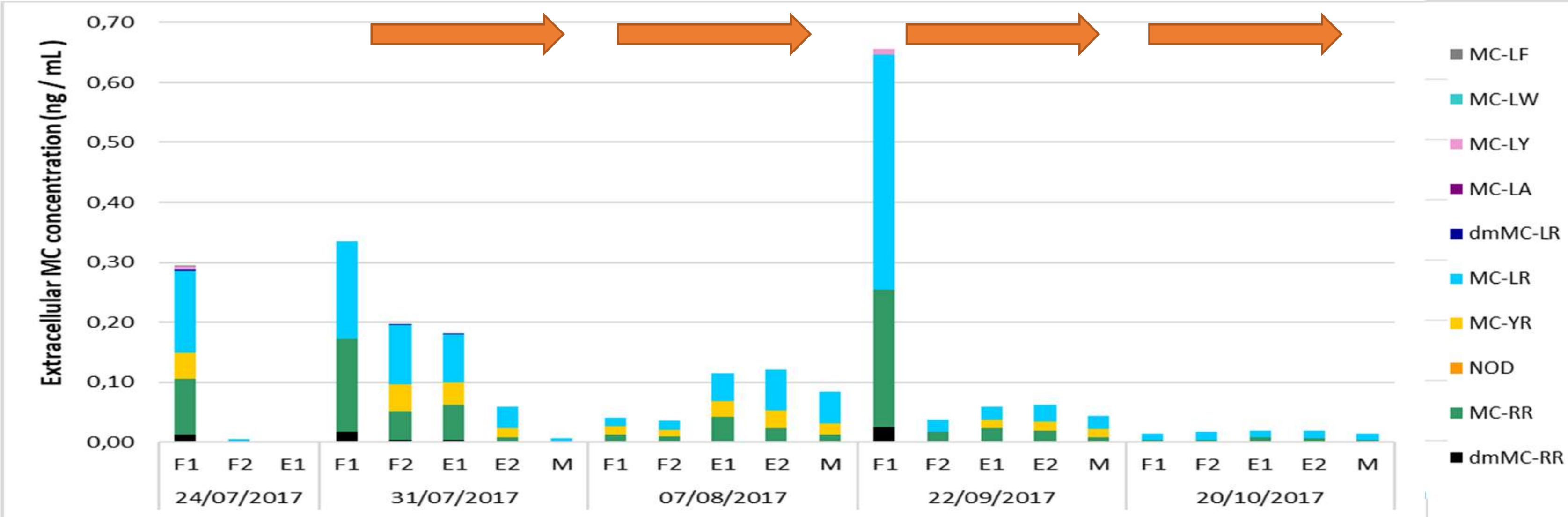
Sites	FO % intracellular MC phytoplankton (June-November)		Max [MC] µg/L
	2016	2017	
F1/F2	**100**	**100**	165
E1	**67**	**87**	1.15
E2	**17**	**38**	0.14
M	**0**	**27**	0.03

- 9 MC variants (mostly MC-LR, MC-YR, MC-RR)

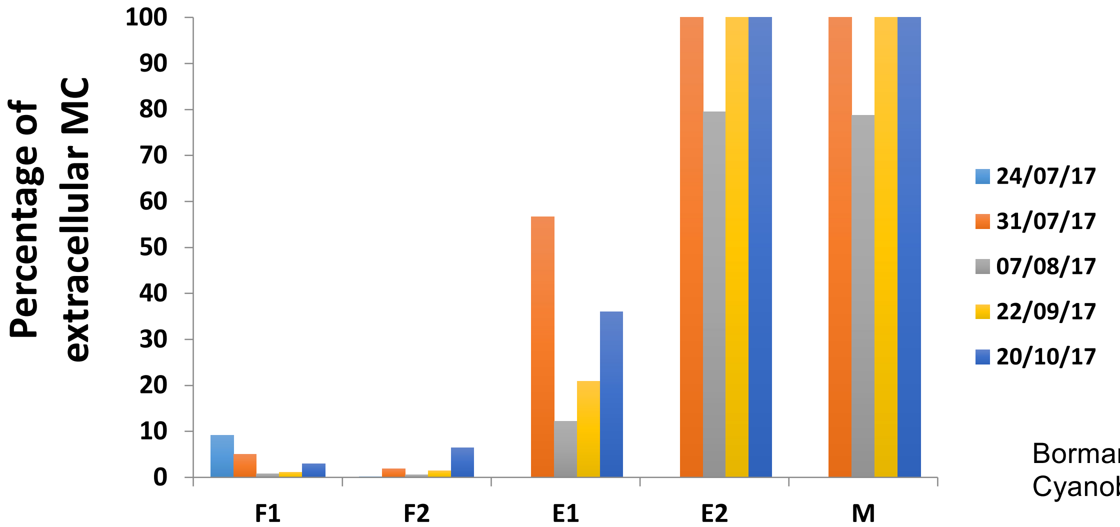


- Transfer of alive cyanobacterial cells producing MCs, with a progressive decrease of intracellular toxin concentration

Extracellular microcystins



Extracellular MC transfer to the estuary and the marine site



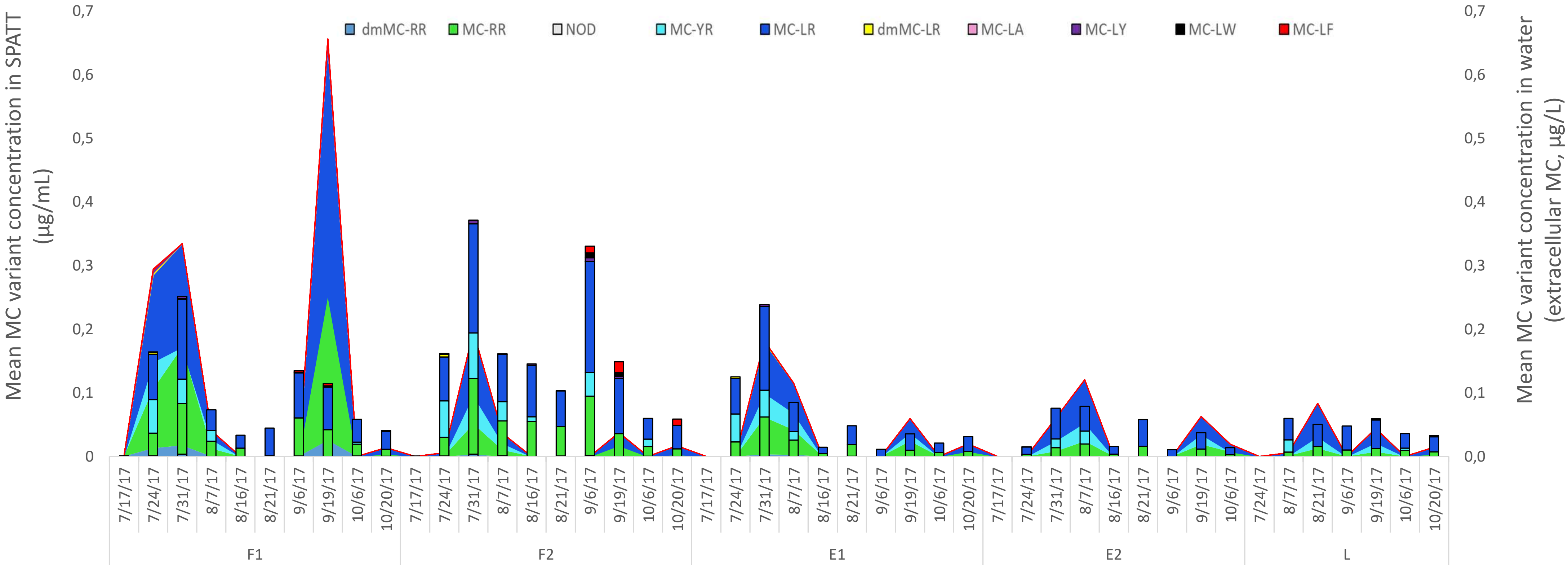
Ratio extracellular / total (extra+ intra) MC : increased along the continuum

Cyanobacterial cell lysing during the transfer

Bormans, M., Savar, V., Legrand, B., Mineaud, E., Robert, E., **Lance, E.**, Amzil, Z., 2020. Cyanobacteria and cyanotoxins in estuarine waters and sediments. **Aquatic Ecology**

Interest of SPATT membrane to reveal the **presence of** dissolved MC

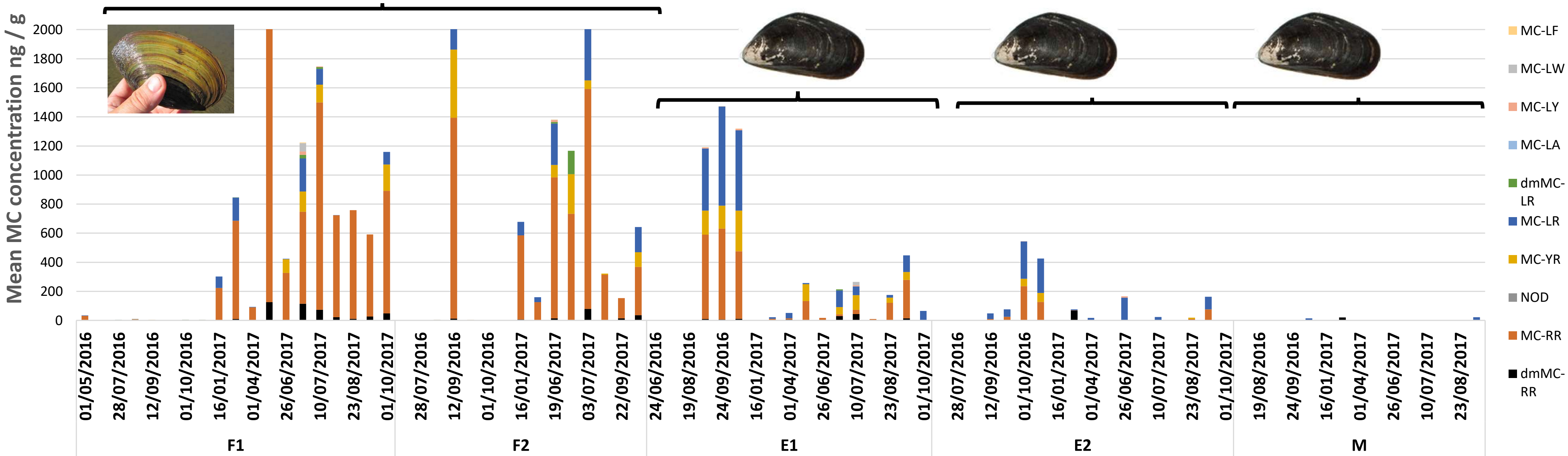
Solid Phase Adsorption Toxin Tracking



Limitations : not quantitative, rapid saturation, temporal integration of few days.

Intracellular and extracellular MCs. Bioaccumulated by mussels?

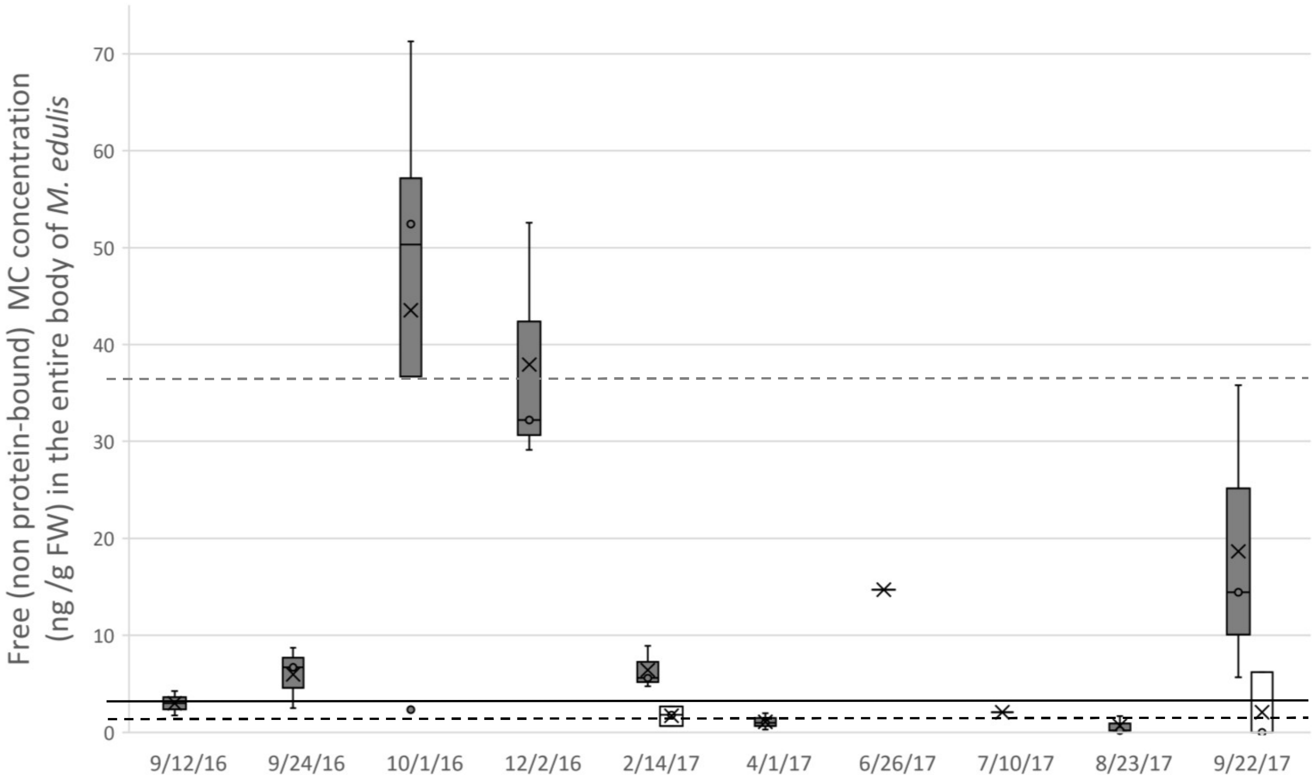
MC bioaccumulation in bivalves



Sites		FO (%)		Mean [MC] ng/g + samples	
		2016	2017	2016	2017
F1	*A. anatina*	85,7	100	7,7	1056,5
F2	*A. anatina*	71,4	100	757,7	856,8

Ability of mussel to integrate MCs between 2 sampling dates and to reveal water contamination levels

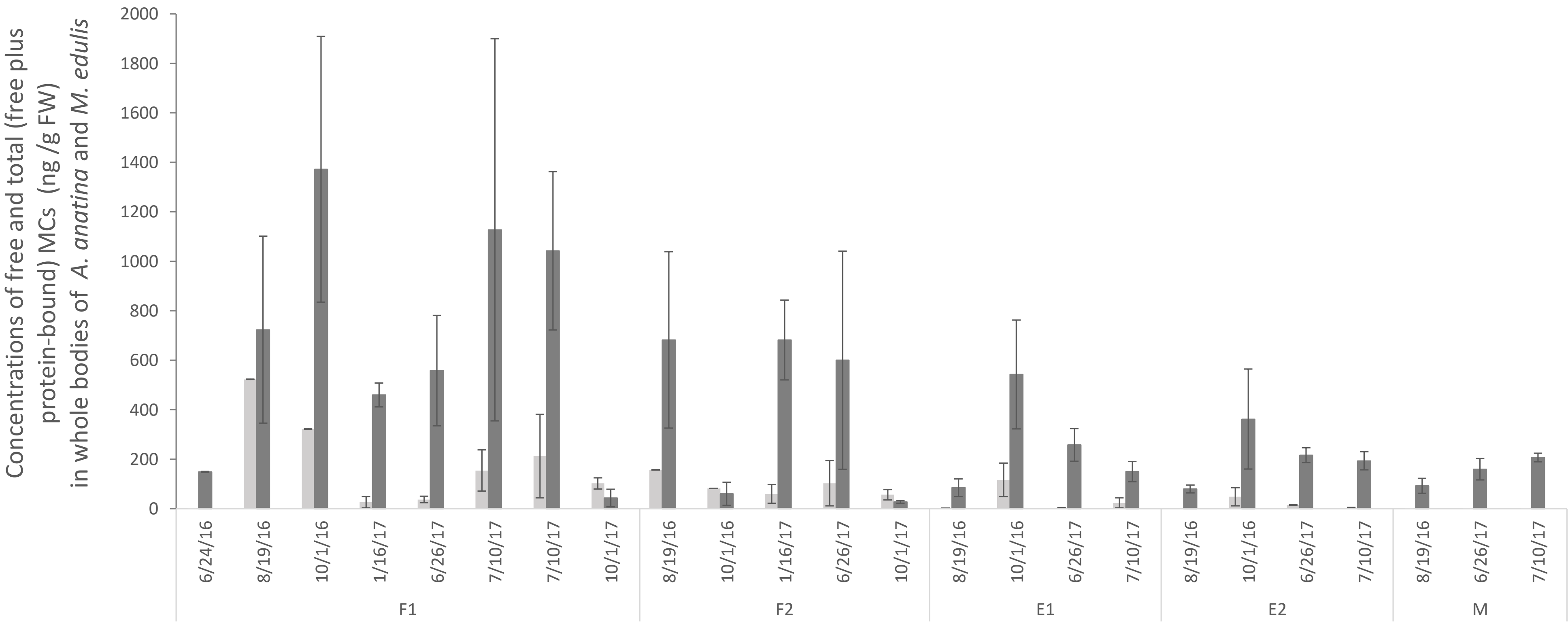
Sites		FO (%) MC bivalve (FO MC phyto)		[MC] ng/g, mean of positive samples	
		2016	2017	2016	2017
E1	*M. edulis*	**57** (67)	**100** (87)	996,0	152,3
E2	*M. edulis*	**57** (17)	**54** (38)	273,7	77,7
M	*M. edulis*	**20** (0)	**27** (27)	14,1	21,3



The safe MC threshold concentrations in mussels were evaluated for low (grey full dash) or high (grey dotted dash) consumers based on the WHO TDI of 40 ng MCs/day/kg, and for low (black full dash) or high (black dotted dash) consumers based on the new French guideline value of ANSES of 1 ng MCs/day/kg BW.

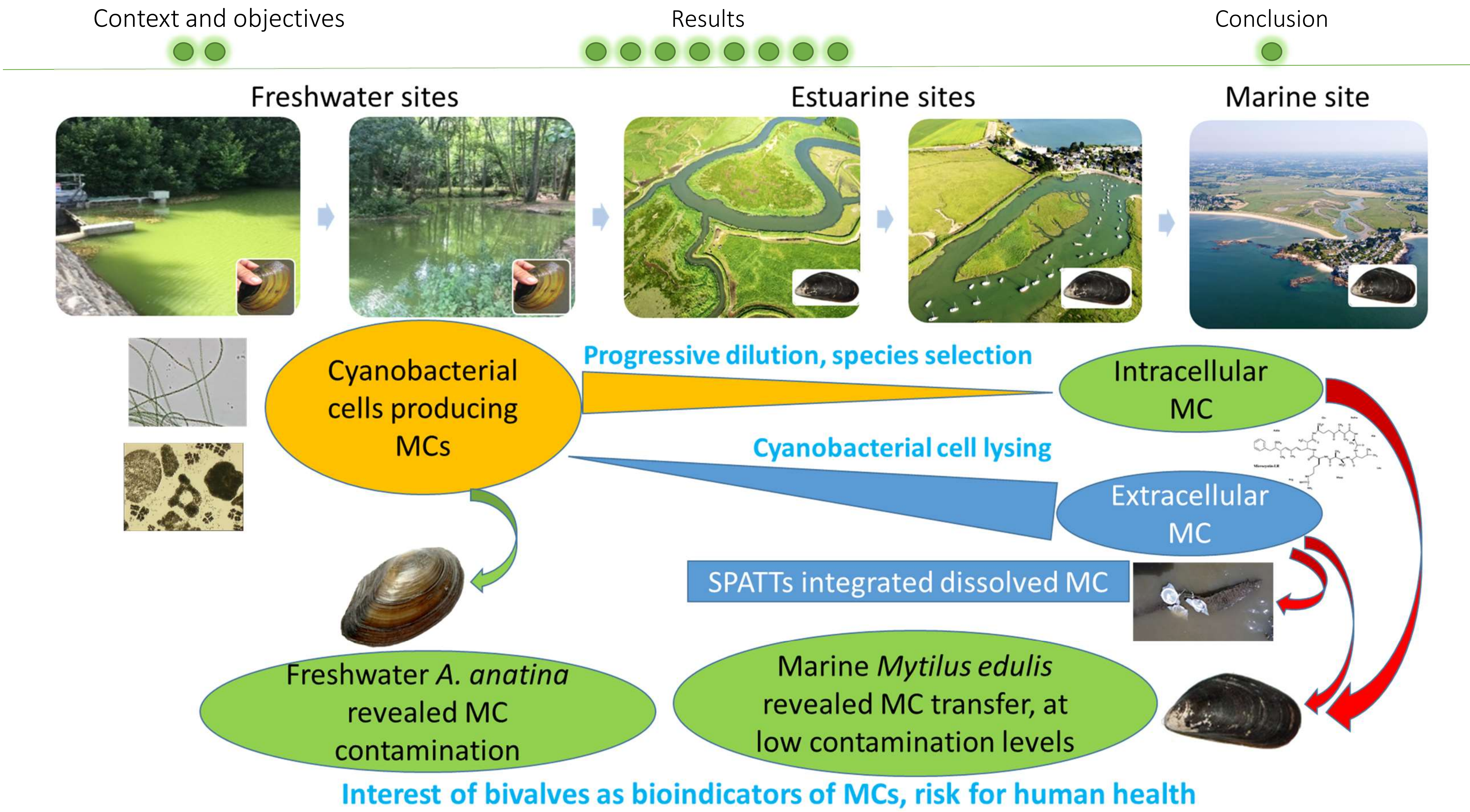
Lance, E., Lepoutre, A., Savar, V., Robert, E., Bormans, M., Zouher, A., 2021. *In situ* use of bivalves and passive samplers to reveal water contamination by microcystins along a freshwater-marine continuum in France. **Water Research**

Free and total MC accumulation in *A. anatina* and *M. edulis*, June 2016 to October 2017



Total MC > free MC : sanitary risk? which bioavailability of protein-bound MC?

Lance, E., Lepoutre, A., Savar, V., Robert, E., Bormans, M., Zouher, A., 2021. *In situ* use of bivalves and passive samplers to reveal water contamination by microcystins along a freshwater-marine continuum in France. **Water Research**


Context and objectives
Results
Conclusion
Freshwater sites
Estuarine sites
Marine site
Cyanobacterial cells producing MCs
Progressive dilution, species selection
Intracellular MC
Cyanobacterial cell lysing
Extracellular MC
SPATTs integrated dissolved MC
Freshwater A. anatina revealed MC contamination
Marine Mytilus edulis revealed MC transfer, at low contamination levels
Interest of bivalves as bioindicators of MCs, risk for human health

THANKS TO FINANCIAL SUPPORT

anses French Agency for Food, Environmental and Occupational Health & Safety

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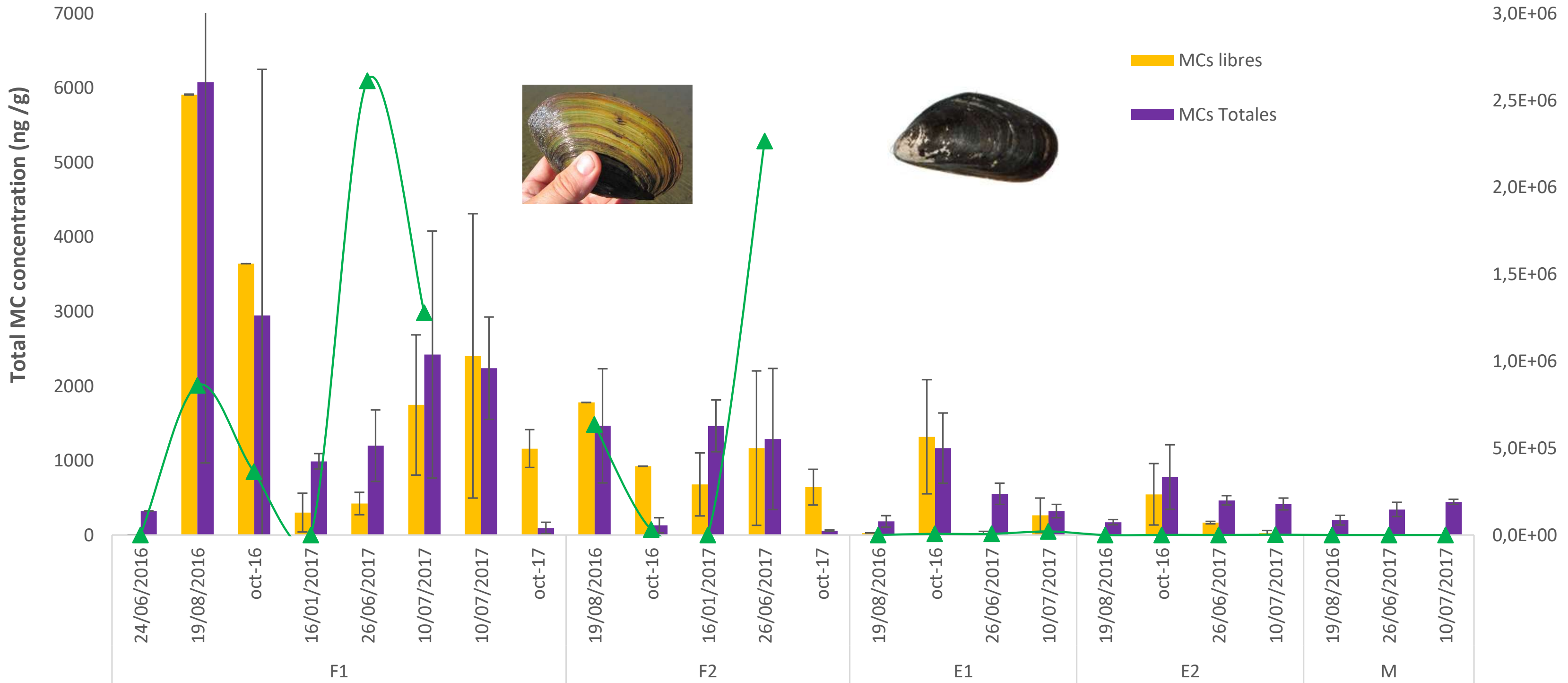
AND THANK YOU FOR YOUR ATTENTION!

Total MC bioaccumulation in bivalves

Sites		**FO MC bivalve** (FO MC phyto)	
		2016	2017
E1	*M. edulis*	**57** (67)	**100** (87)
E2	*M. edulis*	**57** (17)	**54** (38)
M	*M. edulis*	**20** (0)	**27** (27)

FO according to free MC in bivalve tissues probably underestimated
Total MCs = free + protein-bound MCs >>> free MCs at some dates



Mytilus edulis **accumulated extra and intracellular MCs even at low concentration in the medium**