



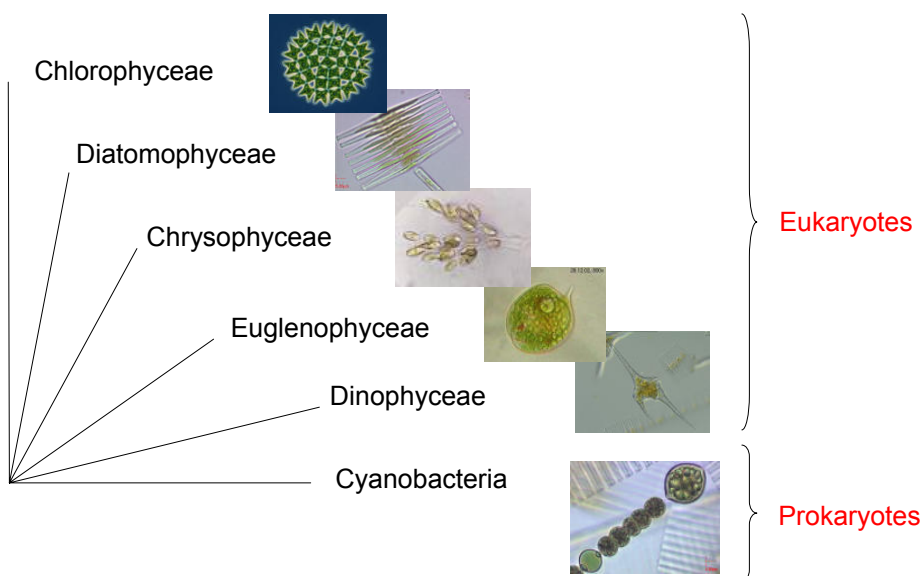
Cyanobacteria ecodynamics and ecotoxicology: an integrative approach applied to water bodies in Paris area

Pr. Cécile Bernard

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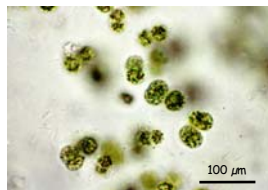
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Microalgae and cyanobacteria



Cyanobacteria

- Prokaryotes, photosynthetic organisms (Chl. a + phycobiliproteins)
- Taxonomy : I. C. Botanical N. / I. C. N. Bacteria
Cyanophytes - Cyanophyceae / Cyanobacteria
160 genus, ≈ 2000 species
- Large morphogenetic diversity



Microcystis aeruginosa



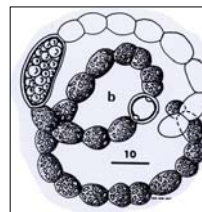
Aphanizomenon ovalisporum

Specific characters

- Motility in the water column : gas vesicles
- Accessory pigments : (allo)phycocyanin, phycoerythrin
- Atmospheric nitrogen fixation: heterocyt
- Resistance forms : akinetes



Competitive characters of cyanobacteria / microalgae



Anabaena flos-aquae



Anabaena solitaria

Cyanobacterial bloom



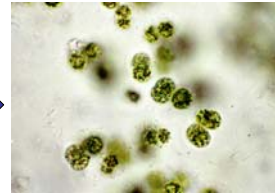
Microcystis sp., Muzillac (France)
Picture © L. Brient



Microcystis sp. (Scotland, UK)
Picture © W. Carmichael



Microcystis aeruginosa, Lac de Villerest (France)
Picture © G. Sarazin



Microcystis aeruginosa

Cyanobacterial bloom



Planktothrix agardhii, Viry-Châtillon (France)
Picture © C. Bernard



Planktothrix agardhii

Cyanobacterial bloom



Cylindrospermopsis raciborskii, Ingazeira (Brésil)
Picture © M. Bouvy



Cylindrospermopsis raciborskii

Environmental impacts

Modification of aquatic ecosystems functioning (Paerl & Huisman PNAS 2008)

- Decrease of the water transparency
- Decrease of biodiversity : microalgae, fish, ...



- Depletion of oxygen: fish killing



Fish death (France)

Economicals impacts

- Visual and olfactive nuisances
- Taste-and-odor compounds:
malodorous or unpalatable drinking water and fish
- Increase treatment costs, loss of aquacultural and recreational revenue
- **Toxins : potent enough to poison animals and humans**



Animals death



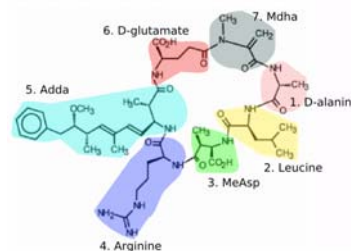
Death of pink flamingo (Kenya)



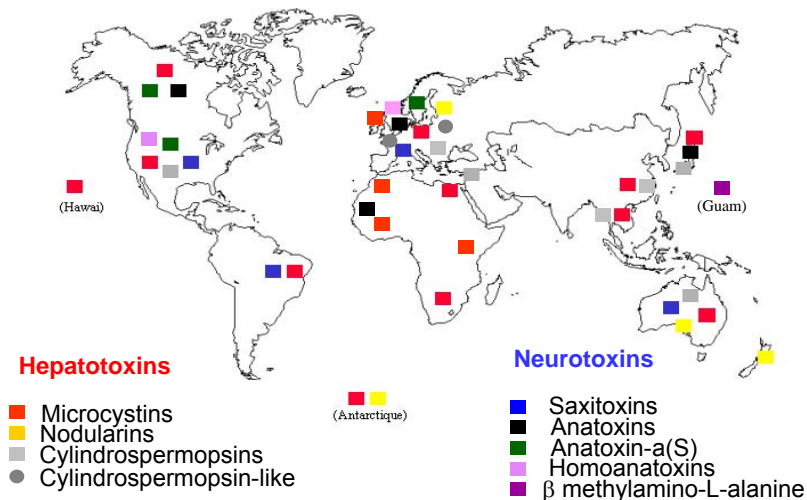
Death of crocodile (Florida)

Health risks ?

- Some cyanoabacteria produce **cyanotoxins** (cf G. Codd presentation)
 - toxicity
 - hepatotoxicity (e.g. microcystins)
 - neurotoxicity (e.g. anatoxin-a)
 - dermatotoxicity (e.g. lipopolysaccharides)
 - chemical structure
 - cyclic heptapeptides, pentapeptides (e.g. microcystins)
 - alkaloids (e.g. anatoxin-a)
 - lipopolysaccharides



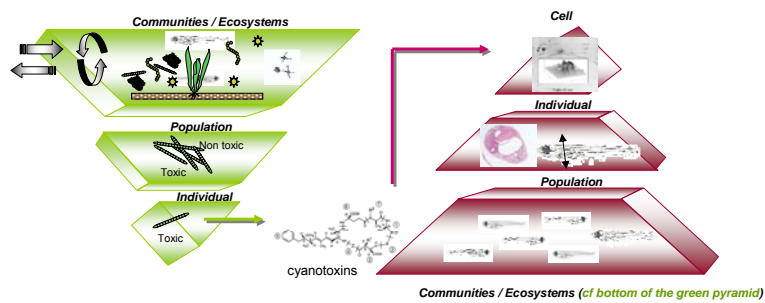
Cyanotoxins occurrence ?

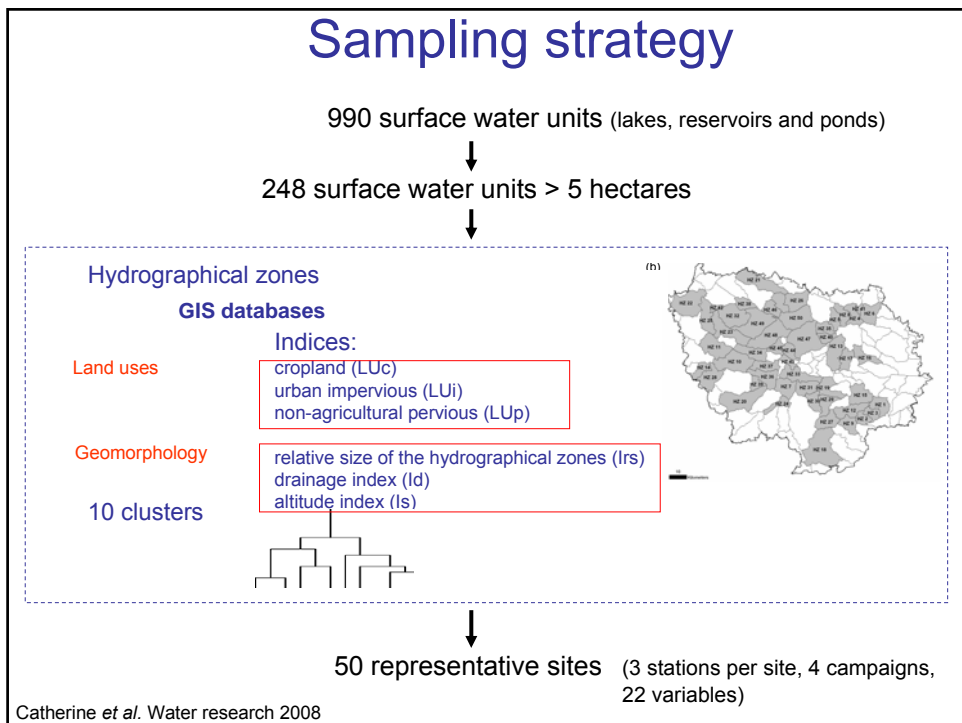
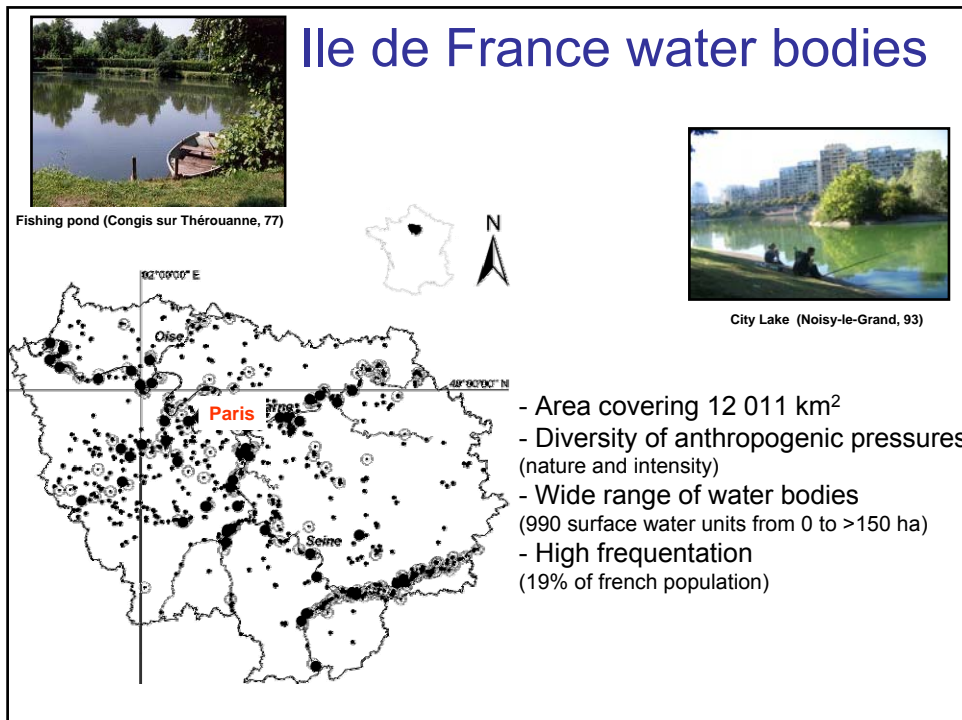


Proliferation of cyanobacterial blooms: causes and consequences ?

- What are the controlling factors responsible for cyanobacterial proliferation ?
- Which processes/mechanisms are involved in responses to cyanotoxins ?

➔ **Integrative approach:** *diversity, dynamics* of cyanobacteria and *toxicology*, in their environmental context





Ile-de-France waterbodies

limnological characteristics

- 48 % of waterbodies not connected to the hydrological network
 - Wide range of phytoplanktonic biomass (< 1 to 357 $\mu\text{g equiv. Chla.L}^{-1}$): 30 % of waterbodies are eutrophic and 42 % hypereutrophic
 - Significant positive relationship found between the total phytoplankton and the cyanobacterial biomasses ($r = 0.849$, $p < 0.0001$, $n = 49$)
 - Significant higher phytoplankton AND cyanobacterial biomasses in waterbodies connected to the hydrographical network
- ➡ Waterbodies connected to the hydrographical network: watersheds with higher areas = higher nutrients inputs

Catherine *et al.* Water research 2008

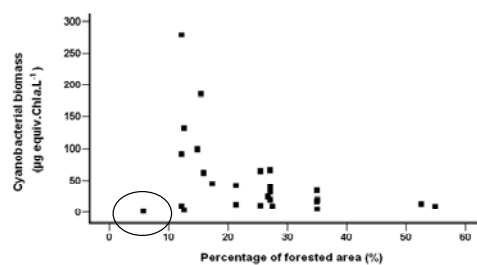
Phytoplankton & cyanobacterial

distribution vs watershed land uses

- All waterbodies: no significant relationship between trophic status and global land use groups
- Connected waterbodies to the hydrological network, significant negative correlation (non-parametric spearman correlation) between:

✓ % forest cover and total phytoplankton
($r_s = -0.589$, $p < 0.01$, $n = 26$)

✓ % forest cover and cyanobacterial biomass
($r_s = -0.497$, $p < 0.05$, $n = 26$)



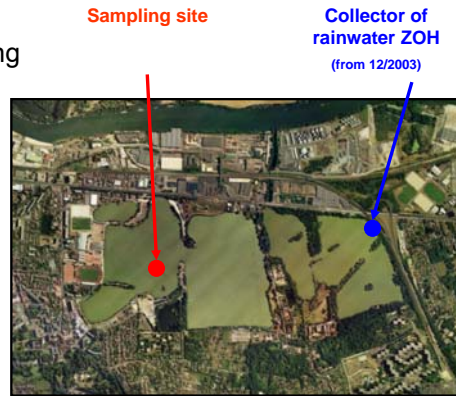
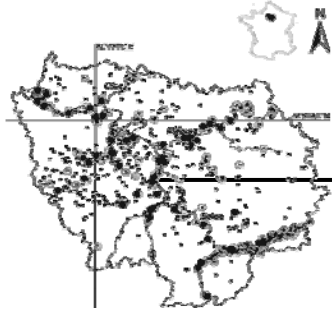
- ➡ Watershed with low impact of human activities generated low nutrient inputs

Catherine *et al.* Water research 2008

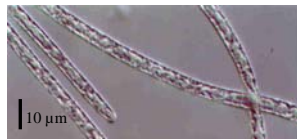
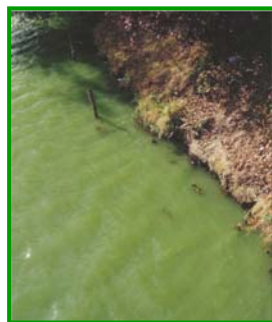
Proliferation of cyanobacterial bloom: causes at a local scale?

Viry-Châtillon Lake worksite

- 96 ha
- 5 connected lakes
- Shallow (mean depth 2.8 m, max. 5.3 m)
- Diffuse and point source inputs (810 ha watershed)
- Connected to the Seine river
- Activities : fishing, sailing, water-skiing



Proliferation of *Planktothrix agardhii* Lake of Viry-Châtillon



In situ survey (2002)

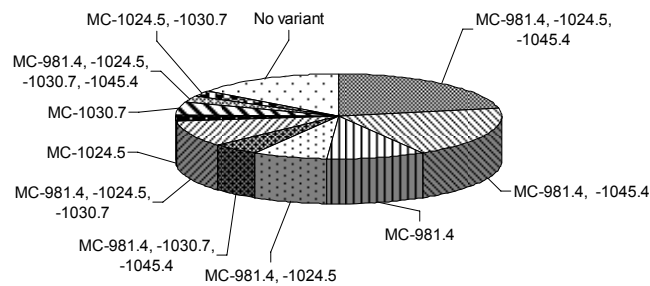
- *Planktothrix agardhii* bloom (95 % of phytoplanktonic biomass)
- 5 variants of microcystins : MC-deRR, MC-RR, MC-YR, MC-deLR, MC-1030.7 *m/z*
- MC concentrations: < 0.05 to 5 μg equiv. MC-LR l⁻¹

Briand et al. Water Research 2002

Diversity of *Planktothrix agardhii* genotypes & chemotypes diversity

Isolation of 64 strains of *P. agardhii* (Paris Museum Collection: PMC)

- genotypes diversity: *mcyB*⁺ and *mcyB*⁻
- 16 MC chemotypes (MC-*m/z*)



Briand et al. Water Research 2002; Yéprémian et al. Water Research 2007

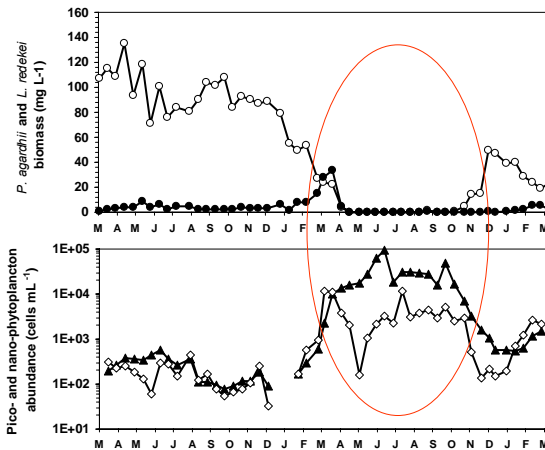
Dynamics of *Planktothrix agardhii* : controlling factors ?

National programm ECODYN (03-2004 → 03-2006)

- 22 variables : **meteorological** (T°C water, T°C air, rainfall amounts, light...)
physico-chemical (NH₄, NO₃, SRP, total N, total P, O₂...)
biological (phytopk, cyanos., zoopk, toxins conc....)
- 48 sampling dates over 2 years (every two weeks)
- 3 replicate samples were taken 0.5 m below the water surface at a distance of ≈ 3 m.

Dynamics of *P. agardhii*

relationship between *P. agardhii*, pico- and nanophytoplankton

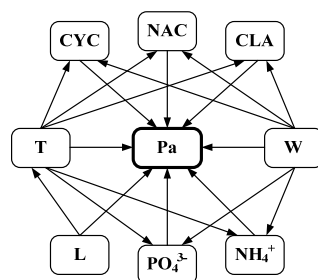


- Significant **decreasing trend** in *P. agardhii* biomass (65% of the overall variance)
- Remarkable **negative relation** between *P. agardhii* biomass and pico- and nano-phytoplankton abundances

Catherine *et al.* FEMS 2007

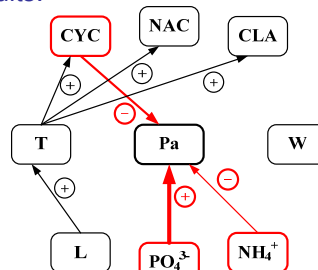
Controlling factors (path analysis modeling)

Hypothesis:



T = temperature;
L = solar radiation;
W = wind speed;
NH₄⁺ = ammonium concentration;
PO₄³⁻ = orthophosphate concentration;
NAC = copepod nauplii abundance;
CYC = cyclopoid copepod abundance;
CLA = cladocerans abundance

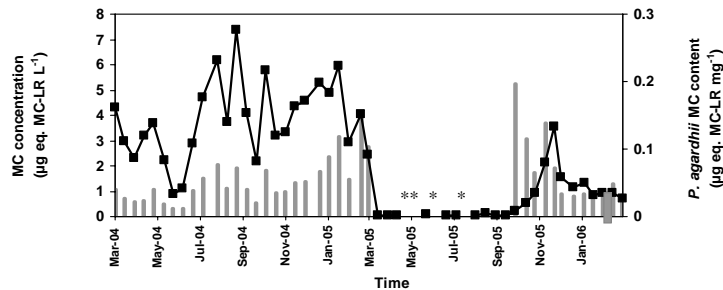
Results:



- Path-analysis model explained **66%** of *P. agardhii* biomass changes
- Decreasing trend in *P. agardhii* biomass was significantly related to a **decrease in the PO₄³⁻ concentration**
- Residual component was related to **zooplankton dynamics** (cyclopoid abundances)

Catherine *et al.* FEMS 2007

Dynamics of MC concentration



- 49 % of changes in MC concentration could be explained by *P. agardhii* biomass changes

- Opposite trends existed between MC content per *P. agardhii* biomass unit and biomass: highest toxin content when *P. agardhii* biomass is low

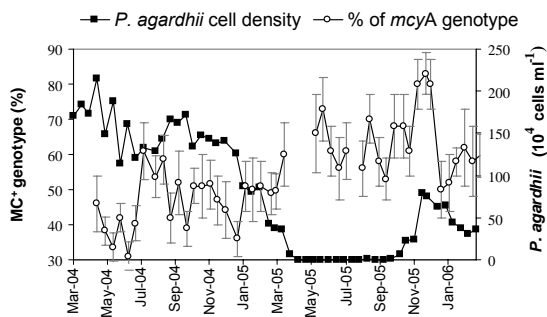
➡ Changes in % of MC-producing and non-MC-producing subpopulations
Changes in the physiological status of cells

Catherine et al. FEMS 2007

Dynamics of MC concentration:

P. agardhii sub-populations

- The relative proportions of potentially MC-producing clones in the *Planktothrix* population were estimated by quantitative real-time PCR
MC⁺ sub-population and whole *P. agardhii* population



- Variation of MC⁺ sub-population proportion between 31 to 83 %

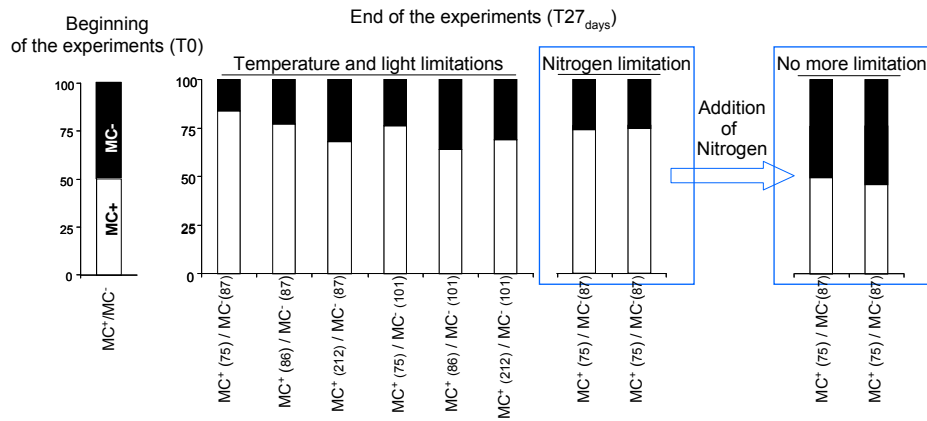
- When total biomass decrease, MC⁺ sub-population increase

➡ MC⁺ sub-population better adapted to adverse conditions than MC⁻ ?

Briand et al. AEM 2008

MC production: controlling factors (*in vitro*)

- Limiting growth conditions (e.g. light, T°, nutrients)
 - low cell growth rate
 - low MC production rate
 - dominance of MC⁺ genotype in co-culture



Briand *et al.* Environmental Microbiology 2008

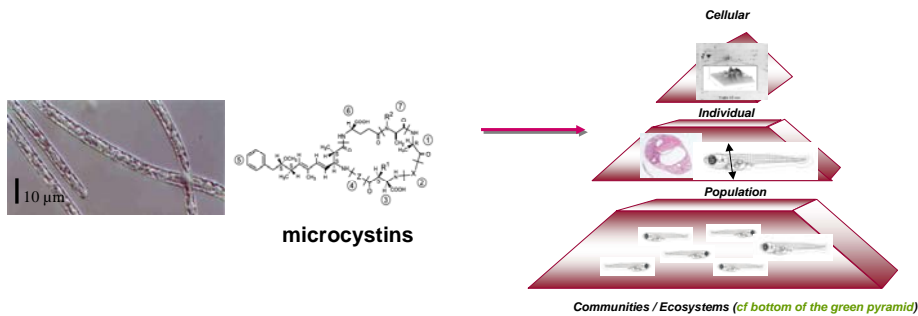
Controlling factors of *P. agardhii* and microcystins ?

- MCs production is controlled by several biological processes:
 - indirect: presence/absence of potentially toxic cyanobacteria
 - biomass dynamics
 - genotypes selection
 - direct: growth rate
 - toxin production rate

➡ all being dependent of environmental factors

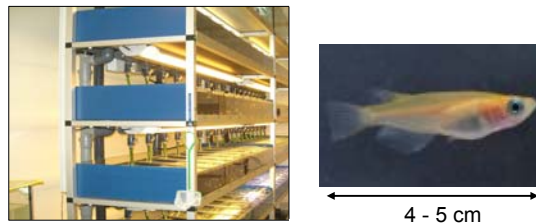
Proliferation of cyanobacterial bloom: consequences ?

Integrative approach: *diversity, dynamics* of cyanobacteria and *toxicology* in an environmental context



- Main target in vertebrates: liver
- Symptoms: vomiting, diarrhea, fatigue, hepatic bleeding
- Tumor promoter in rodent (Dawson, 1998; Codd et al., 1999)

Model in Environmental Toxicology: Medaka, *Oryzias latipes*



- Easy breeding
- Model in oncology, developmental biology and toxicology (OCDE, 2006)
- Commonly used in biological risk assessment programs of aquatic contaminants
- Sequenced genome (Kasahara et al., 2007, *Nature*)

Effects of microcystins on fish embryonal toxicity

- Introduction of pure toxin (MC-LR) and cyanobacterial extracts from *Planktothrix agardhii* strains (MC⁺ & MC⁻) directly into the vitellus of late neurula embryos (stage 19) of medaka

- Embryonal toxicity:

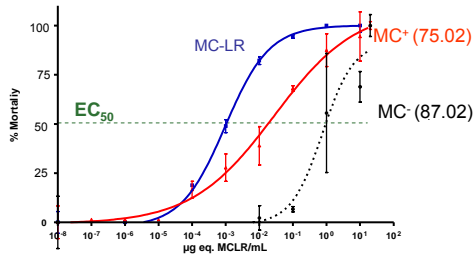
MC-LR (EC₅₀ = 1.93 fg per egg)

> **MC⁺ strain** (EC₅₀ = 32 fg MC-LR eq. per egg or 7.8 µg CE ml⁻¹)

> **MC⁻ strain** (EC₅₀ = 460 µg CE ml⁻¹)



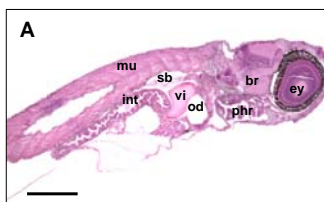
by microinjection (2 nl)



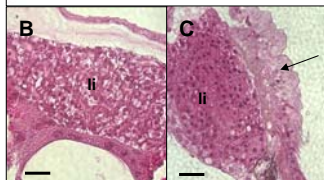
CE : Crude extract

Lecoz *et al.* Toxicon 2008

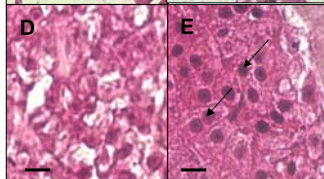
Effects of microcystins on fish embryonic toxicity



- Light microscopy sagittal sections (A), and transverse sections (B–E) of hatched (day 11 pf) **control medaka embryos (A, B, D)** and **embryos injected with 10 µg CE mL⁻¹ of the *P. agardhii* MC⁺ strain extract (PMC 75.02) (C, E).**



- Clair unstained zones in hepatocytes referred as normal glycogen accumulation in control (D) and the **loss of these areas in extract-injected embryos (arrows in E).**



Scale bars: 250 µm (A), 100 µm (B, C), 20 µm (D, E).

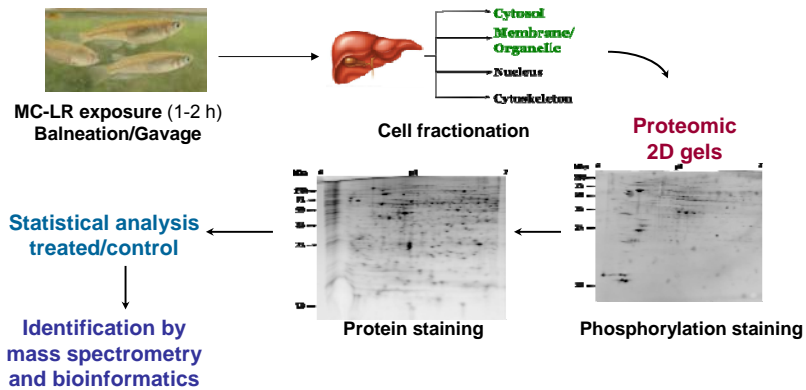
Abbreviations:

br, brain; ey, eyes; int, intestine; li, liver; mu, muscle; od, oil droplet; phr, pharyngeal region; sb, swimbladder; vi, vitellus

Lecoz *et al.* Toxicon 2008

Effects of microcystin-LR on fish toxicoproteomics

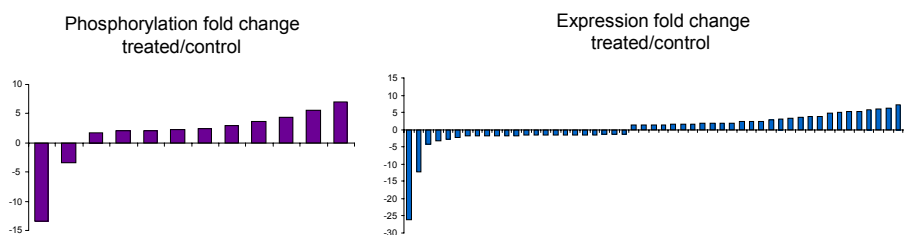
- Trying to better understand microcystin-LR actions: global proteomic approach for assessing **expression and phosphorylation variations** of liver proteins



Mehzoud *et al.* Aquatic Toxicology 2008, Toxicon 2008

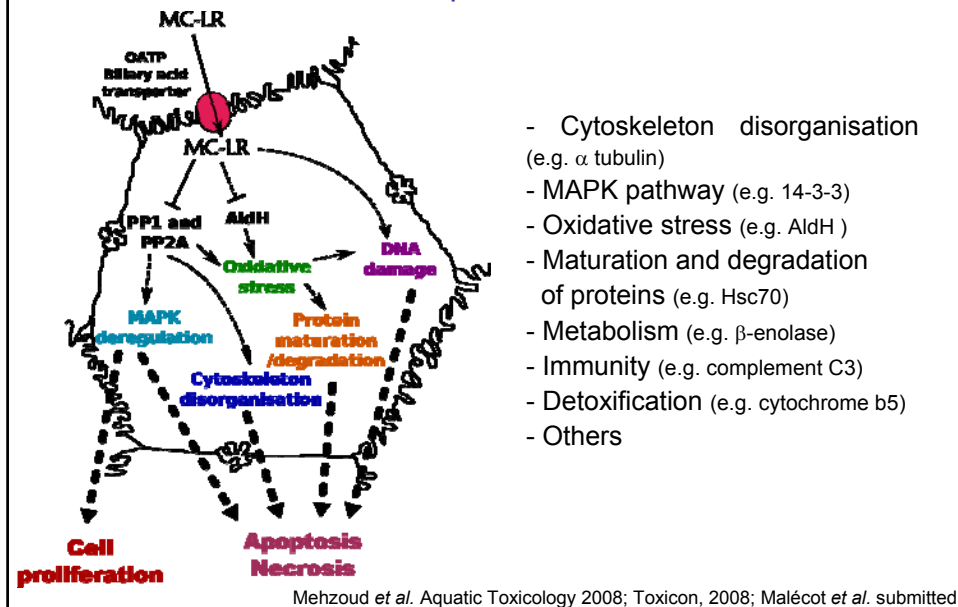
Expression and phosphorylation:

- Maps of liver cells of animals exposed (balneation or gavage) or non-exposed to the cyanotoxin showed modifications either of i) **protein phosphorylation**, ii) **total protein** expression or both iii) **protein expression & phosphorylation**
- **131 spots** showed a significant increase or decrease of their stain signal either in specific phosphoprotein stain or total protein stain
- **40** of these proteins have been **identified by mass spectrometry**



Mehzoud *et al.* Aquatic Toxicology 2008; Toxicon, 2008; Malécot *et al.* submitted

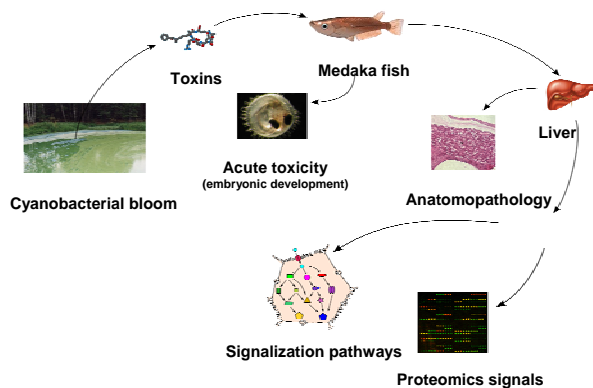
Effects of microcystin-LR on fish toxicoproteomics



- Cytoskeleton disorganisation (e.g. α tubulin)
- MAPK pathway (e.g. 14-3-3)
- Oxidative stress (e.g. AldH)
- Maturation and degradation of proteins (e.g. Hsc70)
- Metabolism (e.g. β -enolase)
- Immunity (e.g. complement C3)
- Detoxification (e.g. cytochrome b5)
- Others

Effects of microcystins on medaka fish

- **Acute toxicity** of *P. agardhii* extracts producing MCs
- Embryonic developments **abnormalities**
- Toxicoproteomics:
 - **Early affected pathways**: oxidative stress response and protein maturation/ degradation leading to apoptosis
 - Develop **diagnostic tools** to monitor water resources



Conclusions

- Controlling factors of MCs production:
 - multi-factorial
 - at different biological regulation levels
 - integration of knowledge (deterministic modeling ?)
 - ➡ to better control the causes
- Functional roles of MCs
 - intracellular roles ?
 - interactions with other microorganisms ?
 - ➡ to better estimate the ecological consequences
- Toxicological effects of MCs ?
 - “universality” of responses mechanisms to MCs ?
 - diversity of responses as function of toxins diversity
 - ➡ to better evaluate the health risks



Ecosystèmes et interactions toxiques
USM505/EA4105, Département RDDM, MNHN



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Ecosystèmes et interactions toxiques
USM505/EA4105, Département RDDM, MNHN



Thanks for your attention

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