Muséum National d'Histoire Naturelle – Paris
http://www.mnhn.fr/mcam/

Effect of cyanotoxins on fish

Pr. Cécile Bernard

National Museum of Natural History – Paris

cbernard@mnhn.fr

MNHN Cyanobacterial team

http://www.mnhn.fr/mcam/CCE

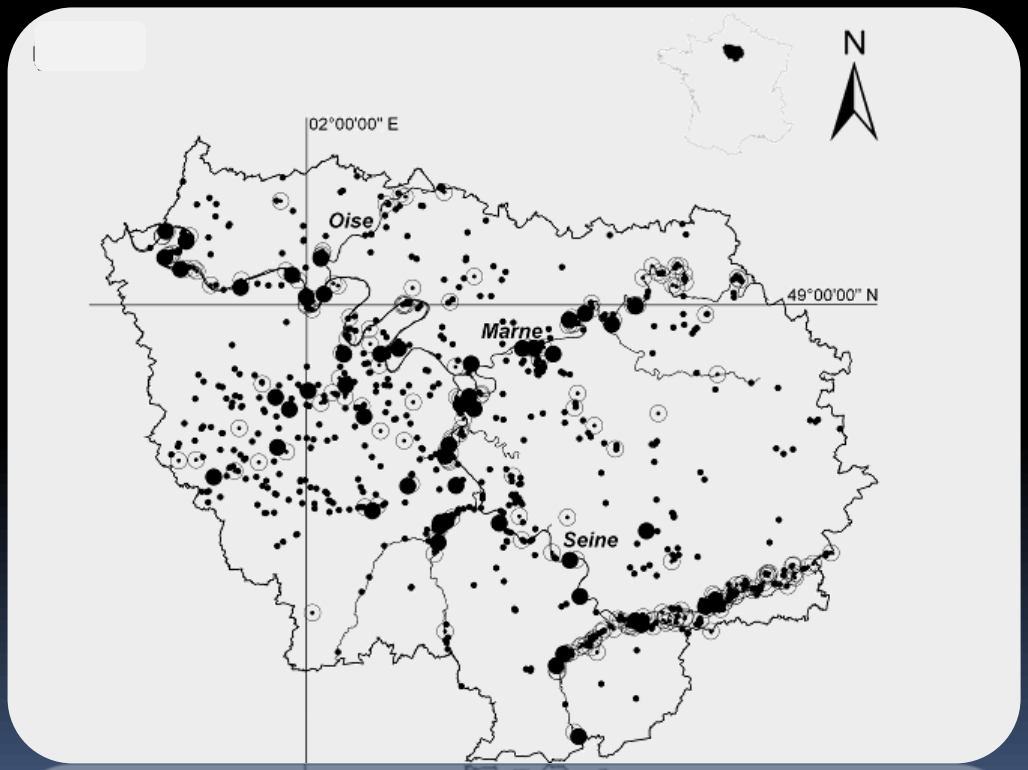
- What are the controlling factors responsible for cyanobacterial toxin production?

- Which processes/mechanisms are involved in responses to cyanotoxins exposure?

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

Ile de France water bodies

City Lake (Noisy-le-Grand, 93)



- Diversity of anthropogenic pressures (nature and intensity)

- Wide range of water bodies (990 surface water units from 0 to > 150 ha)

- High frequentation (19% of French population)

- Regional scale = management and decisions scale

Ile de France and cyanotoxins

Among the 50 sampled water bodies:

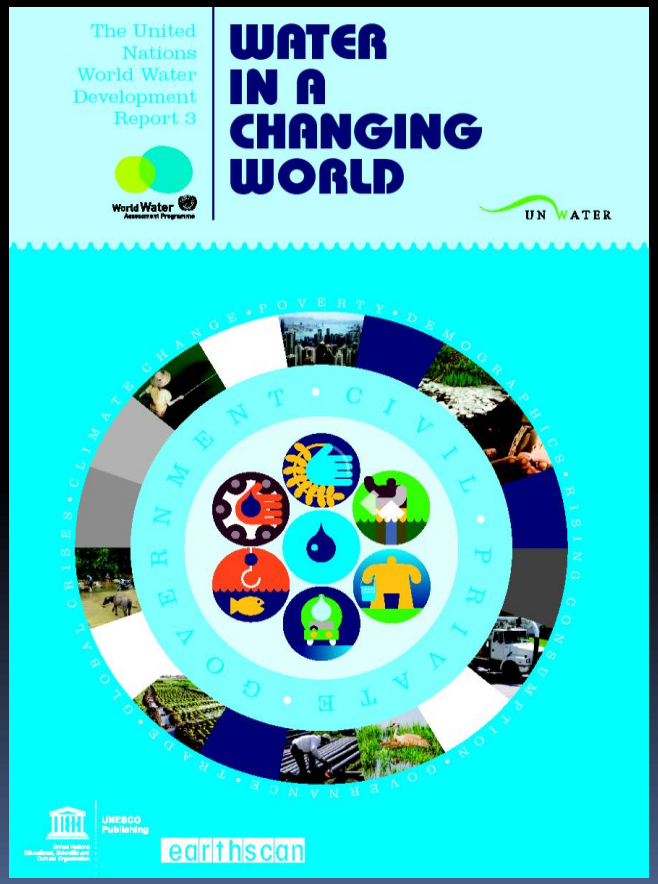
- 15 water bodies with cyanobacterial blooms + MCs
- 2 water bodies with cyanobacterial blooms + STXs (1rst occurrence in France, *Aphanizomenon gracile*)
- 1 water bodies with cyanobacterial blooms + MCs + STXs

Ledreux *et al.* 2010; Catherine *et al.* 2010

Cyanobacterial bloom

- Increase of occurrence, dominance and bloom of potentially toxic cyanobacteria (Huissman, 2009; Ledreux et al. 2010)

- Among the main causes: global (e.g. temperature increase) and local anthropogenic (e.g. eutrophication increase) growing pressures on aquatic ecosystems



Cyanobacterial bloom (Ile de France)

Planktothrix agardhii

10 µm

- Lake « Base Nautique Viry »

- MC concentrations (n = 24)

  mean. 3.5 µg equiv. MC-LR l^{-1}

microcystins *m/z*

981.6 (Asp^{3}MC-LR)

1024.8 (Asp^{3}MC-RR)

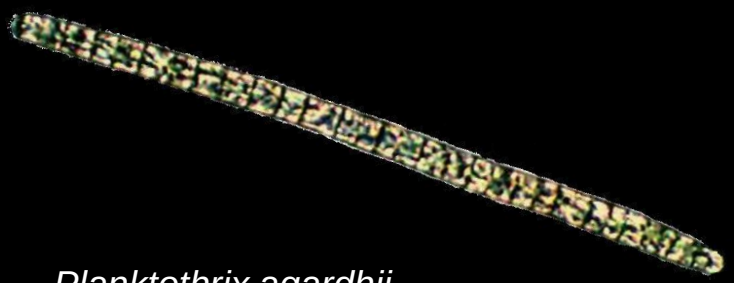
1030.7

1038.6 (MC-RR)

1045.6 (MC-YR)

Briand et al. Water Research 2002; Yéprémian et al. 2007; Catherine et al. 2008

Cyanobacterial bloom (Ile de France)



Planktothrix agardhii

10 µm

- Lake « Grande Paroisse »

- microcystins all the year

mean. 5.2 µg equiv.MC-LR l^{-1}

microcystins *m/z*

981.6 (Asp^{3}MC-LR)

1024.5 (Asp^{3}MC-RR)

1031.6

1042.6

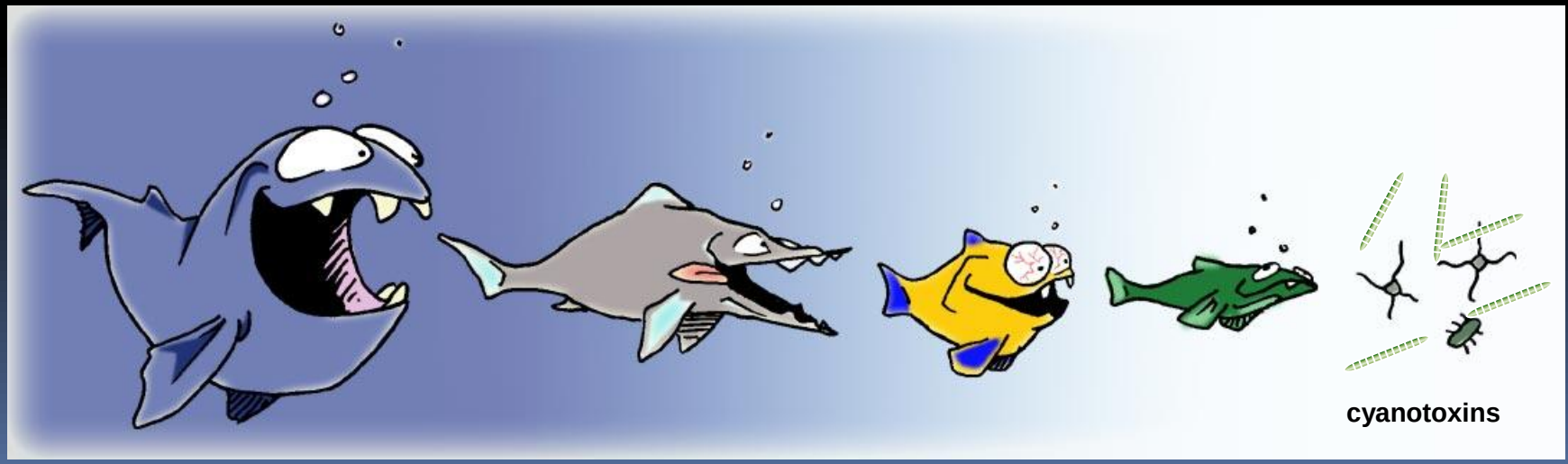
1045.5 (Asp^{3}MC-HtyR)

1097.0 (MC-YR)

Proliferation of cyanobacterial blooms

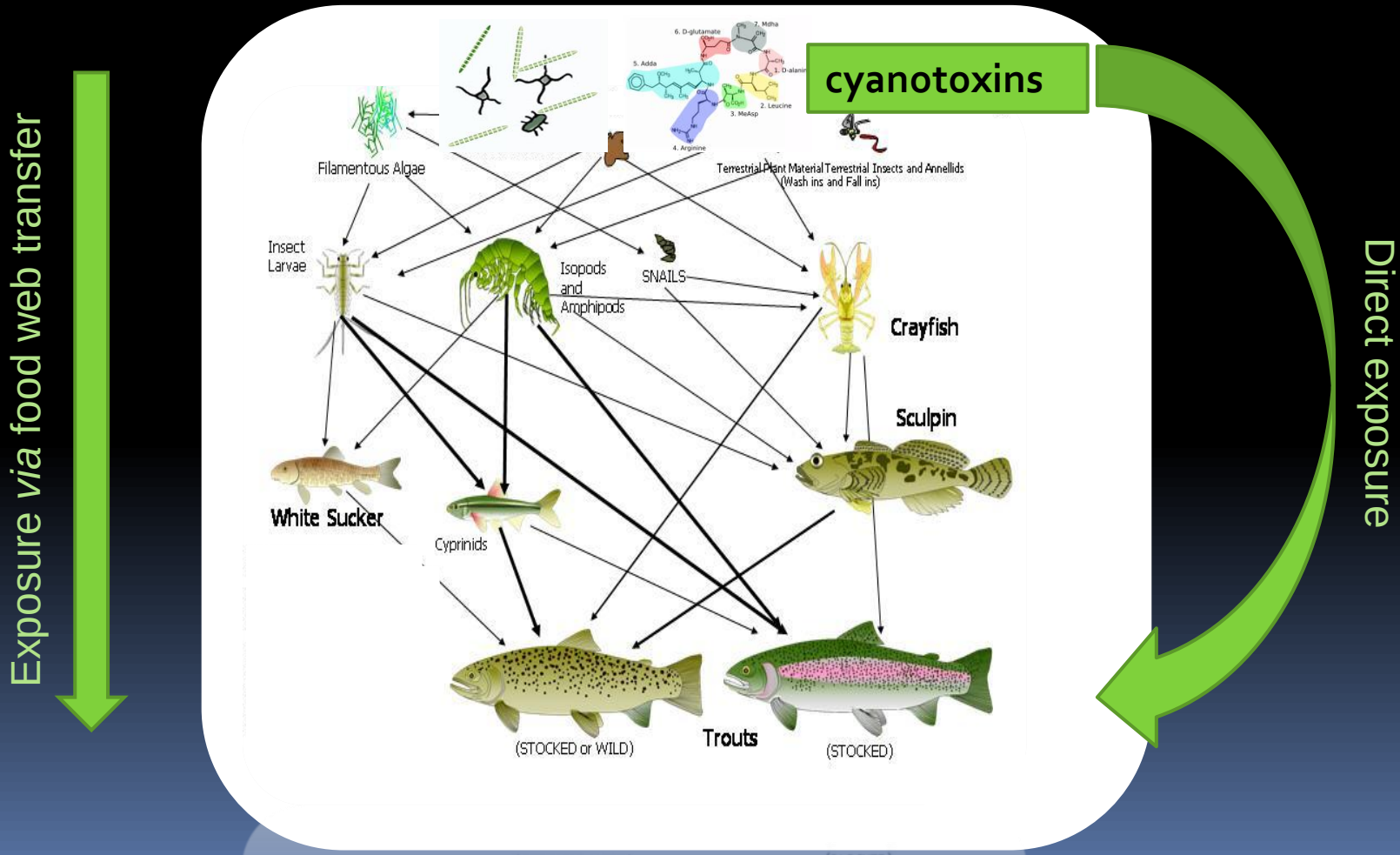
- Cyanobacteria, cyanotoxins in water bodies may have:
  - ➢ Indirect effect on ecosystem functioning (e.g. biomass, dominance of cyanobacteria …)
  - ➢ Direct effect of toxins

- What are the effects of cyanotoxins on fish ?

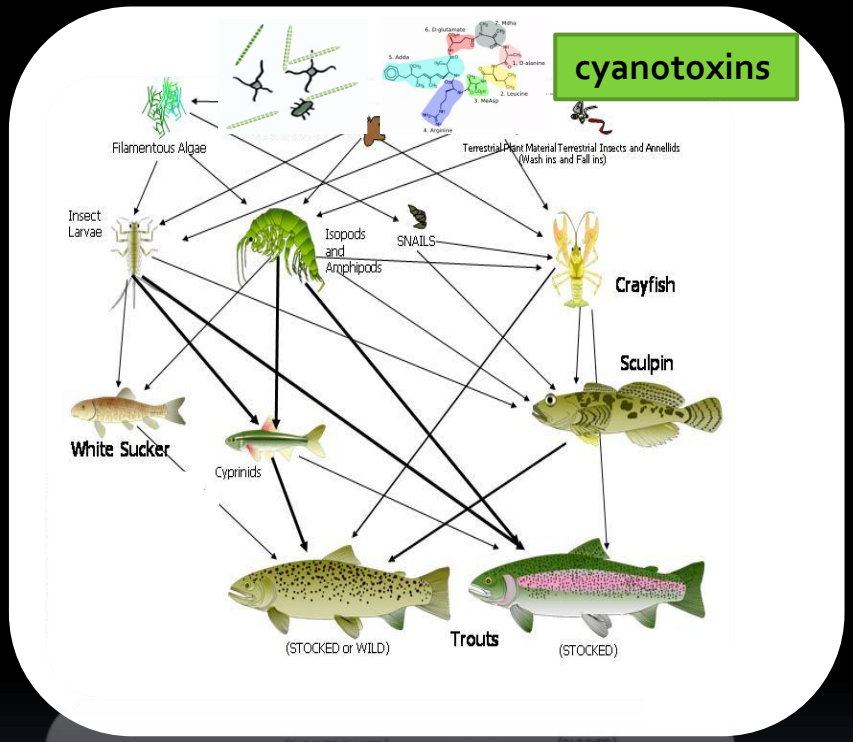


Effects of cyanotoxins on fish

- Two ways of exposure:
  - i) direct contamination = acute or chronic toxicity
  - ii) through the food web: modifications of biological interactions, functional alterations, …



Ways of studying effects of cyanotoxins on fish



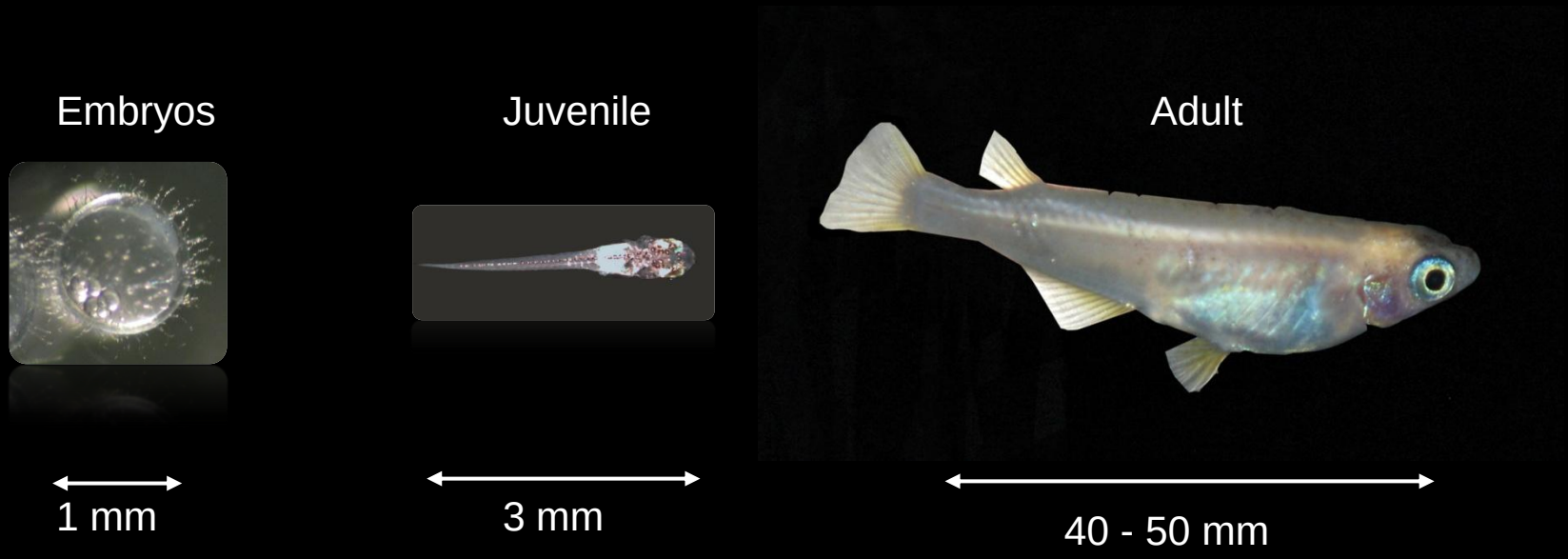
- Alterations of the dynamics of fish populations / communities

Ecosystemic approach
In situ experimentation

- Mechanisms involved in responses to cyanotoxins exposure

In vitro approach

Medaka, *Oryzias latipes*
biological model in Environmental Toxicology



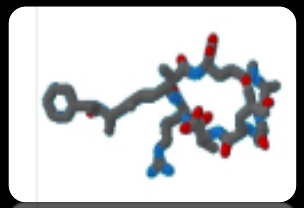
- Easy breeding
- Model in oncology, developmental biology, toxicology

(Organization for Economic Co-operation and Development OCDE, 2006)

- Model in risk assessment programs of aquatic contaminants
- Genome completely sequenced (Kasahara et al., 2007, *Nature*)

Experimental design

Extracts complexity



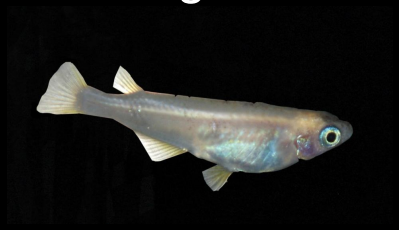
Pure toxin (MC-LR)

Culture of strains

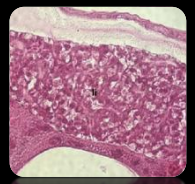
Natural Blooms

Effects on fish

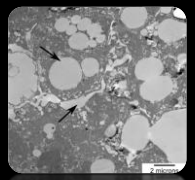
Organism



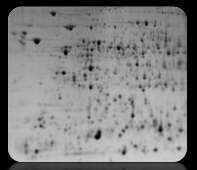
Organ



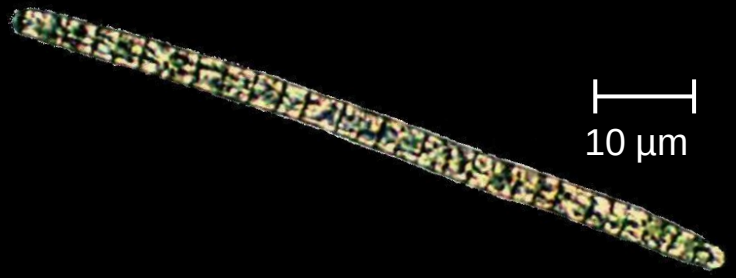
Cell



Proteom



Extracts of *Planktothrix agardhii*



- *P. agardhii*

- Culture of isolated strains and lyophilisation
http://www.mnhn.fr/mcam//Collections/Cyanobacteries.htm

PMC 75.02

microcystins *m/z*

1045.5 (Asp^{3}MC-HtyR)

1024.5 (Asp^{3}MC-RR)

981.5 (Asp^{3}MC-LR)

PMC 87.02

microcystins free

Extracts of *Planktothrix agardhii*

- *P. agardhii*

- Concentration of natural blooms of « Grande Paroisse » and lyophilisation

microcystins *m/z*
981.6 (Asp^{3}MC-LR)
1024.5 (Asp^{3}MC-RR)
1031.6
1042.6
1045.5 (Asp^{3}MC-HtyR)
1097.0 (MC-YR)

Tests on Medaka:

acute toxicity at different stages of development

- Embryos:

  Microinjection (stage 19)

  Embryotoxicity: acute toxicity

- Adults (young adults)

  i) gavage: acute intoxication

  ↪ control of the administered dose

  ii) balneation: acute intoxication

  ↪ close to environmental exposure (high toxic bloom event)

Acute toxicity on embryos

- Injection into the vitellus of late neurula embryos (stage 19) of medaka
  - i) pure toxin (MC-LR),
  - ii) strains (PMC75.02 and 87.02)
  - iii) natural bloom (« Grande Paroisse »)

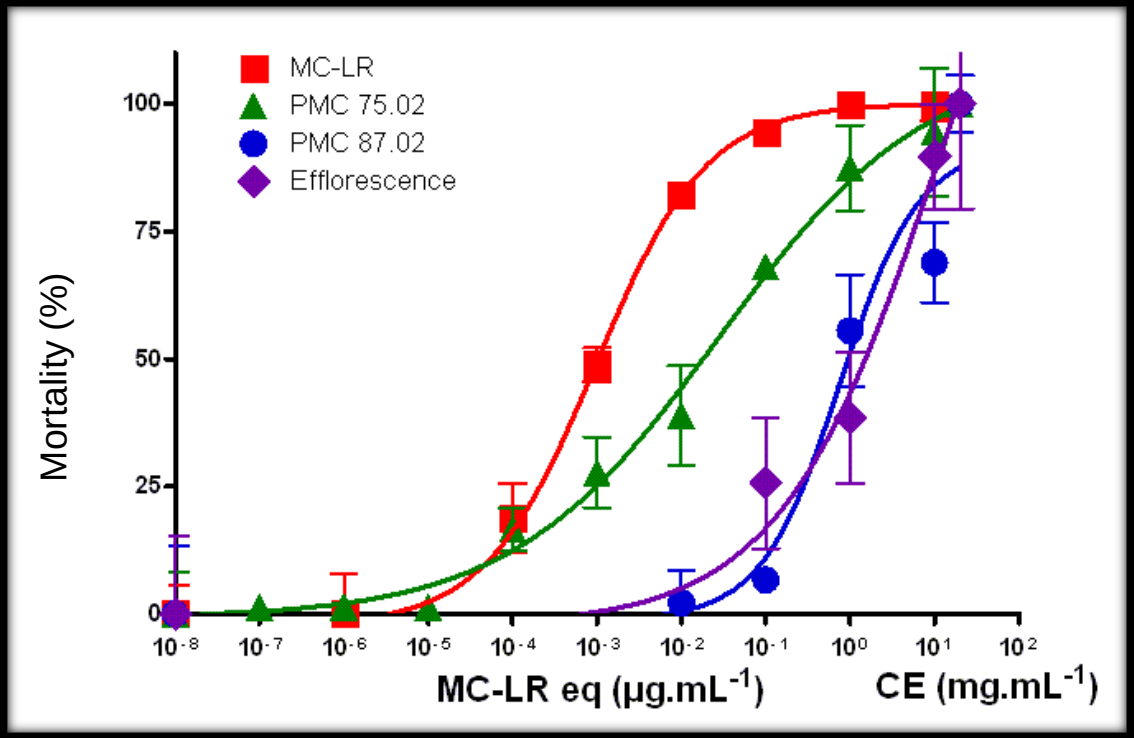
by microinjection (2 nl)

Precoce hatching

But no delay in embryo development

Decrease of survival rate

Embryos survival rate

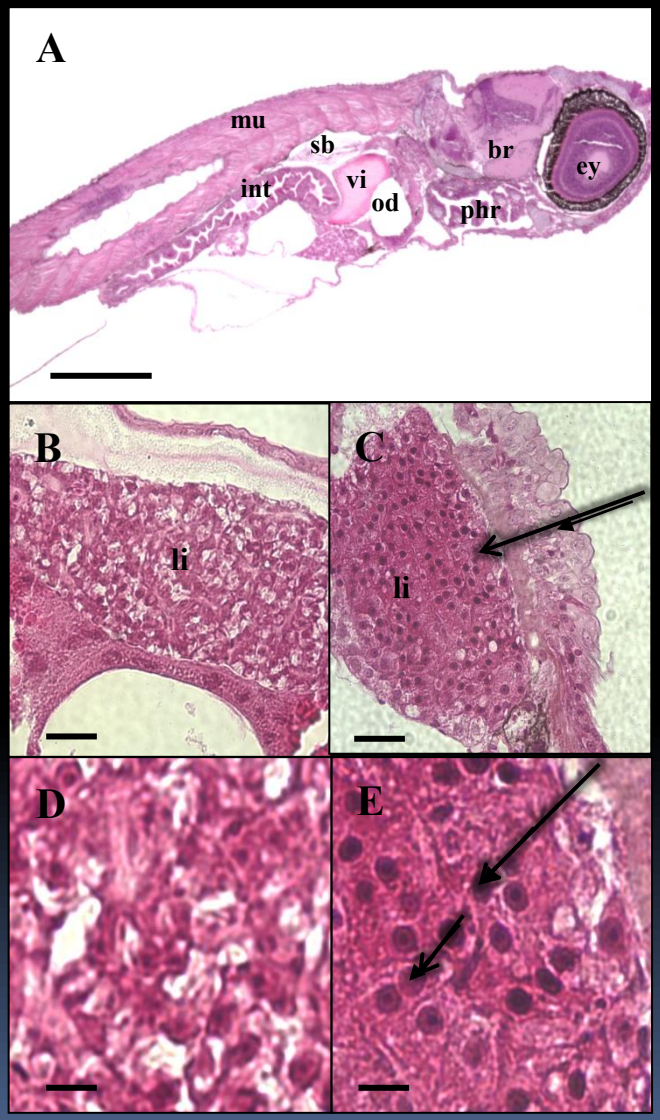


pure toxin (MC-LR) > *P. a.* MC+ (PMC75.02) > *P. a.* natural bloom = *P. a.* MC-free (PMC87.02)

Difference in toxicity related to MCs variants and their diffusion properties into the vitellus

Lecoz *et al.* Toxicon 2008; Malécot 2010

Acute toxicity on embryos



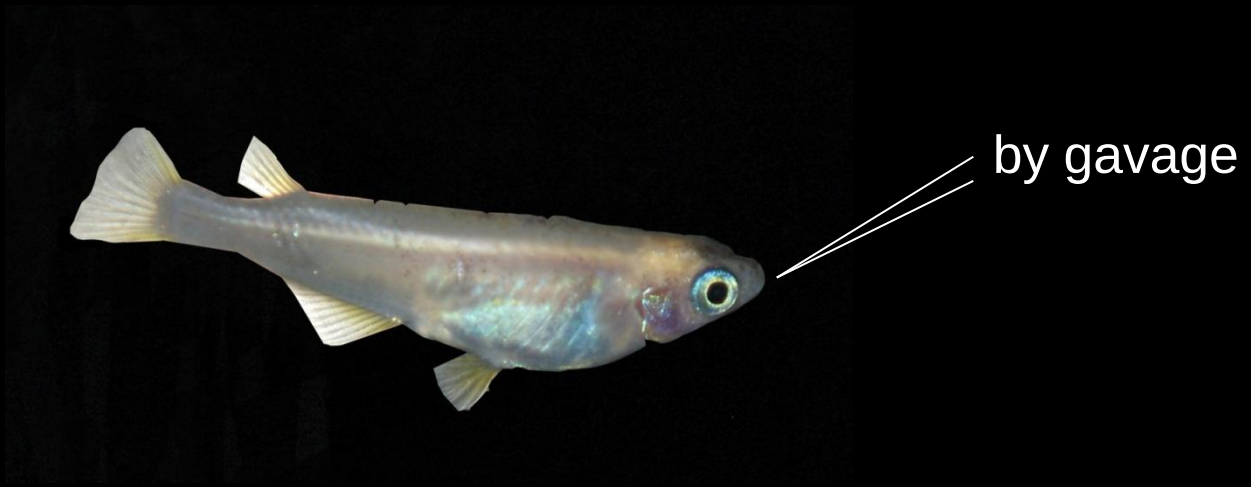
- Light microscopy sagittal sections (A), and transverse sections (B–E) of hatched (day 11 pf) control medaka embryos (A, B, D) and embryos injected with 10 µg CE mL^{-1} of the *P. agardhii* MC^{+} strain extract (PMC 75.02) (C, E).

- 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).

Hepatic hemorrhage
Loss of glycogen storage

Lecoz *et al.* Toxicon 2008

Acute toxicity on adults



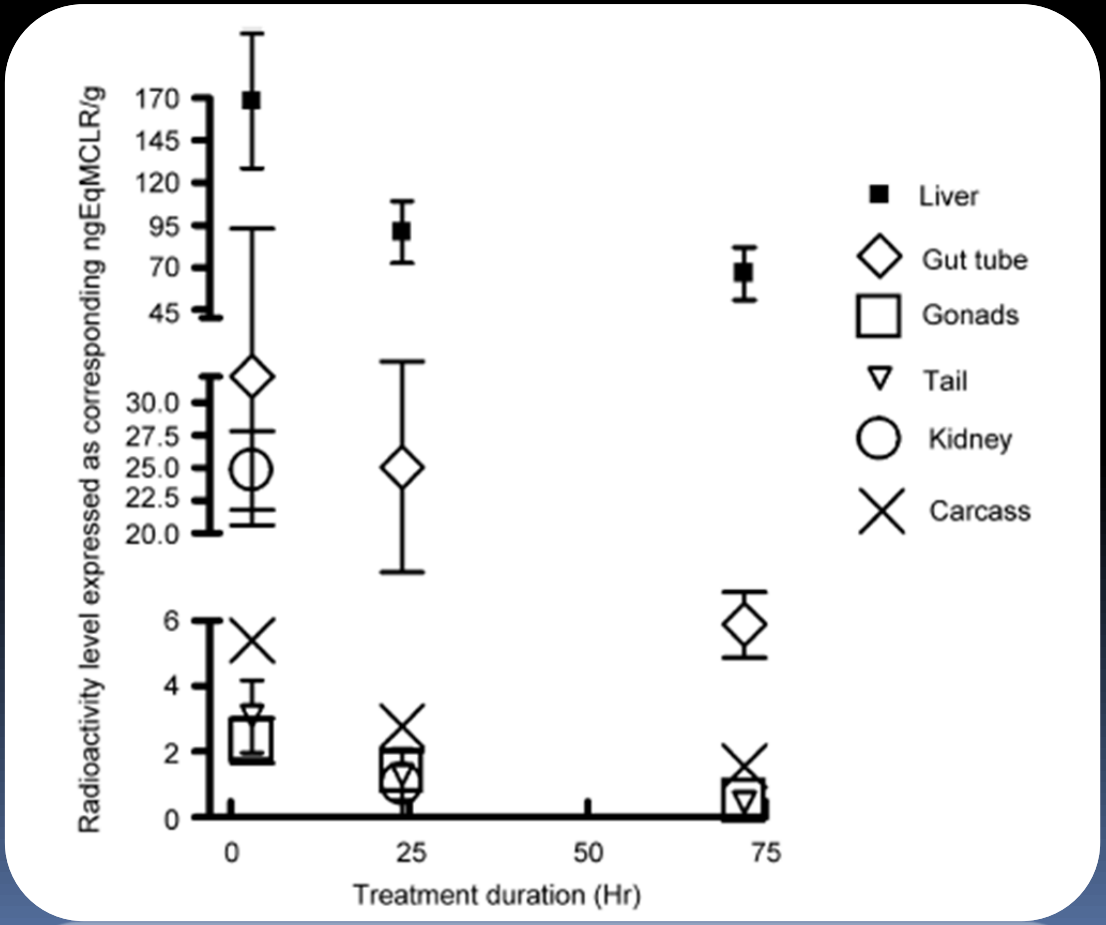
- 1 dose, 2 hours exposure:

  i) MC-LR: 5 µL of 1 µg μL^{-1} MC-LR solution, during 2 h

  ii) & iii) extracts of *Planktothrix agardhii* (culture and bloom): 5 µL of extract at 0.5 µg μL^{-1} eq. MC-LR

Acute toxicity on adults

- Tissue distribution of tritium-labelled dihydroMC-LR administered to adult medaka *via* gavage after different exposure times (2h, 24h, 72h)

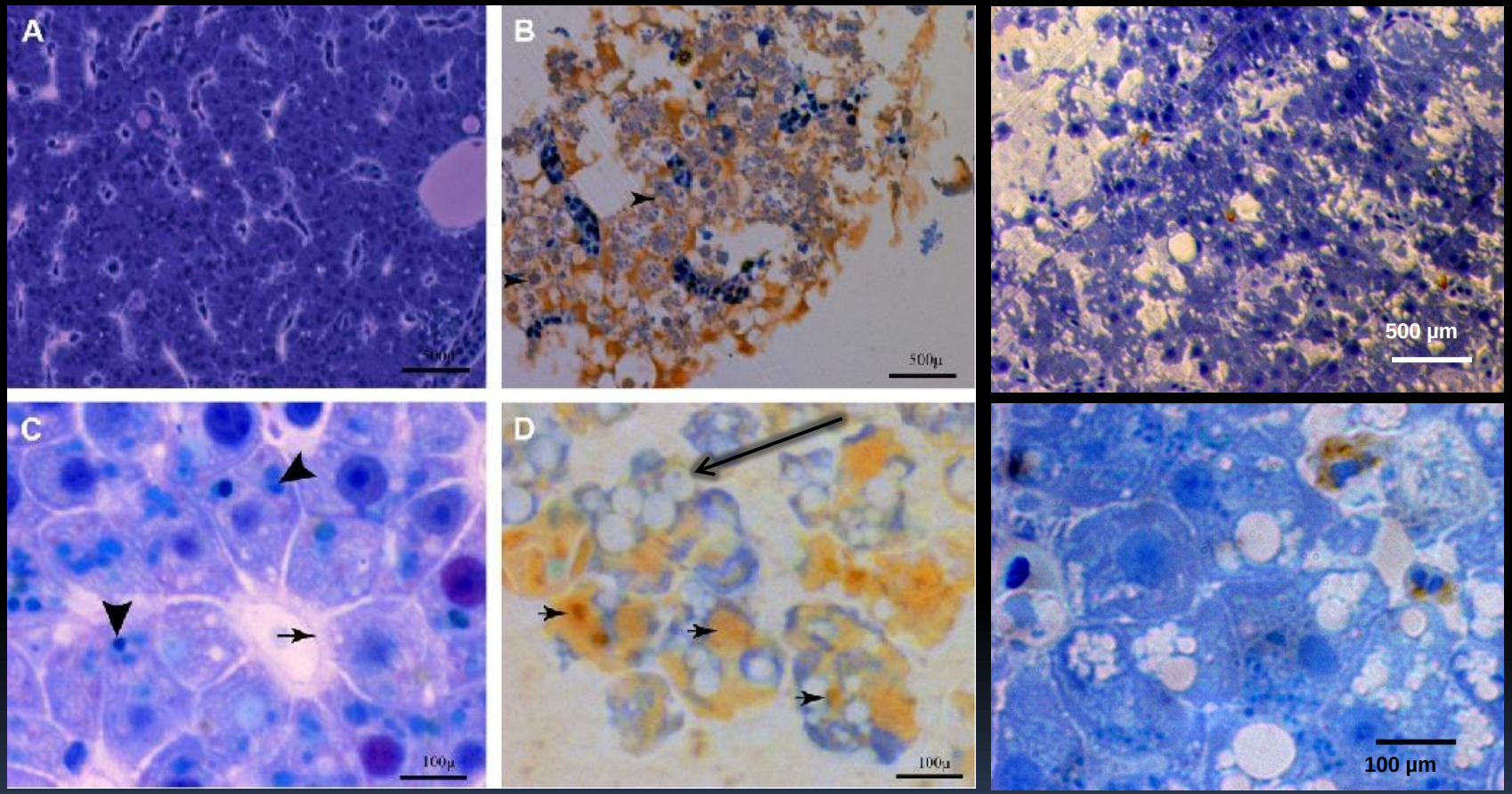


Mainly in liver
70% at 1 hour
90% at 3 days

Mezhoud *et al*., 2008

Effects of microcystins on liver

Control | MC-LR | *P. agardhii* extracts



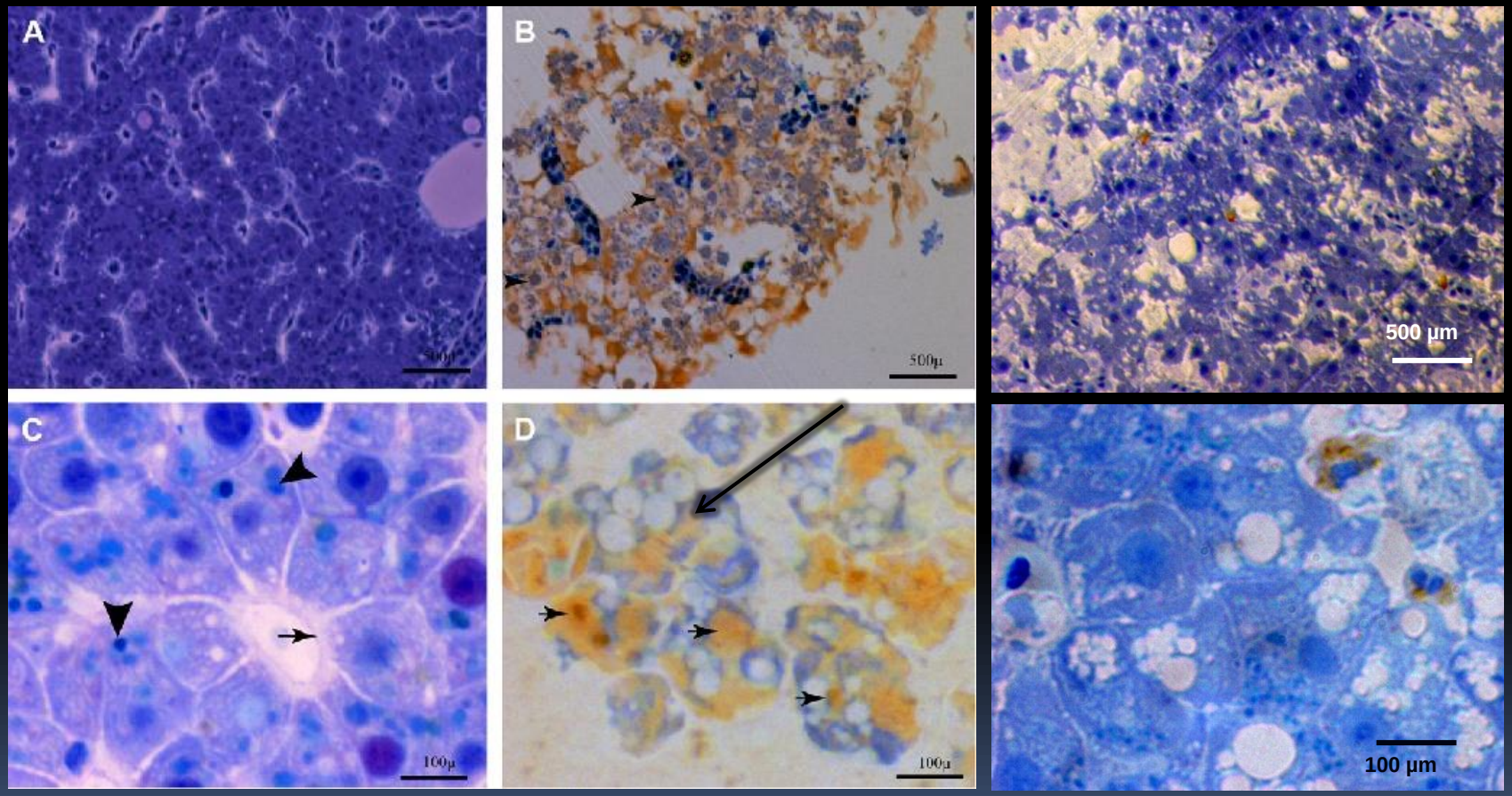
Cell lysis
Vacuolisation of the hepatocytes
Loss of glycogen storage and glycoproteins

Djediat *et al. 2010*

Effects of microcystins on liver

Immunolocalisation of microcystins:

Control | MC-LR | *P. agardhii* extracts



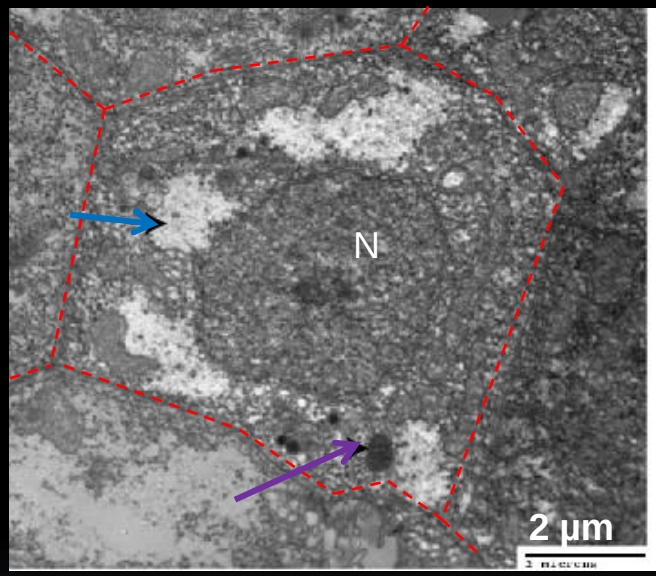
Immunolocalisation of MC in the hepatocytes and the macrophages

Djediat *et al. 2010*

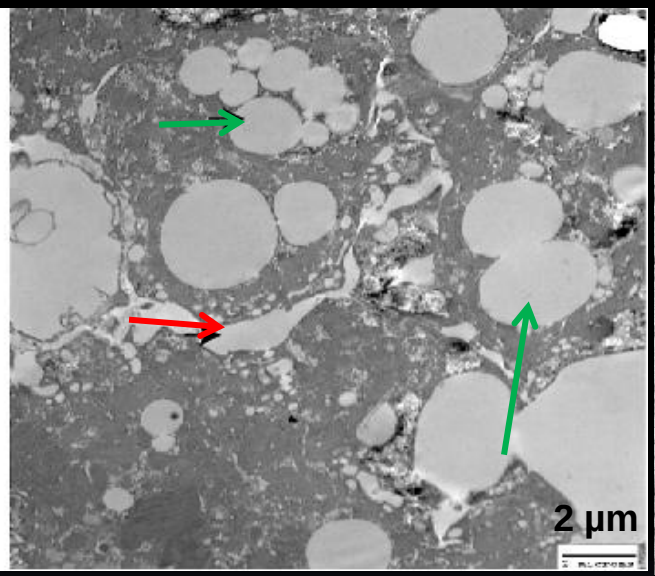
Effects of microcystins on cell liver

Electron microscopy:

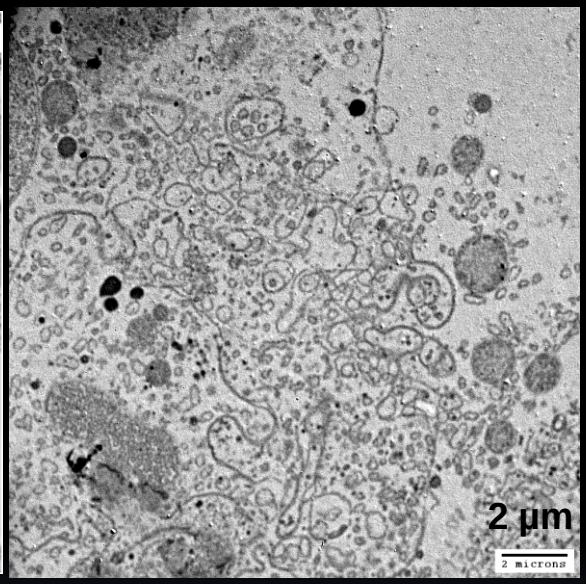
Control



MC-LR



P. agardhii extracts



-

+ +

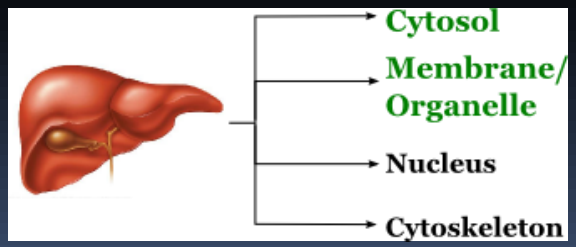
Cells are disrupted: cytoskeleton disorganisation
Loss of the storages of glycogen and glycoproteins
Lipidic droplets
Large beaches of cytoplasm

Effects of MC-LR on liver proteome

- Which proteins are modulated after microcystin exposure ?

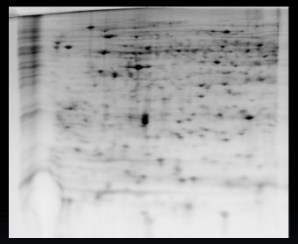
Medaka
(*Oryzias latipes*)

MC-LR exposure (1-2h)
Balneation/Gavage

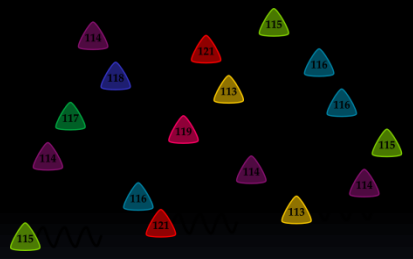


Cell fractionation

Proteomics



2D gels



iTRAQ

Bioinformatic analysis

Effects of MC-LR on liver proteome

42 proteins are modulated significantly
22 down-regulated, 20 up-regulated

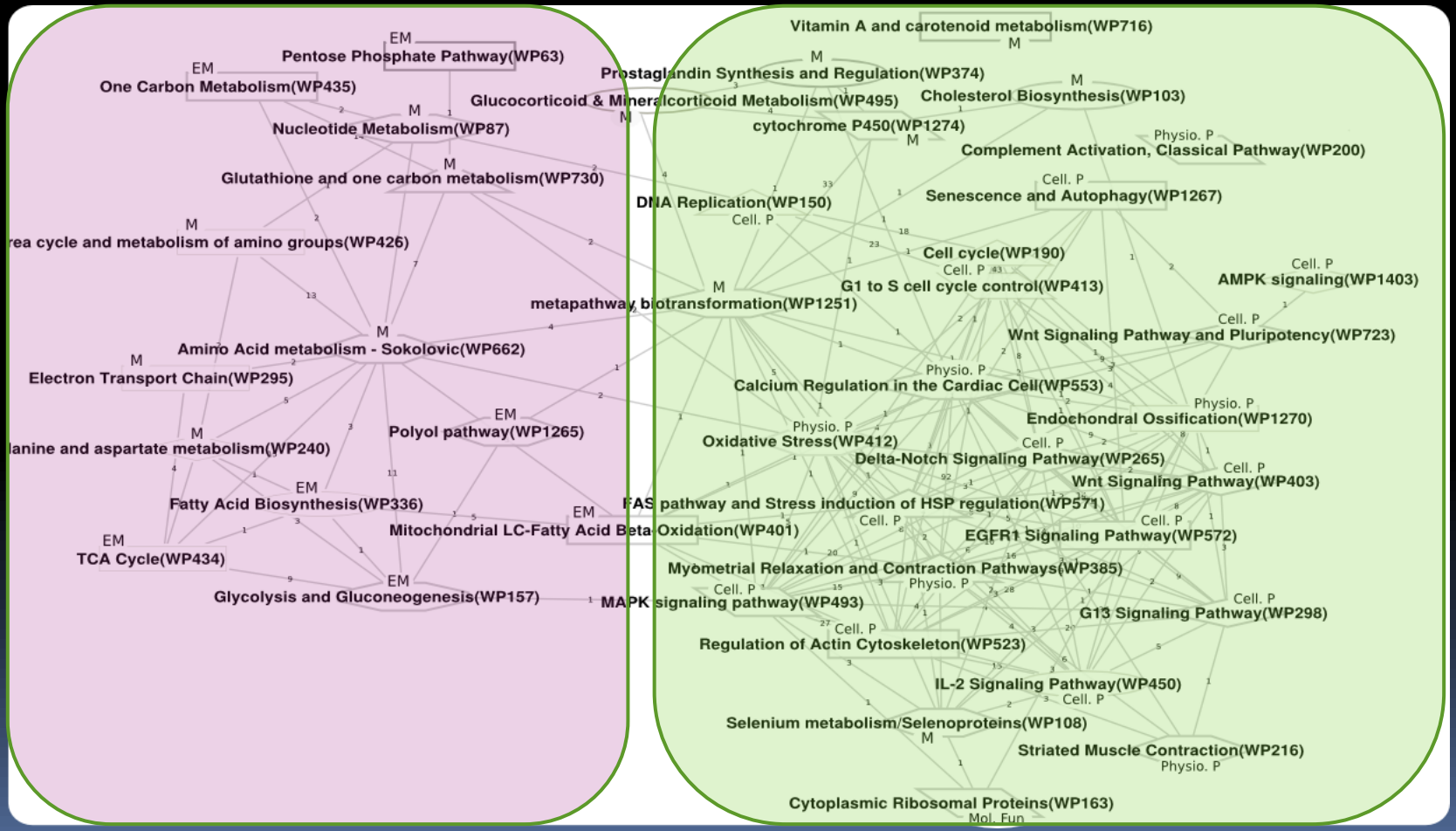
Several functions:

- Sugar, lipids and AA metabolism
- Oxidative stress (e.g. AldH)
- Protein maturation and degradation
- MAPK
- Cytoskeletton (e.g. α tubulin)

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

Effects of MC-LR on liver proteome

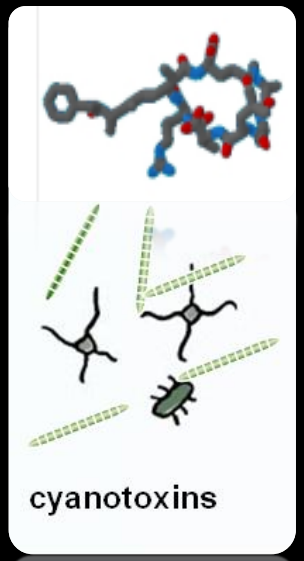
- Interactions network among proteins involved in microcystin responses i) metabolic network (AA, sugar …), ii) physiologic network (stress induction, …) in relation to the cell/organs alterations



Mehzoud *et al.* submitted

Effects of microcystins on fish

- What are the effects of cyanotoxins on fish ?



Acute toxicity

Toxic effects of microcystins (pure, strains and natural bloom) on embryo and adults whatever the exposition (microinjection, gavage & balneation)

Effects of microcystins on fish

Organ level

Liver and intestine : the most altered

Necrosis and cellular lysis

Cell level

Cell disruption

Detoxification with macrophages activation

Decrease of glycogen storage

Increase of lipid droplets

Molecular level

Modifications of proteins expression

Metabolic pathways are first modified

Perspectives

- Chronic *vs* acute intoxication?
  - Impact on male and female gonads?
  - Proteomic studies

- Comparison between *in vitro* exposure results and natural fish exposed to cyanobacterial bloom?

- Does multiple toxins have synergistic or additive effects?

- Transfer of microcystins through the food web to human?

Thanks to the MNHN medaka's team ….

Dr Marc Edery
Dr Karim Mehzoud
Pr Simone Puiseux-Dao
Dr Melodie Malecot
Dr Chakib Djediat
Dr Arul Marie
Charlotte Duval
Isabelle Trinchet
Hélène Huet

Thanks for your attention

Pr. Cécile Bernard
Muséum National d'Histoire Naturelle – Paris
cbernard@mnhn.fr