Cyanobacteria and their toxins: ecological and health risks

Luděk Bláha, Blahoslav Maršálek and co.

Masaryk University, Faculty of Science, RECETOX
& Institute of Botany, Academy of Sciences
Brno, Czech Republic

www.recetox.muni.cz

www.cyanobacteria.net

Research centre
for toxic compounds
in the environment

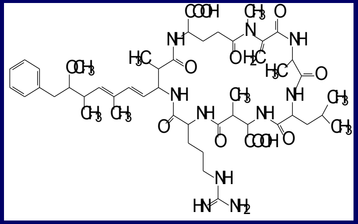
Flos Aquae Foundation

Centrum pro cyanobakterie a jejich toxiny

www.sinice.cz

Centre for Cyanobacteria and Their Toxins

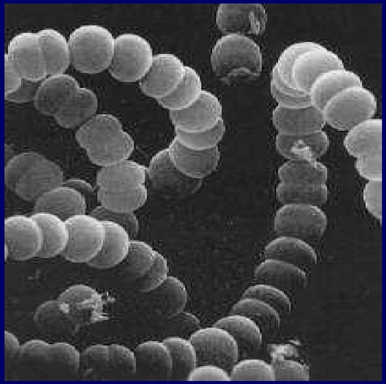
www.cyanobacteria.net

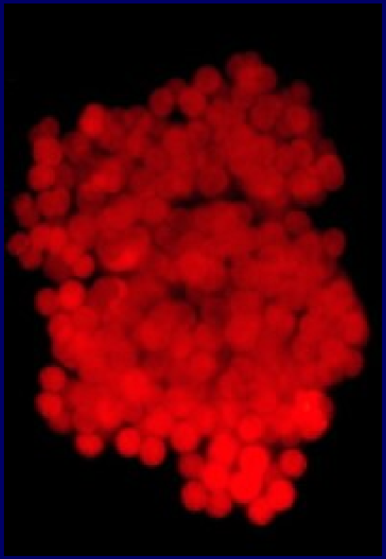


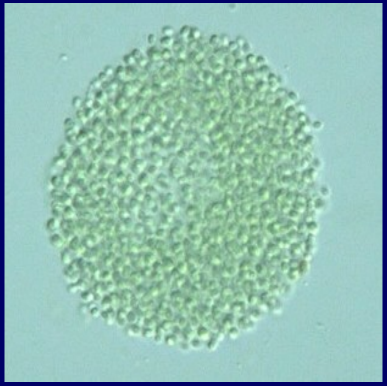
Blue green algae
(CYANOBACTERIA, CYANOPHYTA)

- photosynthetic prokaryota
  - live at various biotops
  (**water**, soil, ice, rocks, lichens …)

- cca 3 x 10^9 years old
- formation of the oxygen atmosphere







Cyanobacteria - current problem

HUMAN ACTIVITIES
(agriculture, waste waters…)

EU/<u>TROPHICATION</u>
(=increased concentration of **<u>nutrients</u>**)

CYANOBACTERIAL MASS DEVELOPMENT




Cyanobacterial water blooms – global problem

Upper Saranac River, USA

Bedetti Lake, Argentina

Neuse River, USA

Baltic sea, Europe

Nové Mlýny, Czech Rep.

Yellow sea, China

Lake Mokoan, Australia

South Africa

Talking about „risks“ of cyanobacteria

- **RISK = probability of the occurrence of HAZARDOUS event**

  - **„Hazardous events“ resulting from eu/trophication of the environment**

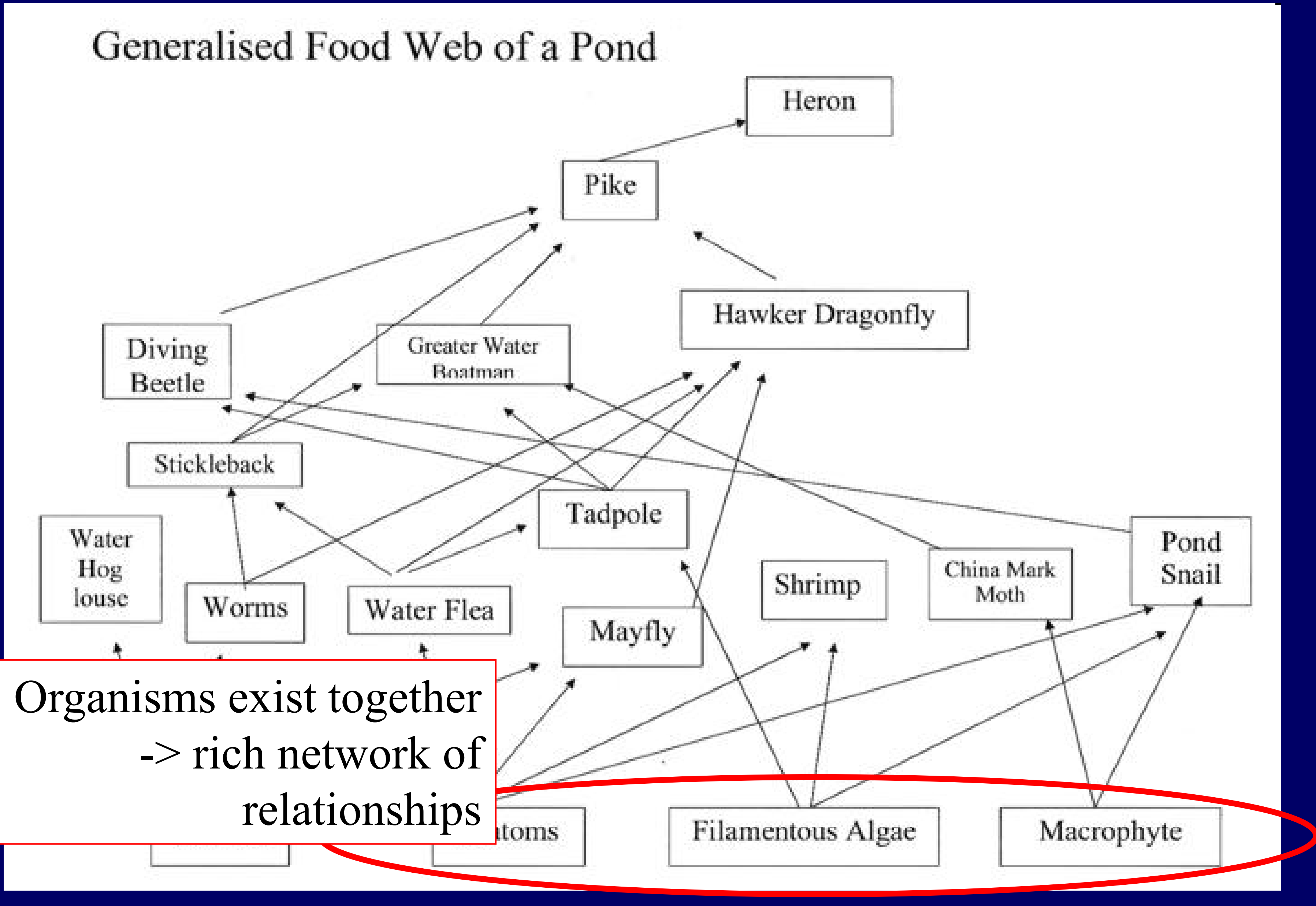
    - **Primary** damage to structure and functioning of ecosystems

    - **Secondary** signs -> ecotoxicity and toxicity

Ecological „stability“

- **Stable and functioning ecosystem**
  - Complex and complicated structure (diversity)
  - Many links (food networks) among organisms = ecosystem functioning
    - *Including „ecosystem services*" *to humans:*
      supplies, regulations, cultural / aesthetic, supporting

Complex ecosystem



Organisms exist together -> rich network of relationships

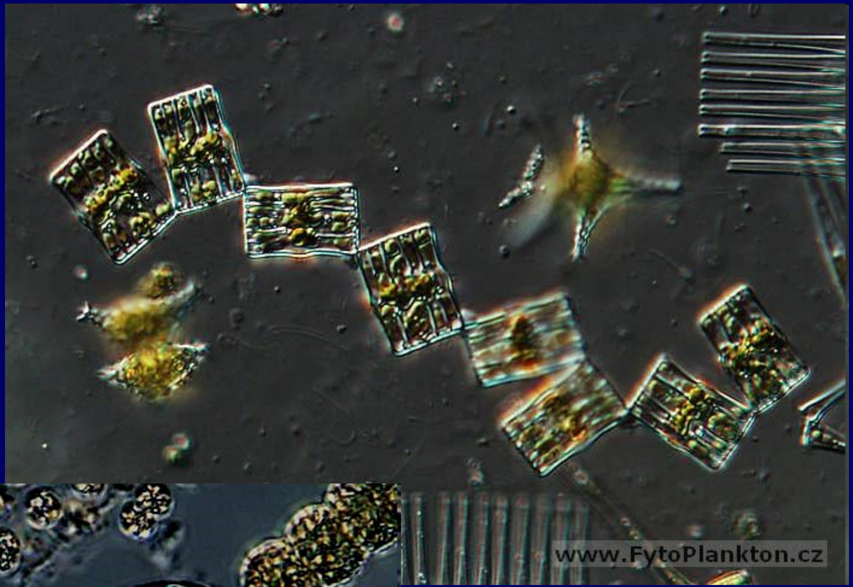
Ecological risk 1: Loss of phytoplankton biodiversity

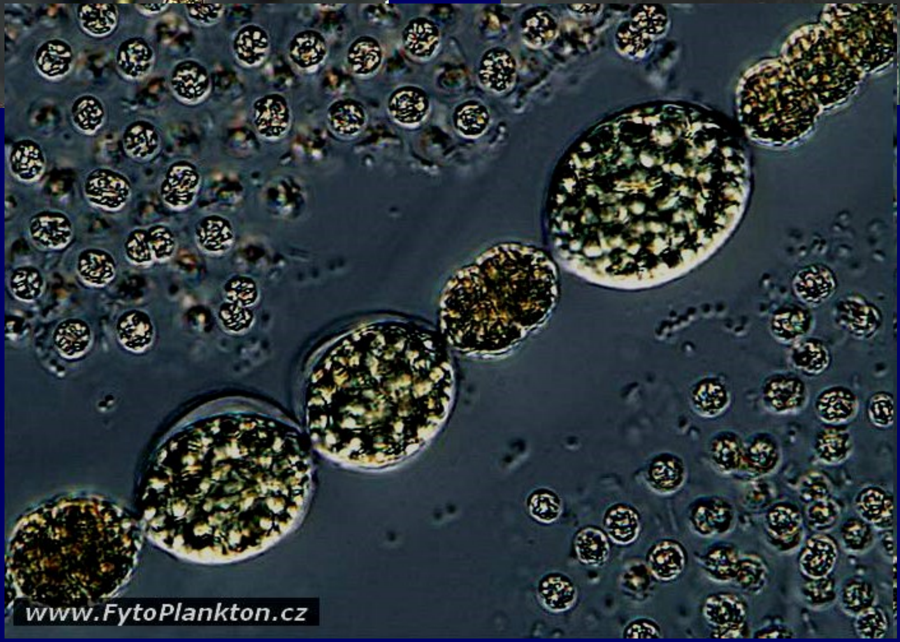
- Anthropogenic changes in the environment (more nutrients - P,N)
  -> advantage for „some" phytoplankton organisms

- Complex communities replaced with „monoculture" (often *Microcystis aeruginosa, Planktothrix sp.*)

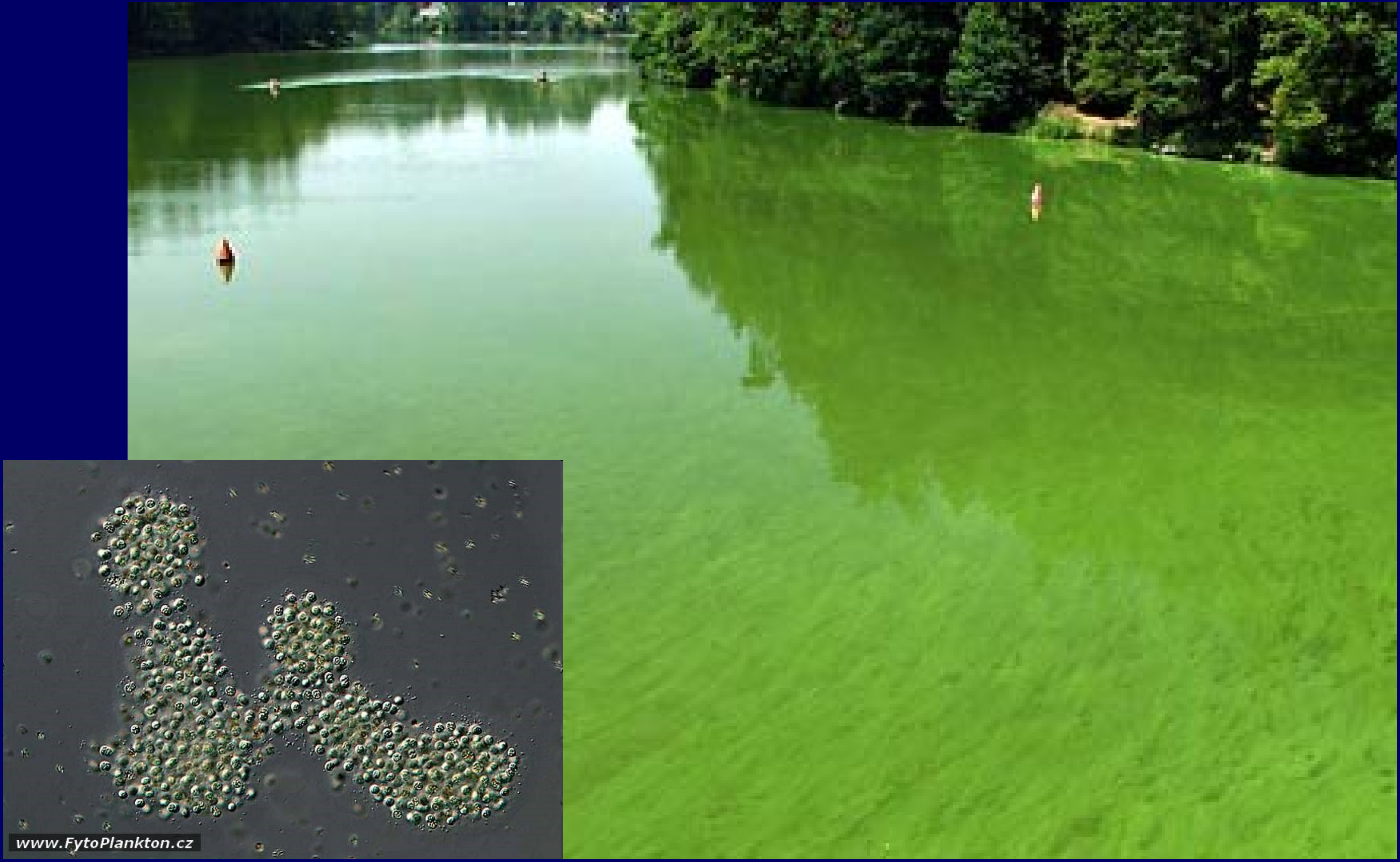
- „Monocultures" **have secondary effects**
  -> changes in hydrochemistry (higher pH, transparency)
  -> further indirect impacts on other organisms

Ecological risk 1: Loss of phytoplankton biodiversity





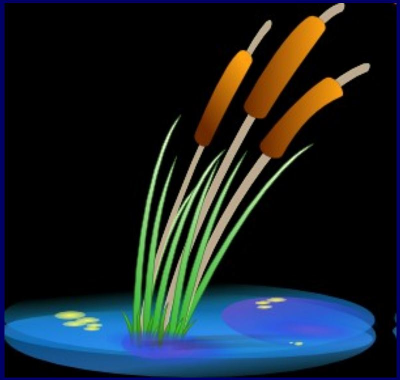
Ecological risk 1: Loss of phytoplankton biodiversity



Ecological risk 2: Further ecosystem changes

- <u>**Phytoplankton -> changes in the whole network**</u>
  - Reported examples …
    - Changes in the consumers communites
      zooplankton -> fish -> …
    - Makrophyte disappearance (reed)
      (shading -> no germination …)

        -> macrophytes
        = substrate for other organisms …



- <u>**New „expansive“ species**</u>
  - cyanobacterium *Cylindrospermopsis raciborskii (?)*
- <u>**Water blooms**</u> = substrate for „associated bacteria“

Ecological risk 3: Ecosystem catastrophes

- Sudden disappearance of the producers „monoculture"
  (*rapid environmental changes, „infections" by viruses/phages*) -> **Ecosystem collapse**

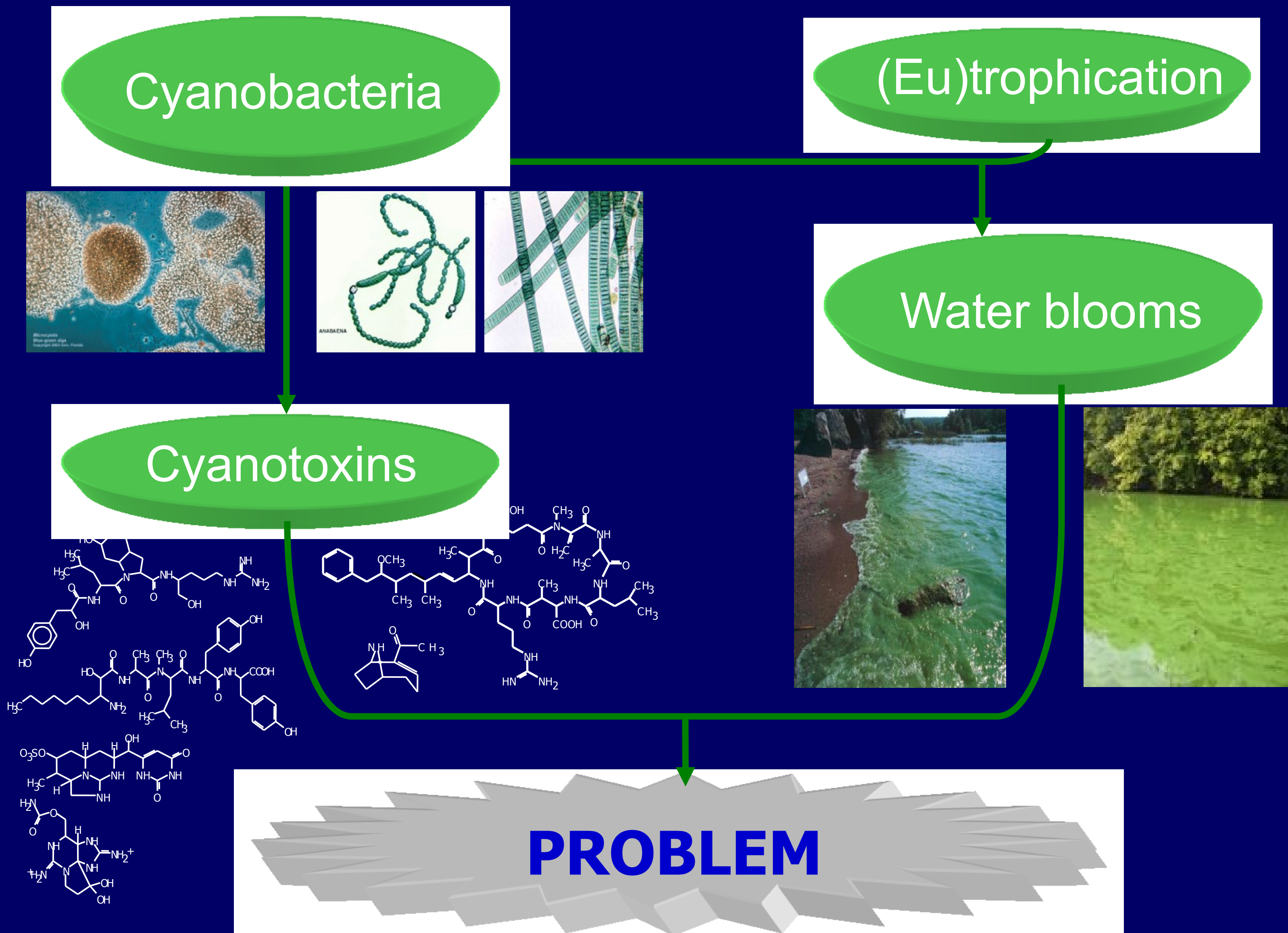
- Seasonal changes
  - Cyanobacterial biomass lysis
    -> bacterial decay -> loss of O_2
    -> **anaerobic conditions - collapse**
  - Deaths of aquatic organisms (fish ...)
  - Pathogens (anaerobic Clostridium botulinum)

Ecological risk 4: Cyanobacterial **toxins**

- **Cyanobacteria** - evolutionary old and important organisms (atmospheric oxygen)

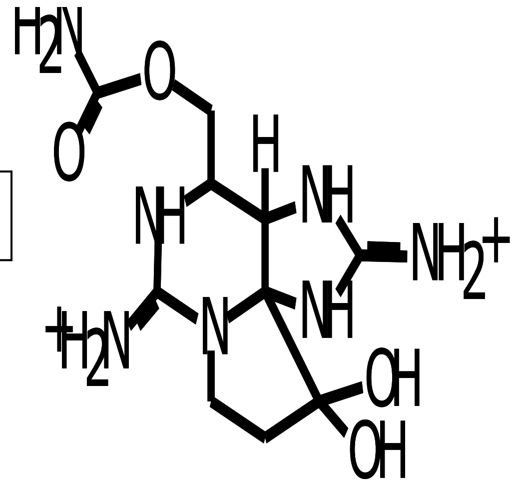
- **G- bacteria** (10 mil. Cells / mL)
  - G- : cell walls contain lipopolysaccharides (LPS, *similar to E. coli, Salmonella sp…)*

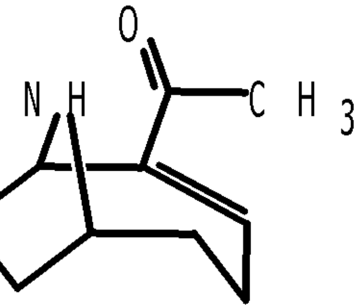
- **Water blooms**
  - several complex problems (see previous slides…)
  - just one of the problems = **toxin production**


Cyanobacteria
(Eu)trophication
Water blooms
Cyanotoxins
PROBLEM

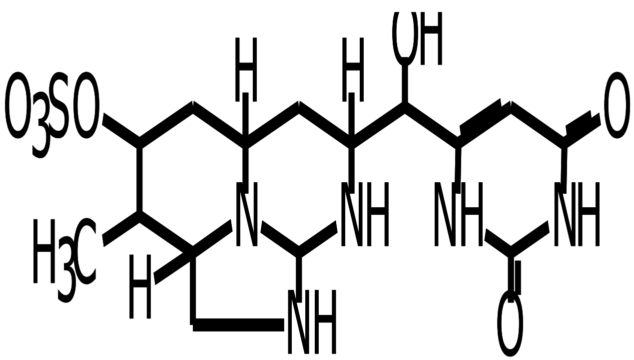
Selected „known“ cyanotoxins

Saxitoxin

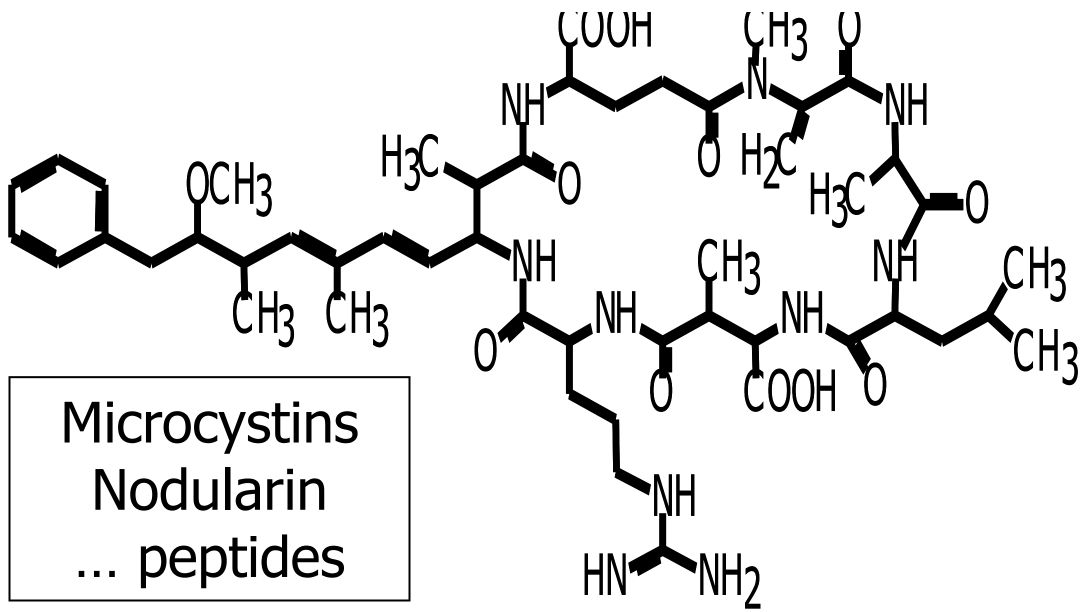




Anatoxin-a



Cylindrospermopsin



Microcystins
Nodularin
... peptides

Categorization of cyanotoxins

1. **According to the chemical structure**

   - cyclic and linear peptids
   - alkaloids
   - lipopolysaccharides

2. **According to biological activity**

   <u>mechanisms of toxicity</u>

   - hepatotoxicity, neurotoxicity, cytotoxicity, irritating, immunotoxicity, genotoxicity ...

TOXIN	STRUCTURE	STRUCTURE VARIATION	LD50* (μg.kg-1)	TOXICITY
Microcystin	cyclic heptapeptide	>60	50-1200	hepatotoxicity, tumor promotion, induction of oxidative stress
Nodularin	cyclic pentapeptide	7	50-2000	hepatotoxicity, tumor promotion
Anatoxin	alkaloide	2	200-250	neurotoxicity
Anatoxin-a(S)	methylphospho-ester N-hydroxy-guanine	1	20	neurotoxicity
Saxitoxin	carbamat alkaloid	19	10	neurotoxicity
Cylindrospermopsin	guanidin alkaloid	2	200**	cytotoxicity, target organs: liver and kidney
Aplysiatoxin		2		dermatotoxicity, tumor promotion
Lyngbyatoxin	modified cyclic dipeptide	1		dermatotoxicity, tumor promotion
Lipopolysaccharide				irritate effect

Cyanobacteria	Toxins produced
Anabaena	Anatoxins, Microcystins, Saxitoxins, LPS's
Anabaenopsis	Microcystins, LPS's
Anacystis	LPS's
Aphanizomenon	Saxitoxins, Cylindrospermopsins, LPS's
Cylindrospermopsis	Cylindrospermopsins, Saxitoxins, LPS's
Hapalosiphon	Microcystins, LPS's
Lyngbia	Aplysiatoxins, Lyngbiatoxin-a, LPS's
Microcystis	Microcystins, LPS's
Nodularia	Nodularin, LPS's
Nostoc	Microcystins, LPS's
Phormidium (*Oscillatoria*)	Anatoxin, LPS's
Planktothrix (*Oscillatoria*)	Anatoxins, Aplysiatoxins, Microcystins, Saxitoxins, LPS's
Schizothrix	Aplysiatoxins, LPS's
Trichodesmium	yet to be identified
Umezakia	Cylindrospermopsin, LPS's

THE COMPARIOSON OF TOXICITY OF THE NATURAL TOXINS

(i.p. injection, acute rat test, **LD50 in μg/kg)**

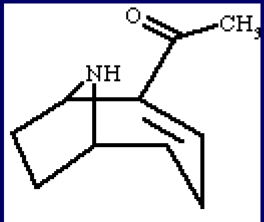
Bacteria-cyanobacteria- animals- fungi- plants

Amatoxin	Amanita phalloides	fungus	500
Muscarin	Amanita muscaria	fungus	1100
Aphanotoxin	*Aphanizomenon flos-aquae*	cyano	10
Anatoxin -A	*Anabaena flos-aquae*	cyano	20
microcystin LR	*Microcystis aeruginosa*	cyano	43
nodularin	*Nodularia spumigena*	cyano	50
botulin	*Clostridium botulinum*	bacteria	0,00003
tetan	*Clostridium tetani*	bacteria	0,0001
kobra	*Naja naja*	snake	20
kurare	*Chondrodendron tomentosum*	plant	500
strychnine	*Strychnos nux-vomica*	plant	2 000

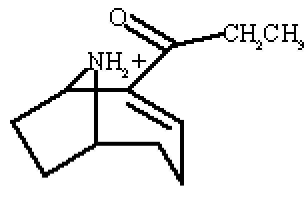



Anatoxin-A, Anatoxin-A(S)

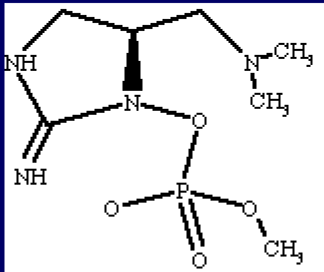
- **neurotoxic alkaloids**
- produced by a number of cyanobacterial genera including *Anabaena*, *Oscillatoria* and *Aphanizomenon*.
- LD50s from 20 µg kg-1 (by weight, I.P. mouse) making them **more toxic than microcystins.**



ANATOXIN-A



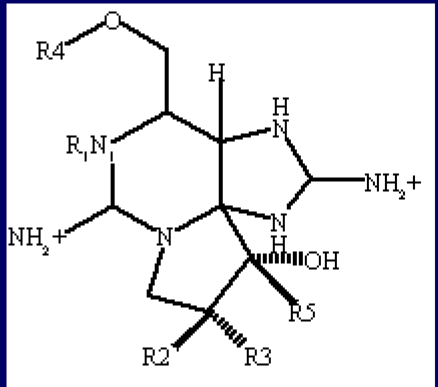
HOMOANATOXIN-A



ANATOXIN-A(S)

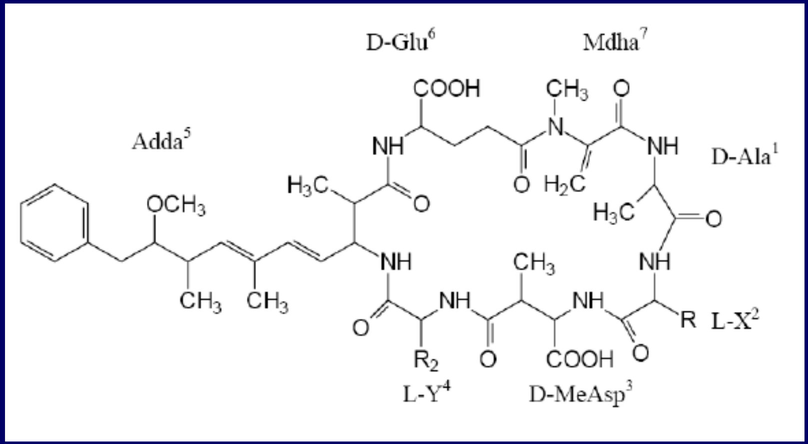
SAXITOXINS

- **neurotoxic alkaloids**
- also known as PSP's - paralytic shelfish poisons - due to their accumulation in seafood
- Produced by marine dinoflagellates and cyanobacteria *(but also in others such as Aphanizomenon sp.)*
- **Number of STX variants exist**



SAXITOXIN - GENERAL STRUCTURE

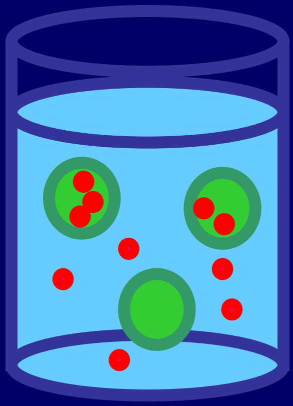
MICROCYSTINS



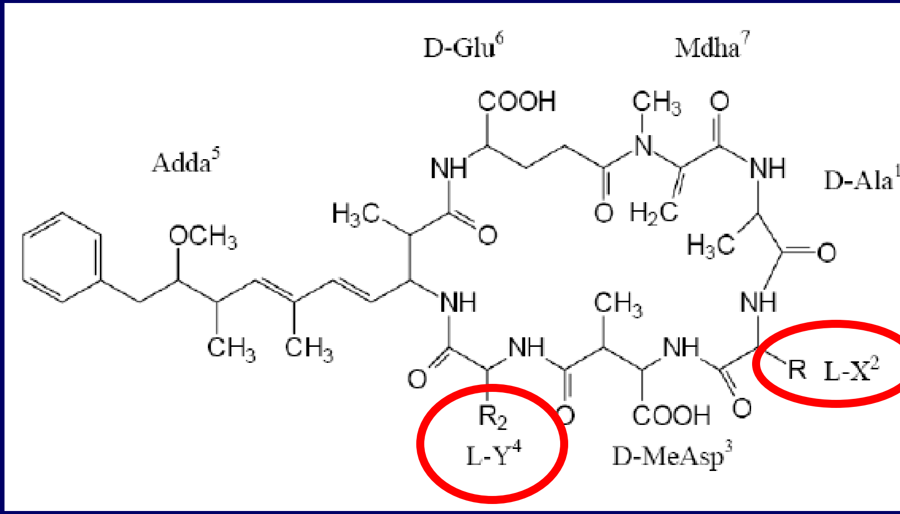
- **The most studied and most important**

- Produced and present inside cells:
  - **Intracellular**:
    - up to 10 mg/g d.w. of biomass

      1% dw -> tons / reservoir
  - **Extracellular** (dissolved): up to 10 ug/L
- Stable in water column, bioaccumulative (?)



MICROCYSTINS



- Inhibit regulatory protein phosphatases
  -> tumor promoter
  -> hepatotoxic

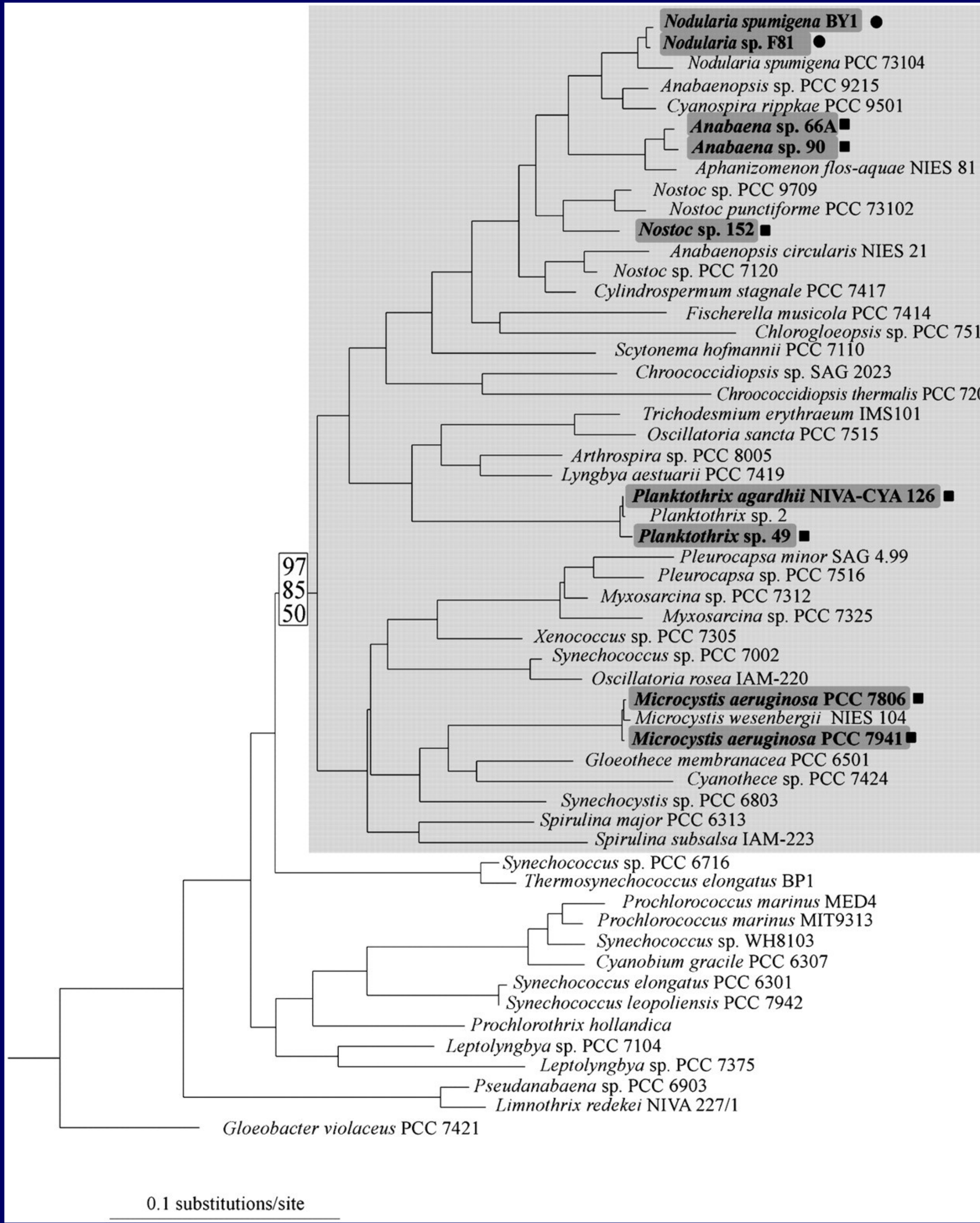
- **70 variants**: MC-LR only considered by WHO
  - chronic TDI: 0.04 ug/kg b.w./day
  - drinking water guidline recommendation: 1 ug/L

- **Highly toxic to mammals** and humans
- Ecotoxicology ? Natural function ?

Microcystin synthesis

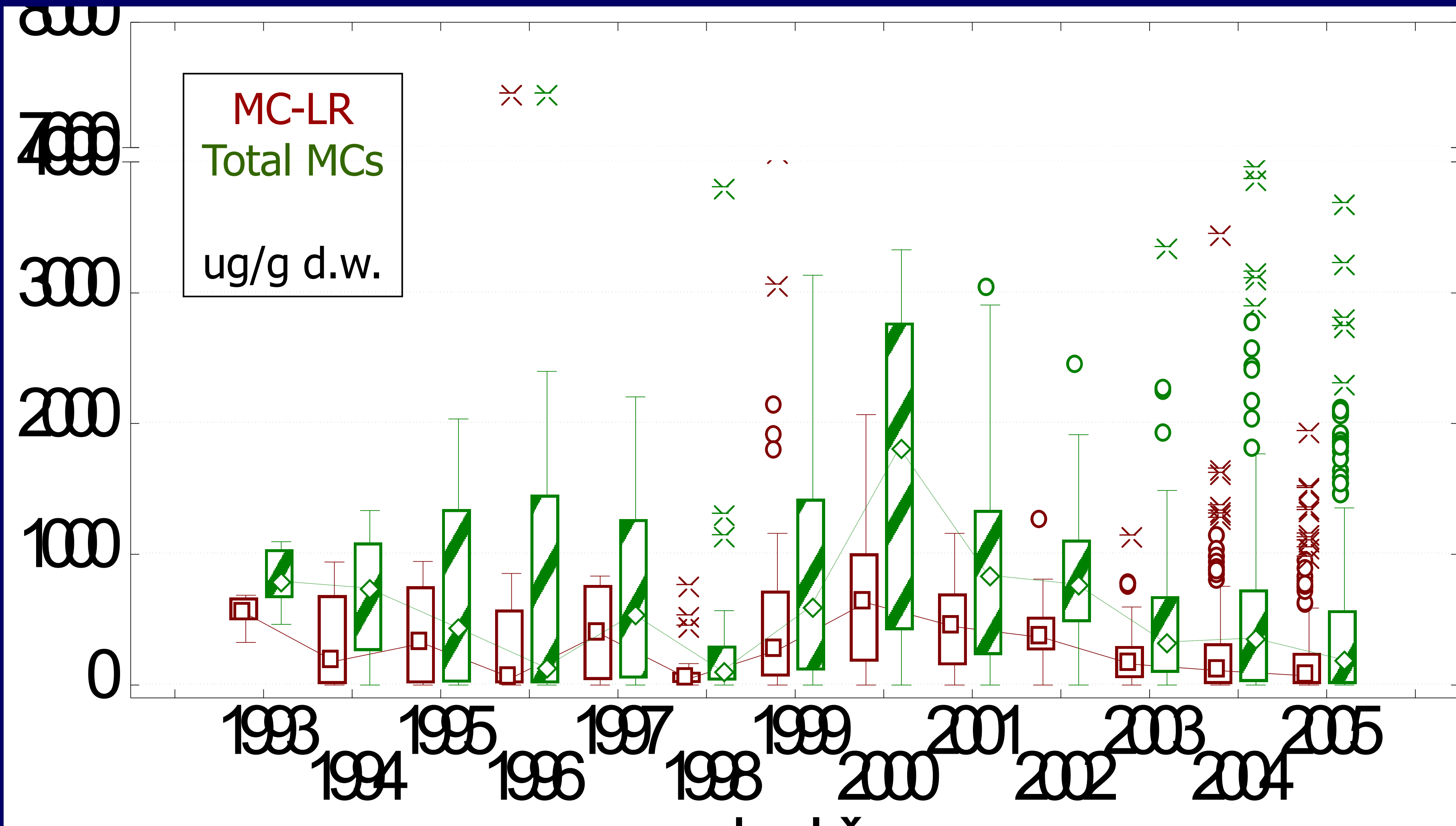
- **Non-ribozomal polyketide synthetases**
- **Evolutionary old genes**
  - ***Why remained?***
- **Horizontal gene transfer**

Rantala et al. (2004) PNAS 101:568



Microcystins in the Czech Rep.

(Water bloom biomass concentrations
… up to several mg/g dry weight)

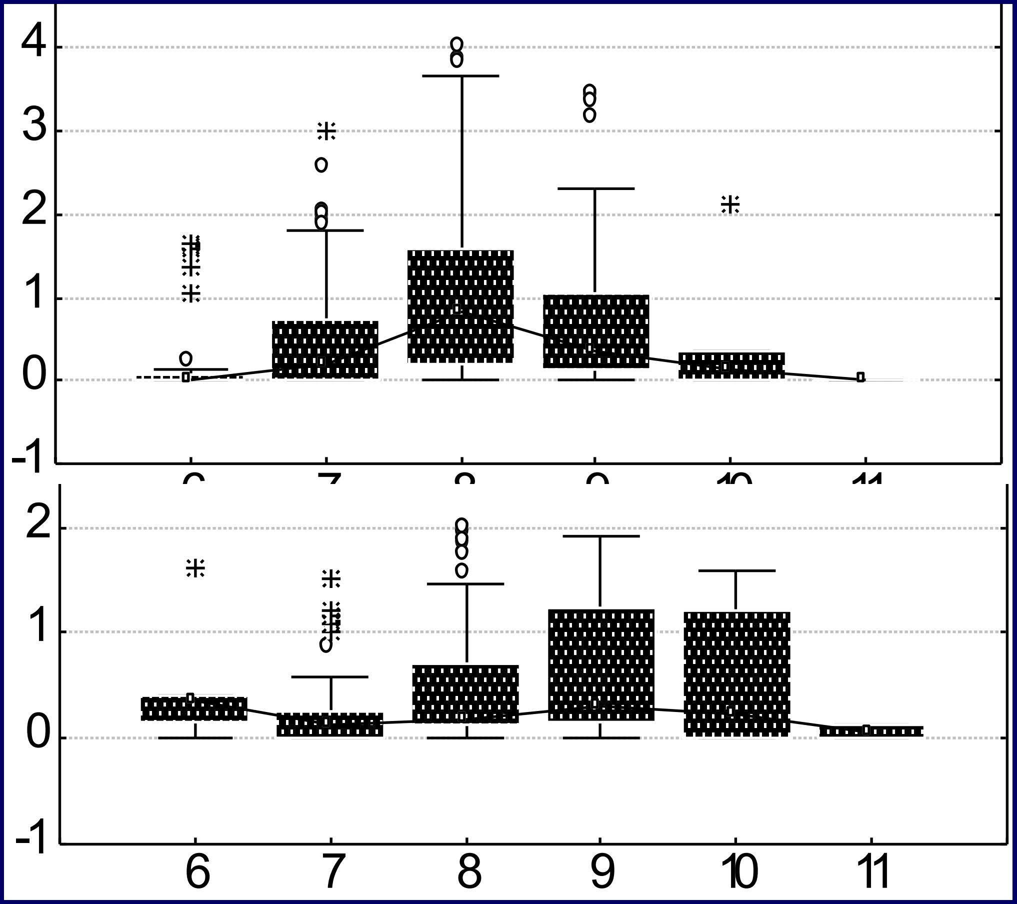


Seasonal variability

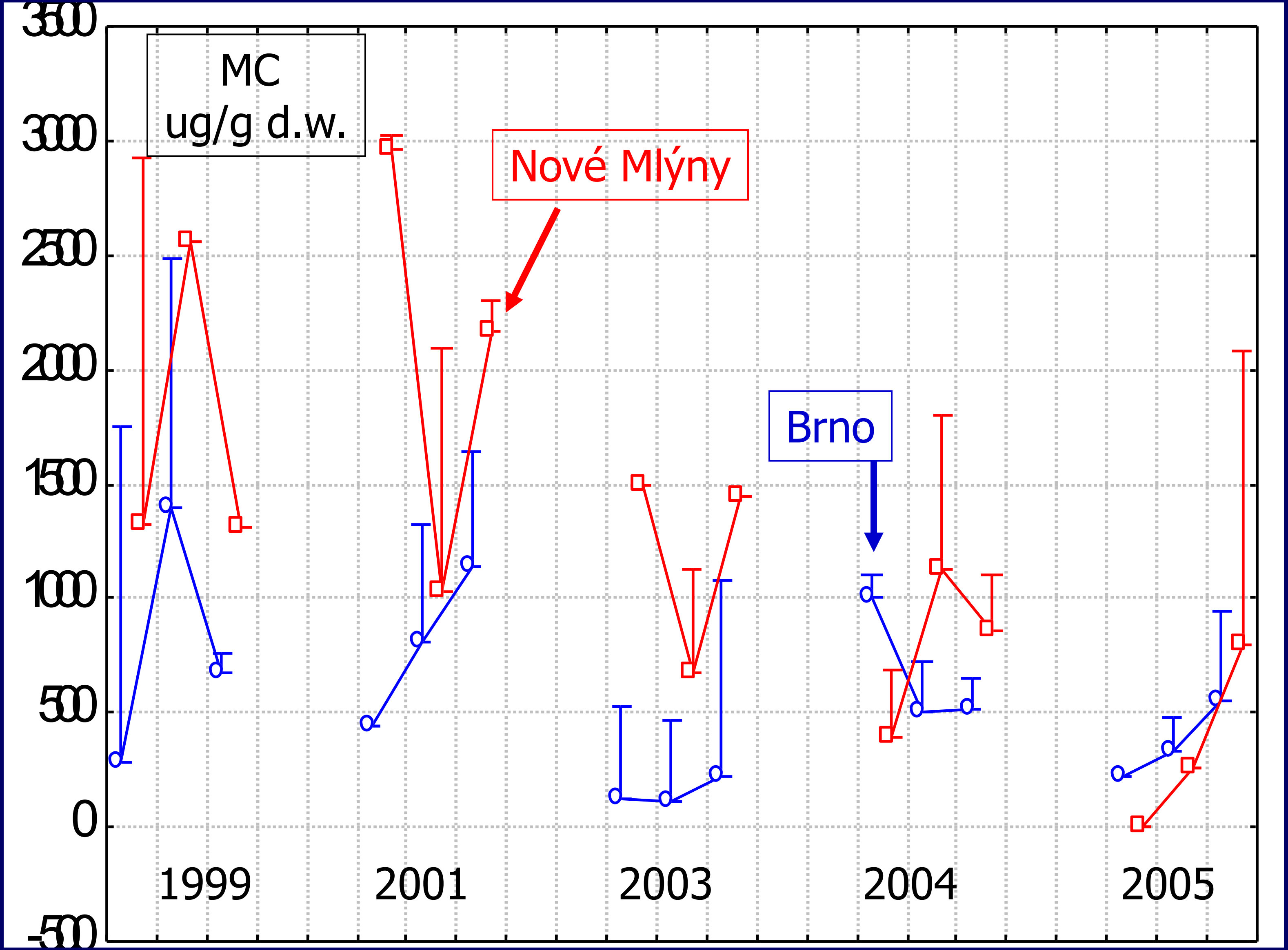
- **dissolved** microcystins in the C.R. (water concentrations)

2004

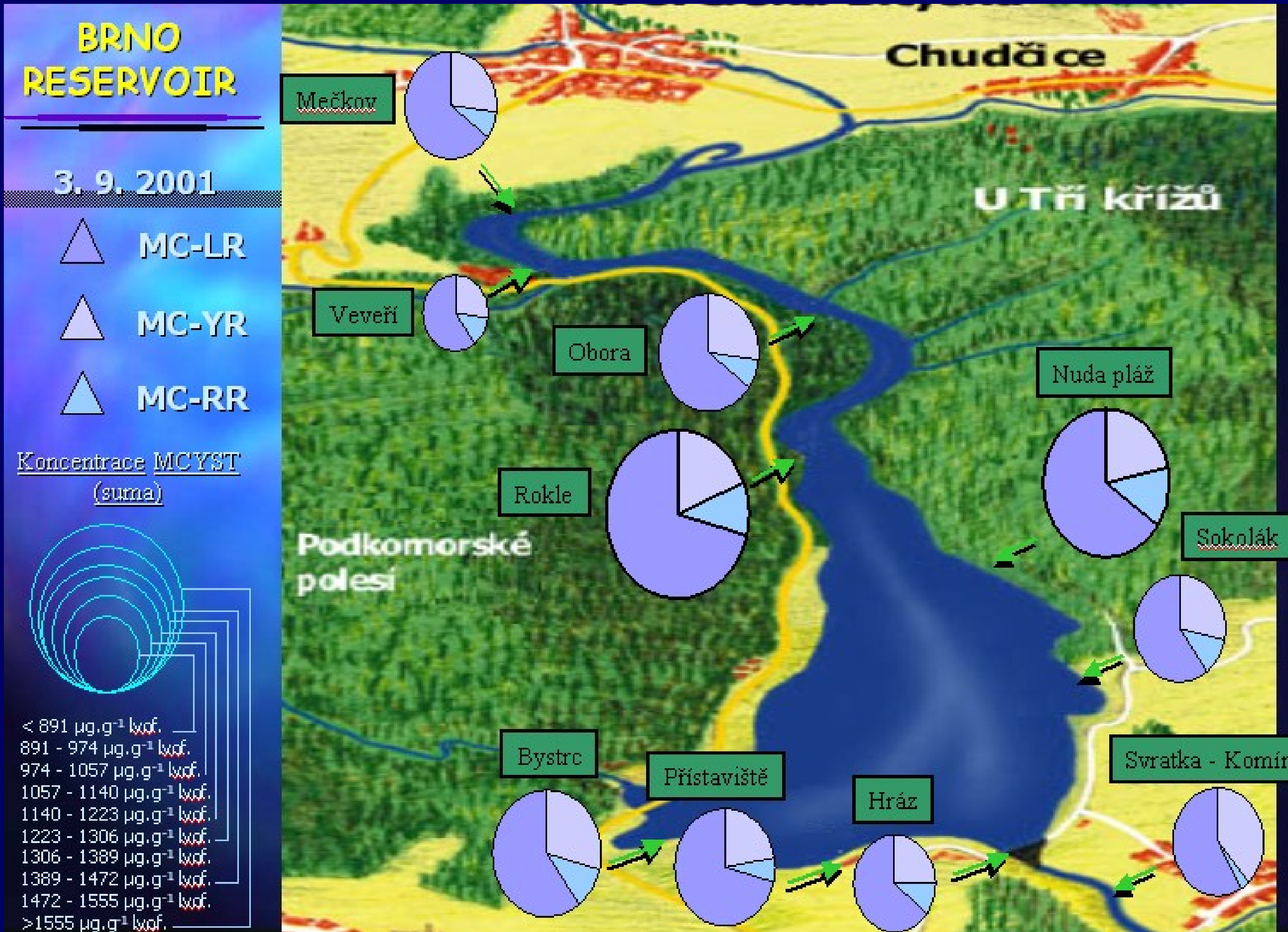
2005



Reservoir seasonal data



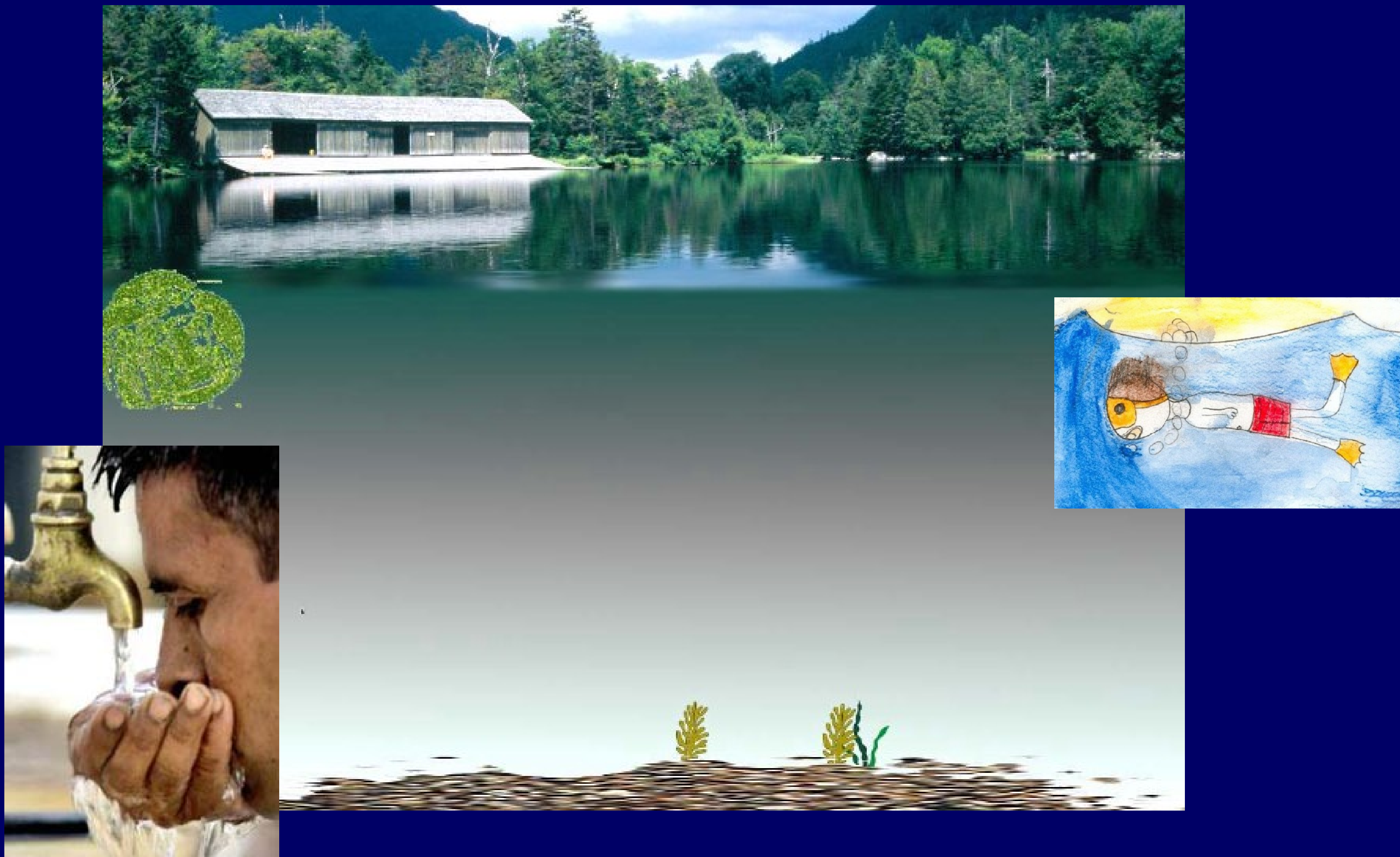
Reservoir spatial variability



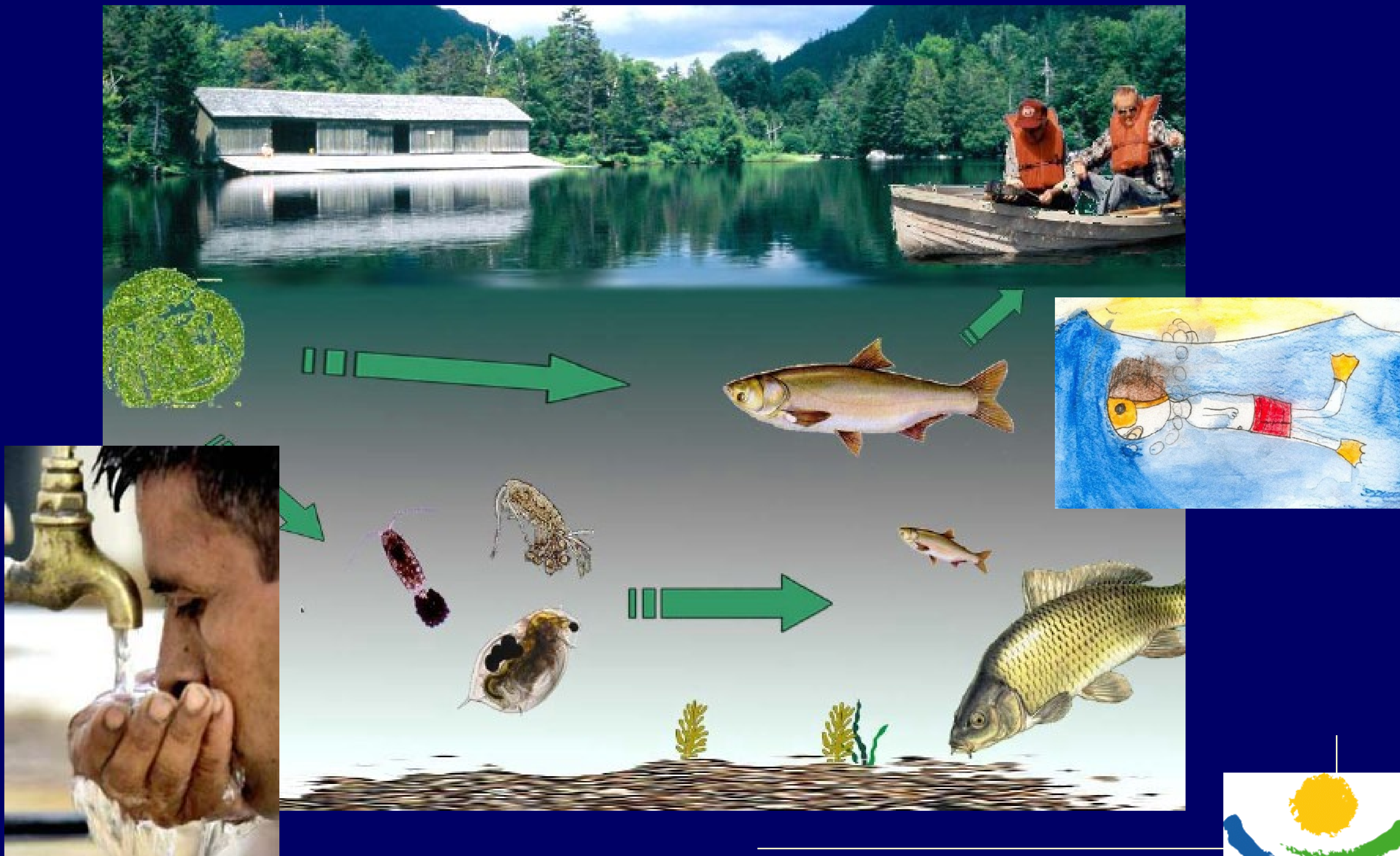
Microcystins

HUMAN HEALTH RISKS

EXPOSURE ROUTES

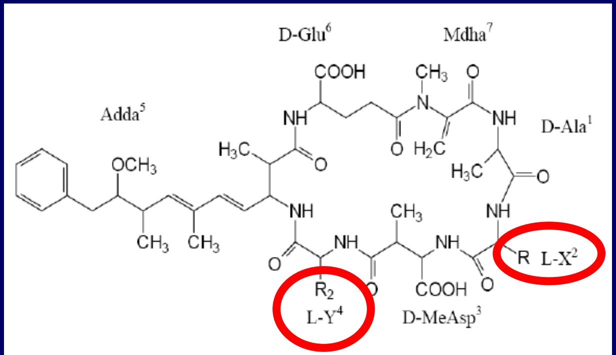


EXPOSURE ROUTES



MICROCYSTINS

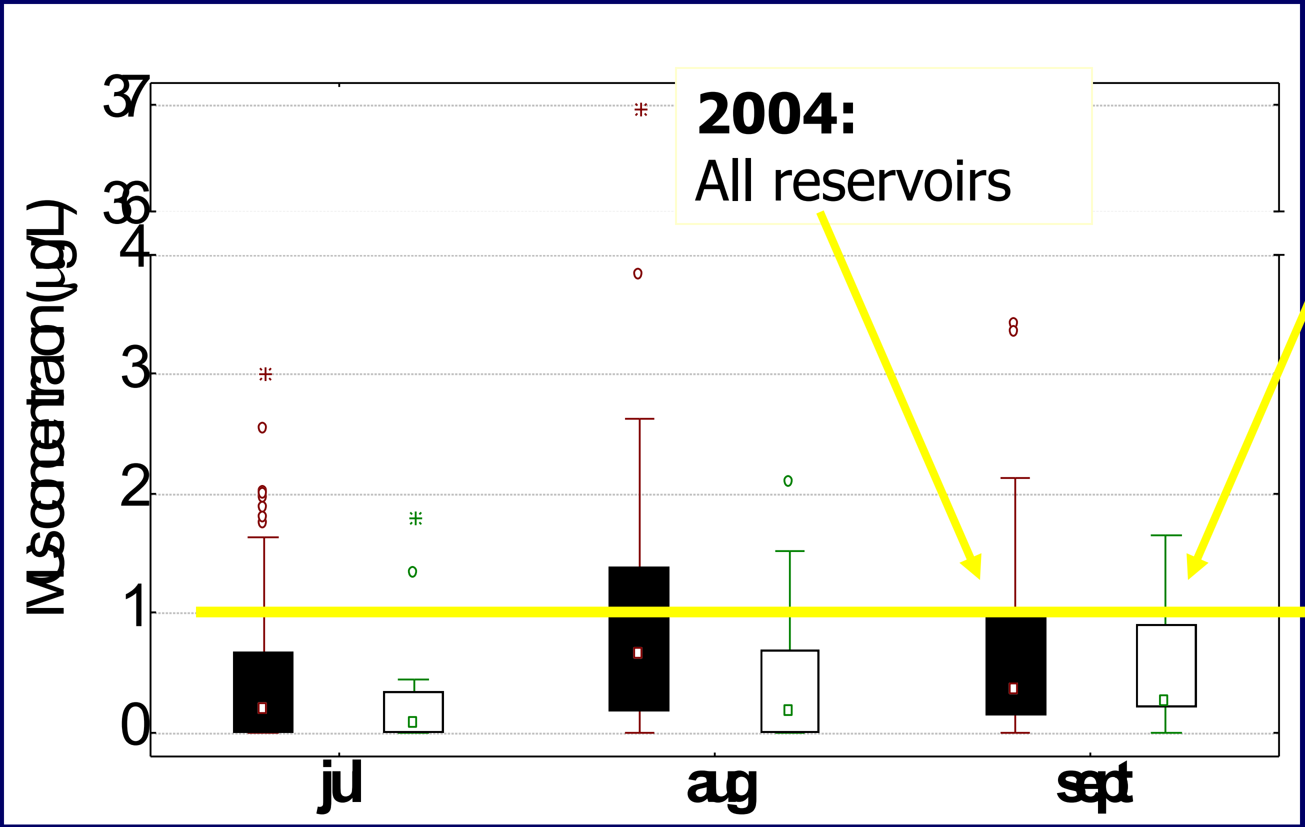
… brief reminder …



- **70 structural variants**:
  MC-LR only (about 30-50% of MCs) considered by WHO
- Human chronic TDI: **0.04 ug/kg b.w. daily**
  - drinking water guideline recommendation: **1 ug/L**
    (usually accepted in national laws worldwide, incl. Czech Rep.)

- **High toxicity** - safety risks: **manipulation regulated**
  United Nations - Bacteriological and Toxin Weapons Convention
  Czech Rep. - Law no. 281/2002 Sb. and 474/2002 Sb.

MCs in drinking water reservoirs



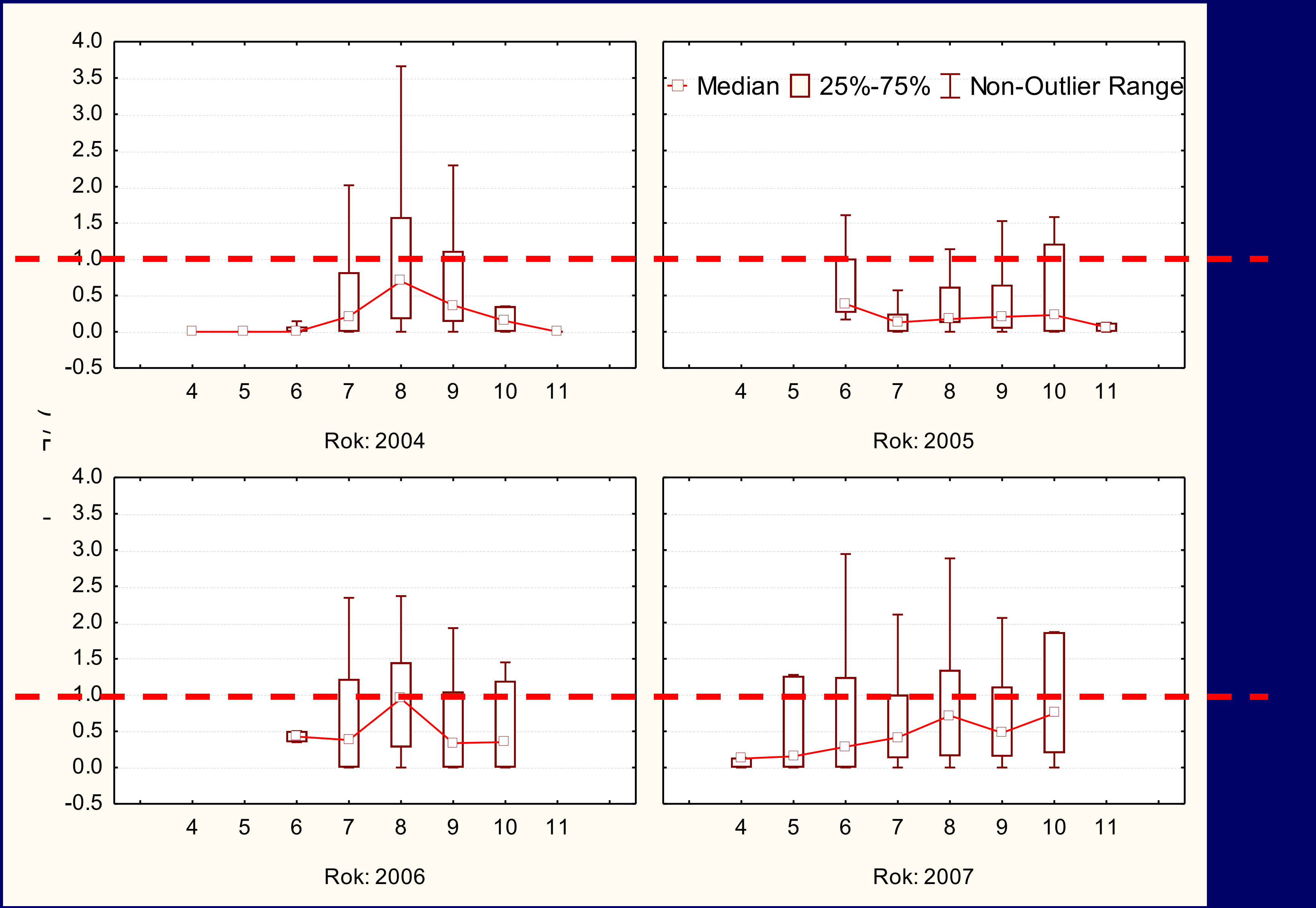
2004:
27 DW reservoirs

WHO recom.
for tap waters
1 ug/L

- **Tap waters up to 8 ug/L (1999)**

Bláha & Maršálek (2003) Arch Hydrobiol

MCs in drinking water reservoirs



"TOP" MCs **in waters** (Czech Rep. 2004-7)

Lokalita	**Datum odběru**	**MC [ug/L]**
Velké Žernoseky (pískovna)	1.8.2004	37.0
Nechranice	31.7.2004	19.0
Dubice, Česká Lípa	8.9.2004	15.1
Prostřední, Lednice	6.9.2005	18.7
Lučina	19.7.2005	17.3
České údolí VN	8.8.2005	9.3
Plumlov	15.8.2006	24.8
Dalešice	14.7.2006	16.3
Hracholusky	21.8.2006	16.3
Nechranice	26.7.2007	29.8
Skalka	22.8.2007	19.9
Novoveský	2.10.2007	16.3

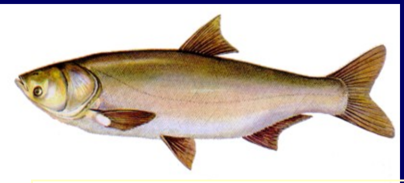
Bláhová et al. (2007). CLEAN - Soil, Air, Water 35(4), 348-354.

Risks of MCs in drinking water supplies

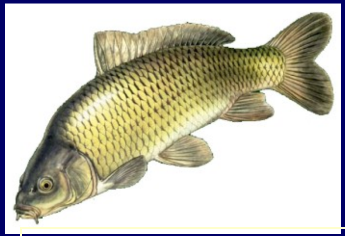
concentration of dissolved MC	20% daily intake from sources of drink.w.		100% daily intake from sources of drink.w.	
	child (25kg)	adult (70kg)	child (25kg)	adult (70kg)
	dose MC(μg.kg^{-1} live wt. day^{-1}) **HI**	dose MC(μg.kg^{-1} live wt. day^{-1}) **HI**	dose MC(μg.kg^{-1} live wt. day^{-1}) **HI**	dose MC(μg.kg^{-1} live wt. day^{-1}) **HI**
median **0.205** µg/L	0.0015 **0.038**	0.0005 **0.014**	0.0075 **0.189**	0.0027 **0.067**
extreme **17.27** µg/L	0.1272 **3.180**	0.0454 **1.136**	0.6359 **15.898**	0.2271 **5.678**

- SIGNIFICANT HEALTH RISKS EXIST !
- To minimize risk
  - Addopt appropriate technologies and treatments
  - Establish routine monitoring of MCs during the season

Accumulation of MCs in fish

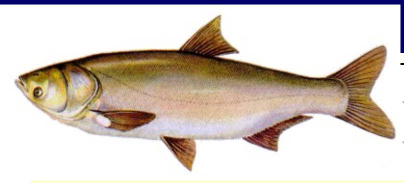


Silver carp

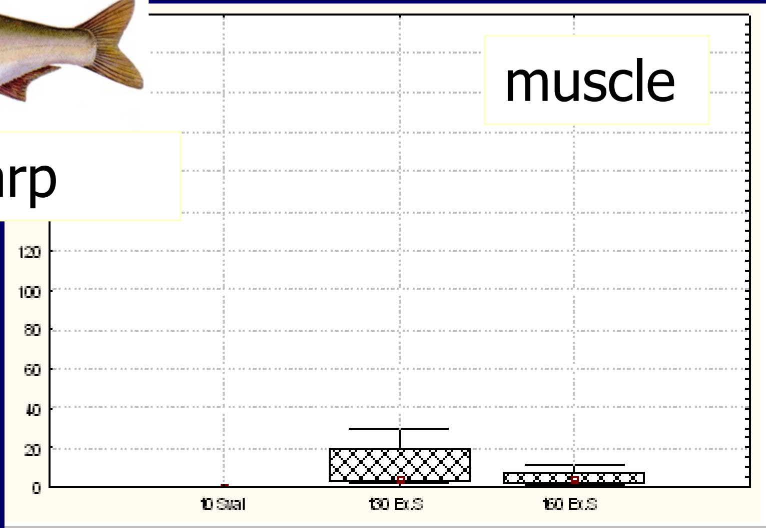


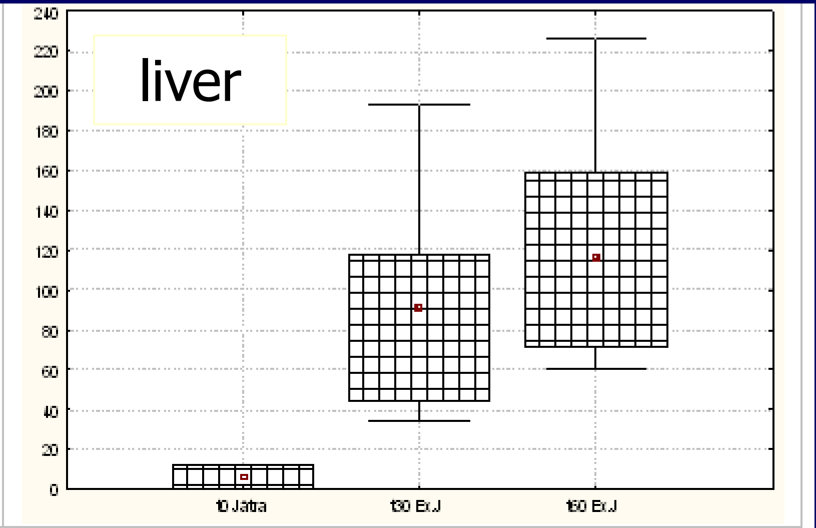
Common carp

Accumulation of MCs in fish

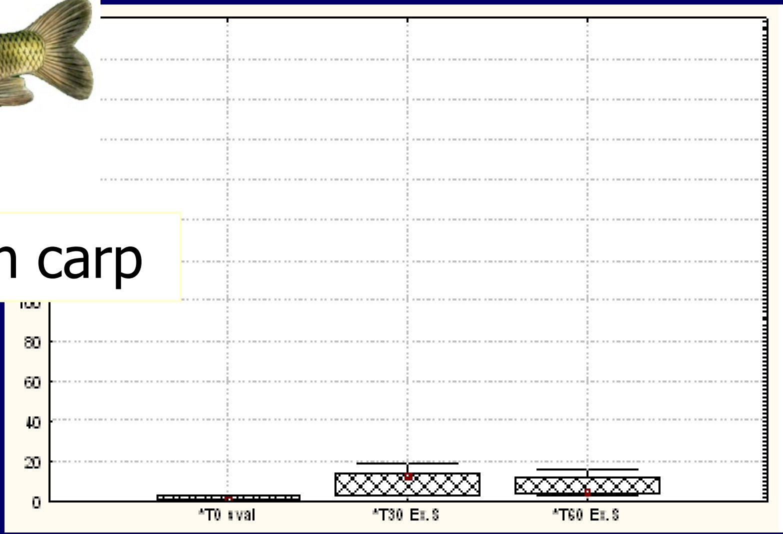


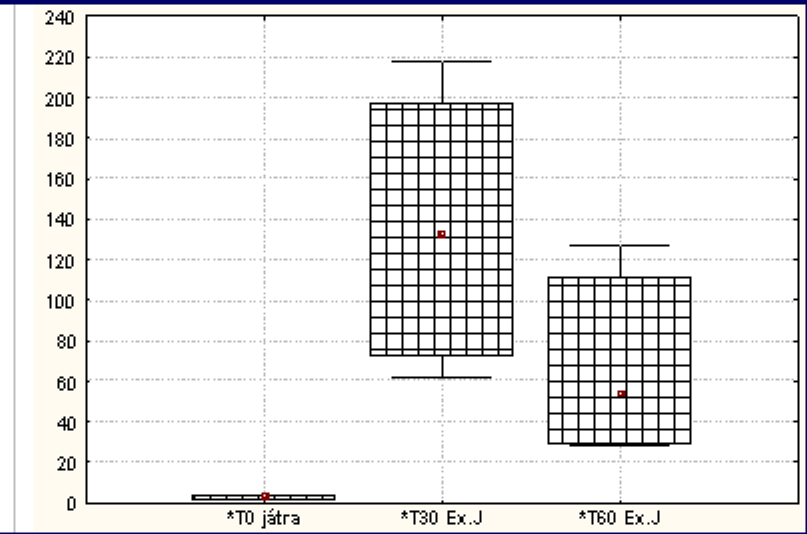
Silver carp





Common carp

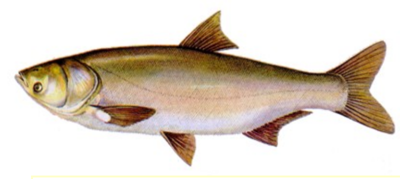




Control	30 d	60 d

Control	30 d	60 d

Risk of MCs in edible fish



Silver carp

Common carp

	Max. conc. (dose)	Max. HI	Average conc. (dose)	Average HI
SC: liver	226 ng/g 68 ug	28	106 ng/g 32 ug	13.2
muscle	29 8.8	3.7	8.4 2.5	1.1
CC: liver	217 65	27	132 39	16.5
muscle	18.8 5.6	2.4	8.5 2.6	1.1

100% of food from the contaminated source

avg. person: 60kg, food - 300g

TDI: 0.04 ug/kg/day

MCs in fish [ng/g f.w.] (Czech Republic reservoirs, 2008)

	Liver		Muscle
	Average	Maximum	
Pike perch	15.6	22.7	0
Amur	2.02	6.1	0
Carp	0.57	1.8	0
Catfish	0	0	0
Silver salmon	4.14	9.5	0

- Exposure to MCs from fish

  Less (if any) significant health risks

RECREATIONAL EXPOSURE

- **Contact dermatitis**

non-specific (!!!!)
responsible agents
(? MCs, LPS?)

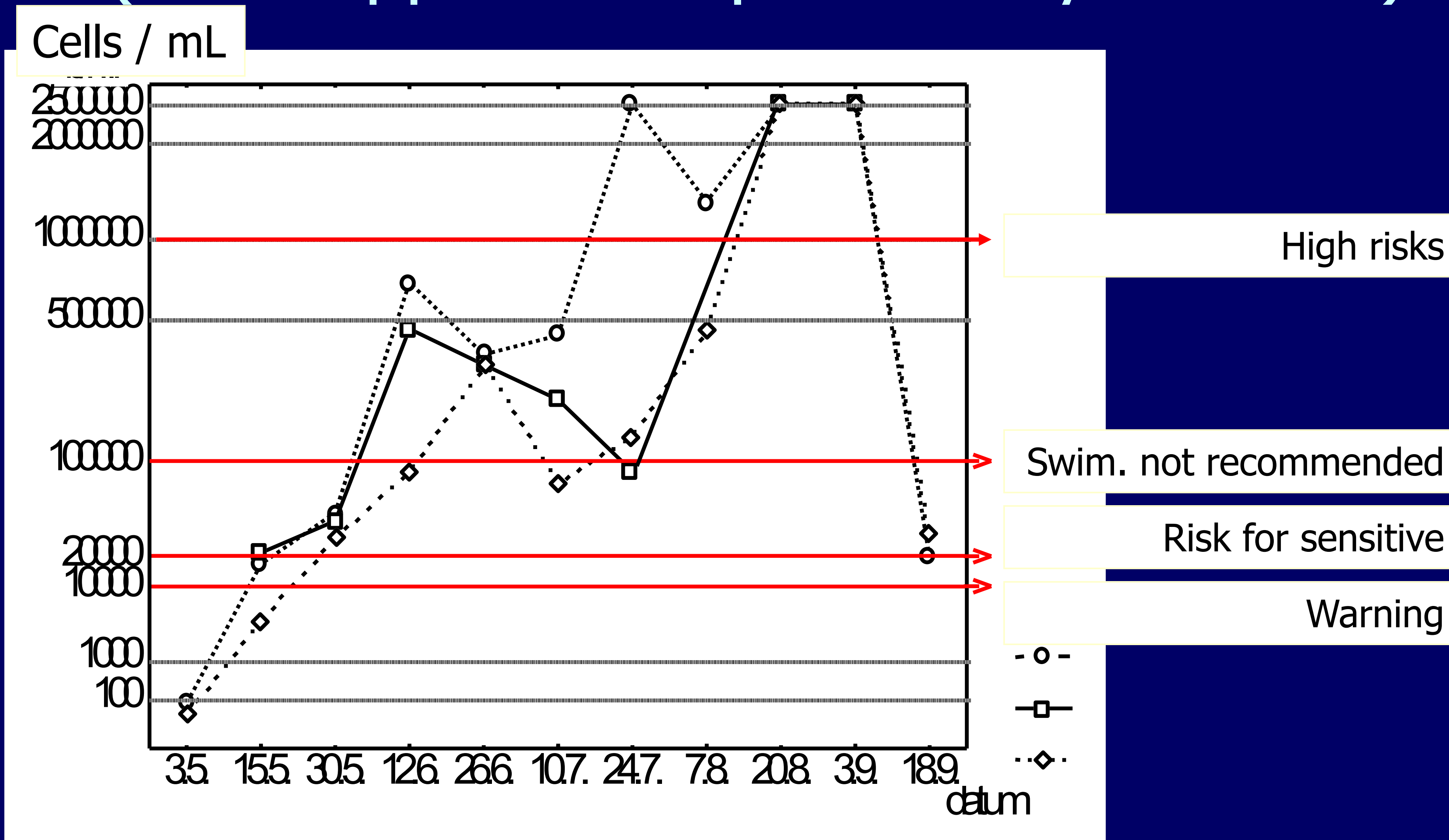
Lipopolysaccharides ?

- **Pyrogenicity of LPS**
  significant in water blooms
  (less in lab cultures)

Sample	Endotoxin activity (EU mg^{-1} d.w.)	Endotoxin activity (EU mg^{-1} LPS)
Green alga		
P. subcapitata	0	0
Cyanobacterial culture		
P. agardhii	301	35 456
A. flos-aquae	426	38 399
M. aeruginosa	257	36 809
T. variabilis	2 518	270 848
Water bloom		
Planktothrix sp.	61	46 959
Aphanizomenon sp.	7 895	918 118
M. aeruginosa	799	199 895
Microcystis sp.	989	449 576
Anabaena sp.	277	48 699
Heterotrophic bacteria		
E. coli	14 692	1 347 959
K. intermedia	1 702	239 770
P. putida	11 392	1 294 592
P. fluorescens	55	6 669

Bernardová et al. 2008 J Appl Toxicol

Toxic cyanobacteria in recreational reservoirs (WHO approach - „preliminary caution“)



RECREATIONAL EXPOSURE

- **Contact dermatitis**

non-specific (!!!!)
responsible agents
(? MCs, LPS?)

- **Toxins enter the body**

(MCs risk assessment possible)

Risks of MCs: recreational exposure
(US EPA R.A.methodology)

biomass-bound MC	7 days per year (chronic exposure)				1 day acute exposure			
	Guidance level 2 100 000 cells/mL		Guidance level 3 2 000 000 cells/ml		Guidance level 2 100 000 cells/mL		Guidance level 3 2 000 000 cells/ml	
	child (25kg/80ml.h^{-1})	adult (70kg/50ml.h^{-1})	**child** (25kg/80ml.h^{-1})	adult (70kg/50ml.h^{-1})	**child** (25kg/80ml.h^{-1})	adult (70kg/50ml.h^{-1})	**child** (25kg/80ml.h^{-1})	adult (70kg/50ml.h^{-1})
	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**	MC dose (μg.kg^{-1}bw. day^{-1}) **HI**
median concentration **348** μg/g dw	0.00019 **0.005**	0.00004 **0.001**	0.00389 **0.097**	0.00087 **0.022**	0.01013 **0.253**	0.00226 **0.057**	0.20268 **5.067**	0.04524 **1.1310**
extreme concentration **3945** μg/g dw	0.00220 **0.055**	0.00049 **0.012**	0.04406 **1.102**	0.00984 **0.246**	0.11488 **2.872**	0.02564 **0.641**	2.29757 **57.439**	0.51285 **12.823**

- Recreation exposure

  -> significant risks of MCs

Summary I - MCs and the health risks

- MCs present in 80-90% of reservoirs
- High MCs concentrations

- **All exposure routes pose significant health risks** under certain scenarios
  ! Recreation, Drinking water
  (MCs accumulated in fish - less important)

Cyanobacterial EKOtoxicity ?

- **Isolated microcystins** - many toxicological studies
- HOWEVER: **Water blooms are more than microcystins**
  - complex mixtures of many compounds (toxins, lipopolysaccharides, non-toxic components…)
  - ? accumulated toxicants (metals, POPs ???)

Many studies:

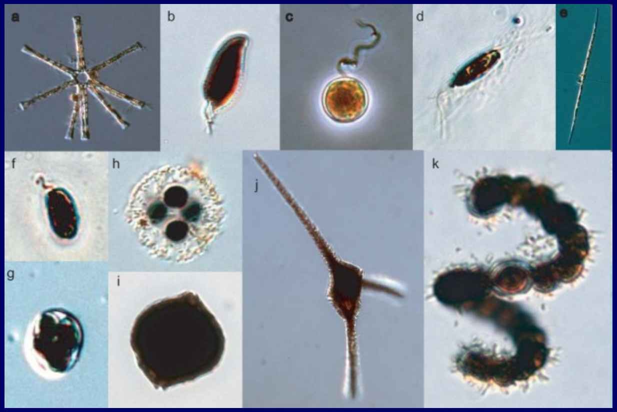
tested complex water blooms BUT interpreted as „MCs"

Ecotoxicity of WATER BLOOMS to **bacterioplankton**

- highly relevant question (MCs are evolutionary old … as well as bacteria)
- only few studies - in general **low toxicity** observed

Ecotoxicity of WATER BLOOMS to **algae (phytoplankton)**

- **Algae = competitors to cyanobacteria**
  - limited data
  - **weak direct toxicity** only at high (nonrelevant) concentrations
  - some studies indicate allelopathy between cyanobacteria & algae (*inhibition of growth, specific effects on dormant stages)*



Ecotoxicity of <u>WATER BLOOMS</u> to **zooplankton**

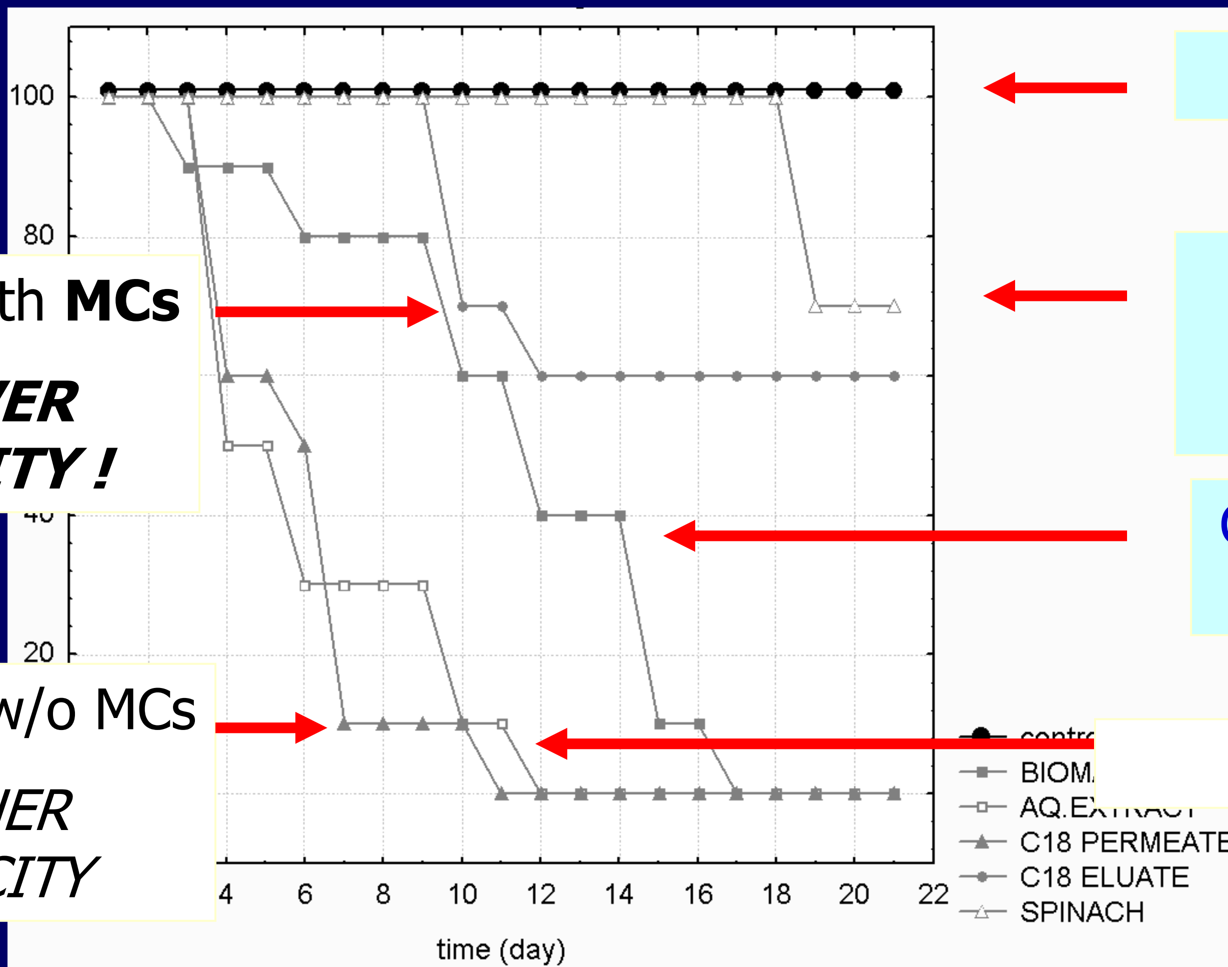
- invertebrates - **lower sensitivity** than vertebrates
- variable sensitivity of different (even closely related) invertebrate species

- one of the first hypotheses: „MCs are against predators“ (not confirmed - several contras…)

BUT: zooplankton prefers nontoxic strains during feeding
(? -> indirect effects on development of toxic blooms ?)

Ecotoxicity of cyanobacteria

Reproduction



Controls

Nontoxic biomass (spinach)

Complex water bloom

Extract

Fraction with **MCs**

! LOWER TOXICITY !

Fraction w/o MCs

HIGHER TOXICITY

Ecotoxicity of WATER BLOOMS to **fish and amphibians**

- Many studies … toxin accumulations
  + several effects observed (histhology, biochemistry…)

! Indirect effects (pH changes, oxygen content) more important in toxicology !

Ecotoxicity of WATER BLOOMS to **birds**

- deaths documented (with toxins in bird tissues)
- limited number of controlled experiments
    - low direct toxicity to model birds

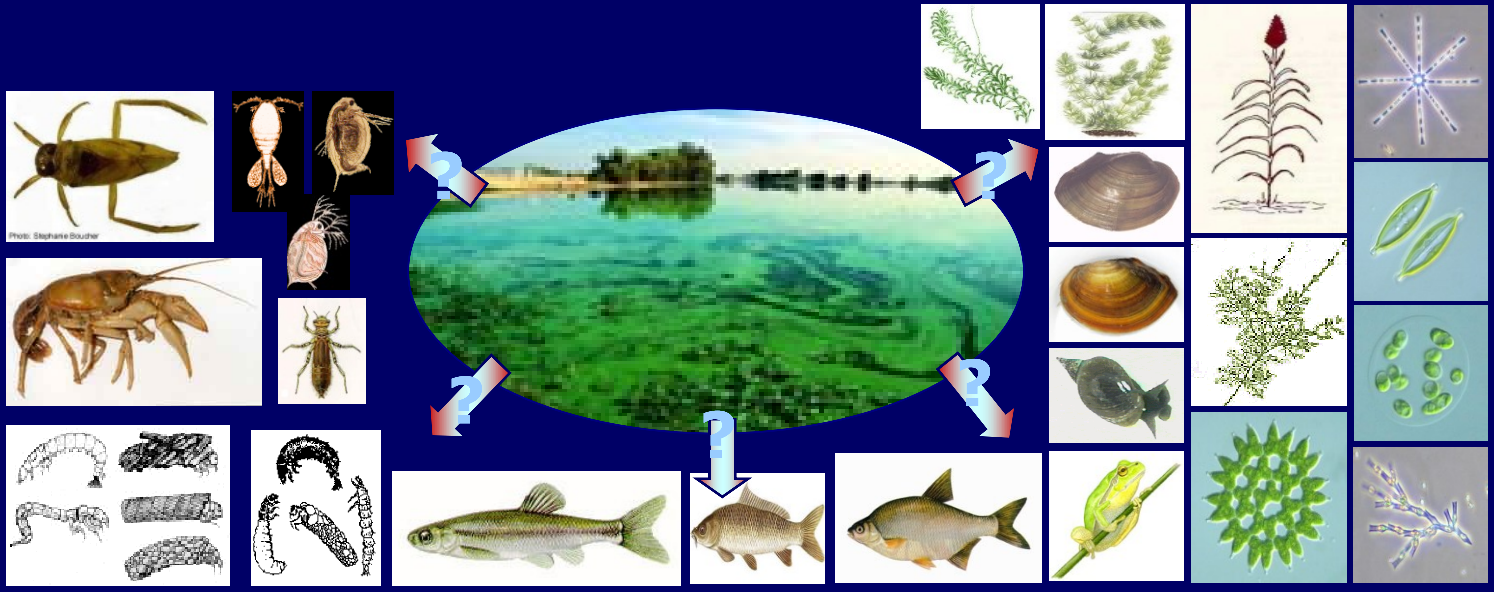
! Water blooms stimulate effects of other agents
(lead toxicity, immunosupressions)

Summary II - Ecotoxicological risks

- **Only MCs studied** *(… results disputable …)*

- **In general: Lower importance of „known" isolated toxins (such as MCs)**

  ! Complex bloom effects are more important !



… emerging toxins

Cylindrospermopsin (CYN)

	MC	CYN
LD50 (acute oral toxicity)	**6000 μg/kg**	**5000μg/kg**
NOAEL	**40 μg/kg/den**	**30 μg/kg/den**
TDI	**0.04 μg/kg**	**0.03 μg/kg**
Limit pro pitnou vodu	**1 μg/L***	**1 μg/L * * 15 μg/L * * ***

- discovered in tropics (Australia, Florida, New Zealand …)
- now reported from Europe … including C.R.

Risks of both MCs and CYN are comparable

(CYN not regulated, concentrations unknown…)

Cylindrospermopsin in the C.R.

nádrž / odběr	tax. složení vodního květu	CYN (µg/L) ELISA
Dubice		
2007-08-27	Apahnizomenon flos-aquae var. klebahnii 5%, Limnothrix redekei 70%, Planktothrix sp. 5%, Microcystis sp. 15%, Anabaena lemmermannii	3.135±0.003
Máchovo jezero		
2007-07-30	Aphanizomenon gracile 10%, Aphanizomenon sp. (10%), Microcystis sp. 30%, Aphanocapsa sp. 10%, Oscillatoriales 20%, Aphanothece sp., Anabaena sp.	0.470±0.032
Svět		
2007-07-25	Aphanizomenon flos-aquae var. klebahnii 5%, Anabaena flos-aquae 40%, Anabaena planctonica 50%, Cylindrospermopsis raciborskii	0.061±0.010

Bláhová et al. 2008 Toxicon

How to manage toxic blooms?

- Limit **nutrient sources** → **river basin (upstream)** / → **in the reservoir**
- **Cyanocides** (chemical, natural - e.g. Humic acids)
- **Flocculants** $Al(OH)_3$ …
- **Biological control** (… planktophagous fish)
- **Others** (mechanical removal, ultrasonic …)

How to manage toxic blooms?

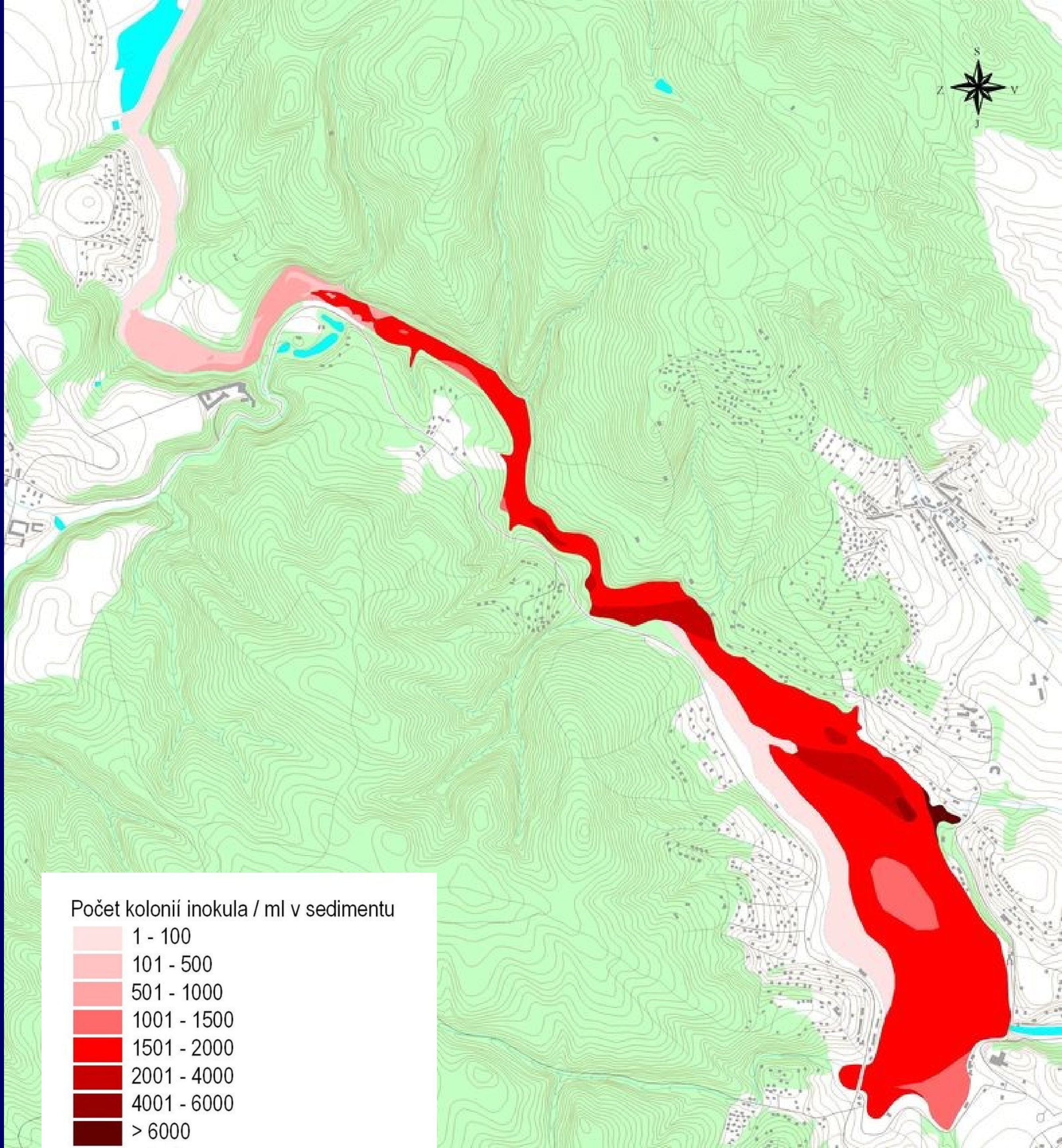
No ideal and universal approach exists

- combinations of methos
- locality-specific approach

Example

Brno reservoir

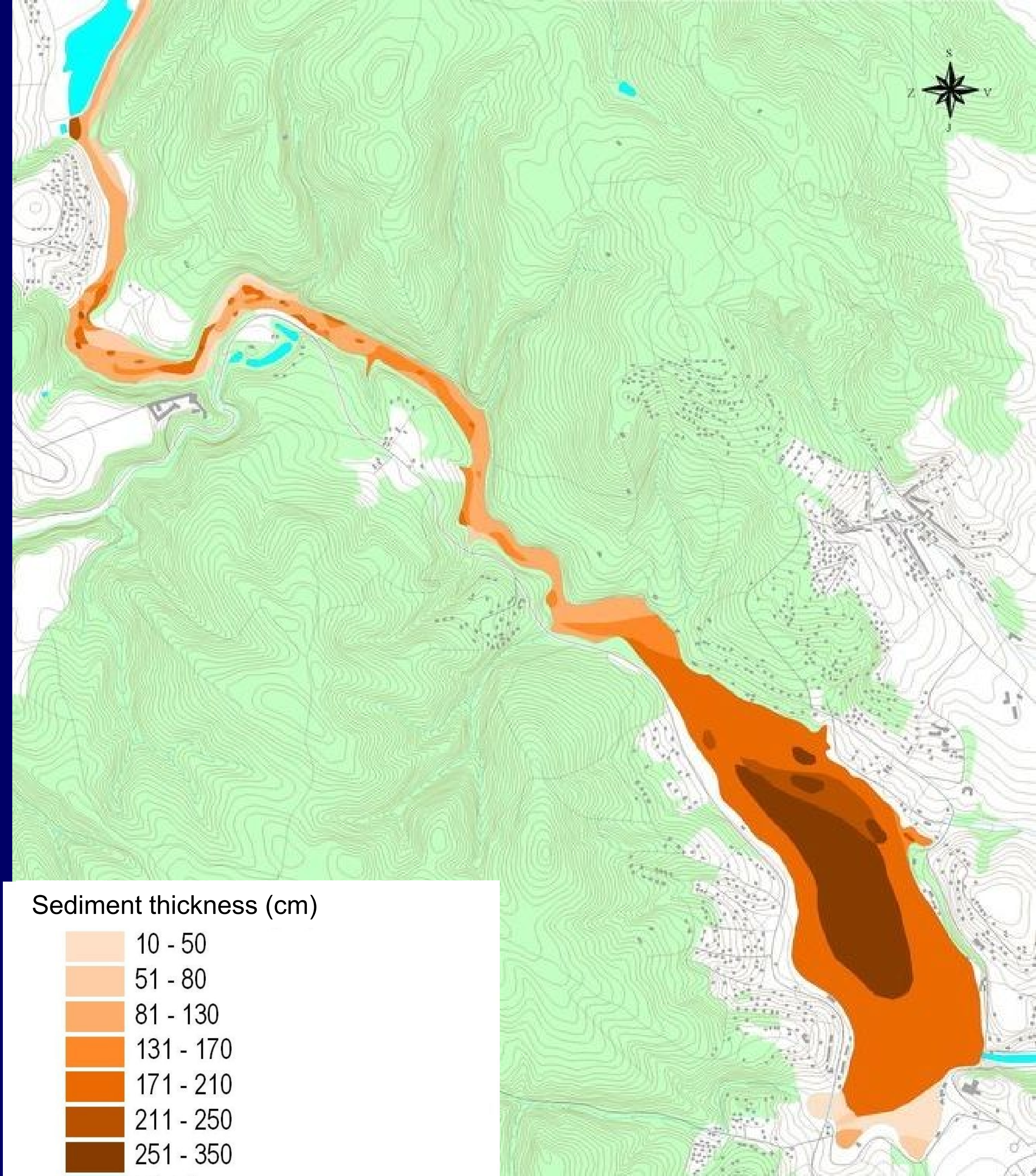
sources of cyanobacteria (colonies in sediment)



Sources of nutrients

... in the reservoir

(sediments up to 3 m thickness)



Sources of nutrients

… upstream

- several small towns & villages (no WWTPs)

CONCLUSIONS

- Eutrophication causes complex risks with complicated management

1) Ecological risks

- **Loss of diversity** … followed by losses of functioning
- **Secondary changes in the environment**

    - hydrochemistry (pH, O_2)

    - loss on natural habitats (makrophytes…)

    - new conditions (associated bacteria - patogenic ?)
- **Susceptibility to catastrophes**

- Direct ecotoxicity of individual (known) cyanotoxins seems to be less important

CONCLUSIONS

2) HEALTH RISKS OF CYANOTOXINS

- **Lower importance** - known toxins (MC) in food chains *(fish)*

- **MC in drinking water** - higher costs needed for management and control

- **Important risk** - recreation !

- **New and less explored risks**

  - new toxins (and their mixtures) - LPS, CYN ...

  - water blooms as „sorbents“ of other toxins (metals, POPs)