GARDEN – Lake GARDa ENvironmental System

2nd International Scientific Workshop – Manerba del Garda, 10 May 2018

Characterization and management of cyanotoxins in Lake Garda

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FONDAZIONE EDMUND MACH

“Idrobiologia” Research Unit

Mission

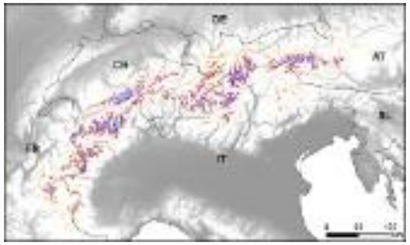
- To understand ecosystems functioning;
- To study the interctions betwwen organims and environment

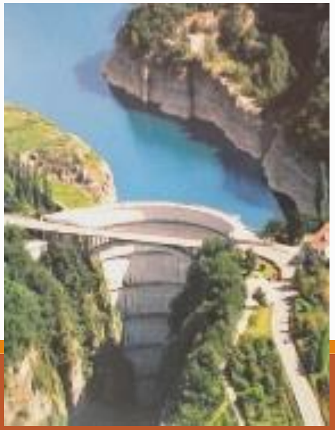
Fields of activity

- Climate changes
- Eutrophication
- Exploitation of the water resources

Effects on…

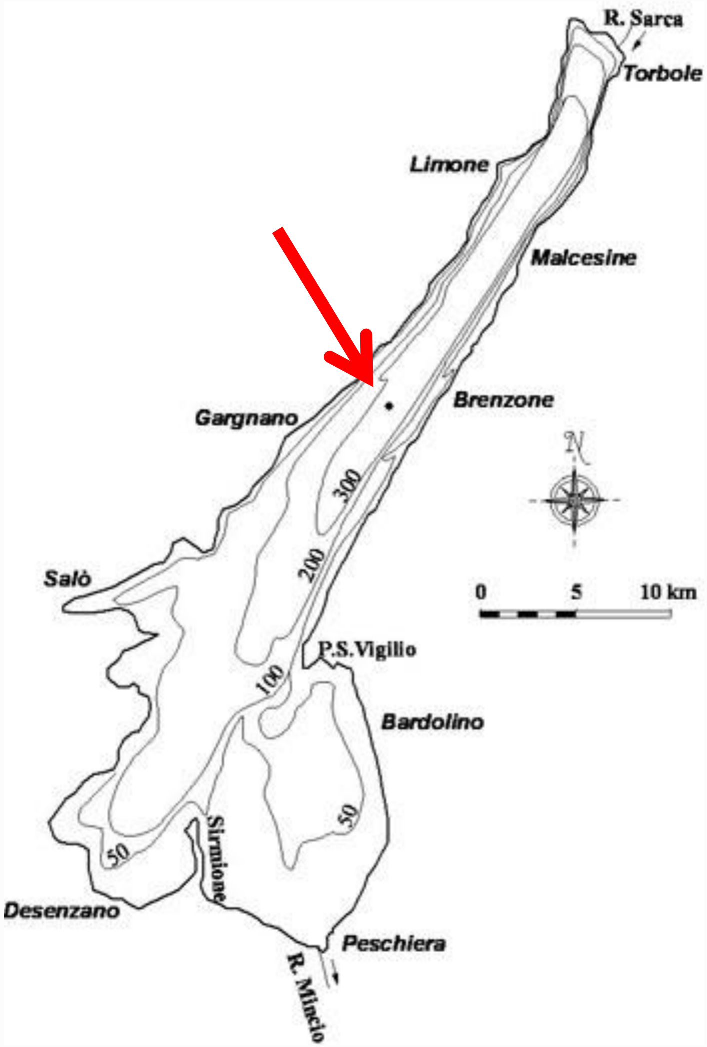
- Natural biodiversity
- Increase of algal biomass
- Deterioration of the water quality



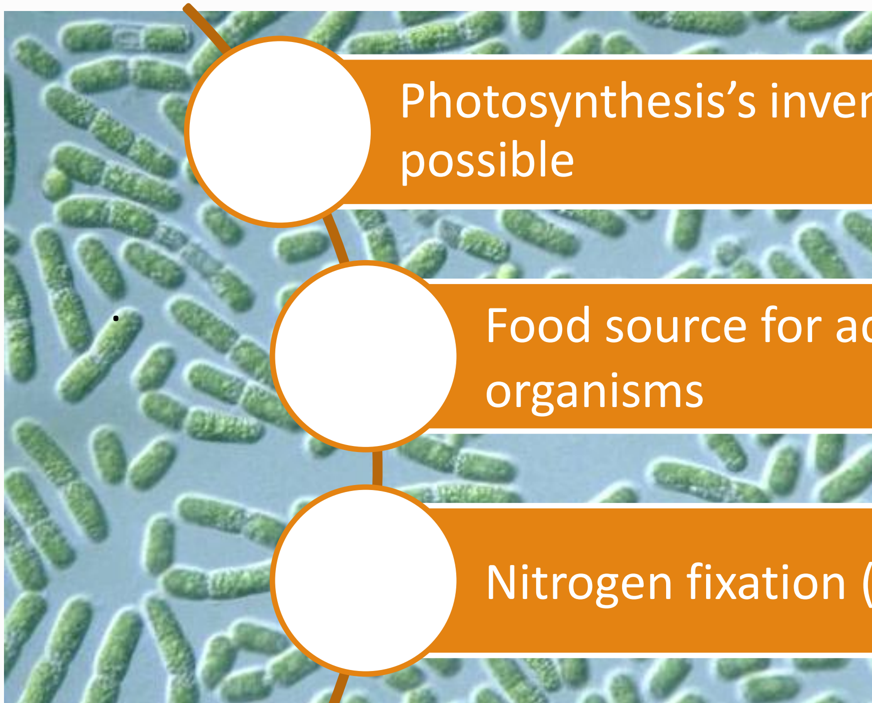


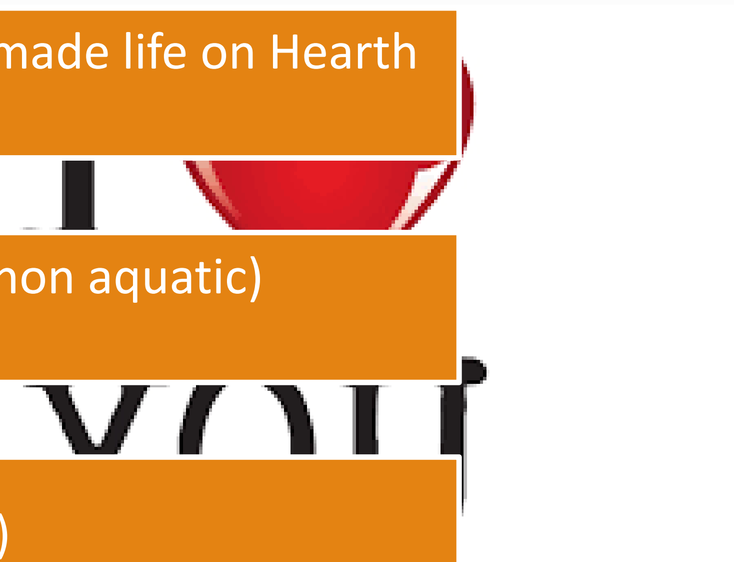
Lake Garda Study Site

Site Name:
Lago di Garda
Site Code:
LTER_EU_IT_044
Year Site Established: 1991



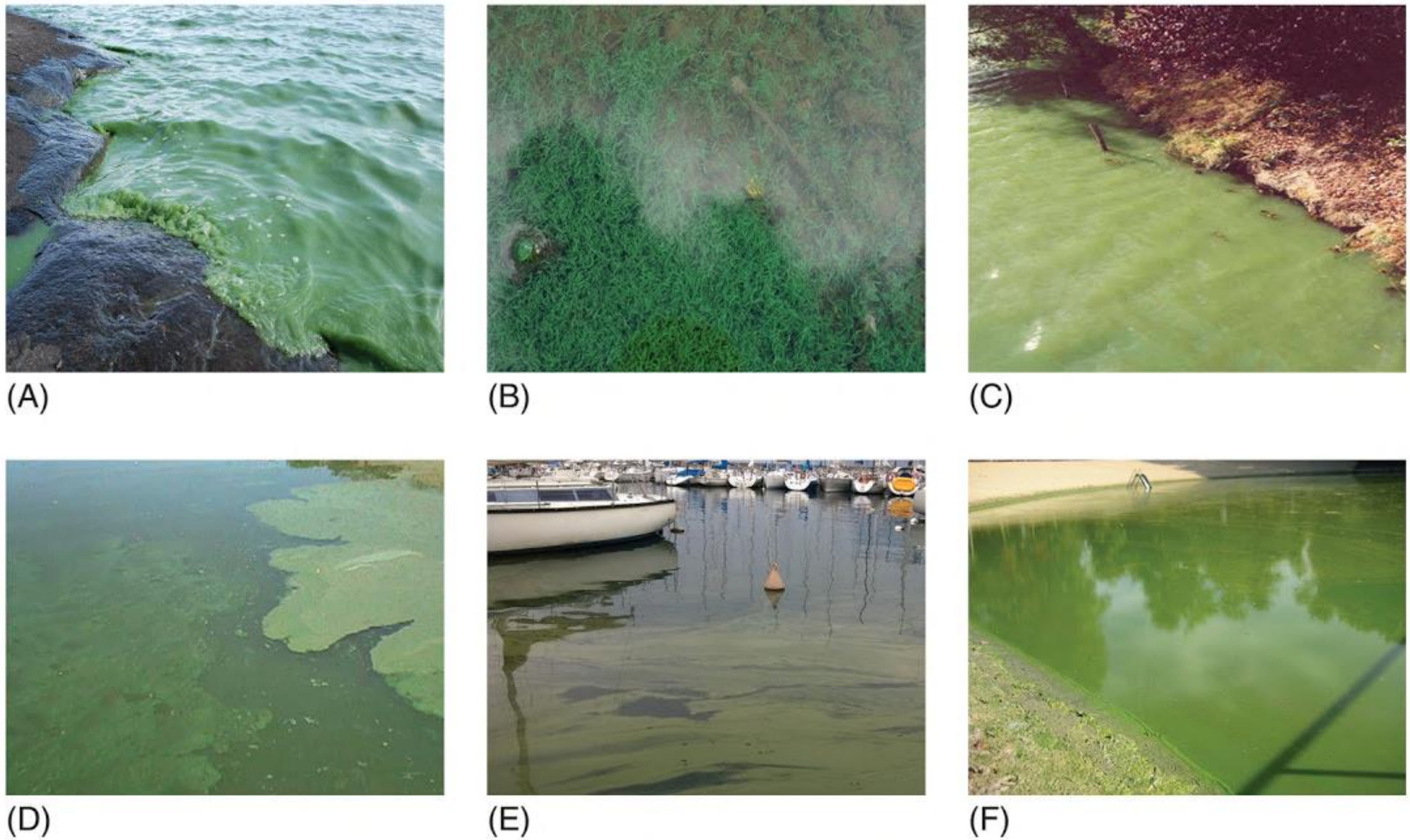
Cyanobacteria: thanks for being there!





- Photosynthesis's inventors. They made life on Hearth possible
- Food source for aquatic (and non aquatic) organisms
- Nitrogen fixation (soil fertility)
- Valuable compounds' producers (for example antibiotic production in *Lyngbya*)

Toxic Cyanobacteria



From: Salmaso et al, 2017. Basic Guide to Detection and Monitoring of Potentially Toxic Cyanobacteria. In: Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. First Edition. Edited by Jussi Meriluoto, Lisa Spoof and Geoffrey A. Codd. John Wiley & Sons, Ltd.

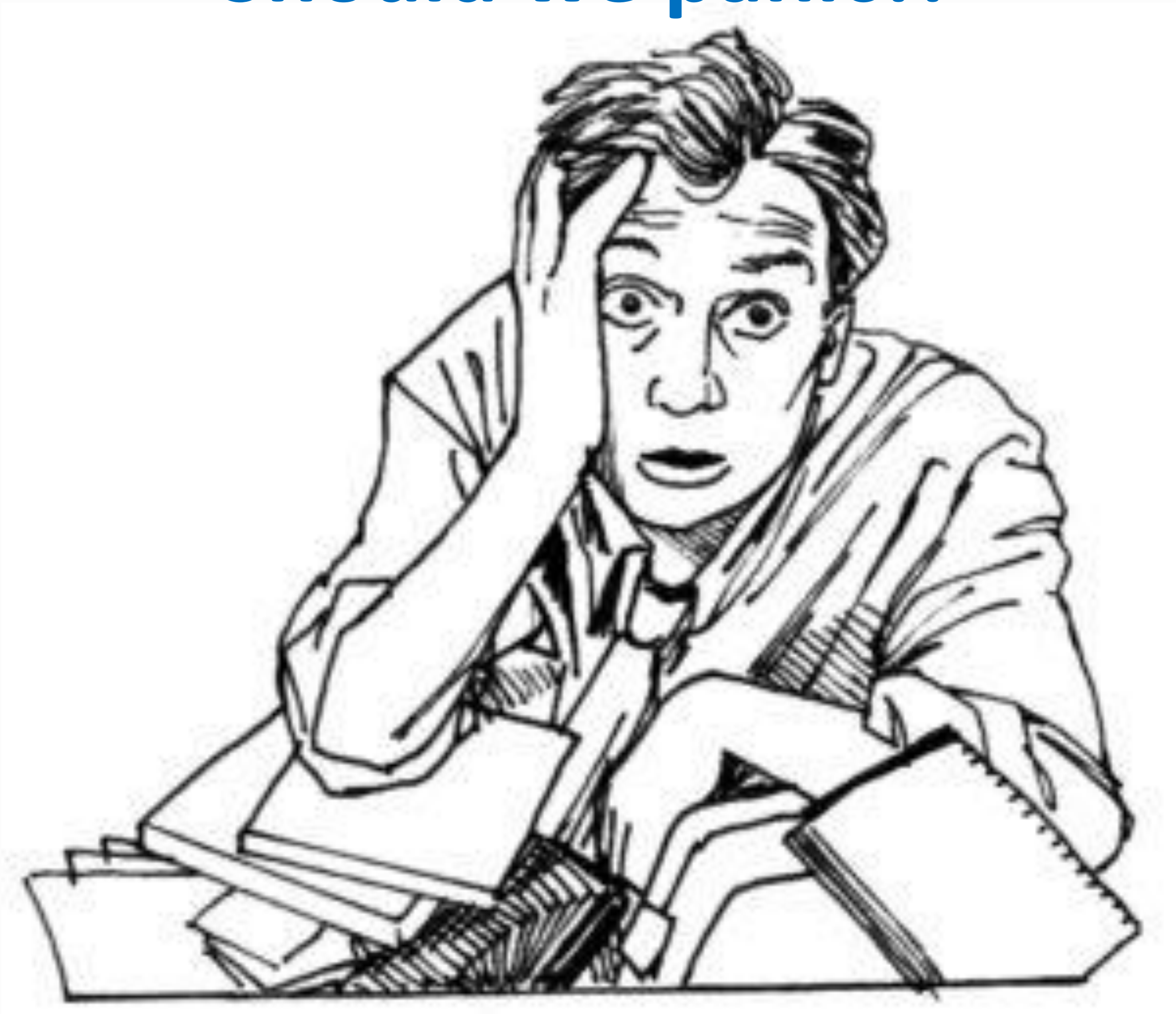
Toxic cyanobacteria, a global concern

(In black: countries where documentd episodes of cyanobacteria toxic blooms have been documented)

Cyanotoxins list

Cyanotoxin	Chemistry/number of congeners	Associated cyanobacteria	Toxicity
MICROCYSTINS	Cyclic peptides/**200**	*Dolichospermum*, *Microcystis*, *Nostoc*, *Plankthotrix*	EPATO-TOXIC, TUMOR PROMOTERS
NODULARINS	Cyclic peptides/**10**	*Nodularia*, *Nostoc*	EPATO-TOXIC
CYLINDROSPERMOPSINS	Alkaloids/**5**	*Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*	TOXIC
ANATOXINS	Alkaloids/**10**	*Dolichospermum*, *Aphanizomenon*, *Tychonema*	NEURO-TOXIC
ANATOXIN-a(S)	Organo-Phosphate/**1**	*Dolichospermum*	NEURO-TOXIC
SAXITOXINS (paralithic toxins, PSP)	Alkaloids/**50**	*Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*	NEURO-TOXIC
BMAA (β-metil-amino-alanin)	Aminoacid/**1**	many	NEURO-TOXIC
LPS (LypoPolySaccharides)	Lypopolisaccharides/**tante**	All	INFLAMMATORY AGENTS
tot	**277**		

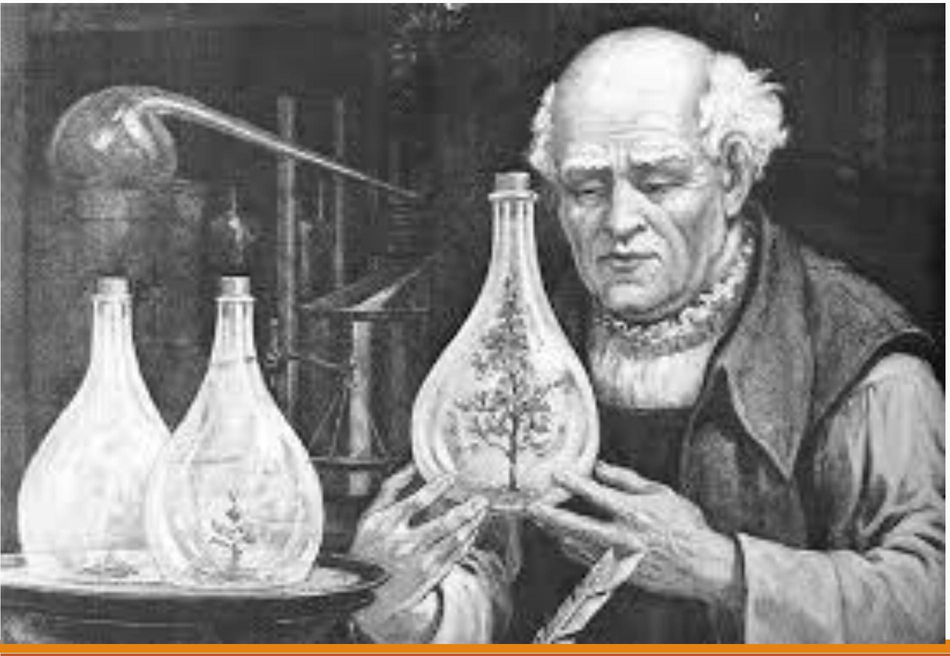
Should we panic!?

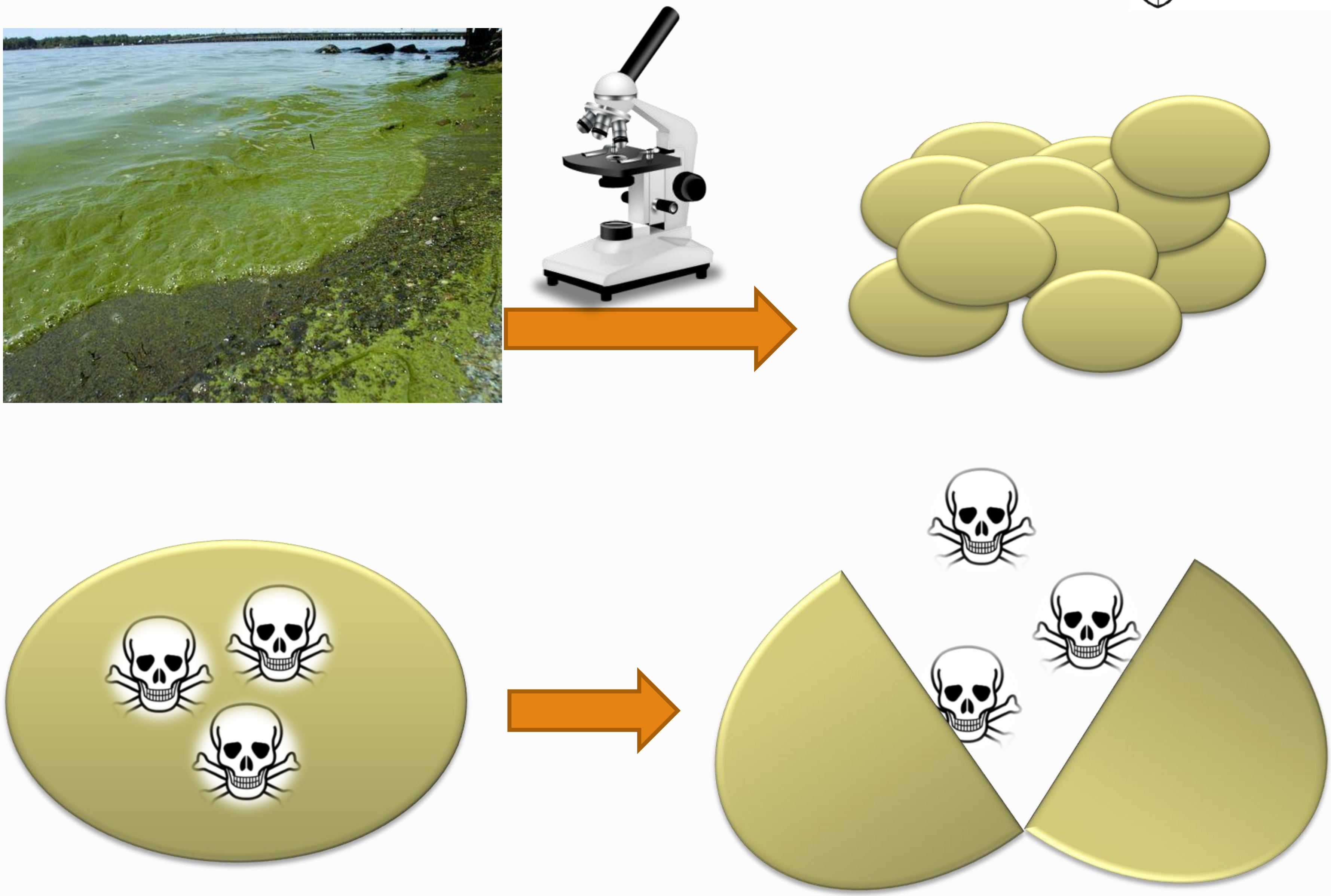


“All things are poison and nothing is without poison; only the dose makes a thing not a poison”

(Paracelsus, 1493-1541)

Philipp Theophrast Bombast von Hohenheim





Health Risk: Exposition routes

- Ingestion of contamined water
- Inhalation/skin contact
- Ingestion of fruit/vegetables irrigated with contaminated water
- Ingestion of fish living in contaminated water
- Food supplements
- Endovenous administration

Tegel Lake (Berlin), june 2017


REINICKENDORF

Neuartige Blaualge im Tegeler See tötete drei Hunde

In drei Hunden wurde das Gift Anatoxin A nachgewiesen. Es stammt von einer neuartigen Blaualge, die auch für Menschen gefährlich ist.

14.06.2017, 12:59 Uhr **Janine Richter**

Auch Jessica Simon trauert am Tegeler See um ihre tote Mischlingshündin

Foto: Frank Lehmann

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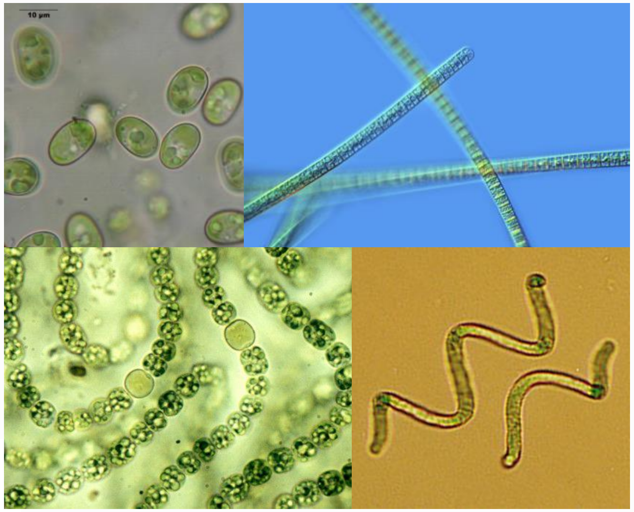
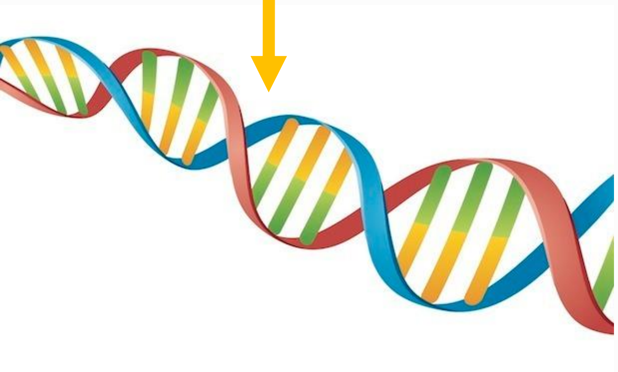
Multidisciplinary approach

microscopy

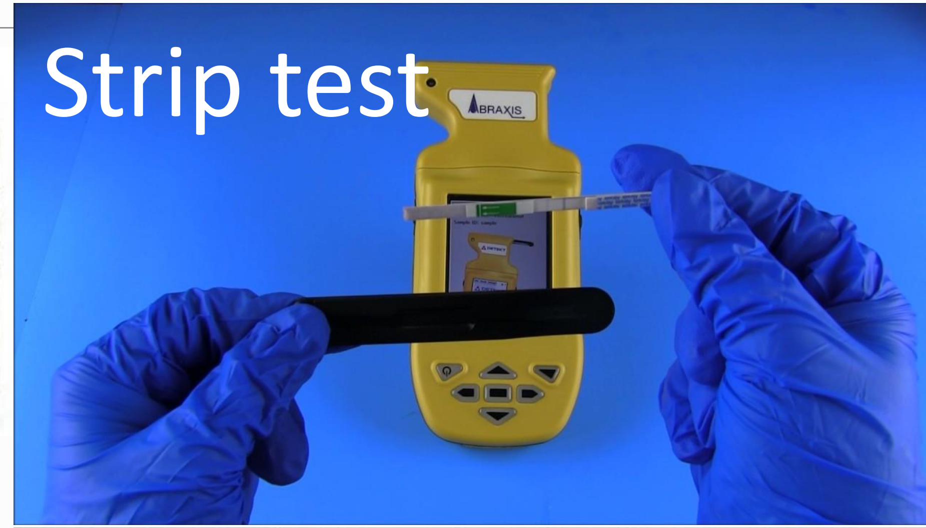


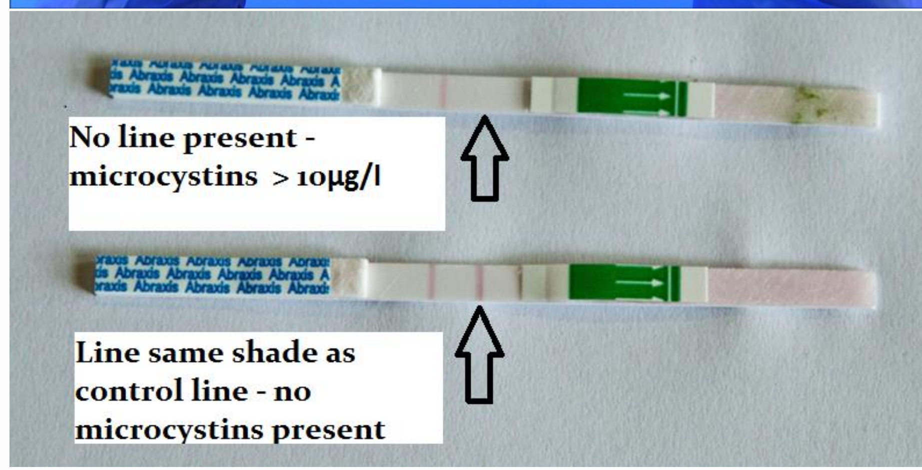
chemistry

Genetics/genomics



Cyanotoxins' analysis





Tegel Lake, June 2017

Anatoxin A concentration in water: 1870 micrograms/liter!

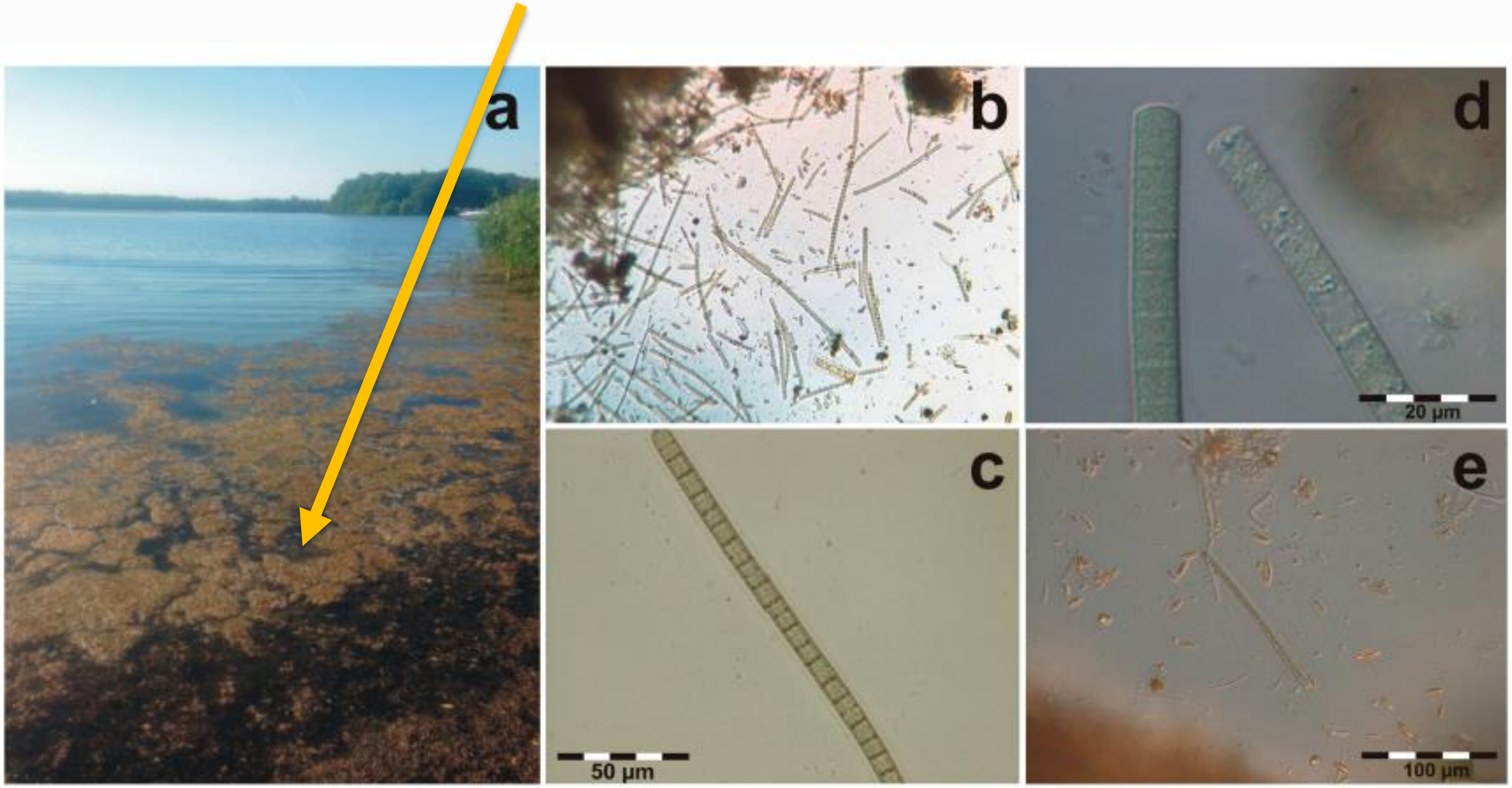
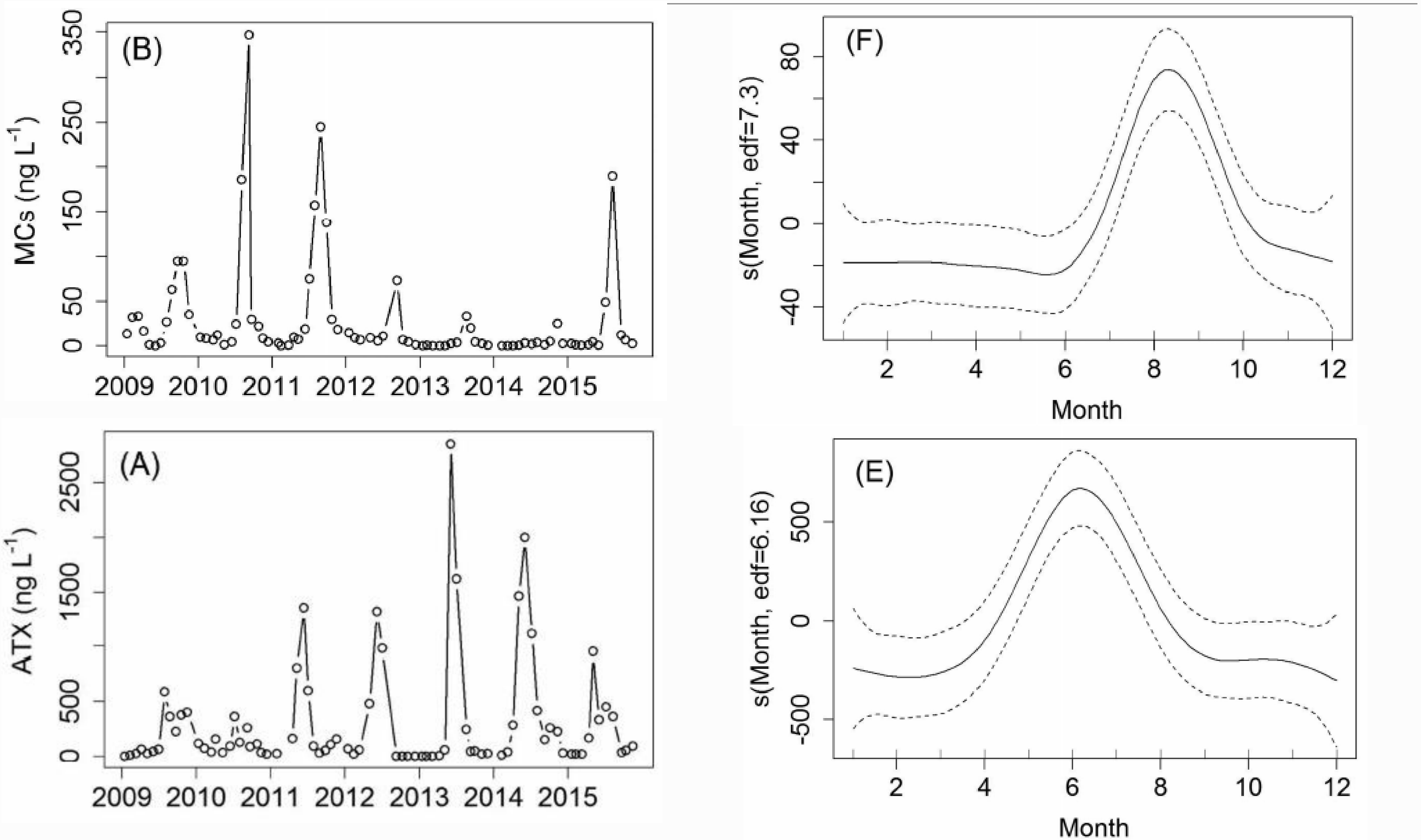


Figure 1. (a) Water moss (*Fontinalis antipyretica*) floating on the surface of Lake Tegel (2 June 2017); (b–d) *Tychonema* sp. filaments within water moss clumps (b: 2 June 2017; c-d: 6 June 2017; b: ×100; c: ×400, d: ×1000); (e) *Tychonema* sp. filament observed in the stomach contents of a dead dog (×200).

Lake Garda - long term studies

Salmaso *et al*. FEMS Microbiology Ecology, 2016.



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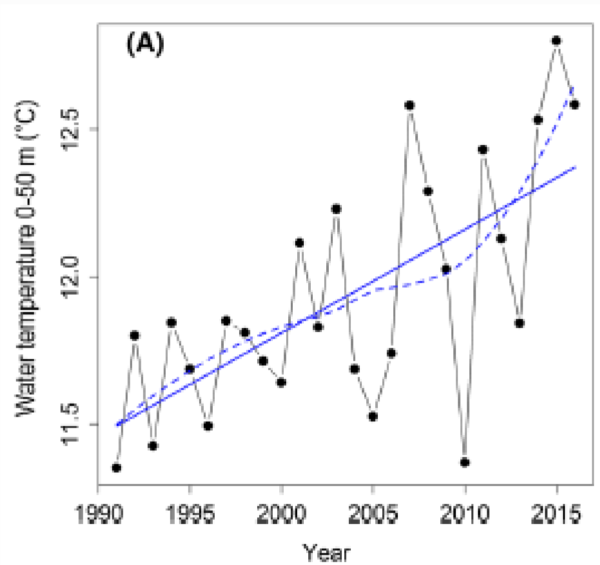
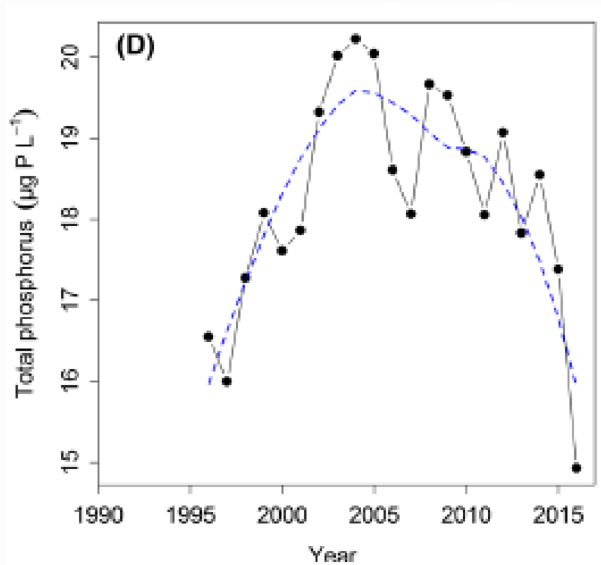
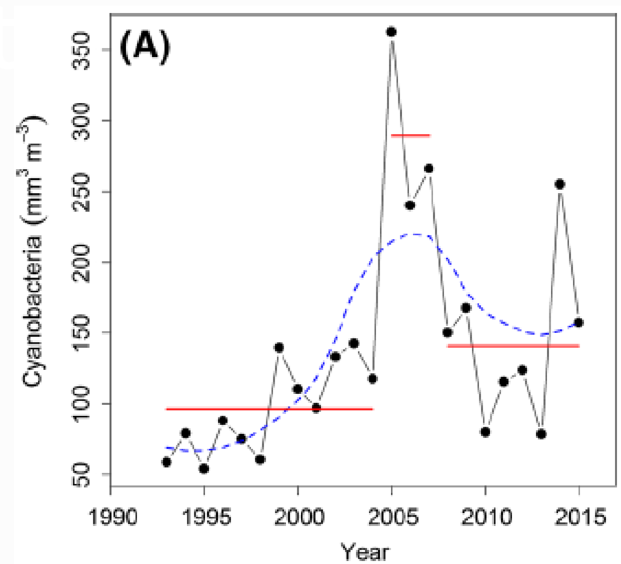
Lake Garda - long term studies

Salmaso *et al*. Hydrobiologia, 2017.







Ongoing ecological shifts are driven by **climate change** and **eutrophication**

Lake Ledro

http://www.floraitaliae.actaplantarum.org/download/file.php?id=67953

Lake Ledro

Boscaini *et al*. Adv Ocean Limnol, 2017.

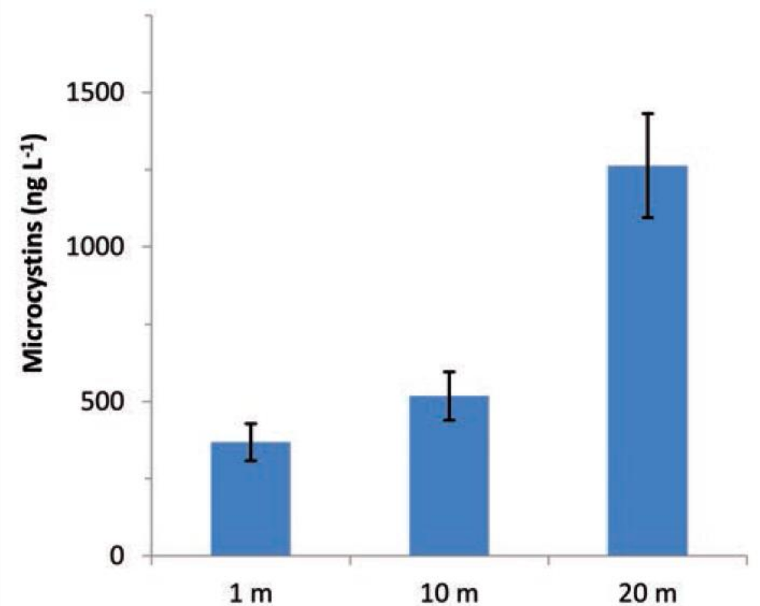
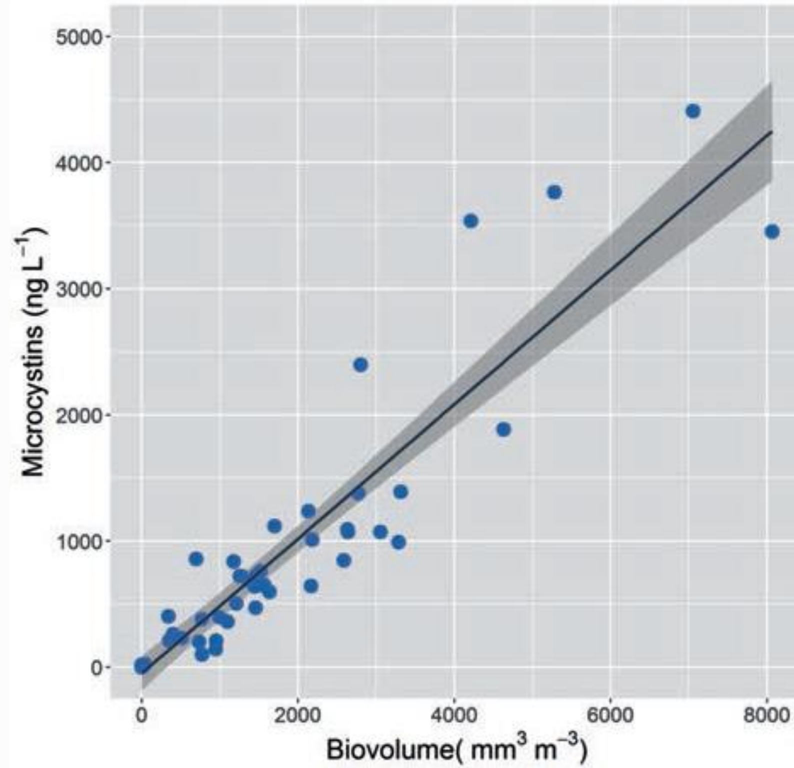


Fig. 8. Mean values of total MC measured at the surface, 10 m and 20 m; data refer to samples collected between June 2011 and December 2012. The vertical bars indicate the standard errors of the means.

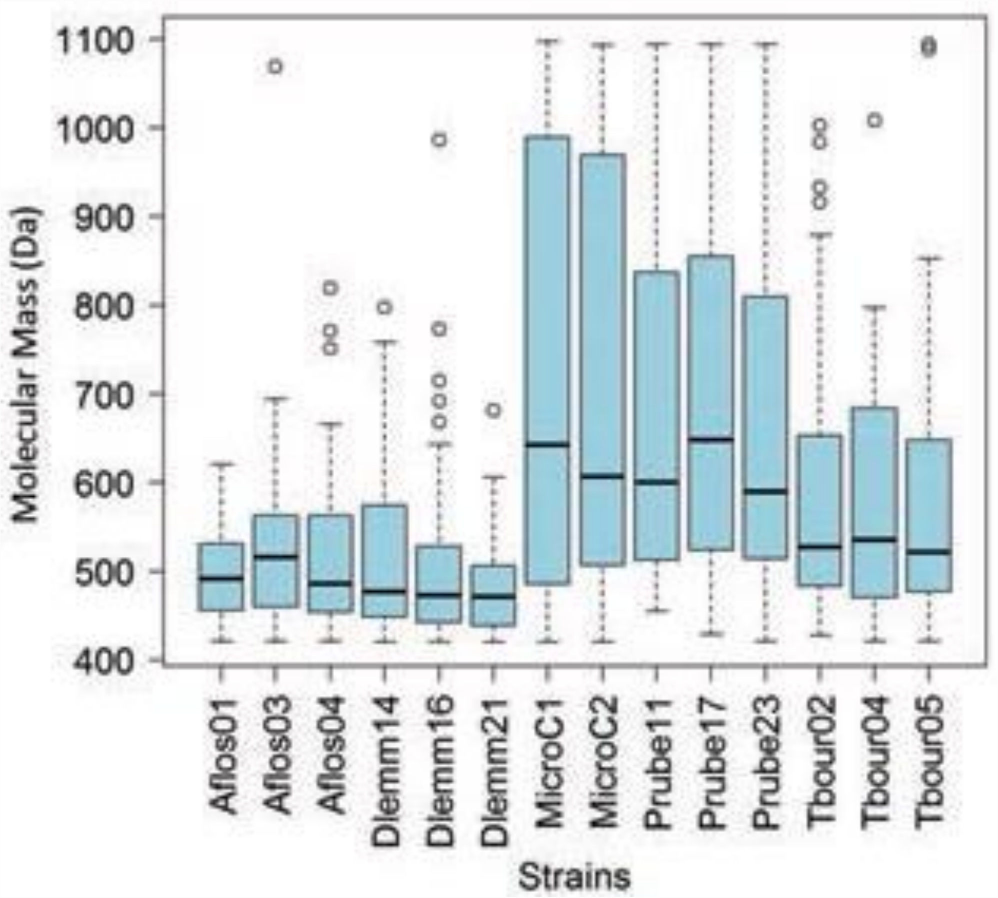


Metabolic profiles of cyanobacteria in southern perialpine lakes

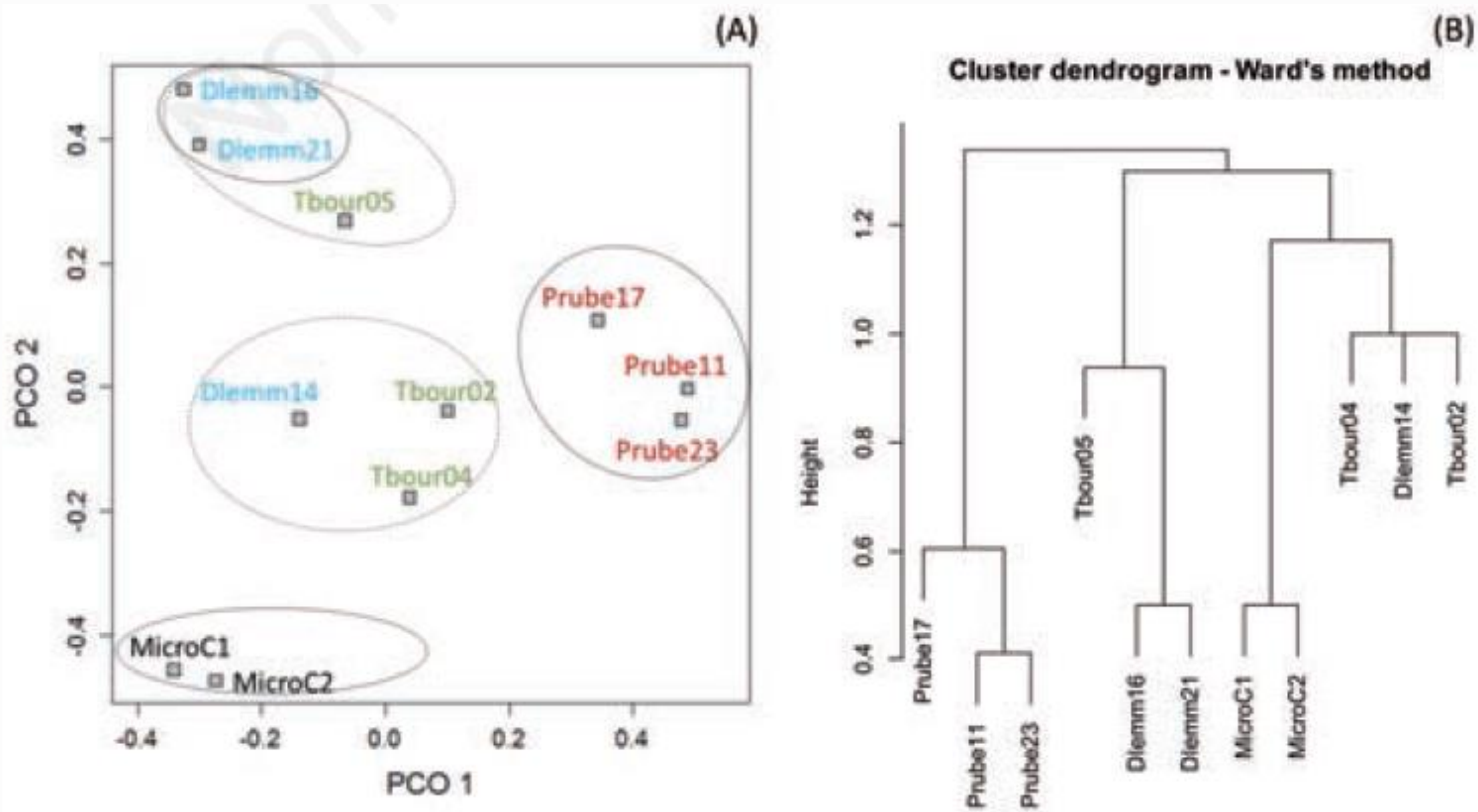
Cerasino *et al*. Adv Ocean Limnol, 2017.

		Targeted analysis		Ur
Species (Lake)	Strain code	Alkaloids	MC (%)	
Aph. flos-aquae (Idro)	Aflos01			
	Aflos03			
	Aflos04			
D. lemmermannii (Garda)	Dlemm14			
	Dlemm16			
	Dlemm21			
M. aeruginosa (Caldonazzo)	MicroC1		*LR (96.6), LRdm (3.2), YR (0.2)*	
	MicroC2		*LR (90.8), LRdm (9.1), YR (0.1)*	
P. rubescens (Garda)	Prube11		*RRdm (83.1), LRdm (16.6), HtyrRdm (0.2), RR (0.1)*	
	Prube17		*RRdm (89.4), LRdm (10.1), RR (0.3), HtyrRdm(0.1), LR (0.1)*	
	Prube23		*RRdm (99.6), LRdm (0.3), RR (0.1)*	
T. bourrellyi (Garda)	Tbour02	*ATX, C3/4(?)*		
	Tbour04			
	Tbour05	*ATX, C3/4(?)*		

Metabolic profiles of cyanobacteria in southern perialpine lakes



Metabolic profiles of cyanobacteria in southern perialpine lakes



Molecular mass	Observed *m/z* and RT	Putative compound	Strain	Diagnostic fragmentation peaks#
592.3	593.3 at 1.37	Aeruginosin	Prube11, Prube17	140, 120,
642.4	643.4 at 4.89	aeruginosin 101	Dlemm16, Tbour05	309, 221, 86
650.4	651.3 at 1.92	aeruginosin 102	Prube11, Prube23	150, 140, 86,
698.3	699.2 at 0.83	anabaenopeptin	Tbour04, MicroC2, Prube11	120, 74
714.3	715.3 at 1.23	aeruginosin 126B	Prube17, Prube23	164, 150
816.3	817.3 at 4.82	anabaenopeptin	Prube11, Prube17, Prube23	120, 72
830.3	831.3 at 1.09	anabaenopeptin	MicroC1	243, 150, 120
836.4	837.4 at 1.25	anabaenopeptin B	Prube11	201, 175
850.3	851.3 at 1.57	anabaenopeptin F	Prube17, Prube23	201, 175
855.3	856.3 at 5.03	anabaenopetin	MicroC2	243, 120
983.4	984.4 at 4.06	cyanopeptolin	MicroC1, MicroC2	243, 150
987.4	988.4 at 7.54	Microcystin Asp3Dhb7-LY	Prube11	375, 213, 135, 107, 86
996.4	997.4 at 3.21	microcystin (L-MeAla7)LR	Prube17	375, 213
997.4	998.4 at 4.61	cyanopeptolin	MicroC1, MicroC2	243, 150, 120
1008.5	1009.5 at 5.17	Microcystin	MicroC1, MicroC2	375, 213, 135
1010.6	506.8 at 5.41*	cyanopeptolin	Prube17, MicroC2	243, 215, 150, 120
1011.4	1012.4 at 3.56	cyanopeptolin	Prube17	243, 150, 120
1023.5	1024.5 at 4.15	cyanopeptolin	MicroC1, MicroC2	150, 120
1030.5	1031.5 at 5.91	Microcystin	Prube11	375, 213, 135
1039.5	520.7 at 4.20*	cyanopeptolin	MicroC2	150
1093.5	1094.5 at 2.53	cyanopeptolin	Prube11, Prube17, Prube23, Tbour05	150, 107, 84
1107.6	554.8 at 3.60*	Peptide	Prube11, Prube17, Prube23	164, 107, 84
1121.7	561.8 at 4.18*	Peptide	Prube23, Prube11	339, 164, 107
1123.7	562.3 at 3.04*	Peptide	Prube17	150, 120, 84
1163.6	582.8 at 3.85*	Peptide	Prube17	164
1179.6	590.7 at 3.40*	Peptide	Prube11, Prube17	164
1182.7	592.3 at 0.88*	Peptide	MicroC2	120

The Eco-AlpsWater project

(April 2018 – April 2021)

Partner	
Partner Abbreviation	Country
FEM	ITALIA
LFUI	ÖSTERREICH
ARPAV	ITALIA
NIB	SLOVENIJA
ARSO	SLOVENIJA
INRA	FRANCE
LfL	DEUTSCHLAND
AGES	ÖSTERREICH
LfU	DEUTSCHLAND
AFB	FRANCE
ISPRA	ITALIA

Title: "Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers"

Objective:
Next Generation Sequencing (NGS) to analyze environmental DNA in waterbodies:
-> rapid species identification at low. costs (from fish to bacteria)

GOAL: upgrading traditional WFD/WPO methods

The AlgaeCeuticals project

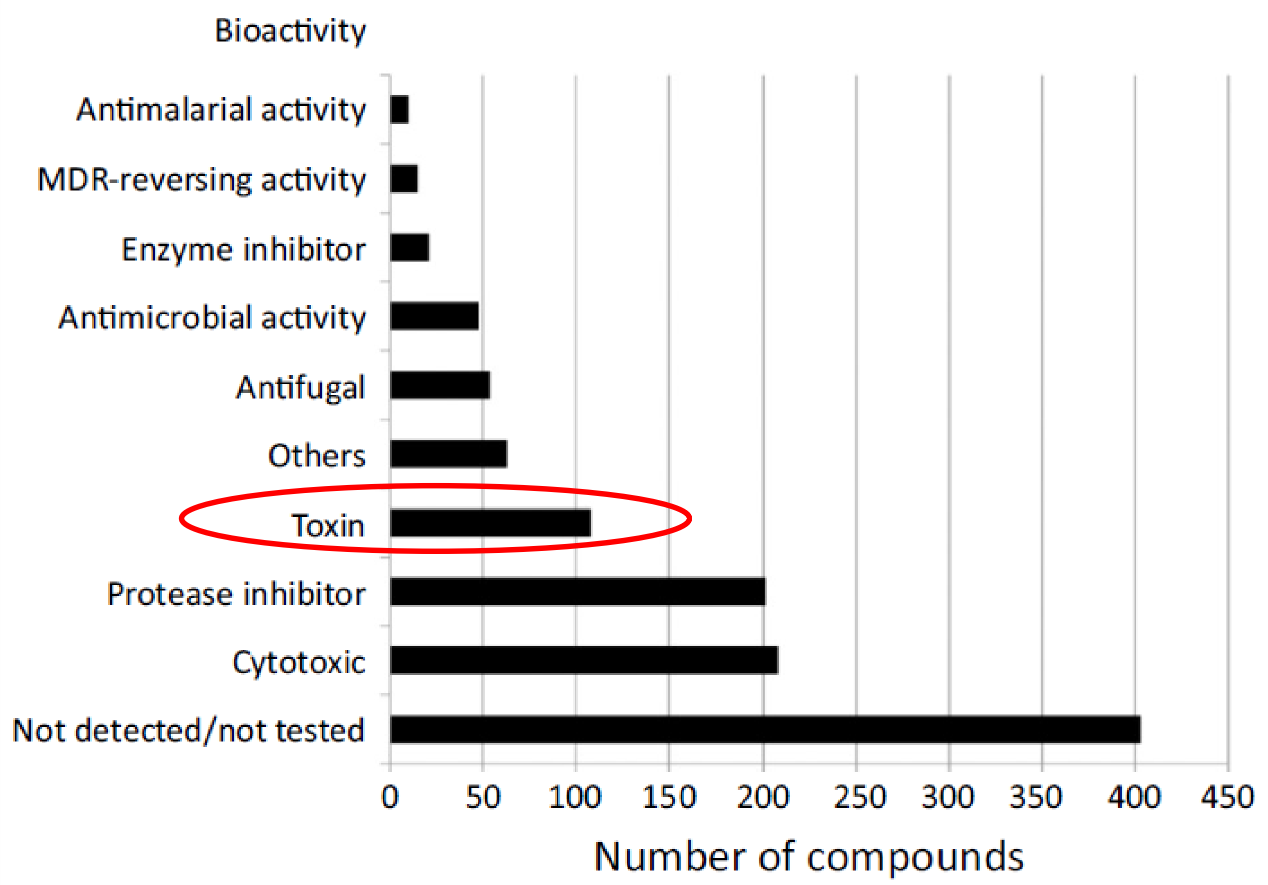
(January 2018 – December 2021)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778263

Title: "Development Of Microalgae-based Natural Uv Sunscreens And Proteins As Cosmeceuticals And Nutraceuticals"

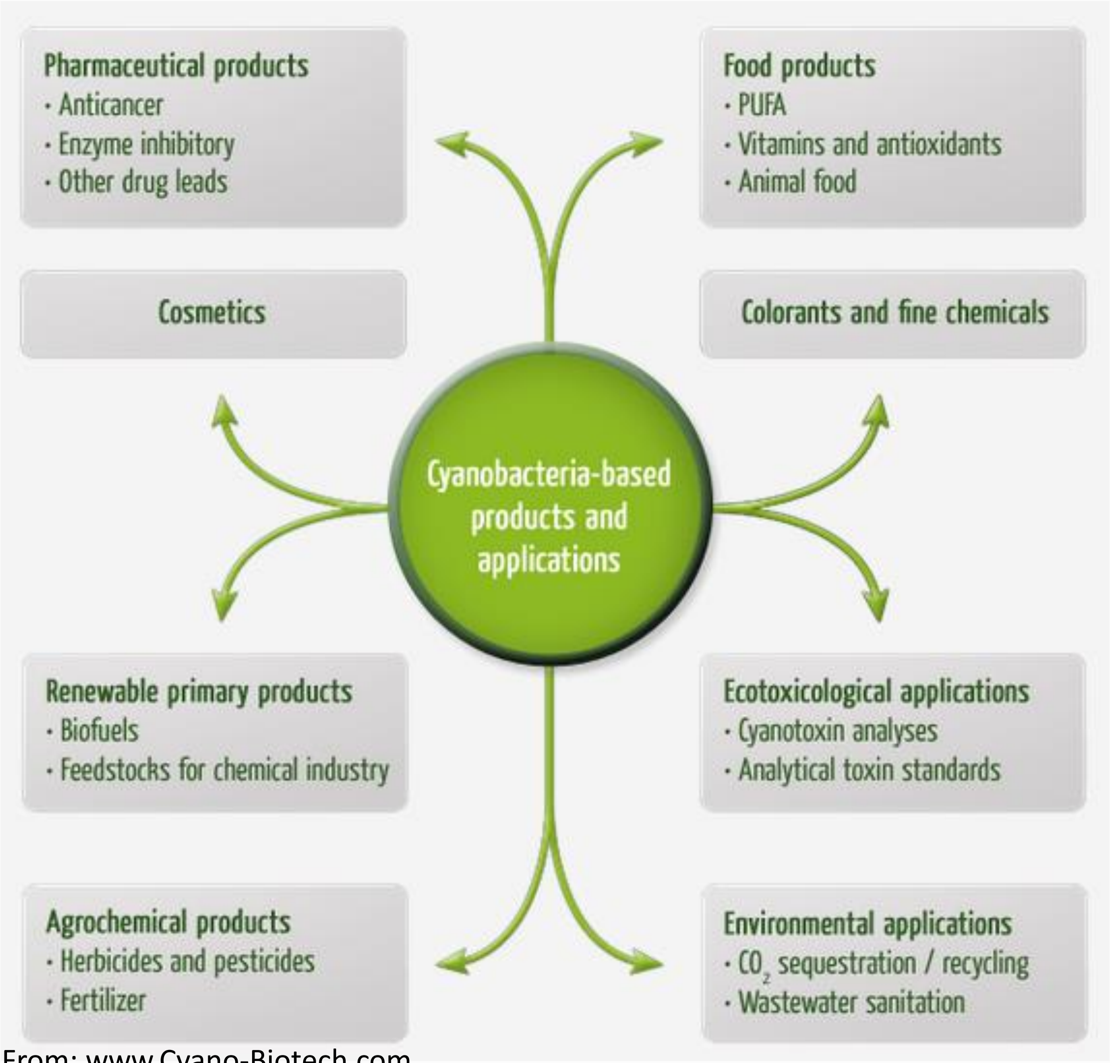
FONDAZIONE EDMUND MACH

Bioactive compounds produced by Cyanobacteria



Source: Dittmann et al, 2015. Trends in Microbiology

Cyanobacteria: biotech applications



From: www.Cyano-Biotech.com

Grazie per l’attenzione

Narcissus, by Fran

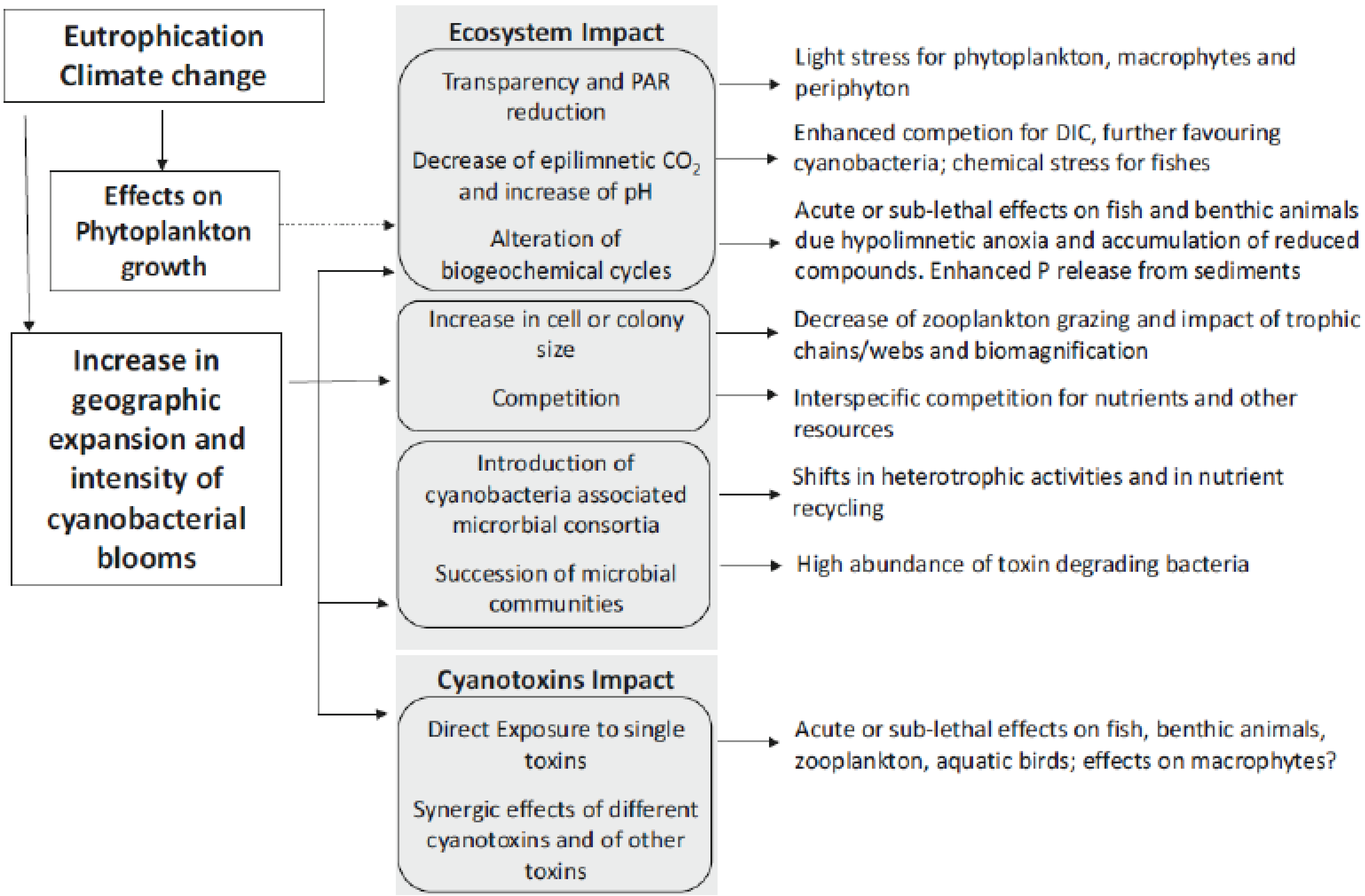
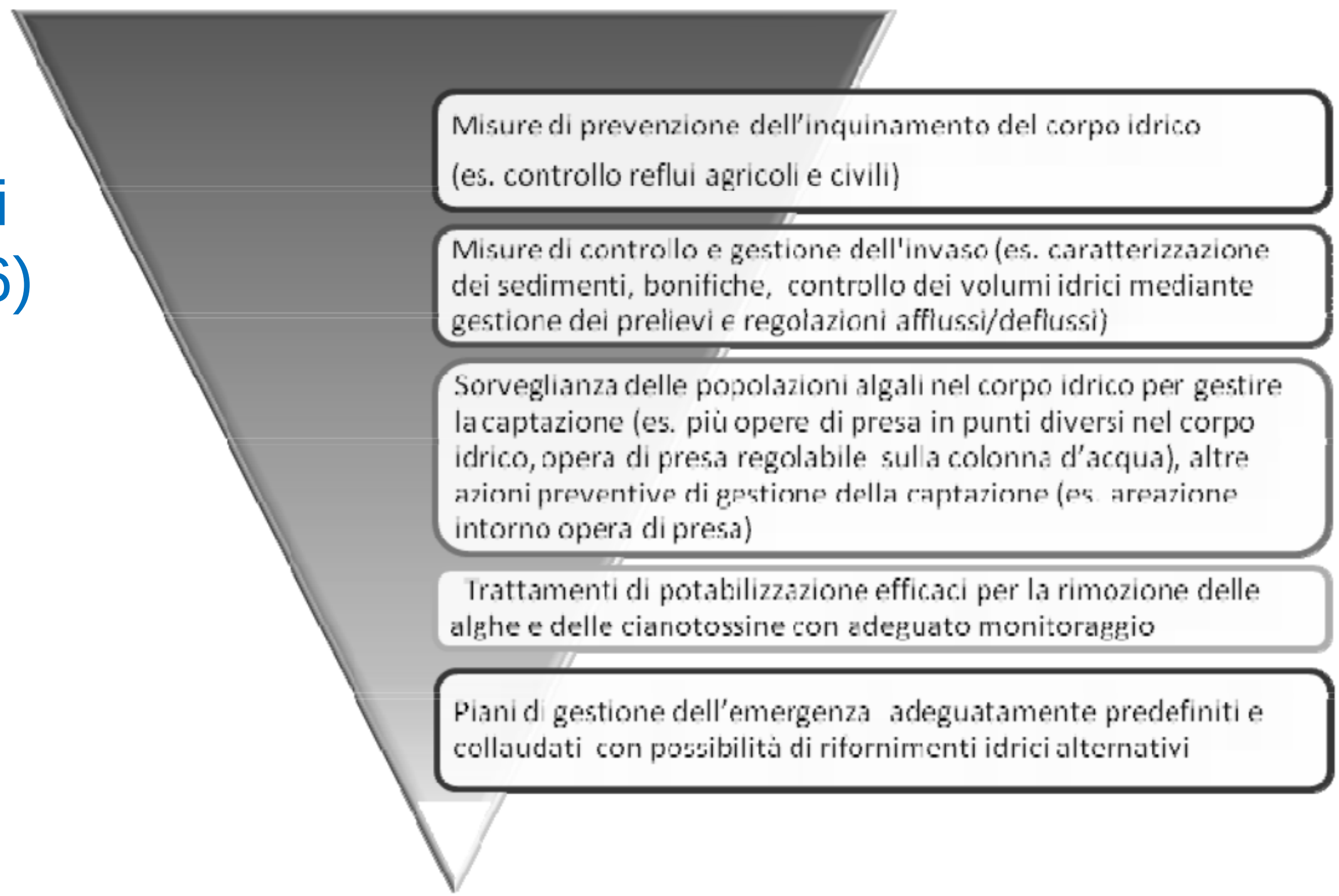


Fig. 1 Schematic presentation of the consequences and impacts of the invasion of toxic cyanobacteria on various components of the aquatic ecosystem

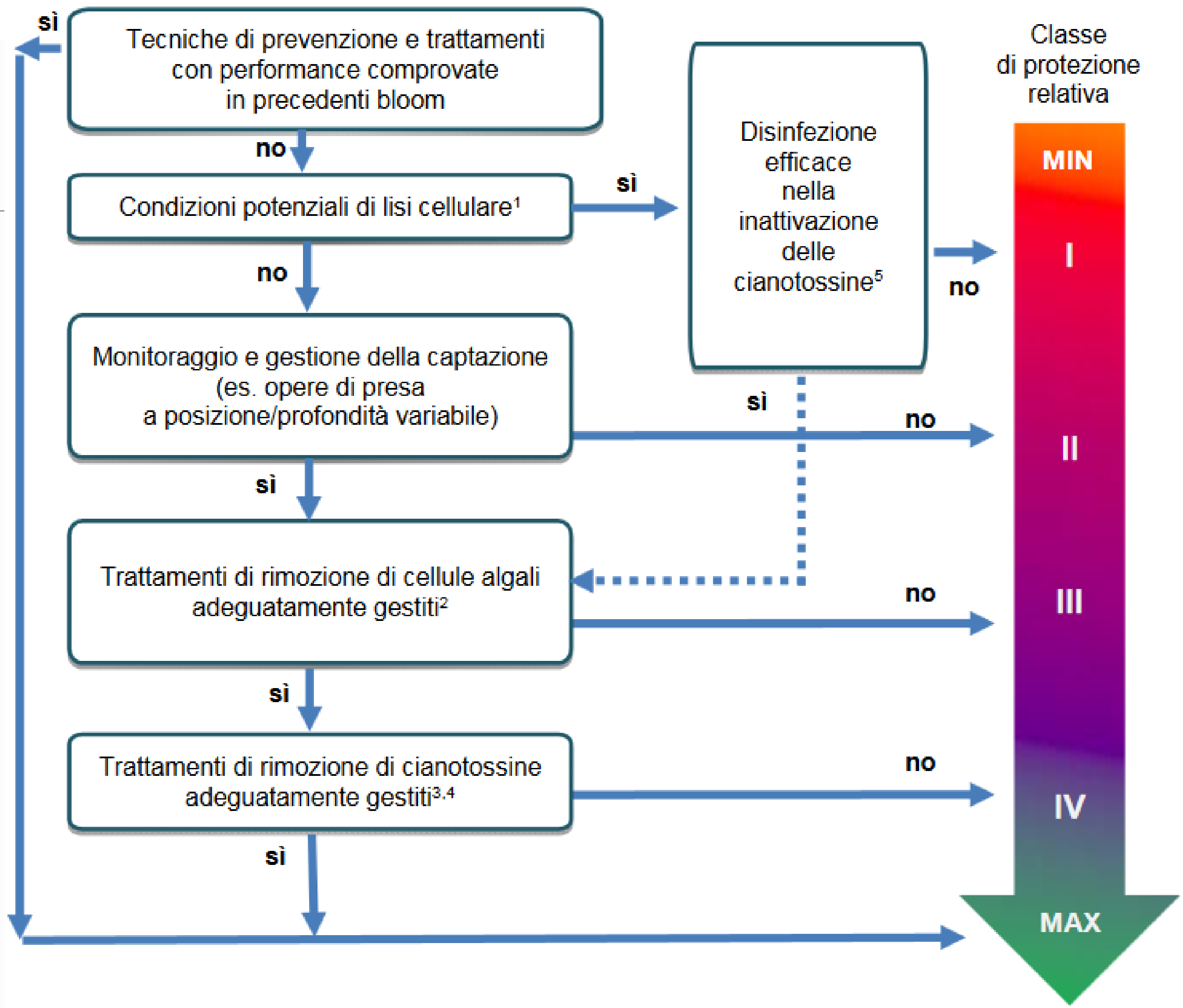
Sukenik, Quesada & Salmaso, 2015

Linee Guida e Valori di riferimento

Acque potabili
(D.L.152/2006)



(da ISTISAN 11/35: Cianobatteri in acque destinate al consumo umano)



(da ISTISAN 11/35: Cianobatteri in acque destinate al consumo umano)

Rete di Riserve Alpi Ledrensi

FONDAZIONE EDMUND MACH

Tabella A1. Schema riassuntivo dei livelli di rischio e della loro gestione adottati nel sistema di sorveglianza

Base decisionale	Definizione della soglia e rischio configurabile[1]	Azioni raccomandate	Provvedimenti ed eventuali limitazioni d'uso[2]
0 Livello di rivelazione			
Rivelazione della presenza di cianobatteri potenzialmente tossici nel corso del monitoraggio[3,4]	Specie potenzialmente tossiche[4,6]: 500-2.500 cell/mL oppure Clorofilla cianobatterica: 1-2,5 µg/L *Rivelazione di cianobatteri a ridotte concentrazioni, non configurabili rischi sanitari immediati.*	Intensificare ispezione visiva sull'invaso. Implementare monitoraggio regolare almeno su base quindicinale del conteggio algale o clorofilla cianobatterica[6,11,12]	-
1 Livello di allerta			
Allerta per possibile rischio sanitario: conteggio algale associato a potenziale presenza di cianotossine nelle acque da destinare al consumo (prima della filiera di potabilizzazione) a livelli pari al valore massimo ammissibile[7,8]	*P. rubescens:* 2.500 cell/mL[4,6] *oppure* Altre specie tossiche: 5.000 cell/mL[4,6] *Insediamento e sviluppo della popolazione di cianobatteri nel corpo idrico tale da configurare in condizioni di peggiore scenario[7] un potenziale di produzione di tossina nelle acque da destinare a consumo a concentrazioni nell'intorno del valore massimo ammissibile (1,0 µg/L MC-LR[8])*	Notifica ad autorità sanitaria locale in circostanze dove i fenomeni non sono ricorrenti/sistematici e adeguatamente gestiti, a conoscenza dell'autorità sanitaria[5]. Implementare monitoraggio regolare su base quindicinale o, preferibilmente, settimanale mediante conteggio algale[6,11,12] almeno sulle acque in entrata e uscita dall'impianto. Se il livello di protezione del sistema è considerato inadeguato[9] implementare analisi settimanale[9,10,11,12] di cianotossine nelle acque in entrata e, se necessario, in uscita dall'impianto e/o in distribuzione[13]. Ottimizzare per quanto possibile le misure di mitigazione nella filiera di potabilizzazione[9,14] Assicurare un'adeguata clorazione[15]	Limitazioni d'uso[2] in seguito a riscontro di concentrazioni di tossine superiori ai valori massimi ammissibili nelle acque in distribuzione[8,13]
2 Livello di allerta elevato			
Allerta elevato per possibile rischio sanitario: conteggio algale associato a potenziale presenza di cianotossine nelle acque da destinare al consumo (prima della filiera di potabilizzazione) a livelli pari a 10x del massimo ammissibile[7,8] nelle acque destinate al consumo umano	*P. rubescens:* 25.000 cell/mL[4,6] *oppure* Altre specie tossiche: 50.000 cell/mL[4,6] *Insediamento e sviluppo della popolazione di cianobatteri nel corpo idrico tale da configurare in condizioni di peggiore scenario[7] un potenziale di produzione di tossine nelle acque da destinare a consumo a concentrazioni nell'intorno di 10x il valore riferimento massimo ammissibile (1,0 µg/L MC-LR[8]). Misure di prevenzione e trattamento adeguate devono essere implementate per mitigare i rischi, altrimenti sono richiesti provvedimenti di limitazioni d'uso e implementazione di piani di risposta all'emergenza[16] e adeguata informazione e comunicazione[17]*	Notifica ad autorità sanitaria[5] Monitoraggio regolare settimanale o preferibilmente bisettimanale mediante conteggio algale[6,11,12] Determinazione cianotossine frequenza settimanale o preferibilmente bisettimanale[11] su acque in entrata, in uscita dal potabilizzatore e in distribuzione[8,10,11,12,13] Ottimizzare e/o potenziare le misure di mitigazione nella filiera di potabilizzazione[9,14] Assicurare un'adeguata clorazione[15] Predisposizione dei piani di emergenza[16] informazione e comunicazione[17]	Limitazioni d'uso[2] in seguito a riscontro di concentrazioni di tossine superiori ai valori massimi ammisibili nelle acque in distribuzione[8, 13]

(da ISTISAN 11/35: Cianobatteri in acque destinate al consumo umano

Acque di balneazione

(D.L. 116/2008 + decreto attuativo 30 marzo 2010)