TEXTE

63/2012

Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries

Umwelt Bundes Amt

Für Mensch und Umwelt

| TEXTE | 63/2012

Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries

compiled and edited by

Dr. Ingrid Chorus
Federal Environment Agency, Germany

UMWELTBUNDESAMT

This publication is only available online. It can be downloaded from
http://www.uba.de/uba-info-medien-e/4390.html.

ISSN 1862-4804

Publisher: Federal Environment Agency (Umweltbundesamt)
Wörlitzer Platz 1
06844 Dessau-Roßlau
Germany
Phone: +49-340-2103-0
Fax: +49-340-2103 2285
Email: info@umweltbundesamt.de
Internet: http://www.umweltbundesamt.de
http://fuer-mensch-und-umwelt.de/

Edited by: Section II Drinking Water and Swimming Pool Water Hygiene
Dr. Ingrid Chorus

Dessau-Roßlau, December 2012

Table of Contents

INTRODUCTION

Ingrid Chorus[1]

[1] Federal Environment Agency, Germany; **ingrid.chorus@uba.de**

The VIIIth International Conference on Toxic Cyanobacteria (ICTC), held in September 2010 Istanbul, Turkey, included a session in which scientists and regulators reported approaches to controlling hazards from toxic cyanobacteria implemented or discussed in their country, as well as awareness of the issue. Presentations demonstrated substantial recent progress in the perception of cyanotoxins as risk to human health and in risk management, particularly when comparing the current status of regulatory approaches to that reported six years earlier at the VIth ICTC in Bergen, Norway. Again, differences and similarities between countries in the approaches to managing this risk proved very much worth sharing, and it became clear that the booklet of regulatory approaches compiled after the Bergen conference should be updated, particularly with contributions from countries who have implemented regulatory approaches since then.

This booklet therefore compiles the responses to a post-conference call mailed to all participants asking for updates or new contributions. Although the contributions included span a wide range of regulatory approaches to cyanotoxins, coverage is neither globally balanced nor comprehensive. Also, contributions do not represent authorized government positions, but rather the personal views of the authors, the majority of which are scientists.

Overall, the progress in regulatory approaches to the assessment and management of cyanotoxin risks illustrates how research results can effectively feed into policy development, particularly where scientists invest time and energy in making their results useful for that purpose. It is therefore hoped that this compilation will again be useful as trigger and as support for national discussions on assessing health hazards from cyanotoxins and on the best management approaches to protect human health from this hazard.

Contributions in this booklet are organised alphabetically by country. In some countries, regulations have changed little since the publication of the first edition of this booklet in 2005, and readers are referred to that for information on the approaches in Brazil, the Czech Republic, Denmark, Germany, Hungary and South Africa as well as to Tables 1 and 2 below for updates of detail. The 2005 edition remains available on the web under:
http://www.umweltbundesamt.de/wasser-und-gewaesserschutz/cyanocenter.htm.

Tables 1 and 2 give an overview of how cyanotoxins are regulated in drinking-water and in water used for recreational activities in the countries covered in the following chapters or in the first edition. They indicate whether or not a risk management framework is in place, which parameters are used for assessing situations and which values guide responses to blooms.

Whether or not countries implement a legally binding standard or rather some type of guidance value usually relates to central versus federal structures: where the legislative power for surveillance is with state governments, the central national level sometimes cannot issue standards but can recommend guideline values. Also, '*names for numbers*' vary: across the countries covered in this booklet, besides '*guideline values'* or '*standards'* they include '*maximum acceptable values'*, '*maximum acceptable concentrations'* and '*health alert levels'*, sometimes explicitly designated as '*provisional*' just like the WHO Guideline-value for Microcystin-LR. While these differences in terminology reflect different legal situations and regulatory cultures, the underlying scientific considerations are very similar. For the purpose of orientation when planning regulatory approaches, their comparison is therefore worthwhile.

The role of risk management frameworks for regulating the safety of drinking-water as well as recreational use of water-bodies is increasing in many countries. Typically, risk management frameworks assess the likelihood for potentially hazardous concentrations to occur as well as the efficacy of the measures already in place or of those which could be implemented to control them. In face of the rapid shifts in cyanobacterial blooms and cyanotoxin concentrations, risk management frameworks are a highly appropriate approach particularly for this hazard. For drinking-water, those currently implemented follow the concept of developing site-specific *Water Safety Plans* as proposed by WHO (2004), or similar concepts. For recreational water-body use, the European Union Bathing Water Directive[1] requires the establishment of a '*bathing water profile'*, i.e. the assessment of the potential for contamination. While it emphases microbial contamination, it also explicitly addresses cyanobacterial blooms and their causes, and countries on other continents follow similar approaches. Risk management frameworks may trigger the assessment and improved management of the causes for cyanobacterial blooms (primarily nutrient loading and sometimes also flow regime management), and they may interface effectively with environmental policy addressing the reduction of eutrophication (e.g. in the European Union with the local implementation of the Water Framework Directive).

Particularly in the context of risk management frameworks, some countries use parameters reflecting the concentration of cyanobacterial biomass, i.e. cell numbers, biovolumes or pigment concentrations (e.g. chlorophyll-a attributable to cyanobacteria or a specific cyanobacterial pigment detected by fluorometry), with specific values set to guide responses (such as intensified monitoring) or interventions (e.g. upgrading drinking-water treatment or banning recreational site use). Using biomass parameters as basis for situation assessment and as triggers for responses has the advantage of encompassing all cyanotoxins, including those yet unidentified or for which the toxicological data are insufficient for the derivation of a guidance value. The downside is that where toxicity of the cyanobacteria present is low, responses such as restrictions of the use of a site may be more restrictive than necessary for health protection.

For drinking-water, the provisional WHO Guideline-value for Microcystin-LR of 1 µg/L or the underlying TDI of 0.04 µg/kg have been widely used as basis for national standards or guideline values (Table 1). For Microcystins, some countries have used the TDI, but have adapted other factors in the calculation to their national circumstances, e.g. body weight or amounts of water consumed, thus reaching somewhat higher guidance values or standards. Also, a few countries have only regulated Microcystin-LR while others implicitly or explicitly use this as default value for the other microcystins as well, in some cases explicitly designated as 'equivalents' or 'toxicity equivalents'. The values are similar across all countries, ranging between 1.0 and 1.5 µg/L.

[1] in which 'bathing' means any water-related recreational activity; see the 1st edition of this booklet (http://www.umweltbundesamt.de/wasser-und-gewaesserschutz/cyanocenter.htm) for a detailed discussion of this directive's requirements in relation to cyanobacteria

Table 1: Examples of guidance values or standards and other national regulations or recommendations for managing cyanotoxins in drinking-water

(from individual country contributions in this booklet and its 1st edition from 2005)

* RMF: risk management framework (e.g. WSP, HACCP, PHRMP); **S: Standard; (P)GV: (provisional) guidance value; (P)MAV / (P)MAC: (provisional) maximum value or concentration; HAV: health alert level

Country /source document	RMF* required	Cyanotoxins and/ or Cyanobacteria explicitly regulated	S, (P)GV, (P)MAV, (P)MAC or HAL**	Comments; specific action in case of derogation
WHO	√	Microcystin-LR	PGV: 1 µg/L	Depends on setting; strong emphasis on assessing cyanotoxin risks in relation to other risks
Argentina	Requirements neither for Risk Management Framework nor for cyanotoxin surveillance, but some water utilities have implemented either or both; these refer to the provisional guideline by WHO for Microcystin-LR of 1 µg/L			
Australia	√	Microcystin (toxicity equivalents of MCYST-LR); equivalent to 6500 cells/mL or or a biovolume of 0.6 $mm^3\ L^{-1}$ of a highly toxic strain of *Microcystis aeruginosa*	GV: 1.3 µg/L	The Australian Drinking Water Guidelines (2011) are a set of national guidelines which include fact sheets with information on key cyanotoxins; Health Alert can be triggered by the toxin concentrations or the equivalent cell or biovolume concentrations. Trigger levels for each of the 4 key toxin-producing species are also provided for immediate notification to the health authority; Individual states/territories use the national framework as the basis for their specific regulatory requirements.
		Nodularin HAL at 40 000 cells mL^{-1} or a biovolume of 9.1$mm^3\ L^{-1}$ of a highly toxic strain of *Nodularia spumigena*	No value	
		Cylindrospermopsin equivalent to 15 000 – 20 000 cells mL^{-1} or a biovolume of 0.6 – 0.8 $mm^3\ L^{-1}$ of *Cylindrospermopsis raciborskii*	HAL: 1 µg/L	
		Saxitoxins (toxicity equivalents to STX); equivalent to 20,000 cells mL^{-1} or a biovolume of 5 $mm^3\ L^{-1}$ of a highly toxic strain of *A. circinalis*	HAL: 3 µg/L	
Brazil (2005)		Cyanobacteria	GV: 10 000 – 20 000 cells/ml or 1 mm^3/L biovolume	at >10 000 cells/ml weekly monitoring is required; at > 20 000 cells/ml toxicity testing and/or quantitative cyanotoxin analysis in drinking-water are required
		Microcystins	S: 1 µg/L	
		Clindrospermopsin	GV: 15 µg/L	
		Saxitoxin	GV: 3 µg/L (STX equiv.)	

Country /source document	RMF* required	Cyanotoxins and/ or Cyanobacteria explicitly regulated	S, (P)GV, (P)MAV, (P)MAC or HAL**	Comments; specific action in case of derogation
Canada		Microcystin-LR	*MAC:* 1.5 µg/L	MAC for Microcystin-LR is considered protective against exposure to other microcystins; monitoring frequencies driven by bloom occurrence – more frequent where there is a history of bloom formation
		Anatoxin-a	PMAC: 3.7 µg/L	ATX regulated only in Quebec
Czech Republic		Cyanobacteria in raw water (as cell counts or biomass or concentration of chlorophyll-a)	≥ 1 colony/ml or ≥ 5 filaments/ml	<u>Vigilance Level</u>: quantification of cyanobacteria in the raw water s at least once per week; visual observations of the abstraction point (water blooms at the surface of water level)
			≥ 2 000 cells/mL or ≥ 0.2 mm3/L biovolume or ≥ 1 µg/L chlorophyll-a	<u>Alert Level 1</u>: attempt reduction by changing abstraction depth. If that is not possible, ascertain that treatment sufficiently reduces cyanobacteria and toxins (data from operational parameters, if necessary also toxin analyses)
			≥ 100 000 cells/mL or ≥ 10 mm3/L biovolume or ≥ 10 µg/L chlorophyll-a	<u>Alert Level 2</u>: as Alert Level 1, but with stronger emphasis on treatment efficacy and microcystin monitoring
		Microcystin-LR in treated water	S*:* 1 µg/L	Monitored once per week in treated water
Cuba No regulatory requrement, but framework currently being tested		**Phytoplankton** cells	< 20 000 cells mL^{-1}	Monthly visual inspection and sampling at least four months a year
		Cyanobacterial cells	< (or only slightly above) 1500 cells mL^{-1}	
		Phytoplankton and **proportion of cyanobaceria**	20 000 – 100 000 cells mL^{-1} ; >50% cyanobacteria	Alert: increased sampling (weekly and more sites); daily inspection; notification to public health unit and local managers; report to local government; warning of the public
		Cyanobacteria known as potentially toxic	At least one of the species	
		Any report of **toxic effect** (humans or animals)		Action (in red): as for “Alert”, but with increased actions for public communication and water use restrictions
		Scum consistently present; confirmed bloom persistence		
Denmark		No specific cyanotoxin drinking water regulation as almost all drinking water originates from ground water. In specific cases where surface waters has supplemented drinking water supply, only less than 10 % were added to the groundwater, applying the provisional WHO guideline for Microcystin-LR of 1 µg/L if needed.		
France	√	**Microcystins** (sum of all variants)	S: 1 µg/L	Analysis required in raw water and at the point of distribution only when cyanobacteria proliferate (visual observation and/or analytical results)

Country /source document	RMF* required	Cyanotoxins and/ or Cyanobacteria explicitly regulated	S, (P)GV, (P)MAV, (P)MAC or HAL**	Comments; specific action in case of derogation
Finland	√	Potentially toxic Cyanobacteria in raw water (as cell counts or biomass; biomass is equal to biovolume assuming a 1:1 ratio of volume to mass)	>5000 cells/ml or >1 mg/L biomass	microcystin monitoring; enhanced treatment
		Potentially toxic Cyanobacteria in raw water	>100 000 cells per ml, >20 mg/L biomass	change of abstraction site and/or restrictions of water use; information to the water users, particularly if microcystins are found in finished drinking water
		Microcystins (sum of all variants) in raw water	>1 µg/L	
			GV: >1 µg/L	Restrictions of water use; unlikely on basis of experience
		Microcystins (sum of all variants)in finished drinking-water	GV: >10 µg/L	Ban on water use; highly unlikely
Germany		No specific cyanotoxin regulations as only about 20% of water supply is from surface water, and that mostly from well protected reservoirs. However, for non-regulated chemicals the Drinking-water Ordinance requires that they do not occur in hazardous concentrations. On this basis, where cyanobacteria do occur, the WHO GV can be applied for microcystins. National guidance for substances with incomplete toxicological evidence proposes <0.1 µg/L if carciongenisis cannot be excluded (until data are generated that allow higher levels), and this can be applied to Cylindrospermopsin.		
Hungary		Drinking-water legislation includes “biological parameters” to be monitored by microscopy, e.g cyanobacteria		frequency of examination based on amount of water supplied and source of drinking water (cyanobacteria if source is surface water); at least once a year for every network for all biological parameters
Italy		National decree includes “algae” as accessory parameter to monitor if local authorities presume a risk, based on the provisional WHO GV for Microcystin-LR.		
Netherlands		No specific regulations for cyanotoxins in drinking water, although about 40% of water supply is from surface water, mainly from well protected reservoirs and infiltration basins. However, concentrations of micro-organisms may not exceed levels which may have adverse consequences for public health. For the production of drinking water, barriers in the treatment process to prevent cyanobacterial cells and microcystins from reaching finished drinking water. In case this should happen, the Netherlands would aplly WHO guidance, i.e. for cells < 4.700 mL^{-1} as described in Chorus and Bartram (2000) and for microcystins the WHO GV of 1µg/l.		
New Zealand	√ PHRMPs	Microcystins (as MC-LR equivalents)	PMAV 1 µg/L	Effective implementation of the protocols required by Public Health Risk Management Plans (PHRMPs) has prevented concentrations > PMAV from reaching the consumers
		Cylindrospermopsin	PMAV 1 µg/L	
		Saxitoxin (as STX equivalents)	PMAV 3 µg/L	
		Anatoxin-a	PMAV 6 µg/L	
		Anatoxin-a(s)	PMAV 1 µg/L	
		Homoanatoxin-a	PMAV 2 µg/L	
		Nodularin	PMAV 1 µg/L	

Country /source document	RMF* required	Cyanotoxins and/ or Cyanobacteria explicitly regulated	S, (P)GV, (P)MAV, (P)MAC or HAL**	Comments; specific action in case of derogation
Singapore	√	Microcystin-LR in free and cellbound forms	S: 1 µg/L	Every supplier of piped drinking water is legally required to prepare and implement a water safety plan to ensure that the piped drinking water supplied complies with the piped drinking water standards (stated as 1 µg/L for total microcystin-LR, in free and cellbound forms).
Spain		Microcystins	S: 1 µg/L	to be analysed when eutrophication is evident in the water sources (one known case of exceedance; Quesada 2012)
Turkey (proposed for 2014 at the time of this publication)		Cyanobacteria	>5000 cells/ml or >1 µg/l Chlorophyll-*a*	monthly analysis if in raw water; if exceeded, weekly sampling (of water column) and toxin analysis
		Sum of all microcystins	1 µg/L MC-LR-equivalents	If >1 µg/l, toxin analysis in treated water and advanced treatment (ozonation or active carbon) or alternative water supply
Uruguay		Microcystin-LR	S: 1 µg/L	Decree: "*Drinking water should not contain amounts of cyanobacteria that could affect water characteristics or human health*"
South Africa		Microcystin-LR	GV: 1 µg/L	supported by guidelines for chlorophyll-a and cyanobacterial cell counts
United States of America		No national requirements, but action taken by many of the States – see Table 1 in the USA Chapter on p. 139 for the approaches pursued by each of 21 States.		

Table 2: Examples of national regulations or recommendations for managing cyanotoxins in water-bodies used for recreation

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
World Health Organization		Cells or Chlorophyll-a with dominance of cyanobacteria	20 000 cells / mL or 10 µg/L Chl.-a	Information to site users and to relevant authorities
			100 000 cells / mL or 50 µg/L Chl.-a	Information to site users and to relevant authorities; watch for scums; restrict bathing and further investigate the hazard
		Scum	Observation in bathing area	Action to prevent scum contact; possible prohibition of swimming and other water contact activities; public heath follow-up investigation; information of relevant authorities
European Union	Bathing Water Directive (EU BWD)	Requires 'bathing water profiles' indicating – among other parameters – the potential of the site for cyanobacterial proliferation; monitoring based on the bathing water's history and regional climatic conditions; conformity as a matter of appropriate management measures and quality assurance, not merely of measuring and calculation. Applies to any element of surface water where a large number of people to practice bathing and bathing is not prohibited or advised against (termed "bathing water"). Article 8: 1) When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks. 2) When cyanobacterial proliferation occurs and a health risk has been identified or presumed, adequate management measures shall be taken immediately to prevent exposure, including information to the public.		

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
Australia	Annual assessment of susceptibility to cyanobacterial growth in categories of Very Poor, Poor, Fair, Good, Very Good	Cells or Biovolume	≥500 to <5000 cells mL^{-1} *M. aeruginosa* or biovolume >0.04 to <0.4 $mm^3\ L^{-1}$ for the combined total of all cyanobacteria	Green level Surveillance mode: • Regular monitoring
			≥5000 <50 000 cells mL^{-1} *M. aeruginosa* or biovolume ≥0.4 to <4 $mm^3\ L^{-1}$ for the combined total of all cyanobacteria with a known toxin producer dominant or ≥0.4 to <10 $mm^3\ L^{-1}$ for the combined total of all cyanobacteria where known toxin producers are not present	Amber level Alert mode • Notify agencies as appropriate • Increase sampling frequency • regular visual inspections of water surface for scums • Decide on requirement for toxicity assessment or toxin monitoring
			≥10 $\mu g\ L^{-1}$ total microcystins or ≥50 000 cells mL^{-1} toxic *M. aeruginosa* or biovolume ≥4 $mm^3\ L^{-1}$ for the combined total of all cyanobacteria with a known toxin producer dominant or ≥10 $mm^3\ L^{-1}$ for total biovolume of all cyanobacterial material where known toxins are not present. or cyanobacterial scums consistently present	Red level Action mode • Continue monitoring as for alert mode • Immediately notify health authorities for advice on health risk • toxicity assessment or toxin analysis (if this has not already been done) • Health authorities warn of risk to public health (i.e. the authorities make a health risk assessment considering toxin monitoring data, sample type and variability)
Canada	Bloom risk management programs in some provinces	Microcystin-LR; or Cell Counts	≤ 20 µg/L ≤ 100,000 cells/mL	If either of guideline values is exceeded, a swimming advisory may be issued by the responsible authority. Contact with waters where an advisory has been issued should be avoided until the advisory has been rescinded

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
Cuba	No regulatory requirement, but framework currently being tested	Phytoplankton	<1500 cells mL-1	Monthly visual inspection and sampling at least four months a year
		Cyanobacteria	<500 (or only slightly above) cells mL^{-1}	
		Phytoplankton cells; proportion cyanobaceria	20 000 – 100 000 cells mL^{-1} ; >50% cyanobacteria	Alert: increased sampling (weekly and more sites); daily inspection; notification to public health unit and local managers; report to local government; warning of the public
		Cyanobacteria known as potentially toxic	At least one of the species	
		Any report of toxic effect (humans or animals)		Action (in red): as for "Alert", but with increased actions for public communication
		Scum consistently present; confirmed bloom persistence		
		Benthic mats	Similarly, three Alert levels for benthic cyanobacteria defined by coverage of surfaces (<40% with any cyanobacteria; > 20% with toxicogenic cyanobacteria; >50% with potentially toxicogenic cyanobacteria, particularly where they are visibly detaching and accumulating as scum: Responses similar to those for plankonic cyanobacteria	
Czech Republic	70% of sites used for recreation tend to develop blooms	Cells and/or Chlorophyll-a	>20 000 cells/mL	1st warning level
			>100 000 cells/mL	2nd warning level: closure for public recreation
Denmark	National implementation of EU BWD by risk assessment in response to larger blooms	Visual inspection	Scums in bathing area	Relevant authorities are informed and decide when and how the public should be informed; warnings include signs, media and contact to local user groups such as kindergardens, scouts, water sports clubs.
		Microscopy; Chlorophyll-a	High chlorophyll > 50 µg/L and cyanobacteria dominate	
		Toxin content	Some regions perform toxin analysis and include the results in their risk assessments	

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
Finland	National implementation of EU BWD For the Baltic: monitoring with automated sensors on commercial ships; visual observations submitted by the Finnish Border Guard from the air; with satellite images	Visual inspection, including by trained volunteers Note that "algae" includes cyanobacteria	No algae on the water surface or on the shore line. Water transparency (Secchi depth) is not affected by algae.	Level 0: not detected
			Greenish flakes detected in the water or when taken into a transparent container, or narrow stripes on the shore. The Secchi depth is reduced by algae.	Level 1: detected Possibly microscopical examination and even toxin analysis if there is a specific cause such as very popular beach or reports of adverse health effects or animal deaths
			The water is coloured by algae, small surface scums or cyanobacterial mass on the beach are detected.	Level 2: high amount Preferably microscopical examination; toxin analysis; warning of the public is compulsory
			Wide and heavy surface scums or thick aggregates of cyanobacteria are detected on the shore.	Level 3: very high amount Preferably microscopical examination; toxin analysis; warning of the public is compulsory
France	National implementation of EU BWD	Visual inspection	visible bloom, scums, change in water colour	Microscopy examination. If cyanobacteria are absent: no further action. If present: counting and genus identification
		Cyanobacteria	<20 000 cells/ml ± 20 %	Active daily monitoring. Counting at least on a weekly basis. Normal recreational activity at the site
			20 000 – 100 000 cells/ml ± 20 %	Active daily monitoring. Counting on a weekly basis. Recreational activities are still allowed; the public is informed by posters on site.
		Cyanobacteria; Microcystins	> 100 000 cells/mL ± 10 %. 25 µg/L MC-LR equivalent ± 5 %	if MC < 25 µg/L bathing and recreational activities are restricted. if MC > 25 µg/L bathing is banned and recreational activities are restricted. In either case, public is informed.
		Scums or foam	Appearance in recreational or bathing area	All water activities in this area are prohibited. Restrictions do not necessarily apply to the whole recreational site. Other areas without scum may still be open.
Germany	National implementation of EU BWD The water body's capacity for bloom development is	Transparency in combination with an "indicator" for cyanotoxin potential: Chlorophyll-a with domi-	Secchi Disk reading >1 m and <40 µg/L Chl.a or <1 mm³/L BV or <10 µg/L MCYST	Monitor further cyanobacterial development

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
	assessed on the basis of total phosphorus concentration (i.e. >20-40 µg/L) and transparency (Secchi Disk). Microcystin monitoring is optional, intended as basis for de-warning if use of site is to be continued in spite of high cyanobacterial density	nance of cyanobacteria or **Biovolume** (BV) or **Microcystins** (MCYST)	Secchi Disk reading < 1 m **and** >40 µg/L Chl-a **or** > 1mm³/L BV **or** > 10 µg/L MCYST	Publish warnings, discourage bathing, consider temporary closure
		Scums **and / or Microcystins**	observation of heavy scum **and/or** >100 µg/L MCYST	Publish warnings, discourage bathing, temporary closure is recommended
Hungary	National implementation of EU BWD	**Chlorophyll-a** with dominance of cyanobacteria or **Cell counts** or **Microcystin-LR** equivalents	<10 µg/L or <20 000 cells/ml or < 4 µg/L	excellent
			<25 µg/L or <50 000 cells/ml or <10 µg/L	good
			<50 µg/L or <100 000 cells/ml or <20 µg/L	acceptable
			>50 µg/L or >100 000 cells/ml or >20 µg/L	unacceptable
Italy	National implementation of EU BWD The water body's capacity for bloom development is assessed on the basis of transparency (<1m), concentrations of total phosphorus (>20 µg/L) and chlorophyll-a (>20 µg/L)	**Cell counts** combined with identification of genus and, if possible, species	< 20 000 cells/mL	If possible, daily visual observation; weekly counting
			20 000 – 100 000 cells/mL	Daily visual observation; at least weekly counting; information to the public; quantification of microcystins
			>100 000 cells/mL	Bathing prohibited until quantification of microcystins; information to the public; at least weekly counting
		Scums	presence	Bathing prohibited until quantification of microcystins; warning notice; scum drift monitoring
		Microcystins If other cyanotoxins are present, case by case risk assessment	>25 µg/L	Bathing prohibited
Netherlands	National Water Authority and National implementation of EU BWD Samples are taken at fortnightly intervals at bathing locations and cyanobacteria	**Surface scum intensity** and/or **Biovolume** or **Chlorophyll** attributable to cyanobacteria	scums category **I**; cyano-chl-a <12.5 µg/L **or** cyano-biovolume < 2.5 mm³/L	<u>Surveillance level:</u> continue fortnightly monitoring
			or scum category **II** **or** cyano-chl-a 12.5 – 75 µg/L **or** cyano-biovolume 2.5 – 15 mm³/L	<u>Alert Level 1</u>: weekly monitoring and issue warning (by site operator) for duration of that week: "Toxic blue-green algae. Risk of skin irritation or intestinal problems". In case of daily site inspection, re-evaluate the warning on a daily basis

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
	quantified. Presence and intensity of surface scums is noted		scums category III or cyano-chl-a > 75 or cyano-biovolume > 15 mm³/L If 80% dominance of MC–producers and MC < 20 µg/L, revert to Alert Level 1.	Alert Level 2: weekly monitoring and advice against bathing (by public authority): "You are advised not to bathe in this water"; prohibition by local authority is possible.
New Zealand	Guidelines include identifying high-risk water bodies, sampling and site surveys (including benthic cyanobacteria), a list of laboratories, examples for media releases, warning signs and sampling forms, photos of blooms and . Photographs of cyanobacteria blooms and benthic mats are provided to assist samplers with minimal cyanobacterial expertise to collect the correct samples. This has proved particularly useful for benthic cyanobacteria.	Cells	<500 cells/ml	Surveillance: Where cyanobacteria are known to proliferate, weekly or fortnightly visual inspection and sampling between spring and autumn
		Biovolume	0.5 to < 1.8 mm³/L of potentially toxic cyanobacteria or 0.5 to < 10 mm³/L total biovolume of all cyanobacterial material	Alert: increase inspection and sampling to weekly, including multiple sites; notify the public health unit
		Microcystins or Biovolume or Scums	≥ 12 µg/L total microcystins or biovolume ≥ 1.8 mm³/L of potentially toxic cyanobacteria or ≥ 10 mm³/L total biovolume of all cyanobacterial material or consistent presence of scums	Action: Continue monitoring as for alert; if potentially toxic taxa are present, consider testing samples for cyanotoxins Notify the public of a potential risk to health.
		Benthic mats	Similarly, three Alert levels for benthic cyanobacteria defined by coverage of surfaces (<20%, 20-50% and > 50%) and by river flow (as this can detach benthic cyanobactera); responses similar to those for plankonic cyanobacteria	
Poland	National implementation of EU BWD	Sampling of bathing sites not less than 4 times per season (the interval between sampling does not exceed one month), including responses to cyanobacteria if blooms are observed		

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
Singapore	Guidelines adopted from WHO Guidelines; applied to water bodies used for primary contact activities (e.g. swimming, skiing, wakeboarding) with frequent immersion of body, face or trunk, water ingestion likely	Chlorophyll-a	≤ 50 µg/L for 95% of a 3-year rolling period	Status of the sites reviewed annually. If the assessment is that the water body is unsuitable for primary water contact activities, the public is notified.
Spain	National implementation of EU BWD Classification of sites by probability of cyanobacterial proliferation (basis: 2 years of intensive monitoring)	Probability for cyanobacerial proliferation	Low probability	Criteria for assessment of health risk and response are set locally; some health authorities use WHO scheme, others include further risk parameters (such as number of users, type of use); temporary closure has occasionally occurred based on the abundance of cyanobacteria
			Medium probability	
			High probability	
Turkey	Under discussion for implementation in 2014 at the point of publication of this document	Cells or Microcystin-LR or Chlorophyll-a (if largely from cyanobacteria)	< 20 000 cells/mL or <10 µg/L Microcystin-LR equivalents or <10 µg/L chlorophyll-a	Level 1: recreational activities are allowed to continue and users are informed by posters on site. Monitoring (sampling, counting and species identification) should be done fortnightly.
		Cells or Microcystin-LR	20 000 – 100 000 cells/mL or >25 µg/L Microcystin-LR equivalents	Level 2: At >20 000 cells/mL, microcystins are analysed. If MC-LR equivalents >25 µg/L, immediate action to inform relevant authorities and public. Discourage users from swimming and other water-contact activities by advisory signs on site.
		Scums in bathing area	Visual inspection	Level 3: all activities in the water may be prohibited
United States of America	No national requirements, but action taken by many of the States – see Table 1 in the USA Chapter on p. 139 for the approaches pursued by each of 21 States.			

Some countries have included guidance values for further cyanotoxins – i.e. Cylindrospermopsin, Saxitoxins, sometimes Anatoxin-a and even Anatoxin-a(s). Values for Saxitoxins can be based on toxicological data from exposure through shellfish consumption. The demand for a guidance value for Cylindrospermopsin is increasing, as surveys from a growing number of countries – including from temperate climates – are showing this toxin to occur frequently, with a high extracellular proportion and slow rates of biodegradation.

For recreational water-body use, most countries base guidance values on a parameter reflecting cyanobacterial biomass (Table 2) in order to include symptoms observed in epidemiological studies but not clearly attributable to any of the known cyanotoxins. However, typically the levels at which such biomass values are set are also guided by limits for the concentration of Microcystins, with the target of avoiding exposure to potentially hazardous concentrations of this toxin. This is justified by the frequency at which Microcystins have been found in high concentrations (particularly in scums). Some countries also directly include Microcystin levels which trigger interventions, and where this is the case, values range from 12.5 to 100 µg/L (though triggering different levels and types of responses). Scientific debate is ongoing about the need to include neurotoxins in such considerations because they are more acutely toxic (as demonstrated by animal deaths; the problem is the lack of data for their acute oral toxicity), and if there is a risk of acute intoxication after accidental ingestion of larger water volumes containing scum material (e.g. in the context of capsized sailboats), this might be through neurotoxins rather than through Microcystins.

An emerging issue is the potential for intoxication from benthic mats of cyanobacteria which grow on stream sediment surfaces and at times may be detached by currents and perturbations. These may contain highly toxic cyanobacteria, as demonstrated by animal deaths, and awareness of their potential hazard to human health is growing. Two countries have included them in their approaches to protect recreational site users (implemented in New Zealand and under discussion in Cuba; see Table 2).

As first outcome of this comparison of regulatory approaches, it is proposed that the International Conferences on Toxic Cyanobacteria dedicate a full session to sharing information about the approaches taken in different countries, the scientific rational behind them, the 'numbers' chosen to trigger certain interventions and experience with their implementation.

ARGENTINA: Cyanobacteria and Cyanotoxins: Identification, Toxicology, Monitoring and Risk Assessment

Silvia Otaño[1], Graciela Salerno[2], Marcia Ruiz[3], Anabella Aguilera[2], Ricardo Omar Echenique[4]

[1] Laboratorio Central. Aguas de Corrientes. S.A., Gobernador Pampín 115, 3400 Corrientes, Argentina; silviaotano@gmail.com
[2] Fundación para Investigaciones Biológicas Aplicadas-CONICET, Vieytes 3103, Mar del Plata, Argentina.
[3] INA-CIRSA, Córdoba, Argentina.
[4] Facultad de Ciencias Naturales y Museo (UNLP), Argentina-CIC.BA.

The water quality problems in reservoirs used for public supply, irrigation and recreation are an alarming fact in our country and around the world. In recent years there has been an increase in the occurrence of cyanobacterial blooms in different river systems in the Southern region of South America (named CONOSUR). With that concern, "Workshops on Toxigenic Cyanobacteria in the CONOSUR" have been organized in Argentina in 2005, 2007, 2008 and 2010, with the aim of finding multidisciplinary strategies to determine the scope and impact of the bloom to develop prevention and management of risks. Discussions focused on the problem of the presence of cyanobacteria in water, the potential production of cyanotoxins and associated problems. Experts and other actors interested in the issue from different Argentinean provinces and neighboring countries presented the current state of knowledge and described the actual situation in each location. Thus, these meetings allowed the exchange of experience about the presence of cyanobacteria and cyanotoxins in waters of different parts of Argentina, Uruguay, Brazil and Paraguay.

One of the goals of the workshops was to develop a theoretical and practice guide for laboratories and institutions working on water quality in order to harmonize techniques for the detection and characterization of toxins and the identification of toxigenic cyanobacterial species. Also, concepts and practices for bloom management were outlined. The handbook entitled "Cyanobacteria and Cyanotoxins: Identification, Toxicology, Monitoring and Risk Assessment" (Giannuzzi 2009) was published in 2009. It constitutes a significant contribution towards the identification of strains and management of toxigenic blooms in the region.

Approaches to risk assessment and management of cyanobacteria

Although several toxigenic species of Cyanobacteria have been reported and associated with algal blooms in Argentina, the most common genera are *Microcystis, Dolichospermum* (*Anabaena*), *Cylindrospermopsis* and *Raphidiopsis,* and the most common cyanotoxins detected are microcystins (Table 1).

In Argentina there are no regulations on drinking and recreational water quality regarding cyanobacteria and cyanotoxins. Most treatment plants have not yet incorporated analyses of cyanobacteria or toxins as a routine, although in recent years the request for such analyses has increased and plant operators follow the value established by the World Health Organization for microcystin-LR as a guide level.

Table 1: Cyanobacteria, metabolites and management actions.

Data from Echenique & Aguilera 2009, and from Otaño & Román 2008[3], Otaño 2009a[1], 2009b[3], Otaño 2012[2], Bogarín *et al.* 2012[3]

Site	Species	Toxins and other metabolites	Actions
Buenos Aires - River de la Plata	*M. aeruginosa* *M. viridis* *M. flos-aquae*	Microcystins Microcystin-LR Microcystin-YR	Samples every two weeks Early alert level of 2000 cells/mL in two drinking water supplies Communication to drinking water operators Prohibition of fishing and bathing
Buenos Aires – Paso de las Piedras Dam	*M. aeruginosa* *D. circinalis*	Microcystins	Monthly sampling (increasing at blooms) Early alert level of 2000 cells/mL in drinking water supply Powder Activated Carbon (PAC) to raw water.
Chaco – River Salado[1]	*C. raciborskii* *P. agardhii* *R. curvata* *R. mediterranea*	Saxitoxins	Monitoring water quality intended for drinking water supply (project). Change the water source to another waterbody
Chaco – Reservoirs for drinking water supply[2]	*M. aeruginosa* *D. spiroides* *C. raciborskii* *R. curvata* *R. mediterranea* *Aph. favaloroi*	Microcystins	Occasional monitoring of Cyanobacteria. Algae control with lime in some water bodies.
Chubut – Zeta Lagoon		Microcystin-RR Microcystin-YR Microcystin-LR 2 microcystins not identified	Identification of environments with harmful Cyanobacteria issues
Córdoba – San Roque Dam	*Microcystis* sp. *Anabaena* sp.	Microcystin-RR Microcystin-YR Microcystin-LR	PAC and ozone. Aeration of Lake
Corrientes – River Uruguay[3]	*M. aeruginosa* *M. wesenbergii* *D. spiroides* *D. circinalis* *D. planctonicum* *D. viguieri* *C. raciborskii* *Aph. schindleri*	Microcystins Geosmin 2-MIB	Weekly monitoring Alert Levels PAC Analysis of Microcystins, Saxitoxins and Cylindrospermopsin. Mouse bioassays Sensory analysis
Entre Ríos – River Paraná	*M. aeruginosa*	Microcystins	Two sewage treatment plants upstream the dam (project)
Mendoza – Lake Parque General Belgrano	*M. aeruginosa*		
Neuquén – Ramos Mexía Dam		Hepatotoxins Neurotoxins	Monitoring during spring and autumn. Alert system and communication Weekly reporting to drinking water supplies
Salta – El Tunal Dam	*D. spiroides*		
Santa Fe – River Paraná	*M. aeruginosa*		

Only a few operators of water treatment plants and people responsible for the environment and water quality control use a model of management and monitoring following various alert levels for the presence of cyanobacteria and toxins (Figure 1). This model is based on critical control points in the categories of source protection and on the procedure proposed by Chorus & Bartram (1999) (Ruibal *et al.* 2009).

Determination of saxitoxin and cylindrospermopsin is carried out in a few treatment plants whereas anatoxin is analytically determined by HPLC-MS-MS only in Córdoba, where that toxin is detected in San Roque Dam. Argentinean guidelines for water quality are under revision and there is a strong movement of researchers and some governmental authorities to include cyanobacteria and cyanotoxins as a new parameter to be considered in water quality control.

References

Bogarín C., Otaño S. & Román N. 2012. Cianobacterias en el Río Ururguay con determinaciones y ensayos de adsorción de cianotoxinas y geosmina. XXXIII Congresso Interamericano de Engenharia Sanitária e Ambiental. Salvador, Brasil

Chorus, I. & Bartram, J. 1999. Toxic Cyanobacteria in Water. A Guide to their Public Health Consequences, Monitoring and Management. World Health Organization. E. & F. N. Spon, London. 416 pp.

Echenique R. & Aguilera A. 2009. Floraciones de Cyanobacteria toxígenas en la república Argentina: antecedentes. En: Gianuzzi L. (Ed.). Cianobacterias y Cianotoxinas. Identificación, Toxicología, Monitoreo y Evaluación de Riesgo. Instituto Correntino del Agua y Administración de Obras Sanitarias de Corrientes. Moglia S.R.L., Corrientes, Argentina. p. 53-65.

Gianuzzi, L. 2009. Cianobacterias y Cianotoxinas. Identificación, Toxicología, Monitoreo y Evaluación de Riesgo. Instituto Correntino del Agua y Administración de Obras Sanitarias de Corrientes. Moglia S.R.L., Corrientes, Argentina. 237p.

Otaño S. 2009a. Saxitoxins in Argentinean inland waters. Harmful Algae News 39:19.

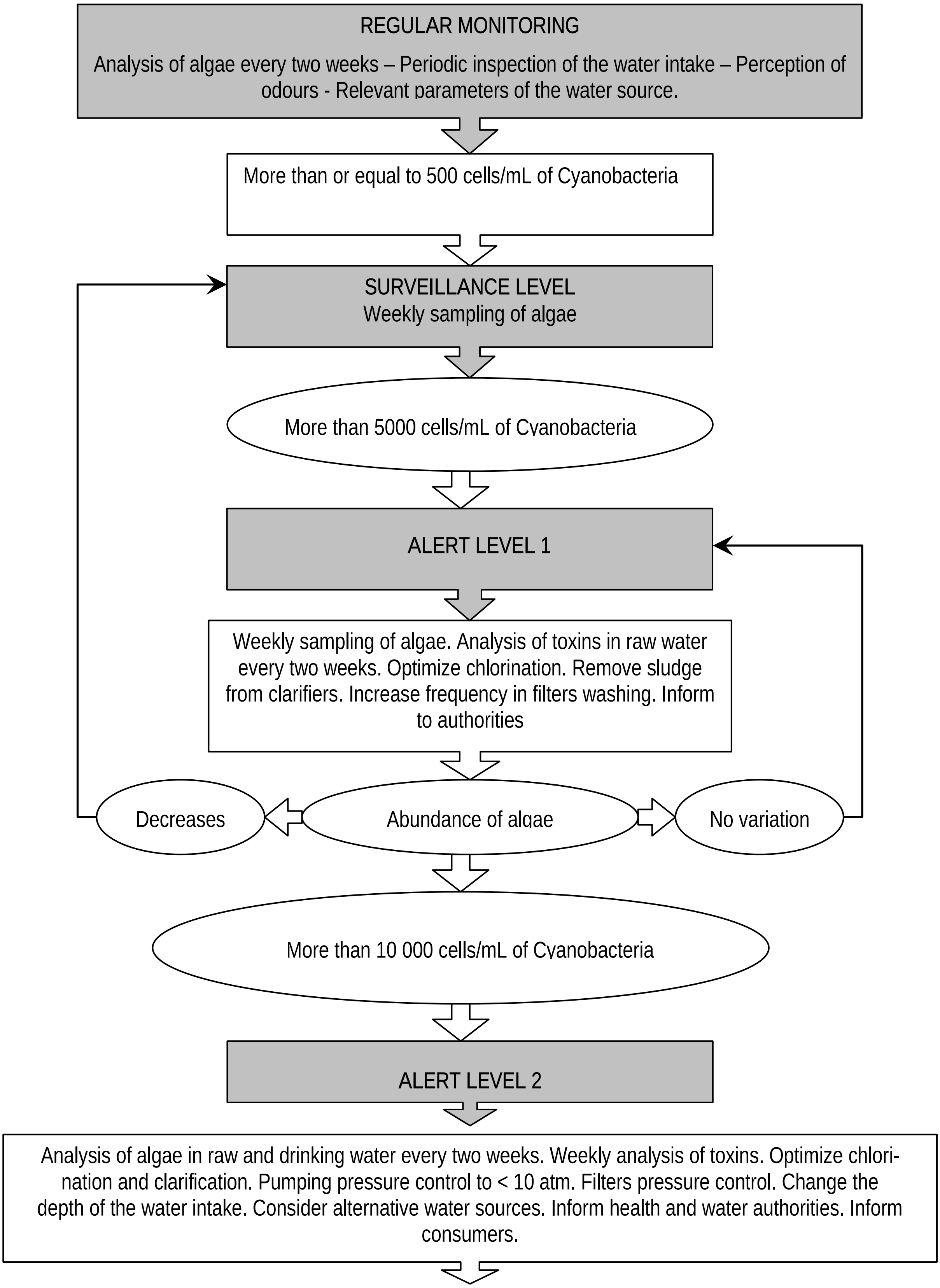
Otaño S. 2009b. First report of the cyanobacterium *Aphanizomenon schindleri* (Nostocales, Cyanophyceae) in River Uruguay, Argentina. Algolog. Stud**.** 131: 35-42.

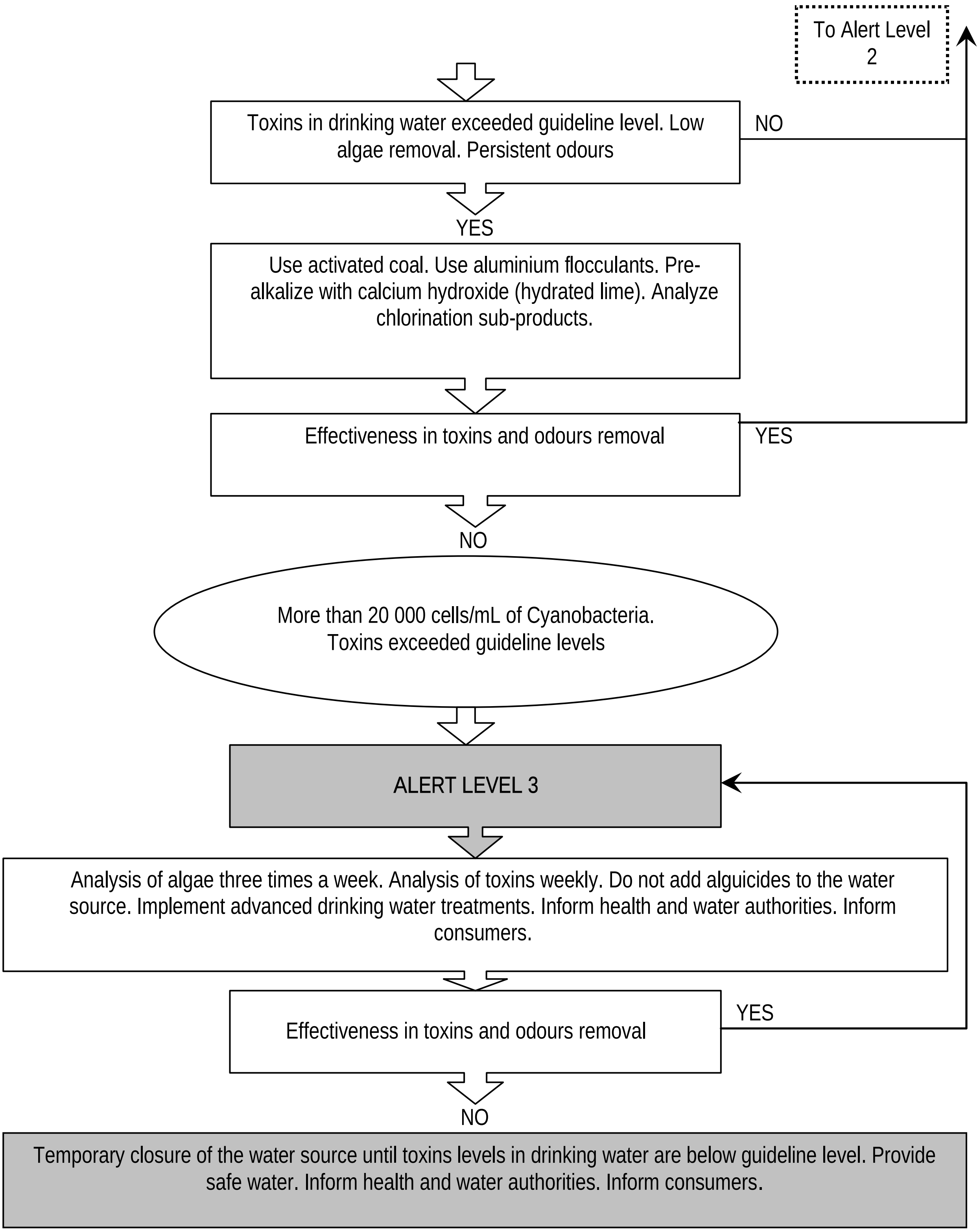
Otaño S. 2012. *Aphanizomenon favaloroi* spec. nov. (Cyanobacteria, Nostocales), a new planktic species from Argentina. Algolog. Stud. 138: 27-36.

Otaño S. & Román N. 2008. Floración de cianobacterias sobre la costa del Río Uruguay Verano del 2008. V Taller Internacional sobre Enfoques Regionales para el Desarrollo y Gestión de Embalses en la Cuenca del Plata. Itaipú (Paraguay- Brasil). Instituto Argentino de Recursos Hídricos. 12 pp

Ruibal A.L., Ruiz M. & Otaño S. 2009. Enfoques para la Evaluación y Manejo del Riesgo de cianobacterias. Niveles de alerta. En: Giannuzzi L. (Ed.). Cianobacterias y Cianotoxinas. Identificación, Toxicología, Monitoreo y Evaluación del Riesgo. Instituto Correntino del Agua y Administración de Obras Sanitarias de Corrientes. Moglia S.R.L., Corrientes, Argentina. p 159-191.

Figure 1: Alert levels for the presence of harmful Cyanobacteria in Argentina
(from Ruibal *et al.* 2009)




To Alert Level 2
Toxins in drinking water exceeded guideline level. Low algae removal. Persistent odours
NO
YES
Use activated coal. Use aluminium flocculants. Pre-alkalize with calcium hydroxide (hydrated lime). Analyze chlorination sub-products.
Effectiveness in toxins and odours removal
YES
NO
More than 20 000 cells/mL of Cyanobacteria. Toxins exceeded guideline levels
ALERT LEVEL 3
Analysis of algae three times a week. Analysis of toxins weekly. Do not add alguicides to the water source. Implement advanced drinking water treatments. Inform health and water authorities. Inform consumers.
Effectiveness in toxins and odours removal
YES
NO
Temporary closure of the water source until toxins levels in drinking water are below guideline level. Provide safe water. Inform health and water authorities. Inform consumers.

AUSTRALIA:
Guidelines, Legislation and Management Frameworks

Vanora Mulvenna[1] and Philip T. Orr[2]

[1] Department of Health, Melbourne, VIC 3001 Australia; vanora.mulvenna@health.vic.gov.au
[2] Seqwater. 240 Margaret St, Brisbane, QLD 4000 Australia; philip.orr@bigfoot.com

Background

Regulation and management of toxic cyanobacteria in Australia occurs at multiple levels of government. The Federal Government does not have the legal authority to mandate regulatory frameworks because this power resides with individual Australian states and territories. However, federal councils provide the overarching framework that allows states and territories to manage cyanobacteria through three key documents:

- The **Australian Drinking Water Guidelines** (NHMRC and NRMMC, 2011) (ADWG) are published jointly by the National Health and Medical Research Council and Natural Resource Management Ministerial Council;
- The **Guidelines for Managing Risks in Recreational Water** (NHMRC 2008) (GMRRW) are published by the National Health and Medical Research Council.
- The **Australian and New Zealand Guidelines for Fresh and Marine Water Quality** (ANZECC and ARMCANZ, 2000) (ANZECC) were originally published jointly by the Agriculture and Resource Management Council of Australia and New Zealand and the Australian and New Zealand Environment and Conservation Council. These Australian and New Zealand intergovernmental agencies have now been abolished and responsibility has been vested in the Standing Council on Environment and Water. This council incorporates the National Environment Protection Council.

These documents are periodically reviewed, revised and updated to reflect current knowledge about cyanobacteria and their management. They are technical and scientific documents that provide the frameworks for management of cyanobacteria and cyanotoxins but are not legislative documents. Each state and territory defines its own regulatory environment based on the scientific information in the above Guidelines.

The Australian Drinking Water Guidelines

The ADWG provide the framework for managing drinking water quality in Australia. The framework covers all aspects of water quality (including cyanobacteria and cyanotoxins) and is written from a catchment to tap risk management perspective. Information on individual classes of cyanotoxins including toxicity of cyanotoxins known to occur in Australian drinking water supplies is provided in Fact Sheets that form part of the ADWG. The Fact Sheets provide general information on each cyanotoxin group, their significance in an Australian context, methods of treatment, methods of identification and detection, health considerations, worked examples of guideline derivation and recommendations for notification protocols. Table 1 summarises the ADWG status for each class of cyanotoxin.

Table 1: Australian Drinking Water Guideline status of Cyanotoxins

Cyanotoxin	Guideline Status	Concentration
Microcystins	Formal Guideline	1.3 μg (MCYST-LR toxicity eq) L^{-1}
Saxitoxins	Health Alert Level	3 μg (STX toxicity eq) L^{-1}
Cylindrospermopsins	Health Alert Level	1 μg L^{-1}
Nodularin	NIL	–

- **Cylindrospermopsins** – No guideline value is presented for cylindrospermopsin or for organisms that produce it. It is recommended that health authorities be notified when *Cylindrospermopsis raciborskii* or other producers of cylindrospermopsins are detected in sources of drinking water. An initial health alert level of 1 μg L^{-1} is suggested in the ADWG equivalent to between 15,000 and 20,000 cells mL^{-1} or a biovolume of 0.6 – 0.8 mm^3 L^{-1}.
- **Microcystins** – Drinking water microcystin concentrations should not exceed 1.3 μg L^{-1} expressed as microcystin-LR toxicity equivalents (TE). An indicative equivalence to 6,500 cells mL^{-1} of a highly toxic strain is provided. For health risk assessment, toxin determination is required.
- **Nodularin** – No guideline value is presented for nodularin due to the lack of adequate data. The relevant health authority should be advised immediately if blooms of *Nodularia spumigena* are detected in sources of drinking water.
- **Saxitoxins** – No guideline value exists for saxitoxins due to the lack of adequate data. The relevant health authority should be advised immediately if blooms of *Anabaena circinalis* or other producers of saxitoxins are detected in sources of drinking water. A health alert level of 3 μg (saxitoxin TE) L^{-1} – equivalent to 200 μg (saxitoxin TE) $person^{-1}$ – is suggested based on consumption of shellfish contaminated with Paralytic Shellfish Poisons. This is equivalent to 20,000 cells mL^{-1} or a biovolume of about 5 mm^3 L^{-1} of a highly toxic strain of *A. circinalis*.
- Trigger levels for initial notification to health authorities for a range of known toxin-producing cyanobacteria are included in the ADWG. These trigger levels have been summarised in a Report (WQRA 2010), and are detailed below in Table 2.

Guidelines for Managing Risks in Recreational Water

The GMRRW provide a framework for managing the risks associated with cyanobacteria in recreational waters. The framework suggests water bodies should be graded on an annual basis based on prior assessment of cyanobacteria and on historical information on physicochemical conditions. This gives an indication or susceptibility to cyanobacterial growth of Very Poor, Poor, Fair, Good and Very Good categories. For the Poor, Fair and Good categories, this then initiates a more intensive short term assessment to define three Alert Levels:

- “Green Level” is called Surveillance mode
- “Amber Level” is called Alert Mode
- “Red Level” is called Action Mode

For each Mode there are guideline cell concentration and/or biovolume trigger levels as well as recommended actions. Within the Action Mode there are two further Levels.

- Level 1 – is based on the probability of adverse health effects from ingestion of known toxins and based on *Microcystis aeruginosa* cell counts, biovolume or concentration of microcystins.

- Level 2 – is based on total cyanobacterial biovolume or, the presence of surface scums where known toxins are not present. Level 2 accounts for the probability of increased likelihood of nonspecific adverse health outcomes from exposure to elevated cell concentrations or biovolume of cyanobacterial material.

These guideline cell concentration/biovolume trigger levels are detailed in Table 3.

Table 2: Cyanotoxin drinking water levels triggering action

Cyanobacterial Species	Notification		Alert	
	Cell concentrations (cells mL^{-1})	Biovolume (mm^3 L^{-1})	Cell concentrations (cells mL^{-1})	Biovolume (mm^3 L^{-1})
M. aeruginosa	2 000	0.2	6 500	0.6
A. circinalis	6 000	1.5	20 000	5
C. raciborskii	4 500	0.18	15 000	0.6
N. spumigena	12 000	2.7	40 000	9.1

The framework also provides recommendations on sampling protocols for site assessment along with a list of recommendations that should be provided to the public in information packages.

Australian and New Zealand Guidelines for Fresh and Marine Water Quality

The ANZECC water quality guidelines provide the basis for assessing and managing the quality of marine, estuarine and freshwaters. Specifically for cyanotoxins this document:-

- does not currently provide trigger values for use of cyanobacteria contaminated irrigation water,
- provides a value of 11,500 cells mL^{-1} of *M. aeruginosa* (or a trigger value for microcystin-LR toxicity equivalents of 2.3 μg L^{-1}) above which "an increasing risk to livestock health is likely." This however conflicts with additional data elsewhere in the guidelines that provide a range of cell concentrations from 11,500 – 81,500 cells mL^{-1} for various livestock species.
- provides an interim guide, that direct contact should be avoided when 15,000 – 20,000 cells mL^{-1} are present, depending on the algal species.

A revision of the ANZECC water quality guidelines is currently underway that will have implications for how cyanobacteria and their toxins are reported in the future. The review will also ensure that the guidelines are better aligned with the other two guideline documents.

Table 3: Cyanotoxic recreational guideline levels triggering action

Green level Surveillance mode	Amber level Alert mode	Red level Action mode
≥500 to <5 000 cells mL^{-1} *M. aeruginosa* or biovolume equivalent of >0.04 to <0.4 mm^3 L^{-1} for the combined total of all cyanobacteria.	≥5 000 to <50 000 cells mL^{-1} *M. aeruginosa* or biovolume equivalent of ≥0.4 to <4 mm^3 L^{-1} for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume[a]. or[b] ≥0.4 to <10 mm^3 L^{-1} for the combined total of all cyanobacteria where known toxin producers are not present.	Level 1 guideline: ≥10 μg L^{-1} total microcystins or ≥50 000 cells mL^{-1} toxic *M. aeruginosa* or biovolume equivalent of ≥4 mm^3 L^{-1} for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume. or[b] Level 2 guideline: ≥10 mm^3 L^{-1} for total biovolume of all cyanobacterial material where known toxins are not present. or cyanobacterial scums are consistently present[c].

Source: GMRRW

[a] The definition of 'dominant' is where the known toxin producer comprises 75% or more of the total biovolume of cyanobacteria in a representative sample.

[b] This applies where high cell concentrations or scums of 'nontoxic' cyanobacteria are present, ie where the cyanobacterial population has been tested and shown not to contain known toxins (microcystin, nodularin, cylindrospermopsin or saxitoxins).

[c] This refers to the situation where scums occur at the recreation site each day when conditions are calm, particularly in the morning. Note that it is not likely that scums are always present and visible when there is a high cell concentration, as the cells may mix down with wind and turbulence and then reform later when conditions become stable.

Implementation of the framework at a state and territory level

Australia's states and territories have implemented the framework for management of cyanobacteria and their toxins in different ways, as outlined below.

Management and contingency planning

Due to the large range of issues posed by cyanobacterial toxins to the environment, human and livestock health and food safety, a multitude of government agencies are involved within each Australian state and territory in cyanobacterial management and contingency planning. As a result, many states have developed overarching algal management strategies and have set up multi-agency algal coordinating committees to manage risks at a regional or local level. Examples include the South Australia State Blue-green Algal Task Force and the Western Australia State Algal Management Strategy (WA Department of Water draft 2010). These overarching strategies refer to the health guideline values and alert level frameworks specified within the relevant national guidelines detailed above.

Cyanobacterial blooms of significant magnitude may also be managed under relevant state emergency management arrangements in some circumstances.

Drinking water

Most states and territories in Australia administer legislation to manage risks to drinking water supplies, however the degree of implementation as well as the nature of legislation varies. Some states have legislation that requires drinking water providers to prepare and implement risk management plans which address risks to drinking water supplies, including those posed by cyanobacterial toxins. Examples include Victoria's *Safe Drinking Water Act 2003*, Tasmania's Drinking Water Quality Guidelines 2005, South Australia's *Safe Drinking Water Act 2011* and Queensland's *Water Supply (Safety and Reliability) Act 2008*. An alternate example is Western Australia's *Water Services Licensing Act 1995*, which requires that drinking Water Service Providers obtain an operating license. A condition of the operating license is that the Water Service Provider enters a memorandum of understanding (MOU) with the Western Australian Health Department. The MOU requires the Provider to establish various protocols, one of which relates to cyanobacteria.

States that do not have legislative frameworks covering risks posed by cyanobacterial toxins rely on strategies that detail management arrangements. An example includes the New South Wales Algal Management Strategy (NSW Office of Water draft, 2009). The majority of states and territories in Australia refer to the health guideline values within the ADWG as a basis for managing risks to drinking water supplies in their respective areas.

Recreational Water

With the exception of Tasmania, which manages risks to recreational water users through public health legislation (i.e. the Tasmanian Recreational Water Quality Guidelines 2007) all states and territories in Australia manage risks through guidance rather than through specific legislative oversight. Recreational water blooms are typically managed by local water managers, with the assistance of regional algal coordinators and multi-agency committees in the event of significant large-scale blooms. The GMRRW are generally adopted within state and territory-based frameworks, however in some cases, these guidelines are adapted at a local level. For example, within the Australian Capital Territory (ACT) Guidelines for Recreational Water Quality (ACT Health, 2010), the GMRRW have been adapted as follows:

- Medium alert level: If >20000 cells mL^{-1} (>1.6 mm^3 mL^{-1} biovolume equivalent), change warning signs to indicate increased risks for skin irritation, gastro illness
- High alert level: If >50000 to <125000 cells mL^{-1} *M. aeruginosa* (>4 to <10 mm^3 L^{-1} biovolume equivalent), close water body for primary contact recreation
- Extreme alert level: >125000 cells mL^{-1} *M. aeruginosa,* 40,000 cells mL^{-1} *Anabaena sp.*, or scums consistently present, advise of increased risk for secondary contact users

Additional advice is also provided within the ACT guidelines on:

- Risks to primary contact recreational users from *Tychonema sp.*
- Risks from blue-green algae through exposure to aerosols from jet fountains (>50000 cells mL^{-1} *M. aeruginosa* or >4 mm^3 L^{-1} biovolume equivalent)

Livestock and irrigation

As detailed above, the ANZECC water quality guidelines do not currently provide trigger values for the use of cyanobacteria contaminated irrigation water, and those specified for livestock health are not considered to be sufficiently robust by some states.

For these reasons, the Victorian state Blue-green Algae Circular (Department of Sustainability and Environment, 2011) recommends that water managers undertake a risk assessment for cyanobacterial blooms in water bodies used to supply water for irrigation and livestock drinking purposes to determine whether the water is potentially hazardous.

The Queensland and New South Wales state governments both reference the livestock drinking water guideline for *Microcystis aeruginosa* specified within the ANZECC water quality guidelines, but acknowledge the lack of sufficient toxicological trials and data to support guideline development for this end use. The NSW Algal Management Strategy (NSW Office of Water draft, 2009) also adopts the *Anabaena circinalis* livestock alert of 25,000 cells mL^{-1} specified by Orr and Schneider (2006). The adoption of these guidelines in NSW is an interim measure, pending review and update of ANZECC guidelines.

In terms of managing cyanobacterial risks in water bodies used for irrigation, several states including Victoria and New South Wales recommend that water contaminated with blue-green algae should not be spray irrigated on vegetables and fruit, or come in contact with plants being grown for food.

Seafood

With the exception of a Food Standards Australia New Zealand standard for saxitoxins in shellfish, there are currently no national guidelines in Australia that advise on safe levels of cyanobacterial toxins in seafood. In order to define acceptable levels of cyanobacterial toxins in seafood, the Victorian Department of Health convened a scientific advisory group to carry out a risk assessment regarding seafood safety in the Gippsland Lakes - a system of coastal lagoons in Victoria, which are an important commercial and recreational seafood source. Health guideline values derived through this assessment (based on seafood consumption by the 2–16 year age group) are detailed in Mulvenna et al (2012) and are summarised in Table 4:

Table 4: Cyanotoxin food guideline values

Toxin	Health guideline value (μg kg^{-1} of whole organism sample)		
	Fish	Prawns	Mussels or Molluscs
Cylindrospermopsin and deoxyCYN	18	24	39
Microcystin-LR* or equivalent toxins, incl. nodularin	24	32	51
Saxitoxins	800	800	800

* The guideline value represents the sum value of all microcystins and nodularin present

Apart from their application in the state of Victoria, the New South Wales State Algal Advisory Committee recently adopted the above guidelines as an interim measure for use in New South Wales.

Acknowledgements

The authors would like to acknowledge the contribution of the following individuals:

- Glenn McGregor, Queensland Department of Science, Information Technology, Innovation and the Arts
- Natasha Ryan and Lee Bowling, NSW Office of Water
- Cameron Dalgleish, Tasmanian Department of Health and Human Services
- Michelle Wittholz and David Cunliffe, SA Health
- Brian Jones, ACT Health
- Karen Beirne, Northern Territory Department of Health and Families
- Jared Koutsoukos, WA Health
- Rachael Poon, Victorian Department of Health
- Andrew Humpage, Australian Water Quality Centre
- Michael Burch, Australian Water Quality Centre

References

ACT Health 2010. ACT Guidelines for Recreational Water Quality. http://www.health.act.gov.au/c/health?a=sendfile&ft=p&fid=1281404492&sid=

ANZECC and ARMCANZ. 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. The Australian and New Zealand Environment and Conservation Council and The Agricultural Resource Management Council of Australia and New Zealand. ISBN 09578245. http://www.mincos.gov.au/publications/australian_and_new_zealand_guidelines_for_fresh_and_marine_water_quality

DERM. 2009. Queensland Water Quality Guidelines, Version 3. pp167. Department of Environment and Resource Management, Brisbane, Australia. ISBN 978-0-9806986-0-2. http://www.derm.qld.gov.au/environmental_management/water/queensland_water_quality_guidelines/index.html

Department of Sustainability and Environment. 2011. Blue-green Algae Circular 2011–12. http://www.ourwater.vic.gov.au/environment/algae/blue-green-algae-circular

Humpage, A.R. and Falconer, I.R. 2003. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of No Observed Adverse Effect Level for deriving a drinking water guideline value. *Environmental Toxicology* 18(2) 94–103.

Mulvenna, V., Dale, K., Priestly, B., Mueller, U., Humpage, A., Shaw, G., Allinson, G. and Falconer, I. 2012. Health Risk Assessment for Cyanobacterial Toxins in Seafood. *Int. J. Environ. Res. Public Health* 9(3) 807-820.

NHMRC 2008. Guidelines for Managing Risks in Recreational Water. pp215. National Health and Medical Research Council, Canberra. ISBN Print: 1864962666, ISBN Online: 1864962720. http://www.nhmrc.gov.au/publications/synopses/eh38.htm

NHMRC and NRMMC 2011. Australian Drinking Water Guidelines. National Health and Medical Research Council, Canberra. http://www.nhmrc.gov.au/guidelines/publications/eh52/

NSW Office of Water 2009 (draft). New South Wales Algal Management Strategy. Unpublished.

Orr, P.T. and Schneider, P.M. 2006. Toxic Cyanobacteria Risk Assessment Reservoir Vulnerability and Water Use Best Practice. pp61. A Report to SEQWater. Brisbane.

Tasmania Department of Health and Human Services 2007. Recreational Water Quality Guidelines.

Tasmania Department of Health and Human Services 2005. Drinking Water Quality Guidelines.

WA Department of Water 2010 (draft). Western Australia State Algal Management Strategy. Unpublished.

WQRA. 2010. Management Strategies for Cyanobacteria (blue-green algae): a Guide for Water Utilities. Research Report 74. pp100. Water Quality Research Australia. Adelaide, Australia. http://www.wqra.com.au/publications/report74_management_strategies_BGA.pdf

CANADA: Cyanobacterial Toxins: Drinking and Recreational Water Quality Guidelines

Michèle Giddings[1], Rocio Aranda-Rodriguez[2], Gordon Yasvinski[1], Sue B. Watson[3], Ron Zurawell[4]

[1] Water, Air and Climate Change Bureau, Health Canada, 269 Laurier Ave West, Ottawa, ON, K1A 0K9, Canada; michèle.giddings@hc-sc.gc.ca
[2] Chemistry Research Division, Health Canada, Tunney's Pasture, 0800C, Ottawa, ON, K1A 0L2, Canada
[3] Aquatic Ecosystem Management Research Division Environment Canada, CCIW Burlington ON
[4] Environmental Assurance Division, Alberta Environment, Edmonton AB

Introduction

In Canada, the provision of safe drinking and recreational water requires the cooperation of all governments. The Federal-Provincial-Territorial (FPT) Committee on Drinking Water, made up of representatives from Health Canada and provincial and territorial departments responsible for drinking water quality, develops the *Guidelines for Canadian Drinking Water Quality* (GCDWQ). These Guidelines establish health and/or aesthetic limits for a variety of microbiological, chemical, physical and radiological parameters in Canadian drinking water (Health Canada, 2010). The GCDWQ are either embodied in provincial or territorial legislation to become enforceable standards, or used by the provinces and territories as the basis for assessing the quality of drinking water supplies.

An *ad hoc* FPT Working Group on recreational water was established by the Federal-Provincial-Terrritorial Committee on Health and the Environment to develop the *Guidelines for Canadian Recreational Water Quality* (Health Canada, 2012). The Guidelines address the health hazards associated with the quality of recreational water (regarded as any natural fresh, marine or estuarine body of water that is used for recreation). The document establishes guideline values and aesthetic objectives for recreational water quality parameters, and provides an approach to safe recreational water management. The Working Group is composed of representatives from Health Canada, Environment Canada, and provincial and territorial departments responsible for recreational water quality. These guidelines and aesthetic objectives are used within the provinces and territories to guide the safe management of recreational waters.

One issue of concern in Canada in both drinking and recreational waters is the presence of cyanobacteria (or blue-green algae) and their toxins. In 2002, Health Canada approved a drinking water guideline for microcystin-LR which was deemed protective of total microcystins. More recently (2012), a guideline for recreational water (microcystin toxin and total cells) was approved. This paper briefly summarizes the guideline development process, risk management programs across Canada, and Health Canada's work on the use commercially-available field test kits for the on-site determination (presence-absence) of cyanobacterial toxins within a water supply.

Background

As the second largest country in the world, Canada covers a vast area (9,984,670 km^2) and faces jurisdictional and logistical challenges in providing safe drinking and recreational waters and ensuring drinking water contaminants are at levels below those known to cause adverse health risks. Almost 10% of the land is covered by freshwater, which span 20 ecozones and temperate to subarctic or arctic climates. Some of the largest waterbodies (e.g. the Great Lakes) share binational (Canada-US) shorelines and catchments with varied basin development, water usage demands and nutrient related impacts. The 10 Canadian provinces and 3 territories have significant differences in population density (Table 1) and associated impairments to surface waters. More than 2 million lakes, reservoirs, ponds and rivers show a wide range in size, hydrology, chemistry and nutrients; comparatively few have been characterised for their susceptibility to cyanobacterial blooms.

The issue of cyanobacterial blooms developing in Canadian water bodies and impacting their uses was first detected in the prairie provinces. In late August 1993, a large algal bloom developed in the Deacon Reservoir, the main drinking water storage facility for the city of Winnipeg, Manitoba. The Deacon Reservoir stores water that is transported through an aqueduct from Shoal Lake, located 135 km away. Toxin-producing blue-green algae were found to be present in Shoal Lake, but not in the Deacon Reservoir. Microcystin-LR was detected in Shoal Lake and within the distribution system, but was not present at detectable levels (>0.05 µg/L) in the Deacon Reservoir. Maximum microcystin-LR concentrations measured in the raw water of Shoal Lake and in treated tap water were approximately 0.45 µg/L and 0.55 µg/L, respectively. In response to the 1993 bloom, Manitoba Environment, in cooperation with the City of Winnipeg, continued to monitor for microcystin-LR in Winnipeg's water supply. A comprehensive study of the quality of surface water supplies in rural southwestern Manitoba was also conducted. The study found Microcystin-LR to be widely distributed across all water supply categories, with concentrations occasionally exceeding 0.5 µg/L.

Presently, blooms are reported to be a concern for most Canadian provinces, though they remain particularly prominent in the prairie provinces (Alberta, Saskatchewan, Manitoba) and in the province of Quebec. In 2010, Health Canada analysed samples from recreational waters, drinking water sources, and treated drinking water (unpublished data in four Canadian provinces. Total microcystins were detected in many of the blooms tested, with concentrations in most samples ranging from 0-1.5 µg/L in 71% of 138 samples. A small proportion of samples (4%) had concentrations of total microcystin between 1.5 and 5 µg/L while 25% of samples had concentrations greater than 5 µg/L (levels as high as 2000 µg/L). Microcystin was not detected in treated water. It is generally the smaller shallow, warm, slow-moving or still bodies of water common in rural areas across the country that are subject to repeated blooms. Uses for these water supplies are typically for domestic purposes and for livestock watering.

While the number of water bodies reported to be impacted by blooms has been on the rise in recent years, some of these increases can be attributed to greater monitoring and an increased awareness of this problem. The cyanobacterial genera associated with toxin formation that are known to occur in Canada are the same ones that have been most frequently encountered in other countries, namely *Anabaena*, *Aphanizomenon*, *Microcystis* and *Planktothrix.*

Guideline for Canadian Drinking Water Quality for Microcystin-LR

Health Canada's (2002) risk assessment review classified microcystin-LR as "possibly carcinogenic to humans" placing it in Group IIIB (inadequate data in humans, limited evidence in experimental animals). For compounds in Group IIIB, the lowest-observed-adverse-effect level (LOAEL) or no-observed-adverse-effect level (NOAEL) from the most suitable chronic or subchronic study is divided by an appropriate uncertainty factor, to derive a tolerable daily intake (TDI). Such an approach was used for microcystin-LR, which remains the only microcystin for which there is sufficient information available to derive a guideline value.

A TDI of 0.04 µg/kg body weight per day was derived from a NOAEL of 40 µg/kg body weight per day for liver changes in a 13-week mouse study (Fawell et al., 1994), using an uncertainty factor of 1000 (×10 for intraspecies variation, ×10 for interspecies variation and ×10 for the less-than-lifetime study).

A maximum acceptable concentration (MAC) of 0.0015 mg/L (1.5 µg/L) for microcystin-LR was calculated from the TDI by assuming a 70-kg adult consuming 1.5 L of water per day, as well as allocating 80% of the total daily intake to drinking water[2] (the major route of exposure to these toxins is via drinking water). Although the MAC is derived for microcystin-LR, it is considered to be protective against exposure to other microcystins (total microcystins, i.e., free plus cell bound) that may also be present. It is a conservative value, as it is derived on the basis of daily consumption of microcystin-LR over a full year (Health Canada, 2002). In Canada, due to climatic conditions, toxic blooms are not a year-round problem and toxins are not be expected to be present in water supplies more than 3-4 months per year, thus exposure is non-continuous. Additional contributing factors include spatial and temporal variations in the levels of microcystins within supplies, and that toxins are generally confined to within the bloom borders, while water intakes for most municipal supplies are generally positioned well beneath the surface and at a distance from shore. A flow chart illustrating those factors that should be considered by water purveyors and health and environment authorities during bloom events and actions that may be taken to address the issue is shown below (Health Canada, 2002). Presently, Health Canada's microcystin guideline has been identified for re-evaluation as part of the Guidelines for Canadian Drinking Water Quality's rolling revision process.

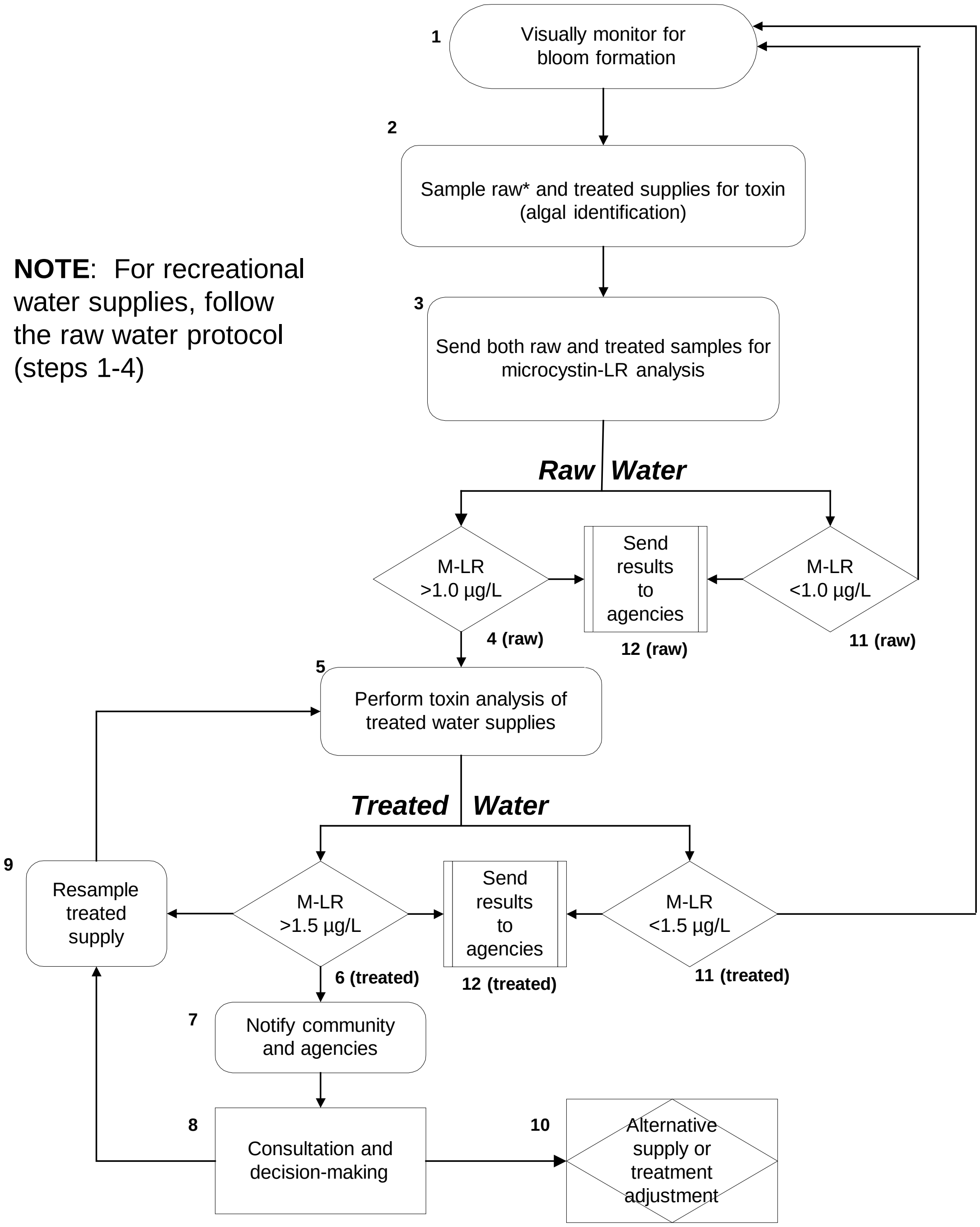
Recreational Water Quality Guideline Development Process

In 2004, Health Canada began the process of updating the Guidelines for Canadian Recreational Water Quality (Health Canada, 2012). Increasing public awareness of algal blooms prompted the development of guideline values for cyanobacteria and their toxins in Canadian recreational waters. These guidelines provide protection for recreational water users against both the risk of exposure to microcystins through inadvertent ingestion as well as from other harmful effects that may be possible following exposure to high densities of cyanobacterial cells.

[2] The MAC of 1.5 µg/L established by Health Canada differs slightly from the WHO's provisional guideline value of 0.001 mg/L (1 µg/L). Both approaches are based on the same TDI derived from the Fawell et al. (1994) study. The difference stems from the reference values used by the respective organizations for adult body weight (HC – 70 kg; WHO – 60 kg) and average daily drinking water consumption (HC – 1.5 L/d; WHO – 2 L/d)

Cyanobacterial Toxins -- Microcystin-LR
Flow Chart
- Water Supplies for Human Consumption -

NOTE: For recreational water supplies, follow the raw water protocol (steps 1-4)



April, 2002

* A field kit could be used for screening. A validation sample should be send to a laboratory for confirmation of actual levels following a positive field test.

The FPT working group tasked with developing the guideline assessed approaches used by other jurisdictions worldwide, and concluded that developing guideline values for cyanobacteria (i.e., the cells) and for cyanobacterial toxins (i.e., microcystins) in recreational waters was the preferred approach for the Canadian situation. A guideline value of 20 µg/L for total microcystins in Canadian recreational waters was established using a similar approach to that used in developing the Canadian drinking water quality guideline (Health Canada, 2012).

A TDI of 0.4 µg/kg body weight per day was derived from the NOAEL of 40 µg/kg body weight per day for liver changes in the same key study (Fawell et al., 1994) that was used in the drinking water assessment. An uncertainty factor of 100 was used (x10 for intraspecies variation, x10 for interspecies variation). An additional uncertainty factor for less-than-lifetime study was judged to be unnecessary since the types of exposures being considered were short-term and episodic in nature. The guideline value of 20 µg/L for total microcystin-LR was calculated from the TDI by assuming a 13 kg child consuming 0.25 L per day through accidental ingestion while swimming. These assumptions reflected some risk management decisions. Children were selected as the exposure group as they engage in recreational activities more frequently than adults and typically spend a longer time in the water per recreational event. Children are also likely to swallow greater volumes of water through accidental ingestion. The 0.25 L/per day is likely to be an overestimate of the amount of water that could be consumed by a child during extensive recreational activity was (Health Canada, 2012).

For total cyanobacterial cell density, a guideline value was calculated using the derived total microcystin guideline and a recognized reference value of 2 x 10^{-7} µg/cell for the total toxin content per cell for microcystin-producing cyanobacteria (WHO, 2003; NHMRC, 2008). The guideline established for cyanobacteria is ≤ 100,000 cells/mL.

In the event of bloom development, in order to fully characterize the extent of the risk posed by the cyanobacterial population, authorities are advised to conduct sampling during, and after the collapse of, the bloom. Should either of the guideline values be exceeded, a swimming advisory may be issued by the responsible authority. Contact with waters where an advisory has been issued should be avoided until the advisory has been rescinded.

Health Canada’s guideline document emphasizes that guideline values are but one important part of an overall risk management approach to safe recreational water quality. The responsible authorities are encouraged to use guideline values together with the appropriate technical documentation for assessing recreational water quality hazards and managing human health risks.

Cyanobacteria Risk Management Programmes in Canadian Drinking and Recreational Water

As with other countries worldwide, monitoring program coverage and timely response to bloom events are challenged by geographic constraints and cost and logistics, particularly in areas with low population densities. Specific designs of drinking water treatment systems and the associated cost of operation varies widely from jurisdiction to jurisdiction (Statistics Canada, 2010). Most jurisdictions use the MAC of 1.5 µg/L microcystin-LR from the Guidelines for Canadian Drinking Water Quality in treated drinking water (Health Canada, 2002). As noted above, this guideline is considered protective of human health against exposure to other microcystins (total microcystins) that may also be present within a drinking water supply. As a general rule, microcystin-LR is not a parameter with regularly scheduled monitoring frequencies, and most municipal drinking water treatment plants monitor for algal toxins only when a bloom occurs. However, municipalities with a history of bloom formation will typically monitor their water supplies with greater frequency. At a national level, very few (~4-5%) municipal water treatment plants have identified or responded to cyanobacteria as a source water issue (Statistics Canada, 2010).

For recreational waters, some of the more densely populated provinces or regions with greater economic and technical resources have developed their own bloom risk management programme (BMRP), largely based on the Health Canada (2012), and/or WHO (2003) frameworks. Many regions, however, have no formal monitoring or risk management programme in place. Table 1 summarises the national and regional drinking and recreational water quality guidelines and standards currently in place across Canada.

British Colombia is Canada's third largest province. It borders the west coast of the country and has a diverse geography and a wide variance of climate. The most heavily populated coastal regions rarely experience freezing temperatures and can have extended bloom seasons, particularly in areas with more intense anthropogenic impacts. Northern and interior lakes in British Columbia have few occurrences of blooms in drinking or recreational water supplies. A regionalized provincial surface water management system is coordinated from the Health ministry and regional offices, but responsibility for sampling generally rests with the water supplier. A draft BRMP of vigilance, sampling, analyses and advisories has been developed to provide standardised first-level response and long term risk management, with particular vigilance for problematic waterbodies. To increase cost effective coverage of the numerous waterbodies across the province, it proposes the following surrogate or rapid screening methods: i) spring total phosphorus (TP) levels of 20 µg/L and 10 µg/L as threshold indicators of moderate and low risk of cyanotoxins, respectively, to guide monitoring effort; and ii) field test kits to screen water and bloom material for toxins and assist in decisions on risk management.

Alberta is the most densely populated of the three Prairie Provinces. The landscape is characterized by mountains, boreal lowlands and prairie grasslands with a wide diversity of waterbodies which range dramatically in productivity and human impact and use. Though pristine in the mountains, the majority of lakes are naturally eutrophic and in the more populated central parkland and southern grassland regions, this is exacerbated by intense agriculture and other human activities. Water supply, drought and extensive irrigation play a major role in southern Alberta. In recognition of this inherently high risk, the province has conducted cyanotoxin research to identify at risk lakes and reservoirs and the toxins most prevalent including various microcystin analogues, neurotoxins, cylindrospermopsin and BMAA. Methods for rapid determination of toxin levels are under study. Provincial regulations require all regulated drinking water treatment plants drawing surface water to test for microcystin-LR during July-August which is the high risk bloom-period. Testing for total microcystin was introduced in 2005 and has become a routine water quality parameter in all government funded lake monitoring programs. Alberta was also one of the first provinces to develop a standardized BRMP (Hrudey and Watson, 2006). Under this programme, which follows the Canadian recreational water quality guideline of 20 µg/L total microcystins, The province supports a pilot public beach monitoring program for total microcystins during the open water season (June-August) in select, heavily used recreational lakes. A centralized public advisory system is also under development.

The prairie province of Saskatchewan also follows the Canadian drinking water guideline for microcystin-LR for their treated municipal drinking water supplies, but has no formal BRMP. Recreational water quality alerts are usually based on the presence/absence of visible blooms. No routine recreational water testing is conducted, although recreational waters are surveyed every 5 years in some regions. Bloom reports are investigated or referred to applicable agencies as required.

Manitoba has more than 110,000 lakes including Lake Winnipeg, currently considered the 6th largest and most eutrophic North American Great Lake. This prairie province has an unmitigated continental climate with temperature extremes, extensive risk of floods, drought and intense storms. Lakes and rivers receive drainage from intensive farming operations across very large catchments often extending across both in the US and Canada. Agricultural activities and human settlement are concentrated in the south and west regions, where there is also greatest surface water impairment and risk of blooms. Manitoba applies the Canadian drinking water guideline for microcystin–LR for municipal supplies and the Canadian recreational water guideline for cyanobacterial cells and microcystin. In addition, the province has developed a provisional protocol on algal bloom and toxin monitoring/reporting again based largely on the Health Canada (2012) and WHO (2003) frameworks, and a public website of updated advisories at a number of public beaches.

Ontario is the most populated of the Canadian provinces. As the 2nd largest province (1,076,395 km^2), approximately 15% of Ontario is covered by freshwater. Drinking water is regulated by Ontario drinking water quality standards as prescribed under the provincial Safe Drinking Water Act. This includes a drinking water standard of 1.5 µg/L for microcystin-LR. Provincial water quality objectives for recreational water quality currently do not include values for algal toxins; however, the province has developed a response guide for field staff responding to blooms events, which outlines roles and responsibilities of the departments and the local Health Units. It is currently being updated to reflect new information and lab screening methods. A second document is being developed as a resource to assist Health Units in making decisions on public health issues associated with blooms.

Quebec is the largest of the ten provinces with a total area of 1,542,056 km^{2,} of which approximately 11 % is covered by surface water. As the second most populous province, Quebec has recently experienced negative impacts on surface water quality (e.g., eutrophication) associated with intense agriculture and other human activities. Blooms are being reported in both lakes and large rivers, many of which being used for recreational purposes or drinking water sources. Toxin-producing species have been identified, though toxin concentrations measured in treated drinking water supplies to date, have been below the Canadian drinking water quality guideline value. Quebec has been proactive in developing a provisional BRMP and has adopted guidelines for total microcystins in drinking water (1.5 µg/L) and recreational water (16 µg/L) that are consistent with the Canadian values. In addition, guidelines for concentrations of anatoxin-a in drinking water (3.7 µg/L) and recreational water (40 µg/L) have also been developed. Though provisional, public consultation on proposed changes to the drinking water regulation has taken place.

Table 1: National and regional drinking and recreational water quality guidelines and standards currently in place across Canada

Jurisdiction	Population (a)	Drinking Water Treatment Plants(b)					Water Quality Guidelines		
		Cyanobacteria identified as source water issue (%)	Cyanobacteria-related treatment modifycations (%)	Amount of surface water treated (m^3 10^6)	Treatment costs (CDN $) per 10^3 m^3	% households on municipal supply [c]	Drinking Health Canada: MC-LR: 1.5 µg L^{-1}	Recreation Health Canada; MC-LR: 20 ug L^{-1}; Cells: 100 000 ml^{-1}	Bloom Risk Management Program in place
Canada	34 278 400	3.9-4.7		5186	124		✓	✓	✖
Alberta	3 742 800	4.4-5.4	1,8	485	196	64	✓	✓	✓ (provisional)
British Columbia	4 554 100	6.9-7.4	6.9-7.4	731	61	69	✓	✓	✖
Manitoba	1 243 700	6,2	6	106	211	50	✓	✓	✖
New Brunswick	753 200	0-5	0	80	97	58	✓	✖	✖
Newfoundland and Labrador	509 100	ND	ND	132	114	72	✓	✓	✓ (under development)
Nova Scotia	943 400	0	0	101.6	158	73	✓	✖	✖
Yukon, NW Territories	43 600 (NWT) 34 300 (YK)	0	0	6.2	1144	ND	✓	✖	✓
Ontario	13 282 400	4.6-5.1	4.6-5.1	1733	139	54	✓[d]	✓	✖
Prince Edward Island	143 500	0	0	0	0	ND	✓	✖	✓
Quebec	7 943 000	1.3-3.7	1.3-3.7	1697	96	60	✖ (provisional) MC_{total}[e]: 1.5 µgL^{-1} ATX[f]: 3.7 µgL^{-1}	✖ (provisional) MC_{total}[e]: 16 µgL^{-1} ATX[f] : 40 µgL^{-1}	✓ (provisional)
Saskatchewan	1 052 100	7.6-8.5	6.9-7.1	114	327	70	✓	✓	✖
Nunavut	33 300	ND							

[a] Statistics Canada 2010; [b] Statistics Canada, 2009, Environment Accounts and Statistics Division, Survey of Drinking Water Plants (survey number 5149; 2007 Households and the Environment Survey relating to drinking water decisions of Canadian households); [c] for the census metropolitan area around a major urban core with a total population of at least 100 000; [d] In Ontario, this is a provincial Standard, not a guideline; [e] sum of all microcystins; [f] ATX: anatoxin-a

With the exception of New Brunswick, the Atlantic Provinces (Nova Scotia, Prince Edward Island, Newfoundland and Labrador) have few bloom-related issues identified in municipal drinking water sources or recreational waters and BRMPs have not been rigorously developed. Most drinking water in these provinces is derived from groundwater. In particular, Prince Edward Island derives all of it's drinking water from groundwater sources. In New Brunswick, an apparent recent increase in bloom events has been addressed under the provincial Clean Water Act which states that "the water shall be free of algae blooms that impair use of habitat for aquatic life, or use for primary or secondary contact activities". A provincial BRMP was developed by the province to respond to specific events, in addition to routine monitoring of selected lakes. Sampling and advisories are issued based on the presence of a bloom (from a visual inspection) and identification of cyanobacteria in samples.

Detection and Analysis of Microcystin

About 90 microcystin variants have been identified, including a number of microcystin-LR homologues, some of which have similar toxicity to microcystin-LR. The current analytical methods rely on the availability of authentic microcystin congeners for identification and quantification. Previous studies have shown the presence of additional microcystin congeners, in some instances at higher concentrations than microcystin-LR. However, to date only 6 variants (-LR, -RR,-LA, YR -LW and -LF) have been commercially available, thus limiting the number of microcystins that can be monitored. Other microcystins can be only tentatively identified and their quantities estimated.

The microcystins that are commercially available are general reagent grade rather than analytical standards; thus, their purity has to be checked by HPLC-photodiode array (PDA) and liquid chromatography–mass spectrometry (LC-MS). A study found that microcystin-RR purity ranged from 70 to 80%; some lots contained a mixture of microcystin-RR and its demethyl variant [Dha7]microcystin-RR, while other lots contained [Dha7]microcystin-RR only (Kubwabo et al., 2004).

Canadian laboratories can obtain certified reference materials for microcystin-LR and microcystin-RR from the National Research Council Canada (Halifax, NS, Canada). Laboratories must be accredited to analyze microcystins. The two methods used most commonly in Canada for monitoring purposes are Enzyme-Linked Immunosorbent Assay (ELISA) and liquid chromatography with UV detection (LC-MS). The Protein Phosphotases Assay (PPA) and liquid chromatography with mass spectrometry detection (LC- MS/MS) methods are used less frequently. Both of the commonly used methods (ELISA and LC-MS) have detection limits in the sub-parts µg/L levels; however, the ELISA provides the sum of all microcystins that cross react with the antibodies used in the kit, whereas LC-/MS provides the concentration of target microcystin variants. Health Canada has developed an innovative and efficient method for the isolation of microcystin from cells based on accelerated solvent extraction (Aranda-Rodriguez et al., 2005).

The use of field test kits offers the potential for rapid results at a lower cost than laboratory analysis, and may be used to screen the water samples for toxin formation throughout a bloom episode. In 2010, Health Canada conducted a study to evaluate the use and limitations of field test kits in providing evidence for preliminary action by the responsible authorities. In this study, 9 end-users collected and tested samples in their location/municipality during July-October 2010.

The following important considerations for end-users were raised during the study:

- The detection of free versus cell-bound toxin: All the kits are able to detect free microcystins, and analysis of total microcystins can be obtained by the use of a lysing agent (QuikLyse™, Abraxis LLC – note that the effect of using reagents with kits produced by different manufacturers must be verified).
- Qualitative vs semiquantitative results: The field test kits provide qualitative (presence/ absence information within their detection limits. Semi-quantitative results can only be obtained when microcystin-LR standards of a known concentration are used and the results are compared to those standards.
- The range of concentrations in which a response is observable: the kits are designed to detect microcystin concentrations between 0 - 5 µg/L (Abraxis, PA, USA) or 0 - 3 µg/L (Envirologix Qualitube). For recreational water quality, the Abraxis strip test kit for recreational water can be used (analytical range of 0 - 10 µg/L). To assess results against the proposed Canadian recreation guideline of 20 µg/L, samples must be diluted when the kit results are greater than 10 µg/L.
- The interpretation of field test kit results, which depends on a visual analysis, varies greatly between end users. It is therefore very important to have a training program in place and to add controls.

Overall, the study demonstrated that the field kits can provide a presence/absence response with reasonable confidence. In cases where false positives are observed, the interpretation still protects the public as the results would trigger additional testing/monitoring in an accredited laboratory. Thus, with careful end-user training, the commercially available field test kits appeared to be a simple and inexpensive way to screen water samples (both raw and treated) for the presence of microcystin toxins.

Emerging Issues

While most of the work in Canada has focused on microcystin, the possible presence of toxins such as anatoxin-a and cylindrospermopsin cannot be ignored. The cyanotoxins (microcystin, anatoxin-a, cylindrospermopsin) are structurally and biologically different, such that concurrent analysis poses significant challenges. Anatoxin-a, an alkaloid-like neurotoxin, degrades within 24 hours under high pH and in the presence of pigments; therefore, it is important to look for degradation products when analysing for anatoxin-a. A method for the concurrent determination of microcystin and anatoxin-a has been developed recently and focuses on the extraction of toxins from cyanobacterial cells and detection by LC-MS/MS.

Cylindrospermopsis raciborskii, a species of subtropical and tropical origin, is new to North America and dominates 23% of lakes in Florida, USA. This cyanobacterial species was recently detected in a lake near Ottawa, Ontario, Canada. It is reported that the majority of *C. raciborskii* or *Aphanizomenon ovalisporum* produce the cytotoxin cylindrospermopsin, a cyclic guanidine alkaloid. There is no Canadian guideline for cylindrospermopsin in drinking water or in recreational water, as there are insufficient toxicological studies available with which to derive guidelines at this time.

Conclusions

Despite geographic constraints, cost and logistics, all of which continue to challenge monitoring program coverage and timely response to bloom events in Canada, Canadian drinking water and recreational water quality guidelines for cyanobacterial toxins (based on microcystin-LR) have been developed through federal, provincial and territorial co-operative efforts. This has involved significant collaboration and communication between F-P-T partners. In addition to the guidelines, improved monitoring tools (field test kits) and analytical methods have been assessed and developed respectively to assess the presence of cyanobacterial toxins in blue-green algae blooms. For more information on Health Canada's involvement in activities related to drinking and recreational water quality in Canada, please refer to Health Canada's water quality website at www.healthcanada.gc.ca/waterquality.

References

Aranda-Rodriguez, R., Tillmanns, A., Benoit, F.M., Pick, F.P., Harvie, J. and Solenaia, L. 2005. Pressurized liquid extraction of toxins from cyanobacterial cells. J Environmental Toxicol. 20(3): 390- 396

Fawell, J.K., James, C.P. and James, H.A. 1994. Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. Report No. FR 0359/2/DoE 3358/2, Water Research Centre, Medmenham, UK. 46 pp.

Giddings, Michèle. 2002. Cyanobacteria Toxins: The development and evaluation of methods to determine microcystin levels in Canadian water supplies. Proceedings of Health Effects of Exposure to Cyanobacteria Toxins: State of the Science. Sarasota, Florida., August 13-14, 2002. Florida Department of Health, the Center for Disease Control, Mote Marine Laboratory, and the University of South Florida, Editors: David Johnson, M.D., M.S., Raymond D. Harbison, Ph.D.

Health Canada. 2002. Guidelines for Canadian Drinking Water Quality: Supporting Documentation — Cyanobacterial Toxins — Microcystin-LR. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. [Available at: www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/cyanobacterial_toxins/index-eng.php]

Health Canada 2010. Summary of Guidelines for Canadian Drinking Water Quality. Health Canada, Ottawa, Ontario. [Available at: www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/2010-sum_guide-res_recom/index-eng.php]

Health Canada. 2012. Guidelines for Canadian Recreational Water Quality. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. [Available at: http://www.hc-sc.gc.ca/ewh-semt/water-eau/recreat/index-eng.php]

HMSO. 1998. The determination of microcystin algal toxins in raw and treated waters by high performance liquid chromatography. Methods for the Examination of Waters and Associated Materials. London, UK.

Kubwabo, C., Vais, N. and Benoit, F.M. 2004. Identification of microcystin-RR and [Dha^{7}]microcystin-RR in commercial standards by electrospray ionization mass spectrometry. Journal of AOAC International 87(4):1028–1031.

NHMRC (2008). Guidelines for managing risks in recreational water. National Health and Medical Research Council of Australia, Government of Australia, Canberra. WHO (2003). Guidelines for safe recreational water environments. Vol. 1. Coastal and fresh waters. World Health Organization, Geneva

CUBA:
Toxic cyanobacteria risk assessment, research and management

Liliana Gomez[1], Inaudis Álvarez[1], José Carlos Rodríguez[2]

[1] Laboratory of Environmental Services and Ecotoxicology, Universidad de Oriente, Santiago de Cuba. Ave de la Américas s/n Esqu. I CP 90400. Santiago de Cuba; lilimagl@yahoo.com
[2] Toxicology and Experimental Medicine Centre. Santiago de Cuba. Autopista Nacional km 1 ½ Apto. Postal 4033. Santiago de Cuba

Introduction

In Cuba, information on the occurrence of cyanobacteria and cyanotoxins is emerging from scientific research, i.e. from initial surveys and ongoing monitoring programmes. Some of these were funded or sponsored by public authorities interested in an initial overview, by water management institutions, or by research laboratories involved in academic programmes. Interest in monitoring programmes also arises in the context of phytoplankton studies or in response to some problems concerning to health conditions of the population served by local drinking-water reservoirs. Universities and research centres develop some monitoring programmes as an own initiative, chiefly with specific projects.

Progress in awareness includes a monograph of ten years of cyanobacteria monitoring, mainly of drinking-water reservoirs, by the "Universidad de Oriente" in Santiago de Cuba (Gomez et al., 2011). This research group has experience in harmful algae bloom detection, characterization and management, not only in freshwater reservoirs, but also in estuaries and coastal areas. The monograph came to the attention of Public Health Care Ministry and was awarded a National Prize. This, as well as publication in a medical profile journal (Gómez et al., 2010) was an important step of the Ecotoxicology Group in creating awareness in the Public Health Care sector.

In the eastern region of Cuba, where Santiago de Cuba is located, the main environmental factors which favour the cyanobacteria growth and lead to bloom occurrence are nutrient enrichment (largely phosphorus but also nitrogen) of aquatic systems, warm temperatures and calm stable water conditions. There are some natural or emergent wetlands which reduce nutrient loads and contribute to ecosystem equilibrium. In all the reservoirs, aquaculture is developed, with a contribution to the nutrient enrichment of their water.

While some measures to protect public health from cyanotoxins are taken locally, formal regulations are not implemented in Cuba, and there is no national initiative to manage cyanotoxins risks. An important precedent of local initiative was a project in Havana, in collaboration with Brazil in 2004 which provided the experience in the surveillance of cyanobacteria to include them in water quality evaluations. The main goal of this project was building capacities and qualification of the Cuban technicians in laboratory techniques for cyanobacterial detection and cyanotoxin analysis (IF-BRASCUB-200405).

For drinking-water quality in general, since 1962 Cuba organized a national surveillance system to evaluate systematically the water quality for human consumption, as a mandatory function of institutions who work together: The National Institute of Water Resources and the National Centre of Sanitation and Epidemiology (the latter belongs to Public Health Care Ministry). The functional structures for water management in Cuba could support any protocol for cyanotoxins risk assessment and management, provided this was initiated.

With regulations lacking at the national level, the World Health Organisation's guidance may be used for the assessment of health impacts, management and surveillance, both for drinking-water and for recreational water use. A point of departure is phytoplankton data, which indicate cyanobacterial dominance and amounts. While this not included as a routine in water analysis, a local initiative in Santiago de Cuba has implemented phytoplankton investigations for some water reservoirs. This was developed by the research team of the "Universidad de Oriente" since 2003 and is being conducted in collaboration with TOXIMED (Public Health Care Ministry) since 2008.

Potentially toxic cyanobacteria in Santiago de Cuba: research initiatives

From November 2008 to March 2010 this monitoring programme focused on three reservoirs which supply water to more than 80% of the population (approximately 480 000 inhabits) to Santiago de Cuba municipality: Chalóns, Paradas and Charco Mono. Phytoplankton density ranged between 5 and 25 x 10^4 cells mL^{-1} in the majority of the water samples but five cyanobacterial blooms were detected during this period with a heavy blue-green or brown – reddish colour in two reservoirs.

The 73 species of phytoplankton identified include 9 potentially toxic cyanobacteria species: *Microcystis viridis, Oscillatoria chalybea, Oscillatoria limosa, Oscillatoria tenuissima, Anabaena torulosa, Planktothrix* sp*., Lyngbya* sp*., Synechococcus* sp. and *Gomphosphaeria* sp*.* These represent 38% of the total cyanobacterial numbers, with *Microcystis* spp. being the most frequent, followed *Aphanothece minutissima* and *Oscillatoria chalybea* (Gómez et al., 2010).

The reservoirs more vulnerable to algal blooms occurrence was Chalóns and Charco Mono, with high species diversity (56 and 34 species, respectively), the higher number of toxic cyanobacteria species (8 and 9, respectively) and bloom reports. *Microcystis, Planktothrix, Lyngbya, Oscillatoria* and *Anabaena* were the main dominant bloom-forming genera in these reservoirs. They co- dominated these water bodies. Interestingly, *Microcystis* usually dominated in all waters. It was detected in 93% of 480 analyzed samples, sometimes as part of a bloom in combination with diatoms and chlorophyta. Some of the species identified in the reservoirs could contain anatoxins (neurotoxins) and microcystins (hepatotoxins).

While the first study faced limitations in cyanobacteria toxicity determination and furthermore, the accessibility to Charco Mono was limited due to the lack of permits for frequent analysis, during 2011, a new monitoring programme was developed focused in two reservoirs: Parada and Chalóns. It includes more sampling points for reservoir data, as well as the protein phosphatase inhibition assay for toxicity. It allowed identifying other species of cyanobacteria; *Oscillatoria limosa* and *Microcystis aerugionosa* were isolated and monoclonally grown as cultures in the laboratory. This monitoring programme is still active. Samplings are taken in March, May, September and November. The goal is to identify the potential toxic cyanobacteria and to evaluate the potential risk of cyanotoxins with the protein phosphatase inhibition assay (PPI), considering that it is easy to perform and it is sufficiently sensitive to detect even low concentrations, e.g. at the onset of a bloom. Furthermore, PPI has potential for routine use in the screening and monitoring of microcystins from recreational waters and drinking water supplies (Rapala et al., 2002). In order to reduce the experimental cost, PPI was applied only in those samples with a high number of cyanobacteria cell counts and/ or high phycocyanin: chlorophyll ratio (which indicates cyanobacerial dominance); focusing on November when in Cuba the risk of cyanotoxin occurrence is greatest.

Both reservoirs showed positive responses in the assay. The average inhibition percentages in the assay were higher in Paradas reservoir (23.33 ± 3.10%) as compared to Chalóns (17.62 ±6.67%).

In both reservoirs 95 phytoplankton morphospecies were identified in 2011, which belong to 62 genera; 19 of them were Cyanobacteria (20%). They belong to 14 genera, and 12 are potentially toxic (Annadotter, 2006; Ballot et al., 2010; Ballot et al., 2005; Codd, 1999; Codd et al., 2004; Codd et al., 1997), which represent 40% of the total cyanobacteria species identified as potentially toxic. The number of morphospecies in both reservoirs was quite similar in 2011. Comparing the results in both periods, the total amount of cyanobacteria species was 22 for Chalóns and 18 for Paradas; but Chalóns has a higher number (and thus diversity) of toxic species (Table I).

Table I: Species of cyanobacteria in three reservoirs in Santiago de Cuba municipality: Chalóns, Paradas and Charco Mono (2010[a]-2011). * indicates potentially toxic species.

Cyanobacteria	Chalóns 2008-2010	Chalóns 2011	Paradas 2008-2010	Paradas 2011	Charco Mono 2008-2010
Total Number of species	**13**	**16**	**6**	**16**	**16**
1. *Anabaena* sp.	X				X
2. *Anabaena torulosa**					X
3. *Anacystis* sp.*	X	X		X	X
4. *Aphanocapsa delicatissima*					X
5. *Aphanothece minutissima*	X	X	X	X	X
6. *Aphanothece clathrata*		X		X	
7. *Chroococus dispersus*			X		
8. *Chroococus limneticus*	X	X	X	X	X
9. *Cyanotetras crucigenielloides*		X			
10. *Eucapsis* sp.		X		X	
11. *Gloeocystis bacillus*				X	
12. *Gloeocystis vesiculosa*		X		X	
13. *Gloeothece sp.*		X		X	
14. *Gloeocapsa* sp.	X		X		X
15. *Gomphosphaeria* sp.*	X	X		X	
16. *Lyngbya* sp.*					X
17. *Lyngbya majuscula**		X			
18. *Microcystis aeruginosa*		X	X	X	X
19. *Microcystis viridis**	X	X		X	X
20. *Oscillatoria chalybea**	X	X	X	X	X
21. *Oscillatoria limosa**	X	X		X	X
22. *Oscillatoria princeps*				X	
23. *Oscillatoria tenuissima**	X				X
24. *Planktothrix* sp.*	X				X
25. *Pseudoanabaena limnetica*	X				
26. *Radiocystis geminata*					
27. *Raphidiopsis* sp.					
28. *Spirulina* sp.		X		X	X
29. *Synechocystis aquatilis**		X		X	
30. *Synechococcus* sp.*	X				X
Number of toxic species (*)	**8**	**7**	**1**	**6**	**9**

Some research was carried out in a riparian system (San Juan River), not only on phytoplanktonic species of cyanobacteria, but also benthic mat-forming ones. The latter proved widespread throughout the whole ecosystem and are found in a wide range of water-quality conditions. The common mat-forming benthic cyanobacteria genus are *Phormidium* and *Oscillatoria*. During stable flow conditions *Phormidium* can proliferate, at times forming expansive black-brown mats over the river's substrate (see Table II for taxa of cyanobacteria identified in San Juan River).

Concerning estuarine ecosystem, research results in the Santiago de Cuba bay showed a total of 18 morphospecies of cyanobacteria, some of them associated with mats or biofilms with a homogeneous spatial distribution; potentially toxic species represent around the 76.4% of the total number (Table III).

Table II: Benthic and planktonic species of cyanobacteria in San Juan River, Santiago de Cuba (2008-2010).

Anabaena flos-aquae
Anabaena lemmermannii var. Minor
Anabaena maxima
Anabaena torulosa
Anacystis sp.
Aphanothece minutissima
Arthrospira fusiformis
Chroococcus dispersus
Chroococcus limneticus
Cylindropermopsis curvispora
Cylindropermopsis sp.
Gloeocapsa sp.
Gloeocystis sp.
Lyngbya mayuscula
Lyngbya sp.
Merismopedia glauca
Microcystis aeruginosa
Microcystis botrys

Microcystis flos-aquae
Microcystis viridis
Microcystis wesenbergii
Oscillatoria chalybea
Oscillatoria limosa
Oscillatoria moniliforme
Oscillatoria princeps
Oscillatoria rubescens
Oscillatoria sp.
Phormidium sp.
Planktolyngbya limnetica
Planktolyngbya sp.
Planktothrix sp.
Pseudoanabaena moniliforme
Romeria simplex
Spirulina sp.
Trichodesmium erythraeum
Tychonema bornetii

Table III: Benthic and planktonic species of cyanobacteria in Santiago de Cuba Bay (2009-2011)

Anabaena torulosa
Anabaena lenmermani
Anabaena moniliformis
Aphanizomenum sp.
Aphanothece clarthrata
Aphanothece minutissima
Chrococcus limneticus
Chroococcus sp.
Lyngbya majuscula
Lyngbya sp.
Microcystis spp.
Oscillatoria chalybea
Oscillatoria limosa
Oscillatoria princeps
Oscillatoria sp.
Phormidium spp.
Planthotrix sp.
Pseudoanabaena moniliforme
Radiocystis sp.

Spirulina subsalsa
Synechocystis sp.

Cyanotoxin risk perception in Santiago de Cuba

The perception of the risk is an important factor as decision basis for the implementation of management strategies. Risk perception in particular depends on who are the ones affected, the probability of damage, main features of the risk, the history of the phenomenon which determines the risk itself, and its intensity duration and extent. In Cuba, specifically in Santiago de Cuba, there are no socio-cultural elements that favour the perception of risks due to cyanobacteria. With the objective to evaluate the existing knowledge in key sectors related to cyanobacteria health impact or their risk management, surveys were applied in Santiago de Cuba to people in public health care, public services and academia: 284 people were interviewed, 18% belong to the academic sector, 17% to services and 65% to the public health sector. The selection of those interviewed was carried out at random in different institutions. 38% of those interviewed were men and 62% were women; the most frequent age group was 31-40 years (31±0.37%). People from different municipalities were included in this study.

As result, 49% of the people interviewed affirmed to know cyanobacteria and 51% identified that they can be harmful for the human health, while 45% specifically knew them to have toxic potential. Academics demonstrated a better knowledge about cyanobacteria and their implications (93%) and toxic potential (80%); in contrast, the other sectors showed a limited awareness of the topic. Sectors of public services have a lower knowledge about implications (50%) and toxic potential (57%), and public health care sector showed 51 and 42% awareness, respectively.

About the perception of some indicators of exposure to cyanobacterial blooms, 45% of the people interviewed affirmed to have been exposed to waters with green, yellow, white or brown colours coming from rivers (18%), pools (18%), dams (13%), coastal zones (9%) and lagoons (4%). Some people explained having been exposed to drinking waters that show discolouration in water containers like tanks (13%) or others storage reservoirs (1%); 35% do not associate any symptom after being exposed to discoloured waters or scum; for the rest of individuals, the most frequent symptom was pruritus or itch (25%); other symptoms associated with the exposition to coloured water were diarrhoeas, headache (5%), vomits with abdominal pain (3%), fever (4%) or paresthesia (1%).

When we asked about the potential services that could be affected by cyanotoxins, 37% affirmed that the presence of these microorganisms can be risky for hospital services like haemodialysis (6%), dermatology or gastroenterology (4%); paediatric services, rehabilitation, intensive care units (3%) and nephrology (1%). 30 % assert that it can be risky for other services including gastronomy (12%), educational institutions (6%), and other public services (4%); the rest of the individuals do not associate the risk with any service.

Screening, monitoring and management

So far, data are available only for the south-east of Cuba. Further, they do not include cyanotoxin identification and concentrations, and lastly, the need to cover freshwater, brackish and marine waters and both climatic periods (drought and rain).

In the south-eastern region of Cuba, bathing and drinking water studies indicate a potential for cyanobacteria proliferation, depending on seasonal conditions and the trophic status of the reservoir. In Santiago de Cuba, drinking water comes mainly from surface water supplies, and these (rivers, ponds, even pools) are used extensively for a range of recreational activities, too. Since 2009 a decision tree (chart) was set up at local level in the context of a research initiative supported by academic activities; it is not yet widely used by local managers of the water bodies. It is now a proposal for local implementation, conciliated with local actors in 2012 but still need to be approved. It is only implemented in Chalóns and Paradas as a pilot experience.

The procedure it proposes is to sample the chosen pilot reservoirs in March, May, September and November, but if there was any bloom, an additional sample is taken. In case of persistently high cyanobacterial concentrations, a tighter monitoring system is implemented, based on visual and microscopic observations. Three levels of management measures are identified according to levels of planktonic or benthic cyanobacteria. The procedure also includes establishing an adequate strategy of communication or public information.

Level 1: the total number of phytoplankton cells is between 20 000 and 100 000 cells mL^{-1} with an important contribution of cyanobacteria or around 1 500 cell mL^{-1} of cyanobacteria (even non toxic): recreational activities are still allowed and the public is informed by posters on site. Visual inspections are intensified. Sampling for counting and species identification should be performed. Drinking water could contain amounts of cyanobacteria that could affect water quality and the water suppliers are alerted.

This level is based on the total number of phytoplankton cells, not only cyanobacteria, for the following reasons: massive phytoplankton development causing high turbidity is often the basis for a shift in species composition towards cyanobacteria and thus favours their blooms. Furthermore, massive phytoplankton development affects water quality for potable and recreational use, and it is detrimental to the environmental quality of a water-body ('ecosystem health'). The increase of phytoplankton cell density can be the first ecosystem symptom to be considered in order to implement early actions against eutrophication in order to prevent the development of a situation conducive to cyanobacterial blooms.

Level 2: if the total number of phytoplankton cells is higher than 100 000 cells mL^{-1}, with a majority of cyanobacteria or when toxic species of cyanobacteria are present: bathing and recreational activities are restricted. The use of water for agricultural purposes (irrigation of leafy vegetables) is restricted too. In either case, the public is informed. Leafy vegetables irrigated with such water would be analysed for cyanotoxin contamination. Drinking water could contain amounts of cyanobacteria that could affect water quality or human health and the water suppliers are alerted with a recommendation to change the depth or site of the water inlet.

Level 3: Cyanobacterial scum appears in bathing areas or drinking water reservoirs or any bloom of toxic specie is confirmed: water activities are banned. The recommendations of level 2 are important but it is necessary to restrict the use or reservoir and implement and epidemiological surveillance plan. The stakeholders' integrated action is relevant at this level. Drinking water could contain amounts of cyanobacteria that could affect human health. The water suppliers and community are alerted with a recommendation to stop the water supply and alert to drinking water treatment plant.

These three levels support a decision chart framework proposed for planktonic and benthic cyanobacterial risk management for recreational water-body use, but considering the lack of legal rules to limit cyanotoxins in drinking water; it is also useful to apply this to reservoirs for drinking water supply. The decision charts are proposed for local implementation as a local initiative. They consider three stages depending on the alert level: surveillance, alert and action in red (Table IV-V). Each one also denotes certain action to be carried out by the research team alongside local water managers in the validation stage of the proposal.

Table IV: Decision chart for planktonic cyanobacteria to drinking water reservoirs

ALERT LEVEL	ACTIONS
Surveillance *Condition A:* Cell concentration of total phytoplankton is between 20 000 and 100 000 cells mL^{-1} and there are some cyanobacteria, not reported as potentially toxic *Condition B:* Cell concentration of total cyanobacteria is in the range of 1 500 cells mL^{-1} (i.e. <1 500 or only slightly above 1 500 cells mL^{-1})	• Undertake monthly visual inspection of water bodies. • Sample at least four months a year.
Alert *Condition A:* the number of cells is higher than 100 000 cells mL^{-1} with more than 50% of the cells being cyanobacteria *Condition B:* At least one species of cyanobacteria is reported as potentially toxic	• Increase sampling frequency (at least weekly). • Implement daily visual supervision. • Notify to the public health unit and local managers. • Report to local government. • Inspect and sample more sites. • Set a warn sign in the reservoir indicating the potential hazard.
Action in red *Condition A:* Any report of toxic effect documented (humans or animals) *Condition B:* Cyanobacterial heavy scum consistently present. *Condition C:* Confirmed bloom persistence and negative effects.	• Increase sampling frequency (at least weekly). • Implement daily visual supervision. • Notify to the public health unit and local managers about the potential risk to health. • Increase actions for public communication. • Set a warning sign at the reservoir indicating the potential hazard and restrictions.

Table V: Decision chart for benthic cyanobacteria to drinking water reservoirs

ALERT LEVEL	ACTIONS
Surveillance Condition A: Up to 40% coverage of cyanobacteria attached to substrate.	• Undertake monthly visual inspection of water bodies where mat proliferation occurs. • Sample at least four months a year.
Alert Condition A: 20% coverage of potentially toxigenic cyanobacteria attached to substrate.	• Increase sampling to weekly. • Implement daily visual supervision. • Notify to the public health unit and local managers about the potential risk to health. • Increase the number of surveillance sites. • Provide adequate public information by means of set warning signs in the reservoirs indicating the potential hazard.
Action in red *Condition A:* More than 50% coverage of potentially toxigenic cyanobacteria attached to substrate. *Condition B:* Up to 50% where potentially toxigenic cyanobacteria are visibly detaching from the substrate, accumulating as scum.	• Notify the public health unit. • Notify the government and local authorities. • Provide adequate public information by means of set warning signs at the reservoir indicating the potential hazard and restrictions.

A general protocol proposed for the integrated cyanotoxin risk management defines three stages: i) visual supervision, ii) analysis, iii) verification and official report (Gomez et al., 2011); however, the last stage is critical considering the lack of resources to implement it; furthermore regulations are necessary to ensure implementation (Figure 2).

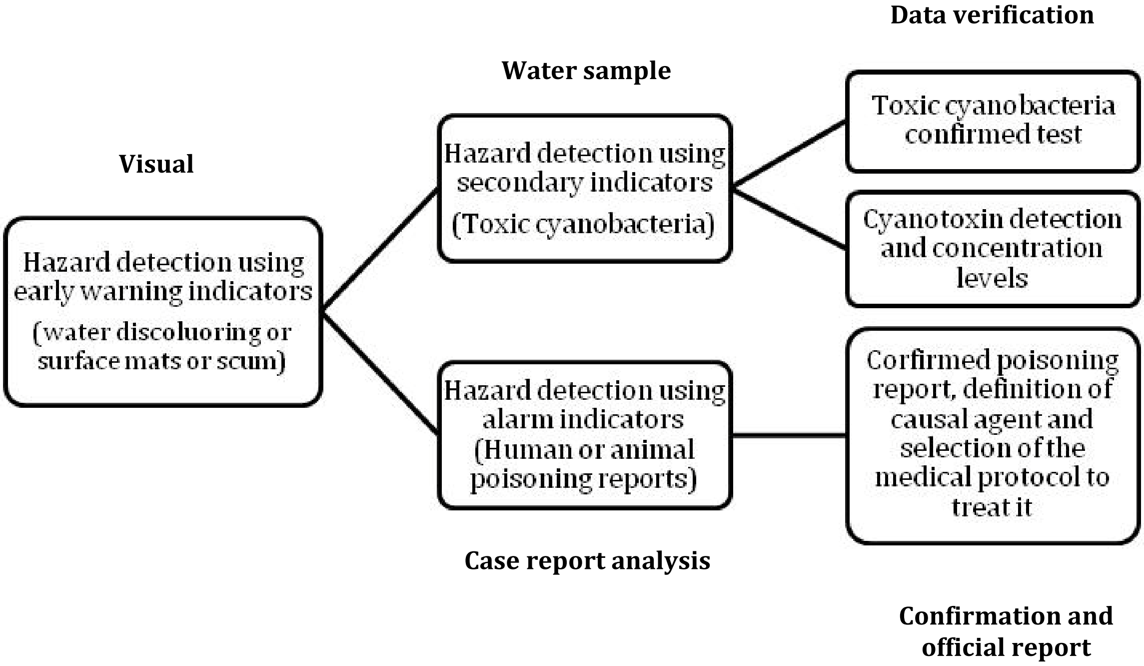


Figure 2. General protocol for integrated cyanotoxin risk management

Conclusions

Our experience in monitoring is a valuable information basis, but its regulatory value towards implementing regulations is still a limited endeavour to recognize the cyanobacteria blooms as a potential risk in Cuba. No human poisoning has been attributed to cyanotoxins in Cuba to date. However, in consequence of the cyanobacteria identified it is important to assess the risks associated with cyanobacteria and cyanotoxins in Cuba and to implement cyanotoxin management regulations for waters reservoirs, including drinking water and recreational ones. Local and isolated initiatives are not sustainable. It is necessary to develop comprehensive and functional management plans to ensure these controls work, supported by an adequate emergency and contingency strategy at the national level. This includes a clear public communication strategy and effective documentation; all of which could be summarised in an integrated risk management system. Cyanobacteria-cyanotoxin risk management is a key topic to include in the integrated water management focusing on the prevention of cyanobacteria blooms, rather than their impact mitigation. This involves proactive activities, in particular a strategy of pollution management, waste treatment, environmentally wise practices, clean technologies, stakeholder involvement.

References

Annadotter, H., 2006. Mass developing cyanobacteria in temperate and tropical freshwater bodies. Ecology, toxins and effects on human health Institute of Biology. University of Copenhagen, Copenhagen, pp. 176.

Ballot, A., Fastner, J., Wiedner, C., 2010. Paralytic shellfish poisoning toxin-producing cyanobacterium Aphanizomenon gracile in northeast Germany. *Applied and environmental microbiology*, 76, 1173-80.

Ballot, A., Krienitz, L., Kotut, K., Wiegand, C., Pflugmacher, S., 2005. Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful Algae*, 139-150.

Biagini, M., 1980. Valutazioni dell´inquinamento delle acque correnti per mezzo del fitoplancton. *Biologi Italiani*, X, 20.

Codd, G.A., 1995. Cyanobacterial toxins: occurrence, properties and biological significance. *Water Science Research*, 32, 149-156.

Codd, G.A., 1999. Cyanobacterial toxin: their occurence in aquatic environments and significance to health. *Bulletin del'Institut Océanographique du Monaco*, 483-500.

Codd, G.A., Morrison, R.F., Metcalf, J.S., 2004. Cyanobacterial toxins: risk management for health protection. *Toxicology & Applied Pharmacology*, 203, 264-272.

Codd, G.A., Ward, C.J., Bells, S.G., 1997. Cyanobacterial toxins: occurrence, modes of action, health effects and exposure routes. *Archives of Toxicology* 19 399-410.

Chorus, I., 2005. Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries Federal Environmental Agency (Umweltbundesamt)

Gómez, L.M., Álamo, B., Rodríguez Tito, J.C., 2010. Riesgo de contaminación con cianobacterias en tres embalses de agua de Santiago de Cuba. *Medisan*, 14.

Gomez, L.L., Álvarez, H., I, Rodríguez, T.J.C., Álamo, D.B., 2011. Cianobacterias: un riesgo emergente en embalses de agua de Santiago de Cuba. in: E. Universitaria (Ed.). Universidad de Oriente, Santiago de Cuba, pp. 76.

IF-BRASCUB-200405-16, 2004. Vigilancia em Salud Ambiental Informe Final del Proyecto CTP OPS, Brasil/ Cuba.

Rapala, J., Erkomaa, K., Kukkonen, J., Sivonen, K., Lahti, K., 2002. Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography–UV detection and enzyme-linked immunosorbent assay Comparison of methods. *Analytica Chimica Acta*, 213-231.

WHO, 2003. Cyanobacterial toxins: microcystin-LR in drinking-water. 2nd ed Guidelines for drinking-water quality. Health criteria and other supporting information. World Health Organization, Geneva.

DENMARK: Occurence, Monitoring, and Management of Cyanobacteria and their Toxins in Danish Water Bodies

Kirsten S. Christoffersen and Trine P. Warming

Freshwaterbiological Laboratory, University of Copenhagen, DK-3400 Hillerød, Denmark; kchristoffersen@bio.ku.dk

Introduction

Denmark is a cultured landscape comprised mostly of farmland (>60% of total area), forests (ca. 12%) and urban areas (ca. 10%). Approximately 2% of the area is occupied by lakes, ponds and streams, which drain off mostly farmland. Eutrophication in both inland waters and coastal areas has consequently been a problem for many years. The increased amount of wastewater treatment implemented via the Action Plan for the Aquatic Environment 1 and 2 in the 80'ies and 90'ies have reduced the load of inorganic nutrients to the aquatic environment dramatically, but the load of especially inorganic nitrogen from agricultural activities remains high. Additionally, many lakes and coastal areas still suffer the effects of internal phosphorus load, and the effects of eutrophication are not yet under control. Many inland and coastal water bodies are subject to annually recurring cyanobacterial blooms, especially during calm weather periods in the late summer (July-August), which in some cases cause anoxia and/or the occurrence of cyanobacterial toxins. Most aquatic sites are open to the public for swimming, boating, sports fishing and similar recreational activities. A small number of lakes are used for drinking water uptake, although the vast majority (99%) of the drinking water consumed in Denmark is groundwater. The main routes of public exposure to cyanobacterial toxins are therefore either via recreational activities or consumption of aquatic animals (mainly mussels and oysters) which have accumulated the toxins.

Occurrence

The most frequently occurring cyanobacterial genera in Danish lakes and coastal areas are *Microcystis* (6-8 potentially toxic species), *Anabaena* (at least 3 potentially toxic species), *Aphanizomenon* (2 potentially toxic species), *Planktothrix* (2 potentially toxic species), and *Nodularia* (1 potentially toxic species). However, while surveys on the occurrence of cyanobacteria are carried out on a regular basis (see below), the cyanobacterial toxin concentrations are not routinely monitored.

Since cyanobacterial blooms occur in both lakes and coastal waters in Denmark almost every year and many of these blooms contain toxic strains, it is obvious that a monitoring and management strategy is needed. However, cases of illness among humans are rare, and there have as yet been no deaths reported due to contact with water containing cyanobacterial toxins. Deaths among pets and livestock are annually recurring however, mainly caused by the animals coming into contact with toxic surface scums.

Monitoring of lakes and coastal areas

A national monitoring and assessment programme for the aquatic and terrestrial environment has been in place in Denmark since 1998, and serves to describe the development in our country's environmental conditions. The current programme (named NOVANA 2011-2015) is run by the Danish Nature Agency, and includes surveys on the occurrence of phytoplankton, in addition to numerous other biological, chemical and physical parameters.

The lake monitoring programme under NOVANA 2011-2015 serves to describe environmental conditions in Danish lakes, and includes (among many other things) analyses of nutrient concentrations as well as phytoplankton composition and biomass. The number of monitored sites has varied through the years, and the current monitoring program for lakes is carried out at two different levels, 18 representative lakes are monitored at a high level of detail each year, while ca. 150 lakes are monitored less intensively over the 4-year period. There is no special emphasis on the monitoring of cyanobacteria in the programme, and toxin concentrations are not routinely measured.

Coastal areas are monitored under the NOVANA programme too. Since none of the Danish coastal areas have achieved the classification of “good ecological status” as defined by the European Water Framework Directive, all sampling stations are monitored intensively with a focus on eutrophication and anoxia. The phytoplankton composition and biomass are monitored routinely at 20 sampling stations (9 in fiords, 11 in open water). Again, there is no speciific focus on cyanobacteria or their toxins.

The data from these surveillance programmes is used for management of the environment by the local municipalities, and are also compiled into a national archive of environmental data (the Danish Nature and Environment Portal http://www.miljoeportal.dk/). These data are summarized and presented to the public in reports, the latest contains data from 2010 and is available online here: http://www.naturstyrelsen.dk/Udgivelser/Aarstal/2011/NOVANAsammenfatning2010.htm.

Management

In 2007, the municipal structures of Denmark underwent a major reform. The 13 counties were abolished, and their previous tasks were outsourced and shared between various governmental agencies and the 98 new, larger municipalities (previously 271). For many tasks (e.g. the management of lakes, streams, and bathing water), the new municipal structure means that the Danish Nature Agency has the highest, i.e. national responsibility, but that the day-to-day management is carried out by the municipalities.

Drinking water

Since only a very small part (1%) of the drinking water consumed in Denmark originates from surface water, and thus the risk of coming into contact with cyanobacterial toxins via drinking water is limited to those few settings. Drinking water is supplied by local waterworks, which are monitored by the local municipalities. In certain areas (mainly in the eastern part of the country), surface water is used occasionally to supplement the drinking water supply in dry seasons. The lakes that provide this water are normally routinely monitored for cyanobacterial toxins by the local municipalities, but there are no formal requirements for this by the higher authorities (the Danish Nature Agency). The authors know of no occurrences of illness of either humans or animals caused by ingestion of cyanobacterial toxins from drinking water in Denmark.

Bathing water

With a few exceptions, all coastlines, lakes and streams in Denmark are open for bathing. Normally, the bathing water quality is high throughout the bathing season (June 1st – September 1st), but certain events, such as heavy rainfalls resulting in spillage from wastewater treatment plants, may temporarily compromise water quality. Potentially toxic cyanobacterial blooms also occur locally almost every year, especially during calm weather periods in the late summer.

The national authority for bathing water is The Danish Nature Agency. Since the municipal reform in 2007, the municipalities have taken over the responsibility for local bathing water surveillance and management, which formerly lay with the counties. The municipalities are responsible for monitoring the bathing water quality (i.e. the water is analyzed routinely for presence of pathogenic bacteria), but there are no formal requirements for any registration of cyanobacterial blooms other than visual assessment. There are no official guidelines for the tolerable amount of (potentially) toxic algae in bathing water, but if an algal bloom is suspected to be toxic, the local authorities will post signs at the waterfront warning the public to stay out of the water. Warnings may also be issued in local newspapers and on local webpages. For instance, a prognosis for the bathing water quality in Northern Sealand – a water forecast – is available from this website: http://oresund.badevand.dk/ (Figure 1).

In addition, the Danish Nature Agency hosts a website (http://www.naturstyrelsen.dk/Vandet/Badevand/) which informs the public of the general conditions of the bathing water, and which also contains a rule of thumb for bathing in water where toxic algal blooms may occur. The rule is low-tech in the extreme – simply wade into the water to knee-depth. If you cannot see your feet, you should not bathe in the water (Figure 2). The website also warns the public not to get themselves or their children or pets into contact with scums on water surfaces, as these may contain algal toxins.

The public is also guided by the international "Blue Flag" eco-label, which signifies among other things that the bathing site has a high bathing water quality. The certification is managed locally by the n on-governmental organization the Danish Outdoor Council (http://www.blaaflag.dk/), and most major Danish bathing sites are certified to fly the Blue Flag. When, for whichever reason, a bathing site is deemed to pose a health risk, the blue flag is taken down until water conditions return to their normal state.

Mussels

There are many sites in Denmark where blue mussels (*Mytilus edulis*), common cockle (*Cardium edule*), surf clam (*Spisula* spp.), scallop (*Pecitinidae* spp.), and oysters (*Ostrea edulis*) are produced and harvested commercially for consumption. The harvested mussels as well as the harvesting sites have been monitored weekly, specifically for toxic algae and cyanobacteria (including species of *Nodularia* and *Anabaena*) and algal toxins (including cyanotoxins) since 1990. If concentrations are found (determined by HPLC or LC-MS analysis and mouse tests) that exceed the accepted levels, the harvesting site is closed off, and the harvested mussels are destroyed. A closed harvesting site I s re-opened when two samples taken at least 48 hours apart show that toxin levels have decreased to acceptable levels again. The surveillance of the mussel production and harvesting sites is run by the Danish Veterinary and Food Administration.

Figure 1: Example of a water forecast for the coastal areas around Copenhagen and Northern Zealand

Figure 2: Examples of the rule-of-thumb regarding algal blooms in bathing water

The picture on the left shows conditions where contact with the water is inadvisable. The picture on the right shows conditions where no restrictions apply

Private mussel gatherers are advised to avoid gathering mussels when conditions favor blooms of toxic algae, and to keep themselves informed of local water conditions by the relevant authorities (for instance the abovementioned water forecasts etc.).

References

Denmark's National Environmental Research Institute, Natur og Miljø 2009: http://naturogmiljoe.dmu.dk/ (In Danish, English summary).

Christoffersen, K. (1996). Ecological implications of cyanobacterial toxins in aquatic ecosystems. Phycologia 36(6 suppl.): 42-50.

Christoffersn. K. (2000). Are blue-greens a problem in Danish lakes?. Vand og Jord 3:104-109 (in Danish)

Gyldendals Danish Encyclopedia http://www.denstoredanske.dk/

The Danish Veterinary and Food Administration http://www.foedevarestyrelsen.dk/Foedevarer/Naturlige_giftstoffer/Algegift/Sider/forside.aspx

The Danish Veterinary and Food Administration, 2007. Shellfish monitoring and management 2007. Food Safety. Available online at: http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/Toksiske%20Alger%202007%20rapport%2014%20oktober%202010.pdf

FINLAND: Guidelines for monitoring of cyanobacteria and their toxins

Jarkko Rapala[1], Jaana Kilponen[2], Marko Järvinen[3] and Kirsti Lahti[4]

[1] Ministry of Social Affairs and Health, Department for Promotion of Welfare and Health, Environmental Health, P.O. Box 33, 00023 Helsinki, FINLAND; jarkko.rapala@stm.fi
[2] National Supervisory Authority for Welfare and Health, P.O. Box 210, 00531Helsinki, FINLAND; jaana.kilponen@valvira.fi
[3] Finnish Environment Institute, Freshwater Centre, Monitoring and Assessment Unit, Survontie 9, 40500 Jyväskylä, FINLAND; marko.jarvinen@ymparisto.fi
[4] Water Protection Association of the River Vantaa and Helsinki Region, Asemapäällikönkatu 12 B, 00520 Helsinki, FINLAND; kirsti.lahti@vesiensuojelu.fi

Systematic monitoring since 1998

Data on nuisance 'algae' have been collected in Finland since 1967. The respective species have traditionally been determined microscopically, and the results recorded in the database of harmful algae provided by the national environmental administration.

In summer 1998 a nation-wide observation system was launched to monitor cyanobacteria systematically throughout the country. The aim was to provide an up-to-date overview of the cyanobacterial situation and information about their spatial and temporal variation during the summer months. Since then, the monitoring programme has been a joint-venture of local and regional authorities, the Finnish Environment Institute (SYKE) and the Finnish Institute of Marine Research (since 2009 Marine Research Centre of SYKE).

Observation sites and methods

Observation sites (n = approximately 300) have been selected by regional environment authorities (Centres for Economic Development, Transport and the Environment) to represent various water types: eutrophic waters with frequent cyanobacterial mass occurrences, mesotrophic waters with less frequent water blooms and oligotrophic waters with no or few observations of nuisance algae or cyanobacteria, and coastal waters of the Baltic Sea. Several observation sites are situated in the vicinity of public beaches or cities.

During summer cyanobacterial abundance is monitored weekly at the pre-determined sites. Municipal health protection or environmental authorities or volunteer citizens visit and estimate the cyanobacterial abundance by visually examining the water area from the shore. In order to harmonize the estimates the observers have received prior pre-training by SYKE (Table 1).

Collection and reporting of the weekly data

The regional authorities collect the observation data and update the information on weekly bloom situation at Lakewiki web service (see below). In addition, in the open Baltic Sea areas cyanobacteria are monitored by using unattended recording, automatic sampling by commercial ships, visual observations submitted by the Finnish Border Guard from the air, and also by using satellite images. Based on this recent information SYKE prepares the weekly report. The report consists of four different parts:

Table 1: The observations are classified into four classes (0-3), and the term 'algae' is used to encompass both algae and cyanobacteria

Classes	The observation
0) Not detected	No algae on the water surface or on the shore line. The transparency of water (Secchi depth) is not affected by algae.
1) Detected	Greenish flakes detected in the water or when taken into a transparent container, or narrow stripes on the shore. The Secchi depth is reduced by algae.
2) High amount	The water is clearly coloured by algae, small surface scums or cyanobacterial mass on the beach are detected.
3) Very high amount	Wide and heavy surface scums or thick aggregates of cyanobacteria are detected on the shore.

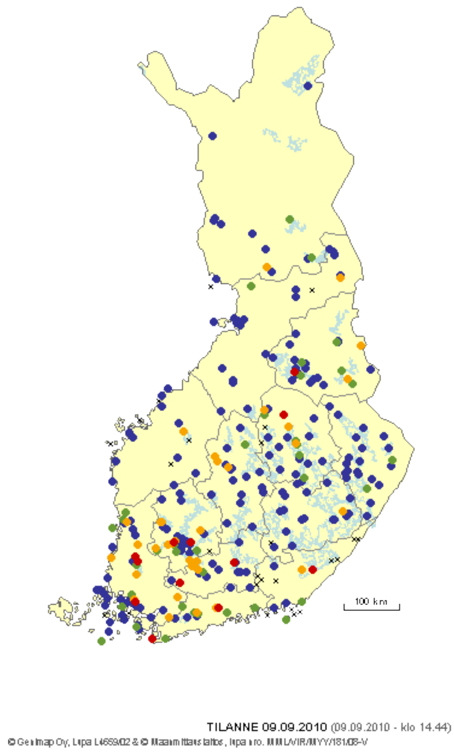
1. The summary which is a short one-page description of the weekly situation for lakes and the coastal and open Baltic Sea.
2. The map which shows by colour codes the situation at each observation site (Fig. 1).
3. The cyanobacterial "abundance barometer" which allows a comparison of the current situation to previous years (Fig. 2). The barometer is calculated as the balanced mean of the observation sites and cyanobacterial abundance.
4. Further descriptions of regional situations may be included in the report.

The report is published at the Internet pages of SYKE (http://www.environment.fi/SYKE), the Lakewiki web service (www.jarviwiki.fi; also in English) and in a press release. Data on the Baltic Sea areas are also published in more detail at the Baltic Sea Portal (www.itameriportaali.fi/en_GB/).

If the abundance of cyanobacteria is estimated as high or very high a water sample is taken and sent to SYKE or to the regional centres for further microscopical investigation. The species composition of the cyanobacterial mass occurrences is recorded in the national database of harmful algae.

LakeWiki

In 2011 SYKE launched LakeWiki ('JärviWiki'), a web service open to all (www.jarviwiki.fi). Lakewiki was created with the aim of sharing information on Finland's lakes, to raise awareness and promote the protection of our waters. At present, it contains basic information of all the lakes larger than one hectare in area in Finland (n = 55 821) from the authorities' registers. Citizens have been invited to update the information, especially with their observations and photographs on cyanobacterial occurrences, but also on water temperature, ice conditions and selected invasive species. Since 2011 the visual nuisance algae monitoring of SYKE has been carried out using LakeWiki where the bloom situation reports, bloom distribution maps and abundance barometers are presented on-line.



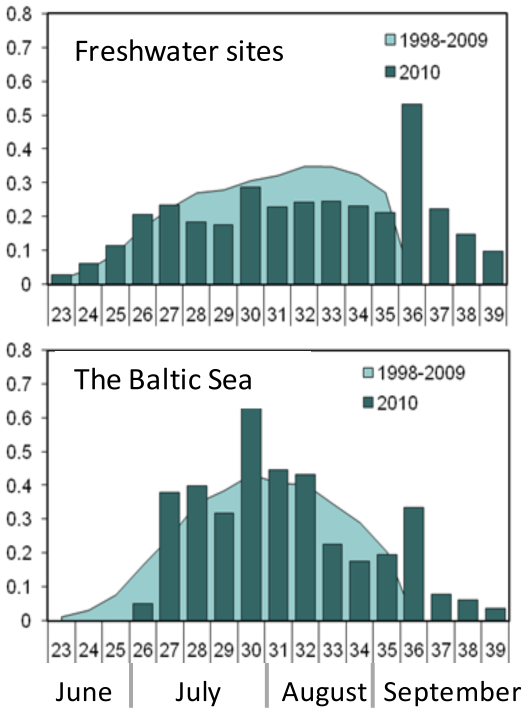


Fig. 1: Example of the weekly map of cyanobacterial abundance.
•detected
•abundant
•very abundant

Fig. 2: The cyanobacterial "abundance barometer" in 2010 in the freshwater sites and at the coastal Baltic Sea area in Finland.

Monitoring of toxins

Two large-scale studies on the occurrence of cyanobacterial toxins in water blooms in Finland have been conducted. The first was conducted in the middle on 1980's (Sivonen et al., 1990). In that study, toxins (microcystins, nodularin and anatoxin-a) were studied from 215 fresh and brackish water samples, and they were detectable in 44% of the samples.

Another study was conducted in 2003-2005 (Le pistö et al. 2005, Rapala 2007, Table 2). Particularly due to more developed analytical methods than in 1980's a higher proportion of toxic samples than previously was detected. Saxitoxin and cylindrospermopsin were analyzed and found for the first time (Rapala et al. 2005, Spoof et al. 2006), and the indirect acetylcholinesterase inhibition assay suggested that also anatoxin-a(S) may exist in Finland. The detected toxin concentrations were occasionally very high.

Table 2: Cyanobacterial toxins, their relative occurrence and the maximum concentrations detected in cyanobacterial mass occurrences in Finnish freshwaters and brackish waters in 2003−2005

Toxin	% of samples (n studied)	Maximum Concentration mg/L
Microcystins and nodularin	68 (589)	44
Anatoxin-a	12 (295)	0.86
Saxitoxin	9.9 (181)	10
Anatoxin-a(S)	1.6 (385)	detected
Cylindrospermopsin	0 (46)*	-

*One *Anabaena lapponica* strain producing cylindrospermopsin was isolated from a lake, but no analysis for cylindrospermopsin was conducted in that lake.

Bathing Waters

Cyanobacteria have been monitored in the bathing waters of Finland since 1985, first by the guidance document given by the National Board of Health and later by the Degree of the Ministry of Social Affairs and Health. Together with adoption of the Directive 2006/7/EC of the European Parliament and of the Council concerning the management of bathing water quality, which requests monitoring of cyanobacterial proliferation, the central authority National Supervisory Authority for Welfare and Health gave more detailed guidelines (Fig. 3). The monitoring is largely based on visual inspection on the site, as described above (Table 1).

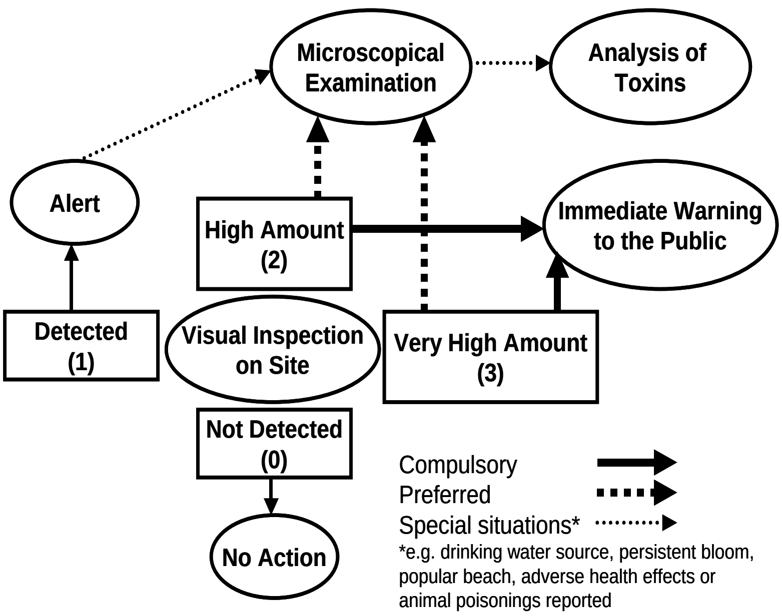


Fig. 3. Monitoring of cyanobacteria in bathing waters and in raw water supplies for drinking water.

The monitoring scheme is based on scientific research and risk assessment, and the cost of such monitoring is relatively low. In the monitoring of recreational waters the importance of immediate warning of water users of the risk caused by cyanobacteria should be the main target. This target can be achieved only by rapid and thus relatively simple means. Immediate warning is achieved by obligation of the municipal health authorities to place warning signs to the public beaches if visual inspection shows that cyanobacteria exist in high or very high number in the bathing water. Warning signs were recommended already in the guidance document for authorities in 1984. Later, in 1996, this obligation was put into the national legislation. After publication of the implementing decision by the European Commission establishing symbols for information to the public on bathing water classification and any bathing prohibition or advice against bathing (European Commission 2011), the same symbols (Fig. 4), supplemented by additional information on adverse health effects by cyanobacteria and their toxins, are used for warning of the bathers of the risk caused by cyanobacteria.

Fig. 4: Bathing prohibited -symbol in Finnish according the Commission implementing decision 2011/321/EU.

According to research results, more than half of cyanobacterial mass occurrences are toxic. Thus, the presence of cyanobacteria alone is a strong indication of potential health hazard. Analysis of specific toxins from bathing water samples is too time-consuming in order to meet the need of rapid assessment and action. If e.g. microcystins were analysed from the water samples, there might still be other hazardous compounds present in the water, because cyanobacteria produce a variety of different toxins and yet still unidentified potentially toxic compounds. Therefore, we recommend analysis of specific toxins only in special situations.

During the study in 2003-2005 (Table 2) also adverse health hazards linked with exposure of bathers (n=159) to cyanobacteria were studied. The data was gathered from the telephone calls of the 24-hour telephone service of the Poison Information Centre at Helsinki University Hospital. This service provides medical advice for persons in acute poisonings of any kind. Yearly, approximately 100 telephone calls are received concerning adverse health effects suspected to be caused by cyanobacteria, but during summers of high cyanobacterial occurrence there have been more than 350 contacts. Data for the health symptoms (Fig. 5) after exposure to cyanobacteria were collected by the telephone calls and structured interviews of the patients. Two interviews were made, one immediately when the exposed persons contacted and another after approximately two weeks after the exposure. Samples were collected from the exposure sites as soon as possible after the telephone call of the exposed persons (normally within one day), and the water was analyzed for all the known cyanobacterial toxins and bacterial lipopolysaccharide endotoxins. While these data indicated no correlation between the symptoms and the toxin concentrations they showed that gastrointestinal symptoms occurred among more than 50% of those who called the Poison Information Centre, and that fever, skin irritations and symptoms of head, ear, eyes, nose and throat were reported by more than 30% of the persons (Rapala 2007, Berg et al. 2011). Musculoskeletal and neurological symptoms that were reported by less than 10% of the persons were tentatively associated with the presence of the cyanobacterial genus *Anabaena lemmermannii* (Lepistö et al. 2005).

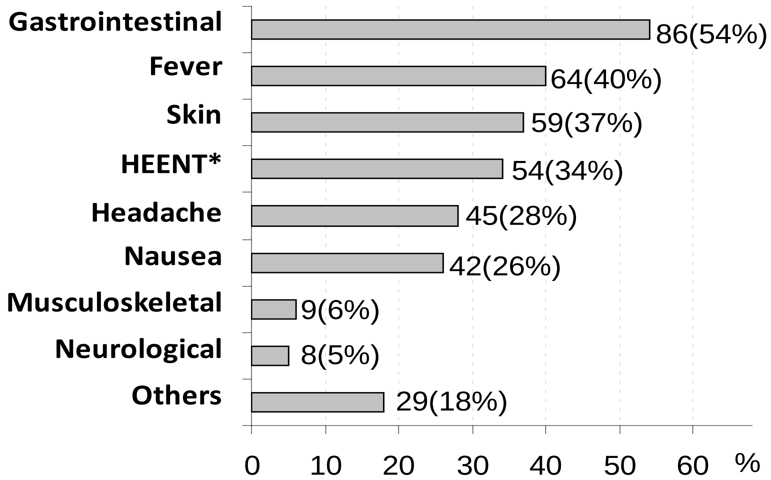


Fig. 5: Number of bathers (and percentage) that reported the specific symptoms after recreational exposure to cyanobacteria in the monitoring study in 2003–2005. The X-Axis shows the percentage of bathers that reported the specific symptoms in relation to the total number of bathers that reported symptoms. *HEENT: symptoms of head, ear, eyes, nose and throat.

Drinking water

The monitoring scheme presented for environmental samples and bathing waters is also suitable for risk management purposes in drinking water supplies. Scientific studies on the occurrence of cyanobacteria and their toxins have been conducted since 1990's from approximately 30 drinking water treatment plants that use surface water as their raw water (Lahti et al. 2001, Rapala et al. 2006). Toxins have been measured from the incoming raw water, after different treatment processes and from treated drinking water. The drinking water treatment plants included surface water plants as well as bank filtration and artificial groundwater plants. Although cyanobacteria mainly exist in great masses during summertime, high numbers of cyanobacteria (*Planktothrix agardhii*) have caused problems in at least one surface water plant (Fig. 6) and one artificial groundwater plant during the winter. High numbers of cyanobacteria in the raw water have for example clogged the filters of the drinking water treatment plants, and obliged them to enhance the water treatment processes and extend the monitoring period of the toxins year-round.

The guidelines given by the central authority National Supervisory Authority for Welfare and Health for monitoring cyanobacteria in incoming raw water and in distributed drinking water, the actions to be undertaken by the water treatment facility and by the authorities and the communication strategies are summarized in Table 3. The guidelines have been partly modified from those presented by WHO and by the Australian National Health and Medicinal Council, and they are also based on the wide research conducted at the Finnish drinking water treatment plants.

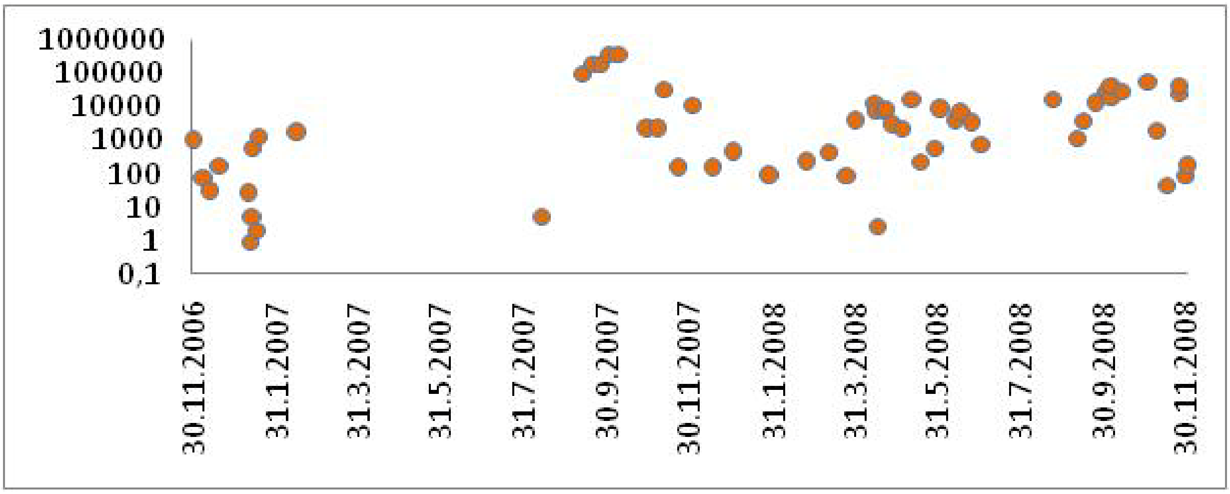


Fig. 6: *Planktothrix agardhii* cell numbers (cells/ml) of the incoming raw water of a drinking water treatment plant. Dotted lines show the reference points for actions (500, 5000 and 100 000 cells/ml, Table 3). Note that high numbers of cyanobacteria existed also during the winter. Microcystins were detectable occasionally in the treated water with a maximum concentration of 0.04 µg/L at the end of September in 2008.

According to the studies conducted at the drinking water treatment plants, the reference point where the microcystin concentration exceeds 1 µg/L in the incoming raw water is relatively common at a few drinking water treatment plants. However, in the few cases when microcystins have been detected in the treated drinking water, the highest concentrations have been well below the WHO provisional guideline value of 1 µg/L. When microcystin was detected in drinking water cyanobacterial cells were also present, and in these cases it is likely that the consumers complained about the taste and odour of the water. Situations with > 1 µg/L microcystins in the treated drinking water (i.e. concentrations higher than the reference point) and delivered to consumers are thus highly improbable. The extreme scenario of > 10 µg/L microcystins in the last row of Table 3 has never been encountered and is not expected to be; nonetheless a planned response is included in the strategy.

Telephone Services

During summer months of 1998-2009 there was a continuous (Monday–Friday, 8.00–16.00) telephone service of SYKE ("Citizens Algaline") from which the public could get information about cyanobacteria. On the average 350 telephone calls were received each summer, but concerning algal situation during summer (Monday-Friday, 13.00-15.00). Further, the 24-hour telephone service of the Poison Information Centre at Helsinki University Hospital remains available for those who are concerned that they might have been exposed to hazardous cyanotoxin levels.

Table 3: The points for action, actions to be undertaken and communication strategies to be adopted in the event that toxin-producing cyanobacteria (including *Microcystis* sp., *Anabaena* sp. and *Planktothrix*sp.) and microcystins are identified at facilities supplying water intended for human consumption.

Reference point	Actions	Communication strategy
Incoming water: >500 cells/ml or >0.1mg/L biomass	• Increased monitoring of incoming water	• Prepare to respond to enquiries
Incoming water: >5,000 cells/ml or >1 mg/L biomass	• Identify the genus of cyanobacteria in volved and assess levels in incoming water • If toxin-producing cyanobacteria are detected, the microcystin concentration in the incoming water is to be determined • Potential health risks are assessed by municipal health protection authority	• The facility should inform the municipal health protection authority of the presence of cyanobacteria in the incoming water and the measures taken to manage the situation • Stand by to inform the wider public
Incoming water: toxin-producing cells >100,000 cells/ml or >20 mg/L biomass or >1µg/L microcystin	• Identify cyanobacteria and toxin concentrations in drinking water • Potential health risks are assessed by municipal health protection authority • Ensure the effectiveness of water treatment processes, e.g. enhance coagulation/clarification or activated carbon filtration • Change water intake site, depth or source	• Following the investigations, the facility must publish information on water quality with regard to cyanobacteria and toxins
Drinking water: Toxin-producing cells or <1.0 µg/L microcystin	• Identify cyanobacteria and toxin concentrations in incoming and drinking water • Potential health risks are assessed by municipal health protection authority • Change water intake site or source • Enhance water treatment processes	• The facility must publish information on drinking water quality and toxin levels jointly with the municipal health protection authority
Drinking water: >1.0 µg/L microcystins	• Identify cyanobacteria and toxin concentrations in incoming and drinking water • If the concentration exceeds the limit as a one-off occurrence, the decision on whether the water can continue to be used for drinking and cooking purposes is to be taken on a case-by-case basis • In the event that the concentration remains elevated for a sustained period of time, the use of water for cooking or drinking purposes will be prohibited and the facility will organise the distribution of alternative water supplies to the affected population • Change water intake site, depth or source • Enhance water treatment processes	• The facility will communicate the restrictions on water usage and water quality with regard to cyanobacteria and toxins jointly with the municipal health protection authority • Information provided on the health effects of microcystins • Information to include details of alternative water distribution arrangements
Drinking water: >10.0 µg/L microcystins	• Identify cyanobacteria and toxin concentrations in incoming water and drinking water • Water not to be used for domestic purposes except for toilet flushing	• Communicate ban on water use • Communicate alternative water distribution arangements

Conclusions

Visual monitoring of cyanobacteria in water bodies allows a cost-effective, rapid and sufficiently reliable warning of water users for risk management purposes. In Finland, a cost-effective monitoring network has been developed. It has arisen as a consequence of public awareness and interest, actions taken by the authorities and targeted national research funding. The network produces information for health authorities for decision making, information on the ecological condition of the water bodies, and it also meets the needs of informing the public. Health authorities have been given guidelines since the late 1980's to monitor cyanobacteria in public beaches, drinking water sources and treated drinking water.

References

Berg, K. A., Lyra, C., Niemi, R. M., Heens B.. Hoppu, K., Erkomaa, K., Sivonen, K. and Rapala, J. 2011. Virulence genes of *Aeromonas* isolates, bacterial endotoxins and cyanobacterial toxins from recreational water samples associated with human health symptoms. Journal of Water and Health 9:670-679.

European Commission. 2011. Commission implementing decision (2011/321/EU) of 27 May 2011 establishing, pursuant to Directive 2006/7/EC of the European Parliament and of the Council, a symbol for information to the public on bathing water classification and any bathing prohibition or advice against bathing. Official Journal of the European Union L 143 pp. 38-40.

Lahti, K., J. Rapala, A.-L. Kivimäki, J. Kukkonen, M. Niemelä, and K. Sivonen. 2001. Occurrence of microcystins in raw water sources and treated drinking water of Finnish waterworks. Water Science and Technology 43:225-228.

Lepistö, L., Rapala, J., Lyra, C., Berg, K. A., Erkomaa, K. and Issakainen, J. 2005. Occurrence and toxicity of cyanobacterial blooms dominated by Anabaena lemmermannii P. RICHTER and Aphanizomenon spp. in boreal lakes in 2003. Algological Studies 117 (Cyanobacterial Research 6):315-328.

Rapala, J. 2007. Exposure to cyanobacteria – an epidemiological study. 7th International Conference on Toxic Cyanobacteria. Rio de Janeiro, Brazil, 5.-10.8.2007.

Rapala, J., Robertson, A., Negri, A. P., Berg, K. A., Tuomi, P., Lyra, C., Erkomaa, K., Lahti, K., Hoppu K. and Lepistö, L. 2005. First report of saxitoxin and possible associated human health effects in lakes of Finland. Environmental Toxicology 20:331-340.

Rapala, J., Niemelä, M., Berg, K. A., Lepistö, L. and Lahti, K. 2006. Removal of cyanobacteria, cyanotoxins, heterotrophic bacteria and endotoxins at an operating surface water treatment plant. Water Science and Technology 54:23-28

Sivonen, K., Niemelä, S. I., Niemi, R. M., Lepistö, L., Luoma, T. H. and Räsänen, L. A. 1990. Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal waters. Hydrobiologia 190:267-275.

Spoof, L., Berg, K. A., Rapala, J., Lahti, K., Lepistö, L., Metcalf, J. S., Codd, G. A. and Meriluoto, J. 2006. First observation of cylindrospermopsin in Anabaena lapponica isolated from the boreal environment (Finland). Environmental Toxicology 21:552-560.

FRANCE: Regulation, Risk Management, Risk Assessment and Research on Cyanobacteria and Cyanotoxins

GRISCYA, the French informal scientific interest group on cyanobacteria

Corresponding author: Nathalie Arnich

French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 94701 Maisons-Alfort, France; nathalie.arnich@anses.fr

The cyanotoxins formally identified in France are microcystins and anatoxins[1], and more recently cylindrospermopsin (in 2006) and saxitoxins (in 2008), often in mixture of several variants (Afssa/Afsset, 2006; Brient *et al.,* 2009; Ledreux *et al.,* 2010). The death of dogs has been reported due to the presence of anatoxin-a (Gugger *et al.,* 2005, Cadel-Six *et al.* 2007).

Regulation and risk management

Drinking-water:

Since 2001 a decree set the maximum limit of microcystin-LR in drinking water at 1 µg/L (applicable since December 2003), following the provisional WHO guideline value. In 2007, an order stated that 1 µg/L is applicable to the sum of all microcystins detected and quantified in the samples.

The program of official sanitary controls for public drinking water network in terms of sampling, analyses and frequency is defined in a separate order (updated in 2010) that applies to all parameters. Regarding microcystins, this order states that microcystins have to be analyzed in raw surface water and at the point of distribution only in the event of cyanobacterial proliferation according to visual observation and/or analytical results.

Water used in food industry (in case its water supply is not from the public network) is covered by another order. Microcystins are not listed in the parameters to be analyzed.

Freshwater recreational sites:

According to the European directive 2006/7/EC concerning the management of bathing water quality, when the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks. Cyanobacterial proliferation means an accumulation of cyanobacteria in the form of a bloom, mat or scum.

In 2003 a decision tree was set up for local managers of the water bodies (circulaire DGS 7a n°2033-270). In case of persistent high algal concentrations, a strict monitoring system is implemented, based on visual and on microscopic observations. Three levels of management measures are identified according to the levels of cyanobacteria:

Level 1: the number of cells is between 20,000 and 100,000 cells/mL ± 20 %, with a majority of cyanobacteria ➔ Recreational activities are still allowed and the public is informed by posters on site. Visual inspections are intensified to a daily basis. Sampling for counting and species identification should be performed at least on a fortnightly basis.

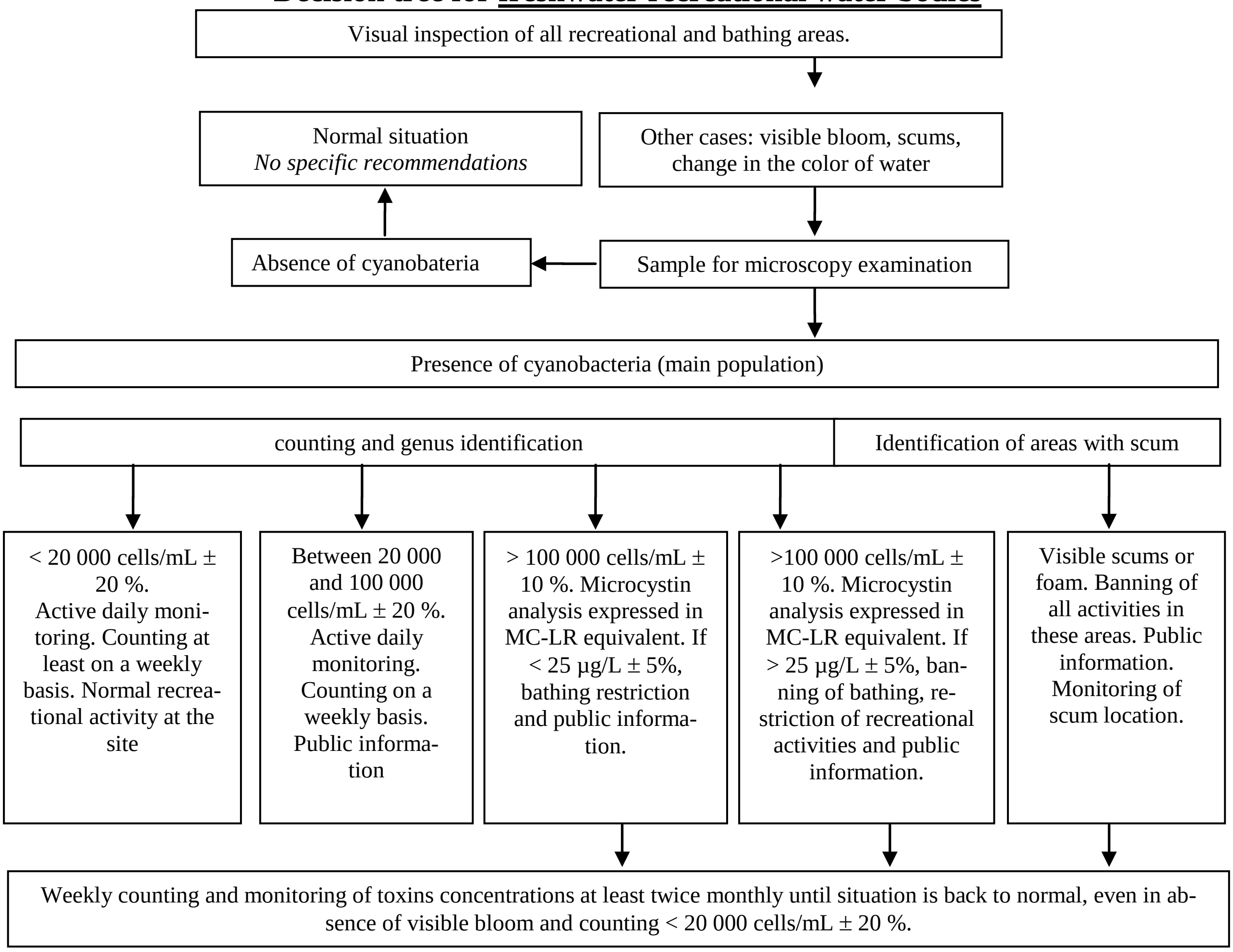
Level 2: if the number of cells is > 100,000 cells/mL ± 10 %, then microcystins are analysed. If the concentration expressed in LR-equivalents is < 25 µg/L ➔ bathing and recreational activities are restricted. If the concentration is > 25 µg/L ➔ bathing is banned and recreational activities are restricted. In either case, the public is informed.

Level 3: cyanobacterial scum appears in bathing areas ➔ all water activities in those bathing areas are banned. Restrictions do not necessarily apply to the whole recreational site. Other areas without scum may still be open. This notion of scum is at the discretion of the manager, particularly when species do not produce blooms such as the genus *Planktothrix.*

<u>Algicide treatments:</u>

Authorization to use an algicide (as copper sulphate) must be requested by the departmental and national authorities for preventive use on raw water only to produce drinking water.

Decision tree for <u>freshwater recreational water bodies</u>



Recommendations of the French Risk Assessment Agencies

To date, no case of human poisoning has been attributed to cyanotoxins in France. However, according to the list of cyanobacteria determined on various bathing water bodies and to some cases of animal poisoning through drinking water in the natural environment, the Directorate General for Health requested the French Agency for Food Safety (Afssa) to assess the risks associated with the presence of cyanobacteria in water for human consumption and the French Agency for Environmental and Occupational Health (Afsset)[3] to assess the risks associated with bathing. Experts from both agencies collected and analyzed data that were previously scattered from various entities, so as to update knowledge on cyanobacteria in France. The joint report has been published in 2006 (Afssa/Afsset, 2006).

Regarding bathing water, data collected from the health monitoring system showed that:

- the representativeness is difficult to evaluate because the data mainly come from the West and the Center part of the country (absence of data from Eastern part and overseas territories),
- data are lacking on the spatial and temporal distributions of blooms potentially toxinogenic and on the production of microcystins,
- data are lacking in the presence of cyanobacteria in brackish and marine waters,
- data are mainly available from July to September,
- the amount of data collected at bathing or water sports' sites between 2002 and 2004 includes 2,680 samples;
- the sub-sample of samples (500 samples) for which there is a result for microcystin level is biased compared to the total number of samples with regard to the abundance of cyanobacterial cells since toxins are only looked for when the cell number is higher than or equal to 100,000 cells/mL;
- data are inadequate to estimate the exposure of the French population to microcystins through bathing water, and their contribution to the total intake compared to drinking water;
- a new threshold was recommended at 13 µg/L ± 5 % eq MCs for banning bathing and recreational activities, to protect the most sensitive population (children between 2 and 7 years old).

In 2009, Afsset issued a report on artificial bathing waters open to the public. Such "natural bathing waters" may be artificial or natural ponds and public swimming pools that are not subject to technical treatment and disinfection; rather their water is treated by biofiltration. Thus they are a hybrid between a bathing site in a natural lake or river and an outdoor pool. The use of such systems has increased these last 10 years in France. They are however not covered by any health regulations and are therefore not subject to any water monitoring obligations. Afsset highlighted the need to set regulations for such bathing sites in order to guide their development and avoid major health risks due to the development of toxic microalgae (including cyanobacteria) or pathogens (Afsset, 2009).

Regarding drinking water, in terms of risk assessment, Afssa's expert group could only issue an opinion on one toxin, microcystin LR, considering that the risk of its presence in water for hu-

---

[3] On 1st July 2010, Afssa and Afsset merged to become Anses (French Agency for Food, Environmental and Occupational Health & Safety)

man consumption is negligible. Moreover, Afssa recommended amending regulations to update the parameters to be looked for using the standardised analytical method of microcystins in public water supplies (Afssa/Afsset, 2006).

The 2006 report concluded that more data are needed on:

- the toxicity of cyanotoxins (short and long term, carcinogenicity) to set health based guidance values for toxins alone and in mixtures
- the monitoring of cyanobacteria and cyanotoxins in water for drinking water production and for recreational activities (freshwater, brackish and marine waters)
- analytical methods and availability of standards (especially for other toxins than MC-LR)
- the understanding of production of toxins
- the distribution of toxins in the environment (water, sediments, aerosol) and in living organisms (animals and plants) and their bioavailability.

The agencies also recommended creating a national database to collect monitoring data.

Regarding food, Afssa issued an opinion in 2008 specifically regarding the contamination of food (fish, shellfish, and crustaceans) related to cyanobacterial proliferation. The opinion mainly concluded that data are lacking on the presence and the toxicokinetics of cyanotoxins in fish tissues as well as on their bioavailability for humans (especially regarding the bound microcystin). Most of the data available on tissue contamination were investigated in Asia, and are therefore difficult to extrapolate for European countries. Nevertheless these data show:

- a contamination of the freshwater mollusks which, even if they are not directly eaten by humans, can be transferred via the food web to upper predators like fish,
- a contamination of marine bivalves mollusks, especially mussels of the common species *Mytilus edulis*,
- a significant contamination of the muscles of freshwater fish
- some studies show that elimination of the toxins in fish and gastropods could take a long time (several days after the end of exposure via contaminated feed). Such data suggest a background level of fish contamination even in absence of cyanobacterial proliferation (or between 2 bloom events).

Based on the Tolerable Daily Intake for chronic exposure of 0.04 µg/kg bw/day set by the WHO, a default body weight of 60 kg, a daily consumption of fish of 86 g (high percentile in the French database) and an intake of water (2 L at 1 µg/L), the limit for adults would be 5.6 µg MCs/kg of fish (or edible part of fish). For children, this value would be 1.4 µg/kg of fish (default body weight of 10 kg and daily consumption of 57 g). If data are available to show the absence of MCs in drinking water, the limits become 28 and 7 µg/kg of fish, respectively.

Afssa reiterated the need for studying the contamination of microcystins in fish and its bioavailability for humans. A study on this topic is currently on-going at the French agency (Anses), jointly with the French National Museum of Natural History from Paris (UMR 7245 MCAM MNHN – CNRS).

In 2008, the French Museum of Natural History (UMR MCAM) reported for the first time in France the presence of saxitoxins (STXs) in a water body used for recreational activities (e.g. bathing) (Ledreux *et al*., 2010). Based on Australian, Brazilian and New Zealand recommendation values, Afssa recommended to do not eat fish from this water body and to set a monitoring of the level of STXs (Afssa, 2009). The co-occurrence of toxins (for example STXs and microcystins) was not considered in these recommendations.

Date	Site	[Chl. A] (µg/L)	**Potentially toxic cyanobacteria** (in per cent of total phytoplankton biomass)	[STXs] (µg eq. STX.L^{-1})
08 September 2008	1	153 ± 0,9	*Microcystis aeruginosa* (1 %) *Microcystis wesenbergii* (2 %) *Aphanizomenon flos-aquae* (36 %) *Aphanizomenon gracile* (6,5 %) *Aphanizomenon aphanizomenoïdes* (< 1 %)	4.8 ± 0,5
08 September 2008	2	208 ± 4	*Microcystis wesenbergii* (1,5%) *Aphanizomenon flos-aquae* (71,5 %) *Aphanizomenon gracile* (7,5 %) *Aphanizomenon aphanizomenoïdes* (< 1 %)	6.7 ± 3,5

In April 2010, around sixty people reported cutaneous and respiratory irritations in Mayotte Island (a French territory in Indian Ocean), probably subsequent to bathing and/or inhalation of spray on the beach. The appearance of these irritating symptoms was concomitant with a massive bloom of *Lyngbya majuscula*, a potentially toxic filamentous and benthic cyanobacteria, in association with marine plants Phanerogamae. A large quantity of these plants contaminated by the cyanobacteria was found on the beach. A collective expertise has been performed by the French Agency for Environmental and Occupational Health (Afsset) on this topic and several recommendations have been proposed for the management of this crisis, (*e.g.* collection of the algal mats using appropriate personal protective equipment (Afsset, 2010).

Recent findings from research

In 2006, the University of Rennes (UMR Ecobio 6553 Rennes Univ. – CNRS) reported for the first time in France the presence of cylindrospermopsin, concomitantly with microcystins (both hepatotoxins) with species *Aphanizomenon flos aquae* et *Anabaena planctonica* (Brient *et al*., 2009).

In 2009, BMAA (β-N-méthylamino-L-alanine), a neurotoxin produced by cyanobacteria, has been detected in raw freshwater used for drinking water production. A network of multidisciplinary scientists has been set involving neurologists, geographers, cyanobacteriologists, ecologists and biochemists to study the assumption of a link between ALS (Amyotrophic lateral sclerosis) and the presence of cyanobacteria and their toxins in the environment and food produced locally (Brient et al., 2010).

In 2011, two French laboratories (ENS Paris and ISARA Lyon) published a paper on the influence of sampling strategies on the monitoring of cyanobacteria in small freshwater ecosystems. They demonstrated that the spatial and temporal aspects of the sampling strategy had a considerable impact on the findings of cyanobacteria monitoring in the small lake studied. In particular, two peaks of *Aphanizomenon flos-aquae* cell abundances were usually not picked up by the various temporal sampling strategies tested. In contrast, sampling once a month was sufficient to provide a good overall estimation of the population dynamics of *Microcystis aeruginosa*. The choice of the location of the sampling points around the pond was also very important for the quality of the estimation. Finally, it appeared from this study that it was impossible to propose a single universal sampling strategy that is appropriate for all freshwater ecosystems and also for all cyanobacteria (Pobel *et al*., 2011). In the same way, it has been shown in another study dealing with *Planktothrix rubescens*, a cyanobacteria proliferating in the metalimnic layer of several deep alpine lakes, that hydrodynamical processes (internal waves) have a major impact on its vertical and horizontal distribution that should be taken in account in monitoring programs for the survey of this toxic species (Cuypers *et al*., 2011). Finally, several French laboratories belonging to GRISCYA have published a paper on the development of a first prototype for an instrumented buoy allowing the *in situ* high-frequency monitoring of *P. rubescens* in Lac du Bourget (Le Vu et al., 2011). This prototype was developed in the framework of the PROLI-PHYC project funded by the ANR (French Agency for Research), which has permitted the development, the validation and the industrialization of a real-time monitoring system (see photo) for the monitoring of cyanobacteria in freshwater ecosystems.

GRISCYA, the French Informal Scientific Interest Group on Cyanobacteria

In 2002, French scientists involved in research projects on cyanobacteria/cyanotoxins set an informal Scientific Interest Group called GRISCYA which brings together 20 national scientific institutions from various fields.

Annual meetings are organized to present and discuss preliminary results of on-going projects and to provide advice to PhD students and post-doctorates. Half a day is dedicated to exchanges with local water managers.
According to the 2011 meeting, current research projects deal with:

- Impact of *Phaeocystis* on coastal ecosystems (pelagic and benthic)
- Persistence of toxic potential and *mcyB* gene transcription in benthic populations of *Microcystis* at different ages
- Genetic diversity of *Microcystis aeruginosa*.
- Physiological cost for production of microcystins by *Microcystis aeruginosa*
- Microcystins interaction with the intestinal barrier using human *in vitro* model (Caco-2)
- Trophic transfer of microcystins from *Lymnaea stagnalis* (*Gastropoda Pulmonata*) to *Gasterosteus aculeatus* (*Teleostei Gasterosteidae*) and impact on fish
- Bioaccumulation of free and bound microcystins in fish
- Accumulation and detoxification of microcystin-LR by fish

- Physiological acclimatisation / adaptation in invertebrates: Are invasive mussels more tolerant against cyanobacteria?
- Chronic toxicity in fish (Medaka model) : histopathology study
- Study of the diversity and the potential toxicity of marine benthic cyanobacterial mats from La Réunion Lagoon.
- Impact of mixing on phytoplankton of a small pond.

References

Afssa (5 juin 2008), Note de l'Agence française de sécurité sanitaire des aliments relative à la consommation de produits alimentaires en présence d'efflorescence de cyanobactéries [10p]. http://www.anses.fr/Documents/RCCP2007sa0219.pdf

Afssa (9 juillet 2009). Note de l'Agence française de sécurité sanitaire des aliments relative à la situation de contamination des plans d'eau de la base de loisirs de Champs sur Marne par des saxitoxines [4p].
http://www.anses.fr/Documents/EAUX2009sa0180.pdf

Afssa/Afsset (juillet 2006). Rapport commun de l'Agence française de sécurité sanitaire des aliments (Afssa) et de l'Agence française de sécurité sanitaire de l'environnement et du travail (Afsset). Evaluation des risques sanitaires liés à la présence de cyanobactéries dans les plans et cours d´eau destinés à la baignade et/ou à d´autres usages [228p].
http://www.afsset.fr/upload/bibliotheque/085391856141331010617707867709/cyanobacteries.pdf

Afsset (juillet 2009). Agence française de sécurité sanitaire de l'environnement et du travail. Risques sanitaires liés aux baignades artificielles. Evaluation des risques sanitaires [197p].
http://www.afsset.fr/upload/bibliotheque/957631159435562010556935879998/baignades_artificielles_avis_rapport_afsset_juillet_2009.pdf

Afsset (juin 2010). Note d'expertise collective de l'Agence française de sécurité sanitaire de l'environnement et du travail relative aux risques sanitaires liés à la présence de végétaux associés à des cyanobactéries sur la plage de N'Gouja à Mayotte [21p].
http://www.afsset.fr/upload/bibliotheque/993914462490329442359547495250/cyanobacteries_mayotte_juin2010.pdf

Arrêté du 11 janvier 2007 relatif aux limites et références de qualité des eaux brutes et des eaux destinées à la consommation humaine mentionnées aux articles R. 1321-2, R. 1321-3, R. 1321-7 et R. 1321-38 du code de la santé publique. NOR : SANP0720201A

Arrêté du 21 janvier 2010 modifiant l'arrêté du 11 janvier 2007 relatif au programme de prélèvements et d'analyses du contrôle sanitaire pour les eaux fournies par un réseau de distribution, pris en application des articles R. 1321-10, R. 1321-15 et R. 1321-16 du code de la santé publique. NOR : SASP0928279A

Arrêté du 11 janvier 2007 relatif au programme de prélèvements et d'analyses du contrôle sanitaire pour les eaux utilisées dans une entreprise alimentaire ne provenant pas d'une distribution publique, pris en application des articles R. 1321-10, R. 1321-15 et R. 1321-16 du code de la santé publique. NOR : SANP0720203A

Brient L., Lengronne M., Bormans M.,Fastner J. (2009). First Occurrence of Cylindrospermopsin in Freshwater in France. *Environmental Toxicology* 24:415-420.

Brient L., Lengronne M., Esperanza M., Bruchet A., Boumediene F. and members of the INSERM network's. Cyanobacteria neurotoxins. (2010). Poster entitled "BMAA and amyotrophic lateral sclerosis (SLA)" at ICTC8, Turkey.

Cadel-Six S, Peyraud-Thomas C, Brient L, de Marsac NT, Rippka R, Méjean A. (2007). Different genotypes of anatoxin-producing cyanobacteria coexist in the Tarn River, France. *Appl Environ Microbiol*. 73(23):7605-14.

Circulaire DGS 7a n°2033-270 du 4 juin 2003, relative aux modalités d'évaluation et de gestion des risques sanitaires face à des proliférations de micro algues (cyanobactéries) dans les eaux de zones de baignades et de loisirs nautiques.

Cuypers Y., Vinçon-Leite B., Groleau A., Tasssin B., Humbert J.F. (2011). Impact of internal waves on the spatial distribution of *Planktothrix rubescens* (cyanobacteria) in an alpine lake. *ISME J* 5: 580-589.

Décret n°2001-1220 du 20 décembre 2001 relatif aux eaux destinées à la consommation humaine, à l'exclusion des eaux minérales naturelles (transposition en droit français de la directive européenne 98-83/CE).

Gugger M., Lenoir S., Berger C., Ledreux A., Druart J.C., Humbert J.F., Guette C., Bernard C. (2005). First identification in a French river of the benthic cyanoacterium *Phormodium favosum* producing anatoxin-a associated with dog neurotoxicosis. *Toxicon*, 45 (7): 919-928.

Le Vu B., Vinçon-Leite B., Lemaire B.J., Bensoussan N., Calzas M., Drezen C., Deroubaix J.F., Escoffier N., Dégrés Y., Freissinet C., Groleau A., Humbert J.F., Paolini G., Prévot F., Quiblier C., Rioust E., Tassin B. (2011). High-frequency monitoring of phytoplankton dynamics within the European water framework directive: application to metalimnic cyanobacteria. Biogeochemistry 106: 229-242.

Ledreux A., Thomazeau S., Catherine A., Duval C., Yéprémian C., Marie A., Bernard C. (2010). Evidence for saxitoxins production by the cyanobacterium Aphanizomenon gracile in a French recreational water body. *Harmful Algae* 10: 88-97.

Pobel D., Robin J., Humbert, J.F. (2011). Influence of sampling strategies on the monitoring of cyanobacteria in shallow lakes: Lessons from a case study in France. *Water Research* 45: 1005-1014.

GREECE: Occurrence, Monitoring and Risk Management of Cyano-bacteria and Cyanotoxins

I.Kagalou[1], K.Kormas[1],T.Papadimitriou [2],M.Katsiapi[3],S.Genitsaris[3] & M.Moustaka-Gouni[3]

[1] Dept. of Icthyology & Aquatic Environment, School of Agricultural Sciences, Univ. of Thessaly, 38446, N.Ionia, Greece; ikagalou@uth.gr
[2] Management Authority of the protected Lake Karla, Greece
[3] Department of Botany, School of Biology, Aristotle Univ. of Thessaloniki, GR 54124, Thessaloniki, Greece

Introduction

Greek freshwaters exhibit extensive eutrophication symptoms including frequent occurrence of cyanobacterial blooms (Vardaka 2001; Cook et al., 2004; Gkelis et al., 2005a, b; Moustaka-Gouni et al., 2006, 2007, 2009; Kagalou et al., 2008; Papadimitriou et al., 2010a; Kormas et al., 2010, 2011). Factors typical of the Mediterranean climate like higher temperatures and frequently occurring summer droughts impose considerable environmental stress on the hydrology of freshwaters, especially lakes, and promote cyanobacterial growth. Consequently, the threat from the occurrence and persistence of toxic cyanobacteria is considered high, and there is a risk that this might be increased by climate change.

Most Greek freshwaters are accessible to the public for boating, sports, fishing and similar recreational activities. A significant number of water bodies are used for irrigation purposes and as drinking water supplies. However, Greek freshwaters are not monitored at the national level. The currently available data on cyanotoxin risks for water body users are solely the outcome of research performed by academic and research institutions (Cook et al. 2005).

Occurrence and Monitoring of Cyanobacteria

In Greece, where screening programmes have been conducted, hepatotoxic cyanobacterial blooms were observed in most of the lakes included in the respective programme (Lanaras et al., 1989; Vardaka 2001; Gkelis et al., 2001; Vardaka et al., 2005). Species known to produce toxins have been found in 36 waterbodies, and in the eutrophic ones they were dominant in blooms (e.g., *Microcystis aeruginosa*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae, Cylindrospermopsis raciborskii and Planktothrix rubescens*). Cosmopolitan species (e.g., *M. aeruginosa*), pantropic (e.g., *Anabaenopsis tanganyikae*) and holarctic species (e.g., *Anabaena flos-aquae*) were observed (Vardaka et al., 2005; Kagalou et al., 2008; Moustaka-Gouni et al., 2007, 2009; Vareli et al., 2009; Kormas et al., 2011). In most of the waterbodies total cyanobacterial biovolumes were higher than the Guidance Level 2 of 100 000 cells per liter given by WHO (2003) for recreational waters, (Gkelis et al., 2001; Vardaka et al., 2005; Katsiapi et al. unpublished data). However, total cyanobacterial biovolumes in some of the water bodies exceeded Guidance Level 3, i.e scum formation in bathing areas for recreational waters, with the potential for acute cyanobacterial poisoning (Gkelis et al., 2001; Katsiapi et al. unpublished data).

In the shallow hypertrophic lake Koronia a mass death of thousands of birds coincided with a *Prymnesium* bloom in 2004, some cyanobacteria and a microcystin concentration of 55 μg L^{-1}) while N-fixing cyanobacteria formed a bloom a month before the bird deaths (Michaloudi et al., 2009). In September 2007, a mass death of flamingos (*Phoenicopterus ruber*) coincided with an extremely dense bloom (biovolume > 500 μL L^{-1}) of *Arthrospira fusiformis* and *Anabaenopsis* *arnoldii* (Moustaka-Gouni et al. 2008). The *Arthrospira-Anabaenopsis* bloom was preceded in June by a heavy *Microcystis aeruginosa* bloom (5.6 x 10^9 cells L^{-1}) with its epiphyte *Pseudanabaena mucicola* (12.4 x 10^9 cells L^{-1}). A *M. aeruginosa* bloom (1.0 x 10^9 cells L^{-1}) in 2010 coincided with a limited number of dead birds in Lake Koronia (Moustaka-Gouni et al. unpublished).

Lake Karla has been drying out up due to human activity since 1962, and approximately one third of this lake is now under reconstruction since 2009. First investigations since the spring of 2010 (based on both morphological and molecular analyses of phytoplankton) point towards considerable abundances of known toxin-producing genera such as *Anabaenopsis*, *Planktothrix* and *Microcystis* along with high microcystin concentrations (Oikonomou et al. 2012; Katsiapi et al., unpublished, Papadimitriou et al.2012b).

The urban Lake Kastoria used for recreational and fishing activities has attracted considerable scientific interest. It is a highly eutrophic system with intense fishing and recreational activities and is included among the hotspots for bird nesting in Greece. Morphological and molecular analysis showed the presence of the genera *Microcystis*, *Anabaena*, *Aphanizomenon*, *the Cylindrospermopsis-Raphidiopsis* group and *Limnothrix* spp. (Gkelis et al., 2005b; Moustaka-Gouni et al., 2007, 2009; Kormas et al., 2010, 2011). In summer 2010, an extremely dense bloom (biovolume reaching 175 µL L^{-1}) of *Microcystis* species (Katsiapi et al. unpublished) presented a potential hazard for wildlife and human health in the urban lake.

In 2007, the Marathonas Reservoir which contributes to the drinking water supply of the City of Athens was monitored for the presence of toxic cyanobacteria and results showed that *Microcystis aeruginosa* was the dominant cyanobacterial species (Lymperopoulou et al., 2011). The contribution of cyanobacteria to phytoplankton biomass of 50-88% indicates a moderate ecological water quality (Katsiapi et al., 2011). In addition, the total biovolume of cyanobacteria (2.5 µL L^{-1}) as well as the dominance of the known toxin-producing *M. aeruginosa* in the reservoir's phytoplankton may indicate a potential hazard for human health (Katsiapi et al., 2011). In 2003, the Polyphytos Reservoir, that contributes to the City of Thessaloniki drinking water supply, was monitored for phytoplankton and the presence of known toxic cyanobacteria. *Microcystis aeruginosa* formed a water bloom (maximum 378 x 10^6 cells L^{-1}) in autumn (Chrisostomou et al., 2009). Experimental evidence was provided that over distances of < 1 km, the wind was an important agent for airborne dispersal of *M. aeruginosa* from Polyphytos Reservoir, enabling the spread of water bloom into other waterbodies.

A recent scientific effort has been directed to investigating the presence of airborne cyanobacteria in Greece. Cyanobacteria can contribute significantly in the total air particle load. In the air of the City of Thessaloniki Genitsaris et al. (2011) identified 12 cyanobacterial taxa within a year, including potentially harmful ones. During a day with the maximal measured abundance of airborne photosynthetic microorganisms a person could inhale at least 2500 potentially harmful microorganism cells per day. Studies on the relationship between airborne cyanobacteria abundance and respiratory-related outbreaks or other related incidents, especially in urban areas nearby to water bodies with harmful cyanobacterial blooms, are currently largely lacking. Annadotter et al., (2005) have proposed respiratory exposure to airborne toxic cyanobacteria during bloom events as cause of acute health problems.

Occurrence and Monitoring of Cyanotoxins

In Greece, cyanotoxin monitoring is a fairly young field. In Lake Kastoria high cyanobacterial biovolume, clearly from scum formation (exceeding Guidance Level 3 for recreational water use) and microcystin (MCYST) –LR concentrations indicated elevated risks of adverse human health effects (Cook et al 2004). MCYST in 7 different waterbodies of the country were first analysed in 1999 by HPLC, and the total MCYST concentration per scum dry weight ranged from 0.04 to 2.56 ng g^{-1} dry weight (Gkelis et al., 2005a). The most abundant variants are MCYST–LR and MCYST–RR, while MCYST–LA, MCYST–YR and desmethylated derivatives of MCYST–LR and MCYST–RR have also been found (Gkelis et al., 2005a). In 2005, microcystins were analysed in 13 Greek lakes by Papadimitriou et al. (2010a). These lakes are used for different purposes such as fisheries, irrigation, recreation, industrial production and hydropower. MCYST were detected in all the examined lakes, irrespective of their trophic state, morphometry and dominant cyanobacterial species. MCYST concentrations in water ranged between 0.2 µg L^{-1} and 3.8 µg L^{-1}. MCYST concentrations in scum ranged between 1 .1 µg L^{-1} and 16 µg L^{-1} (Fig.1) (Papadimitriou et al., 2010a).

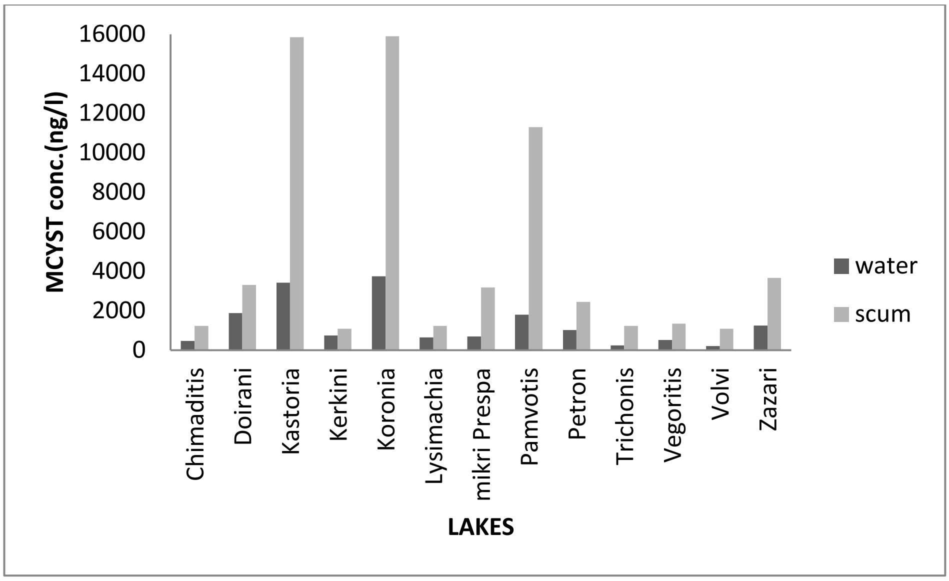


Figure 1: Microcystin concentration in water and scum from Greek lakes (Papadimtriou et al., 2010a)

Thus, the MCYST concentrations in water samples of 6 out of 13 examined lakes were above the provisional WHO Guideline value for microcystin-LR in drinking-water of 1 µg L^{-1}. Using the risk classification proposed by Papadimitriou et al., 2010a for microcystins in water-bodıes with recreational use, the concentrations found in scum of four lakes would pose a low risk of adverse health effects while the scum of three lakes poses a moderate risk of adverse health effects (Fig.2).

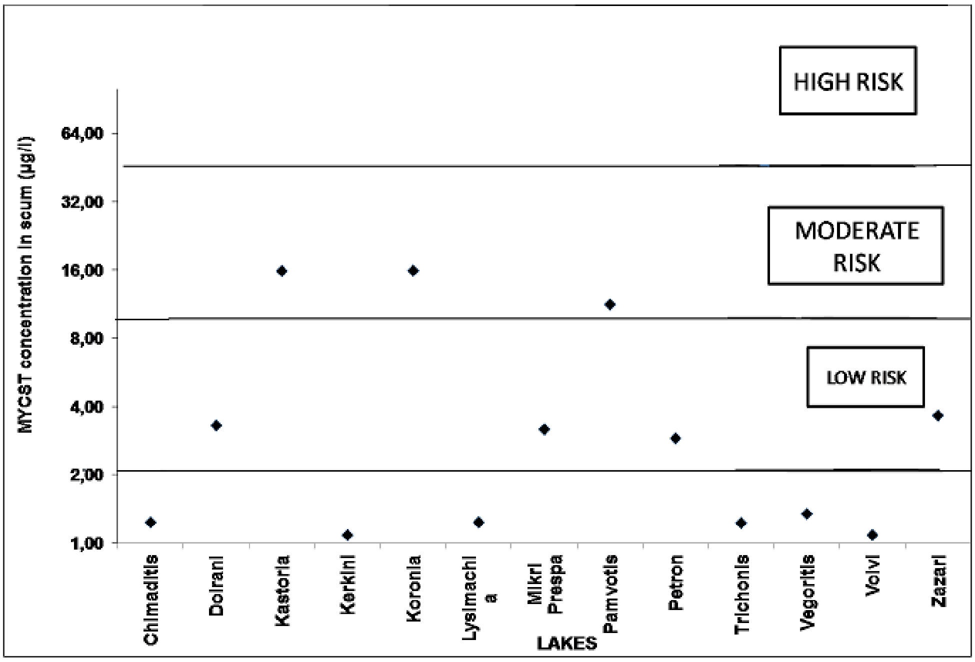


Figure 2: Microcystin concentrations (µg L^{-1}) in scum of Greek lakes compared with guidelines for adverse health risks through recreational exposure (after Papadimitriou et al., 2010a)

Relationships between environmental factors and microcystin dynamics were investigated for Lake Pamvotis by Papadimitriou et al. (2010a). Microcystin concentrations were positively correlated with temperature and Soluble Reactive Phosphorus, and their correlation was stronger with phycocyanin than with chlorophyll *a* (Papadimitriou et al., 2010b; Papadimitriou et al., 2011)

The 13 lake monitoring programme revealed MCYST in fish as well, i.e. in edible and non-edible *Carassius gibelio* tissues). Muscle tissue concentrations in four lakes ranged between 12.92 ng g^{-1} of dry weight and 52 ng g^{-1} of dry weight (Papadimitriou et al., 2010a) while mean muscle tissue concentrations in the cyprinid species *Rutilus panosi* monitored seasonally in lake Pamvotis over one year was found 19±2,5 ng/g (Papadimitriou et al., 2012a). These concentrations would cause a 60 kg person consuming a 300 g serving of *Carassius gibelio* to exceed the TDI (Tolerable Daily Intake) proposed by WHO for daily lifelong consumption (Fig. 3).

The investigation of microcystins in a number of aquatic food web compartments (phytoplankton, zooplankton, crayfish, shrimp, mussel, snail, fish, frog) in Lake Pamvotis (Papadimitriou, 2010c; Papadimitriou,et al., 2012b) showed significant microcystin concentrations in all the aquatic organisms examined during both the warm and cold season, with higher concentrations in phytoplankton and lower in fish species and frogs.

The species *Astacus astacus* and *Cyprinus carpio* are considered as commercial species in many regions of the world. Additionally, the frog *Rana epirotica* is considered as a local food which attracts the liking of many tourists. Estimated Daily Intake of MCYSTs would exceed the TDI on average by six times through the consumption of *Astacus astacus and Rana epirotica* and 14 times through the consumption of *Cyprinus carpio* (Fig. 4) (Papadimitriou, 2012). Therefore, lake products targeted for human consumption should be monitored for MCYST content in order to detect health risks and potentially restrict consumption.

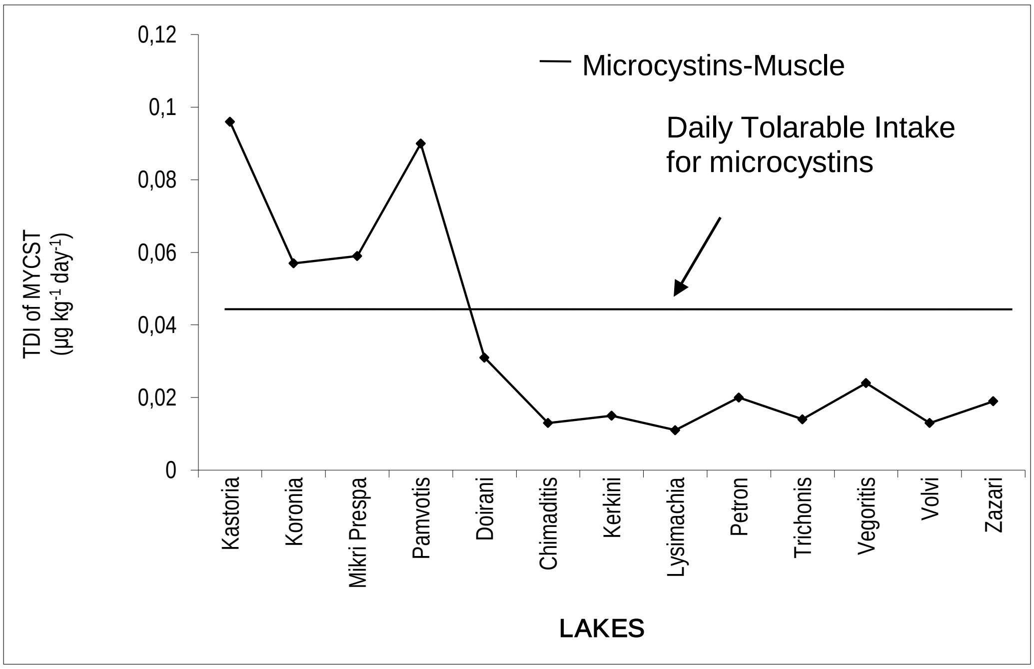


Figure 3: Estimated daily intake (Tolerable Daily Intake, TDI) of microcystins based on a 60 kg person ingesting a 300 g serving of *Carassius gibelio*.The horizontal line represents the provisional tolerable daily intake (TDI) established by WHO (after Papadimitriou et al., 2010a)

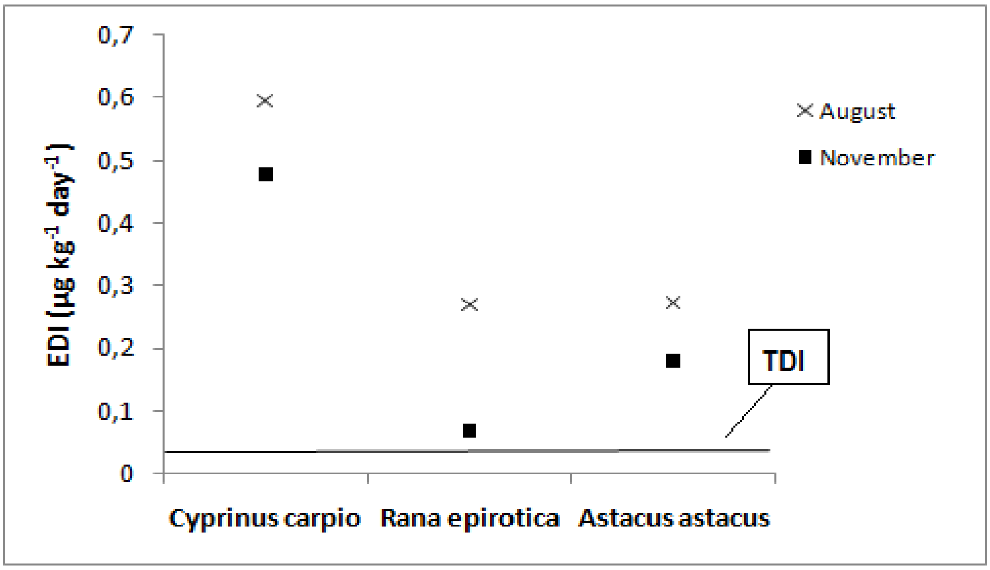


Figure 4: Estimated daily intake (EDI) of microcystins based on a 60 kg person ingesting a 300 g serving of *Cyprinus carpio* or a 50 g serving of *Astacus astacus*, or 50 g of *Rana epirotica* from Lake Pamvotis during November and August of 2008. The horizontal line represents the provisional tolerable daily total intake (TDI) proposed by WHO for Microcystin-LR (after Papadimitriou, 2010)

Risk Management and Regulation

Worldwide, in many countries, measures to protect public health as well as agricultural products and livestock from cyanotoxins have been implemented following the WHO suggestions. In Greece, although cyanobacteria and cyanotoxins have attracted significant scientific interest, the instigation of monitoring programs and legislation by the government at the national level is disproportionate (Cook et al., 2005). Inland water bodies are managed in terms of water usage, but to date cyanotoxin concentrations are not a target of management plans. Furthermore, there are no specific regulations concerning cyanotoxins. Local authorities still need to be convinced that cyanotoxins present hazards and that an afflicted waterbody should be characterized as potentially toxic (Cook et al., 2005). An example is Lake Kastoria which has a long history of toxic cyanobacterial blooms (Lanaras et al., 1989; Moustaka-Gouni et al., 2006). In 2010, no action was taken in face of a massive cyanobacterial scum persisting for months, at times decomposing. This bloom was dominated by *Microcystis aeruginosa* (bearing the *mcyA* part of the microcystin synthesis operon; Katsiapi et al., unpublished) and problems with both odour and sore eyes were referred in mass media.

In Greece Local Departments of the Ministry of Agricultural Development and Food are responsible for the safety of aquaculture products targeted for human consumption (Cook et al. 2005). However, these departments have not included yet the monitoring of commercial aquaculture products despite research evidence for the presence of microcystins in the tissues of lake fauna (Gkelis et al., 2006; Kagalou et al., 2008; Papadimitriou et al., 2010a). In Greece, the management of cyanobacteria is perceived as falling within the scope of the EU Water Framework Directive, which calls for a '*good ecological status*' of the water resources by the year 2015. Indeed, where the natural reference state of water-bodies is a low trophic status, cyanobacteria may still occur occasionally, but not in potentially hazardous cell densities, and thus this piece of EU legislation– if implemented as intended – should help reverse the currently perceived general increase of cyanobacteria. It is currently the only regulatory tool in place in Greece for achieving this target.

Conclusions

Toxic cyanobacteria in Greek freshwaters present a potential hazard for human health and wildlife exposed through the consumption of lake products and or water, and through recreational activities. However, to date there is no legislation in Greece concerning cyanotoxins. Greek freshwaters are affected by human activities, and increasing eutrophication in most of Greek freshwaters under climate change is reflected in their ecological status by increased cyanobacterial blooms and dominance of harmful taxa. It is of great importance for the conservation of freshwaters and for human health protection to include their monitoring with the implementation of Water Framework Directive (2000/60/EC).

References

Annadotter H., Cronberg G., Nystrand R., Rylander R., 2005. Endotoxins from cyanobacteria and gram-negative bacteria as the cause of an acute influenza-like reaction after inhalation of aerosols. Eco-Health 2: 209-221

Chrisostomou A., Moustaka-Gouni M., Sgardelis S., Lanaras T., 2009. Air-dispersed phytoplankton in a Mediterranean river-reservoir system (Aliakmon-Polyphytos, Greece). J. Plankton Res. 31: 877–884

Codd GA, Lindsay J, Young FM, Morrison LF & Metcalf JS, 2005. Cyanobacterial toxins. Harmful Cyano-bacteria (Huisman J, Matthijs HCP & Visser PM, eds), pp. 11–23. Springer, Dordrecht.

Cook, C., Vardaka, E., & Lanaras, T., 2004. Toxic cyanobacteria in Greek freshwaters, 1997–2000: Occurrence, toxicity and impacts in the Mediterranean region. Acta Hydrochemistry and Hydrobiology 32: 107–124.

Cook, C.M., Moustaka-Gouni, M., Gkelis, S., Lanaras, T., 2005. Greece: Cyanotoxin Risk Assessment, Risk Management and Regulation. In: Chorus I (ed) Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries. Series: WaBoLu 02/05. Umweltbundesamt, Dessau (ISSN: 0175-4211). pp. 69-75.

Genitsaris S, Kormas KA, Moustaka-Gouni M., 2011. Airborne algae and cyanobacteria: occurrence and related health effects. Front. Biosci. E3: 772-787

Gkelis S., Vardaka E., Moustaka–Gouni M., Lanaras T., 2001. The two most abundant toxic cyanobacteria in Greek lakes and their impact on water quality. Phycologia 40: 123.

Gkelis, S., Harjunpa, V., Lanaras, T., & Sivonen, K., 2005a. Diversity of Hepatotoxic Microcystins and Bioactive Anabaenopeptins in Cyanobacterial Blooms from Greek Freshwaters. Environmental Toxicology 20: 249–256.

Gkelis, S., Rajaniemi, P., Vardaka, E., Moustaka-Gouni, M., Lanaras, T., Sivonen, K., 2005b. Limnothrix redekei (Van Goor) Meffert (Cyanobacteria) strains from Lake Kastoria, Greece form a separate phylogenetic group. Microb. Ecol. 49: 176–182.

Gkelis, S., Lanaras, T., Sivonen, K., 2006. The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. Aquat. Toxicol. 78: 32–41.

Kagalou, I., Papadimitriou, T., Bacopoulos, V., Leonardos, I., 2008. Assessment of microcystins in lake water and the omnivorous fish (*Carassius gibelio*, Bloch) in Lake Pamvotis (Greece) containing dense cyanobacterial bloom. Environmental Monitoring and Assessment 137: 185–195.

Katsiapi M, Moustaka-Gouni M, Michaloudi E, Kormas KA, 2011. Effects of flushing on phytoplankton and water quality in a Mediterranean drinking-water reservoir (Marathonas Reservoir, Greece). Environ. Monit. Assess. DOI: 10.1007/s10661-010-1851-3

KormasK Vardaka E. Moustaka-Gouni M, Kontoyanni V, Petridou E, Gkelis S, Neofitou .C 2010. Molecular detection of potentially toxic cyanobacteria and their associated bacteria in lake water column and sediment. World J Microbiol Biotechnol, 26: 1473–1482.

Kormas KA, Gkelis S, Vardaka E, Moustaka-Gouni M, 2011. Morphological and molecular analysis of bloom-forming Cyanobacteria in two eutrophic, shallow Mediterranean lakes. Limnologica DOI: 10.1016/j.limno.2010.10.003

Lanaras T., Tsitsamis S., Chlichlia C., Cook CM, 1989. Toxic cyanobacteria in Greek freshwaters. Journal of Applied Phycology 1: 67–73.

Lymperopoulou DS, Kormas KA, Moustaka-Gouni M, Karagouni AD, 2011. Diversity of cyanobacterial phylotypes in a Mediterranean drinking water reservoir (Marathonas, Greece). Environ. Monit. Assess. 173: 155-165

Michaloudi E, Moustaka-Gouni M, Gkelis S & Pantelidakis K., 2009. Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. J. Plankton Res. 31: 301–309.

Moustaka-Gouni, M., Vardaka, E., Michaloudi, E., Kormas, K.A., Tryfon, E., Mihalatou, H., Gkelis, S., Lanaras, T., 2006. Plankton food web structure in a eutrophic polymictic lake with a history of toxic cyanobacterial blooms. Limnol. Oceanogr. 51: 715–727.

Moustaka-Gouni M, Vardaka E, Tryfon E, 2007. Phytoplankton species succession in a shallow Mediterranean lake (L. Kastoria, Greece): steady-state dominance of Limnothrix redekei, Microcystis aeruginosa and Cylindrospermopsis raciborskii. Hydrobiologia 575: 129–140

Moustaka-Gouni M, Cook CM, Gkelis S, Michaloudi E, Pantelidakis K, Pyrovetsi M & Lanaras T, 2004. The coincidence of a *Prymnesium parvum* bloom and the mass kill of birds and fish in Lake Koronia. Harmful Algae News 26: 1–2.

Moustaka-Gouni M, Michaloudi E, Katsiapi M & Genitsaris S, 2008. The coincidence of an *Arthrospira - Anabaenopsis* bloom and the mass mortality of birds in Lake Koronia. Harmful Algae News 35: 6–7.

Moustaka-Gouni, M., Kormas, K.A., Vardaka, E., Katsiapi, M., Gkelis, S., 2009. Raphidiopsis mediterranea SKUJA represents non-heterocytous life-cycle stages of *Cylindrospermopsis raciborskii* (WOLOSZYNSKA) SEENAYYA et SUBBA RAJU in Lake Kastoria (Greece), its type locality: evidence by morphological and phylogenetic analysis. Harmful Algae 8: 864–872.

Oikonomou A., Katsiapi M., Karayanni H., Moustaka-Gouni M., Kormas K., 2012. Plankton microorganisms coinciding with two consecutive mass fish kills in a newly reconstructed lake. TheScientificWorldJournal doi:10.1100/2012/504135

Papadimitriou, T., Kagalou, I., Bacopoulos, V., & Leonardos, I., 2010a. Accumulation of microcystins in water and fish tissues: An estimation of risks associated with microcystins in most of the Greek lakes. Environmental Toxicology 25: 418-427.

Papadimitriou Th., Leonardos I., Kagalou I, 2010b. Seasonal variation of microcystins and phycocyanin concentrations in a Mediterranean shallow lake: link with environmental factors, 8th International Conference on Toxic Cyanobacteria (ICTC8), Turkey, 2010.

Papadimitriou Th., 2010c. Effects of microcystins on aquatic animals. Doctoral dissertation, University of Ioannina, 2010 (in Greek with English summary)

Papadimitriou Th., Leonardos I. & Kagalou I.2011. Environmental factors and phycocyanin associated with microcystin variation in lake Pamvotis- Greece. European journal of Phycology, 46(1), pp 132.

Papadimitriou Th. Kagalou I., Leonardos I.2012a. Seasonally accumulation of microcystins in the various tissues of an endemic and protected fish species (Rutilus panosi) with different sizes. Clean-Soil Air, Water, 40(4): 402-407.

Papadimitriou Th., Kagalou I., Stalikas C., Pilidis G., Leonardos I.2012b. Assessment of microcystins distribution and biomagnification in tissues of the aquatic food web compartments from a shallow lake and potential risks for public health. Ecotoxicology, 21(4): 1155-1166.

Vardaka E., 2001. Toxic cyanobacteria and cyanobacterial toxins in Lake Kastoria and in other freshwaters in Greece. Doctoral dissertation, Scientific Annals of the School of Biology of the Faculty of Sciences, Appendix, Aristotle University of Thessaloniki, 2001 (in Greek with English summary).

Vardaka, E., Moustaka-Gouni, M., Cook, C.M., Lanaras, T., 2005. Cyanobacterial blooms and water quality in Greek freshwaters. J. Appl. Phycol. 17: 391–401.

Vareli K., Briasoulis, E., Pilidis G., and Sainis I., 2009. Molecular confirmation of *Planktothrix rubescens* as the cause of intense, microcystin—Synthesizing cyanobacterial bloom in Lake Ziros, Greece. Harmful Algae 8: 447-453.

WHO, 2003. Algae and cyanobacteria in fresh water. In: Guidelines for safe recreational water environments. Vol. 1: Coastal and fresh waters. World Health Organization, Geneva, Switzerland, pp. 136–158.

ITALY: Management of Potenially Toxic Cyanobacteria

EnzoFunari [1], LianaGramaccioni[2] and SimonaScardala[1]

[1] National Institute of Health, Department of Environment and Primary Prevention, Viale Regina Elena, 299, 00161 Rome (Italy); enzo.funari@iss.it; simona.scardala@iss.it
[2] Ministero della Salute, via Giorgio Ribotta, 5, 00144 Rome (Italy); l.gramaccioni@sanita.it

The Italian legislation for drinking water considers algae as an accessory parameter. No limit values have been set for cyanotoxins in Italian legislation, and the reference value used by local authorities is the provisional WHO guideline of 1 µg/l for MC-LR. The National Institute of Health has published a technical guidance Report about toxic cyanobacteria. (Lucentini et al., 2011).

Recreational Water

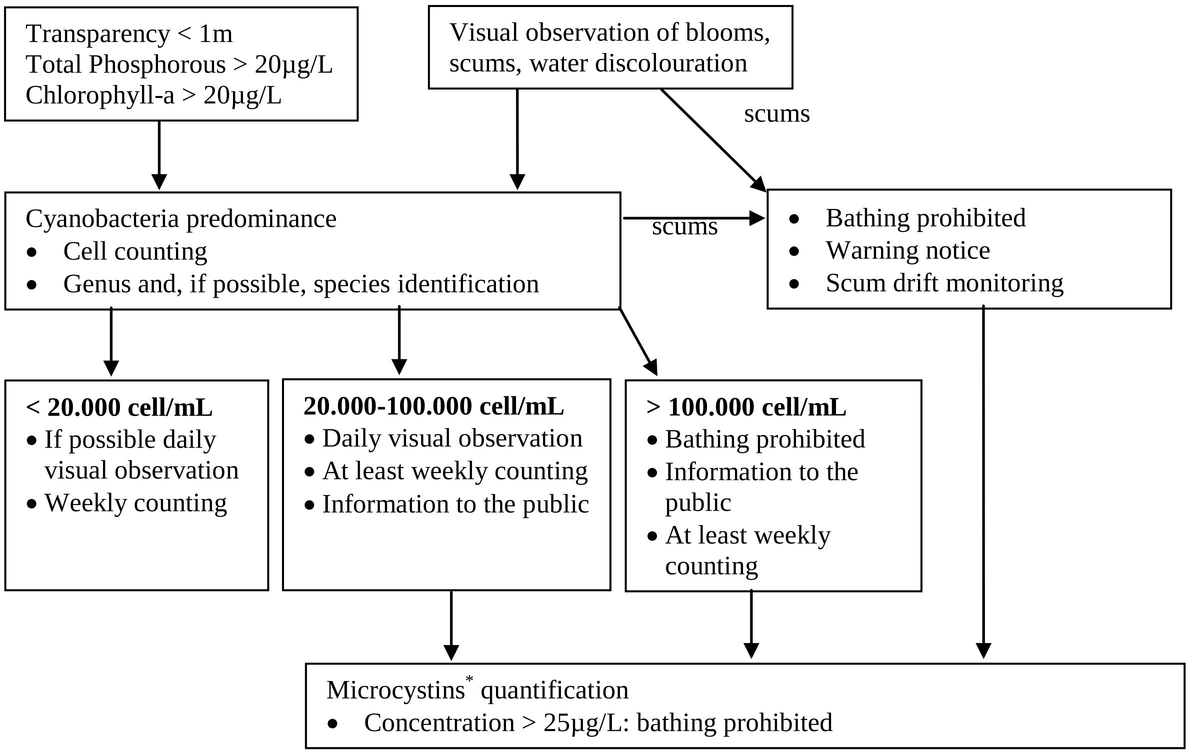
In 2006 the European Parliament issued the European Directive on Bathing Waters 2006/07/CE, concerning the management of bathing water quality and repealing Directive 76/160/EEC. This Directive indicates that “When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring has to be carried out to enable timely identification of health risks” and “When cyanobacterial proliferation occurs and a health risk has been identified or presumed, adequate management measures have to be taken immediately to prevent exposure, including information to the public”.

Italy has transposed the European Directive on Bathing Waters 2006/07/CE with a National Decree (Dlgs 116/08). The Decree has an annexed document, *Procedures for the management of the risk associated to cyanobacterial proliferations in bathing waters,* which is based on the WHO *Guidelines for safe recreational waters* (2003) and the *Bathing Water Profiles: Best Practice and Guidance,* elaborated by the Regulatory Committee under Directive 2006/7/EC ('Bathing Water Committee') and published in 2009.

The document, after introducing the main aspects of the issue represented by cyanobacterial proliferations in bathing waters, defines the following decision tree for cyanobacterial monitoring, based on the 2003 WHO guidelines.

The document also provides guidance for identifying water bodies with a significant potential of cyanobacterial proliferation. This can be done for the water bodies used for recreational activities through a survey which includes:

- evaluation of historical data;
- the analyses of nutrients to define the capacity of the water body for a bloom to grow;
- visual inspection to look for presence/bloom of cyanobacteria;
- transparency evaluation (Secchi disk).



*In the cases cyanotoxins other than microcystins are present a case by case risk assessment should be applied. The National Institute of Healh should be consulted for any technical advice.

All these activities should be performed during the bathing season in representative sites.

From this evaluation three different situations can be characterized:

1. Basins with no cyanobacteria and that are unlikely to support their proliferation. The suggested action is an annual control, based on visual inspection, transparency evaluation and the analyses of nutrients, to assess that nothing has changed.
2. Basins with no cyanobacteria but in which cyanobacteria are possible (transparency less than 2 m, total phosphorous > 0.02 mg/l). The suggested action is a seasonal control based on visual inspection, transparency evaluation and phytoplankton analyses.
3. Basins with cyanobacteria proliferations. The suggested action is a planned monitoring activity.

The document also suggests how to select periods and time for sampling; how to conduct bloom, surface and water column sampling; how to transport and store samples. Advice is further given for the equipment to be used and for protection of the staff taking the samples.

More detailed information about chemical and biological methods for the identification and quantification of cyanobateria and cyanotoxins has published in the guideline “Potentially toxic cyanobacteria: Ecological and methodological aspects and risk evaluation” (Funari et al., 2008) published by the Italian National Institute of Health. This Report summarizes the ecological, chemical, methodological, toxicological and epidemiological information available on this issue and identifies the actual risky exposure scenarios, and, when possible, provides reference values for cyanotoxins other than microcystins or for protection from acute and subchronic exposure Funari and Testai, 2008).

References

European Commission. Bathing Water Profiles. Best Practices and Guidance. December 2009. The document is available as PDF file at http://ec.europa.eu/environment/water/water-bathing/index_en.html .

Funari E, Scardala S, Testai E (Ed.). Cianobatteri potenzialmente tossici: aspetti ecologici, metodologici e valutazione del rischio. (Potentially toxic cyanobacteria: ecological and methodological aspects and risk evaluation) Roma: Istituto Superiore di Sanità; 2008. (Rapporti ISTISAN 08/6).

Funari E. and E. Testai (2008). Human health risk assessment related to cyanotoxin exposure. Critical Review in Toxicology. 38 (2): 97-125

Lucentini L, Ottaviani M per il “Gruppo nazionale per la gestione del rischio cianobatteri in acque destinate a consumo umano” (Ed.). Cianobatteri in acque destinate a consumo umano. Linee guida per la gestione del rischio. (Cyanobacteria in water for human consumption: Guidelines for risk management) Roma: Istituto Superiore di Sanità; 2011. (Rapporti ISTISAN 11/35 Pt. 2)

WHO Guidelines for safe recreational Water Environment Volume1. Coastal and fresh waters. Geneva: World Health Organization; 2003.

NETHERLANDS: Risks of toxic cyanobacterial blooms in recreational waters and guidelines

Bas W. Ibelings[1], Jasper M. Stroom[2], Miquel F.L.L.W. Lürling[3,1] & W. Edwin A. Kardinaal[4]

[1] Netherlands Institute of Ecology (NIOO-KNAW) – Department of Aquatic Ecology, Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands (b.ibelings@nioo.knaw.nl) and Institute FA Forel, University of Geneva, Switzerland;
[2] Waternet, PO Box 94370, 1090 GJ Amsterdam, The Netherlands;.
[3] Wageningen University – Aquatic Ecology & Water Quality Management Group, PO Box 47, 6700 AA Wageningen, The Netherlands;
[4] KWR Watercycle Research Institute, Groningerhaven 7, 3433 PE Nieuwegein, The Netherlands

Introduction

God created the earth, but the Dutch created Holland. This old proverb nicely emphasises the crucial role of water management in The Netherlands. Protection against flooding has always been essential for survival of the country. The earliest regional water boards were already founded in the 14th century. Traditionally the focus of water management has been purely on management of water quantity, but since 1970 water quality management was added to the responsibilities. The current central aim of water management in The Netherlands has been described as: “to obtain and maintain a safe country and to preserve and strengthen healthy, resilient water systems that ensure sustainable use of water”(Nationaal Waterplan 2009-2015). Water management more and more is aimed at closing the water-cycle and reflecting this by integrating groundwater, sewerage, surface- and drinking water all in one organisation. All functions of water systems (e.g. production of drinking water, shipping, recreation, nature) are weighed and balanced. Although 40 % of the drinking water consumed in the Netherlands is abstracted from surface water-bodies, cyanobacteria and cyanotoxins are of no concern, since several steps in the production processes are efficient barriers for cells and toxins (Vernooij et al, 2011). Subject of this paper is the use of surface waters for recreation. Recreational use is thus only one of a number of functions of lakes and rivers in the Netherlands.

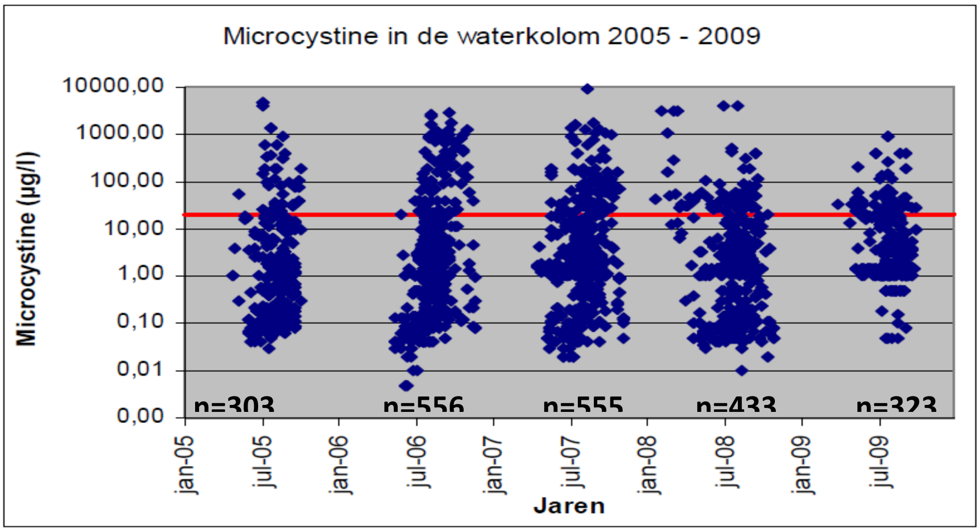
The overall responsibility for the safety of recreational waters, as well as the formal decision to issue warnings and such lies with the provinces and not with the water management authority. The appropriate water management authority has the responsibility to carry out the monitoring and investigate water quality and sources of pollution. In inland small(er) water bodies this is the responsibility of the water boards. The larger lakes and bathing locations along the main rivers fall under the responsibility of the Directorate-General for Public Works & Water Management, the executive arm of the Ministrty of Infrastucture and the Environment. As regulated by the EU bathing water directive and its Dutch legal implementation, the water management authority needs to examine causes of water quality exceedances (reported in bathing water profiles) and propose measures to comply with the standards of the bathing water directive. This does not necessarily mean that implementation and financing of all measures is a responsibility of the water management authority. Implementation depends on the type of measure and the importance of the bathing location to other stakeholders such as the provinces, local municipality and care-takers of bathing areas. The above applies to faecal pollution which is EU-standardized. For cyanobacteria the situation is even less clear since in the EU Water Framework Directive nor the Bating Water directive nor any Dutch legislation imposes an obligation to take specific measures against cyanobacterial blooms. Preventing exposure to cyanobacterial proliferation from the Bathing Water Directive varies with the level of local ambitions. In most cases the approach of controlling cyanobacteria follows the fecal approach. Nevertheless in Dutch waters the problems and costs of cyanobacterial measures by far outweigh the costs of measures concerning fecal pollution.

Most of the lakes in the Netherlands are man-made. In the densely populated western part of the country many shallow lakes are the result of extensive peat-digging in the 19th century, with deeper lakes being of more recent date and resulting from sand and gravel extraction. Yet others, like the large lake IJsselmeer are closed-off former estuaries. Almost all of these lakes are still eutrophic and ecological quality needs further improvement, despite the fact that large and costly efforts have brought down the concentrations of nitrogen and phosphorous. Lake restoration has been successful in some cases, but many lakes are still turbid and without the extensive submerged vegetation that once characterized them. The Ecological Quality Ratio of the Water Framework Directive shows an upward trend over 1987-2008, but this is based upon a general trend of decreasing chlorophyll-a, whilst there is no significant improvement in the decreased occurrence of nuisance algal blooms (Hosper *et al*., 2011). Indeed blooms of cyanobacteria are a conspicuous attribute of many of the lakes in the Netherlands, and concentrations of microcystin often reach high levels (see Fig. 1). Trends seem to indicate that microcystin levels have increased rather than decreased over recent years. In short it is clear that the potential for recreational exposure to (toxic) cyanobacteria in the Netherlands is rather large. We have numerous lakes that are eutrophic and support blooms of cyanobacteria in a densely populated country with a high demand for water related recreational activities (see Fig. 2a). In 2009, the Netherlands had 553 official inland freshwater bathing sites, of which 524 were located at various lakes. In fresh and brackish waters nearly 50 % of these official bathing waters experience the presence of nuisance cyanobacterial blooms (Fig. 2b).

Controlling cyanobacterial blooms is a serious responsibility for water management in the Netherlands, visit http://www.stowa.nl/Thema_s/Cyanobacteri_n/index.aspx for an overview of activities. The general public is relatively well informed about the health risks of recreation (e.g. swimming, (wind)surfing, waterskiing, diving) in lakes. There are special hotlines where information about the water quality of specific water bodies can be obtained (‘Zwemwatertelefoon’) and there are dedicated websites (e.g. http://www.waterland.net/zwemwater) with clickable maps of the provinces, showing whether swimming is unsafe in particular water bodies. Most warnings and almost all closure of lakes are related to blooms of toxic cyanobacteria. In the following we discuss regulations with respect to cyanobacteria and their toxins which have been developed in the Netherlands. We further present how the procedures and guidelines have evolved over the years from a simple protocol, based purely on microcystin measurements to a more complex protocol, which is based upon cyanobacterial biomass. Furthermore, the implementation of the EU Bathing Water Directive (DIRECTIVE 2006/7/EC) in Dutch legislation is addressed.

Guidelines for microcystin in recreational waters

In 2001 the Health Council of the Netherlands published the report: “Microbial risks of recreational waters”. The report gave advice to the government on epidemiological research, control measures (the ‘safety chain’), legislation etc. The focus was not only on cyanobacteria but also on other agents in surface water that may transmit a disease, e.g. *Leptospira, Clostridium*



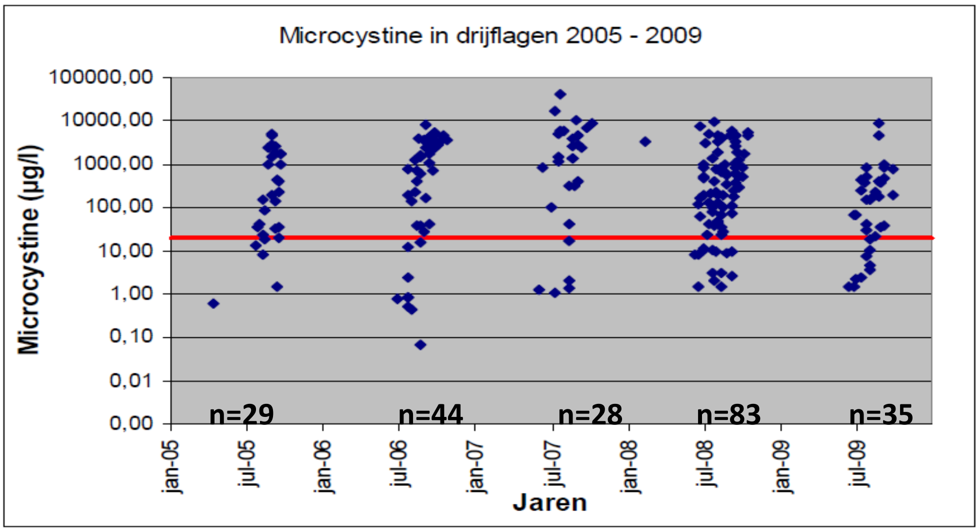


Fig 1: Concentrations of microcystin-LR equivalents in the water column (top-panel) and microcystin in surface scums (lower panel) of surface waters in the Netherlands, 2005-2009 (Kardinaal & de Haan, 2010). The red line indicates a microcystin level of 20 µg L^{-1}. Note the logarithmic scale.

botulinum, Naegleria fowleri or *Acanthamoebae*. The Ministry of Infrastructure and the Environment, provinces, water boards and other stakeholders strive since 2002 to implement standardised national guidelines on risks of exposure to cyanobacteria during recreation.

These guidelines are summarised in a protocol. The protocol is based upon proposals by a working group of cyanobacterial experts. The final version of the protocol, however, is the responsibility of functionaries, organised in NWO (National Water Council). The NWO is the highest council concerning surface water related issues in the Netherlands, and it takes different interests into account than the cyanobacterial experts, sometimes resulting in adjustments to the protocol. The ambition to implement national guidelines – there is no legal obligation to u the protocol – has had some success; most provinces use the guidelines, despite on-going discussion about pros and cons of the evolving versions of the protocol.

The earlier guidelines on mitigating exposure to cyanobacteria during recreational activities (see Ibelings, 2005) were purely based upon the risks of microcystin, in addition to a general guideline on presence of cyanobacterial surface blooms (scums – see Box 1). Since in the Netherlands microcystin (MC) was (and still is) by far the most widely occurring cyanobacterial toxin, the earlier protocol did not include toxins other than microcystin. Based upon the provisional WHO tolerable daily intake (MC-LR < 0.04 µg per kg bodyweight), from which the provisional WHO guideline for drinking water (MC-LR < 1 µg L^{-1}) was derived, and assuming that a swimmer ingests 100 mL of water (and bathes 365 days per year – more likely this would be less than 35 days – see Ibelings & Chorus, 2007 for derivation of a 'seasonal tolerable daily intake') an exposure limit of 20 µg MC-LR per litre of bathing water was derived. In the former protocol the following guideline-values were used (see Fig. 3):

- MC-LR > 10 µg L^{-1}: issue warning;
- MC-LR > 20 µg L^{-1}: issue warning and continue monitoring; if levels are persistently high
- close bathing facility;
- Presence of scums: at least issue a warning and continue monitoring.

Several developments led to a re-assessment of the microcystin based guidelines, in particular:

- The Implementation of the new EU Bathing water directive;
- Use of new analytical methods leading to the detection of cyanotoxins other than microcystin (in particular saxitoxin (variant STX); anatoxin-a) in Dutch surface waters (Faassen & Lürling, 2010, van der Oost, 2010);
- The need to distinguish surface scums of different intensity, instead of scums simply being present or absent, as in the old protocol;
- The need to take dynamics in scum formation, degradation, and transport into account as well as the availability of new approaches to do this;
- Reducing costs associated with the risk-assessment procedure;
- Political pressure to re-asses the protocol, given the large number of warnings issued.

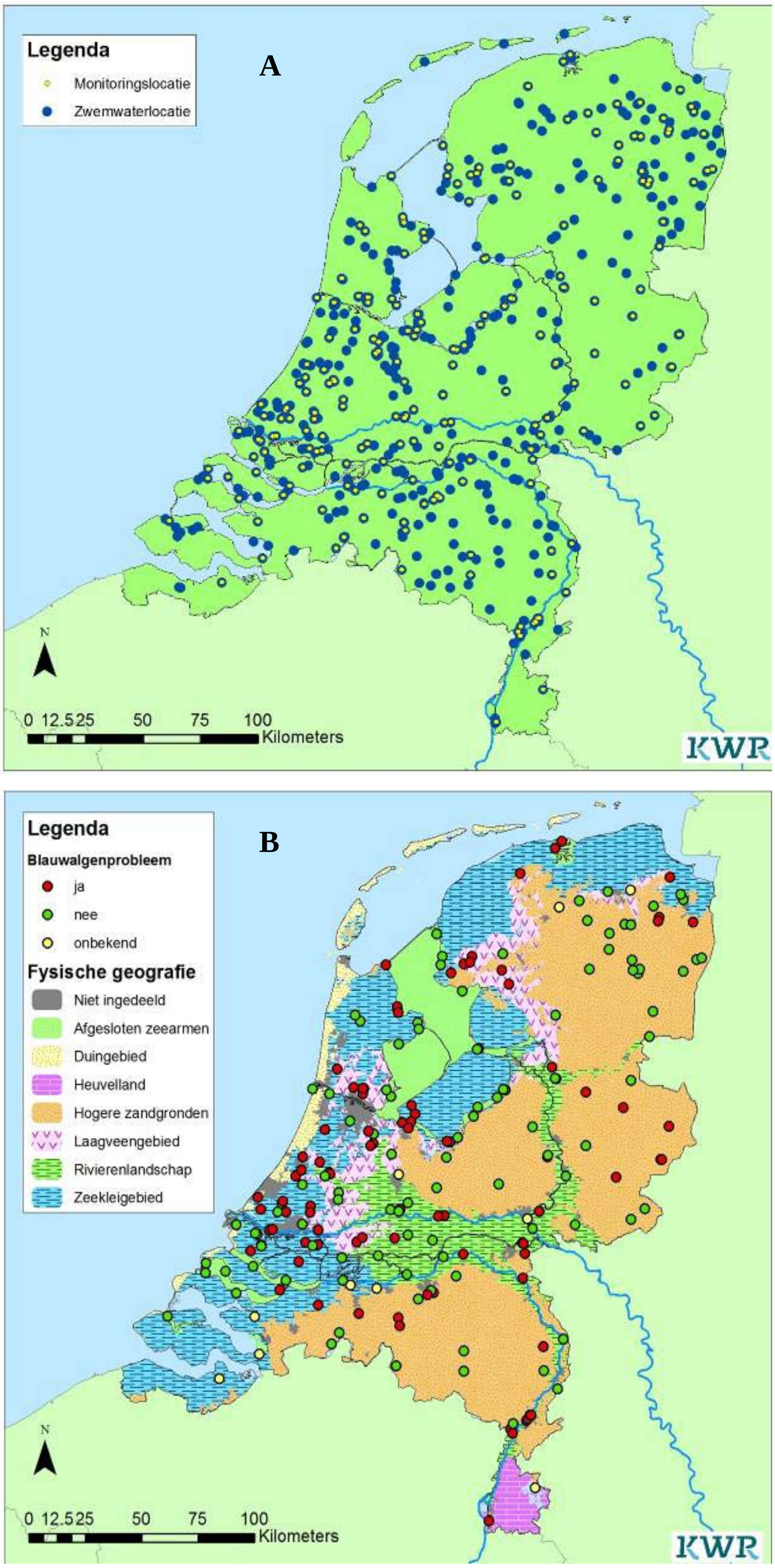


Fig 2: Overview of the officially designated and monitored bathing water locations in fresh and brackish waters, the open circles are screened for the presence / absence of cyanobacterial blooms (panel A); spatial distribution of lakes dominated by cyanobacteria, the solid circles indicate sites at which problems with (toxic) cyanobacterial blooms for recreational use of the lake in question have occurred in the past (panel B) (Kardinaal & van der Wielen, 2011).

Guidelines based on biomass: new protocol

The new protocol (see Fig. 4) provides guidelines for those locations where the bathing water profile[4] or measurements indicate that proliferation of cyanobacteria is to be expected on the basis of historic data or water-body conditions. The new protocol (2011) is more complex than the old protocol shown in Fig. 3, yet leaves more flexibility for on-site inspection and expert knowledge. It distinguishes between two alert-levels. Below alert-level 1 no further action is required. At level 1 the risk of adverse health-effects is presumed to be small or restricted to a small area of the bathing water location, but the risks increase at level 2. Level 1 results in a general warning to the public that blooms of toxic blue-green algae have been observed, and that these may cause skin irritation or intestinal problems. In case of persistent local scums a warning can be issued for the scum area only. This warning can be issued and withdrawn at short notice. Monitoring frequency increases from fortnighly to weekly. Thus data are obtained through a program of fortnightly (below alert level 1) or weekly (alert levels 1 and 2) site visits and sampling. Guidelines for sampling cyanobacteria are available (van der Oost, 2009). Samples must be taken at several locations, depending on whether surface scums are present or not, and whether these are located in- or outside the designated swimming area(s). Samples taken outside scums should always be included in the sampling effort. The distinction between alert-levels 1 and 2 is made upon (i) concentration of cyanobacterial biomass, suspended in the

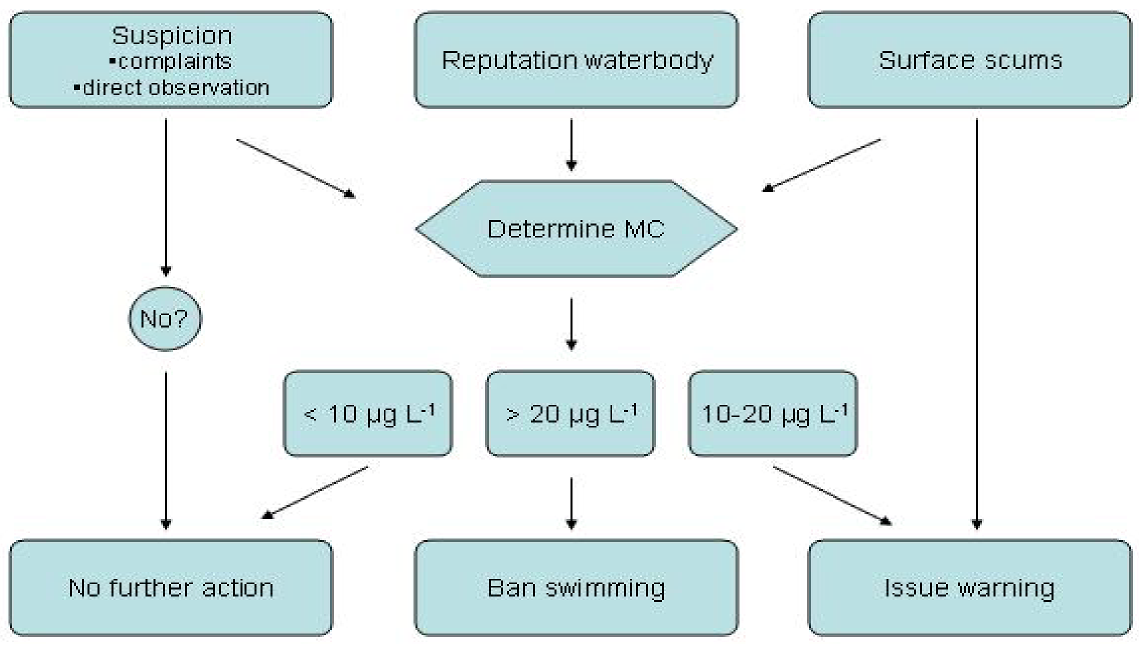


Fig. 3: The 'old' microcystin-based protocol (2002).

water-column, and (ii) presence/absence and intensity of surface scums. The transition from directly measuring microcystins in the old protocol to measuring cyanobacterial biomass ac-

[4] The **bathing water profile** describes the physical, geographical and hydrological characteristics of the bathing water. The profile is an instrument to identify and assess possibilities and causes of pollution. The proliferation of cyanobacteria is explicitly mentioned although the bathing water directive does not give any standards as to what is considered to be a proliferation.

knowledges the potential risks of toxins other than microcystin. For the assessment of cyanobacterial biomass a so-called quick-scan has been developed (Kardinaal, 2009), in which the total cell number of (potentially) toxin producing cyanobacterial genera is counted in quantitative terms, using an inverted microscope. Cell numbers from the quidck scan have to be converted to biovolumes, the reason being that biovolume has a closer relationship to cyanobacterial biomass and thus to toxin concentrations than cell counts.

As an alternative to biovolume, the protocol also allows for the use of fast measuring probes, which determine cyanobacterial biomass (cyanobacterial Chl-a) on basis of a fluorescence signal. In the Netherlands the BBE Moldaenke Fluoroprobe or AlgaeTorch are increasingly used for this purpose. Studies have indicated an acceptable correlation to biovolume, based upon microscopic counts and Fluoroprobe measurements. No correlation between the Fluoroprobe signal and microcystin was found, because of the variable presence of non-toxic strains and species (van der Oost, 2010).

The same study by van der Oost (2010) reports the results of a small interlaboraty comparison for quantifying cyanobacteria on basis of cell counts: three laboratories compared results for four samples composed of laboratory cultures (*Aphanizomenon, Anabaena, Planktothrix, Microcystis), and* standard deviations between their results amounted to up to 59% (for *Microcystis*).

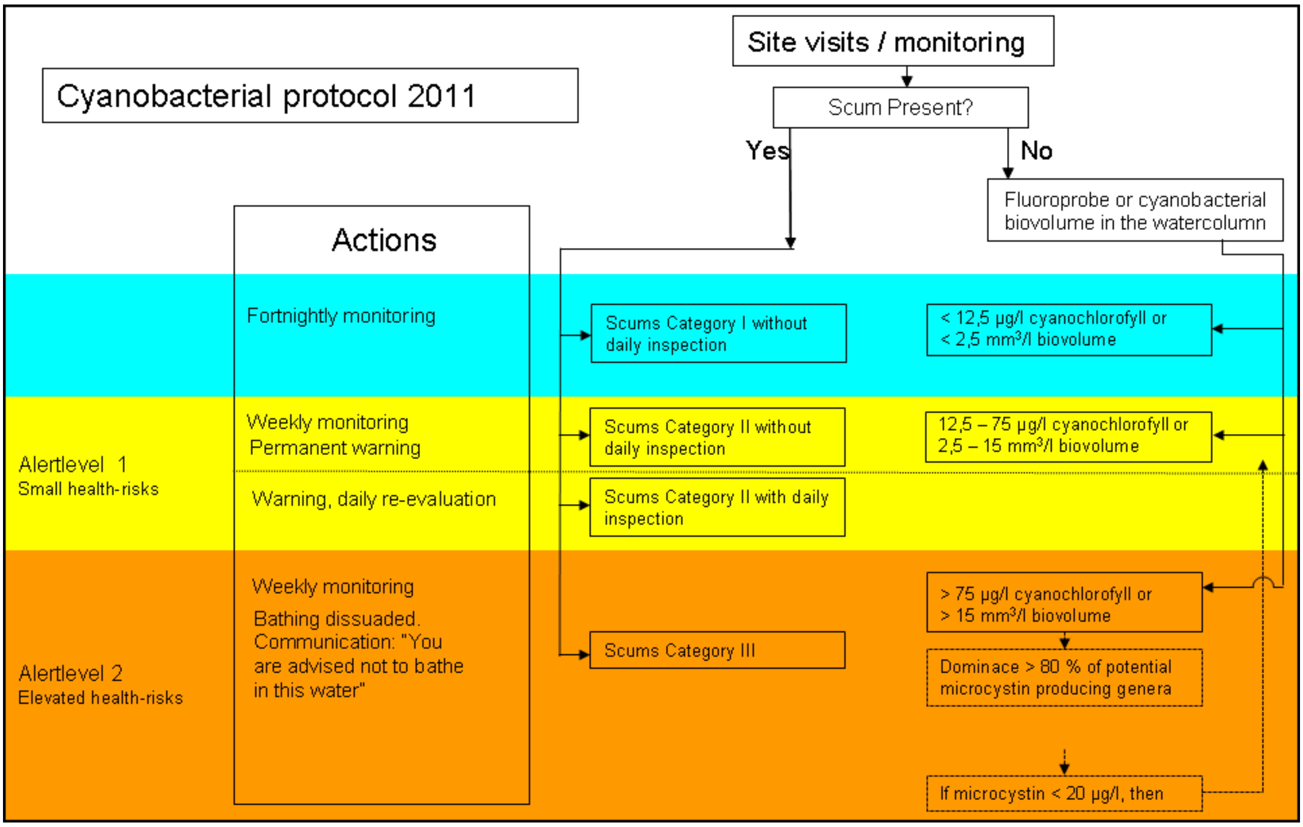
This shows that the uncertainties of cell counts will limit any correlation between this parameter and direct Fluoroprobe measurements, independently of the quality of the Fluoroprobe results. In spite of the uncertainties involved, both give a useful indication of the maximum toxin concentrations to be expected.

Alert-level 1 can be reached when cyanobacterial biomass is in the range 2.5 – 15 $mm^3\ L^{-1}$ of cyanobacterial biovolume, or 12.5 – 75 µg L^{-1} cyanobacterial chlorophyll-a (see Box 2 - Abundance). When biomass exceeds 15 $mm^3\ L^{-1}$ biovolume or 75 µg L^{-1} chlorophyll-a the warning is upgraded to alert-level 2. If microcystin producing genera dominate the cyanobacterial community (> 80 %) an optional microcystin measurement can follow and if microcystin levels are below 20 µg L^{-1} alert-level 2 may be lowered to level 1, however, taking into account that microcystin producing genera may also produce toxins other than microcystin.

Scums are distinguished in 3 categories (see Box 1 - Scums). Presence of category I scums does not lead to alert level 1. When these scums are more obvious and are classified as category II – scums, alert level 1 is issued: i.e. a general warning to the public. For supervised bathing waters this warning can be re-evaluated on a daily basis. When supervision is absent (which is the case for the majority of sites) the warning remains until the next weekly site visit. When scum layers reach category III, this then results in alert level 2: a negative advice discouraging bathing.

EU Bathing Water Directive

The European Bathing Water Directive (DIRECTIVE 2006/7/EC) was fully incorporated into Dutch legislation in December 2009. The Directive's objective is to protect the health of bathers



Permanent warning. Communication “Warning toxic blue green algae. Risk of skin irritation or intestinal problems”

Fig. 4: The ‘new’ current biomass-based protocol (2011)

in surface waters. In 2015, all bathing waters must have an acceptable minimum standard water quality. In accordance with this EU Directive, the Member States must draw up a so-called bathing water profile for each designated bathing location (Article 6). The bathing water profile is a description of the water system and the sources (or causes) of pollution in a wide sense. Criteria are presented in Annex III of the Directive including a section on the assessment of the possible cyanobacterial proliferation (Annex III, section 1C), which is relevant to Article 8 of the Directive.
Article 8:

1) When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks.

2) When cyanobacterial proliferation occurs and a health risk has been identified or presumed, adequate management measures shall be taken immediately to prevent exposure, including information to the public.

In the Netherlands a guide has been developed (RWS Waterdienst, 2008) to draw up the section of the bathing water profile which is relevant for cyanobacteria. In composing this guide, the authors sought to establish a link between the Bathing Water Directive with Dutch government policy and existing water-management policies in the Netherlands. This so-called cyanobacteria report is constructed in several steps, depending on the amount of available information and the presumed risk level. If one of the following bullet-points was 'true' at any time in the past five years (including the current monitoring season) there is a considerable risk that toxic bloom will re-occur in the coming five years:

- Presence of surface scums with detectable microcystin levels;
- microcystin concentration in the aqueous phase exceeding 20 µg L^{-1};
- disease/mortality of animals with a strong indication that cyanobacteria have contribu-ted;
- disease/mortality of swimmers with a strong indication that cyanobacteria have contribu-ted;
- the density of *Microcystis* was higher than 100,000 cells mL^{-1} [5], or belonged to the highest category of other semi quantitative methods ('very many', for example);

If the collected data indicate that toxic blooms are likely to re-occur in the coming five years, a further description of the system must be provided and appropriate checks should be carried out in the coming seasons. The aim of further description is to give better insight into the development of toxic blooms in the particular system at hand. This means describing the underlying causes/factors (hydrology, nutrients, vegetation and fish stocks) that contribute to the development of cyanobacterial blooms. Moreover, this should aid in designing the most effective measures to reduce the likelihood and consequences of cyanobacterial blooms in the system.

Discussion

Since the implementation of the EU Bathing Water Directive authorities, stakeholders and scientists in the Netherlands have been involved in the development of a cyanobacterial protocol, in an ongoing process with yearly adjustments of the protocol. The use of the 2010 version of the protocol (not shown in this chapter, however see below) has been evaluated by means of a survey in 2010 (Bijkerk et al., 2010). The survey was send out to 24 water boards, five regional services of the Directorate–General for Public Works & Water Management and all 12 provinces, together responsible for 480 inland bathing-sites. All but 10 % of these authorities applied the protocol in their management of cyanobacterial risks at bathing sites. Problems with the occurrence of cyanobacteria occurred in all 12 provinces, but the number of times action was undertaken varied greatly. In the two densely populated western provinces (North- and

[5] The limit of 100,000 cells per ml originates from the WHO recommendation in Chorus and Bartram, 1999 and WHO, 2003. See also the 'Blue-green algae in the new European Bathing Water Directive' report.

Box 1 Scums.

Cyanobacterial scums form when a community of floating cyanobacteria accumulates at the lake surface when the water-body is stable, i.e. not mixed. Especially strongly buoyant cyanobacteria like *Microcystis* spp. will dis-entrain easily from weak mixing and rise to the surface (Ibelings et al.., 1991). Scums are highly relevant for judging risks at bathing sites for the simple reason that intracellular cyanobacterial toxins get concentrated manifold in a scum. Surveys in the Netherlands regularly show microcystin concentrations well over 10,000 µg L^{-1} (Fig. 1) in scums, so that volumes as small as a single millilitre may exceed the acute tolerable intake for a child (see table 2 in Ibelings & Chorus, 2007). In the protocol (Fig. 4) a distinction is made between different scum categories, which vary in intensity. In category I there are cyanobacterial cells floating on the surface. In category II these cells form scum layers, but not yet completely covering several adjacent square meters. In category III the scum layer is closed over a greater surface, and scums are persistent; blue pigments from lysed cells may occasionally be visible. Specific instructions, including pictures (see examples below), have been developed for the assessment of scum intensity in the field. The formation and breakdown of surface scums of floating cyanobacteria is highly dynamic, and varies at a time scale of hours. Monitoring these dynamics with routine means is impossible. Ibelings et al. (2003) published a model which predicted the formation - timing and location - of scums in the open water of the large Lake IJsselmeer on basis of modelled cyanobacterial biomass, buoyancy and water column stability, and using the local/regional weather forecast. In the EWACS project (EWACS: Early Warning Against Cyano Scums) this model is being transformed into an online, fully operational tool for water-managers, and should in future provide weekly warning bulletins. It is proving difficult, however, to find the right balance between false-negatives (scums that were not properly predicted, but did show up, potentially putting the public at risk) and false-positives (scums that were predicted, but were not really present, jeopardising confidence in the model). We are currently strengthening our understanding of scum formation by different cyanobacteria by studying the process under controlled levels of turbulent mixing.

Scums of increasing intensity (left: category I, middle: category II and right: category III)

South Holland) warnings were issued 34, respectively 90 times in 2010; whilst bathing was dissuaded in 9, respectively 15 cases. A bathing prohibition was given 2, respectively 7 times. Incidence of warnings was also relatively high in the northern province of Groningen and southern province of Noord-Brabant, but relatively rare in most other provinces. One of the complaints in the survey about the 2010 protocol was ‘too many different alert-levels’. In the protocol 2011 the number of alert-levels has been reduced from four to two. A bathing prohibition (alert-level IV in the 2010 protocol) no longer is part of the protocol in 2011, but actually the possibility still exists, although it is no longer directly linked to specific monitoring results, but left to judgment of the local authorities. The difference between alert level 1 (warning) and alert level 2 (dissuade bathing) might be confusing for the public. It originates from the Dutch legal situation that a warning can be issued by anyone who ‘operates’ a bathing area. An advice against bathing can in the Netherlands only be issued by the responsible authorities. This makes an advice against bathing a slow and inflexible measure. In this case legal and practical aspects are muddling the ‘simplicity’ of the protocol.

Also, where the 2010 protocol still used cell counts, next to biovolume and cyano-chlorophyll-a, the current version has abandoned cell counts. In comparison to the 2002 version, a microcystin-based protocol (see Ibelings, 2005), the incidence of instances where bathing was dissuaded or even prohibited increased with the implementation of the new, cell density/ biomass based protocol in 2010 (Rijnland waterboard made a one on one comparison between old and new protocols based on historical data). Particularly the high cell counts of filamentous species (*Planktothrix* and *Aphanizomenon*) resulted in an increase of cases in which bathing was dissuaded, whilst in fact the stronger scum-formers (like *Microcystis*) seem to pose a bigger threat to human health (see Box 1). It is not clear yet how version 2011 will work out. It should be noted, however, that the use of the fluorescence based *in situ* biomass estimates in The Netherlands is still under debate, because of among probe variability, difficulties in calibration, interference of other compounds with the signal and possible occurrence of large false negative errors.

Apart from legal issues and complying with EU and national directives, the cyanobacteria protocol should provide a sound balance in several aspects:

- <u>Health risks</u>. Current risk assessment for recreational activities in lakes supporting cyanobacterial blooms is mainly based on knowledge of microcystins, and even here the step from microcystin concentrations to risk assessment is not trivial (see discussion in Ibelings & Chorus, 2007). Moreover we have limited knowledge of the presence and potential human risks of other cyanobacterial toxins in the Dutch situation. Additional complications are the high dynamics involved in scum formation (see Box 1) and the variability in toxin concentrations.
- <u>Promoting water recreation</u>. If there’s a warning, bathing is dissuaded or even prohibited, it harms the recreational and economical functioning of a recreational area and in particular the health gains from outdoor water recreation.
- <u>Uncertainties in monitoring</u>. Cell counts and correlated biovolumes are still common practice, although the reproducibility and representativeness of these counts can be rather poor, costs are high, and the procedure is time consuming. Relatively new techniques like fluorescent probes and DNA-techniques are promising but may have their own shortcomings (see Box 2). These ‘new’ techniques may serve as an early warning enabling authorities rapid communication with bathers, but should preferably be supported by an adequate risk assessment based on actual measurement of cyanotoxins (and not only microcystin).
- <u>Feasibility, complexity and costs</u>. Proper monitoring of the risks posed by cyanobacterial blooms is no simple task. For instance, not on all locations a daily check on scums is feasible. The continuation of side-by-side use of biovolume and Fluoroprobe combined with a quick scan – each with their own cut-off levels, in addition to the renewed 2011 version of the protocol, where there is a possibility to take decisions on basis of a microcystin-level < 20 µg L^{-1} (in this respect the 2011 protocol reverts to the situation in the old protocol, shown in Fig. 3), and the difficulty in monitoring scum dynamics still results in a fairly complex set of guidelines and decision-making processes.

- Communication. A proper protocol and the resulting measures should be explainable to the average bather to uphold credibility.

The subject of cyanobacteria and bathing waters calls for balanced approaches. The issue discussed is ‘Safety first’ or more responsibility (or freedom) for local authorities, stakeholders, and the bathers themselves? The general opinion among authorities and stakeholders seems to be that on the one hand the existing protocol is not good enough, but on the other hand that it is hard to drastically improve it with the knowledge and means available. That is also one aspect why the 2011 protocol allows a larger degree of freedom in monitoring and decision making compared to the 2010 protocol. The expectations are that the protocol will continue to have yearly updates and adaptations in response to increasing expert knowledge and new or better monitoring techniques.

Directions for future improvements of the protocol:

- The distinctions between the present scum classification and associated alert levels are considered rather subjective. Dense or less dense accumulation at the water surface does not necessarily imply differential intake of toxins by bathers. Risks should not be extrapolated from visual appearance of floating material without toxin analysis. For ex ample, category II scum material has revealed microcystin concentrations of 260 µg g^{-1} (c. 13 000 g L^{-1}), but also other cyanotoxins such as decarbamoylsaxitoxin (15 µg g^{-1}) and decarbamoylgonyautoxin-2 (26 µg g^{-1}) have been detected in category I and II scum material. In contrast, occasionally scum II and III material may contain hardly any cyanotoxins. Whether this disagreement between scum category and toxin content has a more general bearing remains to be studied.
- The 2011 protocol is based on indirect estimates of cyanobacterial risks based on abundance of presumed toxic and non-toxic species. In the future a more obvious, straightforward and reliable risk assessment through broad spectrum analysis of cyanotoxins could be applied. Nowadays powerful analytical tools that can detect and quantify a wide variety of cyanotoxins are available. In the 2011 protocol, the analysis of microcystin concentration is left as an option: in case the contribution of potentially microcystin producing species to the cyanobacteria community exceeds 80 % microcystins may be quantified and the results used as basis for a decision on warnings or discouragement of use. This 80% is a rather arbitrary cut-off that seems to lack scientific support.
- The protocol’s biomass based approach acknowledges the possible presence in cyanobacteria of cyanotoxins other than microcystins. At present only five potentially toxic genera (*Anabaena, Aphanizomenon, Microcystis, Planktothrix and Woronichinia),* which may indeed be the most common genera in The Netherlands, are recognised in the Bathing Water Directive. In spring 2011, however, three dogs died after playing on beaches where benthic toxic *Phormidium* was present. The assumption that blooms, scums and benthic mats of cyanobacteria other than the listed 5 genera are harmless is unjustified and seems in conflict with the basic principles of the Bathing Water Directive. The protocol should be adjusted at this point as the most rational approach is still the traditional monitoring of bloom development, where exceeding a biomass threshold level, regardless of species composition, is not only followed by a warning, but also by wide array cyanotoxin analysis.

Box 2 Abundance and biomass of cyanobacteria

The new cyanobacterial protocol shown in Fig. 4 focuses on cyanobacterial abundance and biomass rather than on microcystin concentrations. As a result, a discussion started on how to determine these parameters with reliable approaches. The disadvantage of this approach is that no clear relation between a biomass signal and toxins exists because all cyanobacteria contribute biomass or contain pigments, while not all cyanobacteria produce toxins. Thus, measures of cyanobacterial abundance or biomass always provide only an indication of the upper limit of cyanotoxin concentrations to expect. However, this information is very useful for risk assessment.
Different methods to estimate the abundance or biomass of cyanobacteria can be applied:

Chlorophyll a
Method based on spectrophotometric analysis after extraction of the pigments with e.g. ethanol. Advantage of this method is the ease of use. The parameter can be routinely measured and is therefore relatively fast and cheap. Disadvantage is that the method is not specific for cyanobacteria; all phytoplankton contains chlorophyll a. Extraction and analysis of specific cyanobacterial pigments like phycocyanin is less straightforward. However, the analysis of Chlorophyll a can be supplemented by qualitative microscopy, i.e. estimating (without cell counting, usually within 10 minutes or less) whether the phytoplankton seen in the microscopic image largely consists of cyanobacteria or not.

Fluorescence
Method based on different light absorption spectra (excitation wavelengths) of pigments in cyanobacteria, green algae and diatoms. Sensors can be applied in field situations. Advantage of the method is the instant results of the measurements, which can be obtained in-situ. The parameter can be routinely measured and is therefore relatively fast and cheap. Some sensors measure not only Chlorophyll-a, but also a pigment specific to cyanobacteria, i.e. phycocyanin and thus allow their distinction from other phytoplankton.

Microscopic counts / estimates
Method based on the analyses of concentrated cells in a sedimentation chamber, counted under an inverted microscope. Advantage of this method is the direct insight in the composition of the phytoplankton population in general and the possible abundance of potential toxin producing genera / species of cyanobacteria. Disadvantage is that the method is time consuming (and therefore relatively expensive) and a specialist job. The quantification is complicated by growth forms of cyanobacteria, like colonies, filaments or coiled or twisted growth forms. Cell counts between laboratories have been known to show a high level of variation.

Biovolume
Like above (microscopic counts), but in addition the cell numbers are multiplied by the cell volume of each genus / species to provide an estimate of biomass. Advantage of using this method is that the relative biomass of cyanobacteria in relation to other phytoplankton genera can be estimated. Cell sizes are incorporated in the measurements, dominance of phytoplankton genera and species can be easily evaluated.
Disadvantage of the method is comparable to the microscopic counts. Furthermore, this method introduces an additional parameter, with a certain distribution and variation and scope for potential errors, the volume of each cell.

DNA-copy detection
Method based on the extraction of DNA and the multiplication of certain gene targets. Targets are either on the phycocyanin genes or on the genes encoding for the toxin production. Advantage of the method is the objective way of quantification. The targets are genus specific. The method is relatively fast (result within hours), and sensitive, so that low concentrations of cells can be detected. Disadvantage is the need for a well-equipped laboratory. Besides, the relation between cell numbers and DNA copies can be a source of variation. Overestimation of the cell densities may occur. Detection of toxic genes may not relate to actual toxin production and concentrations.

- The cyanobacteria protocol provides not only a tool to authorities for official bathing sites, but is also being used at unofficial sites with cyanobacteria blooms, such as urban waters often with high recreational pressure. Furthermore, several, if not all, authorities use the monitoring data to prioritize water bodies in lake restoration programmes

Conclusion

The use of a standard cyanobacterial protocol by almost all waterboards and provinces is a great improvement compared to the situation in the last century where no guidelines existed and commonly no action was taken to protect the bathers. An additional bonus of the national protocol and of its evolution is that it has sparked much research and discussion and as such has improved our understanding of the risks involved. Oddly, perhaps, after many years of discussion and testing various protocols, the process of finding the optimal protocol has not yet been completed. The 2011 biomass-based cyanobacteria protocol reflects the Dutch approach of making compromises between the various stakeholders and authorities. It might seem tightrope walking between presumed health risks, sampling costs, economic and political aspects of recreational waters and legislation. However, the ongoing discussions and evaluations should ensure that the protocol will evolve to meet local, national and international demands.

Acknowledgements

The working group on cyanobacteria is thanked cordially for their constructive discussions which formed the basis for the new protocol.

References

Faasen E. & Lürling M. (2010). Inventarisatie van cyanotoxines op zwemlocaties in Nederlandse Rijkswateren. Report M354, Wageningen University.

Gons, HJ (1999). Optical teledetection of chlorophyll a in turbid inland waters. Env. Sci. Technol. 33, 1127-1132

Hoogenboezem W, Wagenvoort AJ & Blaauboer K (2004). The occurence of toxic cyanobacteria. In some Dutch surface waters used for the production of drinking water. RIWA report.

Gezondheidsraad (2001) Microbiële risico's van zwemmen in de natuur. Report Health Council of the Netherlands.

Hosper H, pot R & Portielje R (2011). Meren en plassen in Nederland: toestand, trends en hoe verder? H_2O 07-2011, 25-28.

Ibelings BW (2005). Risks of toxic cyanobacterial blooms in recreational waters: guidelines in the Netherlands. *In*: Chorus, I (ed) Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries. Federal Environmental Agency, Dessau, Germany, pp 85-91.

Ibelings BW, Chorus I. (2007) Accumulation of cyanobacterial toxins in freshwater seafood and its consequences for public health: a review. Env. Poll. 150, 177-192.

Ibelings BW, Mur LR & Walsby AE (1991). Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes. J. Plankt. Res. 13, 419-436

Ibelings BW, Vonk M, Los HFJ, van der Molen DT, Mooij WM (2003). Fuzzy modeling of cyanobacterial surface waterblooms: Validation with NOAA-AVHRR satellite images. Ecol. Appl. 13, 1456-1472

Kardinaal WEA & de Haan MW (2010). Blauwalgen in Nederland; Landelijk beeld 2005-2009. In opdracht van DG Water.

Kardinaal, WEA et al. (2009). Voorschrift quick scan telmethode – versie 2 (19 maart 2009). In opdracht van RWS Waterdienst.

Kardinaal WEA & van der Wielen P (2011). Knelpuntenanalyse Zwemwater. Report KWR 2011.044. In opdracht van Deltares.

Oost, van der R (2010). Toepassing van fluorescentie bij de beoordeling van de risico's van giftige blauwalgen. Stowa report 2010-18.

Oost, van der R (2009), Protocol voor het nemen van oppervlaktewatermonsters voor onderzoek van toxines van cyanobacteriën en voor de alyse van de algensamenstelling, Stowa rapport 2009 21a.

RWS Waterdienst (2008) Blauwalgen in het zwemwaterprofiel. Handreiking om het risico op proliferatie van toxische blauwalgen te beoordelen. Report MD-WR20070080.

Stowa (2000). Toxische blauwalgen in binnenwateren. Stowa report 2000-20

Vernooij, S., J. Ogier, M. Nederlof, C. Carpentier & W.E.A. Kardinaal (2011). “Cyanotoxines en Drinkwater”, Rapportnr BTO 2011. 115(s). KWR Watercycle Research Institute, Nieuwegein.

NEW ZEALAND: Regulation and Management of Cyanobacteria

Susie Wood[1], Wendy Williamson[2] and Alexander Kouzminov[3]

[1] Cawthron Institute, Private Bag 2, Nelson 7042. New Zealand. susie.wood@cawthro.org.nz
[2] ESR Ltd, Christchurch Science Centre, PO Box 29181, Christchurch 8540, New Zealand. wendy.williamson@esr.cri.nz
[3] Ministry for Primary Industries, PO Box 2526, Wellington, New Zealand, stasha@vodafone.co.nz

Introduction – Current state of knowledge

Cyanobacterial blooms have been a regular occurrence in many New Zealand lakes since the 1970s. However, they have become increasingly prominent in recent decades, possibly in association with anthropogenic action and eutrophication (Wood et al. 2008a). Planktonic cyanobacteria in New Zealand are now known to produce the following cyanotoxins: microcystins, nodularin, anatoxin-a, cylindrospermopsin, deoxycylindrospermopsin and saxitoxins (Table 1).

Table 1: Summary of known toxin-producing cyanobacterial species in New Zealand

Cyanobacteria genus/species	Cyanotoxin
Anabaena lemmermannii	Anatoxin-a*[1], microcystins[#2]
Anabaena planktonica	Saxitoxins*[3]
Aphanizomenon issatschenkoi	Anatoxin-a [4]
Cylindrospermopsis raciborskii	Cylindrospermopsin*[5], deoxycylindrospermopsin*[5]
Microcystis spp.	Microcystins[6]
Nodularia spumigena	Nodularin[7]
Nostoc commune	Microcystins*[1]
***Oscillatoria* sp.**	Anatoxin-a*[8],microcystins*[8]
Phormidium autumnale	Anatoxin-a*[9,10], homoanatoxin-a*[9]
***Planktothrix* sp.**	Microcystins[11]
Scytonema* cf. *crispum	Saxitoxins[12]

*This result was obtained from testing environmental samples dominated by this species, [#] based on sequencing of a *mcy*E gene from an environmental sample. Benthic species are in bold type. 1. Wood et al. 2006; 2. Wellar 2011; 3. Kouzminov et al. 2007; 4. Wood et al. 2007a, Selwood et al. 2007; 5. Stirling and Quilliam 2001, Wood and Stirling 2003; 6. Wood et al. 2008b; 7. Carmichael et al. 1988; 8. Hamill, 2001; 9.Wood et al. 2007b; 10. Heath et al. 2010; 11. Wood et al. 2010; 12. Smith et al. 2011.

Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and are found in a wide range of water-quality conditions, including oligotrophic waters (Biggs and Kilroy 2000). The most common mat-forming benthic cyanobacteria genus in New Zealand is *Phormidium*. During stable flow conditions *Phormidium* mats can proliferate, at times forming expansive black-brown leathery mats across large expanses of river substrate (**Figure 1**).

Figure 1: *Phormidium* mats in the Waipoua River (North Island, New Zealand).

Dog deaths associated with the consumption of benthic cyanobacteria have become increasingly common around New Zealand (Hamill 2001; Wood et al. 2007). In most instances these deaths have been associated with the presence of the neurotoxins anatoxin-a and/or homoanatoxin-a (Wood et al. 2007b), and this often results in the rapid death of the animal. The production of microcystins by benthic cyanobacteria (*Nostoc* sp. and *Pankthothrix* sp.) in New Zealand has now been confirmed (Wood et al. 2006; Wood et al. 2010), and in at least one instance a dog death was caused by microcystins (Wood et al. 2010). There are several anecdotal reports of humans becoming sick after participating in swimming in rivers with extensive benthic mats. Recently saxitoxins were identified in benthic *Syctonema* in multiple lakes in the South Island (Smith et al. 2011, Smith unpub. data). Known benthic toxin-producing species are given in Table 1.

Despite this recent research we believe that there are still many toxin-producing cyanobacteria that have not been identified in New Zealand. It is therefore recommended that when a species is known to be a toxin producer elsewhere in the world, it should be regarded as potentially toxic in New Zealand until proven otherwise.

In New Zealand, 75% of our drinking water (in terms of total population served) comes from surface water supplies and our freshwater bodies are used extensively for a range of recreational activities. Cyanobacterial blooms and benthic proliferation can have significant economic, health and social impacts. This paper summarizes the documents that guide the regulation and management of cyanobacteria in water bodies used for recreation and drinking water in New Zealand.

Recreational Guidelines

New Zealand guidelines for managing cyanobacterial risk in water used for recreational purposwere developed in 2009 (Ministry for the Environment and Ministry of Health 2009). The guidelines are available under: http://www.mfe.govt.nz/publications/water/guidelines-for-cyanobacteria/index.html. Their aim was to help agencies responsible for managing cyanobacteria in recreational use water bodies develop monitoring protocols appropriate for local conditions and circumstances and to encourage the adoption of a nationally unified approach to monitoring. The guidelines set out a monitoring framework for establishing the public health risk from cyanobacteria in lakes (mainly planktonic cyanobacteria) and rivers (mainly benthic cyanobacteria). A multi-tiered framework is used that incorporates a monitoring and management action sequence which regulators can use for a graduated response to the onset and progress of a cyanobacterial bloom or benthic proliferation. They can also be applied when responding to an unexpected cyanobacterial event. A major change (for planktonic cyanobacteria only) from current standard practice in New Zealand was the use of estimates of biovolume as thresholds in the alert-level framework instead of cell concentrations. This is in response to a recent increase in the reported high concentrations of picocyanobacteria in some New Zealand water bodies.

Toxic benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and continue to cause many management issues. To our knowledge the inclusion of thresholds for benthic cyanobacteria in the New Zealand guidelines is the first attempt to develop quantitative guidelines for benthic cyanobacteria internationally. The benthic guidelines include a three-tier alert level framework that uses cyanobacterial abundance and the occurrence of mats visibly detaching from the substrate to determine the alert level status.

The guidelines also include sections on: identifying high-risk water bodies, how to collected representative samples and undertake site surveys (including for benthic cyanobacteria), correct sample storage, general information on cyanotoxins and a list of laboratories that offer cyanobacterial identification and cyanotoxin analysis. They also give examples for text of media releases for water bodies affected by cyanobacteria, text for warning signs and sampling forms. Photographs of cyanobacteria blooms and benthic mats are provided to assist samplers with minimal cyanobacterial expertise to collect the correct samples. This has proved particularly useful for benthic cyanobacteria.

Planktonic cyanobacteria

The alert-level framework for planktonic cyanobacteria is given in Decision Chart 1. Three levels of monitoring have been identified: surveillance (green mode), alert (amber mode) and action (red mode). The cell concentrations, or biovolumes that define the levels apply to samples of the recommended type (i.e., composite 50 cm hose-pipes) that are taken at a representative location(s) in the water body (i.e. the likely or designated recreational areas). The guidelines include a section on selection of sampling sites and guidance on how to collect representative samples.

In recent years there has been an increase in reports of pico-cyanobacteria (< 2 µm; e.g., *Aphanothece* sp. and *Aphanocapsa* sp.) in some regions of New Zealand (e.g., the Rotorua lakes, North Island), and basing health warning solely on cell counts has in some instances resulted in their unnecessary issuing. Therefore the guidelines now rely on biovolumes. As it is time consuming and impractical to measure and calculate a biovolume for every individual species in routine counting, a standardized species lists with fixed biovolumes of common problematic species is given in the guidelines and additional information on how to measure and calculate biovolumes for species not in this list is provided.

Decision Chart 1: Alert-level framework for planktonic cyanobacteria

Alert level	Actions
Surveillance (green mode) *Situation 1:* The cell concentration of total cyanobacteria does not exceed 500 cells/mL[a] *Situation 2:* The biovolume equivalent for the combined total of all cyanobacteria does not exceed 0.5 mm^3/L	• Undertake weekly or fortnightly visual inspection[b] and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn.
Alert (amber mode) *Situation 1:* Biovolume equivalent of 0.5 to < 1.8 mm^3/L of potentially toxic cyanobacteria; **or** *Situation 2*[c]***:*** 0.5 to < 10 mm^3/L total biovolume of all cyanobacterial material.	• Increase sampling frequency to at least weekly.[d] • Notify the public health unit. • Multiple sites should be inspected and sampled
Action (red mode) *Situation 1:* ≥ 12 µg/L total microcystins; **or** biovolume equivalent of ≥ 1.8 mm^3/L of potentially toxic cyanobacteria; **or** *Situation 2*[c]***:*** ≥ 10 mm^3/L total biovolume of all cyanobacterial material; **or** *Situation 3*[e]***:*** cyanobacterial scums consistently present.	• Continue monitoring as for alert (amber mode).[d] • If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins.[f] • Notify the public of a potential risk to health.

a. A cell count threshold is included at this level because many samples may contain very low concentrations of cyanobacteria and it is not necessary to convert these to a biovolume estimate.
b. In high concentrations planktonic cyanobacteria are often visible as buoyant green globules, which can accumulate along shorelines, forming thick scums. In these instances, visual inspections of water bodies can provide some distribution data. However, not all species form visible blooms or scums; for example, dense concentrations of *Cylindrospermopsis raciborskii* and *Aphanizomenon issatschenkoi* are not visible to the naked eye.
c. This applies where high cell densities or scums of 'non-toxigenic' cyanobacteria taxa are present (i.e., where the cyanobacterial population has been tested and shown not to contain known toxins).
d. Bloom characteristics are known to change rapidly in some water bodies, hence the recommended weekly sampling regime. However, there may be circumstances (e.g., if good historical data/knowledge is available) when bloom conditions are sufficiently predictable that longer interval sampling is satisfactory.
e. This refers to the situation where scums occur at the recreation site for more than several days in a row.
f. Cyanotoxin testing is useful to provide further confidence on potential health risks when a health alert is being considered; enable the use of the action level 10 mm^3/L biovolume threshold (i.e., show that no toxins are and show that residual cyanotoxins are not present when a bloom subsides).

Based on animal toxicological studies, a threshold for exposure to microcystins via ingestion has been developed for the action level (red mode) – situation 1. The value of 12 µg/L total microcystins is extrapolated from animal experiments on the basis of various assumptions about exposure, and it includes uncertainty factors. The tolerable daily intakes (TDIs) for microcystins are calculated based on data from two separate animal toxicological studies: a 13-week mouse study (Fawell et al, 1999) conducted with purified microcystin-LR via gavage, and a 44-day pig study (Falconer et al, 1994). A full description of the derivation of the microcystin threshold is given in Appendix 2 in the guidelines.

Benthic cyanobacteria

The health risks associated with benthic cyanobacteria are less well known than the risks for their planktonic counterparts. To our knowledge the New Zealand guidelines are the first attempts to develop quantitative guidelines internationally. It is acknowledged that the threshold values given in the guidelines are based on preliminary research, and it is anticipated that these will require further refining as knowledge and monitoring tools improve.

Under certain environmental conditions, or as benthic mats become thicker (and bubbles of oxygen gas become entrapped within them), mats will detach from the substrate and may accumulate along river edges. During these events the risk to human and animal health is increased due to the accessibility of the cyanobacterial mats to river users. The highest risks to water users are likely to be via ingestion of water containing detached mats and/or direct contact with these cyanobacterial mats. The risk associated with both types of contact is likely to rise as the abundance and/or number of detachment events increases. Thus the guidelines use cyanobacterial abundance and the occurrence of mats visibly detaching from the substrate to determine the alert level status.

Recently extracellular toxins (toxins in the water column) have been detected in a river with extensive benthic mat coverage (Wood et al. 2011). This confirms that toxins can be released from mats and that the toxins pose a potential risk when just the water is consumed.

The alert-level framework for benthic cyanobacteria is given in Decision Chart 2.

A correlation between benthic cyanobacterial mat abundance, water temperature and a lack of ’flushing flow’ conditions has been observed in some rivers (Milne and Watts 2007; Wood, et al. 2007b; Heath et al. 2011). In some instances, the length of time since a flushing flow event can be used as an early warning of elevated risk of benthic cyanobacterial proliferations. However, the flow velocity required to shift cyanobacteria from the river bed will vary depending on factors such as the river bed substrate type and size. For example, a river with a sandy substrate will require a markedly smaller flow to flush benthic cyanobacteria compared to a river with a large cobble substrate. In addition, the length of time required for cyanobacteria to proliferate following a flushing flow event will vary. So, although there is no 'one size fits all', on a regional basis experts could use periphyton coverage records, flow data and local knowledge to develop warning systems for cyanobacterial proliferation risk.

A correlation between benthic cyanobacterial mat abundance, water temperature and a lack of ’flushing flow’ conditions has been observed in some rivers (Milne and Watts 2007; Wood, et al. 2007b; Heath et al. 2011). In some instances, the length of time since a flushing flow event can be used as an early warning of elevated risk of benthic cyanobacterial proliferations. However, the flow velocity required to shift cyanobacteria from the river bed will vary depending on factors such as the river bed substrate type and size. For example, a river with a sandy substrate will require a markedly smaller flow to flush benthic cyanobacteria compared to a river with a large cobble substrate.

In addition, the length of time required for cyanobacteria to proliferate following a flushing flow event will vary. So, although there is no 'one size fits all', on a regional basis experts could use periphyton coverage records, flow data and local knowledge to develop warning systems for cyanobacterial proliferation risk.

Decision Chart 2: Alert-level framework for benthic cyanobacteria

Alert level[a]	**Actions**
Surveillance (green mode) Up to 20% coverage[b] of potentially toxigenic cyanobacteria attached to substrate.	• Undertake fortnightly surveys between spring and autumn at representative locations in the water body where known mat proliferations occur and where there is recreational use.
Alert (amber mode) 20−50% coverage of potentially toxigenic cyanobacteria attached to substrate.	• Notify the public health unit. • Increase sampling to weekly. • Recommend erecting an information sign that provides the public with information on the appearance of mats and the potential risks. • Consider increasing the number of survey sites to enable risks to recreational users to be more accurately assessed. • If toxigenic cyanobacteria dominate the samples, testing for cyanotoxins is advised. If cyanotoxins are detected in mats or water samples, consult the testing laboratory to determine if levels are hazardous.
Action (red mode) *Situation 1:* Greater than 50% coverage of potentially toxigenic cyanobacteria (see Table 1) attached to substrate; **or** *Situation 2:* up to 50% where potentially toxigenic cyanobacteria are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as the river level drops.	3) Immediately notify the public health unit. 4) If potentially toxic taxa are present then consider testing samples for cyanotoxins. 5) Notify the public of the potential risk to health.

a The alert-level framework is based on an assessment of the percentage of river bed that a cyanobacterial mat covers at each site. However, local knowledge of other factors that indicate an increased risk of toxic cyanobacteria (eg, human health effects, animal illnesses, prolonged low flows) should be taken into account when assessing a site status and may, in some cases, lead to an elevation of site status (eg, from surveillance to action), irrespective of mat coverage.

b A description on how to undertake a site survey is provided in the guidelines.

The guidelines provide an example of an automated river flow-based warning system for benthic cyanobacterial proliferation risk that is currently used regions of New Zealand. The automated river warning system for cyanobacterial proliferation risk has two complementary alert levels based on flow conditions (see Decision Chart 3).

Drinking-water Standards, Water Safety Plans and Guidelines

The two key tools used by the New Zealand Ministry of Health support the provision of safe drinking water in New Zealand are the *Drinking-water Standards for New Zealand* 2005 (Revised 2008) (*DWSNZ*) and public health risk management plans (PHRMPs), sometimes called water safety plans in other countries and by WHO. A third tool is the *Guidelines for Drinking-water Quality Management for New Zealand* (*DW-Guidelines*). This third document contains information to augment and further explain the requirements of the *DWSNZ* and how they can be met.

Decision Chart 3: Flushing flow alert framework for benthic cyanobacteria

Flow alert level	Monitoring suggestion
Alert mode 1 No flushing flow[1] for 2 weeks.[2]	Survey known problematic sites to assess cyanobacterial cover, as per surveillance level (green mode)
Alert mode 2 No flushing flow for 2 weeks and river flows are low (set at lowest 10th percentile flow for each river).[3]	Increase frequency (e.g., to weekly) of surveys of known problematic sites to assess cyanobacterial cover, as per alert level (amber mode)

[1] In most parts of New Zealand a flushing flow is defined as three times the median flow.

[2] This value is specific to certain rivers and was determined following a summer of monitoring to be an appropriate (and conservative) duration.

[3] The justification for a low flow alarm is that water temperatures may be elevated (promoting algal growth), and any cyanobacterial growths may become exposed or near exposed at the river edges.

Together, the *DWSNZ*, *PHRMPs* and *DW-Guidelines* provide a comprehensive multi-barrier control approach to water supply management, complemented by monitoring that will trigger remedial action where necessary. The aim of this management system is to provide a high degree of confidence in the safety of the water for all drinking-water supplies across New Zealand.

Each of the tools is discussed below with respect to the part each plays in assessing and managing the risk of toxic cyanobacteria in drinking-water supplies.

Drinking-water Standards[6]

The *DWSNZ* have three functions, they

- establish maximum acceptable values (MAVs) or water quality standards
- set out compliance criteria and reporting requirements
- identify remedial actions that must be taken in the event that monitoring shows a MAV to have been exceeded.

To minimize monitoring costs without compromising public health, determinands (the constituents being monitored in the water) are assigned to one of four priority classes (Priority 1 to Priority 4) for each water supply. The basis of the classification is the risk presented to public health, and depends on the consequences of exposure to the determinand and the likelihood of that exposure. For compliance with the *DWSNZ*, only Priority 1 and Priority 2 determinands need to be monitored.

The Priority 1 class is the highest priority class and contains the determinands *E. coli* and protozoa. Microorganisms are ubiquitous in the environment, and consequently all water supplies are assumed to carry the threat of infectious microorganisms and are therefore required to be monitored for Priority 1 determinands. On other hand, Priority 2 determinands are supply-specific and require monitoring when known to be present at health significant levels (50% of the MAV). Although the Priority 2 class contains chemical (including cyanotoxins),

[6] http://www.health.govt.nz/publication/drinking-water-standards-new-zealand-2005-revised-2008-0

microbiological and radiological determinands, in practice to-date, Priority 2 determinands have all been chemical. But unlike other Priority 2 (chemical) contaminants, cyanotoxins can increase in concentration rapidly as the source fluctuates with the environmental conditions.

When cyanotoxins are present in a distribution zone at more than 50% of their provisional maximum acceptable values (PMAVs), they are assigned to that water supply as Priority 2 determinands. For cyanotoxins determinands, PMAVs are used rather than maximum acceptable values (MAVs). With the exception of microcystins the cyanotoxins in the DWSNZ are not yet included in WHO guidelines. For consistency all cyanotoxins determinands are PMAVs irrespective of the WHO values. Where WHO values are not available the PMAV is informed by current scientific understanding, albeit limited in some instances.

Drinking Water Assessors (operating under the auspices of the Ministry of Health), on the basis of data collected by the water supplier, have responsibility for determining when a cyanotoxin should be assigned as a Priority 2 determinand to a supply distribution zone. To date, there have been no water supplies to which cyanotoxins have been assigned as Priority 2 determinands, with the accompanying requirement for monitoring in the *distributed* water. There have been cases when the *source* water has contained cyanotoxins at more than 50% of their PMAVs (Table 2), and in these cases, the *PHRMP* protocols have, so far, been effective at mitigating the impact on water in the *distribution zone* so the problem has been managed well before the consumer.

Table 2: Provisional Maximum Acceptable Values (PMAVs) for cyanotoxins in the *DWSNZ*.

Toxin	PMAVs (mg/L)
Anatoxin	0.006
Anatoxin-a(S)	0.001
Cylindrospermopsin	0.001
Homoanatoxin-a	0.002
Microcystin MC-LR toxicity equivalents	0.001
Nodularin	0.001
Saxitoxins (as STX equivalent)	0.003

Public Health Risk Management Plans for Water Supplies

PHRMPs encourage the use of risk management principles from the water’s source, through treatment and up to the consumer’s property boundary, so that monitoring to demonstrate compliance with drinking-water standards is not the only means by which consumers are protected from waterborne contaminants. *PHRMPs* are prepared by a water supplier and approved by a Drinking Water Assessor. They are expected to be “living documents” that are regularly updated.

For cyanobacteria-cyanotoxins, a water supply’s *PHRMP* should emphasize managing the source water to prevent the development of cyanobacteria blooms, rather than steps to try to mitigate the consequences of bloom development. As knowledge about cyanobacteria dynamics in a water source changes, the PHRMP should be changed to reflect the new knowledge.

A matrix-based approach is used in *PHRMPs* which provides a set of modules for each individual stage of a generalized drinking-water supply system, including barriers, preventive measures and corrective actions against water contamination by cyanotoxins.

The Ministry of Health has prepared various resources to help develop *PHRMPs*, including information for *Small Drinking-water Supplies*, *A Framework on How to Prepare and Develop Public Health Risk Management Plans for Drinking-water Supplies*, various training material and guides on the specific components of a water supply. (http://www.moh.govt.nz/drinkingwater)

The key steps in developing a *PHRMP* for an individual water supply include:

a) Stage – Identification of the barriers to cyanotoxin contamination (Checklist of barriers present)

A water supplier should identify the barriers to cyanobacterial contamination of the supply. Water quality is best protected by having several barriers to the entry of cyanotoxins. These barriers may include the following examples, e.g., stop contamination of raw water with nutrients; removal of cyanobacterial cells by water treatment; destruction or removal of cyanotoxins. The barriers need to:

- prevent cyanobacterial cells and cyanotoxins from entering the treatment plant, that is, preventing bloom development;
- remove cyanobacterial cells and cyanotoxins from the water;
- destroy cyanotoxins in the water.

The multi-barrier approach for safe drinking-water continues into the PHRMP preparation where the water supplier uses the ‘Barriers to Contamination’ module (guide)[7] to identify the barriers in place to consumers from cyanobacteria and cyanotoxins. The guide helps recognise which barriers are in place, and also provides instruction on what actions or supply elements contribute to these barriers.

b) Stage – Estimating Risk (Risk Information Table)

This module includes guides that can help to identify (a) possible causes of each event, (b) preventive measures to avoid each event, and (c) corrective actions to use if preventive measures fail. A matrix for estimating risk is based on five categories of likelihood (i.e., rare, unlikely, possible, likely, almost certain) and five of consequence (i.e., insignificant, minor, moderate, major, catastrophic), including their detailed descriptions.

[7] http://www.health.govt.nz/publication/framework-how-prepare-and-develop-public-health-risk-management-plans-drinking-water-supplies

c) Stage – Preparing Contingency Plans (Set of Contingency Plans for each supply element)

A supplier should look through the *Contingency Plans* provided in the guide for each *supply element*, and decide which may be useful in their situation. The *Contingency Plans* help to identify the reasons for the failure of the system.

d) *Stage – Performance Assessment* (Set of instructions for review of the performance of the Plan)

The *PHRMP Performance Assessment* section of the guides can be used as a basis for preparing instructions for reviewing the operation of a supplier's overall Public Health Risk Management Plan.

e) Stage – Decision on communication policy and needs

A supplier should identify and record to whom reports concerning the management of public health risk for the supply need to be made, what information they are to receive and how often.

The above-mentioned planning will provide public confidence regarding strict control of drinking-water safety in respect to cyanobacteria and cyanotoxins in New Zealand.

Guidelines for Drinking-water Quality Management

Comprehensive information and guidance about cyanobacteria and cyanotoxins in drinking-water sources and supplies is in the *DW-Guidelines*.

The *DW-Guidelines* provide details on how to mitigate and prevent potentially toxic cyanobacterial blooms. They provide details on sampling and enumeration of cyanobacteria and the characterisation of cyanotoxins. The principle of the *DW-Guidelines* is to ensure barriers that prevent cyanotoxins entering the drinking-water supply are working properly. This is done by:

- identification of the causes of bloom formation
- providing information on health significance of cyanotoxins
- use of preventive measures to reduce the risk associated with cyanotoxins
- monitoring of water quality to assess the effectiveness of the preventive measures
- responding to failures in preventive measures, which are shown by monitoring results or routine operational checks.

An alert level framework is outlined in the *DW-Guidelines*. This is a matrix-based monitoring and management action sequence that water suppliers use to provide a graduated response to the onset and progress of a cyanobacterial bloom event. Criteria are based on biovolumes or cell counts in the source water and/or cyanotoxins in the distribution zone. A flow chart in the *DW-Guidelines* indicates trigger levels and some management options, including:

- preventive and remedial measures
- treatments to removal of cyanobacterial cells and cyanotoxins
- management of the source water or reservoir.

Where a *PHRMP* indicates a drinking-water supply is susceptible to cyanobacteria blooms, routine monitoring of cyanobacteria is suggested at a minimum frequency of fortnightly for algal cell counts and identification of the organisms, if inspection of the source water shows:

- the development of scum on the surface or
- the development of algal growths just below the surface or
- any other evidence of algal growth.

The *DW-Guidelines* are currently being updated and a revised document is expected 2013.

Acknowledgements

We thank Dr Chris Nokes (Institute of Environmental Science & Research Ltd (ESR)) and David Ogilvie (Ministry of Health) for comments during preparation of the drinking-water section of this paper. We thank the Ministry of Health and the Ministry for the Environment for developing national guidelines and standards, and for their support during the preparation of this paper.

References

Biggs BJ, Kilroy C. 2000. Stream periphyton monitoring manual. Christchurch: National Institute of Water and Atmospheric Research. Christchurch, New Zealand. 229 p.

Carmichael WW, Eschedor JT, Patterson GML, Moore RE. 1988. Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium *Nodularia spumigena* Mertens emend. L575 from New Zealand. *Applied and Environmental Microbiology* 54: 2257−-2263.

Falconer I, Burch M, Steffensen D, Choice M, Coverdale O. 1994. Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Journal of Environmental Toxicology and Water Quality* 9: 131–139.

Fawell JK, Mitchell RE, Everett DJ, Hill RE. 1999. The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR. *Human Experimental Toxicology* 18: 162–167

Hamill KD. 2001. Toxicity in benthic freshwater cyanobacteria (blue-green algae): first observations in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 35: 1057−1059.

Heath M, Wood SA, Ryan K. 2010. Polyphasic assessment of fresh-water benthic mat forming cyanobacteria isolated from New Zealand. *FEMS Microbiology* 73: 95-109.

Heath M, Wood SA, Ryan K. 2011. Spatial and temporal variability in *Phormidium* abundance and anatoxin production in the Hutt River, New Zealand. *Aquatic Microbial Ecology* In Press.

Kouzminov A, Ruck J, Wood SA. 2007. New Zealand risk management and regulatory approach for toxic cyanobacteria in drinking water. *Australian and New Zealand Journal of Public Health* 31: 275–281.

Milne J, Watts L. 2007. *Toxic Benthic Cyanobacteria Proliferations in Wellington's Rivers in 2005/06*. Wellington: Greater Wellington Regional Council.

Ministry for the Environment and Ministry of Health. 2009. New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters – Interim guidelines. Prepared for the Ministry for the Environment and the Ministry of Health by SA Wood, DP Hamilton, WJ Paul, KA Safi, WM Williamson. Wellington: Ministry for the Environment. 89 p.

Smith F, Wood, SA, Ginkel, Rv, Broady P, Gaw S. 2011. First report of saxitoxin production by a species of the freshwater benthic cyanobacterium, *Scytonema* Agardh. *Toxicon* 57: 566–573

Stirling DJ, Quilliam MA. 2001. First report of the cyanobacterial toxin cylindrospermopsin in New Zealand. *Toxicon* 39: 1219−1222.

Wellar DI. 2011. Detection, identification and toxigenicity of cyanobacteria in New Zealand lakes using PCR-based methods. *New Zealand Journal of Marine and Freshwater Research. In Press.*

Wood SA, Stirling DJ. 2003. First identification of the cylindrospermopsin producing cyanobacteria *Cylindrospermopsis raciborskii* in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37: 821−828.

Wood SA, Stirling DJ, Briggs LR, Sprosen J, Holland PT, Ruck JG, Wear RG. 2006. Survey of cyanotoxins in New Zealand waterbodies between 2001 and 2004. *New Zealand Journal of Marine & Freshwater Research* 40: 585-595.

Wood SA, Rasmussen JP, Holland PT, Campbell R, Crowe ALM. 2007a. First report of the cyanotoxin anatoxin-a from *Aphanizomenon issatschenkoi* (Cyanobacteria). *Journal of Phycology* 43: 456−465.

Wood SA, Selwood AI, Rueckert A, Holland PT, Milne JR, Smith KF, Smits B, Watts LF, Cary CS. 2007. 2007b. First report of homoanatoxin-a and associated dog neurotoxicosis in New Zealand. *Toxicon* 50: 292–301.

Wood SA, Jentzsch K, Rueckert A, Hamilton DP, Cary SC. 2008a. Hindcasting cyanobacterial communities in Lake Okaro with germination experiments and genetic analyses. *FEMS Microbiology Ecology* 67: 252−260.

Wood SA, Rhodes L, Adams S, Adamson J, Smith, K, Smith J, Tervit R, Cary SC. 2008b. Maintenance of cyanotoxin production by cryopreserved New Zealand culture collection cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 42: 277−283.

Wood SA, Holland PT, MacKenzie, LA. 2011. Development of Solid Phase Adsorption Toxin Tracking (SPATT) for monitoring anatoxin-a and homoanatoxin-a in river water. *Chemosphere* 82: 888-894.

POLAND: Management and Regulation of Toxic Cyanobacteria

Joanna Mankiewicz-Boczek[1,2], Justyna Kobos[3], Ilona Gągała[2,1], Katarzyna Izydorczyk[1], Anna Toruńska[3]

[1] European Regional Centre of Ecohydrology u/a UNESCO, PAS, Tylna 3, 90-364 Łódź, Poland; j.mankiewicz@erce.unesco.lodz.pl; www.erce.unesco.lodz.pl
[2] Department of Applied Ecology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland
[3] Laboratory of Biochemical Ecology of Microorganisms, Department of Marine Biology and Ecology, University of Gdańsk, Al. Marszałka Piłsudskiego 46, 81-378 Gdynia, Poland, www.sinice.pl; www.facebook.com/pages/Sinice-Pracownia-Ekologii-Biochemicznej-Mikroorganizmów

Occurrence of cyanotoxins-producing cyanobacteria in Poland

Fresh water bodies

Studies conducted by Polish researchers indicate that the occurrence of toxic cyanobacteria in Poland is of concern for human health. Regular blooms with a dominance of *Planktotrhrix agardii* with the highest microcystins (MCs) concentrations of 212.7 µg/L, 65.2 µg/L and 173.8 µg/L were reported in water bodies studied in the Northern, Western and Eastern parts of Poland, respectively (e.g. Grabowowska, Pawlik-Skowrońska 2008; Mazur-Marzec et al. 2010, Mankiewicz-Boczek et al. 2011a). The dominance of *Microcystis aeruginosa* with maximum concentration of microcystins of 5.8 µg/L was observed in selected water bodies in Central Poland (e.g. Izydorczyk et al. 2008; Mankiewicz-Boczek et al. 2011b). In turn, the highest concentration of microcystins of 305 µg/L in the heavy bloom dominated by *M. aeruginosa* was measured in Northern Poland (Mazur-Marzec et al. 2008). Polish water bodies also contain significant amounts of other toxic cyanobacterial species. In lakes of Northern Poland *Anabaena* and *Aphanizomenon* species including a Scandinavian species, *A. skujae* were observed (Mazur et al. 2003, Kobos at al. 2005; Mankiewicz et al. 2005). The highest concentration of MCs (26 mg/L) was determined during mono-species bloom of *Anabaena lemmermannii* (Kobos 2007, Kobos et al. 2007). Neurotoxin-producing cyanobacteria were observed in Northern and Southeastern Poland (e.g. Pawlik-Skowrońska et al. 2004; Sierosławska et al. 2010). Analysis of surface scum samples during *Anabaena circinalis* dominance revealed the presence of anatoxin-a at a high concentration of 1.04 mg/L. Cylindrospermopsin (with maximum 1.8 µg/L) has been reported from Western Poland, which might be associated with the *Aphanizomenon gracile* occurrence (Kokociński et al 2009; Mankiewicz-Boczek et al. 2012). Additionally, although none of the known toxins were detected during *Gloetrichia echinulata* occurrence in the lakes of Northern Poland, skin irritations were reported by local people (Mazur-Marzec et al. 2010, personal communication).

Coastal zone

In the coastal waters of the Gulf of Gdańsk *Nodularia spumigena* and *Aphanizomenon flos-aquae* are the main bloom-formers (e.g. Pliński 1996; Mazur-Marzec et al. 2006; Mazur- Mazur-Marzec, Pliński 2009). Hepatotoxic nodularin is detected each summer, with the highest concentration of 50 mg/L determined in the *N. spumigena* bloom samples collected at the bathing site in Gdynia (Mazur-Marzec et al. 2010). Apart from nodularin, in the samples where *Microcystis sp.* made up about 10% of the phytoplankton population, microcystins were detected. The highest concentration of MCs reached 2.5 µg/g d.w. (Mazur et al. 2003). In the samples were *Anabaena* spp. made up about 20% of the phytoplankton population, anatoxin-a was found (Mazur et al. 2003).

Management

Monitoring of cyanobacteria

The application of integral procedures of toxigenic cyanobacteria monitoring is indispensible for estimation of the status of water ecosystems with respect to cyanobacterial bloom occurrence and their potential health hazards.

In Central Poland, the long-term research on Sulejów Reservoir made it possible to propose and apply a system for monitoring of microcystins-producing cyanobacteria which can be recommended for other fresh water bodies (Fig. 1). So far the cyanobacteria responsible for the production of mentioned freshwater hepatotoxins are most common in Poland; therefore attention is focused on assessing the threat from this type of cyanobacteria. The system was developed in Department of Applied Ecology (University of Lodz) with collaboration of European Regional Centre for Ecohydrology u/a UNESCO in Lodz, described in detail by Mankiewicz-Boczek (2008) and is widely presented on conferences and workshops to representatives of the Regional Inspectorates of Environment Protection and other institutions dealing with water quality and supply. This system is evaluated positively, however, institutions responsible for water quality are based mainly on existing Polish laws, which do not have clearly defined procedures for monitoring of cyanobacteria and their toxins. Currently the monitoring system described in the paper is used by DAE and ERCE in case of annual monitoring of Sulejów Reservoir as part of a long-term studies on the variability of the different genotypes of cyanobacteria and the cyanotoxins production, in terms of the impact of abiotic and biotic factors. However, at the request or order of the institutions responsible for the quality of bathing water and drinking water quality, the proposed monitoring is carried out for other water bodies, including an assessment of risks to human health according to WHO guidelines.

The scheme of the monitoring of microcystins-producing cyanobacteria includes: (1) analysis of water quality including concentrations of two main nutrients: nitrogen and phosphorus, (2) determination of chlorophyll *a* concentration as an indicator of phytoplankton biomass, (3) analysis of quantity and quality of phytoplankton, and if cyanobacterial species occur (4) determination of toxigenic cyanobacteria which are able to produce microcystins, (5) quick estimation, by different screening methods a total concentration of microcystins as well as their toxicity and after that (6) quantitative and qualitative monitoring of microcystins.

The application of molecular markers of the *mcy* gene cluster provides, as the only method, early identification of toxigenic cyanobacterial strains. Detection of *mcy* genes at the beginning of summer indicated the potential toxicity of environmental samples and the possibility of microcystins production in the next period of monitoring (e.g. Mankiewicz-Boczek et al. 2006, 2011ab). A recent study of reservoirs in Central Poland observed the lack of toxic strains capable of microcystins production despite the presence of *Microcystis aeruginosa* (Gągała et al. 2012). In addition, knowledge of the dynamics of toxigenic strains of the total population of cyanobacteria in the water body together with the genetic analysis of the existence of other microorganisms is essential for further planning action to reduce risks through the use of the natural capacity of the environment according to the concept of Sumino et al. (2008).

Although the 1-3 point monitoring (Fig. 1) is the same for all cyanobacteria, regardless of the type of produced toxins, the next steps of monitoring differ by: (4) type of the analyzed genes specific for different groups of cyanotoxins, (5) type of screening test, such as ELISA, for determination of cyanotoxins concentration and (6) the conditions of chromatographic analysis for sensitive quantification of cyanobacterial toxins.

Spring/Summer
Determination of physico-chemical parameters including nutrients concentration:
P-PO_4, TP (>0.1 mg/l*), N-NO_3, N-NH_4, TN (>1.5 mg/l*) Chlorophyll *a* (> 10 µg/l)**
Phytoplankton analysis

Occurrence of *Microcystis, Planktothrix, Anabaena*
Detection of toxigenic (potentially toxic) strains of cyanobacteria
PCR amplification of *mcy* genes
(*polymerase chain reaction*)

Occurrence of microcystins
Application of screening tests:
determination of microcystins concentration – ELISA
(*enzyme-linked immunosorbent assay*)
determination of microcystins toxicity – PPIA
(*protein phosphatase inhibition assay*)

Confirmation of microcystins if ELISA showed > 2.5 µg/l
Quantitative and qualitative analysis of microcystins – HPLC
(*high performance liquid chromatography*)

ITransdisciplinary interpretation of results
Following the first and second principle of **Ecohydrology**, the identification of cause-effect relationship with comparative studies of the lake/reservoir typology, hydrochemistry, phytoplankton diversity and water toxicity are fundamental for developing a **strategy to reverse eutrophication**.
(Zalewski 2000; Wagner et al. 2009)

Fig. 1: Proposed integral procedure of microcystin-producing cyanobacteria monitoring for bathing water quality. Note: * critical values for eutrophication recommended by OECD (1983); ** relatively low probability of adverse health effect recommended by WHO (2003)

In Northern Poland, systematic studies and analyses of over 30 lakes and coastal areas of the Baltic Sea are carried out by the Laboratory of Biochemical Ecology of Microorganisms (www.sinice.pl). This institution was founded in 2006 and operates within the structure of the University of Gdańsk (Northern Poland). Apart from monitoring, the Centre regularly organizes workshops and conferences on taxonomy and toxicity of cyanobacteria. These events are addressed to both scientists and sanitary officers.

Mitigation of toxic cyanobacterial blooms

The integrative monitoring show in Fig. 1 provides the basis for understanding the factors and processes that predetermine the appearance of cyanobacterial blooms and production of their toxins. This kind of comparative and integrative research is a fundamental component of the Ecohydrology concept (e.g. Zalewski 2000; Wagner et al. 2009), according to which the understanding of the relationship between biotic / abiotic processes and dynamics should be applied to restoration, sustainable use of water resources and preventing toxic cyanobacterial blooms. Toxic cyanobacterial blooms can be mitigated on two levels: firstly by decreasing nutrient availability through reduction of external and internal nutrients load, and secondly by regulation of the structure of trophic chains. Both these aspects are analyzed and developed in research conducted at Sulejów Reservoir (Central Poland). First of all, reduction of the point sources is needed, which can be done by optimisation of the effectiveness of wastewater treatment plants. The next step is to use of ecohydrological tools for reduction of phosphorus and nitrogen. Recognition of the pattern of nutrient transport and dynamics created a basis for the design of a method to reduce reservoir external loading. The method was based on diverting the highest pollutant loads transported by the reservoir's tributaries into floodplain areas upstream the reservoir. The nutrient load may be reduced by sedimentation of the river's suspended matter in the floodplain, and assimilation of dissolved nutrients by wetland vegetation during the processes of primary production and growth (e.g. Kiedrzyńska et al. 2008). Increased effectiveness of these processes is possible by optimising conditions for physical sedimentation of the transported material and also by shaping the spatial distribution and composition of plant communities on the floodplain based on its hydrological characteristics (e.g. Kiedrzyńska et al. 2008).

After the reduction of the *bottom-up* driver (i.e. nutrients supply), restoration measures in water bodies can include the control of biotic components using e.g hydrobiomanipulation.

The key idea of this method is enhancement of filtering large zooplankton by water level control toward reduction of fish recruitment; thus a *top down* food chain effect is initiated by hydrological management instead of predatory fish stocking. The method is under calibration, however the promising results were received in 2006: the decreasing of young-of-the-year (YOY) fish density was observed after regulation of water level during spawning, and as result of low YOY fish pressure high biomass of larger-bodies filter-feeding zooplankton was observed. The high grazing activity of zooplankton has contributed to the phytoplankton reduction, and delayed the growth of cyanobacteria in spite of physical-chemical conditions which were optimal for their increase: the average cyanobacterial biomass decreased to the level characteristic for twice lower phosphorus concentration, which was estimated using the regression model based on long-term data (e.g. Wagner et al., 2009).

Polish legislation

In 2002, the Polish Ministry of Health added the assessment of cyanobacteria to requirements for monitoring bathing water quality, and accordingly the appearance of cyanobacterial blooms should be controlled by observation of water colour, turbidity and/or odour. However, this law has been repealed by the Act from March 4th 2010 (Water Law 2010) and the final consolidated version the Act was announced February 9th 2012 (Water Law 2012). The new article in the Water Law regulation states that the schedule of sampling, collection and analysis should be no less than four samples during the bathing season, so that the interval between the tests will not exceed a month. Additionally, if determined by internal or official examination that the bathing water does not meet the requirements, in particular because of short-term pollution or excessive cyanobacterial blooms, the state district sanitary inspector requires the organizer to determine the cause of pollution and take action to protect human health and improve water quality. This change in bathing water regulation is consistent with the provisions of European Directive 2006/7/EC concerning the management of bathing water quality.

It seems that more precise and structured recreational water regulations would be desirable, including the determination of cyanobacterial biomass and toxicity together with recommendations for methods. Only full knowledge of the problem will enable the development of methods to prevent and reduce the occurrence of toxic blooms of cyanobacteria.

For drinking water, the Guideline value of 1 µg/L of Microcystin-LR has been excluded from Polish legislation by the Ministry of Health in 2007. The new regulation without a guideline value of microcystin was based on European Directive 98/83/EC which does not state such a requirement. However, some water treatment plants that have a surface water intake monitor the probability of microcystin occurrence on their own initiative. They then use of the provisional guideline by WHO for microcystin-LR of 1 µg/L.

References

Gagala I., Izydorczyk K., Jurczak T., Mankiewicz-Boczek J. 2012. The key parameters and early warning methods to identify presence of toxigenic blooms dominated by *Microcystis aeruginosa* in the Jeziorsko Reservoir (Central Poland). *Fresen. Environ. Bull.* 21(2) 295-303.

Grabowowska M., Pawlik-Skowrońska B., 2008. Replacement of *Chroococcales* and *Nostocales* by *Oscillatoriales* caused a significant increase in microcystin concentrations in a dam reservoir. *Oceanological and Hydrobiol. Studies* 37(4), 23-33.

Izydorczyk K., Jurczak T., Wojtal-Frankiewicz A., Skowron A., Mankiewicz-Boczek J., Tarczynska M. 2008. Influence of abiotic and biotic factors on microcystin content in *Microcystis aeruginosa* cells in a eutrophic temperate reservoir. *J. Plankton Res.* 30(4), 393-400.

Kiedrzynska E., Wagner I., Zalewski M. 2008. Quantification of phosphorus retention efficiency by floodplain vegetation and a management strategy for a eutrophic reservoir restoration. *Ecol. Eng.* 33, 15-25.

Kobos J., Mazur-Marzec H., Dittmer M., Witek B., Pliński M., 2005. Toxic cyanobacterial blooms in the Kociewskie Lasek (Northern Poland), *Ocean. Hydrobiol. Stud.* 33 (Supl. 3), 77-84.

Kobos J., 2007, Characteristic of toxic and potentially toxic cyanobacteria occurring in the Gulf of Gdańsk and selected lakes from the Radunia River drainage basin, PhD. Thesis, Univ. Gdańsk, Gdynia, 174 pp., (in Polish with English summary).

Kobos J., Błaszczyk A., Pliński M., 2007, Hepatotoxic microcystins and neurotoxin anatoxin-a in cyanobacterial blooms in Lakes in Pomorskie Province, Poland, 7th International Conference on Toxic Cyanobacteria, August 5-10, Rio das Pedras, Brazil.

Kokociński M., Stefaniak K., Mankiewicz-Boczek J., Izydorczyk K., Soininen J. 2010. The ecology of invasive cyanobacterium *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyta) in two hypereutrophic lakes dominated by *Planktothrix agardhii* (Oscillatoriales, Cyanophyta). *Eur. J. Phycol.* 45(4): 365-374.

Mankiewicz-Boczek J. 2008. Diagnosis and prognosis of toxigenic cyanobacterial blooms occurrence to achieve a good ecological status for water - Habilitation thesis, University of Lodz. (in Polish).

Mankiewicz J., Komarkova J., Izydorczyk K., Jurczak T., † Tarczynska M., Zalewski M. 2005. Hepatotoxic cyanobacterial blooms in lakes of North Poland. *Environ. Toxicol*. 20, 499-506.

Mankiewicz-Boczek J., Gagała I., Kokociński M., Jurczak T., Stefaniak K. 2011a. Perennial toxigenic *Planktothrix agardhii* bloom in selected lakes of Western Poland. *Environ. Toxicol.* 26(1), 10–20.

Mankiewicz-Boczek J., Izydorczyk K., Romanowska-Duda Z., Jurczak T., Stefaniak K., Kokociński M. 2006. Detection and monitoring toxigenicity of cyanobacteria by application of molecular methods. *Environ. Toxicol.*21, 380-387.

Mankiewicz-Boczek J., Palus J., Gągała I., Izydorczyk K., Jurczak T., Dziubałtowska E., Stępnik M., Arkusz J., Komorowska M., Skowron A., Zalewski M. 2011b. Effects of microcystins-containing cyanobacteria from a temperate ecosystem on human lymphocytes culture and their potential for adverse human health effects. *Harmful Algae* 10, 356-365.

Mankiewicz-Boczek J., Kokociński M., Gagała I., Pawełczyk J., Jurczak T., Dziadek J. 2012. Preliminary Molecular Identification of Cylindrospermopsin-producing Cyanobacteria in Two Polish Lakes (Central Europe). *FEMS Microbiol. Lett.* 21(2) 295-303.

Mazur H., Lewandowska J., Błaszczyk A., Kot A., Plinski M., 2003. Cyanobacterial toxins in fresh and brackisch waters of Pomorskie Province (Northern Poland). *Oceanological and Hydrobiological Studies* Vol. XXXII, No.1, 15-26.

Mazur-Marzec H., Błaszczyk A., Błońska M., Cichowska A., Kobos J., Sutryk K., Toruńska A., Pliński M. 2010. Cyanobacterial blooms and cyanotoxin production in the Baltic Sea and the lakes of Pomeranian Province [in:] edit. Olańczuk-Neyman K., Mazur-Marzec H. *Microorganisms in the environment and environmental englineering. From ecology to technology*. Monografie Komitetu Inżynierii Środowiska PAN vol. 64, 159-170.

Mazur-Marzec H., Krężel A., Kobos J., Pliński M., 2006. Toxic *Nodularia spumigena* blooms in the coastal waters of the Gulf of Gdańsk: a ten-year survey. *Oceanologia* 48(2), 255-273.

Mazur-Marzec H., Pliński M., 2009. Do toxic cyanobacteria blooms pose a threat to the Baltic ecosystem?*. Oceanologia* 51(3), 293-319.

Mazur-Marzec H., Spoof L., Kobos J., Pliński M., Meriluoto J., 2008. Cyanobacterial hepatotoxins, microcystins and nodularins, in fresh and brackish waters of Pomeranian Province, northern Poland. *Oceanolo. Hydrobiol. Stud.* 37(4), 1-19.

OECD. 1983. Eutrophication of water. Monitoring assessment and control - technical report. Environment Directorate, OECD, Paris. p 154.

Pawlik-Skowrońska B. Skowroński T., Pirszel J., Adamczyk A. 2004. Relationship between cyanobacterial bloom composition and anatoxin-A and microcystin occurrence in the eutrophic dam reservoir (SE Poland) *Pol. J. Ecol.* 52(4), 479-490.

Pawlik-Skowrońska B., Pirszel J., Kornijów R. 2008. Spatial and temporal variation in microcystin concentrations during perennial bloom of *Planktothrix agardhii* in a hypertrophic lake. *Ann. Limnol. – Int. J. Lim.* 44(2), 63-68.

Pliński M. 1995. Phytoplankton of the Gulf of Gdańsk in 1992 and 1993. *Oceanologia* 37 (1), 123-135.

Sierosławska A., Rymuszka A., Kalinowska R., Skowroński T., Bownik A., Pawlik-Skowrońska B. 2010. Toxicity of cyanobacterial bloom in the eutrophic dam reservoir (Southeast Poland). Environ. Toxicolo. Chem. 29(3), 556-560.

Wagner I., Izydorczyk K., Kiedrzynska E., Mankiewicz-Boczek J., Jurczak T., Bednarek A., Wojtal-Frankiewicz A., Frankiewicz P., Ratajski S., Kaczkowski Z., Zalewski M. 2009. Ecohydrological system solutions to enhance ecosystem services: the Pilica River Demonstration Project. *Ecohydrol..Hydrobiol.* 9(1), 13-39.

Water Law, 2010, Dz.U. z 2010 r. Nr 44, poz. 253.

Water Law, 2012, Dz. U. z 2012 r. Nr 0, poz. 145.

WHO, 2003. Algae and cyanobacteria in fresh water. In: Guidelines For Safe Recreational Water Environments. Volume 1, Costal and Fresh Water, WHO, Geneva, p. 136-158.

Sumino, T., Ogasawara, T., Park, H.D. Method and equipment for treating microcystin-containing water. [US 7,425,267 B2]. 2008.

Zalewski, M., 2000. Ecohydrology—the scientific background to use ecosystem properties as management tools toward sustainability of water resources. *Ecol. Eng.* 16, 1–8.

SINGAPORE: Occurrence, Monitoring and Management of Cyanobacterial blooms

Y. T, Wong, M. H. S. Tay, K. N. Tiew & M. H. Lim

PUB, Water Quality Office, WaterHub, 82 Toh Guan Road East, #C4-03, Singapore 608576;
WONG_yueat_tin@pub.gov.sg

Singapore is a small island nation, situated in the equatorial rain belt, with a mean daily minimum air temperature of 25°C and maximum of 31°C recorded in 2011, and a total land area of about 710km^2. Due to the limited natural freshwater resources, Singapore needs to harvest stormwater[8] on a large scale. Singapore now has a total of 17 reservoirs and two-thirds of its land area is water catchment (PUB, 2011a).

Most of the catchments are urbanized, situated in densely populated areas where human activities occur. The water flowing through these urban stormwater catchments can contain relatively higher nutrient concentrations than protected catchments, and these higher nutrient concentrations favour the growth of aquatic life including cyanobacteria.

Occurrence and Monitoring of cyanobacteria and their toxins

The tropical climate and the nutrient-rich waters in Singapore reservoirs are conducive to the production of rich crops of 'algae', i.e. including cyanobacteria. The national water agency, PUB, is responsible for the collection, production, distribution and reclamation of water in Singapore. PUB has a regular phytoplankton monitoring programme in reservoirs for operational requirements. The programme started in 1974 and has since detected (by microscopy) potentially toxic cyanobacteria including *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Cylindrospermopsis raciborskii*, *Microcystis*, *Planktolyngbya*, *Planktothrix*, *Pseudanabaena*, and *Raphidiopsis* in the reservoir waters.

A monitoring programme for Microcystin-LR was introduced in 2003. The programme has now included three other microcystin variants and two other types of cyanotoxins – Cylindrospermopsin and Anatoxin-a. The results of analysis of the cyanotoxins are provided in table 1. The four variants of microcystins (microcystin-LR, microcystin-RR, microcystin-LA and microcystin-YR) and cylindrospermopsin were tested on a monthly basis in treated waters for the period late 2009 to late 2010. None of the listed toxins were detected in our treated waters during this period and also in subsequent monitoring when the testing frequency was reduced to annual testing. The monthly testing of anatoxin-a lasted for 4 months and was reduced to annual testing due to the low concentration of the algal progenitors (e.g. *Anabaena* sp.).

Although a number of our reservoirs are dominated by cyanobacteria, most of the cyanobacteria blooms are non-toxic and the toxin concentrations are very low. The most frequently detected toxin with the highest concentration appears to be cylindrospermopsin.

[8] Singapore's stormwater and surface runoff is separated from sewage. The pollutant loads in stormwater runoff are low and the water quality of the stormwater storage reservoirs can be similar to that of a protected upland reservoir (Lim et al., 2011).

Table 1: Dissolved cyanotoxins monitoring results (Data from 2003 to 2011)

Cyanotoxin test Using LC/MS/MS	Year started	Results for reservoir & catchment waters	
		Range (µg/L)	Frequency of detection (%)
Microcystin-LR	Mar 2003	<0.025 – 1.65	7.9 (1314 tests)
Microcystin-RR	Dec 2006	<0.025 – 3.34	23.7 (514 tests)
Microcystin-LA	Dec 2006	<0.025 – 0.05	0.8 (513 tests)
Microcystin-YR	Dec 2006	<0.025 – 0.27	1.2 (513 tests)
Cylindrospermopsin	May 2009	<0.1 – 5.16	58.2 (466 tests)
Anatoxin-a	May 2010	<0.1	0 (104 tests)

Reservoir Management and Water Treatment

Singapore adopts a holistic approach to water resource management. Land use is well coordinated and all proposed developments within the water catchment areas are scrutinized to ensure that they are compatible with developments permitted in the water catchment to prevent pollution at an early stage. An integrated catchment water management plan is in place which also looks into the control of nutrients at source for a long term and sustainable effect.

The more effective algal management measures undertaken by PUB to control the risk of cyanotoxins include artificial de-stratification (using aeration units) and selective abstraction of water at different intake points or reservoirs. Chemical treatment or the use of algaecides and/or algaestats is not recommended in reservoirs.

The water from reservoirs and various sources are conveyed to the waterworks for treatment to produce potable water. Most treatment plants use chemical coagulation and rapid gravity filtration to remove the intact algal cells in the raw water (PUB, 2011b). The waterworks are further equipped with disinfection systems such as ozone and chlorine which can remove the dissolved cyanotoxins. Our treatment facilities are adequate to remove cyanotoxins to below detection limits in drinking water and there is currently no immediate risk of cyanotoxin in drinking water.

Regulation of cyanotoxins in Singapore

Singapore recently developed its regulation on microcystin-LR in drinking-water under the Environmental Public Health (Quality of Piped Drinking Water) Regulations (regulated by the National Environment Agency). The Environmental Public Health (EPH) Regulations came into effect on 1 August 2008. The piped drinking water standards set out under the Regulations are generally based on the World Health Organization (WHO) drinking-water guidelines. Under the EPH Regulations, piped drinking water must comply with the water quality standard which is stated as a maximum prescribed quantity of 0.001mg/L total microcystin-LR, in free and cellbound forms (National Environment Agency, 2010). There is currently no standard for cylindrospermopsin under the EPH Regulations. Every supplier of piped drinking water is also required to prepare and implement a water safety plan under the EPH Regulations to ensure that the piped drinking water supplied complies with the piped drinking water standards.

Singapore's recreational water quality guidelines for fresh water bodies are also adopted from the World Health Organization (WHO) Guidelines. The guidelines are used to assess the suitability of a recreational beach or fresh water body for primary contact activities (e.g. swimming, skiing and wakeboarding) which involve frequent immersion of the whole body or the face and trunk or for which it is likely that some water will be swallowed. Chlorophyll-a is one of the parameters used to assess the water quality and the guideline is that 95% of the time, over a 3-year rolling period, chlorophyll-a concentration should be less than or equal to 50 µg/L (National Environment Agency, n.d.). At higher levels of chlorophyll-a, there is a greater chance of algal toxins produced which could increase health risks to water users. If the results do not meet the recreational water quality guidelines, the fresh water bodies could be assessed to be unsuitable for primary activities and the public would be notified accordingly.

References

Lim, M. H., Leong, Y. H., Tiew, K. N. & Seah, H. (2011). Urban Stormwater Harvesting: A Valuable Water Resource of Singapore. Water Practice & Technology, 6:4. Retrieved from http://www.iwaponline.com/wpt/006/wpt0060067.htm

National Environment Agency. (n.d.). Recreational Water Quality. Retrieved from http://app2.nea.gov.sg/topics_rwq.aspx

National Environment Agency. (2010). Environmental Public Health (Quality of Piped Drinking Water) Regulations 2008. Retrieved from http://app2.nea.gov.sg/data/cmsresource/20101220736114746909.pdf

PUB. (2011a). PUB: Local Catchment Water. Retrieved from http://www.pub.gov.sg/water/Pages/LocalCatchment.aspx

PUB. (2011b). PUB: Water Treatment. Retrieved from http://www.pub.gov.sg/general/watersupply/Pages/WaterTreatment.aspx

SPAIN: Cyanobacteria and Cyanotoxins – Legislation and Current Situation

Antonio QUESADA[1], Caridad DE HOYOS[2], Guadalupe MARTÍNEZ[3]

[1] Dpt. Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain, Email. antonio.quesada@uam.es
[2] Centro de Estudios Hidrográficos, CEDEX, Paseo Bajo Virgen del Puerto, 328005 Madrid, Spain, Email. Caridad.dehoyos@cedex.es
[3] Instituto de Ciencias de la Salud, Ctra. Extremadura, km. 114,100, 45600 Talavera de la Reina,Spain, Email. gu-martinez@jccm.es

Introduction

Since the last edition of this booklet, the European legislation has changed remarkably regarding cyanobacteria and cyanotoxins. The European Directive on Bathing Waters 2006/07/CE, requires considering the potential occurrence of cyanobacteria in bathing waters as one of the parameters, among others, posing risks for bathers' health. This European Directive was transposed to the Spanish Legislation and here we will explain how. Further, we explain the current Spanish legislation on drinking water for cyanobacteria and cyanotoxins. Preceding this legal framework we will introduce a recent analysis about the country's situation regarding cyanobacteria and cyanotoxins.

Surface water in Spain is the main source of drinking and recreational waters in the country. Most of this water is stored in reservoirs. Lakes are not abundant and rivers are mostly dammed to store water for the summer months when rain is very scarce or virtually absent in some regions. There are more than 1000 reservoirs throughout the country and drinking water shortages only occur sporadically, during extreme drought events in some areas of Spain. Storing the water in reservoirs, together with the high temperatures and in some cases high levels of eutrophication, can enhance cyanobacterial blooms. De Hoyos et al (2004) and Quesada et al. (2004) showed that the problem may be widespread, affecting at least 25% of the Spanish reservoirs, although only a portion of them is devoted to drinking-water abstraction. In a recent study carried out in CEDEX with data from the Environmental Ministry's programmed samplings to meet WFD requirements (2006-2009), 40% of the reservoirs showed high presence of cyanobacteria (higher than 0.2 mm^3/L) when considering the maximum record of all the samples per reservoir (http://www.magrama.gob.es/es/agua/formacion/Jornada-Gestion-Riesgo-Ambiental-Aguas-Bano-Continentales.aspx)

In recent years it has been demonstrated that microcystins (MCs) are quite widespread in the country (see below), but cylindrospermopsin (CYN) and paralytic selfish toxins (PSTs) are also present in a relevant number of Spanish waterbodies (around 10 waterbodies in each case so far, but the number may increase as more waterbodies are investigated). Anatoxin-a (ANA), however, has only been found, so far, in one water reservoir out of several hundred analysed.

Current cyanobacteria and cyanotoxin situation in the country

The situation of the Spanish reservoirs as reflected by data from 2006 to 2009 for cyanobacteria quantity (Table 1) has not changed compared to the situation in 1999-2001 (De Hoyos et al, 2004). The percentage of reservoirs with cyanobacterial biovolume higher than 0.2 mm^3/l (considering the maximum value of the summer samples of the studied years) in both cases was similar, 38 % in the first study (18 reservoirs out of 47) and 41 % in the second one (114 reservoirs out of 278). Geographically, cyanobacterial problems are more intense in the reservoirs sited on siliceous lithologies of west Spain (De Hoyos et al, 2004).

WHO Guidelines levels	Cyanobacteria (mm^3/l)	Reservoirs (%)
< Vigilance Level	< 0.02	33.1
> Vigilance Level	0.02-0.2	25.9
> Alert level I	0.2-2	20.8
> Guidance Level I	2-10	10.1
> Guidance Level II Alert level II	>10	10.1

Table 1: Percentage of Spanish reservoirs in the different intervals of cyanobacteria biovolume (2006-2009), classified according to the WHO Guidelines levels for recreational waters (Guidance Levels) and drinking waters supplies (Alert Levels). The data included 278 water reservoirs in all the Spanish geography. (Source: CEMAS DB of the Environment Ministry. Data analysis: CEDEX)

In a 7 year-survey carried out by CEDEX (2004-2010) in 49 reservoirs of different trophic degrees, a significant relationship was found between the chlorophyll *a*, the biovolume of cyanobacteria, the percentage of cyanobacteria in the phytoplankton total biovolume and the concentration of microcystins (analysed by ELISA). This study shows that in the reservoirs with higher chlorophyll *a* concentration cyanobacterial biomass dominated the community (biovolume >75% of the total phytoplanktonic community). 67 % of the samples with chlorophyll *a* concentrations higher than 75 µg/l were dominated by cyanobacteria and 86 % of them had cyanobacteria biovolumes higher than 2 mm^3/L. Cyanobacterial dominance decreases at lower chlorophyll *a* concentrations (Figure 1): 53 % of the sites showed cyanobacteria percentages higher than 50 % of total biovolume at chlorophyll *a* concentrations between 25 and 75 µg/L, 17 % at chlorophyll *a* concentration between 8 and 25 µg/L and only 9 % in samples below 8 µg/L (Figure 1).

The concentrations of microcystins (MCs) were directly related to the chlorophyll *a* concentration; thus at chlorophyll *a* concentration higher than 75 µg/L, 47% of the samples showed MCs concentrations above 1 µg/L. This proportion was lower with lower chlorophyll *a* concentration (Figure 2): in the chlorophyll *a* concentration range 25-75 µg/L the proportion of samples above 1 µg/L was 29%, and the proportion was 0% in the samples with chlorophyll *a* concentration below 8 µg/L. Interestingly some of the samples (4%) had MCs concentrations above 1 µg/L at relatively low chlorophyll a concentrations between 8 and 25 µg/L.

The concentrations of microcystins (MCs) were directly related to the chlorophyll *a* concentration; thus at chlorophyll *a* concentration higher than 75 µg/L, 47% of the samples showed MCs concentrations above 1 µg/L. This proportion was lower with lower chlorophyll *a* concentration (Figure 2): in the chlorophyll *a* concentration range 25-75 µg/L the proportion of samples above1 µg/L was 29%, and the proportion was 0% in the samples with chlorophyll *a* concentration below 8 µg/L. Interestingly some of the samples (4%) had MCs concentrations above 1 µg/L at relatively low chlorophyll a concentrations between 8 and 25 µg/L. These results clearly indicate that in Spain MCs are quite abundant, and when chlorophyll *a* concentration is high the probability of finding them is quite high, while at relatively low chlorophyll *a* concentration these compounds can also be found, albeit at low concentrations (Figure 2). Martínez and Merino (2008) published a complete study on the health surveys related with cyanobacteria in drinking and recreational waters.

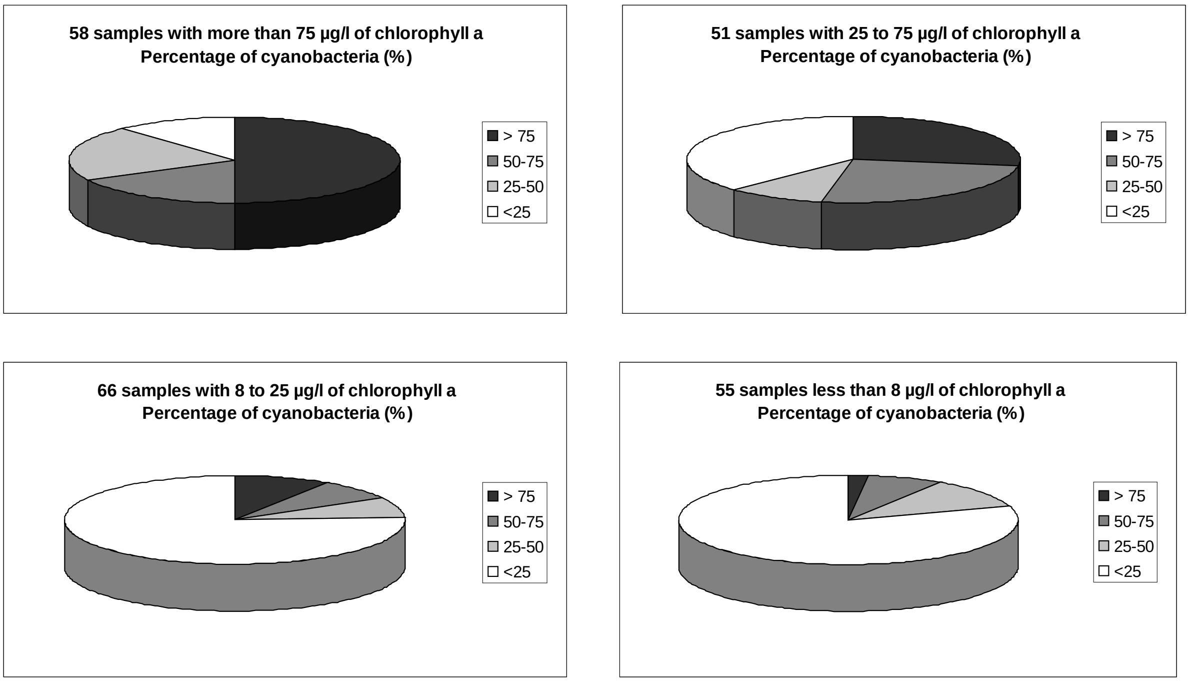


Figure 1: Dominance of cyanobacteria in samples classified by their chlorophyll *a* concentration. The values make reference to the biovolume percentage of cyanobacteria in relation to that of the total phytoplanktonic organisms (Source: CEDEX).

These empirical observations are extremely useful to prepare the management policies for ecological and health risk assessment, based on real data from Spanish aquatic ecosystems.

Legislation

Legislation needs to differentiate between drinking and recreational waters, because the responsibilities and actions are different.

a) Drinking water:

The present legislation in Spain, at a national level, regarding cyanotoxins and cyanobacteria is the *Real Decreto* 140/2003 (7 February 2003). This is a general law establishing the sanitary criteria for water quality for human consumption. This law represents the incorporation of the European Directive 98/83/CE into Spanish law. In general terms, this legislation includes the WHO recommendations for safe drinking water.

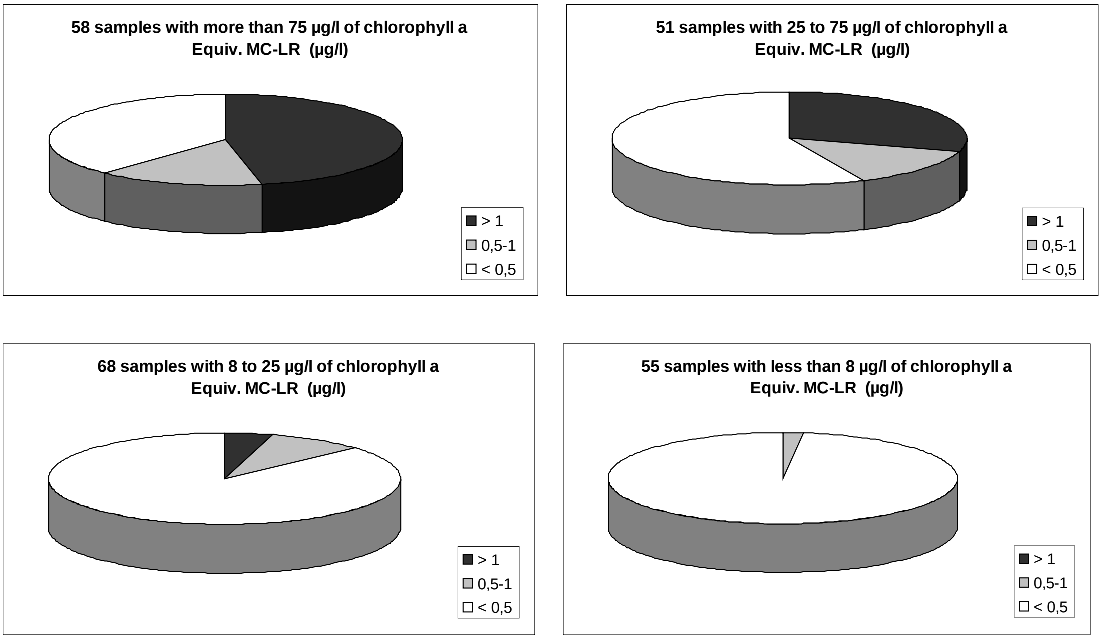


Figure 2: Presence of microcystins in samples classified by their chlorophyll *a* concentration. The values make reference to the MC (equivalents MC-LR) concentrations and are given as percentages of the number samples in each category (Source: CEDEX).

The *Real Decreto* makes reference exclusively to drinking water at the site where the water is available for the consumer, and does not include any concept relative to raw waters in terms of monitoring or analyses. Moreover, recognizing that drinking water may become scarce in some regions of the country, the law allows some flexibility in quality when the water distribution cannot be assured from any other source, provided there is no potential risk for human health.

This law requires an Official Information System (http://sinac.msc.es), which collects all the data from the different water companies and municipalities from the entire national territory and produces the Official Public Report about the drinking water quality which is delivered it to the European Commission.

In terms of cyanotoxins the law does not allow the final treated water to be used for human consumption if there is more than 1 µg/l Microcystin. However, microcystin concentration is required to be measured only when eutrophication is evident in the water sources. This legal limit for cyanotoxin is routinely revised every 5 years.

The water quality control establishes three different levels:

a) Company control: the responsibility of the water company covers all the chemical and physical analyses related to the water quality. The minimum sampling frequency depends upon the size of the distribution network in terms of the population served.

b) Sanitary surveillance: the responsibility of the sanitary authority to organize periodic inspection, samplings and analyses. The sampling frequency will depend upon the circumstances, and it is aimed to complement company analyses.

c) Tap control: the responsibility of the municipality to organize simple analyses except when there is possibility of a particular pollutant, in which case that pollutant is also to be analyzed. The minimum sampling frequency for a large city of about 4 million people is about 200 samples per year.

The ultimate responsibility for drinking-water quality lies with the water supplier and municipalities although the sanitary authority has the supervision responsibility and can ban or restrict the water uses to minimize the potential risks for the population's health. This responsibility of the authorities is conveyed to the water companies and municipalities who are requested to submit periodically the quality data of the water for human consumption. Moreover, the water companies and the municipalities have to publish the information about the quality of water that they are distributing, and to make those reports public.

The law does not consider the creation of a water quality commissariat with the power to sanction companies or municipalities who have failed to meet these sanitary requirements. It does, however, prohibit water distribution when the quality does not fulfil the quality criteria established in this law, and indeed there have been restrictions to the delivery of water exceeding the limit of 1 µg/L of microcystins for a city – an important news item in the local media.

The legislation does not consider any other of the known cyanotoxins as cylindrospermopsin, anatoxin or PSTs although they have been found in the raw waters of the country (Quesada et al., 2006; Carrasco et al., 2007 Wormer et al., 2011).

b) Recreational (bathing) waters

The legislation in Spain regarding bathing water transposes the European directive directly in the *Real Decreto* 1341/2007 without many changes. This legislation establishes the sanitary criteria at national level applicable to bathing waters. Cyanobacteria and cyanotoxins in this Real *Decreto* are considered as a further parameter to be analysed. The legislation obliges to prepare before the bathing season the profiles of every official site indicating – among other parameters – the tendency of the site to produce massive cyanobacterial growths. For preparing these profiles the Ministry of Environment organized the sampling of every official bathing site three times during the bathing season for two consecutive years (2008 and 2009). This sampling included physical and chemical parameters, as well as biological, measuring the chlorophyll *a* concentration, the proportion of cyanobacteria, the presence of potentially toxic taxa and the occurrence of MCs, CYN and ANA. These data are publicly available from the Health Ministry Webpage. http://nayade.msc.es/Splayas/ciudadano/ciudadanoZonaAction.do.
This represented the most widespread and the most intense sampling on cyanobacteria done in the country so far. With all the data collected and following the decision scheme presented in Figure 3 every site was classified according to one of three proliferation probability levels: high, moderate and low. The results indicated that 20% of the sites (43 out of 212) had a high probability of cyanobacterial blooms, 50% of the sites showed a moderate probability and about 30% of the sites (61 out of 212) had low probability.

In Spain, the sanitary authority is responsible for the appropriate sanitary requisites of the bathing waters in the country. The Ministry of Health has prepared a webpage of public information regarding the official bathing sites called Náyade (http://nayade.msc.es/Splayas/home.html) to which the quality data are continually uploaded and assessed. The ministry also elaborates annual reports for the public and for the European Union.

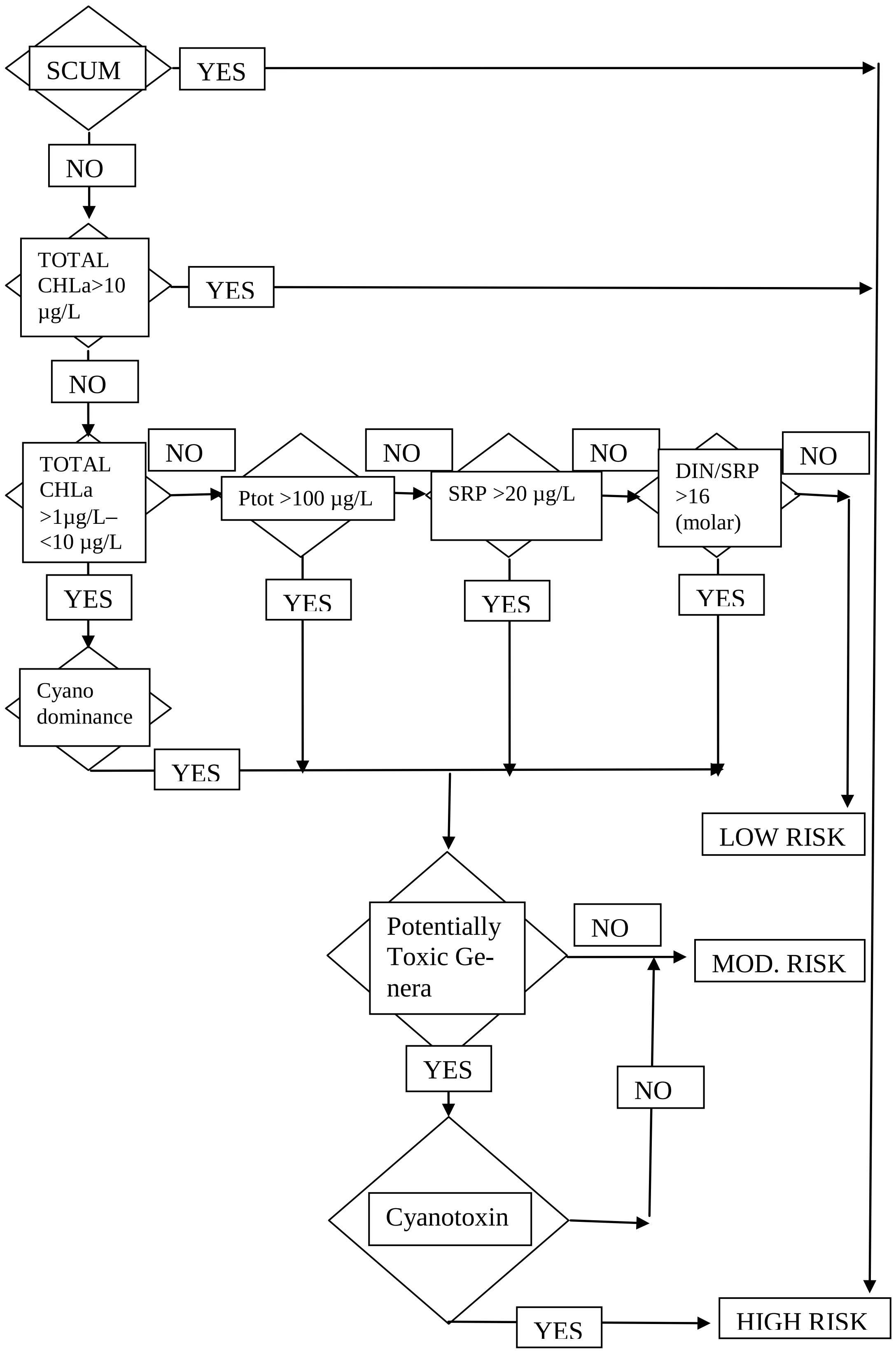


Figure 3: Decision scheme for the probability of cyanobacterial blooms

Practically administration of bathing water quality requires a high degree of coordination, because while the Environmental Administration and the responsibility of providing the profiles and the environmental data to the Health Authorities is on a national level, the health authority is regional (i.e. part of the so-called “autonomous regions”) and can decide to use different criteria to regulate the bathing waters under their responsibility. Some regional health authorities have decided to follow the WHO guidelines adapted to the regional characteristics and others have decided to use a more health risk assessment oriented procedure considering thresholds but also other parameters (number of users, potential utilization, etc.). There is no common strategy in the country regarding the health risk assessment due to cyanobacteria and cyanotoxins in bathing waters. Up to now some regional health authorities have temporarily closed several bathing sites based on the abundance of cyanobacteria and/or on the total chlorophyll *a* concentration.

Discussion

The Spanish legislation on drinking waters includes the provisional guideline value proposed by the WHO of 1 µg/L microcystin-LR, and sets this as the legal limit for drinking water. As for any law intended to improve public health, it is a positive step towards protecting the consumer. However, there is room for improvement and the following concerns will need to be addressed in the future.

Probably the concept that is most lacking in this law is the idea that raw waters are biologically active and dynamic systems. This is a technical law that considers drinking water as a final product and does not consider the treatment process or the water origin. In this regard, it would be very useful to use the information collected in the surveillance monitoring required by the WFD. From our point of view considering the water source as an ecosystem would allow the cyanobacterial problem to be treated more effectively as a whole and not just as ‘microcystin producing units’. This law is also somewhat out of date by focusing on microcystins, given the other potential hazards from cyanobacterial massive growths, which are now known and present in the country such as anatoxin-a, PSTs or cylindrospermopsin. These later problems are also likely to occur in some of the freshwaters of Spain as in other countries.

Focusing on microcystins, there is a problem in the terms used in the law. Specifically, the law uses the term ‘*microcistina*’ (microcystin), which is not defined. Apparently, it refers to microcystin-LR, as it is based on the WHO guideline value, however, such as it is written, it is not possible to be measured since there are many microcystins without any commercial standard, which hampers determination of their concentrations. This problem is also compounded considering that the law does not recommend or establish any methodology for the measurement of ‘*microcistina*’. This apparently minor problem can become quite important. For instance, assuming the likely case that a water company performs its periodic measurements by HPLC and at a given moment measures, for example, 0.5 µg/L of microcystin LR, and 0.4 µg/L of microcystin RR, plus an important peak, with the highest absorbance, of an unidentified compound, probably an unidentified microcystin, the laboratory director could certify this water as safe drinking water, because he/she cannot identify properly that compound as a microcystin. Even if he/she considers unidentified peak likely to be a microcystin (e.g. based on its PDA-characteristics), he/she cannot determine the concentration of that compound. In this case the water would be drinkable from the legal point of view but could exceed the level targeted from a health perspective.

This problem would have been solved easily if the legislation would have included the concept of microcystin LR-equivalents, or recommended a series of methodologies to evaluate the risk associated to cyanobacteria in drinking waters. Water authorities in Spain should work together towards establishing such protocols and guidelines.

In reference to the bathing waters, the profiles identify clearly that 20% of the official bathing sites are prone to produce massive cyanobacterial growths, and at least those sites will require a monitoring strategy and most probably a risk assessment procedure. However, in some cases the regional Health Authorities may not be ready to develop such studies with non-pathogenic organisms (as cyanobacteria) and would delegate the responsibility to the national environmental authority which can deal with the ecological perception of the problem but perhaps they may not be willing to take on this costly monitoring. The national environmental authority and several regional health authorities are well aware of these health issues, although a quite high coordination level is required. Perhaps one of the main problems that may appear in terms of recreational waters is the 50 % of the sites that resulted in a moderate probability of producing cyanobacterial blooms. It has been proposed that those sites at this moderate level should be monitored for cyanobacteria at weekly or bi-weekly frequency with low-cost tools as hand-held fluorometers, to take decisions for each site during the bathing season regarding the health risk associated to cyanobacteria and cyanotoxins. However, regional health authorities are still organizing the strategies for this monitoring and at the moment it is unknown how this will be coordinated.

Another important point refers to the non-official bathing sites. Many very popular bathing sites in the country are not located on the official list of bathing sites. In some cases they have infrastructures and even belong to municipalities, but are not officially designated as bathing sites and thus no bathing site profile has been developed for them. Thus, in some cases they are not monitored according to legislation, nor are site users informed that the site may pose a potential risk to their health.

Summary

The Spanish legislation for safe drinking water adopts the provisional WHO guideline of 1 µg/l microcystin as the legal limit to accept the water for human consumption. This law only considers the water at the point where it is distributed to the consumer. The aspects related to raw water are covered by the EuropeanWater Framework Directive. The law establishes a series of quality control analyses and responsibilities, but in the case of microcystin does not define the term, nor recommend methodology for its analysis. In any case, the Sanitary Authorities occasionally have closed the water delivery in some localities when treatment was not effective enough to reduce the MCs concentration below the legal limits.

Regarding recreational waters, Spanish legislation has transposed the European Directive and has produced the profiles of the official bathing sites. In these profiles 20% of the sites were prone to produce cyanobacterial blooms, 50 % showed a moderate probability to generate cyanobacterial blooms and 30% showed low probability. Some regional health authorities have temporary closed several bathing sites based on the abundance of cyanobacteria and/or on the total chlorophyll *a* concentration.

References

De Hoyos C., Negro A.I. and Aldasoro J.J. (2004). Cyanobacteria distribution and abundance in the Spanish water reservoirs during thermal stratification. Limnetica 23: 119-132

Carrasco, D., E.Moreno, T.Paniagua, C.de Hoyos, L. Wormer, D. Sanchis, G.A. Codd and A. Quesada (2007). Anatoxin-a occurrence and potential cyanobacterial anatoxin-a-producers in Spanish reservoirs. J. Phycology.43: 1120-1125

Martínez, G & I. Merino (2008) Health surveys of Cyanobacteria in drinking and recreational water. Rev. Salud Ambiental 8(1): 1-7.

Quesada, A., Sanchis D. and Carrasco D. (2004). Cyanobacteria in Spanish reservoirs. How frequently are they toxic? Limnetica 23: 111-120

Quesada A., E. Moreno, T. Paniagua, L. Wörmer, C. De Hoyos and A. Sukenik (2006). Toxicity by Aphnizomenon ovasliporum (Cyanobacteria) in a Spanish water reservoir. European J Phycology. 41:39-45.

Wörmer, L., S. Cirés, R. Agha, M. Verdugo, C. de Hoyos, y A. Quesada (2011). First detection of cyanobacterial PSP (Paralytic shellfish poisoning) toxins in Spanish freshwaters. Toxicon. In press

TURKEY:
Occurence of Toxic Cyanobacteria and Guidelines for Monitoring Cyanotoxins in Drinking and Recreational Waters

Reyhan Akçaalan[1*], Meriç Albay[1**], Tacettin Kakilloğlu[2] & Talat Bahçebaşı[3]

[1] Istanbul University Fisheries Faculty Ordu Cad. No: 200 34470 Laleli, Istanbul / Turkey, *rakcaalan@gmail.com; **albay.hermano@gmail.com
[2] Ministry of Health, Primary Health Care, General Directorate Mithatpaşa Cad. No:3 06434 Sıhhiye, Ankara / Turkey
[3] Kocaeli University, Medicine Faculty, Dept. of Public Health, Konuralp yerleşkesi, Düzce

Introduction

Awareness of the issue of the presence of potentially toxic cyanobacteria started to develop in Turkey in 1997 following heavy fish kill in Sapanca Lake. After this episode, Istanbul University Fisheries Faculty, Department of Inland Waters started to examine the cyanotoxins in lakes, drinking water reservoirs and lagoons. A number of the toxin producing cyanobacteria (particularly *Planktothrix rubescens, Planktothrix agardhii, Microcystis aeruginosa*, and *Nodularia spumigena*) demonstrate mass development during late spring, summer and early autumn (Fig 1).

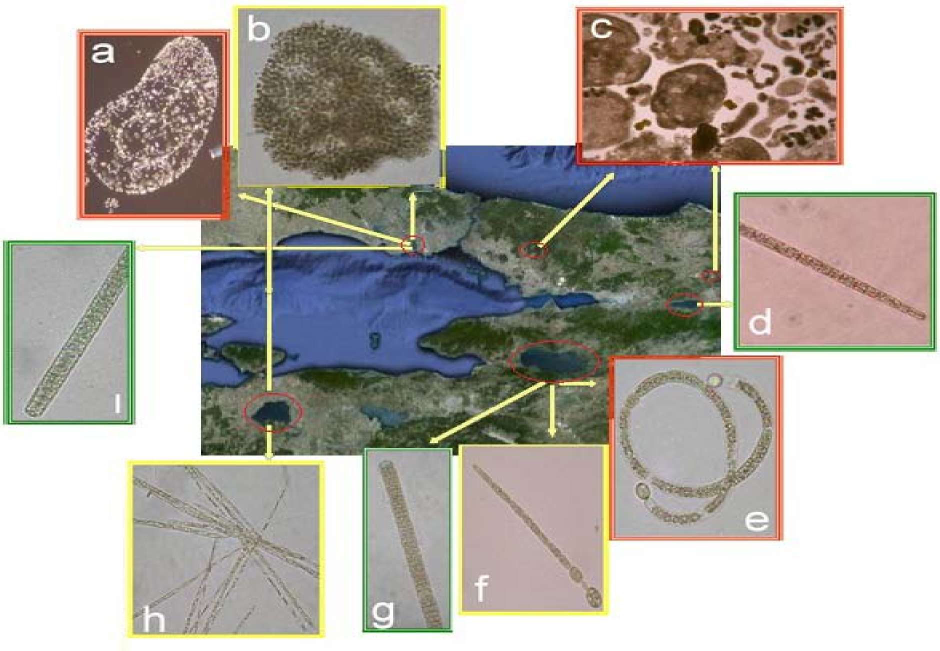


Figure 1: Cyanobacteria species isolated from drinking water reservoirs, lakes and lagoons in Marmara region. a: *Microcystis flos-aquae*, b: *M. aeruginosa*, c: *Microcystis* spp., d: *Planktothrix rubescens*, e: *Anabaenopsis* sp., f: *Sphaerospermopsis aphanizomenoides*, g: *Nodularia spumigena*, h: *Cylindrospermopsis raciborskii*, ı: *Planktothrix agardhii*

Cyanotoxins reached 72 µg/L Microcystin-LR equivalents in Sapanca Lake, 26 µg/L Microcystin-LR equivalents in Küçükçekmece Lagoon, 6 µg/L Microcystin-LR equivalents in Ömerli Drinking water reservoir and over 122 µg/L Microcystin-LR equivalents in Elmali Drinking water reservoir (Istanbul). Thus, all the studied water bodie contained toxic cyanobacterial blooms.

Although there have been numerous descriptions of bird and fish mortalities associated with exposure to cyanobacteria (Albay et al., 2003a,b; Albay et al., 2005; Akcaalan et al., 2009), currently there are no national guidelines for drinking waters, and Ministry of Health is planning to put into effect the monitoring of recreational hazards associated with cyanobacteria in Turkey.

Regulations and Guideline values

Drinking Water:

In Turkey, the quality of surface waters used as a drinking water is regulated by a national Decree (79/869/AB and 75/440/AB). Some microbiological, physical and chemical parameters are defined and limit values set for each of them in the Decree. However, monitoring of algae and cyanotoxins are not considered up to now. Research done since 1998 showed a potential risk hazard of cyanotoxins in drinking waters and in the lakes, mainly in Marmara region. Turkey is a Mediterranean country facing water scarcity because of decreasing rainfall. Cyanotoxins were so far studied mainly in the Marmara region, there is less work done in the other parts of Turkey which also have similar problems, and more research on cyanotoxin occurence throughout Turkey would be important.

In recent years, with increasing blooms and efforts to create awareness on cyanotoxin health risks in administrative bodies, cyanotoxins are being discussed as one of the potential criteria for drinking water quality for the new updated Decree planned to come in to effect in 2014. The guideline prepared and discussed for this purpose is based on the provisional WHO guideline value of 1 µg/l Microcystin-LR for finished drinking-water. This would be applied to the sum of all microcystins, i.e. it would use equivalent concentrations. The decision on when toxin analysis should be done would be based on the results of monitoring cyanobacterial populations in the raw water on a monthly basis as long as levels remain below 5000 cells/ml (or >1 µg/l Chlorophyll-*a*). If this threshold is exceeded, weekly sampling and toxin analysis should be done. Samples should be taken from not only surface but also whole water column especially in the depth of water intake as some toxic cyanobacteria (e.g. *Planktothrix rubescens*) can proliferate in deeper parts of the reservoir. If the toxin concentration is above 1 µg/l in the raw water, then toxins should be analysed in treated water, and advanced water treatment methods (ozonation or active carbon) should be put into service, or it should be considered to use another water supply where available.

Recreational waters:

The Ministry of Health is aiming at putting into effect following recommendation in 2014 in collaboration with the water managers: The following levels show the probable risk of adverse human health effects based on measurements of cyanobacterial abundance, microcystin-LR concentration and chlorophyll-a concentration. Three levels of management responses are planned:

Level 1: At < 20 000 cells/mL or <10 µg/L Microcystin-LR equivalents or <10 µg/L chlorophyll-a of which the majority are cyanobacteria, recreational activities are allowed to continue and users are informed by posters on site. Monitoring (sampling, counting and species identification) should be done fortnightly.

Level 2: At between 20 000 and 100 000 cells/mL, microcystins are analysed. If the concentration Microcystin-LR equivalent is > 25 µg/L, immediate action are taken to inform relevant authorities and public. Users are discouraged from swimming and other water-contact activities by risk advisory signs on site.

Level 3: When cyanobacterial scum appears in bathing areas, all activities in the water are possibly prohibited.

References

Albay, M., Akcaalan, R., Aykulu, G., Tufekci, H., Beattie, K.A.& Codd, G.A. (2003) Occurrence of Toxic cyanobacteria before and after cupper sulphate treatment in a water reservior, Istanbul, Turkey. *Archiv für Hydrobiologie Suppl. Algological Studies,* 109: 67 – 78.

Albay, M., Akcaalan, R., Tufekci, H., Metcalf, J. S., Beattie, K. A. & Codd, G.A. (2003) Depth profiles of cyanobacterial hepatotoxins (microcystins) in three Turkish freshwater lakes *Hydrobiologia,* 505: 89-95.

Albay, M., Matthiensen, A. & Codd, G. A. (2005) Occurrence of Toxic Blue-Green Algae in Kucukcekmece Lagoon (Istanbul, Turkey). *Environmental Toxicology* 20: 277– 284.

Akcaalan, R., Mazur-Marzec, H., Zalewska, A. & Albay, M. (2009) Phenotypic and toxicological characterization of toxic *Nodularia spumigena* from a freshwater lake in Turkey. *Harmful Alga*e 8; 273–278.

URUGUAY:
Occurrence, Toxicity and Regulation of Cyanobacteria

Leticia Vidal[1] & Anamar Britos[2]

[1] Grupo de Ecología y Fisiología de Fitoplancton, Sección Limnología, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, Universidad de la República (Uruguay),: leti.vidal.bertola@gmail.com
[2] Sección Oceanografía y Ecología Marina, Facultad de Ciencias, Universidad de la República (Uruguay)

Cyanobacterial blooms in Uruguay

Freshwater systems of South America account for half of the world´s water supply (UNESCO 2009). Uruguay is located in the southeastern part of South America (Figure 1) and has a total area of 318.413 km^2. It has a terrestrial area of 176.215 km^2, a jurisdictional water area of 16.799 km^2 and a territorial sea of 125.057 km^2 (Bellagamba & Broggi, 2011). It has a dense hydrographic network, formed mainly by rivers, streams, lakes and lagoons. The "Rio Uruguay" river is the border to the west and to the south the estuary of "Rio de la Plata" (southwest) and the Atlantic Ocean (southeast). The "Río Negro" river runs through the middle of the country from east to west, and contains a chain of three reservoirs (*Baygorria, Rincón del Bonete and Palmar*). There is another reservoir on the "Rio Uruguay" river (Salto Grande Dam), which is bi-nationally managed in conjunction with Argentina.

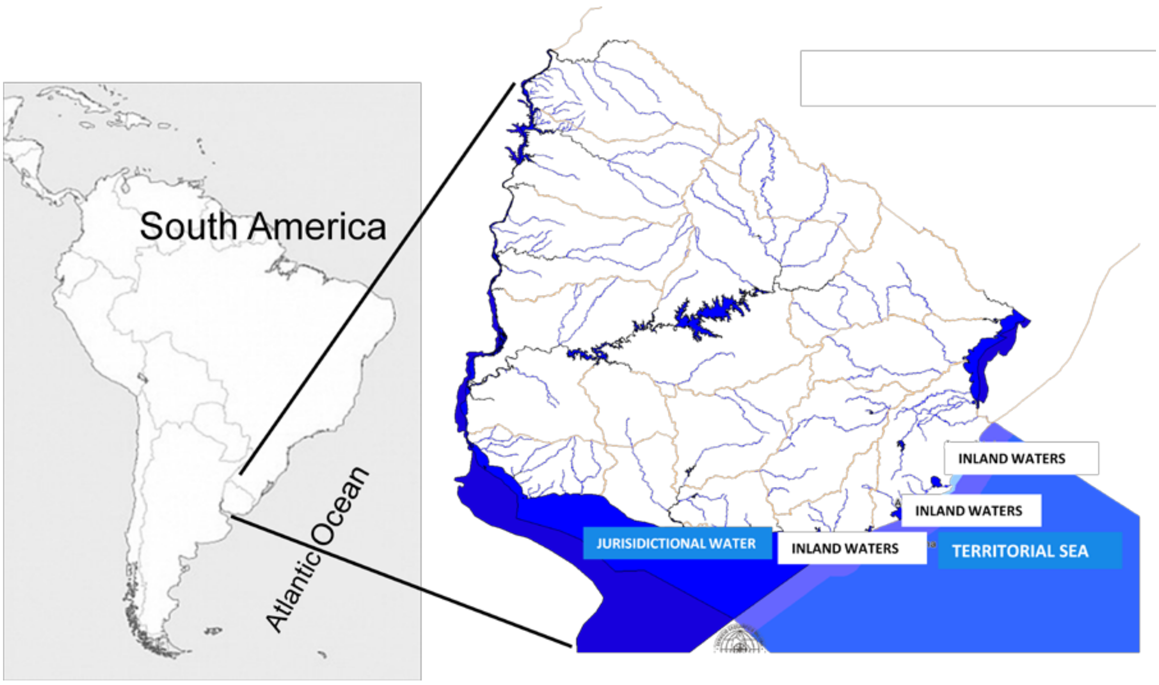


Figure 1: Map of Uruguay. Location in South America

Blooms of cyanobacteria have been registered in rivers, estuaries, reservoirs, lakes and lagoons in Uruguay since 1982. These blooms frequently occurred in eutrophic systems and generally during summer (Bonilla *et al*. 1995; Pérez *et al*.,1999; Ferrari & Méndez 2000; De León & Yunes 2001; Kruk *et al*. 2002; Sienra & Ferrari 2006; Feola *et al*. 2007, 2008; Vidal & Kruk 2008; Ferrari *et al.* 2009; Chalar 2009; Bonilla *et al*. 2012). Since most of these systems are used for drinking supply and recreational purposes, research on cyanobacteria and its toxicity has been intensified in the last few years (UNESCO 2009).

Bloom forming species of cyanobacteria belonging to the orders Chroococcales, Nostocales and Oscillatoriales were found in lentic as well as in lotic systems of Uruguay (Table 1). The most frequently encountered taxa were *Microcystis* sp., *Dolichospermum* sp. (ex *Anabaena* sp.) *and Planktothrix agardhii* (UNESCO 2009). In recent years, species of the Nostocalean order such as *Cylindrosermopsis raciborskii*, *Aphanizomenon* sp. and *Cuspidothrix issatschenkoi* have been more frequently registered in blooms (Vidal & Kruk 2008; UNESCO 2009; Fabre *et al*. 2010; Pacheco *et al*. 2010; Bonilla *et al*. 2012).

Table 1: Bloom-forming species of cyanobacteria registered in limnic and brackish systems in Uruguay (taken and modified from UNESCO 2009).

Type of water system	Order	Species registered
Rivers, streams	**Chroococcales**	*Microcystis aeruginosa, Microcystis novacekii, Microcystis flos-aquae, Microcystis panniformis, Microcystis protocystis*
	Nostocales	*Aphanizomenon gracile*, *Cuspidothrix issatschenkoi, Dolichospermum circinalis*, *Dolichospermum crassum*, *Dolichospermum planctonicum*, *Dolichospermum viguieri, Dolichospermum circinalis, Dolichospermum spiroides,*
	Oscillatoriales	*Pseudanabaena* sp.
Reservoirs, coastal lagoons, natural and artificial lakes	**Chroococcales**	*Merismopedia tenuissima, Microcystis aeruginosa*, *Microcystis novacekii*, *Microcystis panniformis*, *Microcystis protocystis, Aphanocapsa delicatissima*, *Sphaerocavum brasiliense, Aphanothece stagnina*
	Nostocales	*Anabaenopsis elenkinii*, *Aphanizomenom gracile*, *Dolichospermum circinalis*, *Dolichospermum viguieri, Dolichospermum planctonicum*, *Dolichospermum solitarium*, *Dolichospermum spiroides, Cylindrospermopsis raciborskii, Nodularia spumigena, Raphidiopsis mediterranea*
	Oscillatoriales	*Limnothrix redekeii*, *Planktolyngbya limnetica, Planktothrix agardhii*, *Pseudanabaena mucicola, Pseudanabaena moniliformis*

Concentrations of total microcystin between 8.4 µg/L and 58 µg/L were detected in dispersed blooms in coastal beaches (Río de la Plata) that were dominated by *Microcystis* sp. and *Dolichospermum* sp. (De León & Yunes 2001; Brena *et al*. 2006; Feola *et al*. 2010). In some of these systems, higher total microcystin concentrations were detected in scums, i.e. up to 21000 µg/L. Concentrations of total microcystin between 0.2 µg/L and 10.5 µg/L were found in rivers (Gravier *et al*. 2009; Vidal *et al*. 2009), reservoirs (Gorga *et al*. 2002; DINAMA, 2011a; Gianuzzi *et al*. 2011) and lakes (Vidal *et al*. 2009; Méndez *et al*. 2010), with predominance of *Dolichospermum* sp., *Microcystis* sp., *Aphanizomenon gracile* and *Cuspidothrix issatschenkoi*.

Saxitoxin has been recently recorded in rivers and lakes, where *Aphanizomenom gracile, Cylindrospermopsis raciborskii* and other Nostocalean species were dominant (Vidal *et al*. 2009; Vidal *et al.* 2010; Piccini *et al*. 2011). Cylindrospermopsin was also recently registered in low concentrations in rivers in which *Dolichospermum crassum* and *Aphanizomenon* sp. were the dominant species (Vidal *et al*. 2009).

Recreational water regulation approaches in Uruguay

The regulation of recreational water in Uruguay is managed by the National Environmental Management (DINAMA), which is a department of the Ministry of Housing, Regional Planning and Environment (MVOTMA). In 1990, DINAMA developed a project to assess the water quality of Uruguayan coastal beaches through a weekly monitoring program that is carried out during the summer at the most touristic beaches along the coasts of the Rio de la Plata and the Atlantic Ocean (DINAMA 2011b). In 1997, the Departmental Government of Montevideo (IMM) began a more intensive year-round monitoring of coastal water bodies of Montevideo, where nearly half of the population lives. In 2000, the IMM included cyanobacterial quantification and microcystin analysis in the monitoring of coastal water bodies of Montevideo (Sienra *et al*. 2009; IMM, 2011).

At the moment, DINAMA is awaiting for the approval of a legislation modification (decree 253/79) which intends to introduce cyanobacteria guidelines for bathing in recreational waters (DINAMA 2011b). These criteria would classify beaches as acceptable or not acceptable for bathing based on chlorophyll-*a* and cyanobacteria concentrations (DINAMA 2011b) in addition to other microbiological parameters.

Nowadays, DINAMA warns the local authorities and the general public if visible cyanobacteria blooms are detected in recreational waters (DINAMA 2011b). In Montevideo, a warning flag (green cross on a red background) is displayed at the lifeguard´s stand to alert the beach attendants about visible cyanobacterial blooms (IMM 2011, Municipal resolution 1324/10).

Approaches to drinking water regulations in Uruguay

Uruguay has an estimated population of 3.356.000 of which 98% have access to treated drinking water. The State-owned Water Utility (OSE, which is a dependence of the MVOTMA) had been providing all drinking water services in Uruguay until 1996, when some supply services were privatized through concession contracts. In 2004, inconformity with the management and services provided led to a national referendum, where a National Constitutional Amendment was voted by 62.75% of the constituents. Sanitation and drinking water policies were established in this amendment, in wich the main principles stated were: "*Access to drinking water and sewerage system services are fundamental human rights*" and "*The public service of sewerage and the public service of water supply for human consumption will be served exclusively and directly by state legal persons*". After the referendum, OSE began to regain drinking water supply services, reaching control of almost 100% of these services. Nowadays, the Energy and Water Services Regulatory Unit (URSEA) is in charge of controlling drinking water services (Law 17.598, December 2002).

Until November 2011, when the decree 375/11 came into force, there were no national regulations on cyanotoxin levels in drinking water in Uruguay. The decree created by the Executive branch of the Government, establishes to follow the Technical National Standard UNIT 833:2008, which determines the microbiological, chemical and biological requirements for drinking water and the methods to evaluate its quality. The standard was developed by technical representatives of the most relevant national institutions related to water resources and health: URSEA, Ministry of Public Health, National Management of Water (DINAGUA), OSE, Faculty of Medicine and Faculty of Chemistry, among others. The UNIT Standard 833:2008 established a maximum concentration of 1 µg/L of Microcistyn-LR in treated drinking water (measured by the method ISO 20179:2005). It also established that "*Drinking water should not contain amounts of cyanobacteria that could affect water characteristics or human health*". However, the quantitative value of abundance (or biovolume) of cyanobacteria that should be considered a risk for human health is not included in this decree.

Although the decree 375/11 is very recent, the state-owned water utility (OSE) has been concerned with the presence of cyanobacteria in the country for many years. For example, in 2006 OSE defined a maximum microcystins concentration of 1 µg/L and the absence of potential toxic cyanobacteria in 25 mL of drinking water as acceptable (OSE 2006). Phytoplankton and cyanobacteria analyses in drinking water and raw water are performed at the Hydrobiology Area at the Central Laboratory of OSE. Between 2006 and 2008 immunoassays for microcystin, saxitoxin and cylindrospermopsin determination (commercial ELISA kits) were added (Britos *et al*. 2009). Microcystin analyses of drinking water are carried out when potentially toxic species reach 2000 cell/mL or a biovolume of 0.2 mm^3/L in treated water or when dense blooms are observed in raw water (Britos *et al*. 2009). In order to establish the abundances or biovolumes that trigger drinking water toxin analyses of other toxins (saxitoxin and cylindrospermopsin), it is necessary to know their toxin concentration per cell or per unit biovolume (cell quotas). Research about this topic is currently being developed by the Hydrobiology Area of the Central Laboratory of OSE (Vidal *et al*. 2010).

Cyanotoxin analysis have also been performed in other laboratories in the country: The Technological Laboratory of Uruguay (LATU) carries out microcystin-LR analysis through HPLC and ELISA method for total microcystin analysis which was locally developed at the Faculty of Chemistry (University of the Republic) by Brena *et al.* (2006).

Aknowledgements

We thank Sylvia Bonilla of the Limnology Section of Faculty of Sciences (University of the Republic) for her comments and also to Patricia Viera and Soledad Morales for the English review.

References

Bellagamba, J.L. & Broggi, G. (2011). *Recursos Hídricos: Uruguay*. Rafael Bernardi & Marcel Achkar (Eds.). Centro del Agua para América Latina y el Caribe. 70p [on line] http://issuu.com/_cda_/docs/rh_uruguay

Bonilla, S., Perez, M.C. & De León, L. (1995). Cyanophyceae planctónicas del lago Ton-Ton, Canelones, Uruguay. *Hoehnea,* 22: 183–190.

Bonilla, S., Aubriot, L., Soares, M. C. S., González-Piana, M., Fabre, A., Huszar, V. L. M., Lürling, M., Antoniades, D., Padisák, J. & C. Kruk (2012) What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*?. *FEMS Microbiology Ecology*, 79: 594–607

Brena, B. M., Díaz, L., Sienra, D., Ferrari, G., Ferraz, N., Hellman, U., Gonzalez-Sapienza, G. & Last, J. A. (2006). ITREOH building of regional capacity to monitor recreational water: development of a non-commercial microcystin ELISA and its impact on public health policy. *International Journal of Occupational and Environmental Health,* 12: 377-385

Britos, A., Gravier, A. & Vidal, L. (2009). Métodos de análisis de cianotoxinas en los servicios de agua potable de OSE. *Resúmenes del 1º Encuentro Uruguayo. Cianobacterias: del conocimiento a la gestión*. Octubre 2009. http://limno.fcien.edu.uy/CDCIANO2009/Cronograma280909_archivos/PDF/RESUMENES-CIANO2009.pdf

Chalar, G. (2009). The use of phytoplankton patterns of diversity for algal bloom management. *Limnologica*, 39: 200–208.

De León, L. & Yunes, J.S. (2001). First report of a Microcystin-Containing Bloom of the Cyanobacterium *Microcystis aeruginosa* in the La Plata River, South America. *Enviromental Toxicology*, 16(1): 110–112.

DINAMA (2011a). Monitoreo y evaluación de calidad de agua. Plan para la definición de una línea de base del Río Negro. (Agosto 2011). *Informe Técnico. Departamento de Calidad Ambiental. División Evaluación de la Calidad Ambiental. 35 p [on line].* http://www.mvotma.gub.uy/biblioteca/documentos-de-ambiente

DINAMA (2011b). Monitoreo de Playas Temporada 2010 – 2011. *Informe Técnico. Departamento de Calidad Ambiental. División Evaluación de la Calidad Ambiental*. [on line]. www.dinama.gub.uy Publicaciones/Documentos, Informes técnicos, Calidad de Playas.

Fabre, A., Carballo, C., Hernández, E., Píriz, P., Bergamino, L., Mello, L., González, S., Pérez, G., León, J G., Aubriot, L,. Bonilla, S. & C. Kruk (2010). El nitrógeno y la relación zona eufótica/zona de mezcla explican la presencia de cianobacterias en pequeños lagos subtropicales, artificiales de Uruguay. *Pan-American Journal of Aquatic Sciences*, 5:112-125

Feola, G., Brena, B., Risso, J., Sienra, D., Saona G. & Echezarreta M.E. (2010). Programa de Monitoreo de Agua de Playas y Costa de Montevideo. *Informe temporada estival: 2009-2010. Montevideo, Laboratorio de Calidad Ambiental. Depto. de Desarrollo Ambiental. Intendencia Municipal de Montevideo IMM*. 143pp. [on line] http://www.montevideo.gub.uy/sites/default/files/articulo/Playas__Evaluacion_calidad__aguas_2009-_2010.pdf

Feola, G., Brena, B., Risso, J. & Sienra, D. (2007). Programa de monitoreo de agua de playas y costa de Montevideo. *Informe temporada estival 2006-2007. Departamento de Desarrollo Ambiental, Servicio Laboratorio de Calidad Ambiental, IMM.* 84pp.[on line]. http://www.montevideo.gub.uy/sites/default/files/articulo/monitoreoagua_estival0607_0_0.pdf

Feola, G., Brena, B., Risso, J., Sienra, D. & Echezarreta M.E. (2008). Programa de Monitoreo de Agua de Playas y Costa de Montevideo. *Informe temporada estival 2007-2007. Departamento de Desarrollo Ambiental, Servicio Laboratorio de Calidad Ambiental, IMM*. 118pp. [on line]. http://www.montevideo.gub.uy/sites/default/files/articulo/monitoreoagua_estival0708_0_0.pdf

Ferrari, G. & Méndez, S. (2000). Report of phytoplankton species producing coastal water discoloration in Uruguay. *Iheringia, Série Botâni*ca, 54:3-18.

Ferrari, G., Cea, J., Miguez, M. & Saizar, C. (2009). Monitoreo de floraciones algales en el Río Negro, Uruguay. *Resúmenes del 1º Encuentro Uruguayo. Cianobacterias: del conocimiento a la gestión -*

.Octubre 2009. [on line]. http://limno.fcien.edu.uy/CDCIANO2009/Cronograma280909_archivos/PDF/RESUMENES-CIANO2009.pdf

Giannuzzi, L., Sedan D., Echenique, R. & Andrinolo, D. (2011), An Acute Case of Intoxication with Cyanobacteria and Cyanotoxins in Recreational Water in Salto Grande Dam, Argentina. *Marine. Drugs,* 9:2164-2175.

Gorga, J., Paradiso, M., De León, L., Brugnoli, E., Mandiá, M. & Conde, D. (2002). Identificación de las problemáticas potenciales que afectan los usos de los embalses del Río Negro: entre lo posible y lo necesario. *2da Reunión Internacional de Eutrofización de Lagos y Embalses y II Taller de Ecología y Manejo de los Embalses de Uruguay: Bases para la Gestión Integrada de los Recursos Hídricos*. Montevideo – Uruguay. [on line] http://rele.fcien.edu.uy/talleres/Gorga2.html

Gravier, A., Vidal, L. & Britos, A. (2009). Cianobacterias como organismos interferentes en el servicio de de agua potable de Bella Unión (Uruguay). *Resúmenes -del 1º Encuentro Uruguayo. Cianobacterias: del conocimiento a la gestión. Octubre 2009*. [on line]

http://limno.fcien.edu.uy/CDCIANO2009/Cronograma280909_archivos/PDF/RESUMENES-CIANO2009.pdf

IMM, (2011). Floraciones algales, [web site]. http://www.montevideo.gub.uy/ciudadania/desarrollo-ambiental/playas/floraciones-algales .

ISO 20179:2005. Water quality -- Determination of microcystins -Method using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

Kruk, C., Mazzeo, N., Lacerot, G. & Reynolds, C.S. (2002). Classification schemes for phytoplankton: a local validation of a functional approach to the analysis of species temporal replacement. *Journal of Plankton Research*, 24(9):901-912.

Méndez, G., Lagomarsino, J.J. & Alcorta, R. (2010). Mitigación de las consecuencias adversas en el proceso de potabilización. p 89 – 94. *En: Seffen M & H. Inda Eds., Bases técnicas para el manejo integrado de la Laguna del Sauce y cuenca asociada. Uruguay.* ¨[on line] http://issuu.com/institutosaras/docs/laguna-del-sauce

OSE (2006). Norma Interna de Calidad de Agua Potable. Administración de las Obras Sanitarias del Estado.Uruguay. [on line] http://www.ose.com.uy/descargas/reclutamiento/norma_interna_de_calidad_agua_potable_de_OSE.pdf

OSE (2011). Resolución de Directorio Nº1784/11. Administración de las Obras Sanitarias del Estado. Uruguay. [on line] http://www.ose.com.uy/acuerd/index.html

Pacheco, J. P., Iglesias, C., Meerhoff, M., Fosalba, C., Goyenola, G., Teixeira-de Mello, F., García, S., Gelós, M., García-Rodríguez, F. (2010). Phytoplankton community structure in five subtropical shallow lakes with different trophic status (Uruguay): a morphology-based approach. *Hydrobiologia*, 646 (1):187-197.

Pérez, M. C., Bonilla, S., De León, L., Smarda, J. & Komárek, J. (1999). A bloom of *Nodularia baltica-spumigena* group (Cyanobacteria) in a shallow coastal lagoon of Uruguay, South America. *Algological Studies,* 93:91-101.

Piccini, C., Aubriot L., Fabre A., Amaral, V., González-Piana, M., Giani,, A., Figueredo, C.C., Vidal, L., Kruk, C. & Bonilla, S. (2011). Genetic and eco-physiological differences of South American *Cylindros-permopsis raciborskii* isolates support the hypothesis of multiple ecotypes. *Harmful Algae*, 10:644–653.

Sienra, D. & Ferrari, G. (2006). Monitoreo de cianobacterias en la costa de Montevideo (Uruguay). In: Menafra, R., Rodríguez-Gallego, L., Scarabino, F. & Conde D. (Eds). *Bases para la conservación y el manejo de la costa uruguaya*, Montevideo, 403-420.

Sienra, D., Brena, B.M. & Feola, G. (2009). Monitoreo de Cianobacterias en Playas de Montevideo *Resúmenes del 1º Encuentro Uruguayo. Cianobacterias: del conocimiento a la gestión-. Octubre 2009.* [on line]. http://limno.fcien.edu.uy/CDCIANO2009/Cronograma280909_archivos/PDF/RESUMENES-CIANO2009.pdf

UNESCO, (2009). Cianobacterias Planctónicas del Uruguay. Manual para la identificación y medidas de gestión. Sylvia Bonilla, (Ed). Documento Técnico PHI.LAC, N° 16. 94p. [on line] http://www.unesco.org.uy/phi/biblioteca/bitstream/123456789/473/1/Cianobacterias+Planctonicas+del+Uruguay.pdf

UNIT (833:2008). Agua Potable Requisitos. Norma Técnica. [on line]. http://www.ecotech.com.uy/pdf/UNIT.pdf

Vidal, L. &. Kruk, C. (2008). *Cylindrospermopsis raciborskii* (Cyanobacteria) extends its distribution to Latitude 34°53'S: taxonomical and ecological features in Uruguayan eutrophic lakes. *Pan-American Journal of Aquatic Sciences*, 3:142-151

Vidal, L., Britos, A. & Gravier, A. (2009). Cianobacterias planctónicas en aguas superficilaes destinadas para consumo humano en Uruguay: taxonomía, distribución y toxicidad. *Resúmenes del 1º Encuentro Uruguayo. Cianobacterias: del conocimiento a la gestión-. Octubre 2009.* [on line] http://limno.fcien.edu.uy/CDCIANO2009/Cronograma280909_archivos/PDF/RESUMENES-CIANO2009.pdf

Vidal L., Britos A. & Gravier A. (2010). Saxitoxin to biovolume ratio in natural population of *Cylindropermopsis* complex of Uruguay. *Official Program and Abstract Book. The International Conference of Toxic Cyanobacteria (ICTC8).* Istanbul, Türkiye. 2010. p73

UNITED STATES OF AMERICA: Historical Review and Current Policy Addressing Cyanobacteria

H. Kenneth Hudnell[1], Lorraine C. Backer[2], Joel Andersen[3], Dionysios D. Dionysiou[3]

[1] Medora Environmental, Inc. SolarBee & GridBee; University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering, 105 Serrano Way, Chapel Hill, NC 27517; kenhud@SolarBee.com
[2] National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway NE MS F-57, Chamblee, GA 30341; lfb9@cdc.gov
[3] University of Cincinnati, Environmental Engineering and Science Program, 705 Engineering Research Center, Cincinnati, OH 45221-0012; ander2jl@mail.uc.edu; dionysios.d.dionysiou@uc.edu

Background Legislation

Federal legislation enacted in the U.S.A. to restore and protect surface waters began with the Rivers and Harbors Act of 1899. That Act set policies concerning pollutant discharges to navigable waters of the United States, which includes most freshwater bodies. The Federal Water Pollution Control Act, enacted in 1948, authorized or encouraged states to act to protect interstate waters by giving them options including forming interstate compacts, identifying water pollution as a public nuisance, and suing the polluters. The first mandatory federal water quality standards came with the Water Quality Act of 1965. Amendments enacted in 1972 significantly reorganized and expanded the Act. Those amendments included the establishment of: 1) requirements for states, territories, and tribes (jurisdictions) to set Water Quality Standards and develop Implementation Plans in Section 303; 2) the Clean Lakes Program (CLP) for restoring publicly-owned lakes and reservoirs in Section 314, and; 3) the National Pollution Discharge Elimination System (NPDES) permit program for the regulation of point-source pollutants in Section 402. The Act commonly became known as the Clean Water Act (CWA) through amendments enacted in 1977. CWA amendments enacted in 1987 established the Nonpoint Source Management Program through Section 319 to address pollutant runoff. The U.S. Environmental Protection Agency (EPA), established in 1970, was charged with administrating the CWA and other environmental acts (EPA, History of the Clean Water Act).

The goal of the CWA is “to restore and maintain the chemical, physical, and biological integrity of the Nation’s waters.” CWA Sections 303 and 304, Water Quality Standards and Criteria, define the objectives for a waterbody to be: 1) restore and protect appropriate designated uses such as drinking source water, aquatic biota and wildlife habitat, recreation, and commerce; 2) set water quality criteria protective of the designated uses that are based on scientific information concerning pollutant concentrations and adverse effects on ecosystems and human health, and; 3) establish policies and implement strategies to meet the water quality criteria (EPA, Surface Water Standards and Guidance).

Many water quality criteria originally were written in narrative form with non-quantitative expressions of the impairment, causative pollutant, and restrictions on that pollutant. Numeric criteria, pollutant concentrations that will not cause impairment, are now required when measurable for priority and toxic pollutants (EPA, Numeric and Narrative Criteria). The EPA designated phosphorus and nitrogen as priority pollutants due to freshwater eutrophication, harmful algal blooms (HABs), and associated toxic (Hudnell, 2005) and hypoxic conditions

(EPA, Nutrient Pollution and Numeric Standards). The requirement for TMDLs, particularly numeric nutrient criteria, has been highly contentious (EPA, Litigation Status; EPA, Necessary Criteria: Addressing Priority Pollutants). Proponents of the requirement sued the EPA, demanding the Agency set numeric nutrient criteria for jurisdictions that had not done so. Jurisdictions sued the EPA charging that the Agency's criteria were technically unsound or without cost-benefit merit (Marten Law, EPA faces lawsuits). A U.S. district court judge ruled in 2012 that the EPA was correct in determining that numeric nutrient standards were needed for Florida freshwaters. The judge upheld numeric nutrient criteria developed by the EPA for Florida's lakes and springs, but overturned those for streams. Criteria recently developed for streams by Florida propose the same numeric values as the EPA's, but are triggered only after a HAB occurrence and ensuing study. The judge upheld the process for variances, or site-specific alternative criteria (Bloomberg BNA, Federal Court Issues Mixed Ruling On Criteria for Florida Nutrient Standards). Although the rulemaking process is incomplete, final rules may include some criteria developed by the Agency and some by the state (EPA, Water Quality Standards for the State of Florida's Lakes and Flowing Waters).

Congress appropriated approximately $85 billion through 2012 to help jurisdictions acquire point-source control technologies (CRS, 2012). CWA Section 303(d) requires states, territories, and tribes to identify, list, and prioritize impaired waters, waters that remained too degraded to meet water quality criteria after implementing required point-source control technologies to meet NPDES limits. A total maximum daily load (TMDL) must be calculated for each pollutant causing a waterbody to be impaired (EPA, Impaired Waters and Total Maximum Daily Loads). A TMDL quantifies the maximum amount of a pollutant that a waterbody can receive and still meet its water quality standards and criteria. TMDLs must account for all point and nonpoint source loading, including internal loading and air deposition when applicable. Indirect air-deposition loading through storm water runoff can be managed under CWA authority with nonpoint source best management practices (BMPs), but reducing the direct-air deposition of pollutants such as nitrogen into waterbodies typically requires authority other than the CWA. Waterbodies are prioritized by the jurisdictions for TMDL development. Perhaps 10-20% of the more than 41,000 impaired U.S. waterbodies have TMDLs developed for all their impairing pollutants (EPA, Watershed Assessment, Tracking & Environmental Results). Jurisdictions then develop and implement watershed management plans, including nutrient management strategies for eutrophic waterbodies, for point and non-point pollutant inputs to achieve TMDLs (EPA, Implement the Watershed Management Plan).

Clean Lakes Program and Waterbody Management

The CLP was established to provide federal financial and technical assistance to jurisdictions for the restoration of publically owned surface waters using waterbody management technologies. Waterbody management is the complement of Watershed Management; whereas watershed management attempts to prevent point- and nonpoint-source pollutants from entering receiving waters, waterbody management uses technologies to mitigate the adverse impacts of pollutants that do enter receiving waters. However, prior to the latter part of the Twentieth Century, mitigation options to address adverse impacts such as pathogens, toxins, and excessive nutrient loads and low water-flow/mixing rates that stimulate HABs were expensive, inadequate or caused adverse impacts themselves (Hudnell, 2008). Technological options included unsustainable chemical applications, aeration limited by small areas of influence and excessive electrical-grid power use, flushing limited by inadequate supply, dredging, and macrophyte harvesting. Only about $145M of Section 314 grants were awarded since 1976, and none since 1995. Although the Section 314 CLP was reauthorized in 2000, the EPA has not requested funding authorizations. That year the EPA encouraged jurisdictions to use at least 5% of the Section 319(h) grant program for activities that might have been funded in previous years through Section 314 grants (EPA, Clean Lakes Program). However, in 2002 the Agency increased focus of the Section 319 Program on TMDL implementation for nonpoint sources (EPA, Supplemental Guidelines). The vast majority of Section 319 funds, about $3.2B total, and $200M per year since 1999, were used to implement Watershed Management BMPs for nonpoint source pollutant containment (MRSC, Best Management Practices). BMPs vary greatly in effectiveness and cost efficiency (Chesapeake Bay Program, Non-point Source BMPs and Efficiencies). Many are difficult to implement over large areas and expensive, do not address internal loading or direct atmospheric deposition, and typically are not expected to restore Designated Uses for 2-3 decades.

Because the CLP has not been implemented to a significant degree, current CWA policy essentially consists of Watershed Management only. Technologies are used to control point-source inputs and meet NPDES limits. BMPs are implemented for non-point source runoff control. Together these strategies are designed to attain TMDL limits and meet Water Quality Criteria. The long-term goal is to meet Water Quality Standards so that Designated Uses are restored and protected. However, EPA data indicate that Watershed Management alone may be insufficient for meeting the CWA goal.

Watershed Management: Impairment, Eutrophication, and HABs

Approximately 44% of river and stream miles, and 64% of lake and reservoir acres in the U.S. are impaired pursuant to CWA Section 303(d) (Hudnell, 2010). Impairment due to eutrophication appears to be increasing rapidly. Chlorophyll-a concentrations in multiple sites of each lake sampled in EPA's National Eutrophication Survey of 1972 indicated that 10-20% of lakes and reservoirs were eutrophic (Gakstatter and Maloney, 1975; USGS, Review of Phosphorus). The Agency's National Lake Assessment of 2007 indicted that about 50% to 58% are now eutrophic or hypereutrophic based on chlorophyll-a concentrations and Secchi depths, respectively (EPA, National Lake Assessment). National Lake Assessment cyanobacterial cell densities and chlorophyll-a concentrations indicated that health risk from exposure to cyanotoxins was moderate to high in 27% to 41% of lakes and reservoirs, respectively, when evaluated relative to the World Health Organization's guidelines for cyanotoxin exposure in recreational waters. The cyanotoxin class of microcystins was detected in about 30% of the sampled waterbodies (EPA, National Lake Assessment). The presence of other cyanotoxins such as anatoxins, cylindrospermopsins, saxitoxins, nodularins, and β-N-methylamino-L-alanine (a.k.a. BMAA) was not assessed. However, the U.S. Geological Survey recently reported that saxitoxins and cylindorspermopsin were present in 8% and 5% of stored samples, respectively, from the 2007 National Lake Assessment (Loftin, 2012).

The National Lake Assessment likely underestimated the prevalence of eutrophication and moderate-to-high health risks from exposure to microcystins and other cyanotoxins in the target-population lakes. The estimates were based on measurements of nutrients, chlorophyll-a, water clarity, and microcystins obtained from single water-grab samples usually collected near the middle of the waterbodies. The survey relied on the assumption that the indicators are homogeneous across space in freshwater bodies. Water samples collected at multiple sample sites within a lake have shown this not to be the case (Blue Water Satellite, 2012). Exclusion criteria applied to 389,005 lakes and reservoirs resulted in 49,546 waterbodies eligible for sampling, of which 1,028 were sampled. The samples, collected during summer, integrated water from just below the surface to a depth of 1.5 m at the deepest point in each waterbody. Satellite data indicate that HABs usually begin in shallower water near lake perimeters where nutrient inputs and concentrations are highest and most recreation occurs (Blue Water Satellite, 2012). Cyanobacterial cell densities and chlorophyll-a concentrations are typically higher, and Secchi depth values are lower, near shorelines than mid-lake areas as blooms develop. If HABs spread to mid-lake areas, cells near the surface are often blown to shoreline areas. Because of the single sample and central location limitations, the results only indicate the probability of eutrophication and moderate-to-high health risk in the central area of target-population lakes on any given day during summer. A sampling design representative of the entire lake is required to estimate each lake's average status of eutrophication and health risk at the time of sampling. The average status for each lake must be known to estimate the average status of the target population. Furthermore, although beyond the scope of the National Lake Assessment, the survey does not indicate the percentage of target-population lakes that experience eutrophication and moderate-to-high health risk at some point during the spring-to-fall period when HABs typically occur. More spatially and temporally intensive sampling, such as that available through satellite monitoring, is required to estimate the percentage of affected lakes and reservoirs.

Despite the limitations of the National Lake Assessment, the data indicate that freshwater eutrophication has increased rapidly during the last 3-4 decades, questioning our ability to maintain a sustainable supply of usable freshwater. Nutrient inputs from point source discharges declined by 1984 to about 5-10% of total input, whereas non-point source inputs accounted for approximately 90-95% of total input (Gianessi and Peskin, 1984). Eutrophication prevalence, health risk from cyanotoxin exposure, and non-point source BMP limitations indicate that the current policy of Watershed Management only is insufficient for protecting and restoring Designated Uses.

Complementing Watershed Management with Waterbody Management

Waterbody management is the application of technologies, biological principles, and hydrologic manipulations within waterbodies to restore and protect their designated uses. A policy that complements watershed management with waterbody management may suppress HABs and reduce nutrient and other pollutant levels in the near term at overall reduced costs. This complementary policy would acknowledge that an impaired waterbody is analogous to an ill person in need of supportive therapy to reduce stress on impaired biochemical processes and enable recovery.

HAB occurrence requires quiescent or stagnant water as well as excessive nutrient loads. Enhanced water mixing through artificial circulation, including environmentally beneficial solar-powered circulation, hydrologic flushing, or aeration prevents HAB-organism predominance and promotes a balanced algal assemblage even in nutrient-enriched waters (Hudnell, et al. 2010). Nutrients, unlike toxic substances and pathogens, are not inherently dangerous. The elimination of HABs without algaecide use enhances fisheries by enabling nutrients to ascend the trophic levels of the food web. Enhanced mixing also reduces pathogen cell densities by repeatedly exposing the organisms to ultraviolet sunlight at the water surface, and increases oxygen levels in the water column. Oxygenated water supports fisheries, prevents the methylation of elemental mercury to toxic methylmercury, precipitates metals such as manganese and iron from the water column, converts toxic and malodorous hydrogen sulfides to nontoxic and odorless sulfates, and promotes the degradation of other toxic compounds (Hudnell, 2010; Hudnell, et al., 2011). Other waterbody management methods include the use of bacteria, floating islands and mats, side stream flow-ways, and flocculants to reduce nutrient or other pollutant levels, or degrade toxic substances.

The Clean Lakes Program, National Pollution Discharge Elimination System (Point Source) Program, and Nonpoint Source Management Program can be viewed as the three pillars that conjoin waterbody and watershed management into a systems approach to surface water protection and restoration (Hudnell, et al., 2008). The combination of waterbody and watershed management may provide the flexibility to optimize management strategies so that each impaired waterbody can be treated in the most effective and cost efficient manner. This ecologically-based, systems-approach to freshwater management could reverse the trends of increasing eutrophication, HAB occurrence, and impairment by other pollutants, restore water quality and designated uses in the near term, and ensure a sustainable supply of useable freshwater at costs less than that now spent on non-point source BMPs alone (Hudnell, 2010).

Protecting Health

The U.S. federal government has not established regulations or guidelines for cyanobacteria or cyanotoxins. Comprehensive data on cyanotoxin occurrence, dose-response relationships, and cost-effective risk management strategies are needed to develop health-based regulations or guidelines (Hudnell, et al., 2008a; Hudnell, 2010a). The EPA placed cyanobacteria and their toxins on the first Drinking Water Contaminant Candidate List (CCL) in 1998 for potential regulatory determination, and cyanotoxins remain on the current CCL3 list (EPA, CCL3). The Agency also drafted toxicological reviews (EPA Toxicological Reviews - draft) to identify levels of cyanotoxin exposure that do not cause adverse-health effects (Hudnell, 2010a). Extensive sampling in a single state, Florida, indicated that cyanotoxins were often present in finished drinking water when HABs occurred in the source waters, sometimes at levels higher than in the source waters (Williams et al., 2006). The Agency may implement the Unregulated Contaminant Monitoring Rule to more thoroughly assess the occurrence of cyanotoxins in drinking water, but cyanotoxins were not included in the UCMR3 initiated in 2012 (EPA, UCMR3).

Many states and other jurisdictions rely on guidelines published by the World Health Organization (WHO, 2003) to manage waterbodies in which HABs occur. Other states derived their own risk assessments to develop guidelines to support public health decision-making, such as posting advisories or closing water bodies. For example, the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency issued guidance on six cyanotoxins in 2012 (Butler et al., 2012). The report provides action levels that may be applied by local, regional, state, or tribal entities to reduce or eliminate exposure of people and animals to algal toxins (see Butler et al., 2012).

Oklahoma was the first state in the US to pass legislation limiting exposure to freshwater algae (LegiScan). The new law requires the Oklahoma Tourism and Recreation Department to maintain a public website, Oklahoma Lake Conditions, which provides data collected by the Oklahoma Department of Environmental Quality, the U.S. Army Corps of Engineers, and municipal authorities. The law also requires that any agency with authority to manage recreational waters shall post signs directing people to the website for information. Finally, the law formalizes the responsibility for warning people about algae and algal blooms and sets the health-related warning thresholds, i.e., tourism officials are responsible for warnings and will warn lake users only if algae cell counts exceed 100,000 cells/mL (compared with the previously used concentration of 20,000 cells/mL) and microcystin concentrations exceed 20 µg/L (Carter 2012a). These levels are not universally accepted (see Carter 2012b) because cell concentrations of 100,000 cells/mL are considered by the World Health Organization (WHO) to be associated with a high probability of health effects (WHO). Table 1 summarizes the current State guidelines for algae and algal toxins in drinking and recreational waters.

Table 1: State Guidelines and Regulations for Cyanobacteria and Cyanobacterial Toxins in Drinking and Recreational Waters in the USA

State	Toxin or species	Drinking water	Recreational waters
California (Butler et al, 2012)	Microcystins (LA, LR, RR, YR)		*Action levels* Human recreational uses: 0.8 µg/L (water) Human fish consumption: 10 ng/g ww[3] (fish) Livestock: Water 3 µg/L, crusts 0.2 mg/L Pets: Water 7 µg/L, crusts 0.02 mg/kg Fish: Water 13 ng/g *Monitoring Guidance* 40,000 to 10,000 cells/ml Microcystin ≥ 8 µg/L Scum associated with toxigenic species
	Cylindrospermopsin		*Action levels* Human recreational uses: 4 µg/L Livestock: Water 10 µg/L, crusts 1 mg/L Pets: Water 40 µg/L, crusts 0.1 mg/kg Fish: 66 ng/g
	Anatoxin-a		*Action levels* Human recreational uses: 90 µg/L Livestock: Water 100 µg/L, crusts 10 mg/L Pets: Water 400 µg/L, crusts 1 mg/kg Fish: Water 1100 ng/g *Monitoring Guidance* ≥ 70,000 cells/ml, ≥ 14 µg/L
Florida	Microcystins	1 ug/L chronic; 10 ug/L 90 day HAL (technical derivation available)	Event Based Response: FDOH Common Sense Approach; no recreational activities in blooms (Press Release available)
Indiana	Microcystins		Event-based response: ≥ 20 µg/L Very low/no risk: < 4 µg/L Low to moderate risk of adverse health effects: 4 – 20 µg/L Seriously consider avoiding contact with water until levels of toxin decrease: >20 µg/L
Iowa	Microcystins		*Routine monitoring:* Advisory/closure ≥ 20 µg/L

State	**Toxin or species**	**Drinking water**	**Recreational waters**
Kansas	Microcystins		*Health advisory:* ≥ 4 µg/L *Health warning:* ≥ 20 µg/L
	Total cyanobacteria cells		*Health advisory:* Cell count ≥ 20,000 cells/mL *Health warning:* Cell count ≥ 100,000 cells/mL
Maryland	Microcystins or Planktothrix		*Routine monitoring:* Cell counts: ≥40,000 cells/mL Only Microcystins: ≥ 14 µg/L
Massachusetts	Microcystins		*Guideline: avoid water contact* Humans: 14 µg/L Cell counts: ≥70,000 cells/mL *Monitoring Guidance* Cell counts: ≥70,000 cells/mL; ≥ 8 µg/L
Michigan			Each county handles incidents on a case-by-case basis.
Nebraska	Microcystins		*Routine monitoring*: Advisory/closure ≥ 20 µg/L
New Hampshire			*Routine Monitoring* > 50% toxigenic cyanobacteria
New York			Each county handles incidents on a case-by-case basis.
North Carolina			Routine monitoring in specific waterbodies, monitoring as needed in others
Ohio	Microcystins	*Regulation (MCL)* 1 µg/L	*Health advisory:* ≥ 6 µg/L "*No contact" advisory:* ≥ 20 µg/L
Oklahoma	Total cyanobacteria cells		*Action level:* Health advisory 100,000 cells/mL
	Microcystins		*Action level:* Health advisory: 20 µg/L
Oregon	Total cyanobacteria cells		*Action level:* Health advisory ≥ 100,000 cells/mL Scum associated with toxigenic species
	Microcystis or *Plantothrix*		*Action level:* Health advisory ≥ 40,000 cells/mL
	Anatoxin-A	3 µg/L	*Health advisory:* 20 µg/L
	Cylindrospermopsin	1 µg/L	*Health advisory:* 6 µg/L
	Microcystins	*TBD* 1-12 µg/L	*Health advisory:* 8 µg/L
	Saxitoxin	3 µg/L	*Health advisory:* 100 µg/L
Rhode Island	Total cyanobacteria		*Health advisory:* "Evidence of a visible cyanobacteria scum or mat."
	Total cyanobacteria cells		*Health advisory:* Cell counts: ≥70,000 cells/mL
	Microcystins		*Health advisory:* ≥14 µg /L
Texas	Any cyanobacteria		*Health advisory* Cell counts: ≥20,000 cells/mL
			Health advisory Visual identification

State	**Toxin or species**	**Drinking water**	**Recreational waters**
Vermont	Microcystins		*Health advisory/closure* Microcystin ≥ 6 µg/L
Virginia	Microcystins		No guidelines Decisions are made by the individual charged with assessing the situation. Two or more data points from water quality monitoring sites, generally spaced 1-2 miles (1.6-3.2 km) apart in small to medium sized segments, achieving > 50,000 cells per milliliter *Microcystins* and subsequently measuring > 10 ug/L microcystin toxin would suggest an extensive bloom and significant impairment status due to human health risks." (EPA, 2007).
Washington	Microcystins		*Provisional guideline for warning to avoid exposure(CAUTION):* ≤6 µg/L *Provisional guideline for warning to avoid exposure(WARNING):* ≥6 µg/L *Provisional guideline for warning to avoid exposure(DANGER):* ≥6 µg/L + high toxicity report of illness or pet death TDI= 0.04 µg/kg-day, BW = 15 kg child, IR = 0.05 L/h, assuming 2 h/d
	Anatoxin-a		*Provisional guideline for warning to avoid exposure(CAUTION):* ≤1 µg/L *Provisional guideline for warning to avoid exposure(WARNING):* ≥1 µg/L *Provisional guideline for warning to avoid exposure(DANGER):* ≥1 µg/L + high toxicity, report of illness or pet death Short-term RfD = 0.003 mg/kg-day, BW = 15 kg child, IR = 0.05 L/h, assuming 2 h/d
Wisconsin	Any cyanobacteria		May close beach if: Cell counts: ≥ 100,000 cells/mL

References

Text:

Bloomberg BNA (2102) Federal Court Issues Mixed Ruling On Criteria for Florida Nutrient Standards. http://www.bna.com/federal-court-issues-n12884907984/, accessed 7-27-12.

Blue Water Satellite (2012) Whole-lake satellite monitoring for total phosphorus, cyanobacteria and chlorophyll-a. NALMS 21st Southeastern Lake & Watershed Management Conference, Columbus, GA, May 14, 2012.

Butler N., Carlisle J., Linville R. (2012) Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, 119 pp.

Carter, S. (2012a) Tourism department launches website on water quality. The Journal Record, Oklahoma City, OK, June 18, 2012.

Carter, S. (2012b) Expert: New law for monitoring blue-green algae inadequate for public protection. The Journal Record, Oklahoma City, OK, June 19, 2012.

Chesapeake Bay Program, Non-point Source BMPs and Efficiencies, http://www.chesapeakebay.net/publications, accessed 7-27-12.

CRS (2012) Water Infrastructure Financing: History of EPA Appropriations, http://www.fas.org/sgp/crs/misc/96-647.pdf, accessed 7-27-12.

EPA (2012), Clean Lakes Program, http://water.epa.gov/type/lakes/cllkspgm.cfm, accessed 7-27-12.

EPA (2012) Contaminant Candidate List3- CCL, http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm#chemical, accessed 7-27-12.

EPA (2012) History of the Clean Water Act, http://www.epa.gov/lawsregs/laws/cwahistory.html, accessed 7-27-12.

EPA (2012) Impaired Waters and Total Maximum Daily Loads, http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/index.cfm, accessed 7-27-12.

EPA (2012) Implement the Watershed Management Plan, http://water.epa.gov/type/watersheds/datait/watershedcentral/plan2.cfm, accessed 7-27-12.

EPA (2012) Litigation Status. http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/lawsuit.cfm, accessed 7-27-12.

EPA (2012) National Lake Assessment, http://water.epa.gov/type/lakes/lakessurvey_index.cfm, accessed 7-27-12.

EPA (2012) Numeric and Narrative Criteria, http://water.epa.gov/learn/training/standardsacademy/mod3/page6.cfm, accessed 7-27-12.

EPA (2012) Nutrient Pollution and Numeric Standards, http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/memo2007.cfm, accessed 7-27-12.

EPA (2002) Supplemental Guidelines for the Award of Section 319 Nonpoint Source Grants to States and Territories in FY 2002 and Subsequent Years, http://water.epa.gov/polwaste/nps/success319/fy2002.cfm, accessed 8-01-12.

EPA (2012) Surface Waters Standards and Guidance, http://water.epa.gov/scitech/swguidance/index.cfm, accessed 7-27-12.

EPA Toxicological Reviews - draft, http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=161263, accessed 7-27-12.

EPA (2012) Unregulated Contaminant Monitoring Rule 3 (UCMR3), http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/index.cfm, accessed 7-27-12.

EPA (2012) Water Quality Standards for the State of Florida's Lakes and Flowing Waters, http://water.epa.gov/lawsregs/rulesregs/florida_factsheet.cfm, accessed 7-27-12.

EPA (2012) Watershed Assessment, Tracking & Environmental Results, http://ofmpub.epa.gov/waters10/attains_nation_cy.control, accessed 7-27-12.

Gakstatter, J.H. and Maloney, T.E. (1975) Potential impact of a detergent phosphorus ban on eutrophication in selected American lakes and streams. U.S. Environmental Protection Agency, Corvallis, OR, pp. 19.

Gianessi, L.P. and Peskin, H.M. (1984) An overview of the RFF Environmental Data Inventory—Methods and preliminary results: Resources for the Future, Washington, D.C., pp. 111.

Hudnell, H.K. (2005) Chronic biotoxin-associated illness: multiple-system symptoms, a vision deficit, and effective treatment. Neurotoxicol Teratol 27: 733–743. http://science.medoraco.com/peer-reviewed-articles, accessed 7-27-12.

Hudnell, H.K. [Ed] (2008) Cyanobacterial Harmful Algal Blooms (ISOC-HAB): State-of-the-Science and Research Needs, New York, Springer Press: Advances in Experimental Medicine and Biology, 619: 1-949. http://www.epa.gov/cyano_habs_symposium/, accessed 7-27-12.

Hudnell, H.K., Dortch, Q., (2008) A synopsis of research needs identified at the interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB). In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, Adv Exp Med Biol, 619, Chapter 2. Springer Press, New York, pp. 17–43. http://www.epa.gov/cyano_habs_symposium/, accessed 7-27-12.

Hudnell, H.K., Dortch, Q., Zenick, H. (2008a) An overview of the inter- agency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB): advancing the scientific understanding of freshwater harmful algal blooms. In: Hudnell, H.K. (Ed.), Cyano- bacterial Harmful Algal Blooms: State of the Science and Research Needs, Adv. Exp. Med. Biol., 619, Chapter 1. Springer Press, New York, pp. 1–16. http://www.epa.gov/cyano_habs_symposium/, accessed 7-27-12.

Hudnell, H.K. (2010) Within water-body management: a needed but neglected complement to watershed management. Clean Technol Environ Policy 12:205-207. http://science.medoraco.com/peer-reviewed-articles, accessed 7-27-12.

Hudnell, H.K. (2010a) The state of U.S. freshwater harmful algal blooms assessments, policy and legislation, Toxicon, 55: 1024-1034. http://science.medoraco.com/peer-reviewed-articles, accessed 7-27-12.

Hudnell, H.K., Jones, C., Labisi, B., Lucero, V., Hill, D.R. and Eilers, J. (2010) Freshwater harmful algal bloom (FHAB) suppression with solar -powered circulation (SPC). Harmful Algae, 9: 208-217. http://science.medoraco.com/peer-reviewed-articles, accessed 7-27-12.

Hudnell, H.K. Green, D., Vien, R., Butler, S., Rahe, G., Richards, B.A., Bleth, J. (2011) Improving wastewater mixing and oxygenation efficiency with solar-powered circulation. Clean Tech Environ Policy, 13:731–742. http://science.medoraco.com/peer-reviewed-articles, accessed 7-27-12.

LegiScan (2012) Legislative Detail: OK Senate Bill 259 - 2012 Regular Session. http://legiscan.com/gaits/view/366438.

Loftin K. (2012) USGS presentation to the EPA's Office of Research and Development, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC, September 5, 2012.

Marten Law (2011) EPA faces lawsuits http://www.martenlaw.com/newsletter/20110210-florida-water-nutrient-criteria, accessed 7-27-12.

MRSC (2012) Best Management Practices http://www.mrsc.org/subjects/environment/water/sw-bmp.aspx, accessed 7-27-12.

Oklahoma Lake Conditions (2012), http://www.travelok.com/checkmyoklake/, accessed 7-27-12.

USGS (1999), Review of Phosphorus Control Measures in the United States and Their Effects on Water Quality. DW Litke, Water-Resources Investigations Report 99–4007, Denver Colorado. http://pubs.er.usgs.gov/publication/wri994007, accessed 8-01-12.

WHO (2003) Guidelines for safe recreational water environments—Volume 1: coastal and fresh waters, Geneva: World Health Organization, http://www.who.int/water_sanitation_health/bathing/srwe1/en, accessed 7-27-12.

Williams, C.D., Burns, J.W., Chapman, A.D., Pawlowicz, M. and Carmichael, W (2006) Assessment of cyanotoxins in Florida's surface waters and associated drinking water resources. Final Annual Report to the Florida Harmful Algal Bloom Task Force, Florida Marine Research Institute, St. Petersburg, Fla. http://www.Greenwaterlab.com/articles/Assess of Cyano 2006.pdf, accessed 8-06-12

Table:

Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

WaBoLu-Hefte. 2005. Current Approaches to cyanotoxin risk assessment, risk management and regulation in different countries. Federal Environmental Agency, Berlin. 122 pp.

World Health Organisation 2003: Guidelines for safe recreational waters, Volume 1 – Coastal and fresh waters, Chapter 8: Algae and cyanobacteria in fresh water. WHO Publishing, Geneva, pp. 136-158.

California: Butler N., Carlisle J., Linville R. 2012. Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento. 119 pp.

Florida: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

Sekerke, J. Microcystin Health Advisory Level. Florida Department of Health, Division of Environmental Health. Internal Memorandum. November 11, 2005

Indiana: Indiana. 2008. Blue-green algae toxin level guide. http://www.in.gov/idem/algae/2345.htm, accessed 7-26-12.

Iowa: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

Kansas: Kansas Department of Health and Environment. "Guidelines for Addressing Harmful Algal Blooms in Kansas." Accessed May 8 2012. http://www.kdheks.gov/algae-illness/download/HAB_policy.pdf, accessed 5-8-12..

Maryland: Personal correspondence. May 10 2012. (410) 260-8630 Department of Natural Resources Tidewater Ecosystem Assessment Division.

Massachusetts: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

Commonwealth of Massachusettes https://www.neiwpcc.org/neiwpcc_docs/protocol_MA_DPH.pdf, accessed 7-27-12

Michigan:http://web.archive.org/web/20080725124425/http://www.miseagrant.umich.edu/upwellings/issues/07june/07june-article4.html, accessed 7-27-12. The original link is: http://www.miseagrant.umich.edu/upwellings/issues/07june/07june-article4.html.

Nebraska: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

New Hampshire: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

New Hampshire Department of Environmental Services. 2008. http://des.nh.gov/organization/divisions/water/wmb/beaches/cyano_bacteria.htm, accessed 7-27-12.

New York: Personal correspondence. 5-May 9-12 2012. 518-402-7820 NYS Department of Health.

North Carolina: Algal and aquatic plant assessments. http://portal.ncdenr.org/web/wq/ess/eco/algae, accessed 7-27-12.http://portal.ncdenr.org/web/wq/ess/eco/algae

Ohio: Ohio Department of Health. "Harmful Algal Blooms." Accessed May 3 2012 http://www.odh.ohio.gov/features/odhfeatures/algalblooms.aspx, accessed 5-3-12.

Oklahoma: Legiscan. http://legiscan.com/gaits/view/366438, accessed 7-27-12.

Oregon: Oregon Health Authority. http://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines.pdf, accessed 7-27-12.

Rhode Island: Rhode Island Department of Health. "Sampling for Cyanobacterial Blooms." Accessed May 8 http://www.health.ri.gov/publications/policies/SamplingForCyanoBacteriaBlooms.pdf, accessed 5-8-12..

Vermont: Graham J, Loftin K., Kamman N. 2009. Monitoring Recreational Freshwaters. Lakeline, Summer:19-24.

Virginia: U.S Environmental Protection Agency (EPA). 2007. Ambient Water Quality Criteria for Dis-solved Oxygen, Water Clarity and Chlorophyll a for the Chesapeake Bay and Its Tidal Tributaries. (available at: http://www.chesapeakebay.net/content/publications/cbp_13142.pdf, accessed 7-27-12).

Washington: Hardy, J. 2008. Washington State Recreational Guidance for Microcystins and Anatoxin, Washington State Department of Health. http://www.doh.wa.gov/Portals/1/Documents/4400/334-177-recguide.pdf, accessed 7-26-12.

Wisconsin: State of Wisconsin Department of Health and Family Services. "Cyanobacteria and Human Health." http://city.milwaukee.gov/ImageLibrary/Groups/healthAuthors/DCP/PDFs/CyanobacteriaLHD.pdf, accessed 5-8-12.