ISTITUTO SUPERIORE DI SANITÀ

Workshop

Le fioriture di alghe tossiche nelle acque dolci: emergenza sanitaria e misure di controllo

Istituto Superiore di Sanità
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Atti a cura di
Serena Melchiorre, Emanuela Viaggiu e Milena Bruno

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Negli ultimi trenta anni le conseguenze dell'effetto serra e l'eccessiva immissione di nutrienti nelle acque hanno determinato in tutto il mondo l'aumento delle fioriture algali, spesso tossiche, anche in bacini d'acqua dolce utilizzati per scopo potabile. Questo problema nel territorio italiano viene spesso affrontato con difficoltà, a causa della scarsità di strutture e competenze nella gestione del rischio sanitario specifico e nel recupero dell'ambiente interessato. Le relazioni presentate al workshop organizzato dall'Istituto Superiore di Sanità nel 2000 hanno riguardato alcuni degli aspetti scientifici più problematici nelle fioriture algali tossiche da cianoficee, numericamente le più frequenti in Italia, tra tutte quelle tipiche delle acque eutrofiche. I problemi dell'identificazione tassonomica, della variabilità genetica tra ceppi nell'ambito della stessa popolazione, l'organizzazione dei monitoraggi, gli effetti acuti e cronici delle tossine e l'efficienza dei sistemi di depurazione sono stati trattati dal punto di vista dell'esperienza dei maggiori laboratori di ricerca internazionale.
Parole chiave: Alghe tossiche, Cianoficee, Acqua dolce, Fioriture algali

Istituto Superiore di Sanità
Workshop. Freshwater harmful algal blooms: health risk and control management. Istituto Superiore di Sanità. Rome, 17 October 2000.
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During the last thirty years the consequences of the "green house effect" and the excessive nutrient input in waters have been determining a worldwide increase in algal blooms, often toxin producers, even in freshwater basins destined for drinkable uses. These problems are often faced with difficulty in the Italian regions, due to the shortage of structures and expertise in the specific health risk management and in the restoration of the affected environment. The papers presented at the workshop organized by the Istituto Superiore di Sanità (Italian National Institute of Health) in 2000 deal with some of the most problematic scientific aspects of toxic algal blooms from cyanobacteria, mostly recurrent in Italy, particularly those typical of eutrophic waters. The problems of taxonomic identification, genetic variability among strains of the same population, monitoring organization, acute and chronic effects of toxins and the efficiency of depuration plants were discussed from the point of view of the main international research groups.
Key words: Toxic algae, Cyanobacteria, Freshwater, Algal blooms

Il rapporto è accessibile online dal sito di questo Istituto: www.iss.it/pubblicazioni.

INDICE

INTRODUZIONE

Le fioriture di alghe tossiche appartenenti alle Cianoficee nelle acque dolci destinate al consumo umano stanno diventando un problema crescente in tutto il mondo, a seguito della contaminazione dei corpi d'acqua con eccessivi livelli di nutrienti primari (fosforo ed azoto).

In Italia esse ormai interessano ogni anno gli invasi di sette Regioni su venti, e le tossine più frequentemente riscontrate, le microcistine, sono a tutti gli effetti nuove sostanze di rischio oncogeno da seguire nel loro "destino" ambientale ed in tutti i passaggi della catena alimentare acquatica.

Decine di morti umane causate da intossicazioni acute per queste sostanze sono state segnalate in Brasile nel 1988 nello stato di Bahia, e nel 1996 nella cittadina di Caruaru, nello stato del Pernambuco.

Indagini epidemiologiche in Cina hanno dimostrato il ruolo chiave delle microcistine nella promozione del tumore epatico primario umano.

Studi sulla somministrazione di microcistine in topi per via intranasale hanno dimostrato che singole dosi sub-acute, non tossiche, dopo 7 giorni di somministrazione giornaliera danno luogo ad un effetto cumulativo, con aumento della massa epatica pari a quello causato da una sola dose 14 volte maggiore.

Recenti dati sperimentali *in vitro* hanno evidenziato il loro ruolo di interferenza nella produzione di vari ormoni nel ratto: cellule di pancreas, incubate con microcistina-LR, raddoppiano il rilascio basale di insulina ed innalzano il livello di Ca^{2+} citosolico; le microcistine dereprimono la sintesi ed escrezione del progesterone nel corpo luteo, e deprimono la formazione dell'aldosterone nelle cellule della zona glomerulosa della cortex.

Nei leucociti polimorfonucleati dell'uomo esse causano l'aumento fino al 28% dell'aderenza precoce spontanea deprimendo l'attività immunitaria: le dosi attive (10^{-11} M), paragonabili a quelle di alcuni ormoni umani (neuropeptidi), sono molto al di sotto dei limiti di sicurezza raccomandati dall'Organizzaizone Mondiale della Sanità (10^{-9} M), e simili invece ai limiti indicati per evitare il rischio cronico tumorale. Le microcistine, come le altre tossine algali, non vengono bloccate nei processi depurativi comuni: specifici filtri o trattamenti, spesso costosi, sono necessari per evitarne l'ingresso in rete.

I rischi derivanti dall'ingestione di queste tossine nel nostro paese non sono ancora indagati, nonostante l'estensione dei territori interessati, anche a causa della mancanza di competenza degli organi di controllo locali in una materia del tutto nuova. Attualmente lo stesso Ministero della Sanità ha diramato a riguardo solo limiti inerenti la balneazione.

Il convegno organizzato nella presente occasione intende offrire una panoramica ampia sugli aspetti ambientali e le conseguenze sanitarie del fenomeno. La manifestazione è stata divisa in quattro sessioni nelle quali ad alcuni dei maggiori esperti mondiali del settore sono stati affiancati ricercatori provenienti da diverse realtà italiane che hanno posto in evidenza le problematiche quotidianamente affrontate e le soluzioni adottate.

A conclusione dei lavori i partecipanti hanno avuto la possibilità di comunicare le loro esperienze ed esprimere le perplessità anche in merito alla legislazione attualmente vigente in Italia. Ritenendo tale dibattito parte integrante del convegno viene riportata nell'Appendice al presente volume la sua trascrizione integrale.

DIFFUSIONE DELLE FIORITURE ALGALI TOSSICHE NELLE ACQUE INTERNE ITALIANE

Milena Bruno
Laboratorio di Igiene Ambientale, Istituto Superiore di Sanità, Roma, Italia

In Italia, il fenomeno delle fioriture algali nei laghi e negli invasi non è cosa nuova (è segnalato come tale da più di due secoli), però è sempre stato considerato sotto l'aspetto della dinamica di popolazione delle alghe e mai sotto i possibili risvolti dal punto di vista tossicologico. Eventuali morìe di fauna ittica che vi si trovavano associate erano sempre state viste come una conseguenza dell'anossia determinata nei fondali dal decadimento delle fioriture. Il governo italiano iniziò a preoccuparsi dal punto di vista legislativo dell'aspetto delle fioriture algali nel 1982 quando emanò un decreto legge (il 470/1982) riguardante tra l'altro l'obbligo di eseguire analisi quantitative e qualitative sulle alghe e sui loro prodotti nel caso che in un corpo d'acqua adibito alla balneazione, fosse esso lago oppure costa marina, si superassero i livelli di determinati parametri, fondamentalmente ossigeno disciolto e colimetria.

Successivamente, ulteriori integrazioni di questo decreto legge definirono sempre più precisamente il tipo di analisi che doveva essere svolto fino a comprendere anche la ricerca delle tossine algali.

Per quanto riguarda l'aspetto delle acque potabili, la prima legge che menzionava l'eventuale necessità del controllo era il DL n. 236/1988, che ora sta per essere sostituito dal recepimento della Direttiva Comunitaria in merito. Questo decreto legge definiva le alghe come un parametro accessorio; vale a dire un parametro su cui andavano eseguiti dei controlli occasionali la cui frequenza era del tutto dipendente dal giudizio insindacabile nemmeno degli operatori, ma delle autorità competenti; non solo quelle di controllo tecnico sul territorio ma anche quelle di gestione amministrativa.

Nel 1985 iniziarono ad essere segnalati all'Istituto Superiore di Sanità casi di fioriture a sospetta produzione tossica, a carico dei laghi artificiali sardi Medio Flumendosa e Mulargia.

Allora non erano disponibili standard di tossine algali di nessun tipo, perché questi erano appannaggio di laboratori che se li procuravano purificandoli essi stessi, e non veniva effettuata commercializzazione. Però, una serie di analisi effettuate su questa fioritura (di *Planktothrix rubescens*) mise in evidenza la presenza di una sostanza epatotossica, con una LD50 di 100 mg/kg di peso corporeo e con le classiche stimmate delle microcistine, ossia danni epatici rilevanti, eventuali emorragie polmonari e anche renali.

Da quel momento le segnalazioni di fioriture tossiche continuarono a pervenire sempre più frequentemente al nostro laboratorio, così fu decisa l'istituzione nel 1993 di una banca dati che riunisse tutti gli eventi di fioriture algali segnalati sia nel mare che nelle acque dolci.

Considerando i dati finora disponibili (dal 1993 al 1999) si può notare (Tabella 1) che nelle 9 regioni settentrionali 26 presidi segnalarono nel 1993 12 fioriture d'acqua dolce, mentre nel 1999 i presidi che fornirono risposta furono la totalità (46) con 25 fioriture segnalate; lo stesso incremento è evidente nel centro Italia (5 regioni) dove si passa da 7 presidi con 3 fioriture segnalate nel 1993 a 22 presidi (la totalità) con 29 segnalazioni; nelle 6 regioni meridionali si passa da 12 presidi con 3 segnalazioni nel 1993 a 30 presidi che ne hanno segnalate 10 nel 1999.

Tabella 1. Frequenza delle segnalazioni dei presidi riguardanti fioriture algali d'acqua dolce (1993-99)

Area geografiche	Presidio (fioritura)					
	1993		1997		1999	
9 regioni settentrionali	26	(12)	46	(24)	46	(25)
5 regioni centrali	7	(3)	22	(12)	22	(29)
6 regioni meridionali	12	(3)	30	(16)	30	(10)

Circa un terzo di queste fioriture è a carattere tossico.

Dalle segnalazioni che ci sono pervenute e anche dalle situazioni che abbiamo personalmente esaminato in questi anni, abbiamo visto che in Italia le specie tossiche più diffuse e quelle che danno più frequentemente origine a fioriture sono: *Microcystis aeruginosa*, *Planktothrix* (ex *Oscillatoria*) *rubescens* e *Anabaena flos-aquae.*

Non ci sono nel nostro territorio areali particolarmente votati all'una o all'altra: le fioriture intercorrono a tappeto su tutto il territorio con una alternanza di specie.

Su quattro Regioni campione (Lazio, Sardegna, Lombardia e Umbria) che nel 2000 hanno segnalato la presenza di fioriture richiedendo il nostro intervento, abbiamo riscontrato la presenza di *Microcystis aeruginosa* nel lago Liscia in Sardegna, nel Trasimeno in Umbria e a Canterno nel Lazio; la presenza di un ceppo tossico di *Anabaena affinis* nello stesso lago di Canterno e quella di *Planktothrix rubescens* nel lago di Iseo in Lombardia e nel lago di Albano, nel Lazio. Nei laghi italiani, sia per il carattere di movimentazione delle acque che per il grado particolare delle percentuali di nutrienti presenti, la specie con incidenza maggiore è la *Planktothrix rubescens*, seguita da *Microcystis aeruginosa*.

La *Microcystis aeruginosa* che siamo riusciti ad analizzare come quantitativo e tipo di tossine prodotte è stata rilevata per la prima volta nel 1991 nel lago Liscia in Sardegna e nel lago Liscione, un invaso artificiale del Molise (Tabella 2).

Contemporaneamente negli stessi anni, è stata esaminata la produzione di tossine in fioriture di *Planktothrix rubescens* segnalate in varie regioni (Tabella 3), ed in una fioritura di *Anabaena planctonica* in un invaso sardo (Tabella 4).

Tabella 2. Alcuni dei ceppi di *Microcystis aeruginosa* esaminati dal Laboratorio di Igiene Ambientale dell'Istituto Superiore di Sanità dal 1991 al 2000

Provenienza	Tipo di tossina	Quantità prodotta	Estrazione da*
1991			
Liscia (Sardegna)	-LR	380 µg/g	peso secco
Liscione (Molise)	-LR	15,76 µg/g	peso secco
1998			
Cedrino (Sardegna)	-RR	0,5 µg/g	peso umido
Polverina (Marche)	-LR	50 µg/g	peso umido
2000			
Trasimeno (Umbria)	-RR	39 µg/g	peso umido
Massaciuccoli (Toscana)	-LR	20 µg/g	peso umido
	-YR	150 µg/g	peso umido
Canterno (Lazio)	-YR	94 µg/g	peso umido

*stato delle cellule all'estrazione

Tabella 3. Alcuni ceppi di *Planktothrix rubescens* esaminati dal 1991 al 2000 in varie regioni

Provenienza	Tipo di tossina	Quantità prodotta	Estrazione da	Rapporto azoto/fosforo
1991				
S.Puoto (Lazio)	-YR	1,16 mg/g	peso secco	N/P 10
1998				
Fiastrone (Marche)	- RR	32 mg/g	peso umido	N/P 25
2000				
Iseo (Lombardia)	- RR	107 ng/g	peso umido	
Albano (Lazio)	- RR	74 μg/g	peso umido	N/P 20

Tabella 4. Ceppo di *Anabaena planctonica* esaminato nel 1990 in varie regioni

Provenienza	Tipo di tossina	Quantità prodotta	Estrazione da
1990			
Mulargia (Sardegna)	anatoxin-a	100 μg/g	peso secco

Per le fioriture di S. Puoto, del Fiastrone e di Albano è stato possibile calcolare il rapporto azoto totale/fosforo totale presente nelle acque al momento della fioritura. In un recente lavoro di Teubner *et al*. nel 1999 (1), che ha studiato il momento in cui le fioriture di *Planktothrix rubescens* succedono a quelle di *Aphanizomenon flos-aquae*, o *Microcystis aeruginosa*, più esigenti per il fosforo, viene rimarcato che questo avviene quando il rapporto Azoto/Fosforo supera il livello di 16. Come si può notare, questo dato trova conferma anche nei laghi italiani, con la sola eccezione del lago S. Puoto. A questo proposito, però, c'è da osservare che il dato a noi pervenuto era puntiforme, e poteva essere riferito al declino della fioritura.

Per quanto riguarda l'*Anabaena planctonica*, questo è il caso più eclatante a noi capitato di una specie non nota come tossica che si rivela tale all'esame dei fatti. Questo ceppo è stato ritrovato in fioritura nel lago Mulargia, in Sardegna, gli estratti della fioritura, risultati tossici al MICROTOX ed esaminati in gas-cromatografia e spettrometria di massa rivelarono la presenza di anatossina-a in buona quantità.

Questo tipo di sorprese è relativamente frequente nelle Cianoficee, di cui attualmente circa il 40% delle specie risulta produttore di tossine.

Come si può evincere dal complesso dei dati, la situazione della trofia delle acque e delle fioriture tossiche nel nostro paese si annuncia non preoccupante ma certamente già seria. Gli invasi di cui sono state qui descritte le caratteristiche tossicologiche sono tutti adibiti alla potabilizzazione e agli usi di balneazione, e quello che è peggio è che questi eventi si stanno verificando anche nei grandi laghi, anche se per ora non sull'intera superficie ma in settori oppure in branche, grandi laghi che servono dal punto di vista dell'acqua potabile città e territori notevoli.

L'Istituto ha quindi giudicato opportuno sottolineare l'importanza e anche la necessità di dedicare un'attenzione particolare a questo genere di fenomeni, che si pensa diventeranno sempre più frequenti ed estesi in Italia, a causa dell'effetto serra, che interessa il clima generale del globo terrestre, e a causa anche dell'aumentato sfruttamento delle fonti d'acqua, che contribuisce insieme all'effetto di cui sopra ad abbassare il livello degli invasi, causando oltre tutto una concentrazione dei nutrienti presenti in determinate stagioni e favorendo molto di più rispetto al passato l'innesco di queste fioriture.

A questo problema si aggiunge la generale impreparazione dei gestori locali a trattare le acque che hanno questo genere di tossine al loro interno, tossine che possono essere bloccate non con i trattamenti convenzionali ma con determinate aggiunte o usando clorazioni spinte che poi richiedono necessariamente un periodo di stasi delle acque prima di poterle convogliare negli acquedotti centrali.

Si tratta quindi di aver a che fare con un problema di addestramento delle unità territoriali di controllo ed anche delle gestioni pubbliche o semiprivate che sovrintendono all'immissione delle acque nelle reti acqueduttali.

Bibliografia

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PROBLEMS IN CYANOBACTERIAL TAXONOMY: IMPLICATION FOR MOST COMMON TOXIN PRODUCING SPECIES

Jirí Komárek
Trebon, Czech Republic

Introduction

The modern taxonomy of cyanobacteria is complicated by three sets of more or less incompatible data. They are:

1) Knowledge of wide diversity of cyanobacteria in nature, based on traditional morphological criteria. The diversity of morphotypes ("traditional species") is described only partly in identification manuals.

2) Still fragmentary data, based on biochemical and molecular methods and evaluations, which are considered often not to be conform with descriptions of phenotype taxonomy.

3) The old idea survives, that all cyanoprokaryotes can occur elsewhere.

Therefore, the names of traditional species, based usually on restricted and insufficiently defined morphological characters are used arbitrary for various populations, and the identification of such populations is superficial. The consequence is that: a) the same types are designated by different names in literature, or, *vice versa*, b) the same name is used in various concepts. The very restricted ecological demands in cyanobacterial populations (and "species") were proved, but the ecological characters are still commonly underestimated.

The main problems of the present cyanobacterial taxonomy, following from this situation, are discussed in this review.

Problems of modern cyanobacterial taxonomy

Conformity of genotypes derived from molecular sequencing with phenotype taxonomy

The molecular approach was introduced in cyanobacterial taxonomy particularly by Stanier's team about 30 years ago (1-6), and numerous other studies followed their first important data. All these results explained lot of unclear and enigmatic problems in cyanobacterial biology. The first attempt to synchronize the modern molecular data with phenotype cyanobacterial taxonomy was published by Anagnostidis and Komárek (7-10), who revised particularly the phenotype generic diagnostic characters, respecting the up to date knowledge of cytomorphological variability, as well as modern molecular results.

The recent molecular analyses confirmed perfectly phenotypically revised stable taxa on the generic level.

A lot of presentations (11-20) yielded important material for this classification. Examples of such revised and confirmed genera are:

- The separation of the genera *Cyanobium*, *Synechococcus* and *Cyanothece*, all classified sooner into the complex genus *Synechococcus*.
- The revision of the genus *Microcystis*, which comprises only the types with gas vesicles.
- The separation of the filamentous genera *Spirulina* and *Arthrospira* with screw-like coiled trichomes is quite justified according to molecular data as well as according to ultrastructural and phenotype characters.
- The cyanobacterial "LPP-group B", defined by Rippka (3) and comprising cluster of traditional species from the botanical genera *Lyngbya*, *Phormidium* and *Plectonema* (all *exclusive typo*), is well recognizable and definable according to phenotype characters, and was described according to botanical nomenklatoric rules under the generic names *Leptolyngbya* and related *Planktolyngbya* respectively.
- The cyanobacterial "LPP-group A", again re-defined by Rippka (3), represents morphologically clearly delimited group of filamentous, aheterocytous cyanobacteria, corresponding to related genera *Oscillatoria*, *Lyngbya*, *Blennothrix* and *Plectonema*, but only in the original concept of their authors. All species, which were described later and do not correspond to the original generic diagnoses, must be transferred in other genera.
- The genus *Planktothrix*, sooner classified into the genus *Oscillatoria*, is just one from the genera, which must be separated in a special taxonomic unit.

The good conformity of molecular data with revised phenotype characters was found also in suprageneric taxa.

The separated cluster of heterocytous genera (order *Nostocales*), baeocytic types, or the mentioned definition of the family Oscillatoriaceae (LPP-group A) were supported already by first published phylogenetic trees (21) and proved several times by later authors.

The phylogenetic tree is given as example (Figure 1), derived from internet data base (state of October 2000), in which the separation of newly defined suprageneric taxa and genera is well recognizable.

Only the cluster of simple filamentous oscillatorialean and coccoid types is polyphyletic (18) and still unclear, but also here the markers leading to the satisfactory solution of taxonomic problems exist. One of the main complicating factors is the manipulation with names of strains of the simplest cyanobacterial types (Figure 2).

It is possible to summarize that the stable generic phenotype features are in conformity with molecular studies.

The molecular sequencing confirmed perfectly the phenotypic genera, derived from the "Geitlerian system" after re-evaluation of morphological and cytological features.

The disagreement was never confirmed; it is usually proclaimed according to very preliminary results.

However, it means also in practice, that several traditional genera must be divided or joined together, and, very probably, that new genera must be established according to modern combined phenotype and molecular revisions.

The selection and revision of cytomorphological characters on the "species" level, which are in concordance with genetic bases, must be studied.

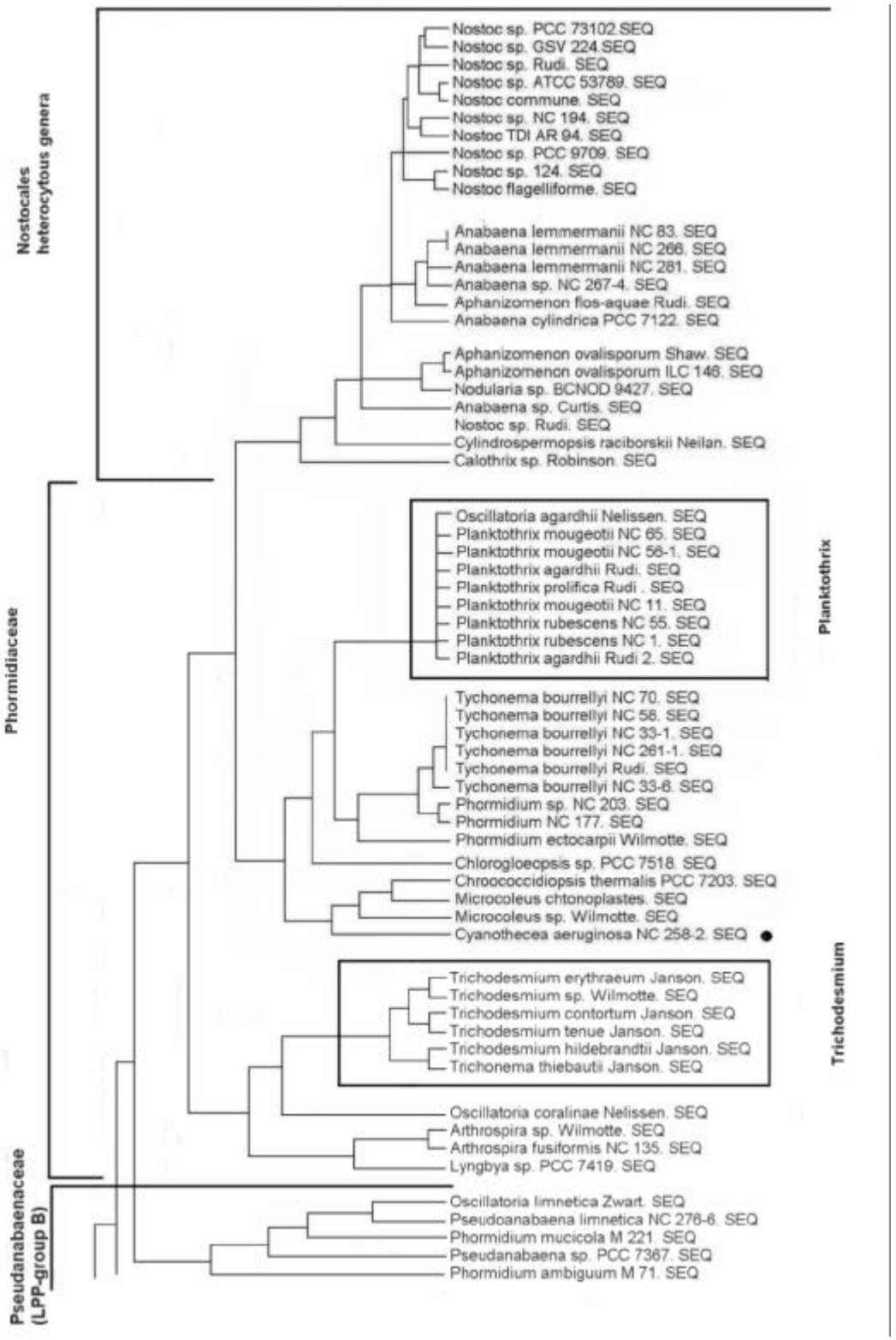
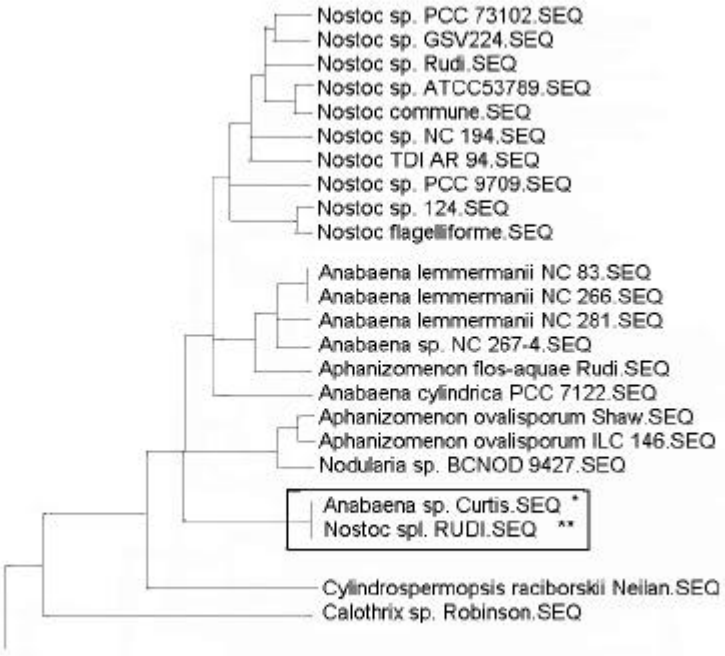


Figure 1. Part of a phylogenetic tree of cyanobacteria derived from Internet database, proving the conformity of phenotypically defined genera with sequencing data. All mistakes in taxonomic names and strain identifications are not corrected



NCBI *Entrez* **Nucleotide QUERY** BLAST Entrez ?

Other Formats: FASTA Graphic

Links: MEDLINE Related Sequences

```
LOCUS       NSZ82803      479 bp    DNA             BCT       21-JUL-1997
DEFINITION  Nostoc sp. 16S rRNA gene (strain NIVA-CYA 246/PCC 7120).
ACCESSION   Z82803
NID         g1668769
VERSION     Z82803.1  GI:1668769
KEYWORDS    16S ribosomal RNA; 16S rRNA gene.
```

*** Anabaena PCC7120**
Ligon P.J., Meyer K.G., Martin J.A, Curtis S.E. (1991): Nucleotide sequenze of a 16S rRNA gene from Anabaena sp. Strani PCC 7120. *Nucleid Acids Res* 19(16), 4553.

**** Nostoc sp. NIVA-CYA 246**
Rudi K., Skulberg O.M., Larsen F., Jakobsen K.S. (1997): Strain characterisation and classification of oxyphotobacteria bin clone cultures on the bases of 16S rRNA sequences from the variable regions V6, V7. *Appl Environ Microbiol* 63(7), 2593-2599.

Figure 2. Part of an Internet cyanobacterial phylogenetic tree, proving the confusions following from different strain identifications. The identity of framed strains does not prove the incompatibility of molecular sequencing with traditional generic characters; both strains are identical, but designed by different generic names in two different collections

Experimental studies of infraspecific diversity (within populations and strains)

Detailed molecular analyses proved the extremely wide infraspecific diversity (within populations and between clonal strains). Similar diversity was found also by precise morphological studies of natural populations. Within genera (genotypes) exists enormous variation; they comprise the endless number of morpho- and ecotypes, which can stabilize in culture. Almost all precisely studied populations or strains differ one from another. Numerous examples were published:

- The wide variation range (particularly if separated characters are used for evaluation) was found in populations of the planktic genus *Microcystis*, in which only indistinctly the units on the traditional specific level are recognizable. Almost all studied populations and strains were found different one from another (22, 23).
- The same diversity in isolated strains was presented within the complex of simple genera *Cyanobium/Synechococcus/Cyanobacterium* studied in isolated strains (6), or in oceanic picoplankton (24).
- The diverse strains were found also in several oscillatorialean species, e.g. in *Limnothrix redekei* (25;26), or in *Phormidium autumnale* (27), (unpublished data) (Figure 3).

From these results follows that very wide diversity not only between populations, but also within one and the same population exists inside of well separated genotypes in cyanobacteria, where almost all individuals represent a continual line of small deviations, which can be stabilized in culture (Figure 2) (16).

Stability of phenotype (traditional) species

The previous conclusions put forward the question, if any taxonomic category ("species") between genotypes-genera and clones (populations) can be defined. It is known that the traditional taxonomy is based on units, which represent stable morphotypes well recognizable in samples from nature, which occur repeatedly in time and sometimes in very distant localities (in the similar ecological situations).

Different species (or, at least, their clusters) are accepted without doubts in few morphologically distinct and diversified genera (e.g., several species of *Hyella, Nostoc, Gloeotrichia, Rivularia*, and others), but hesitated in many others. Such species are recognizable morphologically, several of them were supported by careful ecophysiological or biochemical studies, but only few of them were proved by molecular sequencing.

Examples:

- Several species are recognizable in the genus *Microcystis*, but they represent one homogeneous cluster (Figure 4) in up to date molecular analyses (19). The simple morphology, occurrence of many not identifiable stages and wide variability complicate the morphological delimitation of different types. Several specialists hesitate therefore about the existence of various species within this genus (28, 29), in spite of numerous existing studies describing different morpho- and ecotypes with different physiological characters (22-32).
- The same situation is, in the genus *Planktothrix* (19). Always only one cluster of very close strains was found in phylogenetic trees (Figure 1), in spite that, Skulberg *et al*. (13) recognized four well separated types from Norwegian lakes.
- The molecular data about infraspecific diversity of *Anabaena* are still rare, but Li and Watanabe (15) and Li *et al*. (14, 16) proved, by combined biochemical and morphological criteria, the existence of almost all morphologically defined species, differing sometimes by very small differences in their phenotypes (form of akinetes, type of coiling, etc.).

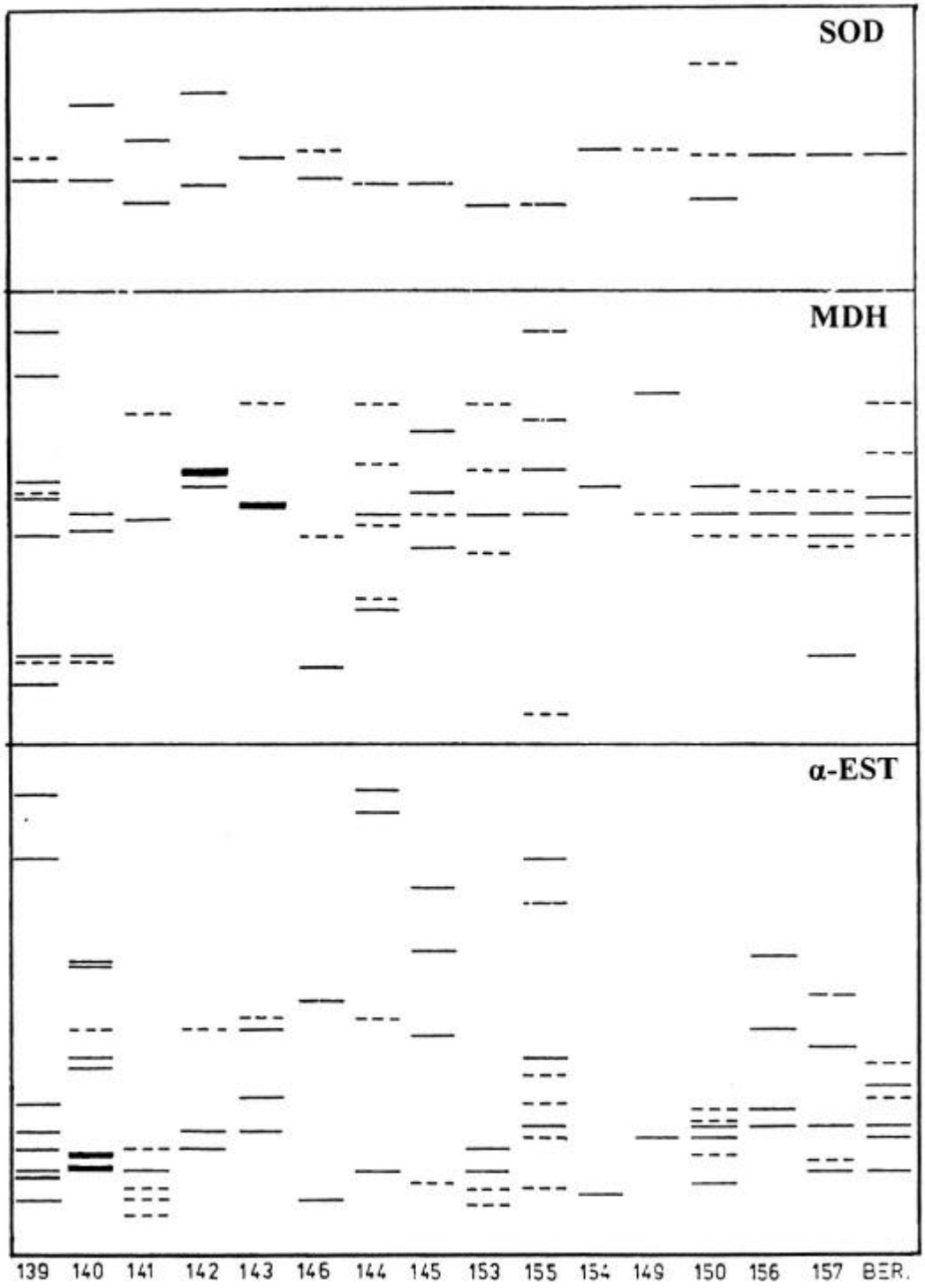


Figure 3. Allozyme analysis, illustating the diversity in the cluster of morphotypes related to *Phormidium autumnale*. SOD: superoxid dismutase. MDH: malate dehydrogenase. α-EST: α-esterase, numbers represent different, morphologically similar strains (and populations)

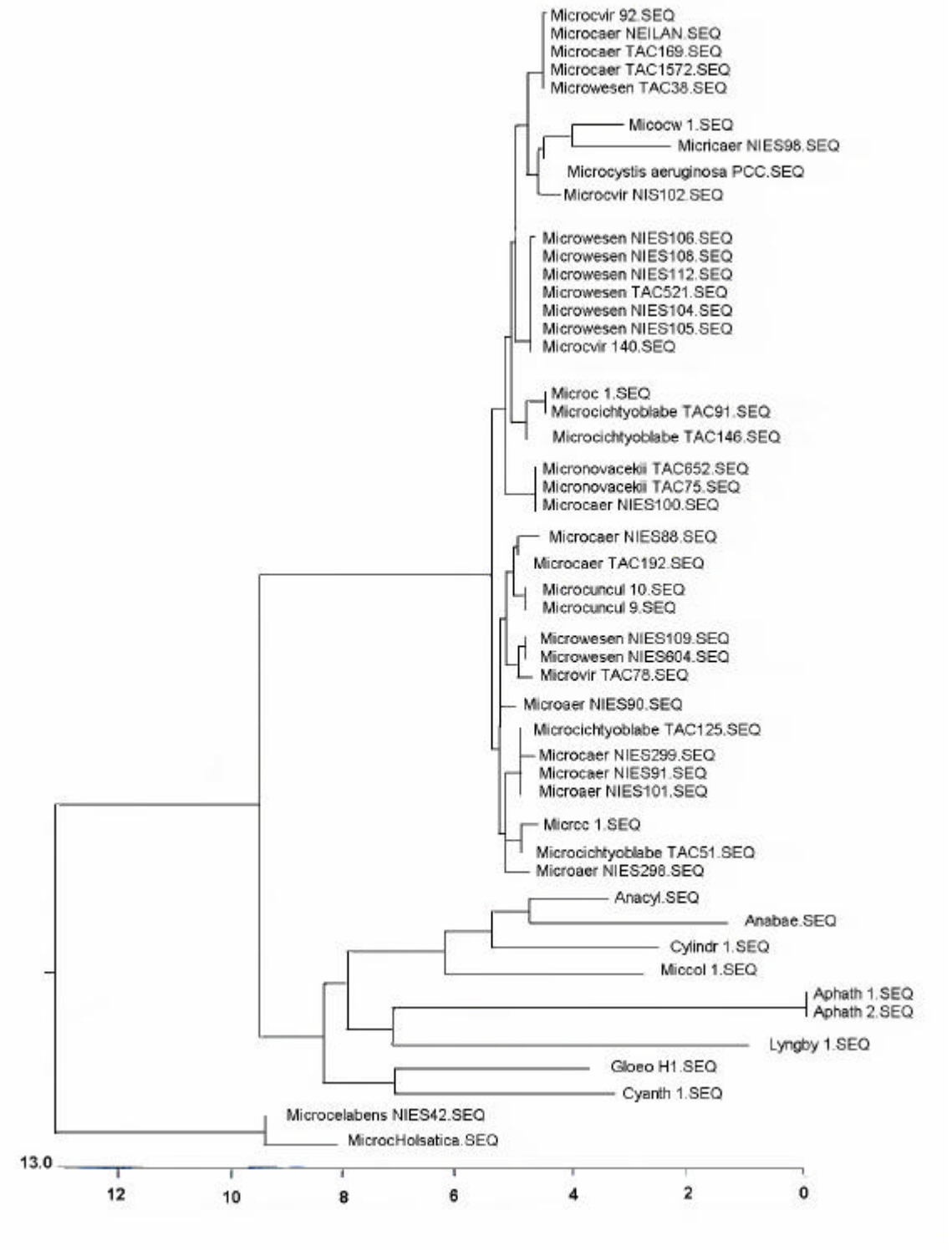


Figure 4. Part of an Internet cyanobacterial phylogenetic tree, proving the relatedness of all morphospecies of *Microcystis*. *Microcystis elabens* and *M. holsatica* from the bottom part of the tree were transferred in other genera (*Aphanothece* and *Aphanocapsa*) according to phenotype taxonomic revisions several years ago, but mainly ignored by later authors

The stable morphotypes (traditional species) are sometimes different only in few stages of their life cycle, which complicates their identification.

The existence of morphologically almost identical stages in various types does not mean that they belong to one and the same genotype. Several stages are not recognisable one from another, but sometimes the whole life cycle must be studied for their determination (33,34). Belong not only the mentioned discussed genera to such still *Microcystis*, *Planktothrix*, but also *Nodularia*, *Arthrospira*, and others.

Ecological specificity and geographical separation of genotypes

Numerous stable morphotypes (traditional "species") exist in nature, which are ecologically restricted and recognisable one from another. It was found after detailed comparison of cyanobacterial microflora from various regions, that:

- all cyanobacterial, morphologically recognisable stable types, are delimited ecologically;
- morphotypes with cosmopolitan distribution exist (or cosmopolitan with dispersed localities), but only in corresponding habitats;
- numerous morphotypes/ecotypes, which cannot be identified according to present literature, exist particularly in specialised tropical and extreme habitats (Table 1). The number of such not identifiable types increases with specificity of the biotope.

Table 1. Percentage of unidentifiable morphospecies in various extreme biotopes

Region and biotope	Identified species ("cosmopolitan")	Identified, endemic species	Unidentifiable species
Volcanic lake in Mexico (Puebla state)	21.4%	15.1%	63.5%
Tolantongo hot springs (central Mexico)	7.2%	3.0%	89.8%
Freshwater biotopes (central Mexico)	26.2%	0.0%	73.8%
Freshwater species in Brazil (San Paulo state)	24.0%	20.0%	56.0%
Tropical lake planktic species	20.0%	45.0%	35.0%
Freshwater *Anabaena* species Cuba	5.9%	70.6%	23.5%
Okawango swamps, S. Africa	12.2%	30.6%	57.2%
S. Shetland island (Antarctica)	19.0%	38.0%	43.0%

This situation could be solved by several possible ways. The new types can be described as new species, but this approach, together with hesitations about species concept in cyanobacteria, is not preferred (in respect to above discussed "species" problems). However, the effort to identify all the found morphotypes exists; many authors use usually the available old identification manuals, and select the name of taxon, which is, according to their meaning, the most similar to their material.

The result of this practice is that now lot of cyanobacterial names exist, which are used in various concepts for very different cyanobacterial types, and that very different populations from various biotopes are designated by the same name. The present cyanobacterial taxonomy is very complicated by this practice, which is, unfortunately, used also for identification of isolated strains and in strain collections (see Figure 2). Everybody can persuade of this situation, if he compares the set of strains from various laboratories, designated by the same name.

It is interesting, the fact that the biochemical methods proved sometimes differences in morphologically almost identical populations from distant regions. This fact is probably in close coincidence with the diversification of populations in lot of small deviations, able to stabilize in cultures. Data on this kind of geographic differentiation were presented, by Kaftan (35) about *Nostoc commune* (central European and Antarctic populations), Bolch and Blackburn (36) about *Nodularia spumigena* (Australian and Baltic populations), and others.

Current questions in modern cyanoprokaryotic taxonomy

The fact that the molecular studies proved (i) the existence of natural clusters of populations (which correspond more or less to the traditional concept of genera or "macrospecies") but also (ii) the wide diversity inside of traditional species (two quite identical strains were not yet found) reflects the basic questions for the present taxonomy of cyanobacteria.

Diversification and speciation

The diversification process within cyanobacteria seems to be very specialised and very effective, which enabled to survive this whole group for millions years without principal changes in their structure, and without lost of their viability. The study of these questions, therefore, is now essential for understanding the cyanoprokaryotic diversity. The exchange of genetic material in natural populations (18, 19) can explain the differentiation in widely distributed morphotypes (only among phyletically related strains?). However, several regulations of this process must exist, and particularly the limit between "phyletically related" and "phyletically less related" strains should be explained. The stable occurrence of numerous morphotypes over long time periods indicates the restricted and regulated action of similar processes. The easy adaptability under changing conditions and next stabilisation of new developed eco- and morphotypes in new stabilised niches seems to be another common type of cyanobacterial differentiation. The question, "what is species?" in cyanobacteria, is now an urgent problem. Species is important, but an conventional category in a certain degree, and now particularly unclear in cyanobacteria. From all present knowledge follows, that the cyanoprokaryotic species could be defined as group of populations (+ strains), which belong to one and the same genotype (genus), they are characterised by stable phenotype (definable and recognisable) features (with distinct limits of variation), and by the same ecological demands. They should occur repeatedly (in different time intervals) in various localities with the same ecological conditions (or in one locality with very specialised life conditions). However, a lot of present papers work with infrageneric classification arbitraryly and not respecting the "botanical" type characters, coincidences in genetic and phenotype features, both botanical and bacteriological rules for nomenclature, etc. This approach complicates the solution of modern cyanobacterial classification (37, 38).

Summary of the main current problems

- Diversification and speciation processes.
- Relations between genotype (molecular) and stable phenotype characters. Molecular evaluation must be combined with evaluation of phenotype and ecological characters.
- Recognition of life cycles, ecological plasticity (and limits) and morphological variation in natural populations and in culture.
- Definition of the conventional criteria of "species" (and other taxonomic categories); this problem is connected with numerous "formal" questions (typification, naming, etc.). It is necessary to tend to such definition, which can be designated as evolutionary and phenotypically well characterised natural units, recognisable in nature and/or in culture.

Diversity in main planktic genera with species producing cyanotoxins

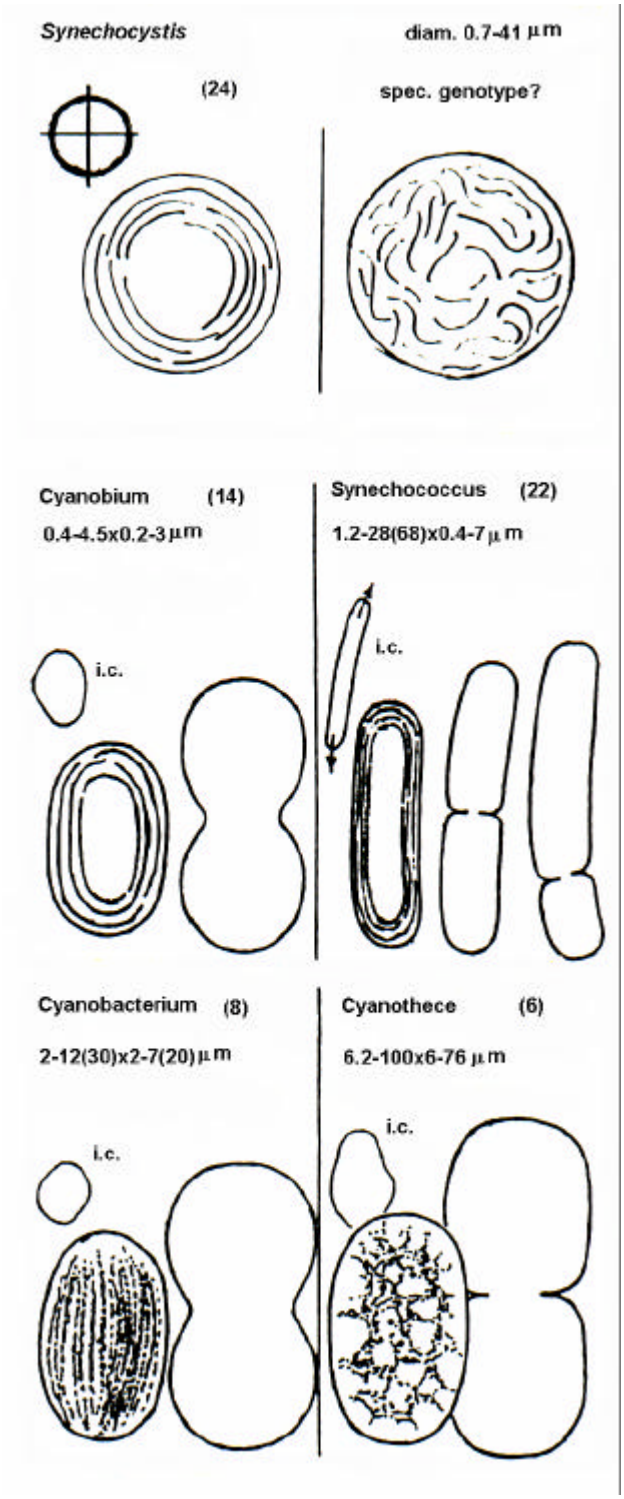
Picoplanktic genera

The cyanoprokaryotic genera, comprising the unicellular (not colonial) forms, occur in plankton of freshwaters and oceans, in soil, thermal springs, in littorals of various types of water bodies and in subaerophytic localities. They started to be intensely studied, if their importance as picoplanktic populations was recognised. The morphology is very simple, but the old traditional classification (*Synechocystis*: spherical cells, *Synechococcus*: elongated cells) was revised (3, 39-41). Type of cell division, ultrastructure (thylakoid patterns, type of nucleoids, cell wall structure, type of S-layers), GC-content, and molecular sequencing were used as intergeneric features. The review of generic character was summarised by Komárek (42) (Table 2, 3).

Table 2. Intergeneric characters in unicellular, solitary living cyanobacteria. Important diacritical features are marked by asterisks (41)

Genus – Number of species	Shape of cells	Type of cell division *	Thylakoid pattern *	Type of nucleoids *	Type of binary fission	Involution cells *	Type of S-layer	GC [mol %]	Cell dimensions [µm]
Synechocystis 21	spherical						p6	30–48	0.7–15(30) diam.
Cyanobium 13	oval to short rod-like	symmetric	parietal		pinching	irregular	(p4?)	(36) 49.1–71	0.4–4.5(10) × 0.2–3(6)
Synechococcus 24	cylindrical	symmetric or asymmetric		band-like	cleavage	filamentous	p4	47–56	1.2–28(68) × (0.4)1–6(11)
Cyanobacterium 8	cylindrical to widely oval		lengthwise	irreg. granular to net-like		irregular to shortly filamentous		39–41	(2)3.4–12(30) × (1)2–12(20)
Cyanothece 6	cylindrical to widely oval	symmetric	radial, infrath. spaces	net-like		irregular	p2	± 41	(6)7–70(100) × (6)7–52(76)

Table 3. Main generic characters of cyanobacteria living in solitary cells



Synechocystis
(type species:*S. aquatilis* Sauv. 1892)
comprises picoplanktic species:

- spherical cells
- division of cells in two planes in successive generations
- parietal position of thylakoids (in all species?)
- type of binary fission=cleavage (revision!)
- type of S-layer=p6 (in all species?)
- diameter of cells=0.7-30(?)µm

Cyanobium
(type species:*C. gracile* Rppka,Cohen-Bazire 1983)
comprises majority of picoplanktic species:

- widely oval cells
- division of cells in one plane in successive generations
- parietal position of thylakoids (1-4)
- type of binary fission=pinching
- type of S-layer=p4 (in all species?)
- length of cells=0.4-4.5(10?)µm

Synechococcus
(type speciers: *S. elongatus* Nag.1849)
comprise picoplanktic species :

- cylindrical, rod-like cells
- division of cells in one plane in successive generations
- parietal position of thylakoids (few to many)
- type of binary fission=cleavage
- type of S-layer=p4 (revision!)
- length of cells=(1.2)3-28(68)µm

Cyanobacterium
(type species:*C. stanieri* Rippka, Cohen-Bazire 1983)
mainly metyaphytic and terrestrial species:

- widely cylindrical to oval cells
- division of cells in one plane in successive generations
- lengthwise position of thylakoids (numerous!)
- type of binary fission=pinching (revision!)
- type of S-layer=p4 (in all species?)
- length of cells=(2)3.4-12(30)µm

Cyanothece
(type species: *C. aeruginosa* Nag, Kom.1976)
only metaphytic species:

- widely oval to cylindrical-oval cells
- division of cells in one plane in successive generations
- radial position of thylakoids (numerous!), with tendency to form intrathylakoidal spaces
- type of binary fission=cleavage (revision!)
- type of S-layer=p2 (in all species?)
- length of cells=(6)7-70(100) µm

The five genera (*Cyanobacterium, Cyanothece, Synechococcus* and *Synechocystis*) are well recognisable and characterised, but the original two names (*Synechocystis/Synechococcus*) are conservatively still commonly used in world literature.

The particular problem is with the genus *Cyanothece*, which was defined by Komárek (42) and Komárek, Cepák (40), and the specificity of which was supported by 16S rRNA sequencing data (Figure 1, black point).

However, this genus is treated very widely and heterogeneously in later papers (usually omitting the up to date definitions), which complicate the necessary revisions based on combined molecular and phenotype approaches (37,38,43).

The greatest problems are in species identification.

In numerous papers, describing the diversity of picoplanktic types from various planktic biotopes, the name "*Synechococcus* sp. div." is usually used without endeavour to classify the different lower taxonomic units.

A good example of this situation is the genus *Cyanobium* (Table 4).

Table 4. Review of the genus *Cyanobium* comprising the majority of picoplanktic types, identified usually as "*Synechococcus*"*

Described species**

C. gracile (type)	from culture, thermophilic
C. amethystinum	thermal springs
C. eximium	thermal springs
C. gaarderi	metaphytic, brackish waters
C. bacillare	planktic, brackish waters
C. diatomicola	freshwater, epiphytic, metaphytic
C. oceanicum	oceanic plankton (green)
C. parvum	freshwater, benthic
C. plancticum	freshwater, planktic (blue-green)
C. roseum	thermal springs (red)
C. rubescens	freshwater, planktic (red)
C. virga-rosea	freshwater, planktic (red)
C. waterburyi	oceanic plankton (red)

Undefined populations***

- ⇨ Several populations from clear freshwater lakes were recorded from Wales, Germany and Italy in lago Maggiore
- ⇨ Several different populations described from lake Arthur, NE. USA
- ⇨ One population recorded from brackish lagoons in Italy, Sardinia, near Cagliari
- ⇨ Two populations (red and green) described from plankton of Baltic Sea
- ⇨ Several types described from the oceanic plankton from North Atlantic

* For data about agglomeration of cells of picoplanktic species, or about colonial species in which the picoplanktic cells liberate from colonies, see reference: 47-49,51

** Several unrevised species from the genus *Synechococcus*

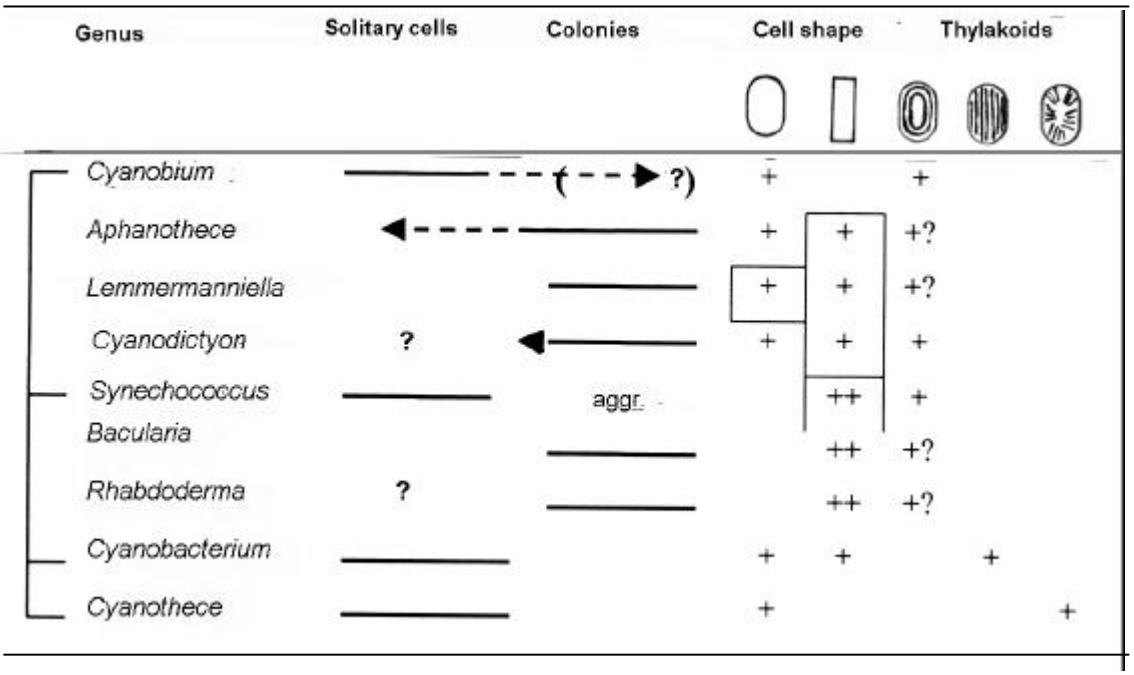
*** Numerous data about picoplanktic populations exist from oceanic, marine and antarctic habitats

In spite of the mentioned difficulties, the identification of picoplanktic cyanoprokaryotes on the subgeneric level begins to be very important.

The toxicity of picoplanktic cyanobacteria was already proved (44,45); because the elimination of picoplanktic populations from water is a serious technological problem, the danger of intoxication by picoplanktic populations exists.

The taxonomy of picoplanktic cyanobacteria is complicated also by the fact that colonial benthic or periphytic genera exist, from which separate the solitary cells in plankton, living as picoplankton for a part of life cycles (46-50). The cells from colonies of various *Aphanothece* (subg. *Anathece*) and *Cyanodictyon* species can escape from colonies in plankton (Table 5).

Table 5. Review of coccoid cyanoprokariotic genera with elongated cells, living solitaries and/or in colonies; several colonial genera can liberate the solitary cells in picoplankton



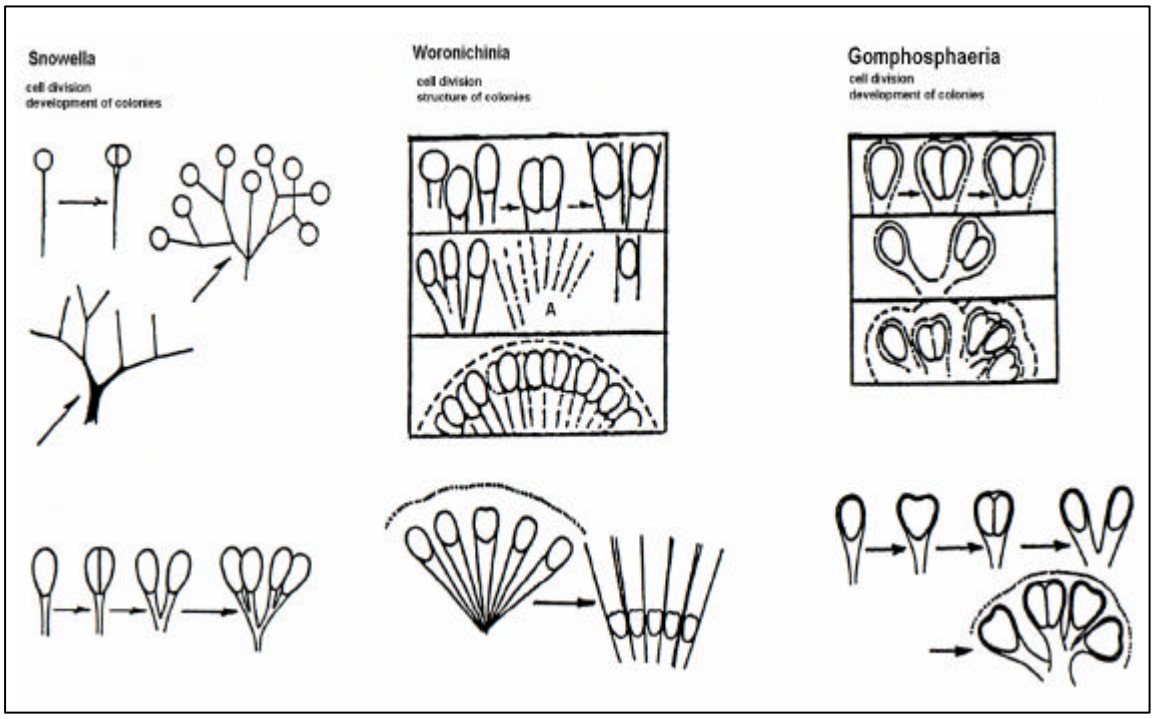
Main problems in taxonomy of picoplanktic cyanobacteria are:

1. How many and which distinct genotypes exist among picoplanktic cyanobacteria?
2. Is the type of binary fission (one/two division planes; pinching/cleavage) conform with genotype characters?
3. Ultrastructural variability in various picoplanktic types.
4. Do distinct ecological differences between various picoplanktic types exist?
5. Do typical picoplanktic types ("species") exist, or do they have (all?) the colonial stages in their natural life cycle?
6. Which molecular/biochemical/ultrastructural/phenotype characters can be used for taxonomic classification of unicellular cyanobacteria?
7. Can the separate taxonomic units ("species"?) be distinguished among picoplanktic cyanobacterial types?

Woronichinia and associated genera

The original genus *Gomphosphaeria* Kütz. 1836 (spheroidal colonies, different types of gelatinous stalk systems inside colonies, cell division in two planes perpendicular to the colonial surface) was divided on the generic level according to different strategies of cell division and different colonial structure (Table 6).

Table 6. Diacritical intergeneric features between gomphosphaerioid genera *Snowella* / *Woronichinia* / *Gomphosphaeria**



*Common features: coccoid colonial cyanobacteria
spherical mucilaginous colonies
cells divide into two planes, perpendicularly to the colonial surface
cells connected by mucilaginous s talks.

Toxicity was recognised mainly in water-bloom forming species *Woronichinia naegeliana.*

However, this cosmopolitan species occurs in numerous morphotypes over the world (Table 7), and their characterisation and identification of potential toxicity is therefore the urgent problem.

Microcystis

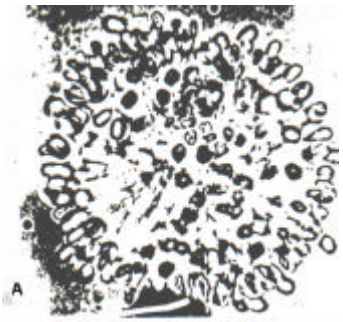
The genus *Microcystis* Kütz. ex Lemm. 1907 belongs to the most important water-bloom forming cyanobacteria, in which the majority of toxicological studies were performed.

The infrageneric taxonomy is extremely difficult. Several morphotypes are well recognisable in nature, if they are well developed, but very often populations occur, in which the phenotype delimitation is unclear.

The genotypic characterisation is quite uniform according to up to date sequencing data (19), (see Figure 4).

Table 7. Review of the main morphotypes of the cosmopolitan, planktic species *Woronichinia naegeliana.* A: *Woronichinia* cf. naegeliana with gas vescicles

population	cell form	cell size µ m	distribution
morphotype with narrow cells		5.4-8.2x1.4-2.3	northern regions (Canada, Japan, Scandinavia)
"typical populations"		5-7x2.5-3.5(5)	mainly temperate zones,(cosmopolitan?)
morphotype with reddish cells		4.5-6.5x2.3-3.5(4)	Canada (northern lakes)
var. *lemmermannii*		4-4.5x1.5-2.5	temperate zones, Europe
Woronichinia hungarica without distinct stalks		3.2-6.5x3.1-4.8	Hungary
Gomphosphaeria wichurae (sensu Drouet,Daily 1952)		3-5x3-4.8	temperate zones?
Woronichinia fremyi (composed colonies)		3-4.5x2.2-3.8	central America, Africa
Woronichinia cf. *fremyi* (simple colonies)		2.4-3.5x1.6-2	pantropical?



Several traditional species (*M. wesenbergii*, *M. viridis* and *M. botrys*) are well recognisable (15, 23, 30, 32) and also in the cluster of less separated species (*M. aeruginosa*, *M. flos-aquae*, *M. ichthyoblabe*) characteristical life cycles exist (33).

A wide diversity of *Microcystis aeruginosa*–like types exists particularly in tropical regions, the identification of which is difficult and possible often only after precise knowledge of the life cycle (51) (Table 8).

Table 8. Comparison of similar *Microcystis* species, differing morphologically distinctly only by life cycle

***Microcystis* species**	**Form of colonies**	**Size of cells** (diameter)
Microcystis aeruginosa *	dense arrangement of cells, holes in colonies	4-9.4 μm
Microcystis protocystis **	cells arranged scarcely, colonies without holes	3-7.2 μm
*Microcystis flos-aquae**	dense arrangement of cells, colonies without holes	3.5-4.8 μm
*Microcystis panniformis***	dtto	2.5-4-8 μm

* cosmopolitan species of *Microcystis*
** tropical species of *Microcystis*

This situation is complicated by the enormous variability inside of populations of one and the same morphotype (23) (Figure 2). Of course, the existence of morphologically similar stages does not prove the identity of "species", and the whole life cycle must be studied.

The recognition of various species is important particularly from ecological and toxicological point of view (Table 9), and the taxonomic studies of this genus are therefore particularly important.

Table 9. Review of *Microcystis* species with number of strains/populations, in which cyanotoxins were identified*

Species	**Toxins identifications**	**No. of types of microcystins**
M. aeruginosa	40	11
M. viridis	10	4
M. cf. botrys	3	-
M. flos-aquae	1 negat. 1	1
M. wesenbergii	2 negat. 3	1
M. ichthyoblabe	1	-
M. protocystis	1	-
M. panniformis	1	-

* The relations between number and types of cyanotoxins and *Microcystis* morphotypes is still not proportional

Main problems in the taxonomy of *Microcystis* are:

1. Do all morphotypes belong (traditional "species") to one and the same genotype?
2. Do the *Microcystis* types, which differ by the specific stages during their life cycle, exist? How should these specific stages be explained?
3. Does different toxicity (microcystins production) exist in various types?
4. How should the various stable *Microcystis* types, recognizable phenotypically and repeatedly occurring in specialised or common biotopes, be taxonomically classified?

Limnothrix/Pseudanabaena/Romeria/Planktolyngbya/Leptolyngbya

The family Pseudanabaenaceae is well defined by phenotype (Figure 5) as well as molecular characters (Figure 6).

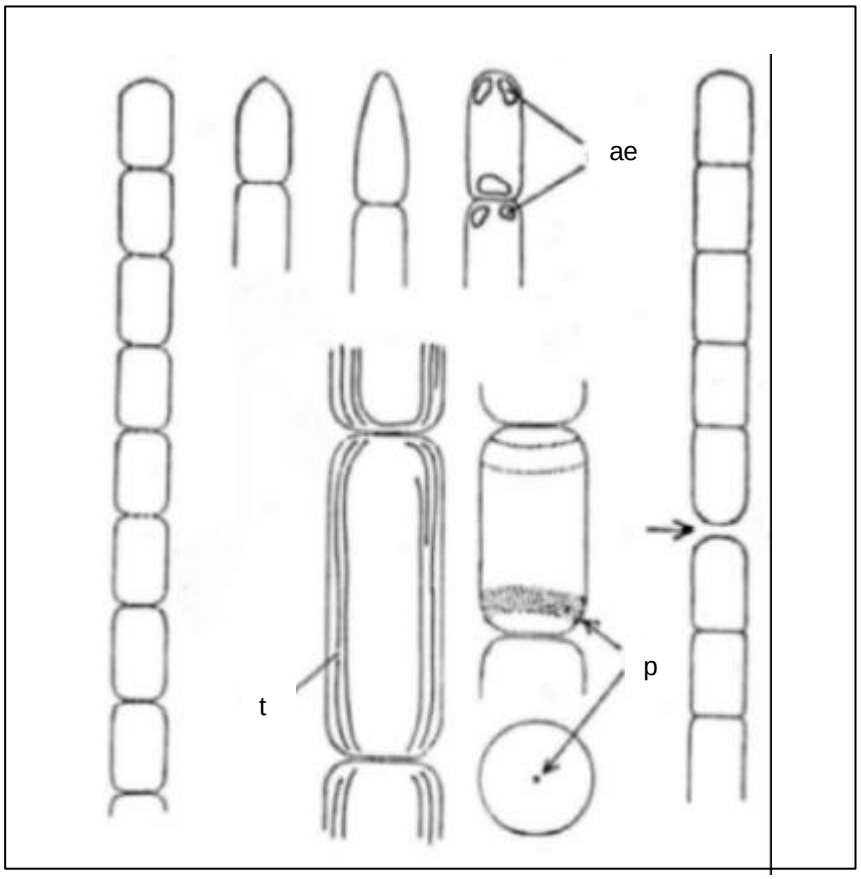


Figure 5. Main cytomorphological characters of simple filamentous family Pseudanabaenaceae, which is closely related with numerous coccoid cyanobacteria according molecular data (compare Figure 6) (ae: aerotopes, p: pores; t: thylakoid)

The first delimitation of this group was published by Rippka *et al.* (3) under the designation "LPP-group B". The intergeneric and interspecific features are less clear.

Several important members of the genera *Limnothrix, Pseudanabaena, Romeria* and *Planktolyngbya* occur in masses in plankton of eutrophic water bodies. However, while *Planktolyngbya* is mostly distributed in tropical regions, and *Pseudanabaena* and *Romeria*-species develop only rarely in massive populations, the genus *Limnothrix* Meffert 1988 (especially *L. redekei*) (Figure 7) plays an important role in mesotrophic reservoirs, particularly during the colder seasons in temperate zones.

The toxicity of this species is little known, but its study is urgent in respect of its massive development in reservoirs serving for water supply.

The water blooms caused by pseudanabaenacean species appear common in last years, and the identification of different species and their potential toxicity start to be an urgent problem.

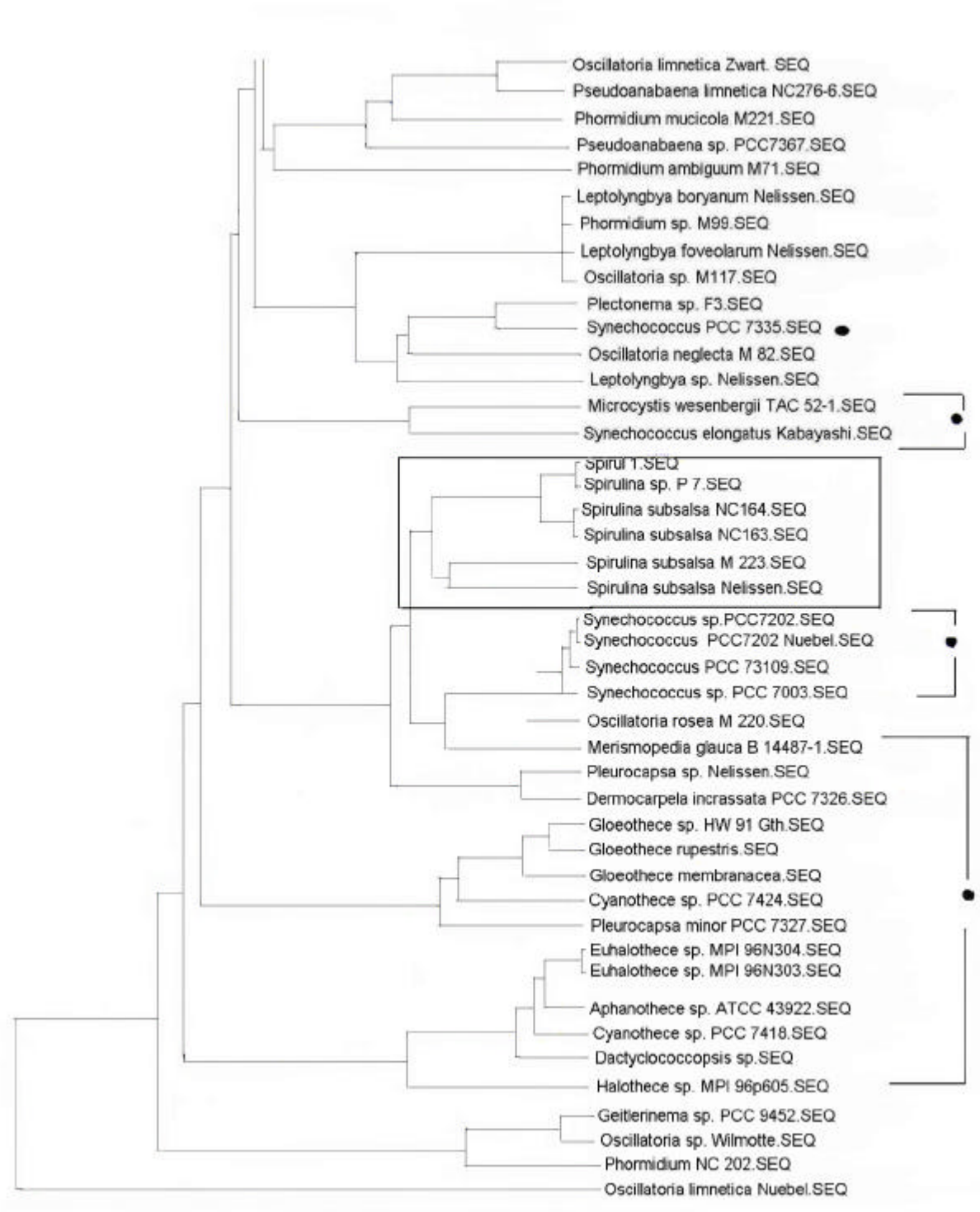


Figure 6. Part of an Internet cyanobacterial sequencing database with a mixture of simple filamentous (pseudanabaenacean) and numerous coccoid (black point) cyanobacteria. However, several phenotypically distinct genera from this group are detected. The names are not changed in spite of numerous evident mistakes in identifications

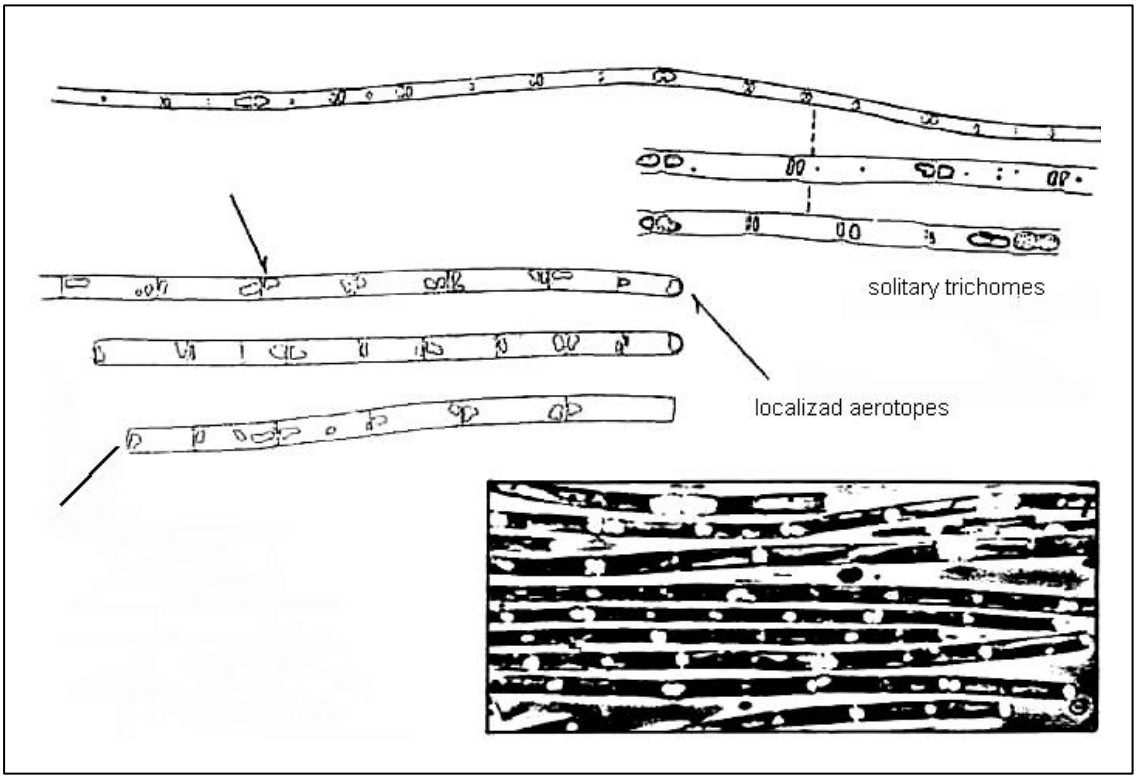


Figure 7. *Limnothrix redekei*, a characteristical planktic cyanobacterium, occurring in numerous modifications (in one and the same population), which can stabilize in culture

Main recent taxonomic problems in the family *Pseudanabaenaceae* (LPP-group B sensu Rippka *et al*. 1978) are:

1. Which genotypes are recognisable within this family?
2. Which phenotype (cytomorphological) characters are in coincidence with genotype (ability to produce gas vesicles, localisation of aerotopes, sheaths, necridic cells, life form)?
3. Which criteria could be used for characteristics of various "species" within various genera (genotypes)?
4. Are different ecotypes distinguishable also phenotypically and according to molecular criteria?

Planktothrix

The genus *Planktothrix* Anagn. et Kom. (8) belongs to important planktic water-bloom forming cyanobacteria. It was originally classified into the genus *Oscillatoria*, because it grows mainly in solitary trichomes without sheaths, heterocytes and akinetes. In cells are gas vesicles gathered in aerotopes, the biology and diversity is still little known. This genus is well separated from all other oscillatorialean genera by its ultrastructure, by life strategy, phenotypically, and mainly according to molecular 16S rRNA sequencing (Figure 1). The massive developments of various species are very common and toxic strains were repeatedly detected.

While the genus is well separated, the infrageneric diversity is not yet solved, and the problematic is similar as within *Microcystis*. Molecular sequencing did not separated any infrageneric taxa (19), but 14 "species" were described, which are recognizable in stagnant water bodies over the world and several other planktic *Oscillatoria* species belong evidently to this genus (Table 10).

Table 10 Review of the genus *Planktothrix* with selected main morphological characters*

Species	Distributions	Trichome width (μm)	Note
P. zahidii	Pakistan	2.5-3.5	straight trichomes
P. suspensa	temperate zones	2.0-4.0	calyptrate
P. prolifica	northern Europe, central North America	2.0-5.8	red ("red agardhii"), calyptrate
P. clathrata	northern parts of temperate zone	5.0-6.0	
P. agardhii	temperate and tropical zones	(2.8?)4.0-6.0(9.5?)	narrowed ends, calyptrate
P. cryptovaginata	eastern Europe	(4.0)5.0-8.5	facultative sheaths
P. rubescens	central and north Europe, central North America	(4.0)6.0-8.2(9.0)	red, calyptrate
P. mougeotii	Cosmopolitan (excl. Polar regions)	(5.0)5.5-9.7(10 ?)	Cylindrical ends
P. planctonica	Eastern Europe	8.0-10.0	Slightly coiled, cylindrical trichomes
P. geitleri	Scarcely in tropical and southern temperate zone	(6.0)7.0-10.0(11.0)	Narrowed straight ends
P. raciborskii	Eastern Asia, tropical and subtropical Asia, Africa	6.0-9.0(11.0)	Narrowed and curved ends

***** Problematic species: *Oscillatoria lutescens* (?syn. to *P. mougeotii*); *O. mehrai* (India); *O. miyadii* (Japan); *O. pseudomugeotia* (India); *O. rileyi* (USA); *O. setigera* (Russia); *Planktothrix arnoldii* (Russia, Aralian Sea); *P. perornata* (northern Europe); *P. compressa* (northern Europe).

They occur repeatedly in various localities in stable morphotypes, and several taxa on the species level are therefore acceptable. One from the most serious studies of natural populations, resulting into definition of four distinguishable types representing the stable modifications, was published by Skulberg *et al*. (13) from Norwegian lakes.

In spite of it, in the specific characters are still many open questions.

The most common *P. agardhii* and *P. rubescens* differ, e.g., mainly by their ecology and stable ratios of phycobilins (PE:PC), but without any proof of their genotype separation (Figure 1, 8).

The studies of infrageneric diversity belongs therefore to most urgent problems in this genus.

The phenotypically most similar genus *Trichodesmium* (growing in fascicle-like colonies and comprising mainly the marine species) differs clearly according 16S rRNA molecular analyses (Figure 1), but in two freshwater species of this genus their generic classification is still an open problem (52).

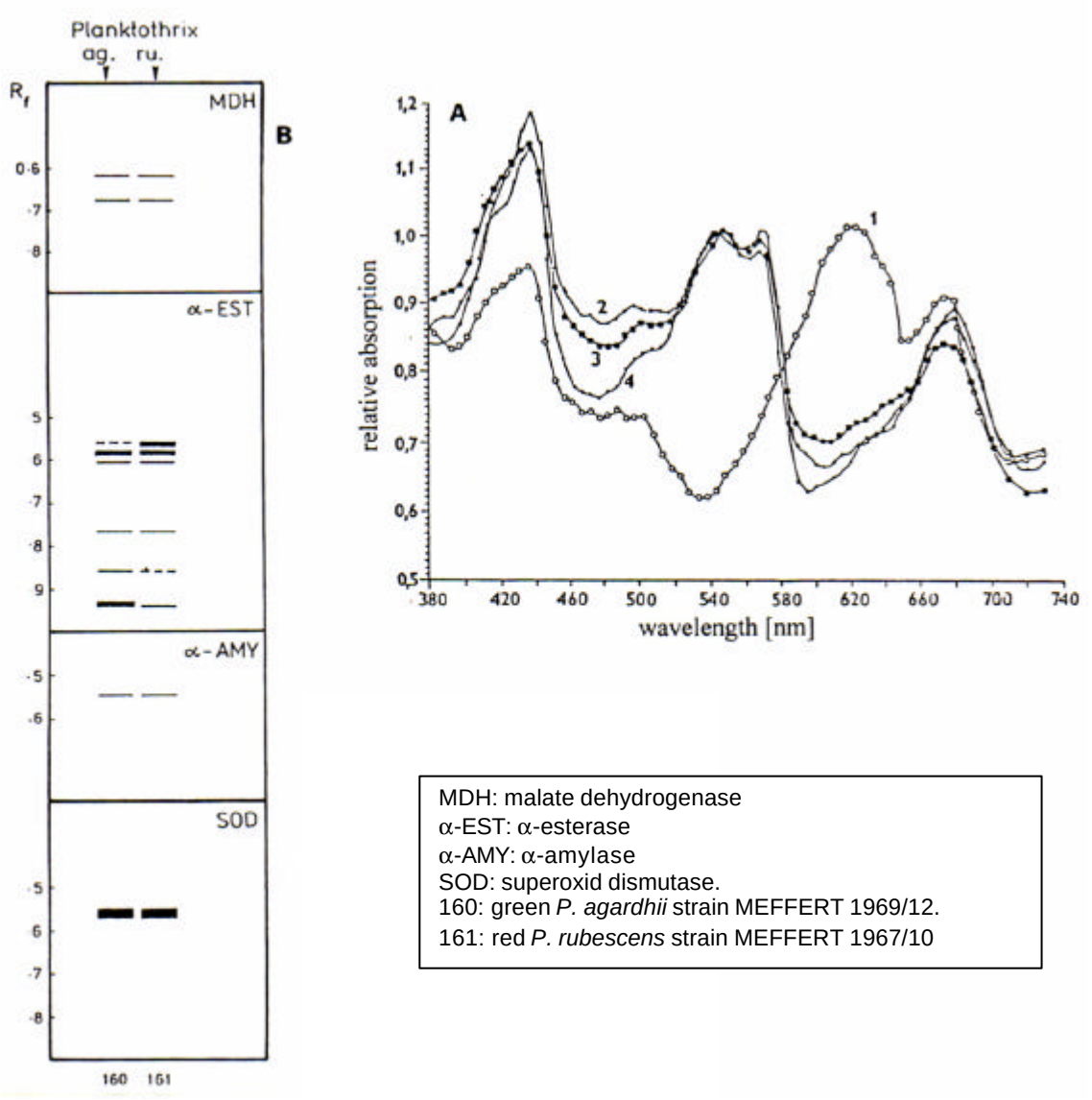


Figure 8. Comparison of stable characters (A: composition of phycobilins) of two related species: green *Planktothrix agardhii* (strain 2-4) and red *P. rubescens* (strain 1), with their almost identical allozyme analysis (B);

Main taxonomic problems in the genus *Planktothrix* are as follows:

1. Generic characters (molecular + phenotypic), delimitation of the genus. Is the genus *Planktothrix* unique and delimited in genotype?
2. Which characters can be used for classification of various types ("species"?) within the genus? Comparison of Skulberg and Skulberg's (13) criteria with genetic uniformity. Is it possible to characterise the various "species" in *Planktothrix*?
3. Are the morphological features (not very distinct, but stable) conform with other biochemical and molecular characters?
4. Does different toxicity exist in various types, recognisable in natural populations?
5. Are ecological (and geographical) limits of various morphotypes supported by molecular analyses?

Arthrospira

The generic delimitation of *Arthrospira* and particularly its separation from the genus *Spirulina* were proved quite doubtlessly by molecular sequencing (12) (Figure 1, 6). However, the both genera are well separated and recognisable also according phenotype and cytological characters. Two groups of species are included within the genus *Arthrospira sensu stricto*: benthic and periphytic species without aerotopes in cells and living in mats (*A. jenneri, A. platensis*), and planktic species with gas vesicles in cells, living in solitary trichomes and forming heavy water blooms, particularly in mineral lakes with high content of salts (mainly in desert and volcanic lakes).While the benthic species are not very common and their mats occur only sporadically in higher quantity, the intense water blooms of planktic species are very distinct in tropical regions. They often form almost monospecific populations in lakes with very extreme salinity. It is interesting, that toxic strains were not yet found in planktic types, and that numerous strains are used for biotechnological mass cultivation. The biomass is used in several tropical regions to alimentation.

The species identification is complicated by the fact that the strains, used for mass cultivation, are commonly designated by the name *"Spirulina platensis"*. This name was wrongly used for identification of planktic populations by Geitler (53) and Rich (54), and up to now this incorrect name is used for commercial purposes. The generic designation *"Spirulina"* concerns another valid genus, and the specific epitheton *"platensis"* concerns another, non-planktic *Arthrospira*–species.

The taxonomy is not yet solved definitely. Both groups, benthic and planktic, are well characterized one from another, but the species are recognizable only with difficulties. From several planktic described species, the two main types (*A. maxima* and *A. fusiformis*) differ phenotypically mainly by the type of spirality (Figure 9), which is surprisingly very wide in *A. fusiformis*, but more delimited in *A. maxima*. In fact, both species differ phenotypically mainly only by the type (variability) of spirality (a similar case is in the genus *Nodularia*). They were not proved by molecular criteria, and they are commonly misinterpreted. From other planktic, less distributed morphospecies were described several species with smaller dimensions, which occur only sporadically and their cultivation and taxonomic status is very little known (e.g., *A. massartii, A. khannae*).

In planktic *Arthrospira* species are several interesting and unexplained other problems. One of them is the tendency to form irreversible straight trichomes, which occur only in cultures and never were found in natural populations. Another problem is the status of *A. indica*, described by Desikachary and Jeeji-Bai (55) from India. The same morphotype was found, as accessoric (without morphological transitions) in one population of *A. maxima* in a volcanic lake in Mexico (Figure 10). The taxonomic status of such morphotype can be solved definitely only by careful studies of isolated strains and their comparison with natural populations.

Main taxonomic problems in the genus *Arthrospira* are:

1. Does a clear genotypic difference exist between planktic (with gas vesicles) and benthic (without gas vesicles) species?
2. Morphological variation limits in various types (traditional species).
3. Do stable and genotypically recognisable morphotypes ("species"?) exist within both *Arthrospira* groups?
4. Does toxin production occur in some types? In which? Under which conditions?

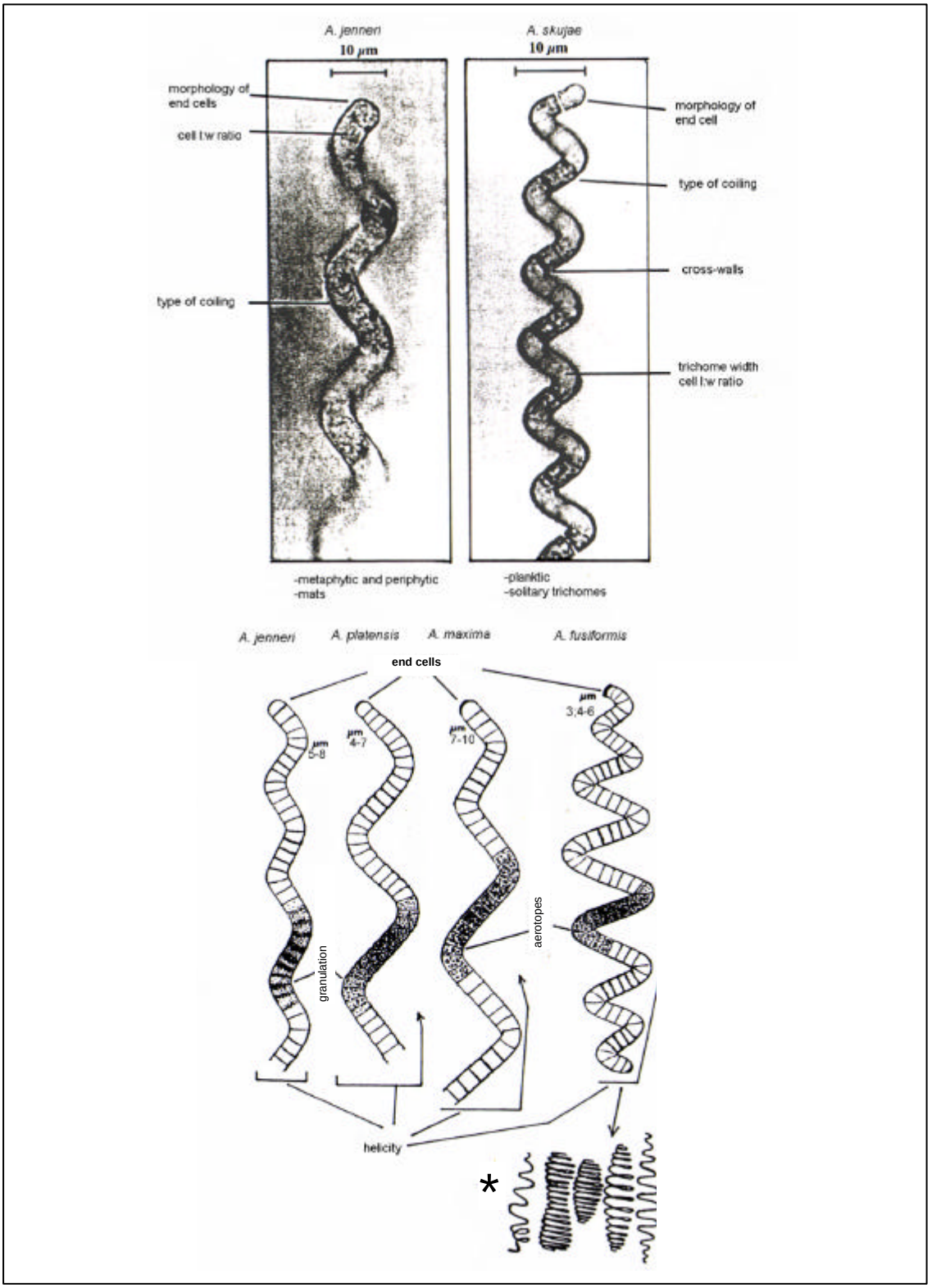
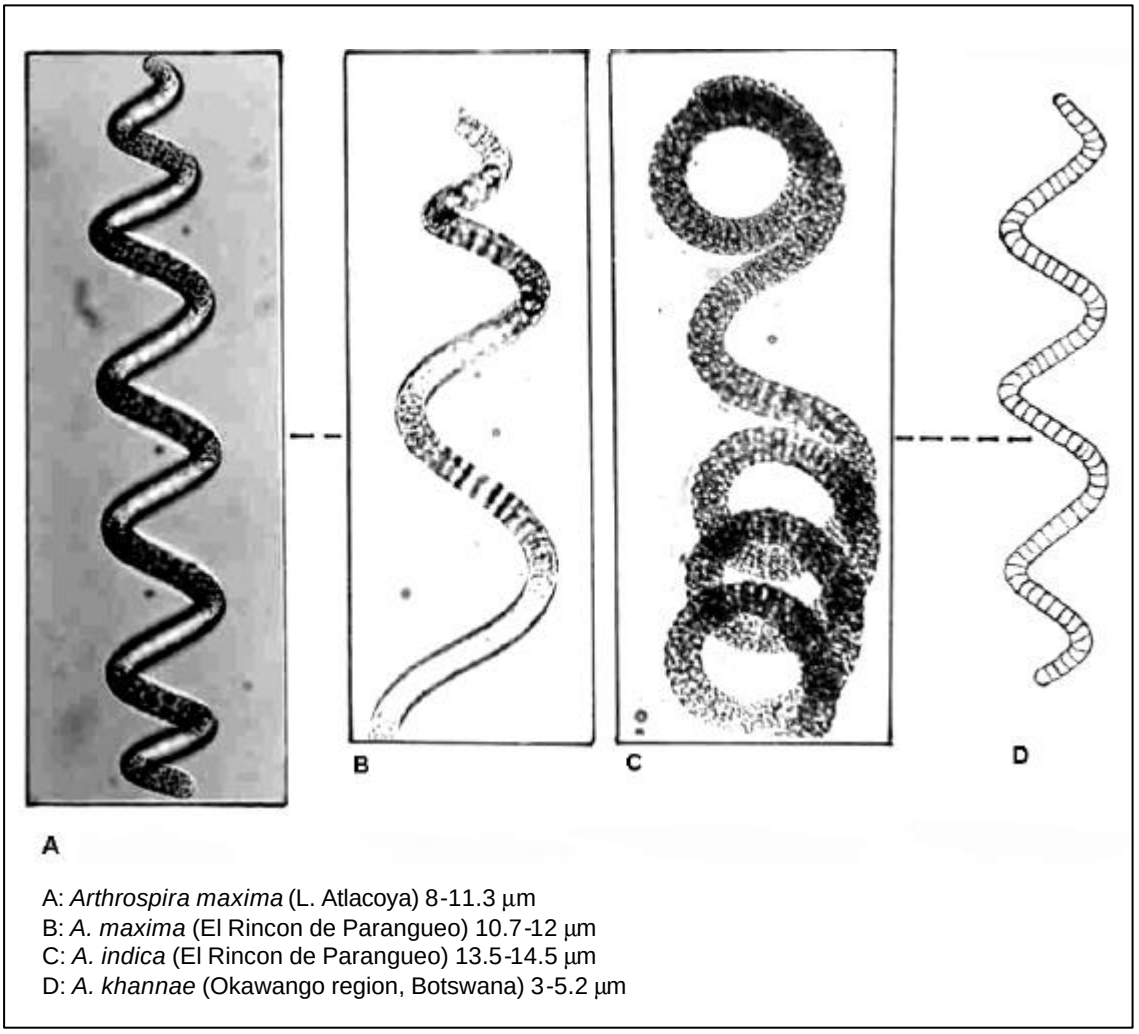


Figure 9. Comparison of two benthic *Arthrospira* morphospecies from Brazil (*A. jenneri, A. skujae*) with two main benthic and two planktic morphospecies derived from literature. The extreme variation in spirality (asterisk) occurs exclusively in the planktic *A. fusiformis*



A: *Arthrospira maxima* (L. Atlacoya) 8-11.3 µm
B: *A. maxima* (El Rincon de Parangueo) 10.7-12 µm
C: *A. indica* (El Rincon de Parangueo) 13.5-14.5 µm
D: *A. khannae* (Okawango region, Botswana) 3-5.2 µm

Figure 10. Comparison of *Arthrospira maxima* from two localities in Mexican volcanic lakes (A and B), with special morphotype corresponding to *A. indica*, occurring accessorily (but without morphological transition) in one Mexican locality, and with morphologically similar, but thinner *A. khannae* from southern Africa

Phormidium, Oscillatoria, Lyngbya

The group of thicker (± over 3 µm wide) filamentous cyanobacteria without heterocytes and akinetes comprise several traditional genera, which are classified according to molecular (3) as well as ultrastructural (8,56) criteria in two well separated groups:

i. *Phormidium* and related genera (*Microcoleus, Symploca*). This group (family *Phormidiaceae*) comprises a large cluster of benthic, periphytic and terrestrial cyanobacteria with filaments ± 3-10 µm wide, and special unique organization of thylakoids in cells. The internal taxonomy is difficult. Several hundreds of taxa were described on the basis of phenotype and ecological characters, but the identification of natural populations and strains is extremely difficult and sometimes problematic. They also exist data about the variation in populations of diverse species (Figure 11,12).

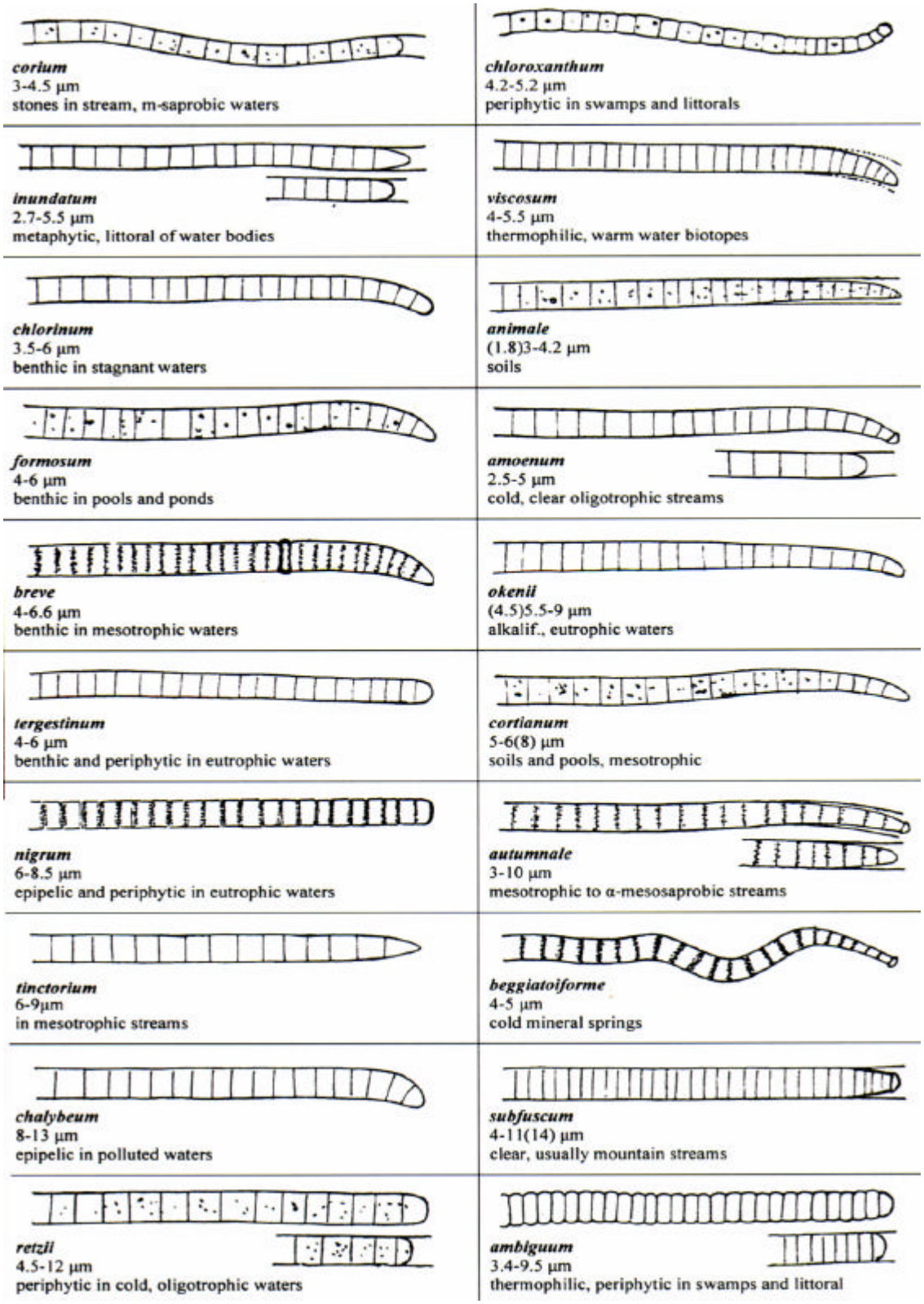


Figure 11. Review of the main traditional *Phormidium* species, described according to morphological characters. The intraspecific features are unclear

strains	width of filaments *	length of filaments *	granulation at the cross-walls	width of filaments * 4 8 12
MARVAN 1967/15	(1.6)-5.0-10.0-10.5	1.0-10.0	- to+	
HINDAK 1963/159	2.0-10.5	1.2-10.0	+	
HINDAK 1963/163	3.5-7.8-(9.0)	1.0-9.6	-	
MARVAN 1966/10	4.0-9.5	1.5-8.0	-	
MARVAN 1963/4	4.0-9.0-(10.0)	1.5-8.4	- to +	
HINDAK 1967/41	4.0-10.0-(10.5)	1.0-15.6	- to +	
HINDAK 1967/1	(4.2)-5.0-9.5-(10.0)	1.0-11.0	- to (+)	
HINDAK 1965/33	4.5-10.0	1.2-11.5	- to (+)	
HINDAK 1963/157	4.8-10.5-(11.5)	1.0-14.0	- to (+)	
MARVAN 1966/20	5.0-10.5-(13.0)	1.2-8.0	(-) to +	

Figure 12. Variation in trichome width in various strains of *Phormidium autumnale* (* in µm)

ii. The cluster of *Oscillatoriaceae sensu stricto* (*Oscillatoria, Lyngbya, Blennothrix, Plectonema* [type!]) was originally defined as a unique taxonomic entity as "LPP-group A" by Rippka *et al*. (3). It is well defined also by phenotype characters (trichomes mainly 8-20 [>60] µm wide, special type of thylakoid arrangement, special type of cell division).

Anabaena, Anabaenopsis

The heterocytous genus *Anabaena* Bory ex Born. et Flah. 1886 (subg. *Dolichospermum*) belongs to the most interesting planktic cyanobacteria, comprising more or less in straight or coiled types.

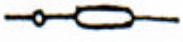
It is unclear, in what a degree can the coiled types produce the straight morphotypes (particularly in culture).

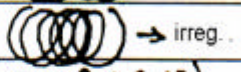
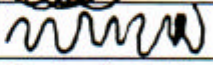
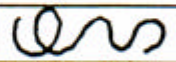
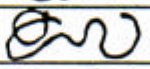
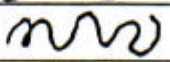
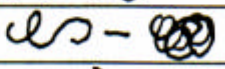
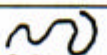
The various morphotypes differ sometimes only in "small" morphological characters (spirality, width of trichomes, form and size of akinetes, position of akinetes), but all studies suggest that they are stable, and can be considered as various species (3,15,16).

The short reviews of planktic species, occurring in Europe, are in Table 11.

The structure of trichomes is metameric (Figure 13); this important character is identical as in another related genus *Anabaenopsis* (Wolosz.) Mill. 1923, but the strategy of heterocyte development clearly differs in both genera.

Table 11. Schematic review of *Aphanizomenon* species

epitheton **	trichome width µm	cell form	akinetes	distribution
affinis in fascicles!	(3)4.5-7(8)			temp. zones
heterospora	4.3-7.2			temp. zones
viguieri	(5)6-7(9?)			cosmopolitan
danica	5-7			N temp. zone
planctonica	(8)9-15			cosmopolitan
smithii	(8)8.5-15			temp. zone?
solitaria	(6.5)8-12(13.5)			temp. zones
macrospora	5-6.5(8)			temp. zones
zinserlingii	4.6-7.2			N Eurasia

epitheton ***	trichome width µm	cell form	coiling	distribution
compacta	2.4-4(5)			N temp. zone
flos-aquae	(2.5)4-5.5(9.5?)			cosmopolitan?
perturbata	(6)7-9(10)			cosmopolitan?
circinalis	(7)8-11			cosmopolitan*
spiroides	6-8(9)			cosmopolitan?
crassa	8-14(15)			cosmopolitan*
curva	7.2-10.2			N temp. zone
reniformis	3.5-5.5			N Europe
longicellularis	5-6			Baltic region
sigmoidea	2.5-5			cosmopolitan?
farciminiformis	3.2-4.5			N Europe
lemmermannii	(2.5)5.2-7			temp. zones?
mendotae	2.3-4.5			temp. zones

* Disjuntive area
** Straight trichomes
*** Coiled trichomes

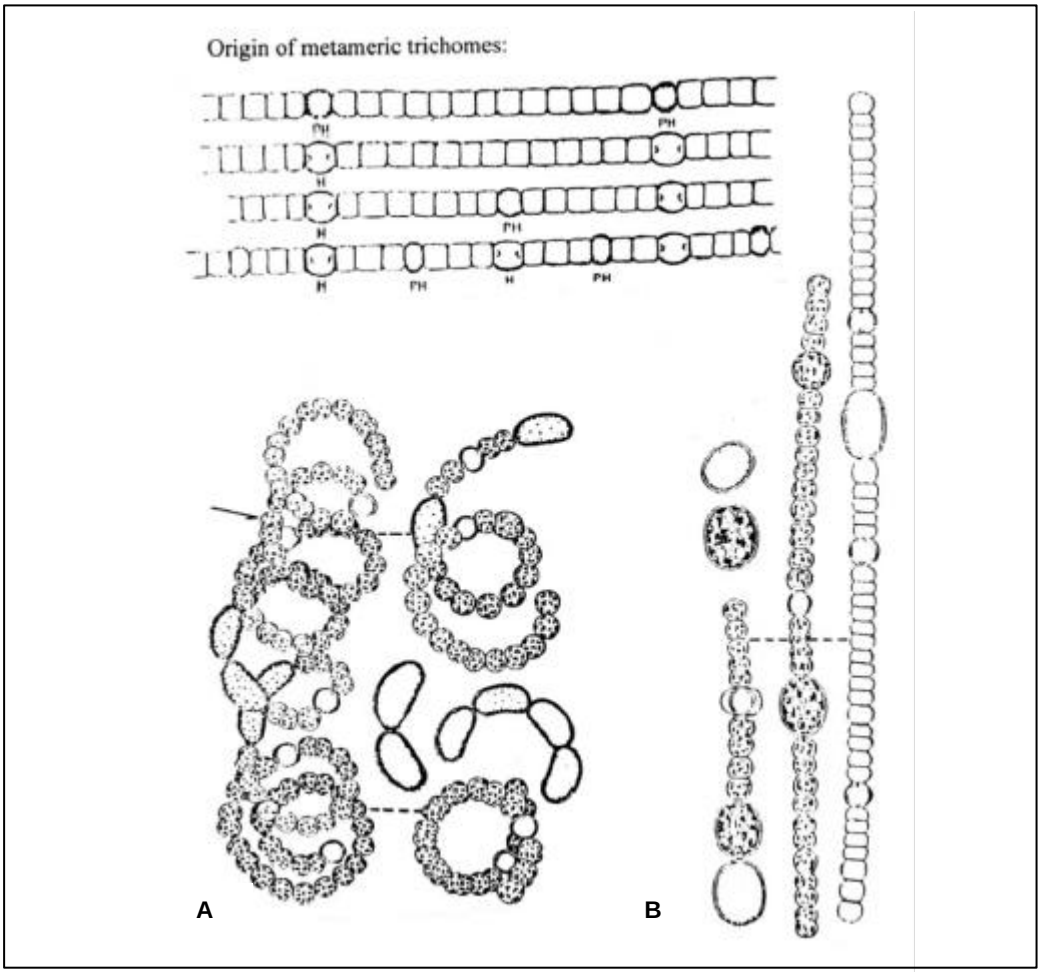


Figure 13. Example of two morphospecies from the genus *Anabaena*, subg. *Dolichospermum* (A: *A. perturbata*, B*: A. viguieri*) with straight and coiled trichomes; both types are well distinguishable in natural populations, without transitional stages. The scheme illustrates the development of anabaenaceam metameric trichomes.

Aphanizomenon

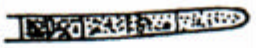
Another nostocalean, heterocytous and exclusively planktic genus, but with subsymmetrical trichomes, is *Aphanizomenon* Morr. ex Born. et Flah. 1986.

The type species, *A. flos-aquae*, grows in characteristical macroscopic, floating, fascicle-like colonies (distributed exclusively in temperate zones), but only three other species form fascicles; numerous other species occur only in solitary trichomes. A special problem represents a cluster of species with narrowed ends of trichomes and more or less pointed apical cells. Such forms represent a transitional group to *Anabaena* species with narrowed ends of trichomes, the taxonomy of which is unclear (57).

The genus *Aphanizomenon* seems to be therefore heterogeneous, and the infrageneric taxonomy must be solved by molecular criteria. However, several species are well distinguishable and delimited according to morphological characters.

The list of up to date distinctly described species is included in Table 12.

Table 12. Schematic review of *Aphanizomenon* species

epitheton	diagnostic features			distribution
	dimensions	trichome ends	life form	
flos-aquae	4.8-8(9)		fascicles	temper. zone
klebahnii	3.3-5.8		fascicles	temper. zone
balticum	2.4-5.2		fascicles	Baltic Sea
yezoense	2.5-4.0		fascicles	temper. zone
flexuosum	2.6-3.7		solitary coiled fil.	temper. Eurasia
paraflexuosum	2.8-4.5		solitary coiled fil.	Japan
skujae	2.6-4.0		solitary flexuous fil.	northern parts of temp. zone
gracile	2.2-3.6		± straight solit. fil.	temper. zones (subtrop.?)
schindleri	1.6-4.2		± straight sol. fil.	Canada
hungaricum	3.2-6.0		± straight sol. fil.	scarcely, temper. zone
issatschenkoi	2.4-4.0		solit. fil.	temp. zone (subtropical?)
tropicale	2.3-3.8		solit. fil.	trop. Eurasia, Africa
ussaczevii	2.7-3.3		solit. fil.	Caspian Sea
elenkinii	3.0-6.0		solit. fil.	rarely temper. zone
capricorni	2.0-3.2		solit. fil.	southern Africa

* with + or – cylindrical cells
** with pointed terminal cells

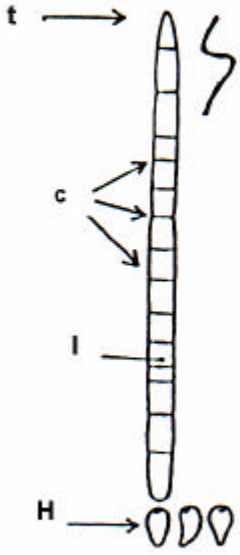
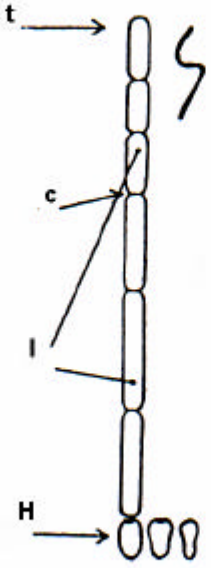
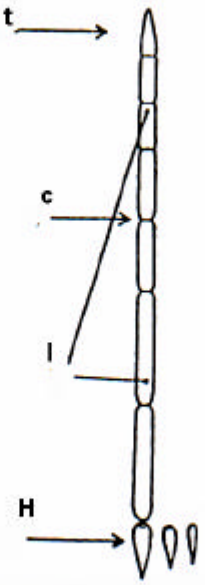
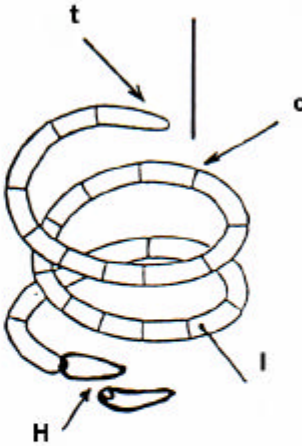
Cylindrospermopsis

Interesting nostocalean, planktic genus, comprising eight different species, well recognizable morphologically (Table 13).

All species occur in tropical regions and several of them form rich populations, dominating in phytoplankton community in tropical reservoirs. Up to know, only one species (type-species, *C. raciborskii*) is well known and studied, evidently from two reasons: this pantropical species has tendency to invade warm regions of temperate zones (in Europe, it is well known from Mediterranean countries, south France, Pannonian region), where it forms also water blooms. The second reason of its study is the intense production of cyanotoxins (cylindrospermopsin).

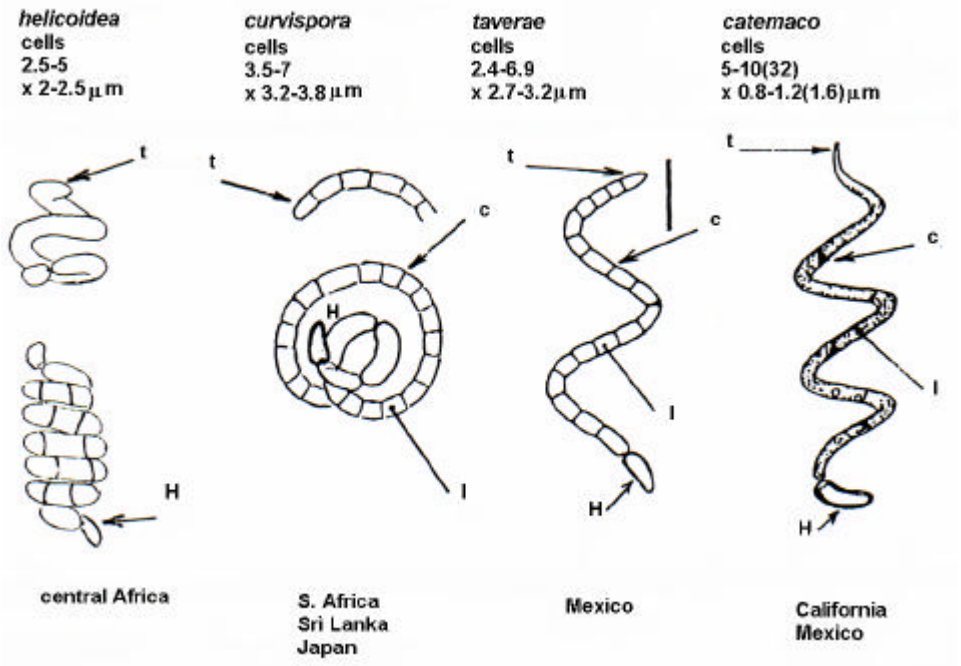
The genus is clearly defined (phenotypically), and the natural species are well identifiable. However, several interesting problems are still open. E.g., *C. raciborskii* grows mainly in straight or slightly curved and waved trichomes, but coiled trichomes also occur.

Table 13. Review of the Cylindrospermopsis species. t: terminal cells, c: constrictions at cross walls, l: cell length, H: heterocytes

raciborskii	*africana*	*cuspis*	*philippinensis*
cells 2.5-12(16) x(1.7) 2-4 µm	cells 4.2-22.4 x 1-2.5 µm	cells 4.2-19.5 x 0.8-1.2 µm	cells (3.6)6-18(22) x(1.5)1.9-3(5)µm
			
pantropical and subtropical; occasionally in S. parts of temperate zone (warm seasons)	E. and S. Africa	E. Africa Mexico	Cuba Indonesia Mexico Philippines

to be continued

continues



In contrast, in populations of the coiled *C. philippinensis* occur sometimes straight trichomes. In both species the populations of *C. raciborskii* with almost all coiled trichomes and of *C. philippinensis* with almost all straight trichomes occur. If we isolate the straight or coiled trichomes in culture, they stabilize in culture in the form of original trichomes. The same variation was found in *C. africana* and *C. taverae*. This type of variability complicates the identification of strains and species.

It seems, that this genus is distributed and diversified particularly in tropical regions, where various populations commonly occur in water bodies. The descriptions of further phenotypic species of *Cylindrospermopsis* are expectable.

Nodularia

Nodularia Mert. ex Born. et Flah. 1886 (Figure 14) is an attractive nostocalean genus, from which mainly the water bloom forming populations are known.

The genus is well recognizable, in spite that it is based mainly on quantitative characters (length: width ratio of cells), particularly in comparison with the genus *Trichormus* (Figure15).

In fact, the taxonomic classification is similar like in *Arthrospira*: two clusters of species within this genus are well separated.

The benthic, metaphytic and terrestrial species occur exclusively without gas vesicles in cells and represent a little known cluster of taxa (with exception of *N. harveyana* and *N. willei*).

The second group of planktic types contains populations, forming heavy water blooms in coastal or inland waters with higher salinity.

They are known from numerous, but very dispersed localities over the world.

Their taxonomy is complicated, and they are usually identified as one traditional species, *N. spumigena.*

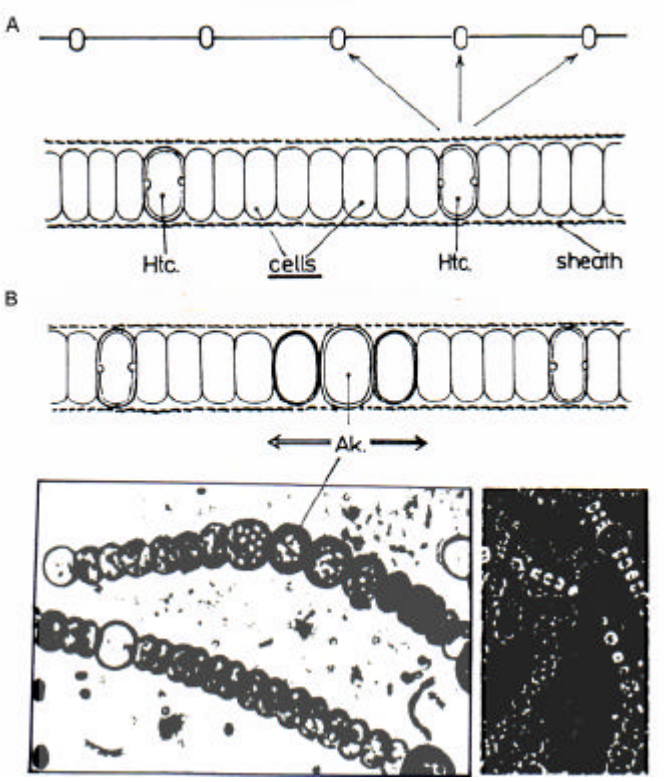


Figure 14. Generic character of the genus *Nodularia*
(A: metameric structure of filaments; B: apoheterocystic formation of akinetes.)

The recent localities of planktic *Nodularia*-blooms are known mainly from Baltic Sea, coastal bays in SW Australia, coastal lakes in Uruguay, and inland lakes with higher salinity in Australia, North America (e.g., Nevada), and Mexico (volcanic lakes). The review of these populations with corresponding citations is summarized on Table14.

The problem is that the available data are incompatible.

Several strains were studied by molecular sequencing, other only on the base of morphology, or combined with ultrastructural characters.

The morphological variability complicates the studies. E.g., *N. spumigena* from Baltic Sea has very wide variation of filament coiling, and occurs in straight to very intensely coiled trichomes, *N. spumigena* from Mexico grows exclusively in very straight trichomes, *N. spumigena* from Uruguay only in very long, slightly coiled filaments.

The molecular data from strains isolated from central parts of Baltic Sea belong to one genotype (which corresponds evidently to variable populations of *N. spumigena;* (58), but other two types, recognizable by Komárek *et al*. (59) were probably not yet revised and identified.

The Australian populations are morphologically similar to Baltic specimens, but different according to sequencing data (36).

The solution of species diversity is therefore possible only on the basis of careful combination of phenotype, ultrastructural and molecular studies, in collaboration of specialists in all methods available.

However, this approach is highly desirable and the only possible in all future taxonomic studies.

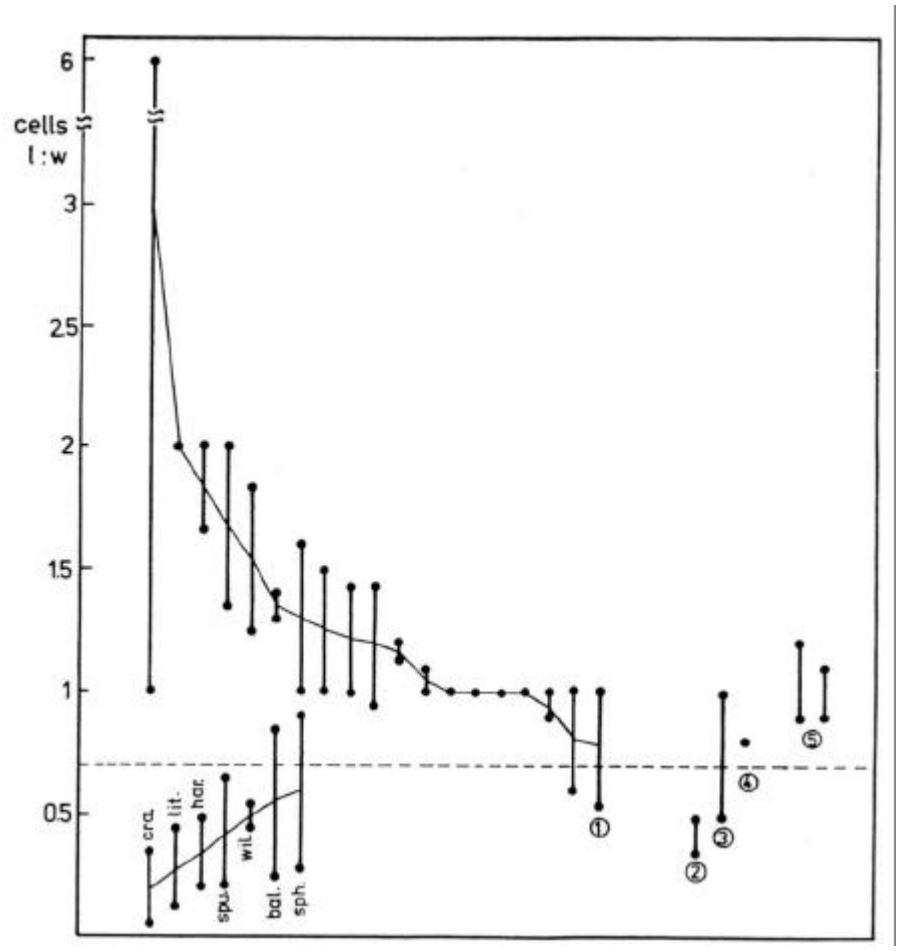


Figure 15. Cell length: width ratio in species of the genera *Trichormus* (up) and *Nodularia* (down). The generic identification of populations 1-5 is unclear. The both genera are closely related according to life strategy and akinete formation, in spite of numerous *Trichormus*-species classified originally into the genus *Anabaena*

The molecular analyses must be decisive for taxonomic evaluation, but without parallel careful study of phenotype variation, life cycles, ultrastructure and ecology are without sense for future progress in knowledge of natural diversity (and *vice versa*, of course).

Main taxonomic problems in *Nodularia* are:

1. Are both groups, planktic (with gas vesicles) and benthic (without gas vesicles) clearly different in genotype?
2. Are the species in benthic group clearly distinguishable morphologically and ecologically? Is it in conformity with molecular characters?
3. In what degree are various characterizable planktic morphotypes from Baltic Sea related? Do they belong to one or to more genotypes?
4. In what degree are the various, clearly distinguishable morphotypes (occurring in very distant regions – Baltic Sea, inland lakes in western part of N America, volcanic lakes in central Mexico, coastal lakes in Uruguay, Australia –, which are all classified as "*N. spumigena*"-group) related?

5. Do any differences exist in toxicity (and other biochemical markers) between planktic populations from various regions?

Table 14. Review of the recently studied planktic *Nodularia populations, with aerotopes**

Region	No. tax. Units	Morphological difference*** taxonomic identity?
Baltic Sea	3**	
→ Komarek *et al.* 1968 (cytomorphology, ecology, popul.+strains)	1-(2)**	
→ Hanley *et al.* 1997 (sequencing-RNA)	3**	
→ Lehtimaki *et al.* 1998 (sequencing-DNA)	1**	
→ Bloch *et al.* 1998 (sequencing-DNA)		
SW Australia	1	
→ Komarek *et al.* 1968 (morphology)- inland lakes	1**	
→ Bloch *et al.* 1968 (sequencing-DNA)- coastal lagoons		
America (N+S)	2**	
→ Nordin, Stein 1956 (morphology, ecology, popul.+strains)	1**	
→ Tavera, Komarek 1997 (morphology)- volc. lakes Mexico	1**	
→ Perez *et al.* 1999 (cytomorphology)- coastal lakes Uruguay		

* The type identified as *Nodularia spumigena* occurs in all papers, but the results (and also the described morphotypes) are incompatible
** at least one species identified as "*N. spumigena*"
***the arrows indicate the "taxonomic incompatibility" between populations, derived from cited papers

Conclusions

1. Molecular approach is decisive for future evaluations in cyanobacterial taxonomy.

However:

2. The stable phenotype features are in conformity with molecular studies.
   - The molecular studies proved (i) the existence of natural clusters, which correspond more or less to the traditional concept of genera or "macrospecies", but also (ii) the very wide diversity inside of traditional species (two quite identical strains/populations were not yet found).
   - Numerous stable infrageneric morphotypes exist in nature, which are ecologically restricted and well recognizable and different from one another.

Therefore:

- Molecular evaluation must be combined with evaluation of phenotype and ecological characters.

- The species concept is always more or less conventional, and now in cyanobacteria unclear; it is necessary to tend to such definition, which can be designated as "evolutionary and phenotypically natural units among cyanoprokaryotes", recognizable in nature and/or in culture.
- The study of diversification, variability, stability and ecological plasticity of natural populations is highly desirable and necessary for ecological and taxonomic research.

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FATTORI INFLUENZANTI LO SVILUPPO DEI CIANOBATTERI NEI LAGHI PROFONDI

L'esempio del Lago di Garda e degli altri grandi laghi a sud delle Alpi

Nico Salmaso
Dipartimento di Biologia, Università di Padova, Padova, Italia

Introduzione

I laghi profondi posti al margine meridionale delle Alpi costituiscono una delle più grandi riserve di acqua dolce in Europa. I più grandi di questi laghi includono – da est a ovest – il Garda, l'Iseo, il Como, il Lugano e il Maggiore (Figura 1).

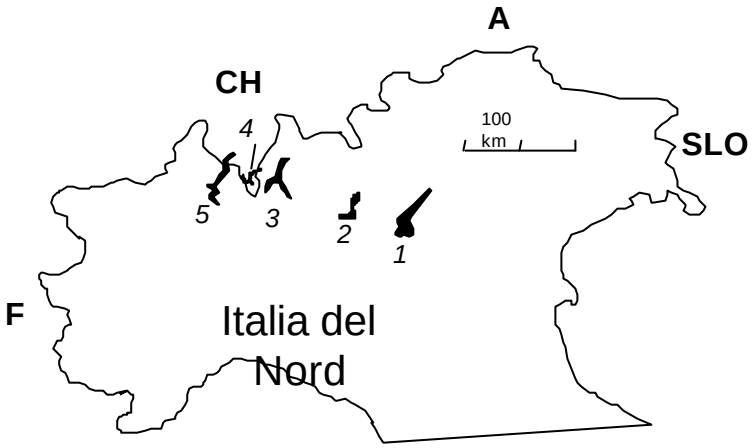

Figura 1. Localizzazione dei laghi sudalpini nell'Italia del Nord

Sulla base dei dati riportati dall'IRSA (Istituto di Ricerca Sulle Acque) (1) e da Marchetti *et al.* (2), i laghi profondi sudalpini hanno un volume complessivo di oltre 120 miliardi di m^3, pari a circa l'80% di tutto il patrimonio idrico invasato nei laghi naturali e artificiali italiani.

Nell'Italia centrale le riserve di acqua superficiale sono raccolte in larga parte nei laghi di Bolsena e Bracciano (14,25 miliardi di m^3), mentre nell'Italia meridionale e insulare le riserve idriche (circa 4 miliardi di m^3) derivano quasi esclusivamente da invasi artificiali (2).

Originariamente i laghi profondi sudalpini erano oligotrofi; successivamente, con il progredire dello sviluppo economico e l'aumento della pressione turistica, soprattutto a partire dagli anni '60, questi bacini hanno subito un progressivo processo di eutrofizzazione, testimoniato da studi di paleolimnologia (3) e dall'aumento delle concentrazioni di nutrienti algali (4).

Attualmente, una evidente tendenza verso condizioni originarie di oligotrofia è rilevabile solo nel Lago Maggiore (5). Sulla base dei più recenti studi il Garda è in una condizione tra l'oligotrofia e la mesotrofia (6, 7), l'Iseo e il Como sono mesotrofi (8-10), mentre il Lugano,

dove i primi segnali di eutrofizzazione erano stati documentati già negli anni ’40, presenta le condizioni peggiori, collocandosi tra la mesotrofia e l’eutrofia (11, 12).

L’aumento del livello trofico nei laghi determina una lunga serie di effetti negativi sulla qualità delle acque. I più evidenti comprendono l’incremento della biomassa algale, le modificazioni a carico della struttura delle comunità vegetali e animali, il deterioramento dell’ambiente chimico (es. anossia ipolimnetica) e delle caratteristiche estetiche delle acque.

In particolare, per quanto riguarda la componente fitoplanctonica, uno dei più evidenti effetti dell’aumento del livello trofico è costituito da una generale tendenza all’aumento dei cianobatteri (13, 14). Questi organismi sono in grado di produrre un’ampia varietà di tossine, costituendo un motivo di seria preoccupazione per i possibili rischi per la salute umana connessi con l’utilizzo di acque contaminate da una loro eccessiva presenza (15).

Lo scopo di questo contributo è di riportare una descrizione della composizione e dinamica stagionale dei cianobatteri dominanti nel più grande ed esteso lago italiano (Lago di Garda) fornendo, nel contempo, alcune indicazioni sui fattori influenzanti lo sviluppo di questo particolare gruppo di organismi nei bacini profondi.

Infine, le popolazioni di cianobatteri del Garda saranno confrontate con quelle identificate negli altri laghi profondi sudalpini.

Caratteristiche del Lago di Garda e lineamenti della ricerca

Le principali caratteristiche morfometriche e la mappa batimetrica del Lago di Garda sono riportate nella Figura 2.

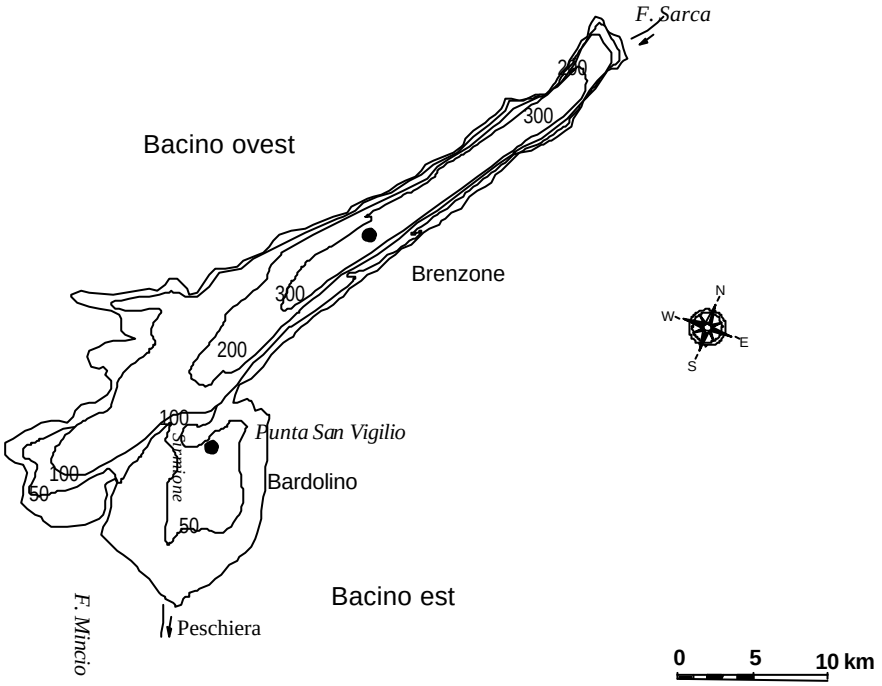

Figura 2. mappa batimetrica del Lago di Garda e ubicazione delle stazioni di campionamento

Il lago può essere suddiviso in due bacini separati da una dorsale sommersa che congiunge Sirmione con Punta San Vigilio. Il bacino occidentale è il più grande e il più profondo (Z_{max}=350 m), mentre quello orientale, di forma conica e con una profondità massima di ca. 80 m, costituisce solo una modesta frazione del volume complessivo (meno del 7%).

Una caratteristica idrologica importante e propria di tutti i laghi profondi sudalpini è costituita dalle modalità con le quali avviene il mescolamento verticale, il quale assume la sua massima estensione nel periodo nel quale la colonna d'acqua presenta le minori differenze di temperatura tra la superficie e il fondo, tra la fine dell'inverno e l'inizio dei mesi primaverili. Tuttavia, la profondità dei laghi sudalpini è tale che la completa omogeneizzazione verticale delle acque (*olomissi*) non sempre riesce a completarsi (16). Nel bacino più profondo del Lago di Garda la completa circolazione è un processo che avviene solo in coincidenza di inverni particolarmente rigidi, mentre situazioni di mescolamento verticale solo parziale (*oligomissi*) possono persistere anche per lunghi periodi.

I dati utilizzati in questo lavoro sono stati ottenuti nell'ambito dei campionamenti mensili effettuati tra il 1991 e 2000 in due stazioni (ubicate al largo di Brenzone e Bardolino) rappresentative delle condizioni dei due bacini del Garda (vedi Figura 2). Le attività sul campo comprendono la rilevazione di alcuni importanti parametri chimico-fisici sulla colonna d'acqua (temperatura, pH, conducibilità e ossigeno disciolto) mediante sonde multiparametriche e la raccolta di campioni per la determinazione dei parametri chimici e della componente fitoplanctonica. I metodi di indagine sul campo e in laboratorio sono riportati in letteratura (6, 17, 18).

Caratteristiche chimiche, fisiche e biologiche del Lago di Garda

Ossigeno, nutrienti e biomassa algale

Nella Figura 3 è riportato l'andamento delle concentrazioni di O_2 in differenti strati d'acqua del Garda dal 1996 fino al mese di agosto 2000.

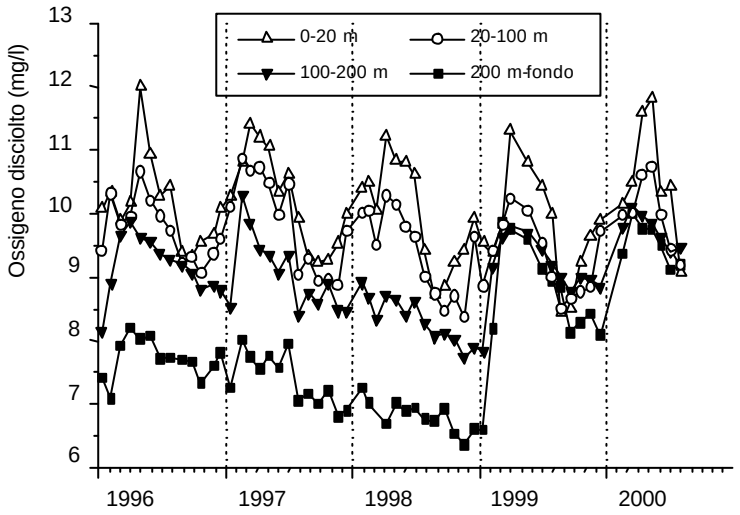

Figura 3. Ossigeno disciolto in differenti strati nel punto di massima profondità (concentrazioni medie ponderate sui volumi nella stazione di Brenzone)

Dal 1996 al 1998 la presenza di un evidente gradiente, con concentrazioni di O_2 da 200 m al fondo sempre inferiori a quelle degli strati sovrastanti, indica uno stato di isolamento delle acque ipolimnetiche profonde da quelle superficiali. Al contrario, le osservazioni condotte nei mesi primaverili del 1999 e del 2000 dimostrano la presenza di concentrazioni di ossigeno omogenee su tutta la colonna d'acqua, conseguenza di una completa circolazione delle acque.

I profili di temperatura, pH, conducibilità e ossigeno disciolto rilevati con sonde multiparametriche sin dal primo anno di indagine (1991) hanno permesso di ricostruire con un sufficiente dettaglio l'andamento della profondità dello strato rimescolato nel bacino più profondo. Nel decennio considerato sono stati individuati tre mescolamenti completi (nel 1991 oltre che nel 1999 e 2000), mentre tra il 1992 e il 1998 la circolazione ha interessato uno strato compreso tra 140 e 200 m (19).

I processi di mescolamento hanno profonde ripercussioni sulla distribuzione verticale dei nutrienti algali. La Figura 4 riporta le concentrazioni di fosforo totale rilevate tra il 1996 e il mese di luglio 2000. Negli anni nei quali il mescolamento è incompleto il fosforo totale presenta maggiori concentrazioni nelle acque profonde del lago, con valori, nel periodo considerato, compresi tra 25 e 35 µg P/l nello strato 200 m-fondo tra il 1996 e il 1998. Al contrario, negli anni di completa circolazione (1999-2000) il fosforo presenta concentrazioni omogenee in tutte le profondità campionate. Analoghi gradienti di concentrazione caratterizzano anche gli altri nutrienti algali (silicati e, in minor misura, nitrati),(19).

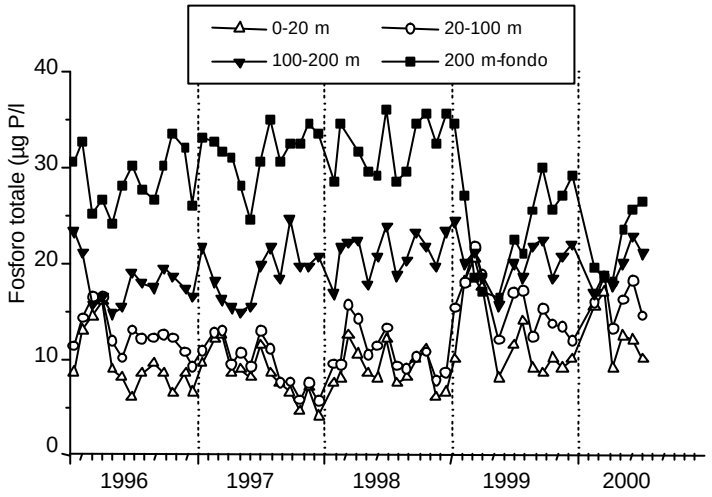

Figura 4. Fosforo totale in differenti strati nel punto di massima profondità (concentrazioni medie ponderate sui volumi nella stazione di Brenzone)

Nei laghi profondi, quali il Garda, il rifornimento primaverile di fosforo (che costituisce l'elemento limitante la crescita algale in questo bacino) rappresenta una fonte significativa di nutrienti per la crescita primaverile ed estiva del fitoplancton. Tale rifornimento, tuttavia, è massimo solo negli anni nei quali il mescolamento è completo. Nella primavera del 1999 e del 2000, le concentrazioni superficiali (0-20 m) di P-totale hanno raggiunto i loro massimi storici, con valori fino a 20 µg P/l.

Tali considerazioni possono essere verificate considerando la relazione diretta tra le concentrazioni epilimnetiche di fosforo totale alla circolazione nelle due stazioni e i volumi

lacustri interessati dal mescolamento primaverile (20). La relazione tra le due variabili, per gli anni tra il 1995 e il 2000, è altamente significativa (r=0,82; P<0,01), evidenziando l'importanza del rifornimento epilimnetico di nutrienti nei laghi profondi nel corso dei mesi più freddi.

La maggiore o minore disponibilità di fosforo negli strati eufotici, a seguito di mescolamenti totali o parziali, ha profonde ripercussioni sulla crescita annuale del fitoplancton. In entrambe le stazioni, nel periodo per il quale i dati sono disponibili (tra il 1992 e il 1999), è stata trovata una significativa correlazione tra i volumi di lago circolati nei differenti anni (i quali determinano la disponibilità di fosforo epilimnetico) e lo sviluppo algale stimato come valori medi annuali di clorofilla *a*, densità e biovolumi fitoplanctonici tra 0-21 m; più specificatamente, la profondità di mescolamento sembra avere avuto un impatto evidente soprattutto sulla crescita dei cianobatteri dominanti, in particolare *Planktothrix* (18) (vedi Figura 6a, b). Questi organismi, come abbiamo già avuto modo di notare, sono tra i primi a rispondere positivamente ad un aumento di disponibilità di fosforo. Nella prossima sezione l'evoluzione temporale dei cianobatteri dominanti sarà analizzata nel contesto dello sviluppo annuale del fitoplancton.

Evoluzione annuale del fitoplancton e cianobatteri dominanti

La Figura 5 riporta l'evoluzione temporale dei biovolumi fitoplanctonici suddivisi per classi algali nello strato epilimnetico (0-21 m) dal 1995 al 1999. I risultati si riferiscono alla stazione di Brenzone e possono essere considerati, se si escludono alcune piccole differenze nei singoli anni, rappresentativi dell'intero lago. Nel complesso, i gruppi algali dominanti nel Garda sono costituiti da Conjugatophyceae, Bacillariophyceae e cianobatteri. Altre classi algali che possono raggiungere livelli di biovolume non trascurabili, limitati però a periodi circoscritti, includono le Dinophyceae e le Chrysophyceae.

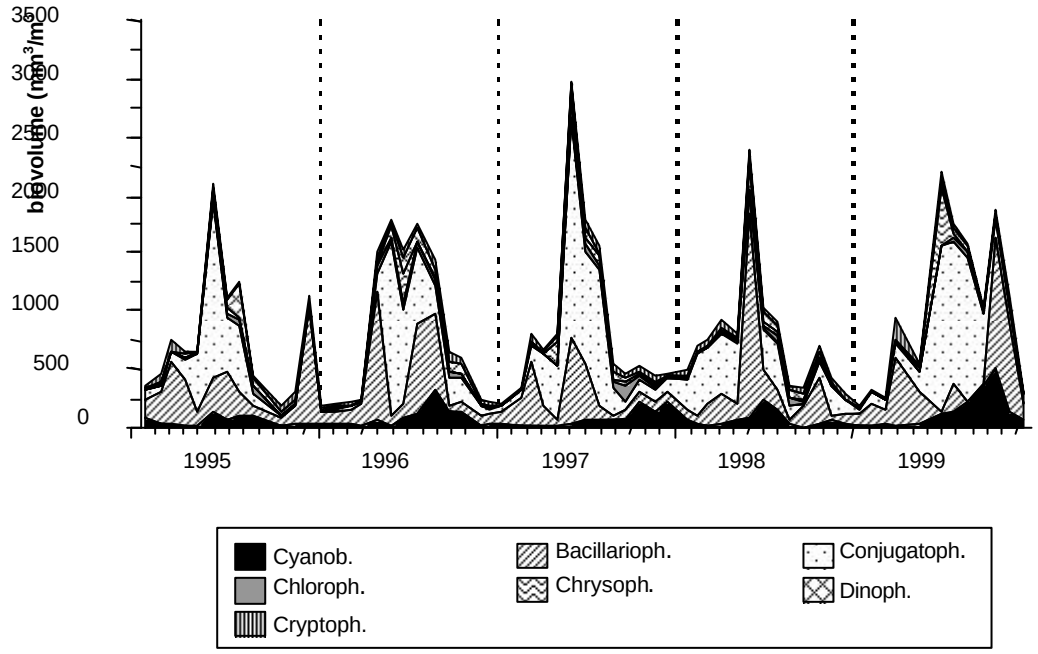

Figura 5. Variazioni temporali del biovolume suddiviso per gruppi algali nello strato tra 0-21 m nella stazione di Brenzone

I picchi di biovolume nei mesi primaverili sono dovuti allo sviluppo di grandi diatomee pennate e centriche (perlopiù *Fragilaria crotonensis*, seguita da *Aulacoseira islandica* e *A. granulata*). I mesi tardo primaverili sono caratterizzati dalla dominanza di *Mougeotia* sp.. Nei mesi estivi la comunità presenta una maggiore diversificazione, con il contributo di *Mougeotia* sp., piccole diatomee centriche (tra 4-8 µm, ascrivibili al genere *Cyclotella*), numerose Chlorococcales (in particolare *Coelastrum* spp.) e alghe verdi coccali di difficile attribuzione, Chrysophyceae (*Dinobryon sociale* e *D. divergens*), Dinophyceae (*Ceratium hirundinella*) e Chroococcales. I cianobatteri, con le Oscillatoriales, assumono una maggiore importanza soprattutto nei mesi tardo estivi e autunnali. Analisi in dettaglio delle successioni stagionali e delle specie dominanti la comunità fitoplanctonica nei due bacini del Garda sono riportate in letteratura (6, 18, 21).

La Tabella 1 riporta l'elenco dei principali cianobatteri planctonici del Lago di Garda. I differenti *taxa* sono stati inseriti sulla base dei massimi valori di biovolume rilevati nei campioni raccolti negli strati tra 0-2, 9-11 e 19-21 m nelle due stazioni di campionamento tra il 1995 e il 1999.

Tabella 1. Valori massimi di biovolume, e rispettivi picchi di densità, rilevati negli strati integrati tra 0-2, 9-11 e 19-21 m tra il 1995 e il 1999 nelle stazioni di Brenzone e Bardolino (l'elenco include solo gli organismi rinvenuti con biovolume massimo maggiore di 20 mm^3/m^3)

Organismo	Biovolume (mm^3/m^3)	Densità (cellule/ml)
Planktothrix rubescens/agardhii	710	8874
Planktolyngbya limnetica	293	29322
Anabaena lemmermannii	190	948
Limnotrichoideae indet.	151	3780
Microcystis aeruginosa	110	1740
Aphanothece spp.	90	44762
Coelosphaerium kuetzingianum	56	7016

Nella Tabella, che include solo gli organismi con picchi di biovolume maggiori di 20 mm^3/m^3, sono stati inoltre riportati anche i rispettivi picchi di densità. Sulla base dei dati ottenuti, i cianobatteri più importanti sono costituiti da Oscillatoriales (in particolare da filamenti ascrivibili al complesso *Planktothrix agardhii/rubescens*, seguiti da *Planktolyngbya limnetica*); lo sviluppo di queste due specie è limitato ai mesi autunnali o, limitatamente agli strati metalimnetici, ai mesi estivi. Altri importanti cianobatteri includono *Anabaena lemmermannii* e una ulteriore Oscillatoriales, costituita da filamenti di difficile collocazione, simili a *Limnothrix*, e per il momento ascritti pertanto alle Limnotrichoideae (22). Le rimanenti specie si sviluppano con biovolumi inferiori, e sono costituite da Chroococcales tipiche dei mesi tardo estivi o autunnali (*Microcystis aeruginosa*, *Aphanothece* spp. e *Coelosphaerium kuetzingianum*).

Le specie che possono maggiormente costituire un motivo di preoccupazione per la qualità delle acque, sia per la riconosciuta potenzialità a svilupparsi con ceppi tossici, sia per i livelli di biomassa raggiunti nel periodo considerato, sono rappresentate da *P. agardhii/rubescens* e *A. lemmermannii*. L'evoluzione temporale di questi due cianobatteri nei campioni integrati raccolti tra 0-2, 9-11 e 19-21 m nei due bacini del Garda è riportata nella Figura 6. Nei grafici si è preferito riportare i valori di densità, dato che questa variabile è quella attualmente in uso nelle diverse normative.

I filamenti di *Planktothrix* (Figura 6a, b) presentano il loro massimo sviluppo in tutti gli strati campionati nei mesi autunnali o, limitatamente agli strati metalimnetici, nei mesi estivi.

Tra il 1995 e il 1998 le densità hanno sempre presentato valori inferiori a 5000 cellule/ml, con l'esclusione di un picco metalimnetico rilevato nell'estate 1998, con valori tra 5000-7000 cellule/ml. Nel 1999 lo sviluppo di *Planktothrix* ha raggiunto i massimi livelli, con densità attorno a 6000 cellule/ml nel bacino nord (in tutti i livelli campionati) e tra 8000-9000 cellule/ml nel bacino sud (tra 0 e 10 m). Il maggior sviluppo di questa specie è avvenuto, come abbiamo visto nella sezione precedente, in coincidenza con una maggiore disponibilità di fosforo negli strati epilimnetici dopo la completa circolazione delle acque del 1999.

L'addensamento metalimnetico di *Planktothrix* nei mesi estivi, al limite inferiore della zona eufotica, è un fenomeno che è stato osservato in molti laghi (23, 24), indicando l'adattamento di questo genere a condizioni di bassa energia luminosa. In generale, come osservato da Reynolds (25), le specie ascrivibili a *Planktothrix*, *Limnothrix* e *Planktolyngbya* sono caratterizzate da elevate capacità di sviluppo in condizioni di bassa illuminazione, riuscendo a mantenere, nello stesso tempo, una densità cellulare tale da garantire un efficace controllo della posizione verticale.

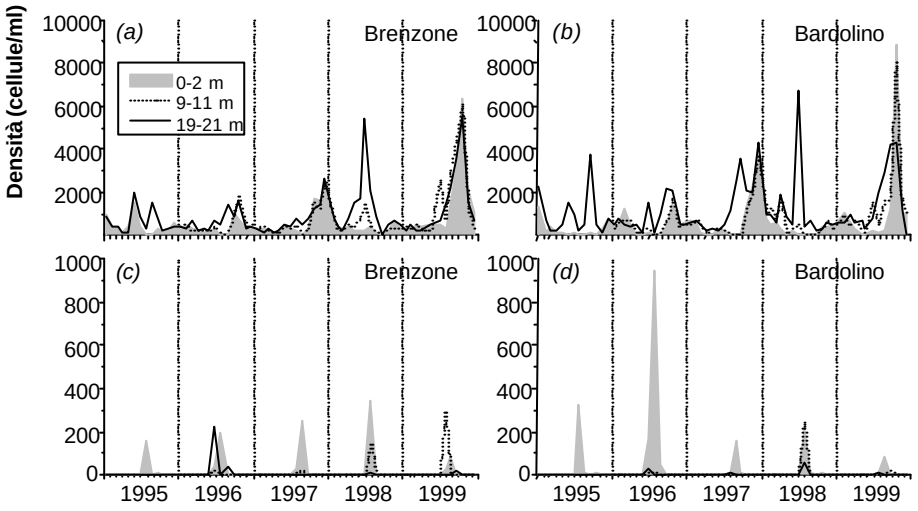

Figura 6. Variazioni temporali dei valori di densità di *Planktothrix agardhiilrubescens* (a, b) e *Anabaena lemmermannii* (c, d) nelle stazioni di Brenzone e Bardolino negli strati integrati tra 0-2, 9-11 e 19-21 m (per il 1995 non sono disponibili le analisi relative allo strato 9-11 m)

Nei campioni raccolti tra 0-2, 9-11 e 19-21 m *Anabaena lemmermannii* (Figura 6c, d) è sempre stata rilevata con densità limitate, in genere mai superiori a 300 cellule/ml, con l'eccezione di un picco di circa 900 cellule/ml rilevato nello strato 0-2 m a Bardolino nel luglio 1996. *A. lemmermannii* presenta una spiccata stagionalità, comparendo nei mesi tra luglio e settembre.

Le colonie di *Anabaena* sono state rilevate anche sotto forma di fioriture osservate sotto forma di sottili strati e strie giallo-verdi sulla superficie dell'acqua, nei primi 2 cm circa (18, 26) (Figura 7). I fenomeni sono stati osservati in particolar modo nel 1991 (luglio), 1992 (luglio e agosto), 1996 (luglio) e 2000 (luglio-settembre). Altri casi, di minore entità, sono stati segnalati nei mesi di luglio del 1993 (dal Presidio Multizonale di Prevenzione, ora Agenzia Regionale per la protezione dell'Ambiente di Verona), 1995 e 1999 e nell'agosto del 1997. I fenomeni, fino al 1999, hanno interessato perlopiù il bacino est; nell'estate 2000, per la prima volta, le fioriture

(seppur di minore entità) sono state invece osservate sull'intera superficie del lago. Prima del periodo preso in esame, nell'autunno del 1990, è stata rilevata anche una estesa fioritura di *Microcystis aeruginosa* (26).

Le fioriture sono causate da un rapido accumulo sulla superficie di popolazioni già presenti nella colonna d'acqua. Una tale localizzazione può dare una falsa impressione di abnorme sviluppo algale (27). Questa osservazione trova conferma nei sempre modesti valori di densità rilevati nei campioni raccolti di routine negli strati integrati (Figura 6c, d). Le fioriture possono svilupparsi in modo evidente sulla superficie dei laghi solo in presenza di una colonna d'acqua stratificata e stabile, condizione tipica dei mesi più caldi. In presenza di moto ondoso e di regime turbolento i meccanismi che controllano il posizionamento verticale delle cellule diventano rapidamente inefficaci. Questo requisito contribuisce a spiegare il motivo per cui le fioriture sono state osservate, con l'eccezione del 2000, solo nel bacino orientale, il più riparato dai venti, in condizioni di lago calmo e forte stratificazione termica. Al contrario, la forma allungata del bacino occidentale, incassato tra rilievi montuosi, favorisce l'azione di forti venti ed elevato moto ondoso, tali da prevenire la formazione di ammassi superficiali facilmente visibili a occhio nudo, nonostante la presenza di un sufficiente numero di cellule diluite nei primi metri d'acqua.

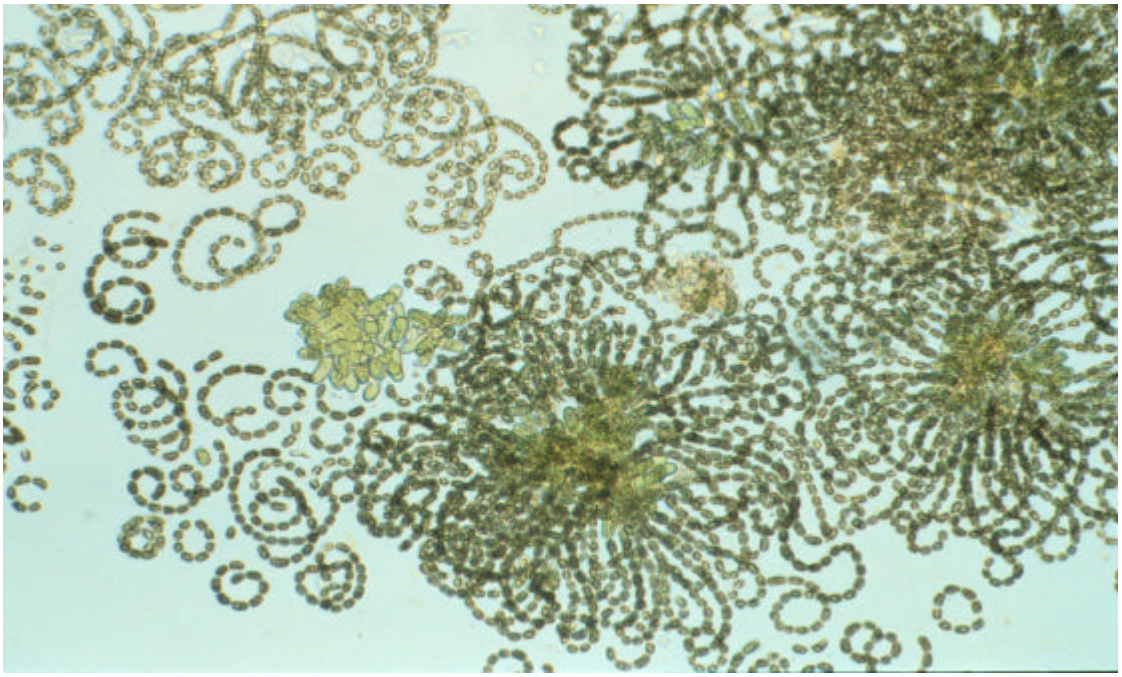

Figura 7. Lago di Garda: *Anabaena lemmermannii* (200x)
(le cellule vegetative hanno una lunghezza massima attorno a 8-10 µm;
sono ben evidenti alcuni gruppi di acineti, isolati o al centro delle colonie)

La ragione della comparsa di strie superficiali di *Anabaena* anche nel bacino occidentale nell'estate del 2000 potrebbe, stante le considerazioni precedenti, dipendere da una diminuzione della quantità di vento filato nella parte settentrionale del lago; una indagine preliminare sui dati meteorologici sembrerebbe confermare questa ipotesi. D'altra parte, nelle condizioni attuali, lo

sviluppo di *Anabaena* (e dell'intera componente algale in genere) è fortemente limitato dalle mai elevate concentrazioni di fosforo negli strati produttivi superficiali. Per questi motivi le fioriture osservate nel Garda possono essere classificate come *fioriture oligotrofe*.

Cianobatteri dominanti nei laghi profondi sudalpini

Un confronto sinottico sui cianobatteri dominanti nei principali laghi profondi sudalpini, basato sulla letteratura più recente, ha dato i risultati presentati in Tabella 2.

I differenti *taxa* sono stati selezionati sulla base dei picchi di biovolume rilevati negli strati epilimnetici (tra 0-20 m, con l'eccezione dell'Iseo, tra 0-10 m). Tali valori si riferiscono a determinazioni effettuate direttamente su campioni integrati o a valori medi calcolati da campioni discreti. Nella tabella i laghi sono stati ordinati sulla base delle concentrazioni medie di fosforo totale calcolate, per l'intera colonna d'acqua, nel periodo della massima circolazione primaverile. Lo stato trofico, dedotto dalle concentrazioni medie epilimnetiche di fosforo totale e clorofilla *a* (28), varia dall'oligo-mesotrofia dei laghi Maggiore e Garda alla meso-eutrofia del Lago di Lugano. Alcuni elevati valori di clorofilla *a* nel Maggiore sono dovuti a un intenso sviluppo di diatomee, causato da eccezionali condizioni meteorologiche, osservato nei mesi invernali del 1996 (29). Nei 5 laghi considerati, i cianobatteri, assieme alle diatomee e alle cloroficee, costituiscono uno dei gruppi più rappresentativi nell'ambito della comunità fitoplanctonica. Altri gruppi possono assumere importanza in periodi ristretti (es. le crisoficee e le dinoficee nel Garda).

Tabella 2. Nutrienti e cianobatteri dominanti nei principali laghi profondi sudalpini[a]

Nutrienti	**Maggiore o-m**	**Garda o-m**	**Como m**	**ISEO m**	**Lugano m-e**
P-totale (Epilimnio/*colonna d'acqua*) (µg/l)	10/*10*	10/*17*	16/*47*	15/*55*	≅20/*155*
Clorofilla *a* Epilimnio (Media/*max.*) (µg/l)	4,0/*12,3*	3,3/*8,7*	3,8/*6,6*	5,4/*12*	8/*15*
Gruppi dominanti (epilimnio)	Diatomee Cianob.	Diatomee Clorof. Cianob.	Diatomee Clorof. Cianob.	Diatomee Clorof. Cianob.	Diatomee Clorof. Cianob.
Cianobatteri					
Planktothrix rubescens/agardhii	+		♦	♦	♦
Planktolyngbya limnetica		+	(+)		∗
cf. Pseudanab./Limnotrich.(**)	∗		(+)		∗/♦
Chroococcales	+/∗				
Anabaena spp.		(fioriture)		(fioriture)	
Aphanizomenon flos-aquae					+/♦

[a] Lo stato trofico è indicato con o (oligotrofia), m (mesotrofia) ed e (eutrofia). I picchi di biovolume nello strato epilimnetico sono raffigurati con simboli: ♦ per > 1500 mm^3/m^3; ∗ per 500-1500 mm^3/m^3; + per 100-500 mm^3/m^3; (+) per *taxon* considerato come un importante costituente dai singoli autori, ma senza riportare valori quantitativi.

(**) Pseudanabaenoideae e Limnotrichoideae: da Anagnostidis e Komarek nel 1988 (22)

Con l'eccezione di *Aphanizomenon flos-aquae*, segnalato con numerose colonie solo nel Lugano, i differenti *taxa*/gruppi di cianobatteri riportati nella Tabella 2 hanno almeno uno specifico rappresentante in tutti i laghi considerati. D'altra parte, è possibile osservare un aumento della biomassa algale totale e una modificazione nella distribuzione quantitativa dei differenti cianobatteri all'aumentare delle concentrazioni di fosforo totale. Più specificatamente, i confronti indicano un evidente aumento dell'importanza delle Oscillatoriales (in particolare *Planktothrix*) all'aumentare dello stato trofico. Studi a lungo termine condotti nel Lago Maggiore hanno dimostrato una notevole diminuzione del biovolume totale e dei filamenti di *Planktothrix* (dominante fino alla metà degli anni '80) verso gli ultimi stadi del processo di oligotrofizzazione (29).

Le Chroococcales (costituite da numerose colonie di *Aphanothece*, ma anche da *Microcystis*), presentano una distribuzione meno interpretabile. Attualmente le fioriture di *Anabaena* sono segnalate solo nel Garda e nell'Iseo, evidenziando così la mancanza di un evidente legame con lo stato trofico. Comunque, in questi due laghi, le fioriture si sono manifestate, più che per uno sviluppo abnorme di *Anabaena*, a causa di un accumulo di colonie negli strati superficiali (per il Lago di Garda vedi pag. 52) o sotto forma di ammassi, probabilmente trasportati dal vento, lungo la costa (per il Lago di Iseo si fa riferimento a quanto espresso da Letizia Garibaldi in una comunicazione personale). La considerevole presenza di *Aphanizomenon* nel Lugano può essere interpretata, invece, come una caratteristica distintiva propria del lago profondo più eutrofico a sud delle Alpi.

Osservazioni conclusive

Nei laghi profondi il rifornimento di fosforo ipolimnetico verso gli strati eufotici costituisce una importante sorgente di nutrienti per lo sviluppo del fitoplancton.

Nel Lago di Garda, negli anni presi in considerazione, è stato possibile documentare una significativa relazione tra l'estensione del mescolamento primaverile da una parte, e concentrazioni epilimnetiche di fosforo, livelli di biomassa algale e abbondanza di cianobatteri (in particolare *Planktothrix*) dall'altra. I risultati indicano come, in linea di principio, la definizione dei fattori favorenti lo sviluppo dei cianobatteri debba necessariamente tenere conto anche delle specifiche caratteristiche – non solo trofiche, ma anche morfometriche ed idrologiche – dei corpi lacustri esaminati.

I risultati osservati sul Garda sono compatibili con i numerosi studi indicanti una tendenza verso un aumento dell'importanza del fitoplancton e dei cianobatteri all'aumentare dello stato trofico (14, 31, 32). Questi risultati trovano inoltre conferma anche considerando la distribuzione quantitativa dei cianobatteri nei grandi laghi a sud delle Alpi. In questi bacini infatti, a fronte di una composizione omogenea dei cianobatteri dominanti (seppur con significative eccezioni), è stato possibile documentare un aumento significativo delle Oscillatoriales (in particolare *Planktothrix*) all'aumentare dello stato trofico e la comparsa di *Aphanizomenon* nel Lugano, il bacino maggiormente eutrofico. Confronti di questo tipo, per un preciso complesso di laghi, divengono importanti soprattutto per quanto riguarda l'aspetto predittivo in risposta a modificazioni ambientali o a futuri scenari ipotizzabili sulla base di trend attuali.

I risultati esposti sottolineano l'urgenza di provvedere al risanamento dei grandi laghi sudalpini maggiormente compromessi (Lugano) o di invertire il trend negativo osservato per altri (in particolare Iseo e, in minore misura, Como e Garda). Gli studi limnologici svolti finora indicano in modo chiaro che, in assenza di serie misure di contenimento dei carichi di nutrienti algali, si andrà inevitabilmente incontro ad un aumento non solo della biomassa algale

complessiva, ma anche dei cianobatteri, in particolare *Planktothrix*, con conseguenze negative sulla qualità e utilizzabilità di una delle più importanti risorse d'acqua dolce in Europa.

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FIORITURE ALGALI: UN'ESPERIENZA NELLA PROVINCIA DI BRESCIA

Sergio Carasi, Carmelo Scracella, Fabrizio Speziani
ASL di Brescia, Italia

Nell'ambito della Provincia di Brescia sono ubicati tre grandi laghi (Garda, Iseo e Idro) due dei quali (Garda e Iseo) sono rispettivamente al confine delle provincie di Verona e Bergamo.

Attorno a questi laghi vive un gran numero di abitanti e la popolazione specialmente durante la stagione estiva si incrementa notevolmente (fino a 5 volte quella di partenza) soprattutto lungo le rive del lago di Garda, sicché in alcuni momenti circa 1.000.000 di persone complessivamente insite sulle sponde lacustri.

L'acqua dei laghi è impiegata per differenti scopi, ma i più importanti sono:

- turismo;
- pesca;
- riserva d'acqua per l'irrigazione di colture poste in vicinanza degli emissari (Fiumi Mincio, Chiese e Oglio);
- collegamento fra i diversi centri ubicati lungo le coste;
- approvvigionamento idrico (di solito integrativo ad altre fonti).

Nel corso degli ultimi anni l'ASL si è occupata in particolare modo della situazione del Lago di Iseo.

Esso è situato ai confini delle provincie di Brescia e Bergamo e piuttosto largo e profondo, giunge fino ad un massimo di 250 m circa. La sua acqua si ricambia completamente in un periodo stimato in 4.1 anni. Nel suo mezzo è ubicata un'isola stabilmente abitata da circa 1800 persone che vivono in un gran numero di piccoli villaggi siti su tutta la sua superficie.

L'immissario del lago (Fiume Oglio) proviene dalla Valle Camonica, anch'essa fittamente abitata soprattutto nelle zone di fondo-valle da circa 80.000 persone con molti insediamenti industriali e terreni coltivati.

Le acque usate in tutta la valle si riversano nell'immissario e solo negli ultimi anni è iniziata la loro raccolta ed il loro trattamento depurativo.

Sull'intera superficie dell'isola non esistono pozzi o sorgenti (a parte una piccola polla altamente ferruginosa e pertanto inutilizzabile a scopo umano). L'acqua è, pertanto, pompata direttamente dal lago da una presa ubicata alla profondità di 35 m.

La stessa acqua subisce un trattamento di disinfezione con biossido di cloro e con raggi UV ed è poi capillarmente distribuita alla popolazione attraverso un acquedotto che si avvale anche di cinque serbatoi.

L'ASL controlla sia l'acqua distribuita dall'acquedotto che quella del lago da cui l'acquedotto si rifornisce, anche ai fini della balneazione.

Le analisi degli ultimi anni hanno permesso di riscontrare nelle acque del lago:

- indicatori batterici di contaminazione fecale;
- *Flagilaria* spp.;
- *Creatium hirundinella*;
- *Oscillatoria rubescens*;
- *Microcystis* sp.

L'analisi chimica ha poi consentito di riscontrare nitrati alla concentrazione di 3-4 mg/l.

I medesimi microrganismi sono stati ritrovati anche nell'acqua distribuita dall'acquedotto di Montisola ad eccezione degli indicatori di contaminazione fecale eliminati dal trattamento di disinfezione.

A fronte della situazione sopra descritta, meritevole di ogni approfondimento, si è stipulato per l'anno in corso un accordo convenzionale con l'Istituto Superiore di Sanità. Obiettivi specifici di tale accordo sono:

- identificazione delle specie tossiche durante le fioriture algali
- disamina dei fattori determinanti le fioriture algali
- proposizione di correttivi da inserire in un progetto di risanamento
- assistenza all'ASL per l'istituzione nell'ambito del laboratorio di Sanità Pubblica di una struttura in grado di eseguire le analisi microbiologiche e di tossicità del caso.

In applicazione all'accordo appena illustrato, con frequenza all'incirca quindicinale, campioni di acqua potabile e di lago sono stati inviati al laboratorio dell'ISS che ha rinvenuto nelle acque del lago specie algali tossiche (*Planktothrix rubescens)* ad una concentrazione inferiore a 5.000.000 cellule/l ed anche la tossina, microcistina – RR alla concentrazione di 107 pg/mg rinvenuta all'analisi con HPLC.

Quanto sopra indicato ha indotto la Direzione dell'ASL a chiedere al Sindaco di Montisola l'adozione di primi provvedimenti a tutela della salute degli utilizzatori dell'acqua distribuita dal civico acquedotto.

Gli interventi richiesti, fra di loro alternativi si possono così riepilogare:

- collegamento dell'acquedotto di Montisola ad un altro acquedotto ubicato su una delle due coste bresciana o bergamasca in grado di fornire con una tubazione sommersa un sufficiente approvvigionamento proveniente da fonte sicura
- trattamento dell'acqua attinta dal lago attraverso le seguenti fasi:
- filtrazione primaria
- disinfezione con ozono o acido peracetico
- filtrazione secondaria
- passaggio su carboni attivi (granulari o in polvere)
- disinfezione finale con biossido di cloro
- disinfezione finale di tutta la rete con acido peracetico.

Naturalmente si è tenuto a sottolineare nel corso di incontri operativi con il Sindaco di Montisola che l'ipotesi alternativa è soggetta a costi elevati di gestione e manutenzione nel tempo.

Si è infine offerta al Sindaco di Montisola la collaborazione per incontri con la popolazione finalizzati a spiegare agli abitanti la natura e l'entità del fenomeno in discussione e si è offerta alla neo-costituita Agenzia Regionale per la Protezione Ambientale la collaborazione per la gestione dell'evento, non dimenticando che fioriture algali interessano anche altri laghi lombardi.

THE POPULATION GENETICS OF TOXIC CYANOBACTERIA

Paul K. Hayes, Gary L.A. Barker, Barbara A. Handley, Panmuk Vacharapiyasophon
School of Biological Sciences, University of Bristol, Woodland Road, Bristol, UK

The fact that cyanobacteria are able to accumulate in large numbers tells us that these organisms are extremely effective competitors within the planktonic environment. The fossil record tells us that they have been successful over many hundreds of millions of years, suggesting that they are adaptable enough to cope with shifting physical, chemical and biological conditions – i.e. they have evolved to meet the challenges of a changing environment. Competitive success on either short or geological timescales ultimately depends on interactions between the genome and the environment. On short timescales it will phenotypic plasticity resulting from changing patterns of gene expression that is important, on longer timescales it will be the evolution of new genes and of novel combinations of existing genes that will determine success or failure.

This presentation describes our attempts to unravel the genetic structure of populations of one particular toxic cyanobacterium and its ability to adapt to changing environmental conditions. What I hope that you will realize at the end, is that the genetic structure that we have observed has profound implications both for the design molecular probes for use in identifying toxic cyanobacteria and for the general applicability of findings arising from study of the physiology of these organisms in pure culture.

The population concerned is the one that develops in the Baltic Sea each summer. The most visible component of this community is comprised of gas vacuolate, filamentous diazotrophs, mainly *Nodularia* and *Aphanizomenon*, but these are by no means the only genera present: significant numbers of picocyanobacteria (< 2 μm diameter), diatoms, prymnesiophytes, dinoflagellates, cryptophytes and chlorophytes can also be found. Our work concentrates on the *Nodularia* component of these populations.

To produce a quantitative description of the structure of the *Nodularia* populations our initial experimental approach was rather traditional:

1. establish clonal isolates in culture and assign to a taxonomic group on the basis of phenotypic characters;
2. search for regions in the genome that differed between isolates i.e. polymorphic gene loci;
3. develop AS-PCR primers (Allele Specific) as a rapid means of identifying which sequence variant is present in any individual isolate.

By looking at the phenotype of the clonal isolates that we had available in culture (and some uncultured, field collected material) we found that:

1. there was considerable morphological diversity within the *Nodularia* population: this diversity took the form of variation in trichome diameter and shape (straight, coiled, supercolied).
2. there were differences in the type of gas vesicles accumulated by individual clonal isolates.

Some clonal isolates established from these mixed populations have wide (90 nm diameter), weak gas vesicles, collapsing at a pressure of about 0.5 MPa, whereas others have narrow (65 nm diameter), strong gas vesicles, collapsing at a pressure of about 1.0 MPa. Largely based on these differences in gas vesicle morphology/strength, we could assign each of our cultured

isolates to one of two taxa: *N. spumigena* (weak/wide gas vesicles) and *N. litorea* (strong/narrow gas vesicles).

Initial attempts to characterise genetic variability between Baltic Sea *Nodularia* isolates involved the analysis of sequence variation in three largely non-coding regions of the genome: PC-IGS (phycocyanin intergenic spacer), rDNA-ITS (rDNA internal transcribed spacer) and *gvpA*-IGS (*gvpA* intergenic spacer). The PC-IGS could be used to divide the isolates into three groups as could the rDNA-ITS; the *gvpA*-IGS produced 2 groups of isolates. What we see with just this small number of isolates is that there is no absolute relationship between phenotypic characters, such as gas vesicle strength, and any of the genotypic characters examined. From this we are forced to conclude that *N. spumigena* and *N. litorea* are not distinct species: this is more or less confirmed by the 16S-rDNA sequence which is identical in strains BC Nod-9402, -9408 and –9427.

What is more there is some indication that there is horizontal exchange of genetic information between individuals within the population. This suggests is that we do not have a population of clonal isolates with purely vertical inheritance, i.e. no reassortment of genetic information, but rather a population where genes can be exchanged between lineages, i.e. a population where there is something akin to sexual reproduction. However, the number of isolates included in this analysis was very small: we need to look at many hundreds of isolates to get a meaningful description of the population structure.

For this larger-scale analysis we developed a PCR-based approach to distinguish between the different sequence variants (alleles) at each of the three gene loci.

a. The PC-IGS, i.e. the non-coding spacer between *cpcB* and *cpcA*. The region that we look at is just over 500 bp in length of which about 5% of nucleotide positions are variable. The three sequences types at this locus fall into two main sequence clusters. Fortunately the base differences responsible for the split into these two clusters are concentrated in two regions. This meant that we were able to design two primers, one specific for each sequence class. When used in combination with a common upstream primer these allele specific PCR primers allow us to tell which sequence variant is present in any *Nodularia* isolate from the length of the PCR product. This means that we can assign any isolate to the appropriate sequence cluster (allele) without the need to perform sequence analysis. There is some additional variation within one cluster (I/II) (about 1% of bases differ between type I and type II) but this cannot be resolved using this methodology.

b. The rDNA-ITS, i.e. the ribosomal internal transcribed spacer. *Nodularia*, like numerous other cyanobacteria, has multiple rRNA operons. Initial attempts to amplify the rDNA-ITS resulted in the production of two products that differed in size by about 300 bp. In our population analysis we have concentrated on just the smaller of these two products. There is far less sequence variation at this locus than at the PC-IGS with only 1.1% of nucleotide positions showing any variability. However, the variable nucleotides are clustered in just one part of the sequence, so we have been able to design AS-PCR primers that can distinguish between the different rDNA-ITS alleles. This requires the use of two separate PCR reactions: isolates are assigned to each of three classes on the basis of the presence or absence of products in each of the reactions. The fact that some isolates yield products with both AS-primers tells us that they have two non-identical copies of the smaller rDNA locus.

c. The *gvpA*-IGS. Like many gas-vacuolate organisms *Nodularia* has two copies of the gene encoding the main gas vesicle protein, *gvpA* and these are separated by a non-coding spacer region. Based on the length of this spacer region (they differ through the presence or absence of a 96 bp region) isolates can be assigned to one of two genotypes.

Using this PCR approach to distinguish between alleles we can determine a multilocus genotype for any individual isolate without recourse to sequencing, restriction analysis etc.

When we use this technique on a larger collection of cultured isolates (86 in total) we get much more convincing evidence that we are dealing with a single population within which there is extensive genetic recombination: 9 of the 12 possible genotypes are present.

To get a statistically meaningful description of the population structure we need to look at many more than 86 isolates. Using this method we can collect the genetic data very easily, the problem that we have is with culture establishment.

Firstly it is difficult to maintain large numbers of clonal isolates. Secondly there is a potential problem relating to the low success rate in clone establishment – it may be that the few filaments that survive the transition into culture are not representative of the population as a whole.

To address both problems we have developed a technique that allows us to use individual trichomes picked directly from the natural population to provide the template for multiple PCR reactions. In this way we can get multi-locus genotypes for large numbers of individual population members without the need to resort to culturing.

Our first large scale population analysis was in the summer of 1998 when we took part in two cruises and collected 500 individual filaments from each of 20 sampling locations over most of the central Baltic basin over a total period of about 3 weeks.

Of the filaments collected we have performed our PCR based analysis on just over 4000. Of these 56% gave results at all three loci, 38% at one or two loci and 6% failed to yield any products.

Why did 44% of filaments fail to give results? This question is impossible to answer, but it needs to be stressed that we are working very close to the lower limit in terms of the amount of template available for amplification: in some cases the DNA template comes from just 20 or so cells and this is divided into several different reactions. For those filaments that failed to give a full set of products it is possible that there was just too little template available for amplification.

The following analysis is based on the results obtained from the 2336 filaments that yielded data for all three loci. Overall the results show that all 12 combinations of alleles at the three loci tested are present in the population, albeit at very different frequencies. The most parsimonious explanation for this observation is that there is horizontal exchange of genetic information between individuals within the population. To go beyond this rather obvious statement requires the calculation of various population genetic parameters. The one that we have concentrated on thus far is the "Index of Association", I_A.

This method of analysis involves calculating a genetic distance for every pairwise combination of individuals within the population. I_A is calculated from the ratio of the variance associated with the observed distribution of genetic distances between individuals and the variance that would be expected if the population was panmictic, i.e. freely recombining.

$$I_A = (V_{obs}/V_{exp}) - 1$$

For this 1998 population of *Nodularia* the value of I_A that we calculate is 0.2. If there was no linkage between any of our three loci in the population then we would expect I_A to be 0. What

we need to know is does our value of 0.2 differ significantly from 0 – if it does then we have some degree of linkage between loci, although it can't be complete linkage because all possible genotypes are present. If it doesn't then we have a population in panmixis i.e. there is free, unconstrained horizontal exchange of genetic information. To calculate the confidence interval on this I_A value we used a Monte Carlo simulation to generate multiple data sets (500) for a panmictic population with the same overall allelic composition as our original set. For each of these simulated data sets we then calculated I_A. When we do this we get a normally distributed set of I_A values (-0.0575 to 0.0586) – clearly our value of 0.2 for the real population lies way outside of this distribution.

This tells us that although we have gene transfer within the population it is not unconstrained i.e. there is some linkage between particular loci. It needs to be said that it is possible that the large preponderance of one genotype influences the calculation, but we do not think that this accounts for the significant deviation of I_A from zero.

We can calculate the frequencies of each genotype that we would expect in a panmictic population from the overall allelic composition. If we do this then it seems that there is some linkage between *gvpA* and the rDNA-ITS: the combination *gvpA* type II and ITS type I is over-represented in the real population whereas the combination *gvpA* type I and ITS type I is slightly under-represented. At this stage it is not clear why this linkage exists. Both of the regions that we are dealing with are non-coding and it is therefore unlikely that they are subject to selective pressure. However, it is possible that each of these loci is closely linked to genes that are under strong selective pressure.

The calculations that we have looked at so far tell us a little about the structure of the population as a whole, but we can go a lot further with the information and look at the population structure at each sampling location. What is clear is that the population structure does vary across the area sampled, e.g. the most common genotype (9) accounts for anywhere between 59 and 99 % of the population. These differences in population structure are significant.

Taken as a snapshot in time these data could be telling us that there are differences in the relative fitness of the different genotypes and that the outcome of the competition between them produces a different result in different locations. What we need to know is what factors influence this final outcome, but to date we have been unable to link the success of any on genotype with any particular set of conditions within the water column.

What we have done is to calculate a parameter called "gene diversity" at each sampling location: this is just a simple measure of total genetic diversity within each sample. We can then calculate a genetic distance between each pair of sampling locations across the Baltic and look at the way in which this genetic distance changes as a function of the physical separation between the samples. We initially thought that there would be a positive correlation between these two distances, i.e. the genetic distance would increase as the physical distance between sampling locations increased. No such relationship was found, but interestingly what can be seen here is that for the most distant sampling locations the genetic distance between them is always low.

This led us to plot the "gene diversity" for each individual sampling location against the distance of that location from the population centre. When this is done there is a significant negative correlation – samples located at the extremes of the area sampled have lower genetic diversity than those at its centre. This would be expected from work on many other populations if the limits of our sampling reflect the limits of the population distribution at that time.

In addition to looking at spatial heterogeneity we have just started to get enough information to look at the way the population changes in time. If we compare the relative abundance of the twelve different genotypes for 1998 and 1999 then what we find is that although the same two

genotypes still dominate the population (9 & 3 = 91% in 1998 and 79% in 1999) there is a far flatter distribution in 1999 than in 1998. Again we do not have an explanation for this observed change and we can not distinguish between what might be due to true differences between successive years and what might result from looking at populations at different stages in their annual development. Although on this latter point it should be noted that the samples were collected at the same time of year in both cases.

In conclusion, we can state that:

1. monospecific populations of *Nodularia* are not clonal.
2. populations are spatially and temporally heterogeneous.
3. as for other bacterial groups we now have a strong indication that horizontal exchange of genetic information does occur within cyanobacterial populations.

In relation to the last point, what we don't know is how that exchange takes place, but our guess is that it involves viral transduction or transformation by small fragments of naked DNA. The other alternative is conjugation, but as a result of some very recent work we do not think that this mechanism of gene transfer is important.

It is now worth considering what these findings mean when it comes to the study of toxin production and for the development of molecular probes that are supposed to indicate the presence of toxin producers within the water column.

There have, and continue to be many studies where the aim is to elucidate the relationship between particular environmental variables and the production/accumulation of toxins within cells. What I have in mind are studies that look at the influence of light or nutrient availability on toxin concentrations. In an effort to ensure that such results can be interpreted reliably it is essential to work with clonal, and preferably axenic cultures. But as we have shown natural populations are not clonal. By examining just three gene loci we find that there are 12 different genotypes present in the population. There are many more than three polymorphic gene loci, there are probably hundreds, which means that there are many thousands of different genotypes within the population. Each of these different genotypes will differ in the details of their physiology. So even if they are all toxin producers, differences in the genetic background against which toxin production occurs will almost certainly mean that the kinetics of toxin production under any given set of conditions will vary amongst individuals. What this means is that the details of what one finds in culture experiments may not be universally applicable, especially if only a single clonal isolate has been examined. This may have implications when attempts are made to devise management strategies that will limit the growth of toxin producers.

To give a simplistic illustration of this, it may be found that in culture a particular *Nodularia* strain fails to accumulate nodularin if the available phosphate concentration is kept below 0.3 mg l^{-1}. This finding may form the basis of a management strategy that essentially comprises reducing phosphate concentrations to below 0.3 mg l^{-1} as a means of ensuring no nodularin accumulation within the water column. Such a strategy could be a waste of time if in reality the threshold concentration varies significantly amongst a diverse range of *Nodularia* genotypes.

There is a large amount of gene exchange taking place between members of the Baltic Sea *Nodularia* population. Our evidence is that this exchange seems to involve DNA from con-specifics, but whole genome sequencing studies show that segments of DNA can move between widely separated taxa. Because of this gene exchange it is not possible to be certain that if organism has DNA sequence X that it will also have sequence Y – i.e. there is no absolute link between gene loci. To put this in slightly more specific terms you cannot be absolutely confident that because a *Nodularia* filament has an rDNA-ITS type that is identical to that in a non-toxic cultured strain that it too will be non-toxic. The take home message from this is basically if you want to know whether cells in your population contain the gene encoding the nodularin peptide synthase, then probe for that specific gene. Even this may not tell you

everything that you need to know – it is not the presence of the gene that is the problem it is the expression of the gene that is important. Therefore it may be better to test cells/filaments/colonies for the presence of the toxin and not just the gene.

This presentation has been based on what we have found for populations of *Nodularia* in the Baltic Sea. Are all cyanobacterial populations like this? The answer is that we do not really know, as there have been no other comparable studies. Information that is available in the literature suggests that this model with spatial heterogeneity, horizontal gene exchange and epidemic growth of particular genotypes will be widely applicable.

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IMAGE ANALYSIS FOR QUALITATIVE AND QUANTITATIVE EVALUATION OF PLANKTIC CYANOBACTERIA

Patrizia Albertano
Department of Biology, University of Rome "Tor Vergata", Rome, Italy

Introduction

Cyanobacterial blooms are widespread around the world, mostly in freshwater and brackish environments. Several species of cyanobacteria produce toxins and the increasing number of reports on the associated health risks have resulted in an increased level of awareness. Monitoring of cyanobacteria, particularly in rivers, lakes and reservoirs has become critical for the use of waters for domestic supply, agri- and aquaculture and recreation (1) (see also the papers by Carmichael and Falconer in this volume). Detailed data recording on the cyanobacterial species causing blooms is, therefore, necessary to define the extent of their occurrence and to understand the dynamics that lead to their development. In recent years, the requirement of automated methods to quantify and identify phytoplankton species has been increasing and computer mediated image analysis has been applied for the enumeration of chroococcal and filamentous cyanobacteria as well as of cells within aggregates.

Although microscopical measurements are still considered by some authors to provide the most suitable estimates of linear dimension and taxonomic resolution (2, 3), microscopical image-analysis for rapid microbial cell size and abundance estimation has started to be largely employed (4-6).

Cyanobacteria present in the smallest phytoplankton fraction have been studied using image analysis coupled to epifluorescence (EIA) in order to investigate cell morphometry and to assess the distribution of populations characterised by different cell size (7). Picocyanobacteria with cell diameter lower than 2.0 μm are mostly present in the samples as individual cells, but might form aggregates in which numerous cells are held together by a mucilage. These aggregates as well as colonial species are usually a problem for conventional cell count methods because of the small cell dimensions and the difficulty in distinguishing cyanobacterial cells within the exopolymeric matrix. Nevertheless, the use of EIA has provided the means for the enumeration and analysis of this phytoplankton component that is usually neglected or underestimated by a variety of methods, including combined systems (8), the use of parametric discriminants (9, 10), electronic particle counting (11) and flow cytometry (12, 13).

Up to date, the quantification of filamentous species has always been problematic and only few works focussed on length determination and distribution of filamentous cyanobacteria as the method based only on epifluorescence microscopy for the measurement of total length of straight filaments of *Oscillatoria* (*Planktothrix*) *rubescens* and *Anabaena flos-aquae* (14) or the use of a digitising tablet, combined with a computer and a mouse (15).

Recently, a new approach has been developed to determine the biovolume of individual taxa of filamentous cyanobacteria present in mixed net samples (16). By this method it has been possible to discriminate and quantify the presence of diazotrophic, heterocystous trichomes of *Anabaena, Aphanizomenon* and *Nodularia*. The data allowed to evaluate the variation in

biomass of filamentous cyanobacteria in the euphotic zone, at different sampling time and in relation to hydrographical condition.

Experimental approach

Epifluorescence microscopy is a critical tool for discriminating cyanobacteria in mixed-taxa samples. In addition to chlorophyll *a*, all cyanobacteria possess phycobiliproteins as accessory photosynthetic pigments. The blue-coloured phycocyanin and the red coloured phycoerythrin are responsible for the characteristic natural pigmentation of cyanobacteria. Both these pigments autofluoresce under green light excitation by typically emitting bright orange or red fluorescence from the whole cell, while the photosynthetic pigments of the other phytoplankters do not. In addition, due to the prokaryotic nature of cyanobacteria, in which the photosynthetic membranes holding the pigments are spread in the cytoplasm, the phycobiliprotein autofluorescence allows to detect shape and dimension of intact cells except for the external exopolymeric envelopes, i.e. capsule and sheath. Thus it is possible to evaluate at a first glance not only the presence but also the morphology of cyanobacteria in natural mixed samples.

Field collections

Samples can be collected along transects using plankton nets and bottles according to any standard procedure. Aliquots of the samples are immediately fixed in glutaraldehyde, previously diluted in filtered seawater or freshwater, to a final concentration of 2.5% (v/v), kept in tubes or bottles at 4°C and transferred to the laboratory. All samples have to be processed within 5 weeks from collection in order to prevent fluorescence loss.

Filtration

In the laboratory, aliquots of fixed samples are diluted in water in order to avoid excess of material and filtered onto 0.45 or 0.2 µ pore-sized, 25 mm diameter, Nucleopore filters, under vacuum below 50 mm Hg.

Epifluorescence microscopy-image recording

Membrane filters, mounted in immersion oil, are immediately observed under epifluorescent microscope provided with a 25x, 40x and 100x objectives, 50 or 100 W mercury lamp and a green filter set (excitation wavelength 546 nm, cut-off of 580 nm). For each sample, epifluorescence images from 30 non-overlapping optical fields (located on a zed line), were transferred, after enhancing the brightness on dark background, to a high definition grey level camera (sensitivity range 1/105 lux) connected to a PC. The brightness setting is maintained constant throughout the image acquisition, using the video camera facility *Gain*, for gain level, to standardise the procedure. Images are acquired, by means of Image ProPlus facility *Acquire/Video-Digital* and stored as .tif files (Figure 1). When aggregates or coiled cyanobacterial filaments (Figures 1b and 1d) are found on the fields of view, extra images are recorded to ensure reliability of the records.

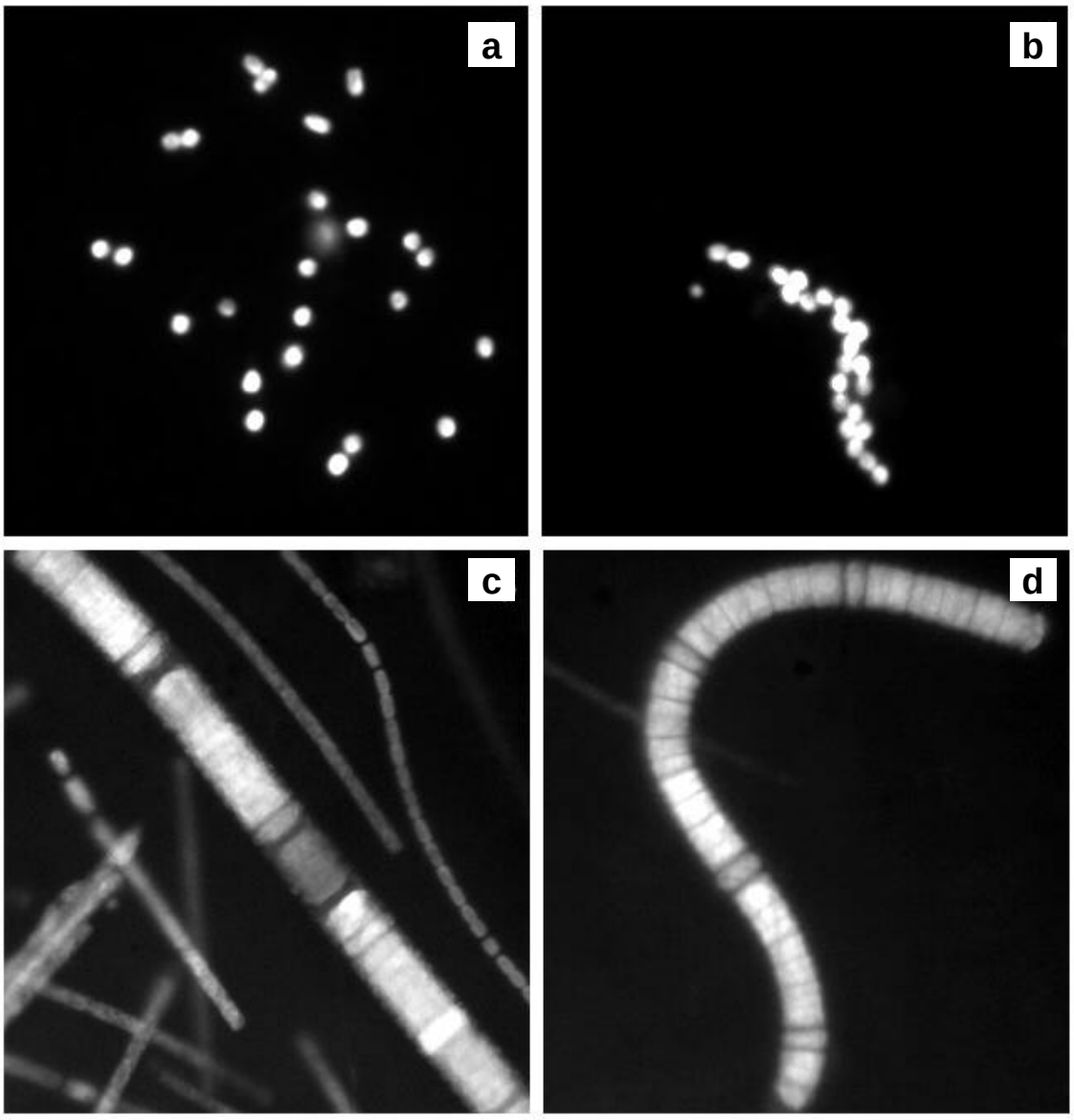

Figure 1. Epifluorescence recorded images of Baltic cyanobacteria: a) picocyanobacteria of the *Synechococcus* and/or *Cyanobium* group; b) aggregate of picocyanobacterial cells held together by a mucilaginous matrix (not visible in epifluorescence); c) Mixed population of filamentous cyanobacteria: *Nodularia* (diagonally in the center), *Anabaena* (on the right) and *Aphanizomenon* (on the left); d) slightly curved trichome of *Nodularia*

Image elaboration

Image analysis of one set of images consists in the selection of useful parameters as minimum and maximum diameter, area, roundness index, etc. that will allow to distinguish between cyanobacterial types. For instance, minimum and maximum diameter are defined, by

largely used softwares, as the length of the shortest and longest line joining two points of the object's outline and passing through the centroid. To convert directly the software measurements into microns, a calibration procedure is run before the start of the analysis. In case of picocyanobacterial populations, a fixed range for the minimum diameter is fixed between 2.3 and 0 μm in order to exclude from the analysis any object exceeding the maximum cell diameter of phototrophic picoplankton. When aggregates are present, the aggregate area is divided by the average area of cells present in the same optical field of view in order to allow enumeration and biovolume calculation of the aggregate-forming cells. For filamentous cyanobacteria, the procedure includes manual interactions and mathematical corrections, that are required during the elaboration process to overcome filament coiling and overlapping on the membrane filters (16). In the case of curved, coiled or supercoiled filaments, the recorded images of trichomes are splitted in order to obtain, sections as straight as possible. Overlapping areas of cyanobacterial filaments, that appeared brighter because of the autofluorescence enhancement, are outlined by drawing parallel lines, noted and added to the final calculation of the biovolume. Crossing and overlapping of filaments can be initially reduced by diluting samples before filtration. Spurious highlighted objects are removed. In the end, all data are eventually saved as .xls files.

Biovolume determination

Biovolume determination for chroococcal cyanobacteria is obtained using the formula for calculating the volume of a sphere, a sphaeroid or a cylinder with hemisphaerical ends (7, 17) For filamentous cyanobacteria the biovolume is calculated using the formula for the volume of a cylinder and by recording, as diameter and height, the values of minimum and maximum diameter, respectively, measured by the PC for every trichome or section of trichome in the image (16).

The biovolume value is calculated for each image and summed to the others of the same sample in order to estimate the total biomass due to cyanobacterial taxa. Conversion into carbon (C) biomass is done assuming a fixed amount of carbon per cell or μm^3 of cell volume for picocyanobacteria and filamentous cyanobacteria, respectively (18, 19).

Two case studies from the Baltic Sea

Picocyanobacteria

EIA analysis of picoplanktic unicellular cyanobacteria collected in the Central Baltic Sea in different summer periods showed that the total number of cells, at the various stations and depths, was different during the time and between the sampling sites (7). It was possible to recognise 4 different types of aggregates, that were present in the pico-fraction mainly at certain stations.

The results of morphometrical evaluation of picocyanobacteria revealed that within the Baltic population three size classes and cell shapes were distinguishable and which of them was the most frequent. Statistical analysis of the whole data set allowed to describe horizontal and vertical distribution of the morphologically different types of picocyanobacteria and to assess their contribution in terms of photosynthetic biomass using a conversion factor of 0.294 pg C $cell^{-1}$.

Filamentous cyanobacteria

For the image analysis of diazotrophic heterocystous cyanobacteria collected at a drift station in the Central Baltic Sea, the EIA computer-based procedure for the estimation of filament biovolume was developed to solve the problems arising from the presence of coiled, curved and overlapping trichomes of *Nodularia*, the fluorescence patchiness of *Aphanizomenon* and the presence of cell wall constrictions in *Anabaena*. The sequence of the operations required is described in details by Congestri *et al.* (6). On the basis of the measured morphometrical parameters of the virtually split parts of trichomes, the biovolume of individual genera was calculated using a conversion factor of 0.11 pg C per μm^3 of cell volume. The results were used to evaluate the fluctuation of the diazotrophic cyanobacterial biomass over three diel cycles along three water layers. In addition, statistical elaboration of all measurements obtained by EIA analysis evidenced a unimodal frequency distribution of trichome diameters of *Anabaena* and *Aphanizomenon*, but a multimodal distribution for *Nodularia* filaments. Variability in trichome morphometry within natural populations of *Nodularia* could be, therefore, further analysed in order to assess the possibility to distinguish different morphotypes.

Conclusions and perspectives

The EIA analysis method allows biovolume estimation of chroococcal and filamentous cyanobacteria and the discrimination of different genera and types in mixed natural samples. The results so far obtained have shown the accuracy and practicality of the method that allows to store large sets of measurements and a precise biovolume evaluation. In particular, the database of all the elaborated images can be statistically analysed in order to identify different "morphometrical types" within the cyanobacterial populations and to assess their distribution and fluctuation in the water mass in relation to any environmental parameter. In addition, this technique offers more advantages being less time consuming than optical microscopy, namely avoiding the use of micrometer and decreasing the operator errors. Moreover, the method is safer because it reduces the operators exposure to fixatives.

Up to date, EIA analysis has been applied to study the smallest non-toxic chroococcal cyanobacteria and some toxin-producing filamentous cyanobacteria. A further application to colonial chroococcal cyanobacteria as *Microcystis* spp. might be very convenient to monitor these widespread epatotoxin producers, the morphological inter- and intraspecific variability of which makes their taxonomical classification and biomass quantification rather difficult.

Finally, the use of fluorochromes, in addition to the natural fluorescence of phycobiliproteins, could further expand the application of EIA analysis of cyanobacteria. Fluorescent probes specific for gene sequences or nucleic acids or proteins and enzymes or sugar polymers can offer the mean of using multiple markers for the identification of taxa as well as of structural and functional properties of cyanobacterial cells.

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HEALTH EFFECTS OF TOXIN PRODUCING CYANOBACTERIA: "THE CYANOHABS"

Wayne W. Carmichael
Wright State University, Department of Biological Sciences, Dayton, Ohio, USA

Introduction

Toxic cyanobacteria poisonings (CTPs) are responsible for almost all known cases of fresh and brackish water intoxication involving phycotoxins (1, 2). Toxic water blooms can be found in many eutrophic to hypereutrophic lakes, ponds and rivers throughout the world and are responsible for sporadic but recurrent episodes of wild and domestic animal illness and death. They are also implicated in human poisonings from certain municipal and recreational water supplies. A timeline of studies for CTPs is given in Table 1.

The primary types of toxicosis include acute hepatotoxicosis, peracute neurotoxicosis, gastrointestinal disturbances, and respiratory and allergic reactions. It is not known whether the latter toxicoses are caused by the hepato- or neurotoxic agents or by other chemical groups. It has been suggested, but so far unproved, that lipopolysaccharide (LPS) endotoxins are involved with some gastrointestinal disturbances. Known poisoning incidents have been summarized by several authors (2-4).

Types of toxic cyanobacteria

Toxic cyanobacteria species display a remarkable diversity of forms. The unicellular cyanophycean forms have spherical ovoid or cylindrical cells that reproduce by binary fission. They occur single celled, or may aggregate into irregular colonies. The morphology of the colony will vary with the environmental growth factors. A filamentous morphology is typical for a number of cyanophyceans. Repeated cell divisions--occurring in a single plane at right angles to the main axis of the filament – give rise to a multicellular structure called a trichome. Variations in trichome morphology are prominent – straight, coiled, etc. Specialized features include differentiation of certain cells into heterocysts (nitrogen-fixing structures) and akinetes (resting structures).

Cell shape and cell size play a special role in the classification of cyanophyceans. Due to the reproductive patterns, the diameter of a cell is a more stable property than its length. Morphometric relations provide important data for distinguishing species or strains. The present known toxigenic cyanobacteria include about 40 species (2, 5, 6). Table 2 is a listing of known cyanotoxin producers.

Not all toxigenic species or toxic water blooms will be toxic at all times. Many cyanobacterial blooms are apparently not hazardous to animals. This can be due to: low concentrations of toxin within strains and species comprising the water bloom; low biomass concentration of the water bloom; variation in animal species sensitivity; amount consumed by the animal; age and sex of the animal; and amount of other food in the animal's gut. Since toxic and non-toxic blooms of the same species can be found, it is not always possible to attribute clinical responses to the presence of a bloom of a toxigenic species.

Table 1. Dates and events in toxic cyanobacteria research

Date	Event
1978	Poisonous australian lake
1949-60	Toxic waterbloom report, Minnesota
1958-64	Isolation and culture of toxic cyanobacterial clones
1964,1968	International conference: ▪ Algae and Man, 1964; ▪ Algae and the Environment, 1968
1971-80	Papers by a number of authors on: ▪ *flos-aquae* neurotoxin production; ▪ *flos-aquae* production of several neurotoxins ; ▪ world-wide occurrence of toxic *M. aeruginosa*; ▪ *Aph. flos-aquae* neurotoxin production; ▪ gastroenteritis outbreaks in human due to cyanobacteria; ▪ characterization of LSP endotoxin from cyanobacteria
1980	International conference on toxic cyanobacterial proceedings, The Water Environment, Algal Toxin and Health, 1981 Dayton, Ohio USA
1981-86	▪ Continuing cases of animal deaths; human gastroenteritis and contact irritation ▪ Research programs on toxic blue-green algae in Norway, Scotland, Germany, Finland, S. Africa, Australia, Japan, U.S.S.R., China and India ▪ Structure of microcystin determined by D. Botes and colleagues(CSIRO, Pretoria, RSA) ▪ Inclusion of toxic cyanobacterial research in several international symposia, especially IUPAC and IST
1987-92	▪ Continuing cases of animal deaths, human contact irritation and gastroenteritis ▪ Association of cyanotoxins in human drinking water supplies with hepatoenteritis and primary liver cancer ▪ Structure determination of numerous microcystins and identification of nodularin ▪ Structure determination of anatoxin-a(s) and cylindrospermopsin
1993-94	Organization of International Symposia in Bath, United Kingdom, and a workshop in Adelaide, Australia. **First exclusive cyanotoxin conference.**
1995	International Conference on Toxic Cyanobacteria, Ronne, Bornholm,Denmark,August 20-24
1996	First confirmed human fatalities: cyanotoxins, including microcystins and cylindrospermopsins; Caruaru, Brazil
1998	▪ International Conference on Toxic Cyanobacteria, Beaufort, North Carolina, USA, Sept. 27- 1 Oct. ▪ US EPA lists Cyanobacteria and Cyanotoxins as a Research Priority on the Drinking Water Candidate Contaminant List (Federal Register March 1998)
2000	Number of Peer Reviewed Publications on Toxic Cyanobacteria Exceeds 2200

Factors influencing formation and toxicity of toxic cyanobacteria blooms include:

- Genetic-There are distinct biotoxin and non-biotoxin producing strains.
- Growth-Factors that lead to good growth (as measured by chlorophyll) lead to optimum toxin production.
- Toxicity of a freshwater population (bloom) depends on the ratio of toxin producers to non-toxin producers and the factors which lead to high biomass - surface scums - (bioaccumulation is not a significant factor).
- Toxicity of a marine population (bloom) depends less on high biomass and more on bioaccumulation factors to compensate for the greater mixing in marine waters.

Table 2. Name and producer organism for the cyanotoxins

Name	Produced by
Neurotoxins	
Anatoxin-a	*Anabaena, Aphanizomenon*,
Homo-Anatoxin-a	*Oscillatoria (Planktothrix)*
Anatoxin-a(s)	*Anabaena, Oscillatoria (Planktothrix)*
Paralytic Shellfish Poisons (Saxitoxins)	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Liver toxins	
Cylindrospermopsin	*Aphanizomenon, Cylindrospermopsis,Umezakia*
Microcystins	*Anabaena, Aphanocapsa, Hapalosiphon, Nostoc, Microcystis, Oscillatoria (Planktothrix)*
Nodularins	*Nodularia* (brackish water)
Contact irritant-dermal toxins	
Debromoaplysiatoxin, Lyngbyatoxin, Aplysiatoxin	*Lyngbya* (marine) *Schizothrix* (marine)

Structure and activity of cyanotoxins

Microcystins and nodularins: the cyclic-peptide hepatotoxins

Most poisoning by cyanobacteria involves acute hepatotoxicosis caused by a structurally similar group of small molecular weight cyclic hepta- and penta- peptides referred to as microcystins (MCYSTs) and nodularins (NODLNs) (7). Sixty five MCYST variants are now described (2 ,8-12). MCYST's are also produced by *Microcystis viridis* (13), *Anabaena flos-aquae* (14), *Oscillatoria agardhii* (15), *Nostoc* sp. (16), *Aphanocapsa cumulus* (17) and *Oscillatoria tenuis* (11). These cyclic heptapeptides are composed of five common amino acids with most variations occurring within a pair of L-amino acids. Desmethyl derivatives have been reported (18), in which methyl groups of *N*-methyldehydroalanine and *N*-methyl aspartic acid are replaced by hydrogen atoms. Of the peptide toxin-producing genera, *Microcystis* is the main genus found world-wide, and of the three toxic species identified to date, i.e. *M. aeruginosa, M. viridis,* and *M. wesenbergii*, only *M. aeruginosa* has been used in toxicosis studies. The clinical events can be outlined (19) as a sequence leading to animal deaths. First, the toxin is released from the cyanobacterial cells in the stomach and taken up preferentially in those areas of the intestine, i.e. the ileum, which have higher levels of bile acid carriers, causing injury to the intestinal cells. Second, the hepatocytes, which in all animals affected are the primary target cells, concentrate the toxin again via bile acid carriers. Third, hepatotoxin-induced alterations of the actin microfilaments and intermediate filaments in the cell's cytoskeleton lead to changes in the cell shape and loss of cell to cell adhesion. Finally, with the loss of the cell architecture and destruction of the parenchymal cells and sinusoids of the liver, lethal intrahepatic hemorrhage (within minutes or hours) or hepatic insufficiency (within several hours to a few days) occurs.

In general, therapies for algal toxicosis in livestock have not been investigated in detail. The most likely agents to be of some benefit are powdered charcoal and cholestyramine. Although cholestyramine is more effective, activated charcoal is more readily available and less

expensive. Cholestyramine has been reported successful in treating certain *Pfiesteria* Estuarine Associated Syndrome (PEAS) cases, where unidentified toxins produced by *Pfiesteria piscicida* or other similar organisms may be the etiological agent of illness (20). Therapeutic support measures in poisoned animals might also include administration of whole blood and glucose solutions. Certain chemicals have also been used experimentally to reduce MCYST hepatotoxicity in laboratory animals. These include cyclosporin-A, rifampin, and silymarin. These antagonists have been most successful when given before or administered along with the toxin. These antagonists may affect MCYST toxicity through inhibition of toxin uptake by the hepatocyte. These types of therapies have not been investigated for the other cyanotoxins and represents a needed area for future research.

Cylindrospermopsins: the alkaloid hepatotoxins

The most recent cyanobacterial taxa to have its cyanotoxins chemically characterized is the heterocystous *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju. It was first implicated in a harmful algal bloom event that occurred in Solomon Dam, Palm Island, North Queensland of Australia in 1979 that led to severe hepatoenteritis to 138 people (21,22). The structure of cylindrospermopsin (CYN) (molecular weight=416 M+H) was elucidated by Ohtani *et al.* (23). Since then CYN has been found in *Umezakia natans* (24) and *Aphanizomenon ovalisporum* (25).

Beginning about 1995 *C. raciborski* was identified in eutrophic Florida lakes (26). Isolation of this species and examination of its toxin from certain Florida strains has revealed that this species is also producing CYN in at least some Florida lakes (27)

Anatoxins and paralytic shellfish poisons: the neurotoxins

Produced by strains of *Anabaena* and *Oscillatoria (Planktothrix)*, the alkaloid neurotoxin anatoxin-a (antx-a) is a potent post-synaptic depolarizing neuromuscular blocking agent. This toxin causes death within minutes to a few hours depending on the species, the amount of toxin ingested, and the amount of food in the stomach. Clinical signs of antx-a poisoning follow a progression of muscle fasciculations, decreased movement, abdominal breathing, cyanosis, convulsions and death. In addition, opisthotonos (rigid "s"-shaped neck) is observed in avian species. In smaller laboratory animals, death is often preceded by leaping movements, while in field cases, larger animals collapse and sudden death is observed. No known therapy exists for antx-a, although respiratory support may allow sufficient time for detoxification to occur followed by recovery of respiratory control. Recent dog poisonings in Scotland, UK were shown to be due to consumption of *Oscillatoria* containing antx-a.

Another neurotoxicosis involving cyanobacteria is from the cholinesterase inhibitor termed anatoxin-a(s) (antx-a(s)), where (s) means salivation factor. Antx-a(s) is a guanidinium methyl phosphate ester (molecular weight 252). Antx-a(s) is very toxic (i.p. mouse LD50 20 μg/kg) but is somewhat unstable and becomes inactivated with elevated temperatures (>40 ^{o}C) and under alkaline conditions. Toxicosis associated with antx-a(s) has been observed in the field in cases involving dogs, pigs and geese. Clinical signs of antx-a(s) toxicosis in pigs include hypersalivation, mucoid nasal discharge, tremors and fasciculations, antaxia, diarrhea, and recumbency. In ducks, the same symptoms occur, plus regurgitation of algae, dilation of cutaneous vessels in the webbed feet, wing and leg paresis, opisthotonos and clonic seizures prior to death. Clinical signs in mice include lacrimation, viscous mucoid hypersalivation, urination, defecation, and death from respiratory arrest. Rats exhibit the same clinical signs plus

chromodacryorrhea (red-pigmented "bloody" tears). At the LD50, survival times are 5-30 minutes.

Some strains of *Aphanizomenon,* so far found only in the state of New Hampshire, produce the potent paralytic shellfish poisons (PSP) saxitoxin and neosaxitoxin (28). Recent research in Australia has shown the widespread occurrence of saxitoxins and related neurotoxins in blooms of *Anabaena circinalis* in rivers and water storage reservoirs. The very extensive water bloom of *Anabaena* in the Darling River in 1990 which caused deaths of cattle and sheep was found to be neurotoxic, and the toxins identified as sodium-channel blocking saxitoxins and other paralytic shellfish poisoning compounds (29). In addition, it is now known that the freshwater mat-forming cyanobacteria *Lyngbya wollei* can produce these neurotoxins. This cyanobacterium is found in several lakes and reservoirs of the southern and south central United States (28,30). Most recently *Cylindrospermopsis* from Brazil has been shown to produce PSP (31). These sodium channel blocking agents inhibit transmission of nervous impulses and lead to death by respiratory arrest.

The evidence for human intoxications by cyanobacteria

As a general statement, acute lethal toxicosis in humans from cyanotoxins should not occur. Only in those cases where accidental ingestion of a heavy water bloom occurs should a lethal dose of toxins be ingested by humans. Normal filtration, coagulation and other treatment processes in municipal water supplies should remove toxic cells and released toxins to levels below that necessary to cause acute lethal effects. However in cases of heavy cyanobacteria blooms and where the normal water treatment process is inadequate or not properly operated, toxic cells and free toxins have been present in finished drinking water supplies (32). There are reports, for the USA and Australia, that cyanotoxins have been implicated in human illness (i.e. acute non-lethal or chronic toxicity) from municipal water supplies, especially after the water bloom has been treated by copper sulfate to lyse the cells and release more of the toxins into the distribution system. In these and other cases involving accidental ingestion, the symptoms reported include abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia and elevated liver enzymes in the serum (especially gamma-glutamyl transferase) (2). Although in most of these cases, the cyanobacteria involved and sometimes the toxins involved have been identified, the levels of toxin associated with illness have not be established in any of the outbreaks. With the newer more sensitive methods of detection available, especially for the hepatotoxins, it is now possible to conduct epidemiological studies that can evaluate and set the risk for microcystins and other cyanotoxins.

The only confirmed route of exposure for acute human toxicity from cyanotoxins is from dialyses water used in a medical facility. The recreational use of lakes and rivers (oral and dermal route), the consumption of drinking water and algal health food tablets (oral route) would be the second most common route of exposure-although no confirmed fatalities are known by this route. In addition, a minor route of exposure is from the use of showers (inhalation). Yearly duration of exposure is shorter in those countries (i.e. U.S. and Canada) where the water bloom growth season is shorter (3-5 months) compared to those with milder climates such as Australia and South Africa (6-10 months).

The confirmed outbreak of cyanotoxin human toxicosis occurred at a dialysis center (termed Clinic A) in Caruaru, Brazil (8o 17' S, 35o 58' W), located 134 km from Recife the state capital of Pernambuco. At Clinic A 116(89%) of 130 patients experienced visual disturbances, nausea and vomiting following routine hemodialysis treatment between February 13-20, 1996.

Subsequently 100 patients developed acute liver failure and of these 70 died. As of October 1997, 53 of the deaths could be attributed to a common syndrome now called "Caruaru Syndrome". This syndrome included:

a. symptoms: painful huge enlargement of the liver, jaundice and a bleeding diathesis manifested by hemorrhagic spots, bleeding from the nose and methrorrhagia,
b. laboratory picture: elevated transaminases, variable hyperbilirubinemia, prolonged prothrombin time and severe hypertrigliceridemia,
c. histopathology: light microscopy – disruption of liver plates, liver cell deformity, necrosis, apoptosis, cholestasis, cytoplasmic vacuolization, mixed leukocyte infiltration and multinucleated hepatocytes; electron microscopy – intracellular edema, mitochondrial changes, rough and smooth endoplasmic reticulum injuries, lipid vacuoles and residual bodies (33).

This case received a large amount of media and public health authority attention in Brazil and has been reported in many countries. The history of this case and summary of the epidemiology has been published (34, 35). Newly published information on this outbreak provides evidence for the cyanotoxins present and the cyanobacteria that produced them (36)

Risk associated with consumption of drinking water

Toxic cyanobacteria are now recognized as the group of HAB organisms that are primarily responsible for HAB related events in fresh and brackish waters. HABs are now being accorded a priority for assessment of health risk that is almost equivalent to other major environmental events such as:

- endocrine disrupter chemicals;
- persistent bioaccumlating toxicants;
- ambient ozone changes;
- global atmospheric changes;
- health & integrity of coastal environments

However, it is difficult to assign reliable estimates of health risk from cyanotoxin exposure since risk requires an estimate of the expected frequency of undesirable effects caused by exposure to these toxicants. Estimation of risk involves establishing relationships between actual exposures and the expected response. While research is slowly accumulating that will give suitable numbers for estimating risk from toxic cyanobacteria, the term risk should be used in discussion of cyanotoxins only when quantities of toxic cells are available, warranting use of the term risk. Otherwise, the term hazard should be used to designate a general threat from exposure to cyanotoxins.

The most likely route for humans to be exposed to cyanotoxins is via drinking water, medical dialyses, recreational waters or consumption of cyanobacterial health food products (37). For a discussion of human risk and exposure to cyanotoxins see references (2, 4, 38-40).

Another important factor in assessing risk of the cyanotoxins, especially the hepatotoxins is that the microcystins and nodularins are potent tumor promoters through their inhibition of protein phosphatases. This added risk factor (i.e. tumor promotion) can now be factored into calculations of risk, along with data obtained from animal exposures and other toxicological and chemical data. Indirect evidence supporting tumor promotion and human liver cancers from MCYST exposure comes from the studies of Yu (41) in China.

Monitoring of cyanobacteria blooms

Cyanobacterial waterblooms can present an intermittent or continuous problem in water supplies. In addition to toxicity these blooms can cause taste and odor episodes in drinking and recreational waters. The blooms, especially when they accumulate on the water surface, are often easily visible making it possible to visually (or even by remote satellite sensing) monitor them.

Several US and Australian states, and some European and Asian countries have organized and advertised an "algae watch" to monitor waterbloom formation. In Australia the New South Wales Blue-green Algae Task Force (42) have adopted a three-level alert system based on blue-green algal cell counts in water. Level 1 is 500-2000 cells ml/1, when water authorities are alerted and sampling increased. Level 2 is 2000-15,000 cells ml/1, when toxicity testing is carried out, water filtration plant operators advised to take precautionary action, agriculture agencies advised. Level 3 is above 15,000 cells ml/1 of toxic blue-green algae in a persistent bloom. In this situation if activated carbon is not available the water may be declared unsafe for human consumption.

A number of tools are available for managing recreation in situations where toxic blooms are likely to occur. These include:

- the placement of permanent warning signs in situations where blooms are known to regularly recur;
- the erection of temporary warning signs during known bloom events;
- the placement of permanent signs with event-specific information;
- newspaper and media announcements;
- closure of specific facilities;
- policing of recreational restrictions/warnings;
- restriction of public access (closure of the waterbody);
- relocation of recreational facilities to areas with lower potential for bloom development/accumulation.

Currently the only international effort to establish guidelines for acceptable levels of cyanotoxins in water supplies and for assessing the risk of toxic cyanobacteria in recreational or drinking water supplies has been the World Health Organization (2, 43).

To date several countries, including Australia, Brazil, Canada, Germany and Great Britain, are moving to establish no-adverse or maximum acceptable levels for MCYSTs in drinking water supplies (Table 3).

Several other countries are reviewing their local situations with regards to CTPs, with a view towards beginning the establishment of MACs for certain cyanotoxins.

To date no guidelines are being developed for the cyanobacterial neurotoxins because they do not appear to pose the same degree of risk from chronic toxicity, including tumor promotion, as the MCYSTs, NODLNs and CYNs do.

However, it is very likely as more areas experience problems from toxic cyanobacteria, and human as well as animal exposure to cyanotoxins becomes better documented it will necessary to develop guidelines for exposure, risk assessment, risk management as well as the management of cyanobacteria water blooms.

Table 3. Summary of cyanotoxins, producer organism, chemical and toxicological characteristics, health guidelines and water treatment procedures

Cyanotoxin & producer organism	Chemical/toxicological characteristics	Health guidelines/ water treatment
Anatoxins *Anabaena* *Aphanizomenon*[1] *Planktothrix (Oscillatoria)*	***Anatoxin-a*** Secondary amine alkaloid, one natural analog homoanatoxin-a. Neurotoxic by depolarizing acetylcholine receptors, especially nicotinic, leading to respiratory arrest. ***Anatoxin-a(s)*** Organophosphate neurotoxic by inhibiting breakdown of acetylcholine (anticholinesterase).	No official guidelines but anatoxin-a has a guidelines value of 3µg/l Watershed management, carbon (PAC, GAC) chlorine, ozone have not been evaluated
Saxitoxins *Aphanizomenon* *Anabaena* *Cylindrospermopsis* *Planktothrix (Oscillatoria)* *Lyngbya*	Also known as PSP's includes the following sulfate and nonsulfated carbamoyl alkaloids: saxitoxin, neosaxitoxin, C-toxins and gonyautoxins. Neurotoxic by inhibition of Na channel conductance.	No official guidelines, but Australia is considering 3 µg STX-eq/l be used based upon data from shellfish toxicity. This toxin level could be associated with cell counts of 20,000 cells/ml. Watershed management, filtration, carbon (GAC, PAC) boiling, ozone (chlorine less effective)
Cylindrospermopsins *Cylindrospermopsis* *Umezakia* *Aphanizomenon*	Cyclic guanidine alkaloid cytotoxin included one analog, deoxycylindrospermopsin predominately a hepatotoxin but also cytotoxic to kidney, spleen, thymus, heart and eye. Mechanism of action unknown, but does inhibit protein synthesis. Bioactivation may occur. Some evidence for covalent binding to DNA (genotoxic)	No official guidelines, but Australia is considering 1 and 15 µg/l (1.5 ng/l if genotoxic) as a guidelines value. Watershed management, filtration[2], chlorine, ozone, carbon (GAC, PAC)
Microcystins *Microcystis* *Anabaena* *Planktothrix (Oscillatoria)* *Nostoc* *Anabaenopsis* *Hapalosiphon* Certain cyanobacterial picoplankton	Family of 65 cyclic heptapeptides. Molecular weight from 909-1037. Main one is microcystin-LR (leucine/arginine). Liver toxic by inhibition of protein phosphatases. Tumor promoter.	MAC WHO 1.0 µg/l Australia 1.3 µg/l Canada 0.5 µg/l[3] New Zealand 1 µg/l 0.1 µg/l[4] Oregon, USA 1 mg/g[5] Watershed management, chlorine, filtration, ozone, carbon (PAC, GAC)[6]
Nodularins *Nodularia*	Family of 3 cyclic pentapeptides. Molecular weight about 800. Liver toxic by mechanism similar to microcystins. Brackish water occurrence	Since it is a brackish water organism, no MAC has been set although presence should be regarded as a health risk. Watershed management, filtration, chlorine, ozone, carbon (PAC, GAC)

[1] can produce antx-a but not known to produce antx-a(s)
[2] compared with other cyanotoxins, cylindrospermopsin can be found free in water even when cells are healthy
[3] based upon a 60 kg person consuming 1.5 l/day
[4] tumor promotion factor
[5] algae health food products (Gilroy *et al.* 2000) (37)
[6] cell lysis promotes toxin release and distribution

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WHY AND HOW TO FIGHT CYANOBACTERIA

Nicola Pacini (a), David Harper (b)
(a) Ministero dell'Ambiente, Rome, Italy
(b) Leicester University, UK

Cyanobacterial blooms are a common problem impairing the quality of water resources worldwide. Whether fresh, brackish or saline, many waterbodies on earth witness Cyanobacteria playing an influential role in plankton dynamics.

In lakes, Cyanobacterial blooms tend to form under conditions of high nutrient loading, nonetheless Cyanobacteria are also common in oligotrophic waters and successfully compete with other phytoplankters for low nutrient supplies. Some of them are able to withstand extreme habitat conditions (high temperature, high salinity, high pH) under which algae are out-competed. Beside waterbodies, Cyanobacteria are able to develop on the surface of soils, on the bark of trees, on moist rocky substrates such as in waterfalls, on urban monuments and virtually any habitat providing light and moisture.

Although Cyanobacteria are among the most ancient forms of life on the planet and attracted the curiosity of biologists relatively early in the history of science, their taxonomic classification remains uncertain. Since Cyanobacteria assemble characters common to both prokaryotic and eukaryotic organisms, they have been located in a separate phylum. Thus, strictly speaking, Cyanobacteria are separate from *Algae* and should not be referred to as "Cyanophytes"; a usually accepted common name is "bluegreens".

It is only during the last 20 years that new experiments revealed the potential of cyanotoxins in affecting the biotic interactions between bluegreens and other aquatic organisms as well as their effects onto higher vertebrates including mankind. In temperate regions where waterbodies are under the long-term impact of anthropogenic eutrophication, the presence of toxic Cyanobacteria is common. A National River Authority survey conducted in the United Kingdom in 1989 (1), revealed that the 87% of 915 waterbodies sampled had Cyanobacteria as the dominant phytoplankton group. Sixty-eight percent of the waterbodies tested for the presence of cyanotoxins gave positive results (2).

Methods to control Cyanobacterial development are necessary to protect the quality of freshwater resources allowing their multiple potential uses and to preserve healthy food-chains in lakes preventing the negative effects of eutrophication.

General characteristics explaining the competitive advantage of bluegreens

Among the phytoplankton, bluegreens are generally slow-growing, large sized "K"-strategists that owe their competitive advantage over other phytoplankters primarily to their adaptability to low light environments (3). They are able to grow under low irradiances (competitive advantage in well-mixed water bodies) but can also migrate actively through the water column by means of "aerotopes" (gas vesicles) and position themselves within water-layers characterized by optimal nutrient and light availability (competitive advantage in stratified water bodies).

Bluegreens are very sensitive to temperature due to their low growth rates and many species are slow in adapting to changing environments. Thus, they tend to slowly build up high populations after more "r"-selected taxa have waxed and vaned. In northern temperate regions it is common to observe the establishment of the first cyanobacterial colonies in summer following a spring diatom bloom. Their growth performance is thought to depend on the availability of nutrients not consumed by previous phytoplankton growth; this factor explains the common appearance of Cyanobacteria in eutrophic systems (4).

With the progression of summer, as temperature rises and nutrients become depleted, bluegreens strengthen their competitive advantage. They are more efficient in growing under high biomass densities due to their adaptation to lower light levels. They preferentially utilise CO_2 as a carbon source and are more efficient than other phytoplankton taxa at obtaining it at low CO_2 concentrations; a condition which prevails at the elevated pH often reached in lakes, in summer, as a result of intense photosynthesis. Bluegreens may also obtain CO_2 directly from the atmospheric pool. Buoyancy regulation mechanisms allow them to float at the water surface and replenish in atmospheric carbon. Protection from light damage at the water surface is provided by reverse migration (over a nycthemeral cycle) and by the development of carotenoid pigments which protect them from UV damage and enhance photosynthetic efficiency. Once bluegreen populations have reached high biomass, they effectively shade out other phytoplankters, exert allelopathic effects upon their competitors, take advantage of their low sedimentation rates, and become increasingly difficult for zooplankton to graze extensively.

The formation of akinetes by many Cyanobacteria of the order of the Nostocales such as *Anabaena* and *Nostoc*, enables bluegreens to survive unfavourable habitat conditions. Akinetes are thick wall reproductive structures which can be deposited in lake sediments and constitute real "seed banks" able to germinate even if the water body undergoes partial desiccation (5).

Not only do Cyanobacteria show a remarkable adaptability to a wide range of environmental conditions, recent studies have highlighted their ability in conditioning the physico-chemical and biological characteristics of the waterbody under their dominance to the enhancement of their competitive advantage. By contributing to pH increase by means of their high photosynthetic activity, by lowering the total irradiance due to the formation of dense colonies and scums at the surface, by increasing water column stability and stratification due to the formation of dense layers of colonies and aggregates positioned at the water surface or at the lower boundary of the photic layer (exploiting optimal light and nutrient gradients), by fostering hypolimnetic anoxia due to their strengthening of water column stability, bluegreens promote a series of positive feed-back mechanisms which tend to boost the progressive eutrophication of the waterbody culminating with cyanobacterial blooms.

Cyanobacterial communities tend to create self-regulating populations (6) characterized by a high degree of resilience that prevent ecosystem recovery to lower biomass levels. Recovery is delayed in stratified lakes where bluegreens tend to accumulate at the clear, nutrient rich metalimnion (7). In these lakes eutrophication recovery needs a higher reduction in nutrient loading than in fully mixed wetlands were bluegreens do not gain the competitive advantage of being able to exploit the deeper, nutrient rich layers.

Most Cyanobacteria are capable of storing large amounts of phosphorus to be used at times of scarcity. The stored phosphate enables them to perform up to 4 cell divisions significantly increasing the biomass even after total depletion of the available phosphorus pool. Also for this reason, although dissolved phosphate is commonly the nutrient limiting phytoplankton growth, it is not possible to predict potential growth from in-lake phosphate availability (8).

Reductions in nutrient loading tend to decrease cyanobacterial biomass, however responses are quite unpredictable. Restoration projects aimed at reducing blooms may in fact strengthen the dominance of those cyanobacterial groups adapted to low nutrient levels, particularly if

nutrient reduction causes N limitation which contributes to advantage nitrogen fixers. Some bluegreen groups form differentiates cells, called "heterocytes" (9), capable of fixing atmospheric nitrogen. However, it has been documented that, under favourable conditions, even species without specialized cells are able to fix nitrogen.

Due to their unpalatability, their toxicity to a wide range of other life forms and their specific property of impairing zooplankton grazing by clogging filtering organs, Cyanobacteria typically tend to create trophic "bottle-necks" by re-circulating nutrients within the phytoplankton and microbial loops rather than sustaining high trophic levels. Nonetheless equilibria seem to switch over at some point along the trophic gradient; observations conducted in 210 hypereutrophic Danish lakes suggest that cyanobacterial dominance is less prominent in shallow water bodies under high phosphorus loading where they can be replaced by faster growing chlorophytes (10).

Strategies for the control of bluegreens

Nutrient supply management

External nutrient load reduction may not completely resolve cyanobacterial blooms but it certainly does contribute to the reduction of the potential phytoplankton standing stock and by enhancing chances of success during the application of other remedial measures. In order to plan effective nutrient input reductions aimed at a decrease in phytoplankton biomass, it is necessary to undertake an investigation of the environmental parameters (light, nutrients, temperature) which may limit growth potential during different times of the year and particularly at those stages of the yearly succession which later develop bluegreen dominance.

High nutrient loads favour cyanobacterial blooms as these, being generally adapted to low light intensities, are capable of growing under conditions of high standing crop and are therefore competitively advantaged by eutrophy. Phosphorus is the commonest limiting nutrient in inland waters, while nitrogen is commonly limiting in catchments rich in mineral P sources and deprived of the human impacts which cause high N leaching (in particular animal husbandry). While a large number of Cyanobacteria such as *Anabaena* and *Aphanizomenon*, is able to fix gaseous nitrogen, the process seems to be impeded under conditions of high turbidity as it is characterized by high energy requirements. Carbon may become limiting under hypertrophic conditions such as in Czech fertilized fish ponds and in wetlands under high nitrogen loading due to bacterial denitrification processes.

External load reduction will not show its effects on the phytoplankton biomass before threshold levels for nutrient limitation are reached. A rough guide to the relationship between P levels and phytoplankton biomass can be found in the Vollenweider/OECD regressions (11). However, the observed behaviour of a single water body may evade these relationships due to particular conditions which do not fit the general characteristics of the lakes used in dressing the OECD regressions and to delays in the response to nutrient and load reductions. Resilience effects are due to:

- the *phosphate buffer system* by which sediments, through adsorption/desorption equilibria, tend to replenish the in-lake available phosphate pool even when external load decreases;
- the inherent stability and resilience of the dominant phytoplankton community that will naturally tend to oppose shifts in biomass and composition by a series of biotic adaptations to lesser nutrient availability (use of stored P, strengthening competition, production of exozymes capable of scavenging P from particulate matter, production of long-term resistance forms, vertical migration through the water column).

As a result of biotic and abiotic mechanisms sustaining the internal resilience of the phytoplankton community, observed successful recoveries tend to proceed in a stepwise pattern across sequential equilibrium states rather than in a linear manner.

Mixing

Much has been written on the details of the techniques and on the effects of artificial mixing, a solution which has had large scale applications in lake management. Here, only mixing techniques aiming at the direct disruption of cyanobacterial blooms will be addressed. For a broader perspective the reader is referred to specific literature (8, 12, 13).

Vertical mixing has been employed to reduce phytoplankton abundance by increasing the time spent by photosynthesising cells below the compensation depth. Water bodies characterized with a total depth greater than the double of the euphotic depth are well suited (1). Oscillatoriales such as *Planktotrix agardhi* and *P. redekei*, are slow to adapt to changing light environments and can be effectively eliminated by mixing provided that $Z_{eu}<Z_{mix}$ (euphotic depth < depth of the mixed layer) so as to realize an effective reduction in photosynthetic rates (14).

Due their natural adaptation to low irradiance, in shallow lakes, where $Z_{eu}\sim Z_{mix}$, continuous mixing may not be effective in reducing Cyanobacteria, instead these measures will reduce the performance of algae. Intermittent mixing with one to two-week cycles was found to give better results than continuous mixing, in these waterbodies, as bluegreens were slow in adapting to changing conditions. At the onset of stable conditions they would rapidly move to the surface by effect of their vacuoles whose buoyancy had increased as an adaptation to mixing. Here the filaments lysed as a consequence of abruptly entering a high-irradiance environment (15). In relation to other phytoplankters, bluegreens are generally more sensitive to sudden exposure to high irradiances (7).

Other case studies showed that, when intermittent mixing was applied, such permanently changing conditions in the end favoured colonial *Microcystis* which can adapt to this form of light regime and successfully control its migration through the water column to realize optimal light and nutrient conditions (16). In particular *Microcystis*, *Anabaena* and *Aphanizomenon* have been found able to adapt to short term diel light fluctuations (6, 17). Recent observations suggest however, that adaptation to mixing could be more effective in green algae than in *Microcystis* and that the competitive advantage of the latter would be rather due to its unpalatability for grazers and its low sedimentation rate (18).

A mesocosm study in the 'Lund tubes' in Blelham Tarn gave encouraging results under 3-4 week periods of mixing/stratification although the authors questioned the applicability of the technique to large reservoirs and lakes (19). They concluded that low levels of mixing may be more cost efficient as they result in similar conditions by just preventing stratification but do undergo stability fluctuations imposed by weather changes. In the 1980s, insufficient mixing in Rutland Water, a large artificial reservoir in lowland England, occurred during summer anticyclones and caused variable conditions which prevented dominance by bloom-forming taxa and kept chlorophyll levels below potential capacity as calculated from nutrient loads (20). In 1989 however, the occurrence of a series of hot summers increased water column stability in Rutland Water with heavy blooms of Cyanobacteria (1).

A general problem with mixing techniques occurs due to the difficulty of mixing the entire cyanobacterial population of a water body during bloom conditions. In the Biesbosch reservoirs (The Netherlands), a layer of *Microcystis* colonies was observed to remain in the upper water surface undisturbed by water column mixing because of its high buoyancy (21). It is thus clear that successful mixing requires the application of very thorough destratification, or creation of

some other conditions preventing surface scum development. In a detailed study on the physiological responses of *Microcystis* to turbulence and stratification, Visser (22) suggests that continuous, powerful mixing can overcome dominant bloom-forming populations.

Flushing

Short retention times, <10-30 days, (4) appear to be effective in reducing dominance of slow-growing, large, inedible bluegreens, such as *Aphanizomenon* and *Oscillatoria*. Beside the direct export from the waterbody of cyanobacterial colonies, flushing has the advantage of disrupting thermal stratification and reducing cell growth by entrainment into deeper, less illuminated water layers.

For this process to occur efficiently it is recommended to determine optimal flushing frequency in relation to cell growth and flotation speed by measuring such parameters directly from the phytoplankton community residing within the waterbody of interest (23). At still shorter retention times, the beneficial consequences of flushing may engender a negative feedback due to the effects of wash-out on zooplankton population biomass and growth with a consequent loss of top-down control by grazing (24).

Flushing is an effective technique which affects directly the growth of phytoplankton and does not bear the negative impacts of chemical treatment or high equipment costs such as in the case of forced aeration.

However this technique is seldom applicable due to the scarcity of pristine water sources which would flush a lake without increasing the in-lake phosphate concentration. Even when an appropriate source of water can be found, the impact of flushing on water bodies located further downstream may reduce the opportunity of the whole operation.

Although not legal in most European countries, flushing with water containing high CO_2, has been considered as a possible control mechanism in the US and Germany for several decades. Field-scale experiments are under way at the University of Dresden to test the possibility of decreasing Cyanobacteria which are dominant under conditions of high pH and CO_2 depletion, by flushing the epilimnion with CO_2-rich water from the hypolimnion, within the same lake (Benndorf, pers. comm.).

The design of these experiments is based on the observation of natural collapses of *Microcystis* during summer storms causing sudden vertical mixing which bring CO_2 to the surface (25).

Biophysical approaches

Gas vesicle collapse constitutes a more specific approach. Ultrasonic radiation has been shown to be successful in bursting gas vesicles in the laboratory and could by implemented on a large scale (3).

A method designed by Clarke and Walsby (26) to be operated at sewage works, demonstrated the crushing of gas vesicles by circulating water through a pipe sunk deep into the ground. An analogous principle could be applied to a small lake where water would circulate through a pipe at one end and would be put back into the lake at the other end (3).

Explosives provide a fast but drastic method for inducing the Cyanobacteria to sink out but are unlikely to find wide acceptance due to side effects upon recreational users and upon fish, killed by the bursting of swim bladders.

Mechanical removal

Under conditions of massive bluegreen scums it may be desirable to use booms of the type employed to collect oil spills. The units can be dragged by boat and the surface pumped off (1). This temporary remedy however does not prevent further formation of the scum.

Particular techniques had to be designed to eradicate benthic Cyanobacteria from the Biesbosch reservoirs in the Netherlands. High levels of geosmin, a substance produced by benthic Oscillatoriales, were measured in the water column giving strong odour and taste effects. SCUBA-diver investigations revealed the presence of a narrow band of benthic bluegreens (27). A harrow attached to heavy chains was designed to disturb the sediment and prevent growth. The device resulted nearly as effective as copper sulphate addition without the lethal impact on non target benthic organisms. However, disturbance of the top sediment layer is not recommended as a long term strategy as it tends to loosen sediment-water gradients in phosphate concentration bringing nutrient rich interstitial solutions into contact with the water column.

If properly designed, interventions on bottom sediments such as dredging and sediment pumping, can at one time, remove benthic Cyanobacteria, decrease internal P loads, remove contaminated sediments, increase depth below the reach of photosynthetic radiation and clear damped rubbish. The cost of sediment removal often prevents the use of such management techniques to large lakes; the smaller Norfolk Broads (England) are a case in point (28). In large lakes it is necessary to carry out a detailed investigation of sediment composition and of the phosphorus adsorbing capacity of different sediment layers to ensure efficiency in the reduction of internal loading. Dredging activity does cause some sediment resuspension but its effects can be contained. In the design of suction dredgers higher priority should be given to the precision of removing targeted sediment layers than to pumping capacity (29).

The general problem of pumping excessive water faced by conventional dredgers which require the construction of extensive settling ponds was eventually solved by the design of new highly automated sledge suction dredgers able to remove sediment with a high dry matter content. Similar dredgers were designed in Sweden, built under cooperation by Czech and Swedish firms and operated for the first time in the Vajgar fish pond in southern Czech Republic. Accurate positioning of the suction nozzles was obtained by laser technology (30) after the clearance of living roots from the lake bottom by specific techniques and machinery. Details regarding sediment removal operations are cited by Björk (29) who conducted one of the first successful sediment removal experiment at Lake Trummen (Sweden) obtaining an immediate improvement in lake phosphorus concentrations and algal biomass (31, 32).

Chemical treatment

No chemical addition aimed at a direct impairment of phytoplankton blooms by means of interference with their physiology, by direct coagulation or by reduction by dissolved phosphate through precipitation, can be considered without effects on non target biota. The high dosages and the specialised equipment needed for the applications render chemical additions costly and therefore applicable only to small scale waterbodies such as wetlands and in particular small artificial reservoirs which offer the added advantage of tight hydrological control. Added costs of chemical additions are represented by the to need of frequent monitoring of the relevant chemical parameters and their effects on target and non-target biota.

Chemical additions may affect aquatic habitats located downstream the treated waterbody and especially in the case of artificial reservoirs, may build up forming heavily contaminated sediments with the effect of reducing future management options. Nonetheless, in the case of

potentially dangerous contamination with high levels of cyanotoxins, chemical treatment may prove to be cost effective since later water treatment by toxin removal can be more expensive (33). By causing cell collapse and toxin release, algicide treatment may provoke unwanted hazard (34). Waterbody uses have to be interrupted and isolated from the public during the application of chemical additives and for as long as it is necessary for the products to stabilize into less harmful forms (8).

Lime ($Ca(OH)_2$ and $CaCO_3$) is often used as a form of chemical control for its capability of precipitating phytoplankton cells. Applications do not cause cell wall rupture and liberation of cyanotoxins and do enhance the semi-permanent precipitation of dissolved phosphate and its binding within the sediment reducing the potential for phytoplankton growth. Other means of phosphate precipitation such as the addition of Al and Fe salts and clay particles, may also coagulate bluegreen colonies but are less effective and outlawed in several EU countries. Lime is inexpensive but needs to be applied in high doses involving high transport and mechanical dispersal costs. Lime treatment may not work in soft water lakes. Impacts due to the pH shock can be minimized by careful monitoring.

Copper sulphate treatment is uncommon in Europe but it is widely used in Australia and in the USA where it is still the only USEPA approved algicide for use in municipal water reservoirs and fish ponds. Chorus and Bartram (8) indicate that there is no full consensus about the actual risks for humans linked to a long-term copper accumulation in lake sediments; the WHO does not warn against copper sulphate use at low doses (35). Field and laboratory tests indicate that some planktonic bluegreens are more sensitive to copper sulphate than diatoms or green algae (36). The aim of copper application is not to kill the Cyanobacteria but rather to interfere with their growth and with the fixation of N_2 by decreasing their biomass (37). Treatment is most effective if applied during early stages of colony formation. Due to acclimatizing and to the secretion of organic substances able to complex copper salts, actual doses of copper sulphate often have to be higher than predicted by laboratory tests (38). In the Biesbosch reservoirs, additions of copper sulphate were not effective up to a dosage that cleared out the entire benthic population (27). Actual doses may have to be worked out empirically as it is often difficult to estimate the natural copper complexation and inactivation potential of a water body (39).

A whole range of organic compounds is being currently experimented worldwide for their activity against toxic bluegreens. Many compounds are being isolated from natural products after the observation of cyanolytic effects. Such is the case of phenols, quinones, aldehydes and other organic compounds extracted from barley straw after the observation of bluegreens' suppression in reservoirs where barley straw was dumped and left to rot (40). Due to the known complexity of the composition of natural products and to transformations which take place during straw decomposition, it has not been possible to indicate a simple mechanism, or a single compound that would explain algicide activity. Observations of cyanolytic activity in nature may be the result of the synergistic action of a complex cocktail of natural products under specific environmental conditions. Nonetheless, recent developments indicate that future research developments may soon lead to the isolation of highly specific algicides extracted from natural products which will hopefully have very limited impacts on the rest of the biocenosis.

Biological control

Microbiological control methods are often cited in reviews on cyanobacterial control (12, 38) but have never been tried on a large scale. The major advantages of these techniques is the specificity of treatment since only target organisms are affected. Preliminary investigations support the idea that inoculation with cyanophage viruses or bacteria will reduce the

development of extant blooms but cannot be used to prevent the appearance of new blooming since viruses and bacteria tend to develop only in conditions of high densities of cyanobacterial cells. A similar conclusion was reached about the possible use of bacterial *inoculi* (41). Recent reports confirm the potential for the isolation of an increasingly wide range of organisms able to attack bluegreens, among these: viruses, bacteria, fungi, actinomycetes and protozoa (42). Beside the specific cyanolytic activity of different groups, effectiveness of biological control agents depend on physico-chemical parameters. At high pH inoculation may fail as bluegreens seem able to avoid contamination (43). The implementation of biological control requires strategies to optimise microorganism activity. Further problems associated with the technique are the potential rapid development of resistance and the consequences of massive die-off and toxin release in case of successful inoculation. However, beside bacterial strains able to lyse bluegreens, laboratory and *in situ* experiments carried out during the last decade, have confirmed the presence, in lakes and reservoirs, of bacterial strains capable of degrading cyanotoxins into compounds of much lower activity (8).

Ecosystem management by manipulation of trophic chain relationships (biomanipulation) has been applied in some instances, with encouraging results (44). The technique involves the removal of zooplanktivorous fish to facilitate phytoplankton grazing by large zooplankters (in particular Daphnids) by releasing them from predation pressure. At the same time, coarse fish suppression contributes to reduce direct nutrient release by fish bioturbation. Under conditions of external nutrient load reduction and other, less well understood, favourable conditions (for example climatic), large Daphnids are able to graze on early stages of Cyanobacteria preventing bloom development.

Artificial storage reservoirs, such as the Thames Valley Reservoirs in England, posses anti-fish features which naturally prevent the establishment of dense coarse fish populations. This characteristic, combined with high flushing rates, destratification devices and the presence of large Daphnids, prevents the establishment of high cyanobacterial standing crops even under extremely high external loading (44). On the other hand, in shallow natural lakes and wetlands, phytoplankton blooms may be prevented by the promotion of macrophyte stands. Macrophytes compete for nutrients, provide shelters for zooplankton and for predatory fish such as pike, decrease sediment resuspension and mineral turbidity. The establishment of macrophytes is considered essential to stabilise the “clear-water” conditions that are sought by external nutrient reduction.

The twin problems for biomanipulation are lack of human control of the fish community, leading to a dominance of planktivory, and lack of zooplankton grazing control of the phytoplankton, leading to dominance by Cyanobacteria. These two may occur together or they may not, depending upon a complex set of circumstances. The question of the establishment of bluegreens in water bodies is central to the potentiality of biomanipulation as these organisms interfere with grazing by clogging filtering mechanisms (see references cited by Phillips and Moss,45) and in so doing impair the applicability of top-down control. Notwithstanding a good understanding of the key trophic relationships in freshwater food-chains and a growing number of case studies where it has been successfully implemented, the outcome of biomanipulation techniques is considered still too unpredictable to become a standard management technique (2).

Bluegreens management in the Mediterranean region

The character of Mediterranean inland waters is best represented by coastal wetlands, temporary streams periodically swept by intense flush floods and numerous small, artificial water storage reservoirs used for drinking water supply and irrigation.

Cyanobacterial dominance is widespread in artificial reservoirs of the Italian "Mezzogiorno" such as in Sardinia, Calabria and Sicily. Bluegreens are favoured by high temperatures and strong stratification which tends to stabilize the water column despite the relative shallowness of these artificial reservoirs. In many ways, during the six month long Mediterranean summer, habitat conditions and limnological parameters resemble those found in other (climatically) mediterranean and sub-tropical waterbodies such as in Australia, California and South Africa.

Cyanobacterial control in southern Mediterranean reservoirs can be attempted by ecotechnical reservoir management (46, 47), in particular by testing alternative modes of water abstraction. Observations carried out in an impounded lowland river in the Murray-Darling Basin (Australia) suggest that pulsing discharge by sudden increases in flow can be an effective means of disrupting surface cyanobacterial scums by strongly mixing the entire water column (23). Studies on the flotation speed of the resident population of *Anabaena circinalis* allowed the authors to identify an ideal destratification pattern based on the assumption that intervals between water column mixing events should be lower than:

- the ratio of water column depth to flotation speed;
- the ratio of water column depth to the specific growth rate.

The position of water uptake siphons can also contribute to reduce the surface accumulation of scums and can improve the water quality abstracted from the reservoir by selectively abstracting different layers at different times (23). The main difficulty, in modulating water abstraction to prevent cyanobacterial blooms, resides in the option of increasing outflow at time of cyanobacterial establishment while there may be need of storing water instead. Water quantity and water quality are directly related.

In the Mediterranean, recurrent droughts, such as those observed at the end of summer, typically between September and October, give the opportunity to encourage, when possible, sediment desiccation to prevent akinete germination during the following spring (5). If carefully planned, drawdown and desiccation would lend the opportunity of inexpensive sediment and damped rubbish removal. As a counter effect, especially if not all the eutrophic sediment has been extracted, desiccation promotes higher sediment oxidation and a higher nutrient release at the onset of the new floods which tends to enhance eutrophication effects. The option has to be carefully planned according to specific case studies.

High irradiance and high evaporation rates favour the formation of waterbodies with high salt concentrations. Hard and slightly alkaline waters with conductivities above 1000 µS are common among Sicilian reservoirs and carbonatic waters are found in the Apulian karst. Biogeochemical waters such as these would interact with chemical additives applied in the attempt to control toxic cyanobacterial blooms. Recent reports indicate that copper sulphate treatment is impaired by hard waters and that copper chelate algicides are better suited for such waters than $CuSO_4$ alone (8).

Biomanipulation has been seldom attempted in the Mediterranean region. The scarce adaptation of predatory fish species to high temperature waterbodies and the competitive advantage of warm adapted coarse fish, may reduce the potential for fish population control. In addition to this, periodical draughts engender an intensive use of waterbodies by cattle and humans. Major impacts are due to organic pollution, increasing sediment and nutrient loads, damages to ecotonal and aquatic vegetation, increasing sediment resuspension within the waterbody. Due to these factors periodical anoxia accompanied to high ammonia releases and fish kills, is very common in coastal lagoons, wetlands, artificial reservoirs and lowland streams. Under these conditions metazoan communities are replaced by microbial loops, to the direct advantage of organisms such as Cyanobacteria.

Climatic reasons and human impact explain why Mediterranean water bodies appear to be more sensitive to bluegreen dominance. This situation is likely to worsen as a direct

consequence of climate change. Management options should include coordinated interventions in the catchments and water distribution systems. However this may not be enough to reduce cyanobacterial blooms. More emphasis has to be given to in-lake control strategies including mixing, sediment treatment and biomanipulation. From the drinking water supply industry an ability to monitor bluegreen development and quantify toxicity hazards is likely to become urgent in the near future.

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CYANOBACTERIAL TOXICITY, SAFE DRINKING WATER AND HUMAN HEALTH

Ian R.Falconer
Department of Clinical and Experimental Pharmacology, Adelaide University and The Cooperative Research Centre for Water Quality and Treatment, Adelaide, Australia

Introduction

During the late summer period many drinking-water storage reservoirs worldwide are contaminated by blooms of toxic cyanobacteria (1). Rivers are also at risk, particularly those in countries with a Mediterranean climate in which the summers are dry and irrigation agriculture is widely practiced. For example the River Darling in 1991 in New South Wales had a bloom of the toxic cyanobacterium *Anabaena circinalis* of 1,000 km length during conditions of minimal river flow, with the consequence of thousands of livestock deaths. Because of the use of the river for both human and livestock water supply, a State of Emergency was declared and temporary drinking water treatment plants installed by the Australian Army. The toxins present were later identified as paralytic shellfish poisons (saxitoxins and related compounds) (2). These toxins are resistant to boiling or chlorination.

Animal poisoning

The first recorded instance of livestock deaths from drinking a cyanobacterial scum was that by Francis (3) in South Australia. The cyanobacterium was *Nodularia spumigena*, in a brackish-water coastal lake, and horses, cattle, sheep and pigs were reported killed. Later, in 1944, thousands of deaths of sheep and cattle were reported from South Africa, due to *Microcystis* poisoning in a river after dam construction had slowed water flow (4). In the Northern hemisphere the Canadian lakes were the location of deaths of tens of thousands of ducks which appeared to be from cyanobacterial toxicity together with botulism, and of livestock and domestic pets (5,6) In this case the predominant organism in the scum was *Aphanizomenon.* Since these early records similar instances of domestic animal and wildlife poisoning have occurred in North and South America, Africa, Europe, Asia and Australasia (1).

The toxins from these organisms have been isolated and identified, and can be grouped into the hepatotoxins which primarily cause damage to the cells of the liver, the cytotoxins which cause general cell death and the neurotoxins which block nerve transmission. The *Microcystis* and *Nodularia* toxins are selectively injurious to liver cells as a result of transport mechanisms which concentrate the toxins in these cells (7). These toxins are inhibitors of key enzymes in cell function (8). Poisoned domestic animals show symptoms of direct liver injury and also the indirect effects of liver failure such as photosensitization after consuming *Microcystis* (9). *Microcystis* hepatotoxins have been responsible for the majority of livestock deaths and injury worldwide that are due to cyanobacteria (10), as *Microcystis* thrives in standing water, particularly when stratification occurs in summer, solublising sediment phosphorus under the anoxic conditions of the lake bottom.

The cytotoxin from *Cylindrospermopsis* is under intensive investigation at present. It is an inhibitor of protein synthesis (11) which appears to readily penetrate mammalian cells, and causes cell death in a range of organs. The intestinal lining, liver, blood vessels, kidneys and lymphatic cells are injured (12, 13). This cyanobacterium is found commonly in tropical rivers and lakes, but most often in low population densities. Water blooms have been reported in Australia, the USA and in Europe, including in temperate waters (14). Other cytotoxins await characterisation.

The neurotoxins from cyanobacteria have caused livestock deaths in many parts of the world, and were the first group of toxins to be successfully identified and characterised (15). *Anabaena* genus of cyanobacteria are the most abundant source of livestock poisoning by neurotoxins. In the Northern hemisphere a neurotoxic alkaloid named anatoxin-a, which blocks neuromuscular impulse transmission, has caused death of cattle (16) and of dogs (17). This neurotoxin occurs in *Anabaena* scums and in *Oscillatoria* filaments on sediments and rocks in shallow water. A second *Anabaena* neurotoxin is an acetylcholine esterase inhibitor, similar in action to organophosphorus insecticides. This toxin appears to cause bird deaths as well as mammalian poisoning (18). The third well characterised group of cyanobacterial neurotoxins are the paralytic shellfish poisons. These are saxitoxin ,neosaxitoxin, gonyautoxins and C toxins (19). Usually a mixture of these toxins occur together in the same bloom of cyanobacteria. In the Northern hemisphere the most frequent cyanobacterial species causing livestock deaths by paralytic shell fish poisoning is *Aphanizomenon flos-aquae,* whereas in Australia it is *Anabaena circinalis* (2). As noted earlier, this toxicity was the cause of thousands of livestock deaths in the River Darling bloom in Australia in 1991. To summarise, there are continuing livestock mortalities and loss of productivity from the agricultural use of eutrophic waters containing toxic cyanobacteria. These losses are usually from the direct consumption of contaminated water, particularly of wind-blown scums. Pumping of drinking water from such sources can also cause injury to livestock and poultry held under intensive farming conditions.

Human poisoning

Cases of human injury attributed to cyanobacterial toxins have arisen from three sources of exposure. Treated drinking water supplies have been responsible for outbreaks of illness in town populations, whereas individual cases have followed recreational water exposure. One severe single poisoning event which resulted in more than 50 deaths occurred in a renal dialysis clinic.

Domestic drinking water reservoirs are regularly monitored and increasingly the counting of cyanobacterial cells is one of the measures undertaken. Detailed recommendations on monitoring are set out in the recent WHO publication on toxic cyanobacteria (1). The cells of the cyanobacterial species *Microcystis aeruginosa* and *Anabaena circinalis,* which cause the major problems in drinking water in temperate regions, both contain gas vacuoles. Under calm weather conditions these cells accumulate on the surface and can form dense scums of high toxicity. Under these circumstances the capacity of a water treatment plant to remove cyanobacterial cells or toxins may be grossly exceeded, and the toxins can then appear in the reticulated water supply.

All the major outbreaks of poisoning in towns that have been attributed to cyanobacterial toxins in drinking water have occurred in chlorinated supplies. The symptoms reported may resemble those from pathogens and therefore require careful evaluation to exclude bacteria, viruses and other toxins. An illustration of such an outbreak is that reported from Bahia, Brazil. A newly constructed reservoir in the Paulo Alfonso region of the state developed a mixed water bloom of *Anabaena* and *Microcystis* at concentrations of about 100,000 to 1,000,000 cells per

ml. Coincident with this a severe gastroenteritis epidemic occurred with some 1,200 cases receiving hospital treatment, with a mortality in April, 1988 of 23.7% (31 patients) and in May of 45.1% (33 patients) among those hospitalised during the peak of the epidemic. The highest percentage mortality was coincident with dosing the reservoir with copper sulphate twice in May to lyse the cyanobacteria. By the end of May the incidence of gastroenteritis had fallen to normal levels and the concentration of cyanobacteria in the reservoir had declined. Investigation of the outbreak demonstrated that the reservoir water was responsible, no infectious agent or toxic agricultural chemicals or metals were found and cases occurred in patients drinking only boiled or filtered water . It was concluded that cyanobacterial toxins were responsible (20).

A second example of evidence of human injury from the presence of toxic *Microcystis* in a drinking water supply reservoir occurred in Australia in 1981 (21). The reservoir is situated in northern New South Wales and supplies the town of Armidale, with a population about 25,000. Monitoring of cyanobacteria was carried out regularly as the reservoir has a history of repeated cyanobacterial blooms from the 1970's to the present. The reservoir is situated 150 m higher than the treatment plant, and is connected by about 20 km of pipeline. The treatment plant had conventional pre-chlorination, alum flocculation, sedimentation, rapid sand filtration and post-chlorination. The reservoir was monitored in 1981 during the formation of a bloom of toxic *Microcystis* and after the termination of the bloom by the aerial spreading of 1 ppm of copper (as sulphate) in the top metre of water.

A retrospective epidemiological study was carried out on the population consuming their drinking water from this reservoir, as compared to adjacent populations on other water supply sources. All the data on liver function tests carried out by the hospital regional pathology laboratory over the relevant period were analysed. Data from patients receiving drinking water from the Armidale supply was separated from that of patients receiving other water supplies. Tests on blood samples taken during the 6 week time period before the bloom occurred, the 6 weeks of the bloom and its termination with copper, and the 6 weeks after that were assessed. Potentially confounding issues such as alcoholism, hepatitis and major public holidays were eliminated. The results for one indicator of toxic injury to the liver illustrate that a significant rise in the enzyme gamma glutamyl transferase in blood of patients occurred only in consumers of the Armidale water supply during the period of the bloom and copper treatment of the reservoir. The average increase in enzyme activity was indicative of overall minor liver injury, however some individuals within the group showed increases indicating substantial liver injury.

A more severe occurrence of poisoning by toxic cyanobacteria in a drinking water supply reservoir-Solomon Dam- was reported in 1980 from Palm Island in Queensland, Australia. In this case the organism responsible was the predominantly tropical species *Cylindrospermopsis raciborskii*. Complaints of bad taste and odour of the water supply caused the operating authority to dose the Solomon Dam with copper sulphate to control the cyanobacterial bloom. Within a week the first cases of an unusual gastro-hepatitis among children were reported. Ultimately 140 children and 10 adults required hospital treatment, with the most severe cases in intensive care with intravenous therapy. There were no mortalities due to the quality of care given. The symptoms commenced with malaise, anorexia, vomiting, headache and painful liver enlargement, progressing to constipation ,followed by bloody diarrhoea and electrolyte, protein and glucose loss through kidney damage (22).

Since this occurrence the biology of the organism *Cylindrospermopsis raciborskii* and chemistry of the toxin cylindrospermopsin have been studied (23), with considerable current research into the toxicology in progress. The organism occurs occasionally in bloom proportions in the reservoirs supplying water to the city of Brisbane, in which it is closely monitored.

The clearest demonstration of cyanobacterial toxicity from a water supply was the recent poisoning of 117 out of 136 patients at a dialysis clinic in Caruaru, Pernambuco, Brasil. After a

normal dialysis treatment patients complained of nausea, vomiting, painful liver enlargement, visual disturbance and muscle weakness. Of these patients 100 developed acute liver failure and more than 50 died. International investigation uncovered histopathological changes in the livers of patients similar to cyanobacterial toxicity and the presence of *Microcystis* toxins in the dialysis centre's water treatment system and in samples post-mortem of human liver (24,25). The water supply reservoir was not being monitored for cyanobacteria at the time but had a previous history of blooms. It is unclear what treatment the water had received prior to delivery to the dialysis clinic.

These illustrations have been selected from among the better-described episodes of human poisoning attributed to cyanobacterial toxins, a more comprehensive list can be found in Ressom (16).

Cancer risks from cyanobacterial toxins

The first epidemiological evidence that there may be an association between cyanobacterial contamination of water supplies and cancer arose from studies of the incidence of hepatocellular carcinoma in China. Within China there are local regions which have proportionately very high rates of this cancer. These regions used surface water from ditches and ponds for drinking, by contrast immediately adjacent regions using well water supplies had average rates of this cancer (26,27). Changing to well water markedly reduced the rate of hepatocellular carcinoma in regions previously with very high rates. The surface water supplies carry cyanobacteria with detectable toxin contents, but may also be contaminated with agricultural chemicals and naturally occurring carcinogens from other sources.

To clarify these issues experimental models have been used for detection of increased cancer growth on exposure to cyanobacterial toxins, as well as direct measurements of cancer initiation by the toxins. Oral exposure of mice to *Microcystis* toxins has been shown to increase the rate of growth of skin tumours in carcinogen-treated mice (28). In a model using rats, a range of cancer initiators have been shown to result in pre-cancerous lesions of the liver which are stimulated to growth by injection of *Microcystis* toxin (29,30). Investigation of the effects of toxic extracts of cyanobacteria on the growth of tumours in the gut and lymphatic cells of mice are underway in our laboratory to further explore the potential consequences for human populations of exposure to these toxins. Evidence for the stimulation of growth of pre-cancerous foci in the colons of mice by oral extracts of *Microcystis* is shortly to be published.

Recreational exposure

Water contact through recreational use presents a different route of exposure to cyanobacterial toxins. Swimming in cyanobacterial scums can cause injury from ingestion of water as well as direct effects on the skin and eyes.

An illustration of the adverse effects on people of swimming and rolling canoes in a *Microcystis* contaminated lake occurred in the UK, when army trainees were carrying out exercises (31). The blisters round the mouth and sore throat described by these individuals may have been due to direct toxic effects on dermal tissue, whereas the vomiting, abdominal pain and diarrhoea were likely consequences of ingestion of cyanobacterial scum. All the individuals were described as having a dry cough and two had pleuritic pain from pneumonia. Inhalation of

Microcystis toxins is as effective in poisoning as intraperitoneal injection, so it appears possible that the pneumonia arose from cell or toxin inhalation (32).

Epidemiological studies of the association between minor illness and exposure through bathing to cyanobacteria showed a relationship between cell count in the water and symptoms shown by bathers the week after swimming (33). Symptoms increased from 5,000 cell/ml upwards, indicating that even these low cell concentration can have observable effects. Allergic reactions to cyanobacteria are well known and independent of toxic effects (34).

Guideline values

The World Health Organisation established in 1997 an expert group to consider the health effects of toxic cyanobacteria and to make recommendations on Guideline Values for safe drinking water content of cyanobacterial toxins.

Only one Guideline Value was recommended, of 1 µg/L for microcystin-LR in drinking water. Determination of Guideline Values for other toxins are under further consideration, as described in the volume published from the discussions (1).

The derivation of these guidelines is done on the basis of animal oral toxicity, in a similar way to chemical safety in drinking water. A 'Tolerable Daily Intake ' is determined from the No-Observed-Adverse-Effect-Level in rodent experiments, applying a standardised set of safety factors to convert rodent experimental data to human lifetime exposure through drinking water (35). No conclusion was reached on the use of tumour promotion data in rodents, for application to drinking water guideline values.

Australia is in the process of a revision of the Drinking Water Guidelines, with a draft document issued for public comment. The new draft includes a Guideline Value for total microcystins in drinking water of 1.3 µg/L toxicity equivalents to microcystin-LR. When more data is available Guideline Values for cylindrospermopsin and saxitoxins will be considered.

Conclusions

Toxic cyanobacteria in water resources present a risk to water users whether for livestock consumption, human drinking water supply or simply for recreation. Reduction of the cyanobacterial cell numbers by algicides (like copper or chlorine) may aggravate the hazard through liberating toxins into the water. Safe Guideline Values for drinking water content of these toxins are being established by WHO and adopted by several countries at present.

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ACQUA POTABILE E CIANOFICEE: ESPERIENZE E PROBLEMATICHE DI UN SERVIZIO TERRITORIALE DI PREVENZIONE DEL CENTRO SARDEGNA

Maria Francesca Murineddu
Servizio Igiene degli alimenti e della nutrizione, ASL 3 Nuoro

La situazione

L'acqua

La provincia di Nuoro comprende 100 comuni. La popolazione residente ammonta a 270.000 abitanti.

Il 70% circa di tale popolazione è approvvigionato con acqua proveniente da 14 laghi artificiali. Buona parte dell'acqua di tali laghi è di scarsa e, in alcuni casi, di scarsissima qualità, infatti la Classificazione Regionale delle acque superficiali destinate alla potabilizzazione riporta più della metà dei bacini in primo elenco speciale, alcuni in secondo elenco speciale, altri in A2 e A3.

Le alternative all'utilizzo di tali acque sono scarse, alcuni comuni hanno possibilità di miscelarla, durante i periodi invernali, con acqua profonda. Nel periodo estivo all'aumento dei consumi dovuto soprattutto al flusso turistico, si associa la cronica carenza d'acqua dovuta alla piaga ormai periodica della siccità, per cui non solo non è possibile attingere alle fonti profonde che sono per lo più notevolmente ridotte o a secco, ma deve essere razionata anche l'acqua dei bacini che inizia a scarseggiare.

In questa situazione si inserisce un ulteriore elemento di preoccupazione rappresentato dalle periodiche fioriture di Cianoficee.

I dati acquisiti dal nostro servizio, a partire dalla fine del 1998 ad oggi, indicano chiaramente che il fenomeno è presente in tutti i laghi da cui si approvvigiona il nostro territorio. Pressoché costantemente le fioriture iniziano a manifestarsi nel mese di maggio, raggiungono valori massimi tra la fine di agosto e l' inizio di settembre e a volte perdurano fino a novembre. Le cianoficee riscontrate in tali fioriture, appartenenti e di possibile appartenenza a specie tossiche (in quanto individuate solo per genere) sono le seguenti:

- Individuate per genere
  Anabaena sp., *Aphanizomenon* sp., *Coelosphaerium* sp., *Lyngbya sp.*, *Microcystis* sp., *Oscillatoria* sp., *Pseudoanabaena* sp.

- Completamente tipizzate
  Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis aeruginosa, Microcystis viridis, Oscillatoria agardhii rubescens, Pseudoanabaena catenata.

In ogni lago si riscontrano in genere più d'una delle specie di cui sopra e, sebbene spesso l'una prevalga sull'altra in una singola analisi e tali prevalenze si alternino velocemente, si sono anche riscontrate fioriture contemporanee e consistenti di più specie a comprovata tossicità.

Gli impianti

Sono 21 gli impianti che potabilizzano l'acqua proveniente dai 14 laghi di cui sopra e la forniscono alla popolazione della Provincia di Nuoro.

I trattamenti che vi si effettuano comprendono in genere i seguenti processi:

- preossidazione;
- chiariflocculazione;
- filtrazione su letti a sabbia;
- disinfezione finale con biossido di cloro e, in alcuni casi, con ipoclorito.

In alcuni impianti e in particolari situazioni viene utilizzato anche il carbone attivo in polvere, mentre solo 3 impianti dispongono di filtri a carbone attivo granulare.

Per svariati motivi, primo tra tutti l'impossibilità dei nostri laboratori ad eseguire le determinazioni su quantitativi e tipi di alghe presenti e sulla tossicità delle stesse, si è dovuto far riferimento per quanto riguarda le densità algali alle analisi predisposte dagli Enti Gestori e per la tossicità all'Istituto Superiore di Sanità e all'Istituto Zooprofilattico Sperimentale della Sardegna.

Esaminando i dati trasmessi dagli Enti Gestori e relativi soprattutto a campionamenti effettuati nel periodo estivo del 1999 e in alcuni casi anche del 2000, nell'acqua in entrata ed in uscita da 9 impianti di potabilizzazione, la somma delle cianoficee individuate solo per genere con quelle completamente tipizzate (ricadenti tra quelle più sopra elencate) registra i seguenti valori massimi di cellule/l (relative a singole analisi):

- *acqua in entrata*
  - un miliardo circa per un impianto
  - tra 100 e 200 milioni per altri due impianti
  - 25 milioni circa in un altro
  - tra 1 e 5 milioni per i rimanenti cinque

- *acqua in uscita*
  - 150 milioni in un impianto
  - 13 milioni in un altro
  - tra 0 e 700.000 nei restanti sette

Considerando questi dati e i trattamenti generalmente effettuati presso gli impianti è ragionevole pensare che, qualora le cianoficee producano tossine, i quantitativi di queste ultime, riscontrabili nell'acqua in uscita da impianti sprovvisti di filtri a carbone attivo, siano da ascrivere non solo alle cellule presenti nell'acqua potabilizzata, ma anche a quelle presenti nell'acqua grezza in entrata.

Purtroppo per le difficoltà più sopra accennate, che comportano spesso anche notevoli ritardi nell'acquisizione dei dati relativi alle densità algali, i test di tossicità si sono potuti predisporre solo in quattro occasioni, per due di queste il loro esito è stato positivo.

I dati epidemiologici

Anche se la situazione descritta per alcuni versi risulta drammatica, di contro non esistono segnalazioni di patologie verificatesi nella nostra popolazione ed ascrivibili all'ingestione di tali acque, neppure in occasione delle fioriture risultate tossiche.

Per una spiegazione esauriente sarebbero certamente necessari appositi studi epidemiologici, e una verifica di alcune possibili cause come ad esempio le seguenti:

- la maggior parte della popolazione non beve l'acqua di rete (un'indagine effettuata dall'Università di Cagliari sui residenti in tale provincia ha evidenziato che il 70% della popolazione beve altra acqua)
- i sintomi sono lievi e passano inosservati
- i sintomi sono attribuiti ad altre cause
- la tossicità al rubinetto dell'utente, è più bassa di quella in uscita dagli impianti (per la lunghezza della rete di distribuzione, per la miscelazione con altre acque, per la possibile aggiunta di ipoclorito nei serbatoi comunali di accumulo)

Per quanto riguarda il bestiame nessuna segnalazione è pervenuta ai Servizi veterinari dal 1998 ad oggi. Anche in questo caso occorrerebbe fare ulteriori ricerche, sebbene una delle spiegazioni potrebbe essere il fatto che l'acqua prelevata per irrigare ed utilizzata anche per gli abbeveratoi del bestiame, proviene in genere da bocche di presa situate a maggior profondità rispetto a quelle utilizzate per gli impianti di potabilizzazione

Le problematiche

A parte quanto già esposto relativamente all'impossibilità di usufruire dei laboratori dei Presidi Multizonali di Prevenzione per la conta e tipizzazione delle alghe e per le prove di tossicità, sono da segnalare le problematiche connesse con la valutazione del rischio per la salute e dei conseguenti provvedimenti adottabili dalle Autorità competenti.

In particolare tali problematiche sono da ascrivere fondamentalmente all'assenza di limiti normativi e al giudizio d'idoneità d'uso.

I limiti

Il DPR 236/1988 che regolamenta tra l'altro le caratteristiche di qualità dell'acqua destinata al consumo umano, per le alghe non prevede alcun limite.

Dai limiti indicati dal Ministero per le fioriture di alghe tossiche in acque destinate alla balneazione, dalle indicazioni fornite dalla dr.ssa Bruno che, durante le esperienze suddette ha assicurato per conto dell' Istituto Superopre di Sanità il massimo supporto, dalla bibliografia che si è riusciti a reperire sull'argomento si è potuto concludere quanto segue:

- Il limite di microcistine entro il quale non si hanno effetti acuti per ingestione di acqua è di 0,84 μg/l , equivalente a circa 5.000.000 di cellule/l.
- Il limite di microcistine entro il quale non si hanno effetti cronici per ingestione di acqua è di 0,3 μg/l, equivalente a circa 1.700.000 cellule/l.
- Per le alghe che producono neurotossine il limite per non incorrere in danni alla salute derivanti dall'ingestione di acqua è di 5.000.000 di cellule/l.

I problemi relativi che si pongono spesso sono i seguenti:

- nel caso di impianti di potabilizzazione sprovvisti di filtri a carbone attivo i limiti suddetti vanno riferiti all'acqua in entrata o a quella in uscita?
- qualora si utilizzi il numero di cellule ai fini del giudizio di potabilità, in attesa dei risultati dei test di tossicità, nel conteggio vanno comprese anche quelle non completamente tipizzate ed individuate solo con il genere?

– nel caso il giudizio debba invece essere espresso in base ai quantitativi di tossina presenti, qual è il limite applicabile alle neurotossine?

Il giudizio

Come risulta dall'elenco delle specie presenti nei nostri laghi, le tossine che potrebbero essere prodotte, possono causare, nell'uomo, patologie gastroenteriche, epatiche, cutanee, neurologiche, polmonari e a lungo andare anche tumorali.

L'insorgenza di tali patologie potrebbe avvenire attraverso l'ingestione, il contatto e l'inspirazione di aerosol di acqua contenente le tossine algali.

Il problema che si pone spesso è: quando le cellule algali sono presenti a valori superiori ai 5 milioni/L e risultano produttrici di tossine che agiscono per ingestione, contatto e inspirazione di aerosol, dando per scontato che quell'acqua non va bevuta, può essere utilizzata per la preparazione di alimenti e per la pulizia della persona?

Conclusioni

La Sardegna, come accennato nella prima parte, non ha grandi risorse idriche, l'approvvigionamento potabile è assicurato per lo più da bacini superficiali e, nei periodi estivi quando aumentano notevolmente i consumi, anche l'acqua di tali bacini scarseggia. In questa situazione qualora dovessero insorgere fioriture tossiche il divieto ad uso potabile diretto non creerebbe molti problemi sia perché è facile recuperare pochi litri di altra acqua da bere sia perché questo viene probabilmente già fatto indipendentemente dai divieti.

Molti problemi si verrebbero invece sicuramente a creare nel caso i divieti fossero estesi alla preparazione di alimenti e alla pulizia della persona, in tal caso infatti sarebbe difficile per le autobotti reperire i grandi quantitativi di acqua necessaria agli utenti e alle imprese alimentari per tutta la durata dell'emergenza, che pur considerando una singola fioritura ed il suo decadimento, potrebbe durare quasi un intero mese.

In conclusione, in attesa che vengano effettuate le opere di risanamento dei corpi idrici e adeguati gli impianti, risulta di vitale importanza che in uno con i limiti vengano specificati al meglio gli usi da interdire e quelli permissibili o eventualmente gli accorgimenti di emergenza che si possono adottare per prevenire i rischi.

Presidente dell'Istituto Superiore di Sanità
e Direttore responsabile: Enrico Garaci

Coordinamento redazionale:
Paola De Castro e Sandra Salinetti

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