US EPA BENTHIC HABS DISCUSSION GROUP WEBINAR

February 8, 2022, 10:00am – 11:30am Pacific Daylight Time

Webinar registration:

https://zoom.us/webinar/register/WN_t5PYEKJcTjSeHeCqG86BCw

GUEST SPEAKERS:

TRIANTAFYLLOS KALOUDIS, ATHENS WATER SUPPLY AND SEWERAGE COMPANY, GREECE

SZE TEE, RESEARCH ASSISTANT, UNIVERSITY OF AUCKLAND, NEW ZEALAND

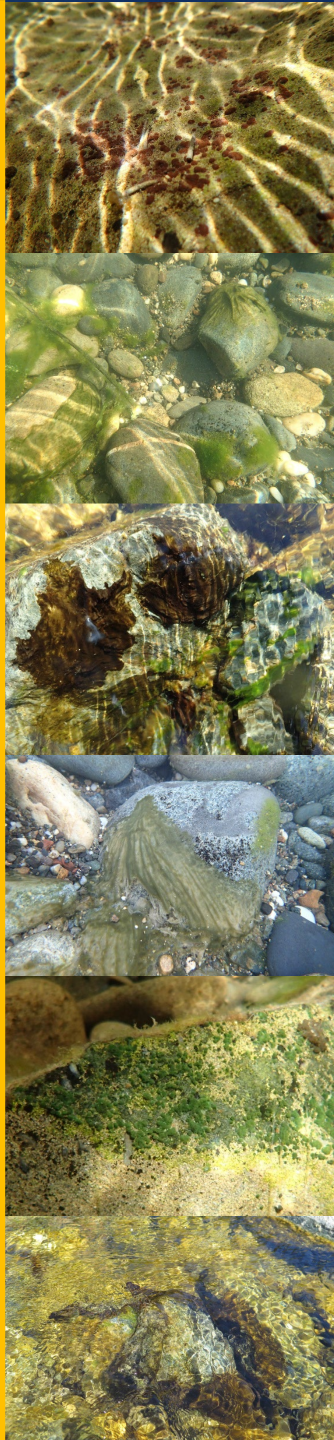
I. AGENDA

I Welcome, Agenda Overview, Announcements, and Introductions
Keith Bouma-Gregson, Margaret Spoo-Chupka, and Eric Zimdars

II Presentation: Seasonal Geosmin Production from Benthic Cyanobacteria in a Freshwater Canal, with Implications for Drinking Water Supplies
Guest Speaker – Triantafyllos Kaloudis

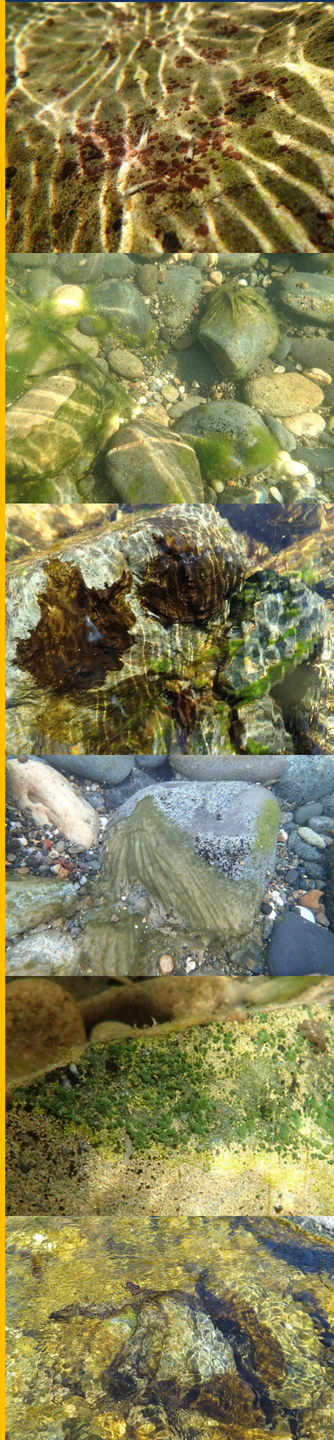
III Presentation: Growth or Toxicity: Genetic Divergence, Flexibility and Secondary Metabolism Distinguishing Toxic and Non-toxic Member of a Widespread Freshwater Cyanobacteria Genus
Guest Speaker – Sze Tee

IV 2022 Schedule, Wrap Up & Next Steps
Facilitators & Benthic HAB members



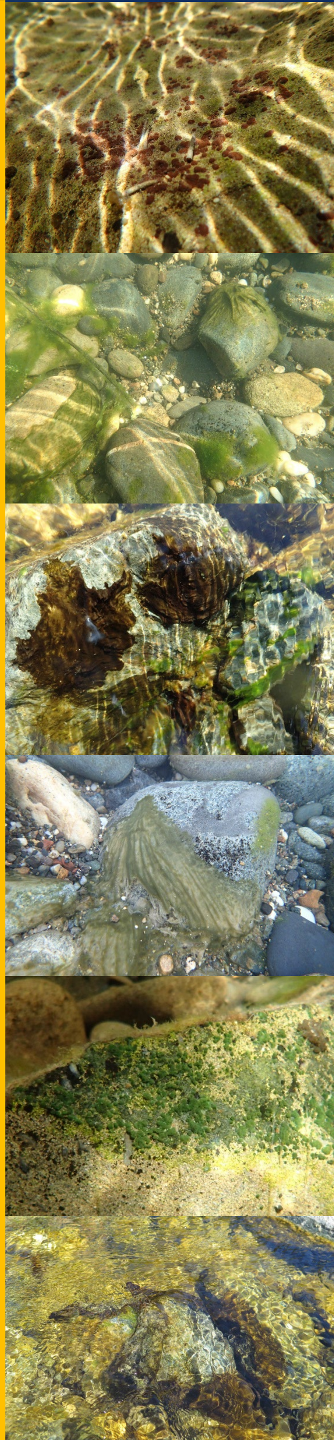
I. INTRODUCTIONS

Name	Affiliation	Contact Information
Margaret Spoo-Chupka	Metropolitan Water District of Southern CA	Phone: 909-392-5127 Email: MSpoo-Chupka@mwdh2o.com
Keith Bouma-Gregson	United States Geological Survey	Phone: 510-230-3691 Email: kbouma-gregson@usgs.gov
Eric Zimdars	United States Army Corps of Engineers	Email:Eric.S.Zimdars@usace.army.mil
Dr. Lesley D'Anglada	US EPA, Washington, DC	Phone: 202-566-1125 Email: Danglada.Lesley@epa.gov



I. ANNOUNCEMENTS

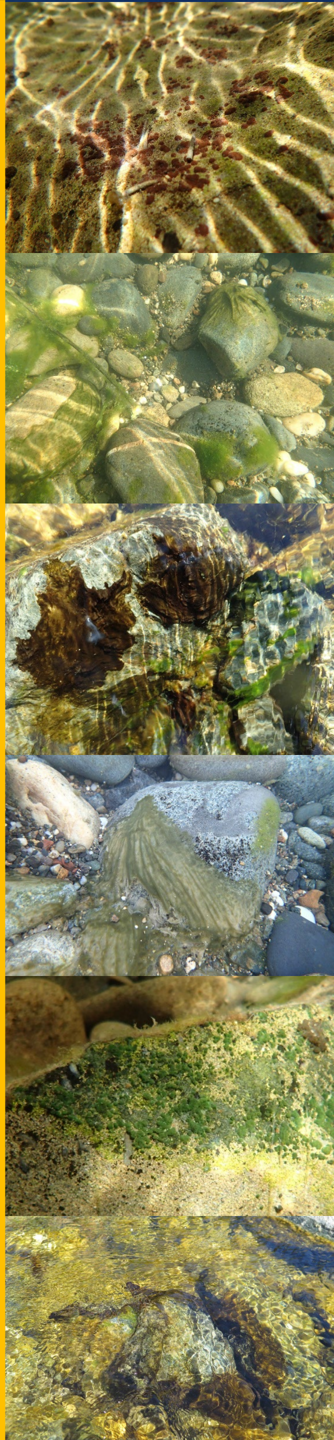
- Upcoming Meetings
  - 12th International Conference on Toxic Cyanobacteria: May 22-27, 2022 Toledo, Ohio Registration March 1, 2022
  - U.S. Symposium on Harmful Algae: October 23-28, 2022, Albany, New York Abstract Deadline: May 6, 2022
- Recent Papers
  - Gaget et al., 2022 *Benthic Cyanobacteria: A Utility-Centered Field Study*
  - Yao et al., 2022 Potential Influence of Overwintering Benthic Algae on Water Quality
- Other News
  - ITRC Benthic HCB (HCB-2) webpages go live in March 2022.
    - https://itrcweb.org/teams/active/hcb



ITEM II

GUEST PRESENTATION: SEASONAL GEOSMIN PRODUCTION FROM BENTHIC CYANOBACTERIA IN A FRESHWATER CANAL, WITH IMPLICATIONS FOR DRINKING WATER SUPPLIES

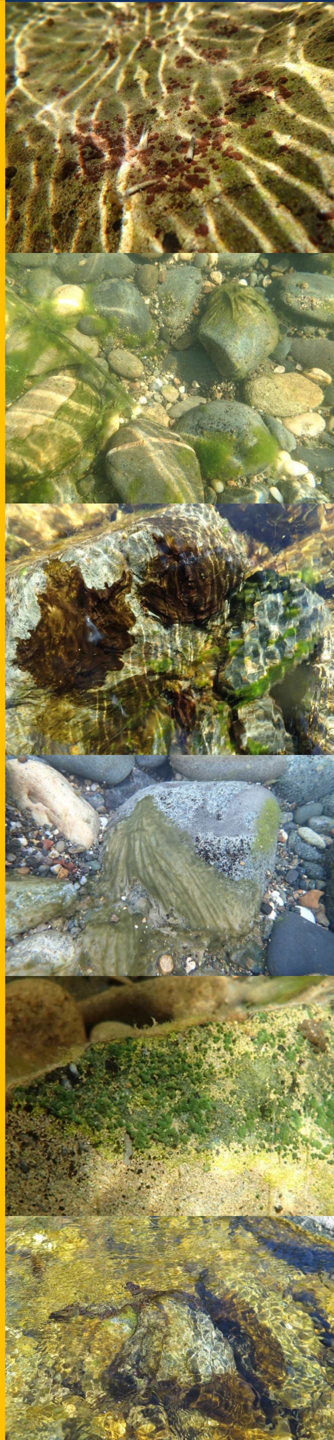
Triantafyllos Kaloudis, Athens Water Supply and Sewerage Company, Greece



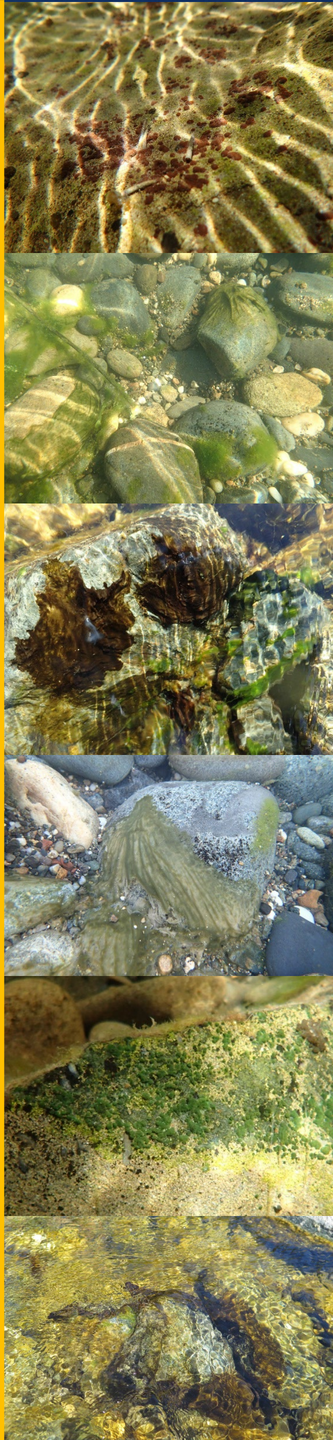
ITEM III

GROWTH OR TOXICITY: GENETIC DIVERGENCE, FLEXIBILITY AND SECONDARY METABOLISM DISTINGUISHING TOXIC AND NON-TOXIC MEMBERS OF A WIDESPREAD FRESHWATER CYANOBACTERIAL GENUS

Sze Tee, Research Assistant, University of Auckland, New Zealand



ITEM IV
2022 Schedule, Wrap Up, & Next Steps
Facilitators & Benthic HAB members



Seasonal geosmin production from benthic cyanobacteria in a freshwater canal, with implications for drinking water supplies.

Triantafyllos Kaloudis[1], Angelika-Ioanna Gialleli[1], Christos Avagianos[1], Sevasti-Kiriaki Zervou[2], Anastasia Hiskia[2], Maria van Herk[3], Petra Visser[3], Nico Salmaso[4], Manthos Panou[5], Spyros Gkelis[5], Nikolaos Deftereos[1], Phani Miskaki[1]

Contact: kaloudis@eydap.gr (TK), nd.iwwc@eydap.gr (ND), miskaki@eydap.gr (PM)

[1] Water Quality Control Department, Athens Water Supply and Sewerage Company (EYDAP SA), Greece
[2] Institute of Nanoscience and Nanotechnology, NCSR Demokritos, Athens, Greece
[3] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands.
[4] Hydrobiology Research Unit, Fondazione Edmund Mach, Trento, Italy
[5] School of Biology, Aristotle University of Thessaloniki, Greece.

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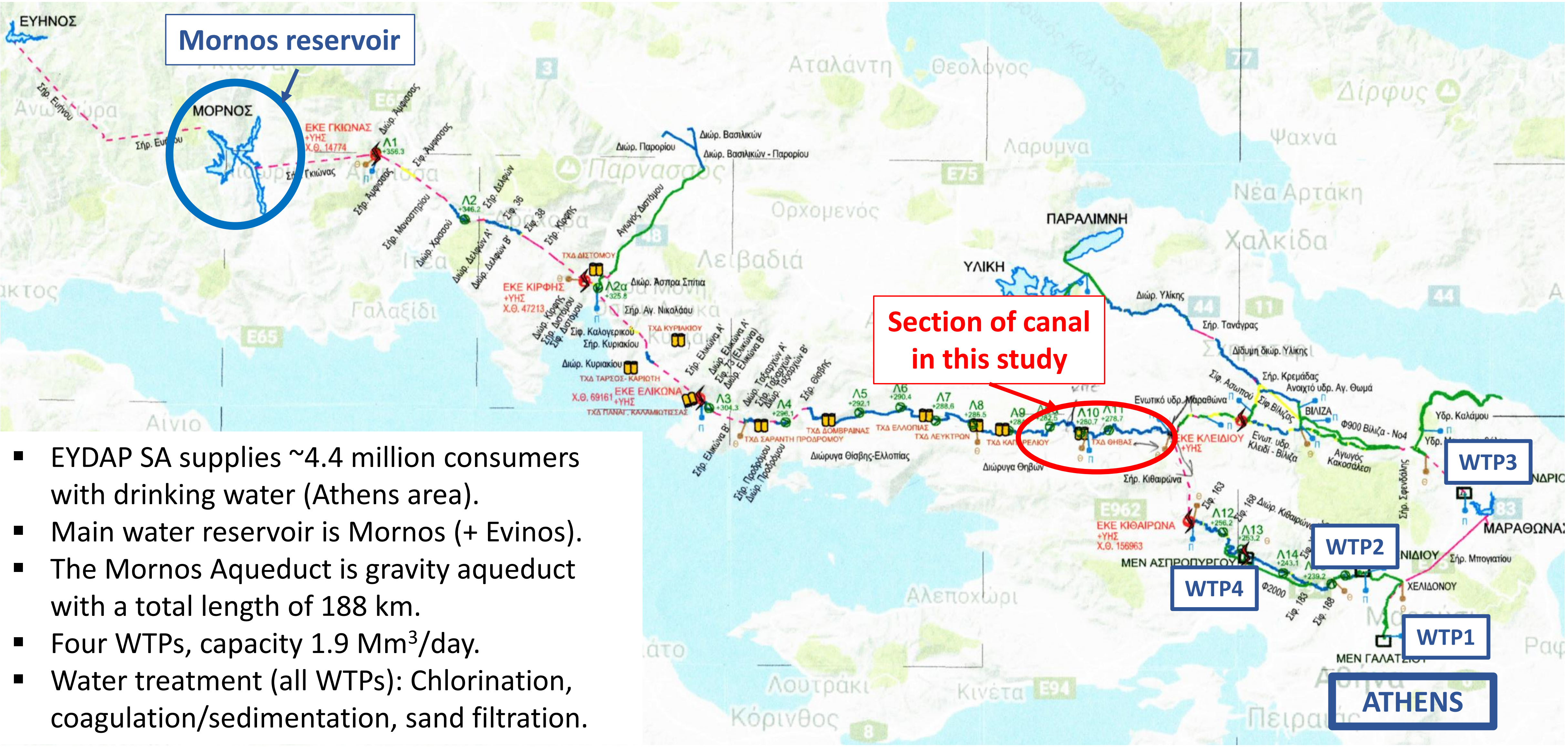
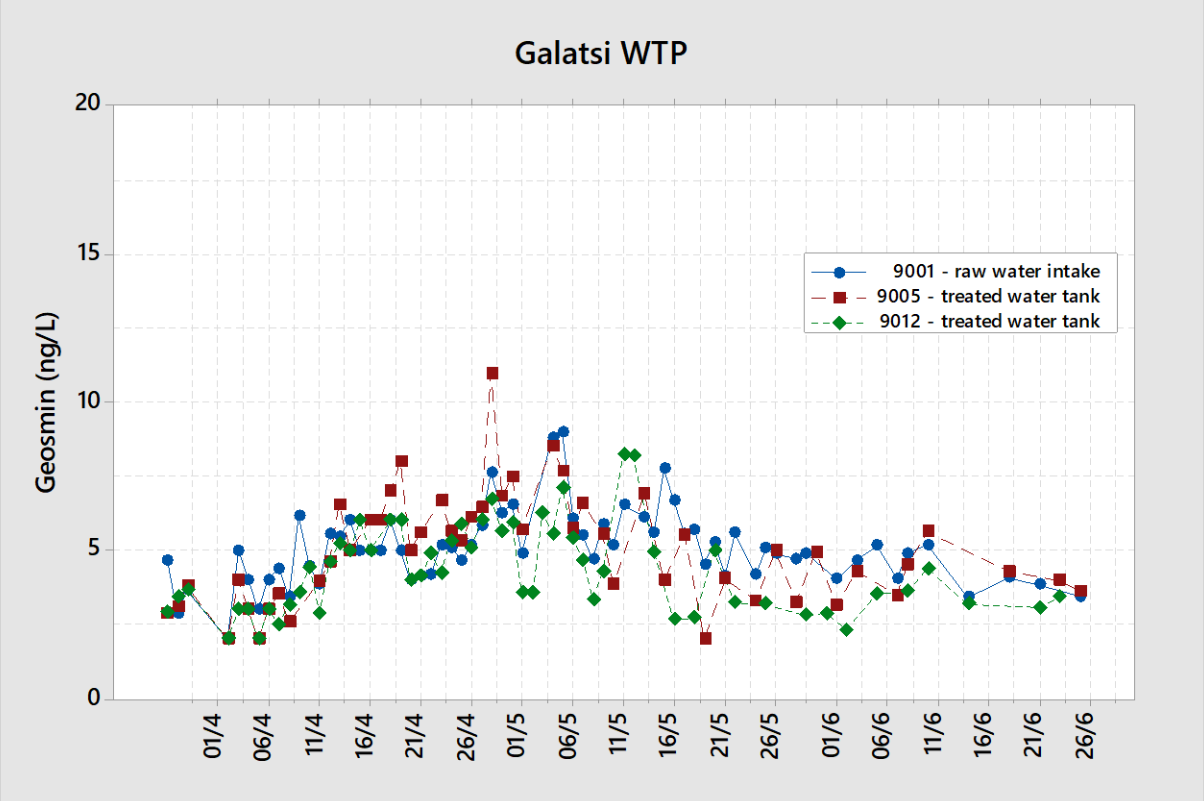
The Athens water supply aqueduct

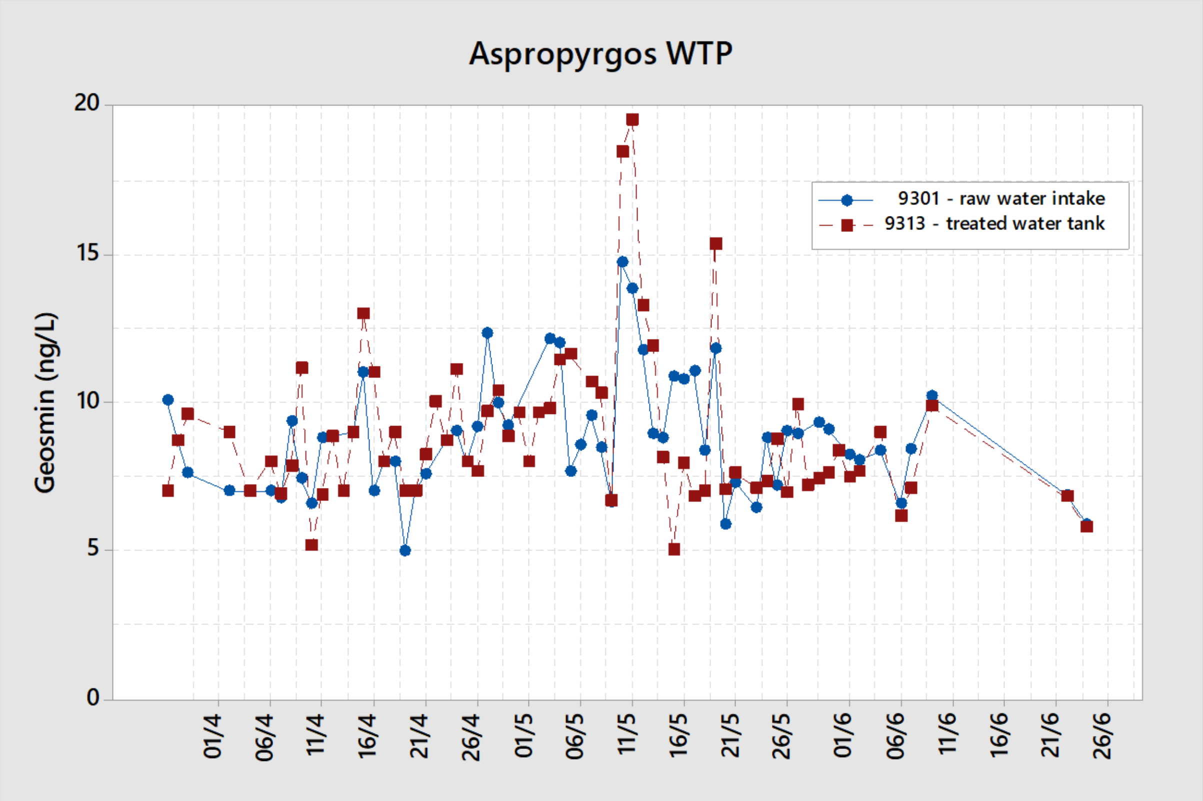


- EYDAP SA supplies ~4.4 million consumers with drinking water (Athens area).
- Main water reservoir is Mornos (+ Evinos).
- The Mornos Aqueduct is gravity aqueduct with a total length of 188 km.
- Four WTPs, capacity 1.9 Mm^3/day.
- Water treatment (all WTPs): Chlorination, coagulation/sedimentation, sand filtration.

Seasonal geosmin episodes in Athens water supplies

- Three (3) consumer complaints on 22 March 2019.
- Samples analyzed (GC-MS), geosmin was 6-7 ng/L.
- Geosmin was found in all WTPs and in DW network.
- No previous history of geosmin occurrence in Athens.
- Wide monitoring scheme initiated.

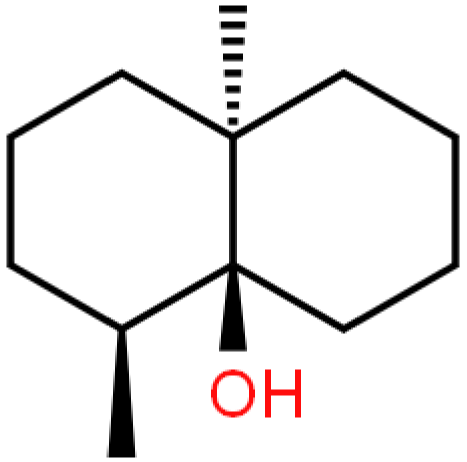




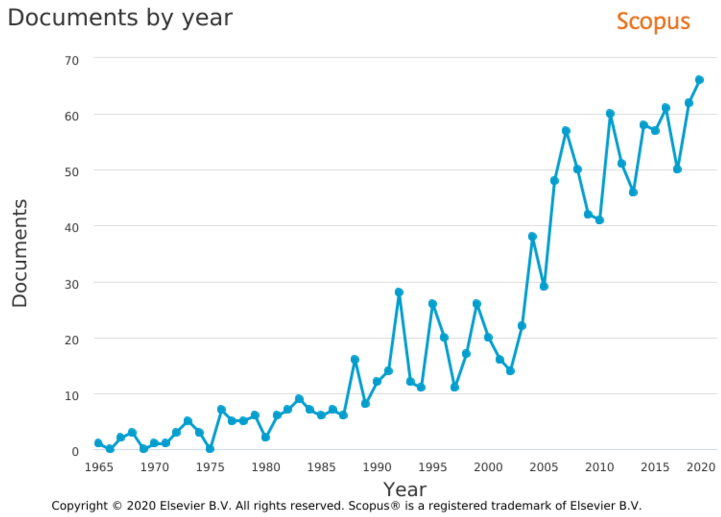
- **Geosmin was the only T&O** compound present in intake and treated water.
- The episode lasted **from March to late summer 2019.**

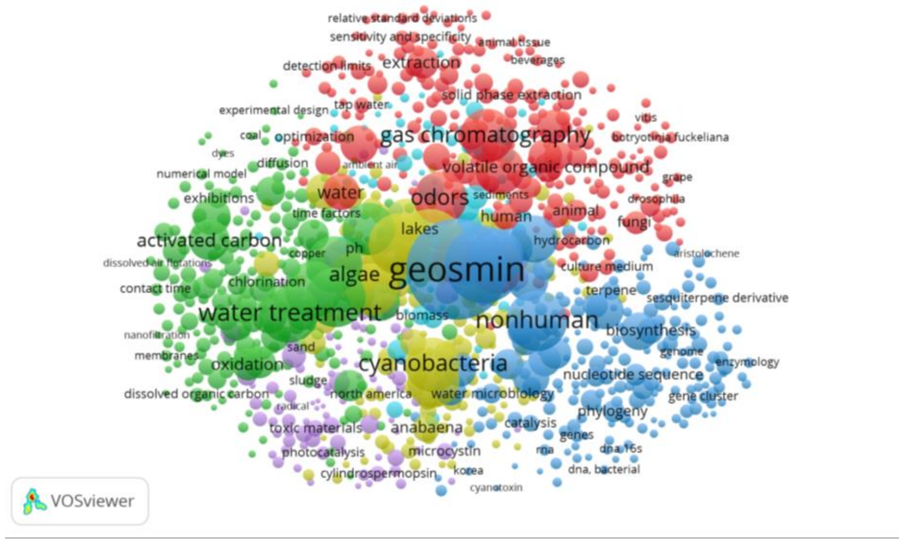
Geosmin

γεω- (earth) + οσμή (smell)



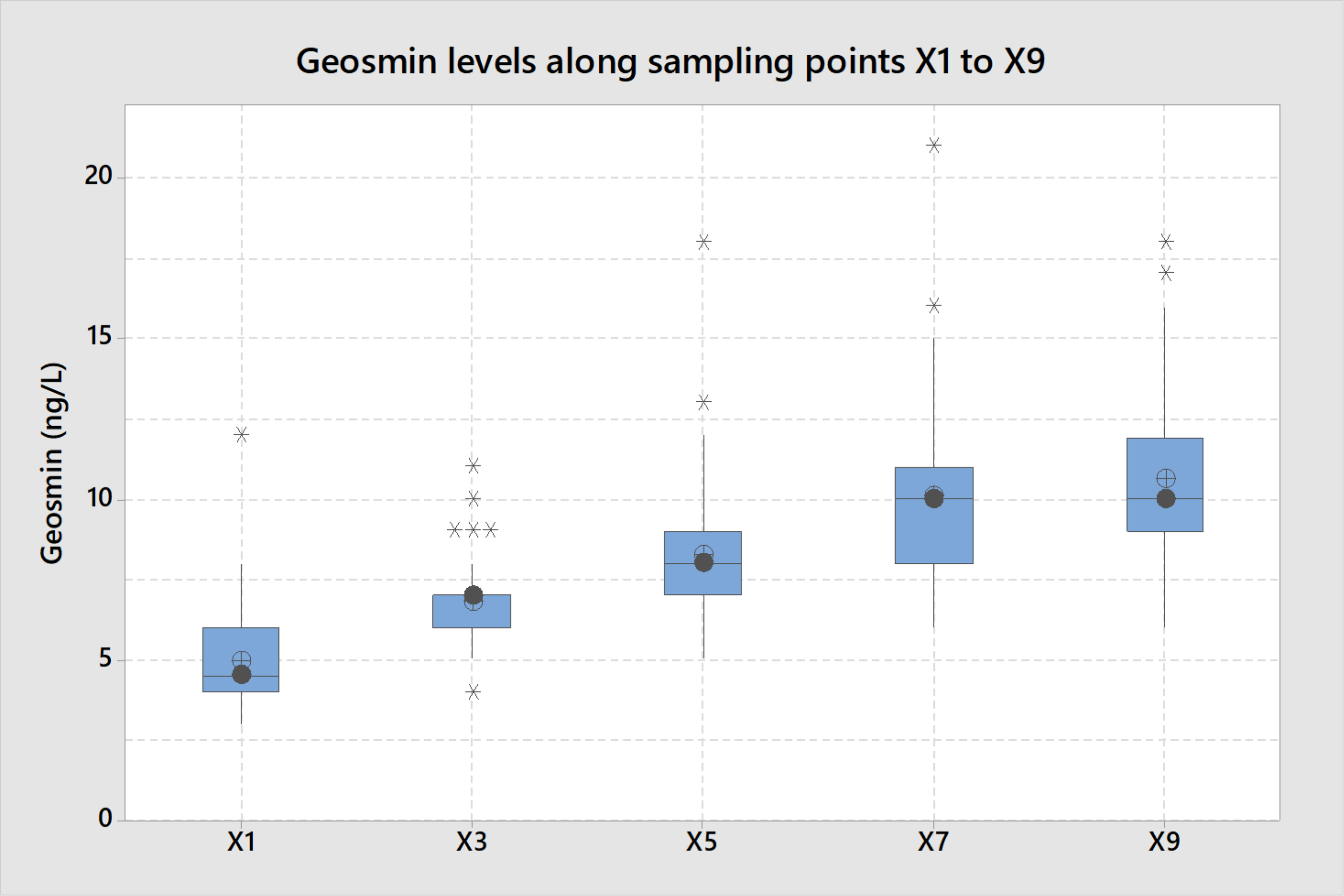
- $C_{12}H_{22}O$, MW: 182.3 g/mol
- Bicyclic terpenoid, **semi-volatile**
- **Earthy odor** (rain on soil)
- Odor Threshold Concentration (OTC) in water : **~ 4 ng/L** *(Young et al., WatRes 1996)*
- Produced by **benthic and planktonic cyanobacteria** *(Watson, J Tox Env Health 2004)*
- Production by **actinomycetes** in soil *(Zaitlin & Watson, Wat Res 2006)*
- Drinking water: main source of **consumer complaints** (second to chlorinous taste)





Production of geosmin in a section of the main water canal

- Geosmin was not detected in the main water reservoir, Mornos Lake.
- Concentrations of geosmin increased downstream a **section (~ 20km)** of the main water canal transferring water to WTPs.
- Sampling points X1 to X9 were established along the problematic section of the canal (X1 to X9 = 20km).

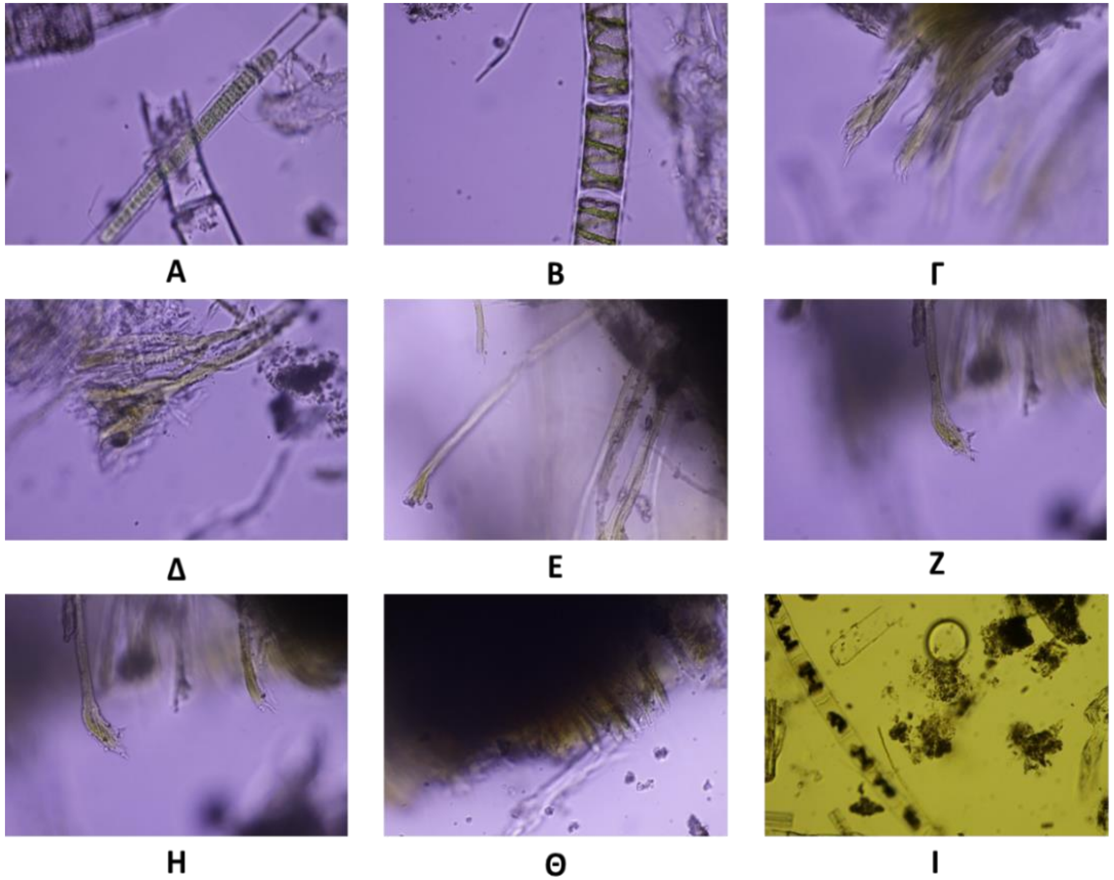


Benthic mats on the walls of the canal

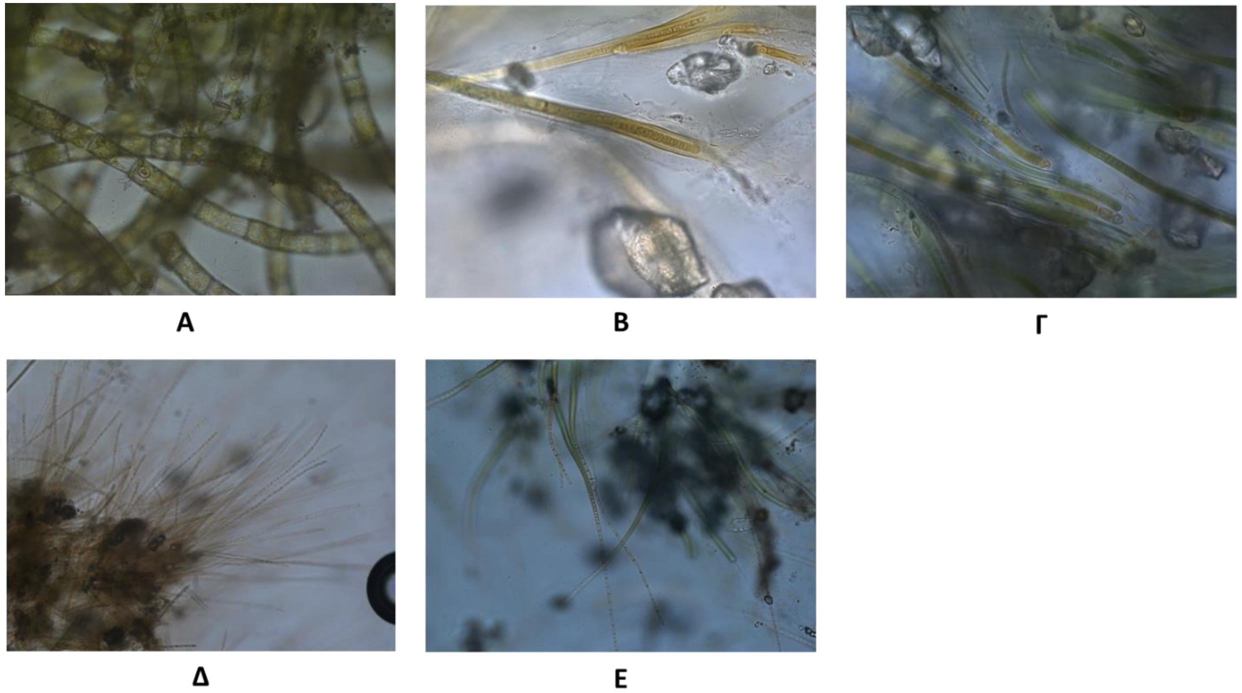
Benthic mats on canal walls, (a waterproofing membrane is used in this part of canal)

Benthic samples in the lab

Benthic cyanobacteria on the walls of the canal



Microscopic examination of benthic mats. (A-B): Spirogyra, (Γ-Θ): Possibly benthic cyanobacteria communities, (I): Spirogyra and organic matter *(photos: Maria vah Herk and Petra Visser, UvA).*



(A): Chlorophyceae & Spirogyra, (B): *Gloeotrichia punctulata*, (Γ-Δ) *Gloeotrichia echinulata*, (E): *Gloeotrichia sp*. *(photos: Manthos Panou & Spyros Gkelis, AUTH).*

Investigation for cyanotoxins and toxin genes (2019)

Samples	CYN	Anatoxin-a	[D-Asp3] MC-RR	MC-RR	NOD	MC-YR	MC-HtyR	[D-Asp3] MC-LR	MC-LR	MC-HilR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
All WTPs, treated	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
All WTPs, intake	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Canal water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benthic samples	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LOD (ng/L)	1	1	2	1	2	4	7	4	4	6	6	3	6	4	5

Summarized results of cyanotoxins analysis. Method: SPE-LC-MS/MS at NCSR Demokritos. LODs refer to water samples.

Samples	Gene copies/ml			
	Cyanobacteria (16s RNA)	MC-NOD *(mcyE)*	CYN *(cyrA)*	SXT *(sxtA)*
X5 – Canal water	3427	ND	ND	ND
X7 – Canal water	4360	ND	ND	ND
X5-Benthic sample	216450	ND	ND	ND

Examples of multiplex-qPCR results (Phytoxigene-CyanoDTec kit)

Additional screening for MCs was carried out with PP2A assay (Abraxis).

Cyanotoxins/cyanotoxin genes were NOT detected.

Metagenomic analysis (Nico Salmaso, FEM)

Sampling and eDNA extraction protocols:

- Rimet et al. 2021, Lake biofilms sampling for both downstream DNA analysis and microscopic counts. https://dx.doi.org/10.17504/protocols.io.br2xm8fn

Library preparation and bioinformatic analyses:

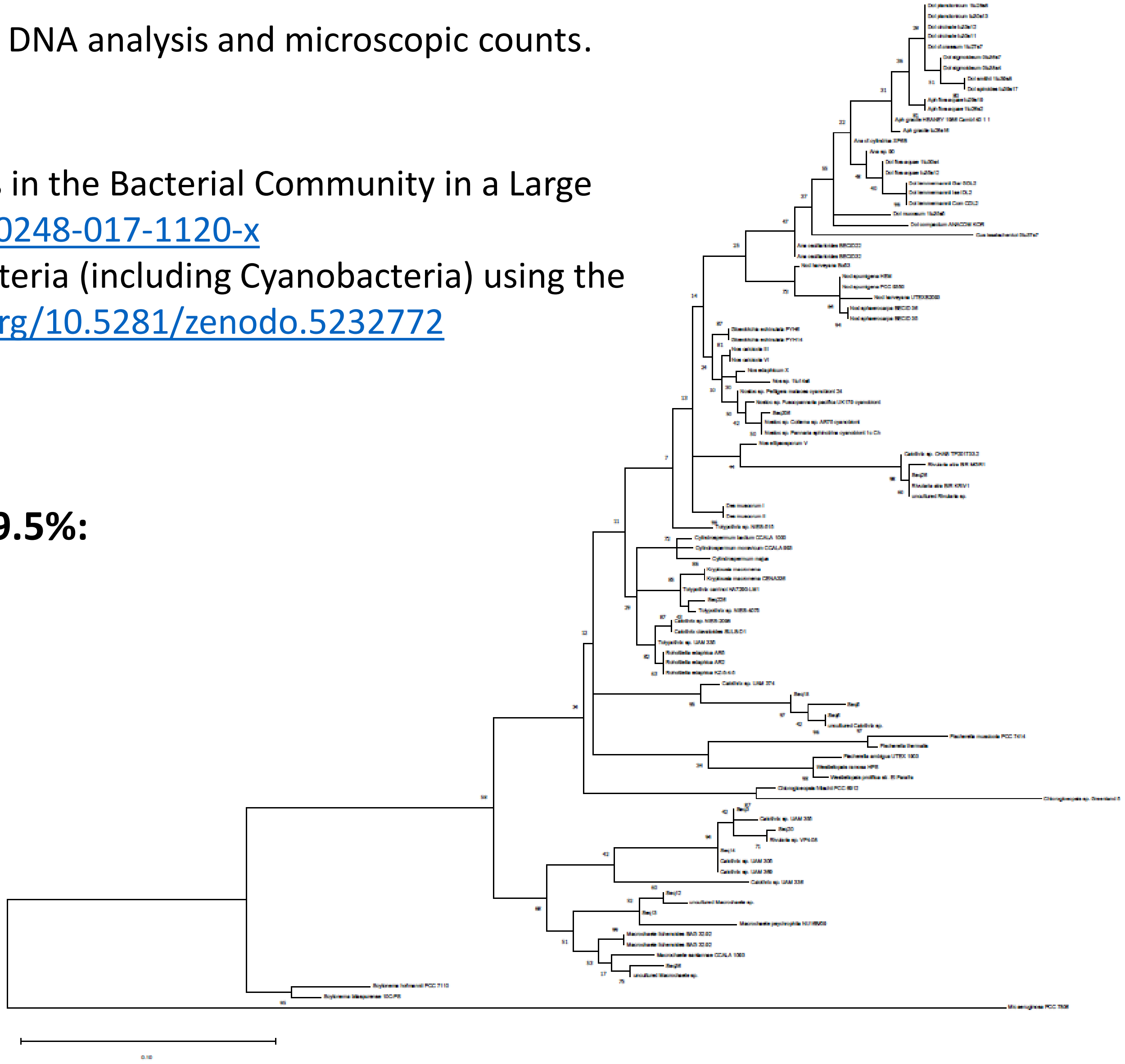
- Salmaso et al., 2018. Diversity and Cyclical Seasonal Transitions in the Bacterial Community in a Large and Deep Perialpine Lake. http://link.springer.com/10.1007/s00248-017-1120-x
- Salmaso et al., 2021. Metabarcoding protocol – Analysis of Bacteria (including Cyanobacteria) using the 16S rRNA gene and a DADA2 pipeline (Version 1). https://doi.org/10.5281/zenodo.5232772

Sampling: 22 March 2021

Main cyanobacteria found with % sequence similarity > 99.5%:

Calothrix spp.
Rivularia spp.
Nostoc spp.
Scytonema spp.
Tolypothrix spp.

Many sequences did not find taxonomic coverage in reference databases (GenBank)



Management and control measures

challenge: the flow must go on…

▪ Canal wall scraping

— Scraping 15km of canal walls (where there was no waterproofing membrane), using excavators.

— Only side walls were scrapped (not the bottom of the canal).

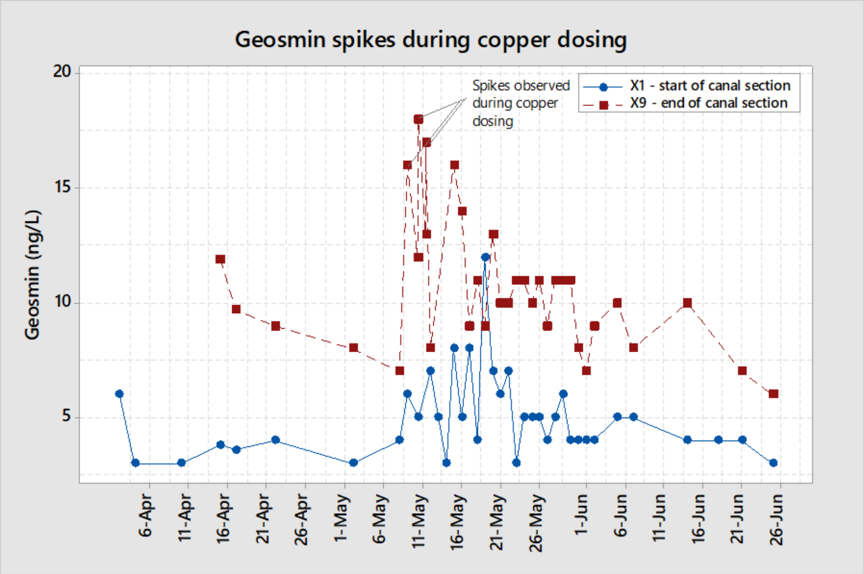
— Care not to damage canal walls – divers to safeguard the process.

▪ Copper sulfate dosing

— Dose: 0.11 ppm, for 3 consecutive days, 10-11 h/day during daytime

— Max Cu concentration measured in water was 0.070 ppm.

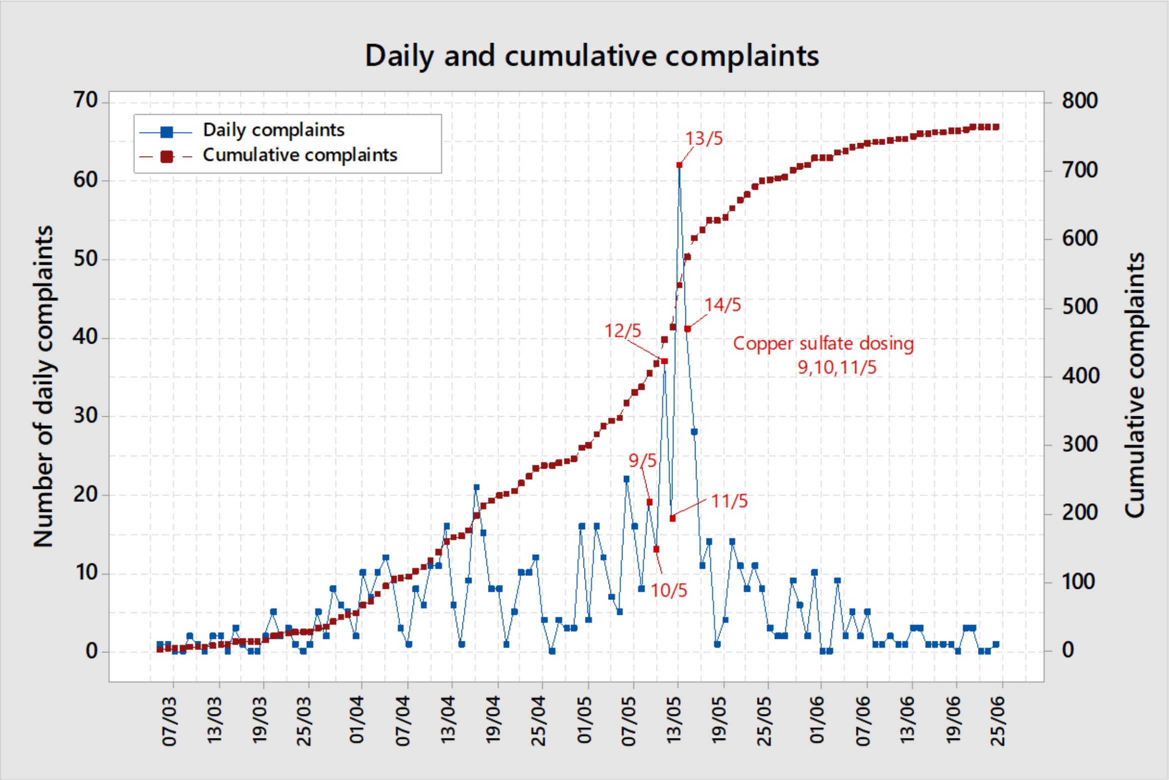
— Spikes of geosmin were observed, as expected from cell lysis.

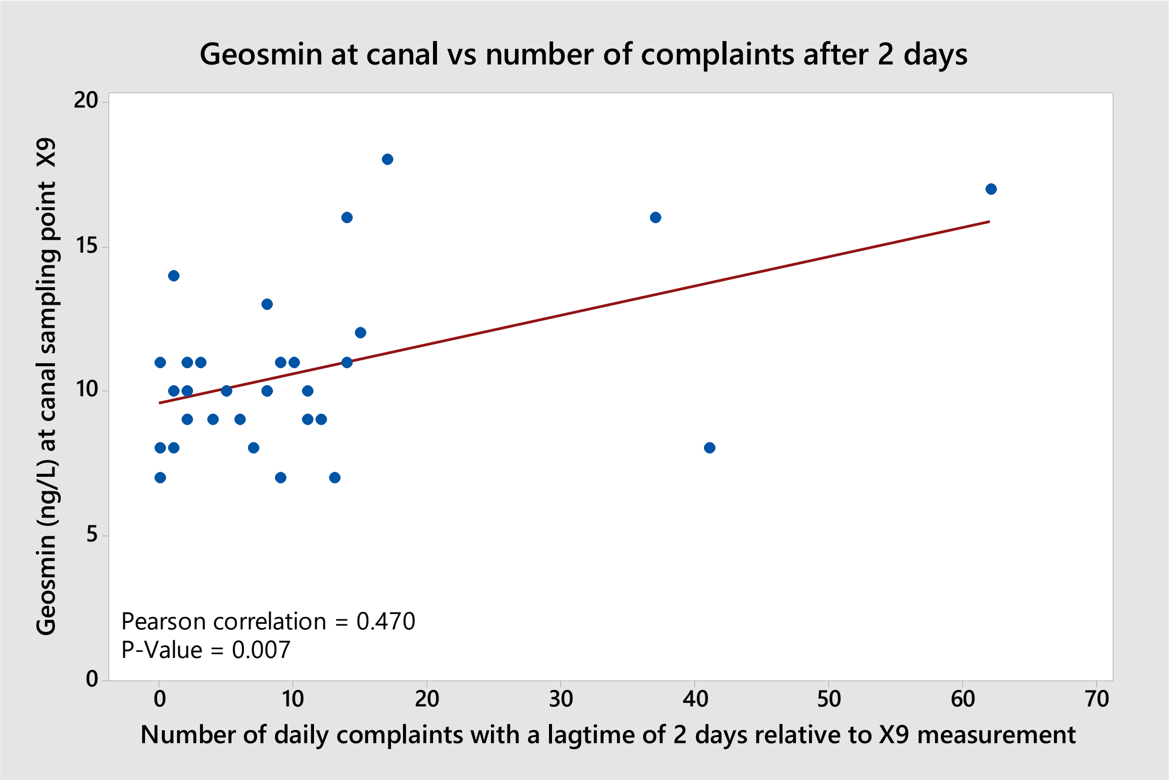


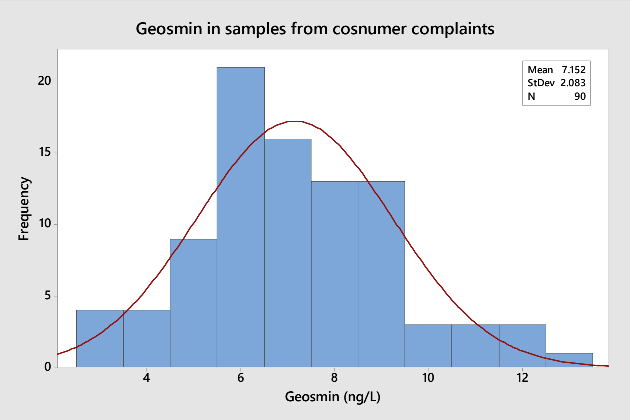
Outcome:

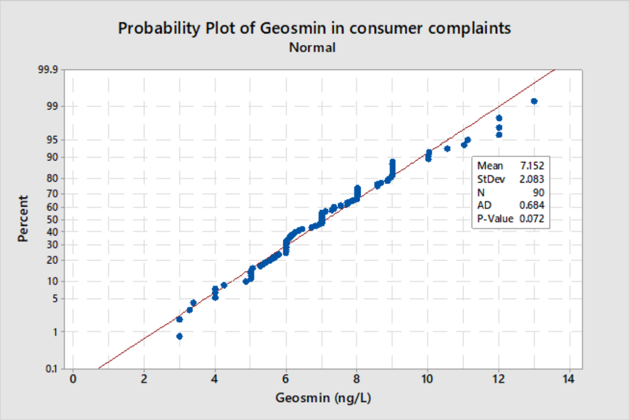
- **No complete elimination** of cyanobacteria/geosmin after wall scraping & copper sulfate dosing.
- Geosmin levels dropped gradually to < 4 ng/L in mid-summer.

Consumer complaints









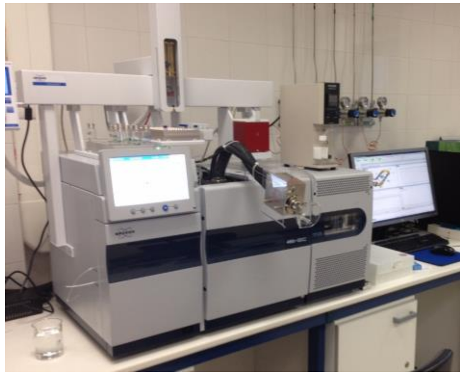
- Complaints spiked during copper dosing.
- Correlation of geosmin in canal with number of complaints after 2 days.
- Training of the call center personnel and personnel handling the complaints and sampling from taps. Q&A list to answer consumers' questions *(Burlingame et al. Chapter 9 in "T&O in Source & Drinking Water" IWA 2019, EPA/600/R-07/027, 2007).*

Methods

Analysis of geosmin and T&O (EYDAP):

- Untargeted analysis of a wide range of T&O with automated HS-SPME-GC-MS.
- HS-SPME-GC-MS & GC-MS/MS methods for geosmin-MIB. (accredited, ISO 17025)

(PAL autosampler - Bruker 456 GC – TQ MS) *(Kaloudis et al., Handbook Cyan. Mon. Cyan. Anal. 2016).*

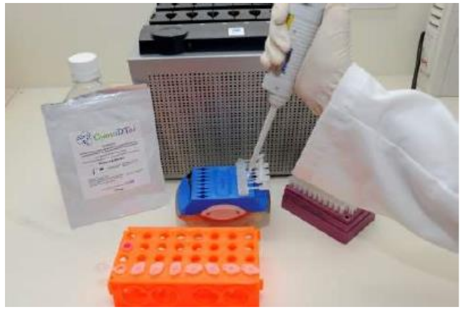


Analysis of cyanotoxins (NCSR Demokritos, EYDAP):

- Targeted determination of MCs, NOD, ATX-a and CYN with SPE-LC-MS/MS (Thermo LC-TSQ-MS) *(Zervou et al., HazMat 2017).* (accredited, ISO 17025)
- Additional screening of MCs-NOD by PP2A (Abraxis) in microplate format (TECAN M200 reader) *(Kaloudis et al., HazMat 2013)*.

Metagenomic analysis (FEM):

- Extraction of eDNA from biofilms/nets *(Rimet et al. 2020; Vautier et al., 2020)*, followed by amplicon sequencing analysis (Illumina MiSeq) of 16S rRNA genes, and determination of amplicon sequence variants (ASVs) using DADA2 *(Callahan et al., 2016; Salmaso et al., 2021)*.



Cyanobacteria and cyanotoxin genes (EYDAP):

- 16s rRNA (total cyanobacteria), mcyE (MCs-NOD), cyrA (CYN), sxtA (SXTs) genes (Phytoxigene - CyanoDTec kit) with multiplex qPCR (Cepheid SmartCycler II), *(McKindles et al., Toxins 2019)*.

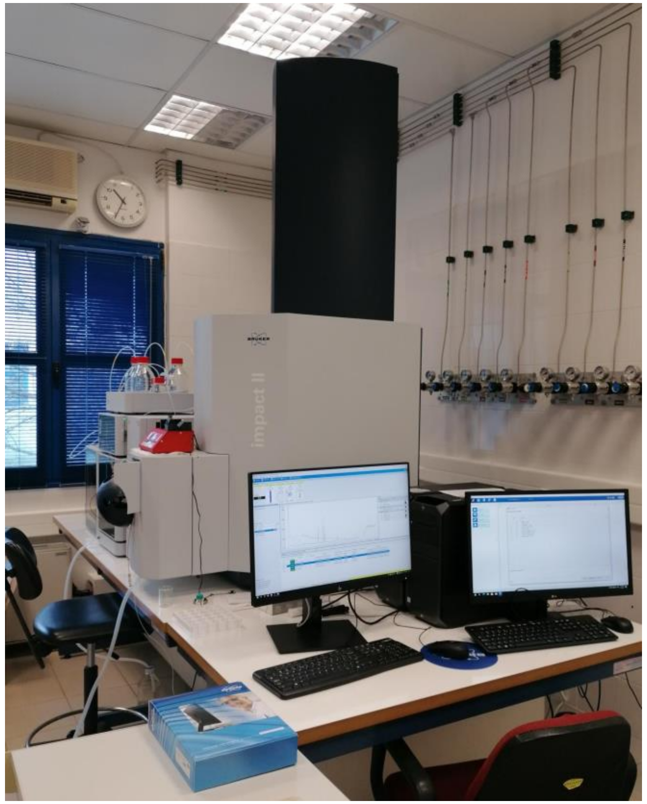
Microscopic analysis (UvA, AUTH):

- Microscopic examination of samples (e.g. Zeiss Axio imager z2 microscope - Axio Cam MRc5 digital camera).

Future research and management plans

- Recurring episodes: 2020 and 2021 (March-September), less consumer complaints.
- March 2022: Pilot project using H_2O_2 and possibly solid peroxides (sodium percarbonate). Collaboration with Petra Visser's group.
  - a) Laboratory scale experiments to test effectiveness and optimal conditions (key parameter: photosynthetic vitality).
  - b) Pilot application of peroxides in the canal.
- Switch to canal brushing instead of scraping.
- Continuation of geosmin and cyanotoxins monitoring.
- Spatiotemporal detection of benthic cyanometabolites (untargeted LC-HRMS).

- Long term: Upgrades in water treatment and light barriers at the canal.



Impact II LC-qToF at LOM-EYDAP

Acknowledgements

EYDAP

Laboratory of Organic Micropollutants

Institute Nanoscience Nanotechnology
Ινστιτούτο Νανοεπιστήμης Νανοτεχνολογίας

LEAD
Laboratory of Environmental Analysis
NCSR Demokritos

University of Amsterdam

Aristotle University of Thessaloniki

Fondazione Edmund Mach
Centro Ricerca e Innovazione

CyanoLab
Aristotle University of Thessaloniki

EPA United States Environmental Protection Agency

Benthic HABs Discussion Group

Now open (till July 2022):

AWWA Water Science
Theory and Practice of Safe, Sustainable Water

Topical Collection on Harmful Algal Blooms

Guest Editors: Donysios (Dion) Dionysiou, Nicole Blute, Tri Kaloudis, Lauren Weinrich, Arash Zamyadi

AWWA Water Science Topical Collection Link

WaterTOP
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Growth or Toxicity

GENETIC DIVERGENCE, FLEXIBILITY AND SECONDARY METABOLISM DISTINGUISHING TOXIC AND NON-TOXIC MEMBERS OF A WIDESPREAD FRESHWATER CYANOBACTERIAL GENUS – *MICROCOLEUS*

Hwee Sze Tee
University of Auckland, New Zealand

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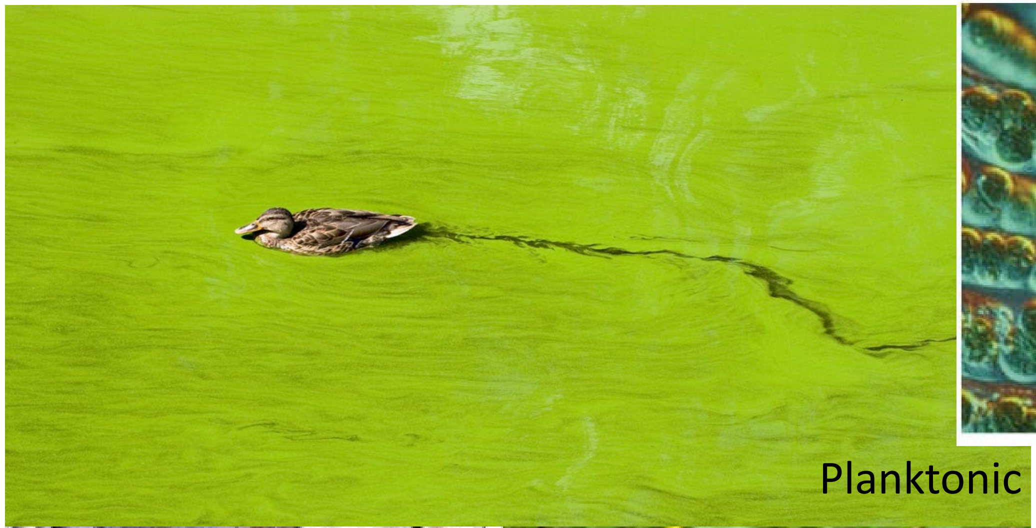
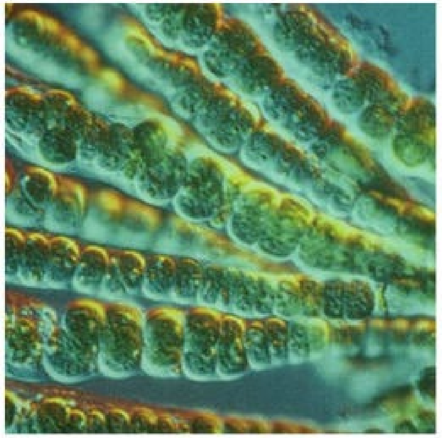
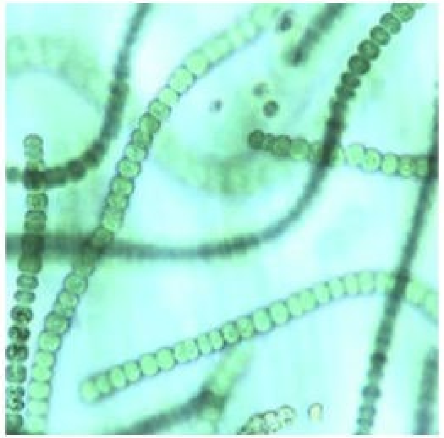
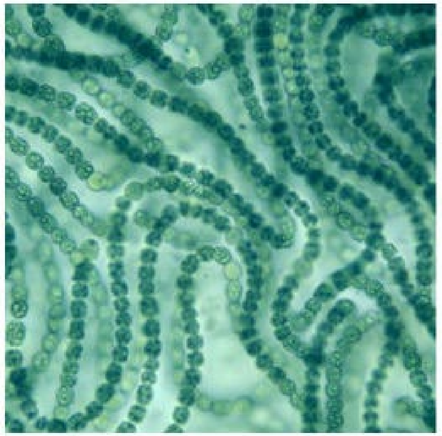
What is cyanobacteria?









- Ancient, 2 billion years ago
- Produce oxygen

Harmful cyanobacterial bloom

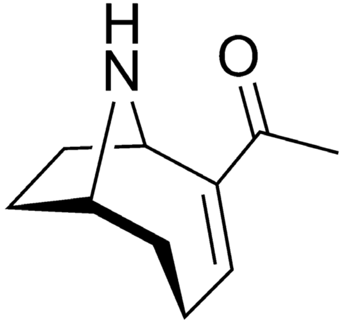
- Disrupt habitat/ Outcompete
- Water quality/eutrophication, oxygen depletion
- Toxins /taste & odour compounds

DANGEROUS!

Microcoleus sp.

-Bloom in low phosphate condition

Some produce cyanotoxin (Anatoxin-a)



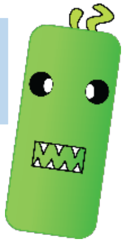
Very Fast Death Factor

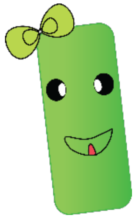
Characterization of toxic and non-toxic strains of *Microcoleus*

Microcoleus bloom toxicity??

Toxic (anatoxin)

found in sites with **higher** nitrogen
(Bouma-Gregson et al., 2019)





Non-toxic (anatoxin-deficient)

found in sites with **lower** nitrogen
(Bouma-Gregson et al., 2019)

Hypotheses:

Toxic *Microcoleus* strains are **less efficient** in nutrient acquisition than non-toxic *Microcoleus* strains, likely due to the cost/investment in toxin production.

Characterization of toxic and non-toxic strains of *Microcoleus*

Total number of *Microcoleus* : 42 (12 toxic + 30 non-toxic)

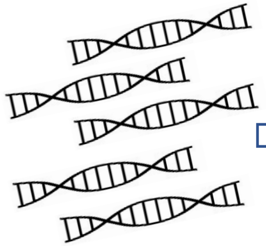
10 NZ isolates
1 US isolate

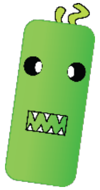
3 US isolates
from NCBI

26 US MAGs

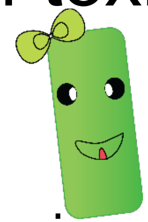
2 NZ MAGs

DNA sequencing
(Illumina Hi-Seq)



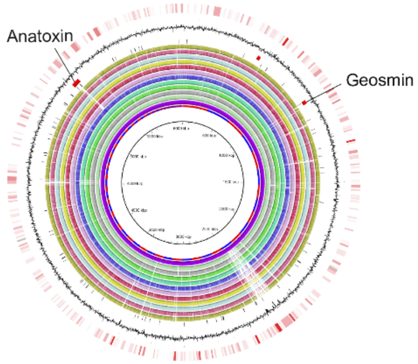


Contigs Assembly



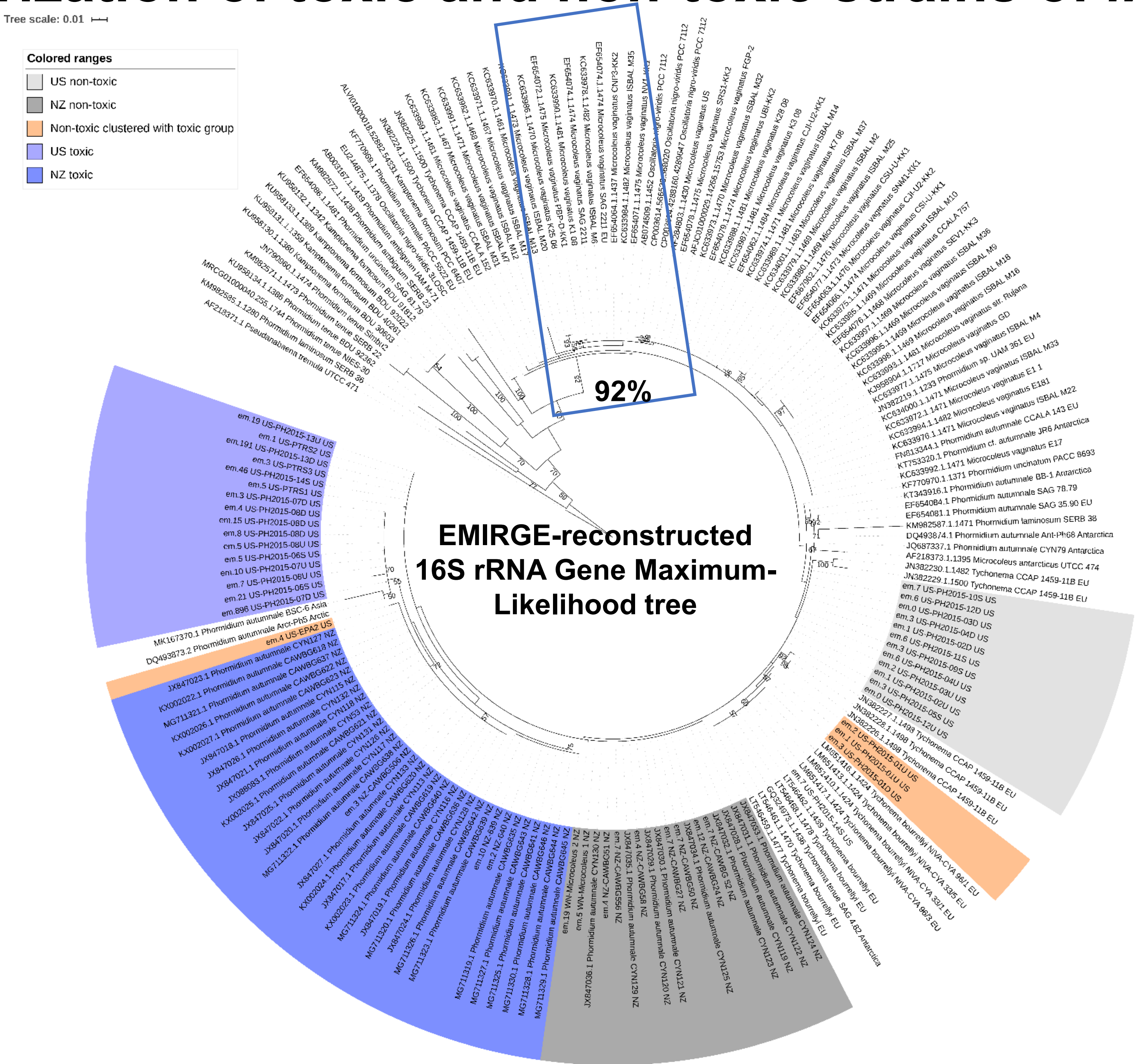
Binning

Comparative genomic analyses



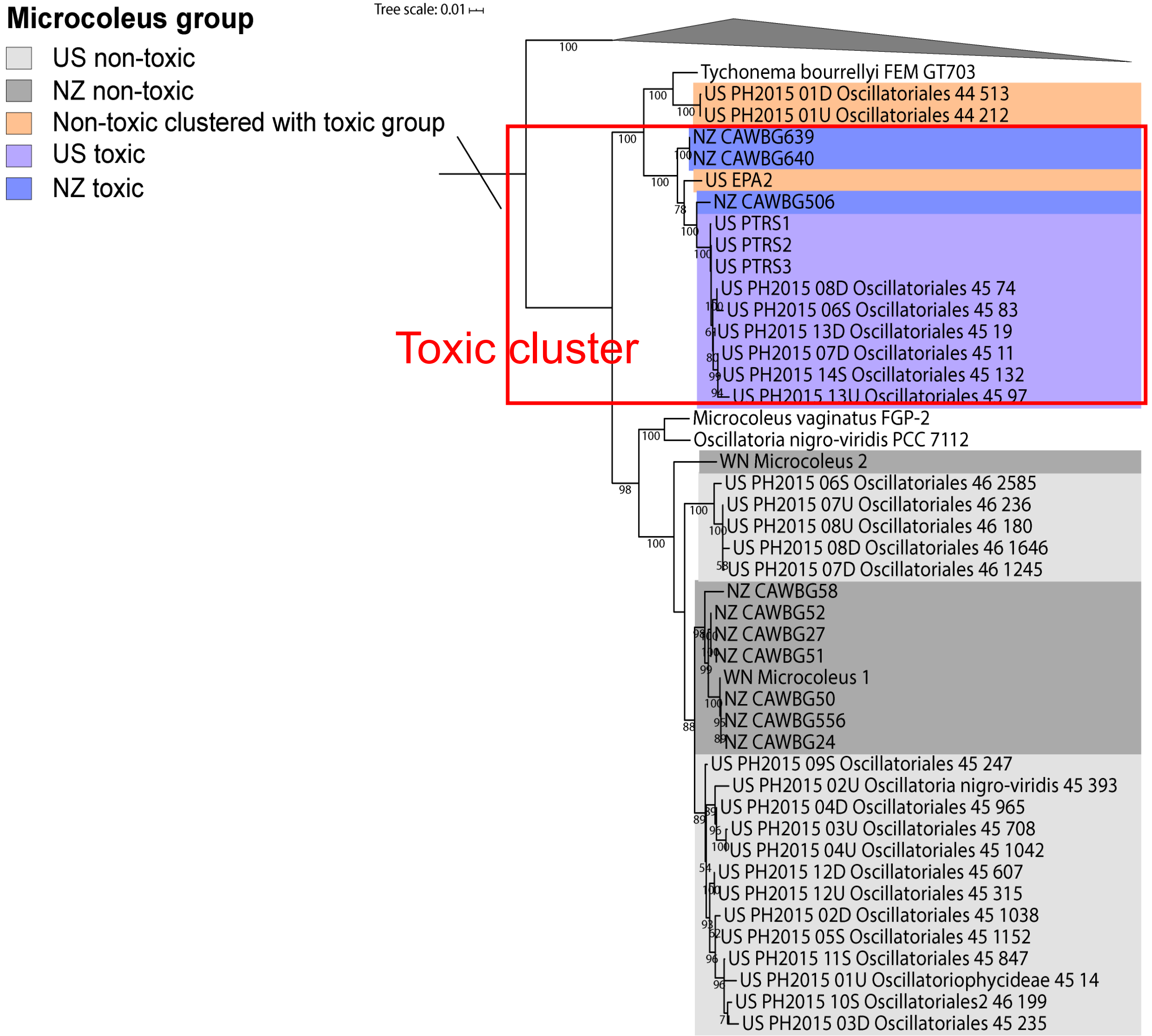
MAGs= Metagenome-assembled genomes

Characterization of toxic and non-toxic strains of *Microcoleus*

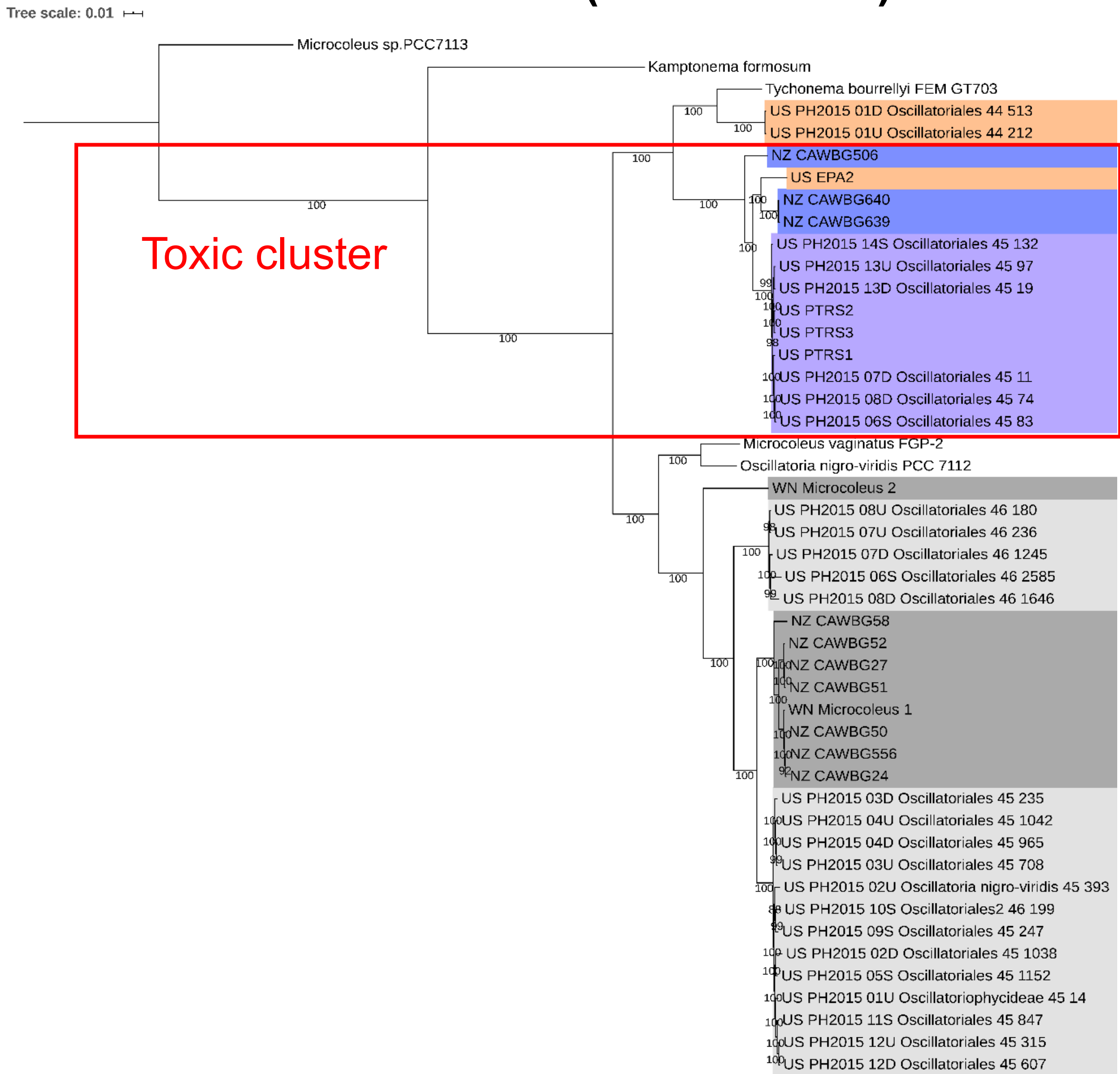


Characterization of toxic and non-toxic strains of *Microcoleus*

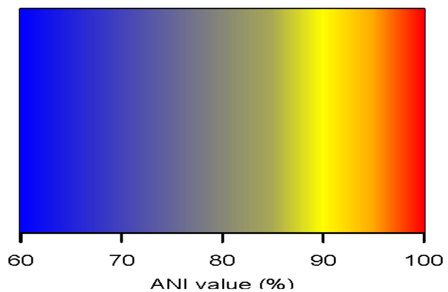
Core Marker Genes Maximum-Likelihood tree (GTDB)



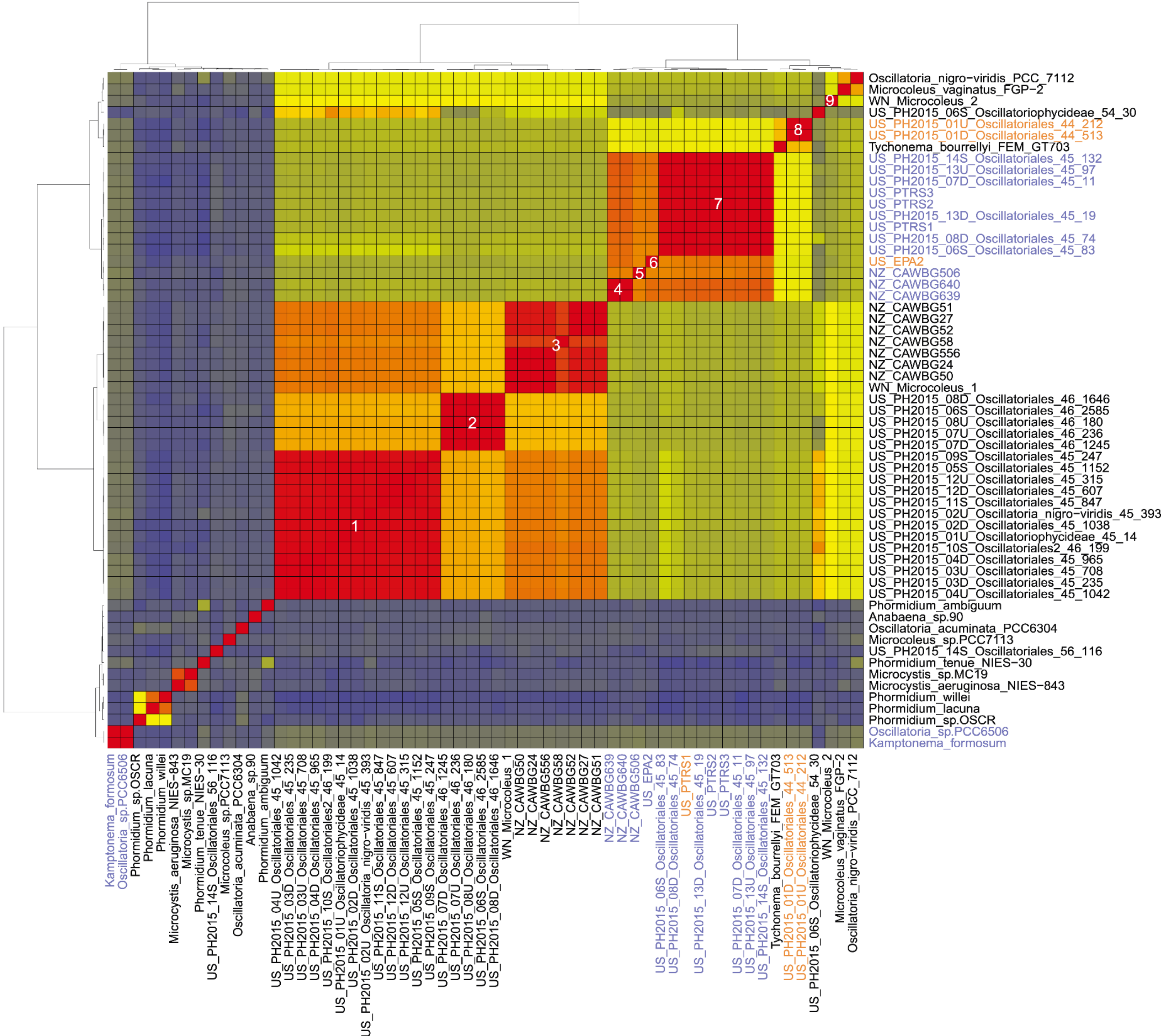
Single-Copy Core Orthologs Maximum-likelihood tree (orthoFinder)



Average Nucleotide Identity

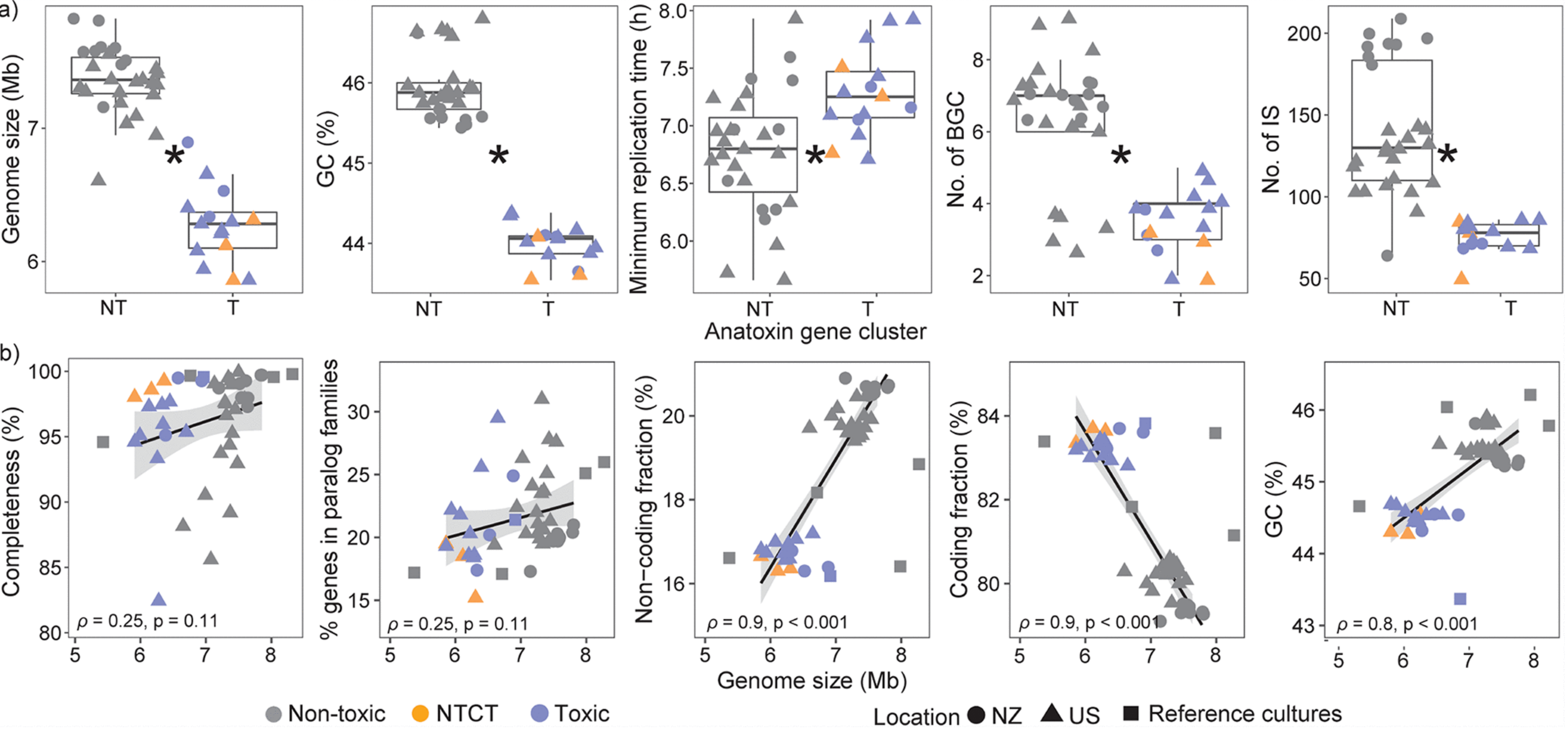


9 species among 42 strains
DDH: >70%
ANI : >96.5%



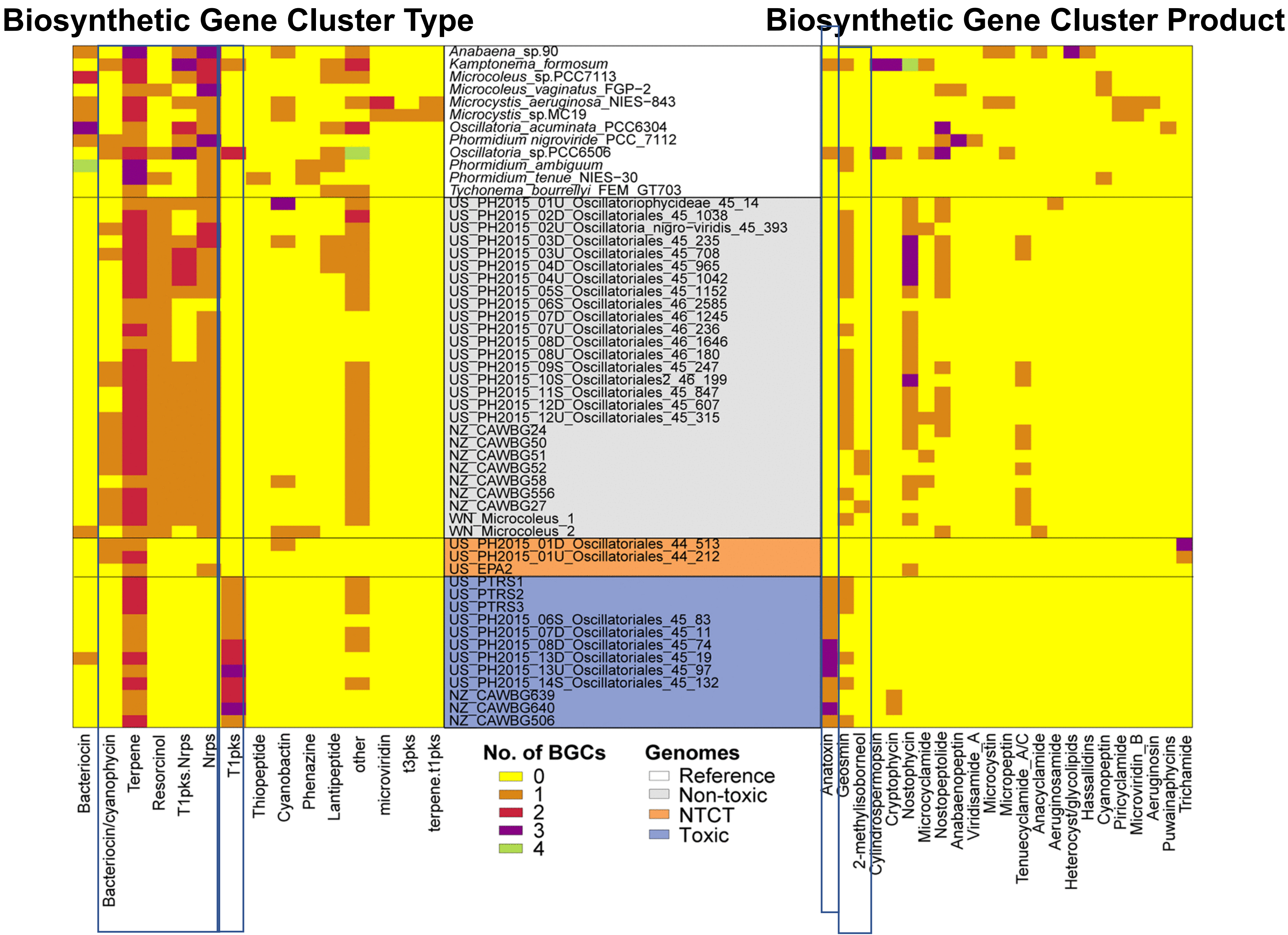
Characterization of toxic and non-toxic strains of *Microcoleus*

BGC= Biosysnthetic gene cluster (Secondary metabolites)   IS= Insertion sequence

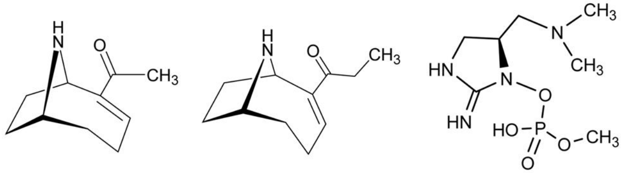


Other cyanobacterial genomes

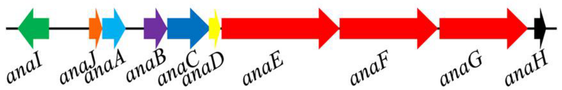
Species	Strain	Toxic/non-toxic	Genome size (Mb)
Cylindrospermopsis raciborskii	CS-506	CYN+	4.1
	CS-505	CYN+	3.9
	CS-509	CYN-	4.0
Cylindrospermopsis raciborskii	CS-505	CYN+	4.2
	CR12	CYN+	3.7
	CENA302	SXT+	3.5
	ITEP-A1	SXT+	3.6
	MVCC14	SXT+	3.6
	CS-508	CYN-, SXT-	3.6
	CENA303	CYN-, SXT-	3.4
Microcystis aeruginosa	NIES-843	MCY+	5.8
	PCC 7806SL	MCY+	5.1
	NIES-298	MCY+	5.0
	NIES-88	MCY+	5.3
	KW	MCY+	5.9
	DIANCHI905	MCY+	4.9
	PCC 7941	MCY+	4.8
	CHAOHU 1326	MCY+	5.3
	PCC 9443	MCY+	5.2
	PCC 9807	MCY+	5.2
	PCC 9808	MCY+	5.1
	PCC 9809	MCY+	5.0
	Sj	MCY+	4.6
	SPC777	MCY+	5.5
	PCC 9806	MCY-	4.3
	PCC 9701	MCY-	4.8
	PCC 9432	MCY-	5.0
	NIES-98	MCY-	5.0
	NIES-1211	MCY-	4.7
	NIES-2549	MCY-	4.3
	NIES-2481	MCY-	4.4
	PCC 7005	MCY-	4.9
	TAIHU98	MCY-	4.8
	NIES-87	MCY-	4.9
	NIES-44	MCY-	4.6


Biosynthetic Gene Cluster Type
Biosynthetic Gene Cluster Product
Anabaena_sp.90
Kamptonema_formosum
Microcoleus_sp.PCC7113
Microcoleus_vaginatus_FGP-2
Microcystis_aeruginosa_NIES-843
Microcystis_sp.MC19
Oscillatoria_acuminata_PCC6304
Phormidium_nigroviride_PCC_7112
Oscillatoria_sp.PCC6506
Phormidium_ambiguum
Phormidium_tenue_NIES-30
Tychonema_bourrellyi_FEM_GT703
US_PH2015_01U_Oscillatoriophycideae_45_14
US_PH2015_02D_Oscillatoriales_45_1038
US_PH2015_02U_Oscillatoria_nigro-viridis_45_393
US_PH2015_03D_Oscillatoriales_45_235
US_PH2015_03U_Oscillatoriales_45_708
US_PH2015_04D_Oscillatoriales_45_965
US_PH2015_04U_Oscillatoriales_45_1042
US_PH2015_05S_Oscillatoriales_45_1152
US_PH2015_06S_Oscillatoriales_46_2585
US_PH2015_07D_Oscillatoriales_46_1245
US_PH2015_07U_Oscillatoriales_46_236
US_PH2015_08D_Oscillatoriales_46_1646
US_PH2015_08U_Oscillatoriales_46_180
US_PH2015_09S_Oscillatoriales_45_247
US_PH2015_10S_Oscillatoriales2_46_199
US_PH2015_11S_Oscillatoriales_45_847
US_PH2015_12D_Oscillatoriales_45_607
US_PH2015_12U_Oscillatoriales_45_315
NZ_CAWBG24
NZ_CAWBG50
NZ_CAWBG51
NZ_CAWBG52
NZ_CAWBG58
NZ_CAWBG556
NZ_CAWBG27
WN_Microcoleus_1
WN_Microcoleus_2
US_PH2015_01D_Oscillatoriales_44_513
US_PH2015_01U_Oscillatoriales_44_212
US_EPA2
US_PTRS1
US_PTRS2
US_PTRS3
US_PH2015_06S_Oscillatoriales_45_83
US_PH2015_07D_Oscillatoriales_45_11
US_PH2015_08D_Oscillatoriales_45_74
US_PH2015_13D_Oscillatoriales_45_19
US_PH2015_13U_Oscillatoriales_45_97
US_PH2015_14S_Oscillatoriales_45_132
NZ_CAWBG639
NZ_CAWBG640
NZ_CAWBG506
Bacteriocin
Bacteriocin/cyanophycin
Terpene
Resorcinol
T1pks.Nrps
Nrps
T1pks
Thiopeptide
Cyanobactin
Phenazine
Lantipeptide
other
microviridin
t3pks
terpene.t1pks
No. of BGCs
0
1
2
3
4
Genomes
Reference
Non-toxic
NTCT
Toxic
Anatoxin
Geosmin
2-methylisoborneol
Cylindrospermopsin
Cryptophycin
Nostophycin
Microcyclamide
Nostopeptolide
Anabaenopeptin
Viridisamide_A
Microcystin
Micropeptin
Tenuecyclamide_A/C
Anacyclamide
Aeruginosamide
Heterocyst/glycolipids
Hassallidins
Cyanopeptin
Piricyclamide
Microviridin_B
Aeruginosin
Puwainaphycins
Trichamide

Anatoxin-a structures and gene clusters

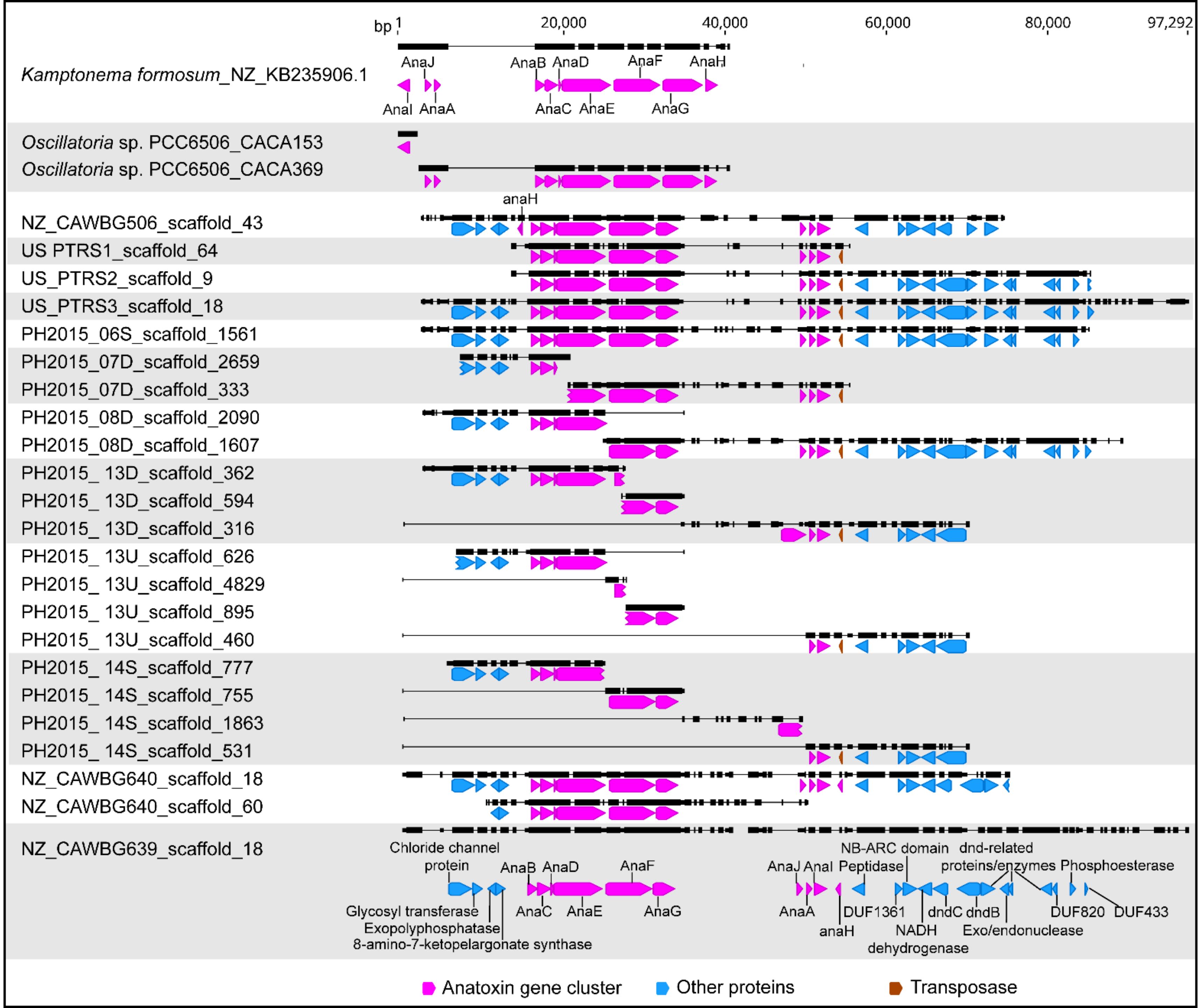


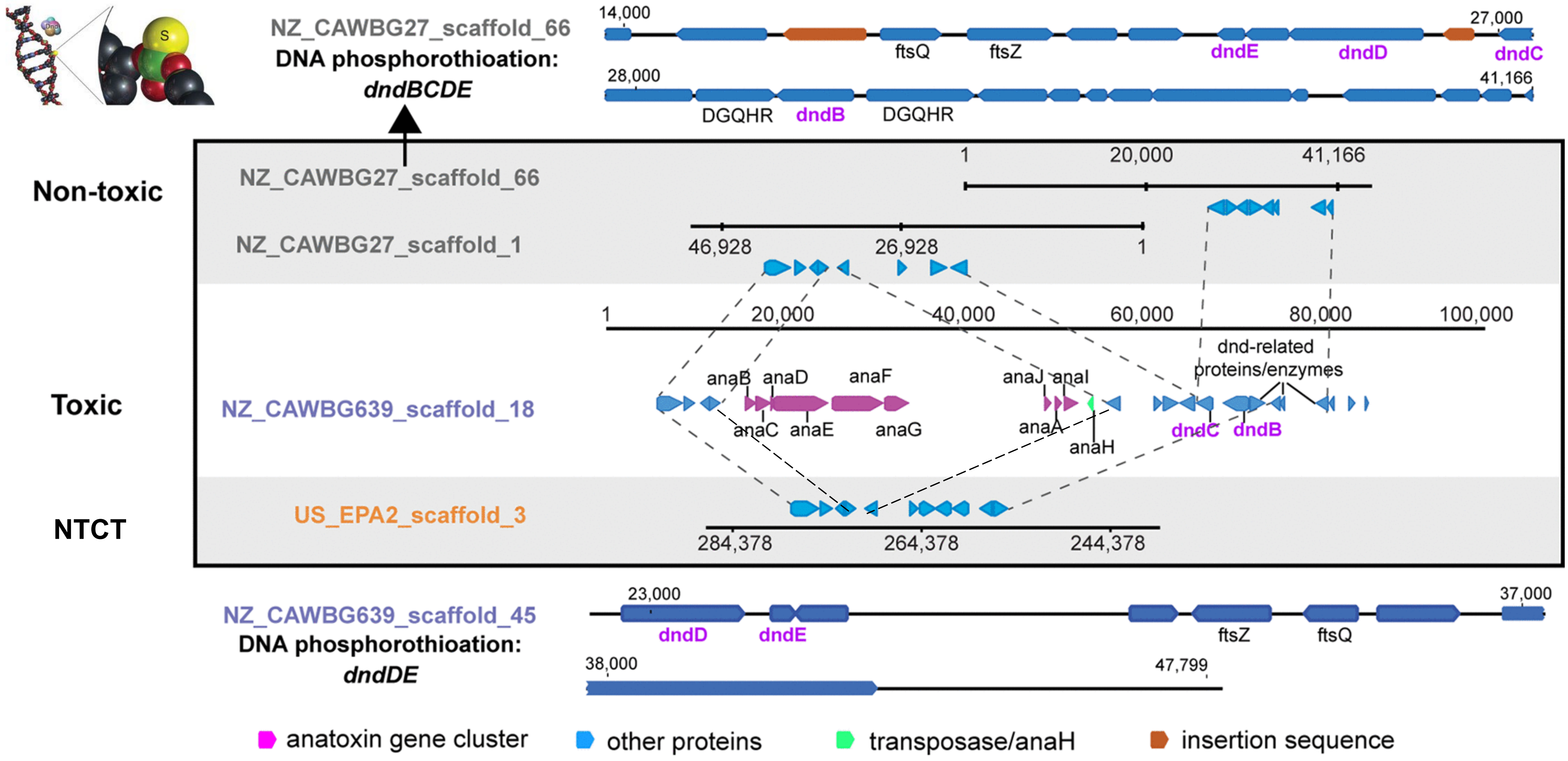
Anatoxin-a Homoanatoxin-a Anatoxin-a(s)



Anatoxin gene (*ana*) cluster

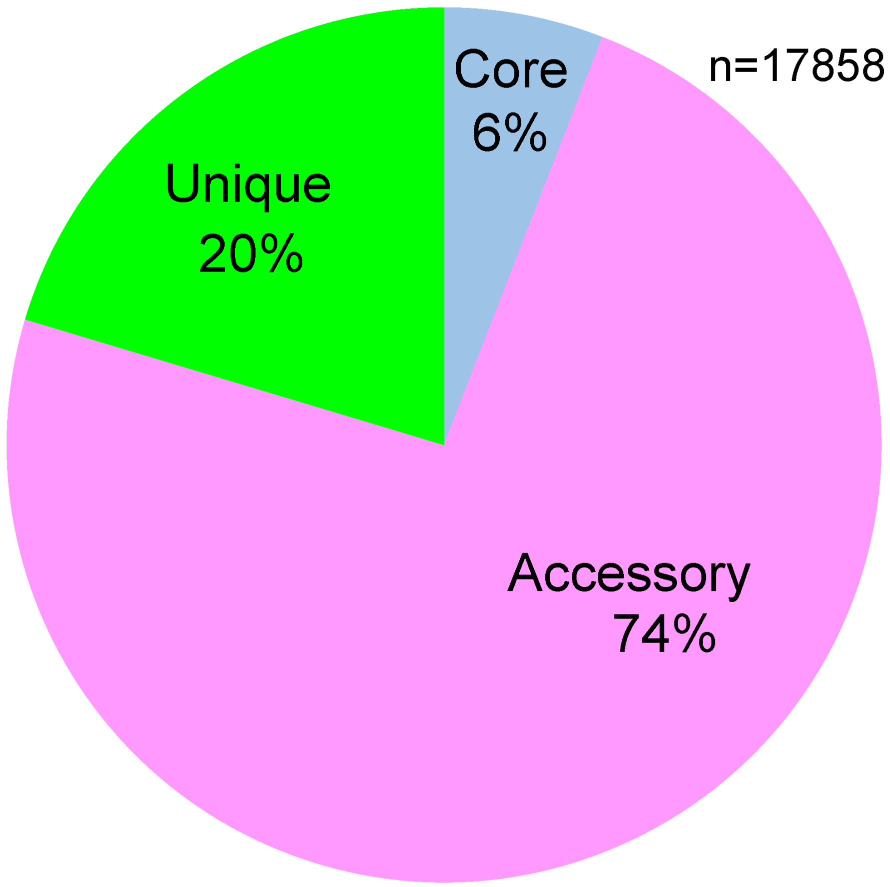
Rastogi et al., 2015



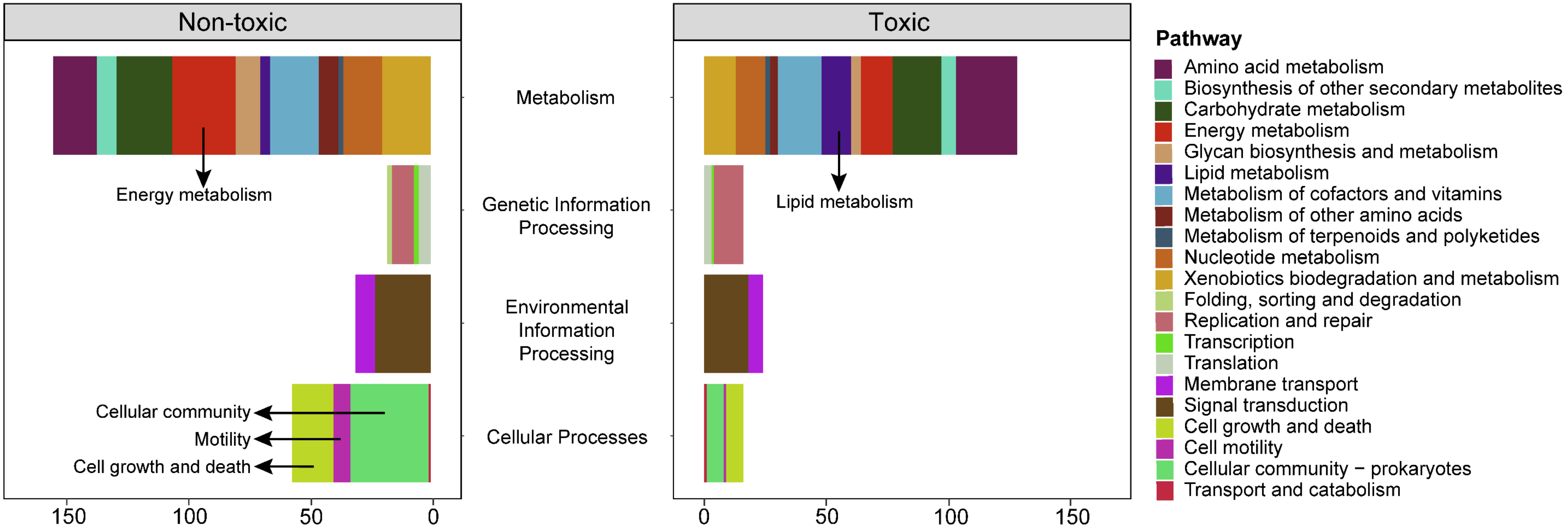


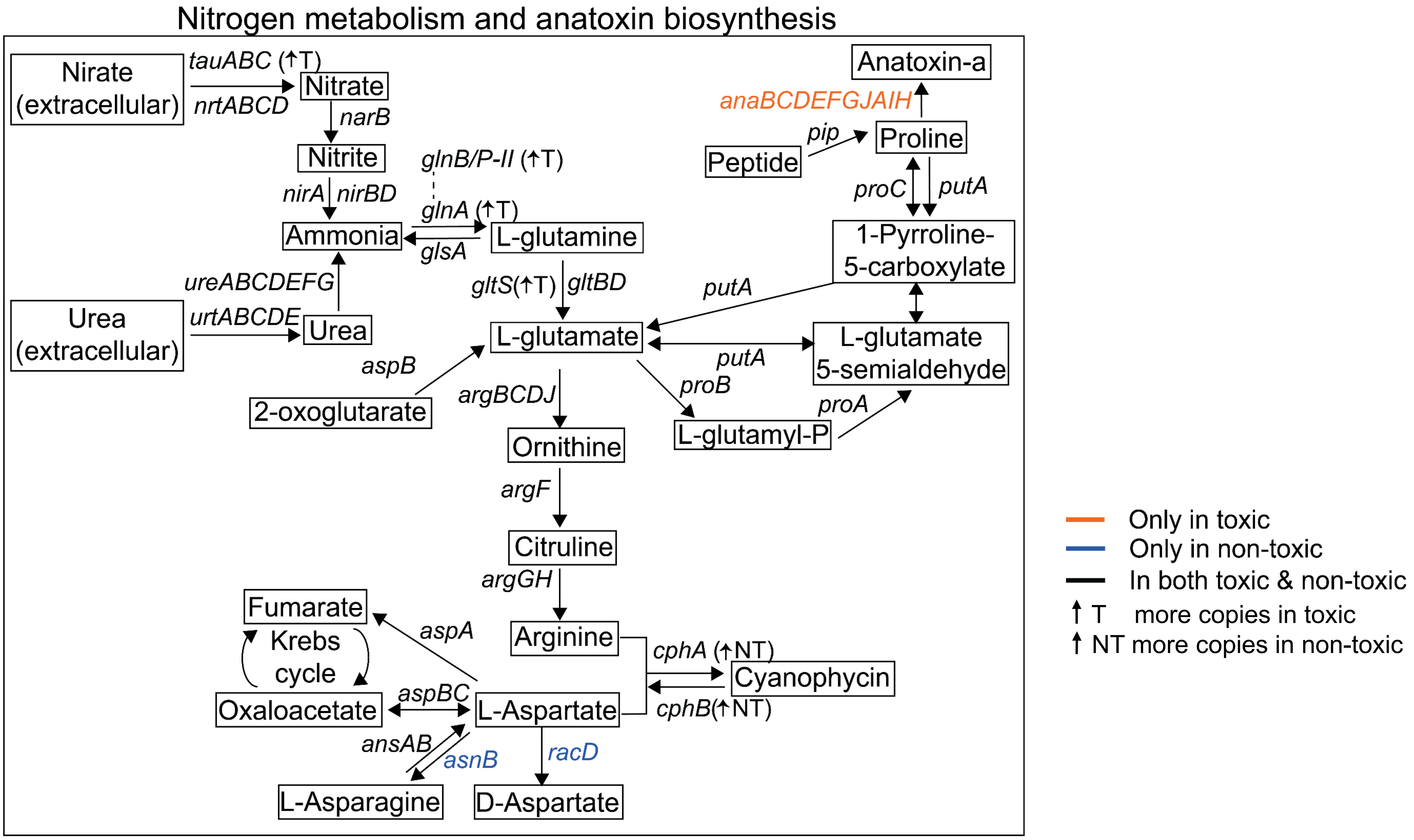
NZ_CAWBG27_scaffold_66
DNA phosphorothioation:
dndBCDE
14,000
27,000
ftsQ
ftsZ
dndE
dndD
dndC
28,000
41,166
DGQHR
dndB
DGQHR
Non-toxic
NZ_CAWBG27_scaffold_66
1
20,000
41,166
NZ_CAWBG27_scaffold_1
46,928
26,928
1
20,000
40,000
60,000
80,000
100,000
dnd-related proteins/enzymes
Toxic
NZ_CAWBG639_scaffold_18
anaB
anaD
anaF
anaJ
anaI
anaC
anaE
anaG
anaA
anaH
dndC
dndB
NTCT
US_EPA2_scaffold_3
284,378
264,378
244,378
NZ_CAWBG639_scaffold_45
DNA phosphorothioation:
dndDE
23,000
37,000
dndD
dndE
ftsZ
ftsQ
38,000
47,799
anatoxin gene cluster
other proteins
transposase/anaH
insertion sequence

Number of Pan-genome Gene Clusters

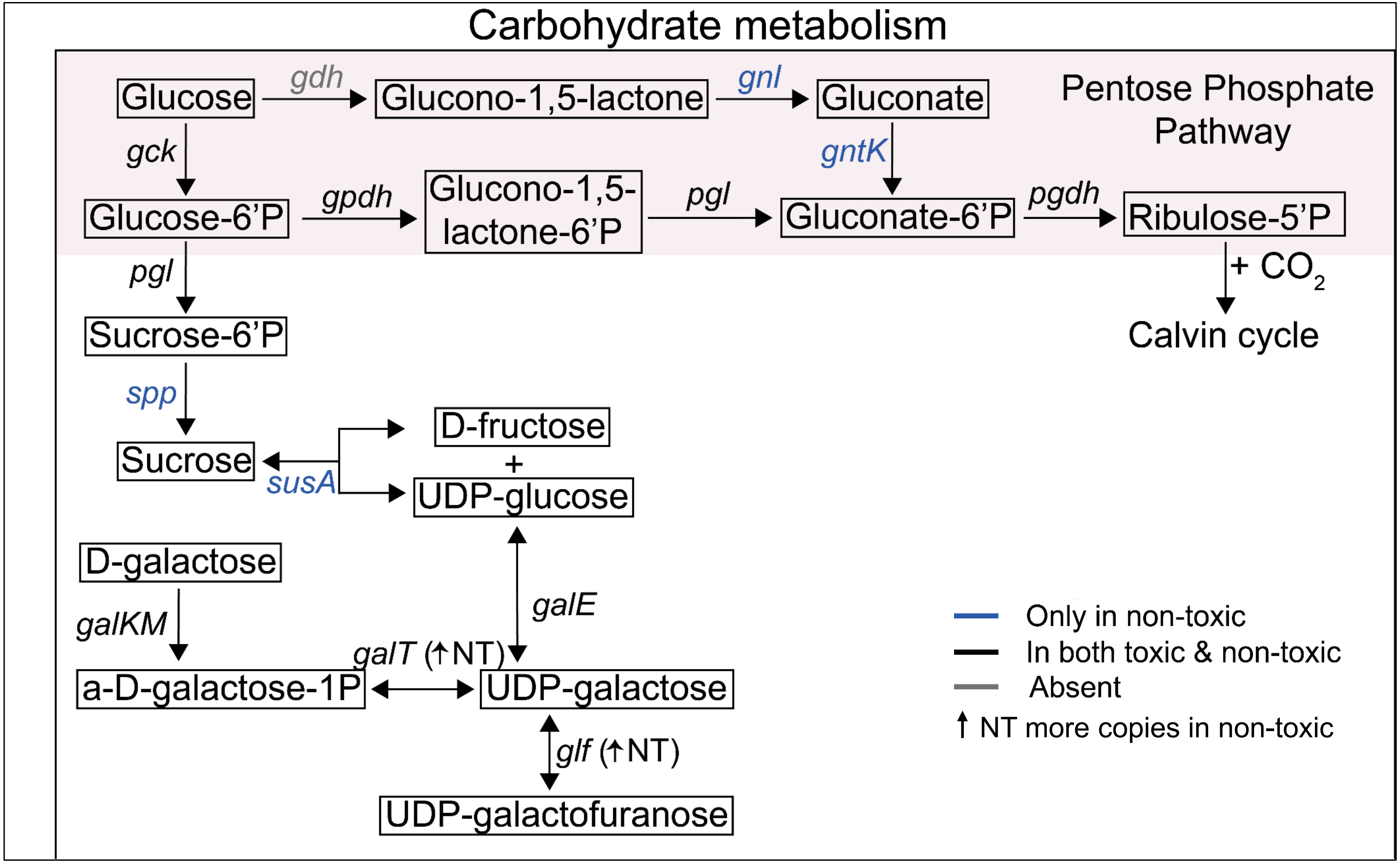


Characterization of toxic and non-toxic strains of *Microcoleus*



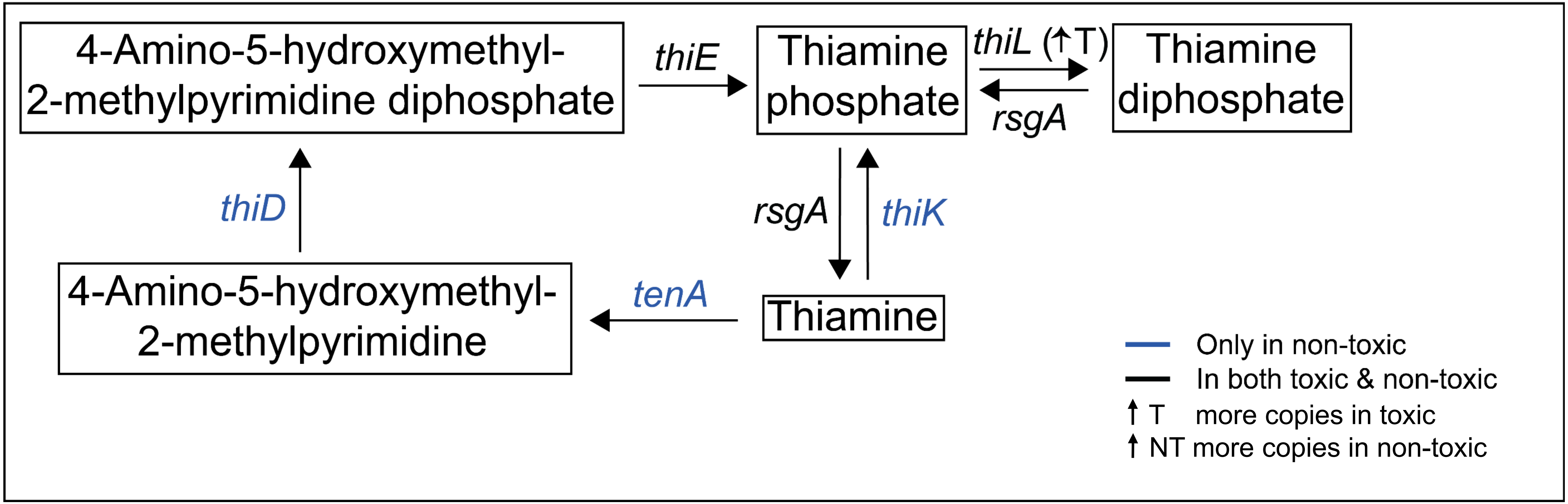


Nitrogen metabolism and anatoxin biosynthesis
Nirate (extracellular)
tauABC (↑T)
nrtABCD
Nitrate
narB
Nitrite
nirA
nirBD
glnB/P-II (↑T)
glnA (↑T)
Ammonia
glsA
L-glutamine
ureABCDEFG
Urea (extracellular)
urtABCDE
Urea
gltS(↑T)
gltBD
L-glutamate
aspB
2-oxoglutarate
argBCDJ
Ornithine
argF
Citruline
argGH
Arginine
Anatoxin-a
anaBCDEFGJAIH
pip
Peptide
Proline
proC
putA
1-Pyrroline-5-carboxylate
putA
putA
L-glutamate 5-semialdehyde
proB
proA
L-glutamyl-P
Fumarate
Krebs cycle
Oxaloacetate
aspA
aspBC
L-Aspartate
cphA (↑NT)
Cyanophycin
cphB(↑NT)
ansAB
asnB
L-Asparagine
racD
D-Aspartate
Only in toxic
Only in non-toxic
In both toxic & non-toxic
↑T more copies in toxic
↑NT more copies in non-toxic



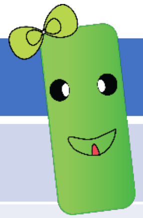
Carbohydrate metabolism
Pentose Phosphate Pathway
Glucose
gdh
Glucono-1,5-lactone
gnl
Gluconate
gck
gntK
Glucose-6'P
gpdh
Glucono-1,5-lactone-6'P
pgl
Gluconate-6'P
pgdh
Ribulose-5'P
+ CO_2
Calvin cycle
pgl
Sucrose-6'P
spp
Sucrose
susA
D-fructose
+
UDP-glucose
D-galactose
galKM
galE
galT (↑NT)
a-D-galactose-1P
UDP-galactose
glf (↑NT)
UDP-galactofuranose
Only in non-toxic
In both toxic & non-toxic
Absent
↑ NT more copies in non-toxic

Thiamine metabolism



Characterization of toxic and non-toxic strains of *Microcoleus*

- Toxic and non-toxic strains are **phylogenetically different** and form distinct clusters

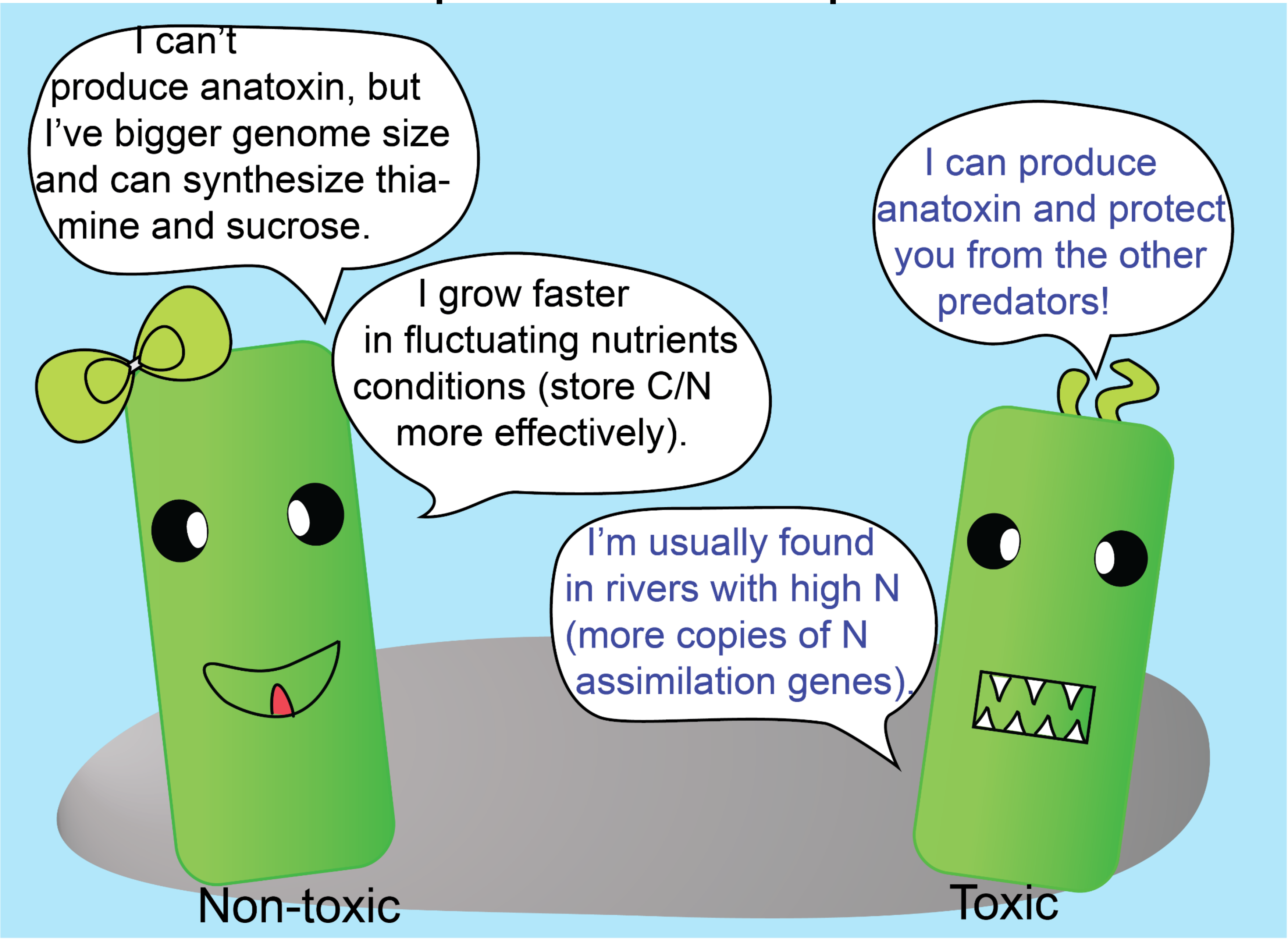


Toxic strains	Properties	Non-toxic strains
Smaller	Genome size	Larger
Fewer	BGC clusters	More
Fewer	Transposases	More
Fewer	Metabolic genes	More
Longer	Minimum replication time	Shorter
Yes	Anatoxin production	No
No	Thiamine and sucrose synthesis	Yes
More N assimilation genes	Nitrogen (N) uptake and storage	More cyanophycin for C/N storage

- Resource allocation **tradeoff** between toxin production and strains proliferation/ growth
- Genome streamlining in toxic strains cause dependencies on non-toxic strains/ co-occurring bacteria

Competition & Cooperation

No man is an island, so does the cyanobacterial biofilm



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Genome Streamlining, Plasticity, and Metabolic Versatility Distinguish Co-occurring Toxic and Nontoxic Cyanobacterial Strains of *Microcoleus*

Authors: Hwee Sze Tee, Susanna A. Wood, Keith Bouma-Gregson, Gavin Lear, Kim M. Handley | AUTHORS INFO & AFFILIATIONS

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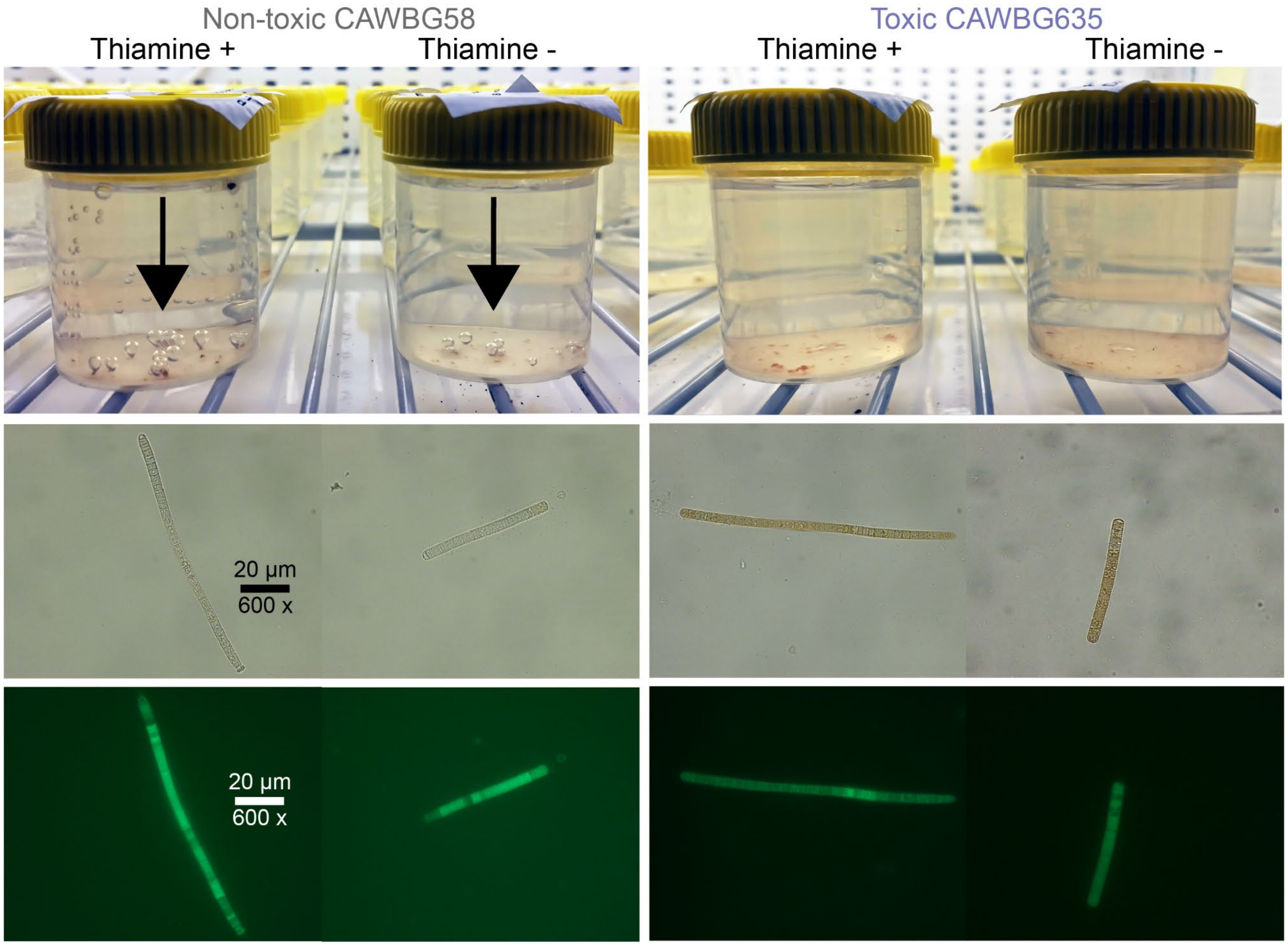
ABSTRACT

Harmful cyanobacterial bloom occurrences have increased worldwide due to climate change and eutrophication, causing nuisance and animal deaths. Species from the benthic cyanobacterial genus *Microcoleus* are ubiquitous and form thick mats in freshwater systems, such as rivers, that are sometimes toxic due to the production of potent neurotoxins (anatoxins). Anatoxin-producing (toxic) strains typically coexist with non-anatoxin-producing (nontoxic) strains in mats, although the reason for this is unclear. To determine the genetic mechanisms differentiating toxic and nontoxic *Microcoleus*, we sequenced and assembled

Future work


Non-toxic CAWBG58
Thiamine +
Thiamine -
Toxic CAWBG635
Thiamine +
Thiamine -
20 µm
600 x
20 µm
600 x

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Laura Kelly (cawthron Institute)

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Handley's Lab (University of Auckland)

Genomics Aotearoa

Dini (NeSI)

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