“Learning from the past to predict the future”

Scientific Programme

Kraków, Poland
05-10 May 2019
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of contents</td>
<td>2</td>
</tr>
<tr>
<td>Welcome letter</td>
<td>3</td>
</tr>
<tr>
<td>Scientific Committee/Local Organizing Committee</td>
<td>4</td>
</tr>
<tr>
<td>Scientific Sessions/Speakers</td>
<td>5</td>
</tr>
<tr>
<td>Special Issues</td>
<td>6</td>
</tr>
<tr>
<td>Guidelines for Authors</td>
<td>8</td>
</tr>
<tr>
<td>Conference Venue</td>
<td>10</td>
</tr>
<tr>
<td>Social Programme</td>
<td>13</td>
</tr>
<tr>
<td>Agenda</td>
<td>14</td>
</tr>
<tr>
<td>Conference Programme</td>
<td>16</td>
</tr>
<tr>
<td>Abstracts – Oral Presentations</td>
<td>25</td>
</tr>
<tr>
<td>List of Poster Presentations</td>
<td>124</td>
</tr>
<tr>
<td>Abstracts – Poster Presentations</td>
<td>132</td>
</tr>
<tr>
<td>Organizers and Sponsors</td>
<td>274</td>
</tr>
</tbody>
</table>
Dear Colleagues and Friends,

On behalf of the Organizing Committee and my own, I would like to warmly welcome you to the historic city of Krakow for the 11th International Conference on Toxic Cyanobacteria, ICTC11.

During this edition of the conference, 220 participants from 45 countries will present their work and exchange ideas on the most recent scientific discoveries and achievements in the field of toxic cyanobacteria, their ecology, physiology, diversity and toxicity. The presentations will be delivered by experienced, distinguished researchers but also by young, rising stars of cyanoscience.

The theme of the conference is “Learning from the past to predict the future”. With this, we hope that the knowledge gained throughout the years, since the first ICTC meeting in 1980, will improve our understanding of the connections between toxic cyanobacteria and other environmental and social problems such as climate changes, the changes in the structure and diversity of organisms, limited access to high quality drinking water resources and new water management strategies.

We are convinced that the scientific programme of the conference and the contribution of all the presenters will inspire us to further research and hopefully help us find answers to many intriguing scientific questions. We believe that the younger generation will be encouraged to continue the directions set during this meeting.

What is equally important, the conference, together with the social events will provide an excellent platform to build and strengthen our relationships and make this meeting truly unforgettable.

We would like to express our gratitude to everyone who has supported this conference, especially to our Scientific Committee with Professor Wayne Carmichael and Professor Geoffrey Codd as chair persons, our Sponsors and also the Jagiellonian University for hosting this event.

We wish you all a great time in Krakow, a city with a long tradition in science and arts.

Dariusz Dziga

Chair of the Local Organizing Committee
**SCIENTIFIC COMMITTEE**

Maria Antoniou, Cyprus
Sandra Azevedo, Brasil
Ludek Blaha, Czech Republic
Michele Burford, Australia
Wayne Carmichael, USA
Geoff Codd, Scotland
Elke Dittmann, Germany
Tim Downing, South Africa
Dariusz Dziga, Poland
Ambrose Furey, Ireland
Elizabeth Hilborn, USA
Jean F. Humbert, France
Tri Kaloudis, Greece

Rainer Kurmayer, Austria
James Metcalf, USA
Jussi Meriluoto, Finland
Shin-ichi Nakano, Japan
Philip Orr, Australia
Hans Paerl, USA
Antonio Quesada, Spain
Lirong Song, China
Sigitas Šulčius, Lithuania
Nico Salmaso, Italy
Kaarina Sivonen, Finland
Zorica Svirčev, Serbia
Petra Visser, The Netherlands

**LOCAL ORGANIZING COMMITTEE**

Dariusz Dziga, Jagiellonian University, Kraków
Iwona Jasser, University of Warsaw, Warszawa
Mikołaj Kokociński, Adam Mickiewicz University, Poznań
Joanna Mankiewicz-Boczek, University of Łódź, Łódź
Hanna Mazur-Marzec, University of Gdańsk, Gdańsk
Barbara Pawlik-Skowrońska, University of Life Sciences, Lublin
Anna Toruńska-Sitarz, University of Gdańsk, Gdańsk
SCIENTIFIC SESSIONS

- Detection, identification and diversity of toxic/invasive cyanobacteria
- Toxic cyanobacteria in the context of climate changes
- Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production (2 parts)
- Secondary cyanometabolites – structure, biosynthesis, physiological function, environmental significance and biotechnological application
- Cell physiology and molecular biology of cyanobacteria
- Toxicity and harmful effects of cyanobacteria and their metabolites
- Risk identification, water management and toxin removal
- New tools, new methods, most original findings and hypotheses

SPEAKERS

**Muriel Gugger**  
Head of the Collection of Cyanobacteria, Institut Pasteur

**Jef Huisman**  
Department of Freshwater and Marine Ecology (FAME), University of Amsterdam

**Diana Kirilovsky**  
Group Leader in the Institute of Integral Biology of the Cell (I2BC, CNRS) in Gif sur Yvette, France.

**Linda Lawton**  
Professor of Environmental Microbiology - Robert Gordon University

**Hanna Mazur-Marzec**  
Head of the Division of Marine Biotechnology, University of Gdańsk

**Kaarina Sivonen**  
Professor of Microbiology, Department of Food and Environmental Sciences Division of Microbiology and Biotechnology, Helsinki University

**Steven Wilhelm**  
Department of Microbiology, The University of Tennessee

**Susie Wood**  
Senior Scientist at the Cawthron Institute in Nelson, New Zealand

**Bojana Žegura**  
Assistant professor in Toxicology at the University of Ljubljana, Slovenia
SPECIAL ISSUES
The Local Organizing Committee agreed with three journals (Limnologica, Toxins and Process Safety and Environmental Protection) to call for a special issue related to the research presented during ICTC 11.

LIMNOLOGICA
Call for papers for a special ICTC XI issue of Limnologica on cyanotoxin occurrence
Our understanding of cyanotoxin occurrence has major gaps:

1. published data on cyanotoxin occurrence in tropical and subtropical settings, generated with solidly described methods;
2. published data relating intra- and extracellular cyanotoxin concentrations to measures of biomass – either biovolume or chlorophyll-a (plus phycocyanin) – preferably to both biovolume and pigment concentrations taken from one-and-the same sample – and preferably from a wide range of water-body types, climates, continents;
3. published data on occurrence and dominance of cyanobacterial taxa or even genotypes in relation to environmental conditions, presented with a sound understanding of phytoplankton ecology – of how the drivers of species dominance actually work over time (not mere correlations or CCA analyses).

Limnologica will be happy to publish a special volume on the occurrence of cyanobacteria and cyanotoxins, with contributions drawing on ICTC XI (but not restricted to those who actually attended). This will be an opportunity to collate contributions with a rather descriptive scope – with the added value of generating a better understanding of occurrence of toxins in relation to cyanobacterial species and biomass.

Contributions will need to meet quality criteria, specifically:

- solidly described methods, both for the analyses of cyanobacteria and their toxins;
- if the influence of environmental factors is discussed, inclusion of total P and preferably also total N or DIN as well as an understanding of water-body mixing;
- if data are analysed with statistical tools, the associations need to be discussed on the basis of a sound understanding of the possible mechanisms causing them.

TOXINS
Selected Papers from the 11th International Conference on Toxic Cyanobacteria (submission deadline – 30th November 2019)
The submitted articles should contain recent and most important findings discussed during the conference including: the occurrence of toxic/invasive cyanobacteria in the context of climate changes; ecology of cyanobacteria with special emphasis on abiotic and biotic factors which regulate their growth and/or toxin production; physiological function, environmental significance and biotechnological application of secondary cyanometabolites; physiology and molecular biology of cyanobacteria; toxicity and harmful effects; risk identification and water management.

Additional links to the special issue which you may find useful:
PROCESS SAFETY AND ENVIRONMENTAL PROTECTION

Special Issue - Novel practices for monitoring, treating, and preventing cyano-HABs

Scope:
This special issue aims to address several aspects related to the monitoring, prediction of formation, treatment (at source and in water treatment plants) and toxicity of cyano-HABs as well as the health risks posed from exposure and use of cyanotoxin contaminated water in crop irrigation, aquatic cultures, and inadequately treated water for human consumption. Special emphasis will be given to the effects that each treatment process has for the remaining ecosystem when applied at source and the residual toxicity of the transformation by-products when applied in a treatment train. Publications on the uptake of toxins from crops, fish, and mussels are also welcome. The main topics covered by this Special Issue are:

- Novel at source treatments for the removal of cyanotoxins and cyanobacteria from surface water
- Novel physico-chemical treatments for the removal of cyanobacteria and cyanotoxins from drinking water.
- Preventative practices for minimizing bloom formation
- Toxicity of transformation products from the treatment of cyanotoxins
- Toxicity and uptake of cyanotoxins in aquatic organisms.
- Effects on cyanobacteria and cyanobacterial toxins on crop irrigation (including soil)
- Up-take, accumulation, transportation and transformation of cyanotoxins in crops
- Human exposure of cyanotoxins via inhalation and ingestion of contaminated food and water.

Purpose:
This Special Issue will be published to cover a lack of scientific research in relation to the monitoring, treatment (at source and in treatment facilities) and health risks from coming in contact directly (recreational activities) and indirectly (food chain and potable water) with cyanotoxins and toxic cyanobacteria. This Special Issue will become a key publication for engineers, scientists, competent authorities, public health practitioners and policy makers in the field of water monitoring/treatment/remediation of cyano-HABs, as well as the toxicity of the by-products formed during treatment, and the effects of cyanobacterial water on aqueous organisms and crops.

Submission of manuscripts:
All special issue submissions should stem from authors participated at ICTC11 (irrespective whether the work was presented at the conference or not). Please see https://www.journals.elsevier.com/process-safety-and-environmental-protection/ for details of the journal’s aims and scope to ensure that your paper is within scope. To meet the journal requirements for a full research paper your extended paper should be a minimum of 6000 words excluding figures and tables. The manuscript will be subjected to the standard journal peer review process. Submissions to the special issue will need to be received via https://www.evise.com/profile/api/navigate/PSEP by 15 September 2019. To ensure that all manuscripts are correctly identified for inclusion into the special issue, please select ‘VSI: Cyanos’ when choosing the ‘Article Type’.

To indicate your expression of interest in submitting a paper to this special issue please notify Managing Editor Catherine Cliffe at ccliffe@icheme.org as soon as possible please, but no later than 30 June 2019. Please supply details of a provisional title and author names where possible.
GUIDELINES FOR AUTHORS

ORAL PRESENTATIONS:

We kindly ask you to keep the time limit. It means that your oral presentation should not exceed 12 minutes. Minimum 3 minutes should be saved for discussion! Please remember that the font used on your presentation should not be smaller than 20 pt.

Depositing of file:
A speakers’ preview room will be located on the ground floor of Auditorium Maximum (follow signs on site) to coordinate the overall running of the conference sessions and to assist speakers with any requests.
All speakers are requested to use the PREVIEW ROOM to provide his/her PowerPoint presentation in advance, that will be transferred to the conference room on time. Speakers will not have access to the projection rooms or the possibility of connecting their own laptop.

Your file must be handed over to the staff of the PREVIEW ROOM, as far in advance as possible and at least one hour BEFORE the beginning of your session.
In the PREVIEW ROOM, you will be assisted by a technician, who will help you to transfer your presentation to the internal network. You will also be able to review your presentation and to verify that it has been transferred correctly to the network. All presentations will be considered as confidential by our staff and removed from the system at the end of the conference.

The opening hours of the PREVIEW ROOM:
Sunday 5 May 2019: 16:00 - 18:00
Monday 6 May 2019: 08:00 - 18:00
Tuesday 7 May 2019: 08:00 - 16:00
Wednesday 8 May 2019: 08:00 - 18:00
Thursday 9 May 2019: 08:00 - 12:00
Friday 10 May 2019: 08:00 - 14:00

Presentation:
Official Language: all presentations and questions must be delivered in ENGLISH.
Format of Presentation: Only Presentations for PC’s (Windows latest versions) will be accepted.
Your presentation should be saved in the .pdf or .pptx format only (PowerPoint for Office 365, PowerPoint 2019, PowerPoint 2016, PowerPoint 2013, PowerPoint 2010, PowerPoint 2007). Other formats will not be accepted. The PC on the lectern is programmed in 16:9 and is linked to a video-projector.
POSTER PRESENTATIONS:

Poster exhibition will take place in the Exhibition room on the second level of Auditorium Maximum.

- The posters will be discussed in front of the poster board during the dedicated session slots.
- The authors are kindly asked to hang their posters one-two days before the appropriate session.
- Posters should be displayed as follows:
  • Poster session No 1 – from Monday morning to Tuesday evening
  • Poster session No 2 – from Wednesday morning to Friday afternoon
- In the dedicated session slot, the presenting author must be available next to his/her dedicated panel to present and discuss the poster.

Poster dimension and format: the maximal size must not exceed 130 cm in height and 90 cm in width

Language: posters must be written in ENGLISH

Content: each poster should contain: title, full names and affiliation of contributing authors.

Materials: suitable fixing materials will be provided by the Conference organizers. There will be a poster helpdesk close to the poster area, where staff will be happy to assist you.
CONFERENCE VENUE

ICTC 11 will be held at Auditorium Maximum, a modern conference and teaching facility located in close proximity to the city centre and most hotels. The oral sessions and round table discussions will take place in the Medium hall, whereas the poster sessions, welcome party, closing ceremony, coffee breaks and lunches will be organized in the Exhibition room. Fluid Imaging workshop will take place in the Conference room, whereas the sponsors booths will be located in the Exhibition room.

Location:
Ground floor (level 0): Registration desk and Medium hall (entrance form the level 1 also possible)
Second floor (level 2): Exhibition room and Conference room

How to find the conference venue:
Auditorium Maximum, ground floor (level 0)
Auditorium Maximum, second floor (level 2)
Welcome Party – Sunday, 5th May, 18:00
This event will take place in the Exhibition Room (2nd floor) of Auditorium Maximum. It is free and open to all the registered participants, accompanying persons and sponsors.

Old Town Excursion – Tuesday 7th May, 16:45
The excursion will start at the front of the conference venue and is free of charge for all the participants. Groups of maximum 30 people will be shown around the historic centre of Krakow by English speaking tour guides. The tour includes the Royal Tract, a picturesque route leading through some of the best-known streets of the historic centre of Krakow, Barbican and St. Florian’s Gate, The Main Square, St. Mary's Basilica with its distinctive two towers, the small Church of St. Adalbert whose history dates back to the pre-Romanesque period, and the Cloth Hall. You will also see Wawel Castle – the home of the kings of Poland in the days of greatest glory until the 17th century.

Half-day Excursion – Thursday, 9th May, 13:10
This event is available only for those registered participants who made an additional payment of PLN 100; reservation in advance is necessary). The tour will start at the front of the conference venue.

Cultural event and Banquet – Thursday, 9th May, 18:30
This event is available for free to all the registered participants and will take place in a traditional restaurant Krakowiacy i Górale (Głogoczów, about 20 km from Krakow). It will include a spectacular folk show (great music, dance and songs) as well as traditional food and drinks.
Those of you who participate in the half-day excursion will be transferred to the venue directly after the tour. Other participants will be picked up at the front of the conference venue at 17:45.
Return transfer to Krakow will be organized by buses at at 21:00, 22:00, 23:00 and 24:00

Closing ceremony and dinner – Friday, 10th May, 20:00
This last meeting will take place in the Exhibition Room (2nd floor) of Auditorium Maximum. It is free and open to all the registered participants, accompanying persons and sponsors.
<table>
<thead>
<tr>
<th>Sunday, 5th May 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00-18:00 Workshop</td>
</tr>
<tr>
<td>15:00-18:00 Registration</td>
</tr>
<tr>
<td>18:00-21:00 Welcome party</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monday, 6th May 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00-09:00 Registration</td>
</tr>
<tr>
<td>09:00-09:30 Opening ceremony</td>
</tr>
<tr>
<td>09:30-11:30 Session 1 Detection, identification and diversity of toxic/invasive cyanobacteria</td>
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<tr>
<td>11:30-12:15 Coffee break</td>
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<tr>
<td>12:15-13:45 Session 1 Detection, identification and diversity of toxic/invasive cyanobacteria</td>
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<tr>
<td>13:45-14.30 Lunch</td>
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<tr>
<td>14:30-15:45 Session 2 Toxic cyanobacteria in the context of climate change</td>
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<tr>
<td>15:45-15:55 Sponsor presentation</td>
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<tr>
<td>15:55-16.20 Coffee break</td>
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<tr>
<td>16:20-17:20 Session 2 Toxic cyanobacteria in the context of climate change</td>
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<tr>
<td>17:20-17:40 Coffee break</td>
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<tr>
<td>17:40-18:40 Round table discussion I</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tuesday, 7th May 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00-10:15 Session 3 Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/1</td>
</tr>
<tr>
<td>10:15-10:45 Coffee break</td>
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<tr>
<td>10:45-11:45 Session 3 Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/1</td>
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<tr>
<td>11:45-12:45 Lunch</td>
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<tr>
<td>12:45-14:00 Session 4 Secondary cyanometabolites – structure, biosynthesis, physiological function, environmental significance and biotechnological application</td>
</tr>
<tr>
<td>14:00-14:30 Coffee break</td>
</tr>
<tr>
<td>14:30-15:30 Session 4 Secondary cyanometabolites – structure, biosynthesis, physiological function, environmental significance and biotechnological application</td>
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<tr>
<td>15:30-15:45 Sponsored presentation (parallel to poster session)</td>
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<tr>
<td>15:30-16:45 Poster session I</td>
</tr>
<tr>
<td>16:45-19.45 Excursion (Old Town)</td>
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</tbody>
</table>
### Wednesday, 8th May 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00-10:30</td>
<td>Session 5</td>
<td>Cell physiology and molecular biology of cyanobacteria</td>
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<tr>
<td>10:30-11:15</td>
<td>Coffee break</td>
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<tr>
<td>11:15-12:15</td>
<td>Session 5</td>
<td>Cell physiology and molecular biology of cyanobacteria</td>
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<td>12:15-13:15</td>
<td>Lunch</td>
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<tr>
<td>13:15-14:30</td>
<td>Session 6</td>
<td>Toxicity and harmful effects of cyanobacteria and their metabolites</td>
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<td>14:30-15:00</td>
<td>Coffee break</td>
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<tr>
<td>15:00-16:15</td>
<td>Session 6</td>
<td>Toxicity and harmful effects of cyanobacteria and their metabolites</td>
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<tr>
<td>16:15-17:30</td>
<td>Poster session II</td>
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<tr>
<td>17:30-18:30</td>
<td>Round table discussion II</td>
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### Thursday, 9th May 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topic</th>
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</thead>
<tbody>
<tr>
<td>09:00-10:30</td>
<td>Session 7</td>
<td>Risk identification, water management and toxin removal</td>
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<tr>
<td>10:30-11:00</td>
<td>Coffee break</td>
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<tr>
<td>11:00-11:30</td>
<td>Session 7</td>
<td>Risk identification, water management and toxin removal</td>
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<td>11:40-11:40</td>
<td>Sponsor presentation</td>
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<tr>
<td>11:40-12:25</td>
<td>Session 7</td>
<td>Risk identification, water management and toxin removal</td>
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<tr>
<td>12:25-13:10</td>
<td>Lunch</td>
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<tr>
<td>13:10-18:30</td>
<td>Excursion</td>
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<tr>
<td>18:30-19:30</td>
<td>Cultural performance</td>
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<tr>
<td>19:30-22:30</td>
<td>Banquet</td>
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### Friday, 10th May 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Topic</th>
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<tbody>
<tr>
<td>09:00-10:30</td>
<td>Session 8</td>
<td>New tools, new methods, most original findings and hypotheses</td>
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<td>10:30-11:00</td>
<td>Coffee break</td>
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<tr>
<td>11:00-12:30</td>
<td>Session 8</td>
<td>New tools, new methods, most original findings and hypotheses</td>
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<td>12:30-13:15</td>
<td>Lunch</td>
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<tr>
<td>13:15-14:30</td>
<td>Session 9</td>
<td>Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/2</td>
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<tr>
<td>14:30-15:00</td>
<td>Coffee break</td>
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<tr>
<td>15:00-16:00</td>
<td>Session 9</td>
<td>Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/2</td>
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<tr>
<td>16:00-16:30</td>
<td>Coffee break</td>
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<tr>
<td>16:30-17:15</td>
<td>General conclusions, closing lectures</td>
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<tr>
<td>17:15-18:00</td>
<td>ICTC12 Candidate Presentations and Voting</td>
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<tr>
<td>20:00-23:00</td>
<td>Closing ceremony and dinner</td>
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### Sunday, 5th May 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>15:00-18:00</td>
<td><strong>Workshop</strong></td>
<td>Fluid Imaging: Semi-automated method for detecting and counting cells of cyanobacterial colonies and filaments</td>
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<tr>
<td>15:00-18:00</td>
<td><strong>Registration</strong></td>
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<tr>
<td>18:00-21:00</td>
<td><strong>Welcome party</strong></td>
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### Monday, 6th May 2019

<table>
<thead>
<tr>
<th>Time</th>
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<th>Details</th>
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<tbody>
<tr>
<td>08:00-09:00</td>
<td><strong>Registration</strong></td>
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<tr>
<td>09:00-09:30</td>
<td><strong>Opening ceremony</strong></td>
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<tr>
<td>09:30-13:45</td>
<td><strong>Session 1</strong>: Detection, identification and diversity of toxic/invasive cyanobacteria</td>
<td>Chairs: Sandra Azevedo, David Fewer</td>
</tr>
<tr>
<td>09:30-10:15</td>
<td><strong>Opening lecture</strong>: Kaarina Sivonen (Finland)</td>
<td>Chemical, molecular and omics analyses of cyanobacteria.</td>
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<tr>
<td>10:15-10:45</td>
<td><strong>Invited lecture</strong>: Muriel Gugger (France)</td>
<td>From phylogeny (and thus naming the taxa differently) to natural products of cyanobacteria, another way for this phylum.</td>
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<tr>
<td>10:45-11:00</td>
<td>Te S.H., Xu Z., Goh Y.F., He Y., Gin K.Y.-H. (Singapore, China)</td>
<td>Assessing the effectiveness of 16S rRNA amplicon sequencing for cyanobacterial studies.</td>
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<tr>
<td>11:00-11:15</td>
<td>Salmaso N. (Italy)</td>
<td>The hidden diversity of cyanobacteria unveiled by high throughput sequencing approaches.</td>
</tr>
<tr>
<td>11:30-12:15</td>
<td><strong>Coffee break</strong></td>
<td></td>
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<tr>
<td>12:15-12:30</td>
<td>Panou M., Cegłowska M., Szubert K., Toruńska-Sitarz A., Mazur-Marzec H., Gkelis S. (Greece, Poland)</td>
<td>Profiling cyanobacteria diversity: is chemical heterogeneity driven by taxonomic distance?</td>
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<tr>
<td>12:45-13:00</td>
<td>Khomutovska N., Suska-Malawska M., Jasser I. (Poland)</td>
<td>Detection of the toxicity genes in endolithic communities: are cold desert endoliths still able to produce cyanotoxins?</td>
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<tr>
<td>Time</td>
<td>Session</td>
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<tr>
<td>13:45-14:30</td>
<td>Lunch</td>
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<td>14:30-17:20</td>
<td><strong>Session 2: Toxic cyanobacteria in the context of climate change</strong></td>
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<td>Chairs: Zorica Svirčev, Nico Salmaso</td>
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<td>14:30-15:00</td>
<td><strong>Invited lecture: Jef Huisman</strong> (The Netherlands)</td>
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<td></td>
<td>Impacts of rising CO2 and global warming on harmful cyanobacterial blooms.</td>
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<td>15:15-15:30</td>
<td>Rogers Paranhos R., Brandão L., Pereira R., Azevedo S. (Brazil)</td>
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<td></td>
<td>Revisit the past to understand the present: physiological and molecular effects of high CO2 on <em>Cylindrospermopsis raciborskii</em> (Cyanobacteria).</td>
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<td>15:30-15:45</td>
<td>Davis T.W., Doherty O., Gobler C.J. (USA)</td>
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<td>The role of surface water warming in the timing of the <em>Microcystis</em>-dominated cyanobacterial blooms in western Lake Erie.</td>
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<td>15:45-15:55</td>
<td><strong>Sponsor presentation</strong></td>
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<td>Waters: One second screening of cyanobacteria utilizing laser REIMS technology.</td>
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<td>15:55-16:20</td>
<td>Coffee break</td>
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<td>16:20-16:35</td>
<td>Isles P., Bouffard D., Lepori F., Capelli C., Köster O., Pomati F. (Switzerland)</td>
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<td>Forecasting cyanobacteria blooms at multiple timescales using hydrodynamic models and machine learning.</td>
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<td>16:35-16:50</td>
<td>Dietz M., Helmer D., Weisbrod B., Dietrich D., Yohannes E., Martin-Creuzburg D. (Germany)</td>
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<td>Harmful cyanobacterial bloom mediates trophic shifts and enhance carbon source alternation in an artificial reservoir.</td>
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<td>16:50-17:05</td>
<td>Shan K., Song L. (China)</td>
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<td>Analysis of environmental drivers influencing cyanobacterial succession and cyanotoxin production in three large, shallow eutrophic lakes, China.</td>
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<td>Comparisons of cyanobacterial diversity in soil crusts in hot and cold deserts – are there toxigenic taxa?</td>
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<td>17:20-17:40</td>
<td>Coffee break</td>
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<tr>
<td>17:40-18:40</td>
<td><strong>Round table discussion I</strong></td>
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<td>Molecular and morphological concepts in cyanobacterial taxonomy – recent achievements and perspectives.</td>
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<td>Chairs: Muriel Gugger and Nico Salmaso</td>
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<td>Time</td>
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| 09:00-12:00  | **Session 3**: Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/1  
Chairs: Barbara Pawlik-Skowrońska, Philip Orr |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 09:00-09:30  | Invited lecture: Steven Wilhelm (USA) | Metatranscriptomic insight into the effects of viruses on *Microcystis* blooms                                                                                                                                                                                                                                                                                                                   |
| 09:30-09:45  | McKindles K., Manes M., McKay R.M., Bullerjahn G. (USA, Canada)  
Parasites of *Planktothrix*; cyanophages and chytrids as top-down regulators in a Lake Erie embayment. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 09:45-10:00  | Clercin N.A. (USA)  
Bacterioplankton assemblages during seasonal occurrences of microcystins, 2-methylisoborneol and geosmin in a midwestern eutrophic reservoir. |                                                                                                                                                                                                                                                                                                                                                                                                   |
(Republic of Korea)  
Seasonal changes in distinct bacterial modules that drive harmful cyanobacterial blooms. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 10:15-10:45  | Coffee break |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 10:45-11:00  | Dziga D., Barylski J., Maksylewicz A., Maroszek M., Marek S., Antosiak A., Kokociński M.  
(Poland)  
Relations between microcystin producers and degraders in freshwater bodies of western Poland. |                                                                                                                                                                                                                                                                                                                                                                                                   |
(United Kingdom, France)  
Metabolic interactions between *M. aeruginosa* PCC7806 and *D. magna*. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 11:15-11:30  | Svirčev Z., Dulić T., Codd G.A., Palanački Malešević T., Savela H., Faassen E., Meriluoto J.  
(Serbia, Finland, United Kingdom, The Netherlands)  
Biological loess crusts – a special environment influences cyanobacterial toxicity. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 11:30-11:45  | Zhang Y., Whalen J. K., Husk B.R.  
(Republic of Korea)  
Soil cyanobacteria and production of toxins in agroecosystems of South-Central Quebec, Canada. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 11:45-12:45  | Lunch |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 12:45-13:15  | Invited lecture: Hanna Mazur-Marzec (Poland)  
Cyanopeptides from Baltic cyanobacteria – diversity, environmental significance and application. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 12:45-13:30  | Popin R.V., Abreu V.A.C., Rigonato J., Dörr F.A., Pinto E., Sivonen K., Fiore M.F.  
(Brazil, Finland)  
Genomic and metabolomic analyses of natural products in *Nodularia spumigena* isolated from a shrimp culture pond water. |                                                                                                                                                                                                                                                                                                                                                                                                   |
| 13:15-13:30  | Session 4: Secondary cyanometabolites – structure, biosynthesis, physiological function, environmental significance and biotechnological application  
Chairs: Christine Edwards, Rainer Kurmayer |                                                                                                                                                                                                                                                                                                                                                                                                   |
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<th>Time</th>
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<tr>
<td>13:45-14:00</td>
<td>Toruńska-Sitarz A., Panasiak L., Mazur-Marzec H. (Poland) Genotypic and phenotypic diversity of new cyanobactin producers classified to <em>Limnoraphis</em> genus.</td>
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<td>14:00-14:30</td>
<td>Coffee break</td>
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<tr>
<td>14:30-14:45</td>
<td>Alvarenga D.O., Arsin S., Wahlsten M., Jokela J.K., Fiore M.F., Varani A.M., Fewer D., Sivonen K. (Finland, Brazil) Genomic and chemical analyses of novel natural products from <em>Brasilonema</em> spp.</td>
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<td>14:45-15:00</td>
<td>Konstantinou D., Zervou S.-K., Mavrogonatou E., Giannogonas P., Gkelis S. (Greece) A preliminary assessment of the potential of sponge-associated cyanobacteria to produce bioactive compounds.</td>
</tr>
<tr>
<td>15:00-15:15</td>
<td>Pilkaityte R., Overlingé D., Mazur-Marzec H. (Lithuania, Poland) Distribution of cyanobacterial non-ribosomal peptides in the shallow temperate lagoon.</td>
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<tr>
<td>15:30-16:45</td>
<td>Poster session I</td>
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<td>16:45-19:45</td>
<td>Excursion (Old Town)</td>
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**Wednesday, 8th May 2019**

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<th>Time</th>
<th>Session</th>
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<tr>
<td>09:00-12:15</td>
<td><strong>Session 5</strong>: Cell physiology and molecular biology of cyanobacteria</td>
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<td>Chairs: Claudia Wiegand, Dariusz Dziga</td>
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<td>09:00-09:30</td>
<td><strong>Invited lecture</strong>: Diana Kirilovsky (France) Photoprotection in cyanobacteria.</td>
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<td>09:45-10:00</td>
<td><strong>Woodhouse</strong> J.N., Willis A., Grossart H.-P., Burford M., Neilan B.A. (Germany, Australia) Metagenomic diversity of bloom forming cyanobacteria through time and space.</td>
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<td>Time</td>
<td>Speaker(s)</td>
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<td>10:00-10:15</td>
<td>Fewer D.P., Shishido T.K., Jokela J., Popin R., Alvarenga D., Sivonen K. (Finland)</td>
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<td>10:15-10:30</td>
<td>Zhang L., Huang Y., Yang Z. (China)</td>
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<td>10:30-11:15</td>
<td>Coffee break</td>
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<td>12:00-12:15</td>
<td>Aydin E., Akcaalan R., Koker L., Tunc Z., Albay M. (Turkey)</td>
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<tr>
<td>12:15-13:15</td>
<td>Lunch</td>
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<td>13:45-14:00</td>
<td>Pappas D., Gkellis S., Panteris E. (Greece)</td>
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<td>14:00-14:15</td>
<td>Campos A., de Oliveira F.L., Martins J.C., Diez-Quijada L., Caméan A.M., Turkina M.V., Vasconcelos V. (Portugal, Spain, Sweden)</td>
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<td>14:30-15:00</td>
<td>Coffee break</td>
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Ingestion of microcystin and toxic *Microcystis* and its impact on gut microbiome.                                                                                                                                                                                                 |
| 15:30-15:45  | **Kokociński M., Brzozowska A., Falfushynska H., Gagała-Borkowska I., Jurczak T., Mankiewicz-Boczek J., Meriluoto J., Rzymski P. (Poland, Ukraine, Finland)**  
New reports on neurotoxicity of *Raphidiopsis raciborskii* strains.                                                                                                                                                                                                                     |
| 15:45-16:00  | **Downing T.G., van Onselen R. (South Africa)**  
How does BMAA cause progressive slow neurodegeneration?                                                                                                                                                                                                                             |
| 16:00-16:15  | **Babica P., Moosová Z., Hošeková V., Goliášová Z., Vašiček O., Šindlerová L. (Czech Republic)**  
Cyanobacterial LPS exerts biological activity unpredictable by standard endotoxin test.                                                                                                                                                                                        |
| 16:15-17:30  | Poster session II                                                                                                                                                                                                                                                                                                                                 |
| 17:30-18:30  | **Round table discussion II**  
Risk management of cyanobacterial blooms and cyanotoxins: contributions of the ICTC community.  
**Chairs:** Geoffrey Codd and Wayne Carmichael                                                                                                                                                                                                                                        |

**Thursday, 9th May 2019**

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<tr>
<th>Time</th>
<th>Session Details</th>
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| 09:00-12:25  | **Session 7: Risk identification, water management and toxin removal**  
**Chairs:** Maria Antoniou, Ludek Blaha                                                                                                                                                                                                                                             |
| 09:00-09:30  | **Invited lecture:** Linda A. Lawton (United Kingdom)  
Treatment strategies for cyanotoxins in water.                                                                                                                                                                                                                                        |
| 09:30-09:45  | **Shan K., Song L. (China)**  
Managing the harmful cyanobacterium *Microcystis* and cyanotoxin risk by large-scale monitoring and machine learning: a framework of Bayesian network.                                                                                                                                               |
| 09:45-10:00  | **Sukenik A., Wu X., Levy R., Viner-Mozzini Y., Nir S. (Israel, China)**  
Removal of cyanobacteria and cyanotoxins from lake water by composites of bentonite with micelles of organic quaternary ammonium cation.                                                                                                                                               |
| 10:00-10:15  | **Pestana C.J., Capelo-Neto J., Lawton L.A., Oliveira S., Carloto I., Linhares H.P. (Brazil, United Kingdom)**  
The effect of water treatment unit processes on toxic cyanobacterial trichome integrity.                                                                                                                                                                                              |
Exploring morphological deformation of cyanobacteria cell during oxidation.                                                                                                                                                                                                          |
| 10:30-11:00  | Coffee break                                                                                                                                                                                                                                                                                                                                  |
| 11:00-11:15  | **Wiegand C., Gérard G., Dupas R., Le Goffe P., Latouche P., Hernandez S. (France)**  
Payment for ecosystem services – an efficient approach to reduce eutrophication?                                                                                                                                                                                                     |
Control and mitigation of microcystin-producing cyanobacteria occurrence in lowland dam reservoirs.

11:30-11:40  Sponsor presentation
Taronis Technologies: Reduction of blue-green algae using Plasma Arc technology

Genetic engineering of cyanobacteria for degradation of microcystins – prolonging whole cell MlrA activity under extended culturing regimes via application of trc promoter.

11:55-12:10  Joosten E., Milferstedt K., Hamelin J. (France)
Naturally occurring cyanobacteria can form oxygenic photogranules to treat wastewater.

Nanobody ELISA for the determination of microcystins in fish.

12:25-13:10  Lunch
13:10-18:30  Excursion
18:30-19:30  Cultural performance
19:30-22:30  Banquet

Friday, 10th May 2019

09:00-12:30  Session 8: New tools, new methods, most original findings and hypotheses
Chairs: Petra Visser, Jussi Meriluoto

09:00-09:30  Invited lecture: Sussie A. Wood (New Zealand)
Toxic benthic cyanobacteria: new insights into their ecology and toxin production.

09:30-09:45  Godlewska M., Mankiewicz-Boczek J., Izydorczyk K., Jurczak T., Kaczkowski Z., Balk H., Ye S., Długoszewski B. (Poland, Norway, China)
New assessment method of the spatial distribution and biomass of Microcystis bloom using high frequency echosounder.

09:45-10:00  Gonzalez G., Pirez M., Brena B. (Uruguay)
Ultrasensitive detection of microcystins in untreated biological samples by immunoconcentration with nanobody coated nanoparticles and direct quantitative MALDI-TOF analysis.

10:00-10:15  Altaner S., Fotler R., Jaeger S., Zemskov I., Wittmann V., Schreiber F., Dietrich D.R. (Germany)
Microcystin congener specific inhibition of mammalian ser/thr protein phosphatases (PP1, PP2a and PP5) and prediction of inhibitive capacity via machine learning.
LC-HRMS versus LC-TANDEM-MS: a comparative approach for the identification of cyanotoxins in cyanobacterial biomass.

10:30-11:00 Coffee break

11:00-11:15 **Manolidi K., Triantis T., Kaloudis T., Hiskia A. (Greece)**
Dual cartridge SPE method for extraction of different variants of saxitoxins and HILIC-MS/MS analysis.

11:15-11:30 **Beach D.G., Wright E.J., Melanson J.E., McCarron P., Miles C.O. (Canada)**
Untargeted high-resolution mass spectrometry workflow for identifying microcystins.

11:30-11:45 **Schneider M., Rataj R., Kolb J.F., Bláha L. (Czech Republic, Germany)**
Degradation of cylindrospermopsin using advanced non-thermal plasma technologies.

11:45-12:00 **Mercader J.V., Cevallos-Cedeño R.E., Quiñones-Reyes G., Agulló C., Abad-Somovilla A., Abad-Fuentes A. (Spain)**
Development of a rapid immunochemical assay for anatoxin-a analysis.

Use of freshwater bivalves as sentinel species to determine the presence of BMAA in aquatic ecosystems.

12:15-12:30 **Bownik A., Pawlik-Skowrońska B. (Poland)**
Early biomarkers of behavioural and physiological disturbances in *Daphnia magna* exposed to anatoxin-a estimated by video analysis.

12:30-13:15 Lunch

13:15-16:00 **Session 9: Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/2**
*Chairs: Assaf Sukenik, Sigita Šulčius*

Managing the risk of cyanobacteria through water quality characteristics analysis: A case study of two warm Mediterranean reservoirs.

13:30-13:45 **Neves de Lima D., Furlanetto Pacheco A.B., Azevedo S.M. (Brazil)**
Physiological responses of *Cylindrospermopsis raciborskii* (cyanobacteria) strains to water conductivity: effect of sodium and magnesium ions.

13:45-14:00 **Van de Waal D.B., Kraak Y., Liu J. (The Netherlands)**
Impacts of CO₂ and nitrogen availability on the eco-physiology of harmful cyanobacteria.

14:00-14:15 **Georges des Aulnois M., Caruana A., Briand E., Dittmann E., Bormans M., Aizil Z. (France, Germany)**
Salt stress responses of brackish and freshwater strains of *Microcystis aeruginosa*.

14:15-14:30 **Natumi R., Janssen E.M.-L. (Switzerland)**
Environmental photodegradation of emerging cyanopeptides beyond microcystins.
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<td>14:30-15:00</td>
<td>Coffee break</td>
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<tr>
<td>15:00-15:15</td>
<td><strong>Duan Z., Xiao T., Van de Waal D. (China, The Netherlands)</strong> Colony formation: a master trait of <em>Microcystis</em>.</td>
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<td>16:00-16:30</td>
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| 16:30-17:15  | **General conclusions.** **Closing lectures:** **Ingrid Chorus** (Germany) The new World Health Organisation Guidebook: Toxic Cyanobacteria in Water.  
**Jussi Meriluoto** (Finland) From cyanobacterial problems to blue-green solutions. |
| 17:15-18:00  | ICTC12 Candidate Presentations and Voting                            |
| 20:00-23:00  | Closing ceremony and dinner                                          |
Harmful algae blooms are increasing in frequency and intensity. Public safety and conservation agencies demand a replicable and scalable method to rapidly detect and enumerate cells comprising cyanobacterial colonies and filaments. The FlowCam is a proven technology that identifies taxa to the genus level and provides an estimate of the abundance of individual cells. It combines digital imaging, flow cytometry, and microscopy to calculate the dimensions, biovolume and abundance of cells. The FlowCam Cyano leverages recent technological developments – a 633 nm laser – enabling the instrument to distinguish cyanobacteria from other algae in a water sample. The abundance of cells within colonies and filaments are counted using a simple Excel based formula, enabling monitoring agencies and researchers to rapidly enumerate cells in large sample volumes. The FlowCam system facilitates an accurate measurement of cell abundance for large folded colonies because the colonies flatten within the unique flow cell chamber. Here we present an overview of the technology along with HAB field data from freshwater systems that affect drinking water and recreational lakes across North America.
Oral session 1

Detection, identification and diversity of toxic/invasive cyanobacteria
CHEMICAL, MOLECULAR AND OMICS ANALYSES OF CYANOBACTERIA

Kaarina Sivonen

Department of Microbiology, University of Helsinki, Viikinkaari 9, FI-00014 Helsinki, Finland

Corresponding author: kaarina.sivonen@helsinki.fi

Cyanobacteria produce bioactive compounds including cyanobacterial toxins but also biomedically interesting metabolites. Genome mining confirmed cyanobacteria to be one of the major bacterial lineages containing high number of nonribosomal as well as ribosomal biosynthetic gene clusters [1]. One class of ribosomal peptides are cyanobactins. Our research has extend cyanobactins to contain also non-heterocyclic and linear peptides [2]. By bioactivity guided search we detected antifungal macrolide scytophycin and glycolipopeptide hassallidin in a number of cyanobacteria and characterized the biosynthesis of hassallidins [3]. We identified nodularin in Brazilian Nostoc strains [4] and novel toxins, lipopeptide anabenolysins and their biosynthesis in benthic Anabaena cyanobacteria and showed that cyclodextrins produced by the same strains improved the antifungal activity of the anabaenolysins [5]. The 4-methylproline (4-mPro) biosynthetic genes were detected in 30 of the 116 cyanobacteria strains, 12 which were confirmed to produce 4-mPro compounds. Altogether, 11 new nonribosomal cyclic peptides, nostoweipeptins and nostopeptolides, were identified from Nostoc sp. strains. The cell experiments showed that these peptides inhibit the uptake of the microcystin hepatotoxin by blocking the organic anion-transporters [6]. We found the swinholide biosynthetic gene cluster from a terrestrial cyanobacterium Nostoc sp. and anabaenopeptins and namalides to be coded by same gene cluster [7]. The detection methods of toxigenic cyanobacteria were extended to include also odorous metabolites producing cyanobacteria [8]. Our recent finding of Baltic Sea Nodularia spumigena showed that they can utilize natural phosphonates and produce methane [9]. Cyanobacteria continue to be excellent source of new discoveries.

References:


FROM PHYLOGENY (AND THUS NAMING THE TAXA DIFFERENTLY) TO NATURAL PRODUCTS OF CYANOBACTERIA, ANOTHER WAY FOR THIS PHYLUM

Muriel Gugger

_Institut Pasteur, Collection des Cyanobactéries, 28 rue du Dr Roux, 75724 Paris Cedex 15, France_

Corresponding author: muriel.gugger@pasteur.fr

Recent access to 100s of cyanobacterial genomes changes our way of considering these organisms under different angles. Although several efforts are done to decipher a more logical classification with monophyletic group and thus new taxa, it seems that cyanobacteria are still so diverse than the goal is still far to achieved. Two collections of cyanobacteria in France and in Japan are tacking this challenge seriously and aim together to implement a new classification frame via the genomics of their cultures. In addition, genomics in the diversity of the cyanobacteria give a better access to their natural compounds and I will give an example through the recent enlargement of the aeruginoguanidine family from _Microcystis_ [1], a highly studied toxic bloom-forming cyanobacteria.

References:
Amplicon sequencing on 16S rRNA genes is one of the techniques adopted to investigate microbiome of various environments. Recently, many studies on harmful cyanobacterial blooms applied this method to investigate bacterial community as well as identify cyanobacterial taxa present in a bloom. Since a portion of cyanobacterial taxa capable of producing toxic or odourous compounds, correct identification of cyanobacterial taxa is crucial to determine the risk associated with bloom events. However, studies have shown that method optimization is still needed for different bacterial group and applications in order to get the best result. Here, we evaluated the potential source of biases in the analysis workflow designated for cyanobacterial bloom research, including choices of analysis pipeline, hypervariable region and reference database. The effectiveness of each option was assessed using mock communities and natural samples based on taxonomic identity, relative abundance, population diversity, genetic distance and number of operational taxonomic units (OTUs). The optimized data-processing strategy obtained here can be translated to other frameworks that assess microbial taxonomy, composition and diversities for future cyanobacterial bloom studies. In addition, the disparate classification results between phenotypic observation and 16S rRNA was significant when the target concentration was low. This highlighted other underlying biases which should be taken into consideration in experimental design and data analysis.

Acknowledgements:
This research grant is supported by the Singapore National Research Foundation under its Environmental & Water Technologies Strategic Research Programme and administered by PUB, the Singapore’s National Water Agency (Grant number: 1102-IRIS-14-02).
THE HIDDEN DIVERSITY OF CYANOBACTERIA UNVEILED BY HIGH THROUGHPUT SEQUENCING APPROACHES

N. Salmaso

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Corresponding author: nico.salmaso@fmach.it

While the use of microscopy can compromise the correct identification of cyanobacteria due to overlap and lack of a suitable number of diacritical features, the use of genetic approaches can limit the taxonomic identification mostly to the isolable and cultivable species. Advances in metagenomics and metabarcoding techniques have opened new rapidly growing fields of research, allowing better identification of the diversity characterizing planktic communities. For example, one of the most exciting discovery was the recent identification of new non-photosynthetic cyanobacteria (NPC) in a variety of environments, including waterbodies and animal guts. At present, owing to the lack of isolates and microscopic descriptions, these new organisms can be identified only using metagenomic analyses of environmental samples. This contribution will evaluate the efficacy of high-throughput sequencing (HTS, Illumina MiSeq) in the study of cyanobacterial diversity in a group of large and deep lakes south of the Alps (lakes Garda, Como, Iseo, Lugano and Idro). The analyses, based on gene marker amplification metagenomics (16S rRNA gene), were carried out on samples collected during the summer months. Bioinformatic pipelines identified several different amplicon sequence variants (ASVs) that coincided with the most abundant taxa previously characterized using traditional microscopic methods and molecular and phylogenetic analyses based on cyanobacterial isolates (e.g. Tychonema, Planktothrix, Dolichospermum, Microcystis). In addition, HTS allowed identification of many other abundant small Synechococcales, Chroococcales, rare large Nostocales never identified so far with traditional approaches, as well as several ASVs belonging to the new classes of NPCs, i.e. Melainabacteria and Sericytochromatia (formerly ML635J-21).
Numerous reports indicate that cyanobacterial blooms are increasing in frequency and magnitude globally. Here we are presenting the first results of a study started in 2016 spanning four years financed by Genome Quebec and Genome Canada entitled Algal Blooms, Treatment, Risk Assessment, Prediction and prevention through Genomics (ATRAPP). For two summer campaigns six lakes in Canada were intensively sampled overs few months to obtain the profile of “before, during and after” the blooms. The spatial (17 sampling sites) and temporal sampling generated close to 900 samples. The samples were tested by mass spectrometry for toxins concentrations (MCtotal and 17 specific variants [1]), nutrients (total & dissolved nitrogen and phosphorous, nitrite and nitrate, ammonium, soluble reactive phosphorous, DOC and TOC), total metal concentrations (ICP-MS). Taxonomy reports 16S genomics are also available and more advanced genomic analysis underway. The picture emerging from these results supports the complexity of the bloom appearance and toxicity triggers.

References:

Acknowledgements: Megan Larsen and Jason Venkiteswaran, Wilfrid Laurier University, Esther McAlisher and Helen Baulch, University of Saskatchewan.
Cyanobacteria are ecologically versatile microorganisms inhabiting most environments, ranging from marine systems to arid deserts [1]. It is a common perception that chances for the discovery of chemical novelty increase by moving towards rarely screened organisms, such as underexplored microorganisms occupying extraordinary habitats or specific ecological niches [2]. In this context we sampled a number of different environments, from freshwater and brackish ecosystems to terrestrial and anchialine caves, spanning from the Canary Islands and Iceland to Estonia and Greece. Thirty-six strains were isolated and characterised based on their morphology and phylogeny, with taxonomic indices and molecular markers (16S rRNA and cpcBA), respectively. Strains were screened for antimicrobial, cytotoxic, and enzyme inhibitory activity, whilst their ability to produce cyanide was examined. Strains were analyzed in order to detect their peptide content. The phylogeny of genera like Scytonema, Geitleria, and Myxosarcina, amongst others, is discussed whilst we report a new species within the newly established Komarekiella genus and new lineages within the Chroococcales order. Twenty of the examined strains seemed capable of producing cyanide, while some of them posed antimicrobial and cytotoxic activity. In most of the strains we were unable to correlate the responsible bioactive agent due to the absence of known cyanopeptides. Nevertheless, we report the presence of new microcystin congeners from Trichormus variabilis. In this work we demonstrate possible correlation of cyanobacteria chemo- with species diversity, which may have implications on strategic focusing of screening programs on underexploited taxa.

References:
Benthic cyanobacterial mats ( BCMs) are natural components of coral reef communities, but are increasing in abundance, probably due to eutrophication. On shallow reefs (<30 m), BCMs grow over sand and macroalgae, and next to corals. Recently BCMs were observed growing on sandy bottoms between depths of 50 and 90 m off the coast of Bonaire, a phenomenon that has not been described before. Presently, not much is known on the composition of BCMs and their impact on other reef organisms. 16S amplicon sequencing showed that all BCMs sampled on Bonaire and Curacao were diverse bacterial communities consisting of thousands of OTUs. Shallow and deep BCMs consisted of similar cyanobacterial species, but had different physiological characteristics. Shallow BCMs fixed nitrogen, while deep mats did not. Standard ecotoxicological assays showed that extracts from shallow BCMs were toxic, whereas extracts of deep mats were not. The absence of the common cyanotoxins microcystins ([D-Asp3]MC-RR, MC-RR, MC-YR, MC-HtyR, [D-Asp3]MC-LR, MC-LR, MC-HiiR, MC-WR, MC-LA, MC-LY, MC-LW and MC-LF), nodularin, anatoxin-a, cylindrospermopsin, saxitoxin, and neo-saxitoxin suggests that other toxins might be responsible for the toxicity observed in shallow BCMs. Adult coral colonies showed a stress response when bordering BCMs, as indicated by a lower potential photosynthetic yield, but since hardly any overgrowth by BCMs of corals was observed these detrimental effects on adult corals were likely only minor. On larvae, the effects were much more harmful: extracts of shallow BCMs decreased the survival of coral larvae and affected survival and settlement success even when exposure to these extracts was removed.
DETECTION OF THE TOXICITY GENES IN ENDOLITHIC COMMUNITIES: ARE COLD DESERT ENDOLITHS STILL ABLE TO PRODUCE CYANOTOXINS?

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Living under harsh conditions, cyanobacteria colonized the endolithic niche to protect themselves from environmental factors. Cyanobacteria are known to produce about 300 bioactive compounds [1], which can be explained as being among their potential strategies to survive in extreme habitats. Most studies up till now have focused on the taxonomic composition and structure of endoliths, and few on the extremophile`s physiological state. The techniques available today are insufficient to provide full understanding of the ecological meaning of some adaptations. Analyses of functional genes diversity, (i.e. toxicity genes), in comparison with microhabitat parameters, could shed some light on the ecological context of possible adaptations. Studies of toxicity genes concerned mostly aquatic communities. Still, there is a lack of information related to endoliths. In this study we sought to answer two questions: how often toxicity genes occurred in terrestrial endolithic genomes; is there a relationship between the occurrence of toxicity genes and environmental conditions?

The classical PCR was applied using five pairs of universal primers coding mcyA, mcyE, mcyE+ndaF, sxtA and anaC genes. Forty-nine environmental samples of endoliths collected from cold desert (Eastern Pamir) and five cultures, isolated from the endoliths, were analyzed. In this study we describe the toxicity gene distribution as well as the diversity of endolithic cyanobacteria based on NGS of V3-V4 region of 16S rDNA. Although we have not revealed any correlation between the occurrence of toxicity genes in endolithic communities and environmental conditions, we have found toxicity genes in almost one sixth of the samples.

References:

Acknowledgements:
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Large hepatotoxic blooms of *Microcystis aeruginosa* are becoming a yearly phenomenon in central and southern Florida largely as a result of discharges from Lake Okeechobee. These toxic blooms have resulted in water closures and health complaints from the public. Although water discharges from the lake to the eastern coast of Florida down the St. Lucie canal have resulted in massive *Microcystis* blooms, the discharges to the west coast via the Caloosahatchee have ended in the Gulf of Mexico. At the same time a bloom of *Karenia brevis* was causing fish and mammal mortalities due to the presence of brevetoxins. We sampled blooms of cyanobacteria and red tide organisms in marine and freshwater environments to determine the distribution of various toxin groups in south-west Florida. We identified microcystins, brevetoxins and β-N-methylamino-L-alanine in water samples from these freshwater and marine environments. The results indicate that these toxins can co-occur in the same environment and that *Microcystis* and microcystins can be present in saline environments. The implications of the overlapping of such algal and cyanobacterial blooms in relation to human and animal health are discussed.

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DIVERSITY AND DISTRIBUTION OF POTENTIALLY TOXIC PHOTOTROPHIC COMMUNITIES IN LAKE BAIKAL

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Lake Baikal is a UNESCO World Heritage Site and holds 20% of the world’s freshwater reserves. Since 2011 an excessive growth of filamentous algae and cyanobacteria was observed in the Lake Baikal. In some cases, the accumulation of photosynthetic microorganisms reached extremely high values, up to 41.7 g chlorophyll a per m³ of water [1]. We performed an analysis of the satellite data about the content of chlorophyll a in the surface layers of the Lake Baikal. This analysis allowed us to identify the major areas of development of algae in Lake Baikal and showed that the highest concentration of chlorophyll a was observed in the coastal areas of the lake with temporary outbreaks in the central part of the lake. Also, we performed the 16S and 18S rRNA gene and transcriptome analysis of the samples of cyanobacterial bloom and oligotrophic waters collected in different parts of Lake Baikal using Illumina MiSeq. The bioinformatics analysis showed that the samples were dominated by potentially toxic cyanobacteria belonging to Dolichospermum, Cyanobium, Synechococcus and Microcystis genera. Also, the expression of several types of toxins was detected. Our study showed that the excessive growth of potentially toxic cyanobacteria occurs in the coastal areas of Lake Baikal that are popular among tourists and the local population.

References:

Acknowledgements:
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POLYPHASIC TAXONOMY OF RISK CAUSATIVE CYANOPROKARYOTES IN BULGARIA

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The cyanoprokaryotes are common inhabitants of water bodies but when developed in massive they are capable of harming wetlands with serious economic damage and consequences for human and animal health. Therefore, their low-cost monitoring with reliable identification is increasingly important. Bulgaria is not an exception in this case and cyanoprokaryotes have been studied with different methods. During this study for first time in Bulgaria the field sampling design was based on combination of expert chosen waterbodies with subsequent choice for sampling sites in each of them after drone observations. Further taxonomical work was based on combined application of real-time PCR, amplification of genes involved in production of toxins (microcystins, nodularins and cylindrospermopsins), standard microscopical examination of field samples, isolation of potential toxin producing strains and their investigation in clonal cultures combined with HPLC-DAD and ELISA toxin identifications. Studied strains are stored in the Algal Collection of Sofia University “St. Kliment Ohridski” (ACUS). The results obtained on species taxonomy and distribution are used for risk assessment for social significant diseases and national security in Bulgaria.

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Oral session 2

Toxic cyanobacteria in the context of climate change
IMPACTS OF RISING CO$_2$ AND GLOBAL WARMING
ON HARMFUL CYANOBACTERIAL BLOOMS

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Cyanobacteria can form dense surface blooms, which threaten ecosystem functioning and degrade water quality for recreation, drinking water, fisheries and human health in many eutrophic waters around the world. Here, I review increasing evidence that both direct and indirect effects of global warming will favor cyanobacterial blooms. In addition, recent research has shed new light on the possible implications of rising atmospheric CO$_2$ concentrations. In particular, a steeper pCO$_2$ gradient across the air-water interface at elevated atmospheric CO$_2$ concentrations will lead to a greater influx of CO$_2$, which can be intercepted by surface-dwelling cyanobacteria. I will illustrate with several examples how a combination of mathematical models, laboratory studies and field data can be used to improve prediction of the impacts of rising atmospheric CO$_2$ concentrations on cyanobacterial blooms. One of the key challenges in this research is the surprising genetic diversity and phenotypic plasticity in the carbon-concentrating mechanism of bloom-forming cyanobacteria. This variability may lead to rapid acclimation and microevolutionary adaptation to changes in CO$_2$ concentrations, and warn that both rising CO$_2$ and global warming are likely to intensify cyanobacterial blooms.
EFFECTIVE APPROACHES TO IMPROVE PREDICTION OF THE IMPACTS OF GLOBAL CHANGE ON CYANOBACTERIA

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Global changes resulting from human impacts, such as over-enrichment of waterways, as well as climate change, are major drivers of cyanoHAB proliferation and persistence. In order to better predict and manage cyanoHABs in a changing world, there is a need to leverage studies undertaken to date, to undertake experiments, observations, and develop models that more effectively capture the temporal scales of processes driven by eutrophication and a changing climate. Better integration of laboratory culture and field experiments, as well as whole system and multiple-system studies are needed to improve confidence in models predicting impacts of these multiple stressors. Recent studies examining the adaptation of species and strains to long-term perturbations, as well as incorporating multi-species and multi-stressor approaches, emphasize the limitations of only using approaches focused on single stressors and individual species. There are also emerging species of concern, such as toxic benthic cyanobacteria, for which the effects of global change require more detailed study. This talk reviews current approaches, and examples of studies globally tackling the challenging issue of understanding how global changes will affect cyanoHABs. It also identifies critical information needs for effective prediction and management.
REVISIT THE PAST TO UNDERSTAND THE PRESENT: PHYSIOLOGICAL AND MOLECULAR EFFECTS OF HIGH CO₂ ON CYLINDROSPERMOPSIS RACIBORSKII (CYANOBACTERIA)

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CO₂ has received great attention since it is the main cause of the greenhouse effect. It is estimated that by the end of this century the current concentration (400 ppm) will triplicate, causing serious climate impacts and consequently harming the environment and life [1]. Cyanobacteria are one of the oldest groups of organisms and therefore faced a great number of environmental changes including alterations in CO₂ concentration [2]. Considering it, the present study evaluated the physiological response (including saxitoxin and cyanopeptides production) of 4 strains of Cylindrospermopsis raciborskii (2 STX +: LETC-CY-05 and T3 and 2 STX -: LETC-CY-01 and LETC-CY-02) and transcriptomic response of 2 of these strains, submitted to 400 or 4,000 ppm of CO₂. ASM-1 medium, 24°C and 100 µmol photon.m⁻².s⁻¹ of light intensity during 72h were the culture conditions. Strains did not vary growth and chlorophyll concentration between treatments as no differences were observed in STX production. One toxic strain (T3) and one non-toxic (LETC-CY-01) presented variation in cyanopeptides profiles between CO₂ conditions. Transcriptomes allowed observing variations in RNA expression of LETC-CY-01 (STX-) and LETC-CY-05 (STX+) in face of high CO₂ and between strains. The results showed that elevated concentrations of CO₂ did not provoke alterations in growth and photosynthesis but differences in metabolic profiles of cyanopeptides and RNA expression. Became evident that each strain behaved physiologically different in response to the challenge imposed. Therefore, it can represent a concern to the management and modeling for predicting cyanobacteria blooms in scenarios with CO₂ increasing.
THE ROLE OF SURFACE WATER WARMING IN THE TIMING OF THE MICROCYSTIS-DOMINATED CYANOBACTERIAL BLOOMS IN WESTERN LAKE ERIE

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In Lake Erie, the smallest and shallowest of the Laurentian Great Lakes, cyanobacterial harmful algal blooms (cyanoHABs) in the western basin (WLE), dominated by potentially toxigenic Microcystis spp., have intensified over the past 15 years. It is well known that spring nutrient loads, primarily from the Maumee River watershed, plays a key role in determining the magnitude of the annual cyanoHAB. Furthermore, shifts in regional climatology, specifically warming and more extreme hydrologic events, leading to increased nutrient loading, is predicted to exacerbate the WLE cyanoHAB [1]. However, one factor that is often overlooked is the potential shift in the initiation and duration of the bloom that may accompany earlier increases in spring water temperatures and warmer waters later into the fall, respectively. Currently the WLE cyanoHAB initiates around mid to late-July, peaks in August/September and dissipates by mid-October. However, if climate shifts cause warmer springs, the timing of bloom initiation could be shifted earlier in the summer. Furthermore, warmer fall temperatures may allow the blooms to persist longer than they currently do. We used high-resolution lake surface temperature retrievals from 2002-2017 and temperature-dependent growth rates of multiple Microcystis strains to evaluate recent changes in the phenology and growth rates. Our findings suggest that if the biogeochemical conditions for bloom formation in WLE persist, rising temperatures will indeed lead to an earlier onset and longer growing season for the Microcystis-dominated blooms with potential negative impacts for water managers.

References:
ONE SECOND SCREENING OF CYANOBACTERIA UTILISING LASER REIMS TECHNOLOGY

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Laser desorption- rapid evaporative ionization mass spectrometry (LA-REIMS) is a recent addition to the field of ambient analysis techniques which allows chemical information to be obtained directly from an object with minimal sample preparation[1]. Here, we report the first instance of the application of this technique to a collection of 26 cyanobacterial strains maintained in culture. With cyanobacterial biomass collected onto a glass-fiber filter(Whatman GF/C), each analysis consisted of >five repeats of one second laser pulse, with the aerosol generated passing into the REIMS source attached to a Q-ToF mass spectrometer (Xevo G2-XS, Waters, Wimslow). The resultant chemical fingerprint of the different species and strains were processed and analysed using multivariate analysis. Both unsupervised and supervised methods were employed with clear differences observed for each sample within the group. Upon building a statistically significant dataset within custom written software, it was possible to build a model and classify the cyanobacteria based on their spectral fingerprint in real-time. Using this approach, cyanobacteria of unknown species and origin can be projected into the compiled model to obtain an answer on the one second time scale. Questions regarding biological variation, bacterial toxicity and minimum cell numbers are still under investigation, but this advancement in immediate screening shows huge promise and further work is underway.

References:

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Over recent decades, increased occurrence of potentially toxic cyanobacteria blooms in lakes have been attributed to anthropogenic eutrophication and climate warming; however, the sources of variation in cyanobacterial bloom severity at short and intermediate timescales (from daily to inter-annual) are poorly understood. Incomplete understanding of the sources of temporal variability complicates efforts to produce useful forecasts of cyanobacteria blooms, a major goal of contemporary limnology. Machine learning (ML) approaches may be useful for identifying important driving processes where traditional models have failed, because they are better-able to represent complex interactions between variables, non-linear dynamics, and threshold effects. As more lake monitoring data become available (from long-term monitoring, high-frequency sensors, and remote sensing data sources), ML approaches may also prove to be more effective than process-based ecological models at producing accurate short- or mid-range cyanobacteria bloom forecasts. Here we present early results from our efforts to forecast blooms in 9 Swiss lakes. Using a combination of process-based physical models and data-driven models of plankton ecology, we will (1) apply random forests and self-organizing maps to identify the abiotic drivers of cyanobacterial growth and distinct categories of cyanobacteria blooms using long-term and high-frequency data; (2) generate projections of lake physical conditions based on weather forecasts and climate scenarios coupled to 1-D hydrodynamic models; (3) generate forecasts of cyanobacteria abundance using ML models (random forests, empirical dynamic models, and recurrent neural networks) trained on monitoring data and hydrodynamic model outputs, and (4) generate web-based forecasting tools available to stakeholders and the public.

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The occurrence of cyanobacterial blooms often leads to changes in the zooplankton community composition. Due to the deleterious effects of cyanobacteria on grazing zooplankton, mediated through morphological properties, the production of toxins or the absence of biochemical nutrients, a shift to small-bodied herbivores is often observed. In the reservoir “Schwarzenbachtalsperre” in the Black Forest (Germany) a harmful cyanobacterial bloom occurred for the first time in 2003. From that year onwards, cyanobacterial blooms were observed every year with strong implications for recreational use and other ecosystem services. In most recent years, cyanobacterial blooms were dominated by nitrogen-fixing cyanobacteria of the genus *Dolichospermum*. In order to investigate if and how the dominant nitrogen-fixing cyanobacteria affect the composition of the zooplankton community, we assessed seasonal changes in the zooplankton and phytoplankton community composition and quantified the seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ signatures of various zooplankton taxa and the phytoplankton community in 2018. In addition, we determined seasonal changes in the concentration of microcystins in phytoplankton samples. We found that the seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter (POM) was correlated with the abundance of different algae classes. Our data also revealed shifts in trophic level ($\delta^{15}N$) and carbon diet source of zooplankton during and after the cyanobacterial bloom, suggesting that the zooplankton switched to other dietary sources during the cyanobacterial bloom. The results demonstrate the importance of incorporating the impact of cyanobacteria-induced changes on food web structure in future nutrient cycling studies and ecological risk assessments of cyanobacterial bloom.

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ANALYSIS OF ENVIRONMENTAL DRIVERS INFLUENCING CYANOBACTERIAL SUCCESSION AND CYANO Toxin PRODUCTION IN THREE LARGE, SHALLOW EUTROPHIC LAKES, CHINA

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Non-diazotrophic Microcystis and filamentous N₂-fixing Aphanizomenon and Dolichospermum co-occur or successively dominate freshwaters globally. Previous studies indicate that dual nitrogen (N) and phosphorus (P) reduction is needed to control cyanobacterial blooms; however, N limitation may cause replacement of non-N₂-fixing by N₂-fixing taxa. To evaluate potentially counterproductive scenarios, the effects of temperature, nutrients, and zooplankton on the spatio-temporal variations of cyanobacteria were investigated. Through structural equation modelling, predictor variables were aggregated into 'composites' representing their combined effects on species-specific biomass. The model results showed that Microcystis biomass was affected by water temperature and P concentrations across the studied lakes. The biomass of two filamentous taxa, by contrast, exhibited lake-specific responses. Furthermore, we compared the spatio-temporal variability of cell-bound and dissolved microcystins (MCs), and succession pattern among Microcystis populations in different studied lakes. The best biotic and abiotic variables for predicting high risk of MCs were identified. This study highlighted that understanding of driving forces of the succession and competition among bloom-forming cyanobacteria will help to guide lake restoration in the context of climate warming and N:P stoichiometry imbalances.
In face of climate change and desertification, it is important to learn about the potential threat posed by cyanobacterial communities in biological soil crusts (BSC) and to determine whether they contain toxigenic species and produce toxins.

We studied BSC in cold mountain desert in the Eastern Pamirs and hot desert in California using NGS to study V3-V4 hypervariable region of 16S rDNA. In 18 out of 25 samples of BSCs from the Eastern Pamirs the dominant species was *Thermosynechococcus elongatus*. Its average share in cyanobacterial communities was 43% (up to 76%). In all but one sample, in which *T. elongatus* was not dominating, *Microcoleus vaginatus* took over, averaging 67%. Both dominant species were often accompanied by heterocytous *Nodularia*, *Nostoc* or *Calothrix*, as a second large taxon. In one sample *Calothrix parietina* dominated (29%).

In BSC from California (20 samples) the cyanobacterial communities were more diverse than in the crusts from the cold desert, and with less pronounced domination of a singular species. The dominant species were: *Microcoleus* sp. (23% on average) predominating in five samples, *Dolichospermum affine* (28%), *T. elongatus* (37%), *Chroococcidiopsis* sp. (22%) and *Phormidium* sp. (16%) each in two samples. In the remaining samples *Calothrix parietina* (59%), *D. mendotae* (28%), *Nodularia* spp. (25%), *Nostoc* spp. (20%) or unidentified species dominated.

We have found potentially microcystin-producing species: *M. vaginatus* in the cold desert and *Leptolyngbya frigida* and *C. parietina* in the hot desert. Additionally, genera from which microcystin-producing species came: *Nostoc*, *Dolichospermum* and *Geitlerinema*, were found in both environments. We haven’t detected species potentially producing anatoxins and saxitoxins.

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Oral session 3

Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/1
Viruses are considered active agents of mortality in microbial communities. Collectively, virus particles are thought to infect cells across the microbial spectrum, with estimates suggesting that as much as one-quarter of photosynthetic carbon flow is redirected through the “viral shunt” back into the pool of organic carbon. Yet information on viral effects on specific populations remains less clear. To address this, we have coupled metatranscriptomes of active, ongoing infections of microbial communities with informatics targeting specific viral and host groups to assess interactions between known hosts and their viruses. This approach has illustrated, for example, that during a bloom in Lake Erie increases in lytic virus-infection of Microcystis occurred concomitant with the detection of microcystin in potable water supplies. In contrast, this approach has demonstrated that viruses indirectly shape cyanobacterial proliferation by acting as potential predators on competing populations. For example in the case of China’s Lake Tai (Taihu), cyanobacterial blooms are accompanied not only by strong evidence for increased lysogen establishment in Microcystis populations entering bloom-maintenance phase, but also by increased representation of viruses consistent with pathogens for competing communities (e.g. diatoms). We present the above information couched within the cautionary context of emerging observations demonstrating regionalism in gene sequences associated with virus populations. The goal of this presentation is to highlight how modern molecular biology can (or cannot) explain the direct and indirect effects of virus activity that shape cyanobacterial bloom dynamics and microbial community succession.
PARASITES OF PLANKTOTHRIX; CYANOPHAGES AND CHYTRIDS AS TOP-DOWN REGULATORS IN A LAKE ERIE EMBAYMENT

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Planktothrix agardhii consistently dominates the harmful algal bloom population in Sandusky Bay, Lake Erie (USA) from May until September each year. This filamentous cyanobacterial genus has a few known parasites; the cyanophage PaV-LD (Lake Dunghu, China), and the chytrid fungal species Rhizophydium megarrhizum sp. Chy-Kol 2008 (Lake Kolbotnvatnet, Norway). The purpose of our work has been to establish how these parasitic interactions affect the host population, specifically during a bloom. Metatranscriptomic data from the 2015 bloom year indicates the presence of endemic variants of both of these pathogens. Utilizing dilution and single filament isolation techniques, 8 chytrid isolates have been obtained for characterization, including host specificity on 20 Planktothrix agardhii isolates and modes of pathogenesis. In parallel work, wild viral stocks were obtained via a modified cation-charged filtration technique. These stocks are then used to establish potential host-viral interactions and for viral purification through multiple rounds of infections. Continuing work will take an ‘omics approach at understanding the spatial and temporal prevalence of phage and chytrid infection as well as relationships between chytrid host range and secondary metabolite production.
BACTERIOPLANKTON ASSEMBLAGES DURING SEASONAL OCCURRENCES
OF MICROCYSTINS, 2-METHYLISOBORNEOL AND GEOSMIN
IN A MIDWESTERN EUTROPHIC RESERVOIR

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The structure and the composition of aquatic bacterial communities depend on climatic, geographic, hydrologic and physicochemical characteristics of water bodies. Eutrophication impairs the chemical balance of water resources and usually leads to cyanobacterial blooms with the generation of toxins and odorous metabolites. Eagle Creek Reservoir, a dimictic eutrophic reservoir in the US Midwest experiences annually frequent blooms of cyanobacteria associated with the production of toxic microcystins, and two odorous compounds: 2-methylisoborneol (MIB) and geosmin. In 2010, the whole bacterioplankton community composition was investigated during seasonal occurrences of these metabolites in spring and fall. The spring bloom was driven by the pelagic \textit{Planktothrix agardhii}, strongly correlated to microcystins (p<0.001), geosmin (p<0.001) and MIB (p<0.05) detections while the benthic \textit{Nostoc} sp. was only correlated to microcystins (p<0.001) and geosmin (p<0.01). During the summer stratification, low detections of metabolites led to weak correlations with thriving cyanobacteria. In early fall, detections of cyanobacterial metabolites were linked to the presence of numerous potential producers but individual contributions were difficult to assess. Constructed association networks of each seasonal bacterioplankton community revealed co-occurrences of known cyanobacterial metabolite-degrading bacteria, such as \textit{Flavobacterium}, \textit{Novosphingobium} and \textit{Pseudomonas} spp. Most of these degraders were found abundant throughout the summer period when metabolites detections were the lowest. The summer and early fall bacterial networks were more complex than the spring’s network. This latter was characterized by elevated toxin and odorous compound detections and, higher levels of nitrate and ammonium in water.
SEASONAL CHANGES IN DISTINCT BACTERIAL MODULES THAT DRIVE HARMFUL CYANOBACTERIAL BLOOMS

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To elucidate the interspecies connectivity between cyanobacterial harmful algal blooms (cyanoHABs) and heterotrophic bacteria, samples were collected from the Nakdong River, Korea, from June 2016 to August 2017, and microbial recurrent association network (MRAN) analysis was performed to overcome the limitations of conventional network analysis. Microcystis blooms were tightly linked with Pseudanabaena in summer and were accompanied by significant changes in the heterotrophic bacterial community composition (hBCC). Riverine bacteria could be clearly separated into modules that were involved in the formation, maintenance, and decomposition of cyanoHABs. Approximately one-fourth of the bacteria that were directly linked with major cyanobacteria acted as connectors or module hubs in cyanoHAB-related modules. The functional profiles of the cyanoHAB-related modules included nitrate reduction, aerobic ammonia oxidation, fermentation, and hydrocarbon degradation during the Microcystis bloom periods. In conclusion, the ecological network in relation to cyanoHABs appears to be highly dynamic; additionally, specific bacterial groups, or modules, contribute to the development and collapse of cyanoHABs. Furthermore, cyanoHABs were directly linked with many ecologically important keystone bacterial species that regulate the overall microbial network. Therefore, to understand cyanoHABs, a modular microbial perspective may be more helpful than a single bacterial species perspective.

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RELATIONS BETWEEN MICROCYSTIN PRODUCERS AND DEGRADERS
IN FRESHWATER BODIES OF WESTERN POLAND

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Microcystins produced by several toxic cyanobacterial strains constitute an important problem for public health. Bacterial degradation of these hepatotoxins may play a key role in natural ecosystems, however the nature of this process is very poorly understood. Thus, a comprehensive description of the relationships between cyanotoxin producers and degraders is of crucial importance. The aim of our study was to investigate such possible relations. Samples collected from several water bodies in western Poland were analysed to determine the chemo-physical parameters, phytoplankton content, bacterial community structure and microcystin-biodegradation potency. A redundancy analysis allowed to document the correlation of the MC-degradation process with some environmental variables (pH, conductivity, chlorophyll content) and MC-producers. A relative abundance of major bacterial phyla was similar in lakes with and without MC-biodegradation potency, however some specific bacteria genera were closely correlated with the strain capable of MC synthesis. Furthermore, the MC biodegradation process was associated with the same bacterial groups. Based on our findings we concluded that: (i) only some specific bacterial classes/orders may be involved in MC biodegradation in the investigated reservoirs; (ii) these bacteria belong to distinct branches of bacteria kingdom and (iii) are closely associated particularly with Microcystis sp.; however they can utilize MC in lakes dominated by other MC-producing cyanobacteria. The presented approach that assumes a more comprehensive analysis of the existing correlations may be helpful in understanding the natural mechanisms of MC elimination involving bacteria such as MC-degraders.

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Due to eutrophication, freshwater ecosystems frequently experience cyanobacterial blooms, many of which produce bioactive metabolites that can affect vertebrates and invertebrates life traits. Zooplankton are able to develop tolerance as a physiological response to cyanobacteria and their bioactive compounds, however, this comes with energetic cost that in turn influence Daphnia life traits and may impair populations. Although, it has been suggested that Daphnia are able to reduce cyanobacterial dominance until a certain cyanobacterial density, it remains unclear whether Daphnia metabolites alone influence the physiological state and bioactive metabolites production of cyanobacteria. The aim of this study is investigating mutual physiological reactions of Microcystis aeruginosa PCC7806 and Daphnia magna. We hypothesize that: a) the presence of D. magna will induce the production of cyanobacterial bioactive compounds, as well as affect growth and stress response in M. aeruginosa and b) The presence of M. aeruginosa will affect physiological responses and life traits in D. magna. In order to test these hypotheses experiments were conducted in a specially-designed co-culture chamber that allows exchange of the metabolites without direct contact. In the presence of the respective other, cyanobacterial growth, photosynthetic activity, production of ROS, and kinetics of cyanobacterial intracellular and extracellular secondary metabolites were monitored and in parallel, Daphnia’s life traits, oxidative stress and energy allocation. Cyanobacterial metabolites reduced survival of D. magna. Simultaneously, presence of D. magna increased production of ROS and impacted dynamics of intracellular and extracellular cyanobacterial metabolites by inhibiting microcystin-LR protective role and increasing excretion of cyanopeptolin A, thus confirming mutual interactions.
Many aquatic cyanobacteria are known toxin-producers. Some cyanobacteria in terrestrial and extreme environments have also been reported as toxic. However, our recent studies provided surprising results, i.e. absence of detectable cyanotoxins and toxin-encoding genes in cyanobacteria and biocrusts of loess sediments. LC-MS/MS and HPLC-DAD analyses performed on 12 cyanobacterial biocrusts and 108 cyanobacterial cultures originating from loess environments of three distinct regions (Serbia, Iran, China) indicated the absence of tested cyanotoxin variants – 8 microcystins, cylindrospermopsin, saxitoxin and BMAA. Furthermore, PCR performed on 60 cyanobacterial strains isolated from these habitats found no microcystin (mcyE), nodularin (nodF), cylindrospermopsin (cyrJ), saxitoxin (sxtA, sxtG, sxtS) or anatoxin-a (anaC) synthetase genes. Our results open many questions for future research on cyanotoxin production in cyanobacteria, concerning e.g. their biological role(s), genetics and the evolution of toxin production. Do environmental conditions applying to loess have a profound effect on the cyanotoxin production? Have cyanobacteria from loess biocrusts lost known cyanotoxin-encoding genes? Or have the genes evolved separately in aquatic species? Do cyanobacteria living in loess crusts produce some unknown toxins? Can the properties of the sediment (e.g. loess) and absence of predators and competitors contribute to the lack of cyanotoxin production? What can we learn from the past of loess-grown cyanobacteria and how can we use the knowledge in controlling their toxicity in the future? Our future eco-physiological, biochemical, molecular and phylogenetic studies will assess a greater number of isolates and loess biocrusts from different regions to form a wider picture on cyanotoxin production by loess cyanobacteria.
Soil is a habitat for cyanobacteria. The abundance and diversity of soil cyanobacteria found in biological crusts of undisturbed arid environments is well documented, but there is very little information on the soil cyanobacteria inhabiting cultivated agroecosystems in the cold humid temperate regions. In this environment, soil cyanobacteria have access to ample nutrients from fertilizers, which may stimulate their growth and production of cyanotoxins. The purpose of this study was to document the presence of cyanobacteria and cyanotoxins in soil from agroecosystems in the cold humid temperate region of south-central Quebec, Canada. Soils were collected from agricultural fields and a nearby forest on a working dairy farm. The presence of soil cyanobacteria was confirmed metagenomics analysis and cyanotoxin concentrations in soil detected with ELISA kits. The concentration of cyanotoxins analyzed by ELISA kits were validated by LC-MS/MS. All tested cyanotoxins were detected in water and soil samples which indicated the presence of indigenous cyanotoxins from native soil cyanobacteria. This study is a first, original report of indigenous cyanotoxins released by soil-inhabiting cyanobacteria. Furthermore, we will present a conceptual model describing how this habitat supports an active cyanobacteria community and stimulates cyanotoxin production in agroecosystems of south-central Quebec.
Oral session 4

Secondary cyanometabolites – structure, biosynthesis, physiological function, environmental significance and biotechnological application
PEPTIDES FROM BALTIC CYANOBACTERIA - DIVERSITY, ENVIRONMENTAL SIGNIFICANCE AND APPLICATION

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Cyanobacteria-derived peptides and peptide-containing compounds are a structurally diverse group of metabolites with a broad spectrum of biological activities. They are synthesized through either ribosomal or nonribosomal pathways. Numerous classes of the compounds and their structural variants have been identified in species representing different taxonomic groups and isolated from different geographical regions. The profile of the peptides was found to be a stable and unique feature of a cyanobacterial strain and was deemed useful as a taxonomic marker.

The presentation focuses mainly on cyanopeptides produced by the Baltic filamentous cyanobacteria, both the bloom forming species and the rarely recorded ones. The profiles of the metabolites were used to determine the intraspecies differences within the Baltic populations. In case of \textit{N. spumigena}, it was proven that the same two subpopulations, characterized by different peptide profiles, have coexisted in the sea for millennia. The analysis of nodularin and anabaenopeptins in deep sediment samples from the Baltic Sea revealed massive occurrence of \textit{N. spumigena} approximately two thousand years ago. The application of the same tools led to a discovery that at least one of the Baltic \textit{N. spumigena} subpopulations occurred in the Norwegian coastal waters even earlier.

The bioactive cyanopeptides are also widely explored as promising drug leads. In case of peptides produced by the Baltic cyanobacteria, the cytotoxic and proteases inhibiting activities have been most frequently observed. Despite extensive research on biological activity and the environmental significance of cyanopeptides, the role of these secondary metabolites in life and functioning of their producer is not yet fully understood.

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GENOMIC AND METABOLIC ANALYSES OF NATURAL PRODUCTS IN NODULARIA SPUMIGENA ISOLATED FROM A SHRIMP CULTURE POND WATER

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The bloom-forming cyanobacterium Nodularia spumigena CENA596 isolated from a shrimp production pond supplied with water pumped from Southeast Atlantic Ocean was found to be a potential prolific source of natural products. Genome-guided approach revealed biosynthetic gene clusters (BGCs) of the known natural products nodularins, spumigins, anabaenopeptins/namalides, aeruginosins, mycosporin-like amino acid and scytonemin, as well as the terpenoid geosmin. Chemical investigations confirmed the production of these metabolic compounds, except for the alkaloid scytonemin. The large number of NRPS and PKS genes predicted in the CENA596 genome indicates many unknown BGCs. Comparative genomic analyses of N. spumigena CENA596 and its three closely related Nodularia strains, two from Baltic Sea and one from Japanese marine sediment, revealed that the number of BGCs in planktic strains was higher than in the benthic strain, and also the geosmin, a volatile compound with unpleasant taste and odor, was unique to the Brazilian strain CENA596. The phylogenomic analysis of N. spumigena CENA596 based on 31 conserved protein sequences positioned this strain together in the same branch with marine Nodularia strains with 100% bootstrap value. The identities between the 16S rRNA gene sequences of the strains are high (99% identity, 100% coverage). Automatic annotation of the genomes using subsystems technology revealed a related number of coding sequences and functional roles. Analysis of orthologous groups identified a large number of shared genes between the Nodularia genomes. The comparative analysis between planktic Nodularia strains showed that their genomes are considerably similar despite their geographically distant origin.
EFFECT OF CYANOPHAGE INFECTION AND LYSIS ON TOXIN NODULARIN IN NODULARIA SPUMIGENA

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The effect of cyanophage infection and lysis on the dynamics of the hepatotoxin nodularin (NOD) and other non-ribosomal peptides (NRPs) produced by cyanobacteria is barely understood. In this study, we assessed changes in concentration of NOD and other NRPs during cyanophage infection of the filamentous cyanobacteria Nodularia spumigena. Viral infection and lysis was associated with significant reduction (93% at the 96 hours post infection) of N. spumigena cell density. While no correlation between N. spumigena abundance and total intracellular concentration of NOD (ng mL⁻¹) was observed, cellular NOD quota (ng cell⁻¹) has gradually increased in the remaining cyanophage resistant N. spumigena subpopulation. Lysis of N. spumigena resulted in substantial increase (>57 times) of extracellular NOD concentration in the culture medium. The relative concentration of other cyclic (anabaenopeptins) and linear (aeruginosins, spumigins) NRPs produced by N. spumigena also increased in response to cyanophage addition. This study highlights the importance of cyanophage infection on the population toxicity of filamentous cyanobacteria and demonstrates a significant contribution of virus-mediated cell lysis on the conversion of NOD from its intra- to extracellular state.

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Cyanobacterial genus *Limnoraphis* was delineated in 2013 and since that time species from this genus have been identified in fresh and brackish waters worldwide [1]. However, no data on the genetic and metabolic diversity of *Limnnoraphis* are available. In this study, we analyzed four *Limnoraphis* strains isolated from surface waters of the Gulf of Gdańsk (Baltic Sea) and deposited in the Culture Collection of Northern Poland (CCNP1314-16, 1324). The molecular phylogenetic analyses were based on sequences of 16S rRNA gene and the adjacent intergenic transcribed spacer (ITS), phycocyanin intergenic spacer region (PC-IGS) and genes from cyanobactin-encoding cluster. The phenotypic analysis comprised cell and filament morphology and the profile of cyanopeptides (LC-MS/MS). The isolated *Limnoraphis* strains showed similar morphological features. They also exhibited 100% similarity of 16S rRNA sequences and formed monophyletic group with other strains from this genus. Genetic variability was much higher when other investigated sequences were considered and correlated well with the metabolic diversity determined based on peptide profiles. Three of the analyzed *Limnoraphis* strains produced several linear cyanobactin variants called aeruginosamids. Cyanobactins are ribosomally synthesized and post-translationally modified peptides (RiPP) with mild cytotoxic activity [2]. Besides aeruginosamide C, which was detected in strain CCNP1324, other identified cyanobactins represent new structural variants of the peptides. The strain CCNP1314 carries the cyanobactin genes, but does not produce any of the peptides identified in other analyzed *Limnoraphis* strains. This is the first report on the production of cyanobactins by cyanobacteria classified to *Limnoraphis* genus.

**References:**

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GENOMIC AND CHEMICAL ANALYSES OF NOVEL NATURAL PRODUCTS FROM BRASILONEMA SPP.

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Brasilonema is a pantropical cyanobacterial genus found on the surface of mineral substrates and plants such as bromeliads, orchids and eucalypts. Although Brasilonema strains have been obtained from several environments and different countries, natural products synthesized by this genus are mostly unknown. This work aimed to identify gene clusters putatively involved in the biosynthesis of secondary metabolites in Brasilonema spp. and to evaluate their natural products expressed under culturing conditions. We sequenced and assembled the genomes of strains B. octagenarum UFV-E1, B. sennae CENA114, B. octagenarum UFV-OR1, B. bromeliae SPC-951 and Brasilonema sp. UFV-L1, isolated from Brazilian environments, using the Illumina HiSeq platform. Predictions for secondary metabolite genes estimated 25 clusters in the genomes of strains UFV-E1 and CENA114, 33 in UFV-OR1, 12 in SPC-951 and 22 in UFV-L1. While most gene clusters did not present significant similarity to references from known biosynthetic pathways, clusters putatively encoding pathways for known molecules included geosmin, microviridin, marinostatin, plesiocyn, shinorine, nostopeptolide and anabaenopeptin as likely products. Methanol extracts of B. octagenarum UFV-E1 were then analyzed in the Waters SYNAPT G2-Si mass spectrometer with a Kinetex 1.7 µm 100Å C8 column, which confirmed the production of two anabaenopeptin variants and new microviridin and mycosporine-like amino acid variants, along with yet unidentified molecules. These results show that Brasilonema spp. are interesting targets for the discovery of novel natural products, with potential to produce a considerable number of currently unknown compounds.

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A PRELIMINARY ASSESSMENT ON THE POTENTIAL OF SPONGE-ASSOCIATED CYANOBACTERIA TO PRODUCE BIOACTIVE COMPOUNDS

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Marine cyanobacteria are considered a rich source of bioactive natural products with potential biotechnological and pharmacological applications [1]. However, information on production of novel natural compounds from sponge-associated cyanobacteria is scarce. The present study aims to provide a preliminary assessment on the potential of sponge-associated cyanobacteria isolates [2] representing different taxonomic groups to produce bioactive compounds and the biological activity of their extracts. PCR screening for the presence of genes encoding non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) was performed [3]. Agar disc diffusion assays were used to determine the ability of cyanobacterial extracts to inhibit the growth of four pathogenic bacteria (Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus). For cytotoxicity screening, cyanobacteria extracts were tested against several cancer cell lines. Furthermore, a multi solvent extraction protocol was developed for the thorough fractionation of the cyanobacterial extracts. Then, LC-MS/MS analyses of the fractions were carried out to search for specific metabolites of interest and their analogues. PKS and NRPS genes were detected in the majority of the strains tested, indicating the metabolic potential of the isolates. Sponge-associated cyanobacteria strains belonging to Leptolyngbya, Pseudanabaena, and Synechococcus were found to have activity against E. coli, P. aeruginosa and S. aureus. Extracts with the most promising bioactivity deserve further investigation in order to isolate and identify bioactive molecules. Results of the present study could constitute a valuable starting point for the biodiscovery of natural compounds with biomedical value from sponge-associated cyanobacteria.

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Mass occurrences of cyanobacteria in the shallow Curonian lagoon (SE Baltic Sea) are associated with the presence of cyanotoxins and other bioactive metabolites. In the previous studies, cyanotoxins were found in water, insects, mollusks, fish tissues, sediments during cyanobacterial blooms [1,2,3]. They were detected even in spring, possibly, as a result of a decay of cyanobacterial biomass [1,3]. This study is focused on both spatial and temporal patterns of distribution of cyanotoxins and other cyanobacterial metabolites in the Curonian Lagoon. We analyzed regular water samples during cyanobacteria vegetation period in 2013-2017 from five stations in the Lithuanian part of the lagoon. Additionally, two stations were sampled also during the winter season in 2016-2017. The presence of secondary metabolites was determined using LC-MS/MS. During the investigation period microcystins (MCs), nodularin, anatoxin-a, anabaenopeptins (APs), aeruginosines (AERs), aeruginosamides (AERMD), cyanopeptolins (CP), microginins (MRG) were detected. MC-RR and -LR were present in samples all year round in the central part of the lagoon. Other peptides, such as Osc-Y, AP-A, AERMD, were commonly detected in winter samples together with small biomass of *P. agardhii*, *Microcystis* spp. MC-RR and Asp³MC-RR were found at the highest concentrations (respectively 15.6 and 22.2 µg/l. After RDA analysis, environmental factors (water temperature, salinity) in combination with observed biomass of main potentially toxic cyanobacteria (*Aphanizomenon*, *Microcystis*, *Planktothrix*, *Dolichospermum*) accounted for about 50% of the variance in MC concentrations. We did find a close relationship between concentrations of demethylated microcystins (Asp³MC-RR and Asp³MC-LR) and biomass of *Planktothrix agardhii*, while MC-RR, -LR, and -YR were mostly associated with the abundance of *Microcystis* spp.

References:
UNDERSTANDING DIFFERENTIAL PARALYTIC SHELLFISH TOXIN PROFILES BETWEEN TWO STRAINS OF SCYTONEMA CRISPUM

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Of all known cyanotoxins, Paralytic Shellfish Toxins (PSTs) – that is saxitoxin and related analogues – are the most toxic. While the genetics underlying PST biosynthesis were initially proposed in 2008, biochemical validation of the putative pathway remained non-existent until 2018. This study investigated two PST-producing strains of the cyanobacterium Scytonema crispum, CAWBG524 and CAWBG72, isolated in New Zealand. Genome sequencing of both strains revealed the largest saxitoxin biosynthesis (sxt) clusters described, with the highest abundance in transposable elements [1]. While genomes sequences of the two strains show high similarity, their toxin profiles are vastly distinct. S. crispum CAWBG524 produces 10 PSTs (2 which are sulphated at the C11 position) while S. crispum CAWBG72 produces 22 PSTs (13 which are sulphated at the C11 and/or N21 position) [2]. Comparative genomics correlated these variations to transposase insertions that inactivated genes encoding the tailoring enzymes SxtN, SxtO and SxtDIOX, in S. crispum CAWBG524. These enzymes were predicted to be responsible for the production of sulphated PSTs. To validate this hypothesis, we investigated the function of the putative N-sulphotransferase SxtN via substrate binding assays and in vitro biochemical assays. Our results confirmed that SxtN acts as a N-sulphotransferase on the N21 position of saxitoxin yielding the monosulphated PST gonyautoxin-5, thus explaining the lack of N-sulphated analogues in S. crispum CAWBG524 [1]. This study is the first to correlate biochemical characterisation of PST biosynthesis to the toxin profile within cyanobacteria.

References:

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TAXONOMY, TOXICITY AND ECOLOGY OF THE CYANOBACTERIUM LEPTOLYNGBYA SP. ISOLATED FROM ALGAL MATS FROM GEOTHERMAL PONDS IN UNIEJÓW (POLAND)

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The thermal pool complex in Uniejów is a famous and very fast developing tourist attraction in central Poland located on the banks of the River Warta. Its spectacular tourist success is based on the use of hot ground water. The water is pumped from a depth of over 2000 meters and has a temperature of 68 °C. It contains a high concentration of minerals with the high conductivity of about 12 mS.cm⁻¹ and average pH about 7.5. Due to reach mineral content thermal brine from Uniejów finds application in the treatment of many different diseases and production of some food and cosmetics.

An additional attraction for scientists are thick algal mats growing in artificial ponds situated next to the pool complex where hot groundwater is partly discharged. In contrast to planktic species, our knowledge of the toxicity and ecology of benthic cyanobacteria is much poorer. The aim of this study was therefore to investigate the morphological diversity, taxonomic composition and toxicity of cyanobacteria forming mats. The collected mats were distinct layered and composed mainly of thin filaments surrounded by mucilage sheets. Based on the morphological characteristics of the cyanobacterium from natural material and culture, this taxon was classified to Leptolyngbya group. Molecular analysis confirmed the close relationship of the isolated strain to Leptolyngbya genus. Preliminary toxicity analysis using HPLC-DAD methods did not show the presence of microcystins, cylindrospermopsin or anatoxin-a. Chemical analysis of cyanobacterial exudates showed a high content of polyphenols what makes benthic cyanobacteria potential natural source of bio-products.
Oral session 5

Cell physiology and molecular biology of cyanobacteria
Light, which is essential for photosynthetic organisms, becomes dangerous for them when the energy arriving at the photochemical reaction centers exceeds the energy consumption by multiple cellular processes. This occurs under high irradiance but also under nutrient starvation conditions. In these cases, the entire photosynthetic electron transport chain becomes reduced and reactive oxygen species (ROS) leading to severe damages of the cells. Thus, it is crucial for photosynthetic organisms to be able to constantly sense and adapt to environmental changes.

Plants, algae and cyanobacteria developed acclimatory responses that involve protein synthesis and degradation to maintain the balance between the energy absorbed and the energy used, to decrease the accumulation of ROS or to scavenger them. In addition, they possess mechanisms that sense the quality and quantity of incident light and rapidly induce a reorganization of the photosynthetic apparatus in order to decrease the energy arriving at the photochemical reaction centers and/or balance the activity of each photosystem. The mechanisms differ in cyanobacteria from those existing in plants and algae due to the special cyanobacterial antenna, the phycobilisome. In this conference, I will specially describe two photoprotective mechanisms: The Orange Carotenoid Protein (OCP)-related Non-Photochemical-Quenching (NPQ) and state transitions. The OCP is a photoactive protein that sense light intensity and induces thermal dissipation of excess excitation energy by interacting with the phycobilisome. The OCP-related NPQ is induced only under high irradiance independently of the redox state of the electron transport chain. In contrast, the redox state of the plastoquinone pool regulates state transitions by inducing a restructuration of the photosynthetic apparatus. This leads to a rebalance of the photosystem activities.
Toxic Microcystis spp. blooms constitute a serious threat to water quality worldwide. Aeromonas veronii was isolated from Microcystis sp. colonies collected in Lake Kinneret. Spent Aeromonas media inhibits the growth of Microcystis aeruginosa MGK isolated from Lake Kinneret. The inhibition was much stronger when Aeromonas growth medium contained spent media from MGK suggesting that Aeromonas recognized its presence and produced secondary metabolites that inhibit Microcystis growth. Fractionations of the crude extract and analyses of the active fractions identified several secondary metabolites including lumichrome in Aeromonas media. Application of lumichrome at concentrations as low as 4 nM severely inhibited Microcystis growth. Inactivation of aviH in the lumichrome biosynthetic pathway altered the lumichrome level in Aeromonas and the extent of MGK growth inhibition. Conversely, the initial lag in Aeromonas growth was significantly longer when provided with Microcystis spent media but Aeromonas was able to resume normal growth. The longer was pre-exposure to Microcystis spent media the shorter was the lag phase in Aeromonas growth indicating the presence of, and acclimation to, secondary MGK metabolite(s) the nature of which was not revealed. Our study may help to control toxic Microcystis blooms taking advantage of chemical languages used in the interspecies communication.
METAGENOMIC DIVERSITY OF BLOOM FORMING CYANOBACTERIA THROUGH TIME AND SPACE

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Cyanobacteria occur within phytoplankton populations as dense assemblages of multiple species often exhibiting a high degree of inter- and intra-specific phenotypic and genomic variation. This high level of variation allows for co-occurring species and strains to inhabit diverse environmental niches. In recent years research has started to analyse this high genotypic and phenotypic plasticity of cyanobacteria by coupling culture-dependent approaches with whole genome sequencing [1,2]. Whilst it is possible to describe the high intra-specific diversity that occurs within a single sample, extrapolating these approaches over time and space are not yet feasible. We have sought to evaluate whether intra-specific genomic diversity could be evaluated via the metagenomic analysis of samples collected over time and space. Towards this end we compiled pan-genomes from sequenced genomes of toxic bloom-forming cyanobacteria and created individual searchable databases for each organism. For each organism we identified, from multiple metagenomes, sets of core genes shared by all members of the population and sets of variable genes that occur infrequently throughout the population. We will present results of cyanobacterial intra-specific diversity spanning time and space. Specifically, we highlight metabolic processes intra-specific diversity and whether this changes over multiple temporal scales. The research emphasises both the intra-specific genomic diversity of cyanobacteria and the stability of these complex cyanobacterial assemblages, demonstrating the role of intra-specific genomic variation for population success and decline under changing environmental conditions.

References:
Microcystins and nodularins are potent toxins produced by a range of distantly related cyanobacteria [1] and are responsible for human poisonings as well as the deaths of wild and domestic animals around the world. They are assembled on large enzyme complexes encoded in the microcystin (mcy) and nodularin (nda) biosynthetic pathways [2-4]. There are over 100 chemical variants of microcystin and nodularin reported to date [4]. Here we traced the distribution of mcy and nda biosynthetic pathways across the cyanobacterium phylum. We identified 35 complete mcy and nda gene clusters from public sequence repositories and obtained a further 11 new mcy gene clusters from the UHCC culture collection for this study. We reconstructed the evolutionary history of the mcy and nda biosynthetic pathways and examined the roles of common ancestry, convergent evolution, horizontal gene transfer, and recombination in promoting the chemical diversity of these toxins. Recombination events appears to the primary determinant of chemical diversity but the gain and loss of biosynthetic enzymes as well as gene fusions play an important role in shaping the diversification of this ancient pathway. This analysis lead to the discovery of new homo-amino acid containing microcystin variants and uncovered cross-talk between different natural product pathways. Together this information provides a new consensus on the origins, distribution and evolution of the biosynthetic pathways in cyanobacteria. We used this information to redesigned PCR-based strategies to detect microcystin and nodularin biosynthetic pathways from environmental samples in order to access the diversity of over-wintering cyanobacterial communities from the Baltic Sea.

References:

Acknowledgements:
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TRANSCRIPTOMIC AND METABOLOMIC ANALYSES TO REVEAL THE MOLECULAR MECHANISM OF RESISTING AND DEGRADING MICROCYSTIN IN MIXOTROPHIC OCHROMONAS

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Toxic Microcystis blooms are a worldwide environmental issue with a large quantity of negative influences on aquatic ecosystem. Especially, microcystin is highly toxic and is an easily accumulated secondary metabolite of toxic Microcystis that threatens water safety. Biodegradation of microcystin by protozoan grazing is a promising and efficient biological method, but the mechanism in this process is still unclear. In this study, to seek the potential process of degrading microcystin in flagellates, we preformed comparative transcriptomic and metabolomic analyses of mixotrophic Ochromonas fed on toxic Microcystis (TM) and non-toxic Microcystis (NM). The results showed there were 999 differently expressed genes (DEGs) and 443 differently expressed metabolites (DEMs) between TM and NM treatments. These DEGs and DEMs were mainly involved in translation, carbohydrate metabolism, energy metabolism and cell proliferation. Integrating transcriptomic and metabolomic analyses suggested strong resistance of mixotrophic Ochromonas to microcystin was related to enhanced antioxidant and repair activities in the cell and extracellular matrix protection strategy; microcystin degradation in Ochromonas benefited from metabolic activity in lysosome, (strong oxidation of ROS) and the detoxification of GST and GSH. The present study provided a better understanding of transcriptomic and metabolomic responses of flagellates to toxic Microcystis as well as highlighted a potential mechanism of biodegrading microcystin by flagellate Ochromonas, which served as a strong theoretical support for control of toxic microalgae by protozoans.

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SUBCELLULAR LOCALIZATION OF TOXIC PEPTIDES IN BLOOM-FORMING CYANOBACTERIA REVEALS A DISTINCT COMPARTMENTATION

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Harmful algal blooms formed by colony-forming cyanobacteria threaten our water resources by the production of cyanotoxins that mostly occur intracellularly. The mechanisms enabling high intracellular concentrations remain unknown. We aim to visualize toxic microcystin (MC) and anabaenopeptin (AP) - peptides under noninvasive conditions on single cell level. Our approach is enabled by the promiscuity of certain key enzymes during the respective nonribosomal peptide biosynthesis pathway. These key enzymes can use non-natural functional groups as precursors (e.g. amino acid-alkynes) enabling subsequent labeling by an azide-modified fluorophore through a copper-catalyzed click chemistry reaction. We demonstrate that the filamentous genus Planktothrix and the unicellular genus Microcystis revealed a significant incorporation of unnatural amino acids resulting in modified AP or MC-peptides carrying the incorporated alkyne moiety, which were subsequently labeled in the cells. Counting cells carrying peptide signals suggested that peptide labeled subpopulations could be reliably differentiated. Sensitive in vivo techniques such as laser scanning confocal microscopy revealed that toxic peptides occur as distinct entities inside the cell with a cellular wide distribution. The observation that peptide signals are concentrated in distinct compartments rather than homogeneously distributed across the cell volume has implications for our understanding on the mechanism of storage in the cell.
Microcystis aeruginosa is well known for its ability to form dense blooms at the water surface. The frequent production of microcystin and its impact on the bloom-forming life-style are poorly understood. The toxin was demonstrated to covalently bind to the key-enzyme of the Calvin-Benson cycle, RubisCO. We studied the response of low light adapted M. aeruginosa PCC7806 to a high light shift to get a better understanding how it adapts to fluctuating light conditions. Subcellular fractionation studies and fluorescence and electron microscopy revealed that a substantial portion of RubisCO is not associated with carboxysomes after a few hours of high light treatment, although carboxysome localization of RubisCO is considered as a pivotal component of the carbon concentrating mechanism of cyanobacteria. The exposure to high light radiation leads to a re-localization of RubisCO from the cytosol towards the cell membrane. There it accumulates in spots without carboxysomes. The re-localization of RubisCO could be an effective way to fixate CO2 without the need to assemble carboxysomes under fluctuating light conditions. Degree and dynamics of the re-localization process significantly differ between the microcystin-producing strain PCC7806 and its microcystin-lacking ΔmcyB mutant. Furthermore a significantly higher amount of 2-phosphoglycolate is produced in ΔmcyB in comparison to the wild type. These differences suggest that microcystin interferes with RubisCO assembly and localization and modulates its substrate affinity. We hypothesize that microcystin binding enables an extreme versatility of RubisCO that involves secretion of RubisCO products and promotes the mutual interaction of Microcystis with its heterotrophic microbial community.
RESPONSES OF CYANOBACTERIA TO HYDROGEN PEROXIDE AT ELEVATED CO\textsubscript{2}

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Cyanobacterial blooms have been considered to be a common environmental issue in many parts of the world. At the same time, atmospheric CO\textsubscript{2} concentrations are rising globally. Rising CO\textsubscript{2} levels will probably intensify the blooms in eutrophic and hypertrophic waters. Recently, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) a strong oxidant chemical, was proposed as a potent algaecide for cyanobacterial bloom control in lakes. H\textsubscript{2}O\textsubscript{2} is non-polluting as it decomposes into H\textsubscript{2}O and O\textsubscript{2} within 1-2 days. To find out how cyanobacteria response to H\textsubscript{2}O\textsubscript{2} under elevated CO\textsubscript{2}, Microcystis PCC7806 was cultured in chemostats under 150 ppm and 1500 ppm CO\textsubscript{2}. Subsamples were treated with different concentrations of H\textsubscript{2}O\textsubscript{2} in batch cultures under different light intensities (15 and 100 \textmu mol photons m\textsuperscript{-2} s\textsuperscript{-1}) and the response was monitored during 24 h. Microcystis cultured under high CO\textsubscript{2} showed a higher biovolume, higher cell count, and higher microcystin-LR content, but a lower photosynthetic activity and a lower pH compared to the cultures under low CO\textsubscript{2}. Results showed that Microcystis was more sensitive to H\textsubscript{2}O\textsubscript{2} when grown under C-limitation. Exposure to high light after H\textsubscript{2}O\textsubscript{2} addition also increased the sensitivity and microcystin-LR decreased much faster under high light during H\textsubscript{2}O\textsubscript{2} treatment. The results imply that more H\textsubscript{2}O\textsubscript{2} will be needed for cyanobacterial blooms suppression when atmospheric CO\textsubscript{2} increases in future.
EFFECTS OF ROXITHROMYCIN ON MICROCYSTIN PRODUCTION
OF M. AERUGINOSA

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Roxithromycin (ROX) is a widely used antibiotic detected various surface waters. To reveal its interaction with toxic cyanobacteria, *Microcystis aeruginosa* PCC7806 strain was exposed to ROX at two different concentrations (0.05 µg/L and 10 µg/L). All exposed samples along with control group are incubated for 96h at 24°C with 18:6 light/dark periods in triplicates. Methanolic extracts of intracellular metabolites were analysed with Thermo Q-Exactive high resolution mass spectrometer. The data is acquired in a fashion that enables us to investigate global metabolism (non-targeted analysis), microcystin production (targeted analysis), and microcystin like compound production (suspect analysis). Data were analysed with Thermo Compound Discoverer 2.1. Low concentration (0.05 µg/L) of ROX did not exert growth inhibition, while high (10 µg/L) concentration caused approximately 50% growth inhibition over 96h period. Unsupervised learning (principle component analysis) showed that metabolites in low dose ROX exposure started to be different than control group after 24 hours unlike growth inhibition results. The difference in metabolisms was specifically due to inhibition on carotenoid biosynthesis and lipid synthesis pathways for ROX exposed groups. ROX concentrations affected the production of microcystin (MC) variants (MC-LR and [d-Asp3]MC-LR) produced by *M. aeruginosa* PCC7806. Low concentration has no particular effect on MC production, on the other hand, high concentration lowered MC-LR production when promoting the production of [d-Asp3]MC-LR. Moreover, two metabolites of MC-LR and [d-Asp3]MC-LR, namely MC-LR-alanine and [d-Asp3]MC-LR-sarcosine, were detected in control group. Our findings indicate even low levels of environmental contaminants may alter microcystin production and metabolism of it in toxic cyanobacteria.

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Oral session 6

Toxicity and harmful effects of cyanobacteria and their metabolites
Cyanobacteria produce a wide range of bioactive compounds including highly toxic cyanotoxins, which are of particular concern as they can affect human and animal health as well as ecosystems. In humans, acute intoxication with cyanotoxins is not very likely; however, they are of high human health concern due to their potential long-term adverse effects (genotoxic, carcinogenic, reproductive) at chronic exposures to low concentrations, which occur through consumption of contaminated water and food, dermal exposure and/or inhalation. The mechanisms behind the toxic effects of cyanotoxins are different as they are structurally diverse chemicals and defined by their potential toxicological activity they fall into different groups ranging from hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, irritant toxins, gastrointestinal toxins and many yet unknown biologically active metabolites. To identify the threat for human and animal health, it is important to evaluate their potential toxicological in particular genotoxicological risk as genotoxic compounds interfere with the function of DNA and genetic material resulting in cancers and other chronic diseases, reproductive effects, heritable disease, and as shown by more recent studies also neurodegenerative disorders. Therefore, the assessment of the genotoxic potential of cyanotoxins represents an important component for the safety assessment of cyanotoxins, which is relevant for the protection of human health and the environment. The presentation will discuss genotoxic and potential carcinogenic properties of cyanotoxins, which will be supported by the in vitro data obtained for two in freshwater environment ubiquitously present cyanotoxins with different mechanisms of action, namely microcystin-LR and cylindrospermopsin.
FRESHWATER CYANOBACTERIAL COMPOUNDS AND THE PLANT CYTOSKELETON – A "LOVE TO HATE" STORY?

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Freshwater cyanobacteria produce a wide range of bioactive compounds, such as cyanotoxins, a chemically diverse group of water-soluble secondary metabolites, often released into the aquatic environment [1, 2]. Cyanotoxins (microcystins, among them) are harmful to many eukaryotic organisms, including several plant species [3], but are only produced by certain strains of cyanobacteria and therefore comprise a small part of the total amount of cyanobacterial bioactive compounds. This study aims to highlight the effects of both toxic (microcystin-producing) and non-cyanotoxin-producing freshwater strains on the plant cytoskeleton (microtubules and F-actin). Due to their commercial value and concerns over consumer health, widely consumed crop species (rice, maize, wheat), potentially exposed to water contaminated by such strains via irrigation, were chosen for experimental purposes. Four- to five-day-old seedlings were treated for various time periods (30 min - 24 h) with methanolic (evaporated and resuspended in distilled water) extracts from 10 different strains of microcystin-producing (Microcystis flos-aquae TAU-MAC 1410 and 1510, Microcystis sp. TAU-MAC 1910 and 2410) and non-cyanotoxin-producing (Calothrix sp. TAU-MAC 0399, Synechococcus sp. TAU-MAC 0499, Chlorogloeopsis fritschii TAU-MAC 0599, Jaaginema sp. TAU-MAC 0110 and 0210, Cylindrospermopsis raciborskii TAU-MAC 1414) strains isolated from Greek freshwaters. Confocal laser scanning microscopy (CLSM), epifluorescence microscopy and transmission electron microscopy (TEM) on treated root tips revealed that all extracts induced cytoskeletal alterations in root cells. Considering the fact that microcystins are known to disrupt microtubules [3], this is the first report of non-cyanotoxin-producing freshwater strains affecting the plant cytoskeleton. Effects on chromatin and subcellular components are also discussed.

References:
PROTEOMIC RESPONSES OF THE MARINE MUSSEL *MYTILUS GALLOPROVINCIALIS* TO TOXIC FRESHWATER CYANOBACTERIA BY QUANTITATIVE PROTEOMICS

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Many freshwater ecosystems worldwide are affected by the proliferation of toxic cyanobacteria. Likewise, ecosystems at the land-sea interface are also affected whenreceive inland waters contaminated toxic cyanobacteria. This study aimed to disclose the effects of freshwater toxic cyanobacteria on the marine mussel *Mytilus galloprovincialis*. For this investigation marine mussels were fed with three different microalgae species (105 cells/ml) twice a day for 15 days with the toxic cyanobacteria *Microcystis aeruginosa* and *Chrisosporum ovalisporum*, and the non-toxic microalgae *Parachlorella kesseleri*, followed by 15 days of depuration. Feeding mussels with toxic cyanobacteria led to several responses at the proteome level in mussels hepatopancreas, as revealed by shotgun proteomics. In total 39 proteins were affected during mussel exposure. The proteomic responses differed in respect to the conditions of exposure and mussel diet. Among the proteomic responses detected we highlight those related with the regulation of protein activity (changes in PDIA, Calr and YWHAE), when mussels were fed *M. aeruginosa* cells, and cytoskeleton structure and function (ACTB, Paramyosin, TPM, ACTN, COL1A2, LCP1, FSCN1), gene transcription/translation (ribosomal protein, RNA-binding protein, RPS5), regulation of protein activity (HSP90, RPN1) and protein catabolism (CTSB, MEP1A, CTSD) when mussels were fed simultaneous with *M. aeruginosa* and *C. ovalisporum* cells. Moreover, the depuration allowed mussels to partially recover from the effects of toxic cyanobacteria. In conclusion synergistic effects of toxic microalgae at the proteomic level should be considered to evaluate the impacts in mussels and likely the co-occurrence and feeding on different toxic cyanobacteria could be particularly adverse to these organisms.

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SYNTHETIC MiR92b-3p DELIVERY AND THE EFFICACY OF GENE EXPRESSION SILENCING IN WHITEFISH: TOWARDS UNDERSTANDING MiR92b-3p FUNCTION IN MICROCYSTIN-LR–INDUCED LIVER INJURY IN FISH

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Successful applications of synthetic miRNAs to resolve problems pertinent to fish biology and immunology prompted us to use RNA interference methods to investigate patterns of microcystin-LR–induced liver injury (MILI) in a teleost fish, whitefish (Coregonus lavaretus). In that fish, repeated exposure to microcystin-LR (MC-LR) results in severe liver damage, followed by an unexpected resilience to further exposures to the toxin and regeneration of the damaged liver structure [1]. In these aberrant processes, we found expression of one microRNA, MiR92b-3p, to be substantially reduced in the challenged group [2]. To study the potential biological function of MiR92b-3p in MILI in fish, juvenile whitefish were exposed to a synthetic analog of MiR92b-3p suspended in Invivofectamine 3.0 transfection reagent [3]. Our results indicate that the MiR92b-3p mimic is effectively delivered via intraperitoneal injection to the spleen and the liver of whitefish, and that it likely achieves functionality without causing any apparent toxic effects in the challenged animals. We also report the novel finding that the MiR92b-3p mimic reduced the in vivo liver mRNA expression levels of its putative pro-apoptotic targets (p53, cdkn1a and pcna), and important metabolic genes, e.g. cdo1. This methodology for delivering short snippets of RNA has recently been applied in a study on a larger scale, which included long-term exposure of juvenile whitefish to the mimic or the inhibitor of MiR92b-3p alone, or in combination with MC-LR. To understand the role MiR92b-3p in MILI, an array of different methods are currently being used to explore the effects of fish treatments with these synthetic RNA agents on different levels of biological organization, from cells to individuals.

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CELL TYPE-DEPENDENT EFFECTS OF CYANOBACTERIAL HEPATOTOXINS CAN CONTRIBUTE TO THE DEVELOPMENT OF CHRONIC LIVER DISEASES – EVIDENCE FROM STEM CELL-BASED 2D AND 3D HEPATIC IN VITRO MODELS

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Parenchymal hepatocytes are the most abundant liver cell type and best-recognized target of hepatotoxic cyanotoxins microcystin-LR (MC-LR) and cylindrospermopsin (CYN). However, development, function, regeneration, and repair of the liver depends also on the other cell types, including adult (tissue resident) liver stem cells/liver progenitor cells. These cells could be also affected by hepatotoxic cyanotoxins. Therefore, we utilized hepatic stem cell-based in vitro models to investigate cell type- and differentiation-stage specific effects of cyanotoxins. Specifically, human embryonic stem cells (hESCs) [1] and immortalized adult human liver stem cells HL1-hT1 were used in a monolayer [2] or 3-dimensional hepatospheroid cultures [3]. Hepatic cells were becoming gradually more sensitive to cytotoxic/necrotic effects of both CYN and MC-LR in the hepatospheroid cultures of HL1-hT1 or during hepatic differentiation of hESCs towards mature hepatocyte-like cells. Subcytotoxic concentrations of CYN selectively eliminated HNF4A+ cells from the population of differentiating hESC-derived liver progenitor cells, induced lipid accumulation in immature hepatocytes, and impaired their differentiation into mature hepatocyte-like cells. In undifferentiated HL1-hT1 cells, CYN did not increase oxidative stress or DNA damage but activated MAPKs ERK1/2 and p38, as well as stress-inducible transcription factor ATF3. MC-LR also induced various cell type- and differentiation stage-specific effects. The observed interactions of MC-LR and CYN with liver stem and progenitor cells vs. differentiated cells suggested several mechanisms leading to disruption of liver tissue homeostasis in response to cyanotoxins, which might represent key events contributing to the development of adverse health outcomes, such as chronic liver toxicity, steatosis or liver cancer.

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The impacts of MC and *Microcystis aeruginosa* lysate ingestion on the gut microbiome were studied using a preclinical exposure model. Ninety-six C3H/HeJ, male mice were assigned into 4 groups (negative control with pure water; positive control with phenobarbital, a tumor-promoter; MC group with water + 10 µg/L MC-LR; and Lysate group with water + *M. aeruginosa* lysate containing 10 µg/L total MCs). At 4 weeks of age, all mice were injected with 5 µg/mg diethyl-nitrosamine, initiating liver carcinogenesis. Following a 4-week recovery period, mice were administered water, phenobarbital, MC-LR, or lysate. Fecal samples were collected at 4, 12, 20, 28 and 36 weeks. DNA was extracted and microbial community was analyzed by targeting V4–V5 regions of 16S rRNA genes. Aerobic and anerobic bacterial counts were also determined. At 36 weeks of age animals were euthanized and necropsied. Overall longitudinal taxon change was an increase in *Firmicutes* and a decrease in *Bacteroidetes* in the entire groups. The abundance of *Firmicutes* in the positive control and lysate groups was much higher than the negative control and MC groups. Exposure to MC-LR or lysate was associated with significantly decreased bacterial richness and diversity (p<0.05). Moreover, a principal coordinate analysis plot based on Bray-Curtis dissimilarity showed a distinct separation between the negative control and MC-LR/lysate groups. Bacteria colony counts were significantly lower in all the treated groups than the negative control (p<0.05). Phenotypic diversity of bacteria (size, color and shapes of colonies) from the MC/lysate-treated groups was dramatically lower than the control group.
NEW REPORTS ON NEUROTOXICITY OF RAPHIDIOPSIS RACIBORSKII STRAINS

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Cyanobacteria blooms are considered as a serious threat to humans and wildlife due to high risk of simultaneous occurrence of cyanotoxins. However, neurotoxic compounds are less frequently reported, their exposures can lead to acute and fatal outcomes. Moreover, human-driven eutrophication and climate changes accelerated expansion of some potentially toxic cyanobacteria including *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*). This cyanobacterium is a major producer of cylindrospermopsin in Australia and saxitoxin in Brazil. In contrast, European strains of this species have not been reported to produce any known cyanotoxins. Recent studies revealed however, that exudates of some strains of *R. raciborskii* showed cytotoxic [1] and neurotoxic effects [2]. Therefore the aim of the study was to assess capability of *R. raciborskii* strains to produce anatoxin-a (ATX), cylindrospermopsin (CYN) and microcystins (MCs). Six strains were isolated from natural, eutrophic lakes in Western Poland and two strains were isolated from an artificial reservoirs in Ukraine in the Galicia-Volyn area. The potential to produce mentioned cyanotoxins was determined by specific genes amplification (*anaF* – ATX; *cyrA* and *cyrJ* – CYN; and *mcyE* – MCs). The presence of toxins was detected using HPLC-DAD and HPLC-MS/MS methods. Results of this study showed none of the investigated strains was capable to produce MCs or CYN. Molecular analysis detected *anaF* gene in two of these strains and chromatographic analysis detected compounds with characteristic for ATX spectrum of absorption. These results may contribute to better understand recently reported neurotoxic effects of these strains [3].

References:
HOW DOES BMAA CAUSE PROGRESSIVE SLOW NEURODEGENERATION?

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A hypothetical role for the cyanobacterial metabolite β-N-methylamino-L-alanine (BMAA) in the aetiology of sporadic neurodegenerative diseases (ND’s) has not been widely accepted, in part due to a lack of a feasible mechanism of toxicity that could result in the pathology associated with ND’s. Excitotoxicity was initially suggested because BMAA was discovered while looking for β-N-Oxalylamino-L-alanine (BOAA), the causative agent of lathyrism. BMAA is, however, a relatively weak excitotoxin, incapable at environmental levels of producing neurodegeneration, and subsequent attempts to invoke prolonged excitotoxicity by virtue of a slowly released endogenous pool in order to explain the ‘slow-toxin’ nature of BMAA, have not been substantiated [1, 2]. The more recent hypothesis of misincorporation into proteins has largely been discredited [3, 4, 5, 6] and would not, in any case, result in such specific neurotoxicity. However, the very strong BMAA-protein association, although not primary in nature, does offer an alternative mechanism [7] that might, in conjunction with the reported rapid localization, specifically in the hippocampus and dopaminergic systems, explain the observed patterns of pathology and behaviour seen in the BMAA-neonatal rodent model [8]. This strong intermolecular association appears to be due to side chain hydroxyl binding by the charged BMAA amino group. We report here on the modes of toxicity (based on this mechanism) that result in progressive neurodegeneration, and on the protection offered by molecules postulated to have protective effects based on side chain hydroxyl binding by BMAA and/or by modulating the developmental neurotransmitter imbalances that result from BMAA exposure.

References:

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Harmful algae blooms (HAB) are a source of lipopolysaccharides (LPS) which are released into the water during cell division and lysis. Bacterial LPS is known to be a potent pro-inflammatory agent but the cyanobacterial LPS is not studied well. To study its ability to induce pro-inflammatory responses, LPS extracts from HAB biomasses dominated by different species as well as from axenic and non-axenic cultures of the same species were prepared. First, pyrogenicity of these LPS was tested using Pyrogene assay. There was a wide range of pyrogenic activity among all samples, from dozens to millions of EU/mg LPS. Further, production of pro-inflammatory cytokines was detected using murine macrophages RAW264.7 and human intestinal epithelial cells Caco-2 and HT-29. In intestinal cells, level of interleukin 8 was significantly increased also after exposure to LPS from HAB with low pyrogenicity. Moreover, LPS from axenic culture of *Aphanizomenon flos-aquae* PCC7905 and *Planktothrix agardhii* PCC7805 significantly increased levels of tumour necrosis factor α in macrophages despite the fact that their pyrogenicity was very low. Since LPS is generally recognized as a ligand of TLR4, its antagonist was used but it did not affect pro-inflammatory effects of studied LPS.

We can conclude that pro-inflammatory potency of cyanobacterial LPS may not be predicted by Pyrogene test and can be significantly underestimated. Moreover, we showed that not only environmental mixtures but also pure cyanobacterial LPS can exert significant biological properties possessing risk to human health. Further, these effects seem not to depend on TLR4.

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Oral session 7

Risk identification, water management and toxin removal
TREATMENT STRATEGIES FOR CYANOTOXINS IN WATER

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It is now widely accepted that cyanotoxins contaminate many of our freshwater supplies world-wide with a significant potential threat to human health. Eliminating the risks associated with the occurrence of harmful cyanobacteria in water used for domestic, industrial and recreational activities is challenging and depends greatly on a wide array of variables. These include the cyanotoxins present, associated biomass, nature of waterbodies (from single household wells to lakes), and resources available, underpinned by reliable monitoring, and the end use of the water. Cyanobacteria and cyanotoxins represent a unique water treatment challenge since the toxins often, but not always, enter a treatment system within living cells. The choice of treatment steps, order they are performed and our own assumptions will influence final cyanotoxins concentrations. Understanding water availability, bloom and toxin persistence, along with treatment practices around the globe is important to ensure a well inform approach. During this presentation we will explore the challenges of preventing exposure to cyanotoxins through contaminated water, assessing the range of treatment strategies available (conventional, advance and nature-based) and look to the future of affordable/reliable treatment practices suitable for application world-wide.
MANAGING THE HARMFUL CYANOBACTERIUM MICROCYSTIS AND CYANOTOXIN RISK BY LARGE-SCALE MONITORING AND MACHINE LEARNING: A FRAMEWORK OF BAYESIAN NETWORK

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The frequency of high concentration events of cyanotoxin has increased in lakes and reservoirs, because cyanobacteria dominance increases worldwide with eutrophication and climate warming. Most site-specific studies were conducted on exploring the dynamics of toxic cyanobacteria population and toxin production, to evaluate the potential effects from different anthropogenic pollutants, all with the objective of informing options for water quality management. Considering the heterogeneity in environmental conditions, predictions by empirical models based on appropriate data across large spatial scales for estimating widely applicable relationship are limited. In the current study, data-driven and knowledge-supported Bayesian networks (BNs) were developed for evaluating the probability of microcystins (MCs) risk. The development and use of BNs model incorporating observation error, within a Gibbs sampler and Monte Carlo simulation for estimating water quality targets under a range of possible nitrogen (N) and phosphorus (P) windows, is described. The framework of BN herein can avoid discretizing continuous variables, and allow to be updated with a lake-specific data. When separating the joint effects of different forms of N and P concentrations, critical nutrient thresholds were found to be lake-dependent and be counteracted by the effects of water temperature. The framework provided a useful screening tool to evaluate cyanotoxin in three studied lakes in China, and it can also be used in other lakes suffering from cyanobacterial blooms dominated by Microcystis.
Cyanobacteria and their toxins present potential hazard to consumers of water from lakes, reservoirs and rivers, thus their removal via water treatment is essential. The capacity of nano-composites of octadecyltrimethyl-ammonium (ODTMA) micelles complexed with bentonite clay to remove cyanobacteria and their toxins from laboratory cultures and from lake water, was evaluated using column filters packed with granulated clay-ODTMA complex. Using laboratory cultures we demonstrated a significant reduction in the concentration of cyanobacteria cells or filaments and their corresponding toxins. Fluorescence measurements and microscopic observatins demonstrated that cyanobacteria cells and filaments disintegrated and lost their metabolic activity (photosynthesis) upon exposure to the granulated micelle (ODTMA)–bentonite complex (ED$_{50}$ estimated at 1 g L$^{-1}$), or to ODTMA monomers (estimated ED$_{50}$ ranged between 0.05 and 0.1 mM for different cyanobacteria species). Other organic quaternary ammonium cation (QAC) such as hexadecyltrimethyl ammonium (HDTMA), tetradecyltrimethyl ammonium (TDTMA) and dodecyltrimethyl ammonium (DDTMA) had similar inhibitory effect but varied by their ED$_{50}$ values. The granulated micelle (ODTMA)–bentonite complex efficiently removed cyanobacteria toxins (microcystins and cylindrospermopsins) with an exceptional high removal capacity for microcystins. The effectiveness of the granulated micelle (ODTMA)–bentonite complex in elimination of cyanobacteria was further demonstrated with lake water containing cyanobacteria and other phytoplankton species. These results and model calculations suggest that filters packed with granulated composites can secure the safety of drinking water in case of an event of toxic cyanobacteria bloom.

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THE EFFECT OF WATER TREATMENT UNIT PROCESSES ON TOXIC CYANOBACTERIAL TRICHOME INTEGRITY

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Many toxic cyanobacteria appear in nature as trichomes. Water treatment can be optimized to keep cyanobacterial cells intact and avoid the release of toxic compounds, many physical and chemical stresses encountered during the treatment process may result in trichome truncation, decreasing treatment efficiency allowing single cells or short trichomes to reach the product water. This makes it possible for harmful compounds to enter the distribution system. Investigations in a pilot and three full-scale water treatment plants elucidated the degree of trichome truncation across different unit processes. It was found that genera (Pseudanabaena, Planktolyngbya) with short trichomes (<10-12 cells per trichome), are hardly affected by the unit processes (loss of one to four cells respectively), while genera (Planktothrix, Geitlerinema, Dolichospermum) with longer trichomes (30+ cells per trichome) suffer from high degrees of truncation (63, 30, and 56 cells per trichome respectively). The presence of a rigid sheath and/or mucilaginous layer appears to offer protection from truncation. It was observed that certain unit processes alter the sensitivity or resilience of trichomes to disruption by physical stress. Some genera (Planktothrix, Geitlerinema) were sensitive to pre-oxidation making them more susceptible to shear stress, while Dolichospermum sp. appears more robust after pre-oxidation. While the potential of toxicogenic genera breaking-through into the product water is a real danger, in the current study no toxicogenic cyanobacteria were observed. This work stresses the need for plant operators to study the incoming cyanobacterial composition in order to adjust treatment parameters and thus limit the potential of toxic compound breakthrough.

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EXPLORING MORPHOLOGICAL DEFORMATION OF CYANOBACTERIA CELL DURING OXIDATION

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Direct oxidation of toxic cyanobacteria cells can occur at various stages of water treatment and several unknowns involve the understanding of phenomena governing oxidation process. The general objective of this work was to determine and model the extent of naturally occurring cyanobacterial cells lysis, toxins and organic compounds release and oxidation. The specific objectives of this study were as follows: (1) To assess morphological deformation of 7 cyanobacteria strains during oxidation and (2) To evaluate impact of organic matter type and concentration on oxidation efficiency of cyanobacteria cell. To the best of our knowledge, this study presents the first assessment of morphological deformation of cyanobacteria (using Enhanced dark field Microscopy/Hyperspectral Imaging) alongside organic matter fractioning analysis during cyanobacteria oxidation. Varying chlorine, ozone, KMnO₄ and H₂O₂ exposure (CT) values were studied. Hyperspectral images were captured under optical microscope equipped with dark-field illumination to assess the cell membrane integrity. Qualitative images of cyanobacteria morphological deformation during oxidation were captured using a Scanning Electron Microscope. Organic carbon fractionation based on their size and hydrophobicity was conducted using Liquid Chromatography - Trace Organic Carbon Detector (LC-OCD). Toxin measurements helped to compare toxin oxidation rates with cell lysis rates. This work presents much needed unit cellular chlorine demand which could be used to adjust the oxidation capacity to satisfy the total oxidant demand associated with the presence of cells. Furthermore, a novel successive reaction kinetics model was developed using the kinetics of the reaction with cyanobacterial cells and cell-bound toxins.

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PAYMENT FOR ECOSYSTEM SERVICES – AN EFFICIENT APPROACH TO REDUCE EUTROPHICATION?

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More than 50 % of the waters entering the Atlantic Ocean between France and Great Britain (Channel) are below Good Ecological Status due to sedimentation, low oxygen levels, or excess nutrient load, which promotes cyanobacterial dominance. Economical losses in cyanobacteria infested lakes concern drinking water production, fishing and tourism activities. The most effective ways to improve water quality are to reduce nutrient losses from lake catchments, but these reductions are, however, associated with costs. The project CPES (Channel Payments for Ecosystem Services) aims to develop PES (Payment for Ecosystem Services) schemes to improve water quality by reducing nutrient (both, nitrogen and phosphorus) losses with a catchment wide approach. The PES schemes will include land management interventions, ecosystem quality monitoring plans, economic models, commercial agreements, and stakeholder engagement plans. The project comprises 6 pilot studies within the British and French side of the Channel area. One of which is the multi-purpose reservoir Lac au Duc (touristic, drinking water, fishing), frequently suffering cyanobacterial blooms and its catchment in France. We are studying aspects of the PES approach from catchment assessment (hydrology, land use, nutrient emissions), aquatic ecology (dynamics of cyanobacteria and zooplankton, eutrophication), and economy (evaluation of public policies applied to agriculture and environmental economics, and design of PES). Aim is to test the efficacy of incentive supports by applying a ‘the user pays’ approach in complementation of the ‘the polluter pays’ approach that has seen its limitations.
CONTROL AND MITIGATION OF MICROCYSTIN-PRODUCING CYANOBACTERIA OCCURRENCE IN LOWLAND DAM RESERVOIRS

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Abstract

Elaboration of effective methods for mitigating the impact of microcystin-producing cyanobacterial blooms (MCB) requires, according to the ecohydrological approach, long-term research to identify complex biotic-abiotic interactions and the related environmental processes. Nitrogen and phosphorus reduction strategy at the catchment scale is the most realistic and effective long-term approach to eutrophication management to achieve reduction of bloom intensity. It could be realized, inter alia, by creation, restoration and protection of riparian buffer zones and their enhancement with additional solutions such as denitrification or limestone barriers (www.ekorob.pl). In such case, a reduction of nitrate load on the level of 67% was observed. However, genetic analysis of the relationship between the abundance of MCB (16S rRNA) and toxinogenic genotypes (mcyA) showed that under stressful conditions (abiotic factor) cyanobacteria mobilize the mentioned genotypes capable of producing microcystins (MCs) and despite the decrease in total MCB, the activity in MCs synthesis is still high. Therefore, there is a need for better understanding of biotic interactions in regulation of the MCB occurrence. Among the biotic factors are cyanophages dedicated to MCB and bacteria capable of degradation of cyanobacteria cells and/or cyanotoxins. This research aimed to characterize and isolate bacterial strains of Aeromonas and Sphingosinicella genera capable of degrading MCs and to trace cyanophages from Myoviridae family with possibility to phycobilisomes degradation. The determination of periods of increased microbial activity will enable the utilization of the potential of natural aquatic ecosystems to reduce the toxicity induced risk of MCB.
Harmful Algae Blooms (HABs) initiated by fertilizers, septic and agricultural waste into aquatic ecosystems can have severe impacts on ecosystem functions, causing fish kills, the decline of submersed plant life, and affect oxygen concentrations in the water. In addition, the production of toxins (cynotoxin) increase human and animal health hazards. Although control measures for phosphorous and nitrogen rich fertilizers can help reduce the likelihood of HABs, there exist few safe and reliable ways of immediately treating a blue-green algae bloom in situ.
GENETIC ENGINEERING OF CYANOBACTERIA FOR DEGRADATION OF MICROCYSTINS – PROLONGING WHOLE CELL MLRA ACTIVITY UNDER EXTENDED CULTURING REGIMES VIA APPLICATION OF TRC PROMOTER

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Microcystins, the most commonly observed toxins associated with freshwater harmful algal blooms, are observed to be increasing in intensity, duration, and distribution on a global scale. Chemical-based methods for remediation are costly, and are generally less sustainable than the primary natural route via microbial activity. Previous work has shown that heterologous expression of MlrA enzyme, capable of eliminating microcystin toxicity, allowed for generation of an E. coli isolate with over 6800-fold increased expression of MlrA compared to a natural gene host [1]. Unfortunately, the E. coli chassis cannot survive prolonged freshwater incubation, thus we have investigated MlrA expression within a cyanobacterial chassis [2], and have previously completed proof-of-principle, with generation of a Synechocystis 6803 strain showing about 3 times higher whole cell degradative activity than a naturally occurring gene host (Sphingomonas sp. ACM 3962). Furthermore, utilization of a photoautotrophic chassis resulted in prolonged stability of MlrA activity when cultured under seminatural conditions (using lake water), in comparison with heterologous MlrA biocatalytic activity of the E. coli culture disappearing after 4 days. Here, we report utilizing the non-native trc promoter as a means to maintain strong MlrA expression over an extended period and compare it with the MlrA expression construct utilizing the native cpcB560 promoter of our initial success. The new construct with trc promoter displayed advantage in MC decomposition during long-term cultivation which documents further success with an optimization strategy focusing on prolonging MlrA activity under extended culturing regimes.

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NATURALLY OCCURRING CYANOBACTERIA CAN FORM OXYGENIC PHOTOGRA NULES TO TREAT WASTEWATER

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Oxygenic photogranules (OPGs) are roughly spherical aggregates with diameters of several millimeters containing a syntrophic community of heterotrophic bacteria and cyanobacteria, predominantly of the order Oscillatoriales [1]. OPGs may potentially be used in wastewater treatment because their in-situ produced oxygen can replace costly mechanical aeration for pollutant removal [2]. Toxin production could however nullify their application.

OPGs can be produced from “activated sludge”, the aerobic microbial aggregates used for treating wastewater. This sludge is transferred into unagitated vials and exposed to light. Over the course of several weeks, the unconsolidated sludge transforms into one photogranule per vial. Curiously, in replicates using the same sludge, a granule does not always form: sometimes microbial mats result or the material remains unconsolidated. We therefore aim to better understand the initial conditions for photogranulation, specifically the role of filamentous cyanobacteria. We hypothesize that the behavior of Oscillatoriales is key to photogranulation and that cyanobacteria type and their relative abundance determine granulation success, i.e., OPG formation.

We therefore investigate whether the formation of microbial mats, well-formed photogranules and intermediates, correlates to the presence of a specific microbial community. We performed nine granulation experiments which resulted in 135 qPCR corrected amplicons from both partial 16S (all bacteria) and 23S (cyanobacteria and algae) rRNA genes from samples on a gradient of granulation success. From sequence analysis we learnt that several Oscillatoriales species are able to form photogranules. For an application in wastewater treatment, we now need to evaluate whether operational conditions may lead to toxin production.

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ELISA is a well-established method for the determination of microcystins in water. However, its validity for risk assessment in animal tissues has been questioned [1]. One of the main concerns for the analysis tissue extracts is matrix effect. Since nanobodies display a fine-tuned specificity, we tested the performance of our recently developed llama nanobody MC-ELISA [2] in fish muscle extracts. The method was sensitive (LOQ=1.8 ng MC-LR/g fresh tissue), showed excellent accuracy (spiked recoveries 78-98%) and precision (CV < 20%) in the range 1.8 - 30 ng MC-LR/g. Another possible problem with ELISA for MCs in animal tissues is that these toxins could be conjugated to glutathione and other thiols [3]. These compounds cross-react with the antibodies but their toxicity is much lower than that of free MCs, leading to an overestimation of risk. To evaluate this problem, we analyzed fish taken from a dam with a Microcystis sp. bloom containing MCs. ELISA results ranged from non-detected to 29 ng MC-LR equivalents/g. Pre-concentration on magnetic beads with immobilized nanobody and internal standard, allowed the quantification of MCs by MALDI-TOF, which showed the presence of MC-LR and [L-MeLan]MC-LR (conjugation product of MC-LR and Cystein). Free MC-LR was prevalent over the conjugate (average LR 72% of total MCs). Moreover, the correlation ELISA-quantitative-MALDI-TOF for MC-LR was excellent (r Pearson= 0.98, n=16) which highlights the potential of the nanobody ELISA as a screening tool to minimize the number of samples analyzed by reference methods such as LC-MS/MS.

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Oral session 8

New tools, new methods, most original findings and hypotheses
Proliferations of the benthic cyanobacteria appear to be increasing in streams, rivers and lakes worldwide. This is of particular concern when these waterbodies are used as drinking water sources or for recreation. In New Zealand, *Microcoleus* (previously *Phormidium*) forms mats that can smother the entire bottom of cobble-bedded streams and may stretch for many kilometres. The *Microcoleus* that dominates these mats commonly produces anatoxins which have killed numerous dogs and led to health warnings which advise against contact with the water been issued long hundreds of kilometers of streams. Over the last decade our team has explored what makes streams susceptible to *Microcoleus* proliferations at a national scale, and factors that promote proliferations at a regional, river and mat scale [1,2]. Toxin concentrations within mats vary markedly between and within streams. A suite of chemical and molecular techniques, coupled with laboratory and field studies have been used to explore this phenomena [3,4].

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NEW ASSESSMENT METHOD OF THE SPATIAL DISTRIBUTION AND BIOMASS OF MICROCYSTIS BLOOM USING HIGH FREQUENCY ECHOSOUNDER

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Microcystis are one of the major components of cyanobacterial blooms worldwide. They can produce microcystins (MCs), which are toxic to many aquatic organisms, including fish. It is expected that the frequency and severity of cyanobacterial blooms will increase in future due to the climate change and human-accelerated eutrophication. Therefore it is of primary importance to have an appropriate tool to study bloom development and its propagation in time. The aim of this study was to investigate the possibility of using a fishery echosounder to study biomass and distribution of cyanobacterial bloom. Simultaneous measurements of cyanobacterial bloom distribution using the on-line phycocyanin detection by Turner Design 10AU Field Fluorometer and Simrad EY60, 200 kHz echosounder were performed in years 2013 - 2015 in the shallow lowland Sulejów Reservoir. Genetic analyses confirmed presence of Microcystis (based on a specific fragment of 16S rRNA gene) including toxigenic genotypes with potential to MCs production (based on a specific fragment of mcyA gene). The highest copy number of 16S rRNA was detected in 2015 with max. $5.89 \times 10^5$, while in 2013 the maximum was $3.93 \times 10^5$. Results indicated that the biomass values of cyanobacterial bloom estimated by acoustic and fluorometric methods were highly correlated ($R^2 = 0.88$) and the maps produced (based on average values for 100 m) were practically identical (differences were no significant at $p = 0.01$). The additional advantage of acoustic method is that simultaneously fish distribution is measured, allowing to study the relationship between fish and cyanobacterial bloom.

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ULTRASENSITIVE DETECTION OF MICROCYSTINS IN UNTREATED BIOLOGICAL SAMPLES BY IMMUNOCONCENTRATION WITH NANOBODY COATED NANOPARTICLES AND DIRECT QUANTITATIVE MALDI-TOF ANALYSIS

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Monitoring of microcystins (MCs) has been challenged by the growing knowledge on the number of MC variants, the low detection limits imposed by current legislation, and the requirement of laborious procedures for their analysis in complex matrices. In this study a new analytical method was developed using immunoconcentration coupled to quantitative MALDI-TOF analysis that in minutes allows the identification and highly sensitive detection of MC congeners, being particularly suitable for direct analysis of untreated biological samples. Immunocapture is mediated by magnetic beads loaded with an in vivo biotinylated nanobody of broad microcystin cross-reactivity that is partially bound to an easily synthesized internal standard for MS quantitation. After capture, the beads are directly dispensed on the MALDI target and analyzed. Since sample contaminants are removed during the concentration step, no clean-up or other samples treatment are needed. The method was validated with water, serum and fish muscle samples with excellent recovery at quantitation limits of 0.025 ppb.
MICROCYSTIN CONGENER SPECIFIC INHIBITION OF MAMMALIAN SER/THR PROTEIN PHOSPHATASES (PP1, PP2A AND PP5) AND PREDICTION OF INHIBITIVE CAPACITY VIA MACHINE LEARNING

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Microcystins (MC) represent a family of cyanobacterial toxins that represent a considerable health threat to humans. MCs toxicity is characterized by their ability to specifically inhibit ser/thr-protein phosphatases (PPP). Risk assessment of MCs is challenging due to the large number of structural variants (congeners) and the fact that the targets (PPP) belong to a family of enzymes encompassing several members. The aim was to establish a dataset of the inhibitive capacity of MC congeners in ser/thr-PPP (PPP1, PPP2A and PPP5) and to develop a machine learning approach for MC congener toxicity prediction based on this dataset. The PPP inhibition capacity (toxicity) of 18 structurally diverse MC congeners (naturally occurring and synthetic variants) was determined using ser/thr-PPP (rPPP1, hPPP2A and hPPP5) in a colorimetric protein phosphatase inhibition assay. Results were classified into three categories (toxic, less toxic, non-toxic) and employed together with chemical structures and protein sequence in a machine learning approach using two models. Inhibition data revealed, that the different MC congeners show different activities towards the three tested PPP albeit generally the same patterns were observed. hPPP5 was less susceptible to MC congeners compared to rPPP1 and hPPP2A. Finally, the machine learning approach provided predictions that allowed correct grouping of MC congeners into toxicity classes based on their 2D chemical structure. The data obtained demonstrated that the current risk assessment for MC, which is based solely on MC-LR, may under- or overestimate actual risk, especially as toxicodynamics (data presented) and toxicokinetics [1] for the various MC congener differ dramatically.

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LC-HRMS<sup>n</sup> VERSUS LC-TANDEM-MS: A COMPARATIVE APPROACH FOR THE IDENTIFICATION OF CYANOTOXINS IN CYANOBACTERIAL BIOMASS

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Cyanobacteria, commonly referred to as blue-green algae, are photosynthetic prokaryotes naturally occurring in a variety of aquatic environments including lakes, rivers, ponds and estuaries worldwide [1]. Over 35 genera, including *Microcystis*, *Planktothrix* and *Anabaena*, are known to be toxic [2] as they produce a large number of structurally related compounds, named cyanotoxins often implicated in accidental human and animal poisonings along lake and estuarine shores during massive cyanobacterial proliferation. Among cyanotoxins, there are many bioactive metabolites (anabaenopetins, anabaenopeptilides, cyanopeptolins, micropeptins, microviridins) whose toxicological or eco-toxicological effects are only partially. On the other hand, microcystins (MCs), cyclic hepta-peptides containing the unusual φ-aminoacid ADDA, are the most frequently occurring cyanotoxins and also the most toxic ones being potent inhibitors of serine/threonine protein phosphatases 1 and 2A, with hepatocytes as final molecular target. To date, more than 248 different MC congeners have been characterized, and the number is continuously increasing.

In this study, we analyzed a cyanobacterial biomass sample collected in September 2014 from Lake of Kastoria (Greece) by using two different analytical approaches: a targeted analysis by LC-tandem mass spectrometry [3] and an untargeted approach by LC-high resolution LTQ Orbitrap MS<sup>n</sup>. A good quantitative correlation between the two approaches emerged even if the untargeted approach revealed the presence of additional MC variants, some cyanopetolins and anabaenopeptins that were missed by the targeted approach. These findings underscore the importance of a careful and comprehensive of cyanotoxins in environmental samples for a correct risk evaluation.

References:
DUAL CARTRIDGE SPE METHOD FOR EXTRACTION OF DIFFERENT VARIANTS OF SAXITOXINS AND HILIC-MS/MS ANALYSIS

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Saxitoxins (STXs) are potent neurotoxins presenting association with human intoxications [1]. Nevertheless, only a limited number of countries set guidance values for STXs in drinking water to prevent possible human health effects [2]. STXs can be produced by marine dinoflagellates and freshwater cyanobacteria [1]. They are relatively polar and soluble in water [3], which makes their extraction and chromatographic separation a challenging issue. On top of that, similar structural properties of various analogues of STXs, lead to co-elution and therefore false positive results. On the other hand, even though they have similar properties, not all of the analogues can be retained by one solid phase extraction (SPE) cartridge at once, which imposes a whole new experimental task. Saxitoxin analysis from shellfish has been thoroughly investigated [4].

However, their analysis from water samples is largely understudied. In this study, a sensitive analytical method has been developed for the simultaneous determination of nine analogues of STXs (including 6 gonyautoxins) from water. Successful extraction of STXs was performed by using a dual SPE cartridge assembly with weak and strong cation exchange sorbents. Separation was achieved using a HILIC column while identification of each toxin was achieved by using tandem mass spectrometry (LC-MS/MS). Method trueness, as % recovery ranged from 50-95%, while for all measured STXs the detection limit was equal to 0.03 μg/L. RSDs measured under repeatability and reproducibility conditions ranged up to 10.9% and 14.9%, respectively. Optimized SPE-HILIC-MS/MS method was successfully applied to surface water samples obtained by Greek lakes.

References:

Acknowledgements:
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More than 240 different microcystin (MC) congeners have been reported [1], and new congeners are being discovered regularly. In addition to methods for measuring total MCs or targeting known congeners, improved methods are needed for identifying new putative MCs. Untargeted high resolution tandem mass spectrometry (HRMS/MS) allows for simultaneous analysis of thousands of known and unknown chemicals in complex biological or environmental samples. Typically, HRMS/MS identification of MCs involves only analysis in positive ionization mode with detection of a characteristic fragment from Adda$^5$ at $m/z$ 135.0804. However, some MCs contain modified Adda-moieties, or are otherwise modified to change their MS/MS behavior, and are not readily detected in this manner. We recently showed that derivatization of the Mdha$^7$/Dha$^7$ group in MCs is a highly effective method for identifying novel MCs in complex sample by LC–MS [2]. Here, we present a new approach using commercial metabolomics software for semi-automated detection of novel MCs based on mercaptoethanol derivatization, together with accurate mass detection of precursor and characteristic product ions in positive and negative ($m/z$ 128.0353 from $\gamma$-D-Glu$^6$) ionization modes. Taken together, this approach targets any molecules containing Adda$^5$, $\gamma$-D-Glu$^6$ or Mdha$^7$/Dha$^7$, one or more of which is present in every microcystin reported to date, and which appear to be most relevant to toxicity. We demonstrate the power of this workflow with the identification of numerous novel MCs in culture samples as well as a blue–green algal matrix reference material [3].

References:
DEGRADATION OF CYLINDROSPERMOPSIN USING ADVANCED NON-THERMAL PLASMA TECHNOLOGIES

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Non-thermal plasmas (NTPs) have received much attention for their application in wastewater and air purification. Classified as Advanced Oxidation Processes, plasmas ignited in water or at the air-water interface efficiently generate a vast range of reactive species. Although plasmas have been shown to even degrade recalcitrant organic pollutants such as pharmaceuticals, available information for their application in drinking water treatment and cyanotoxin degradation is limited.

In the present research, six different plasma sources – corona, surface, spark, gliding arc and dielectric barrier discharges (DBD) and a plasma jet – were compared for their efficiency to degrade a cyanobacterial extract containing cylindrospermopsin. Two plasma types were then selected for further in-depth study of the efficiency and degradation mechanisms. The spark discharge showed the most energy-efficient degradation, followed by the other sources showing similar efficiencies, while the plasma jet was least efficient. The follow-up detailed studies included the corona-like discharge and the DBD. For the corona-like plasma, the degradation efficiency increased with increasing voltage and solution pH. After 15 min of plasma treatment at pH ≥ 7.5 degradation even progressed without further plasma application. This pH-dependent effect was not observed in the DBD reactor, whose degradation efficiency increased with decreasing voltage. Degradation in the corona-like plasma is primarily promoted by hydroxyl radicals, whereas the DBD reactor mainly produces ozone and NOx.

The application of NTPs appears to be an innovative and promising approach for efficient removal of cyanotoxins such as cylindrospermopsin from drinking water.

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DEVELOPMENT OF A RAPID IMMUNOCHEMICAL ASSAY FOR ANATOXIN-A ANALYSIS

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Public health risks due to harmful algal blooms include exposure to toxic metabolites through consumption of contaminated drinking water, fish, shellfish or algae, and by recreating on or in contaminated waters. Anatoxin-a, formerly known as VFDF (Very Fast Death Factor), is a very potent neurotoxin that can be found in superficial waters as a consequence of cyanobacteria blooms from a variety of genera, including Anabaena, Aphanizomenon and Cylindrospermum [1]. Nowadays, immunochemical methods constitute sensitive, fast, portable, and economic approaches to analyze chemical contaminants; however, no antibodies to anatoxin-a were available so far. In order to generate high-affinity and specific antibodies to such a small molecule, the synthesis of functionalized analogues (haptens) and the correct conjugation to proteins are mandatory. (+)-Anatoxin-a is an enantiomeric molecule characterized by a homotropane framework. In this study, three different anatoxin-a haptens were synthesized based on the initial preparation of a common enoltriflate intermediate, using an amine-epoxide ring-opening reaction and an amine-alkene transannular cyclization as key steps for the construction of the characteristic ring system. Each hapten was functionalized with a carboxylated spacer arm, and was conjugated to three proteins (BSA, OVA, and HRP) using the corresponding purified N-hydroxysuccinimide ester. Mice were immunized and high-affinity enantioselective monoclonal antibodies to natural (+)-anatoxin-a were raised for the first time. Enzyme-linked ImmunoSorbent Assays (ELISA) in different formats were optimized and characterized. These immunoreagents have enabled the development of a commercial ELISA kit for the analysis of anatoxin-a in environmental water samples, with IC₅₀ values in the low nanomolar range.

References:
USE OF FRESHWATER BIVALVES AS SENTINEL SPECIES TO DETERMINE THE PRESENCE OF BMAA IN AQUATIC ECOSYSTEMS

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The environmental neurotoxin β-methylamino-L-alanine (BMAA) has trigged an emerging interest because of its potential link with the development of neurodegenerative pathologies. Although the etiological link has not been formally proven so far, human exposure has been suggested to occur through the ingestion of BMAA-containing food. BMAA has been detected in animals destined to human consumption (fish, crustaceans and bivalves) that may have ingested BMAA-producing phytoplankton. However, direct BMAA quantification in water samples can be tenuous, preventing to perform reliable risk assessment studies. Hence the interest of using integrative tools such as bivalves, to facilitate the analysis of BMAA in fresh waters. Here we studied the kinetics of BMAA accumulation and detoxification by two filter-feeders freshwater bivalves: Anodonta anatina and Dreissena polymorpha, through laboratory studies supported by an in situ monitoring. Both mussel species were exposed to 1, 10 and 50 µg/L of dissolved BMAA for 21 days, followed by 42 days of depuration. The BMAA concentration in whole D. polymorpha and digestive glands of A. anatina was analyzed weekly by LCMS/MS. Moreover, BMAA has been analyzed in caged mussels during two monitoring (summer 2016 and 2017) in three stations, presenting contrasting level of cyanobacterial proliferations, in a French lake. Results are presented in terms of the use of bivalves as sentinel species as they showed that BMAA accumulation was concentration and time dependent in whole D. polymorpha from 10 µg/L, and that the digestive glands of A. anatina were only able to reveal the presence of BMAA from 50 µg BMAA/L.

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EARLY BIOMARKERS OF BEHAVIOURAL AND PHYSIOLOGICAL DISTURBANCES IN DAPHNIA MAGNA EXPOSED TO ANATOXIN-A ESTIMATED BY VIDEO ANALYSIS

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The majority of reports on the toxic effect of cyanobacterial metabolites on the Cladocera is based on determination of two endpoints: mortality or immobilization. However, detection of sub-lethal effects requires more sensitive biomarkers. The aim of the present study was to evaluate the applicability of digital-video analysis of behavioural and physiological biomarkers in the assessment of effects caused by the cyanobacterial neurotoxin, anatoxin-a (ANTX) at a broad range of its concentrations (0.5-50 µg/mL) on swimming speed (SS), heart rate (HR), oxygen consumption (OC), thoracic limb activity (TLA) and abdominal claw movement (ACM) of Daphnia magna.

Swimming speed and abdominal claw movements were determined by digital analysis of video clips by Tracker® software; OC by Oxygraph Plus System®. HR, TLA and ACM were evaluated by digital frame-by-frame analysis of video clips of microscopic view with the use of a media player software.

The experimental study showed a concentration- and time-dependent decrease of SS, HR, OC, TLA and ACM as early as after 2 h of the exposure of D. magna to ANTX. Further inhibition of these parameters was also noted after 24 h exposure. On the other hand, stimulation of ACM was noted at the lower (0.5 and 2.5 µg/mL) concentrations of ANTX after both 2 h and 24 h of exposure.

The results indicated that behavioural and physiological biomarkers measured by video analysis may be a valuable tool for an early determination of toxic effects induced by cyanobacterial metabolites in zooplankters.
Oral session 9

Ecology of cyanobacteria, abiotic and biotic factors in the regulation of cyanobacterial growth and/or toxin production/2
MANAGING THE RISK OF CYANOBACTERIA THROUGH WATER QUALITY CHARACTERISTICS ANALYSIS: A CASE STUDY OF TWO WARM MEDITERRANEAN RESERVOIRS

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The study aimed to correlate the trophic condition of two Mediterranean reservoirs of different typology with their water quality characteristics and identify the key environmental variables driving cyanobacteria blooming and their cyanotoxicity [1]. The two studied cases included a reservoir enriched with tertiary treated wastewater located in Cyprus (Polemidia Dam) and a re-established reservoir in Greece (Lake Karla). Annual data provided by the national waterbodies authorities as well as by regular monitoring were collected and analyzed for both cases. To examine which environmental variables contribute the most to the trophic condition of the reservoirs, PCA analysis (Principal Component Analysis) was applied since it can assess the effect of multiple variables at the same time. In addition, through multiple linear regression analysis, we were able to correlate characteristics of the blooms with nutrients (N, P) and water temperature. As expected, temperature is not a limiting factor [2] for bloom formation for both waterbodies. Among the variables tested, phosphorus (P) was found to be the key element for the growth of cyano-HABs in Lake Karla, while a significant reduction in the TP concentration of the recycled water used to enrich Polemidia reservoir following year 2010 altered the trends of cyano-HABs formation and their characteristics. It is anticipated that the outcomes of this study will assist in identifying the most challenging issues related to cyano-HABs in warm reservoirs due to climate change [3]. By doing so effective management tools can be applied.

References:

Acknowledgements:
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Continental water bodies located at semi-arid environments, such as Brazilian northeastern region, usually have high water conductivity (1000 µS/cm). Previous studies have reported the occurrence of cyanobacterial blooms with the dominance of *Cylindrospermopsis raciborskii*, a potentially saxitoxin producer, in those environments. The response of *C. raciborskii* to conductivity has been studied under culture conditions but generally with one strain. However, blooms consist of a pool of strains with diverse physiological responses. The aim of the present work was to investigate the effects of conductivity (provided by addition of MgCl$_2$ or NaCl) on physiological aspects of four Brazilian *C. raciborskii* strains: two saxitoxin-producers—and two non saxitoxin-producers. The strains were cultured in ASM-1 medium as control (450 µS/cm) or in ASM-1 added with NaCl (10mM) or MgCl$_2$ (5mM) reaching 1500 ± 100 µS/cm. Cultures were maintained at 50 µmol.photon.m$^{-2}$.s$^{-1}$ during 15 days. Growth was accompanied by cell counting in an optical microscope and photosynthetic parameters were monitored using a Phyto-PAM. Saxitoxin content was analyzed by HPLC. Protein profiles were visualized by gel electrophoresis. All strains showed a growth increase at 10 mM NaCl. No significant differences were observed among the strains for photosynthetic parameters. Protein profiles did not show differences in response to salts but differed among the strains. Saxitoxin content was not affected by conductivity but the two toxic strains presented different saxitoxin quotas. In conclusion, intraspecific variability was a pronounced aspect of these responses and must be considered for better understanding of this cyanobacteria species.
Elevated pCO₂ may fuel phytoplankton photosynthesis and thereby promote their growth. Particularly CO₂ fixation in cyanobacteria may be facilitated as they possess a Rubisco (Ribulose-1,5-bisphosphate Carboxylase/Oxygenase) with among the lowest affinities for CO₂ [1]. To compensate for this low affinity, they developed carbon concentrating mechanisms (CCMs), which are cellular mechanisms to enhance CO₂ concentrations in the vicinity of Rubisco [2]. Down-regulation of these CCMs may possibly allow reallocation of energy to nutrient uptake and/or growth [3]. Various freshwater cyanobacteria species are toxic and can proliferate under eutrophic conditions, forming dense harmful blooms. Among the most common toxins produced are microcystins. Consisting of seven amino acids, these are nitrogen-rich compounds of which many variants exist with distinct toxicities as result of two variable amino acid positions [4]. The development of cyanobacterial blooms is accompanied by depletion of resources, including CO₂, light and nitrogen. During bloom development, cells thus experience large shifts in resource availabilities with possible consequences for CO₂ fixation, nitrogen acquisition, carbon:nutrient stoichiometry and toxin synthesis [5]. In this talk, I will explore the extent to which elevated changes in pCO₂ may alter both carbon and nitrogen assimilation, and how this may affect the toxicity of freshwater cyanobacteria.

References:
SALT STRESS RESPONSES OF BRACKISH AND FRESHWATER STRAINS OF MICROCYSTIS AERUGINOSA

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Blooms of Microcystis are most often observed in freshwaters but increasing numbers of bloom events have been recorded in coastal areas. Hydrologic events like storm and droughts are promoting the transfer of Microcystis and microcystins (MCs) across the freshwater-to-marine continuum. Salinity represents one of the main abiotic factor controlling the presence and toxicity of this genus. Therefore, understanding the impact of salinity on Microcystis physiology and toxin production and release is crucial to assess the potential environmental risk. Physiological responses of Microcystis aeruginosa including toxin production were investigated during five days using two toxic strains (PCC 7806 and PCC 7820) respectively from brackish and freshwater origins. After a sudden salt shock from salinity 3.4 to 14.4, PCC 7806 and PCC 7820 presented distinct limits of salinity tolerance. Their growth were inhibited above a salinity of 8.4 and 6.7 respectively. PCC 7806 produced 2 variants of MCs (MC-LR > dmMC-LR), and PCC 7820 produced 6 variants of MCs (MC-LR > MC-LW > dmMC-LR > MC-LY > MC-LF). For both strains, the sudden increase in salinity led to a decrease of MCs concentration per cell with no modification in their toxin profiles. Further experiments are being conducted to compare the short term physiological response to salinity shock in both strains with the relative expression of several genes using qPCR. Genes involved in MCs synthesis (mcyA), in oxidative responses (sod, gshB, groel), photosystem activity (psbC and psbA) and sucrose synthase (sppA and spsA) were evaluated. Also, osmolyte contents are studied using targeted analyses.
ENVIRONMENTAL PHOTODEGRADATION OF EMERGING CYANOPEPTIDES BEYOND MICROCYSTINS

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Intensified cyanobacterial bloom events across the globe are of increasing concern because of the adverse effects associated with the release of cyanotoxins. While it is known that many cyanopeptides are produced simultaneously from one specie, occurrence and environmental fate have not been explored comprehensively for cyanopeptides other than microcystins. As cyanobacterial blooms are dominant in sunlit surface water, photodegradation can be one major fate pathway. This project focused specifically on environmental photodegradation of emerging cyanopeptides beyond microcystins. As most cyanopeptides are not commercially available, we harvested them from cultures of Microcystis aeruginosa, Dolichospermum flos-aquae, and Planktothrix rubescens. We analyzed biomass extracts by suspect screening with LC-HRMS/MS and a vigorous data analysis workflow considering more than 580 structurally known cyanopeptides including cyclamides, cyanopeptolins, anabaenopeptins, microginins, aeruginosins and microcystins. With this, we tentatively identified more than 50 cyanopeptides in purified extracts that were then used for mechanistic fate studies. Exposure to simulated sunlight in the presence of chromophoric dissolved organic matter (CDOM) revealed those cyanopeptides that were rather stable in sunlight while others degraded rapidly also by indirect photochemical reactions. For those compounds, we currently determine the bimolecular reaction rate constants with photochemically produced reactive oxygen species such as singlet oxygen. These constants can later be applied to other exposure scenarios where the oxidant concentrations are known. Our results are among the first to quantify the environmental fate processes of these emerging cyanopeptides. Knowing which cyanopeptides are persistent in surface waters is of crucial importance for the risk assessment of cyanobacterial blooms.
COLONY FORMATION: A MASTER TRAIT OF MICROCYSTIS

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Colony formation is a key cyanobacterial trait and provides flexibility in size of species, for instance Microcystis, thereby affecting the flux of carbon and nutrients through aquatic food webs. Living together also provides Microcystis cells with numerous benefits such as defense and competitive strength [1], facilitating their proliferation in lakes and reservoirs. Relatively little is known about this trait, particularly with respect to its role in shaping the future of cyanobacteria blooms under global environmental change. To this end, we investigated how environmental and climatic changes (i.e. eutrophication and warming) affect colony formation, and assessed the implications for their competitive strength through the role of extracellular polymeric substances (EPS) in nutrient uptake and storage, and the interaction with heterotrophic bacteria [2]. We performed a series of batch experiments using colonial strains isolated from Lake Taihu, China. Our results show that nutrient enrichment and warmer temperatures increased colony size because of an increase in cell densities, but significantly decreased the production of EPS, thereby enhancing the percentage of single cells. Vice versa, nutrient limitation led to more colonies, particularly under nitrogen limitation. We also found that there was no significant cost (with respect to growth rate) for colonial Microcystis, compared to single cells. This might be partly due to the ‘adhesive effect’ during colony formation [3]. Together, our results suggest that cells may secrete EPS as a means to balance the intracellular carbon: nutrient stoichiometry, thereby driving colony formation. Moreover, EPS seems to play a key role in both competitive and beneficial interactions between Microcystis and associated heterotrophic bacteria.

References:
TEMPORAL TRENDS IN CYANOBACTERIAL COMMUNITIES FROM THE WESTERN TREATMENT PLANT, AUSTRALIA

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Cyanobacteria are commonly implicated in the development of algal blooms in waste water treatment plants (WWTPs). The Western Treatment Plant, Victoria, is plagued by annual, potentially toxic harmful algal blooms (HABs) that disrupt the supply of water to the surrounding agricultural districts. It is therefore crucial to develop an understanding of the biological parameters that favour non-toxic bloom formation to reduce disturbances to the water supply. To assess the cyanobacterial community composition we analysed surface water samples from the Western Treatment Plant for three consecutive years (2016-2018) during the bloom period. Weekly sampling of each lagoon was conducted to capture variation in the cyanobacterial community structure using metagenomic 16S rRNA amplicon sequencing. Sequence reads were processed using MOTHUR v1.35.1 and over 1300 operational taxonomic units were classified as cyanobacteria using the SILVA database. Microcystis, Synechococcus and Chroococcidiopsis were the most relatively abundant cyanobacterial genera. The community profile of each seasonal bloom was highly conserved and this inter-seasonal stability may arise from the genetic reservoir of Microcystis within the treatment plant sediment. Significant variation in the community composition occurred within each bloom period observed. Cyanobacterial diversity at the WTP was correlated to nutrient availability and intra-seasonal variation is suggestive of the potential emergence of dominant OTUs throughout different proliferation stages of the algal bloom.
TOXIC CYANOBACTERIA MONITORING IN TURKEY

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In many countries, cyanobacteria and their toxins are main problem and subject for scientific researches. In Turkey, toxic cyanobacterial blooms are common and particularly in drinking and recreational waters pose risks to human health. Because of hazardous effect of cyanobacteria, surveys and monitoring programs generally funded in part by public authorities. Ministry of Forestry and Water Affairs, General Directorate of Water Management in cooperation with Istanbul University Faculty of Aquatic Sciences conducted the ‘SIYANOTOKS’ project which focused on distribution of cyanobacteria and their toxins in all around Turkey. In 2015, 7 natural lakes, 8 reservoirs, 1 lagoon and 2 coastal and transitional waters were monitored in monthly intervals between May to October. And also two sampling was carried out in winter and spring. Selected water quality parameters were also measured. Potentially toxic cyanobacteria species were detected in 14 water bodies and blooms were observed in 57% of them. In general, Microcystis aeruginosa, Aphanizomenon flos-aquae, Cuspidothrix issatschenkoi and Anabaena minderi were most frequently found species in the sampling sites. Total 34 cyanobacteria species were determined in sampling areas and twelve potentially toxin-producing cyanobacteria species were found. Based on cyanobacterial abundance, cyanotoxin and chlorophyll-a concentrations, legislation proposal was prepared for drinking and recreational waters. Data indicate that cyanotoxin production can be a year-round phenomenon in Turkey and can occur at levels that may cause ecological and human health problems. Therefore, implementation of guideline values for cyanotoxins in drinking and recreational waters in our country has a vital importance.
Recent research on lake sediments has provided new insights into the causes of cyanobacterial blooms. In addition to the well-known role of surface sediments in the annual life cycle of bloom forming species like *Microcystis* [1, 2], sediment cores can also harbour information on historic species composition, the occurrence of blooms and toxins. An improved understanding of temporal and spatial patterns of species composition and toxin concentration in sediments is therefore important for interpreting data retrieved from sediments. Lake Rotorua (New Zealand) has experienced severe *Microcystis* blooms in recent years, but there is uncertainty regarding historical bloom events and the associated triggers of bloom formation. The 2016 7.8 magnitude earthquake may have disrupted the sediment surface layer or resulted in external sediment overlay, which could have altered the composition and intensity of cyanobacterial blooms. Twelve spatially separated surface samples and two 60 cm deep sediment cores were collected from Lake Rotorua. High throughput sequencing was used to analyse overall bacteria community and qPCR for enumerating toxic *Microcystis* cells. Sediment surface layers showed significant sampling site-specific clustering of cyanobacteria and heterotrophic bacteria community. However, no *Microcystis* or microcystins were detected. Preliminary analysis from deeper sediment layers revealed *Microcystis* in high numbers, testimony of previous blooming events. Our data suggest that spatial heterogeneity of bacterial composition should be taken into account for core sampling. It also highlights the value of incorporating paleolimnological approaches into cyanobacteria research as it allows to reconstruct how and why cyanobacterial communities and biomass has changed over time.

References:

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CLOSING LECTURES
The Guidebook Toxic Cyanobacteria in Water, published by the World Health Organisation in 1999, has been widely used by regulators to develop policies for protecting people from exposure to cyanotoxins, by practitioners who implement such policies in the field as well as by students and scientists. New developments since 1999 include knowledge about cyanotoxins as well as a regulatory paradigm shift towards promoting a better understanding of drinking-water systems as well as recreational water use. This encompasses assessing the locally specific risks and developing plans for their effective control. The new edition of „Toxic Cyanobacteria in Water“ begins with an introduction into cyanobacteria, their toxins and toxicological Guideline values for their concentration in water used for drinking and recreation. It provides extensive guidance for assessing the risk of cyanotoxin occurrence as well as for managing this risk, i.e. for planning measures in catchments, in water bodies, at points of water use and in drinking-water treatment. It also introduces methods for field and laboratory analyses of cyanobacteria, their toxins and water-body conditions leading to blooms and provides guidance for responses to potentially hazardous concentrations.
FROM CYANOBACTERIAL PROBLEMS TO BLUE-GREEN SOLUTIONS

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This presentation should be regarded as a discussion starter. I would be happy if you disagree with me and give arguments for your thinking. The presentation briefly highlights some recent actions/initiatives related to toxic cyanobacteria and cyanotoxins. These include the COST Actions CYANOCOST and NETLAKE and the revision of the EU Drinking Water Directive. Further, some of the presentations given during the ICTC11 will be discussed. Would a paradigm shift in our efforts be necessary? After some 40 years of intensive research we know quite much. We know the common toxin producers and most toxin structures. Using sophisticated tools we have determined typical toxin concentrations in the environment and we thus have a reasonably good understanding of exposure levels related to the consumption of cyanotoxin-contaminated water and foodstuffs. We have identified the main toxin actions in many organisms. We can attribute some (serious) illnesses and even deaths especially, but not exclusively, to microcystins in environments where some further hazards or bad practices exist. However, the overall picture of cyanobacterial risks is less dramatic than many of us thought earlier. We also have an understanding of the microbiological and chemical degradation of many cyanotoxins, and we are able to produce cyanotoxin-free drinking water if we invest in the right technologies. It is realistic to say that most of the ground-breaking research has been done by now. In my opinion, the only truly unsolved big question is why cyanobacteria produce toxins.

Would it now be time to wrap up and focus more - but of course not solely - on the management of the water environment. Such thinking is also supported by many funding agencies as it is increasingly difficult to get research money for simply pointing out problems - they want solutions. The management actions should of course be based on sound and authoritative science, and they need to be supported by legislation and official guidelines. To establish this we need to get out of our academic circles and actively collaborate with and listen to national stakeholders, international environmental agencies/programmes and legislators. A change of focus to more management-type research would also be welcome at future ICTC conferences.

In general, cyanobacterial problems and opportunities should be approached from a holistic "One Water" perspective in order to avoid segmentation. Toxic cyanobacteria are just one of the problems in the water environment. Personally, I am more worried about the ongoing chemicalization and pharmaceuticalization of the environment than about cyanotoxins. Further, some cyanobacteria should also be seen as wonderful producers of beneficial compounds and raw materials (blue biotechnology), and colonizers of desolate terrains.
1. Antoniou M.G., Brient L., Tsiarta N., Keliri E., Christofi M., Hadjiouraniou G., Sukenik A. (Cyprus, France, Israel)  
Monitoring and treatment of cyanobacterial contaminated surface waters in France and Cyprus.

2. Blahova L., Hilscherova K., Lepsova-Skacelova O., Szmucova V., Sivonen K., Teikari J., Blaha L. (Czech Republic, Finland)  
Anatoxin-a and cylindrospermopsin in the Czech Republic: toxins, genes and producers.

The extreme cyanobacteria bloom in the Gulf of Gdańsk, in 2018.

4. Bober B., Bialczyk J. (Poland)  
Characteristic of newly identified cyanopeptides - anabaenopeptin 899 and cyanopeptolin 1081.

5. Bober B., Bialczyk J., Chropusta-Srebrny E., Duchnik K., Źmudzki P. (Poland)  
Determination of toxins content in cyanobacterium Woronichinia naegeliana (Unger) Elenkin.

Cyanobacteria in aquatic systems of the Americas.

6b. Haakonsson S., Rodríguez M.A., Rodríguez-Gallego L., Arocena R., Pérez M., Carballo C., Bonilla S. (Uruguay, Canada)  
Predicting planktonic cyanobacteria through a novel Bayesian approach.

Cyanobacteria and cyanotoxins in estuarine waters and sediments.

8. Breiholz J., Weisbrod B., Martin-Creuzburg D., Dietrich D. (Germany)  
The role of nitrogenase expression for Anabaena bloom formation.

9. Brient L. (France)  
Cyanobacteria for future cyanobacteriologists.

10. Brient L., Bormans M., Vallet F. (France)  
Phycocyanin: measure in real time benthic and planktonic cyanobacteria by submersible fluorescence sensor.

11. Brzozowska A., Kokociński M. (Poland)  
Influence of temperature on the growth rate of alien cyanobacterium - Raphidiopsis raciborskii.

12. Bubak I., Śliwińska-Wilczewska S., Chlost I. (Poland)  
Allelopathic activity of the picocyanobacterium Synechococcus sp. on a natural plankton community.

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Response of potentially toxigenic filamentous cyanobacteria to climate warming in a deep subalpine lake (Lake Lugano).

Pseudanabaena galeata from the Baltic Sea - toxicity, diversity and light adaptation.

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Bioassay to explore factors influencing de novo synthesis of microcystins by Microcystis aeruginosa PCC7813.

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Determination of cyanotoxins in fish tissue: matrix-interference challenges.

Cyanotoxins production potential in newly isolated cyanobacteria from the Azores.

Source-tracking a toxigenic Microcystis bloom in a major Great Lakes tributary.

20. Druga B., Szekeres E., Buda D., Sicipa C. (Romania)  
The impact of cation concentration on Microcystis (cyanobacteria) scum formation.
21. Fang Y., Gin K. (Singapore)
Isolation and characterization of cyanophages infecting cyanobacterium *Microcystis aeruginosa*
from a tropical reservoir.

*Nostoc edaphicum* - a rich source of bioactive products.

Cyanotoxins presence related with phytoplankton and cyanobacteria biovolume and chlorophyll a concentrations from five reservoirs in temperate climate (South-East Portugal).

24. Grabowska M., Mazur-Marzec H., Więcko A. (Poland)
Toxic cyanobacteria response on extreme weather events in lowland dammed river.

Multistage cloning and heterologous expression of microcystins-degrading genes.

(Co-) Production dynamics of cyanopeptides beyond microcystins.

27. Keliri E., Edwards C., Mazur-Marzec H., Antoniou M.G. (Cyprus, United Kingdom, Poland)
Bioactive metabolites of cyanobacteria detection in a eutrophic dam in Cyprus (Polemidia Dam).

A toxic puzzle - unravelling the relationship between anatoxin production & strain dominance in *Microcoleus autumnalis* (*Phormidium autumnale*).

Culture independent and dependent analysis of bacteria community in the phycosphere of cyanobloom-forming *Microcystis aeruginosa*.

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Diversity of cyanobacterial and cyanotoxins in Küçük Menderes basin, Turkey.

Analysis of deep sediments revealed a thousands-year presence of toxic *Nodularia spumigena* in the Baltic sea and Norwegian coastal waters.

33. Kozak A., Dondajewska R., Kowalczewska-Madura K., Goldyn R. (Poland)
Planktonic cyanobacteria and physicochemical parameters of a small hypertrophic lake.

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Are toxigenic cyanobacteria in microbial mats from cold deserts of Eastern Pamir a real threat?

35. Lee Y.-E., Yoo Y.-S. (Republic of Korea)
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36. LeMoal M., Mineaud E., Briant L., Wiegand C. (France)
Implementation of a hydrogen peroxide curative treatment of cyanobacteria within The Lac au Duc, France.

37. Martin-Creuzburg D. (Germany)
Food quality of cyanobacteria: a first approach towards disentangling multiple nutritional constraints.

Molecular screening of the potential production of cyanotoxins and cyanobacterial natural products in environmental samples from Cabo Verde Islands.

39. McCarron P., Rafuse C., Scott S., Douthwright E., Bruce M.R., Lawrence J., Murphy C., Reith M., Beach D.G. (Canada)
Anatoxins in samples associated with dog deaths in New Brunswick, Atlantic Canada.

40. Menezes C., Valério E., Botelho M.J., Martins O., Dias E. (Portugal)
Successful isolation and cultivation of *Cylindrospermopsis raciborskii* strains isolated from finished drinking water samples.

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Succession of Microcystis genotypes accompanies different microbial modules with recurrent patterns.

Detection of cyanobacteria in the diet composition of crustacean zooplankton: a multiproxy study.

Molecular evaluation of the occurrence of invasive cyanobacteria and their toxin production potential in the Nakdong river, Korea.

Perennial droughts and blooms - cyanobacterial treatment challenges and solutions.

Phytoplankton community shifts after selective removal of cyanobacteria by hydrogen peroxide treatments of lakes.

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Lake Ludos – an aquatic ecosystem with a (cyanobacterial) problem.

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Extracts of *Planktothrix agardhii*-dominated scum samples rich in oligopeptides influenced growth, production of Chl-a and peptide composition of natural *P. agardhii* population.

Defining the mode of interaction between temperature and phosphate in promoting cyanobacterial growth.

Toxin production by microcystin- and saxitoxin-producing cyanobacteria in response to *Daphnia gessneri* infochemicals.

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*Anabaena* bloom dynamics in a hydropower reservoir.

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Study on the cyanobacterial toxin metabolism of *Microcystis aeruginosa* in nitrogen-starved conditions by stable isotope labeling method.

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Elaboration of subchronic toxicity reference values (TRVs) for the oral routes of microcystin-LR and cylindrospermopsin
<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>Microcystins in pond water of rural Bangladesh.</td>
<td>Akter S., Meriluoto J., Lamminmäki A. (Finland)</td>
</tr>
<tr>
<td>69</td>
<td>Application of Electron Paramagnetic Resonance (EPR) for radical identification during the photocatalytic degradation of cyanotoxins with enhanced photocatalysis.</td>
<td>Antoniou M.G., Boraíi E., Pantelides D., Solakidoy M., Deligiannakis Y., Abhishek M., Edwards C., Lawton L. (Cyprus, Greece, United Kingdom)</td>
</tr>
<tr>
<td>70</td>
<td>Acute toxicity and gene responses induced by <em>Cylindrospermum</em> sp. in Zebrafish (<em>Danio rerio</em>) embryos.</td>
<td>Babic O., Mric P., Fent K., Smittal T., Blagojević D., Švirić Z., Simeunović J. (Serbia, Croatia, Switzerland)</td>
</tr>
<tr>
<td>71</td>
<td>Abundance and toxicity of <em>Planktothrix rubescens</em> in the German Osterseen Lake District.</td>
<td>Bauer F., Stix M., Geist J., Raeder U. (Germany)</td>
</tr>
<tr>
<td>73</td>
<td>Early biomarkers of behavioural and physiological disturbances in <em>Daphnia magna</em> exposed to anatoxin-a estimated by video analysis.</td>
<td>Bownik A., Pawlik-Skowrońska B. (Poland)</td>
</tr>
<tr>
<td>75</td>
<td>GlobalHAB: a global initiative to enhance collaboration and communication on HABs.</td>
<td>Burford M.A., Berdalet E., Banas N., Bresnan E., Karlson B., Kudela R., Llana-Ruiz-Cabello M., Catunescu G., Puerto M., Jos Á., Cameán A.M. (Spain)</td>
</tr>
<tr>
<td>76</td>
<td>Intrinsic antibiotic resistance in cyanobacteria – the case of trimethoprim.</td>
<td>Dias E., Manageiro V., Caniça M. (Portugal)</td>
</tr>
<tr>
<td>77</td>
<td>DNA damage induced by cylindrospermopsin in rats.</td>
<td>Diaz-Quijada Jiménez L., Llana-Ruiz-Cabello M., Catunescu G., Puerto M., Jos Á., Cameán A.M. (Spain)</td>
</tr>
<tr>
<td>78</td>
<td>Toxicological evaluation of a binary mixture of cyanotoxins using mutagenicity biomarkers.</td>
<td>Diaz-Quijada Jiménez L., Puerto M., Prieto A.I., Jos Á., Cameán A.M. (Spain)</td>
</tr>
<tr>
<td>79</td>
<td>BMAA causes progressive neurodegeneration typical of Alzheimer’s and Parkinson’s diseases and ALS.</td>
<td>Downing T.G., Scott L.L. (South Africa)</td>
</tr>
<tr>
<td>80</td>
<td>Benthic cyanobacteria and their toxins in Dutch recreational waters.</td>
<td>Faassen E., Demarteau M. (The Netherlands)</td>
</tr>
<tr>
<td>81</td>
<td>Dog fatalities associated with tychoplanktic, anatoxin-a producing <em>Tychonema</em> sp. in a Berlin lake recovering from eutrophication - challenges for surveillance.</td>
<td>Hercog K., Maisanaba S., Filipič M., Sollner-Dolenc M., Žegura B. (Slovenia, Spain)</td>
</tr>
<tr>
<td>82</td>
<td>Anatoxin-a(s) revisited: chemical features and isolation.</td>
<td>Fernandes K.A., Pinto E., Sanz Rodan M. (Brazil)</td>
</tr>
<tr>
<td>83</td>
<td><em>Cynobacteria</em> species recordings through statistics; a proposed TO(X)OLBOX.</td>
<td>Panou M., Giourieva V., Gkelis S. (Greece)</td>
</tr>
<tr>
<td>85</td>
<td>Gene-level modeling of <em>Microcystis</em> growth and toxin production.</td>
<td>Hellweger F. (Germany)</td>
</tr>
<tr>
<td>86</td>
<td>Plastics in cyanobacterial bloom - combined effects of the exposure to cylindrospermopsin and bisphenols.</td>
<td>Hercog K., Maisanaba S., Filipič M., Sollner-Dolenc M., Žegura B. (Slovenia, Spain)</td>
</tr>
</tbody>
</table>
87. Hilscherová K., Večerková J., Jonáš A., Smutná M. (Czech Republic)
Cyanobacterial exudates with retinoid-like activity cause developmental disorders in fish and frog embryos.

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122. Simiyu B.M., Kurmayer R. (Austria)
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Cytotoxic metabolites of Anabaena from Baltic Sea.

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β-N-methylamino-L-alanine is a dopaminergic toxin.

131. Vasconcelos V. (Portugal)
Cyanobacteria and cyanotoxins risks via food. Do we know all the hazards?

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β-N-methylamino-L-alanine interferes with nitrogen assimilation in the cyanobacterium, non-BMAA producer, Synechococcus TAU-MAC 0499.

133. Vick C., Hellweger F.L. (Germany)
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134. Wojtal-Frankiewicz A., Bernasńska J., Frankiewicz P., Jurchak T., Mankiewicz-Boczek J., Gwoździński K. (Poland)
Size-related activity of antioxidant system in zebra mussel (Dreissena polymorpha) during toxic cyanobacterial bloom.

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Health risk assessment of the cyanobacterial neurotoxin β-n-methylamino-L-alanine (BMAA) in freshwater aquaculture ecosystem and the study of its control technology.

Water hyacinth for polluted water remediation and challenges in engineering application.

137. Yamashita R., Arii S., Tomita K., Tsuji K., Bober B., Harada K. (Japan)
Life cycles of Microcystis in late summer: reveal lysis with β-cyclocitral
Poster session I
MONITORING AND TREATMENT OF CYANOBACTERIAL CONTAMINATED SURFACE WATERS IN FRANCE AND CYPRUS

M. G. Antoniou¹, L. Brient², N. Tsiarta¹, E., Keliri¹, M. Christofi¹, G. Hadjiouraniou¹, and A. Sukenik³

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CYANOS is a two-year project with interdisciplinary activities that combines surface water monitoring and on-site water treatment for the restoration of eutrophic surface waters [1]. Specifically, CYANOS aims to monitor seasonal variations in cyanobacterial harmful algal blooms (cyano-HABs) in surface waters of Cyprus and France and to explore emerging onsite treatments to control their formation. In this project the French scientists lead the phytoplankton characterization, while the Cypriot partners are in charge on the application of emerging technologies for the on-site treatment of cyano-HABs. Chemical oxidation with hydrogen peroxide (HP) appears to be a promising in-lake chemical oxidation treatment since it does not generate hazardous byproducts and has minimum effect on other associated aquatic life forms [2]. Initial bench scale experiments have indicated that differences between the antioxidative capacity of the photosynthetic system of eukaryotes (algae) and prokaryotes (cyanobacteria) make the latter, five to ten times more sensitive to HP treatment compared with algae. Therefore, in this project, hydrogen peroxide will be tested for its efficiency to control cyano-HABs in French and Cypriot surface waters. In fact HP treatment is becoming common practice in France for small shallow lakes. In addition we will evaluate the effectiveness of peroxymonosulfate, a chemical that is currently used in swimming pools, for shock oxidation. Overall, we present results from the taxonomy of microalgae and cyanobacteria, and cyanotoxins as well as emerging in-site treatments based on chemical oxidation for the removal of cyanobacteria and cyanotoxins.

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MGA is grateful to the Research Promotion Foundation of Cyprus for funding the Bilateral Agreement FranceCyprus, CYANOS and the Water Development Department for granting permission to take water from the Polemidia dam. L.B. is thankful to the Campus France organization for funding the CYANOS project.
ANATOXIN-A AND CYLINDROSPERMOPSIN IN THE CZECH REPUBLIC: TOXINS, GENES AND PRODUCERS

L. Blahova¹, K. Hilscherova¹, O. Lepsova-Skacelova², V. Szmucova¹, K. Sivonen³, J. Teikari³, L. Blaha¹

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In this paper we present the results of a survey of cyanobacterial blooms dominated by other than Microcystis sp. in the Czech Republic. The focus was on 34 bloom samples dominated by potential producers of anatoxin-a and cylindrospermopsin (CYN) such as Dolichospermum sp. (Anabaena sp.), Aphanizomenon sp. and others. Levels of cyanotoxins were analyzed in -dried biomass using the multi-target UPLC-MS/MS allowing simultaneous determination of microcystin-LR, -RR, -YR, -LF, -LW, -LA, -LY, -WR, nodularin, cylindrospermopsin, deoxycylindrospermopsin, anatoxin-a, homoanatoxin-a, dihydrohomoanatoxin-a, and dihydroanatoxin-a). In addition, toxic lipopeptide puwainaphycin F was also analyzed and revealed in the highest concentration in the bloom 90% dominated by Woronichinia naegeliana. CYN has been confirmed (4.25 microgram/g d.w.) in a single bloom from the pond Pisecensky (South Moravian region close to Slovakia and Austria borders) dominated (30%) by Cylindrospermopsis raciborskii. Other species in the CYN-positive bloom were Cuspidothrix issatchenkoi, SphaerospERMopsis aphanizomenoides (formerly Anabaena aphanizomenoides), Pseudoanabaena limnetica and Planktolyngbya limnetica. Genetic analyses confirmed the presence of cyrJ gene in the studied sample. For the first time, we report the occurrence of anatoxin-a, detected in three Czech samples (South Bohemia region) at concentrations ranging 0.34-2.82 microgram/g d.w. Dolichospermum species were found in all three anatoxin-a positive samples (D. planctonica, D. smithii and D. flos-aquae). The communities of the anatoxin-a positive blooms were generally rich in composition containing also Aphanocapsa sp., Aphanizomenon sp., Microcystis sp., Woronichinia sp., SphaerospERMopsis sp. Genetic analyses were not able to confirm presence of anatoxin-a synthesis gene (anaC) in any of the samples studied.
THE EXTREME CYANOBACTERIA BLOOM IN THE GULF OF GDAŃSK, IN 2018

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In the Baltic Sea, summer blooms of cyanobacteria occur on annual basis. In the Gulf of Gdańsk, cyanobacterial community is usually dominated by toxin-producing Nodularia spumigena Mertens ex Bornet & Flahault. Three distinguishable morphotypes of this organism are recorded: straight, twisted and strongly curled. Last summer (2018), an exceptionally intense N. spumigena bloom occurred. The first, transient bloom was observed on 11-13 July. It was mainly formed by the long, curled N. spumigena morphotype, typical for the Gulf of Gdansk. At that time, the concentration of nodularin (NOD) in coastal waters reached 6750 μg dm⁻³. During the second peak of cyanobacterial bloom (23 July-3 August), the phytoplankton community was dominated by long, straight filaments of N. spumigena, characteristic for the central part of the Baltic Sea. Then, the concentration of NOD in the coastal zone reached even 30,000 μg dm⁻³. For 12 days, all the bathing sites of the Gulf of Gdańsk and Puck Bay were closed due to formation of toxic scums. At the end of the bloom, the contribution of cyanobacteria from Dolichospermum genus significantly increased, and high concentrations of microcystins were determined. During this period, intense blooms of cyanobacteria were also recorded in the central and western parts of the Polish coast.
CHARACTERISTIC OF NEWLY IDENTIFIED CYANOPEPTIDES - ANABAENOPEPTIN 899 AND CYANOPEPTOLIN 1081

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Cyanobacteria are capable to synthesize and release into the aquatic environment many secondary metabolites of various chemical structure and biological interaction. Research has primarily focused on compounds toxic to humans and animals, whereas others cyanobacterial products are poorly studied. Most of these secondary metabolites are oligopeptides containing unusual amino acids in their structure [1]. Taking into consideration that some cyanopeptides show bioactivity and in a consequence possess potential ecological and toxicological importance it is necessary to develop knowledge about these compounds. Preliminary made experiments show that poorly studied freshwater cyanobacterium Woronichinia naegeliana (Unger) Elenkin is a rich source of cyanopeptides [2,3]. Two new oligopeptides, so far not described in the literature, belonging to anabaenopeptins and cyanopeptolins were isolated from W. naegeliana cells. The application of mass spectrometry and nuclear magnetic resonance allow identifying these compounds as anabaenopeptin 899 and cyanopeptolin 1081. Characteristic of cyanobacterial secondary metabolites is important especially in relation to the reservoirs of drinking water and in long term is essential in the search for and development of effective methods of degradation of these compounds. The influence of selected abiotic factors such as pH, temperature, visible and ultraviolet radiation on the stability of newly identified anabaenopeptin and cyanopeptolin was studied. It has been shown that these compounds are oligopeptides of relatively high stability. In view of poor knowledge of cyanopeptides presence in aquatic environment, such studies are needed to reveal their detailed characteristics.

References:

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The progressive eutrophication of water reservoirs affects the increased expansion of cyanobacteria, which are able to synthesize and release into the aquatic environment diverse bioactive compounds. The monitoring of potential threats caused by cyanobacterial blooms relies mainly on confirmation of the presence of toxins such as hepatotoxic microcystins, neurotoxic anatoxin-a or cytotoxic cylindrospermopsin. Cyanobacterium *Woronichinia naegeliana* (Unger) Elenkin appears numerously in surface freshwater in Europe, North America and Australia. Although this species frequently occurs worldwide in reservoirs that constitute a source of drinking water, little is known about it. The aim of the presented study was a determination of cyanobacterial toxins content in *W. naegeliana*. The qualitative and quantitative analysis of extract obtained from lyophilized *W. naegeliana* cells was conducted following the appropriate procedures and using high-performance liquid chromatography and mass spectrometry. The presence of two commonly known cyanobacterial toxins, microcystin-LR and anatoxin-a, was confirmed. The detected concentration of microcystin-LR was 0.66 mg per gram dry weight (d.w.). The lower concentration was denoted for anatoxin-a of 0.21 mg per gram d.w. The previously obtained results indicate that *W. naegeliana* produces also several compounds belonging to the poorly known groups of oligopeptides [1] whose role in the metabolism of cyanobacteria and biological properties has not been clearly defined yet. Taking into consideration a large variety of secondary metabolites synthesized by *W. naegeliana*, understanding their biological role is an interesting issue because the synthesis of these compounds requires the high cost of materials and energy.

References:
The Americas are one of the world’s major natural freshwater reserves, characterized by diverse climates and heterogeneous ecosystem types. In these sites, eutrophication and climate change are drivers for the loss of water quality, triggering the growth of potentially toxic planktonic cyanobacteria. Blooms of these organisms threaten the use of water for many different purposes, often resulting in negative local economic impacts in developing countries. Cyanobacteria are a heterogeneous group of organisms, and major evolutionary differences between taxonomic orders result in diverse physiological and morphological traits and environmental preferences. Studies with a large geographical perspective allow for comparisons of cyanobacteria at different taxonomical levels and across ecoclimatic regions. In this study, we investigate the distribution of planktonic cyanobacteria in lakes around the Americas with a gradient of over 135 degrees of latitude, from Tierra del Fuego, 54°51’S (Argentina) to Ellesmere Island, 82°54’N (Canada). We performed a survey using unpublished and published data from 1300 lakes, including limnological and environmental variables, ecoregion information, phytoplankton and detailed data for more than 150,000 cyanobacterial populations, with researchers from 13 institutions and eight countries. We present preliminary results seeking to identify the main patterns in latitudinal distribution of total cyanobacteria and their major taxonomic orders in relation to trophic state, morphometric and climatic variables. Our results will have important implications for the health of aquatic ecosystems and the human populations that rely on them.
Increasing frequency of cyanohalts engenders the need for predictive models of cyanobacterial abundance. There are three basic challenges in developing these models. First, cyanobacterial abundance is highly variable in time and space. Second, abundance data typically contain many zeros. Third, far from being a homogeneous group, bloom-forming cyanobacterial groups and species differ in their functional traits and environmental preferences. Our objectives were to develop a model relating cyanobacterial biovolume to temperature and salinity, the two main environmental predictors at our selected study area, and to use the model to predict the probabilities of reaching three exposure thresholds implying differing levels of risk to humans (0.2 mm$^3$/L, 1 mm$^3$/L, and 2 mm$^3$/L). We also wanted to model the biovolume of Dolichospermum and Microcystis, the two dominant cyanobacterial genera at our study site, to investigate the differences in their ecological preferences. We developed a Compound Poisson-Gamma (CPG) regression model which can account for continuous data containing a large proportion of zeros and a skewed distribution of the remaining values, which are typical features of cyanobacterial abundance. Parameter estimation was conducted within a full Bayesian framework. We successfully modeled total cyanobacterial biovolume and the probability of crossing the selected risk thresholds, in scenarios of differing temperature and salinity. Total cyanobacterial biovolume responded positively to temperature and negatively to salinity. Dolichospermum and Microcystis always co-occurred; however, we found ecological differences in their responses to temperature and salinity. This novel application shows that the Bayesian CPG approach to modeling cyanobacterial biovolume is a promising tool for predicting cyanobacterial dynamics in response to environmental change.
Only few studies have examined the occurrence of cyanobacteria and cyanotoxins in estuarine waters. During fall 2018, we sampled in the water column and in the sediments, phytoplankton and cyanotoxins at 5 stations along a river continuum, from a freshwater reservoir through an interconnecting estuary to the coastal area in Brittany, France. Cyanobacteria dominated the phytoplanktonic community in the water column with high densities in the freshwater sites, with a cells transfer in estuarine and marine sites and decreasing cell densities associated with flow dilution. Cyanobacterial biomass was dominated by *Microcystis* sp. that survived intermediate salinities. Intracellular MCs were detected in the freshwater samples at high concentrations (up to 28 µg/L), and decreased from upstream to downstream in accordance with cyanobacterial biomass. Extracellular MCs concentrations showed the opposite trend and their contribution to total MCs increased from upstream to downstream in accordance with the lysing of the cells at elevated salinities. Surface sediments samples contained also high densities of *Microcystis* in freshwater, and with decreasing densities along the salinity gradient. Surprisingly, *Microcystis* was detected in the sediments at all sites. Intracellular MCs were detected in the sediments at all sites except at the marine outlet. The mcyB gene was present at all sites while mcyA gene was absent at the marine outlet confirming the presence of toxic strains along the estuary. *anaC* gene was also present highlighting the potential of anatoxins producing species. This study confirmed a cyanobacterial and MC transfer from fresh to marine waters, together with the occurrence of cyanobacterial cells and toxins in the estuary’s sediments.
The role of nitrogenase expression for Anabaena bloom formation

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Anabaena species are among the most common bloom-forming cyanobacteria, some of which also producing harmful secondary metabolites such as microcystins. In comparison to other cyanobacteria taxa, Anabaena species can form blooms also in nitrogen-deficient waters, which has been linked to their ability to fix atmospheric nitrogen via nitrogenases, providing a competitive advantage over eukaryotic algae. The fixation of atmospheric nitrogen is metabolically less efficient than organic nitrogen uptake. Although it is currently assumed that Anabaena is able to control the expression of nitrogenases depending on environmental conditions [1, 2], little is known on how nitrogenase expression is affected by changing nitrogen concentrations. To investigate the influence of varying nitrogen concentration on nitrogenase expression we ran chemostat experiments with Anabaena as mono-cultures and in co-culture with green algae (Chlorella) as nitrogen consumption competitors. We hypothesized that nitrogen limitation in mono-cultures leads to an increased nitrogenase expression. In co-culture we assumed an increased nitrogenase expression under nitrogen limiting conditions, resulting in a growth advantage of Anabaena over Chlorella. To quantify nitrogenase expression we developed a western plot protocol using a nitrogenase-specific antibody. Our results provide new insights into the adaption of Anabaena to nitrogen limitation and help to understand the factors influencing bloom formation in nitrogen-depleted water systems.

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CANOBACTERIA FOR FUTURE CYANOBACTERIOLOGISTS

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Beyond the rules of taxonomy that constantly evolve especially with molecular tools, visual identification by microscopy remains essential today whatever its name or classification. Poster in English available for free in pdf.
PHYCOCYANIN: MEASURE IN REAL TIME BENTHIC AND PLANKTONIC CYANOBACTERIA BY SUBMERSIBLE FLUORESCENCE SENSOR

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For about ten years, limnologists have access to the detection of cyanobacteria by submersible probes by measuring phycocyanin by fluorescence. This relatively new tool has several advantages: it facilitates the identification of cyanobacteria among other groups of phytoplankton [1] when viewing green mats, for example, by water managers responsible for recreational activities; It allows this measurement in real time with simplicity and reliability; It also identifies cyanobacterial presence at depths greater than 10 m on layers only a few centimeters thick, in areas where conventional samples will not highlight them, which is of interest for drinking water plans often collecting in these areas at the limits of the euphotic zone; It is an alarm trigger when installed online in drinking water treatment systems. For measure of phycocyanin to be relevant, the probe must have particular characteristics with respect to the measured parameters (planktonic or benthic cyanobacteria; in or out of the water): in particular the distance between the probe and the object of measurement. Examples of measurements with various characteristics and in different geographical areas will be given.

References:
INFLUENCE OF TEMPERATURE ON THE GROWTH RATE OF ALIEN CYANOBACTERIUM - RAPIDIOPSIS RACIBORSKII

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Among factors determining occurrence and dispersion of Raphidiopsis raciborskii (Nostocales) in freshwater ecosystems is temperature. R. raciborskii was recorded for the first time on the Java island in Indonesia. For many years it has been considered as a tropical and subtropical species. The research aim was to study influence of temperature variants on the growth rate of Raphidiopsis raciborskii strains from Poland and Ukraine.

Water samples were collected during summer of 2017 from 5 lakes from Western Poland, and 2 sites from Seret River from Galicia-Volyn area in Ukraine. Single trichomes of R. raciborski were isolated from environmental samples, in order to obtain monocultures. The experiments was conducted in triplicates in temperatures: 10.0°C, 22.1°C and 31.0°C, per 12 days, in a thermostatic chamber with a 12:12 h (light:dark) regime. Light intensity and nutrients concentration in WC medium were constant. Growth rate was measured daily based on absorbance using a microplates spectrophotometer (λ = 650 nm).

Temperature of 10.0°C appeared to be too low for a significant growth of strains as the only growth of one strain from Lake Żnińskie Małe was observed. At the temperature of 22.1°C, a constant growth of all strains originating from Poland and Ukraine was noticed. The strain from Lake Żnińskie Małe showed a higher growth. However, at the temperature of 31.0°C the best growth of strain from Lake Kierskie Małe was observed. This preliminary study showed some variety in responses of isolated strains from Poland and Ukraine to different temperatures.
Cyanobacteria are known to produce a variety of bioactive compounds, whose toxicity to animals and humans has been clearly demonstrated. Some of these compounds might function as allelochemicals that inhibit the growth of competitors. Allelopathy is considered one of the factors that promote and maintains massive phytoplankton blooms in freshwater ecosystems around the world. Therefore, more efforts have to be done to investigate in depth the mechanisms causing cyanobacterial blooms, like, potentially, their allelopathic activity on coexisting phytoplankton species. We studied allelopathic activity of picocyanobacterium *Synechococcus* sp. on a natural plankton community from four Polish lakes. In this study, the allelopathic activity of the *Synechococcus* sp. on natural plankton community growth, expressed as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and chlorophyll *c* (Chl *c*), as well as total abundance and structure of phytoplankton community were investigated. Our results indicated that *Synechococcus* sp. filtrate had generally an inhibitory effect on all phytoplankton community however, the degree of inhibition was different for each species, causing a change in the phytoplankton abundance and dominance during the experiment. This work demonstrated that the allelopathic activity exhibited by the *Synechococcus* sp. is probably one of the major competitive strategies affecting some of the coexisting phytoplankton species in freshwater ecosystems. To our knowledge this is the first report of the allelopathic activity of *Synechococcus* sp. in the freshwater reservoirs, and one of the few published works showing allelopathic properties of picocyanobacteria on coexisting phytoplankton species.
Climate warming and anthropogenic eutrophication are increasingly driving harmful algal blooms worldwide. In particular, in a warming scenario, it is predicted that filamentous cyanobacteria will benefit from increased water temperature, stronger thermal stratification and weaker mixing during turnovers. Their resulting increased dominance raises wide concerns on water quality and the integrity of lake ecosystems. In the past century, the deep subalpine Lake Lugano was severely affected by eutrophication, which induced meromixis and led to the appearance of blooms of *Planktothrix rubescens*. At the same time, the surface waters of the lake warmed significantly, especially in summer (+0.9 °C per decade since 1972). Although a lake-restoration programme, started in the '80s has considerably reduced nutrient loads, phytoplankton biomass has remained high, and the restoration targets have not been fully reached yet. In this lake, the dominance of cyanobacteria, especially the filamentous species, has changed during the past decades, but the magnitude and direction of these changes, along with the underlying ecological drivers, are poorly understood. In this study, a forty-year record of biological and physicochemical data was analyzed to: i) determine the seasonal and long-term patterns in cyanobacteria community composition; ii) identify the ecological drivers (e.g. solar radiation, water column stability, temperature, nutrients) that control the abundance of potentially toxigenic filamentous species; and iii) assess the potentially hampering effects of climate change on lake-restoration measures. The results will help to develop adaptive management solutions to meet restoration targets and mitigate the spread of toxigenic cyanobacteria under a warmer climate scenario.
PSEUDANABAENA GALEATA FROM THE BALTIC SEA - TOXICITY, DIVERSITY AND LIGHT ADAPTATION

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While the cyanobacteria from Pseudanabaena genus are ubiquitous organisms present in freshwater and marine environments worldwide, the occurrence of Pseudanabaena galeata has been mainly reported in Europe and America. Due to the fact that this species does not form extensive blooms, it might be overlooked. The aim of this study was to characterize the genotype and chemotype diversity, and light adaptation abilities of two P. galeata strains (CCNP1311 and CCNP1313) isolated from the Southern Baltic Sea. Characterization and comparison of the examined organisms were based on analysis of selected DNA sequences (16S rRNA, mcyE and PC-IGS), peptide profiles and pigment composition (chlorophyll-a, carotenoids and phycobilins).

Both strains were cultured in the same conditions, under three different light regimes. Both of them produced zeaxanthin, echinenone, β-carotene and phycocyanin. Myxoxantophyll and pheophytin were only detected in the strain CCNP1311, while phycoerythrin was only produced by CCNP1313. P. galeata CCNP1311 belongs to MC producers, while the strain CCNP1313 produces several unknown variants of peptides. Both strains induced cytotoxic effects in MCF-7 human breast cancer cell line, however, the cell death mechanism of the strain CCNP1313 was determined to be apoptosis, while for the strain CCNP1311 the tested cells showed necrotic changes.

The results showed that the Baltic population of P. galeata is not clonal and shows significant genotype and phenotype differences.

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During last decades, cyanobacteria species known as potent producers of toxic substances have been noted in water reservoirs in Central part of Russia. The Volga is one of the longest rivers in Europe, it is 3,692 km long. Its basin area is 752,443 km². Eleven of the twenty largest cities of Russia, including Moscow, are located in the Volga’s drainage basin. There are 9 water reservoirs along the Volga. In our research, the samples from 9 Volga River Reservoirs collected during research cruises in 2016 were studied. Three methodological approaches were used: light microscopy, molecular methods, and mass-spectrometry. *Aphanizomenon flos-aquae, Microcystis aeruginosa, M. wesenbergii, Aphanocapsa holsatica, A. incerta, Dolichospermum spp.*, *Planktothrix agardhii* has been dominated species in phytoplankton. *Microcystis* and *Dolichospermum* were PCR detected as key microcystin-producing cyanobacteria. The profile of detected cyanotoxins and anabaenopeptines was evaluated. The concentrations of detected intracellular microcystins did not exceed 0,1 mkg L⁻¹ in June, and were in a range 0,3-16,0 mkg L⁻¹ in August.

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BIOASSAY TO EXPLORE FACTORS INFLUENCING DE NOVO SYNTHESIS OF MICROCYSTINS BY MICROCYSTIS AERUGINOSA PCC 7813

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Microcystins are the most common cyanobacterial toxins detected worldwide in freshwater and terrestrial ecosystems in concentrations up to 1300 μg·L⁻¹ [1]. Nevertheless, the factors responsible for fluctuations in concentration remain poorly understood. To resolve this issue, the aim of the current study was to determine de novo synthesis of microcystins. Assay parameters such as cell density and age of the M. aeruginosa inoculum were optimised. We used a stable Na¹⁵NO₃ as the sole nitrogen source in the BG-11 medium to trace the successful incorporation of ¹⁵N isotope into microcystin variants. The data clearly showed that the inoculum size significantly affected the intracellular accumulation of microcystins. The highest de novo MC-LR production rate/cell·day was observed in cultures with an inoculum size of 2.5·10⁶ cells/mL, compared to inocula with higher cell densities up to 8·10⁶ cells/mL. Moreover, the PCC7813 growth rate/day was higher when inoculated with the lower cell density. ¹⁵N-labelling bioassays further demonstrated that the inoculum age significantly influenced metabolic profile. After 10 days of cultivation, the concentration of labelled (¹⁵N) MC-LR synthesized by the oldest studied inoculum, 4-weeks-old, was 217% greater compared to the 1-week-old. Furthermore, the relative abundance of the partially labelled MC-LR (1004.55 g/mol) decreased with the advancing inoculum age reaching 20% and 15% for the youngest and oldest tested cultures, respectively. In addition, 4-weeks-old cells were characterized by the highest biomass accumulation. Further studies are needed to fully understand the factors regulating biosynthesis of cyanotoxins, and stable ¹⁵N isotopes can be ideal tools to explore it.

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Determination of Cyanotoxins in Fish Tissue: Matrix-Interference Challenges

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Cyanobacteria are able to produce various toxic secondary metabolites (cyanotoxins), which could significantly affect aquatic habitats and human health. These compounds present several chemical structures and modes of toxic action \cite{1}. They can be accumulated in fish organs \cite{2}, therefore consumption of contaminated aquatic organisms, poses a potential risk to human health \cite{3}. The determination of toxins in fish tissue presents a challenging task, due to their increased structural diversity, the numerous similar congeners within different toxin groups, the complexity of the analyzed matrices and the extremely low expected concentration of target analytes \cite{4,5}. The scope of this study was the development of efficient and sensitive LC-MS/MS analytical methods for the simultaneous determination of multi-class cyanotoxins (Microcystins, Cylindrospermopsin and Anatoxin-a) in freshwater fish tissue (muscle and liver). The optimization of the sample treatment methods prior to LC-MS/MS analysis included several combinations of extraction solvents at different pH, appropriate procedures to release conjugated and protein-bound fractions of microcystins, protein precipitation and lipid extraction, followed by SPE treatment with various materials, in order to eliminate matrix interferences. During the treatment steps, complementary LC-DAD and LC-MS/MS chromatograms were obtained at identical chromatographic conditions \cite{6}, indicating the co-elution of several matrix components with the target compounds, which revealing the matrix interferences during each step of the treatment method. Optimized pretreatment procedures were proposed, presenting significant elimination of matrix effects and maximum extraction and recovery for each cyanotoxin group. The optimized analytical methods were applied to fish samples from various Greek lakes.

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Title: CYANOTOXINS PRODUCTION POTENTIAL IN NEWLY ISOLATED CYANOBACTERIA FROM THE AZORES

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Cyanobacteria can be found in a variety of habitats, including freshwater, marine, terrestrial and extreme environments (e.g. temperature, salinity). Their presence in the Azores has been reported for more than a century, however, very little is known about their biodiversity and toxin production. Due to environmental factors, such as geographic isolation or physicochemical conditions of the habitat, Azorean cyanobacteria can present differences at taxonomic, ecological or biochemical levels. In order to assess cyanobacteria biodiversity and cyanotoxins production potential various habitats from the Azores islands were sampled: 25 volcanic freshwater lakes, 21 thermal water sampling sites and wetland areas. Cyanobacteria isolation was made by inverted microscopy, after adaptation in BG-11 medium. Saxitoxin, Anatoxin-a and Microcystin genes (sxtA, sxtG, anaC, anaF, mcyC and mcyG) were targeted using specific primer pairs for cyanotoxin production potential identification. Cyanobacteria from 24 genera were isolated, e.g. Microcystis, Mastigocladus and Oscillatoria, in a total of 153 strains. All isolated strains are maintained in unicyanobacterial cultures in the Azorean Bank of Algae and Cyanobacteria created in the framework of the REBECA project (MAC/1.1a/060). Cyanotoxins producing genes were all detected in the tested strains, some in well-known cyanotoxins producers such as Aphanizomenon gracile and Microcystis aeruginosa, and others in non-reported cyanotoxin producers such as Planktolyngbya limnetica and Pseudanabaena minima. This work brings continuously new information regarding cyanotoxins, since these are new isolated strains, and these are the first reports of Saxitoxin, Anatoxin-a and Microcystin producing genes detection in isolated cyanobacteria from the Azores.

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In late September 2017, a cyanobacterial HAB (cHAB) was detected in the estuary of the Maumee River, the largest tributary to Lake Erie. A survey extending 160 km from the river mouth identified Microcystis spp. as the dominant taxa, with bloom biomass extending 10 km from the river mouth and accumulation of microcystins detected by ELISA exceeding Ohio’s Elevated Recreational Public Health Advisory threshold (20 µg/L) at multiple locations.

Coincident with the river cHAB, an expansive Microcystis cHAB covered much of western Lake Erie. Addressing potential continuity between the blooms, samples were screened using high-throughput 16S rRNA gene sequencing to yield amplicon sequence variants (ASVs). Unique Microcystis ASVs identified from the Maumee estuary were found in western Lake Erie, but not in sites sampled upriver.

Likewise, physical evidence supports the advection of cells into the Maumee estuary from Lake Erie. While low river discharge coincident with negligible precipitation prior to the bloom support high-retention in the estuary, easterly winds experienced over the course of the cHAB likely advected surface waters into the river mouth during the low-flow conditions.

These results highlight the need to broaden our understanding of biophysical coupling within freshwater estuaries. This is particularly important in places where these estuaries fall within large metropolitan areas. By combining biological (e.g., -omics and toxin surveys) and physical (e.g., discharge, wind) datasets within these environments, we can achieve a more holistic picture of the mechanisms driving cHABs in lakes and their watersheds.

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THE IMPACT OF CATION CONCENTRATION ON MICROCYSTIS (CYANOBACTERIA) SCUM FORMATION

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Cyanobacterial scums at the surface of the lakes are potentially harmful phenomena with increasing occurrence in the last decades, and the causes that lead to their formation are still an unresolved issue. In order to better understand what triggers the scums, we investigated the effect of several Mg2+ and Ca2+ ion concentrations in promoting them in eight Microcystis aeruginosa strains. The possibility to prevent scum formation by using the ion chelator EDTA was also explored. We found that in some strains the cell aggregation takes place under lower ion source concentrations (20 mM MgSO4 or CaCl2), while in others this phenomenon does not occur even at 60 mM concentration. The scum formation correlated to the amount of extracellular polymeric substances (between 234 and 351 µg/cell). EDTA failed to prevent the scum formation in most strains, and in turn it caused cell lysis followed by the release of cellular content into the culture medium. We emphasize the relevance of these results for cyanobacterial scum formation in the environment and we also suggest that controlling the salinity of the medium (by manipulating the ion concentration) is a potentially efficient method for biomass harvesting in large ponds/tanks.
ISOLATION AND CHARACTERIZATION OF CYANOPHAGES INFECTING CYANOBACTERIUM MICROCYSTIS AERUGINOSA FROM A TROPICAL RESERVOIR

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Cyanophages are one of the important factors which impact the cyanobacterial community in aquatic ecosystems. In this study, we isolated three lytic cyanophages from a reservoir in Singapore using well assay. Two of the isolates were found to specifically infect a toxic local strain of cyanobacterium, *Microcystis aeruginosa*, and one isolate showed broader host range, which was infectious to two strains of *Microcystis aeruginosa*, and one strain of *Pseudanabaena* sp. Transmission electron microscopy showed two distinctive morphological feature of the cyanophage isolates, including the families of the *Myoviridae* (broad range isolate) and *Podoviridae* (narrow range). The growth of *Microcystis aeruginosa* was monitored using optical density, fluorescent intensity and flow cytometry. Results showed that the latent period of the cyanophage was 7 days, and the host abundance decreased by 99% after 20 days.

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Cyanobacteria from Nostoc genus are a rich source of secondary metabolites with a wide range of activities. Among these compounds there are both, acute toxins and metabolites of potential pharmaceutical application. The aim of our study was to characterize the structure and biological activity of metabolites produced by Nostoc edaphicum CCNP1411 isolated from the Gulf of Gdańsk (southern Baltic Sea). Screening experiments showed that some metabolites inhibit proteases and 20S proteasome activity, they are cytotoxic to cancer cells or/and show antibacterial activity. In CCNP 1411, cyanopeptolins (CPs), nostocyclopeptides (NCPs) and cryptophycins (CRPs) were detected and characterized with tandem mass spectrometry, based on their mass fragmentation spectra.

In each class of the peptides, several new structural variants were identified. Experiments performed with application of isolated peptides indicated which parts of the tested molecules are essential for the activities. Although N. edaphicum CCNP 1411 does not produce any of the known cyanotoxins, cell extract of this cyanobacterium exerts harmful effect on small crustaceans.

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South-East Portugal reservoirs are facing eutrophication problems as many countries in temperate climates. Harmful cyanobacterial blooms, reflecting advanced eutrophication, are spreading globally and threaten the sustainability of freshwater ecosystems. Particularly those intended for supply water treatment. Phytoplankton composition structure and cyanobacteria blooms proliferation may give rise to drinking water problems associated with cyanotoxins presence. Valuable and prompt indicators and alerts are widely required from water managers. Phytoplankton biomass indicators were studied for monthly during the period 2016-2018 in five surface water resource from South-East Portugal used as a treated drinking water source. Methods are described for the analyses of phytoplankton including cyanobacteria and cyanotoxins. Methods reference to the international standards and follow laboratories accreditation processes based on ISO 17025. Despite very high cyanobacteria, densities mostly on summer periods cyanotoxins occurrence was low in all five reservoirs studied. Dominant species responsible for cyanobacteria blooms were mostly *Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii*. The relationship between three descriptor of phytoplankton biomass (chlorophyll a, cell abundance and biovolume) during cyanotoxins presence were investigated. Correlations between biovolume and cell density for Phytoplankton and Cyanobacteria were verified. The relationship between chlorophyll a and biovolume throughout the seasonal cycles were analyzed. Strong seasonality in physic-chemical conditions provides a wide range of conditions for the evaluation of those relationships. During bloom periods good approaches between biovolume and cell counts were found but not for chlorophyll a concentrations.
TOXIC CYANOBACTERIA RESPONSE ON EXTREME WEATHER EVENTS IN LOWLAND DAMMED RIVER

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The consequences of altered weather events on phytoplankton community along dammed lowland Narew River (north-eastern Poland) will be presented. The study was carried out during different weather events in hypertrophic Siemianówka Reservoir and outflowing Narew River in summer-autumn periods 2009-2018. The analysis of samples collected during extreme hydrological conditions, such as drought and flood, allowed us to assess the impact of dam on downstream transport of reservoir phytoplankton at a distance of over 130 km. During all study seasons, the strong dominance of toxic Planktothrix agardhii in the total phytoplankton biomass and the presence of many cyanotoxins in shallow dam was recorded. Despite often similar phytoplankton composition, its total biomass and total concentration of microcystins in the reservoir, the significant differences in these parameters at downstream stations were recorded. The biomass of cyanobacteria, the number, and concentration of cyanotoxins decreased with the increasing distance from the dam. The spatial structure of river phytoplankton was largely determined by hydrological factors such as discharge and water level. The most unfavorable impact of the reservoir on the river was noted during drought, when the lowest water level in Narew River resulted in the highest cyanobacteria biomass and the total concentration of microcystins. The reverse situation was observed during floods due to the strongest dilution of the reservoir phytoplankton in the outflowing river.

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MULTISTAGE CLONING AND HETEROLOGOUS EXPRESSION OF MICROCYSTIN-DEGRADING GENES

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Abstract
The main harm of cyanobacterial blooms is to produce and release of carcinogenic microcystins (MCs), which is a very serious threat to ecosystems and human health. Microbial degradation is one of the most efficient ways to remove the MCs which has stable structure. However, the previous research focused on the screening of degrading strains, rarely analyses the characteristics and mechanisms of degrading genes from molecular level. In this paper, a strain named Sphingopyxis sp.USTB-05 was used as the research object. Based on the information of USTB-05 gene cluster (mlr), the degrading genes were multistage cloned and heterologous expressed. The results showed that the cell-free extract (CE) of recombinant E. coli DH5α containing multistage degradation genes had high activity for biodegrading MCs. At the same time, the molecular structures of degradation products were determined by the methods of high performance liquid chromatography - mass spectrometry (HPLC-MS). This work laid a theoretical foundation for building genetic engineering bacteria to degrade MCs efficiently.

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Cyanobacterial bloom events that produce natural toxins occur across the globe, yet information regarding the occurrence and production dynamics of many cyanopeptides is mostly unknown. Cyanobacteria can produce an incredible diversity of hundreds of cyanopeptides beyond the class of microcystins. Identifying the priority candidates of tentatively abundant, persistent, and toxic cyanopeptides would make a comprehensive risk assessments of bloom events more manageable in the future. This project focused specifically on the co-production dynamics of cyanopeptides from common cyanobacterial species in laboratory cultures and from field samples. We use an optimized HRLC-MS/MS suspect screening method targeting more than 580 compounds including anabaenopeptins, cyanopeptolins, aeruginosins, aerucyclamides, microginins, and microcystins. Our data analysis workflow includes an in-house spectral MS library and enables us to identify also those cyanopeptides with high certainty for which no reference standards are commercially available. With this approach, we determined differences of toxin profiles among common cyanobacterial species and field blooms as well as influences of nutrient conditions and growth phase on the production of toxins. These insights of toxin profiles and production dynamics are critical to better understand which peptides and peptide mixtures are present during cyanobacterial bloom events.
Toxic metabolites of cyanobacteria, which are released through cell-death to water, can negatively impact ecosystem and human health. Besides conventional toxins, some genera can produce an array of oligopeptides classified to microginins, cyanopeptolins, aeruginosins and others. Studies showed that oligopeptides may inhibit key metabolic enzyme, mainly protein phosphatases and proteases. Some cyanopeptoline were found to have more lethal effect on small invertebrates than MCs. In years 2014-2018, production and release of conventional cyanotoxins (microcystin, nodularin and cylindrospermopsin) and oligopeptides (microginin, cyanopeptolins and aeruginosins) have been studied in a eutrophic dam located in Cyprus. We focused on the identification of toxic oligopeptides and conventional cyanotoxins by utilizing tandem mass spectrometry technique at different modes. For their detection and quantification of specific cyanotoxins the analysis was conducted in the MRM mode. The concentrations of the conventional cyanotoxins were lower than the method detection limits. An array of oligopeptides was detected such as microcin SF608 (m/z 609.34), anabaenopeptin F (m/z 851.49), and aeruginosin 602 (m/z 603.35). Some of the detected peptides have not been previously reported in the literature. In the assessment of cyano-bloom effects on the ecosystem, the presence of all these bioactive compounds should be included.
A TOXIC PUZZLE – UNRAVELLING THE RELATIONSHIP BETWEEN ANATOXIN PRODUCTION & STRAIN DOMINANCE IN MICROCOLEUS AUTUMNALIS (PHORMIDIUM AUTUMNALE)

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Microcoleus autumnalis (previously known as Phormidium autumnale) is a benthic, filamentous cyanobacteria genus, which under certain environmental conditions can form thick, cohesive mats over large areas of the benthos. Some Microcoleus species can produce spatially and temporally varying amounts of anatoxins that are harmful to both humans and animals. A M. autumnalis-specific Taq-man probe quantitative PCR (qPCR) assay targeting the anaC gene was developed to quantify the number of copies of the anatoxin gene cluster in a sample. The anaC assay and a cyanobacterial 16S rRNA qPCR were then applied to 122 environmental samples collected from 19 sites on 10 streams in New Zealand. The percentage of toxic cells in the environmental samples ranged from 0 to 30.3%, with significant differences between streams. The anatoxin content in mats had a weak, but significant relationship with the percentage of toxic cells. The development of the assay and the verification of its use on environmental M. autumnalis-dominated mats will enable new insights into anatoxin variability and enhance knowledge on how biotic and abiotic parameters influence anatoxin production and the relative abundance on toxic and nontoxic genotypes.
CULTURE-INDEPENDENT AND DEPENDENT ANALYSIS OF BACTERIA COMMUNITY IN THE PHYCOSPHERE OF CYANOBLOOM-FORMING MICROCYSTIS AERUGINOSA

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Roles of epiphytic bacteria on harmful cyanobacterium Microcystis aeruginosa are largely unknown. Confocal and scanning electron microscopic observation confirmed strong bacterial association on the surface of M. aeruginosa cells. DNA-based analysis of 3-μm pore filtered bacterial community (BC) was conducted using two laboratory-grown M. aeruginosa strains and eight newly collected cyanobloom samples. M. aeruginosa was the most dominant species (66 -100 %) within the phylum cyanobacteria. At the genus level, Rhizobium, Hydrogenophaga and Brevundimonas species were commonly found and Flavobacterium species is only present in all environmental samples (ES). Furthermore, Flavobacterium species was dominant genus in four ES, the other four ES showed different primary genus. Two laboratory strains in BC showed that M. aeruginosa FBC2 was similar to M. aeruginosa KW (95.97 %) but, the predominant genus was significantly different. The number of OTUs suggested that BC in the laboratory grown M. aeruginosa strains has less diverse than BC in ES. Total 396 colonies from various samples were screened to reveal that most culturable bacteria belong to Alpha-proteobacteria (19%) including Rhizobium, Brevundimonas, and Porphyrobacter species. The most prevalent genus recovered from culturable members were Rhizobium, Brevundimonas and Pseudomonas species. Genetic variation of M. aeruginosa strains and environmental conditions could lead to have distinct bacterial population in all tested samples. PCR-analyses targeting microcystin genes (mcyA, mcyB and mcyC) showed genetic diversity among strains of M. aeruginosa. Rhizobium sp. MK23 isolated from one ES appeared to promote the growth of axenic M. aeruginosa NIES-298 under co-culture condition and delay to death day of M. aeruginosa NIES-298 compare to uninoculated M. aeruginosa. Our data suggested that epiphytic bacteria such as Rhizobium species could be beneficial to the growth of M. aeruginosa probably due to bacterial stress protection and nutrient supply.
Toxic cyanobacteria are an ubiquitous phenomenon in pelagic zones of lakes, reservoirs, and in marine environments. Less attention has been paid to toxic benthic cyanobacteria, despite their occurrence in many environments. Benthic cyanobacterial toxins have been demonstrated to result in serious issues with wild and domestic animal health and survival and pose an unconsidered risk to human health. We investigated the toxin producing capability of benthic cyanobacterial biofilms in three small stream catchments in Southern Germany (Ammer, Steinlach and Starzach) and in the European Alps i.e. in Ehrwald (Austria, 2100 m a.s.l.), in the Davos area (Switzerland, 2300-2800m a.s.l.) and Lakes Tambo (Switzerland, near Spluegen pass, 2300 m a.s.l.). Mats from the Tambo Lakes were shown previously to contain toxin-producing cyanobacteria and deemed responsible for cattle deaths [1]. Biofilms were analysed for species diversity and the presence of cyanotoxins. We detected microcystin in 8 of 23 samples (2-7 µg/g dw) from Southern Germany and in 3 of 17 alpine samples (0.1-1µg/g dw). However, background noise in the microcystin-specific ELISA (ADDA) was high, precluding detection of very low levels of toxins. To date, the presence of genes involved in microcystin production (mcyE) could be confirmed in 2 samples from Southern Germany and in 3 alpine samples. Although cyanobacterial toxins are still considered a minor component in benthic freshwater habitats in Germany and the European Alps, they may be a hitherto underestimated risk factor that potentially could become more important with progressing climate change [2].

References:
Although cyanobacteria are commonly associated with eutrophic lakes, they are basic components of phytoplankton communities in lakes which have different trophic status. The aim of the study was to evaluate cyanobacteria and their toxins in five reservoirs and two natural lakes in Küçük Menderes Basin which are varying trophic status. Within this scope samples were collected in May and November 2018. Cyanobacterial species were enumerated according to Utermöhl Methods. Cyanotoxin samples were analysed using the LC-HRMS. In order to find the trophic state of the waterbodies, the trophic state index (TSI) developed by Carlson (1977) was used and Total Phosphorus (TP), Chlorophyll-a (chl-a) and Secchi depth (SD) measurements were performed. There were totally 13 cyanobacteria species including potential cyanotoxin producers e.g. Microcystis, Aphanizomenon and Dolichospermum. According to TSI, three reservoirs found mesotrophic and the other four waterbodies had eutrophic-hypereutrophic conditions. Mesotrophic Tahtalı Reservoir which is used as a source of drinking water has the highest cyanobacterial composition (%67.6 of the total phytoplankton biovolumes) and microcystin concentration (9.09 µg/L) in November. Eutrophic Beydağ-ı Reservoir had Dolichospermum mendotae bloom (%93 of the total phytoplankton biovolumes) in May which was not toxic. The cyanotoxin production in the basin was caused mainly by Microcystis species which were detected highest concentrations in November in three sampling areas. Data shown that Microcystis is still the most common bloom-forming freshwater cyanobacterium in Turkey.
ANALYSIS OF DEEP SEDIMENTS REVEALED A THOUSANDS-YEAR PRESENCE OF TOXIC NODULARIA SPUMIGENA IN THE BALTIC SEA AND NORWEGIAN COASTAL WATERS

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The existing knowledge about the occurrence and species composition of cyanobacterial communities is limited to the last several decades. In the current study, the presence of species-specific chemical and genetic markers in deep sediments (4 m) were analyzed to increase the existing knowledge about the history of toxic cyanobacteria blooms in the Baltic Sea (Gdansk Deep) and Norwegian Fjords (Oslofjorden, Ballsfjorden and Trondhaimsfjorden). As chemical markers, nodularin, microcystins and anabaenopeptins were analyzed. From the same sediment samples, DNA was isolated. 16S rDNA gene, the gene involved in biosynthesis of nodularin and phycocyanin intergenic spacer region (PC-IGS) were amplified. Analysis of samples from the Baltic Sea revealed at least four thousands-year presence of toxic Nodularia spumigena. They also indicated that through all this time, the same two sub-populations of this toxic species co-existed. In the Baltic, the biomass of N. spumigena reached its peak approximately 2000 y BP. Analysis of sediment samples from Oslofjorden indicated that in this region N. spumigena was present 10 000 y ago and its highest biomass was recorded approx. 3000 y BC. In Ballsfjorden, the blooms of N. spumigena probably occurred 1500-2000 years earlier. In general, the peaks of nodularin concentrations were recorded in those sections of the deep sediment cores which were deposited in warmer climate periods. Detection of nodularin in sediments deposited thousand years ago and lack of reports on the presence of N. spumigena in Norwegian Fjords today, indicates significant environmental changes in those ecosystems.

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The aim of this study was to determine the response of planktonic cyanobacteria to environmental factors (water temperature, transparency, conductivity, pH, ammonium nitrogen, nitrate nitrogen, nitrite nitrogen and soluble reactive phosphorus). Relationships between cyanobacteria assemblage and water chemistry variables were analyzed with canonical correspondence analysis (CCA).

Uzarzewskie Lake is a small, hypertrophic lake, located in Western Poland. Its surface area covers 10.6 ha, maximum depth is 7.3 m and mean depth 3.4 m. This dimictic and bradymictic lake has short mixing periods. Since 2006 the lake is under restoration approach [1]. Samples were collected monthly from March to November (2008-2015) at the station located in the middle of the lake, from the surface and the depth of 1 and 2 m.

During the research period, 31 taxa of cyanobacteria were noted. Not all taxa were found in each year of the study period. The most important species in case of biomass were *Aphanizomenon gracile*, *A. flosaquae*, *Cuspidothrix issatschenkoi*, *Dolichospermum planctonicum*, *D. flosaquae*, *D. smithii*, *Limnothrix redekei* *Pseudanabaena acicularis*, *P. limnetica*, *Planktolyngbya limnetica*, *Planktothrix agardhii* and *Woronichinia naegeliana*. Among the 162 analyzed water samples, the highest frequency was found for such species as: *P. agardhii*, *Pseudanabaena limnetica* and *A. gracile*. The taxa correlated with the environmental parameters such as NH$_4$-N, NO$_2$-N, temperature, SRP, conductivity, transparency, NO$_3$-N (p < 0.05).

References:
ARE TOXIGENIC CYANOBACTERIA IN MICROBIAL MATS FROM COLD DESERTS OF EASTERN PAMIR A REAL THREAT?

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Toxins produced by cyanobacteria are known for being a health hazard for people, wildlife and domestic animals. The topic of harmful algae has been lately extensively discussed in view of the increased occurrence and intensity of cyanobacterial blooms caused by eutrophication and climate change. Planktonic species were subjects of numerous studies, while benthic populations and their impact on the water quality, especially in regions with limited water resources, still need elucidation. For this study we examined 30 cyanobacterial mats from cold mountain desert of Eastern Pamirs (Tajikistan) in terms of presence of toxin gene-clusters in mat-forming organisms as well as the occurrence of cyanotoxins. The screening of mats by PCR-based methods revealed the presence of mcyE and ndaF in 11 samples and sxtA in one sample. Mats were also analyzed by HPLC to determine the presence of microcystins, nodularin and anatoxins, but surprisingly so far, we have not identified any of them. Furthermore, the hypervariable V3-V4 region of 16S rRNA gene was sequenced by NGS. Cyanobacteria accounted for between 3 and 73% of total bacterial sequences (averaging 28%). On average 10% of cyanobacterial reads were identified at phylum level. In the samples 10 genera dominated, among which Thermosynechococcus dominated in 10 samples, Arthronema in six, Microcoleus and Nostoc in four. Genera known for toxin production (Nostoc, Nodularia, Anabaena, Dolichospermum and Microcystis) were found in almost every sample. In samples with toxin-coding genes, potentially toxic species were found: Calothrix parietina, Planktothrix agarðhii, Microcoleus vaginatus, Nostoc calcicola, N. muscorum and Trichormus variabilis.

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CHARACTERIZATION OF DRINKING WATER TREATMENT SLUDGE (DWTS) ACCORDING TO THE OCCURRENCE OF ALGAL BLOOM

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The increase in population and water usage required more water resources. As a result, artificial structures such as dams were installed in the water system, which affected the flow of rivers, further exacerbated the eutrophication [1,2]. The eutrophication of rivers that used as raw water has brought processing loads to the local water treatment plant. Algal bloom by eutrophication is not just a load on the water treatment process. NOMs, which are metabolites of algae or aquatic microorganisms, and microcystin increase the amount of coagulant and sludge [3]. Previous research on water sludge focused on the possibility of recycling coagulants contained in the sludge, but studies on sludge containing algae and toxic substances by the algal bloom were scarce. Therefore, in this study, we compare the water treatment sludge in the areas where algae are generated with regular water treatment sludge to provide a basis for identifying the differences and presenting alternatives for treatment.

Sludge from the water treatment plant where the algae generated area and sludge from the regular area were dried at 105°C for analysis. Elemental analysis, XRF, and FT-IR were used as analysis equipment. Sludge from algal bloom occurrences region contained 1.4 times higher organic content than the regular region. It also showed higher elution rate of microcystin that produced by cyanobacteria. Water treatment sludge contains microcystin and organic materials may have adverse effects on the surrounding environment when landilled. Therefore, a new alternative is required considering microcystin and organic matters contained water treatment sludge from algal bloom occurrence region.

References:

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Lac au Duc (France) suffers each summer from massive blooms of cyanobacteria, increasing the cost of water purification for potable water and inducing the closure of the bathing zone leading to economical losses. Reducing cyanobacterial dominances requires a long-term reduction of the nutrient load and input, and can be accomplished by acute treatments. These treatments need a thorough analysis of the eutrophication scenario, to meet both efficiency and environmental safety demands. Existing data (2005-2018) were analysed to characterize cyanobacterial dynamics. The cyanobacterial community varied strongly inter-annually. While Plankthothrix was the most abundant genus most years (9 of the 14 monitored years), Microcystis and Anabaena were sometimes dominating and producing toxins. The strength of the wind seems to be stronger than the temperature in explaining these inter-annual variations. Curative treatments with hydrogen peroxide were applied in laboratory experiments and in a delimited area of the lake during summer 2018, and the phytoplanktonic and zooplanktonic communities evaluated. While under laboratory conditions 2.5 mg H$_2$O$_2$/L were sufficient to decrease cyanobacteria abundance for 10 days, in the field the threshold beyond which bathing is prohibited (100000 cells/ml in France) was not permanently achieved. Stronger and more frequent treatments were tested. In the zooplankton community, rotifers and bosmina abundances were strongly reduced, but recovered within a few days. Zooplankton response will be investigated in depht and the treatment adapted to the Lac au Duc specificities.

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The accumulation of cyanobacterial biomass can severely affect food web processes and is often associated with hazards to human health and livestock and reduced recreational quality of water bodies. Studying the role of cyanobacterial carbon within food webs appears crucial for assessing the consequences of cyanobacterial mass developments for ecosystem processes. Cyanobacteria represent nutritionally inadequate food sources for aquatic consumers, because of (1) morphological properties hampering ingestion, i.e. the formation of filaments or colonies, (2) the production of a wide array of harmful secondary metabolites, and/or (3) a deficiency in essential biochemical nutrients, such as sterols and long-chain polyunsaturated fatty acids (PUFA). Disentangling the relative significance of these multiple nutritional constraints may improve our understanding of top-down influences on cyanobacterial bloom formation. This talk aims at highlighting the complexity of food quality constraints that are associated with consuming cyanobacteria.
MOLECULAR SCREENING OF THE POTENTIAL PRODUCTION OF CYANOTOXINS AND CYANOBACTERIAL NATURAL PRODUCTS IN ENVIRONMENTAL SAMPLES FROM CABO VERDE ISLANDS

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The Cabo Verde archipelago is located in the central Atlantic Ocean in the Sahel region of Africa. It is comprised by diverse volcanic islands, formed as a result of eruptions from a hotspot under a submarine platform. Cabo Verde islands represent underexplored habitats in what concerns cyanobacteria and their natural products, including toxins. With the aim of determining the presence of cyanotoxin genes and explore the hidden bioactive potential of marine cyanobacteria from these islands, we have conducted sampling campaigns at different sites in São Vicente (Baía das Gatas, Cova de Inglesa, Calhau and Salamansa) and Santo Antão (Ponta do Sol) islands, during April 2018. A PCR-based screening was performed in the collected environmental samples to evaluate the presence of (1) cyanobacterial and cyanotoxin genes; and (2) genes encoding non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKSs). All the samples contained cyanobacterial DNA whereas cyanotoxin genes related with microcystins (MC)/nodularins (NOD), cylindrospermopsins (CYL), saxitoxins (SXT) and anatoxin (ATX) production were spread by several samples collected in São Vicente Island. The one sample collected in Santo Antão Island exclusively revealed the presence of CYL related genes. Both PKS and NRPS genes were detected in several samples from São Vicente island whereas for the sample collected in Santo Antão island we could not confirm the presence of these genes. The results of this study demonstrate the potential risks of the appearance of cyanotoxins in the Cabo Verde coastal marine areas as well as the potential for the discovery of novel bioactive compounds.

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In July 2018 three dogs - an Australian Shepherd, a Papillion and a Shih Tzu - died after visiting the water’s edge at two different locations on the St. John River near Fredericton, New Brunswick, Canada. All showed signs of toxicosis following exposure, and necropsies on the Australian Shepherd and Shih Tzu revealed non-specific pulmonary edema and multiple microscopic brain hemorrhages. Studies were conducted to establish the role of toxic freshwater algae. Samples analysed included the vomitus of one dog, stomach contents of two dogs, and water and biota from the mortality sites. Targeted and untargeted analysis by liquid chromatography high-resolution mass spectrometry (LC-HRMS) confirmed the presence of anatoxins in all samples. The highest levels were measured in a dried algal mat that two of the dogs had consumed before falling ill, with concentrations of ~330 and ~980 mg/kg for anatoxin-a and dihydroanatoxin-a, respectively. LC-HRMS also showed a rich profile of additional known analogues and previously un-reported ATX conjugates. Cyanobacteria, including species of the genera *Phormidium*, were identified as the causative organism(s) using light microscopy and molecular methods. PCR-based rRNA gene sequencing and subsequent phylogenetic analysis revealed a diverse cyanobacterial community in the benthic algal mats responsible for the poisonings.

This event highlights the need for increased awareness of risks posed by cyanobacteria in Atlantic Canadian freshwater systems. Future efforts will focus on understanding the environmental and physico-chemical factors driving regional toxic cyanobacterial events and implementing a broader range of tools for monitoring based on genetic and chemical analytical techniques.
SUCCESSFUL ISOLATION AND CULTIVATION OF CYLINDROSPERMOSIS RACIBORSKII STRAINS ISOLATED FROM FINISHED DRINKING WATER SAMPLES

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This work presents the successful establishment of Cylindrospermosis raciborskii cultures isolated from water samples collected at the exit point of Water Treatment Plant (WTP). An intense bloom dominated by filamentous cyanobacteria (Aphanizomenon spp., Planktothrix spp., Cylindrospermosis raciborskii, Anabaena spp.) occurred in the summer of 2015 in Roxo reservoir (south Portugal). Several cyanotoxins (microcystins, saxitoxins, cylindrospermopsin) were detected in raw and treated water, although at levels below the corresponding regulatory and/or guideline values. Nevertheless, this bloom caused intense unpleasant odour and taste in the water supplied to the populations and cyanobacterial cells (up to 1000 cells.mL⁻¹) were detected in finished water samples collected at the exit point of WTP. Treated water samples collected at the WTP and at the city water deposit were inoculated in Z8 culture medium and cyanobacterial growth was followed by optical microscopy. After 30 days, cyanobacterial growth was observed showing resistance to the treatment processes and maintenance of reproduction capacity. Interestingly, morphometric and molecular analysis revealed the presence of C. raciborskii. Three isolates of this species were obtained and none were cylindrospermopsin- or microcystins-producers, as confirmed by Enzyme Linked Immunosorbent Assays (ELISA) and by amplification of genes (PS, PKS, mcyA-cd, mcyAB, mcyB) involved in those cyanotoxin synthesis. However, the ELISA for saxitoxins was positive for the 3 isolates and confirmation of this toxin production is in progress. To our knowledge, this is the first report on the establishment of successful cultures of C. raciborskii that survived to conventional water treatment processes.

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The phytoplankton structure in shallow and hypertrophic Lake Łeknenskie is permanently influenced and dominated by *Planktothrix agardhii* during the whole year, in addition to a variety of environmental conditions. Our studies were concerned on defining temporal and spatial variation of the community structure and species richness of phytoplankton during toxic *P. agardhii* blooms in Lake Łeknenskie in hot years. Its ability to produce microcystins was confirmed by HPLC and MALDI-TOF MS analysis. Samples were collected every month during the whole years of 2015 and 2018 in vertical profile (0-3m). Photon irradiance was also measured in our study. Taking into account the temperature gradient, *P. agardhii* proliferated at the shallow depths characterized by higher temperatures, low light availability, and higher concentrations of nutrients with a high oxygen concentration. It often formed thick surface layers in the shallow parts of the lake during summer and autumn, and measurable quantities of microcystins were detected in the water in the range of >5.0 µg l⁻¹ for samples collected in June and >15.0 µg l⁻¹ for samples collected in July-September. Our research provides better understanding of *P. agardhii* ecology and physiology in the wider aspect, inter alia of its ability for toxin production in this shallow, hypertrophic lake. Its tolerance of lower temperatures may allow it to survive during winter months and low-light tolerance can support its growth and proliferation despite heavy self-shading in Lake Łeknenskie.

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DETECTION OF SAXITOXIN-PRODUCING CYANOBACTERIA IN SUBTROPICAL BRAZILIAN DRINKING WATER SUPPLY RESERVOIR BY QUANTITATIVE PCR

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Toxic cyanobacteria in public water supply reservoirs represent a serious health risk since they can release potent cyanotoxins into the water. Among the cyanotoxins are saxitoxin (SXT) and its homologs that form a group of potent neurotoxins called paralytic shellfish toxins. Few studies have focused on occurrence of saxitoxin and the genes involved in the biosynthesis in oligotrophic freshwaters. The aim of this study was to infer the potential toxigenicity of water from the SXT gene (sxtA) copy number in the oligotrophic Itupararanga reservoir, that supplies potable water for approximately 800,000 people in São Paulo State (Brazil). A quantitative PCR (qPCR) approach was successfully developed to quantify the number of SXT-producing cyanobacteria from the sxtA copy number. The sxtA gene varied from $6.76 \times 10^3$ to $7.33 \times 10^5$ copies mL$^{-1}$ and was related to SXT concentrations (detected by ELISA) and the total density of SXT-producing order Nostocales (Cylindrospermopsis raciborskii, Aphanizomenon sp. and Dolichospermum sp.) in Itupararanga reservoir during four samplings over a year. Although the SXT concentrations were generally low (max. 0.20 µg L$^{-1}$), they correlated positively with the sxtA copy number and with the TN:TP ratio, but a negative correlation was found between number of the sxtA gene and nitrate, total P and ortho-P. This confirms the importance of local environmental variables in the overall regulation of saxitoxin production. The qPCR method was sensitive and specific for quantification of saxitoxin gene and should be considered in future monitoring of cyanobacteria and their toxin production.

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Knowing the exact environmental factors that favor toxic cyanobacteria (CB) blooms in freshwater ecosystems is of global interest. In the past decades, Danube Delta lakes experienced such blooms but the factors that favor or precede this phenomenon have not been defined in detail. Therefore, a broad range of investigations of in situ abiotic factors was conducted in 19 lakes in order to establish possible relationships with CB, especially with toxic taxa that could evolve into blooms anytime. This study targeted to ascertain which of the abiotic parameters could represent a key factor that promotes CB presence and dominance, define the response (positive or negative) of each CB genera to these drivers and highlight if nitrogen forms influenced differently the CB. The selected environmental variables had a weakly explanatory power in our case (14.5%), but were statistically significant (p = 0.001). Was clarified that to pH, light, oxygen, conductivity, nitrate and ammonium at least in situ, the CB genera response is individually, also a positive and negative response was displayed. The results revealed also that the environmental predictors are three time more influential on CB community structure comparative with the spatial distribution of the lakes.

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Pampean shallow lakes (Argentina) are affected by blooms of toxic cyanobacteria (CyanoHABs) since the middle of the last century. Although these shallow and typically eutrophic systems are habitats that favor CyanoHABs development, the recent and strong increase in nutrient concentration and the high frequency of extreme drought events (associated with the intensification of agriculture and climate change respectively), have accelerated this problem. The environmental determinants that affect the success of the different species differ according to the morpho-functional features conferring fitness for the acquisition of nutrients and light, among other factors. The objective of this work is to temporarily characterize CyanoHABs in shallow lakes with different limnological characteristics. In the framework of the PAMPA network project (CONICET), monthly monitoring of nine lakes located along a humidity gradient in the Pampa Region was performed between October 2012 and December 2015. The structure of the assemblages of bloom forming planktonic cyanobacteria was analyzed considering both species and eco-strategies abundance and biovolume. Our results indicate that the composition and intensity of the blooms are more likely to be affected by the conditions of nutrients, light and macrophyte development than by the location of the lake in the geographical gradient. The composition and abundance varied annually in close relationship with the water regime. Total microcystin concentration was analyzed in warm periods for six lakes; the toxin was found in all systems, generally at levels below 1 μg / L (WHO guide level); inter-annual differences were found. Morphological variations were analyzed in the Salada de Monasterio Lake, observing relationships between filament length and turbidity that affected the total biomass.
BIOTECHNOLOGICAL POTENTIAL OF SECONDARY METABOLITES PRODUCED BY CYANOBACTERIA FROM CURONIAN LAGOON

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The Curonian Lagoon is the largest and one of the most severely impacted by harmful cyanobacteria blooms in Europe. In summer, cyanobacterial biomass reaches over 100 mg/l [1,2] and is dominated by Aphanizomenon flosaquae, Planktothrix agardhii, Microcystis and Dolichospermum spp. [3]. The goal of this study was to examine the activity of metabolites produced by cyanobacteria from the Curonian Lagoon. Bloom samples collected in 2018 over the season differed in species composition and cyanobacterial biomass. The extracts prepared in 75% methanol were preliminary fractionated, and the obtained material was tested using enzymatic, antibacterial and cytotoxicity assays. The content of the samples was determined using LC-MS/MS. All tested samples inhibited the activity of trypsin and thrombin (mean relative inhibition of 81.5%), however, the strongest activity was observed in samples dominated by Aph. flosaquae. In antibacterial assays, samples dominated by Dolichospermum and Microcystis showed strong (>70%) inhibition of Staphylococcus aureus, Enterococcus faecium and Pseudomonas aeruginosa antibiotic resistant strains. Cytotoxic effects against human breast adenocarcinoma cell line were also observed. LC-MS/MS analysis of active fractions revealed presence of several classes of cyanopeptides, including aeruginosamids, microginins, anabaenopeptins and cyanopeptolins. Preliminary studies indicated that apart from the known toxins, cyanobacteria from Curonian Lagoon produce many bioactive metabolites of potential pharmacological application.

References:

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**SUCCESSION OF MICROCYSTIS GENOTYPES ACCOMPANIES DIFFERENT MICROBIAL MODULES WITH RECURRENT PATTERN**

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*Microcystis*, major bloom forming cyanobacterium, interact with variety of microbes and their association networks have been revealed by recent studies, yet the ecological relationships between genotypes of *Microcystis* and microbes are largely unknown. Here, we investigated the dynamics of cyanobacteria, including genotypes of *Microcystis*, heterotrophic bacteria, and eukaryotes to elucidate the ecological connections among primary producers, consumers, and decomposers during cyanobacterial harmful algal blooms (cyanoHABs). Network analysis revealed that the overall transition patterns and the compositions of modules (microbial clusters) that involved in the same phase of cyanoHABs showed resemblance in three different sites. Distinct clusters of *Microcystis* genotypes and microbes were observed in different types of *Microcystis* bloom, suggesting the alteration of *Microcystis* genotypes could mediate by their specific companions as well as environmental factors. Most of *Microcystis*-related microbes (16S rRNA based) were also directly linked to different genotypes of *Microcystis*. In addition, hidden members of bloom modules were also tightly coupled with genotypes of *Microcystis* and may support communities function profiles during *Microcystis* bloom periods. Therefore, to understand complex ecological interactions during *Microcystis* blooms, we should consider their interactions through a network and modular structures based on the *Microcystis* genotypes. Overall, the distinct modular structures and *Microcystis* genotype based network offer new insight into the dynamic of cyanoHABs.

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MOLECULAR DETECTION OF CYANOBACTERIA IN THE DIET COMPOSITION OF CRUSTACEAN ZOOPLANKTON: A MULTIPROXY STUDY

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The coexistence of bloom-forming cyanobacteria and zooplankton communities has raised a crucial question about the potential role of zooplankton in suppressing harmful algal blooms. We used a combination of different techniques (high-performance liquid chromatography-HPLC, molecular and stable isotope analyses) to study the crustacean zooplankton feeding and selection for phytoplankton taxa in Lake Peipsi (North-East Europe, Estonia). Our special focus was on potentially toxic *Microcystis*, one of the main bloom-forming cyanobacteria in the lake. To assess the general algal diet composition of crustaceans we used HPLC, to identify and quantify the prey of interest, we used genus-specific quantitative polymerase chain reaction (qPCR). To explain the dominant zooplankton and cyanobacterial interactions in food web of Lake Peipsi, stable isotope analysis was used.

Our study showed that colonial cyanobacteria formed a significant part of the diet of cladocerans in Lake Peipsi and genus-specific qPCR, targeting *mcyE* genes showed that potentially toxic *Microcystis* was ingested by all dominant crustacean zooplankton taxa.

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MOLECULAR EVALUATION OF THE OCCURRENCE OF INVASIVE CYANOBACTERIA AND THEIR TOXIN PRODUCTION POTENTIAL IN THE NAKDONG RIVER, KOREA

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Harmful cyanobacterial blooms (cyanoHAB) are one of the largest global issues regarding the water quality of fresh waters. It has been predicted that the amount and duration of cyanoHAB will increase along with recent climate changes. Moreover, global warming is associated with the apparent spread of some nostocalean cyanobacteria from tropical to temperate latitudes including northern Europe, North America and Asia. The Nakdong River is the second largest river in Korea, serving as the agricultural, industrial water source, and the drinking water source for the 13 million people. After the construction of the eight weirs along the river in 2012, there have been cyanoHAB formation every summer. In addition, cyanoHAB were recently observed to persist even during the winter, and the nostocalean cyanobacteria, *Aphanizomenon* spp., were the most dominant. This expansion of amount and duration of cyanobacterial blooms may indicate the possibility of so-called invasive nostocalean cyanobacteria occurrence in the Nakdong River. To confirm the occurrence and distribution of *Cylindrospermopsis raciborskii* and other nostocalean cyanobacteria in the Nakdong River, weekly monitoring of surface water have performed for morphological identification, along with the isolation of *Cuspidothrix issatschenkoi* and *Sphaerospermopsis aphanizomenoides* strains. The isolates were conducted molecular examination for the phylogenetic analysis and cyanotoxins production potential. *C. raciborskii*, *C. issatschenkoi* and *S. aphanizomenoides* have found in low densities in the Nakdong River chiefly in summer. *S. aphanizomenoides* isolates failed to yield PCR products using primers of main cyanotoxins biosynthesis genes including *mcyA*, *mcyB*, *cyrA*, *cyrJ*, *sxtA* and *sxt1*. 
PERENNIAL DROUGHTS AND BLOOMS – CYANOBACTERIAL TREATMENT
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The tropical semi-arid region presents with a unique set of climatic, environmental, and socio-economic conditions that pose a challenge to potable water treatment. The semi-arid region of Brazil, where annual evaporation (in excess of 1500 mm) is greater than annual precipitation, experiences cycles of years-long “green” droughts that lead to water scarcity. Over 50% of potable water reservoirs in the state of Ceará, Brazil, are either completely dry or critically low (< 10% capacity). This fact is compounded by perennial cyanobacterial blooms in hypereutrophic, multiple-use potable water reservoirs. Historic management of the water courses in the region and ad-hoc construction of numerous, small, direct filtration water treatment plants that do not allow for retro-fitting with updated treatment technologies exacerbate the situation. The conventional thinking of blooms occurring over the summer period and then collapsing cannot be applied here. Frequently in excess of 15 different cyanobacterial genera can be observed in a single water body, many of which have been reported to contain toxicogenic strains. Often blooms are not dominated by a single species, but by a consortium of several species. Extensive screening of over 100 reservoirs demonstrated that toxicogenic cyanobacterial strains are present in over half of the reservoirs. The most commonly encountered cyanobacterial toxins were microcystins, saxitoxins, and cylindrospermopsins, either singly or in combination. Other cyanobacterial secondary metabolites were encountered as well (viz a prolonged off-flavour episode in the state’s capital Fortaleza). New approaches have to be taken when conducting investigations in this environment abandoning conventional wisdom.

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PHYTOPLANKTON COMMUNITY SHIFTS AFTER SELECTIVE REMOVAL OF CYANOBACTERIA BY HYDROGEN PEROXIDE TREATMENTS OF LAKES

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Preventing toxic cyanobacterial blooms by reducing internal and external nutrient loads is a preferable method in lakes and reservoirs that cope with these blooms, yet it is usually a long-term process. Methods that work effectively against cyanobacteria on a short term are needed to tackle toxic blooms in acute situations. The major challenge is the selective killing of cyanobacteria while keeping unwanted side effects on non-target organisms to a minimum. Hydrogen peroxide (H₂O₂), a compound that degrades to water and oxygen without leaving chemical traces in the environment, has been shown to be a highly effective and selective cyanocide when applied in low concentrations. To obtain a better understanding of the changes that occur in various ecosystems as a result of a H₂O₂-application, we treated several lakes in The Netherlands with low concentrations of H₂O₂ (2-5 mg/l). The lakes varied in size and depth and were dominated by different cyanobacteria, e.g. Aphanizomenon s. l., Dolichospermum spp., Planktothrix agardhii and Planktothrix rubescens. The blooming cyanobacterial species collapsed almost instantly after a H₂O₂-treatment followed by an increase in dissolved nutrients and light availability which enabled recolonization of either eukaryotic algae or cyanobacteria in the subsequent weeks. We conclude that a treatment with H₂O₂ can be a rapid, highly effective and low-cost method to combat cyanobacterial blooms, but the duration of the post-treatment period with no or low numbers of cyanobacteria varies among lakes.
ACUTE EFFECTS ON DANIO RERIO EXPOSED TO EXTRACTS OF SPHAEROSPERMOPSIS TORQUES-REGINAE (CYANOBACTERIA) (ITEP-24) PRODUCING ANATOXIN-A(S)

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Anatoxin-a(s) [atx-a(s)] is a neurotoxin produced by some species of cyanobacteria, mainly of the genus Anabaena and Sphaerospermopsis. This neurotoxin is known as the only natural organophosphate capable to inhibit acetylcholinesterase. Little information is available on the agonist and cholinergic effects of atx-a(s) on aquatic organisms, especially for the fish group. The aim of this study is to obtain a semi-purified atx-a(s) fraction of S. torques-reginae (strain ITEP-24) and investigate the adverse effects of this toxin as well as other components present in extracts of this cyanobacterium on Danio rerio. For this, LD50 experiments, histopathological and morphological analyses were performed. ITEP-24 strain has been cultivated in the laboratory under controlled conditions, aiming to obtain biomass for the semi-isolation experiments. Six extraction protocols were tested using lyophilized cells from the ITEP-24 strain with different solvents, to evaluate which was better to extract atx-a(s). Samples were analysed by liquid chromatography coupled to the mass spectrometer (LC/MS-QqQ) with ZIC-HILIC chromatographic column. From the extraction protocols tested, the combination of methanol/water (20:80 v/v) in 0.1M acetic acid was better to extract the antx-a(s) from the crude ITEP-24 extract. The same samples for the extraction protocols with crude extract and fresh culture were also analysed in the LC/MS Q-TOF with C18 column, which allowed the identification of other compounds besides the atx-a(s) itself, such as Namalides A and B, Spumigin K, Shinorine (an amino acid of the mycosporine type). After the selection of the extraction and identification method of the atx-a(s), Solid Phase Extraction (SPE) techniques were optimized using reverse-phase and weak polymer-cation exchange (StrataTMX-CW) isolation of the antx-a(s). The use of the StrataTM-X-CW column allowed the elimination of several interferences present in the crude extract of the ITEP-24 strain, at the end, fractions enriched with atx-a(s) were obtained. As there is no commercial standard of atx-a(s), the techniques adopted in the present study can contribute substantially to the identification and the semi-isolation of atxa(s) in water bodies in greater quantity for analytical and toxicological studies. The acute exposure tests were conducted with Danio rerio embryos and larvae at concentrations below 1 mg/mL. The results showed LD50 rate of embryos and larvae in concentrations higher than 0.08 mg/mL and 0.31 mg/mL, respectively, for 96 hours of exposure. Other in vivo exposure tests were performed throughout this study, and aimed to assess not only mortality, but other sub-chronic and chronic effects during the life cycle of this species.

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MICROCYSTIS CHEMOTYPE DIVERSITY IN THE ALIMENTARY TRACT OF BIGHEADED CARP

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Most of the cyanobacterial organisms included in the genus Microcystis can produce a wide repertoire of secondary metabolites. Spreading of cyanobacteria by inert and animate vectors has for long been a focus of interest. In the mid-2010s summer cyanobacterial blooms of Microcystis sp. occurred regularly in Lake Balaton. In this period, we investigated how the alimentary tract of filter-feeding bigheaded carps can deliver and distribute different chemotypes of cyanobacteria with specific peptide patterns. Twenty-five Microcystis strains were isolated from pelagic plankton samples (14 samples) and hindguts of bigheaded carps (11 samples) and three bloom samples were collected from scums of cyanobacterial blooms. An LC-MS/MS based untargeted approach was used for analyzing the peptide patterns; 36 anabaenopeptin, 17 microgigin and 13 microcystin variants were identified. Heat map clustering visualization was used for the comparison of the identified chemotypes. Lack of separation was observed in the case of Microcystis peptide patterns originated from hindguts, water samples and bloom-samples. Except for 13 peptides, all other congeners were detected from the viable and cultivated chemotypes from bigheaded carps. This finding suggests that the alimentary tract of bigheaded carps is not simply an extreme habitat but may also supply the cyanobacterial strains that represent the pelagic chemotypes.
PALEOLIMNOLOGICAL STUDY ON CYANOBACTERIAL COMMUNITY SHIFTS IN SMALL BAVARIAN LAKES (OSTERSEE LAKE DISTRICT) USING SEDIMENTARY DNA

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Nutrient loading and the effects of climate change have been proposed to decrease cyanobacterial diversity between lakes and favour potentially toxic species. In our study, we used ancient sedimentary DNA to detect past cyanobacterial community shifts in small hardwater lakes, in order to establish mechanistic relationships that disentangle the importance of different drivers in relation to their ecological response. One half of sediment cores from small eutrophic lakes served for isotope dating and the other half was used for performing molecular analyses. To reveal the composition of cyanobacterial communities, Illumina MiSeq sequencing of the 16S rRNA gene was performed. Moreover, real-time quantitative PCR using primers specific for potentially toxic genera and cyanotoxins was carried out. The degradation of DNA was determined by assessing the minimum fragment length by applying quantitative PCR using three cyanobacteria-specific primers of different length. An optimized method for DNA sampling and extraction from sediment cores retrieved from small eutrophic lakes was developed. The minimum length of degraded DNA fragments was determined and taken into account for sequencing and PCR. Specific potentially toxic genera, as well as cyanotoxin genes could be detected. This first results show the potential of the molecular paleolimnological approach presented here, to recover historic occurrences of potentially toxic cyanobacteria and shifts in cyanobacteria communities. To assess importance of driving factors like nutrient loading and climate change, additional proxies must be included.

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PREPARATION AND APPLICATION OF CERTIFIED REFERENCE MATERIALS FOR MICROCYSTIN AND CYLINDROSPERMOPsin ANALYSES (15N-LABELED CYANOBACTERIA)

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Cyanobacterial water blooms are often occurred on eutrophicated freshwaters around the world. For the global warming, it is predicted the elongation and broadening of bloom forming are occurred. Cyanobacteria produce many kinds of toxic compounds, cyanotoxin. Microcystin and cylindrospermopsin, the most abundant known cyanotoxin, are recognized as hazardous compounds for human health. To monitor these cyanotoxin in drinking water and/or eutrophicated water bodies, LC-MS/MS has been used because of the selectivity and sensitivity. In general, suppression and/or enhancement of ionization are often occurred as the serious problem by LC-MS/MS analyses. In order to solve the problem, stable isotope-labeled compounds are very useful. We have reported that ¹⁵N-labeled microcystin¹ and cylindrospermopsin² have been prepared and used for the accurate analyses of cyanotoxin. Also, K. Kittler et al³ have shown the availability of ¹⁵N-labeled cylindrospermopsin in the field of biosynthesis research.

To distribute the ¹⁵N-labeled microcystins and cylindrospermopsins, we have prepared the certified reference materials (CRM) of Microcystis and Cylindrospermopsis (Raphidiopsis) cells, which were cultured in ¹⁵N-NaNO₃ containing medium. The CRM of Microcystis cells contain uniform-¹⁵N-labeled microcystins, such as microcystin-RR, YR, LR, WR, FR, dmLR, etc. The CRM of Cylindrospermopsis cells contain ¹⁵Ns-cylindrospermopsin and ¹⁵Ns-deoxycylindrospermopsin. The extract solutions of these CRM can be directly used as stable isotope-labeled internal standards (surrogates) solutions for LC-MS/MS analyses. After determination of the concentrations of ¹⁵N-labeled cyanotoxin, these ¹⁵N-labeled cyanotoxin can be used for isotope-dilution mass spectroscopy analysis. These CRM of ¹⁵N-labeled cyanobacterial cells are very useful for the accurate analyses of microcystins and cylindrospermopsins.

References:
SPATIAL AND TEMPORAL VARIATION IN PARALYTIC SHELLFISH TOXIN PRODUCTION BY BENTHIC MICROSEIRA (LYNGBYA) WOLLEI IN A FRESHWATER NEW YORK LAKE

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Butterfield Lake is a mesotrophic lake in New York State where residents and pets have experienced unexplained health issues. Microseira wollei (basionym Lyngbya wollei) was found at two of 15 sites in Butterfield Lake and analyzed for microcystins, anatoxins, cylindrospermopsins, and paralytic shellfish poisoning toxins (PSTs). Only PSTs and trace levels of anatoxin-a were detected in these samples. This is the first published report of PSTs within a New York State lake. To evaluate the environmental and temporal drivers leading to the observed toxicity, PST content at the two sites was examined in detail. There were distinct differences in the total PST content, filament nutrient, filament chlorophyll, and relationship to environmental drivers between the sites, as well as distinct differences in the total PST content measured using different analytical techniques. A multivariate model containing site, temperature, and filament chlorophyll explained 85% of the variation in PSTs observed over the growing season. This work emphasizes the importance of proper site selection and choice of analytical technique in the development of monitoring programs to protect lake users from the occurrence of benthic cyanobacteria toxins.
ANTIALGAL ASSESSMENT OF DIFFERENT PHENOTYPES OF PICOCYANOBACTERIA STRAINS FROM THE GENUS SYNECHOCOCCUS

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The contribution of picocyanobacteria to summer phytoplankton blooms, accompanied by an ecological crisis is a new phenomenon in Europe. The factors underlying the blooms dynamic are of a great importance in improving the understanding of the ecosystem functioning. In the first section of this study, the allelopathic effect of three Baltic strains of picocyanobacteria Synechococcus sp. BA-120 (red strain), BA-124 (green strain) and BA-132 (brown strain) on co-existing cyanobacteria, green algae and diatoms was investigated. The allelopathic effect was examined with the aid of several parameters analysis. These parameters were: growth, the maximum quantum yield of PSII photochemistry ($F_v/F_m$) and chlorophyll a and carotenoids content. The results of this section demonstrated that three Synechococcus sp. strains revealed allelopathic effect on all mentioned cyanobacteria and microalgae species. In the second section of the study, the most favorable and unfavorable environmental conditions for picocyanobacteria growth were examined. For this to be achieved, the ecological responses of three strains of Baltic Synechococcus sp. to various environmental conditions were studied. These conditions were: temperature from 10 by 5 to 25°C, salinity from 3 by 5 to 18 PSU and Photosynthetically Active Radiation (PAR) from 10 by 90 to 280 µmol photons m⁻² s⁻¹. Results pointed to differences in the ecophysiology between strains. Summarizing, the results of the experiment improved the understanding of allelopathic interactions between picocyanobacteria and other co-existing phytoplankton species in the Baltic Sea as well as provided for the detail characteristics of the Baltic picocyanobacteria ecophysiology under changing environment.
Extracellular polysaccharide (EPS) significantly contributes to colony formation in *Microcystis*. EPS was divided into soluble and bound types. The bilayer structure of bound EPS contained loosely or tightly bound EPS (LB-EPS or TB-EPS), whose roles in shaping the size and tightness of *Microcystis* colonies deserved further investigation. In this study, *Microcystis* colonies was investigated after two types of pre-treatment to obtain LB-EPS retaining or stripped samples. Results showed cells with LB-EPS formed large and loose colonies. The ratios of LB-EPS to TB-EPS in the retaining groups were higher than those in the stripped groups. This study provides new insights into *Microcystis* colonies formation and enlargement, which contributes to better understanding of the bilayer structure of bound EPS. Moreover, EPS is regarded as good sources for bioflocculants preparation. This study also provides valuable information for *Microcystis* collection, separation or removal.

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As primary producers of biomass, cyanobacteria are a major part of the phytoplankton of each water basin. In recent years, they have been often reported as a dominant group in Bulgarian ponds, together with representatives of Chlorophyta and Bacillariophyta. Due to the cyanobacterial blooms and cyanotoxins produced, these organisms are recognized as a threat and ecological risk for the water bodies. Released cyanotoxins may cause death of birds and fish. Lake Burgas is the largest natural lake in Bulgaria. It is located on the Via Pontica migration route of the birds from Europe to Africa. The lake is an important ornithological station for the wintering of a significant number of moisture-loving birds. Out of the 245 registered bird species 71 are included in the Red Data Book of Bulgaria, 105 are of European conservation significance and 9 are protected globally. Since 1997, the western part of the lake is a protected area. The performed study on the phytoplankton composition during the summer months of 2018 in the east and west part of the Lake Burgas determined presence of seven groups (Cyanobacteria, Chlorophyta, Xanthophyta, Bacillariophyta, Euglenophyta, Dinophyta and Cryptophyta), among which Cyanobacteria (74%) were dominant. Twenty cyanobacterial species belonging to 16 genera have been identified. Some of them have been reported as producers of cyanotoxins - *Dolichospermum flos-aquae, Microcystis aeruginosa, Microcystis wesenbergii, Planktothrix agardhii, Raphidiopsis raciborskii*. The toxin producing cyanobacterium *Planktothrix agardhii* was observed in blooming concentrations (with biomass 24.89 mg.L\(^{-1}\)). The measured biomass exceeded twice the WHO-accepted amount as a moderate threshold of risk (10 mg.L\(^{-1}\)). ELISA assays for cyanotoxins showed presence of microcystins (0.3 ppb) and cylindrospermopsin (0.05 ppb). Our data are an indication of warning and demonstrate the need of continuing the observations regarding the cyanobacterial composition, blooming and presence of cyanotoxins in Burgas Lake.

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LAKE LUDOŠ – AN AQUATIC ECOSYSTEM WITH A (CYANOBACTERIAL) PROBLEM

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Lake Ludoš is one of the many aquatic ecosystems in Serbia where cyanobacteria are present and blooming. What sets this ecosystem apart from many others, is that it is known for practically continuous blooming for almost 50 years even though it represents a wetland area of international significance. Previous research has questioned whether protection of this natural habitat in a bad ecological state is justified, since the (cyanobacterial) problem, which can potentially affect every living being in the proximity of this ecosystem, is also preserved. Furthermore, spreading of potentially toxic cyanobacteria by waterbirds and other mechanisms has also been suggested [1]. Most recent investigation regarding the presence of cyanobacteria and cyanotoxins in the Lake Ludoš during 2018 has confirmed many of the previous findings: a) poor ecological state of the lake (e.g. high pH levels); b) presence of potentially toxic (genera Dolichospermum, Microcystis, Planktothrix, Chroococcus, Oscillatoria, Woronichinia and most dominant species Limnothrix redekei and Pseudanabaena limnetica) and invasive cyanobacterial species (Cylindrospermopsis raciborskii); c) detection of several microcystin variants (-LR, -dmLR, -RR, -dmRR) in water samples.

Results indicate that the potential threat to all the organisms in the ecosystem, but also ecosystem itself, is still real and present. Perpetual alarming condition of Lake Ludoš poses a great health risk and a “ticking (cyanobacterial) bomb” that could lead to the destruction of this special nature reserve. Urgent remediation measures are needed to help Lake Ludoš in solving incessant cyanobacterial problem.

References:

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EXTRACTS OF PLANKTOTHRIX AGARDHII-DOMINATED SCUM SAMPLES RICH IN OLIGOPEPTIDES INFLUENCED GROWTH, PRODUCTION OF CHL-A AND PEPTIDE COMPOSITION OF NATURAL P. AGARDHII POPULATION

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Blooms of the cyanobacterium Planktothrix agardhii able to produce microcystins (MCs) and many other secondary metabolites belonging mostly to non-ribosomal oligopeptides are common in shallow eutrophic freshwaters of temperate zone. The present study indicates potential of P. agardhii population to grow and produce several dozen of bioactive compounds in response to compounds of other cyanobacterial communities predominated by this species. The growth, chlorophyll-a production and oligopeptides composition of natural population of P. agardhii were analyzed after 7-day exposure to crude extracts Pa-A and Pa-B of two P. agardhii-dominated bloom samples collected in different lakes and years. Experiments with four concentrations of the extracts, which contained similar high numbers (50 and 55) of oligopeptides but of different structure, were carried out. Generally, aeruginosins (AERs), cyanopeptolins (CLPs) and anabaenopeptins (APs) were the most numerous but only 16% of them were the same in both extracts. The extracts differed also in MCs concentration and profile. Both extracts supported the growth, production of Chl-a and MCs by the P. agardhii exposed; some changes in oligopeptide profiles were also observed. MCs which total concentration was approx. 13-fold lower in the extract Pa-A than in Pa-B, did not inhibit P. agardhii growth neither they had any effect on MCs and Chl-a production. No evidence for bioaccumulation of dissolved oligopeptides was observed in the P. agardhii exposed. Our results suggest that cyanobacterial compounds other than peptides may play a role in the regulation of growth of particular P. agardhii chemotypes of various oligopeptide composition.
DEFINING THE MODE OF INTERACTION BETWEEN TEMPERATURE AND PHOSPHATE IN PROMOTING CYANOBACTERIAL GROWTH

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Increased nutrient loadings in aquatic systems and climate change are among the issues of greatest concern for freshwater science, lake management and public health [1]. Among others, blooms of toxic cyanobacteria have increased over the last decades and are promoted by the direct and indirect effects of high nutrient inputs and increased temperatures. The exact nature of this interaction remains unclear. To better understand the interaction between temperature and phosphorus in promoting cyanobacterial and algal growth, a full factorial experiment in batch cultures was performed. Fourteen species from Cyanobacteria and chlorophytes were incubated under four different temperatures (21.5, 24, 26.5 and 29 °C) and four phosphorus concentrations (2, 10, 50 and 250 HPO₄²⁻ μM). Growth curves were fitted with a logistic model to calculate the maximum growth rate (μmax) of each species. Both phytoplankton groups showed similar μmax under the tested temperatures but not at increasing phosphorus concentration. Our hypothesis that temperature influences all cyanobacteria positively or interacts with phosphorus in promoting cyanobacteria upon chlorophytes was rejected, even though several studies claim that global warming will strengthen the frequency and persistence of cyanobacterial blooms in general [2,3,4,5]. However, for some cyanobacterial species (i.e., Microcystis aeruginosa, Synechococcus leopoliensis), temperature and phosphorus interact significantly, indicating that some species can potentially be favored under warmer, more nutrient rich conditions. Additionally, the group level identity, was found to be a significant factor in the effect of nutrient enrichment, since cyanobacteria exhibited a significantly higher μmax than chlorophytes under higher phosphorus concentrations.

References:
Prey organisms avoid predators through mechanisms which usually involve phenotypically plastic responses. Zooplankton-phytoplankton interaction represents a model to study induced defenses once grazing is a selective pressure which drives functional responses on phytoplankton, especially harmful ones such as Cyanobacteria. We aimed to evaluate the physiological parameters of toxic cyanobacterial strains during indirect exposure to the cladoceran Daphnia gessneri. Experiments were performed with Microcystis aeruginosa LETC-MC-02 (microcystin +) and Raphidiopsis raciborskii T3 (saxitoxins +) strains, which were grown in ASM-1 medium in which deionized water was replaced by filtrate (0.22 μm) of D. gessneri (adults - 60 ind. L-1). Growth, photosynthesis and toxin production were evaluated every two days along six days. No significant differences were observed in photosynthetic responses in both evaluated strains. However, it was evidenced a decrease in R. raciborskii T3 growth when exposed to predator infochemicals. Furthermore, toxin production enhanced (average increase >100%) in response to D. gessneri. These results evidence a chemical induced defense once it comprehends one of the characteristics of cyanobacteria which limit their exploitation by zooplankton.

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Eutrophication of water bodies leads to active vegetation of cyanobacteria and water blooms. Harmful algal blooms occur worldwide, including in water bodies in northwestern Russia. The aim was to analyze the dynamics of structural and functional parameters of phytoplankton, especially cyanobacteria in various sites of a coastal zone of Lake Ladoga around Valaam archipelago and to find environmental factors defining it. Valaam islands are located in the northern part of Lake Ladoga. The phytoplankton samples were collected from 12 - 18 stations around the archipelago during July-August of 2008 - 2017. Integrated phytoplankton samples (volume 1 L) were collected from the surface to the bottom (with interval 0.5 or 1.0 m). The water sampling was performed by a Bogorov’s bathometer; the integral samples were fixed with a Lugol-formalin solution. The biomass was estimated from the total volume of algae according to the counted cell density and measured average cell density. Shannon diversity index was calculated by the biomass of phytoplankton. The total number of taxa identified in the studied material was 185. The discovered species consisted of nine taxonomic groups of algae. Large numbers of species of green algae (30 %), cyanobacteria (19%) and diatoms (18%) are typical for most lakes of northwestern Russia. The potentially toxic species *Aphanizomenon flos-aquae* (L.) Ralfs ex Born. et. Flah., *Planktothrix agardhii* (Gom.) Anagn. et Kom., *Planktolyngbia limnetica* (Lemmerm.) Komark.-Legn., *Woronichinia karelica* Komarek & Komark.-Legn. were observed throughout monitoring period. Density of phytoplankton varied from 0.1 till 56.1 mln cell/dm³, biomass - from 0.1 till 29.5 mg/dm³. The average concentration of chlorophyll a was 4.5 µg/L in the surface of study area. Cyanobacteria and green algae dominated by the phytoplankton biomass. It was shown the spatial heterogeneity of the distribution of phytoplankton abundance and biomass in different years. The maximum values of abundance and biomass were marked in enclosed shallow bays, minimum - in open deep parts of coastal zone. This, along with a significant spatial variability an important role played interannual differences of abundance of phytoplankton. A wide range of the phytoplankton biomass is revealed during the study period. It is showed that the differences in the structure of inter annual phytoplankton primarily are connected with temperature stratification, water dynamics and level.
Cyanobacterial blooms in natural lakes have been intensively studied within the last decades. However, very little is known about bloom triggers and bloom dynamics in artificial water systems. Reservoirs and dams used for hydropower or drinking water supply are subject to major anthropogenic and environmental influences. External water input via pumping activities and disruption of lake stratification may potentially favor harmful cyanobacterial blooms. The Schwarzenbach-Dam in the Black Forest (Germany) is a reservoir used for the production of hydropower. It experienced toxic Microcystis and Dolichospermum blooms since the early 2000s. For three consecutive years we monitored surface waters for water temperature, phosphate, nitrate and toxin (microcystin) concentrations as well as bloom density under different pumping activities. In order to investigate shifts in phytoplankton community during bloom occurrence we used High-Throughput-Sequencing (HTS) of 16S and 18S rDNA sequences. Our data identified non-toxic Dolichospermum as the bloom-forming species. Blooms occurred every year irrespective of pumping management. Intensity of the bloom was mainly determined by water temperature and inorganic nitrogen concentration. Preliminary HTS data suggest that cyanobacterial blooms impact relative abundance of heterotrophic bacteria as well as eukaryotic algae. Our data might help to understand causes of blooms and help to develop sustainable management strategies to prevent or mitigate bloom formation in hydropower reservoirs.

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Previous studies have indicated that certain light and nutrient stress conditions can stimulate synthesis of microcystin (MCs) and enhance the binding of the MCs molecule to proteins [1-3]. In this study, the biosynthesis of MCs was investigated after long-term nitrogen-starved conditions for cyanobacterium Microcystis aeruginosa. The results demonstrated that the algal cells were able to survive at a non-growing state with nitrogen starvation for more than one month. The physiological properties of the algal cells were studied to elucidate the mechanisms of viability under nitrogen-deprivation conditions. After the state of nitrogen chlorosis, new toxins can be resynthesized and tracked by using the 15N-stable isotope labeling nitrogen. Nitrogen starvation of nutritionally replete cells resulted in a significant increase of microcystin-LY (MC-LY), suggesting that MC-LY may undergo catabolism to provide nitrogen or that MC-LY may be produced to play an important role in the cell in response to nitrogen deprivation. The rank order of different types of nitrogen for algal cells assimilation was: N-ammonium > N-urea > N-nitrate > N-alanine. The relationship between the production of toxin variants and various environmental conditions is an interesting issue for future research and may help improve the understanding of the ecological role of cyanobacterial toxins.

References:

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Cyclic heptapeptides Microcystins (MCs) are the most notorious and frequently detected cyanotoxins worldwide. Apart from exposure to contaminated water, humans are susceptible to MCs via consumption of contaminated fish or food supplements (e.g. spirulina) containing cyanobacteria. Due to the great concern regarding effects of MCs on public health, EU is now regulating MC-LR in drinking water (1μg/L) and is further setting performance criteria for the method of analysis i.e. expanded uncertainty <30% [1]. In USA, there is an ongoing wide-scale monitoring of water supplies for six MC congeners as well as “total MCs” against new guidelines that include short-term (10-day) health advisory levels [2]. While all MCs are generally considered toxicologically relevant, the term “total MCs” is loosely defined and “sum of detected MCs” has been proposed instead [3]. In view of those advances, it is important to cross-evaluate methods commonly applied to MC analysis, i.e. enzyme linked immunosorbent assays (ELISA), protein phosphatase inhibition assays (PPIA) and LC-MS/MS. Our study presents results of a head-to-head comparison of commercial ELISA, PPIA assays and LC-MS/MS methods [4,5] for MCs in real and spiked samples of water, cyanobacterial biomass and fish tissues. Results are evaluated in terms uncertainties, biases, matrix effects, false positives/negatives, use of resources and coherent interpretation. The findings showed that although ELISA and PPIA can be useful for screening or providing complementary information in matrices such as drinking water, unambiguous identification and accurate quantitation of LC-MS/MS outbalances those in-vitro assays, particularly with regard to fit-for-purpose of regulatory monitoring and research.

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AMMONIUM LOADING DRIVING BACTERIAL COMMUNITY SHIFT IN BIOFILM ATTACHED TO HYDRILLA VERTICILLATA

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Role of bacteria in biofilm attached to submerged macrophytes remains unclear. In this study, nitrogen removal and bacterial community shift and N-cycling related bacteria were investigated in biofilm attached to Hydrilla verticillata upon increased ammonium concentration (1-16 mg L\(^{-1}\)) and prolonged ammonium exposure time (8 mg L\(^{-1}\) for 21 days). Proteobacteria, Cyanobacteria, Planctomycetes, Bacteroidetes, Actinobacteria, Chloroflexi, Verrucomicrobia, Acidobacteria, Nitrospirae and Firmicutes were dominant phylum occurring at >1% in at least one sample. Relative abundances of Cyanobacteria increased from and from 5% (1 mg L\(^{-1}\)) to 37% (16 mg L\(^{-1}\)) and 2% to 27% in biofilm under 8 mg L\(^{-1}\) ammonium-N for 14 days. These results indicating that ammonium stimulated the Cyanobacteria growth significantly. Meanwhile, relative abundances of N-cycling related microorganisms including nitrifiers Nitrospira, and denitrifiers (including Pseudomonas, Rhodobacter, Hyphomicrobium, Dechloromonas, Hydrogenophaga, Acinetobacter and Flavobacterium) were positively related to the ammonium levels and exposure times. Abundances of N-cycling functional genes such as amoA, nxrA, nirS and norB were promoted by the ammonium loadings while nirK and nosZ showed negative sensitivities to a relatively high ammonium concentration. Our results indicated that ammonium loading disturbed the bacterial community structure and stimulated N-cycling related bacteria. However, the growth of biofilm may cause negative effects on submersed macrophyte growth and provide "algal seeds" for algal burst in water column polluted with ammonium.
BACTERIA AS A FACTOR CONTROLLING THE OCCURRENCE OF MICROCYSTIN-PRODUCING CYANOBACTERIAL BLOOMS

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Harmful cyanobacterial blooms observed worldwide are considered as a major global problem of eutrophication. In order to reduce the threat related to the blooms occurrence it is necessary to find a solution based on understanding the relationships between cyanobacteria and other organisms coexisting in complex bloom structure. Especially important is to identify processes regulating this interactions and, among others, to develop the knowledge about bacteria inhibiting growth or inducing the lye of cyanobacterial cells, i.e. bacteria of algicidal properties - AB. As the activity of AB is usually related to cyanobacterial cell degradation which can cause cyanotoxins release into the water, therefore it is necessary to determine the possibility of simultaneously degradation of cyanobacterial cells and cyanotoxins by AB. In our previous research the MC-degrading activity of the strain JEZ-8L, isolated from lowland dam reservoir- Jeziorisko, was reported. In present work, several AB strains isolated from cyanobacterial bloom sample taken in August and September 2018 from Sulejow Reservoir (dominated by Microcystis genus) exhibited algicidal properties against cyanobacterium Microcystis aeruginosa PCC7806. Their taxonomic characterization (based on 16S rRNA sequencing) classified them as Stenotrophomonas, Chryseobacterium, Pseudomonas and Comamonas genera. An attempt will be made to identify and characterize a potential algicide agents of our bacterial strains. The results of the present study will contribute in filling the gap in the research field related to interactions between AB and freshwater cyanobacteria occurring in temperate climate zone.
ELABORATION OF SUBCHRONIC TOXICITY REFERENCE VALUES (TRVs) FOR THE ORAL ROUTES OF MICROCYSTIN-LR AND CYLINDROSPERMOPSIN

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Cyanobacteria are a group of microorganisms which naturally occur in freshwater and can be found at a higher density in eutrophic or nutrient-enriched water bodies. Many cyanobacteria are able to produce toxins which can impact human health. Exposure to cyanobacteria and their toxins may occur by ingestion of drinking water or during bathing and recreational activities in water bodies with the toxins. Microcystins are the most common type of cyanobacterial cyanotoxins found worldwide in surface waters. Studies in laboratory animals demonstrate liver, kidney and reproductive effects following subchronic oral exposure to microcystin-LR. The French agency for food, environmental and occupational health and safety identified a study by Chen et al (2011) conducted on rats as the critical study in the derivation of a toxicological reference value for microcystins. The critical effect was a sperm quality deterioration.

Cylindrospermopsin can be produced by a variety of cyanobacteria species. Some of them tend not to form visible surface scums and the highest concentrations of cyanobacterial cells typically occur below the water surface. Anses evaluated the health effects of cylindrospermopsin and derived a toxicological reference value. Kidney and liver appear to be the primary target organs for cylindrospermopsin toxicity. The critical study for the derivation of the cylindrospermopsin VTR was conducted by Chernoff et al (2018) based on drinking water exposure to mice. The critical effect was an increase in liver weight.
Poster session II
MICROCYSTINS IN POND WATER OF RURAL BANGLADESH

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We have reported a robust and highly sensitive two-site immunoassay for cyanobacterial hepatotoxins microcystins and nodularin using a unique anti-immunocomplex generic binder [1] isolated from our synthetic antibody library [2]. Located near the Bay of Bengal, Bangladesh is a country of alluvial plain land with many rivers and more than one million ponds. We applied the assay to screen 14 pond water samples collected from a village in Bangladesh during summer 2017. The studied waters, ponds of the “Sayadabad” village, are located in the Kasba subdistrict of district Brahmanbaria. Using microcystin-LR as standard, all samples except two were below 0.15 µg/L for microcystin. The two positive water samples contained microcystin at 2.3 µg/L and 2.8 µg/L. Further investigation of the water samples by LC-MS revealed microcystin-LR and -RR in the first positive sample, and microcystin-RR and -dmRR in the second positive sample. The results corroborate the suitability of our microcystin assay for screening of natural waters as well as justify further ecotoxicological research in this geographical location.

References:

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APPLICATION OF ELECTRON PARAMAGNETIC RESONANCE (EPR) FOR RADICAL IDENTIFICATION DURING THE PHOTOCATALYTIC DEGRADATION OF CYANOTOXINS WITH ENHANCED PHOTOCATALYSIS

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The study utilized Electron Paramagnetic Resonance (EPR) spectroscopy to identify and quantify the type of radicals formed during the enhanced photocatalytic degradation of the hepatotoxins microcystin-LR and nodularin [1]. Conventional UVA/TiO₂ photocatalysis was coupled with sulfate radical generating oxidants (persulfate, PS and peroxymonosulfate, PMS) to enhance and expedite the degradation of the hepatotoxic microcystin-LR and nodularin. Oxidant addition enhanced both the degradation and detoxification rates and followed the order of UVA/TiO₂/PMS > UVA/TiO₂/PS >> UVA/TiO₂. In addition, the transformation products identified at the early stages of degradation were identical for all treatment systems but were occurring at accelerated rates for the enhanced photocatalysis compared with conventional. In order to identify the radicals formed during each treatment, quenching studies with probes such as propanol and tetra-butyl alcohol were performed [2]. The results of the quenching studies were inconclusive, therefore specialized techniques such as EPR spectroscopy was used. EPR spectra were recorded for the photolysis and photocatalysis of each oxidant and compared with UVA/TiO₂. When UVA/TiO₂ was applied there was a sharp formation of short-lived hydroxyl radicals that peaked the first 10 min of irradiation and completed within the first 20 minutes treatment. On the other hand, when oxidants where added there was a continues flow of radicals formed that had a delayed maxima between 20 and 30 minutes depending on the type of radical formed and oxidant used. This confirms that the presence of oxidants prolonged the lifetime of radicals, thus allowed for bulk diffusion and reaction with the cyanotoxins.

References:

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ACUTE TOXICITY AND GENE RESPONSES INDUCED BY CYLINDROSPERMUM SP. IN ZEBRAFISH (DANIO RERIO) EMBRYOS

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Zebrafish (Danio rerio) have been used as model organisms predominantly in developmental biology and molecular genetics, but their importance in toxicology has also been recognized. Embryos of zebrafish as model vertebrates are especially suitable for high-throughput large-scale screening for toxicity of different compounds and for research where the objective is to identify adverse effects of exposure.

This study was conducted to analyse effects on the zebrafish embryos exposing them to a range of extract concentrations of the strain Cylindrospermum sp., as well as to determine changes in the expression of genes involved in oxidative stress (cat), endoplasmic reticulum stress (xbp1 and bip), xenobiotic metabolism (cyp1a1), circadian rhythm (per1a) and estrogenicity (vtg). Quantification of relative changes in the gene expression was evaluated using the real-time quantitative polymerase chain reaction (RT-qPCR).

Exposing zebrafish embryos to the tested extract for 48h, acute toxicity was observed when the concentration reached 5 μgml⁻¹, whereas 50% of embryos died at the extract concentration of 29.3 μgml⁻¹.

According to the obtained data, exposure of embryos to the tested extract resulted in transcriptional alternation of few genes. The affected pathways with significant changes were neurophysiological processes (circadian rhythm) as well as estrogenicity. The relative expression level of the per1a gene involved in the circadian rhythm was up-regulated. The vtg gene involved in estrogenicity was induced by exposure to lower extract concentrations. Concerning other tested genes no significant responses in the expression levels were found.

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ABUNDANCE AND TOXICITY OF PLANKTOThRIX RUBESCENS
IN THE GERMAN OSTERSEEN LAKE DISTRICT

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The presence of the potentially toxic cyanobacterial species Planktothrix rubescens in lakes from the German ‘Osterseen’ district was first described in 1986. In the current study, the local and temporal abundance of P. rubescens in the water column as well as its toxic potential were characterized in relation to hydrophysical and –chemical variables. Moreover, comparisons of its current distribution with data from the 1990s were made to infer possible impacts of climate change. The quantitative determination of the number of P. rubescens specific 16S rRNA gene copies by real-time quantitative PCR and the microscopic analysis of biomass revealed a mass occurrence of P. rubescens in two lakes in 2018. The highest numbers of P. rubescens specific 16S rRNA gene copies were constantly found between the metalimnetic and the hypolimnetic layer, supporting the expected preference of P. rubescens for low light conditions. The quantitative determination of the number of P. rubescens specific mcyA gene copies was highly correlated with the observed P. rubescens distribution patterns. The temporal comparison of data from the 1990s with those from 2018 revealed rather stable cyanobacterial community structures over time with Planktothrix presence observed in the same lakes. In addition, it was possible to determine a conversion factor for the calculation of Planktothrix gene copy numbers to biomass. The findings of this study illustrate the usefulness of long-term data in assessing cyanobacterial community structures in light of climate change.

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THE EFFECTS OF ALGAECIDES AND HERBICIDES ON A MICROCYSTIS WINTER BLOOM IN LAKE OKEECHOBEE, FLORIDA (USA)

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Microcystis-dominated cyanobacterial harmful algal blooms (cyanoHABs) are a reoccurring problem within the Lake Okeechobee Waterway resulting in widespread economic and health impacts. As public awareness on the risks of blooms increases, there is an urgent need for studies on both short-term and long-term management of cyanoHABs. In order to provide science-based best management practices or eradication/treatment options, we tested various concentrations and combinations of algaecides and herbicides. Bloom waters were collected from Lake Okeechobee in November/2018 and were dominated by Microcystis wesenbergii, with some M. aeruginosa and Dolichospermum circinale colonies present. The bloom material was exposed to fifteen different algaecides, herbicides, or combinations, using four different concentrations. Cell abundance and morphology, chlorophyll a/b, phycocyanin and microscopic analyses were undertaken at the time of collection and 24 and 72 hours post-treatment. Microcystin concentrations were measured from the crude bloom, but were determined too low to undertake microcystin degradation analyses. Overall, the effectiveness of the chemicals varied. The most efficacious at treating this bloom included sodium carbonate peroxyhydrate, copper sulfate pentahydrate, copper ethanolamine complex, and combinations of diquat dibromide with endothall, copper gluconate/citrate, and/or copper ethanolamine. Other promising treatment methods included combinations of flumioxazin with copper gluconate/citrate and endothall with liquid H₂O₂. Some chemicals, including liquid H₂O₂ and endothall alone, were unable to deplete cyanobacterial abundance and therefore considered an ineffective treatment option for the treatment of M. wesenbergii-dominated blooms. Future work aims at treating toxic blooms and monitoring cell abundance together with toxin production and release for effective treatments in situ.

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EARLY BIOMARKERS OF BEHAVIOURAL AND PHYSIOLOGICAL DISTURBANCES IN DAPHNIA MAGNA EXPOSED TO ANATOXIN-A ESTIMATED BY VIDEO ANALYSIS

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The majority of reports on the toxic effect of cyanobacterial metabolites on the Cladocera is based on determination of two endpoints: mortality or immobilization. However, detection of sub-lethal effects requires more sensitive biomarkers. The aim of the present study was to evaluate the applicability of digital-video analysis of behavioural and physiological biomarkers in the assessment of effects caused by the cyanobacterial neurotoxin, anatoxin-a (ANTX) at a broad range of its concentrations (0.5-50 µg/mL) on swimming speed (SS), heart rate (HR), oxygen consumption (OC), thoracic limb activity (TLA) and abdominal claw movement (ACM) of Daphnia magna. Swimming speed and abdominal claw movements were determined by digital analysis of video clips by Tracker® software; OC by Oxygraph Plus System®. HR, TLA and ACM were evaluated by digital frame-by-frame analysis of video clips of microscopic view with the use of a media player software.

The experimental study showed a concentration- and time-dependent decrease of SS, HR, OC, TLA and ACM as early as after 2 h of the exposure of D. magna to ANTX. Further inhibition of these parameters was also noted after 24 h exposure. On the other hand, stimulation of ACM was noted at the lower (0.5 and 2.5 µg/mL) concentrations of ANTX after both 2 h and 24 h of exposure.

The results indicated that behavioural and physiological biomarkers measured by video analysis may be a valuable tool for an early determination of toxic effects induced by cyanobacterial metabolites in zooplankters.
LUCIFERASE-BASED REPORTER ASSAY FOR SCREENING CYANOBACTERIAL PEPTIDES TO IDENTIFY INHIBITORS OF MICRONORA-92B-3P

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Cyanobacteria constitute a rich source of biologically active and structurally diverse compounds. The pharmacological potential of these compounds resides, in part, in their ability to control the proliferation and growth of cancer cell lines and potent disease-causing microbial agents. With molecular abilities to modulate regulatory elements of cell processes (proteins and functional RNAs), bioactive cyanobacterial metabolites could be important tools for clarifying the mechanisms of these processes and serve as lead structures for the development of new therapeutic agents. Here, we report the creation of a cell sensor for potential small-molecule regulators of the liver-cancer--specific microRNA MiR92b-3p, which is involved in hepatocellular carcinoma (HCC) development and hepatitis C virus infection. Our small-molecule screen employs a Huh7 human hepatoma cell line stably transfected with a pmirGLO Renilla luciferase sensor for endogenous MiR92b-3p. The assay was optimized and validated using an MiR92b-3p antisense or MiR92b-3p mimicking agent, followed by measurements of luciferase mRNA levels (qPCR) in the engineered Huh7 cell line. This reporter system has been applied for screening cyanobacterial peptides from a de novo library of bioactive and non-toxic compounds, which was prepared on the basis of their activity against key metabolic enzymes (proteases and protein phosphatases) and against selected cell lines (MTT test). Although the first screenings of a few peptides isolated from Nostoc edaphicum (CCNP 1411) with this reporter system failed to identify small molecule inhibitors of MiR92b-3p, the approach provides a means to identify functional miRNA regulators and could be readily extended to other compounds.

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GLOBALHAB: A GLOBAL INITIATIVE TO ENHANCE COLLABORATION AND COMMUNICATION ON HABs


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The Global Harmful Algal Blooms (GlobalHAB) Programme is an international scientific programme on harmful algal blooms (HABs) aimed at fostering and promoting co-operative research directed toward improving the prediction of HAB events, and providing sound knowledge for policy- and decision- making to manage and mitigate HAB impacts in a globally changing planet.

GlobalHAB includes a focus on cyanobacterial HABs (cyanoHABs). The aims of this component are to develop a global perspective in advancing the science and management of freshwater HABs, and cyanobacterial HABs in marine, brackish and freshwater habitats.

- Promote the comparative approach for studying cyanoHABs in contrasting environments.
- Improve communication between scientists and managers working on Freshwater HABs in general and cyanoHABs in all relevant habitats via cross-fertilization of ideas and technologies.
- Identify emerging issues for cyanoHABs across freshwater, brackish and marine habitats, both benthic and pelagic.
- Promote the development of faster, cheaper kits for measuring toxins.
- Synthesize and share existing information on mitigation strategies on Freshwater HABs and cyanoHABs with environmental and resource managers, especially in areas where human populations may be most dramatically affected by contaminated drinking water. Outputs include developing a mitigation manual for cyanoHABs that will provide a summary of mitigation measures trialed throughout the world.
INTRINSIC ANTIBIOTIC RESISTANCE IN CYANOBACTERIA
– THE CASE OF TRIMETHOPRIM

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Antibiotic residues poses an increasing concern both at environmental and public health levels. Besides affecting the structure/functioning/diversity of aquatic communities, antibiotic pollution is responsible for one of the most serious threat to the global health: the emergence/dissemination of antibiotic resistance. Cyanobacteria have been recommended as test organisms in the assessment of environmental contaminants such as antibiotics [1]. However, the species sensitivity should be carefully characterized, since cyanobacteria may present intrinsic resistance to some antibiotics, which can seriously compromise the risk assessment results. We have been studied the putative role of cyanobacteria in aquatic resistome and our studies have demonstrated that strains of Microcystis and Planktothrix genus are not susceptible to some antibiotics, namely trimethoprim (up to 1.6 mg/L) [2,3]. In order to understand the cause of the reduced susceptibility of cyanobacteria to several antibiotics, we performed and analyzed the whole genome sequencing of cyanobacteria strains that exhibits a non-susceptible phenotype. Preliminary results revealed that a M. aeruginosa strain possesses the gene that codifies thymidylate synthase (thyX), an alternative enzyme to dihydrofolate reductase (FolA) in the folate metabolism, which is essential for bacteria replication and survival. As the mechanism of action of trimethoprim in bacteria is the inhibition of FolA, our results strongly suggests that the presence of an alternative pathway to FolA may underlie the intrinsic resistance to trimethoprim in cyanobacteria. The identification of intrinsic antibiotic resistance in cyanobacteria is an important tool for identifying which species should not be included as indicator organisms in environmental risk assessment purposes.

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Cylindrospermopsin (CYN) is a cyanotoxin with hepatotoxic and cytotoxic effects. People and animals can be in contact with CYN by different routes. Thus, it is among the main exposure ways the intake of contaminated water and food. Following the recommendations from the European Food Safety Authority (EFSA), it is necessary the genotoxic evaluation of substances in contact with food, allowing to clarify previous results obtained in its in vitro evaluation. For this reason, in this work, the in vivo genotoxic effects of CYN in liver, stomach and blood were assessed in Wistar rats by the standard and modified comet assays. Animals were exposed by oral gavage to 7.5, 23.7, and 75 μg/kg CYN body weight, and the assays were carried out according to the OECD guideline 489. Comet assay is a genotoxicity test that allow the detection of DNA damage and oxidized bases with the enzymes Endo III (Endonuclease III) and FPG (Formamido pyrimidine glycosylase). Results for the standard assay didn’t show increases in DNA strand breaks at any dose assessed. By contrast, a significant increase in the % of DNA in tail was exhibit with Endo III in liver and blood cells exposed to 75 μg/kg CYN. Besides, in blood cells oxidative DNA damage was shown after treatment with 23.7 and 75 μg/kg CYN in presence of FPG. Therefore, CYN induces oxidative DNA damage in vivo in blood and liver cells of Wistar rats, although more studies are needed to include all the genetic endpoints required by EFSA.

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TOXICOLOGICAL EVALUATION OF A BINARY MIXTURE OF CYANOTOXINS USING MUTAGENICITY BIOMARKERS

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At present, as a consequence of climate change and water eutrophication, an increase in the proliferation of cyanobacteria producing biotoxins is happening. Cylindrospermopsin (CYN) and Microcystin-LR (MC-LR) are among the cyanotoxins whose occurrence is more frequently reported. As the toxicity of mixtures can differ from those of individual toxins due to interaction phenomena it is interesting to evaluate the toxic effects of a combination of CYN and MC-LR, in this case using mutagenicity biomarkers. Therefore, the aim of this work was to assess the mutagenicity and genotoxicity of the CYN-MC-LR mixture by the Bacterial Reverse Mutation Test (Ames test, OECD 471) in presence and absence of S9 in five Salmonella typhimurium strains, the micronucleus test (MN, OECD 487) in presence and absence of S9 in the L5178Y Tk +/- cell line, the standard and modified comet assay with restriction enzymes (Endonuclease III (Endo III) and Formamido pyrimidine glycosylase (FPG)) in Caco-2 cell line and the mouse lymphoma thymidine-kinase assay (MLA, OECD 476) in presence and absence of S9 in the L5178Y Tk +/- cell line. Results obtained in the different mutagenic (Ames test and MLA assay) and genotoxic (MN test and comet assay) in vitro assays revealed that the binary mixture CYN+MC-LR only showed effects with the MN test with S9, indicating the role of metabolism in toxicity. This finding is in agreement with the genotoxicity previously observed for CYN.

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BMAA CAUSES PROGRESSIVE NEURODEGENERATION TYPICAL OF ALZHEIMER’S AND PARKINON’S DISEASES AND ALS

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A possible causative or contributory role for β-N-methylamino-L-alanine (BMAA) in the etiology of sporadic neurodegenerative diseases (ND’s) has been debated for decades. The absence of any relevant pathology in an animal model at environmentally relevant dose regimes has been principally responsible for the dismissal of the so-called ‘BMAA-ALS’ hypothesis [1, 2]. The recent discovery [3, 4, 5] that BMAA is a perinatal developmental neurotoxin that results in dose-dependant behavioural and histopathological characteristic of Parkinson’s (PD) and Alzheimer’s (AD) diseases and Amyotrophic Lateral Sclerosis (ALS) following a single exposure at environmentally relevant concentrations, raises the questions; are these pathologies progressive, and are they presented as they typically occur in clinical cases. We used the BMAA-rat model, as described in [3, 4, 5], to evaluate the long term progression of pathology so as to compare this with human AD, PD and ALS progression and staging. Symptom onset and progression, as measured behaviourally and histopathologically, and proteinopathy and neuronal loss progression and propagation mimicked those observed in AD, PD and ALS for the respective pathologies, as did the late onset progressive muscle atrophy in the lower limbs. These data strongly suggest that BMAA is a causative or contributory environmental factor in AD, PD, ALS and associated diseases, and that exposure at the susceptible age results in lifelong slow neurodegeneration with late clinical symptom presentation.

References:

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BENTHIC CYANOBACTERIA AND THEIR TOXINS
IN DUTCH RECREATIONAL WATERS

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Benthic cyanobacteria can occur in various habitats, including recreational water bodies, such as bathing sites and designated areas for playing children. As fatal dog incidents have occurred in the Netherlands, benthic cyanobacteria have become a concern to the local water managers. However, little is known about the species that occur in the Netherlands, and even fewer data exist on which toxins are produced. To eventually support the water managers in evaluating and managing the hazard posed by toxic benthic cyanobacteria, we started a three year survey. In this survey, we collect benthic cyanobacteria from recreational waters and record environmental parameters. Species are determined by light microscopy and the toxins (microcystins, nodularin, anatoxins, cylindrospermopsins and saxitoxins) are analysed by LC-MS/MS. Here we present the data from the first year of the survey. We will discuss which species are present in the samples testing positive for toxins (microcystins, anatoxins) and whether toxin occurrence can be related to specific environmental conditions.
In May 2017, at least 12 dogs showed signs of acute neurotoxicosis (and several dogs died) after swimming in or drinking from Lake Tegel, a mesotrophic lake in Berlin, Germany. Cyanobacterial surface blooms were absent, but detached and floating water moss (*Fontinalis antipyretica*) harbouring high amounts of *Tychonema* sp., a potential anatoxin-a (ATX) producing cyanobacterium, was found near the beaches where the dogs had been swimming and playing. Necropsies of two of the dogs revealed no specific lesions beside the anamnestic neurotoxicosis. ATX was detected in concentrations up to $8700 \, \mu g \, L^{-1}$ in the stomach contents, while other (neuro)toxic substances were not found. In the aqueous fraction of *Fontinalis/Tychonema* clumps sampled after the casualties, ATX was found in concentrations up to $1870 \, \mu g \, L^{-1}$. This is the first report of a dense population of ATX producing *Tychonema* sp. in stands of *Fontinalis*. The findings emphasizes the need for further investigation of potentially toxic, non-surface bloom forming cyanobacteria in less eutrophic water bodies, but also present a great challenge for appropriate surveillance of bathing sites.
The massive growing of cyanobacteria has been an enormous concern worldwide. Cyanobacteria can produce a variety of secondary metabolites, some of which may compromise water quality, causing adverse effects to aquatic and terrestrial organisms, including human. Among the group of cyanobacteria producing cyanotoxins (toxins produced by cyanobacteria), the anatoxin-a (s) is known as a potent neurotoxin that causes irreversible inhibition of the enzyme acetylcholinesterase. Analytical standards and official methods for its determination are not available. A method for isolation of anatoxin-a(s) in milligram amounts has been developed, involving solid phase extraction (SPE) and semi-preparative scale reversed-phase HPLC and Hydrophilic interaction liquid chromatography (HILIC). The Instability of the molecule of the anatoxina(s) makes difficult its detection and isolation. Therefore, stabilities studies of acid solution o anatoxin-a(s) were also performed to determine its degradation. The isolation was carried out using anatoxin-a(s)-containing extract from Sphaerospermopsis torques-reginae strain ITEP-024, harvested in Tapacurá reservoir, Pernambuco state, Brazil. LC-MS methods to identify and quantify the anatoxin-a (s) from extracts of ITEP-024 strain were developed. Solid phase extraction (SPE) method, using Methanol/Water (20:80 v/v) gradient was effective for pre-isolation of crude extracts. Ion transitions (m/z) for correct detection of this toxin were 253 >58, 253 >159, 235 >98 and 235 >96. High resolution mass spectrometry was employed to determine anatoxin-a(s) identification (LC-MS/Q-TOF). An error below 5 ppm was reached. Chromatographic separation was better achieved under HILIC conditions employing a ZIC-HILIC column. The ion transitions characteristic of anatoxin-a(s) was successfully detected in the extract and fractions of ITEP-024. Anatoxin-a (s) in ITEP-024 was stable after 30, 60 and 120 days of storage at 4 and 20 °C. Since there is no standard solutions commercially available, our findings may substantially assist the isolation of anatoxin-a(s) and its accurate identification by LC-MS/MS.

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Cyanobacteria consist an important group of organisms that on the one hand provide essential ecological services [1] and on the other produce cyanotoxins during blooms, which are intensified by climate change [2]. Blooms that are formed by cyanobacteria consist of toxic and nontoxic strains, yet the mechanisms that result in the occurrence of nontoxic strains are still enigmatic [3]. Biodiversity data provide valuable insights on ecosystem processes. Databases, organized collections of data, constitute an essential tool as they offer a reliable way to store, access and manipulate large scale resources, with some recent examples from the cyanobacteria realm [4, 5]. The aim of this work was to create a database of cyanobacteria species recorded in the Mediterranean basin using Microsoft SQL Server 2017 software and analyze its content using the R programming language via the RStudio integrated development environment. Inter-software connection and initial assessment of the data with R was conducted using the RODBC, dplyr, dbplyr, ggplot2 and ggmap packages. Toxin potential for each species was taken into account and queried through R software with respect to the geographic origin of the different species. The total number of entries was 2155 cyanobacteria taxa. Here we propose the establishment of a public database expected to facilitate efficient investigation of large numbers of toxic species amongst different ecoregions, which could lead us to recognition of patterns regarding different toxins and toxic species. Wider adoption of these tools could provide a missing study material for research, enchasing the effort in understanding the biological role of cyanotoxins.

References:
MICROCYSTINS IN EUROPEAN NOBLE CRAYFISH ASTACUS ASTACUS IN LAKE STEINSFJORDEN, A CYANOBACTERIAL (PLANKTOTHRIX) DOMINATED LAKE

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The Norwegian Lake Steinsfjorden, a major fishery for noble crayfish, is often affected by cyanobacterial blooms caused by microcystin (MC) producing Planktothrix. A recent study demonstrated the presence of microcystins in noble crayfish originating from this lake [1]. However, little is known about the impact of toxic cyanobacteria on crayfish health and crayfish as food source. We have investigated the presence of MC in noble crayfish from Lake Steinsfjorden and elucidated whether MCs are transferred and accumulated in vital organs and the edible parts. In 2015, crayfish were captured each month from June to October. Water samples were taken simultaneously. Tissue samples from tail muscle, intestine, stomach, and hepatopancreas were harvested for MC-analysis using an in-house method for enzyme-linked immunosorbent assay (ELISA). MC-analysis results of the tissues were compared to corresponding cyanobacterial biomass and microcystin analyses in the water samples. Hepatopancreas, stomach and intestine contained the largest amounts of microcystins, with a peak in August-September. The edible muscle contained very low amounts of microcystins, but with an increase in September. Results indicate that a normal portion of boiled crayfish tails (~100 g muscle) from Lake Steinsfjorden in 2015 was well below the tolerable daily intake (TDI) limit for MCs (0.04 µg/kg body weight) for adults. Removal of the intestine more than halve the total microcystin content and seems a reasonable food safety precautionary measure for consumption of crayfish from cyanobacterial dominated waterbodies.

References:

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This study was supported by grant 243907 - “Targeted strategies for safeguarding the noble crayfish against alien and emerging threats” (TARGET) - from The Research Council of Norway.
GENE-LEVEL MODELING OF MICROCYSTIS GROWTH AND TOXIN PRODUCTION

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The problem of harmful cyanobacteria blooms in lakes, for example toxin-producing Microcystis, has a global dimension, increasing trend and dim future projection, and existing control methods are expensive and not always successful. At the same time, advances in microbiology are generating molecular-level understanding of the mechanisms underlying the growth and toxin production of cyanobacteria. Yet, this knowledge is generally not used in our ecosystem models, which limits their utility for research and management. Closing this gap is a grand challenge paramount to understanding and managing toxic cyanobacteria. The aim of this project is to incorporate this vast amount of knowledge into a dynamic, mechanistic, gene-level model of Microcystis growth and toxin production, and then use it to support the management of full-scale ecosystems. In this approach, individual cells are simulated explicitly using agent-based modeling (ABM). Each cell has a number of genes that are expressed to yield transcripts and proteins, which perform metabolism, toxin production and other cellular functions. The model is built using the pattern-oriented modeling (POM), based on a literature meta-analysis. Reproduction of patterns from over 400 laboratory experiments suggests the model cells are realistic representatives of their real-world counterparts. In some cases, the model reproduces and explains mechanisms underlying previously unexplained observed patterns, like the transient increase in microcystin content upon light intensity downshift. The model also reproduces and explains apparent inconsistencies in observations, like the increase/decrease of MC production with NO3 concentration. Present challenges include dealing with the genetic diversity of Microcystis, accounting for interactions with other microbes (allelopathy, Black Queen) and integrating into full-scale ecosystem models.
Cyanotoxins are of progressive concern since their occurrence in the environment is increasing due to water eutrophication and climate change. Cylindrospermopsis (CYN) is lately being considered as an emerging pollutant. At the same time the pollution of water bodies with plastics and their constituents including bisphenols (BPs) is rising. As a result, the environmental exposure to mixtures of contaminants is inevitable. The aim of our study was to investigate the genotoxic effects (additive, synergistic or potentiating) induced by co-exposure to CYN (0.5 µg/ml) and BPs (BPA and its analogues BPS, BPF and BPAF; 10 µg/ml) in HepG2 cells after 24 and 72h. Both CYN and BPs are known to be genotoxic after metabolic activation meaning that they induce metabolic enzymes and thus mutual influence on cell response can be expected. The induction of DNA double strand breaks (DSB) was evaluated by yH2AX assay using flow cytometry, while the influence on the expression of selected genes involved in metabolism of xenobiotics, immediate-early response, and DNA damage response was studied with the quantitative-PCR.

The results revealed that CYN and BPs after 72h-exposure induced DSB, while in combination the effect was compared to CYN alone reduced. In combined exposure, up-regulation of selected genes (e.g. CYP1A1, CDKN1A, GADD45A and GCLC) was more pronounced compared to single compound exposure. The combination of CYN/BPF deregulated gene expression at the highest level. Altogether, the results suggested additive CYN and BPs effects; however, exact mechanisms of action have to be further elucidated.
Recent studies document that next to the known toxins, cyanobacteria can produce new toxic products with retinoic acid-like activity, but there is very limited knowledge regarding their producers, occurrence or potential associated risks. This study conducted extensive screening of exudates (extracellular content) from 50 independent laboratory cultivations of wide spectra of species including 12 cyanobacteria (15 strains) and 4 algae of different taxonomy orders. Exudates of several cyanobacterial species caused activation of retinoic acid receptor, which plays a crucial role in cellular differentiation and early development. Extracellular retinoid-like activity was detected for repeated cultivations of six cyanobacterial species with total all-trans retinoic acid (ATRA) equivalent reaching up to over 2 µg/L. Exudates of two species with high retinoic acid-like activity, coccal cyanobacteria *Microcystis aeruginosa* and filamentous *Cylindrospermopsis raciborskii*, and of one algae with no extracellular activity were selected for detailed study of in vivo effects. The two cyanobacterial exudates caused teratogenicity as well as mortality and effect on growth in both species, while the algal exudate caused no such effects. Retinoic acid-like activity of cyanobacterial exudates determined in vitro bioassay was in a very good agreement with developmental effects in both zebrafish (*Danio rerio*) and frog (*Xenopus laevis*) embryos. Both the effect phenotypes and effective concentrations of exudates corresponded to their content of ATRA equivalents, supporting the hypothesis that the teratogenic effects of cyanobacterial exudates are likely related to the retinoid-like activity. Retinoid-like activity associated with abundant cyanobacterial species can pose risk especially to early developmental stages of aquatic vertebrates.

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EFFECTS OF MICROCYSTIN-LR, CYLINDROSPERMOPSIN AND THEIR COMBINATION ON THE ACETYLCHOLINESTERASE ACTIVITY OF SH-SY5Y CELLS

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Microcystin-LR (MC-LR) and Cylindrospermopsin (CYN) are two cyanotoxins produced by several genera of cyanobacteria. Although they are considered as hepatotoxin and cytotoxic, respectively, some studies manifest their ability to induce damage at different levels, such as the nervous system. In the case of MC-LR, most studies focus on the damage this toxin can exert at the limbic system level. For CYN, the studies are scarcer, but seems to cause some impairment in the acetylcholine levels in several in vivo models, being of importance as this is one of the most common neurotransmitters in the organism. The aim of the present work was to study the alteration both toxins, isolated and in combination, could cause in the acetylcholinesterase activity in undifferentiated and differentiated SH-SY5Y human neuroblastoma cells. Our results showed an increase of the acetylcholinesterase activity in undifferentiated cells only at the highest MC-LR concentration of exposure (37 μg/mL), while the differentiated cultures evidenced a significant decrease after exposure to all the concentrations assayed for CYN (0.075, 0.15 and 0.3 μg/mL). Moreover, an enhancement of the activity after exposure to the combination of MC-LR and CYN at the highest concentrations assayed (45:0.3 μg/mL) was observed in the differentiated and no differentiated cells. These results evidenced a more sensitive response of differentiated SH-SY5Y cells after exposure to CYN, leading to a prolonged stimulation of the post-synaptic cell and, for this, a more lasting response. Moreover, MC-LR and the combination enhanced the AChE activity, shortening the time of synapsis in undifferentiated and differentiated cells, respectively.

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OCCURRENCE OF CYANOTOXINS IN ATLANTIC CANADA

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Very recently, Atlantic Canada has been experiencing an increase in the proliferation of freshwater harmful algal blooms. This can be attributed to a mixture of global change factors including a warming climate,[1] anthropogenic nutrient input and a decrease in atmospheric sulfate deposition.[2] Despite this growing problem, little is known about the occurrence and distribution of cyanobacteria and their toxins in Atlantic Canadian fresh waters. Here, we report results from the analysis of samples from the drinking water reservoirs of Halifax, Nova Scotia and Moncton, New Brunswick, as well as other nearby recreational water bodies. A combination of grab samples and passive samplers using HP-20 resin were used for sample collection. These were analyzed primarily using a novel untargeted high resolution-tandem mass spectrometry method and in some cases results were compared to those from Adda and multihapten [3] enzyme-linked immunosorbent assays (ELISAs). Results showed occurrence of common microcystins (MC-LR, MC-LA, MC-LY) at low levels in some drinking water reservoirs and higher levels in some recreational waters along with low levels of anatoxin-a and homoanatoxin-a. These results will be valuable for guiding the development of cyanotoxin management and monitoring strategies in the region, and the methodology presented is broadly applicable in other jurisdictions where the problem of freshwater harmful algal blooms is emerging and limited information is available on toxin occurrence.

References:
HISTOPATHOLOGICAL DAMAGE IN SH-SY5Y CELLS AFTER EXPOSURE TO MICROCYSTIN-LR, CYLINDROSPERMOPSIN AND THEIR COMBINATION

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Cyanobacteria are capable of producing several secondary metabolites such as cyanotoxins, being Microcystin-LR (MC-LR) and Cylindrospermopsin (CYN) some of the most common. These toxins are relevant for their ubiquity and their damaging properties, not only as hepatotoxin and as cytotoxin, respectively, but at some other levels in the organism, such as the nervous system. As both toxins have proven to exert neuronal damage in in vitro and in vivo models, the aim of the present work was to evaluate the effects of the exposure to these cyanotoxins, isolated and in combination, can exert in the morphology of SH-SY5Y human neuroblastoma cells. For this, light, phase-contrast and electronic microscopies were used. Our results showed that SH-SY5Y cells exposed to both toxins and their combination evidenced cell death characteristics such as cytoplasm fragmentation, nucleolar segregation, presence of heterophagosomes, chromatin condensation, membrane blebbing, apoptotic bodies formation, protein condensation, etc. The majority of these signs appeared at the highest concentrations assayed for MC-LR and the combination. In the case of CYN, the effects appeared even at lower concentrations. More studies are required to investigate if the structural effects observed are accompanied by functional disturbances.

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Recently, cyanotoxins are at the center of attention due to the widespread occurrence of harmful cyanobacterial blooms. Macrophytes inhabit similar ecological niches as cyanobacteria and seem to be great natural factor removing cyanotoxins. The aim of this research was to verify the resistance of the freshwater plant *Lemna trisulca* to various cylindrospermopsin concentrations to evaluate its potential usefulness in the elimination of the cyanotoxin from the water.

*L. trisulca* was cultivated in the BG11 medium at 20±1°C with 80% humidity and 50µmol·m⁻²·s⁻¹ PAR under a 12h photoperiod with the addition of lower (0.1; 0.5µg·mL⁻¹), similar (1.0µg·mL⁻¹) or greater (2.0; 5.0µg·mL⁻¹) CYN concentrations than the highest documented in the environment. The experiments were performed using HPLC, IEC, Clark’s electrode, conductometer, and analytical balance.

The obtained results showed that *L. trisulca* was relatively resistant to the toxic effect of CYN in concentrations ≤ 1.0µg·mL⁻¹ within 15 days of treatment. Under these conditions, we noted an increased synthesis of chlorophyll-a and carotenoids by an average of 9.11%, and 10.67%, respectively, NO₃⁻, Na⁺, NH₄⁺, and Ca²⁺ ions leak and statistically insignificant changes in the fresh weight accumulation and photosynthesis efficiency. During *L. trisulca* cultivation in media with CYN concentrations above 1.0µg·mL⁻¹, we determined a decrease in the fresh weight accumulation and photosynthesis process, leak of all analyzed ions and cell membrane damage.

Data revealed that *L. trisulca* is resistant to the most frequently detected CYN concentrations, but further research is needed to fully verify its toxicity-limiting properties.

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Cyanobacterial blooms are increasing with climate change and anthropogenic eutrophication of waters. Freshwater cyanobacterial blooms in temperate climates are often dominated by the genera Microcystis, Aphanizomenon or Anabaena, that are producing a large set of toxins (e.g. microcystins and anatoxin-a). While anatoxin-a is a known neurotoxin detected in European freshwaters and responsible for several dog fatalities in 2018, there is little information regarding neurotoxicity of microcystins despite their detection in brain tissue. Additionally, there is an increasing evidence of the ubiquitous, genus-independent, production of retinoids like all-trans retinoic acid (atRA) and its derivatives by cyanobacteria. As dietary vitamins (vitamin A) they are indispensable for vertebrate development, but may cause teratogenicity, as shown in frog (X. laevis) and zebrafish studies.

To study the effects of cyanobacterial retinoids (atRA, 9-cis retinoic acid) and cyanotoxins (microcystin-LR, anatoxin-a) on cell viability and altered neurodifferentiation, we set up a model of differentiating neural stem cells.

IC50-values for acute toxicity (4-day exposure) were derived and sub-cytotoxic concentrations were further tested in a 21-day neurodifferentiation assay. Endpoints evaluated were neurite outgrowth, neuron:glia-ratio, and the expression of differentiation-specific markers, including TuJ1, MAP2 and NESTIN.

Our in vitro model for human (developmental) neurotoxicity testing is sensitive to retinoid-induced differentiation, comparable to growth-factor induced differentiation. Additionally, we observed differentiation stage-specific neurotoxicity of anatoxin-a. This in vitro model can be used to assess human (developmental) neurotoxicity of cyanobacterial metabolites and mixtures in vitro and, together with aquatic vertebrate assays, can complement the toxicity characterization for effective risk assessment of cyanobacterial toxins.

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Occurrence of cyanobacterial blooms is fueled by climate change and anthropogenic eutrophication of freshwater ecosystems. Recently, cylindrospermopsin (CYN), produced by the (sub-)tropical cyanobacterium *Cylindrospermopsis raciborskii*, is being detected in temperate climates with increasing frequency. This increases concern of its potential human health hazard and drives scientific effort to investigate health risks linked to CYN-producing blooms.

The primary exposure route for humans to CYN is oral, leading to hepatotoxic effects. Nevertheless, extrahepatic manifestations of CYN toxicity have been reported and cyanobacterial blooms have been linked to pneumonia-like symptoms and other adverse respiratory conditions. Besides respiratory exposure via toxin-containing aerosols, detection of cyanotoxins in loess crust dust raises the question of a potential hazard of human exposure via inhalation.

We investigated the susceptibility and vulnerability of human bronchial epithelial cells (HBE1 and 16HBE14o-1) to 0.1–10 μM CYN in vitro [1]. Cytotoxicity was evaluated morphologically, by real-time cell analysis and by three metabolic assays with EC50 values ranging between 0.7-1.8 μM (HBE1) and 1.6–4.8 μM (16HBE14o-1). Subsequently, the sub-cytotoxic concentration of 1 μM CYN was tested for its impact on intracellular mitogen-activated protein kinase (MAPK) signaling by western blot detection of ERK1/2 and p38 phosphorylation levels. While only a slight increase in p38 phosphorylation was induced in HBE1 cells, ERK1/2 and p38 activation increased gradually and significantly (p38) in 16HBE14o-1 cells upon exposure for 8-48 h. This study implies possible hazards of cyanotoxin inhalation, which may have a severe impact on epithelial barrier integrity and airway inflammation, thus facilitating the manifestation of respiratory diseases.

References:

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EFFECT OF CYANOPHAGE INFECTION AND LYSIS ON NITROGEN FIXATION AND RELEASE IN THE DIAZOTROPHIC CYANOBACTERIUM APHANIZOMENON FLOS-AQUAE

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Filamentous diazotrophic (N₂-fixing) cyanobacterium Aphanizomenon flos-aquae is a harmful bloom-forming species distributed worldwide in fresh and brackish water ecosystems, which significantly contribute to nitrogen input to the environment. Diazotrophically-derived nitrogen plays an important role in the development of cyanobacterial blooms and sustains toxic cyanobacterial growth, prolonging toxic blooms. Many studies have addressed the effects of different environmental factors on nitrogen fixation, however little is known about viral influence on the dynamics of diazotrophically fixed nitrogen. In this study, the effect of cyanophage infection and lysis on nitrogen fixation and ammonium release was experimentally evaluated with the diazotrophic cyanobacterium A. flos-aquae. We found that although growth of A. flos-aquae was suppressed due to viral infection, neither expression of nifH genes nor the rates of nitrogen fixation were inhibited in the infected cells. This suggests that viruses maintain host diazotrophic activity during infection, possibly redirecting newly fixed nitrogen into the production of viral progeny. Infection and lysis were associated with a significantly higher release of ammonium into the medium compared to the control cultures. Altogether, these results suggest that viruses are important biotic factors in the dynamics and fate of diazotrophically fixed nitrogen.
WATERBORNE CYANOBACTERIA IN THE AIR: EXPOSURE AND EFFECTS

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Toxic compounds produced by cyanobacteria pose hazards to wildlife and human health. Detection of cyanobacterial taxa and cyanotoxins in aerosols and dust particles raises the question of potential hazards associated with human exposures via inhalation, especially during water-related recreational activities. Our study focused on the assessment of (i) potential exposure to freshwater cyanobacteria and cyanotoxins via inhalation and (ii) potential adverse effects in human lungs using Beas-2B cell line originating from normal human bronchial epithelium. In the warm and dry summer 2018, the air was sampled at 4 cyanobacteria-contaminated water bodies and one reference locality with background levels of cyanobacteria using a high volume active sampler equipped with TSP (total suspended particulates) inlet. To create an aerosol similar to that people can be exposed during water-related activities (e.g. paddling), we used a water mill-like device specifically developed for this purpose. Furthermore, water and cyanobacterial biomass were sampled. Cyanotoxins, including microcystins, cylindrospermopsin, anatoxin etc. were analyzed in both air and water samples. Different extraction methods were chosen to best estimate the potential exposure of human bronchial epithelium and resulting health outcomes. Intracellular fraction of cyanotoxins and cyanobacterial compounds originating from waterborne cyanobacteria had more profound effects on cytotoxicity of Beas-2B cells than the extracellular fraction. Further, effects of extracellular and intracellular fraction of cyanobacteria-contaminated water, water concentrated by reverse osmosis and cyanobacterial LPS isolated from the biomass on the inflammatory response in Beas-2B will be discussed.

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**THE CHANGES IN NUTRIENTS CONCENTRATIONS FROM ALGAE COAGULATED BY LOESS AND POLYALUMINIUM CHLORIDE**

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The occurrence of harmful algal blooms (HABs) in fresh to estuarine waters is increasing worldwide [1]. There are a number of technologies for removing algae from water and one of the most general approach is using coagulants. In this study, the nutrients concentration changes from algae coagulated by loess and polyaluminium chloride (PACl), which commonly used in Korea to control HABs, were examined for two years. Raw water with chlorophyll-a concentration of 48 μg/L was sampled from Shingal reservoir in Yongin City, Republic of Korea. The optimum doses of loess and PACl (2 g/L and 1 ml/L, respectively) were added to test columns which filled with the raw water. Two coagulated algae columns and a control one without any coagulants dose were stored in quiescent environment at room temperature. The supernatants were sampled each month for pH, turbidity, total nitrogen (TN), total phosphorus (TP), and dissolved nutrients analyses. After two years of analyses, the control column showed a significant TP concentration changes from initial 0.76 mg/L to 10.84 mg/L whereas the values of loess and PACl columns decreased to 0.43 mg/L and 0.12 mg/L respectively. TN concentrations of the control and PACl decreased from 18.2 mg/L to 2.14 mg/L and 11.8 mg/L, but loess showed a slight increase to 20.1 mg/L. Certainly, there were concentration changes during the experiment period and it is likely that the chemical characteristics of coagulants are related to the results. The results of this study highlight the potential nutrients release from coagulated algae that require more environmental algae removal technologies like dissolved air floatation.

**References:**

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CAN PHOSLOCK® BE USED TO BIND AND SEDIMENT MICROCYSTIN-LR IN AQUATIC SYSTEMS?

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Microcystins [and its congener microcystin-LR (MC-LR)] are considered the most common cyanotoxin produced by cyanobacterial harmful algal blooms. The intracellular MC-LR is released into the water column upon cell lysis during treatment or senescence, where it can remain stable for weeks and lead to adverse health effects. The ideal treatment method for MC-LR-producing strains is one that can remove or degrade both cells and toxins from the water column. Many treatment methods result in only cell lysis and consequent release of toxins; therefore, targeted toxin removing methods are desired. We tested the hypothesis that Phoslock® can bind to MC-LR, removing it from the water column by sedimentation. In order to test this, MC-LR was dissolved in deionized water and diluted to 5, 20, 50, 100, and 500 ppb. A stock solution of Phoslock® (1 gL⁻¹) was used and kept homogenized on a magnetic stirrer and applied into solutions containing MC-LR at concentrations of 50, 100, and 150 ppm (in triplicate) including a control without Phoslock®. The tubes were incubated at room temperature for 24-48hr, and then processed for MC concentration using ELISA. Results indicate that MC-LR is removed from the water column when higher concentrations of both Phoslock® (100 and 150ppm) and MC-LR (100 and 500ppb) are present. Future work aims at deciphering the in situ effects of Phoslock®, in combination with other algaecides and flocculants to improve the efficacy of toxin removal.

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AN UNUSUAL PLP-DEPENDENT AMINOTRANSFERASE
PUTATIVELY INVOLVED IN THE SYNTHESIS OF ANATOXIN-A(S)
IN SPHAEROSPERMOPSIS TORQUES-REGINAE ITEP-024

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Anatoxin-a(S) (ANS) is an organophosphate acting as a potent and irreversible inhibitor of acetylcholinesterase in peripheral nervous cells of mammals. Although the structure of ANS was elucidated and some of the biosynthetic steps have been proposed, no candidate genes have been identified. We sequenced and assembled the genome of the ANS-producing Brazilian strain S. torque-reginae ITEP-024 and a gene cluster potentially involved in this toxin biosynthesis was annotated. The hypothetic ANS gene cluster encodes nine biosynthetic enzymes (AnsA-I) and here, we report the structural characterization of AnsB, an unusual aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme that is proposed to catalyze the second step of ANS biosynthesis. The coding sequence (1,242 bp) of enzyme was optimized for heterologous expression in E. coli (GenScript). The synthetic ansB was sub cloned into the pET28a(+) kanamycin resistant expression vector (GenScript), cloned into BL21(DE3) cells and over expressed in TB medium. After protein purification, diffracting crystals were obtained by hanging drop vapor diffusion and the structure was solved to a resolution of 2.1Å. The AnsB protein consists of four monomers (370 residues) and PLP is bounded to Lys219 residue. AnsB has a high protein structure homology with OrfR, which is an enzyme responsible for the cyclization of a dihydroxylated arginine substrate in the biosynthesis of streptolidine. Based on that, we proposed AnsB is a PLP-dependent cyclase that catalyzes cyclization of a modified arginine on route to enduracididine.
INVESTIGATION OF POTENTIAL ENDOCRINE DISRUPTING ACTIVITIES OF CYANOBACTERIAL GENERA MICROCYSTIS AND PLANKTOTHRIX.

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Cyanobacteria produce many bioactive metabolites, potentially including endocrine disrupting compounds. Endocrine disruptors represent potential risks to both environmental and human health, making them a global challenge. Knowledge gaps, including mechanisms of action, still exist. We screened extracts from a collection of 26 Microcystis and Planktothrix strains for agonist and antagonist activity in an estrogen-responsive reporter-gene assay (RGA). The cyanobacterial strains included both microcystin-producing and non-microcystin-producing strains. Cytotoxicity tests (MTT) were performed in parallel. None of the extracts showed a significant estrogenic activity at the nuclear receptor-level at the tested concentrations. Extracts presenting some low estrogenic response included both microcystin-producers and non-microcystin-producers, indicating that the observed weak effects were not only related to these cyanotoxins. We further tested the extracts using the E-screen assay, which is a cell-proliferation assay using MCF-7 cells. The E-screen supported the conclusions that the extracts have a low or no estrogenic effect. Endocrine disruptors can work via a number of mechanisms, not only by acting on receptors. They may also affect the levels of active hormones by interfering with hormone biosynthesis and biotransformation. We thus investigated the possible interference of pure microcystin-LR or a Microcystis aeruginosa (PCC7806) extract on 17-β-estradiol metabolism using human liver microsomes. Preliminary results showed significant impact on the microsomal oxidation of estradiol of both microcystin-LR and the PCC7806 extract, indicating so far that possible endocrine disruptors from cyanobacteria could act via different mechanisms.

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EFFECT OF MICROCYSTIN-LR ON HUMAN PROBIOTICS

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It is well known that humans have symbiotic relationships with trillions of microorganisms, inhabiting specific habitats of the body, which complement our metabolic capabilities. The involvement of human microbiome (MB) in the metabolism of natural and synthetic xenobiotics might modulate their exposure-response relationships through a few general mechanisms. There is also a potential for direct effects of xenobiotics on the composition and functionality of the microbiota itself.

Negative effects of cyanotoxins (CTX) like microcystins (MC) have also been observed on the physiology of heterotrophic bacteria, in a congener- and species-specific manner. Since humans can be exposed to CTX and cyanobacteria (CB) mainly via the oral route, there is a possibility, to be still clearly demonstrated, that CB colonize the intestine. Humans would then be chronically exposed to internal low but repeated doses of CTX.

The role of MB becomes a fundamental aspect to determine individual variability/susceptibility to CTX, which is not only due to genetic polymorphism. Preliminary results from our research group demonstrated the plausibility of the colonization hypothesis, by showing the resistance of Microcystis PCC7806 to gastric pH2, its survival in the dark, at 37°C with Bacillus clausii and the continuous production of MCs. Based on these results, we hypothesize that the MCs produced by CB cells can affect the intestinal MB and in turn can be modified by it, changing their bioavailability.

Preliminary results on the effects of MC-LR on physiological and morphological parameters of commercial human probiotics will be discussed in the framework of CTX risk assessment.
WATER POLLUTION POLICE: ON-LINE SYSTEM FOR CONTROLLING OF (ILLEGAL) WATER POLLUTION EVENTS AS A COMPONENT OF CYANOBACTERIAL WATER BLOOMS PREVENTION

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Management of cyanobacterial water blooms require precise knowledge on the ecology of cyanobacterial dominant species, nutrients control system in catchments and long-term and even high cost measures. However, our experiences around the globe proved, that promises of prevention and order in treatment and nutrients removal are not kept and expensive ecotechnical measures for blooms prevention are depreciated, because accidental events of water pollution occur. Detection and quantification of a random or periodical pollution events is possible by commercially available multiparametric sonds like In-situ, YSI etc., but the insurance is impossible or really expensive and risks of loss is high (experience not only from Africa, or South America, but also Europe). That is why we developed small "invisible sensors", which can be used for on-line sending data from in-situ exposure and prove illegal sources of water pollution. We have 3 models, which are able to measure and send data like temperature, conductivity, oxygen, chlorophyll, phycocyanin, pH or turbidity. Up today we have several evidences of overnight waste pumping in to river, unexpected release of cyanobacterial blooms from fish pond in to drinking water reservoir, or flash-rain signalization for quick switch of dry pond use and similar incidents which can help to preserve good water quality and prevent mass development of cyanobacterial water blooms. Our experience proved, that only a publicity of this system utilization in the region prevent other waste pollution activity. Sensors are flexible and can be also used for precise monitoring of autecology of dominant cyanobacteria forming water blooms or for vertical and spatial distribution of cyanobacterial biomass in reservoirs.
Cyanobacteria and their associated cyanotoxins pose a serious threat to wildlife, pets, livestock and humans exposed to contaminated water, fish and algal dietary supplements. As new regulations and monitoring programs are adopted worldwide there is an increased need for accredited analytical testing of cyanotoxins. A major impediment to the development of accredited test methods has been the limited availability of cyanotoxin certified reference materials (CRMs). CRMs facilitate accurate calibration with traceability in compliance with laboratory testing quality standards (e.g., ISO 17025) and are valuable for method development and validation. Such quality control ensures comparability between measurements carried out at different times or in different laboratories. The development of CRMs for cyanotoxins is particularly challenging because of the large number of toxin variants that must be monitored. The National Research Council of Canada (NRCC) has produced a number of publicly-available cyanotoxin CRMs for instrument calibration including microcystins (MC-LR, [Dha]<sub>7</sub>MC-LR, MC-RR), cylindrospermopsin, anatoxin-a, nodularin and saxitoxins. Feasibility studies have been conducted in support of matrix reference materials for cyanotoxins in environmental samples and dietary supplements. This presentation will provide an overview of the R&D that has gone into cyanotoxin CRMs at NRCC, outlining key steps in their preparation including toxin production and isolation, method development, purity testing, stability testing, and accurate quantitation. Metrological traceability to The International System of Units (SI) and uncertainty are key features of NRCC CRMs. National and international collaborations will be highlighted along with future objectives.
COULD LAKE WINNIPEG BE THE NEXT LAKE ERIE?
A MULTIPLEX ANALYSIS OF SEASONAL VARIATION OF POTENTIALLY TOXIC CYANOBACTERIA IN LAKE WINNIPEG

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Lake Winnipeg (Manitoba, Canada), the world’s 12th largest lake by area, is host to yearly cyanobacterial harmful algal blooms (cHABs) dominated by Aphanizomenon and Dolichospermum. cHABs in Lake Winnipeg are primarily a result of eutrophication but may be exacerbated by the recent introduction of dreissenid mussels. The invasion of dreissenids into Lake Erie has been hypothesized to be one factor promoting the toxic Microcystis blooms currently seen in the western basin. Prior to the invasion, Lake Erie cHABs were a mixed community of Microcystis, Aphanizomenon and Dolichospermum. Using methods to monitor the potential for toxin production in Lake Winnipeg in conjunction with environmental measures, this study defined the baseline composition of a Lake Winnipeg cHAB to measure potential changes due to dreissenid colonization. Surface water samples were collected in 2013 from 23 sites during summer and 18 sites in fall. Genetic and mass spectrometry cyanotoxin profiles identified microcystins (MC) as the most abundant cyanotoxin across all stations, with MC concentrations highest in the North Basin. In the fall, mcyA genes were sequenced to determine which species had the potential to produce MCs, and 12 of the 18 sites were a mix of both Planktothrix and Microcystis. Current blooms in Lake Winnipeg produce low levels of MCs, but the capacity to produce cyanotoxins is widespread across both basins. If dreissenid mussels continue to colonize Lake Winnipeg, a shift in physicochemical properties of the lake due to faster water column clearance rates may yield more toxic blooms potentially dominated by microcystin producers.
ORAL SUBLETHAL MICROCYSTIN-LR DOES NOT AFFECT PULMONARY MECHANICS BUT COMPROMISES MITOCHONDRIAL FUNCTION


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Microcystins (MCs) are cyanobacterial hepatotoxic cyclic peptides reported in human intoxication outbreaks. Among routes of exposure, ingestion is of paramount importance because these toxins might be present in drinking water. Although the lung is not the main MCs target, the toxin can reach the respiratory system after being ingested, absorbed, carried into the liver, and into the inferior vena cava. Interestingly, mitochondria serve as relevant markers of intoxication in several organs. Moreover, mitochondrial dysfunction is usually associated to several pulmonary diseases. Thus, we aimed to assess lung mechanics and mitochondrial function after the administration of sublethal doses of MC-LR during 20 consecutive days. The study was approved by our institutional Ethics Committee on the Use of Animals (082/17). BALB/c mice were divided into two groups (n=7) exposed by oral gavage: TOX (30 μg MC-LR/kg/day in water) and CTRL group (water). The animals were then anesthetized, paralyzed and mechanically ventilated for the determination of lung mechanics. Further, other two groups (n= 4 in each) were exposed to assess mitochondrial function by analysis of the ADP-stimulated respiration, swelling, transmembrane potential, ROS and ATP production. Lung mechanics did not significantly changed in animals exposed to MC-LR. However, mitochondrial ROS increased significantly, mitochondrial respiration fell and the membrane depolarized. Conversely, neither mitochondrial swelling or changes in ATP production were observed. Thus, mitochondrial damage markers revealed that under the present experimental conditions microcystin-LR affected cell bioenergetics and antioxidant defenses, even before jeopardizing lung function.

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This talk is devoted to General Principles and Methods of Mathematical Modeling and Computer Simulations in biology and other sciences according to [1]. This textbook [1] is intended for readers who want to understand the main principles of Modeling and Simulations in settings that are important for the applications without using profound mathematical tools required by most advanced texts. It can be useful for beginning applied mathematicians who use Mathematical Modeling. Our goal is to outline Mathematical Modeling using simple mathematical description that makes it accessible for first- and second-year students (undergraduate courses for bachelor's degrees). This book consists of three parts. Part I "General Principles and Methods" is an elementary introduction to Mathematical Modeling based on the introductory mathematical courses. We think that this is the main part which should be worked out by a beginner in Applied Mathematics and other sciences addressed to Mathematical Modeling. There are general principles, methods and tricks of Mathematical Modeling used in different sciences. Only few textbooks contain such a systematically presented general approach to Mathematical Modeling. First, it is worth to study general principles of Mathematical Modeling and after to discuss examples from mechanics, biology etc. The didactic principle of primary introduction of examples and further of a theory narrows down applications of Mathematical Modeling.


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Cylindrospermopsin (CYN) is a 415 Da alkaloid produced by a range of cyanobacteria. Previous studies have described CYN as a protein synthesis inhibitor and capable of damaging several organs in mammals. Regarding endocrine disruptor activity, CYN was shown to induce estrogenic activity as agonists in yeast. In addition, it was shown to inhibit progesterone synthesis and deregulates the female estrous cycle in mice. However, little is known about its mechanisms of toxicity in fishes. This study aims to characterize the effects of CYN (1000 µg/L) in the male reproductive system. We used an ex-vivo tissue culture of adult zebrafish (Danio rerio) testis to assess morphology integrity as well as the number of spermatozoa cells and, transcript abundance of genes related to spermatogenesis. The results demonstrate that CYN exposure causes a decrease in the number of spermatozoa cells. Furthermore, the results provide evidence that CYN decreases transcript abundances of genes such as 3β-HSD and IGF3 both involved in spermatogenesis. These results provide novel evidence that CYN acts as an endocrine disruptor and inhibits the development of spermatozoa cells.
Freshwater ecosystems have recurrent demands such as drinking, water provision, irrigation and recreation. Cyanobacteria as natural inhabitants of those ecosystems require a continuous vigilance because they may produce dense harmful blooms and insert harmful cyanotoxins in the water representing an important vehicle of contamination to humans and wildlife. Under the scope of a Portuguese national funded project CYANOTOX relevant freshwater ecosystems of Portugal were monitored for the presence of cyanobacteria and cyanotoxins. A two-year sampling campaign has showed through field observation that bloom frequency is higher in warmer years. Molecular and immunological data highlighted that there is still the prevalence of microcystins combined with the proliferation in the North and Center Regions of the recent reported cylindrospermopsins and also of anatoxin-a and saxitoxins. Therefore our data support the evidence that climate change and anthropogenic activities may contribute to increase the risks of exposure to cyanobacteria. Finally our study reinforces the need of intensifying cyanobacteria and cyanotoxins monitoring programs in Portuguese freshwater ecosystems in order to proper implement a demanded Portuguese risk assessment strategy.

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EXPANDING THE HORIZON!
ANALYSIS OF CYANOBACTERIAL TOXINS BY UPLC/MS/MS DETECTION USING A UNISPRAY ION SOURCE

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Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Microcystins, in particular, represent an emerging class of algal toxins of concern to the drinking water industry. Recognizing the potential health risk, the World Health Organization, and other nations throughout the world have established guidelines for the amount of microcystins permissible in drinking water.

Typically electrospray ionization (ESI) is the mode of ionization used for these compounds. In this paper we investigate the use of a new and novel ion source (called UniSpray) which allows for enhanced sensitivity of these compounds (as well as a variety of other compounds). This source allows for greater ionization efficiency as well as providing a rugged interface. Comparison of microcystins, anatoxin-a, and cylindrospermopsin in various matrices to traditional ESI and Unispray ionization will be shown. Data from various toxin blooms in the United States will be shown and discussed using both techniques.
“I SMELL TROUBLE”: CYANOBACTERIAL VOLATILE ORGANIC COMPOUNDS (VOCS) AFFECT THE ROOT OF ARABIDOPSIS THalianA

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Algal volatile organic compounds (VOCs) are metabolites or by-products of cell degradation processes, produced by cyanobacteria and eukaryotic algae. Their presence in water is often detectable by the human senses, olfaction and gustation in particular. Many VOCs are known for their biological activity, affecting other aquatic organisms and ecosystems in general, and thus monitoring of water destined for human consumption, as well as toxicological testing are deemed necessary [1, 2]. Although bacterial volatile compounds have been proven to variably affect plant growth [3] and despite the fact that cyanobacterial VOCs could easily become accessible to crops via irrigation, data on the effects of such compounds on plant cells are currently limited. In the present study, six cyanobacterial VOCs (β-cyclocitrail, β-ionone, 2, 4-decadienial, dimethyl disulfide, cis-3-hexen-1-ol and 6-methyl-5-hepten-2-ol) were tested, by treatment with various concentrations (10, 10², 10³ and 10⁴ μM) on seedlings of the model plant Arabidopsis thaliana, to assess possible effects on root growth and/or cell organelles, such as the cytoskeleton. In some cases, exposure to the highest concentration led to striking effects on root growth and/or cell organelles, such as the cytoskeleton. In some cases, exposure to the highest concentration led to striking effects on root growth and development (such as growth inhibition, or even loss of gravitropism in the case of 6-methyl-5-hepten-2-ol) and/or root cells (such as alterations in microtubule organization), while seedlings appeared to be unaffected by lower concentrations. Ultrastructure of treated root cells was examined with transmission electron microscopy and the results are also discussed.

References:
DYNAMICS OF CYANOBACTERIA AND ENVIRONMENTAL CONDITIONS IN BRAZILIAN SODA LAKES


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Soda lakes occurring in several regions of the world are extreme environments due to elevated pH, salinity and NaHCO₃ content. In Brazilian Pantanal biome, hundreds of shallow soda lakes can be found coexisting side by side with hundreds of freshwater lakes. Unique chemistry and microbiological compositions influenced by the water evaporation/precipitation dynamics determine the different conditions found across these lakes. Here, we evaluated water chemical variables, and microbial composition and functioning patterns (metagenomic sequencing) in three distinct lakes from the sub-region Nhecolândia, state of Mato Grosso do Sul, Brazil. One lake was characterized by permanent cyanobacterial bloom (green water lake - GWL), while the others, black water lake (BWL) and crystalline water lake (CWL), were characterized by the absence of cyanobacterial blooms. The GWL had a dense biomass of Arthrospira sp. and/or Anabaenopsis elenkini, which alter local abiotic parameters such as pH, turbidity, and dissolved oxygen and consequently the overall structure of the microbial community. The variables such as pH, total phosphorus, nitrogen, and electric conductivity were the main factors driving the formation of blooms. Otherwise, the lakes without blooms (BWL and CWL) had mineralized waters, with low nutrient concentration associated with presence of unicellular genus (Synechoccus, Prochlorococcus and Cyanobium). In these two lakes, the microbial community had a high structured taxonomic profile. Distinct signatures in the abundance of genes associated with the cycling of carbon, nitrogen, phosphorus and sulfur were found together with high amount of genes associated with stress condition.
Western Lake Erie (WLE) experiences anthropogenic eutrophication and annual, toxic cyanobacterial harmful algal blooms (cyanoHABs). Recent blooms in 2011 and 2014 had enormous economic impacts estimated at $65 million [1] and caused the 2014 Toledo, Ohio drinking water crisis [2]. While we can forecast the seasonal bloom magnitude and the short-term movement of the bloom, we cannot forecast bloom toxin concentrations. In order to develop a cyanoHAB toxin concentration forecast for the WLE, microcystin production rates are needed. Throughout summer 2018, we conducted 11 microcosm experiments with lake water collected from two locations (nearshore and offshore). In 2.4-L clear polycarbonate bottles, lake water was incubated dock-side under in situ light and temperature at ambient nutrient concentrations (control) and elevated phosphorus (1 µM) and nitrogen (100 µM) concentrations. Bottles were subsampled daily for 72 hours for measurements of microcystins (ELISA and LC-MS/MS), cyanobacteria-chlorophyll a (FluoroProbe), and mcyE and 16S gene copies (qPCR). For all experiments, control chlorophyll production rates ranged from 0.02 to 2.93 µg/L/d, and microcystin production rates ranged from 0.01 to 0.27 µg/L/d. The +P&N enrichment increased chlorophyll production from 0.07 to 9.28 µg/L/d and microcystin production from 0.05 to 1.33 µg/L/d. Overall, production rates decreased throughout summer. Quantification of mcyE will allow us to determine microcystin production rate per toxin gene copy. Our experimental-based approach will be compared to a model-based approach that uses microcystin concentrations measured in the lake. A cyanobacterial bloom toxin concentration forecast will allow water treatment and resource managers to better prepare for toxic water.

References:
Microcystins (MC) are one of the most common toxins associated with drinking water and food supplements, and their proper hazard and risk assessment is of importance for human health. Apical toxicity is characterized by MC toxicokinetics and –dynamics. Although it is known that cellular uptake is facilitated through organic anion transporting polypeptides (OATPs) and toxicodynamics are governed by the specific inhibition of serine/threonine protein phosphatases (ser/thrPPP), toxicokinetics and –dynamics differ greatly between organs and MC congeners. Moreover, toxicokinetic and –dynamic data are available for only a very few of the currently 248 known MC congeners [1]. As a first step we determined the toxicodynamic properties of 18 different (naturally occurring and synthetic) MC congeners i.e. the MC congener inhibition capacity in recombinantly expressed ser/thrPPPs (PPP1-5). Results demonstrate a MC congener specific inhibition of ser/thrPPP, whereby ser/thrPPP differed in their susceptibility to individual congeners. As a second step we studied the effect of single and multi-exposures in HEK293-OATP1B1 and OATP1B3 cells to different MC congeners, whereby apical toxicity, ser/thrPPP inhibition and the role of the intracellular glutathione conjugation as MC detoxification was assessed. As MC congener uptake and MC-GSH conjugation kinetics would compete with the kinetics of ser/thrPPP, the latter should allow to elucidate MC congener specific contributions of the toxicokinetic and –dynamics to the apical toxicity observed.

References:

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SENSITIVITY OF DAPHNIA PROTEIN PHOSPHATASES TOWARDS MC-LR

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Microcystins (MCs) have been shown to inhibit ser/thr protein phosphatases (ser/thr PPP) across habitats and kingdoms [1]. During harmful cyanobacterial blooms, zooplankton is exposed to, and sometimes actively feed on, toxic cyanobacteria and thus are exposed to MCs [2]. Although ser/thr PPP are phylogenetically highly conserved, differences in type and susceptibility of expressed ser/thr PPP to MCs may result in differences in the sensitivity of Daphnia species toward MC intoxication. This may impact population dynamics and species composition during and succeeding harmful algae blooms. To elucidate the latter, we employed DNA sequence analysis of putative ser/thr PPP and a protein phosphatase inhibition assay (PPIA). In a first step we isolated DNA and RNA and generated protein homogenates in parallel from Daphnia magna and Daphnia galeata. Using gene specific primers in PCR and subsequent sequencing we analyzed intron/exon borders of genomic ser/thr PPP. Via RT-PCR and cloning we isolated ser/thr PPP cDNAs and subsequently analyzed amino acid sequence. Activity of ser/thr PPP activity in Daphnia extracts was tested using ser-14-32P-labelled phosphorylase a based assay specific for ser/thr PPP. In a second step we repeated the procedure with Daphnia pulex and Daphnia longispina and compared all four species with regard to their genomic ser/thr PPP, variations in amino acid sequences of the enzymes and susceptibility of the respective ser/thr PPP to inhibitors e.g. MC-LR. Comparing activity and inhibition data with the respective amino acid composition may reveal the reason for specific differences in susceptibility towards MC and its impact on population dynamics.

References:

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INNOVATIVE APPROACHES FOR THE STUDY OF BIODIVERSITY AND WATER QUALITY ASSESSMENT IN THE ALPINE REGION: THE INTERREG ALPINE SPACE PROJECT ECO-ALPSWATER


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The adoption of innovative approaches for monitoring and safeguarding lake and river ecosystems is the object of a new project - Eco-AlpsWater - co-financed by the European Regional Development Fund through the Interreg Alpine Space programme. The project, which began in April 2018, will last three years, involving 12 partners belonging to 6 countries in the Alpine region (Austria, France, Germany, Italy, Slovenia and Switzerland). One of the main objectives is to develop and apply state of the art methods for the monitoring of cyanobacteria and bacteria, microalgae and fish based on the use of High Throughput Sequencing (HTS) techniques, complementing traditional approaches and anticipating the route in the development of new generation water monitoring systems. Owing to the ability to produce a wide variety of toxins, a specific attention is given to the identification of potentially toxigenic cyanobacteria. Traditionally, their monitoring in aquatic ecosystems was based on microscopic examinations. Nevertheless, traditional approaches suffer many drawbacks due to limitations in the correct microscopic recognition of diacritical characters. The new HTS technologies are providing a comprehensive picture of taxonomic composition and biodiversity of cyanobacteria and other biological elements in the Alpine region based on the analysis of samples collected in over 50 lakes and rivers. In order to evaluate general patterns in cyanobacteria composition related to cyanotoxin production, the survey is completed by the concurrent examination of sample aliquots for the determination of a wide variety of cyanotoxins in water samples and biofilms in lakes and rivers.
MULTIHAPТЕN ANTІBODY BASED DETЕCTІON OF МІRCОСYТИНS AND NОDULARIN USІNG ELISA AND IMMUNОАFFІНITY COLUMNs

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Microcystins (MCs) are toxic cyclic peptides produced by cyanobacteria worldwide. Due to their hepatotoxicity, it is important to have cheap, rapid and reliable methods for analysis available to protect livestock and people from acute or chronic exposure. A multihapten approach was used to raise new polyclonal antibodies, with the aim of developing antibodies recognizing as many known and unknown toxic MC congeners as possible with equal affinities. These antibodies, raised in sheep with a carefully selected mixture of microcystins conjugated to cationised bovine serum albumin, were used to develop a new competitive ELISA [1]. Another important ELISA-component was the plate coater [Asp3]MC-RY conjugated to ovalbumin. This approach was chosen to minimize specificity for any particular MC analog and to reduce the detection of ADDA-containing degradation products.

Cross-reactivity studies indicate a broad specificity to microcystins and also detection of nodularin. The limit of quantification is 0.04 µg/L in drinking water, well under the WHO’s maximum recommendation of 1 µg/L. Together, these provide a rapid, sensitive and specific analytical method for screening large numbers of samples. The antibodies have also been used in immunoaffinity columns (IACs) and these may enhance detection and quantitation by LC-MS analysis. Preliminary studies show that IACs using these antibodies were highly effective for sample cleanup, with high specificity for MCs and excellent recoveries.

The use of these antibodies in ELISAs and IACs will be illustrated by analyses of standards, cultures, natural bloom, bird liver, crayfish and dietary algal supplement samples.

References:
COMBATTING HARMFUL CYANOBACTERIA WITH HYDROGEN PEROXIDE IS MORE EFFECTIVE AT HIGH LIGHT

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A promising short-term method to selectively combat harmful cyanobacterial blooms in lakes is the application of a low concentration of hydrogen peroxide (H₂O₂). Cyanobacteria are much more sensitive to H₂O₂ than eukaryotic algae, because cyanobacteria use a Mehler-like reaction that does not produce H₂O₂. We have applied H₂O₂ treatments to several cyanobacteria-dominated lakes in The Netherlands since 2009. Currently, we investigate under which environmental conditions the H₂O₂ treatment method will be most successful.

To study effects of light on the effectivity of the H₂O₂ treatment, we performed lab experiments with the toxic cyanobacterium Microcystis aeruginosa PCC7806. The axenic strain was cultured in chemostats and samples of the steady state cultures were subjected to different H₂O₂ concentrations and light conditions in 24 hour experiments in batch cultures.

Our results show that light has a very strong influence on the effectivity of the H₂O₂ treatment. In the dark, cells were hardly affected by H₂O₂, while cells were increasingly more affected under increasing light conditions. H₂O₂ was not degraded faster by cells exposed to light compared to cells exposed to darkness. Furthermore, extracellular microcystin concentrations increased with increasing H₂O₂ concentrations and higher light intensities, while the sum of intracellular and extracellular microcystins decreased after H₂O₂ addition.

In conclusion, harmful cyanobacteria are more sensitive to H₂O₂ at higher light intensities. Hence, H₂O₂ treatments of lakes should be performed preferably on sunny days, when the treatment is already effective at a low dosage. Conversely, treating lakes after sunset or at night is not recommended.

References:
DETOXICATION OF MICROCYSTINS MEDIATED BY HUMAN GSTs: COMPARISON AMONG VARIANTS WITH DIFFERENT HYDROPHILICITY

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It has been hypothesized that kinetics can be a key determinant of (MC) congener-specific toxicity. The in vitro inhibition potency of PP1/PP2A by single MC is comparable: therefore, the toxicokinetic of MC seems to be the critical point to explain congener-dependent toxicity. Those variants, such as MC-LW and MC-YR, having hydrophobic amino acids (e.g. tyrosine, tryptophan) may be more cell permeable than MC-LR. Glutathione conjugation, occurring either spontaneously or catalyzed by GST, is the accepted main step in MC detoxification. Recently, we characterized the in vitro human conjugation of MC-LR and MC-RR showing some differences in the presence of GSH depletion. This study was carried out to understand possible dependence of detoxication reaction on lipophilicity.

Using single human hepatic recombinant isoforms (GSTA1, A2, A4, M1, T1 T2, P1, and O1) and human liver cytosol (HLC, pool of 200 donors) the kinetic parameters Vmax, Km and Cli were calculated. The efficiencies of recombinant GSTs used are quite similar (0.022-0.066 pmolGS/MC-LW/(μgprot*min*μM and 0.048-0.09 pmolGS/MC-YR/(μgprot*min*μM); the highest Cli were shown by GSTP1 and A1 for MC-LW and P1=O1>A1 for MC-YR. Since GSTA1 is the most abundant hepatic GST, it is expected to give the highest contribution to MCLW and MCYR detoxification. Using the HLC a typical saturation curve was evidenced for MC-LW whilst the reaction was still linear for MC-YR. Besides the differences in the kinetic behavior, comparing in the Cli of MC-LR, MC-LW, MC-YR and MC-RR, the variants which is most efficiently detoxified is MC-RR, which is the least acutely toxic.

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EFFECT OF HYDROGEN PEROXIDE ON A NATURAL PHYTOPLANKTON COMMUNITY AND EVALUATION OF ITS RECOVERY

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Cyanobacterial blooms are compromising water quality worldwide. Their prevention requires the limitation of nutrient inputs. Once established, however, other strategies are used to control the growth of cyanobacteria, such as oxidation processes mediated by H2O2. Some studies have described that cyanobacteria are more sensitive than green algae to this treatment but few studies have assessed the recovery of planktonic communities after H2O2 exposure. Here, we tested the effect of different concentrations of H2O2 (1, 10 and 100 mg L⁻¹) on the survival of a phytoplankton community from a freshwater reservoir dominated by M. aeruginosa. Chlorophyll concentrations [Chl] were determined using Phyto-Pam. Before H2O2 addition, cyanobacteria (29 μg L⁻¹) contributed to most of the [Chl] compared to green algae (14 μg L⁻¹). After 48h, [Chl] decreased in control, 1 and 10 mg L⁻¹ treatments (~10 μg L⁻¹). Cyanobacteria maintained dominance in the 10 mg L⁻¹ treatment (59%). In the 100 mg L⁻¹ treatment, [Chl] decreased to 1.9 μg L⁻¹ with dominance of green algae (86%). After 7 days, only green algae were detected in control, 1 and 10, but not in 100 mg L⁻¹. To test the recovery of the remaining community, after the 7-day treatments ASM-1 was added and [Chl] was followed for two weeks. Green algae were able to grow in the control, 1 mg L⁻¹ and especially 10 mg L⁻¹ treatments, with a higher [Chl]. In contrast, cyanobacteria were more susceptible and unable to grow in all conditions. We have also recovered the heterotrophic bacteria of these treatments to perform metagenomics analysis and evaluate their susceptibility to H2O2 exposure.

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MICROCYSTIN DRIVES THE COMPOSITION OF AN ULTRA-SMALL BACTERIAL COMMUNITY IN A NATURAL LAKE

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Cyanobacterial blooms affect biotic interactions in aquatic ecosystems, including those in microbial communities, but the complexity of these interactions are still little explored. Among these, it is the biodegradation of cyanotoxins by some heterotrophic bacteria specially studied for the cyclic peptide microcystin (MC), due to its widespread occurrence in freshwater. In this study, we investigated MC-biodegradation by the bacterial community of a coastal lagoon historically contaminated by this toxin, focusing in an ultra-small bacterial community (USBc).

Water samples were collected in different dates from Jacarepaguá lagoon (Rio de Janeiro, Brazil). Microbial communities were recovered by centrifugation and separated into two size fractions by filtration (<0.45 μm and <0.22 μm). Each fraction was incubated with MC-LR (~20 ng.mL⁻¹) for 7 days at 25 °C. MC-LR concentrations were determined by LC-MS/MS and the toxin was degraded in all cases. The bacterial communities present at days 0 and 7 were characterized by 16s rRNA sequencing and transmission electron microscopy. As control, we evaluated both fractions maintained for 7 days without MC-LR. Microscopy revealed a few bacteria in each fraction at time 0 and uncommon morphologies in <0.22. In addition, diverse morphologies were observed in each fractions with larger cells and in a higher abundance after 7 days with MC-LR. Sequencing revealed that the presence of MC-LR changed the community composition and led to an enrichment of certain taxa, especially Methylophilales. The same taxa were present in both size fractions. To our knowledge, this is the first study exploring MC-LR biodegradation by USBc.

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MICROCYSTIS AERUGINOSA AS A SUITABLE MODEL SPECIES FOR STUDY OF A RETINOID BIOSYNTHETIC PATHWAY

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Retinoid cyanobacterial products constitute hazardous chemicals with possible teratogenic effects. However, little information is available about their biosynthesis on the genomic level. Hence, the main objective of this study was the identification of suitable model cyanobacteria for study of retinoids biosynthesis. Earlier, we used P19/A15 reporter cell lines for the measurement of total retinoid-like activity in extracts of both freshwater blooms from Czech water bodies and in vitro cultivated cyanobacterial species. Our results showed significant retinoid-like activity in blooms dominated by Microcystis aeruginosa, where maximal retinoid equivalent (REQ) reached 2838 ng of REQ/g dry mass. Analyses of Microcystis aeruginosa in vitro culture showed similar results, whose biomass extract evinced significant retinoid-like activity reaching up to 1837 ng REQ/g dm. We also inspected 22 different cyanobacterial genomes for the presence of genes functionally related to Homo sapiens retinal dehydrogenase (RALDH - an enzyme responsible for the conversion of retinal to retinoic acid). Phylogenetic analysis of found genes revealed only one functionally unknown dehydrogenase in Microcystis aeruginosa PCC 7806. Therefore, our research identified Microcystis aeruginosa as a suitable species for the investigation of retinoids biosynthesis in cyanobacteria. A consequent step in our research is the expression and functional analysis of identified dehydrogenase.

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IMPACT OF WATER CYANOBACTERIAL STRAIN OSCILLATORIA K3 ON GENE EXPRESSION IN ZOOPLANKTON DAPHNIA MAGNA

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Although the fate and ecological impact of cyanotoxins in aquatic environments have gained increasing concern, limited information is provided about toxicity mechanisms of these bioactive compounds to aquatic invertebrates. In the present study, the toxic effects of an intracellular cyanobacterial extract of Oscillatoria sp. strain K3 on Daphnia magna were examined by studying the changes in the gene expression of five genes (vitellogenin (Vtg), glutathione S-transferase gene (GST), P-glycoprotein gene (P-gp), cytochrome P450 360 family (CYP360A8) and cytochrome P450 mitochondrial family 314 (CYP314)). Quantification of relative changes in the gene expression was evaluated using the method of real-time polymerase chain reaction (RT-qPCR).

The results showed that the cyanobacterial strain Oscillatoria sp. strain K3 influenced all of the tested D. magna genes causing disruption of the organism homeostasis. The relative expression of most examined genes gradually changed in the dose-dependent response. Exposure of D. magna to the highest extract concentration led to up-regulated expression of CYP360A8, CYP314 and Vtg genes, while the expression of P-gp and GST genes was down-regulated. The increased expression levels of CYP360A8 and CYP 314 implied that pathways of xenobiotic metabolism and ecdysone biosynthesis were activated, while induced Vtg gene expression indicated that the tested extract contains compounds acting as endocrine disruptors in D. magna. Decreased expression levels of P-gp and GST genes suggested that pathways involved in detoxification and oxidative stress were altered.

The results of this in vivo study indicate that the tested cyanobacterial strain is potentially genotoxic to D. magna what requires further research.

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THE EFFECTS OF HYDROLOGICAL CHANGES ON WATER QUALITY AND CYANOBACTERIAL TOXINS IN NYANZA GULF OF LAKE VICTORIA, KENYA

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In the past few decades Nyanza Gulf of Lake Victoria has shown severe signs of eutrophication by regular occurrence of cyanobacterial blooms dominated by the microcystin-producing buoyant colony-forming genus Microcystis spp.[1]. Cyanobacterial blooms in the Gulf have been associated with shutdown of water treatment, fish kills, and occurrence of microcystins in fish tissue [2]. The eutrophication in the Gulf is partly associated with limited water exchange with the main lake basin after the construction of a Causeway in 1983 which blocked Mbita channel, that links the Gulf to the main basin besides Rusinga channel. In May 2017 the Mbita channel was partly reopened by a 150 m wide entrance to the main basin. It was expected that this dam (re)opening might increase the hydrological connectivity of the Gulf with the main basin and thus reduce phytoplankton growth by increased dilution effects. Since both phytoplankton and cyanotoxin (microcystin) concentration data are available from one year survey performed in 2012 [1] as well as short-term observation periods [2] potential dilution effects through the main basin have been investigated through physical-chemical parameters, phytoplankton composition, and resulting cyanotoxins recording monthly for one year period in 2017/2018. The study reports the changes in physicochemical parameters, spatial phytoplankton composition and cyanobacterial toxin concentrations in relation to hydrological changes in the Gulf. This information provides very important contribution towards understanding water quality assessment and monitoring in relation to hydrological changes.

References:
DIVERSITY OF CYANOPHAGES INFECTING DIAZOTROPHIC TOXIN PRODUCING CYANOBACTERIUM NODULARIA SPUMIGENA FROM THE BALTIC SEA


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The filamentous cyanobacterium Nodularia spumigena is commonly found in the Baltic Sea and other brackish water ecosystems, in which it forms extensive blooms during summer periods. Accumulating evidences suggest that cyanophages significantly influence dynamics and phenotypic traits of N. spumigena. Available studies imply that cyanophages may contribute to the ecological fitness of this species in the natural ecosystems. However, cyanophages infecting N. spumigena are seldom characterized at the genomic level mostly due to the lack of both culture isolates and metagenomic data from the environments dominated by this cyanobacterium.

In this study, we present the genomic analysis of 30 cyanophages infecting filamentous cyanobacterium Nodularia spumigena. All cyanophages were classified as members Siphoviridae with the genome sizes between 136 and 147 kb. All phages showed high genetic similarity, and 6 phages were genetically identical, probably reflecting low diversity of N. spumigena population in the Baltic Sea. Comparative genomics revealed that N. spumigena cyanophages represent a novel group of cyanophages, in which genomes clusters based on the host strain they infect rather than on time or place of their isolation. Apart from sporadically distributed single nucleotide polymorphisms, genetic diversifications were basically located in few variable regions, in which several different transposases were identified and which are highly similar to the homologs found in cyanobacteria. This suggests the possible occurrence of relatively recent gene transfers between cyanophages and their host genomes, and, therefore, indicate that horizontal gene transfer is a major factor in diversification of N. spumigena cyanophages.

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RETINOIDS COMMONLY PRODUCED BY ENVIRONMENTAL CYANOBACTERIAL WATER BLOOMS ACTIVATE RETINOIC-ACID RECEPTOR AND CAUSE TERATOGENICITY IN ZEBRAFISH EMBRYOS

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Cyanobacteria are common inhabitants of surface waters worldwide. They are producers of various types of bioactive compounds, some of which are toxic and may cause embryotoxicity, teratogenicity and adversely affect human health. Our research has documented that various cyanobacterial species can produce retinoids. We have sampled water bloom biomass and surrounding water from independent stagnant water bodies affected by cyanobacterial blooms with different taxonomy composition and various dominant species. Some retinoids were observed in all sampled water bodies. Among the compounds detected in water and water bloom biomass belong all-trans-retinoic acid (ATRA), 9/13cis retinoic acid (RA), 5,6epoxy-RA, 4keto-ATRA, 4keto-retinal, and retinal. There is no information regarding their bioactive and teratogenic potential, even though their structure is very close to some of the well-known teratogens. Their potencies determined in the in vitro assay focused on the activation of retinoic acid receptor, which is an important player in early development, relative to its endogenous ligand ATRA, ranged from 0.02 (retinal) to 1 (ATRA). Teratogenic potency was assessed by analysis of developmental disorders and monitoring of early zebrafish development. All tested retinoids show teratogenic potential. Deformation of spine and tail were the most frequent malformations. Effective concentrations for single compounds are in µg/L range, which is greater than individual compounds levels detected in the environment. Nevertheless, mixtures of retinoids co-occur in water bloom affected aquatic environment together with wide spectrum of anthropogenic and other biologically active compounds, which might together lead to more pronounced adverse effects on the development of aquatic organisms.

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PRELIMINARY ASSESSMENT OF CULTURABLE HETEROTROPHIC BACTERIA ASSOCIATED WITH HEPATOTOXIC CYANOBACTERIAL SCUM

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Although cyanobacterial blooms have the capacity to produce a wide range of toxic compounds, other bacteria are known to be associated with these assemblages. Samples of Microcystis aeruginosa collected in Florida in July 2018, were plated on Blood Agar (BAP) to allow heteroptrophic bacteria to grow. After subsequent isolation and culturing of these bacteria, two of the pure colonies/species underwent analyses using a VITEK 2 Compact (Biomérieux) and identified as Aeromonas sobria and Sphingomonas paucimolis with 93% probability (very good identification) and 98% probability (excellent identification), respectively. Aeromonas, a Gram negative rod shaped genus of bacteria, are known to be able to cause gastroenteritis and wound infections. Sphingomonas paucimolis, also a Gram negative bacterium (although lacking LPS in its outer capsule), is a rod shaped, polar flagellated bacterium with yellow pigmented colonies. It is often associated with nosocomial infections due to contaminated medicinal water, solutions and/or instruments. Both bacteria are ubiquitous in natural environments, including water and can be of particular concern for people with immunodeficiencies. This preliminary work highlights the potential issues caused, not only by cyanobacteria, but also of the associated heterotrophic bacteria. Further work is needed to evaluate the presence and risks of bacteria with potential clinical concern present within cyanobacterial blooms.

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The former planktonic members of genus *Anabaena* now assigned to *Dolichospermum* are known to produce hepatotoxic microcystins and be a component of harmful blooms. However, the toxic potential of benthic *Anabaena* sp. strains is unexplored. There is evidence that in rivers of California members belonging to this species creates buoyant mats producing microcystins and anatoxin-a posing threat to animal and human health [1]. On the other hand, research on the Baltic strains of benthic *Anabaena* sp. revealed that they produce cytotoxic compounds other than microcystins or nodularins [2]. There are numerous reports on extracts from different strains of this cyanobacterium that have an inhibiting effect on cancer and bacterial cells growth or viral infection. However, compounds responsible for these effects remain unknown.

Our aim was to assess the cytotoxic activity of metabolites produced by the Baltic-derived strain of *Anabaena* sp. (CCNP1406, isolated in 2005 from the Gulf of Gdańsk). To achieve this, we tested chromatographically separated fractions (flash chromatography) of methanol extract against breast cancer cell line using MTT assay. Fractions which revealed cytotoxic effect were further separated with preparative chromatography, aimed at obtaining pure active compounds or class of cytotoxic cyanometabolites.

Our results indicate that metabolites isolated from the Baltic *Anabaena* sp. significantly inhibit the proliferation of T47D breast cancer cells in a dose-dependent manner. Active fractions were subjected to LC-MS/MS analyses and their content was preliminarily characterized. Further work is aimed at the identification and more detailed description of active agents produced by the tested strain.

References:

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CLASSIFICATION OF HYDROGEN PEROXIDE, PEROXYMONOSULFATE AND PERSULFATE BASED ON THEIR MODE OF ACTION ON MICROCYSTIS AERUGINOSA STRAIN PCC 7806

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Over the past few decades eutrophication of surface water was increased worldwide because of different anthropological activities, such as land fertilization, disposal of inadequately processed wastewater, in combination with climate change. Excessive amount of phosphorus, a key element to eutrophication, is now detected in freshwater lakes, artificially made reservoirs, and streams [1]. Cyanobacterial nuisance in surface waters is among the most concerning issues in science today as it causes tremendous consequences not only on the system's ecology but also in the social economy. Eutrophic ecosystems host a large range of microorganisms including, the harmful strains of cyanobacteria, such as Microcystis aeruginosa [2]. The latter ones produce bioactive metabolites such as microcystins a group of hepatotoxins that affects both humans and wild life [3]. Investigations on the formation of harmful cyanobacteria in eutrophic lakes and ways to reduce their photosynthetic activity, in order to inhibit their bloom and are being conducted [4,5]. Specifically, this study examined the response of a toxic cyanobacterium species Microcystis aeruginosa PCC7806 in bench-scale experiments to the application of three environmentally friendly oxidants. The effectiveness of hydrogen peroxide (HP), persulfate (PS) and peroxymonosulfate (PMS) was determined by measuring the phytoplankton density of treated samples for 3 days following oxidant addition. The results have shown that HP acts like a cyanocide, PMS inhibits the growth of cyanobacteria or can be a cyanoicide in concentrations higher than 5mg/L equivalent to HP, while PS has no effect on the cells.

References:

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SPRAY IRRIGATION OF SPINACH WITH CYANOTOXINS-RICH WATER: PHYTOTOXICITY, TOXIN BIOACCUMULATION AND LEAF-ATTACHED BACTERIA

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The effects of cyanotoxins-rich irrigation water on growth and anatomical parameters of spinach as well as toxin bioaccumulation were investigated with plants receiving spray and root irrigation. The degraded water was collected from the Karla Reservoir, which is characterized by eutrophic conditions and increased microcystins concentration. The combination of water used and irrigation method resulted in 4 treatments, i.e. irrigation with Karla’s water to the root (KR) or by spraying (KS) and the corresponding controls with tap water (TR and TS). Spinach growth was followed for three months, from the seed to the final harvest at marketable size in a pot experiment. Significant decreases in several growth parameters were recorded at Karla’s groups compared to control, at final harvest. The subsequent statistical analysis revealed an interaction between the two independent experimental factors on leaves fresh weight, root biomass and whole plant biomass. Stomatal density was negatively affected by degraded water, especially in the KS treatment. Plants irrigated with the cyanotoxins-rich water bore a significantly higher concentration of attached bacteria on their leaves, compared to corresponding control plants. Additionally, an interesting profile of toxin bioaccumulation in different plant tissues was evident across various treatments. The results of the present study reveal negative effects of cyanotoxins-rich irrigation water on spinach in terms of phytotoxicity and food safety.
In Brussels, cyanobacterial blooms are quite frequently observed in urban lakes and ponds, as already shown during the B-BLOOMS project in 2007-10[1]. If such a bloom consists of toxic cyanobacteria, this may have an adverse impact on the water quality and poses a threat to public and animal health, mostly during recreation activities. The cyanotoxins may also be ingested by animals (dogs, ducks, fish...) or be transported by aerosols. Thus, the detection of toxigenic cyanobacteria and the produced toxins is a crucial step to assess the risk for public health and to decide on the management measures to limit exposure.

Therefore, we developed a method to detect and quantify intra- and extracellular toxins in the samples by HPCL-MS/MS. We selected 8 microcystin congeners and nodularin for this analysis. These are hydrophobic cyanobacterial toxins, of which microcystin-LR is known to be the most prevalent and most studied cyanobacterial toxin in western Europe.

The method was tested on 4 samples of different ponds in Brussels where visual inspection indicated the presence of a bloom in September. The cyanobacterial genera Microcystis and Planktothrix were identified based on morphology and several microcystin variants were indeed detected in the samples. After separating the cells and lake water, the DNA was also extracted and the presence of mcy genes was tested, as they are responsible for the synthesis of microcystins.

In the future, we intend to implement our analysis in cooperation with the regional authorities to better assess the impacts of toxic blooms.

References:

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The recently published β-N-methylamino-L-alanine (BMAA) rodent model [1,2,3] shows clear Parkinson's disease-type symptoms and neuropathology, with substantia nigra pars compacta neuronal loss, a reduction of tyrosine hydroxylase positive neurons in the hippocampus and striatum, and typical alpha synuclein proteinopathies including Lewy body formation. The published proposed mechanisms of toxicity do not adequately explain the dopaminergic neuropathology observed in the rodent model. The observed neuropathology suggests a reduction in the release of dopamine from the substantia nigra pars compacta and the ventral tegmental area to the various target regions, and a concomitant accumulation of dopamine and/or the dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid in the substantia nigra pars compacta following a single neonatal exposure to BMAA. The vesicular monoamine transporter 2 (VMAT2) is responsible for the uptake of dopamine and serotonin into synaptic vesicles for storage and the subsequent release to the target areas when required. We present data here to show that BMAA inhibits uptake of monoamines via inhibition of VMAT2 to the same extent as reserpine, a known VMAT2 inhibitor. In addition, we show a comparison of in vivo reserpine and BMAA toxicity in which the loss of neurons in the hippocampus, striatum, and substantia nigra pars compacta are comparable, supporting this as a potential mechanism of BMAA neurotoxicity.

References:

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Cyanobacteria and cyanotoxins risks via food. Do we know all the hazards?

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Cyanobacteria toxins and other secondary metabolites not yet well known may accumulate in a diverse array of organisms, from plants to aquatic and terrestrial animals and by that way, enter the human food chain. On the other side, the growing consumption of cyanobacteria and microalgae may pose safety questions that are not yet well assessed. Episodes of human intoxication due to these consumptions are scarce but indicate the risk. The global changes impact all over the world indicates that toxins and toxin producing cyanobacteria are spreading to a wider range of geographic areas. An overview of the main toxins and their risks in terms of human health will be presented, highlighting those toxins that would need a special attention. The main and most important toxin vector will be reviewed and the need for changes in legislation concerning toxins in fish and shellfish discussed. A significant array of new molecules produced by cyanobacteria as secondary metabolites have been unraveled in the last decade, but their environmental and human health impact is far from been known. The problems associated with the production, commercialization and consumption of algae and cyanobacteria based supplements will be pointed out, so as to stress the need for an international regulamentation.

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β-N-METHYLAMINO-L-ALANINE INTERFERES WITH NITROGEN ASSIMILATION IN THE CYANOBACTERIUM, NON-BMAA PRODUCER, SYNECHOCOCUS TAU-MAC 0499

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β-N-methylamino-L-alanine (BMAA) is a non-proteinogenic amino acid, produced by cyanobacteria, diatoms and dinoflagellates [1]. The production of BMAA from cyanobacteria is triggered by nitrogen-starvation conditions [2] and its physiological role, albeit unknown, is associated with nitrogen metabolism [3,4,5]. In the present study, the effect of exogenous BMAA (5 μM) on nitrogen metabolism and physiology of the non-diazotrophic cyanobacterium, non-BMAA producer, Synechococcus TAU-MAC 0499, were investigated. In order to study the combined effect of nitrogen availability and BMAA, the strain was also put in nitrogen-starvation conditions by transferring cells in nitrogen-free medium for five days and subsequently exposing the cultures to BMAA. After short-term treatment (30-180 min) and in the presence of nitrogen, BMAA inhibited glutamine synthetase, which resulted in low concentrations of the amino acids glutamine and glutamate. In the absence of nitrogen, although the effect of glutamine synthase remained unclear, a possible perturbation in nitrogen assimilation is reflected on the significant increase in glutamine along with a decrease in glutamate levels. During the long-term treatment (24-96 hours), growth rate, photosynthetic pigments and total protein were measured. Although BMAA appeared to have a positive impact on Synechococcus TAU-MAC 0499 physiology, the outcome is yet to be elucidated. Results from short-term exposure suggest that BMAA interferes with nitrogen assimilation, in a different way, depending on the presence or absence of combined nitrogen, providing novel data on the effect of BMAA on cyanobacteria that do not produce it.

References:
During a Cyano-HAB, multiple genotypes succeed one another or grow in parallel. Within the planktonic cyanobacteria community, toxic and nontoxic genotypes can be distinguished, e.g. for Microcystis spp. through the mcy gene cluster. Although there are variations in the proportion between these harmful and harmless bacteria, the conditions before and during a bloom seem to favor growth of both inducing co-blooming in most of the cases [1, 2]. This occurrence may be exploited for management.

Under the assumption that the final biomass of the bloom will not change considerably because it is limited by TN or TP, the toxic proportion may be manipulated. The idea of an autologous harmless microbiota boost [cf. 3] by adding precultured nontoxic biomass to outcompete the toxic strains is tested using a model in silico.

We develop a new model to dynamically predict the biomass of toxic and nontoxic Microcystis spp. using variable stoichiometry and a nutrient cycling model [cf. 4]. Microcystin formation and fate is modeled dynamically using variable intracellular quota release and decay in the water column. We apply the model to Meiliang Bay, China, and compare the results to observations. In a boost scenario, nontoxic biomass is added during the onset of the bloom. We estimate the amount when the toxic strain is outcompeted so that the total microcystin concentration falls under the WHO limit of 1 μg/L (from 28 μg/L). This would require a large but feasible mass-culturing infrastructure, suggesting this management strategy should be explored further.

References:
The aim of this study was to investigate the influence of microcystins (MCs) on the seasonal variations in the activity of the antioxidant system of the zebra mussel at different size groups. We examined changes in lipid peroxidation (LPO) levels, glutathione (GSH) content and the catalase (CAT) and glutathione S-transferase (GST) activity of mussels inhabiting the Sulejow Reservoir (Poland) characterised by annual toxic blooms of *Microcystis aeruginosa*. Animals collected in 2015, near the dam (the highest densities), were measured lengthwise as a substitute for age determination. Mussels were classified as: young of the year <15 mm in length (S), and older than 1+ in two size groups: 16-19 mm (M) and 23-25 mm (L). Bioaccumulation of MCs in the tissues of all size classes coincided with the dates of the highest concentration of cyanotoxins in the environment (July and August). However, the lowest MCs concentration was recorded in the largest (L) animals. Simultaneously, the activity of the antioxidant system of the largest thereby oldest mussels was significantly higher than in medium and smallest individuals throughout the season. The values of parameters responsible for detoxification (GSH and CAT) increased in all animals in October. At this time, the MCs concentration in the tissues of all size groups was below detection. The symptoms of oxidative stress (LPO) of mussels were generally the highest during spring, thus they were not associated with the presence of MCs. These findings may indicate a good tolerance of zebra mussel to the toxic bloom conditions.
Cyanobacterial toxin of BMAA which possesses chronic neurotoxicity seriously affects the normal growth, development and reproduction of the cultured species, especially related to the problem of food quality and safety of aquatic products. In this paper, the pollution levels of BMAA in the water, sediment and six aquatic products of the typical freshwater aquaculture ponds in China were investigated, and then the proposed standard limit of BMAA for aquatic products quality and safety was preliminary established based on the situation of our country's, and the potential risk of BMAA existence to human health also was evaluated. Meanwhile, the sustained-release antialgal agent of gallic acid by using graphene oxide modified by L-cysteine as carrier was prepared to study its algae-inhibition ability, control effect of BMAA pollution, and its ecological safety to other aquatic organisms. The results showed that the EDI (Estimated Daily Intake) values of BMAA in the above six aquatic products were much lower than the TDI (Tolerable Daily Intake) values, and the concentrations of BMAA in the six aquatic products were also much lower than the GV (Guidance Values including 12 μg/g for adults and 3 μg/g for children), which posed a relatively low risk to human health. The allelopathic sustained-release antialgal agent concentration of 12.5 mg/L can not only ensure better algae-inhibiting effect, but also effectively control BMAA toxin pollution. The bio-acute toxicity test showed that the sustained-release antialgal agent had good ecological safety and can be applied to the treatment of toxic cyanobacterial blooms in aquaculture ponds.

Acknowledgments:
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Water hyacinth (Eichhornia crassipes (Mart) Solms), a free-floating perennial aquatic macrophyte with hairy root system and nutrient absorption efficiency has gained increasing attention in phytoremediation of many types of wastewater. More than 80% TN and 75% TP can be removed by water hyacinth for sewage purification. Over 90% algae can be removed from algae-enriched eutrophic waters by using water hyacinth. The TN concentration reduced from 13.21mg/L to 1.84mg/L in tailrace of sewage treatment plants with large-scale application of water hyacinth. And also the macrophyte can efficiently remove heavy metals from industrial wastewater. Meanwhile, due to the characteristics of fast propagation, high moisture content and large biological yield some challenges such as ecological risk, timely harvesting, high dehydration and utilization companying with application of water hyacinth in restoration project of polluted waters. To solve the above problems, many methods and technologies have been explored and show excellent effects in water purification and repairment. For example, enclosure facilities have been developed to avoid plants from escaping; specialized equipment have been manufactured for rapid harvesting; equipment for mechanical dewatering have been produced to remove water from plant tissue; technologies for resource utilization (e.g. fertilizer production) have been developed. In future, some new technologies for resource utilization should be developed as well as the strategies for ecological compensation should be established.
LIFE CYCLE OF MICROCYSTIS IN LATE SUMMER: REVEAL LYSIS WITH β-CYCLOCITRAL

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The cyanobacterial blooms have a negative impact on the water environment and the quality of drinking water. Cyanobacteria are able to synthesize some bioactive metabolites and release them into the water regime. Among the Volatile Organic Compounds (VOCs) produced such circumstances, β-cyclocitral is known to be a compound specific to Microcystis[1,2]. β-Cyclocitral is considered to be derived from carotenes and has lysis activity. Easily oxidized and causes the lake characteristic blue with the corresponding acid[3]. Our purpose is to clarify the life cycle of Microcystis genus in which β-cyclocitral is involved and to consider a biological control method based on results. Therefore, we focused on β-cyclocitral and conducted experiments on its production conditions and mechanisms. (1) Production Conditions: High density was suggested that influence in production. The threshold of density was approximately 10⁷ cells/ml. Succeeded in causing blue-lysis by floating densification of lake samples[4]. (2) Production Mechanisms: Solid-phase microextraction GC/MS is used as the analysis condition of β-cyclocitral, but it is not detected by solvent extraction[5]. As a result of the verification of the analysis conditions, it was suggested that β-cyclocitral is formed by heating. Probably Carotenoid Cleavage Dioxygenase (CCD) cuts metabolites of β-carotene to make β-cyclocitral and Microcystis has specific CCD enzyme.

References:
The FlowCam® is an imaging flow cytometer that uses fluorescence and image analysis software to rapidly analyze and differentiate cyanobacteria from other algae. A 1 mL sample is analyzed in 6 minutes.

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“Rapid detection of cyanobacteria and automated sorting of morphotypes... when using FlowCam® greatly relieves operator fatigue compared with...standard light microscopy.” Graham et al. (2018)

“It used to take us 3-4 hours to do algae counts in the summer. Now it takes 15 minutes. We use the FlowCam because it’s quick and easy.” Water Laboratory Supervisor

“In the March 2017 issue of Harmful Algae, scientists from the California Department of Water Resources describe how they estimated cell abundance of colonial Microcystis using a FlowCam.”
Cyanobacteria Technology Highlights
A disruptive cleantech technology, with a massive industry opportunity

Cleaner: All aspects of our technology offer a superior alternative to prevailing industrial solutions when it comes to environmental footprint and health

Safer: Every measurable aspect of our technology also offers a demonstrably safer alternative to the incumbent solutions on the market today

Smarter: We strive to deliver the simplest, most robust technological solutions for the most pressing global needs facing humanity today

Technology Overview – Plasma Arc Sterilization System

- Feedstock is passed through rapidly, ~1-2 times with the sole purpose to maximize sterilization
- Modest quantity of gas produced as by-product and used in the pre-heat process to increase throughput
- Commercial applications in water reclamation, treatment of agriculture, pharma, industrial and manufacturing wastes
- Our technology generates a combination of high heat, ultraviolet radiation, and an intense electro-magnetic field. These powerful mechanisms have the proven ability to:
  1. Break down compounds to simple elements
  2. Reduce NPK and metals
  3. Rupture algae cell walls and reduce toxins
  4. Un-alter the water chemistry of the treated water
- In 2018, the USDA funded test results show a 99.9% reduction in pathogens such as E. coli, fecal coliform, and enterococci.
- The system has a compact physical footprint, can easily fit in the area of a one-car garage. Depending on the volume of waste to process, treatment time can be as little as a few hours. The unit is highly mobile and has flexible configuration options for rural applications.
- Other likely applications include pharmaceuticals and PCBs.
Plasma Arc Treatment of Cyanobacteria

Project Overview

- Taronis was asked to conduct a pilot treatment of a lake sample with an active Harmful Algae Bloom and displaying signs of eutrophication
- 1,600 gallons / 6,000 liters of lake water from the contaminated lake was collected for treatment
- Water was processed using the Taronis Technologies patented 50 KW mobile Venturi Plasma arch system
- Treated water was inoculated with harvested native bacteria which were able to repopulate the sample

Phase Two

1. Florida State University will provide water analysis
2. Florida Gulf Coast University will provide airborne toxin monitoring
3. A portion of the affected lake will be temporarily barrier to prevent algae re-contamination
4. A 300 kW unit will be deployed processing 1,400 gallons / 5,300 liters per hour
5. Estimated current operating cost is approximately $200 / HR

Project Results

- Total nitrogen reduced by 50% through gasification
- Conductivity, salinity, pH remain relatively constant showing that the system is not significantly altering water chemistry.
- ORP shows increasing and/or maintaining of oxygen

- Flower Laboratories and Florida State University provided independent verification
- Cylindrospermopsin reduced 48-62%
- Microcystin reduced 68-100%
- Chlorophyll-a reduced 72-75%
- BOD reduced by 30%
- Phosphorus reduced by 65%

The system kills Harmful Algae Blooms, reduces or eliminates toxins, reduces nutrients that feeds the algae, & the process does not affect the water chemistry.
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Kwadrupolowy spektrometr mas z analizatorem czasu przeletu QTOF łączący najszerszą i najbardziej czystą technologię na świecie z kompaktywą konstrukcją.

LCMS-9030 to produkt posiadający innowacyjne DNA Shimadzu, który wyszedł spod ręki nieobsty Keichi Tanaki. Przyrząd jest dedykowany do ilościowych i jakościowych analiz, zapewniając przy tym rzeczywistą pewność i łatwość pracy. Łączy w sobie niezrównaną szybkość, jakże nietypolową dla analizatorów TOF oraz doskonałą czułość i wysoką rozdzielczość. Znana i sprawdzona w spektrometrach mas Shimadzu technologia UFMS (ultra szybka spektrometria mas) również i w tym przypadku znalazła swoje zastosowanie sprawdzając, że jest to najczęściej i jednocześnie najbardziej czysty spektrometr wysokiej rozdzielczości typu Q-TOF na rynku. LCMS-9030 z łatwością osiąga dokładność masy < 1 ppm i jednocześnie zachowuje dużą stabilność mas, między innymi dzięki unikalnemu układowi termostatującemu analizator czasu przeletu. Q-TOF wyposażono w unikalne technologie takie jak zwiniętadło jonowe iRetOF™, UF-FlyTube™, UFGrating™, UFAccumulation™. Dzięki temu możliwa jest niesiągalna w innych rozwiązaniach szybkość bierania danych aż 100 Hz oraz szybkość zmiany polaryzacji wynosząca jedynie 1 sekundę, co umożliwia uzyskanie lepszych wyników niż w dotychczasowych rozwiązaniach.

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