Contributors

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Assessing Risk

• Effective Public Health Risk Management depends upon accurate Hazard Assessment

• Accurate Hazard Assessment depends upon accurate, reliable data that are representative of the real world situation
  – Representative sampling
  – Accurate and complete quantification of all relevant toxins
  – Adequate toxic potency assessment of all relevant toxins

• Competent translation of Hazard to Risk – understanding of uncertainties

• These stages are often dissociated, leading to potential for misunderstandings and false assumptions
CyanoSurvey - Overarching Questions

Almost 20 years since the last large scale survey.
• New toxic species, changes in water use, changes in climate, taxonomy under review.

• How reliable are our assumptions about:
  – Which species are toxic?
  – Which species are species?
  – Where do various species occur?
  – Why do species prefer certain locations?
  – What is the best means of quantifying toxin risk?

• How might the distribution of species change in a changing climate?
ANNUAL CLIMATE SUMMARY
2010
CyanoSurvey Sampling Sites – 61 in all

Plus 36 “Ad hoc” samples obtained from routine Biology Services submissions.

For genetic taxonomy, these have been supplemented with AWQC culture collection samples from 37 sites.

29 CyanoSurvey samples used for toxicity methods comparison.
Sample processing

10L water sample

Inspect – rough cell count & ID

Filter through GFC to concentrate

Freeze-Dry

Toxin/Toxicity/Tox Gene Analysis

AWQC Analytical Services

Culture

Accurate cell count & ID

HPLC

Water Quality
Nutrient Availability Preferences (incl. 2 WWTP samples)

**Total Nitrogen**

- **Range, all samples**
- **M. aeruginosa**
- **M. flos-aquae**
- **A. circinalis**
- **C. raciborskii**

- Low
- High
- "Optimum"

**Total Phosphorus**

- **Range, all samples**
- **M. aeruginosa**
- **M. flos-aquae**
- **A. circinalis**
- **C. raciborskii**

- Low
- High
- "Optimum"

*Microcystis* are the weeds of the cyanobacterial world!
Occurrence (% of Samples)

Species

% of samples

0 10 20 30 40 50 60

Occurrence (% of Samples)
Occurrence (% of Biovolume)
Capture of a part of a Maximum Likelihood (ML) tree using 3,454 16S rRNA sequences of cyanobacteria showing the monophyletic group of *Microcystis* strains.
Microcystis taxonomy

16S RNA - “species” level discrimination:

- **Microcystis flos-aquae**: 3 μm to 4.5 μm
- **Microcystis aeruginosa**: 5 μm to 6.5 μm

<table>
<thead>
<tr>
<th>BLAST on 16S</th>
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<tbody>
<tr>
<td>μm</td>
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<tr>
<td>4.78</td>
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<td>3.47</td>
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<tr>
<td>5.66</td>
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<tr>
<td>4.60</td>
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</tbody>
</table>

MIC 013B M. aeruginosa / Tox+ / mcy+
I011-0017 M. flos aquae / Tox- / mcy-
FSS3-138/1B M. flos aquae / Tox+ / mcy-
FSS1-152/1B M. aeruginosa / Tox- / mcy-
I010-0037 M. sp. / Tox+ / mcy+

MIC 050D M. aeruginosa / Tox+ / mcy+
FSS3-138/1A M. spp. / Tox- / mcy-
I011-0038 M. aeruginosa / Tox- / mcy-
MIC 309 BC M. aeruginosa / Tox-/ mcy-
I011-0039 M. sp. / Tox+ / mcy-

MIC 029A M. aeruginosa / Tox- / mcy-
MIC 051A M. flos aquae / Tox- / mcy-
MIC 058A M. flos aquae / Tox+ / mcy+
I011-0004 M. spp. / Tox- / mcy-
I010-0058 M. flos aquae / Tox+ / mcy+
I010-0038 M. flos aquae / Tox+ / mcy+
Microcystis Taxonomy

ITS – “ecotype” discrimination:

Treat all Microcystis as potentially toxic
## Toxin/toxicity detection method comparison

<table>
<thead>
<tr>
<th>Assay Class</th>
<th>Type</th>
<th>Endpoint</th>
<th>Detects</th>
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</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>Morphological recognition</td>
<td>Known toxin producers</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Antibody binding</td>
<td>MC/NOD, CYN, STX</td>
<td></td>
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<tr>
<td>Toxin genes</td>
<td>Primer binding, PCR amplification</td>
<td>Cyano16S, MC, NOD, CYN, STX, ANTX-a</td>
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<td>Analytical HPLC</td>
<td>UV absorbance</td>
<td>MC, NOD</td>
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<tr>
<td>LC-MS</td>
<td>Molecular fragmentation</td>
<td>CYN, STX</td>
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<tr>
<td>Toxicity PP2A</td>
<td>Enzyme inhibition</td>
<td>MC, NOD</td>
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<tr>
<td>PSI</td>
<td>Interference with ribosomal translation</td>
<td>CYN, Limnothrixin*</td>
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<td>Neuro-2A (nerve cells)</td>
<td>Cell preservation by Na channel blockade</td>
<td>STX</td>
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<td>Vero (kidney cells)</td>
<td>Mitochondria-dep. metabolism (Resazurin)</td>
<td>Anabaena 131 toxin**, non-specific</td>
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<td></td>
<td>Cell death (cell count)</td>
<td>Anabaena 131 toxin**, non-specific</td>
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<tr>
<td></td>
<td>Cell cycle (G1/G2 ratio)</td>
<td>Non-specific</td>
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**Froscio et al 2011 Toxicon 58: 689-692
Microcystin Analyses

Log-log slope = 0.88, but on average: PP2A = 0.59 x ELISA

No “gold standard” method for mixtures of toxin analogues
Conclusions - MC

• Good +/- agreement between ELISA/PP2A/gene, although some apparent false –ve in gene analyses
• Generally reasonable quantitative agreement (± 2-3-fold) between ELISA, PP2A and HPLC, while cell quota varied greatly.
• *M. flos-aquae* seems to be a fairly consistent MC producer (toxicity highly variable)
• Evidence for an MC producer in most concentrated samples (probably low numbers of *Microcystis*) – ubiquitous occurrence.
Conclusions – All toxins

• While +/- agreement was good between methods for most toxins when cells numbers were high, actual quantification was quite variable
  • variability: STX > MC > CYN. Due to toxin mixtures?
  • understand strengths/weaknesses of the method used
  • cell counts are not a quantitative measure of toxicity (OK as indicator of maximum likely toxicity if right species included)
  • however, assuming worst case may be “safe” but potentially costly in terms of unnecessary closures/remedial actions
  • justifies more comprehensive sampling (temporal/spatial) and more toxin analyses
Conclusions - Overall

• “Usual suspects” still the main threats, although some evidence for other toxic species (*Limnothrix*)

• Sample concentration allowed detection of very low levels of all toxins even when producer cells not observed in cell counts
  • ubiquitous low level distribution of toxin producers even in locations without a history of these species
  • potential for “surprise” blooms when conditions change
A Question of Balance

• Sampling:
  – Maximise for representative hazard identification

• Quantification – no Gold Standard:
  – Minimise cost so as to maximise sampling
  – Maximise rapidity, comprehensiveness
  – Adequate accuracy, precision, sensitivity
  – Use screening assays to filter samples for detailed analysis

• Toxicology:
  – Validate use of biological assays for toxin potency assessment as well as detection

• Overall:
  – Communicate assumptions/compromises/uncertainties to risk managers
Thank you