Australian Approach to Cyanotoxin Analysis

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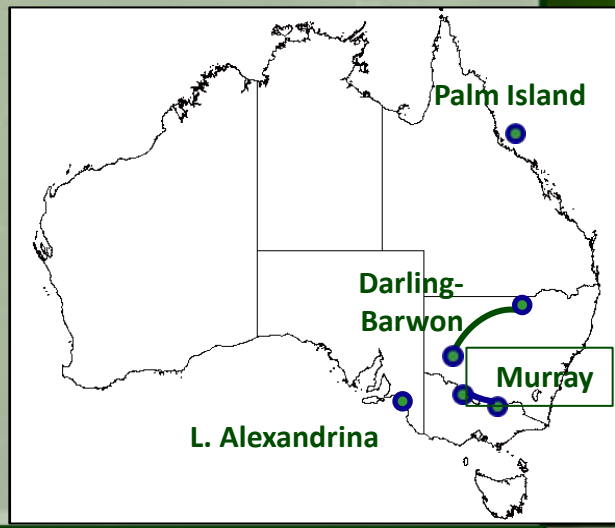
Historical Background

- Blue green algae blooms have occurred in every State and Territory. No human deaths but illnesses including gastroenteritis, dermatitis and liver damage recorded. Stock deaths are more common.
- First record of animal poisoning (livestock) in 1878 at Lake Alexandrina at the end of the Murray-Darling river system. The deaths were caused by *Nodularia* which inhabits brackish waters.
- First recorded human cases in 1979 on Palm Island, Queensland probably from *Cylindrospermopsis raciborskii* involving 149 people with hepatitis syndrome and malaise
- In 1991 a 1000 km bloom affected the Darling and Barwon Rivers. Primarily *Anabaena circinalis* - led to stock deaths and disrupted water supplies. State of Emergency declared
- 2009 and 2010, 300-400 km blooms in the River Murray. Primarily *A.circinalis*



Significant Cyanobacteria

Organism	Toxin	Health Effects
Anabaena circinalis	Saxitoxins (no anatoxins)	Neurotoxin – dizziness, weakness, nausea, muscle paralysis
Microcystis aeruginosa	Microcystin	Hepatotoxin – liver damage
Cylindrospermopsis raciborskii	Cylindrospermopsin	Cytotoxic – liver, kidney and other organ damage
Lyngbya majuscula (marine)	Range of toxins incl. lyngbyatoxin	Inflammatory – cause severe skin irritation and blistering

Monitoring

- General approach is to focus on detecting growth of blue green algae and development of blooms as a first stage. Toxin analysis not undertaken until significant concentrations detected
- A number of approaches are applied including:
  - Routine monitoring (reservoirs, streams, water supply intakes etc)
  - Aerial surveys (rivers, wetlands etc)
  - Boat surveys (rivers, reservoirs etc). Samplers are instructed to collect samples of anything unusual (e.g. surface scums)
  - Physical surveys (all types of water including wetlands, recreational waters)
- Frequency of monitoring governed by decision trees based on risk including consideration of historical results and evaluation of detections

Frequency of Monitoring

- Monitoring programs include two components:
  - a baseline frequency
  - an alert response framework
- Baseline frequency:

Location	Sample point (s)	Frequency
Drinking water reservoir	Offtake and open water sites	Twice weekly (summer) Weekly (winter)
River at intake to drinking water supply	Pump intake	Weekly
Recreational water body	Multiple points	Weekly – fortnightly in season

Alert Response Framework

Low levels:
< 2,000 *M.aeruginosa* per mL
Maintain baseline monitoring

Alert level 1 (Medium):
2,000 – 6,500 *M.aeruginosa* per mL
Increase frequency to at least 2x week. Include depth profiling. Visual inspections
Determine toxicity

Alert level 2 (High):
> 6,500-65,0000 *M.aeruginosa* per mL
Monitor at least 2x week. Include depth profiling. Visual inspections
Measure toxin concentrations in treated water

Alert level 3 (Very High):
>65,0000 *M.aeruginosa* per mL
Monitor at least 2x week. Include depth profiling
Measure toxin concentrations in treated water. Additional monitoring as required.

Guideline Values

Organism	Alert 1 (Med) cells/mL toxin ug/L	Alert 2 (High) cells/mL toxin ug/L	Alert 3 (V high) cells/mL toxin ug/L
Anabaena circinalis	6,000 1	20,000 3	
Microcystis aeruginosa	2,000 0.4	6,500 1.3	65,000 13
Cylindrospermopsis raciborskii	4,500 0.3	15,000 1	150,000 10
Recreational	Surveillance	Amber alert	Action Level
	M.aeruginosa 500-5000 or Biovolume < 0.4 mm^3/L	*M.aeruginosa* 5000-50000 or Biovolume 0.4 - 4 mm^3/L	*M.aeruginosa* >50000 or Biovolume > 4 mm^3/L

Laboratory Analysis

- **Hepatotoxins**
  - Microcystin and nodularin by HPLC – DAD (diode array detection). Detection limit to 0.1 µg/L
- **Saxitoxins**
  - Saxitoxins - about 18 compounds detected by HPLC/post-column derivertisation fluorescence detection with 3 chromatographic runs. Detection limit 0.5 µg/L
- **Cylindrospermopsin**
  - Detected by HPLC/MS/MS. Detection limit 0.1 µg/L
- **Anatoxin (uncommon)**
  - Anatoxin-a HPLC/MS/MS. Detection limit 0.1 µg/L
- **Rapid test (2-3 hrs)**
  - Direct injection into LC/MS/MS. LOR: 0.1 µg/L anatoxin-a, nodularin, mRR, mLR, mLA, MLF, CYN and deoxyCYN, 0.5 µg/L mYR, mLW, mLY

Field Response

- Initial focus of monitoring based on combinations of observation and testing for presence of cyanobacteria. However, observation doesn't work well for all organisms (e.g. *Cylindrospermopsin)*

- As numbers start to increase routine monitoring often augmented by boat surveys.

- Some utilities have field response teams. Ours have:
  - sampling equipment,
  - field monitors (DO, phycotoxin)
  - a microscope,
  - simple cell concentrators (syringes/membrane filter holders)
  - toxin field kits (can measure down to 1ug saxitoxins/1ug microcystin)

- Results phoned to the utility and to the health department the same day (if necessary)

Aerial analysis

- Murray-Darling has a catchment of >1 million square kilometres (about 400,000 sq miles) and a mainstream length of 3700 km (2300 miles
- Population of the basin only about 2 million people. When a large bloom forms or potential bloom conditions exist aerial surveys can provide effective assessments
- Aerial surveys followed up by targeted sampling

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Communication

- In Australia water management is a State issue
- States and territories establish communication protocols to respond to blue-green algal blooms. In Sth Aust we have agreed reporting criteria with our State drinking water utility and with Local Government responsible for recreational waters
- In SA significant blooms dealt with by a Blue Green Algal Task Force chaired by the Health Dept and including the water utility, EPA, Agriculture Dept and water resource agencies (lead varies from State to State)

Communication (contd)

- When blooms cross State borders communication protocols are established to share information and to deal with responses (e.g the River Murray forms much of the border between New South Wales and Victoria)
- In the event of large blooms nationally coordinated blue green algal task forces can be established e.g. 2009-2010
- Extreme events can involve national resources including the army (1991)

Conclusions

- Algal blooms occur in every state and territory. They affect drinking water supplies, recreational activities, livestock and tourism. They are often highly visible and produce distinct odours. They can have large economic impacts

- States have active monitoring programs that start with testing for cyanobacteria combined with observation (land, boat and air)

- The key to assessing impacts is cyanotoxin testing. There are established laboratory methods but testing takes time and laboratory facilities are limited.

- Field kits provide valuable screening tests but it is important that operators/samplers are trained

- Good communication is key to effective responses