3: Phycotoxins (toxins of "algae": cyanobacteria, marine microalgae) [Biological-origin toxins]

CYANOBACTERIAL (CYANOPHYTE, BLUE-GREEN ALGAL) TOXINS

We're Annie*, Noddie†, Mike‡ and Cy§!
Drink us straight down, then wait to die.
Your liver's shot, your nerves are too.
Bid life farewell, the grave's for you!

R.A. McKenzie 5 December 2001

* *Anabaena* † *Nodularia* ‡ *Microcystis* § *Cylindrospermopsis*

Cyanobacteria (cyanophytes or blue-green algae) are prokaryotic unicellular or multicellular micro-organisms, either filamentous or non-filamentous, commonly aquatic (but also terrestrial), and photosynthesising through chlorophyll *a*. Toxicologically-important species are aquatic. Most aquatic cyanobacteria contain gas vacuoles giving them buoyancy and thus the capacity to determine the stratum of the water column that they will inhabit and are thus **planktonic** in habit, floating in or near the surface layers of water bodies. A few species are **benthic**, occurring on the substrate or in the sediment at the bottom of water bodies.

For a perspective on the importance of cyanobacteria in the history of life on earth, read Margulis & Sagan (1987). Ancient symbiotic cyanobacteria are thought to be the origin of the chloroplasts of plant cells. For an overview of forms and the spectrum of habitats occupied by cyanobacteria, see Whitton & Potts (2000). Habitats occupied include geothermal, mats & stromatolites, marine plankton, fresh water, soils, limestones, salts & brines, polar regions, deserts and in symbiotic relationships with plants such as in the coralloid roots of cycads (*q.v.*). The molecular biology of cyanobacteria was reviewed in Bryant (1994).

The cyanobacteria in Australia are somewhat poorly known. Published knowledge of the genera and species occurring here has been gathered by Day *et al.* (1995). They list 323 species in some 67 genera. Aids to identification of cyanobacteria in Australia are Baker (1991, 1992), Fabro & Duivenvoorden (1993) and Entwisle *et al.* (1997).

Cyanobacteria may develop **heterocysts** (thick-walled cells differentiated from the majority of cells in a filament or trichome) which have the capacity to fix nitrogen and spores or **akinetes**, thick-walled cells to promote survival of the organism through dormancy in times of environmental stress.

Rapid population increases (blooms) of planktonic cyanobacteria can occur in fresh, brackish and marine waters. Their occurrence in water bodies has been known for centuries (Griffiths 1938), but their toxicity was first recognised in the modern era in Lake Alexandrina, South Australia (Francis 1878). Hepatotoxicity from cyanobacteria is the most common type of poisoning by these organisms; neurotoxicity is comparatively rare.

Toxin group	Cyanobacterial genera*	Mammalian target organ
Cyclic peptides		
Microcystins	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*	Liver
Nodularin	*Nodularia*	Liver
Alkaloids		

Toxin group	Cyanobacterial genera*	Mammalian target organ
Cylindrospermopsin	*Cylindrospermopsis, Aphanizomenon, Umezakia*	Liver, Kidneys, Heart
Saxitoxins	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis, Planktothrix*	Nerve axons
Anatoxin-a	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*	Nerve synapse
Anatoxin-a(s)	*Anabaena*	Nerve synapse
Lyngbyatoxin-a	*Lyngbya*	Skin, Alimentary tract
Phenolic bislactones		
Apylsiatoxins	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*	Skin
Lipopolysaccharides (LPS)	All	Irritant to any exposed tissue

N.□. toxins are not produced by all species of the listed genera

References:

Baker PD (1991) *Identification of Common Noxious Cyanobacteria. Part I – Nostocales.* Urban Water Research Association of Australia Research Report No.29, Melbourne & Metropolitan Board of Works, Melbourne.

Baker PD (1992) *Identification of Common Noxious Cyanobacteria. Part II – Croococcales, Oscillatoriales.* Urban Water Research Association of Australia Research Report No.46, Melbourne Water Corporation, Melbourne.

Bryant DA (ed) (1994) *The Molecular Biology of Cyanobacteria.* Kluwer Academic Publishers, Dordrecht / London / Boston. ISBN0 7923 3222 9

Day SA, Wickham RP, Entwisle TJ, Tyler PA (1995) *Bibliographic Checklist of Non-Marine Algae in Australia.* Flora of Australia Supplementary Series No. 4. , Australian Biological Resources Study, Canberra. pp. 199-221.

Entwisle TJ, Sonneman JA, Lewis SH (1997) *Freshwater Algae in Australia. A guide to conspicuous genera.* Sainty & Associates, Potts Point NSW.

Fabro LD, Duivenvoorden LJ (1993) *A Photographic Guide to Common Cyanobacteria of Central Queensland.* University of Central Queensland, Rockhampton.

Francis G (1878) Poisonous Australian lake. *Nature* **18**:11-12. [*Nodularia spumigena* in Lake Alexandrina, South Australia]

Griffiths BM (1938) Early references to waterbloom in British lakes. *Proc. Linn. Soc. London* **151**:12-19.

Margulis L, Sagan D (1987) *Microcosmos. Four Billion Years of Evolution from our Microbial Ancestors.* Allen & Unwin, London. ISBN0 04 570015 X

Whitton BA, Potts M (eds) (2000) *The Ecology of Cyanobacteria. Their diversity in time and space.* Kluwer Academic Publishers, Dordrecht / London / Boston. ISBN 0 7923 4735 8 (HB) 4755 2 (PB)

TOXINS OF FRESH & BRACKISH WATER CYANOBACTERIA

☑ Cyanobacterial hepatotoxic cyclic peptides – microcystins and nodularin

Core data

Common sources:

- *Microcystis aeruginosa*
- *Nodularia spumigena*

Toxins:

- microcystins
- nodularin

Animals affected: sheep, cattle, dogs, pigs, birds

Poisoning circumstances (all cyanobacteria)*:*

- *many blooms non-toxic, but all should be considered potentially toxic*

- increased nutrient concentrations (N,P) in water bodies; animal waste, fertilisers
- slow flow and stratification of streams and impoundments
- wind concentration of floating bloom at point of livestock access

Main effects:

- sudden death
- acute liver necrosis

Diagnosis (all cyanobacteria)*:*

- identify cyanobacterium
- demonstrate its toxicity (mouse inoculation; toxin assay)

Therapy:

- prognosis grave
- early activated charcoal

Prevention & Control (all cyanobacteria)*:*

- prevent consumption of bloom:
  - avoidance
  - physical barrier
  - algicides, precipitants (lime, ferric alum, gypsum)
  - barley straw (?)
- reduce nutrient load:
  - revegetate margins of small reservoirs (farm dams)
  - precipitate P
  - aerate bottom layer
  - reduce P inflow (minimise fertiliser use, vegetation buffer zones, minimise sewerage inflow, wetlands at inflow, remove carp)
- improve flow rates and disrupt stratification

Syndrome names:

Cyanobacterial poisoning
"Algal"[*sic*] poisoning

Chemical structure:

Cyanobacteria contain two known types of **peptide hepatotoxins** – the microcystins and nodularin.

Microcystins are monocyclic heptapeptides, at least 70 of which are known. They each consist of a mixture of 7 L- and D-amino acids. The D-amino acids are very stable within the various forms of the microcystins, and include N-methyl-hydroalanine (known as Mdha) and the unique non-polar-linked 3-amino-9-methoxy-2,6,8-trimethyl-10phenyldeca-4,6-dienoic acid (known as ADDA). The L-amino acid components vary between the various forms of the microcystins. ADDA forms a side chain to the molecule and is essential for biological activity, neither ADDA alone nor the cyclic structure alone is toxic. [Botes *et al.* 1982a.b,c; Santikarn *et al.* 1983; Carmichael 1992b]

Microcystins are highly stable at high temperatures for long periods, and are not denatured by boiling. This characteristic has been used as part of biological assay methods to remove other less stable toxins from test samples. They are non-volatile, dialysable, resistant to pH changes and soluble in water, ethanol and acetone. [Ressom *et al.* 1994]

Nodularin is a monocyclic pentapeptide, smaller than the microcystins and comprising the 5 amino acids D-glutamic acid, D-B-methylaspartic acid, L-arginine, D-N-methyl-dehydrobutyrine and ADDA (Rinehart *et al.* 1988)

Sources:

Cyanobacteria (also known as cyanophytes, blue-green algae) are cosmopolitan in distribution [Se14] (Mulhearn 1959; Beasley *et al.* 1991)

Microcystins occur in planktonic (and some benthic) cyanobacteria in fresh water

Microcystis aeruginosa [= *Anacystis cyanea*] (Lahti 1997)
Microcystis flos-aquae

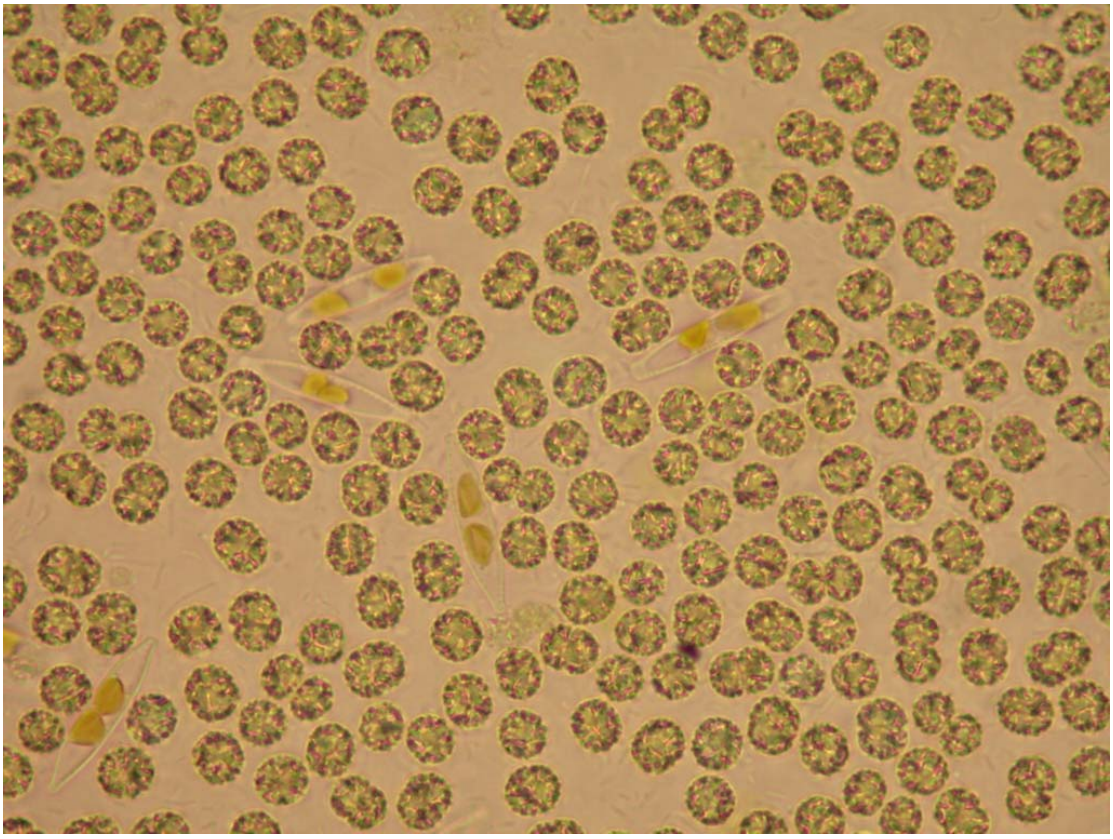
Microcystis viridis (Lahti 1997)
Anabaena flos-aquae (Lahti 1997)
Anabaena spp. (Lahti 1997)
Oscillatoria agardhii (Luukkainen *et al.* 1993)
Oscillatoria limosa (Metz *et al.*1997)
Nostoc sp. (Lahti 1997)
Anabaenopsis milleri (Lahti 1997)

Nodularin occurs in

Nodularia spumigena Mertens (Rinehart *et al.* 1988, Carmichael *et al.* 1988) in salty or brackish water, typically in estuaries and coastal lagoons such as Peel-Harvey Estuary in Western Australia, Lake Alexandrina in South Australia and the Gippsland Lakes in Victoria (Jones *et al.* 1994)

Mussels (*Mytelis edulis*) in estuaries supporting blooms of *Nodularia spumigena* (Falconer *et al.* 1992)

All of these species except *Anabaenopsis milleri* are listed for Australia (Day *et al.* 1995)



Microcystis aeruginosa [Rod Thomas Photo]

Toxicity:

Natural toxicity incidents are recorded in ruminants (cattle, sheep), pigs and dogs. Other species are expected to be susceptible.

Australia:

Microcystis aeruginosa – sheep, NSW (McBarron & May 1966, Jackson *et al.* 1983, 1984)

Microcystis flos-aquae has poisoned cattle in Tasmania (Andrewartha 1998)

Nodularia spumigena

- cattle, sheep, pigs, dogs; Lake Alexandrina, South Australia (Francis 1878, Codd *et al.* 1994)
- sheep; farm dams, south-western Western Australia (Main *et al.* 1977)
- (Runnegar *et al.* 1988)

- human (severe skin rashes, diarrhoea); coastal lagoon, Tasmania (Jones *et al.* 1994)

Potentially toxic cyanobacteria produce hepatotoxins in concentrations ranging from < 100 μg/g cyanobacterial dry weight (essentially non-toxic) to> 5000 μg/g cyanobacterial dry weight (Falconer 1991). Hepatotoxic peptide concentrations in the range 1000-5000 μg/g cyanobacterial dry weight are common in Australian blooms (Jones *et al.* 1994).

Other countries:

Oscillatoria limosa, a benthic species, poisoned cattle drinking from a lake in Switzerland (Metz *et al.*1997)

Nodularia spumigena:

- a perennial bloom in the Baltic Sea has been associated with illness and death of 9 dogs that drank or swam in bloom material along the shoreline (Edler *et al.* 1985).
- cattle have also been poisoned in Germany (Strelasund area) by the Baltic Sea blooms (Gussmann *et al.* 1985)
- dogs exposed to a bloom in a brackish lake on the German North Sea coast (Nehring 1993)

Some crustacea are recorded as susceptible to microcystins, but others are resistant, for example the freshwater crayfish *Procambarus clarkii* which is native to North America and naturalised or cultivated in all continents except Oceania (Vasconcelos *et al.* 2001). Microcystins are concentrated in the intestines and hepatopancreas of *P. clarkii*, representing a source of the toxins for predators on these crayfish (up to 2.9 μg microcystin/g dry weight) , but not for humans who consume only the muscle tissue (Vaconcelos *et al.* 2001). Nodularin is reported in liver and muscle of Baltic sea trout exposed experimentally to *Nodularia spumigena* (Kankaanpää *et al.* 2002).

Mode of action:

Cyanobacterial peptide hepatotoxins appear to damage hepatocyte cytoskeletal components. They inhibit protein phosphatases I and II (Yoshizawa *et al.* 1990).

They also act as tumour promoters if ingested chronically (Yoshizawa *et al.* 1990, Falconer 1991).

Conditions of poisoning:

N.B. Many cyanobacterial blooms are non-toxic to animals, but all should be considered potentially toxic. The means to differentiate toxigenic from non-toxigenic strains are being developed in the form of gene probes (e.g. Tillett *et al.* 2001). The belief that there can be rapid dramatic changes in bloom toxicity from day to day seems to be an artefact of non-quantitative mouse bioassay methods of toxicity testing and is not supported by quantitative data (Jones *et al.* 1993).

Blooms of cyanobacteria result from factors including

- ↑nutrient concentrations (particularly N, P) in water bodies resulting from runoff of livestock or human waste or fertilisers – a process known a eutrophication.
- seasonally-reduced flow rates in inland river systems in Australia leading to strong stratification of the water body, particularly in impoundments behind weirs (Webster *et al.* 2000)
- salinity: *Nodularia spumigena* blooms in estuaries and coastal lagoons are thought to be triggered by declines in salinity which stimulate the germination of akinetes (resting forms of the organism) from sediments. Salinity declines may follow inflows of fresh water after rainfall events or sewerage discharges (Jones *et al.* 1994). Blooms of *Nodularia spumigena* decline at salinities of over 30 g/kg in Australia (Jones *et al.* 1994) and blooms in the Baltic sea develop at salinities of 7-10 g/kg (Kononen 1992).
- Anoxic conditions in the hypolimnion (bottom water layer of water bodies) appear to cause benthic mats of *Oscillatoria* spp. to detach from the substrate and form rafts at the water surface, thus bringing them into contact with terrestrial vertebrates (W. Hosja, personal communication to Jones *et al.* 1994).

Wind may concentrate blooms in stock water sources (earth tanks/dams, streams) at points of access for livestock.

Irrigation with water contaminated with cyanobacterial blooms may deposit toxins on the irrigated crops (Codd *et al.* 1999).

Toxins may be passed to filter-feeding organisms exposed to cyanobacterial blooms, e.g. mussels exposed to *Nodularia spumigena* (Falconer *et al.* 1992), crayfish exposed to *Microcystis aeruginosa* (Vasconcelos *et al.* 2001).

Microcystins contaminating the water supply have killed patients in a medical haemodialysis unit in Brazil (Jochimsen *et al.*1998; Pouria *et al.* 1998).

Clinical signs:

Signs associated with acute hepatic necrosis (*q.v.*)

± blue or blue-green stains around the mouth and on legs

Pathology: acute hepatic necrosis (*q.v.*)

Diagnosis:

- Evidence of access to blooms
- Identification of toxigenic cyanobacteria in bloom material (microscopic examination of bloom samples allows recognition of known toxigenic cyanobacterial species and justifies the expense of toxin assays, mouse toxicity tests or both)
    - For laboratory examination, two (2) samples are required from each bloom:
        - **For Identification of organisms**: 20 ml of bloom material + 1 ml 10% formalin for preservation [N.B. alcohol may produce distortion of cells, so formalin is preferred].
        - **For Toxicity testing**: 1 litre of the most concentrated bloom material (minimum useful quantity is 20 ml) chilled and transported as swiftly as possible; do **not** add preservative to this material.
- Mouse bioassay has been the standard toxicity testing method for cyanobacterial blooms, but is being superseded by assays for specific toxins. Mouse bioassay results should be expressed as LD_{50} or LD_{100} values in units of mg dry weight of cyanobacteria / kg mouse body weight (Jones *et al.* 1993).
- Assay methods for microcystin and nodularin are available for water and cyanobacterial bloom material
    - HPLC
    - ELISA - microcystins (e.g. Nagata *et al.* 1999), nodularin (e.g. Mikhailov *et al.* 2001)

  Microcystin assays available from Queensland Health Scientific Services, 39 Kessels Road, Coopers Plains, Brisbane 4108 @ $215 for the first sample and $80 for subsequent samples in the same submission (Geoff Eaglesham, Senior Chemist, personal communication 27 Aug 01)
- Gene probes are being developed to allow identification of potentially toxigenic strains of *Microcystis* without the need for toxicity testing (Tillett *et al.* 2001).

Therapy:

Prognosis is always poor-to-grave if clinical signs are established

general decontamination techniques should be used including activated charcoal PO to prevent further absorption of toxins

continuing mortalities reported in sheep poisoned by *Microcystis aeruginosa* in Victoria (Carbis *et al.* 1995)

Prevention & control:

Prevent toxicity from an established cyanobacterial bloom by stopping animal intake of the floating bloom material:

- **prevent intake** of intact surface blooms
    - draw water supplies from sites away from blooms
    - use floating booms to separate blooms (top 10 cm of water) from outlet points
- **add algicides** to the water [*Note well*: This will release toxins into the water body. At least 5 days (preferably longer) should elapse before the water is used for stock]
    - copper sulphate
    - other algicides (quinones, organic herbicides) – further work is required on these.
- OR **add precipitants of cyanobacteria** to the water [precipitate cells without rupturing them, thus no toxins released into the water; remove P from the water, thus preventing further blooms]
    - lime
    - ferric alum
    - gypsum
- AND/OR **add barley straw bales** to the water body
    - very effective at clearing algal blooms

- mechanism unknown, but thought to be chemical or a substrate for microscopic crustacea (water fleas – *Daphnia* spp.) which may feed on the bloom material; chemical candidates include quinones derived from oxidised phenols, tannins, products of lignin oxidation (Pillinger *et al.* 1994)

Prevent the conditions required for establishment of blooms:

- Prevent contamination of water sources by nutrients (particularly P)
    - **precipitate phosphates** with alum and iron salts or lime or gypsum (applicable to small reservoirs) or Phoslock®, a CSIRO-developed P-absorbing clay slurry sprayed on the water surface (Sample 2000)
    - **aerate the bottom layer of water bodies** mechanically → prevents stratification within the water body and thus anaerobic mobilisation of phosphate from bottom sediment
    - **reduce nutrient (P) inflow to water bodies** (Catchment control)
        - minimise phosphatic fertiliser use
        - increase forestation & buffer vegetation adjacent to streams
        - minimise soil erosion
        - minimise sewage inflow (human sewage treatment to reduce/remove phosphates; sewage treatment outflows used for irrigation)
        - reintroduce or introduce wetland vegetation or reed beds at inflow regions of reservoirs. This approach has been used successfully for a farm dam in central Queensland by planting local water plants *Ottelia ovalifolia, Juncus* spp., *Potamogeton crispus* and *Ludwigia peploides* at the margins of the dam after fencing stock out. The plants established over an 18 month period, utilised nutrients (denying them to cyanobacteria), and clarified the water, breaking down the stratification caused by suspended clay particles. Blooms of *Microcystis aeruginosa* did not recur despite inflows of rain water containing cattle manure (Armstrong 2001).
        - remove feral fish (European carp) that stir up sediments (and thus P) from the water body
- Water management of weirs on inland river systems to prevent water column stratification from providing ideal conditions for cyanobacterial growth in the surface water layer (Webster *et al.* 2000) through one or several of
    - diurnal mixing of the water column by adjusting discharge volume to suit the depths of water impounded (high discharge volumes for greater depth)
    - pulsed discharge
    - height of discharge (overflow weir vs. underflow weir; siphons for release of surface layer)
    - withdrawal of water for consumption from the base of the water column

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Simple Treatment Protocols for cyanobacterial removal or prevention

Precipitants of cells and P

Lime (calcium carbonate): 100-250 mg/L; precipitates cells without rupturing them (no toxin release)

Ferric alum: Place block of alum in hessian bag and suspend from a float in the water body; rate = 1 kg/10,000 L (100 mg/L; 100 kg/megalitre)

Gypsum: Apply as for ferric alum; 50 kg/megalitre

Algicide

Copper sulphate: target concentration 0.2-0.4 ppm with upper limit of 1.0 ppm; equivalent to 20-40 g / 50,000 L

N.B.

- Cyanobacterial cells are ruptured and toxins are released into the water undamaged; prevent stock access for at least 1 week
- bacteria and protozoa in the water that feed on cyanobacteria are killed, and thus further toxic cyanobacterial blooms are encouraged

- vascular plants in the water are killed, preventing their using nutrients and returning nutrients to the water from their rotting tissues, thus promoting further cyanobacterial blooms

Barley straw

varieties Schooner, Parwon & Clipper have been reported effective; 100 g straw to 1000 L water – spread one third on the dam surface and suspend the remainder in bales across the dam on a rope support

N.B.

Australian experience with the use of barley straw is less positive than that from Europe.

- its use in Australia has not given satisfactory results in controlling cyanobacterial blooms
- its use has promoted the growth in treated water bodies of protozoa (amoebae) that can cause human meningitis (LD Fabbro, personal communication 8 July 2002)
- its rotting tissues provide nutrients that promote further cyanobacterial blooms

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Proposed guidelines for drinking water in Australia suggest an upper limit of 1µg toxin/L water or 5000 cells/ml water (based on *Microcystis aeruginosa* hepatotoxicity)

Degradation of microcystins in water

Titanium dioxide plus exposure to ultraviolet light can degrade microcystin-LR in water (Guterman 1999)

Febton oxidation (hydrogen peroxide in the presence of Fe salts to generate hydroxyl radicals) will degrade microcystin-LR (Gajdek *et al.* 2001).

Biological degradation

This approach is theoretical as a control measure, as to date it has no practical application to bloom control, but has a firm basis in the ecology of aquatic systems and the history of microbial life. Cyanobacteria are food sources for other microbes including viruses (cyanophages) (Padan & Shilo 1973, Safferman & Rohr 1979), bacteria and fungi (Stewart & Daft 1976,1977). Protozoa are also predators of cyanobacteria (Dryden & Wright 1987, Fabbro *et al.* 2001).

Three enzymes in an unnamed species of the bacterium *Sphingomonas* can degrade microcystins; microcystinase breaks the ring structure of the molecule reducing toxicity greater than 100 fold, the other enzymes break the resulting structure down to amino acids; discovered in river water by CSIRO/UQ in 1993 (Anderson 1995).

Integrated catchment management

Strategies such as that proposed by the Murray-Darling Basin Commission (1994) aim to address all predisposing and mitigating factors in the production of cyanobacterial blooms in waterways. Strategies address the management of

plant nutrients – P and to a lesser extent, N – and water flow through the activities of individuals (including primary producers), corporations and government at all levels.

References:

Review literature

Chorus I, Bartram J (eds.) (1999) *Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management.* E & FN Spon (Routledge), London & New York. xv + 416 pp. ISBN 0 419 23930 8

Davis JR (ed.) (1997) *Managing Algal Blooms. Outcomes from CSIRO's Multi-Divisional Blue-green Algal Program.* CSIRO Land & Water, Canberra. xi + 113 pp. ISBN 0 643 06049 9

Duy TN, Lam PKS, Shaw GR, Connell DW (2000) Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Rev. Environ. Contam. Toxicol.* **163**:113-186.

Ressom R, Soong FS, Fitzgerald J, Turczynowicz L, El Saadi O, Roder D, Maynard T, Falconer I (1994) *Health Effects of Toxic Cyanobacteria (Blue-Green Algae)*. National Health & Medical Research Council, Canberra. ISBN 0 644 32908 4

Steffensen DA, Nicholson BC (eds.) (1994) *Toxic Cyanobacteria: Current Status of Research and Management.* Proceedings of an International Workshop, Adelaide, Australia, March 22-26, 1994. Australian Centre for Water Quality Research, Salisbury, South Australia.

General literature

Anderson I (1995) Hungry enzymes thrive on toxic slime. *New Scientist* **146**(1980):17.

Andrewartha R (1998) Sudden deaths in cattle. *Animal Health Surveillance Quarterly (Australian National Animal Health Information System Newsletter)* **3**(2):11.

Armstrong M (2001) Revegetation beats algae. *Queensland Country Life* September 13, p.49.

Beasley VR, Dahlem AM, Cook WO, Valentine WM, Lovell RA, Hooser SB, Suzuki M, Carmichael WW (1989) Diagnostic and clinically important aspects of cyanobacterial (blue-green algae) toxicosis. *J. Vet. Diagn. Invest.* **1**:359-365.

Beasley VR, Lovell RA, Dahlem AM, Haschek WM, Hooser SB (1991) Cyclic peptide hepatotoxins from cyanobacteria. Chapter 22 in Keeler RF, Tu AT (eds) *Handbook of Natural Toxins. Vol.6. Toxicology of Plant and Fungal Compounds.* Marcel Dekker, Inc., New York. pp. 459-495.

Blackburn S, Bolch C, Jones G, Negri A, Orr P (1997) Cyanobacterial blooms: why are they toxic? (The regulation of toxin production and persistence). Chapter 6 in Davis JR (ed.) (1997) *Managing Algal Blooms. Outcomes from CSIRO's Multi-Divisional Blue-green Algal Program.* CSIRO Land & Water, Canberra. pp.67-77.

Botes DP, Kruger H, Viljoen CC (1982a) Isolation and characterisation of four toxins from the blue-green alga, *Microcystis aeruginosa. Toxicon* **20** (6):945-954.

Botes DP, Viljoen CC, Kruger H, Wessels PL, Williams DH (1982b) Configuration assignments of the amino acid residues and the presence of N-methyldehydroalanine in toxins from the blue-green alga, *Microcystis aeruginosa. Toxicon* **20** (6):1037-1042.

Botes DP, Viljoen CC, Kruger H, Wessels PL, Williams DH (1982c) Structure of toxins of the blue-green alga *Microcystis aeruginosa. S. Afr. J. Sci.* **78**:378-379.

Carbis CR, Waldron DL, Mitchell GF, Anderson JW, McCauley I (1995) Recovery of hepatic function and latent mortalities in sheep exposed to the blue-green alga *Microcystis aeruginosa. Vet. Rec.* **137**:12-15.

Carmichael WW, Eschedor JT, Patterson GML, Moore RE, (1988) Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium *Nodularia spumigena* Mertens emend. L575 from New Zealand. *Appl. Environ. Microbiol.* **54**:2257-2263.

Carmichael WW (1992b) Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* **72**:445-459.

Codd GA, Steffensen DA, Burch MD, Baker PD (1994) Toxic blooms of cyanobacteria in Lake Alexandrina, South Australia – learning from history. *Aust. J. Mar. Freshwater Res.* **45**:731-736.

Codd GA, Metcalf JS, Beattie KA (1999) Retention of *Microcystis aeruginosa* and microcystin by salad lettuce (*Lactuca sativa*) after spray irrigation with water containing cyanobacteria. *Toxicon* **37**:1181-1185.

Duy TN, Lam PKS, Shaw GR, Connell DW (2000) Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Rev. Environ. Contam. Toxicol.* **163**:113-186.

Dryden RC, Wright SJL (1987) Predation of cyanobacteria by protozoa. *Can. J. Microbiol.* **33**:471-482.

Edler L, Fernö S, Lind MG, Lundberg R, Nilsson PO (1985) Mortality of dogs associated with a bloom of the cyanobacterium *Nodularia spumigena* in the Baltic Sea. *Ophelia* **24**:103-109.

Fabbro L, (2001) *Paramecium Cylindrospermopsis raciborskii Environ. Toxicol.* **16**:

Falconer IR (1991) Tumour promotion and liver injury caused by oral consumption of cyanobacteria. *Environ. Toxicol. Water Qual.* **6**:177-184.

Falconer IR, Choice A, Hosja W (1992) Toxicity of edible mussels (*Mytilus edulis*) growing in an estuary during a water bloom of the blue-green alga *Nodularia spumigena. Environmental Toxicology and Water Quality* **7**:119-124.

Francis G (1878) Poisonous Australian lake. *Nature (London)* **18**:11-12.

Gajdek P, Lechowski Z, Bochnia T, Kepczynski M (2001) Decomposition of microcystin-LR by Fenton oxidation. *Toxicon* **39**:1575-1578.

Gussmann HJ, Molzahn J, Bicks B (1985) [Poisoning of young cattle by the blue-green alga *Nodularia spumigena*] *Mh. Vet.-Med.* **40**:76-79.

Guterman L (1999) Pure white-out. *New Scientist* **161**(2173):23 citing work by Lawton L, Robertson P *et al*. (1999) *Environmental Science & Technology*, in press.

Jackson ARB, McInnes A, Falconer IR, Runnegar MTC (1983) Toxicity for sheep of the blue-green alga *Microcystis aeruginosa. Toxicon* **3** (Suppl.):191-194.

Jackson ARB, McInnes A, Falconer IR, Runnegar MTC (1984) Clinical and pathological changes in sheep experimentally poisoned by the blue-green alga *Microcystis aeruginosa. Vet. Pathol.* **21**:102-113.

Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CEM, de C. Antunes MD, de Melo Filho DA, Lyra TM, Bareto CSV, Azevedo SMFO, Jarvis WR (1998) Liver failure and death after exposure to microcystins at a haemodialysis centre in Brazil. *New England J. Med.* **338**:873-878.

Jones GJ, Falconer IF, Burch M, Craig K (1993) Algal Toxicity. In *Algal Management Strategy, Technical Advisory Group Reports.* Murray-Darling Basin Commission, Australia. pp.17-32.

Jones GJ, Blackburn SI, Parker NS (1994) A toxic bloom of *Nodularia spumigena* Mertens in Orielton Lagoon, Tasmania. *Aust. J. Mar. Freshwater Res.* **45**:787-800.

Kankaanpää H, Vuorinen PJ, Sipiä V, Keinänen M (2002) Acute effects and bioaccumulation of nodularin in sea trout (*Salmo trutta* m. *trutta* L.) exposed orally to *Nodularia spumigena* under laboratory conditions. *Aquatic Toxicology* **61:** 155-168.

Kononen K (1992) Dynamics of the toxic cyanobacterial blooms in the Baltic sea. *Finnish Marine Res.* **261**:3-36.

Lahti K (1997) Cyanobacterial hepatotoxins and drinking water supplies – aspects of monitoring and potential health risks. Monographs of Boreal Environment Research No. 4 Finnish Environment Institute, Finland.

Luukkainen R, Sivonen D, Namikoshi M, Frdig M, Rinehart KL, Niemel SI (1993) Isolation and identification of eight microcystins from thirteen *Oscillatoria agardhii* strains and structure of a new microcystin. *Appl. Environ. Microbiol.* **59** (7):2204-2209.

Main DC, Berry PH, Peet RL, Robertson JP (1977) Sheep mortalities associated with the blue green alga *Nodularia spumigena*. *Aust. Vet. J.* **53**:578-581.

Metz K, Beattie KA, Codd GA, Hanselmann K, Hauser B, Naegeli HP, Preisig HR (1997) Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. *Eur. J. Phycol.* **32**:111-117.
McBarron EJ, May V (1966) Poisoning of sheep in New South Wales by the blue-green alga *Anacystis cyanea* (Kuetz.) Dr. and Dail. *Aust. Vet. J.* **42**:449-453.
Mikhailov A, Härmälä-Braskén A-S, Polosukhina E, Hanski A, Wahlsten M, Sivonen K, Eriksson JE (2001) Production and specificity of monoclonal antibodies against nodularin conjugated through *N*-methyldehydrobutyrine. *Toxicon* **39**:1453-1459.
Mulhearn CJ (1959) Beware algae! They can poison livestock. *J. Agric., S. Aust.* April Issue, pp.406-408.
Murray-Darling Basin Commission (1994) *The Algal Management Strategy for the Murray-Darling Basin*. Murray-Darling Basin Ministerial Council, Canberra.
Nagata S, Tsutsumi T, Yoshida F, Ueno Y (1999) A new type of sandwich immunoassay for microcystin: production of monoclonal antibodies specific to the immune complex formed by microcystin and an anti-microcystin monoclonal antibody. *Nat. Toxins* **7**:49-55.
Nehring S (1993) Mortality of dogs associated with mass development of *Nodularia spumigena* (Cyanophycae) in a brackish lake at the German North Sea coast. *J. Plankton Res.* **15**:867-872.
Padan E, Shilo M (1973) Cyanophages - viruses attacking blue-green algae. *Bacteriol. Rev.* **37**:343-370.
Pillinger JM, Cooper JA, Ridge I (1994) Role of phenolic compounds in the antialgal activity of barley straw. *J. Chem. Ecol.* **20**:1557-1569.
Pouria S, de Andrade A, Barbosa J, Cavalcanti RL, Barreto VTS, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA (1998) Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *Lancet* **352**:21-26.
Rinehart KL, Harada KI, Namikoshi CC, Harris CA (1988) Nodularin, microcystin and the configuration of ADDA. *J. Am. Chem. Soc.* **110**:8557-8558.
Runnegar MTC, Jackson ARB, Falconer IR (1988) Toxicity of the cyanobacterium *Nodularia spumigena* Mertens. *Toxicon* **26**:143-151.
Sadler R (1994) *Report on Cyanobacteria in Water*. Government Chemical Laboratory, Coopers Plains, Brisbane.
Safferman RS, Rohr ME (1979) *The practical directory to phycovirus literature.* U.S.Environ. Prot. Agency Rep. EPA-600/9-97/013 and Addendum 1981.
Sample I (2000) Clay spray could spell death for algal blooms. *New Scientist* **165**(2221):16. & Robb M (2000) Nonstick clay (correspondence). *New Scientist* **165**(2225):54.
Santikarn S, Williams DH, Smith RJ, Hammond SJ, Botes DP, Tuinman A, Wessels PL, Viljoen CC, Kruger H (1983) A partial structure for the toxin BE-4 from the blue-green algae *Microcystis aeruginosa. J. Chem. Soc. Chem. Commun.* **12**:652-654.
Stewart WDP, Daft MJ (1976) Algal-lysing agents of freshwater habitats. In Skinner FA, Carr JG (eds) *Microbiology in Agriculture, Fisheries and Food*. Academic Press, London. pp. 63-90.
Stewart WDP, Daft MJ (1977) Microbial pathogens of cyanophycean blooms. In Droop MR, Jannasch HW (eds) *Advances in Aquatic Microbiology*. Academic Press, London. **1**:177-218.
Tillett D, Parker DL, Neilan BA (2001) Detection of toxigenicity by a probe for the microcystin synthetase A gene (*mycA*) of the cyanobacterial genus *Microcystis*: comparison of toxicities with 16S rRna and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. *Appl. Environ. Microbiol.* **67**:2810-2818.
Vasconcelos V, Oliveira S, Teles FO (2001) Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii. Toxicon* **39**:1461-1470.
Webster IT, Sherman BS, Bormans M, Jones G (2000) Management strategies for cyanobacterial blooms in an impounded lowland river. *Regulated Rivers: Research & Management* **16**:513-525. [Study of the Maude Weir pool on the Murrumbidgee River, NSW]
Yoshizawa S, Matsushima R, Watanabe MF, Harada K, Ichihara A, Carmichael W, Fujiki H (1990) Inhibition of protein phosphatases by microcystin and nodularin associated with hepatotoxicity. *J. Cancer Res. Clin. Oncology* **116**:609-614.

💣☑ Cyanobacterial alkaloid neurotoxins – paralytic shellfish poisoning (PSP) toxins and anatoxins

Core data

Common source: Anabaena spp.
Neurotoxic "sudden death factors":

- paralytic shellfish poisoning (PSP) toxins: sodium channel blockers
- anatoxin-a & homoanatoxin-a: alkaloid neuromuscular blockers
- anatoxin-a(s): organophosphorus compound

Animals affected: sheep, cattle, dogs, pigs, birds
Poisoning circumstances (all cyanobacteria)*:*

- *many blooms non-toxic, but all should be considered potentially toxic*
- increased nutrient concentrations (N,P) in water bodies; animal waste, fertilisers
- slow flow and stratification of streams and impoundments
- wind concentration of floating bloom at point of livestock access

Main effects: sudden death
Diagnosis (all cyanobacteria)*:*

- identify cyanobacterium
- demonstrate its toxicity (mouse inoculation; toxin assay)

Therapy:

- prognosis grave
- atropine + oximes (OP neurotoxins)
- activated charcoal

Prevention & Control (all cyanobacteria)*:*

- prevent consumption of bloom:
  - avoidance
  - physical barrier
  - algicides, precipitants (lime, ferric alum, gypsum)
  - barley straw (?)
- reduce nutrient load:
  - revegetate margins of small reservoirs (farm dams)
  - precipitate P
  - aerate bottom layer
  - reduce P inflow (minimise fertiliser use, vegetation buffer zones, minimise sewerage inflow, wetlands at inflow, remove carp)
- improve flow rates and disrupt stratification

Syndromes: sudden death

Chemical structure:

Paralytic shellfish poisoning (PSP) toxins (C-toxins, saxitoxins & gonyautoxins) (*q.v.*) are tetrahydropurines. These toxins are the major toxins in *Anabaena circinalis* in Australia (Ressom *et al.* 1994, Negri *et al.* 1995).

Anatoxin-a & homoanatoxin (tropane-related alkaloids; homotropane alkaloids). These toxins have *not* been found in neurotoxic *Anabaena* in Australia (Ressom *et al.* 1994). Dihydro-anatoxin (formed from anatoxin-a) has been found in a mixed bloom of *Tychonema bornetti* [=*Oscillatoria bornetti*] & *Phormidium* associated with death of dogs at Lake Ginniderra in Canberra in September 2001 (Will Andrew, personal communication 18 June 2002)

Anatoxin -a(s) [s = salivation], an organophosphorus compound (*q.v.*) (an N-hydroxyguanidinium methyl phosphate ester or cyclic guanidinium phosphate ester). This toxin has *not* been found in neurotoxic *Anabaena* in Australia (Ressom *et al.* 1994).

Sources:

Cyanophycae (= cyanobacteria or blue-green algae) in fresh water

[neurotoxins isolated: PSP = paralytic shellfish poisons, A = anatoxin-a, HA = homoanatoxin, As = anatoxin-a(s),]

***Anabaena* spp.**

Anabaena circinalis [A (Sivonen *et al.* 1989), PSP (Humpage *et al.* 1994, Negri *et al.* 1994, 1995)] [Darling River bloom 1991/2 = *Anabaena circinalis* (NSWBGATF 1992, Jones & Negri 1995), toxic to bees (May & McBarron 1973), sheep (McBarron *et al.* 1975, Negri *et al.* 1995)]

Anabaena flos-aquae [A (Huber 1972, Devlin *et al.* 1977, Carmichael 1992b), As (Mahmood & Carmichael 1986, Henriksen *et al.* 1997)]

Anabaena spiroides [A (Carmichael 1992b)]

Anabaena lemmermannii [As (Onodera *et al.* 1997a)] – not listed for Australia (Day *et al.* 1995)

Aphanizomenon sp. [A (Codd *et al.* 1997)]

Aphanizoomenon flos-aquae [PSP (Sawyer *et al.* 1968)]

Cylindrospermopsis raciborskii [PSP; detected in South American isolates (Lagos *et al.* 1999)]

Lyngbya wollei [PSP (Carmichael *et al.* 1997; Onodera *et al.* 1997b)] – not listed for Australia (Day *et al.* 1995)

Microcystis spp. [A (Codd *et al.* 1997)]

Nostoc spp. [A (Davidson 1959)]

Oscillatoria spp. [A (Edwards *et al.* 1992), HA (Skulberg *et al.* 1992)]

Tychonema bornetti [=*Oscillatoria bornetti*] + *Phormidium* mixed isolate [A (Will Andrew, personal communication 18 June 2002)]

Planktothrix spp. [PSP (Pomati *et al.* 2000)]

Trichodesmium spp. [A (Hawser *et al.* 1991)]

Toxicity:

Animals affected: sheep (Negri *et al.* 1995), cattle, dogs (Gunn *et al.* 1992), pigs, birds, honey bees

Blooms causing sudden death are most likely to contain peptide-producing, liver necrosis-inducing cyanobacteria (see Acute liver necrosis chapter), but *some* may contain **neurotoxic "sudden death factors"** – those toxins recognised are the paralytic shellfish poisoning [PSP] toxins, anatoxin-a, homoanatoxin-a and anatoxin-a(s).

About half the blooms of *Anabaena circinalis* tested in Australia are toxic in the mouse bioassay; as blooms age, the toxins transform to more toxic types and the bloom becomes more toxic (Jones & Negri 1995)

PSP toxins from *Anabaena circinalis* can be bioaccumulated by Australian freshwater mussels including *Alathyria condola* Iredale (96% of toxins accumulated in the viscera) when low-flow conditions in summer in the southern parts of Australia bring surface blooms in contact with these filter-feeders, thus posing a potential hazard to native water rats and birds and to aquaculture systems (Negri & Jones 1995).

Oscillatoria, a benthic species washed ashore, poisoned dogs scavenging along the shore of a Scottish loch (Gunn *et al.* 1992, Edwards *et al.* 1992, Skulberg *et al.* 1992).

A mixed bloom of *Tychonema bornetti* [=*Oscillatoria bornetti*] and a *Phormidium* sp. was associated with sudden death of 2 dogs (within 20-60 min of onset of signs; collapse, terminal clonic convulsions) and illness of one other (vomiting, recovered) exposed through swimming to floating mats ("pancakes") concentrated by wind against the shore at one point in Lake Ginniderra in Canberra in September 2001. Dihydroxy-anatoxin (formed from anatoxin-a) was detected by MS in bloom samples. (Will Andrew, personal communication 18 June 2002).

Modes of action:

- Paralytic shellfish toxins are sodium channels blockers, the same mechanism as tetrodotoxin (*q.v.*)
- Anatoxin-a and homoanatoxin-a are post-synaptic depolarising neuromuscular blocking agents which mimics the effects of acetylcholine and acts at both nicotinic and muscarinic receptors. Toxicity causes paralysis of skeletal muscle leading to paralysis of breathing.
- Anatoxin-a(s) is which is a potent irreversible cholinesterase inhibitor.

Conditions of poisoning: See section on cyanobacterial peptide toxins under Acute liver necrosis.

Clinical signs:

sudden death

dyspnoea, respiratory arrest

± blue or blue-green stains around the mouth and on legs

± signs of organophosphorus compound poisoning – cholinesterase inhibition (see notes under nervous syndromes)

Pathology: No significant lesions recorded

Diagnosis:

See section on cyanobacterial peptide toxins above.

Saxitoxins (paralytic shellfish poisoning toxins) assays available from Queensland Health Scientific Services, 39 Kessels Road, Coopers Plains, Brisbane 4108 @ $215 for the first sample and $80 for subsequent samples in the same submission (Geoff Eaglesham, Senior Chemist, personal communication 27 Aug 01)

Therapy:

Prognosis is always poor-to-grave if clinical signs are established

General decontamination techniques should be used including activated charcoal PO to prevent further absorption of toxins

There is no generally-recognised effective therapy against anatoxin-a or saxitoxins [PSP], but respiratory support may help. Recent experimental work has revealed that 4-aminopyridine (*q.v.*) is effective for treating the life-threatening effects of saxitoxin (Benton *et al.* 1996, 1998, Chang *et al.* 1996, Cheng *et al.* 1996).

For anatoxin-a(s) toxicity, therapy used for other organophosphorus compound poisonings (*q.v.*) should be used: atropine + oximes

Prevention & Control: See section on cyanobacterial peptide toxins (above)

References:

Benton BJ, Spriggs DL, Capacio BR, Chang F-CT (1996) 4-aminopyridine antagonises the lethal effects of saxitoxin (STX) and tetrodotoxin (TTX). *Toxicon* **34**:335.

Benton BJ, Keller SA, Spriggs DL, Capacio BR, Chang F-CT (1998) Recovery from the lethal effects of saxitoxin: a therapeutic window for 4-aminopyridine (4-AP). *Toxicon* **36**:571-588.

Carmichael WW (1992a) *A status report on planktonic cyanobacteria (blue-green algae) and their toxins.* Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Carmichael WW (1992b) Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* **72**:445-459.

Carmichael WW, Falconer IR (1993) Diseases related to freshwater blue-green algal toxins, and control measures. Chapter 12 in *Algal Toxins in Seafood and Drinking Water*, edited by IR Falconer, Academic Press, London, pp.187-209.

Carmichael WW, Evans WR, Yin QQ, Bell P, Moczydlowski E (1997) Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Forlow ex Gamont) comb. nov. *Appl. Environ. Microbiol.* **63**:3104-3110.

Chang F-CT, Bauer RM, Benton BJ, Keller SA, Capacio BR (1996) 4-aminopyridine antagonizes saxitoxin- and tetrodotoxin-induced cardiorespiratory depression. *Toxicon* **34**:671-690.

Chen H-M, Lin C-H, Wang TM (1996) Effects of 4-aminopyridine on saxitoxin intoxication. *Toxicol. Appl. Pharmacol.* **141**:44-48.

Codd GA, Ward CJ, Bell SG (1997) Cyanobacterial toxins: occurrence, models of action, health effects and exposure routes. *Arch. Toxicol. Suppl.* **19**:399-410.

Conell D, Arthington A, Creighton C, Clewett M, Griffiths D, Hazel C, Neville G, Nimmo P, Rogers RL, Stock E (1992) *Report on Freshwater Algal Blooms in Queensland. Volume 1. Main Report.* Queensland Water Quality Taskforce, Queensland Department of Primary Industries, Brisbane.

Davidson FF (1959) Poisoning of wild and domestic animals by a toxic waterbloom of *Nostoc rivulare* Kuetz. *J. Am. Water Works Assoc.* **51**:1277-1287.

Devlin JP, Edwards OE, Gorham PR, Hunter NR, Pike RK, Stavric B (1977) Anatoxin-a, a toxic alkaloid from *Anabaena flos-aquae* NRC-44h. *Can. J. Chem.* **55**:1367-1371.

Edwards C, Beattie KA, Scrimgeour CM, Codd GA (1992) Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisoning at Loch Insh, Scotland. *Toxicon* **30**:1165-1175.

Gunn GJ, Rafferty AG, Rafferty GC, Cockburn N, Edwards C, Beattie KA, Codd GA (1992) Fatal canine neurotoxicosis attributed to blue-green algae (cyanobacteria). *Vet. Rec.* **104**:301-302.

Hawser SP, Codd GA, Capone DG, Carpenter EJ (1991) A neurotoxic factor associated with the bloom-forming cyanobacterium *Trichodesmium. Toxicon* **29** (3):277-278.

Henriksen P, Carmichael WW, An J, Moestrup Ø (1997) Detection of an anatoxin-a(s) like anticholinesterase in natural blooms and cultures of cyanobacteria/blue-green algae from Danish lakes and in the stomach contents of poisoned birds. *Toxicon* **35**:901-913.

Huber CS (1992) The crystal structure and absolute configuration of 2,9-diacetyl-9-azabi-cyclo[4,2,1] non-2,3-ene. *Acta Crystalog.* **238**:2577-2582.

Humpage AR, Rositano J, Bretag AH, Brown R, Baker PD, Nicholson BC, Steffensen DA (1994) Paralytic shellfish poisons from Australian cyanobacterial blooms. *Aust. J. Mar. Fresh Water Res.* **45**:761-771.

Jones G, Negri A (1995) Blooming algal neurotoxins. *Chemistry in Australia* **62**(6):12-13.

Lagos N, Onodera H, Zagatto PA, Andrinolo D, Azevedo SMFQ, Oshima Y (1999) The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. *Toxicon* **37**:1359-1373.

Mahmood WA, Carmichael WW (1986) Paralytic shellfish poisons produced by the fresh-water cyanobacterium *Aphanizomenon flos-aquae* NH-5. *Toxicon* **24**:175-186.

May V, McBarron EJ (1973) Occurrence of the blue-green alga, *Anabaena circinalis* Rabenh., in New South Wales and toxicity to mice and honey bees. *J. Aust. Inst. Agric. Sci.* **39** (4):264-266.

McBarron EJ, Walker RI, Gardner I, Walker KH (1975) Toxicity to livestock of the blue-green alga *Anabaena circinalis*. *Aust. Vet. J.* **51**:587-588.

Negri AP, Jones GJ, Blackburn SI, Oshima Y, Onodera H (1994) Effect of culture and bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*. *J. Phycol.* **33**:26-35.

Negri AP, Jones GJ (1995) Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola. Toxicon* **33**:667-678.

Negri AP, Jones GJ, Hindmarsh M (1995) Sheep mortality associated with paralytic shellfish poisons from cyanobacterium *Anabaena circinalis. Toxicon* **33**:1321-1329.

NSWBGATF (New South Wales Blue-Green Algae Task Force) (1992) *Blue-green algae. Report of the New South Wales Blue-green Algae Task Force.* NSW Department of Water Resources. Parramatta, NSW.

Onodera H, Oshima Y, Henriksen P, Yasumoto T (1997a) Confirmation of anatoxin-a(s) in the cyanobacterium *Anabaena lemmermannii*, as the cause of bird kills in Danish lakes. *Toxicon* **35**:1645-1648.

Onodera H, Satake M, Oshima Y, Yasumoto T, Carmichael WW (1997) New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei. Natural Toxins* **5**:146-151.

Pomati F, Sacchi S, Rossetti C, Giovannardi S, Onodera H, Oshima Y, Neilan BA (2000) The freshwater cyanobacterium *Planktothrix* sp. fp1: molecular identification and detection of paralytic shellfish poisoning toxins. *J. Phycology* **36** (3): 553 - 562

Ressom R, Soong FS, Fitzgerald J, Turczynowicz L, El Saadi O, Roder D, Maynard T, Falconer I (1994) *Health Effects of Toxic Cyanobacteria (Blue-Green Algae)*. National Health & Medical Research Council, Canberra. ISBN 0 644 32908 4

Sawyer PJ, Gentile JH, Sasner JJ Jr (1968) Demonstration of a toxin from *Aphanizomenon flos-aquae* (L.) Ralfs. *Can. J. Microbiol.* **14**:1199-1204.

Sivonen K, Himberg K, Luukkainen R, Niemelä SI, Poon GK, Codd GA (1989) Preliminary characterisation of neurotoxic cyanobacteria blooms and strains from Finland. *Toxic Assess.* **4**:339-352.

Skulberg OM, Carmichael WW, Andersen RA, Matsunaga S, Moore RE, Skulberg R (1992) Investigations of a neurotoxic Oscillatorialean strain (Cyanophycae) and its toxin. Isolation and characterisation of homoanatoxin-a. *Environ. Toxicol. Chem.* **11**:321-329.

☑ *Cylindrospermopsin*

Core data

Common sources: Cylindrospermopsis raciborskii
Animals affected: livestock (humans)
Mode of action: inhibits mammalian protein synthesis
Poisoning circumstances: chronic ingestion of freshwater bloom throughout the water column
Main effects:

- sub-acute to chronic liver damage
- nephrosis

Diagnosis: syndrome + demonstration of cyanobacterial source, toxin in water
Therapy: nil
Prevention:

- chlorination of water supply removes toxin
- prevent consumption of bloom:
  - avoidance
  - algicides, precipitants (lime, ferric alum, gypsum)
- reduce nutrient load:
  - revegetate margins of small reservoirs (farm dams)
  - precipitate P
  - aerate bottom layer
  - reduce P inflow (minimise fertiliser use, vegetation buffer zones, minimise sewerage inflow, wetlands at inflow, remove carp)
- improve flow rates and disrupt stratification

Chemical structure:

a hepatotoxic & nephrotoxic **alkaloid** (in contrast with peptides in other hepatotoxic cyanobacteria) (Ohtani *et al*. 1992)

Sources:

Blooms of **cyanobacteria** (cyanophytes, blue-green algae) including

Cylindrospermopsis raciborskii (Hawkins *et al.* 1985) – regarded as a tropical/subtropical species, with some occurrences in the temperate zones including in Europe and North America; organisms identified as *Cylindrospermopsis* sp. and cylindrospermopsin have been identified in Lake Waitawa, near Wellington, New Zealand (Stirling & Quilliam 2001)

Umezakia natans [only identified in Lake Mikata, Fukui, Japan (Harada *et al.* 1994, Terao *et al.* 1994)]

Aphanizomenon ovalisporum (Banker *et al.* 1997, Shaw *et al.* 1999) – tends to occur in water bodies with higher-than-normal salt content (LD Fabbro, personal communication 8 July 2002)

Anabaena bergii [= *Aphanizomenon bergii*] (LD Fabbro, personal communication 8 July 2002)

Toxicity:

Only *Cylindrospermopsis raciborskii* has been associated with natural poisoning incidents. Toxin production by *C. raciborskii* reaches a maximum during winter in northern Australia (LD Fabbro, personal communication 8 July 2002)

Presumed cases have been reported in **humans** (Byth 1980, Bourke *et al.* 1983, Hawkins *et al.* 1985, 1997) and **cattle** (Pearce & McKenzie 1993, Thomas *et al.* 1998, Saker *et al.* 1999, McKenzie *et al.* 2003). Confirmation through detection of cylindrospermopsin in affected livers has been achieved only in one bovine case to date where water consumed contained 1.0 mg cylindrospermopsin/L (McKenzie *et al.* 2003).

Cylindrospermopsin has been detected in hepatopancreas (4.3 μg/g dry matter) and skeletal muscle (0.9 μg/g dry matter) of redclaw crayfish cultured in pond water with 589 μg

cylindrospermopsin /L (93% in cyanobacterial cells, 7% in water) (Saker & Eaglesham 1999).

There is speculation that "Barcoo spews", a disease of humans in inland Australia, resulted from cylindrospermopsin toxicity (Hayman 1992).

Mode of action:

Cylindrospermopsin is a potent inhibitor of mammalian protein synthesis (Terao *et al.* 1994).

Conditions of poisoning:

See notes on cyanobacterial peptide toxins

Cylindrospermopsis raciborskii tends not to form floating masses (surface scums), but is usually distributed throughout the water column. The organism avoids high light levels and so can be more prevalent in water with a large sediment loading. *C. raciborskii* blooms discolour water red-brown or green. Red-brown colouration is due to carotenoids produced by the organism in response to light. Populations high in carotenoids tend to be the greatest toxin producers. The toxin remains intracellular with relatively little present in the water. Large volumes of contaminated water would have to be consumed before acute toxicity could occur (oral LD_{50} postulated as 6 mg cylindrospermopsin/kg based on mouse studies). Daily ingestion may lead to chronic hepatotoxicity. (Seawright 1999)

In summer in northern Australia, about 50% of fresh water bodies contain detectable populations of *Cylindrospermopsis raciborskii*; 20% contain blooms (LD Fabbro, personal communication 8 July 2002).

Clinical signs:

cattle - weakness progressing to recumbency and death; clinical course is longer than for peptide cyanobacterial toxins (3 days in the case of McKenzie *et al.* 2001)

Pathology:

cattle: liver, kidney and heart degeneration and necrosis

- sub-acute cases: biliary ductular proliferation, swelling of hepatocytes with foamy vacuolation of cytoplasm, scattered individual hepatocyte necrosis in periacinar areas (Pearce & McKenzie 1993, McKenzie *et al.* 2001); foamy vacuolation and swelling of renal proximal tubular epithelium with protein and cellular casts in tubular lumens; scattered mild focal myocardial degeneration and necrosis (McKenzie *et al.* 2001)
- chronic case: extensive hepatic fibrosis and biliary ductule proliferation with marked reduction in hepatocyte numbers and foamy vacuolation of hepatocyte cytoplasm (Thomas *et al.* 1998)

mice – marked swelling of liver due to fatty infiltration; consistent thymic atrophy and lymphophagocytosis in splenic follicles and lymph nodes; periacinar coagulation necrosis of hepatocytes may occur; focal hepatocyte necrosis and apoptosis may occur; ischaemic acute renal tubular necrosis may occur; at 0.2 mg cylindrospermopsin/kg IP (mouse LD_{50}), deaths can occur at up to 5-6 days. (Seawright 1999)

Diagnosis:

liver and kidney pathology + cyanobacterial identification + mouse inoculation of water (see notes on cyanobacterial peptide toxins) + HPLC/MS assay for cylindrospermopsin (restricted to certain labs)

Cylindrospermopsin assays available from Queensland Health Scientific Services, 39 Kessels Road, Coopers Plains, Brisbane 4108 @ $215 for the first sample and $100 for subsequent samples in the same submission (Geoff Eaglesham, Senior Chemist, personal communication 27 Aug 01).

Prevention & control:

See control & preventative measures described under cyanobacterial peptide toxins (above). Chlorination will rapidly and effectively remove cylindrospermopsin from water supplies, with the effect being reduced with large organic matter content, low pH or both (Senogles *et al.* 2000).

References:

Banker R, Carmeli S, Hadas O, Teltsch B, Porat R, Sukenik A (1997) Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophycae) isolated from Lake Kinneret, Israel. *J. Phycology* **33**: 613-616.

Bourke ATC, Hawes RB, Nielson A, Stallman ND (1983) An outbreak of hepato-enteritis (the Palm Island mystery disease) possibly caused by algal intoxication. *Toxicon Suppl.* **3**:45-48.

Byth S (1980) Palm Island mystery disease. *Med. J. Aust.* **2**:40-42.

Eaglesham GK, Norris RL, Shaw GR, Smith MJ, Chiswell RK, Davis BC, Neville GR, Seawright AA, Moore MR (1999) Use of HPLC-MS/MS to monitor cylindrospermopsin, a blue-green algal toxin, for public health purposes. *Environmental Toxicology* **14**:151-154.

Harada KI, Ohtani I, Iwamoto K, Suzuki M, Watanabe MF, Watanabe M, Terao K (1994) Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. *Toxicon* **32**:73-84.

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.* **50**:1292-1295.

Hawkins PR, Chandrasena NR, Jones GJ, Humpage AR, Falconer IR (1997) Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon* **35**:341-346.

Hayman J (1992) Beyond the Barcoo – probable human tropical cyanobacterial poisoning in outback Australia. *Med. J. Aust.* **157**:794-796.

McGregor GB, Fabbro LD (2000) Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. *Lakes & Reservoirs: Research and Management* **5**:195-205.

McKenzie RA, Noble JH, Fabbro L, Eaglesham G, Shaw GR *et al.* (2003) Cylindrospermopsin toxicity in cattle exposed to blooms of the cyanobacterium *Cylindrospermopsis raciborskii* : epidemiology, pathology and toxin detection. *Aust. Vet. J.* **81**:

Ohtani I, Moore RE, Runnegar MTC (1992) Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J. Am. Chem. Soc.* **114**:7941-7944.

Pearce RJ, McKenzie RA (1993) *Cylindrospermopsis raciborskii* (cyanobacterial) poisoning. *Vet. Path. Report (ASVP Newsletter)* No.38, p.18.

Saker ML, Eaglesham GK (1999) The accumulation of cylindrospermopsin from the cyanobacterium *Cylindrospermopsis raciborskii* in tissues of the Redclaw crayfish *Cherax quadricarinatus. Toxicon* **37**:1065-1077.

Saker ML, Thomas AD, Norton JH (1999) Cattle mortality attributed to the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of North Queensland. *Environ. Toxicol.* **14**:179-182. [Same case as Thomas *et al.* 1998 with cylindrospermopsin assay results and more detail on mouse assay and cyanobacterial isolation added]

Seawright AA (1999) Pathological aspects of cyanobacterial toxicity in Australia. *Proc. Aust. Soc. Vet. Pathol. Conf.* p. 15-16.

Senogles P, Shaw G, Smith M, Norris R, Chiswell R, Mueler J, Sadler R, Eaglesham G (2000) Degradation of the cyanobacterial toxin cylindrospermopsin from *Cylindrospermopsis raciborskii*, by chlorination. *Toxicon* **38**:1203-1213.

Shaw GR, Sukenik A, Livne A, Chiswell RK, Smith MJ, Seawright AA, Norris RL, Eaglesham GK, Moore MR (1999) Blooms of the cylindrospermopsin containing cyanobacterium, *Aphanizomenon ovalisporum* (Forti) in newly constructed lakes, Queensland, Australia. *Environ. Toxicol.* **14**:167-177.

Stirling DJ, Quilliam MA (2001) First report of the cyanobacterial toxin cylindrospermopsin in New Zealand. *Toxicon* **39**: 1219-1222.

Terao K, Ohmori S, Igarashi K, Ohtani I, Watanabe MF, Harada K-I, Ito E, Watanabe M (1994) Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans. Toxicon* **32**:833-843.

Thomas AD, Saker ML, Norton JH, Olsen RD (1998) Cyanobacterium *Cylindrospermopsis raciborskii* as a probable cause of death in cattle in northern Queensland. *Aust. Vet. J.* **76**:592-594.

TOXINS OF MARINE CYANOBACTERIA

Toxins of Lyngbya *(marine filiamentous cyanobacterium)*

Blooms of the filiamentous cyanobacterium *Lyngbya majuscula* (Dillwyn) Harvey have been recorded from coastal waters in southern Queensland in recent time. It has consistently occurred in Hervey Bay for over 20 years and has been known from Moreton Bay for somewhat less time. It is known locally by Hervey Bay scallop fishermen as dick weed, possibly a reflection of its capacity for irritation of sensitive mucosae. (Dredge MCL, personal communication 25 Jan 2001). Blooms have also been recorded in inshore Western Australian waters.

Direct human contact with *Lyngbya majuscula*, found in tropical and temperate regions of the Pacific and Atlantic oceans, is recognised to cause "swimmer's itch" due to **skin irritating toxins** including the phenolic bislactone **aplysiatoxins** (Mynderse *et al.* 1977, Fujiki *et al.* 1990) and the indole alkaloid **lyngbyatoxins** A, B and C (Cardellina *et al.* 1979, Aimi *et al.* 1990). *L. majuscula* grows attached to the substrate, but can tear loose and drift for long distances. The toxins can be concentrated by some marine invertebrates grazing on the cyanobacterium, for example aplysiatoxins are concentrated in, and were first isolated from, the sea hare *Stylocheilus longicaudata* (Aplysiamorpha). (Carpenter & Carmichael 1995)
Lyngbyatoxin A has also been implicated in fatal human poisoning in Madagascar from eating **meat from a green turtle** (*Chelonia mydas*) which species grazes on sea grass, in this case infested by *Lyngbya majuscula* (Yasumoto 1998).

Other toxins reported isolated from *Lyngbya majuscula* include:

- curacins which are antimitotic (Yoo *et al.* 1995, Rossi *et al.* 1997)
- lipopeptides (microcolins) which are immunosuppressive and antimitotic (Koehn *et al.* 1992, Zang *et al.* 1997)
- acyclic lipopentapeptides (majusculamide D, deoxymajusculamide D) which are cytotoxic in cell cultures at 0.2μg/ml (Moore & Entzeroth 1988)
- a lipopeptide (barbamide) which is molluscicidal (Orjala & Gerwick 1996)
- a lipopeptide and its corresponding free acid (malyngamide H & 7-methoxytetradec-4(E)-enoic acid) which is ichthyotoxic (Orjala *et al.* 1995)
- ichthyotoxic neurotoxins (antillatoxin, kalkitoxin) (Berman *et al.* 1999)
- a lactone (tanikolide) which is antifungal (Singh *et al.* 1999)
- citric acid derivatives (Todd & Gerwick 1995)
- indole derivatives (Todd & Gerwick 1995)

References:

Aimi N, Odaka H, Sakai S-I, Fujiki H, Suganuma M, Moore RE, Patterson GML (1990) Lyngbyatoxins B and C, two new irritants from *Lyngbya majuscula. J. Nat. Prod.* **53**:1593-1596.

Berman FW, Gerwick WH, Murray TF (1999) Antillatoxin and kalkitoxin, ichthyotoxins from the tropical cyanobacterium *Lyngbya majuscula*, induce distinct temporal patterns of NMDA receptor-mediated neurotoxicity. *Toxicon* **37**:1645-1648.

Cardellina JH, Marner FJ, Moore RE (1979) *Science* **204**:193.

Carpenter EJ, Carmichael WW (1995) Taxonomy of cyanobacteria. Chapter 19 in Hallegraeff GM, Anderson DM, Cembella AD (eds.) *Manual on Harmful Marine Microalgae*. Intergovernmental Oceanographic Commission (IOC) Manuals and Guides No.33, UNESCO, Paris. p.376.

Fujiki H, Suganuma M, Suguri H, Yoshizawa S, Takagi K, Nakayasu M, Ojika M, Yamada K, Yasumoto T, Moore RE, Sugimura T (1990) New tumor promoters from marine natural products. In Hall S, Strichartz G (eds.) *Marine Toxins, Origin, Structure and Molecular Pharmacology.* Vol 418, American Chemical Society, Washington DC. pp. 232-240.

Koehn FE, Longley RE, Reed JK (1992) Microcolins A and B, new immunosuppressive peptides from the blue-green alga *Lyngbya majuscula. J. Nat. Prod.* **55**:613-619.

Moore RE (1982) *Pure Appl. Chem.* **54**:1919. [cited by Harborne & Baxter 1966, p.240]

Moore RE, Entzeroth M (1988) Majusculamide D and deoxymajusculamide D, two cytotoxins from *Lyngbya majuscula. Phytochem.* **27**:3101-3103.

Mynderse JS, Moore RE, Kashiwagi M, Norton TR (1977) Antileukemia activity in the Oscillatoriaceae. Isolation of debromoaplysiatoxin from *Lyngbya. Science* **196**:538-540.

Orjala J, Nagle D, Gerwick WH (1995)Malyngamide H, an ichthyotoxic amide possessing a new carbon skeleton from the Caribbean cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **58**:764-768.

Orjala J, Gerwick WH (1996) Barbamide, a chlorinated metabolite with molluscicidal activity from the Caribbean cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **59**:427-430.

Rossi JV, Roberts MA, Yoo HD, Gerwick WH, Yoo HD (1997) Pilot scale culture of the marine cyanobacterium *Lyngbya majuscula* for its pharmaceutically-useful natural metabolite curacin A. *J. Appl. Phycology* **9**:195-204.

Singh IP, Milligan KE, Gerwick WH (1999) Tanikolide, a toxic and antifungal lactone from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **62**:1333-1335.

Todd JS, Gerwick WH (1995) Isolation of a cyclic carbonate, a gamma-butyrolactone, and a new indole derivative from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **58**:586-589.

Yasumoto T (1998) Fish poisoning due to toxins of microalgal origins in the Pacific. *Toxicon* **36**:1515-1518.

Yoo HD, Gerwick WH, Yoo HD (1995) Curacins B and C, new antimitotic natural products from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **58**:1961-1965.

Zhang LH, Longley RE, Koehn FE, Zhang LH (1997) Antiproliferative and immunosuppressive properties of microcolin A, a marine-derived lipopeptide. *Life Sciences* **60**:751-762.

Unknown cyanobacterial toxin(s) – prawns

Report of cyanobacterial (Oscillatoriales) involvement in mortality of farmed prawns in NSW. *Oscillatoria corakiana* was the dominant species present, accompanied by species of *Spirulina, Lyngbya, Oscillatoria* and *Nodularia*. PSP toxins, anatoxin-a, homoanatoxin-a and microcystins were not detected in pond water. (Smith 1996)

References

Smith PT (1996) Toxic effects of blooms of marine species of Oscillatoriales on farmed prawns (*Penaeus monodon, Penaeus japonicus*) and brine shrimp (*Artemia salina*). *Toxicon* **34**:857-869.

MARINE MICROALGAL (DINOFLAGELLATE & DIATOM) TOXINS

Most intoxication from these sources are in humans, but increasingly, intoxications of wildlife and species in marine aquaculture are being recognised. This section begins with a brief overview of human poisoning syndromes from consumption of shellfish followed by the instances where poisonings of wildlife and captive species in marine aquaculture have been identified. Daranas *et al.* (2001) briefly review toxins and syndromes from marine microalgae.

References:

Daranas AH, Norte M, Fernández JJ (2001) Toxic marine microalgae. *Toxicon* **39**:1101-1132.

Human shellfish poisoning syndromes related to marine dinoflagellates and diatoms

Cases in domestic animals are not on record in Australia, but toxicity has been induced experimentally in dogs and cats by several of the toxins. It is possible that these toxicities could spill over into the companion animal population where-ever these toxicities occur in humans. Human cases of some syndromes have been documented in southern Australia.
Review literature for this subject includes Falconer (1993), Hallegraeff (1993) and Hallegraeff *et al.* (1995). The latter in particular provides information on taxonomy of source organisms, sampling methods, assay methods, monitoring and management.

Diarrhetic (diarrheic) Shellfish Poisoning (DSP) – okadaic acid +dinophysistoxins

Chemical structure:

- okadaic acid (OA) and the closely-related dinophysistoxins (DTX): OA, DTX-1 and DTX-3 are associated with diarrhoea; OA and DTX-1 have cancer-promoting properties
- other toxins isolated but not involved in the DSP syndrome are pectenotoxins (polyether lactones) + yessotoxin
- structural similarities to polyether ionophore antibiotics (*q.v.*)

Sources: bivalve shellfish that ingested marine dinoflagellates *Dinophysis fortii, D. acuminata, D. acuta, D. norvegica, D. mitra, D. rotundata, Prorocentrum lima, P. minimum, P. concavum* [+ *Protoperidinium oceanicum, P. pellucidum* - probably after feeding on *Dinophysis* spp.]
Distribution: Japan, Europe, Chile, Thailand, Nova Scotia and possibly Indonesia, Tasmania and New Zealand
Toxins:

- phosphatase inhibitors; cause contraction of intestinal smooth muscle

Clinical syndrome: diarrhoea, nausea, vomiting, abdominal pain; no fatalities reported; recovery within 3 days
Shellfish containing > 2 μg okadaic acid and/or 1.8 μg dinophysistoxin-1 / g hepatopancreas unfit for human consumption; mouse bioassays + HPLC and ELISA methods available for OA and DTX toxins

Amnesic Shellfish Poisoning (ASP) – domoic acid

Chemical structure:

Domoic acid is a non-protein amino acid

Sources:

- bivalve shellfish and plankton-feeding fish (e.g. anchovies) that ingested marine pennate diatoms *Pseudonitzschia pungens* f. *multiseries* [= *Nitzschia pungens*], *P. pseudodelicatissima, P. australis* [=*N. pseudoseriata*], *Amphora coffeaformis*
- diatoms produce toxin only after the onset of the stationary growth phase, induced by either phosphate or silicate deficiency

Distribution: North & South America, Japan, Europe, Australia, New Zealand
Toxin: the neuroexcitory amino acid domoic acid is a rigid structural analogue of glutamate; competes with glutamic acid as a neurotransmitter; produces receptor-induced depolarisation and excitation
Toxicity:

- one incident in humans on record; 1987, blue mussels (*Mytilus edulis*) from eastern Prince Edward Island, Canada (Quilliam & Wright 1989, Perl *et al.* 1990)

- seabirds & pinnipeds affected (*q.v.*)

Clinical syndrome:

- abdominal cramps, vomiting, disorientation, memory loss; can be fatal

Paralytic Shellfish Poisoning (PSP) – saxitoxins + others

Chemical structure:

- 18 structurally-related tetrahydropurine (heterocyclic guanidine) toxins (paralytic shellfish poisons, PSPs) including saxitoxin (named after the bivalve *Saxidomu giganteus*, the original source for its isolation)
- saxitoxin = 2 guanidinium functions fused together in a stable linkage (cf. tetrodotoxin (*q.v.*) = a single guanidinium moiety)

Sources:

- bivalve shellfish that ingested marine dinoflagellates in the genera *Alexandrium* [= *Gonyaulax*]*, Pyrodinium* and *Gymnodinium*.
- finfish, coral reef crabs and certain gastropods have been sources in the Asia-Pacific region
- cyanobacteria (*q.v.*)

Distribution: North & South America, Europe, Japan, SE Asia. *Gymnodinium catenatum* recognised in Tasmanian waters; believed introduced in ship's ballast water.

Toxins:

- block the voltage-gated Na channel, but not the voltage-gated K channel (same mode of action as tetrodotoxin (*q.v.*), which has a different molecular structure) → blocks the generation and propagation of action potentials in nerves and muscles

Clinical syndrome:

- virtually identical to tetrodotoxin poisoning (*q.v.*)
- onset 0.5-3.5 hr
- paraesthesia & numbness around the lips and mouth, spreading to the face and neck
- prickly sensations in fingers and toes
- mild headache and dizziness
- ± nausea and vomiting
- paraesthesia progresses to arms & legs + motor weakness
- giddiness, sensation of lightness& floating, incoherent speech
- ataxia, motor incoordination, dysmetria
- dyspnoea (begins as tightness around the throat)
- progressively decreasing ventilatory efficiency + increasing hypoxia, hypercarbia (↑ P_{CO2})
- no hypotension (in contrast with tetrodotoxin poisoning), no effect on pulse

Therapy: experimentally, 4-aminopyridine (*q.v.*) is a promising antidote (Chang *et al.* 1996, Chen *et al.* 1996, Benton *et al.* 1996, 1998)

Neurotoxic Shellfish Poisoning (NSP) – brevetoxins

Chemical structure:

- several (*ca.*9) brevetoxins are known. These are cyclic polyether neurotoxins.

Source:

- bivalve shellfish that ingested the marine dinoflagellate *Gymnodinium breve* [= *Ptychodiscus brevis*] (Florida red tide organism)
- inhalation of sea spray containing fragments of *G. breve* cells ("toxic sea spray")

Distribution: North America (Gulf of Mexico), New Zealand

Toxins:

- specifically induce a channel-mediated Na^+ ion influx → depolarisation
- toxic to fish; blooms → fish kills

Clinical syndrome:

- NSP → nausea, vomiting, diarrhoea, chills, dizziness, numbness or tingling of the face, hands or feet from 3-4 hrs after ingestion
- toxic sea spray → severe irritation of conjunctivae and mucous membranes + persistent coughing and sneezing

Diagnosis: urine analysis for brevetoxins and their metabolites (Poli *et al.* 2000)

Azaspiracid Poisoning (Winter toxicity of mussels)

Source:

- mussels (*Mytilus edulis*) cultivated in Donegal, Ireland

- suspected ultimate source = dinoflagellate, currently unidentified

Distribution: Europe (McMahon & Silke 1996, 1998)

Toxins:

- azaspiracid and 2 analogues currently recognised (Satake *et al*. 1998, Ofugi *et al.* 1999)
- toxic mussels contained *ca.* 0.4 mg azaspiracid + analogues/kg (Ofugi *et al.* 1999)
- mouse IP lethal doses = *ca.* 0.2 mg/kg (Ofugi *et al.* 1999)

Mode of action: undescribed

Clinical syndrome: similar to Diarrhetic shellfish poisoning (above)

- nausea, vomiting
- stomach cramps
- severe diarrhoea

Other shellfish-associated toxins

Gymnodimine (Seki *et al.* 1995)
Pinnatoxins (Uemura *et al.* 1995)
Prorocentrolides (Torigoe *et al.* 1988)
Spirolides (Hu *et al.* 1995)
see also Daranas *et al.* (2001)

References:

Aune T, Yndestad M (1993) Diarrhetic shellfish poisoning. Chapter 5 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 87-104.

Baden DG, Trainer VL (1993) Mode of action of toxins of seafood poisoning. Chapter 3 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 49-74.

Benton BJ, Spriggs DL, Capacio BR, Chang F-CT (1996) 4-aminopyridine antagonises the lethal effects of saxitoxin (STX) and tetrodotoxin (TTX). *Toxicon* **34**:335.

Benton BJ, Keller SA, Spriggs DL, Capacio BR, Chang F-CT (1998) Recovery from the lethal effects of saxitoxin: a therapeutic window for 4-aminopyridine (4-AP). *Toxicon* **36**:571-588.

Brown C (1993) Scombroid poisoning (Letter). *Med. J. Aust.* **158**(6):435-436.

Cembella AD, Todd E (1993) Seafood toxins of algal origin and their control in Canada. Chapter 8 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 129-144.

Chang F-CT, Bauer RM, Benton BJ, Keller SA, Capacio BR (1996) 4-aminopyridine antagonizes saxitoxin- and tetrodotoxin-induced cardiorespiratory depression. *Toxicon* **34**:671-690.

Chen H-M, Lin C-H, Wang TM (1996) Effects of 4-aminopyridine on saxitoxin intoxication. *Toxicol. Appl. Pharmacol.* **141**:44-48.

Daranas AH, Norte M, Fernández JJ (2001) Toxic marine microalgae. *Toxicon* **39**:1101-1132.

Falconer IR (ed.) (1993) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London.

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase (Phycological Reviews 13). *Phycologia* **32**:79-99.

Hallegraeff GM, Anderson DM, Cembella AD (eds.) (1995) *Manual on Harmful Marine Microalgae*. Intergovernmental Oceanographic Commission (IOC) Manuals and Guides No.33, UNESCO, Paris.

Hu T, Curtis J, Oshima Y, Quilliam MA, Walter JA, Watson-Wright WM, Wright JLC (1995) Spirolides B and D, two novel macrocycles isolated from the digestive glands of shellfish. *J. Chem. Soc. Chem. Commun.* 2159-2162.

Hungerford JM, Wekell MM (1993) Control measures in shellfish and finfish industries in the USA. Chapter 7 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 117-128.

Ito E, Satake M, Ofuji K, Kurita N, McMahon T, James K, Yasumoto T (2000) Multiple organ damage caused by a new toxin azaspiracid, isolated from mussels produced in Ireland. *Toxicon* **38**:917-930.

Kao CY (1993) Paralytic shellfish poisoning. Chapter 4 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 75-86.

Lehane L, Olley J (1999) *Histamine (Scombroid) Fish Poisoning. A review in a risk-assessment framework*. National Office of Animal & Plant Health, Canberra. pp. iv + 80.

McMahon T, Silke J (1996) Winter toxicity of unknown aetiology in mussels. *Harmful Algae News* **14**:2.

McMahon T, Silke J (1998) Re-occurrence of winter toxicity. *Harmful Algae News* **17**:12.

Morohashi A, Satake M, Naoki H, Kaspar HF, Oshima Y, Yasumoto T (1999) Brevetoxin B4 isolated from greenshell mussels *Perna canaliculus*, the major toxin involved in neurotoxic shellfish poisoning in New Zealand. *Nat. Toxins* **7**:45-48.

Ofugi K, Satake M, McMahon T, Silke J, James KJ, Naoki H, Oshima Y, Yasumoto T (1999) Two analogues of azaspiracid isolated from mussels, *Mytilus edulis*, involved in human intoxication in Ireland. *Natural Toxins* **7**:99-102.

Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd ECD, Remis RS (1990) An outbreak of toxic encephalopathy by eating mussels contaminated with domoic acid. *New Engl. J. Med.* **322**:1775-1780.

Poli MA, Musser SM, Dickey RW, Eilers PP, Hall S (2000) Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. *Toxicon* **38**:981-993.

Quilliam MA, Wright JLC (1989) The amnesic shellfish poisoning mystery. *Anal. Chem.* **61**:1053A-1060A.

Satake M, Ofugi K, Naoki H, James KJ, Furey A, McMahon T, Silke J, Yasumoto T (1998) Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. *J. Am. Chem. Soc.* **120**:9967-9968.

Seki T, Satake M, Mackenzie L, Kaspar HF, Yasumoto T (1995) Gymnodimine, a new marine toxin of unprecedented structure isolated from New Zealand oysters and the dinoflagellate, *Gymnodinium* sp. *Tetrahedron Lett.* **36**:7093-7096.
Smart DR (1992) Scombroid poisoning. A report of seven cases involving the Western Australian salmon, *Arripid truttaceus. Med. J. Aust.* **157**(11-12):748-751.
Sullivan JJ (1993) Methods of analysis for algal toxins: dinoflagellate and diatom toxins. Chapter 2 in Falconer IR (ed.) *Algal Toxins in Seafood and Drinking Water.* Academic Press, London. pp. 29-48.
Torigo K, Murata M, Yasumoto T (1988) Prorocentrolide, a toxic nitrogenous macrocycle from a marine dinoflagellate, *Prorocentrum lima. J. Am. Chem. Soc.* **110**:7877-7878.
Uemura D, Chou T, Haino T, Nagatsu A, Fukuzawa S, Zheng S, Chen H (1995) Pinnatoxin A: a toxic amphoteric macrocycle from the Okinawan bivalve *Pinna muricata. J. Am. Chem. Soc.* **117**:1155-1156.

Paralytic shellfish poisoning (PSP, saxitoxin) – cetaceans, pinnipeds

Syndrome names: paralytic shellfish poisoning
Sources: marine dinoflagellates in the genera *Alexandrium* [= *Gonyaulax*], *Pyrodinium* and *Gymnodinium*
Toxicity:
- humans (*q.v.*)
- humpback whales in Cape Cod Bay, USA (Geraci *et al.* 1989)
- Atlantic dolphins in a lagoon in Hawaii (Hokama *et al.* 1990)
- monk seals in the Mediterranean Sea (Costas & Lopez-Rodas 1998; Reyero *et al.* 1999)

Mode of action: block the voltage-gated Na^+ channel, but not the voltage-gated K^+ channel, the same mode of action as tetrodotoxin (*q.v.*)
Conditions of poisoning:
- undetermined (dolphins, seals)
- humpback whales (14 beached in a mass mortality incident in 1987-88) consumed Atlantic mackerel (*Scomber scombrus*) which became contaminated with saxitoxin in the Gulf of St.Lawrence, Canada (Geraci *et al.* 1989)

Clinical signs: found dead at sea or washed up on coasts
Pathology: undetermined
Diagnosis:
- saxitoxin detected in Atlantic mackerel livers (but not muscle); toxins with similar characteristic detected in whale stomach contents, liver and kidneys by mouse bioassay, but not by standard HPLC for saxitoxins, suggesting some metabolic transformation of the saxitoxin in the whales (Geraci *et al.* 1989)
- PSP toxins detected by mouse bioassay and immunoassay in water and liver samples and immunofluorescence in brain samples (Costas & Lopez-Rodas 1998)

Therapy: See PSP (human) above
Prevention & control:
References:

Costas E, Lopez-Rodas V (1998) Paralytic phycotoxins in monk seal mass mortality. *Vet. Rec.* **142**:643-644.
Geraci JR, Anderson DM, Timperi RJ, St.Aubin DJ, Early GA, Prescott JH, Mayo CA (1989) Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. *Can. J. Fish Aq. Sci.* **46**:1895-1898.
Hokama Y, Asahina AY, Hong TWP, Katsura K, Shang E, Miyahara JT, Sweeney JC, Stone R (1990) Causative toxin(s) in the death of two Atlantic dolphins. *J. Clin. Lab. Anal.* **4**:474-478.
Reyero M, Cacho E, Martinez A, Vázquez J, Marina A, Fraga S, Franco JM (1999) Evidence of saxitoxin derivatives as causative agents in the 1997 mass mortality of monk seals in the Cape Blanc peninsula. *Natural Toxins* **7**:311-315.

Brevetoxins – sirenians

Florida manatees (*Trichechus manatus latirostris*)
- intoxicated through ingestion of salps (tunicates) containing brevetoxins and attached to seagrass (Anderson & White 1989)
- intoxicated during red tides in spring 1996; rapid deaths; mild encephalitis, acute inflammation of upper respiratory tract, haemolysis; brevetoxins demonstrated in tissues by immunoperoxidase staining (Suzik 1997)

Neurotoxic shellfish poisoning (*q.v.*)
References:

Anderson DM, White AW (eds) (1989) Toxic dinoflagellates and marine mammal mortalities. Woods Hole Oceanographic Institute Technical Report WHOI-89-36 (CRC-89-6). [cited by Hallegraeff GM (1995) Harmful algal blooms: a global overview. Chapter 1 in Hallegraeff GM, Anderson DM, Cembella AD (eds.) (1995) *Manual on Harmful Marine Microalgae*. Intergovernmental Oceanographic Commission (IOC) Manuals and Guides No.33, UNESCO, Paris. pp.1-22.

Bossart GD, Baden DG, Ewing R, Roberts B, Wright S (1998) Brevetoxicosis in manatees (*Trichechus manatus latirostris*) from the 1996 epizootic: gross, histologic and immunohistologic features. *Toxicological Pathology* **26**:276-292.
Suzik HA (1997) Unravelling the manatee mystery. (News report) *J. Am. Vet. Med. Assoc.* **210**:740. [investigation by Dr G Bossart, U Miami School of Med Dept of Pathology]

Domoic acid (amnesic shellfish poisoning – ASP) – sea birds & pinnipeds

Amnesic shellfish poisoning (*q.v.*)
Intoxication incidents:
- sea birds:
  - Monterey Bay, California 1991; brown pelicans (*Pelecanus occidentalis*) & cormorants (*Phalocrocorax penicillatus*) (Work *et al.* 1993); pennate diatoms (*Pseudonitzschia pungens* & *P. australis*) contaminating northern anchovies (*Engraulis mordax*) eaten by the birds
  - Santa Cruz, California 1992 (Work *et al.* 1993); same circumstances as above
  - Baja California, Mexico 1996; brown pelicans (Beltran *et al.* 1997); diatoms (*Pseudonitzschia* sp) contaminating mackerel (*Scomber japonicus*) eaten by the birds; stomachs empty implying recent vomiting, "CNS disorder"
- Californian sea lions:
  - May 1998; 81 stranded along central Californian coast, 48 died/23 recovered; seizures observed, stomachs empty implying recent vomiting; event concurrent with a toxic bloom of *Pseudonitzschia australis* diatoms; domoic acid and *P. australis* frustules detected in northern anchovies (*Engraulis mordax*), a common prey of the sea lions, and in faeces of sea lions with seizures (Lefebvre *et al.* 1999, Scholin *et al.* 2000)
  - 2000, 184 stranded along central Californian coast (Gulland *et al.* 2002)

References:
Beltran AS, Palafox-Uribe M, Grajales-Montiel J, Cruz-Villacorta A, Ochoa J (1997) Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. *Toxicon* **35**:447-553.
Gulland FMD, Haulena M, Fauquier D, Lander ME, Zabka T, Duerr R (2002) Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *Vet. Rec.* **150**:475-480.
Lefebvre KA, Powell CL, Busman M, Doucette GJ, Moeller PDR, Silver JB, Miller PE, Hughes MP, Singaram S, Silver MW, Tjeerdema RS (1999) Detection of domoic acid in northern anchovies and California sealions associated with an unusual mortality event. *Natural Toxins* **7**:85-92.
Scholin CA, Gulland F, Doucette G, Benson S, Busman M, Chavez FP, Cordaro J, Delong R, de Vogelaere A, Harvet J, Haulena M, Lefebvre K, Lipscomb T, Loscutoff S, Lowenstine L, Marin R, Miller PE, McLellan W, Moeller PDR, Powell CL, Rowles T, Silvagni P, Silver M, Spraker T, Trainer V, van Dolah F (2000) Mortality of sealions along the central Californian coast linked to a toxic diatom bloom. *Nature* **403**:80-84.
Work TM, Barr B, Beale AM, Fritz L, Quilliam MA, Wright JLC (1993) Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brant's cormorants (*Phalacrocorax penicillatus*) in California. *J. Zoo. Wildl. Med.* **24**:1:54-62

Chattonella marina – *southern bluefin tuna*

Mortality of 75% of caged southern bluefin tuna near Port Lincoln, South Australia, in April 1996 presented as distressed fish with respiratory embarrassment and excessive mucus production from gills. Histologically there was swelling of respiratory epithelial cells and subepithelial oedema of secondary lamellae. The main aetiological factor was believed to be a bloom of the marine microalgal flagellate *Chattonella marina* (Subrahmanyan) Y.Hara et Chihara 1982 (a member of the Raphidophycae).
References:
Munday BL, Hallegraeff GM (1999) Was a toxic microalga involved in the mass mortality of southern bluefin tuna in Port Lincoln? *Proc. Aust. Soc. Vet. Pathol. Conf.* p.23.

Pfiesteria *spp. - fish, humans*

Toxic *Pfiesteria* complex (TPC) dinoflagellates were first recognised as associated with fish kills and human illness in estuarine waters of the eastern coast of North America in 1988 (Burkholder *et al.* 1992, Glasgow *et al.* 1995). They behaves as ambush-predators of fish, releasing fish-killing toxin into the water, and then feeding on the resulting dead fish.

The toxin is unidentified, but currently believed to be a hydrophilic compound that mimics an ATP neurotransmitter and increases intracellular calcium membrane permeability (in GH4C1 rat pituitary cells) by a mechanism consistent with activation of $P2X_7$ purinoreceptors. $P2X_7$ receptors are

generally restricted to immune cells - macrophages and microglia - and are key effectors of chronic inflammation. This pathogenesis could explain the diverse nature of effects of the intoxication. (Kimm-Brinson *et al.* 2001)

Currently, 2 species of ichthyotoxic *Pfiesteria* (TPC) are recognised:

- *Pfiesteria piscicida* (first recognised North Carolina 1988; first record - Smith et al. 1988)
- *Pfiesteria shumwayae* (first recognised North Carolina 1995; first record - Burkholder et al. 1997)

There are probably other species (Burkholder & Glasgow 1997)

Pfiesteria spp. (Order Dinamoebales; Division Pyrrhophyta, Class Dinophyceae)

- have a complex life cycle involving amoeboid, flagellated and cyst stages
- can be identified by their zoospore stages
- have a strong attraction to live fish and their fresh tissues, secretions and excretions
- are triggered to produce toxin by the presence of live finfish or shellfish
- are very diverse in nutrition - able to take up diverse dissolved organic and inorganic nutrients; heterotrophic, but able to photosynthesise when they retain kleptochloroplasts from algal prey
- have diverse prey spanning the estuarine food web from bacteria to mammalian tissue
- produce aerosols which impair human cognitive, respiratory, gastrointestinal and eye function

Flagellated stages (zoospores) and some lobose amoeboid forms can be ichthyotoxic, the former being the more toxic. Toxic (TOX-A = actively toxin producing & TOX-B = non-toxin-producing but capable of induction) and non-toxic strains (non-inducible) are known (in the ratio 60:40). *Pfiesteria* toxic stages migrate up through the water column towards live fish and settle out to bottom sediments when fish are dead or adverse environmental condition arise.

Experimentally poisoned fish have erosive lesions of the skin and deeper tissue formed typically in < 8 hours after first exposure with haemorrhage and exposure of the viscera. Lesions are also reported in viscera.

Humans exposed to aerosols from fish-killing cultures developed severe cognitive impairment (severe reversible short-term memory loss requiring 3-6 months for recovery), respiratory signs (asthma-like), epidermal lesions unresponsive to antibiotics, severe refractory headaches, joint and muscle pain, vomiting and stomach cramping and problems with vision. Long-term effects have been autoimmune and central nervous system dysfunction and chronic respiratory infections suggestive of immunosuppression (Glasgow *et al.* 1995, Grattan *et al.* 1998).

Pfiesteria-related estuarine fish kills in North America (North Carolina-Maryland) occur

- in quiet, shallow (3-4 m or less), poorly-flushed (complete water exchange every *ca.* 70-100 days, rarely in *ca.* 35-40 days), brackish, eutrophic waters
- during warm seasons (15-33^{o}C, 2-35 psu)

Factors triggering blooms include nutrient enrichment of water bodies with phosphorus and nitrogen, mediated directly through organic nutrient uptake or inorganic nutrient uptake (by kleptochloroplastic strains) or indirectly through algal prey (Burkholder & Glasgow 1997, Burkholder *et al.* 1998)

Criteria used to establish TPC involvement in fish kills (defined as affecting > 1000 fish) (Burkholder *et al.* 2001)

- at least 300 *Pfiesteria*-like zoospores/ml must be present by light microscopy (x600)
- active toxicity of *Pfiesteria* cells collected during the fish kill must be confirmed by fish bioassays wherein cells are tested in the natural sample, then cloned and retested with fish
- species identification must be made from SEM of suture-swollen cells
- both toxicity and species identity are confirmed by independent specialists with demonstrated expertise in dinoflagellate systematics
- no other causative factors can be detected where and while fish are dying

Ulcerated lesions on fish are not used as a reliable indicator of *Pfiesteria* involvement.

Australian situation:

Pfiesteria piscicida has been reported in Tasmanian waters in 2001 and in the Brisbane River in 2002 (positive samples collected from Cockatoo Island on the Wacol reach, the Brisbane-Bremer junction and Bremer pipe input - 2 km upstream from the junction – in October 2001 and subjected to PCR in USA; all standard PCR testing was negative; positive results came from a more sensitive technique - FISH? fluorescent *in situ* hybridisation) (Courier Mail 28 May 2002 p. 1 & 4; 29 May 2002; Queensland Fisheries Service, unpublished data 2002). No confirmed toxicity incidents from these organisms have been recorded in Australia to date.

References:

Review literature

Burkholder JM, Glasgow HB, Deamer-Mella N (2001) Overview and present status of the toxic *Pfiesteria piscicida* (Dinophyceae). *Phycologia* **40**:186-214.

Faith SA, Miller CA (2000) A newly emerging toxic dinoflagellate, *Pfiesteria piscicida*: natural ecology and toxicosis to fish and other species. *Vet. Human Toxicol.* **42**:26-29.

General literature

Burkholder JM, Noga EJ, Hobbs CM, Glasgow HB, Smith SA (1992) New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. *Nature* **358**:407-410, **360**:768.

Burkholder JM, Glasgow HB (1997) *Pfiesteria piscicida* and other toxic *Pfiesteria*-like dinoflagellates: behavior, impacts, and environmental controls. *Limnology and Oceanography* **42**:1052-1075.

Burkholder JM, Mallin MA, Glasgow HB, Larsen LM, McIver MR, Shank GC, Deamer-Melia NJ, Briley DS, Springer J, Touchette BW, Hannon EK (1997) Impacts to a coastal river and estuary from rupture of a large swine waste holding lagoon. *J. Environ. Qual.* **26**:1451-1466.

Burkholder JM, Glasgow HB, Lewitus AJ (1998) Physiological ecology of *Pfiesteria piscicida* with general comments on 'ambush-predator' dinoflagellates. In Anderson DM, Cembella A, Hallegraeff GM (eds.) *Physiological Ecology of Harmful Algae.* NATO ASI Series G: Ecological Sciences Volume 41, Springer-Verlag, Berlin.

Glasgow HB, Burkholder JM, Schmechel DE, Fester PA, Rublee PA (1995) Insidious effects of a toxic dinoflagellate on fish survival and human health. *J. Toxicol. Environ. Health* **46**:501-522.

Grattan LM, Oldach D, Perl TM, Lowitt MH, Matuszak DI, Dickson C, Parrott C, Shoemaker RC, Wasserman MP, Hebel JR, Charache P, Morris JG Jr (1998) Problems in learning and memory occur in persons with environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. *Lancet* **352**:532-539.

Kimm-Brinson KL, Moeller PDR, Barbier M, Glasgow HB, Burkholder JM, Ramsdell JS (2001) Identification of a $P2X_7$ receptor in GH4C1 rat pituitary cells: a possible target for a bioactive substance produced by *Pfiesteria piscicida. Environ. Health Perspectives* **109**:457-462.

Noga EJ, Smith SA, Burkholder JM, Hobbs CW, Bullis RA (1993) A new ichthyotoxic dinoflagellate: cause of acute mortality in aquarium fishes. *Vet. Rec.* **133**:48-49.

Smith SA, Noga EJ, Bullis RA (1988) Mortality in *Tilapia aurea* due to a toxic dinoflagellate bloom. *Proceedings 3rd International Colloquium of Pathology in Marine Aquaculture.* Gloucester, Virginia. pp. 167-168.

☑ *Ciguatoxins (& maitotoxin) (*q.v.*)*