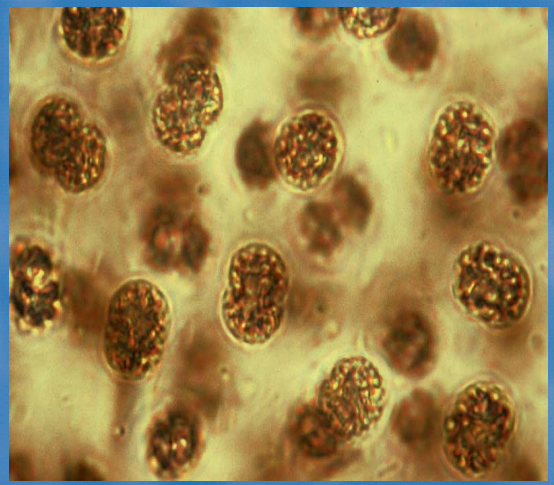
Cyanobacterial Harmful Algal Blooms

An Increasing Risk to Human Health & Ecosystem Sustainability



Ken Hudnell, PhD

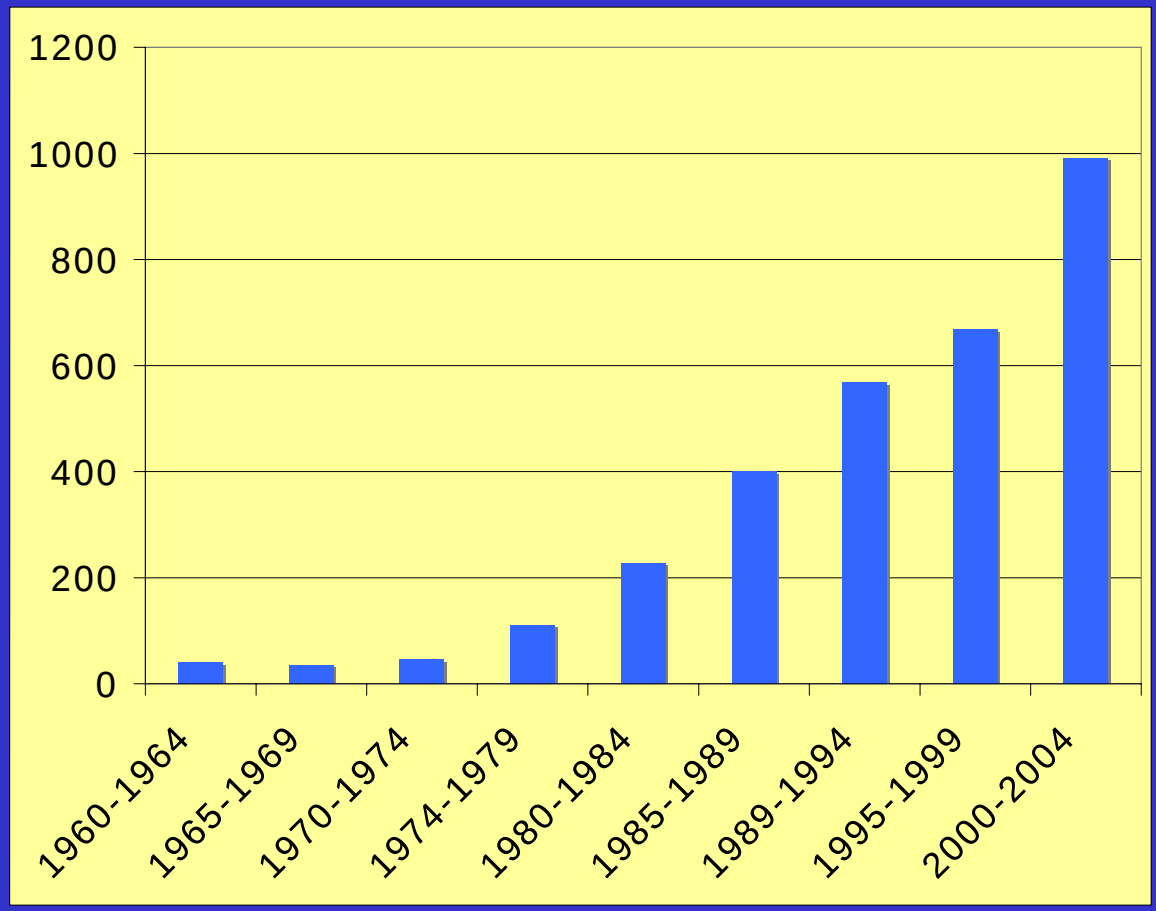
US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Neurotoxicology Division

California and Oregon Workshops on Cyanobacterial Blooms in the Klamath River, November 8&9, 2005

ORD Research Center, RTP, NC

Cyanobacterial Harmful Algal Blooms (CHABS): Recent Area of Science

Number of Articles Cited in CHAB Search 1960-2004



Wayne Carmichael

The Discipline of CyanoHABs

- 1980. First International Conference On Toxic Cyanobacteria. Proceedings entitled: "The Water Environment Algal Toxins and Health". Plenum Press, 1981 (ed. by WW Carmichael).
- 1993. Bath, UK; 1995. Bornholm, DK.
- 1998. 4th ICTC. Beaufort, NC, USA.
- 2001. 5th ICTC. Noosa, Queensland, AUS.
- 2004. 6th ICTC. Bergen, Norway.
- 2005. ISOC-HAB. 1st Government Symposium

Overview

- ISOC-HAB: The Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms, September 6-10, 2005
- Legislative Drivers, Participants & Products
- Theoretical Framework
- Cyanobacteria & their Toxins
  * Microcystin Effects
- Occurrence
- Risk Assessment
- Causes, Prevention & Mitigation
- Future

ISOC-HAB Legislative Drivers, Participants, Topics & Products

ISOC-HAB Legislative Drivers

- **Clean Water Act**
  - * EPA authority to regulate recreational waters
- **Safe Drinking Water Act**
  - * EPA authority to regulate drinking waters
  - - Cyanobacteria & their toxins are on the CCL2
- **Harmful Algal Bloom and Hypoxia Research & Control (HABHRCA) Act Reauthorized & Expanded**
  - - Now Includes Freshwater Harmful Algal Blooms
  - - Mandates Interagency Products on Cyanobacteria

ISOC-HAB Participants & Topics

EPA | NOAA | FDA | USDA | UNC - Chapel Hill IMS Institute of Marine Sciences | CDC | US Army Corps Of Engineers | USGS | National Institutes of Health | NIEHS National Institute of Environmental Health Sciences

- 32 Member Organizing Committee - K. Hudnell, Lead
- 200+ Attendees, 94 Invited Participants, 25 Speakers
  * Speaker Charges - State of the Science
- Six Main Session Topics & Workgroups
  * Workgroup Charges - Identify & Prioritize Research

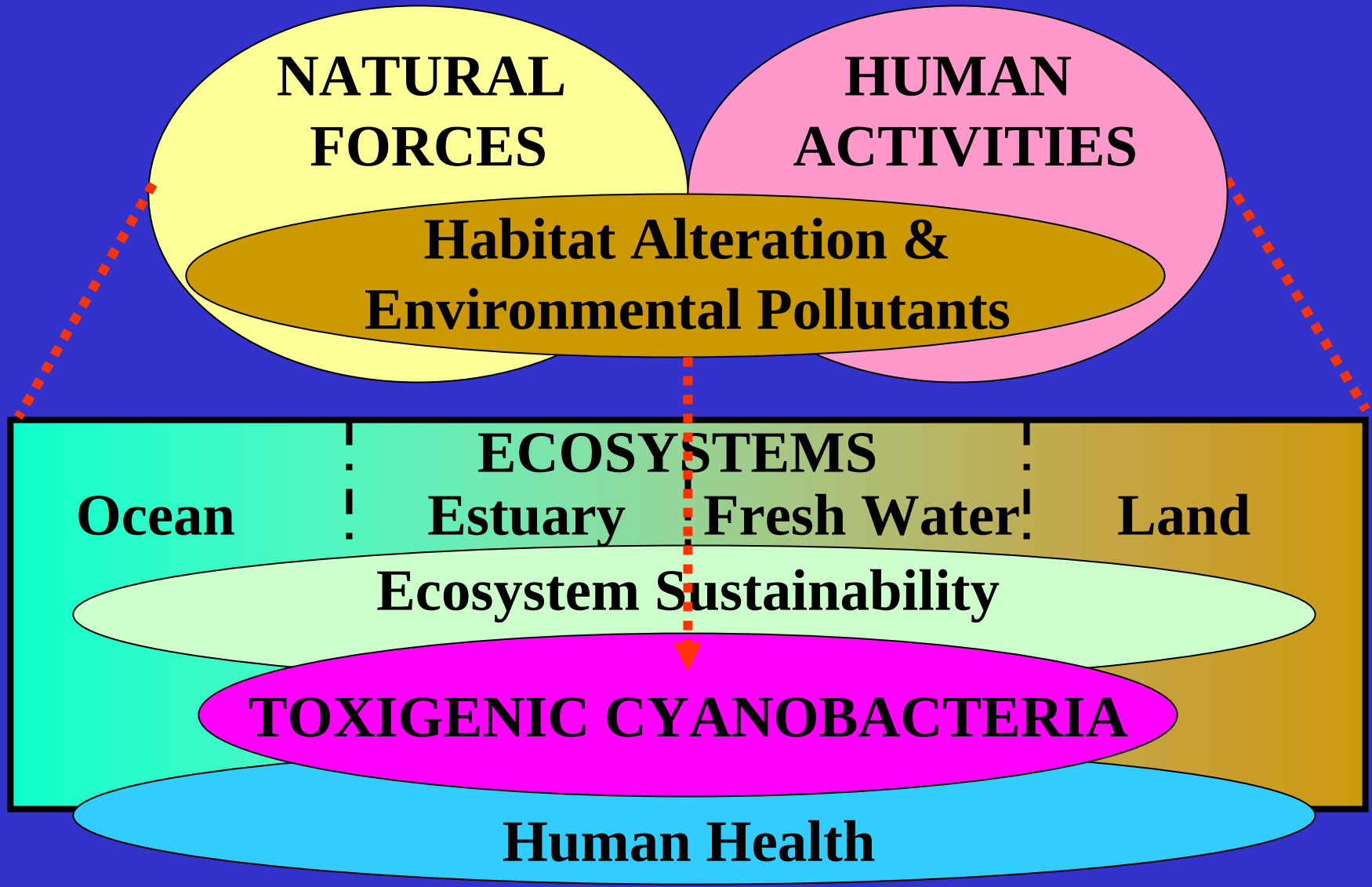
- Causes, Prevention, Mitigation & Treatment
- Toxins
- Effects
- Occurrence of Blooms & Toxins
- Exposure Assessment
- Risk Assessment

ISOC-HAB Products

- Monograph Published by Springer Press in the Series, Advances in Experimental Medicine & Biology, Spring, 2006. Presented to HABHRCA Task Force to Help Meet Mandates
  - Synopsis - National Research Plan on CHABS
  - 6 Workgroup Reports - Research Needs
  - 25 Speaker Papers - State of the Science
  - Multiple Poster Abstracts - Latest Research

Theoretical Framework

Theoretical Framework



Cyanobacteria & their Toxins

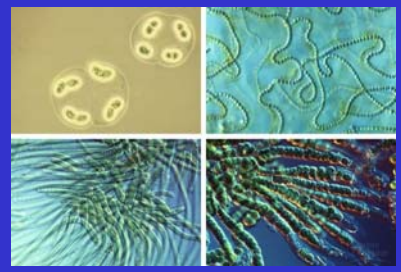
Toxic Cyanobacteria Genera

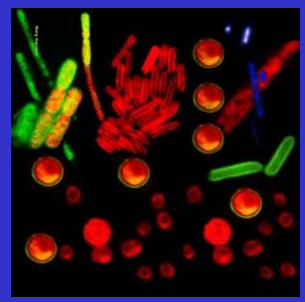
Microcystis
Unicellular, no heterocyst

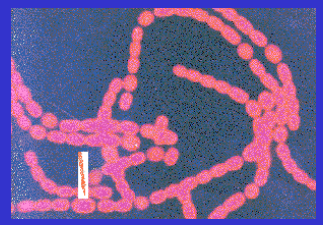
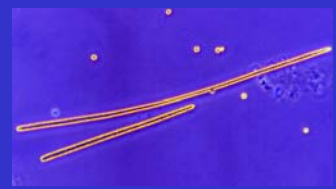
Lyngbya, *Oscillatoria*
Filamentous, no heterocyst

Cylindrospermopsis
Anabaena, *Planktothrix*, *Aphanizomenon*
Filamentous, heterocyst

~3.5 Billion Years Old
Prokaryotic, Asexual





Many Genera Make the Same Toxin
Many Genera Make Multiple Toxins

Anabaena, Aphanocapsa, Microcystis, Nostoc, Oscillatoria, Radiocystis, Hapalosiphon	Cyclic Peptides Microcystins
Anabaena, Aphanizomenon, Oscillatoria	Alkaloids Anatoxin-a
Anabaena, Oscillatoria	Anatoxin-a(s)
Aphanizomenon, Cylindrospermopsis, Umezakia	Cylindrospermopsin
Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya	Saxitoxin Neosaxitoxin

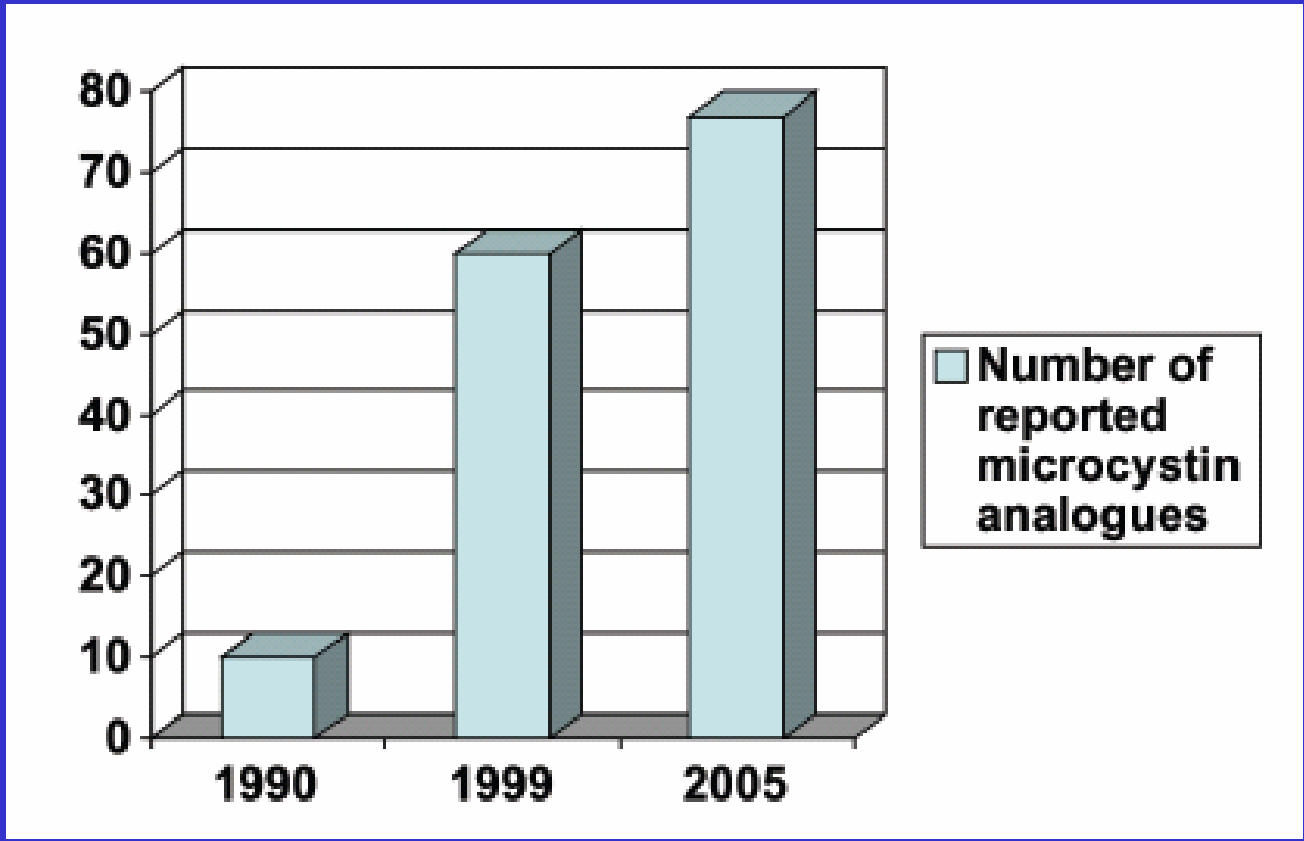
Cyanotoxins are Highly Potent

Compounds & LD_{50} (ug/kg)

Saxitoxin	9	Ricin	0.02
Anatoxin-a(s)	20	Cobra toxin	20
Microcystin LR	50	Curare	500
Anatoxin-a	50	Strychnine	2000

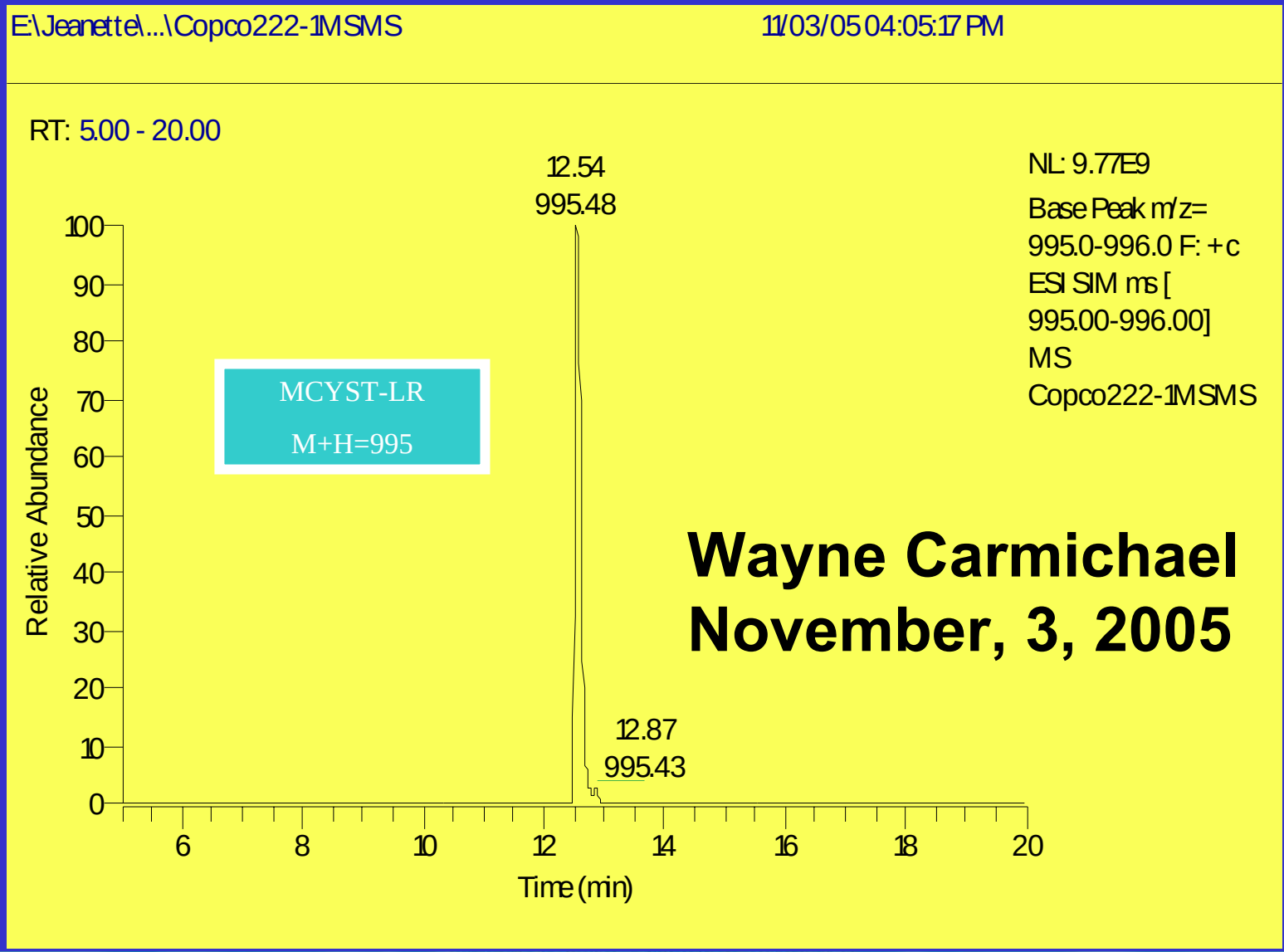
Class	MW	Source
Proteins	10,000-100,000	Culture/Extract/Purify
Cyanotoxins	50-500	Culture/Extract/Purify
Alkaloid Toxins	150-300	Synthesize
Chemical Weapons	<50-300	Synthesize

Many Cyanotoxins Unidentified



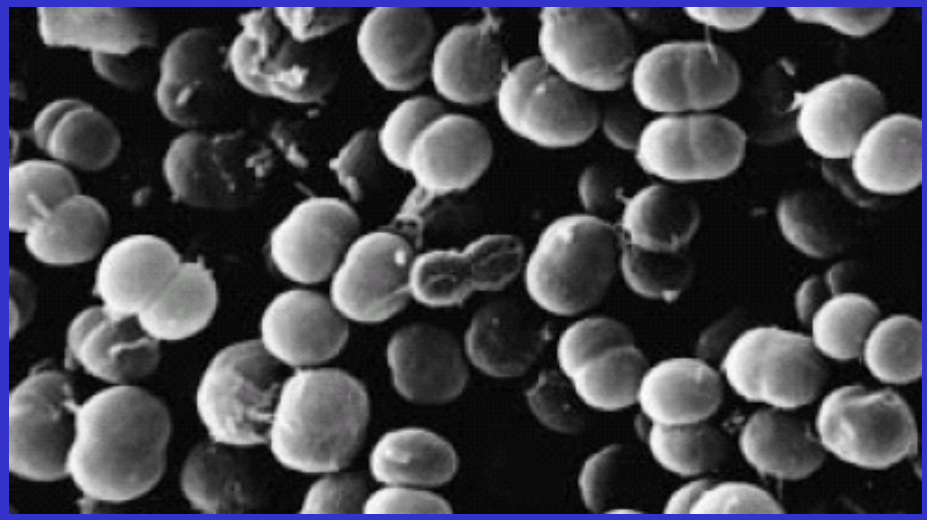
Crude Cell Extracts Always More Toxic than Pure Toxin

MS/MS analysis of Copco Sample 222-1

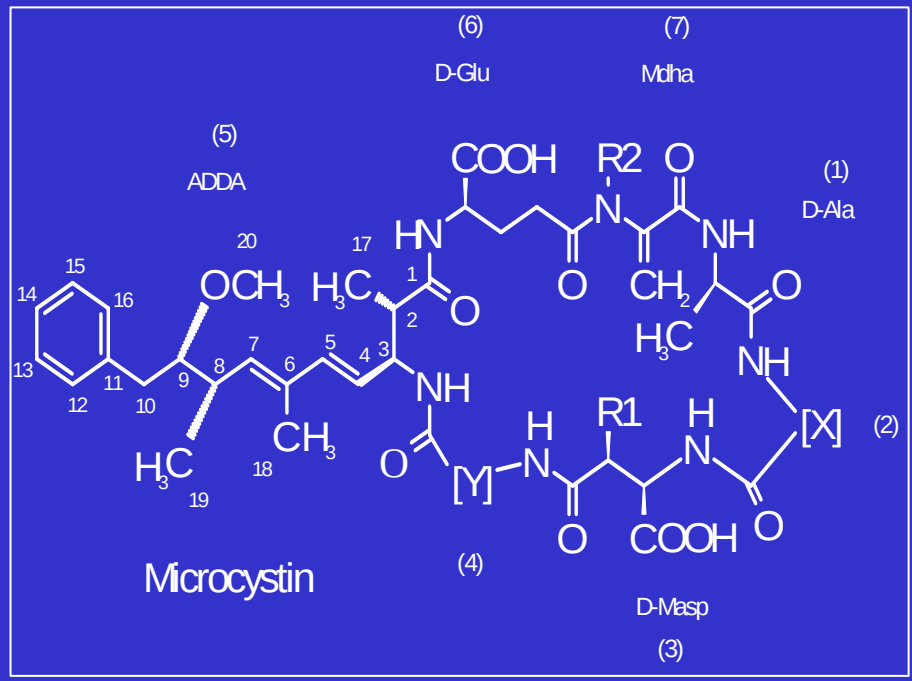


Wayne Carmichael
November, 3, 2005

Microcystis aeruginosa



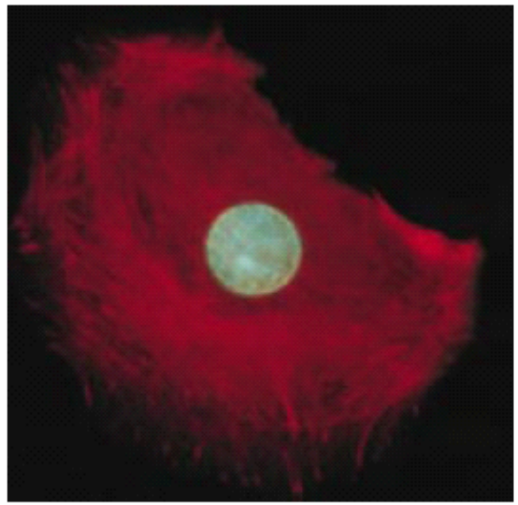
Microcystins

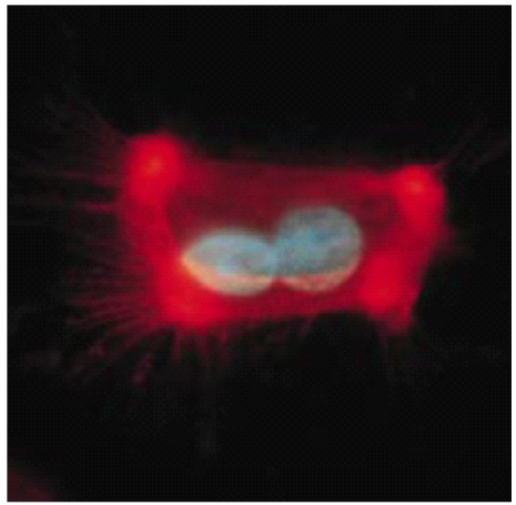


- **~80 analogues**
- **LD50 (ip, mouse, 24hr): 50 µg/kg**
- **Require active uptake by "bile acid transporter" – concentrates the toxin**
- **Inhibit protein phosphatases 1 and 2A**
- **Loss of regulation of cytoskeleton, cell cycle, general metabolism, apoptosis**

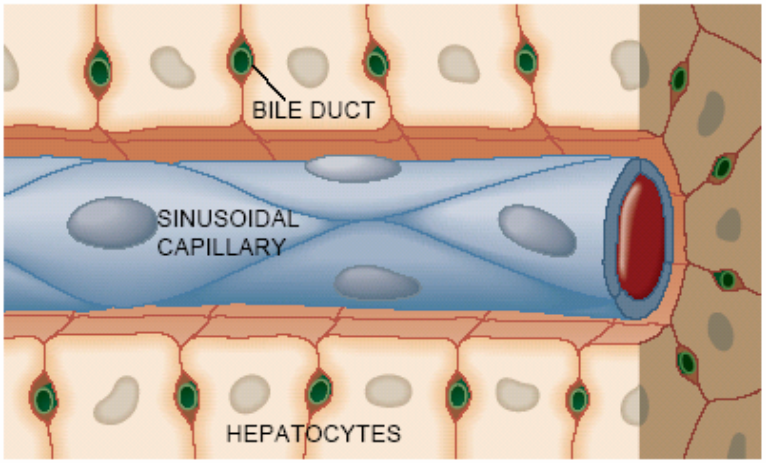
Microcystins and Hepatotoxicity

MICROFILAMENTS (*red threads in micrographs*), structural components of cells, are usually quite long, as in the rat hepatocyte at the left. But after exposure to microcystins (*right*), microfilaments collapse toward the nucleus (*blue*). (This cell, like many healthy hepatocytes, happens to have two nuclei.) Such collapse helps to shrink hepatocytes—which normally touch one another and touch sinusoidal capillaries (*left drawing*). Then the shrunken cells separate from one another and from the sinusoids (*right drawing*). The cells of the sinusoids separate as well, causing blood to spill into liver tissue. This bleeding can lead swiftly to death.

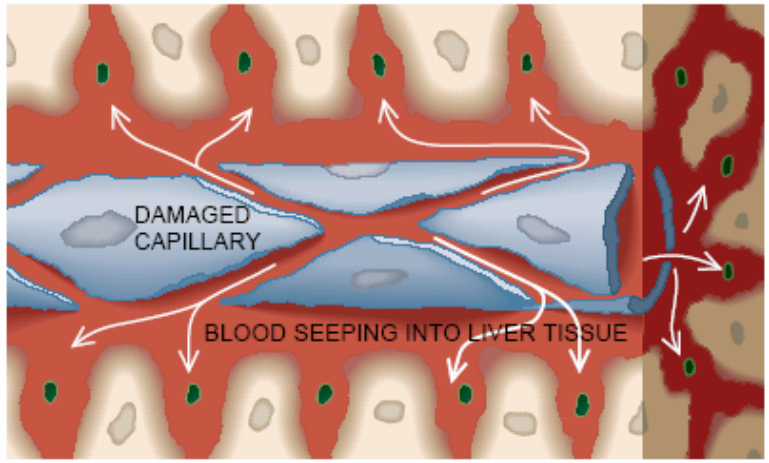




NORMAL LIVER



LIVER AFTER TOXINS ACT



Wayne Carmichael, Scientific American, January, 1994

Human Microcystin Poisonings

- **1931: USA, Illness in 5000-8000 people drinking water from Ohio & Potomac rivers during *Microcystis* Bloom** (Veldee 1931, Tisdale 1931)
- **1959: Canada, *Microcystis & Anabaena* bloom recreational water, animals and humans with multiple-system illness. Organisms isolated from physician's stool sample** (Dillenberg 1960)
- **1981: Australia, *Microcystis* in drinking water & elevated liver enzymes (liver damage) in population** (Falconer 1983)
- **1988: Brazil, *Microcystis* bloom in reservoir. 2000 GI illnesses over 42 Days, 88 Deaths** (Teixeira 1993)
- **1989: England, 10 soldiers with severe illness after swimming/canoeing in *Microcystis* Bloom** (Turner 1990)
- **1994: Sweden, GI illness in 121/304, MC in drinking H20**

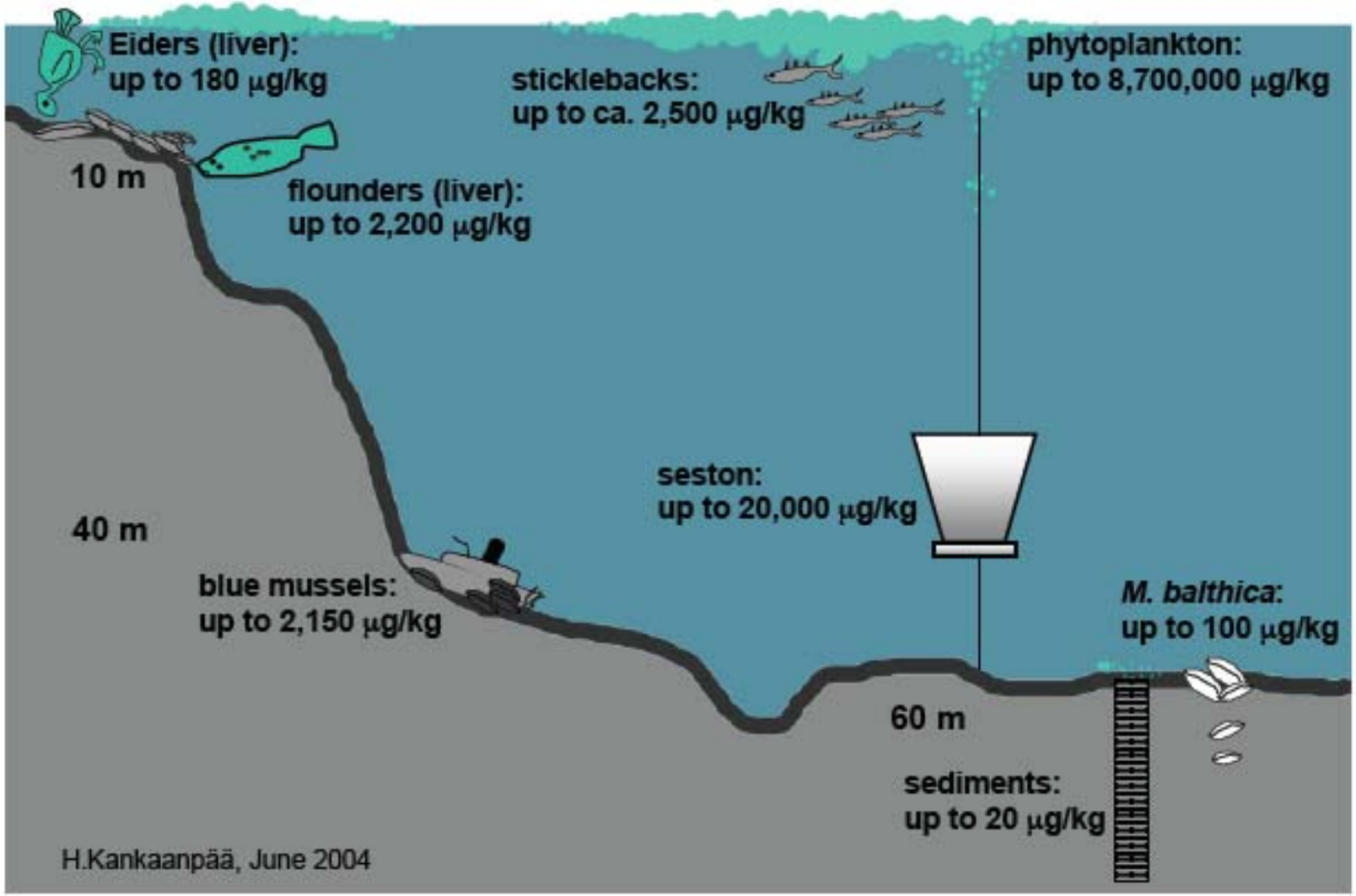
Evidence for Tumor Promotion by Microcystins

- Epidemiology in China:
  - Contaminated drinking water ↔ primary liver and colon cancer.
- Injection of toxin ± initiator:
  - Increased size/number of liver cancer precursors.
- Oral *M. aeruginosa.* extract:
  - Skin papillomas larger/heavier.
  - No effect on duodenal tumours or lymphoma

Colon cancer precursors larger.

Andrew Humpage

Microcystin in Aquatic Environment



Jussi Meriluoto

Microcystin LR Inhibits Plant Growth

Mustard seedlings, one week old, MC-LR 0--20 µg/ml

Jussi Meriluoto

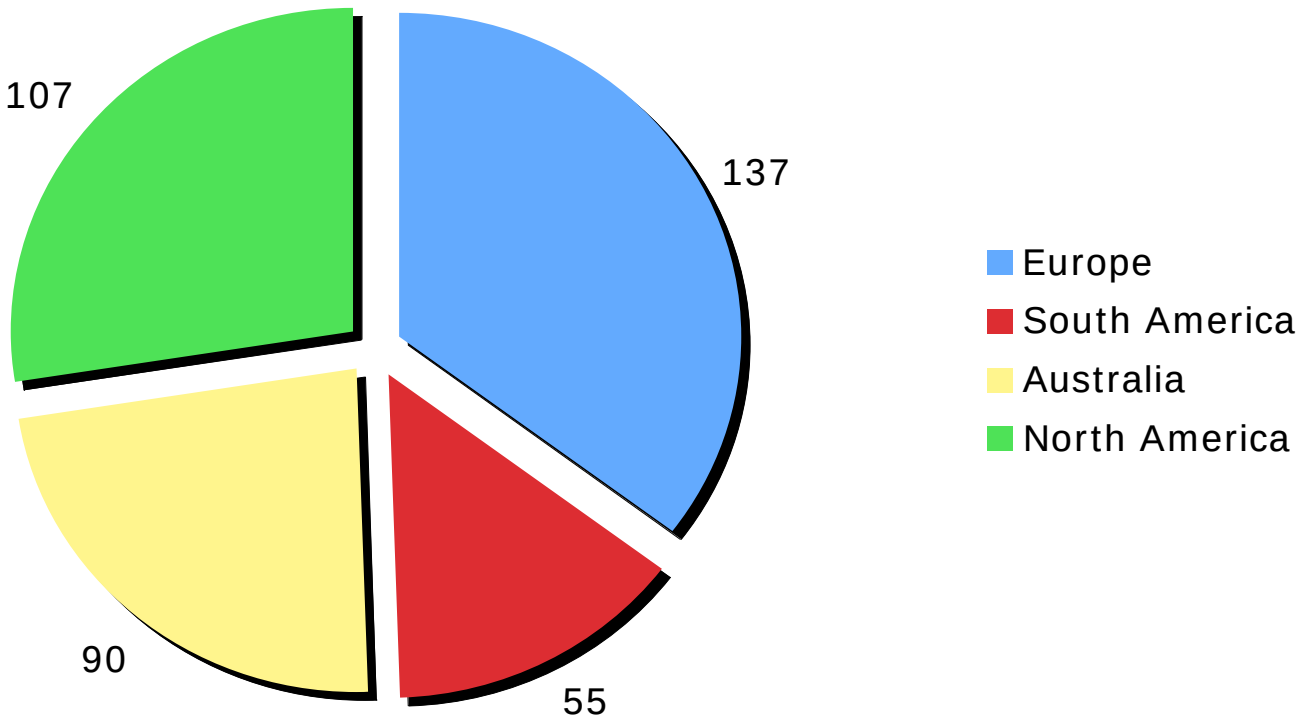
Occurrence

Occurrence Worldwide

Countries Exhibiting One or More Documented CyanoHAB Events

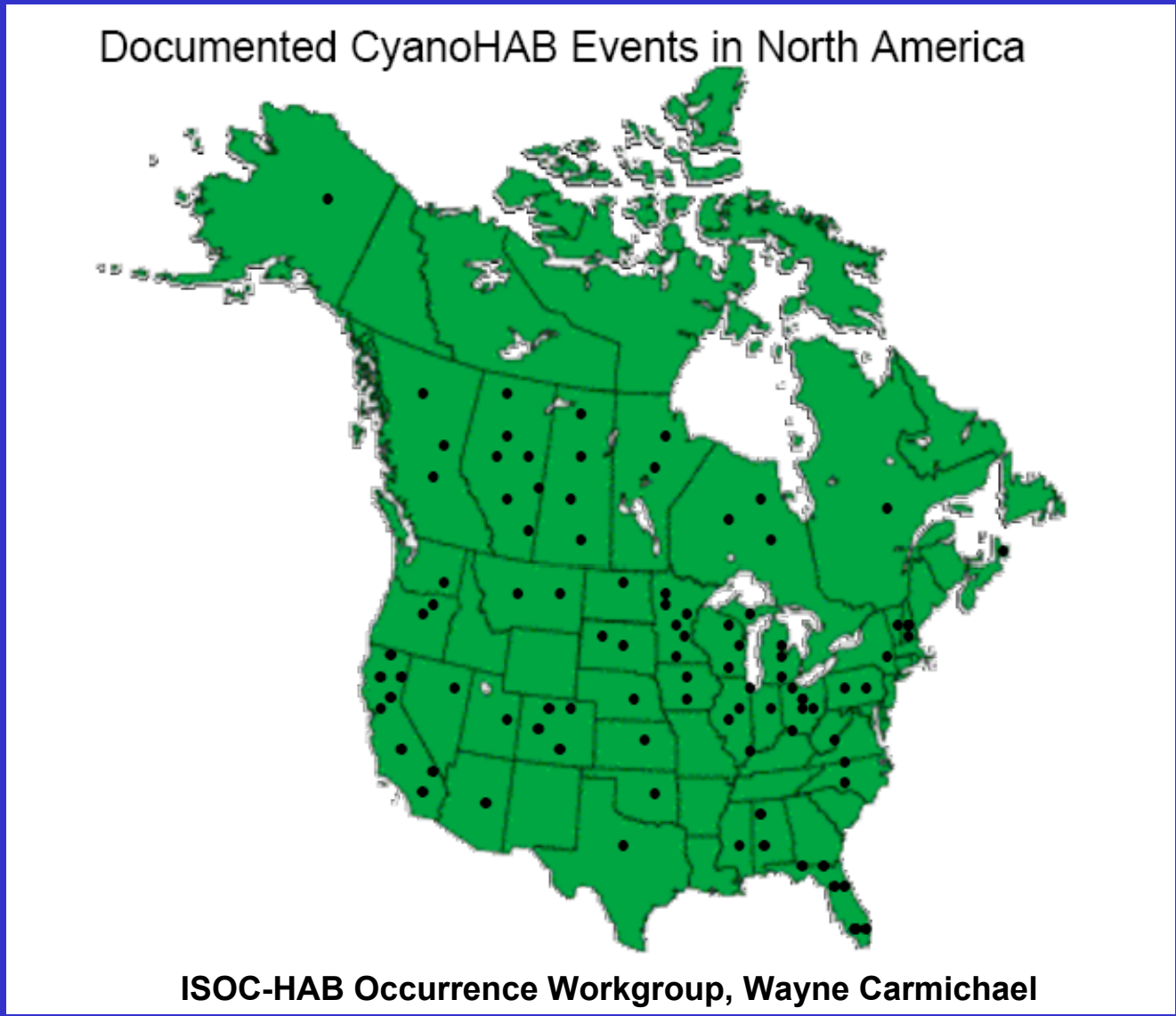
ISOC-HAB Occurrence Workgroup, Wayne Carmichael

Number of CHABS Reported by Continent



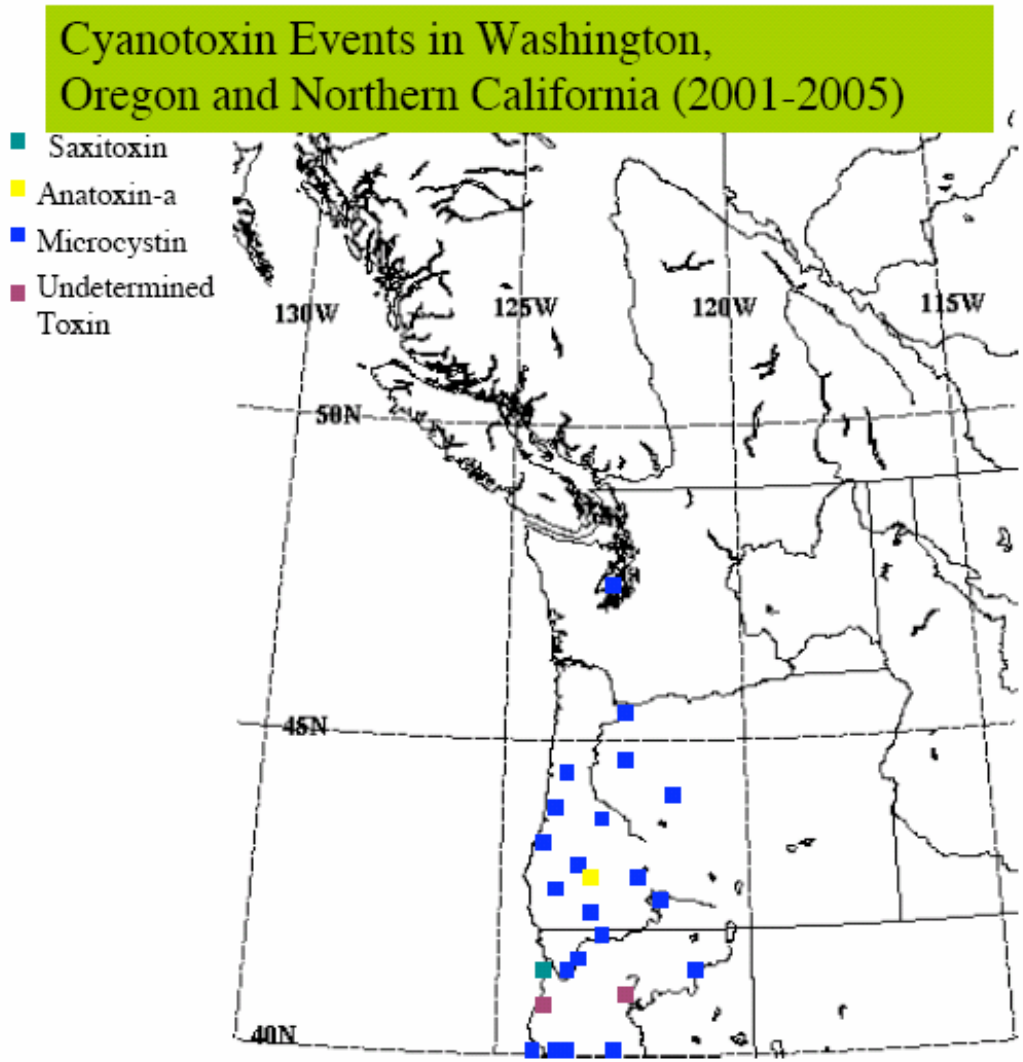
ISOC-HAB Occurrence Workgroup, Wayne Carmichael

Occurrence North America



ISOC-HAB Occurrence Workgroup, Wayne Carmichael

Occurrence Northwest US



ISOC-HAB Occurrence Workgroup, Wayne Carmichael

NEBRASKA EXPERIENCE

Cyanobacterial Harmful Algal Blooms

Steve Walker

402-471-4227

Water Quality Assessment Section

Nebraska Department of

Environmental Quality (NDEQ)

(www.deq.state.ne.us)

Buccaneer Bay
May 4, 2004

- 2 dogs died after drinking water from buccaneer bay lake
- Lake had dense algae bloom
- Investigated by NDEQ and water samples collected
- Microcystin toxin level in water measured at 69.4 ppb
- Autopsy on dog revealed microcystin toxins in lethal concentrations

Steve Walker

Interagency Meetings

- Nebraska Department of Environmental Quality
- Nebraska Game and Parks Commission
- Nebraska Health and Human Services System
- University of Nebraska – Lincoln
- County health departments
- Natural resources districts

Steve Walker

Cyanobacteria Problems Quickly Addressed

- Excellent cooperation and quick action among government entities in Nebraska
- Monitoring and public notification strategies developed within two weeks
- ELISA lab equipment ordered and set up within two weeks

Steve Walker

ELISA Method for Microcystin Analysis

Steve Walker

Microcystin Analysis

Steve Walker

Advantages of ELISA Method for Microcystin Analysis

- Semi-Quantitative Measure of Microcystin
- Quick Turnaround Times
- Relatively Easy Procedure
- Relatively Inexpensive
  - **Approximately $20/test**
- Accurate & Precise
  - **MDL = 0.15 ppb**
  - **Good Duplicate Results**

Steve Walker

Cost Savings Using ELISA Tests

- NDEQ
  - 700 Samples @ $20/Sample X 2 Dilutions = $28,000
- Contract Lab
  - Cost per Microcystin HPLC or LC/MS Analysis = $150/Sample X 700 Samples = $105,000
- Savings
  - $77,000

Cyanobacteria Summary

- 700+ samples analyzed in 2004
  - **111 different lakes**
- Health alerts (microcystins > 15 ppb)
  - **26 different lakes**
- Health advisories (microcystins > 2 ppb)
  - **69 different lakes**
- Longest health alert duration:
  - **Carter Lake - 15 weeks**
  - **Swan Creek Lake (5A) - 14 weeks**
  - **Pawnee Lake - 12 weeks**
  - **Iron Horse Trail Lake – 12 weeks**

Steve Walker

Health Alerts Issued on 26 Lakes During 2004

Steve Walker

Pawnee Lake near Emerald

Steve Walker

PAWNEE LAKE

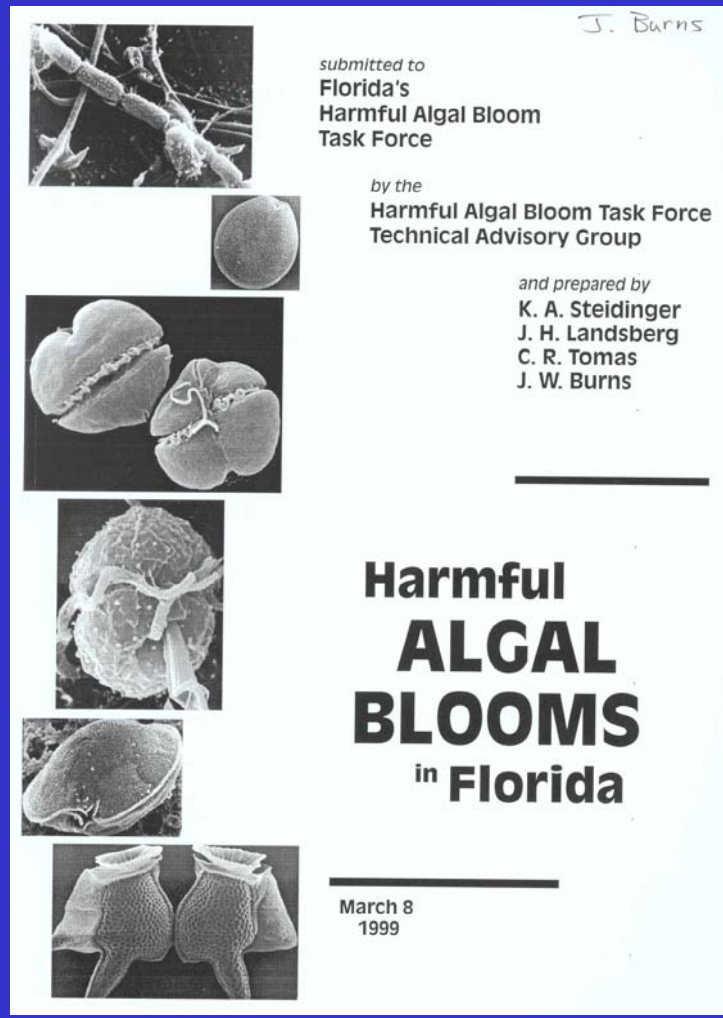
- Observed significant bloom at east swimming beach while collecting bacteria data 7/12/04
- Microcystin analysis > 15 ppb
- Meeting with HHS and G&P
- Signs mistakenly posted at only east beach. People used the west beach and rest of lake
- Following week > 50 reports of people sick with cyanobacteria symptoms

Steve Walker

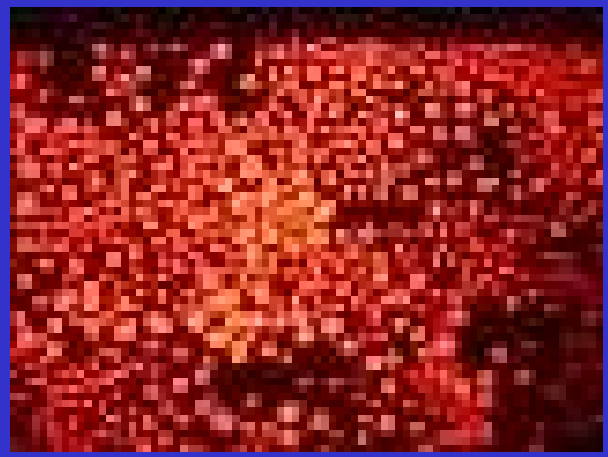
Pawnee Lake near Emerald

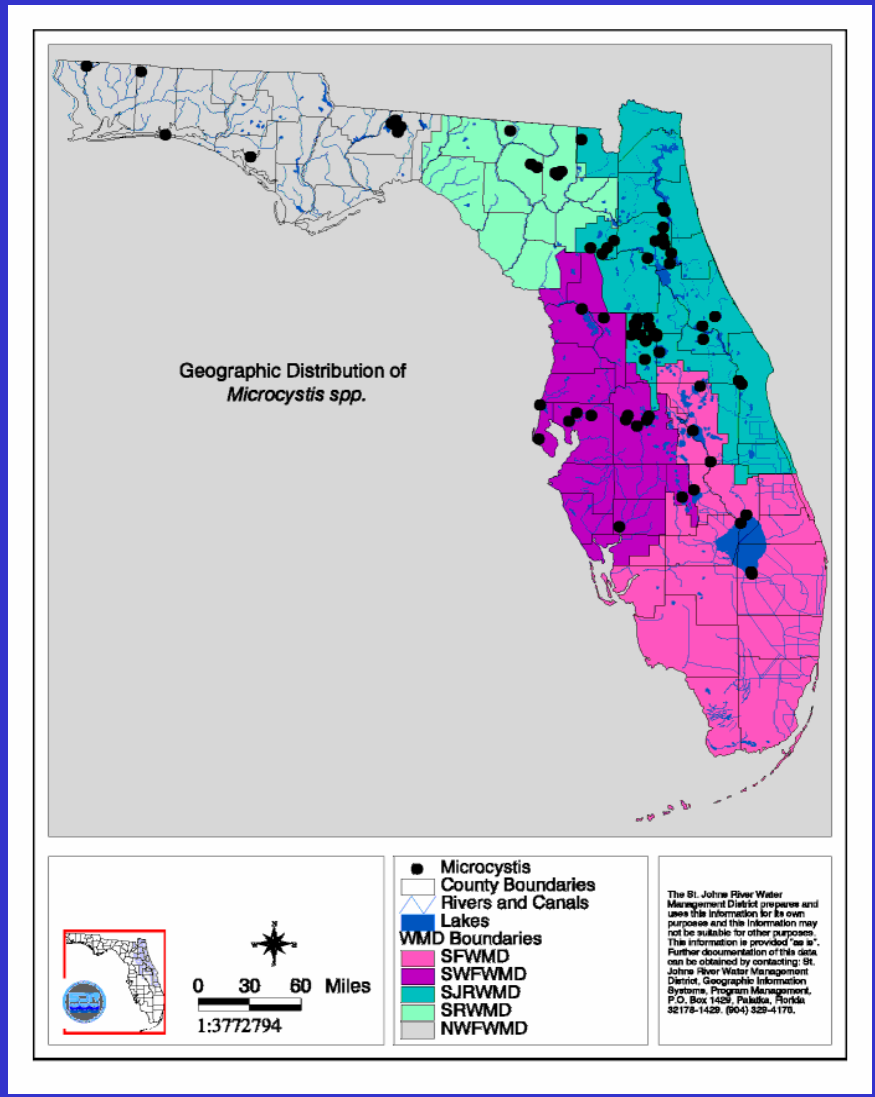
Steve Walker

Florida Survey 1999-2003



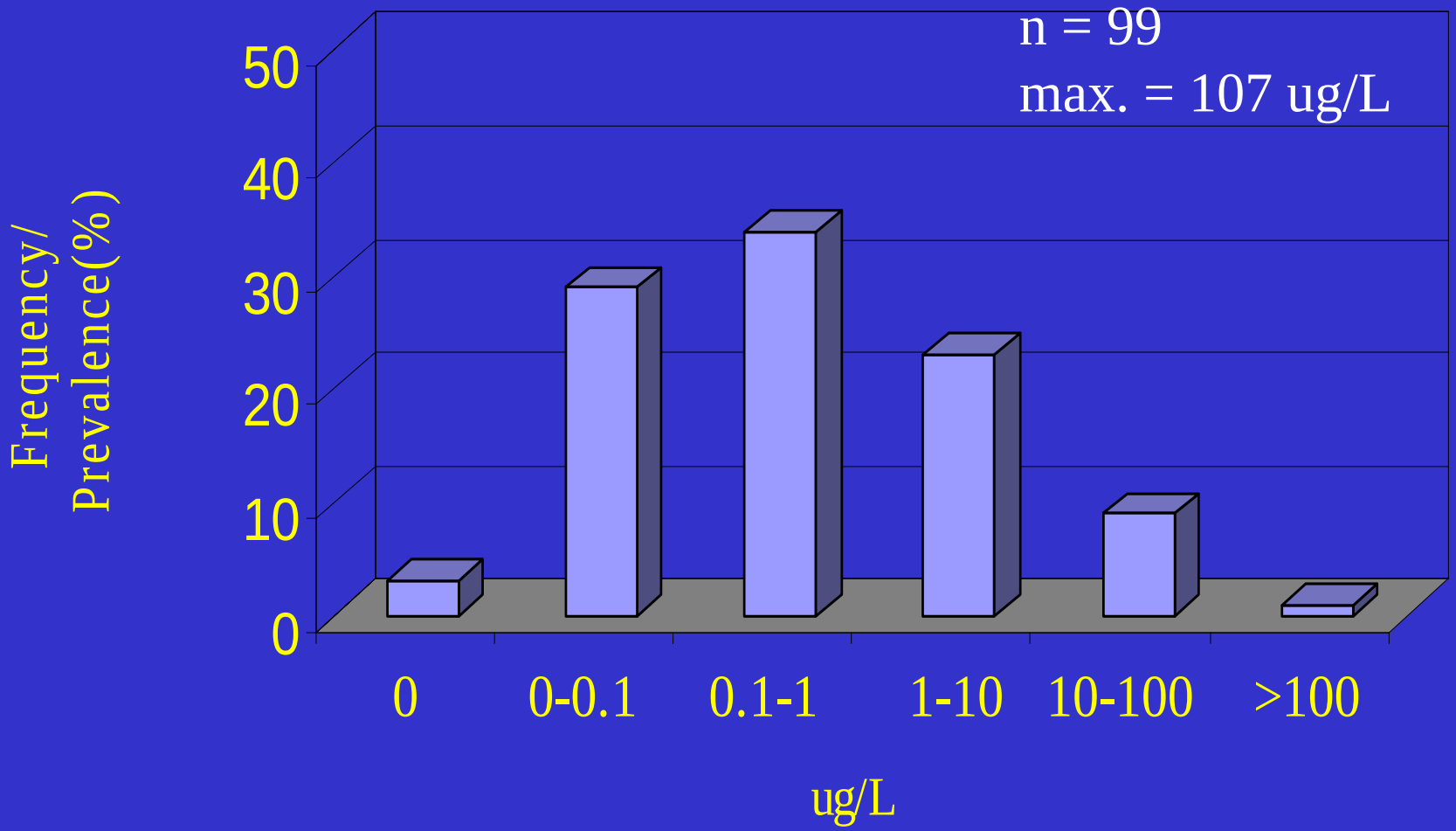
Microcystis Distribution





John Burns

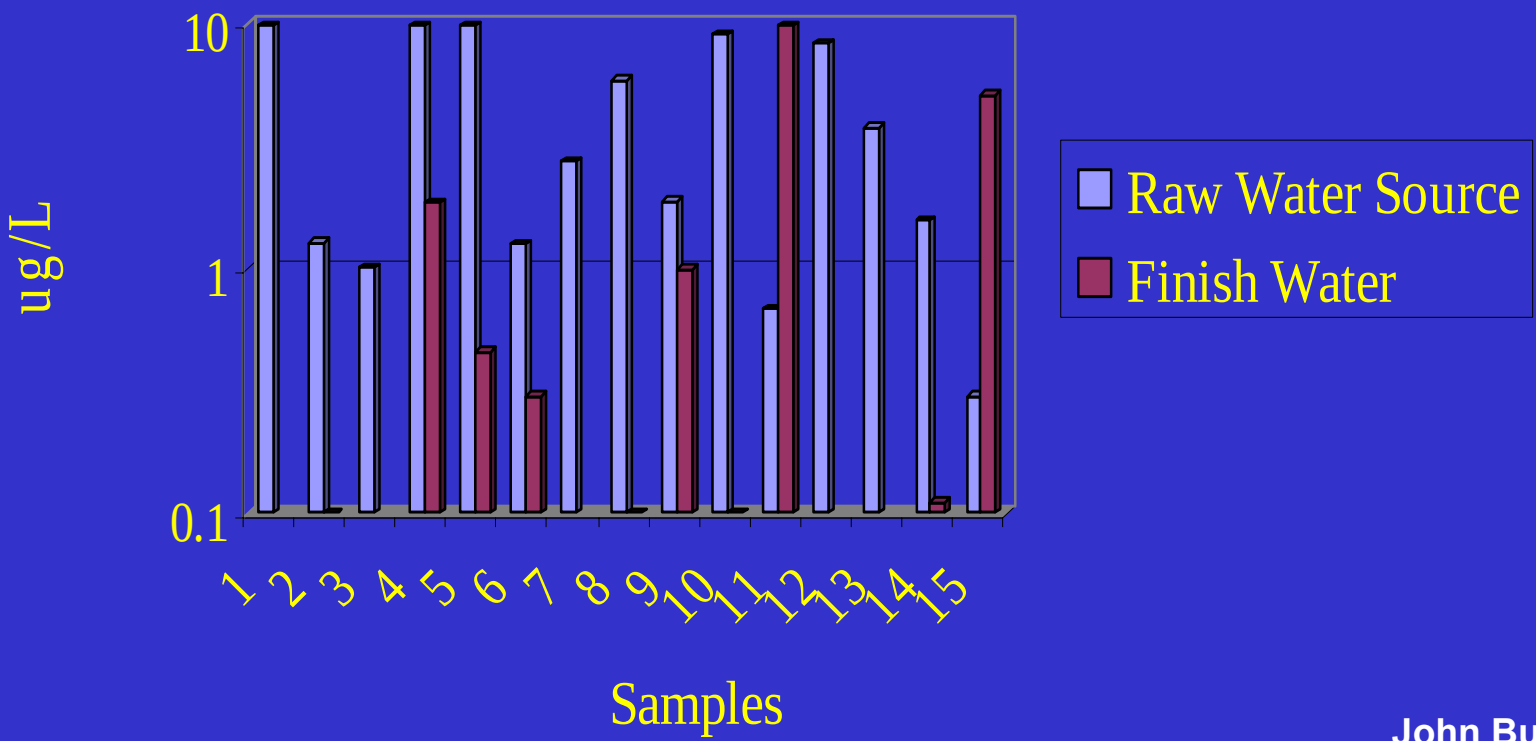
Microcystin YR-2000



John Burns

Microcystins in Drinking Water Resources - Florida

*** Max. environmental value = 106 ug/L**



John Burns

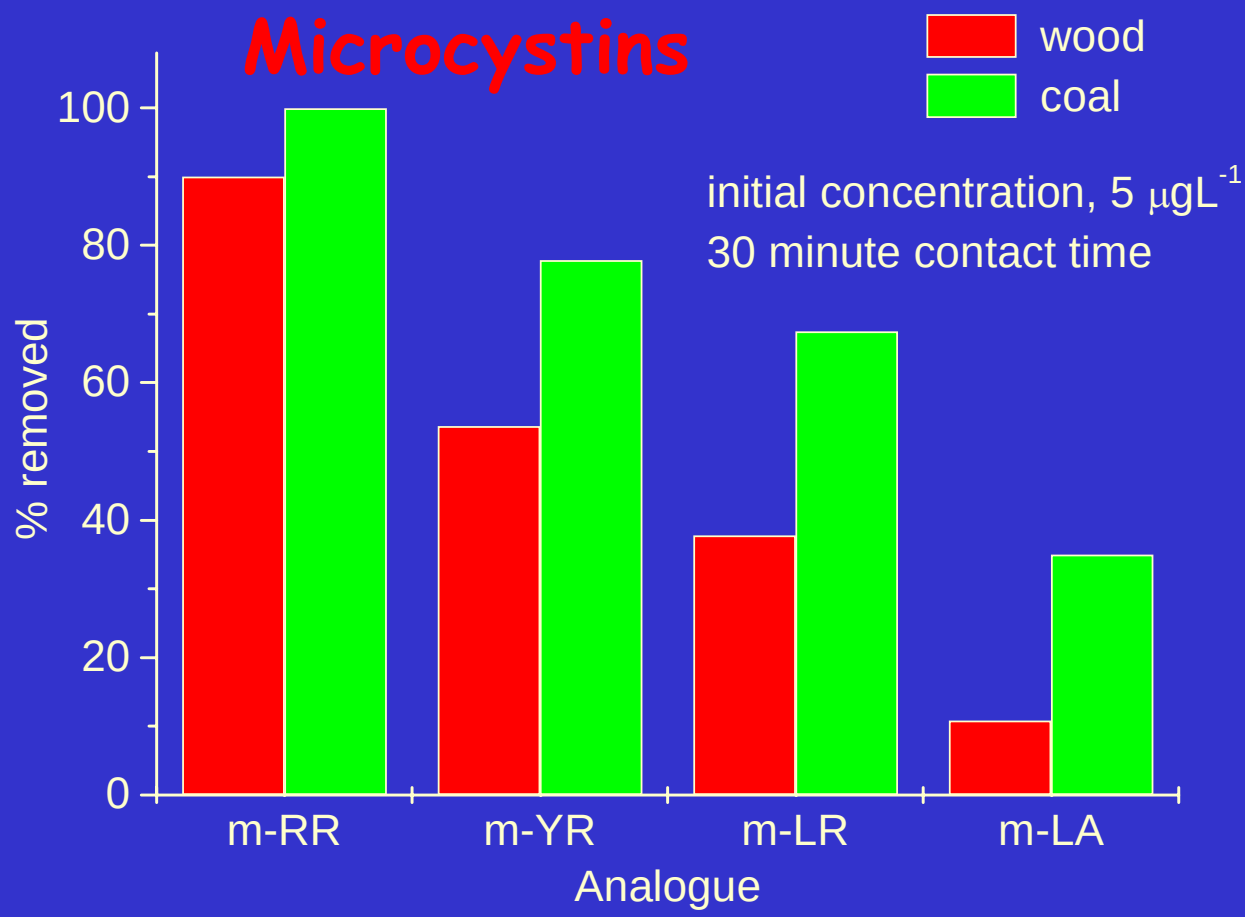
Exposure at the Tap?

Home Filters

Post Chloraminated Water @ WTP

John Burns

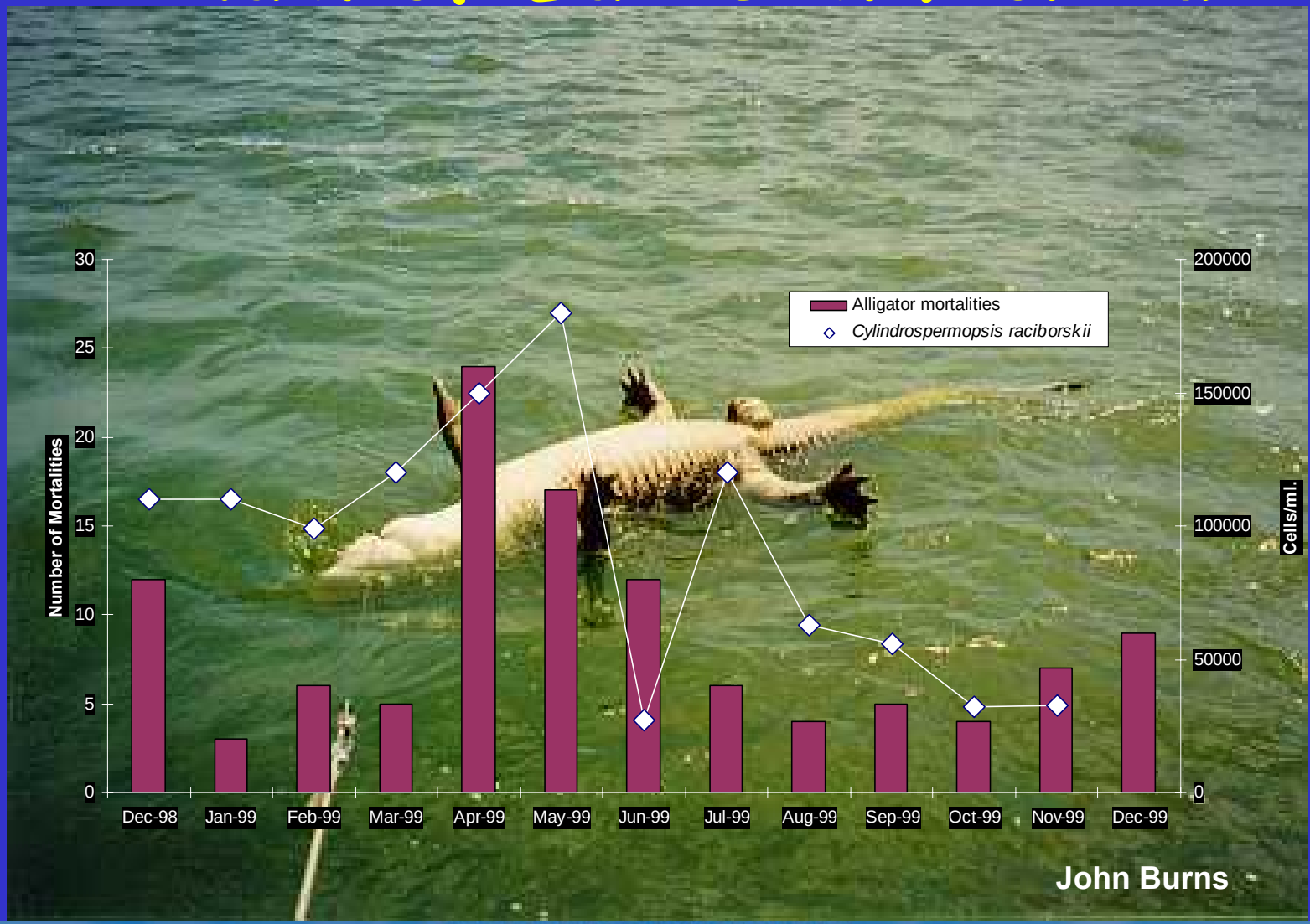
Particulate Activated Carbon



Australian Water Quality Centre

From: Gayle Newcombe, Brenton Nicholson

Wildlife Death – St. John's Chain of Lakes in Florida



Alligator Mortalities

Cylindrospermopsis Cells/ml

Risk Assessment

What are Guidelines & MCLs?

World Health Organization

"….. A guideline value represents the concentration of a constituent that does not result in any significant risk to the health of the consumer over a lifetime of consumption."

US Environmental Protection Agency

"….. The recommended maximum contaminant level must be set to prevent the occurrence of any known or anticipated health event."

Mike Burch

Drinking Water Guidelines

		Microcystins
• WHO	1998	1 µg / L (LR)
• Brazil	2000	1 µg / L **(All, Reg)**
• France	2001	1 µg / L (LR)
• Australia	2001	1.3 µg / L (LR Tox Eq)
• Canada	2002	1.5 µg / L (LR Tox Eq)
• New Zealand	2005	1 µg / L (LR Tox Eq)

Mike Burch

Deriving the WHO Guideline for Microcystin LR

- Microcystin has been treated as a threshold toxicant – *i.e. non-genotoxic, non-carcinogenic*
- Tolerable Daily Intake (TDI) [i.e. Reference Dose (RfD] was calculated from NOAEL (40 µg/kg/day) in 13-week sub-chronic oral mouse dosing study with MC-LR
- Uncertainty Factors: x 10 intraspecies, x 10 interspecies, x 10 for limitations of data – lack of data on chronic toxicity and carcinogenicity = Total UF x 1000
- GV (MCL) = $\frac{\text{TDI x BW x } P_{intake}}{\text{Daily Consumption}} = \frac{0.04 \text{ x } 60\text{kg x } 0.8}{2\text{L}} = 0.96 \sim 1\mu\text{g/L}$

Recreational Water Guidelines

	Cells	Microcystins or Tox Eq
• WHO Level 1	20,000 Cells/L ≃	4 µg/L ≃ 1/5 TDI/100ml
Level 2	100,000 Cells/L ≃	20 µg/L ≃ TDI/100ml
Level 3	Surface Scum ≃	>>>>>>>>>> TDI/100ml

'Immediate action to control scum contact' (Chorus & Bartram, 1999)

- France — Same as WHO

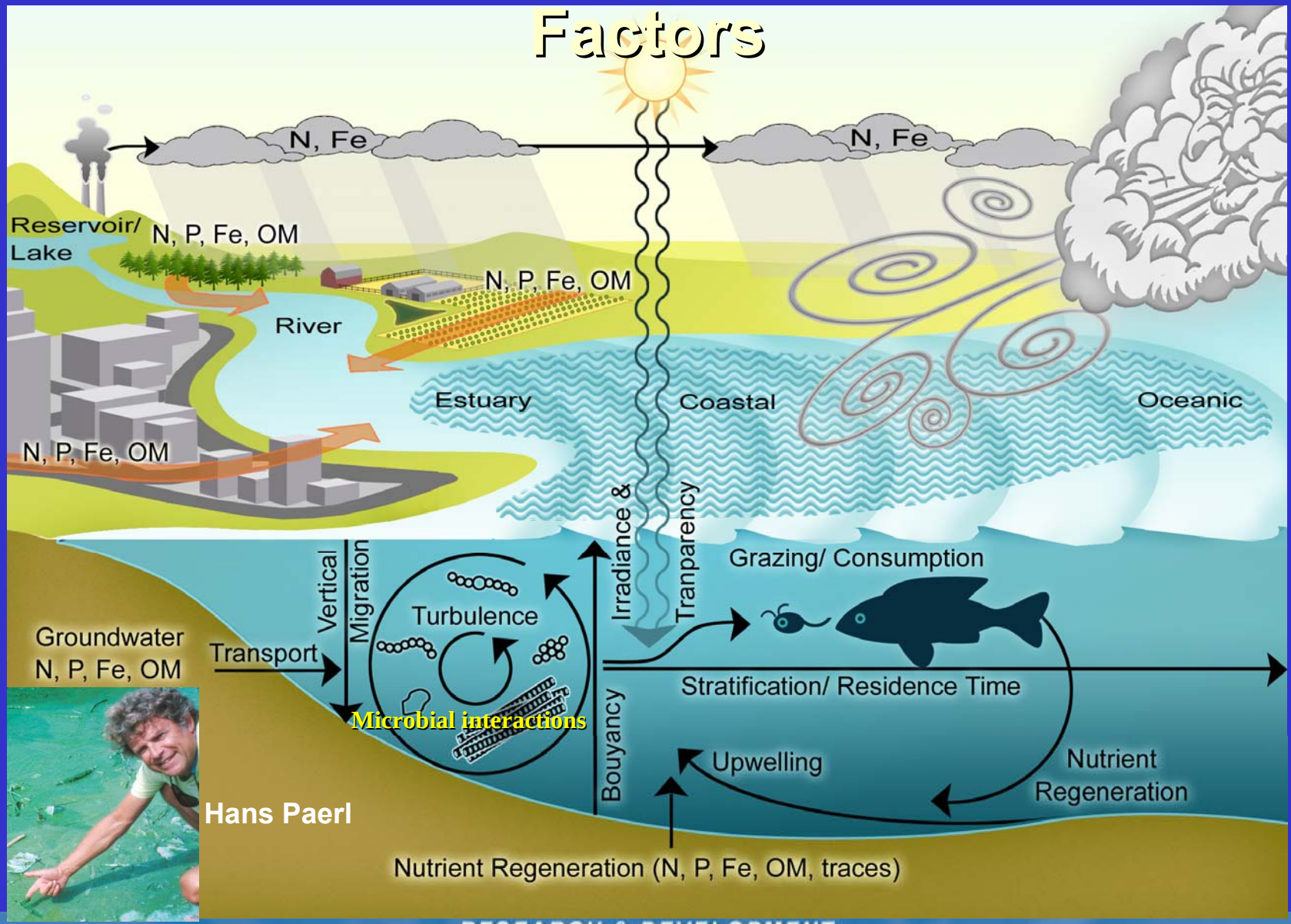
	Biovolume
• Australia Level 1	50,000 Cells/L ≃ 10 µg/L, >4 mm^3/L
Level 2	Biovolume > 10 mm^3/L or Scum

- Netherlands 1 Level 20 µg MCY-LR/L
- Germany Level 1 <10 µg/L, Level 2 >10-<100 µg/L
  Level 3 > 100 µg/L

Mike Burch

Causes, Prevention & Mitigation

CHAB Control: Physical, Chemical & Biotic Factors



Nutrient Issues

N & P enrichments are stimulatory

N:P Input ratios are important

(N:P < 15 favors N_2 Fixers)

Specific chemical forms of N (i.e. nitrate, ammonium, organic N) may regulate algal community composition & toxicity

Other nutrients (Fe, trace elements)?

Hans Paerl

Hydrodynamics

- **Turbulence/Vertical Mixing**

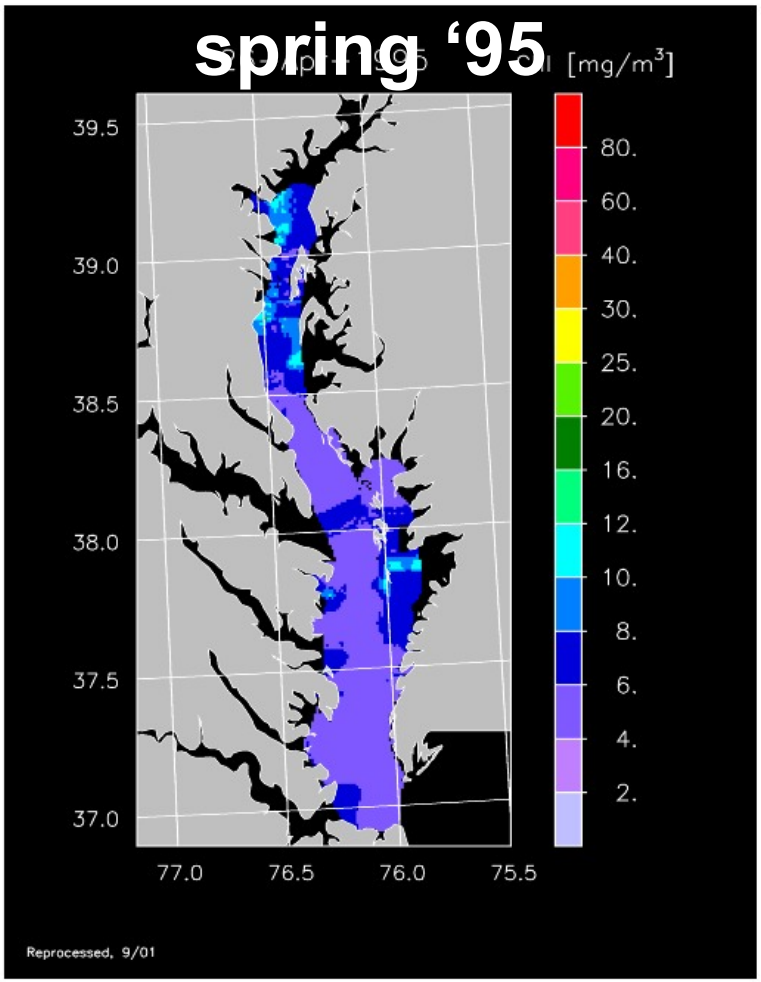
(Low turbulence conditions favor cyanobacteria, especially N_2 fixers)

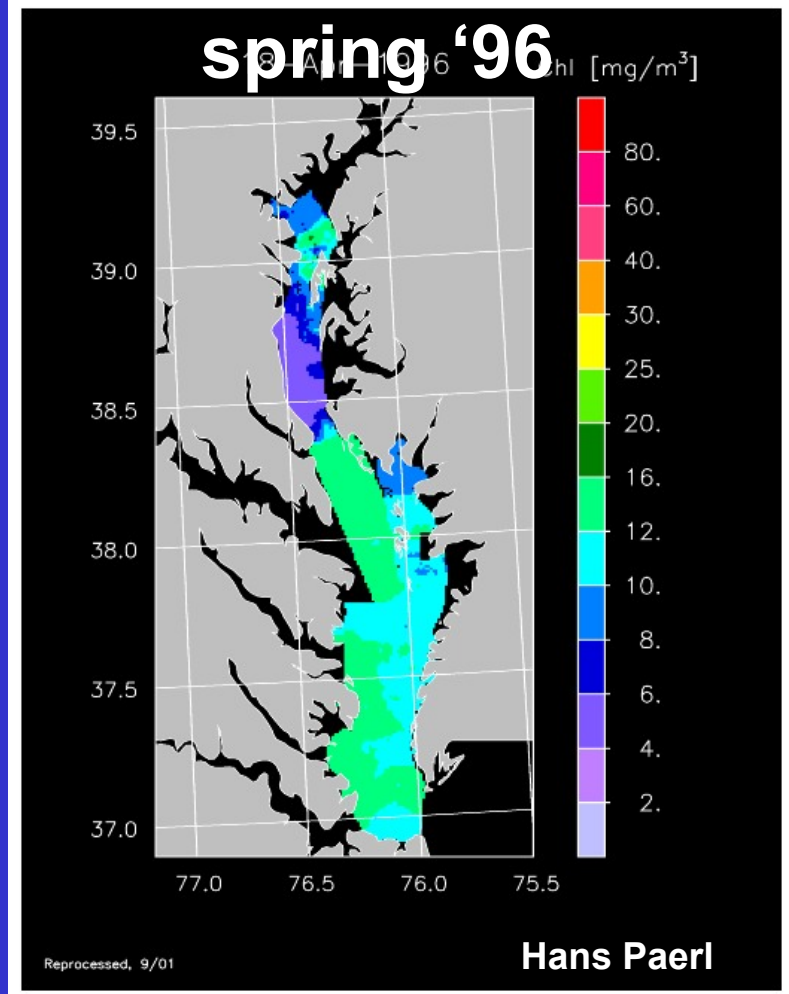
- **Water residence time/flushing**

(long residence time favors cyanobacterial dominance)

Hans Paerl

Chesapeake Bay: Remotely sensed chl-a from SeaWiFS Aircraft Simulator (SAS II) during low flow ('95) and high flow ('96) years





Climatic Factors

- Temperature

(high temperature favors cyanobacteria)

- Irradiance

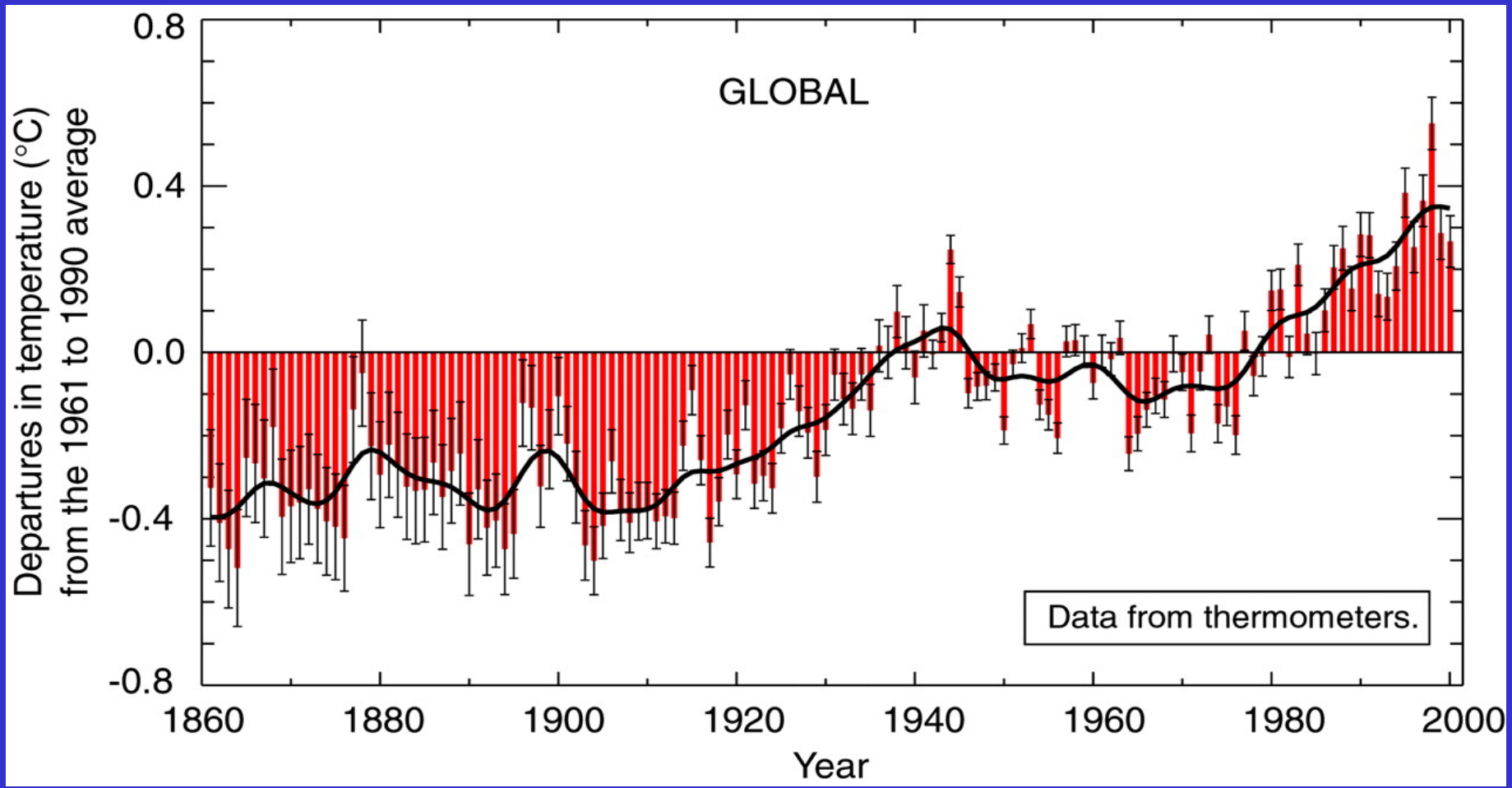
(high irradiance favors *most* cyanobacteria)

Salinity

- Selects for specific taxa

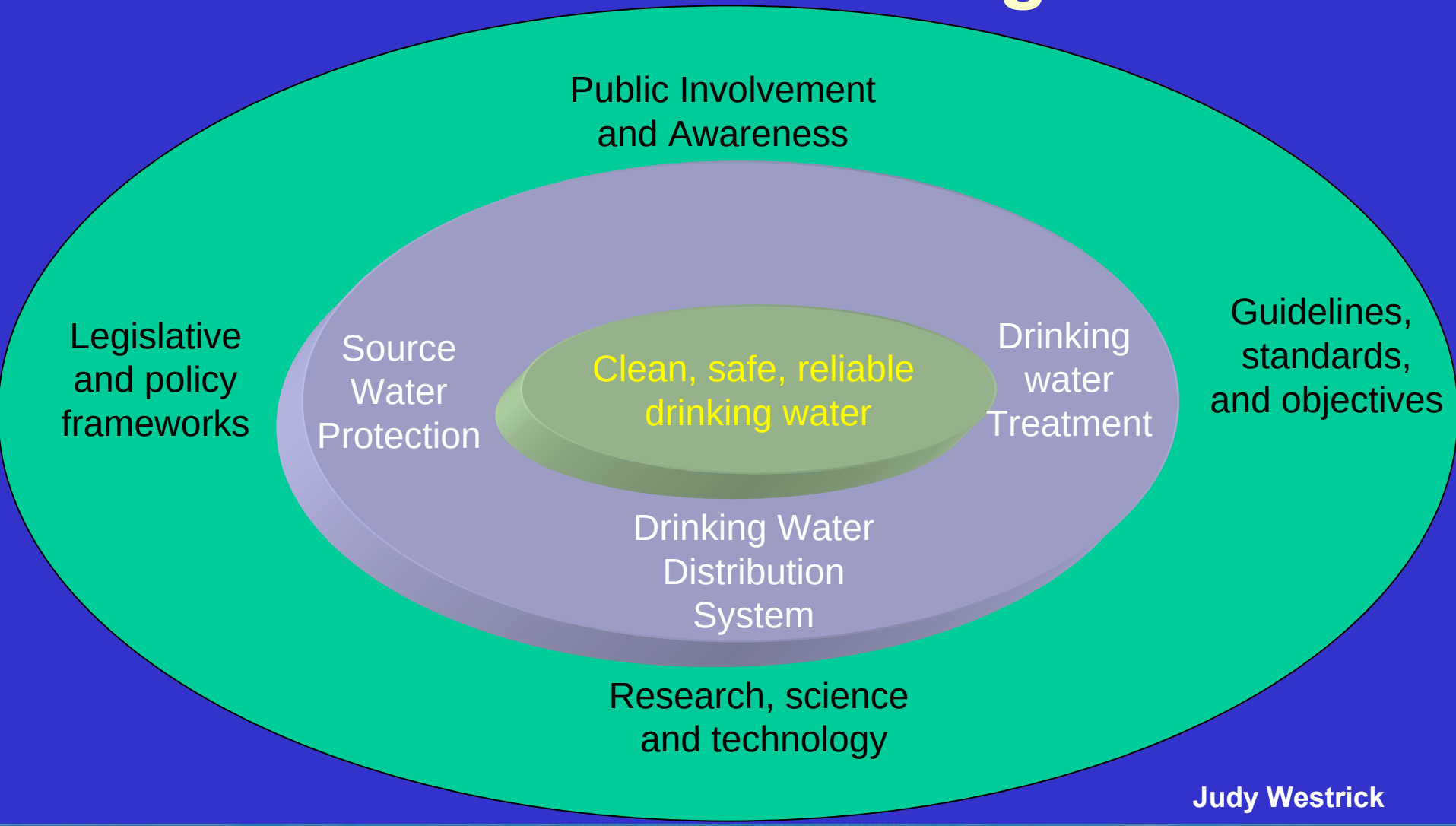
Hans Paerl

Average global surface temperatures from 1860-2000, showing deviation from the baseline 1961-1990 average temperature



Valeria Paul

CHABS: Multi-Barrier Approach To Prevention & Mitigation



Judy Westrick

Watershed Approach

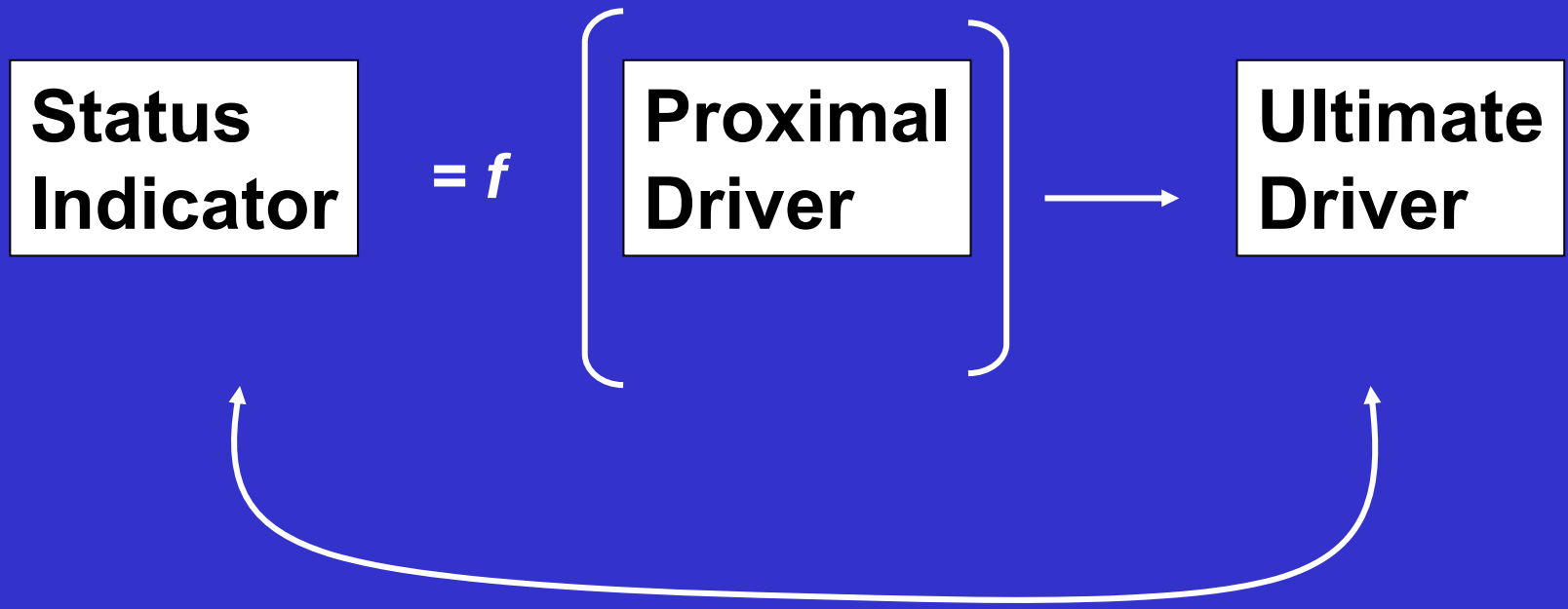
Mike Piehler

A.Partnerships **-- Those people most affected by management decisions are involved throughout and shape key decisions.**

B.Geographic Focus **-- Activities are directed within specific geographic areas, typically the areas that drain to surface water bodies or that recharge or overlay ground waters or a combination of both.**

C.Sound Management Techniques based on Strong Science and Data **--**

i.assessment and characterization of the natural resources and the communities that depend upon them;
ii.goal setting and identification of environmental objectives based on the condition or vulnerability of resources and the needs of the aquatic ecosystem and the people within the community;
iii.identification of priority problems;
iv.development of specific management options and action plans;
v.implementation; and
vi.evaluation of effectiveness and revision of plans, as needed.



CHABS

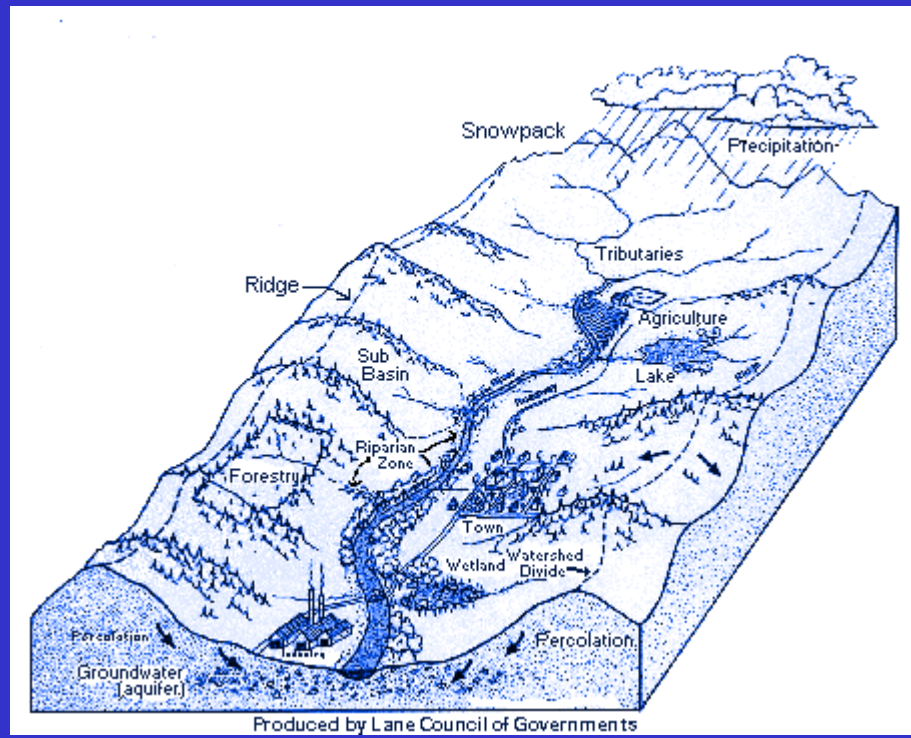
e.g. nutrient concentrations and/or flow regime

e.g. land use

C-HAB prevention

Watershed conservation, restoration and rehabilitation

- Terrestrial
- Land-water margin
- Aquatic
- Atmospheric



Mike Piehler

Recreational Water Treatment

- Watershed Management
- Aeration
- Alum addition
- Electrocoagulation
- Algicides
- Harvesting

Judy Westrick

Drinking Water Treatment

- Treatment to remove extracellular algal toxins
  - Oxidation
  - Biologically active filters
  - Physical removal
- Treatment to remove intracellular algal toxins
  - Membrane technologies
    - Micro and ultra
  - Conventional treatment
    - Coagulation/sedimentation/filtration
    - Dissolved air floatation
  - New technologies

Judy Westrick

Future

ISOC-HAB Product

- State of the Science
  - * EPA - Sufficient for Regulatory Determination?
    - Occurrence Data
    - Health Data
    - Management Options Available
  - * Yes
    - Risk Assessments
    - Produce Guidelines or Regulate
  - * No
- Research Priorities

ISOC-HAB Product

- **Research Priorities**
  - * Occurrence - If Methods Available, Implement Unregulated Contaminate Monitoring Rule
  - * Health - Dose-Response Data
  - * Prevention & Mitigation - Watershed Management
    - Drinking Water Treatment
    - Education
- **National Research Plan**
  - * To CENR Task Force, to Congress
  - * Interagency Implementation

Thank You