Water Quality Perspectives on Inland Harmful Algal Blooms

Bryan W. Brooks

Distinguished Professor of Environmental Science and Biomedical Studies

NIEHS Center for Oceans and Human Health and Climate Change Interactions

Bryan_Brooks@Baylor.edu

UF Water Institute Symposium 2020

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Are Harmful Algal Blooms Becoming the Greatest Threat to Water Quality?

OHC²I

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

Why Global Horizon Scanning?

- The Global Horizon Scanning Project aims to identify important and timely environmental quality research needs
- Research questions identified, if answered, would markedly advance toward more sustainable environmental quality over the next decade
  - Unique partnership with SETAC (and ACS ENVR, AGRO in NA)

Global. Transparent. Inclusive. Multidisciplinary. Multisector.

HHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Inherent Connections among Human Health, Ecosystems and Environmental Quality

Coupled Human and Ecological Frameworks

Integrative Needs for Public Health and the Environment

Which are the environmental variables that trigger the production of algal toxins in the environment? Does exposure through trophic levels threaten human health?

HHC²I

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

What Challenges are Facing Environmental Public Health Professionals in US Health Departments?

Commentary

ehp Environmental Health Perspectives

Environmental Health Practice Challenges and Research Needs for U.S. Health Departments

Bryan W. Brooks,[1] Justin A. Gerding,[2] Elizabeth Landeen,[3] Eric Bradley,[4] Timothy Callahan,[5] Stephanie Cushing,[6] Fikru Hailu,[7] Nancy Hall,[8] Timothy Hatch,[9] Sherise Jurries,[10] Martin A. Kalis,[2] Kaitlyn R. Kelly,[1] Joseph P. Laco,[2] Niki Lemin,[11] Carol McInnes,[12] Greg Olsen,[13] Robert Stratman,[14] Carolyn White,[15] Steven Wille,[16] and John Sarisky[2]

CDC Centers for Disease Control and Prevention

UNCOVER Environmental Health

National Environmental Health Association

Brooks et al 2019 *EHP*; Gerding et al 2020 *AJPH*

https://www.cdc.gov/nceh/ehs/uncover-eh/index.html

OHC2T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

HHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

A Principle of Water Quality

"You only find what you are looking for and you only find it if it is in concentrations high enough to be detected by the method being used to analyze for it."
WT Waller

OHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

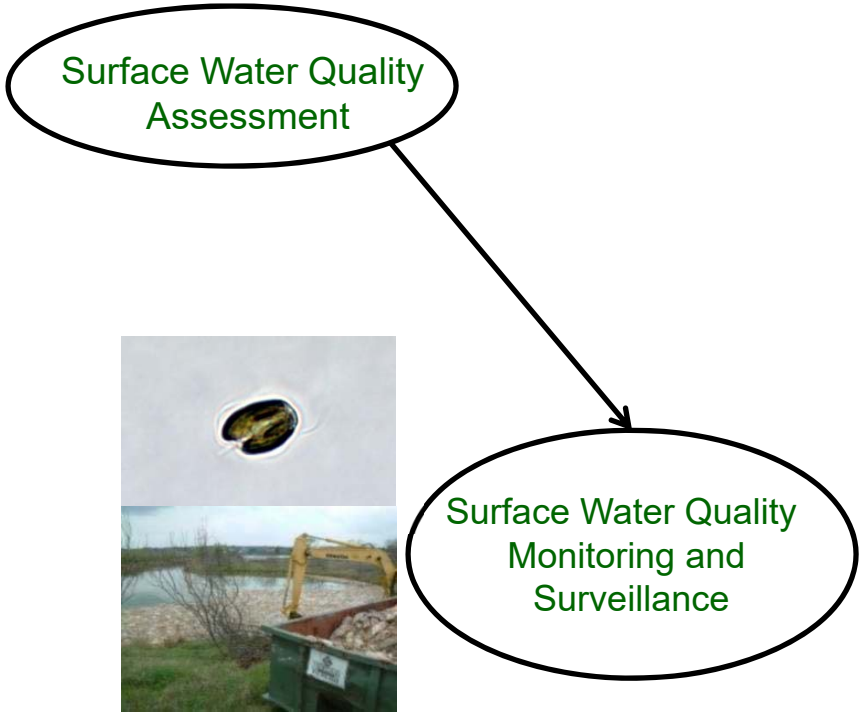
How to Protect and Restore Water Quality?

Surface Water Quality Assessment

Brooks et al 2016. *ETC*

OHC²I

How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Exposure, Exposure, Exposure…

100s of Reservoirs?

100s of Reservoirs?

Recreational waters?

- Swimming, toxin exposure?
- Fishing, food safety?

Agriculture use?

- Toxins in crops?
- Toxins in aquaculture?

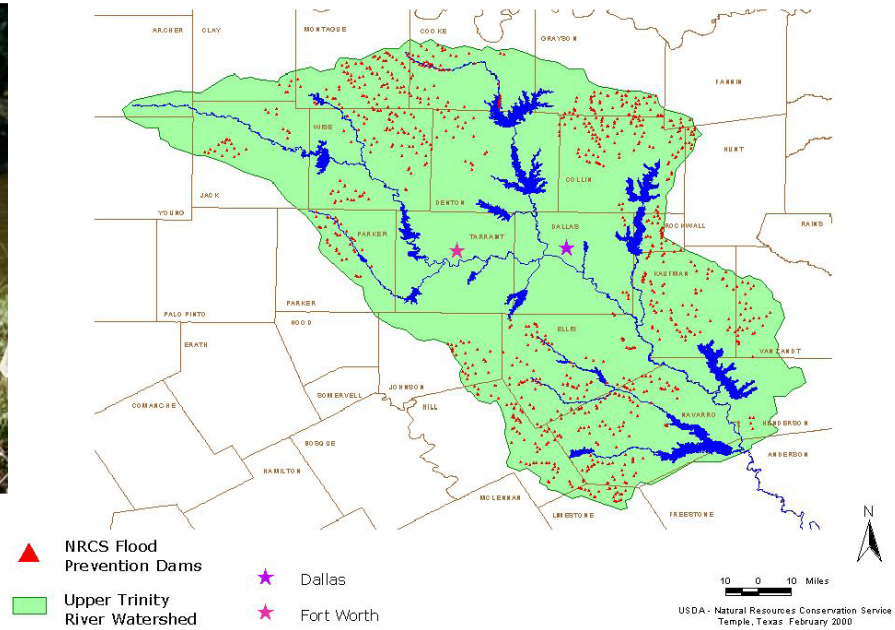
BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

10000s of Stormwater Ponds, Oxbow Lakes?

Stormwater Ponds in South Carolina—Challeng

Proliferating stormwater ponds focus of several S.C. Sea Grant Consorti

USDA - NRCS Dams in the Upper Trinity River Watershed



nrcs.usda.gov

OHC2T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

10000s of Stormwater Ponds, Oxbow Lakes?

Recreational waters?

- Swimming, toxin exposure?
- Fishing, food safety?

Agriculture use?

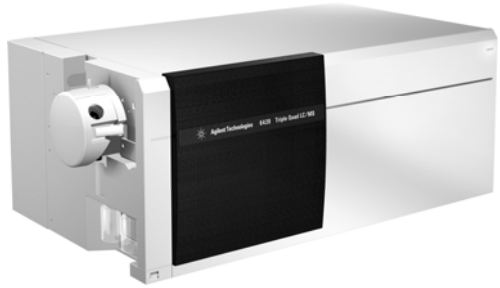
- Toxins in crops?
- Toxins in aquaculture?

HC²T

Methodology: Detection

Instrument: Agilent 6420 Triple Quadrupole Mass Spectrometer

- Isotope dilution LC/MSMS



Multiple Reaction Monitoring (MRM) Transitions

- Identified by Optimizer software (Agilent)

Separation

- Hydrophilic Interaction Chromatography (HILIC)
  - Agilent Poroshell HILIC-Z
    - Anatoxin – a
    - Cylindrospermopsin
    - Saxitoxin
- Reverse Phase Liquid Chromatography (RPLC)
  - Agilent Poroshell SB-C18
    - Microcystins (5)
    - Nodularin

Haddad et al. 2019. *J Chrom A*

Water Extraction Recovery

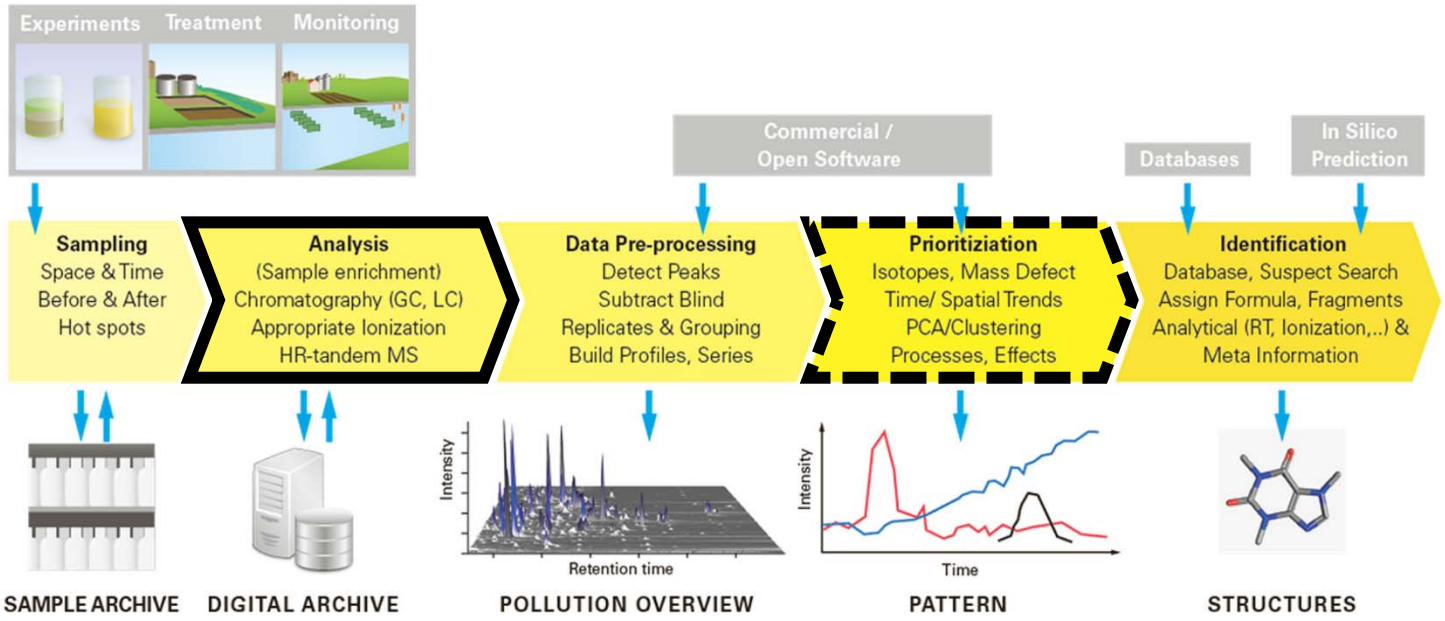
Analyte	Water	
	Percent Recovery (mean ± CV)	MDL (ng/L)
Anatoxin-a	97 ± 1.2	0.08
Cylindrospermopsin	90 ± 2.6	0.43
Saxitoxin	53 ± 2.7	0.22
M-LA	92 ± 3.4	0.6
M-LR	94 ± 2.4	0.83
M-LY	96 ± 9.9	0.38
M-RR	92 ± 2.3	0.91
M-YR	104 ± 9.9	0.8
Nodularin	99 ± 3.5	0.96

Recovery n=3
Method Detection Limits (MDL) n=8

Haddad et al. 2019. *J Chrom A*

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Overview of Non-Target Analysis Workflow



Hollender, J. et al. *Environ. Sci. Technol.*, 2017, *51*, 11505–11512

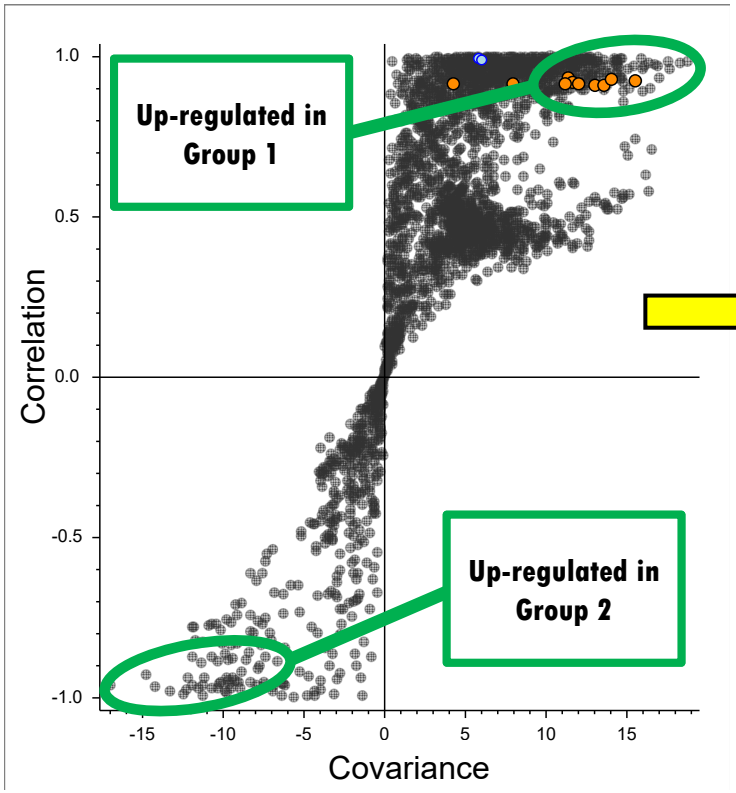
Taylor et al. 2020. *STOTEN*

OHC2T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

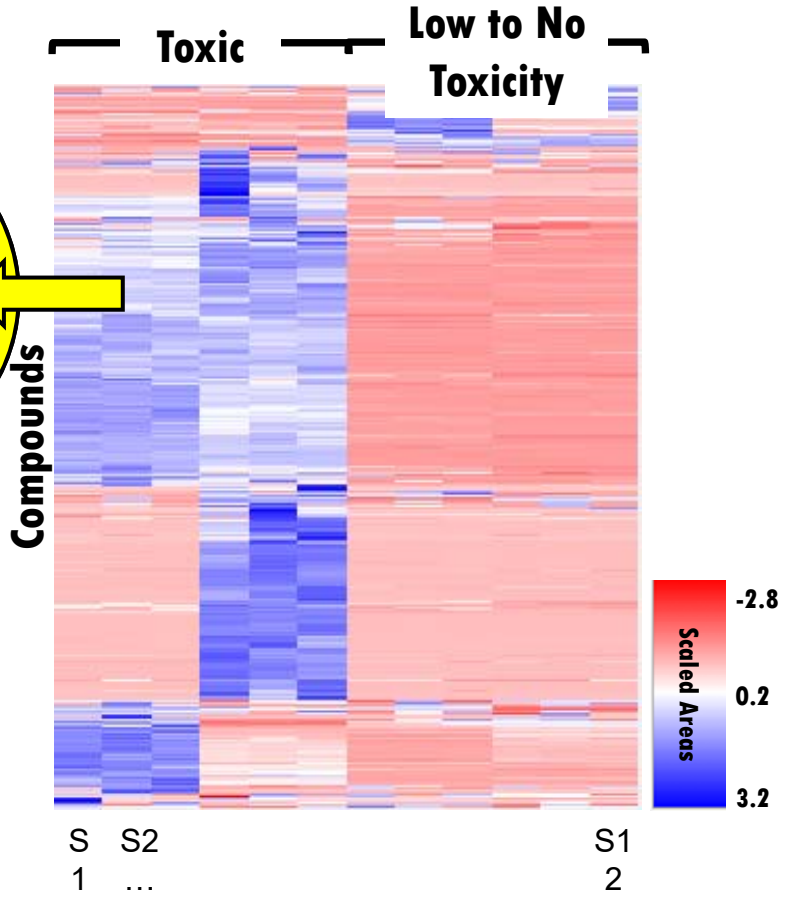
Visualizing Differences Between Groups

S-Plot



Prioritize a subset of compounds to identify

Hierarchical Clustering

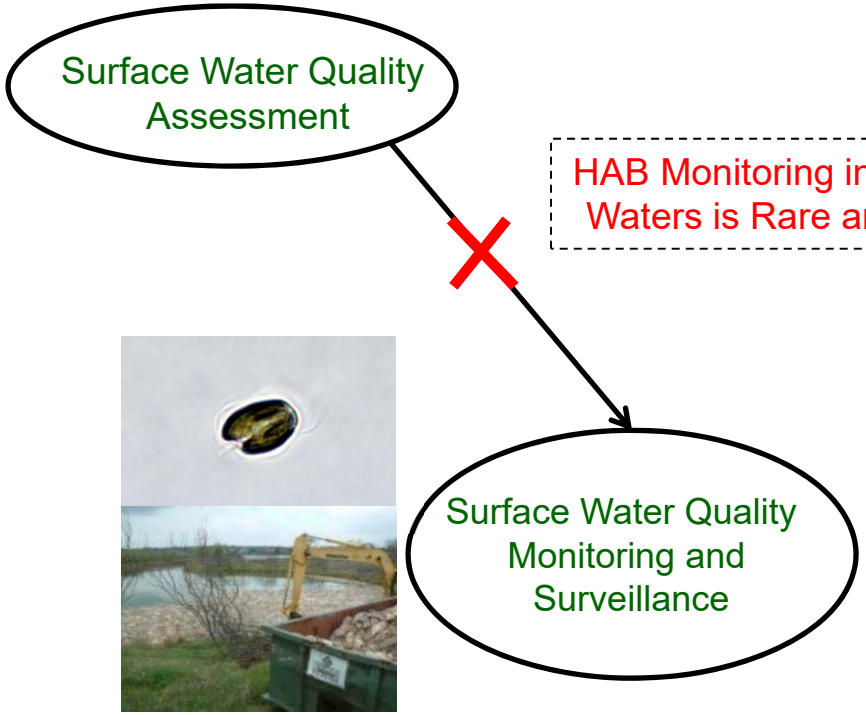


*Each dot represents an unknown compound/peak detected through non-targeted analysis and software algorithms

Taylor et al. 2020. *STOTEN*

HC2T

How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

10 Essential Environmental Public Health Services

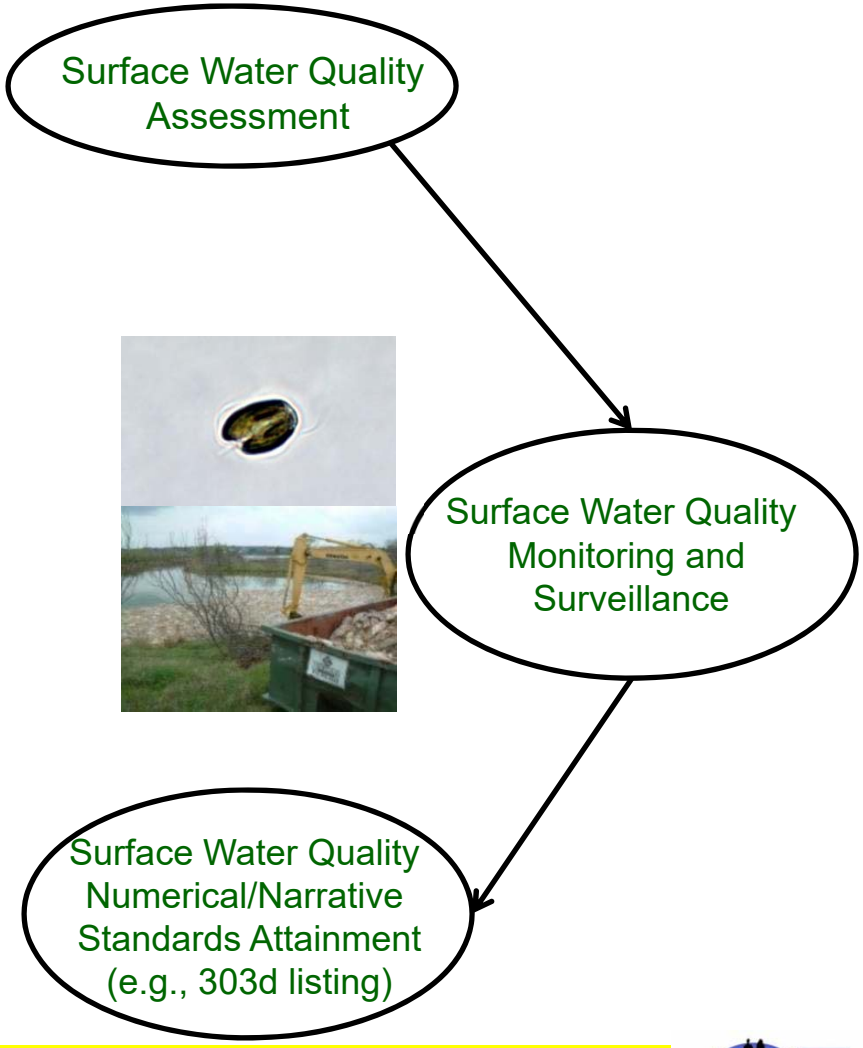
Monitor
Diagnose and investigate
Inform, educate, and empower
Mobilize
Develop policies and plans
Enforce
Link
Assure
Evaluate
Research

www.cdc.gov/nceh/ehs/home/healthservice.htm

OHC²T

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

OHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

So, What *is* Water Quality?

HC²I

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

Water Quality in the USA: Criteria and Standards

I. Section 304(a) of the Clean Water Act in the USA requires the EPA to publish and periodically update ambient water quality criteria (WQC).

Criteria (EPA) — **Recommended** and estimated concentrations of contaminants, based on current scientific information that, if not exceeded, are considered protective for organisms or a defined use of a water body (e.g., fishing, swimming, potable water supply).

II. Water quality standards (WQS) are the foundation of the water quality-based control program mandated by the Clean Water Act (CWA).

Standards (States, Tribes) — **Legal limits** permitted by each state for a specific water body and thought to be sufficient to protect that water body. WQS can also be narrative standards, such as “Free from toxic substances in toxic amounts.”

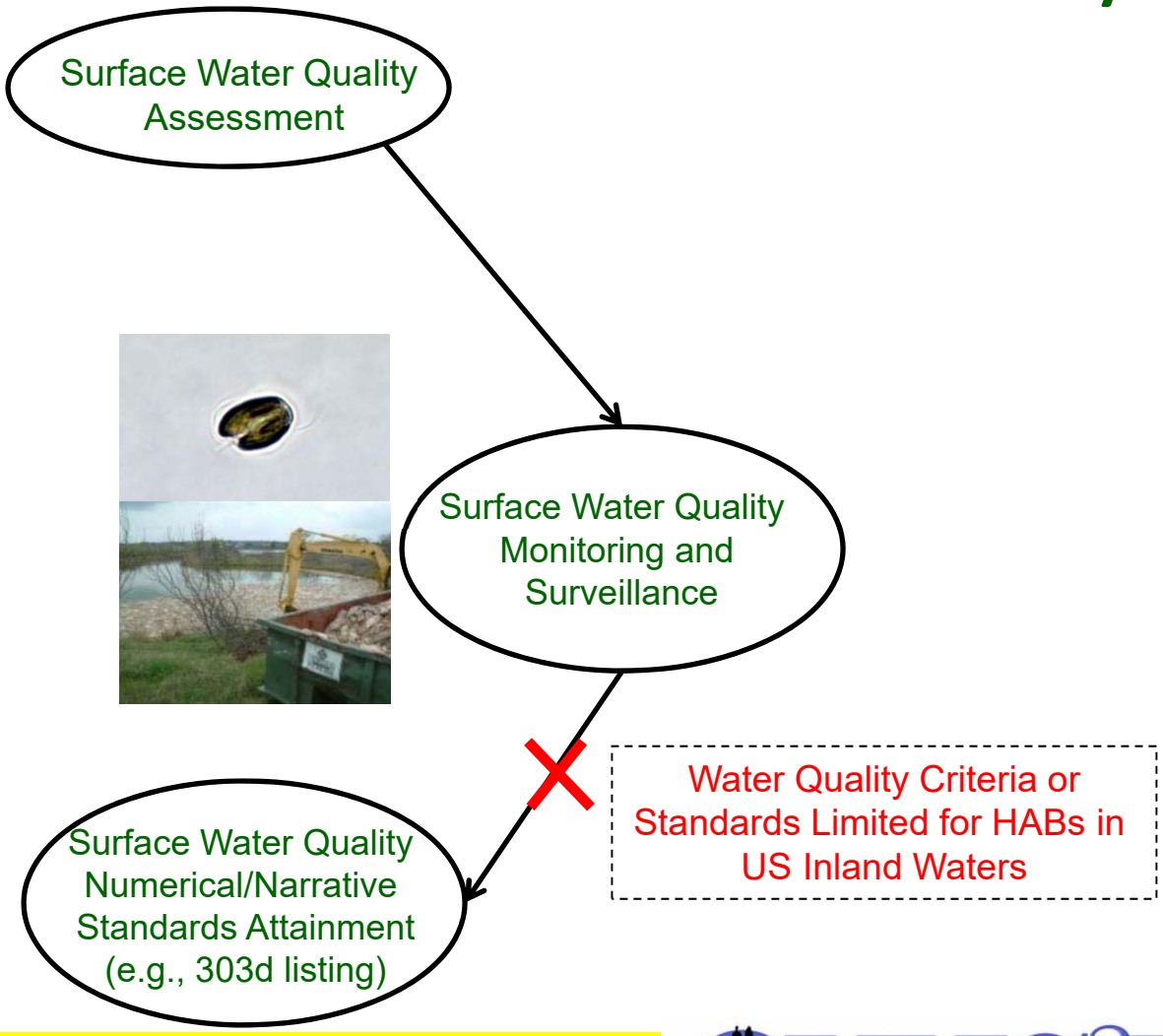
OHC²T

BAYLOR UNIVERSITY
1845
ENVIRONMENTAL SCIENCE

Prymnesium parvum HABs

OHC²I

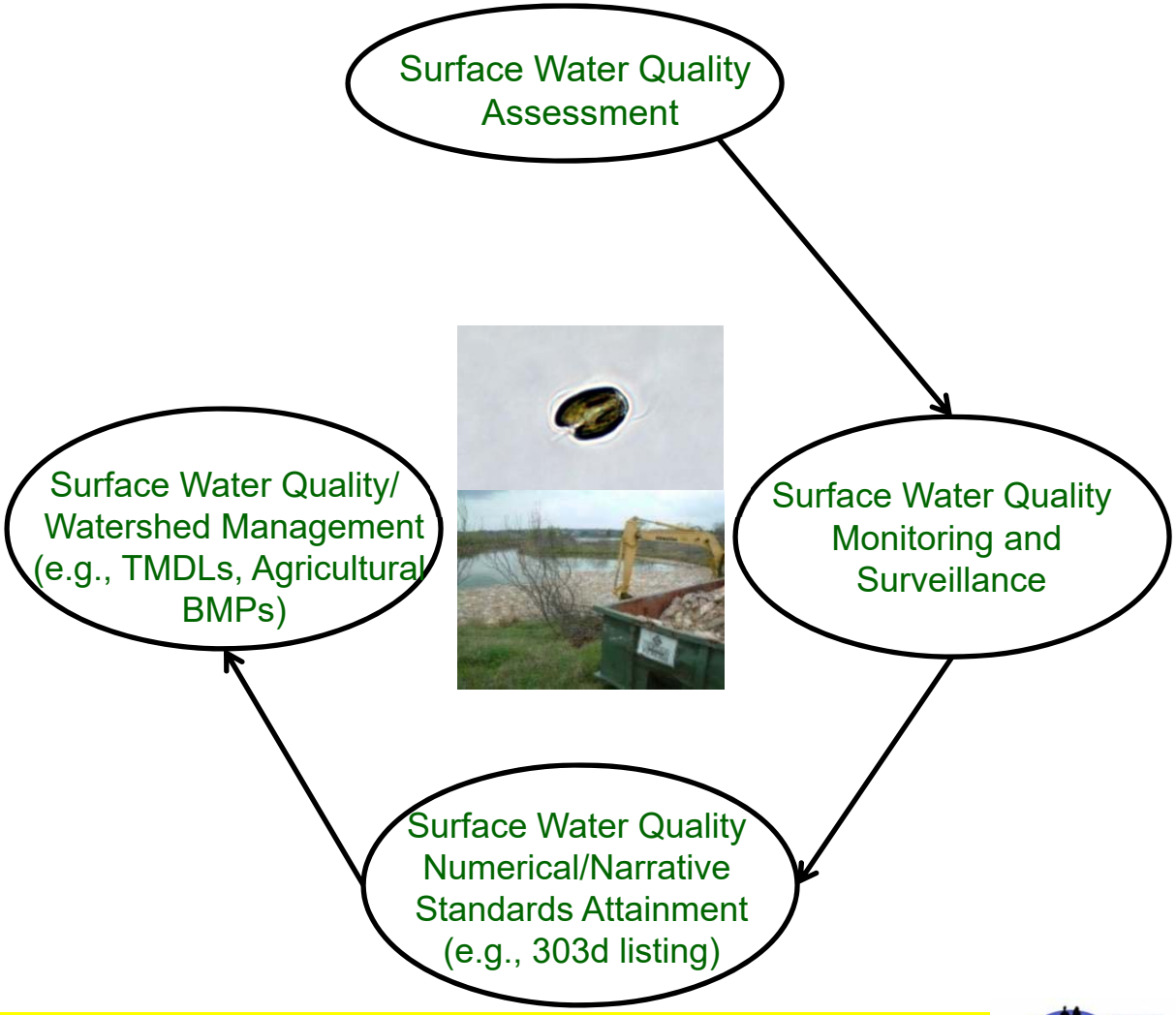
How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

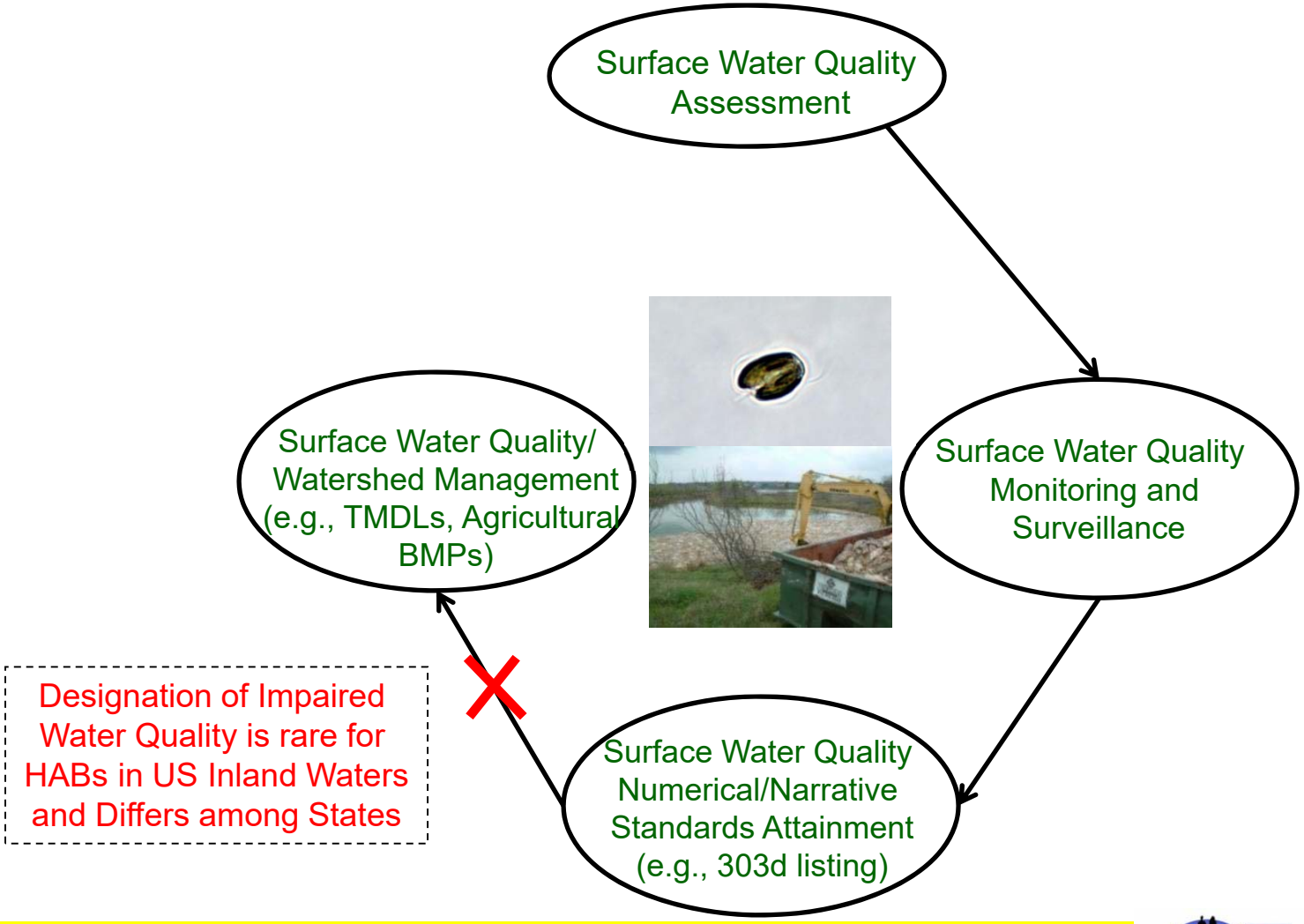
How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

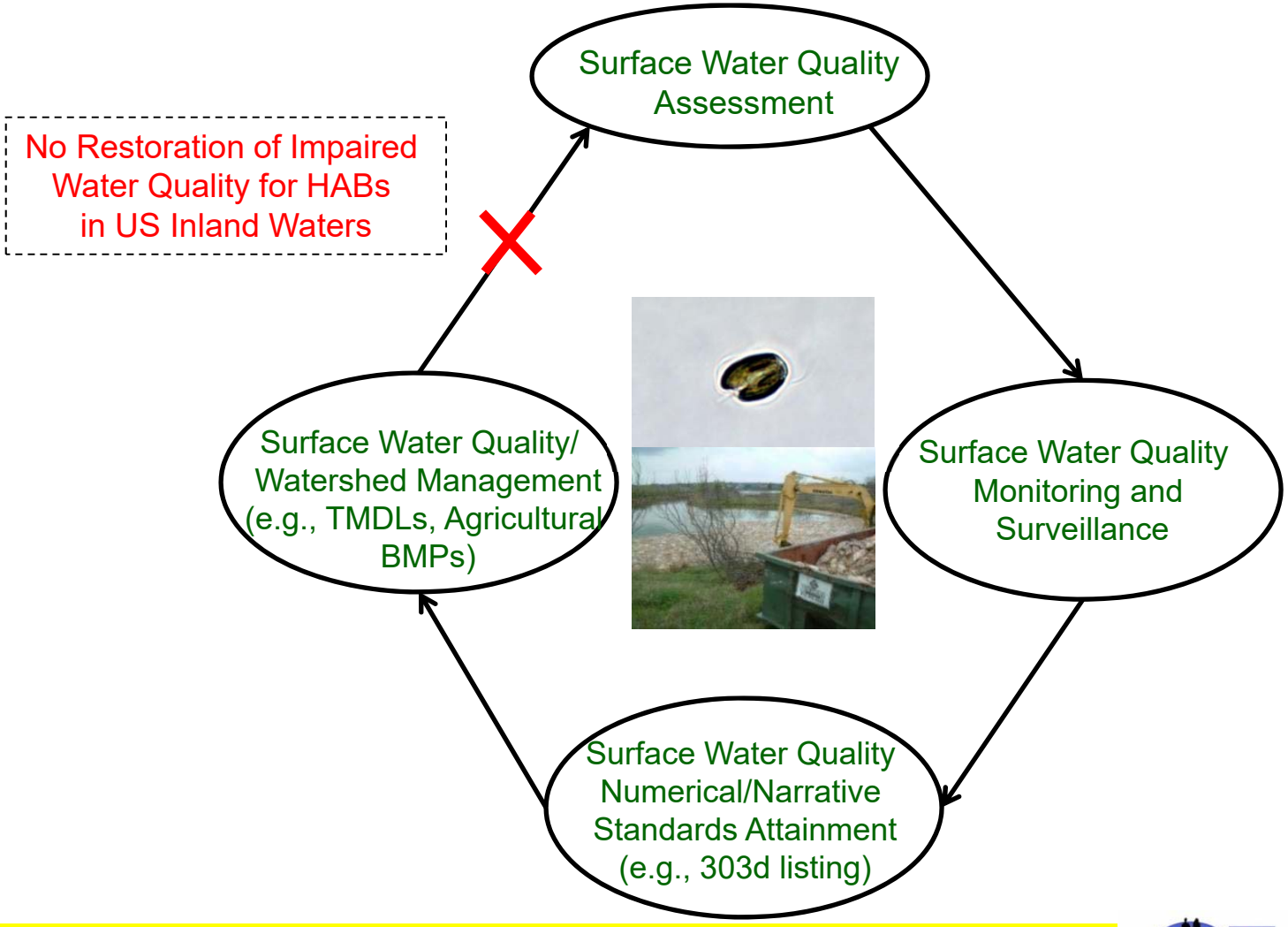
OHC2I

How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

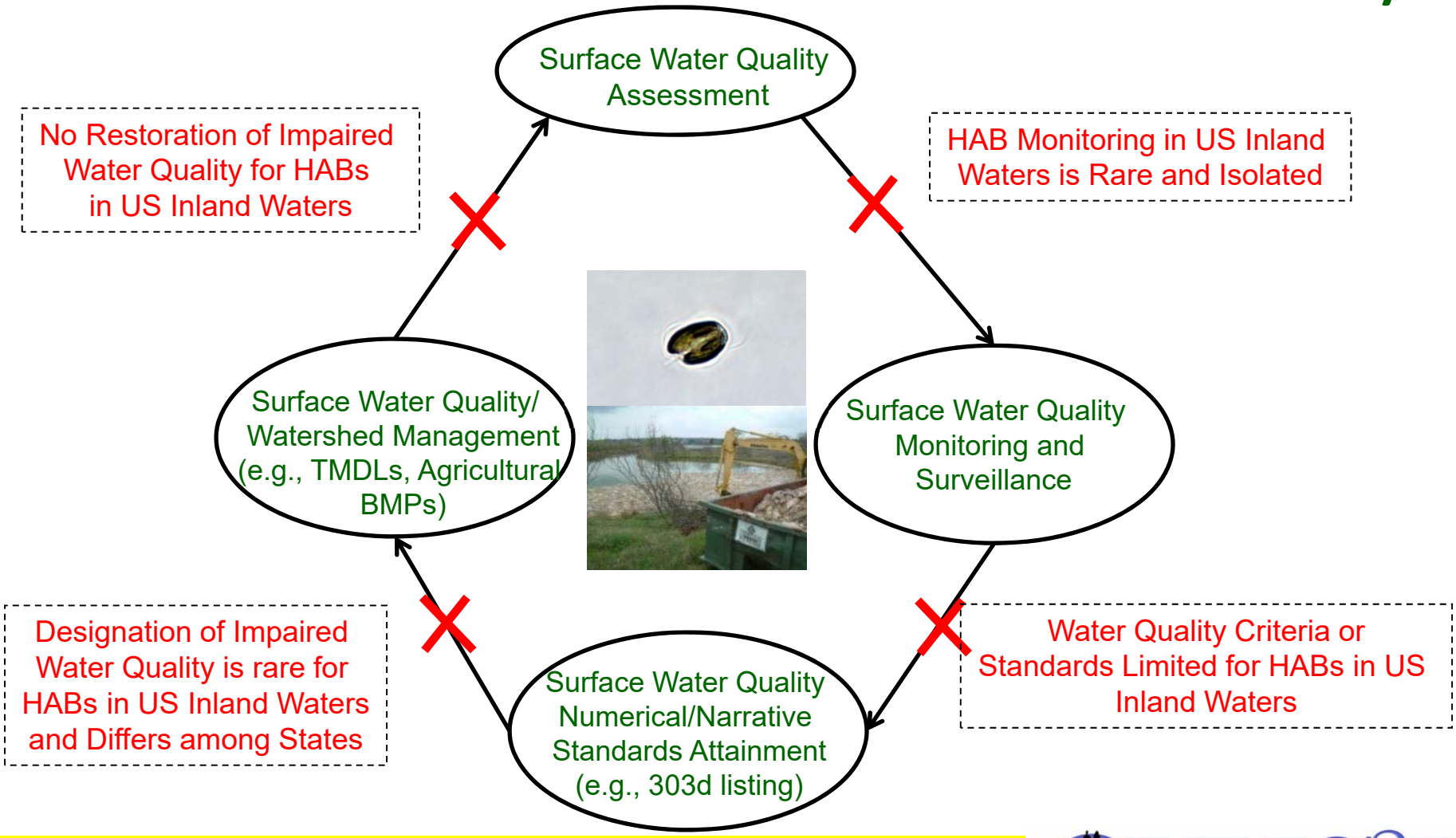
How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

How to Protect and Restore Water Quality?



Brooks et al 2016. *ETC*

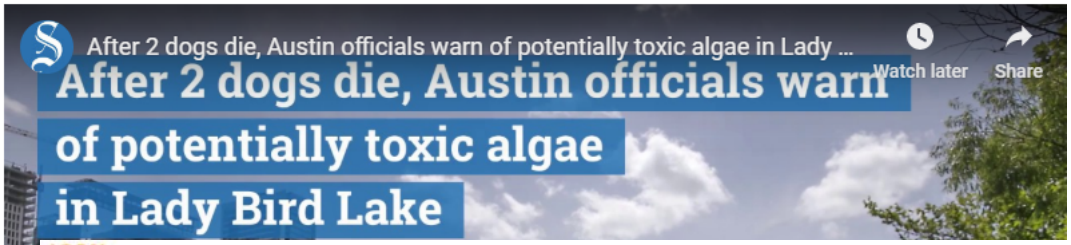
OHC²T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

NO DOGS ALLOWED: Toxic algae risk keeps Lady Bird Lake off-limits

Statesman

KVUE abc



LOCAL

After more reports of dog deaths to algae exposure, expert says nutrients are to blame

Three dogs died recently in Austin after swimming in Lady Bird Lake and more reports of dog deaths are coming in from other states. One expert has a theory.

CBS AUSTIN NEWS WEATHER SPORTS WE ARE AUSTIN CHIME IN

Vet bills for dogs exposed to toxic algae spark awareness for pet insurance

CBS AUSTIN NEWS WEATHER SPORTS WE ARE AUSTIN CHIME IN

Toxic algae blooms that kill dogs are becoming more common, climate change making it worse

FOX 7 Live News Weather Good Day Sports Care Force More

Pet owner warns others after dog dies of algae intoxication

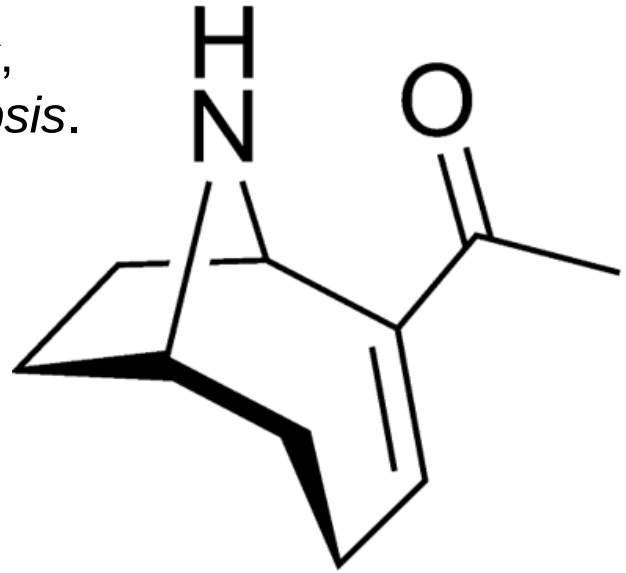
By Casey Claiborne, FOX 7 Austin | Published August 12, 2019 | News | FOX 7 Austin

BAYLOR UNIVERSITY ENVIRONMENTAL SCIENCE

What about Exposure and Aquatic Toxicity of Anatoxin-a?

Physical and Chemical Properties	
Produced by Genera:	*Anabaena, Aphanizomenon, Microcystis, Nostoc, Oscillatoria, Planktothrix*, *Phormidium*, and *Raphidopsis*.
CAS:	64285-06-9
Formula:	$C_{10}H_{15}NO$
Molecular weight:	165.24 g/mol
Log K_{ow}	1.1
Solubility	14 mg/L
pK_a	9.4

Chemical Structure

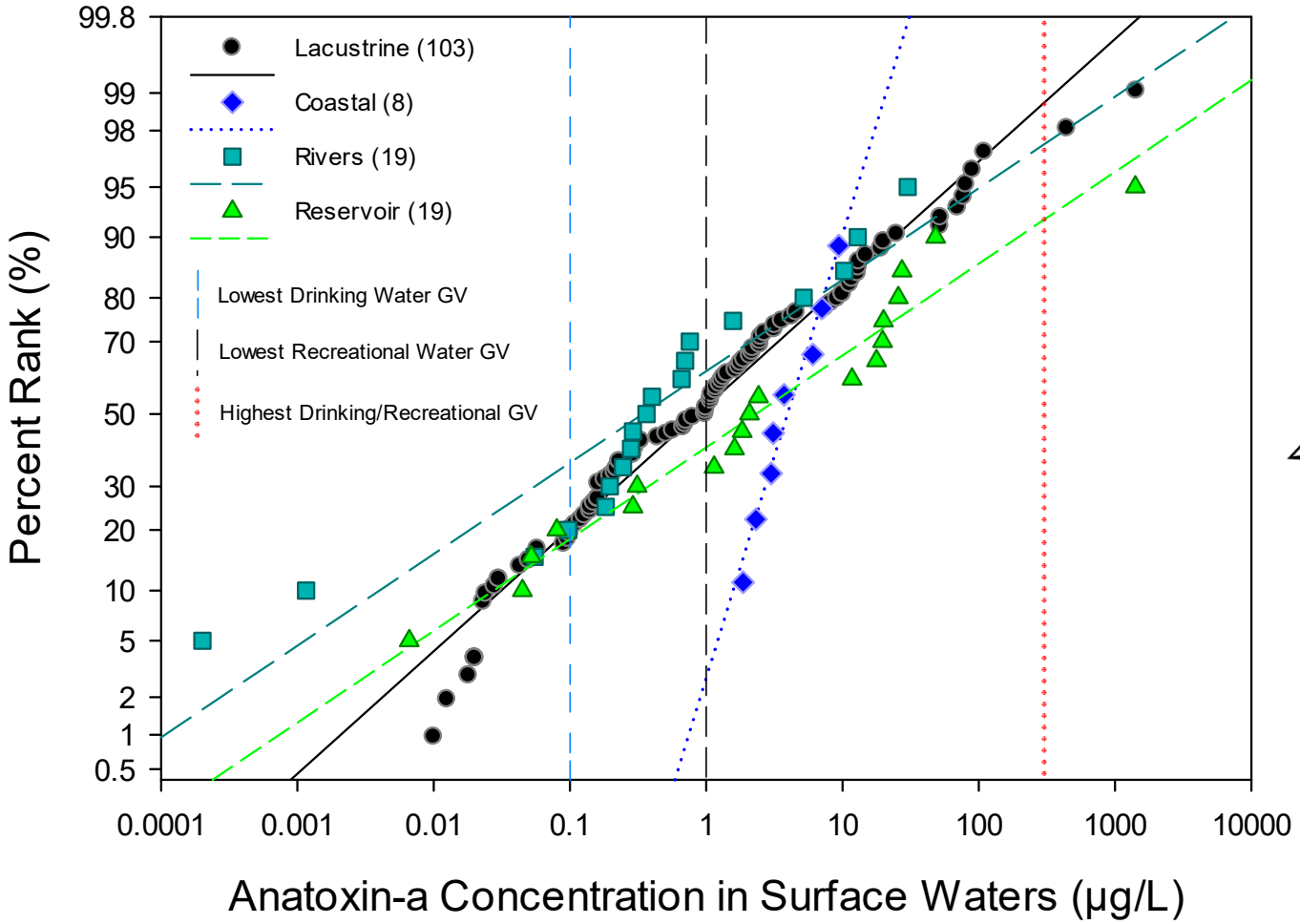


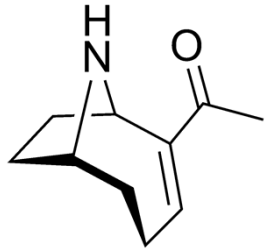
Anatoxin-a

OHC2I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

What are Environmentally Realistic Exposure Levels?

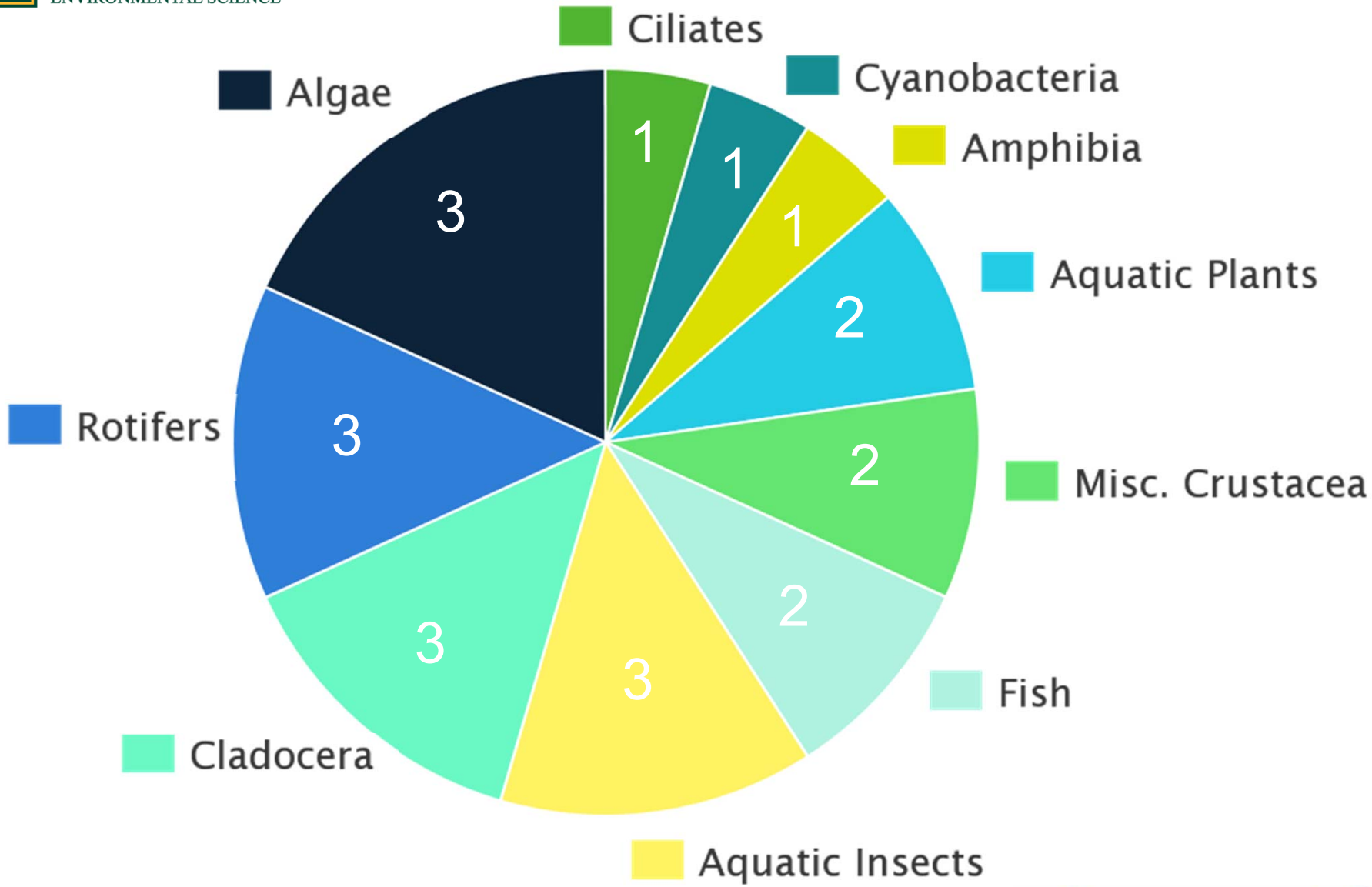




Lovin and Brooks. 2020. *Marine and Freshwater Research*

HC2T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE



Lovin and Brooks. 2020. *Marine and Freshwater Research*

OHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

What about the Aquatic Toxicology of Anatoxin-a?

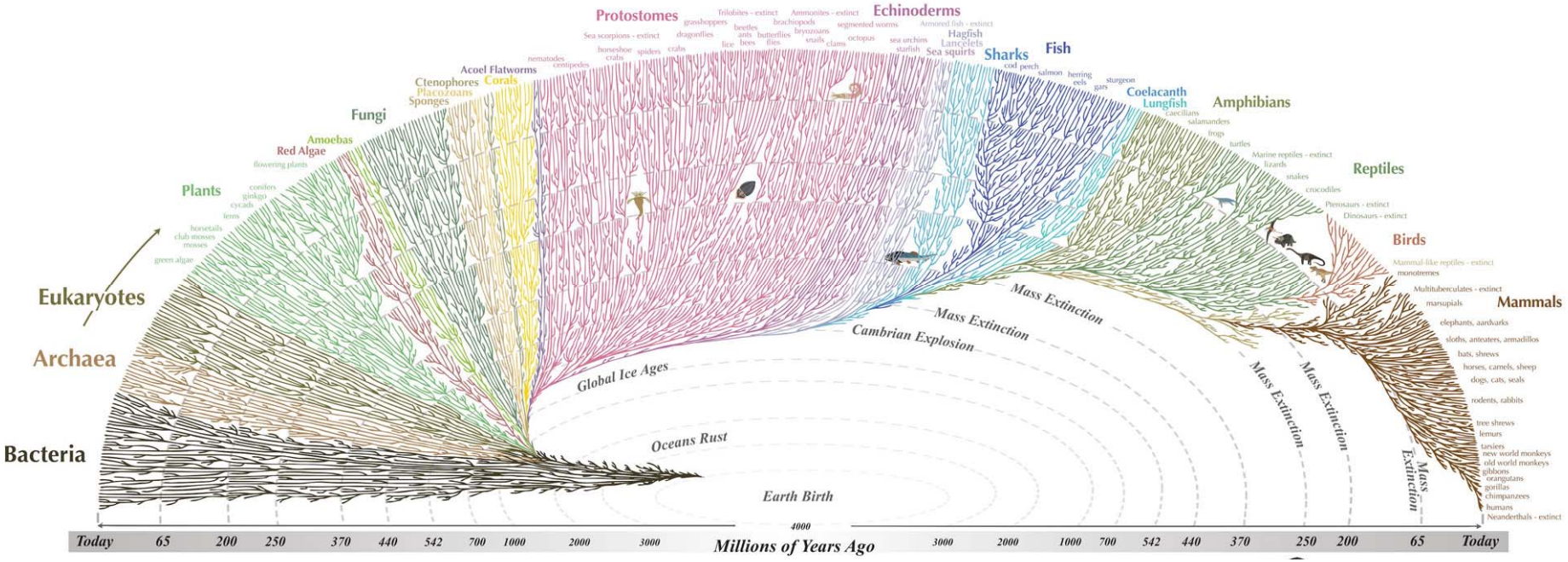
- Only 28.3% of anatoxin-a toxicity studies analytically verified treatment levels.
- Purity of the compound was given in only 5.31% of studies.
- 47.8% of studies used a non-specified enantiomeric mix of anatoxin-a, 23.0% used a racemic mixture, and only 29.2% examined (+) anatoxin-a.

Lovin and Brooks. 2020. *Marine and Freshwater Research*

OHC²T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Comparative Biology…

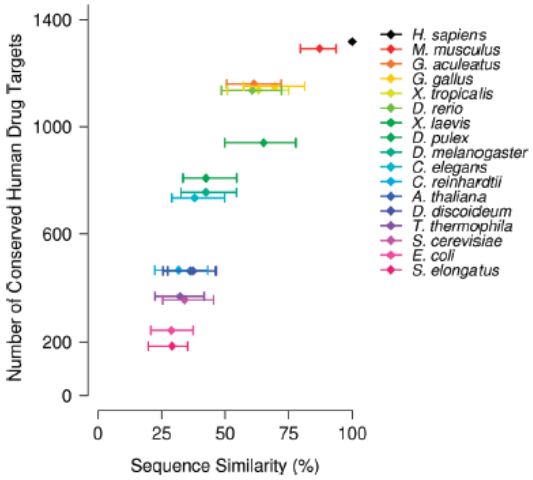


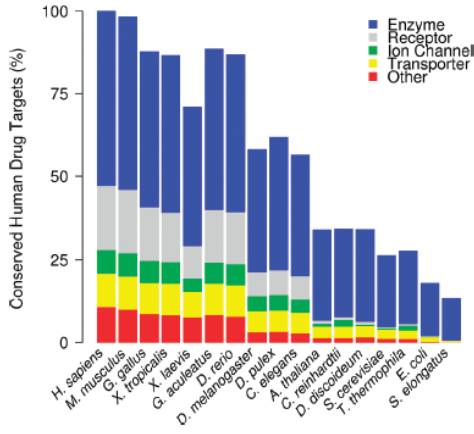
www.evogeneao.com

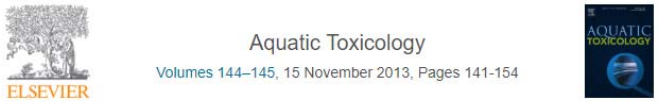
OHC2I

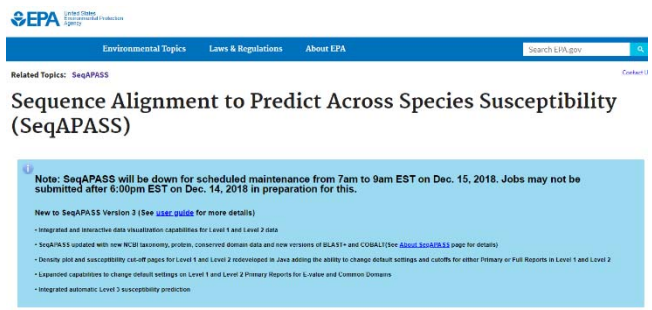
BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

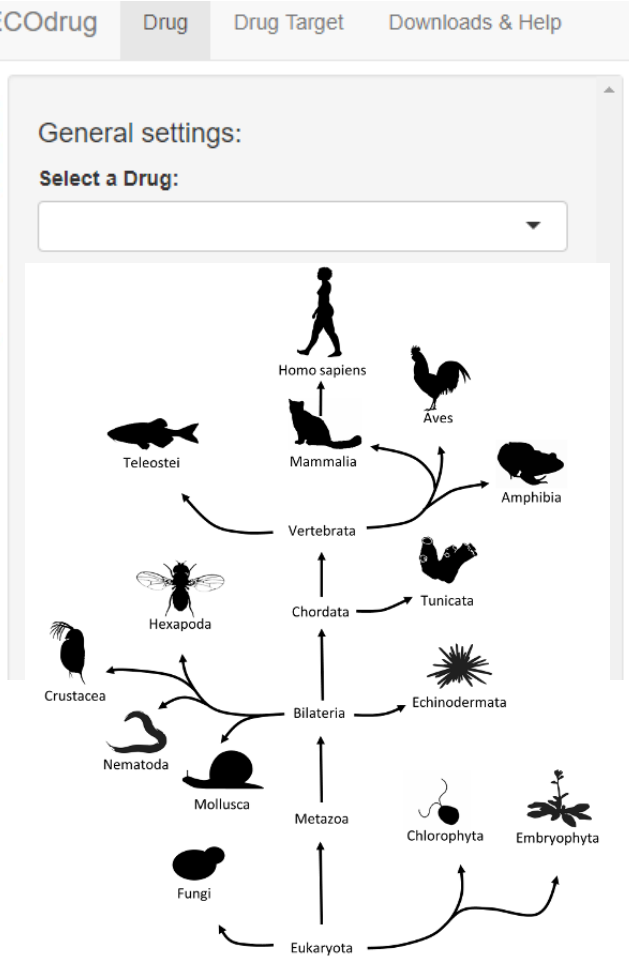
Comparative Pharmacology & Toxicology









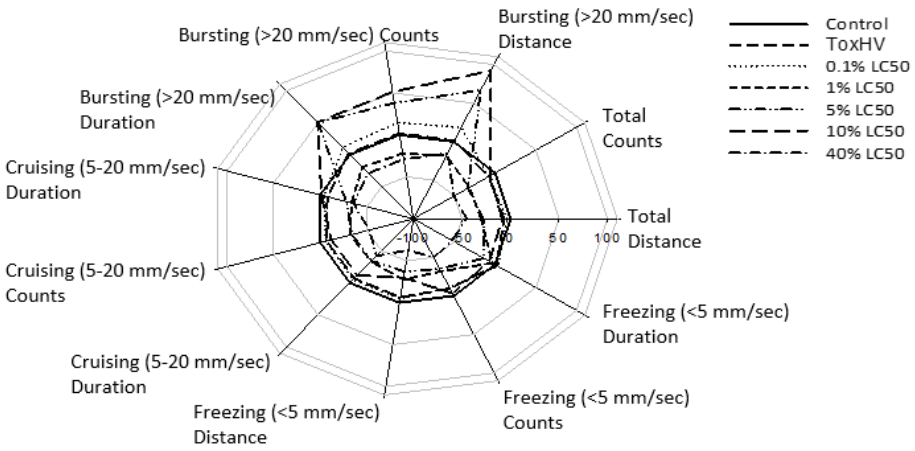


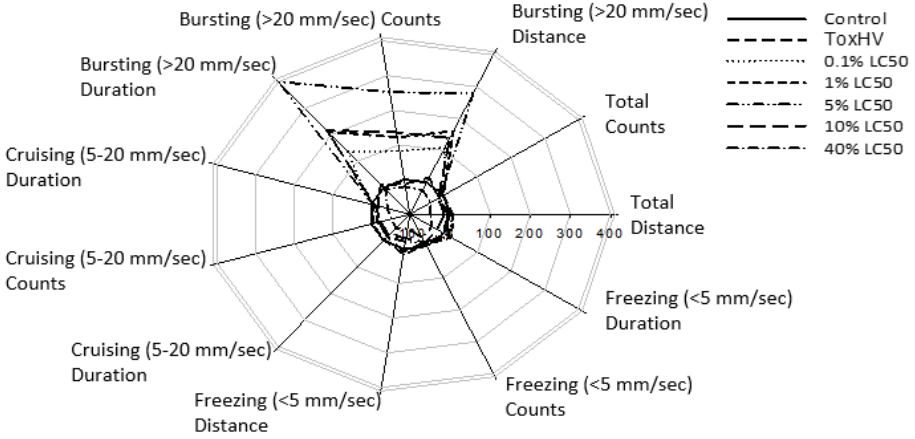
Gunnarsson et al 2008. *ES&T;* Berninger and Brooks 2010. *Toxicol Lett;* LaLone et al. 2014. *Aquat Toxicol;* LaLone et al. 2014. *Phil Trans R Soc B*; LaLone et al. 2016 *Toxicol Sci:* seqapass.epa.gov/seqapass/ Verbruggen et al. 2017. *Nucleic Acids Research*; Ecodrug.org

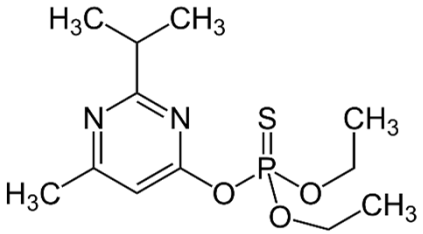
HC2T

Comparative Behavioral Fingerprints for Chemical MOAs

Diazinon: Acetylcholinesterase inhibition

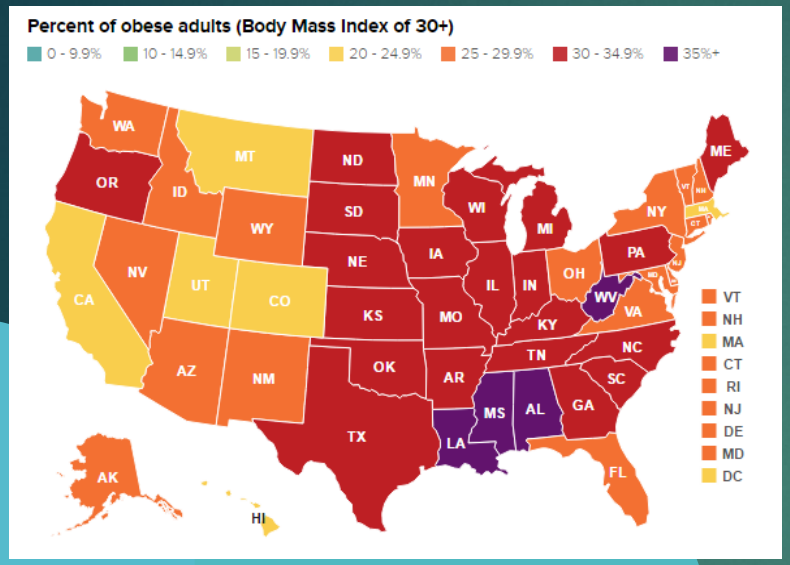


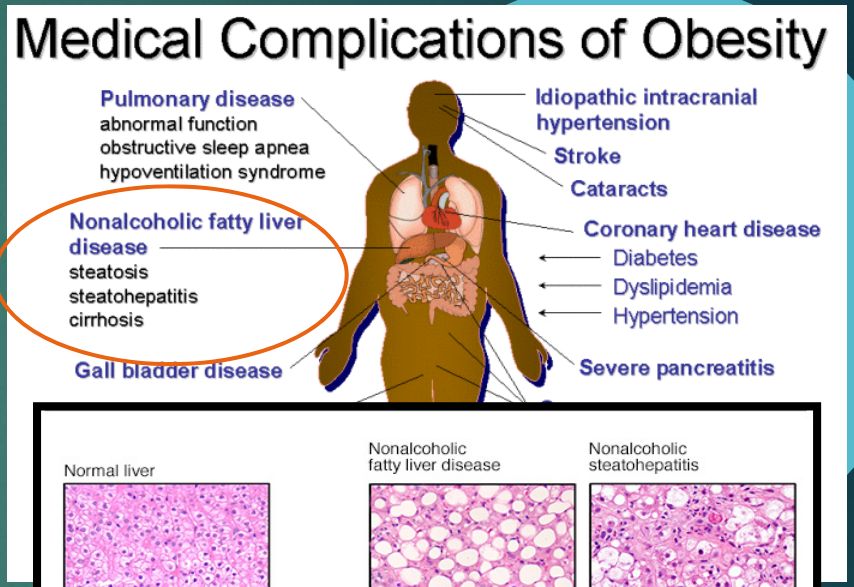


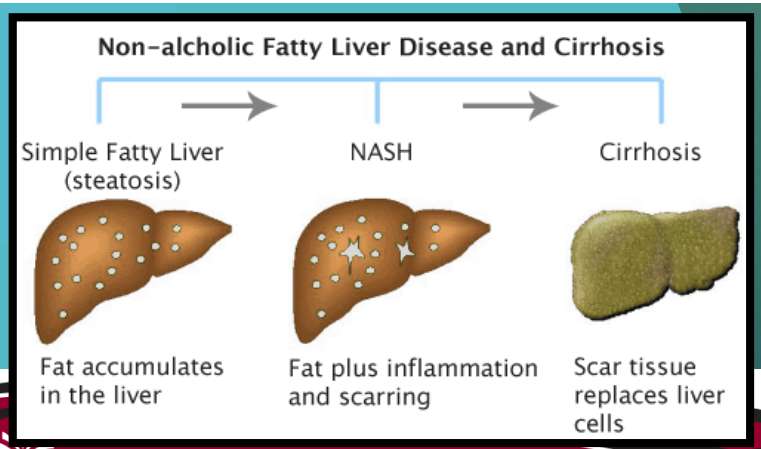


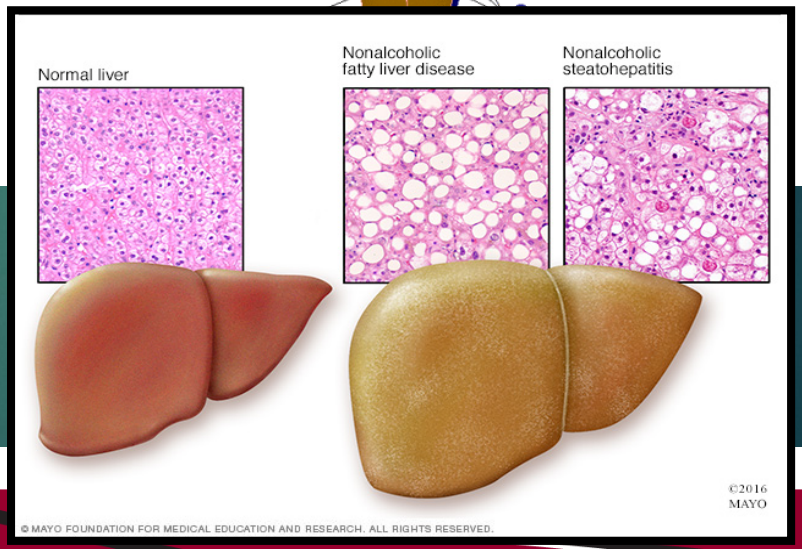
Steele et al 2018. *STOTEN*

HABs, Obesity and NAFLD: They kill silently……









UNIVERSITY OF SOUTH CAROLINA

Exposure in existing disease: Microcystin exposure and Liver Disease updates

american physiological society

AMERICAN JOURNAL of PHYSIOLOGY
Gastrointestinal and Liver Physiology®

JOURNAL HOME | ISSUES | COLLECTIONS | INFORMATION | ABOUT | SUBMIT | SUBSCRIBE | Search this journal

Research Article | Liver and Biliary Tract Physiology/Pathophysiology

Exogenous PP2A inhibitor exacerbates the progression of nonalcoholic fatty liver disease via NOX2-dependent activation of miR21

Muayad Albadrani, Ratanesh K. Seth, Sutapa Sarkar, Diana Kimono, Ayan Mondal, ... Show all Authors

24 SEP 2019 // https://doi.org/10.1152/ajpgi.00061.2019

Figures | References | Related | Information

Volume 317, Issue 4
October 2019

nature > scientific reports > articles > article

a natureresearch journal

MENU

SCIENTIFIC REPORTS

Search | E-alert | Submit | Login

Article | Open Access | Published: 19 June 2019

Environmental microcystin targets the microbiome and increases the risk of intestinal inflammatory pathology via NOX2 in underlying murine model of Nonalcoholic Fatty Liver Disease

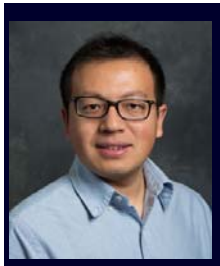
Sutapa Sarkar, Diana Kimono, Muayad Albadrani, Ratanesh K. Seth, Philip Busbee, Hasan

Download PDF

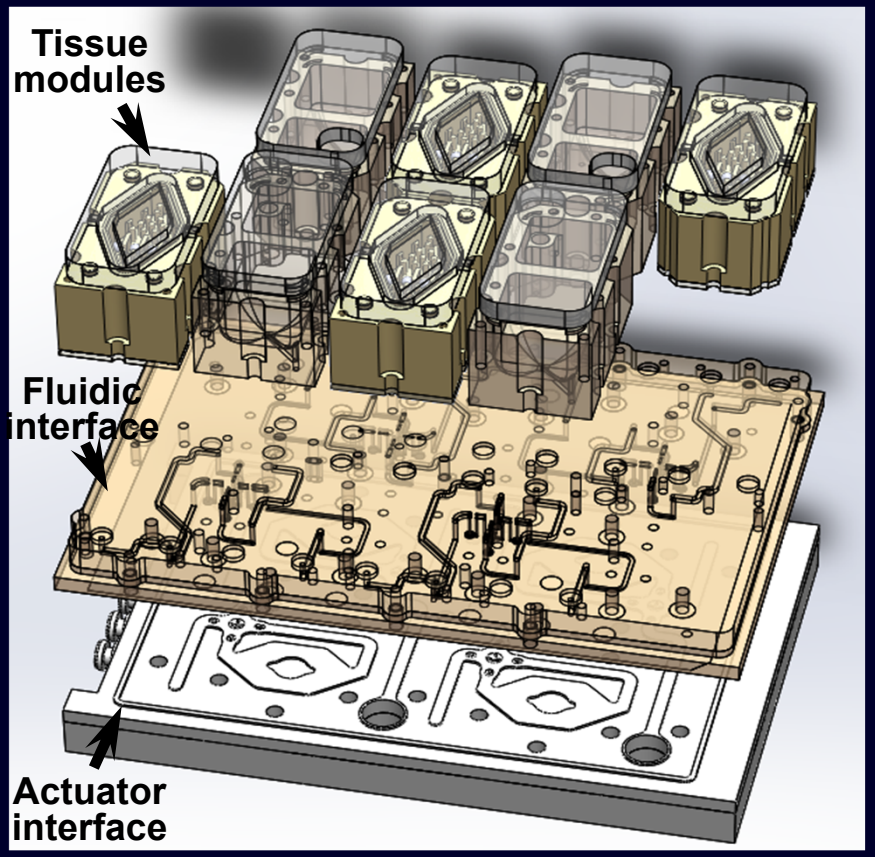
Sections | Figures | References

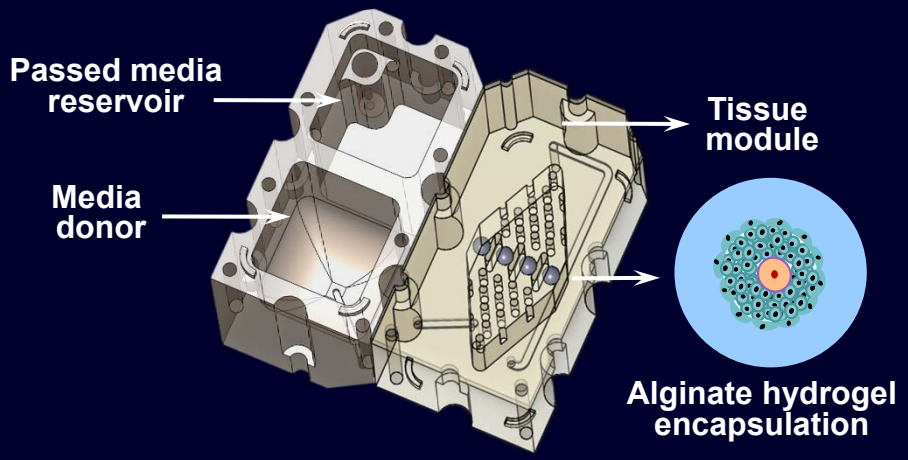
Abstract
Introduction
Materials and Methods
Results
Discussion

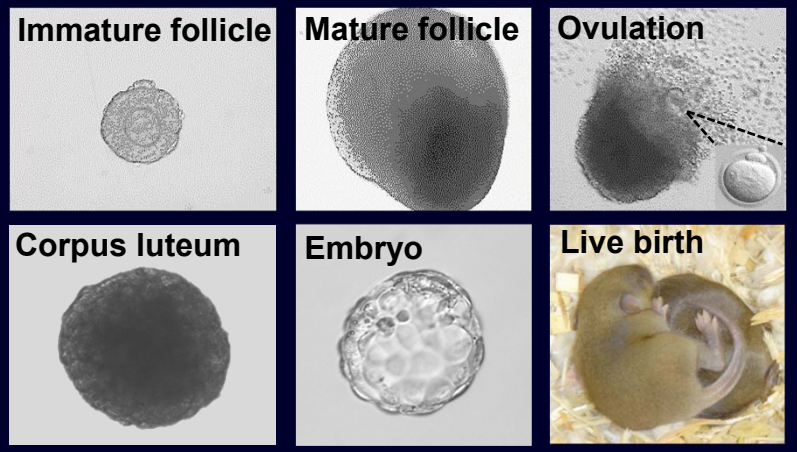
UNIVERSITY OF SOUTH CAROLINA



Ovary-on-a-chip based on microfluidic technology



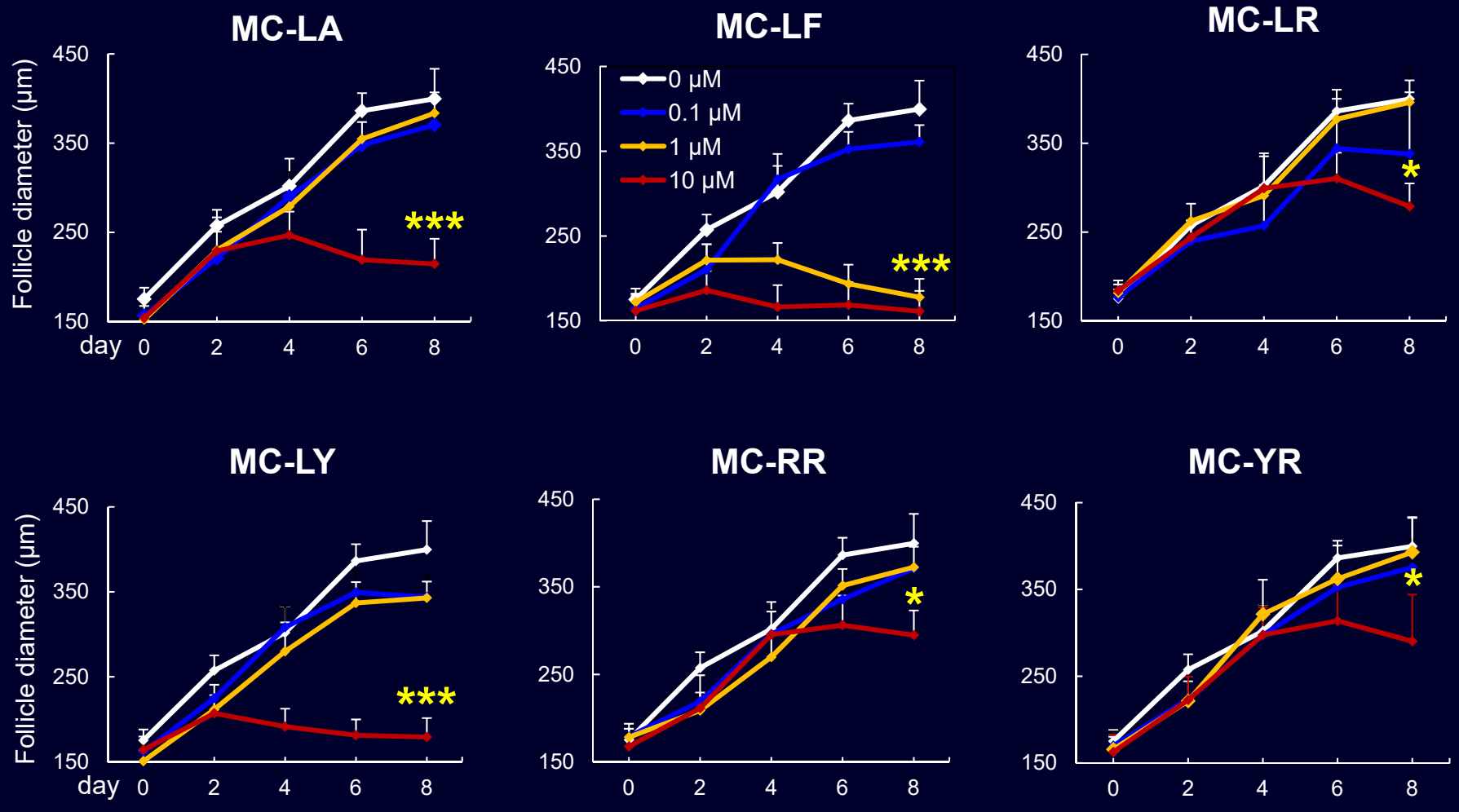




- **Pneumatic actuation technology and programmable fluid flow;**
- **Transport of fresh media and elimination of waste;**
- **Dynamic fluid flow to mimic physiological environment *in vivo*.**

Xiao et al, *Reproduction*, 2015
Xiao et al, *Scientific Reports*, 2016
Xiao et al, *Nature Communications*, 2017
Wang et al, *Toxicological Sciences*, 2018

Ovotoxicity of MCs: Follicle survival & development



Human serum MC-LR: 0.1 – 70 nM

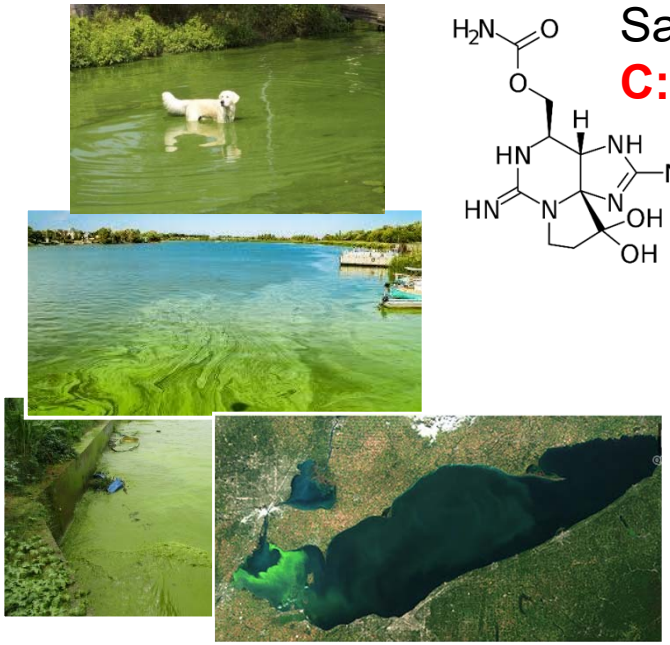
* $p<0.05$; * $p<0.01$; * * * $p<0.001$

Wang *et al*, *Reproductive Toxicology*, 2020

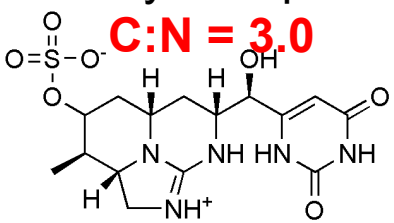
BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Forecasting Toxins Exposure?

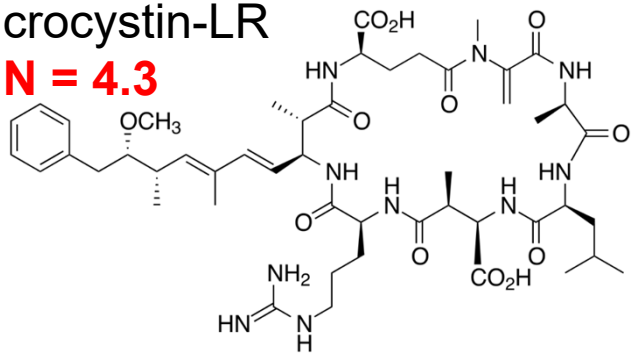
Toxin-producing Cyanobacteria Require High N



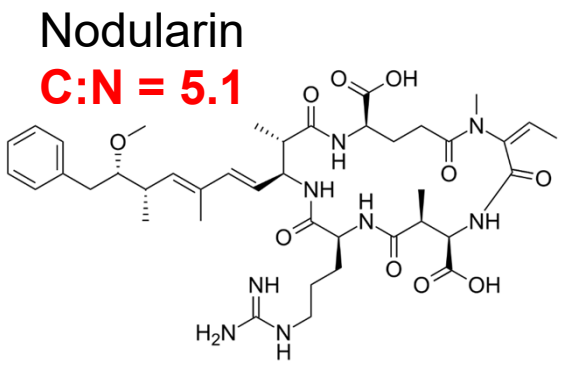
Saxitoxin
C:N = 1.5



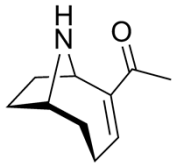
Cylidrospermopsin
C:N = 3.0



Microcystin-LR
C:N = 4.3



Nodularin
C:N = 5.1

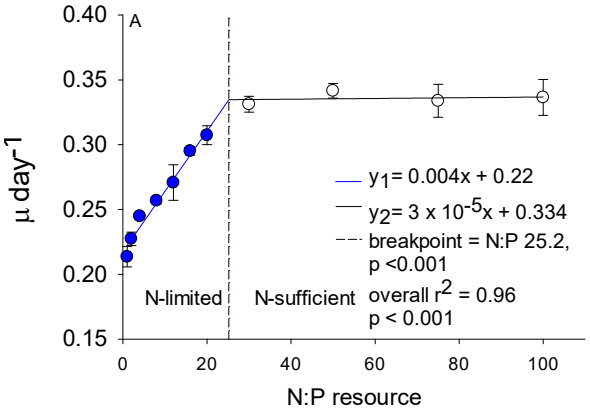


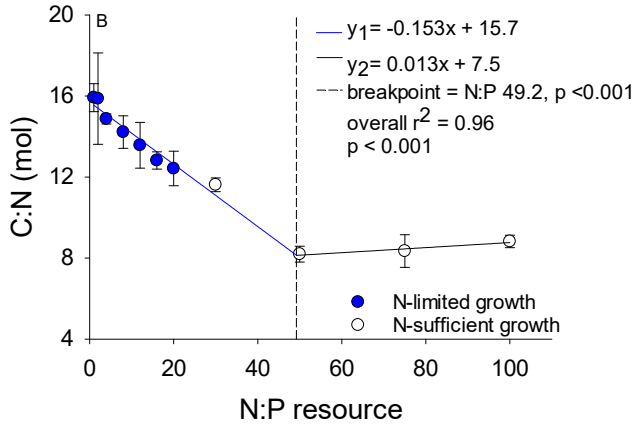
Anatoxin-a
C:N = 10

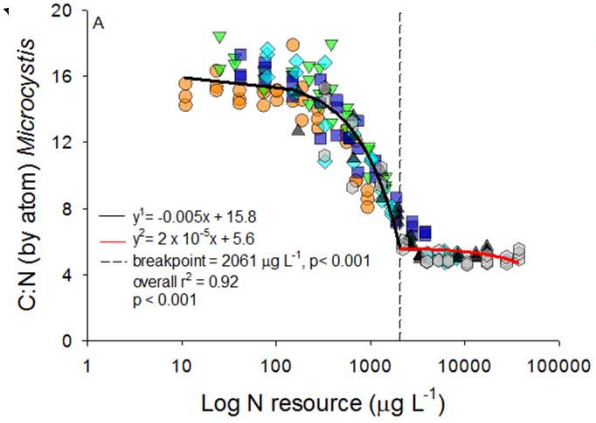
Redfield 106:16:1
C:N = 6.6

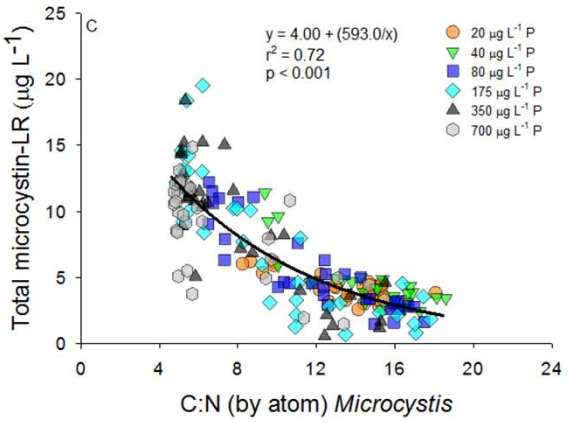
Forecasting Toxins Exposure?

Biological Stoichiometry Regulates Microcystin-LR Production in *Microcystis aeruginosa* (UTEX 2385)

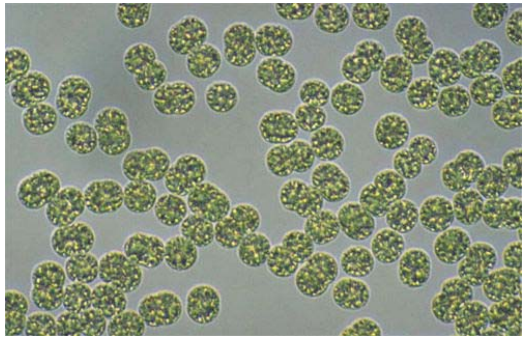








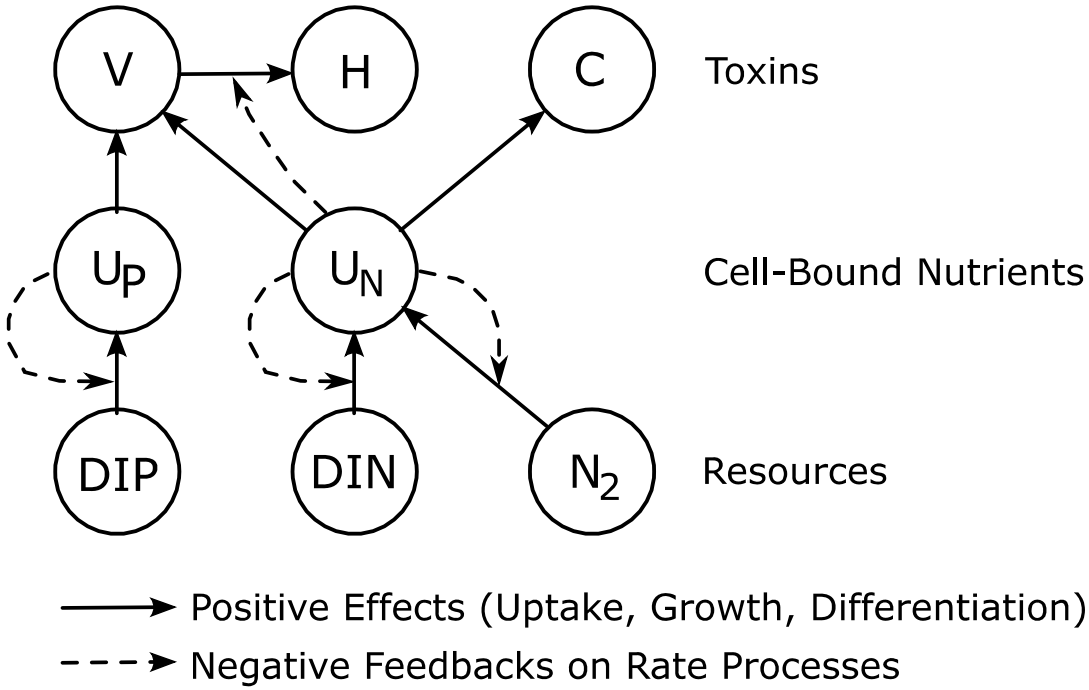
High resource N:P ratios allowed *M. aeruginosa* to decouple microcystin-LR production from growth and generate more toxin than would have been predicted by growth alone

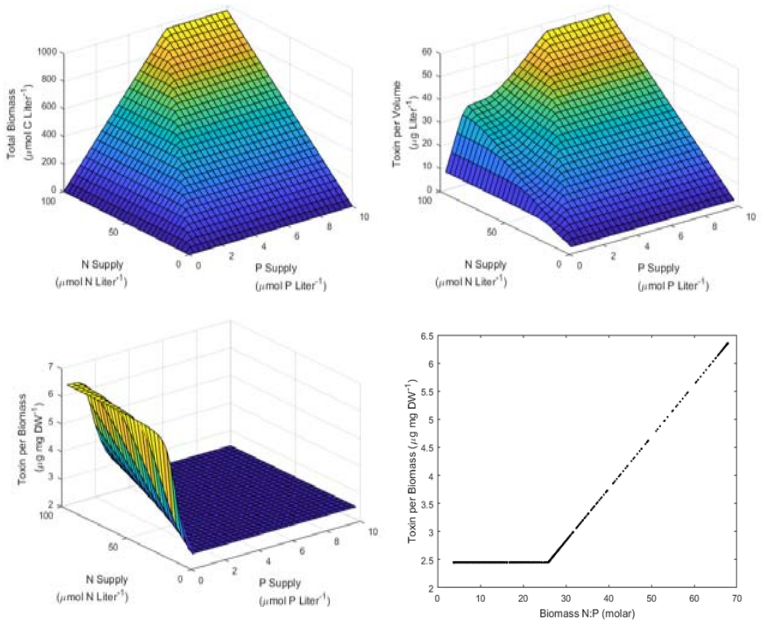


Wagner et al. 2019. *Toxins*

Forecasting Toxins Exposure?

A Stoichiometric Model for CyanoToxins





Grover, Scott, Roelke, Brooks. 2020. *Marine and Freshwater Research*

BAYLOR UNIVERSITY
1845
ENVIRONMENTAL SCIENCE

Are Harmful Algal Blooms Becoming the Greatest Threat to Water Quality?

In come places, in some case and at some times, they already are…

Toxic Algae Closes Utah Lake, Sickens 100

by ASSOCIATED PRESS

A huge toxic algae bloom in Utah has closed one of the largest freshwater lakes west of the Mississippi River, sickening more than 100 people and leaving farmers scrambling for clean water during some of the hottest days of the year.

The bacteria commonly known as blue-green algae has spread rapidly to cover almost all of 150-square-mile Utah Lake, turning the water bright, anti-freeze green with a pea soup texture and leaving scummy foam along the shore.

Bryce Larsen, environmental health director at the Utah County Health Department, looks at dried algae on the shore of Utah Lake on July 20 near Spanish Fork, Utah. Rick Bowmer / AP

"It smells like something is rotting," said Jason Garrett, water quality director for the Utah County Health Department. "We don't have an idea of how long this event will last."

Toxic algae is a problem around the country. An enormous bloom in Florida is now fouling beaches on the Atlantic coast, and a 2014 outbreak at Lake Erie left more than 400,000

Toledo bearing full brunt of Lake Erie algae bloom

People got sick at Pyramid Lake before the state reported toxic algae bloom. Could it have been avoided?

Children play near the shore at Pyramid Lake in Los Angeles County on Friday. Multiple people have reported getting sick after being exposed to a toxic algal bloom there. Stephanie K. Baer — Southern California News Group

RELATED

By Stephanie K. Baer, *The San Gabriel Valley Tribune*

Health & Fitness

Contra Costa County Health: Don't Swim in Discovery Bay

They're testing for a potentially harmful algae. Keep pets out of the water, too.

By California Patch (Patch Staff) - July 20, 2016 2:12 pm ET

This Landsat 8 image captures the extensive algal bloom in Florida's Lake Okeechobee. NASA Earth Observatory

Everglades Foundation launches $10 million prize to clean up toxic algae blooms

By Dianne Lugo | Jul. 21, 2016, 1:00 PM

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Are Harmful Algal Blooms Becoming the Greatest Threat to Water Quality?

In come places, in some case and at some times, they already are…

- Future needs
  - Predictive models
  - Sensors for blooms and toxins
  - Analytical standards
  - Criteria and standards
  - Adaptive management and remedial interventions
  - Engagement across disciplines

HHC²T

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

NIEHS: 1P01ES028942

OHC²I

BAYLOR UNIVERSITY
ENVIRONMENTAL SCIENCE

Global Horizon Scanning

Environmental Toxicology and Chemistry—Volume 37, Number 9—pp. 2281–2295, 2018
Received: 9 April 2018 | Revised: 28 April 2018 | Accepted: 11 June 2018

Critical Review

Toward Sustainable Environmental Quality: Priority Research Questions for Europe

Paul J. Van den Brink,[a,b] Alistair B.A. Boxall,[c,*] Lorraine Maltby,[d] Bryan W. Brooks,[e] Murray A. Rudd,[f] Thomas Backhaus,[g] David Spurgeon,[h] Violaine Verougstraete,[i] Charmaine Ajao,[j] Gerald T. Ankley,[k] Sabine E. Apitz,[l] Kathryn Arnold,[c] Tomas Brodin,[m] Miguel Cañedo-Argüelles,[n,o] Jennifer Chapman,[c] Jone Corrales,[e] Marie-Agnès Coutellec,[p] Teresa F. Fernandes,[q] Jerker Fick,[r] Alex T. Ford,[s] Gemma Giménez Papiol,[t] Ksenia J. Groh,[u] Thomas H. Hutchinson,[v] Hank Kruger,[w] Jussi V.K. Kukkonen,[x] Stefania Loutseti,[y] Stuart Marshall,[z] Derek Muir,[aa] Manuel E. Ortiz-Santaliestra,[ab] Kai B. Paul,[ac] Andreu Rico,[ad] Ismael Rodea-Palomares,[ae] Jörg Römbke,[af] Tomas Rydberg,[ag] Helmut Segner,[ah] Mathijs Smit,[ai] Cornelis A.M. van Gestel,[aj] Marco Vighi,[ad] Inge Werner,[ak] Elke I. Zimmer,[al] and Joke van Wensem[am]

Environmental Toxicology and Chemistry—Volume 38, Number 8—pp. 1606–1624, 2019
Received: 13 February 2019 | Revised: 19 March 2019 | Accepted: 16 May 2019

Critical Review

Toward Sustainable Environmental Quality: Priority Research Questions for North America

Anne Fairbrother,[a] Derek Muir,[b] Keith R. Solomon,[c] Gerald T. Ankley,[d] Murray A. Rudd,[e] Alistair B.A. Boxall,[f] Jennifer N. Apell,[g] Kevin L. Armbrust,[h] Bonnie J. Blalock,[i] Sarah R. Bowman,[j] Linda M. Campbell,[k] George P. Cobb,[l] Kristin A. Connors,[m] David A. Dreier,[n] Marlene S. Evans,[b] Carol J. Henry,[o] Robert A. Hoke,[p] Magali Houde,[b] Stephen J. Klaine,[q,1] Rebecca D. Klaper,[r] Sigrun A. Kullik,[s] Roman P. Lanno,[t] Charles Meyer,[u] Mary Ann Ottinger,[v] Elias Oziolor,[l] Elijah J. Petersen,[w] Helen C. Poynton,[i] Pamela J. Rice,[x] Gabriela Rodriguez-Fuentes,[y] Alan Samel,[z] Joseph R. Shaw,[aa] Jeffery A. Steevens,[bb] Tim A. Verslycke,[cc] Doris E. Vidal-Dorsch,[dd] Scott M. Weir,[ee] Peter Wilson,[ff] and Bryan W. Brooks[m,gg,*]

Integrated Environmental Assessment and Management — Volume 14, Number 3—pp. 344–357
Received: 25 April 2017 | Returned for Revision: 14 August 2017 | Accepted: 14 November 2017

Environmental Policy & Regulation

Toward Sustainable Environmental Quality: Identifying Priority Research Questions for Latin America

Tatiana Heid Furley,† Julie Brodeur,‡ Helena C Silva de Assis,§ Pedro Carriquiriborde,‖ Katia R Chagas,† Jone Corrales,# Marina Denadai,†† Julio Fuchs,‡‡ Renata Mascarenhas,§§ Karina SB Miglioranza,‖ Diana Margarita Miguez Caramés,‖‖## José Maria Navas,††† Dayanthi Nugegoda,‡‡‡ Estela Planes,§§§ Ignacio Alejandro Rodriguez-Jorquera,‖‖‖ Martha Orozco-Medina,### Alistair BA Boxall,†††† Murray A Rudd,‡‡‡‡ and Bryan W Brooks#*

Integrated Environmental Assessment and Management — Volume 15, Number 6—pp. 917–935
Received: 7 March 2019 | Returned for Revision: 26 April 2019 | Accepted: 24 June 2019

Workshop Synthesis

Towards Sustainable Environmental Quality: Priority Research Questions for the Australasian Region of Oceania

Sally Gaw,† Andrew Harford,‡ Vincent Pettigrove,§ Graham Sevicke-Jones,‖ Therese Manning,# James Ataria,†† Tom Cresswell,‡‡ Katherine A Dafforn,§§ Frederic DL Leusch,‖‖ Bradley Moggridge,## Marcus Cameron,††† John Chapman,‡‡‡ Gary Coates,§§§ Anne Colville,‖‖‖ Claire Death,### Kimberly Hageman,†††† Kathryn Hassell,§ Molly Hoak,‡‡‡‡ Jennifer Gadd,§§§§ Dianne F Jolley,‖‖‖‖ Ali Karami,##### Konstantinos Kotzakoulakis,‡‡‡‡‡ Richard Lim,‖‖‖‖ Nicole McRae,† Leon Metzeling,††††† Thomas Mooney,‡ Jackie Myers,§ Andrew Pearson,§§§§§ Minna Saaristo,‖‖‖‖ Dave Sharley,###### Julia Stuthe,†††††† Oliver Sutherland,‡‡‡‡‡‡ Oliver Thomas,§§§§§§ Louis Tremblay,†† ‖‖‖‖‖‖ Waitangi Wood,####### Alistair BA Boxall,††††††† Murray A Rudd,‡‡‡‡‡‡‡ and Bryan W Brooks§§§§§§§*

Furley et al. 2018. *IEAM*; Van den Brink et al. 2018. *ETC*;
Fairbrother et al. 2019. *ETC*; Gaw et al. 2019. *IEAM*

OHC2T