# The Cyanobacterial Neurotoxin L-β-methylamino-n-alanine (BMAA) As An Emerging Public Health Concern In The Columbia River Watershed

Stuart W. Dyer[1,2], Tawnya D. Peterson[2], Joseph A. Needoba[2]

[1]OHSU School of Medicine

[2]OHSU-PSU School of Public Health

April, 2018

CLIMATE

# Blooms Like It Hot

Hans W. Paerl[1] and Jef Huisman[2]

A link exists between global warming and the worldwide proliferation of harmful cyanobacterial blooms.

VOL 320 4 APRIL 2008



FlowCam images, Franz Lake Slough, WA (2016): *Dolichospermum* sp. bloom

# Cyanobacteria are an emerging global health threat to ecosystems and humans

# Cyanobacteria are an emerging local health threat to ecosystems and humans

http://www.opb.org/television/programs/ofg/segment/ross-island/

Ross Island Lagoon (2015)

http://www.kptv.com/story/29568943/health-advisory-still-in-effect-for-ross-island-lagoon-due-to-algae

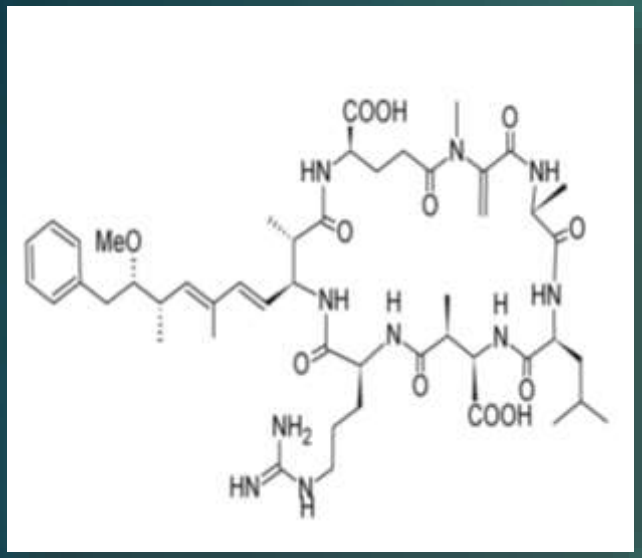
Ross Island Lagoon (2015)

Photo: S. Dyer

Photo: S. Dyer

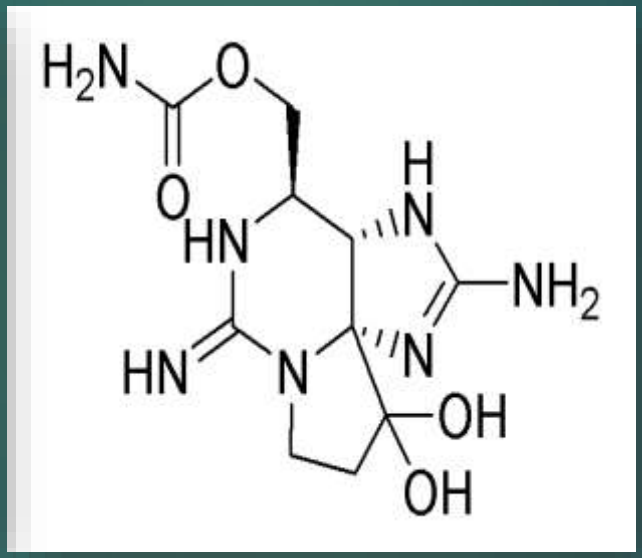
# Cyanotoxins are the main pubic health concern in the management of HABs

## Microcystin



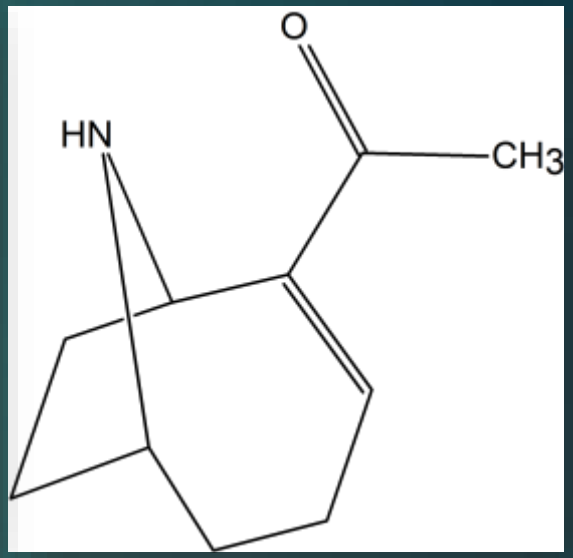

- Hepatotoxic
- 0.3 μg/L (drinking)
- 20 μg/L (no contact)

## Saxitoxin




- Nephero/neurotoxic
- 0.2 μg/L (drinking)
- 3 μg/L (no contact)

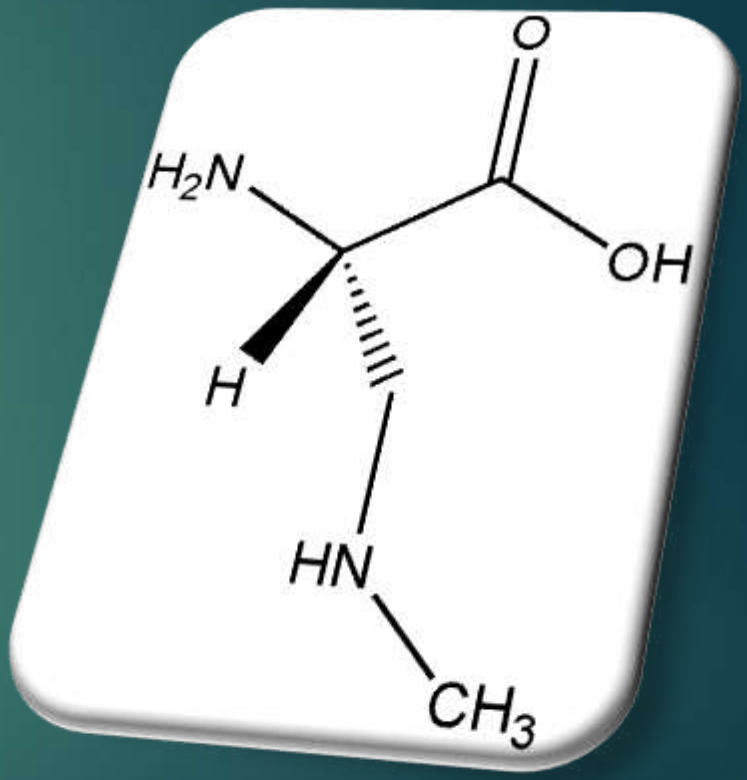
## Anatoxin



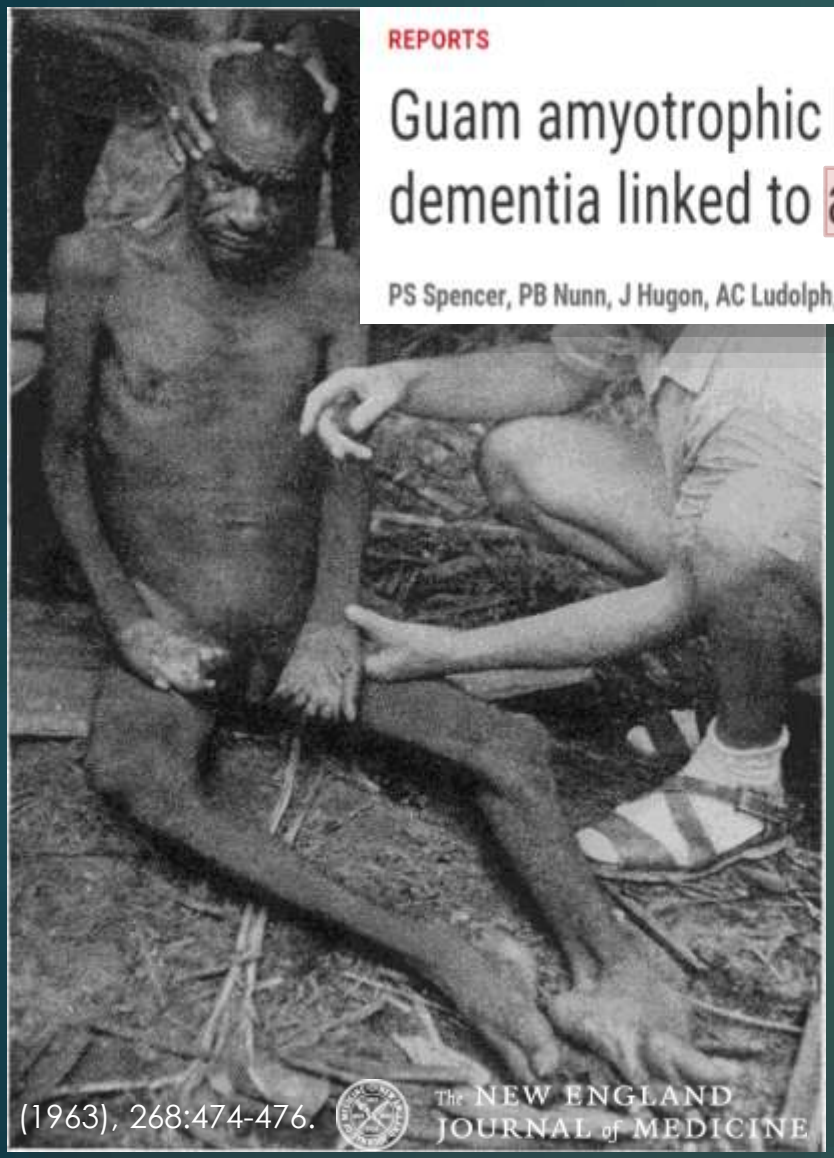

- neurotoxic
- 0.7 μg/L (drinking)
- 3 μg/L (no contact)

# The unique and ubiquitous cyanotoxin: β-N-methylamino-L-alanine (BMAA)

- BMAA detected in representative species of all cyanobacterial morphotypes
- present for duration of bloom forming season
- Innate biochemical and ecological function are unknown



# The developing science of BMAA: 1960-1980




Chamorro native with advanced Guam-PD (Rep Village *ca.*, 1960)

REPORTS

## Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin

PS Spencer, PB Nunn, J Hugon, AC Ludolph, SM Ross, DN Roy, RC Robertson



- BMAA isolated from cycad seeds
- Controversy over dose needed to trigger neuropathology

# ca. 2003

Institution: Oregon Health & Science University

Proceedings of the National Academy of Sciences of the United States of America

PNAS

## Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam

Paul Alan Cox, Sandra Anne Banack and Susan J. Murch

PNAS November 11, 2003. 100 (23) 13380-13383; https://doi.org/10.1073/pnas.2235808100

- First evidence supporting a cyanobacterial origin for BMAA
- First plausible hypothesis for Guam-PD
  - *Pteropus vampyrus* (fruit bat) soup

Adapted from P. Date

wordpress

# ca. 2005

Institution: Oregon Health & Science University
Proceedings of the National Academy of Sciences of the United States of America
PNAS
9734 | PNAS | July 5, 2005 | vol. 102 | no. 27

## Diverse taxa of cyanobacteria produce β-*N*-methylamino-L-alanine, a neurotoxic amino acid

Paul Alan Cox*†, Sandra Anne Banack‡, Susan J. Murch*, Ulla Rasmussen§, Georgia Tien¶, Robert Richard Bidigare¶, James S. Metcalf‖, Louise F. Morrison‖, Geoffrey A. Codd‖, and Birgitta Bergman§

- First evidence indicating that BMAA is a fundamental cyanobacterial metabolite
  - BMAA detected in 95% of axenic cyanobacterial cultures analyzed (n=30)

ca. 2014



Proceedings of the National Academy of Sciences of the United States of America

PNAS



> Current Issue > vol. 111 no. 3 > Mohammad Arif, 1144–1149, doi: 10.1073/pnas.1322614111



# Tau pathology involves protein phosphatase 2A in Parkinsonism-dementia of Guam

Mohammad Arif[a], Syed Faraz Kazim[a,b], Inge Grundke-Iqbal[a], Ralph M. Garruto[c,1], and Khalid Iqbal[a,1]

Author Affiliations

This Issue

January 21, 2014
vol. 111 no. 3
Masthead (PDF)
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ca. 2016

PROCEEDINGS
OF THE ROYAL SOCIETY B
BIOLOGICAL SCIENCES



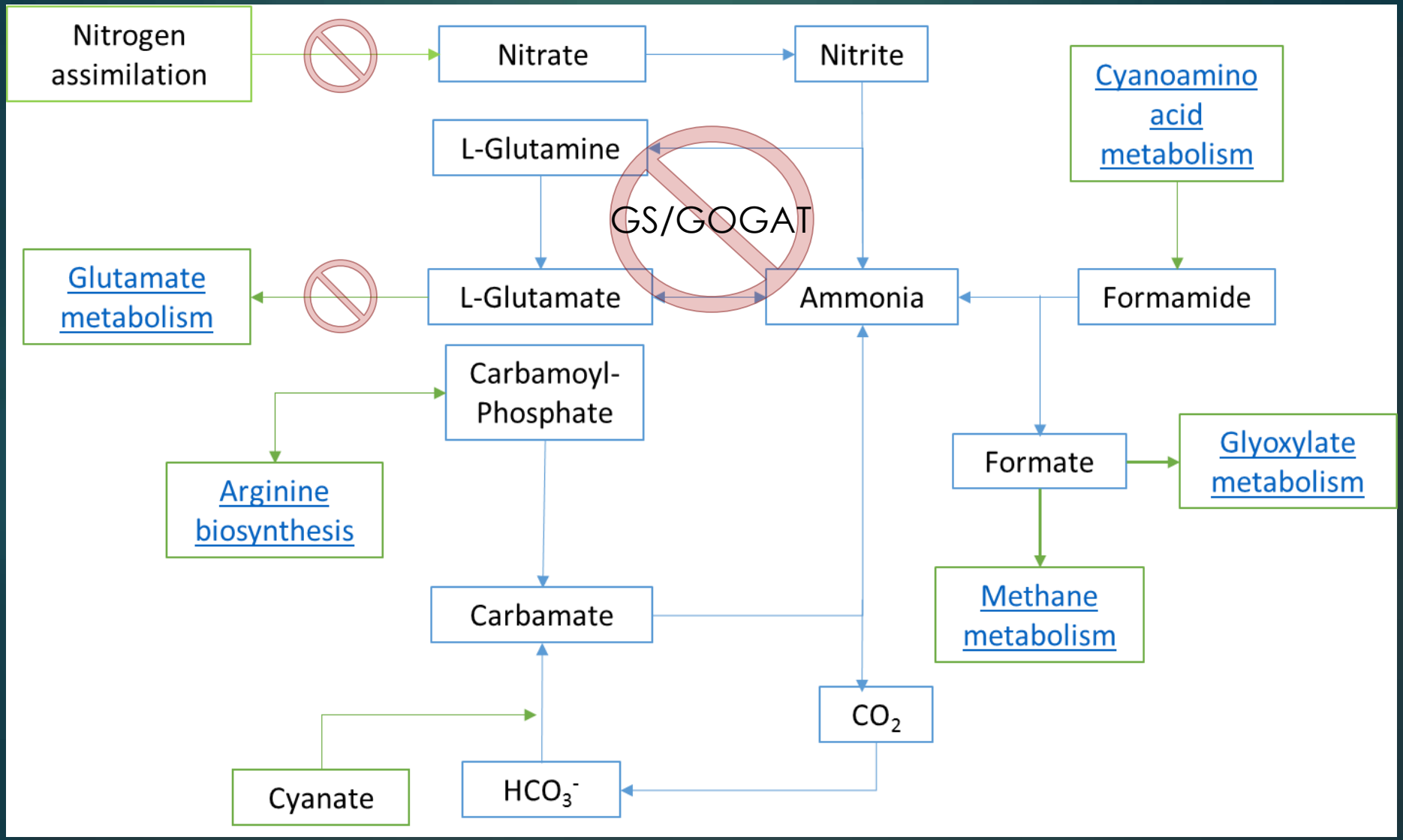


# Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid deposits in the brain

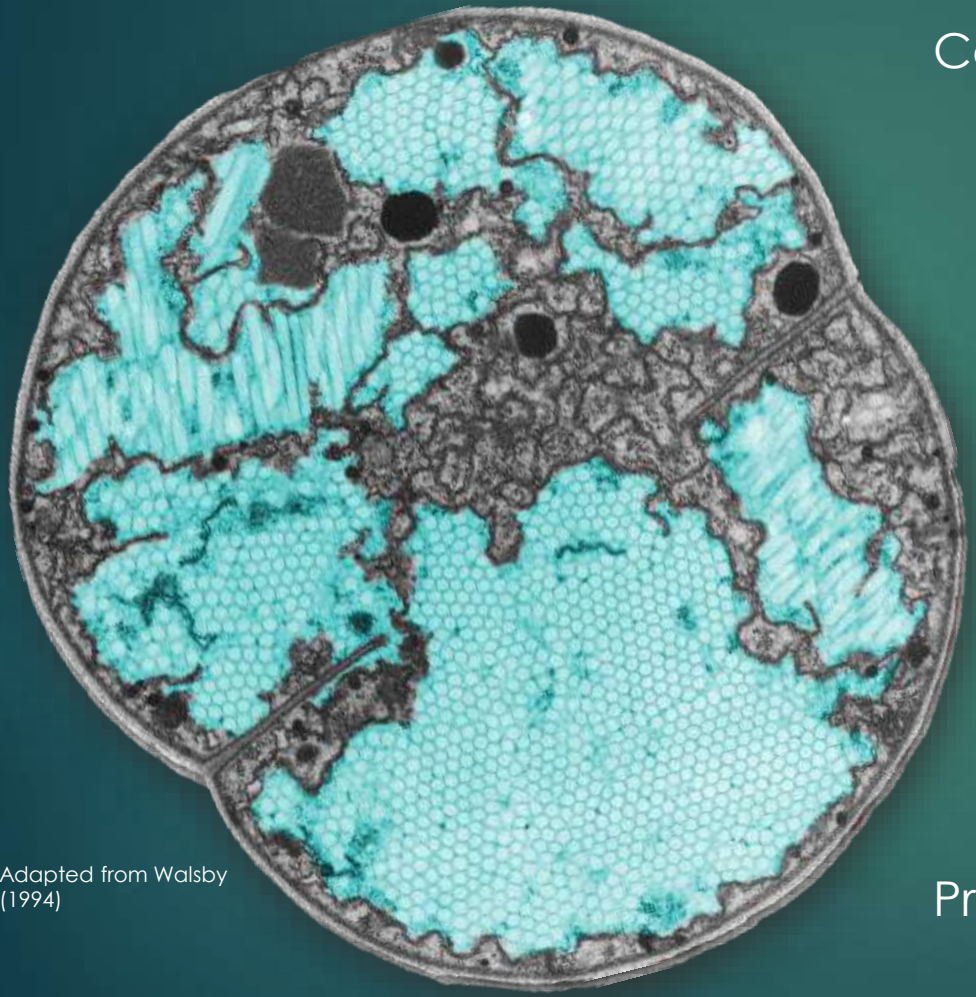
Paul Alan Cox, David A. Davis, Deborah C. Mash, James S. Metcalf, Sandra Anne Banack

Published 20 January 2016. DOI: 10.1098/rspb.2015.2397

# BMAA disrupts the tightly coupled carbon/nitrogen metabolism of cyanobacteria



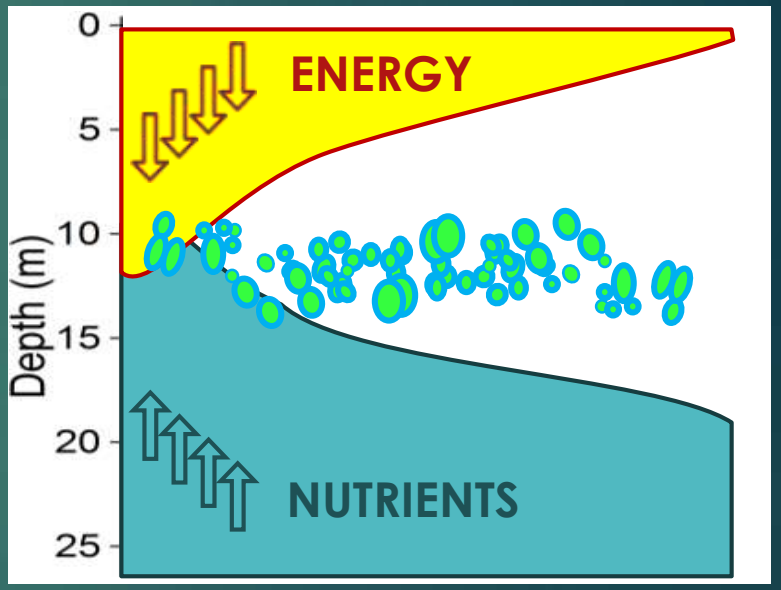
# Amotile cyanobacteria use buoyancy regulation as the physiochemical like between access to energy and nutrient pools



Adapted from Walsby (1994)

Carbohydrate **Ballast**

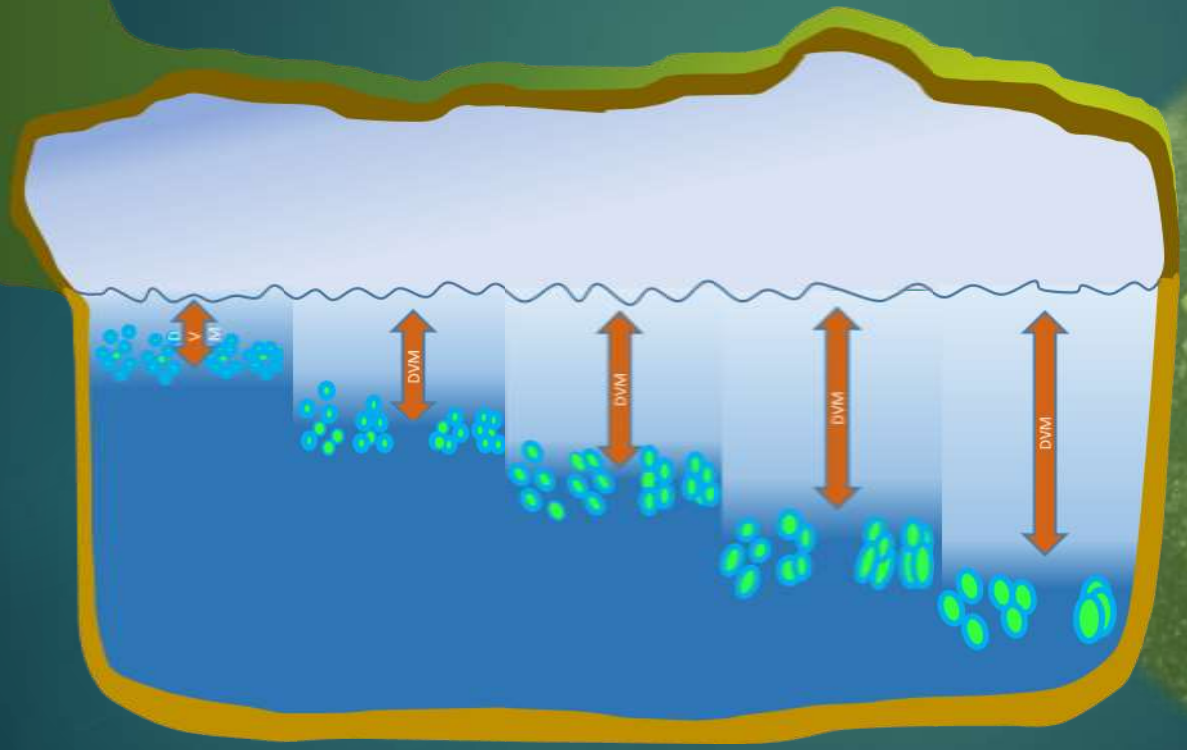
- Promotes downward movement




Proteinaceous **Gas Vesicles**

- Promote upward movement

warm temperatures promote cyanobacterial dominance





Ross Island Lagoon
Willamette River, OR
(2016)

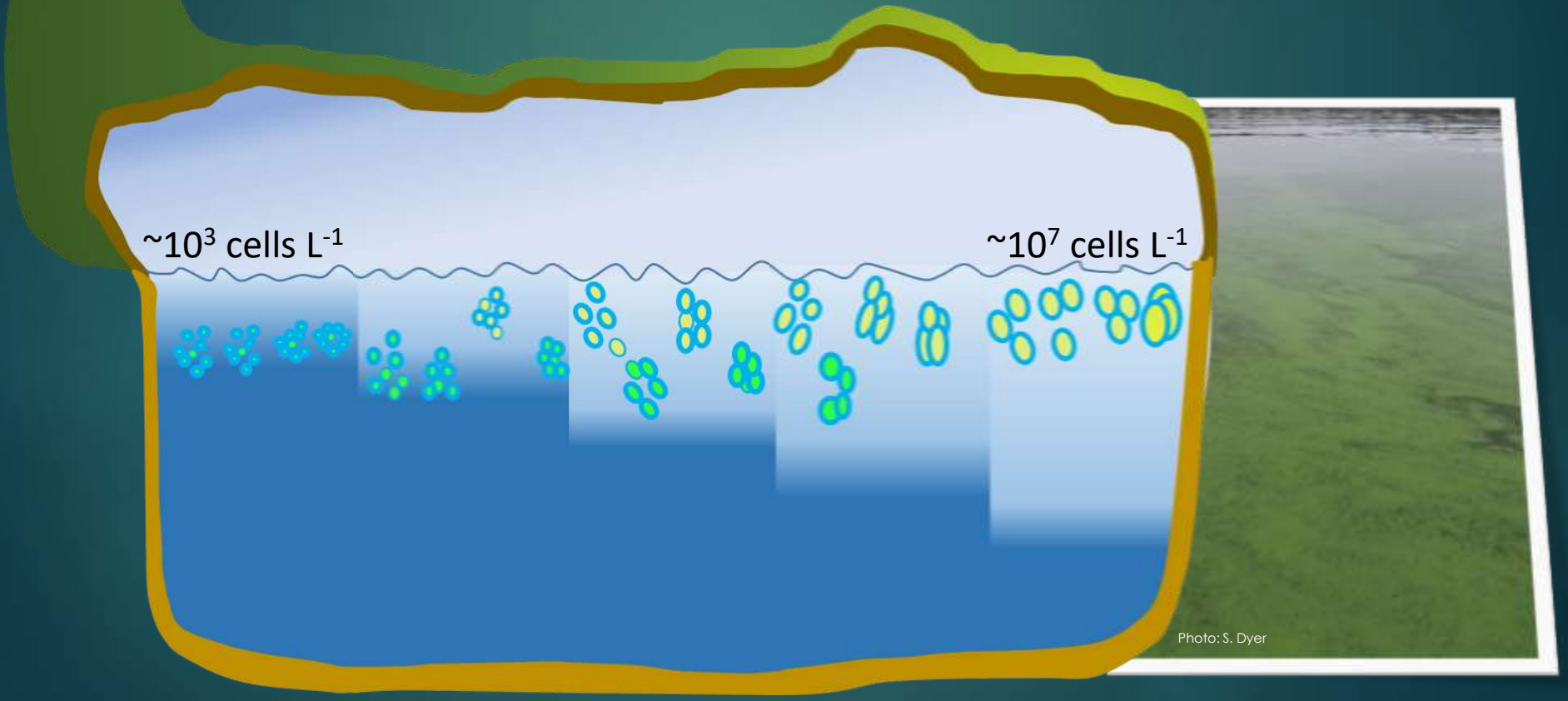
$\left(\frac{mm}{Hr}\right)$ → rate of DVM increases with colony size→ $\left(\frac{m}{Hr}\right)$

# Large initial nutrient inputs promote colonization of the air/water interface

~72 Hrs

rapid doubling time for most planktonic cyanobacteria (< 24 hours), coupled with favorable $CO_2$ uptake kinetics

increased temperature & nutrient loads promote surface trapping



# Surface trapping in HABs drives food-web perturbations and cyanobacterial stress responses

*Dolichospermum* sp., dominant:
Franz Lake Slough (2016)



*Microcystis* sp., dominant:
Ross Island Lagoon (2016)

<u>Foodweb</u>

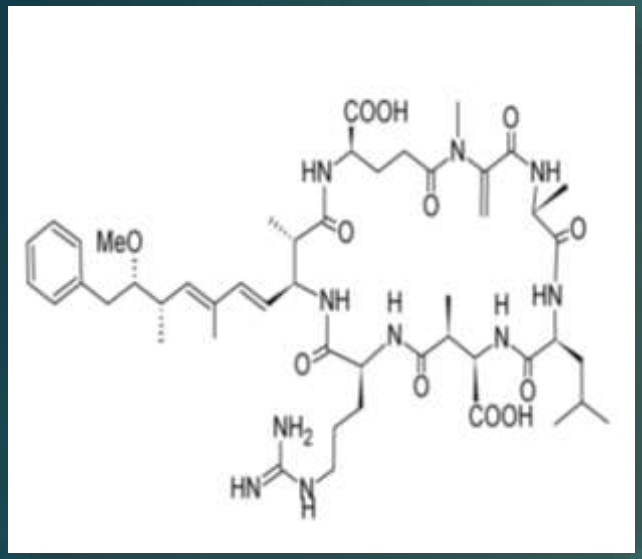
- Oxygen depletion
- Shading
- Toxins

<u>HAB Organisms</u>

- Nutrient limitation
- Increased PAR
- Increased potential for oxidative damage

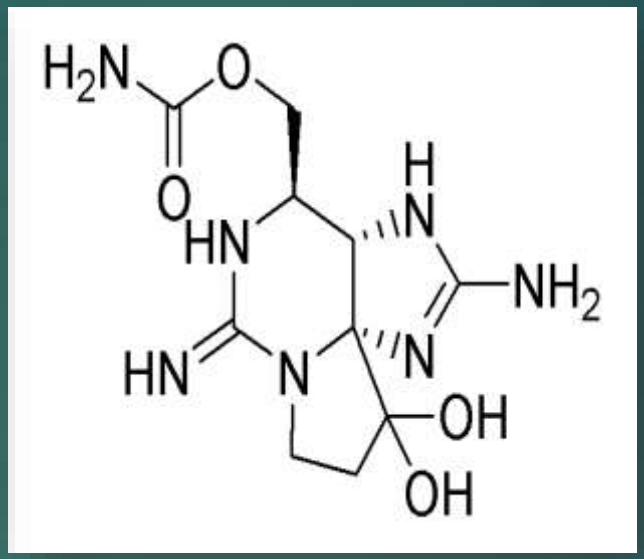
# Cyanotoxins provide a selective advantage

## Microcystin



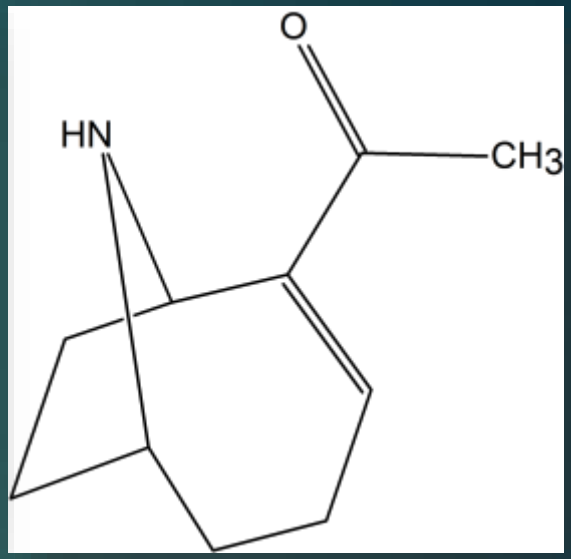

- Increased fitness under high-light/oxidative conditions
- covalent binding to large subunit of RuBisCo

## Saxitoxin



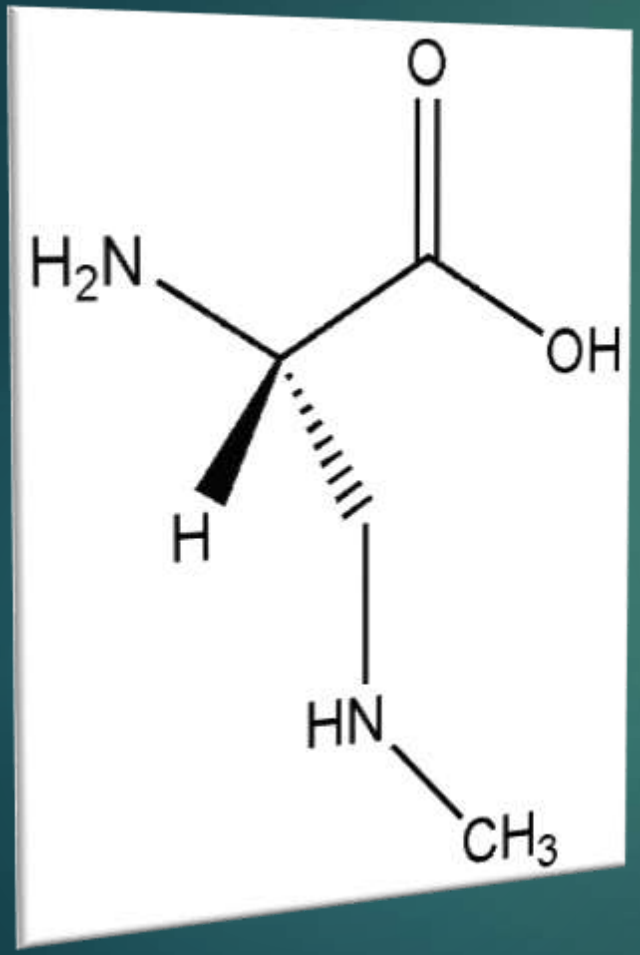

- Increased fitness under periods of osmotic stress
- Bioflim formation decreases $H_2O$ loss and salt stress

## Anatoxin




- Increased fitness due to reduced grazing (?)
- "Very fast death factor".....

# Well then what's the deal with BMAA?

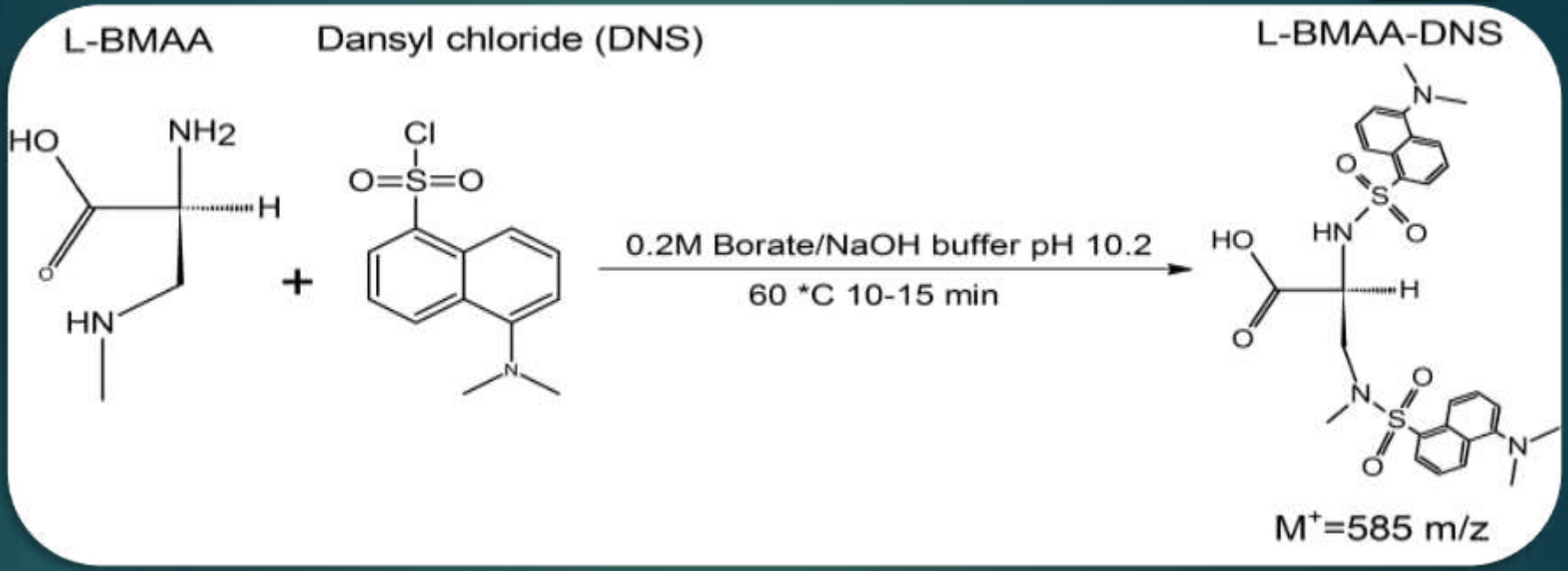


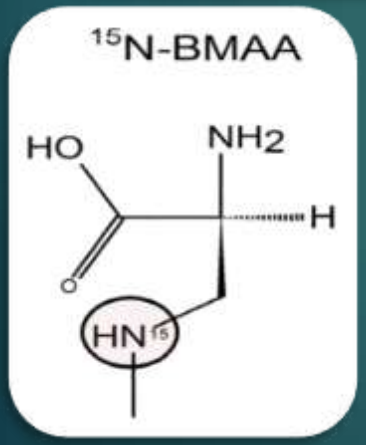

# The Guam-PD hypothesis remains contentious: Chernoff et al., (2017)

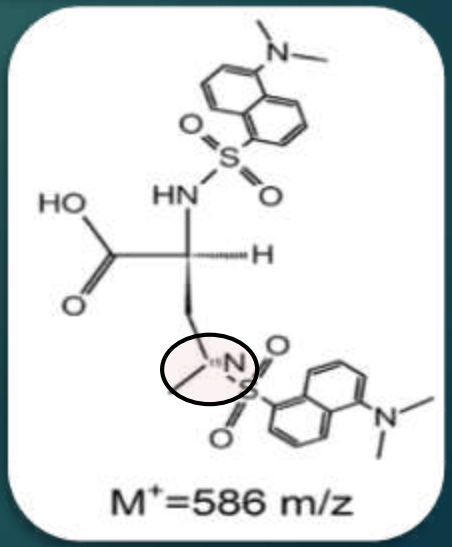
"Before public health actions are taken based on the [Guam-PD] hypothesis, it is imperative that fundamental issues and inconsistencies concerning the central assumptions are discussed and resolved," Including:

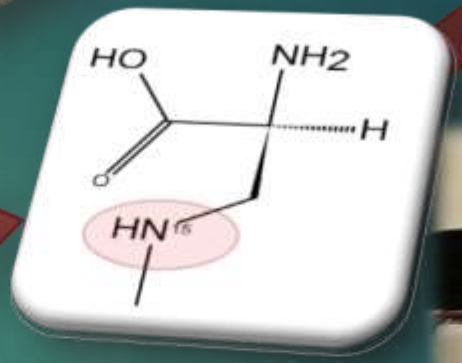
- Issues with detection and quantification

# LC-MS/MS quantification of BMAA within complex environmental samples

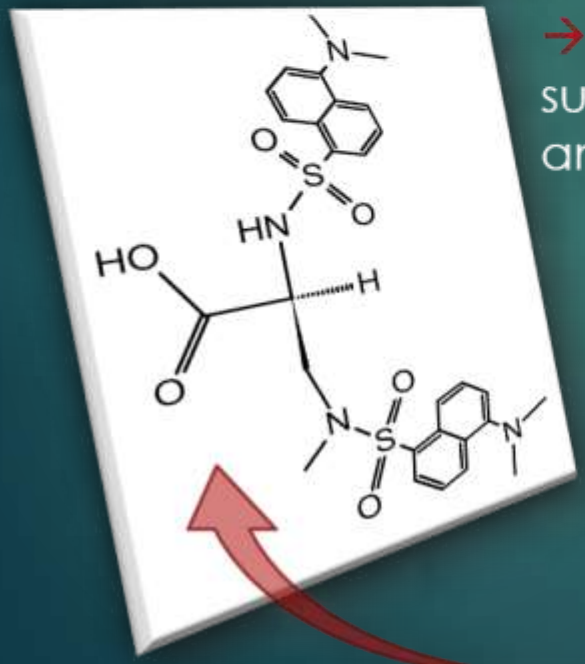


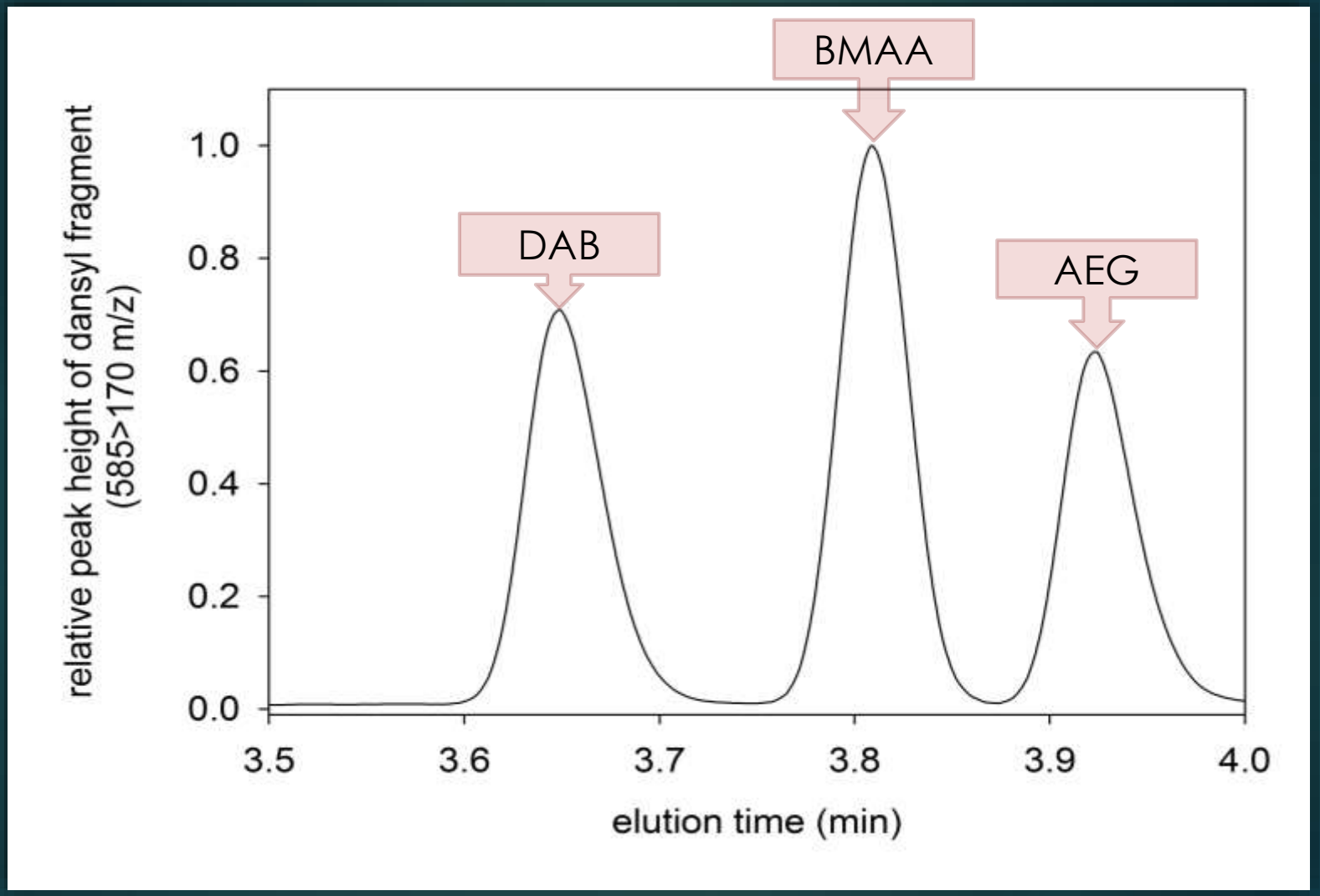






→ derivatized compound submitted for LC-MS analysis

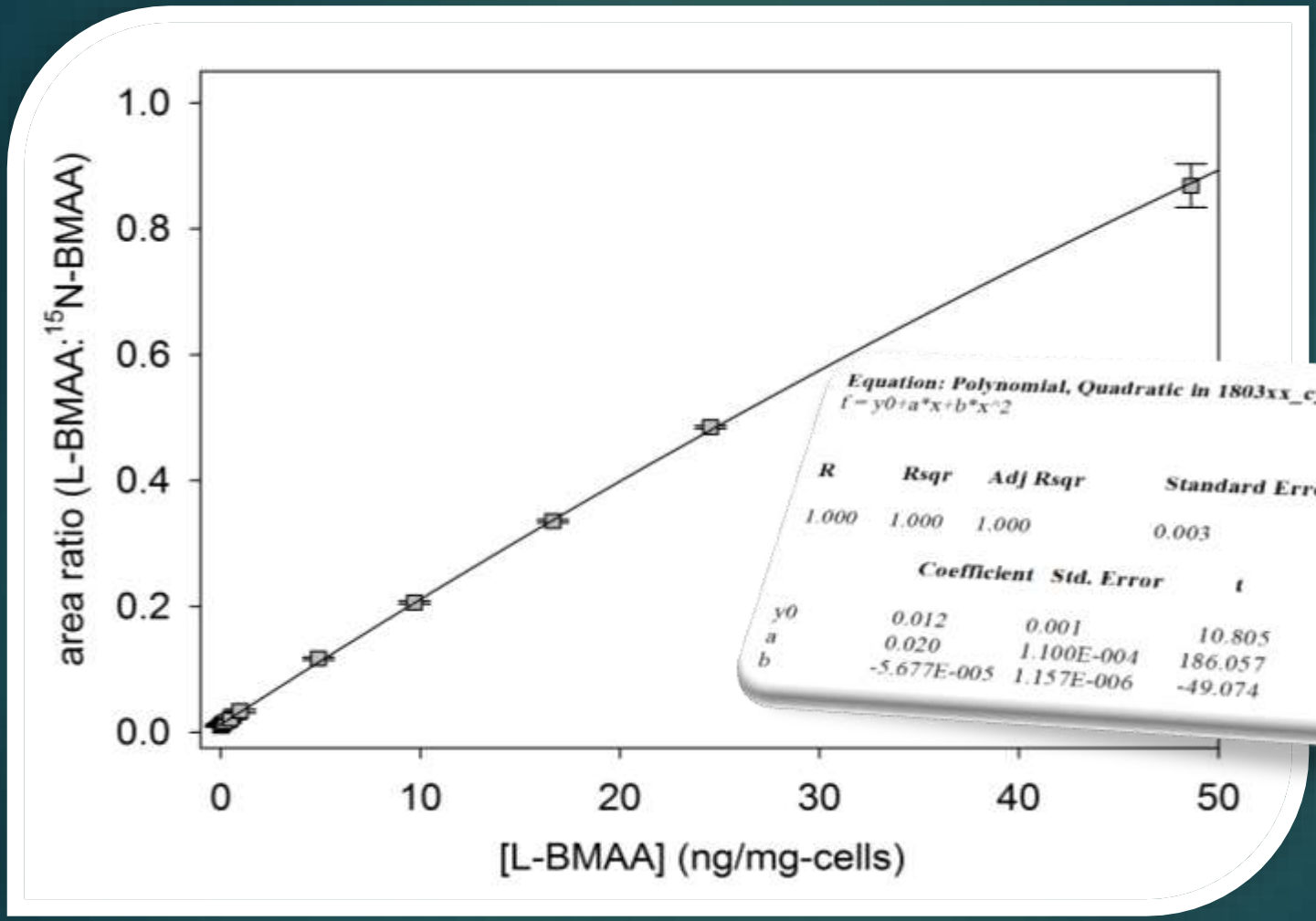



# Disambiguation of BMAA from its isomers DAB and AEG based on elution time



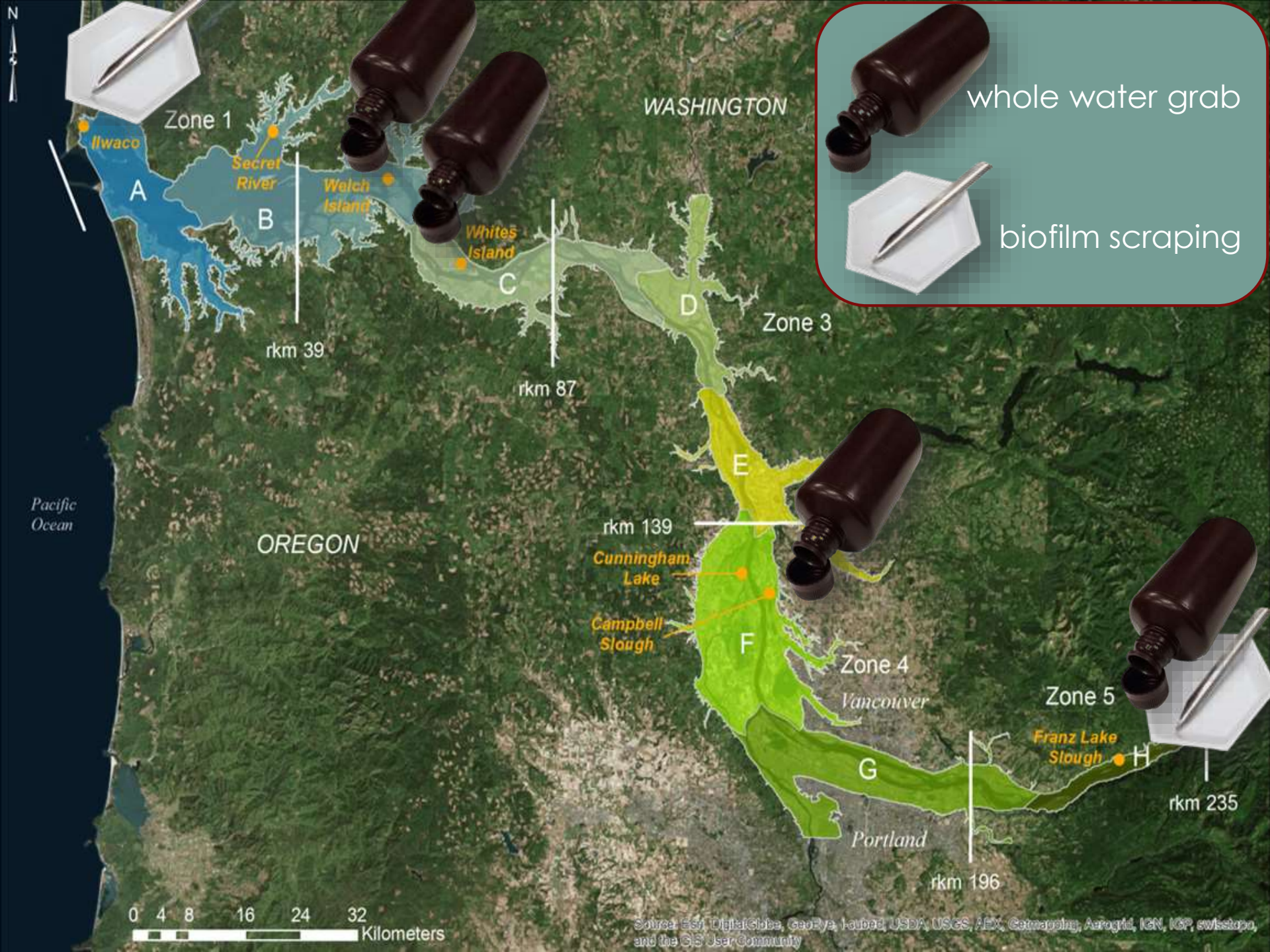

Dyer – unpublished results

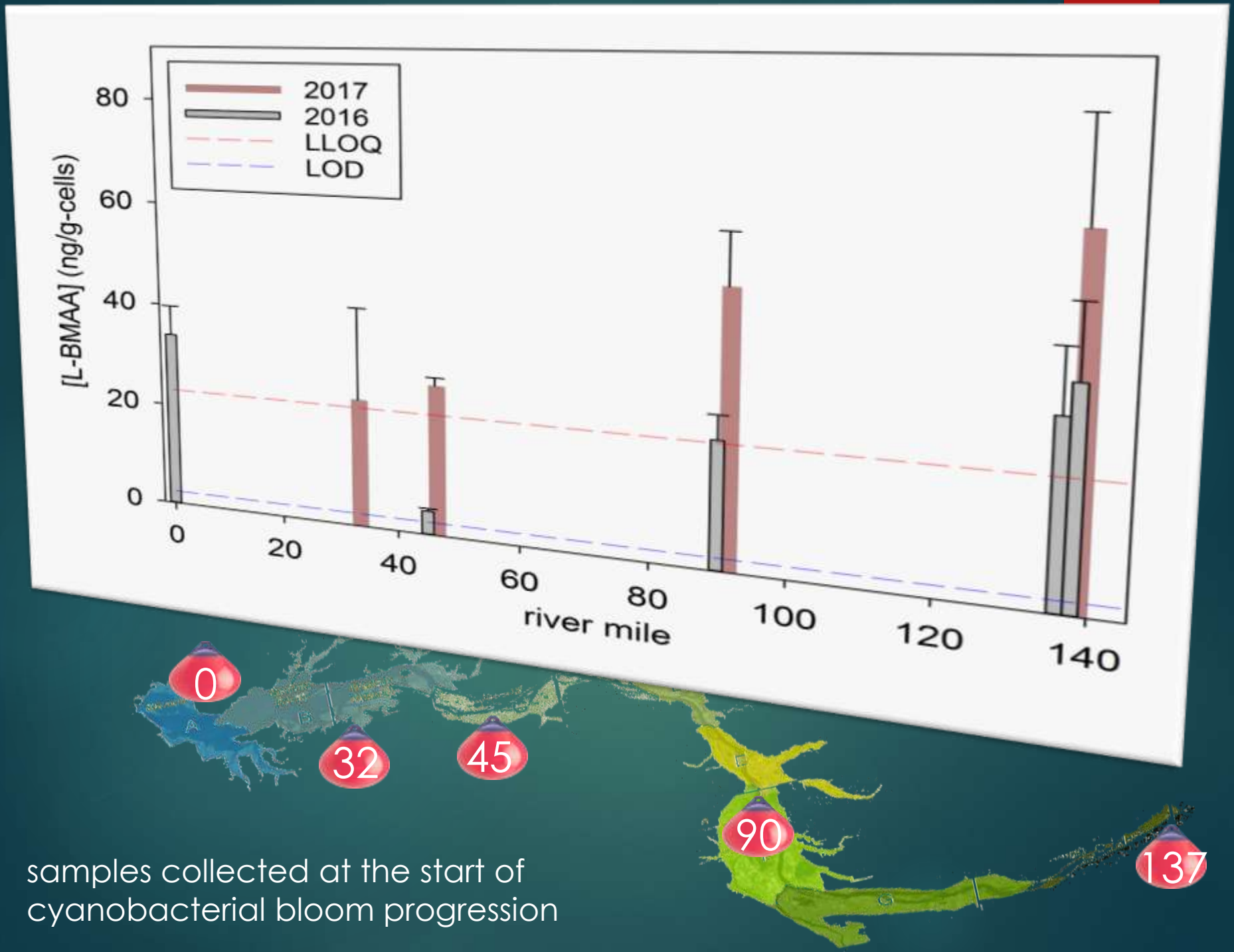
# Quantification based on the area ratio of L-BMAA:$^{15}$N-BMAA




- LOD: 2.4 ng/g-cells
- LLOQ: 23 ng/g-cells

Dyer – unpublished results


whole water grab
biofilm scraping
N
WASHINGTON
OREGON
Pacific Ocean
Zone 1
Ilwaco
Secret River
Welch Island
Whites Island
A
B
C
D
E
F
G
H
rkm 39
rkm 87
rkm 139
rkm 196
rkm 235
Zone 3
Zone 4
Zone 5
Vancouver
Portland
Cunningham Lake
Campbell Slough
Franz Lake Slough
0 4 8 16 24 32
Kilometers
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community




samples collected at the start of cyanobacterial bloom progression

ca. 2018

*Toxins* **2018**, *10*(1), 22; doi:10.3390/toxins10010022 Open Access Feature Paper Article

# A Single Neonatal Exposure to BMAA in a Rat Model Produces Neuropathology Consistent with Neurodegenerative Diseases

Laura Louise Scott * ✉ and Timothy Grant Downing * ✉

- BMAA is a public health concern
- BMAA is present in the Lower Columbia River watershed

# Thank you


Committee Members

- Dr. Joseph Needoba
- Dr. Tawnya Peterson
- Dr. Bradley Tebo
- Dr. John Perona

Lab Members

- Lyle Cook
- Nikolai Danilchik
- Taylor Dodril

Interns

- Desire Povijua

IEH faculty

- Dr. Richard Johnson
- Dr. Mickiko Nakano

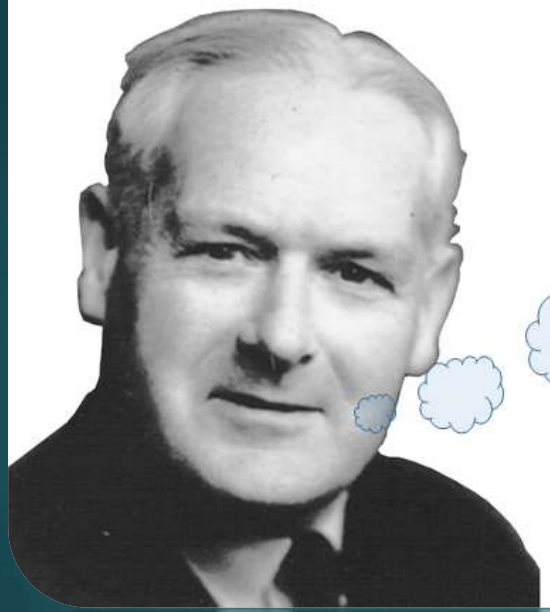
OHSU Bioanalytical Shared Resource/ Pharmacokinetics Core

- Dr. Dennis Koop
- Lisa Bleyle

OHSU Medicinal Chemistry Core

- Dr. Aaron Nilsen

# Questions?



All scientific work is incomplete—whether it be observational or experimental...That does not confer upon us the freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time.

-Sir Austin Bradford Hill

# HOW CAN WE MONITOR NONMONOTONIC CHANGES IN HAB DYNAMICS?

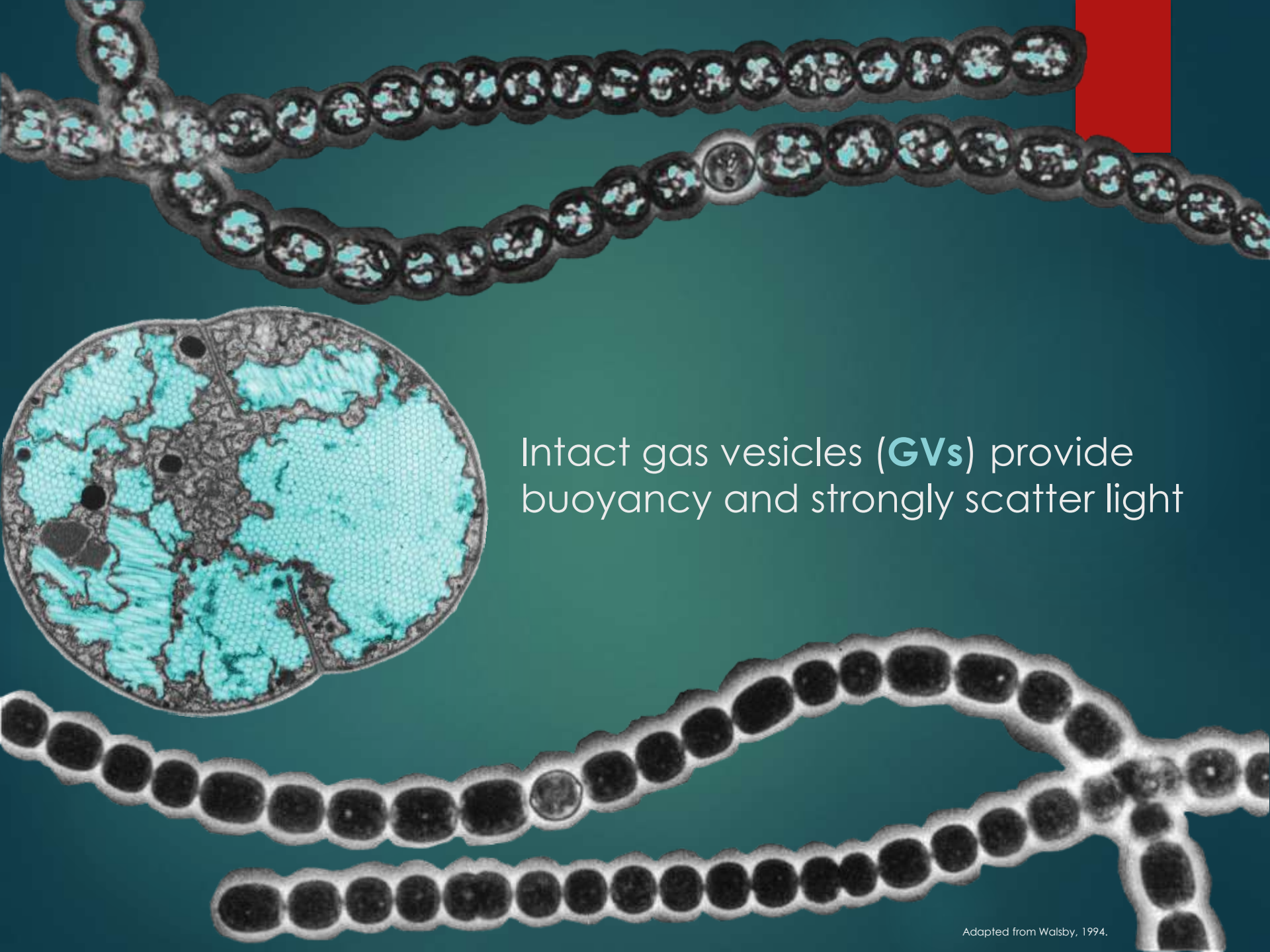
# Nephel-o-metr-ic

→ relating to the measurement of light scattering

# Turbid-i-meter

→ an instrument for measuring the concentration of suspended solids in a liquid medium

➔ An instrument that measures the scattering of light as a proxy for the concentration of suspended solids in a liquid medium

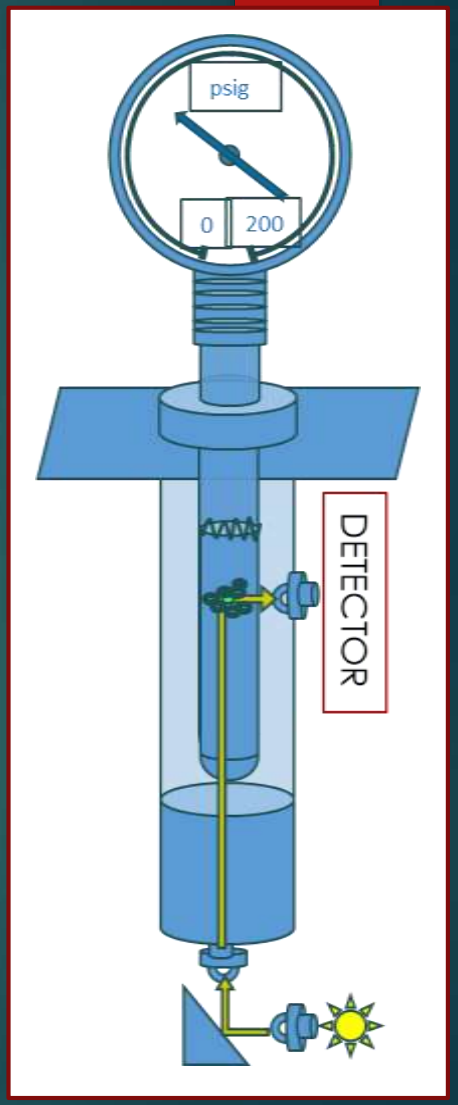


Intact gas vesicles (**GVs**) provide buoyancy and strongly scatter light

Adapted from Walsby, 1994.

# Pressure Nephelometer

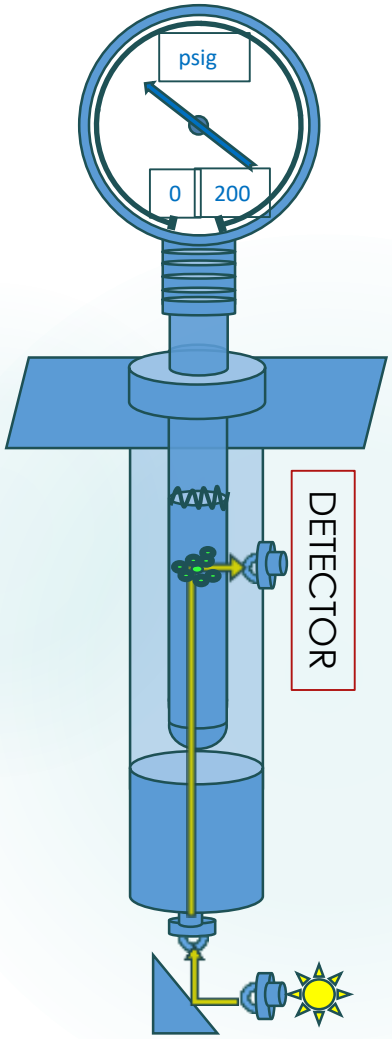
➔An instrument that is able to modulate pressure within a nephelometric cell, measure the resulting scattering of light, and determine pressure/turbidity relationships



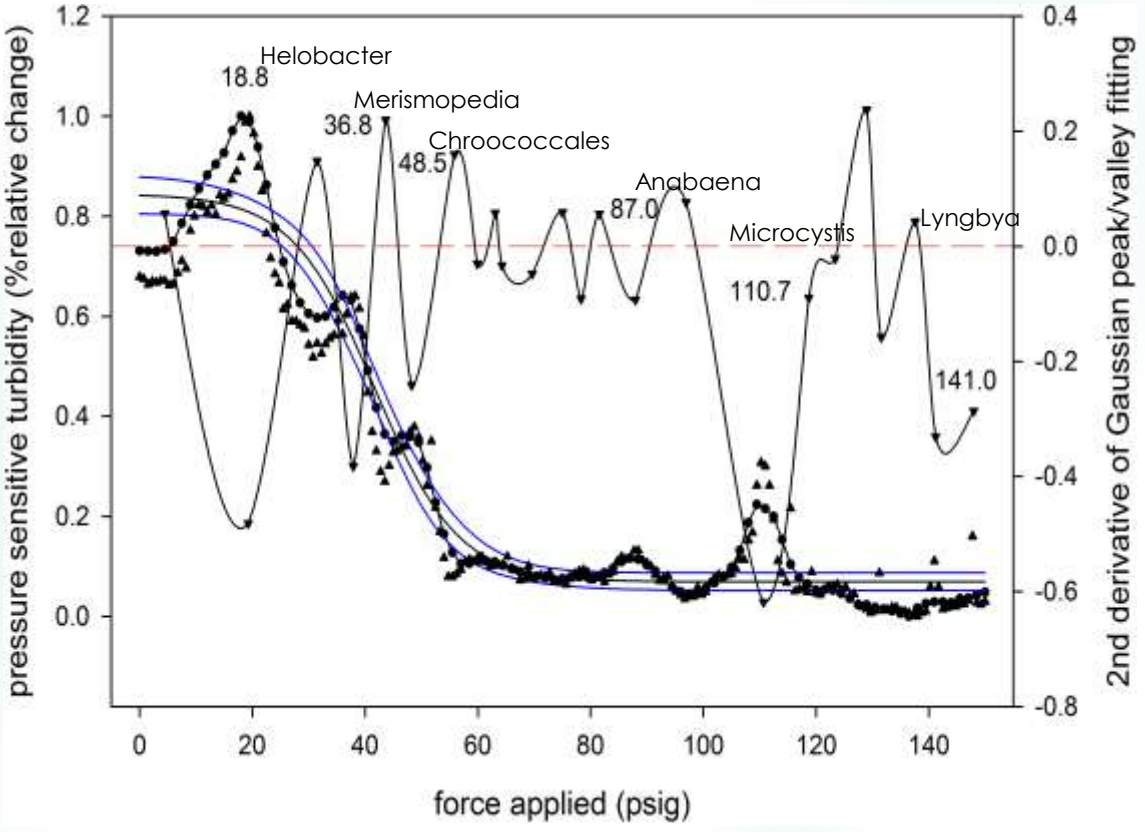
# Critical collapse pressure is species-specific

| Cyanobacteria | Pc (psig) | Pt (psig) | Reference |
|---|---|---|---|
| *Anabaena flos-aquae* CCAP1403/13f | 88.5 | 62.4 | Walsby, 1980 |
| *Aphanizomenon flos-aquae* CCAP1401/1 | 87.0 | 50.8 | Konopka et al., 1978 |
| *Chroococcales* sp. | 47.9 | 11.6 | Walsby & Bleything, 1988; |
| *Microcystis* sp. BC84/1 | 110.2 | 46.4 | Walsby et al., 1983 |
| *Lyngbya/Oscillatoria agardhii* PCC7801 (red) | 143.6 | 55.1 | Walsby & Bleything, 1988 |
| Other Bacteria | | | |
| *Amoebobacter roseus* | 49.3 | 21.8 | Walsby, 1971 |
| *Aebobacter purpureus* | 34.8 | 14.5 | Overmann & Pfennig, 1992 |
| *Ancylobacter aquaticus* | 72.5 | 27.6 | Koch & Pinette, 1987 |
| *Pelodictyon phaeochlathratiforme* | 71.1 | 47.9 | Overmann et al., 1991 |
| *Prosthecomicrobium pneumaticum* | 78.3 | 43.5 | Walsby, 1976 |
| Halobacteria | | | |
| *Halobacterium salinarium* | 13.1 | 0.0 | Walsby, 1971 |

# Generating a high-resolution pressure nephelometry measurement with an environmental sample from the 2017 Ross Island Lagoon HAB

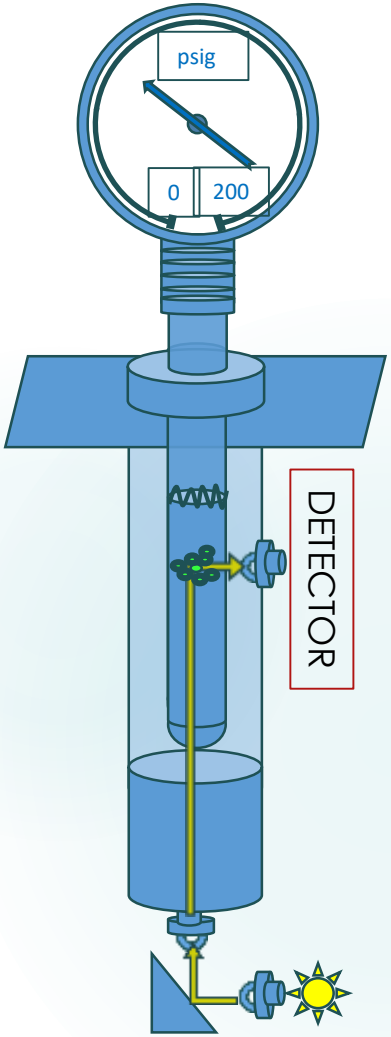



stepwise integration of nephelometric spectrum recorded at each pressure step (ΔP=0.75 psig) yields.....

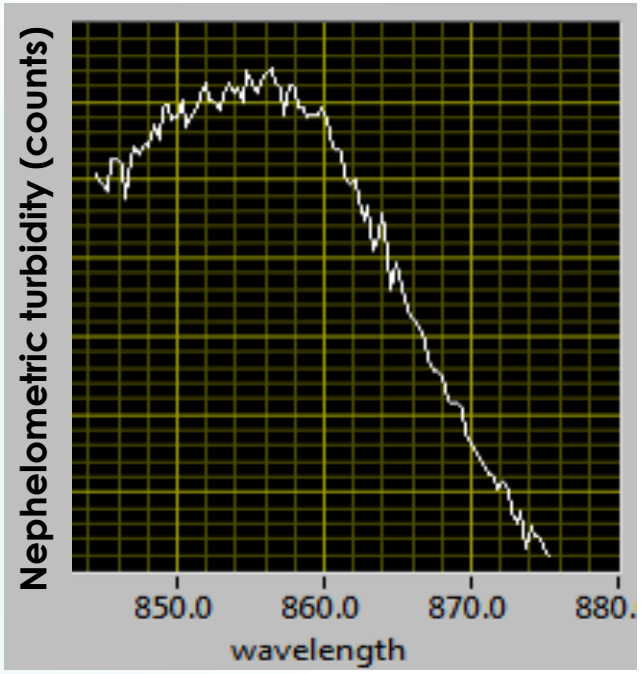



Dyer – unpublished results

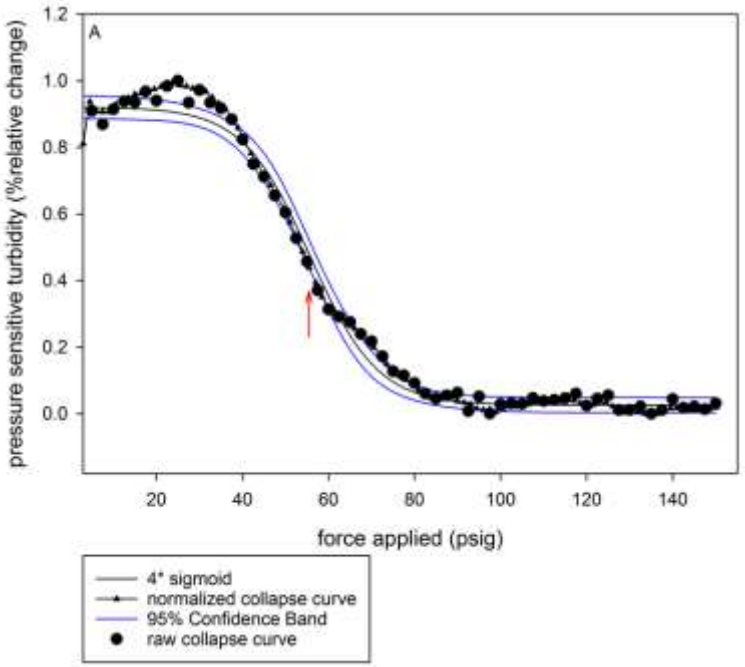
# Generating a traditional pressure nephelometry measurement with an environmental sample from the 2017 Ross Island Lagoon HAB




nephelometric spectrum (845±15 nm) recorded at each pressure step

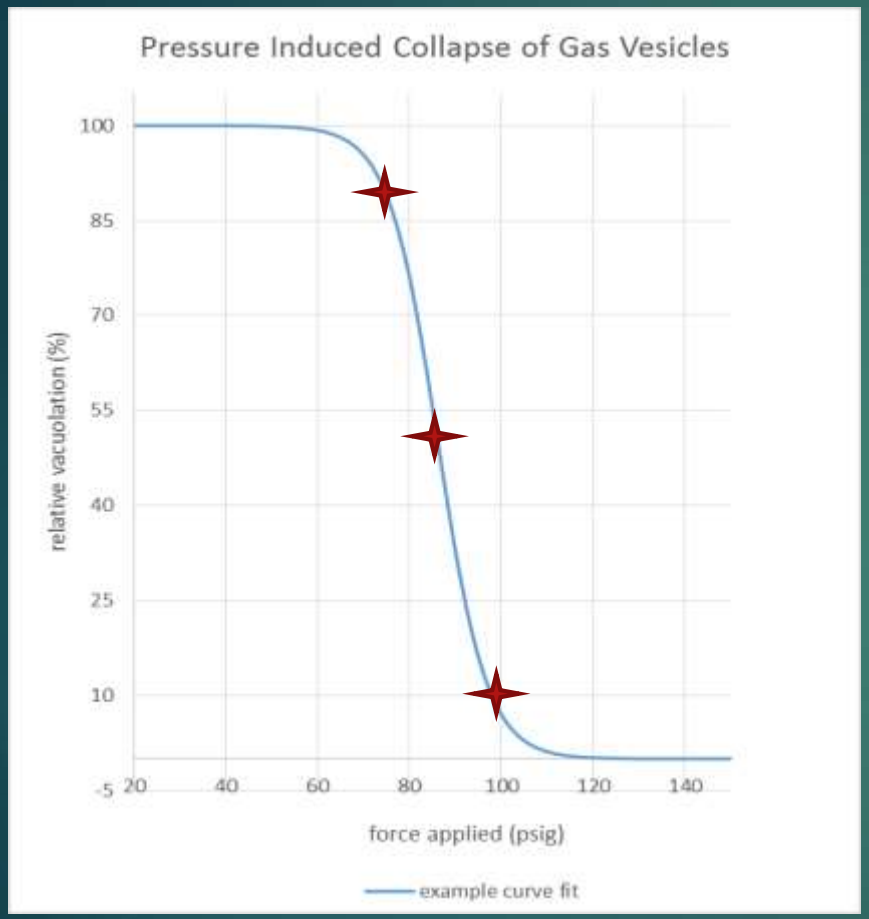



stepwise integration of nephelometric spectrum recorded at each pressure step (ΔP=2.5 psig) yields....




Dyer – unpublished results

# Critical collapse pressure is determined by gas vesicle structure




- GV width is inversely proportional to strength
- Strength is proportional to protein investment
- Changes in GV critical collapse pressure may indicate cellular stress or changes in nutrient status

Dyer – unpublished results

## 2017 – Denial of Petition to Revoke Tolerances

In March 2017, EPA denied a petition that asked us to revoke all pesticide tolerances (maximum residue levels in food) for chlorpyrifos and cancel all chlorpyrifos registrations. The Agency concluded that despite several years of study, the science addressing neurodevelopmental effects remains unresolved and further evaluation of the science during the remaining time for completion of registration review is warranted. As a part of the ongoing registration review, we will continue to review the science addressing neurodevelopmental effects of chlorpyrifos.

PLOS BIOLOGY
FIFTEENTH ANNIVERSARY



OPEN ACCESS

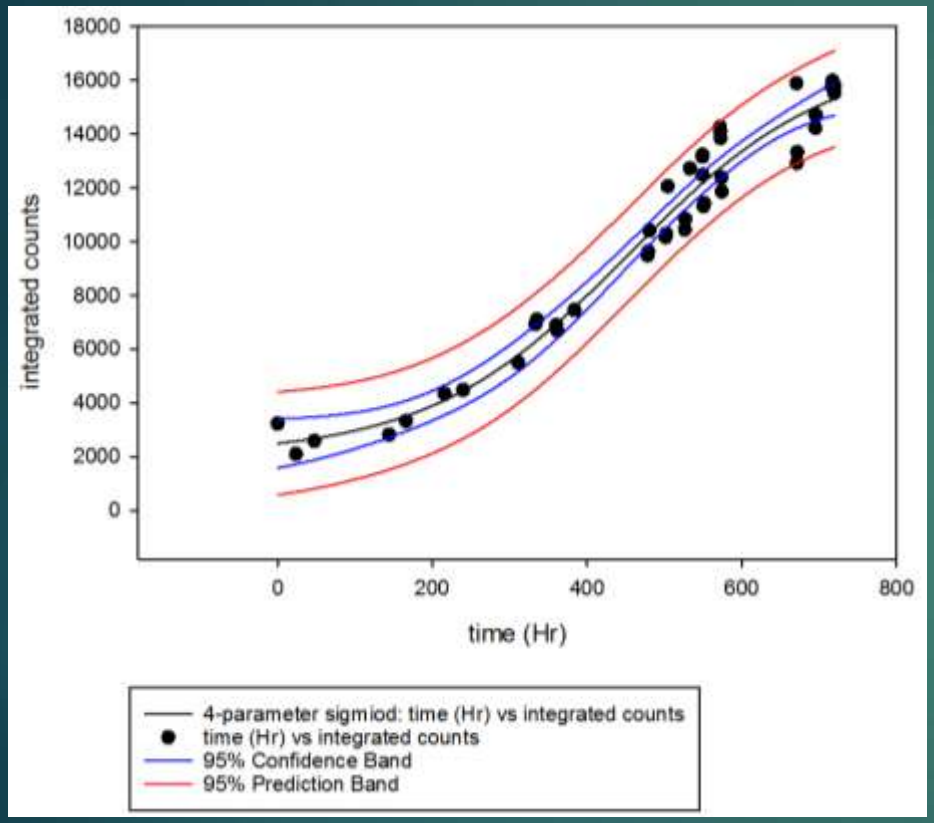
PERSPECTIVE

# When enough data are not enough to enact policy: The failure to ban chlorpyrifos

Leonardo Trasande

Published: December 21, 2017 • https://doi.org/10.1371/journal.pbio.2003671

# Growth Profiling Via Tubidity




**Nonlinear Regression**
**Equation: Sigmoidal**
f = y0+a/(1+exp(-(x-x0)/b))

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9819 | 0.9640 | 0.9612 | 832.4979 |

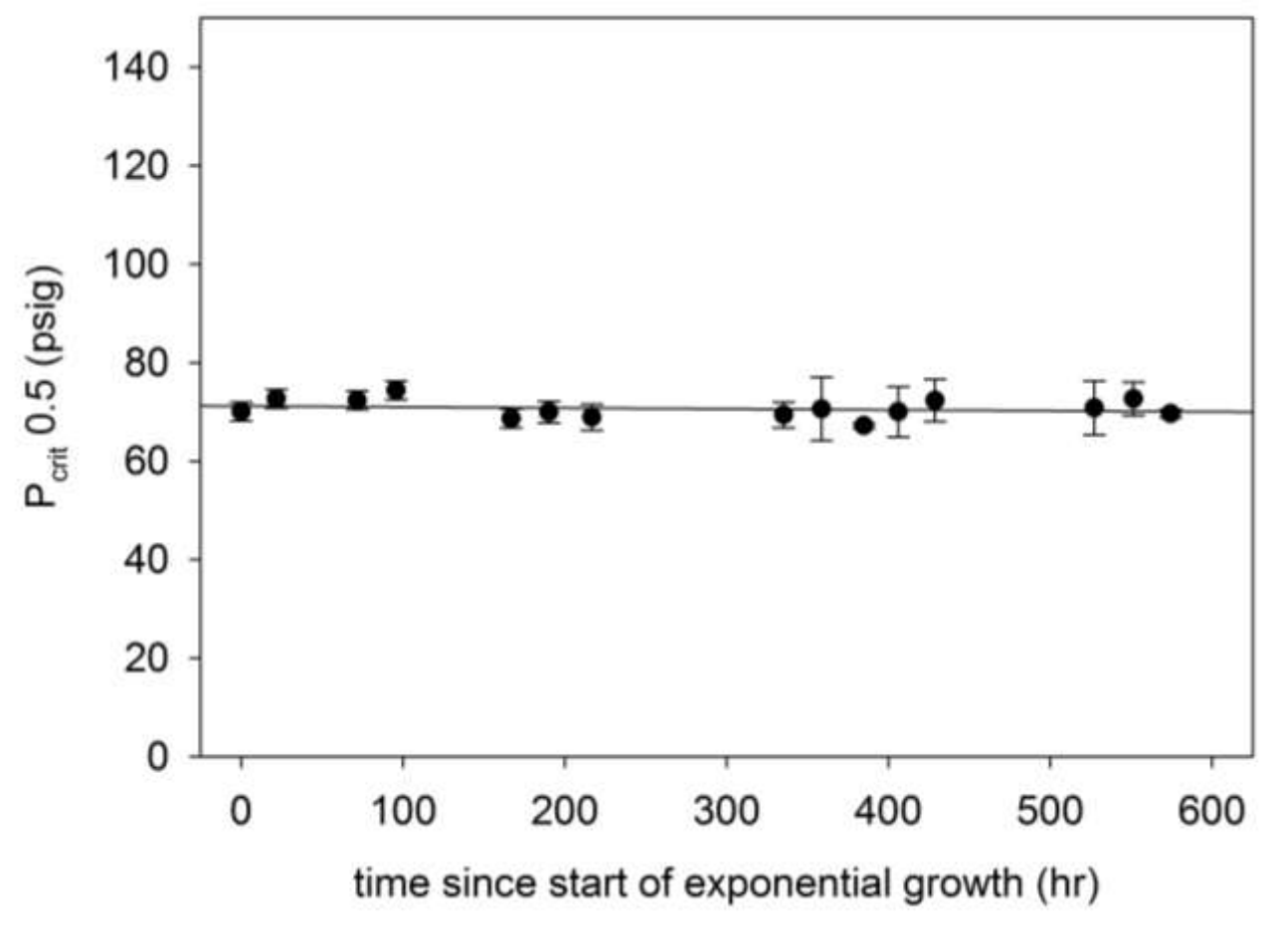
**Statistical Tests:**

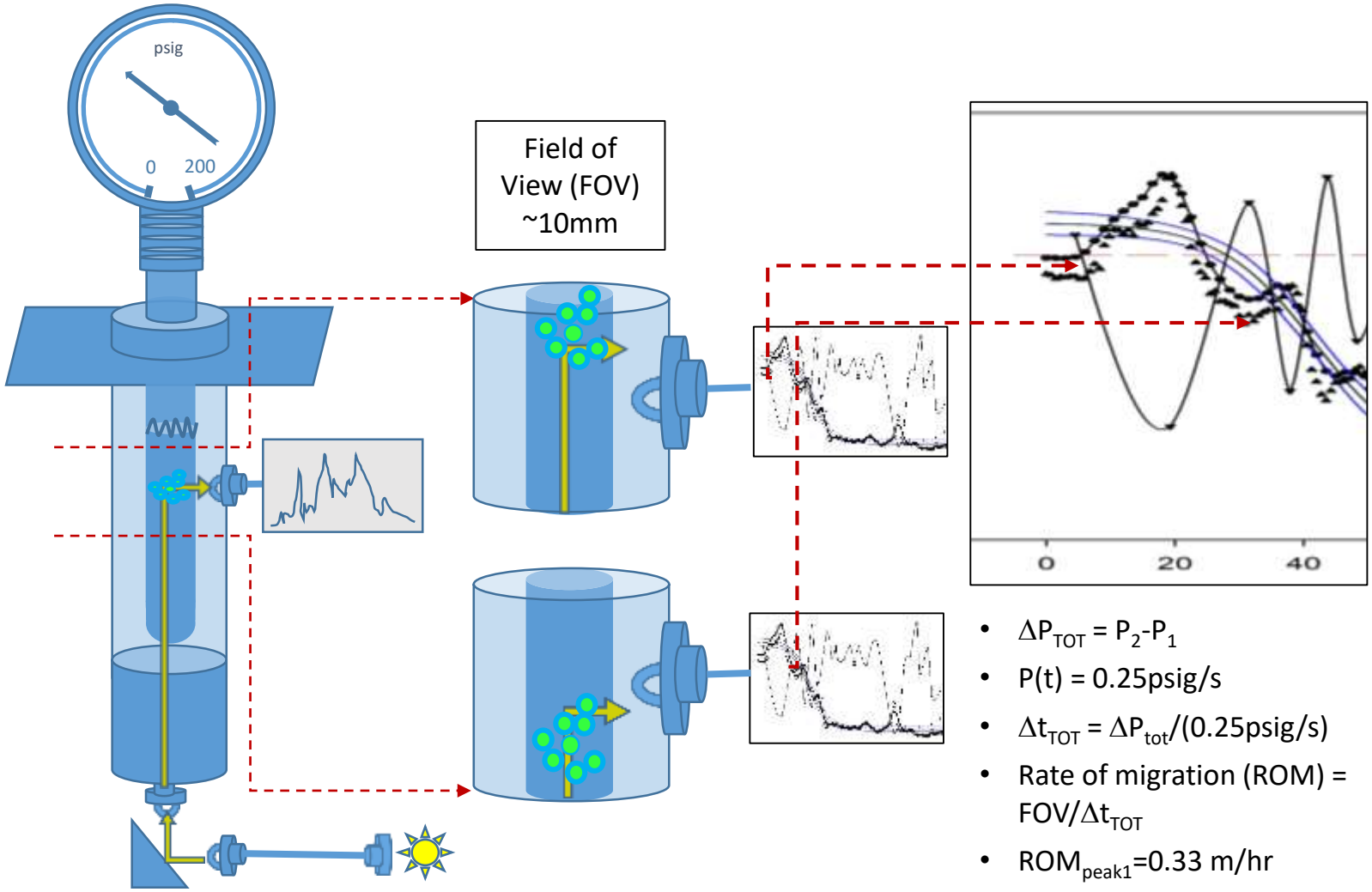
**Normality Test (Shapiro-Wilk)** Passed (P = 0.4713)

W Statistic= 0.9748 Significance Level = 0.0500

**Constant Variance Test** Failed (P = 0.0136)

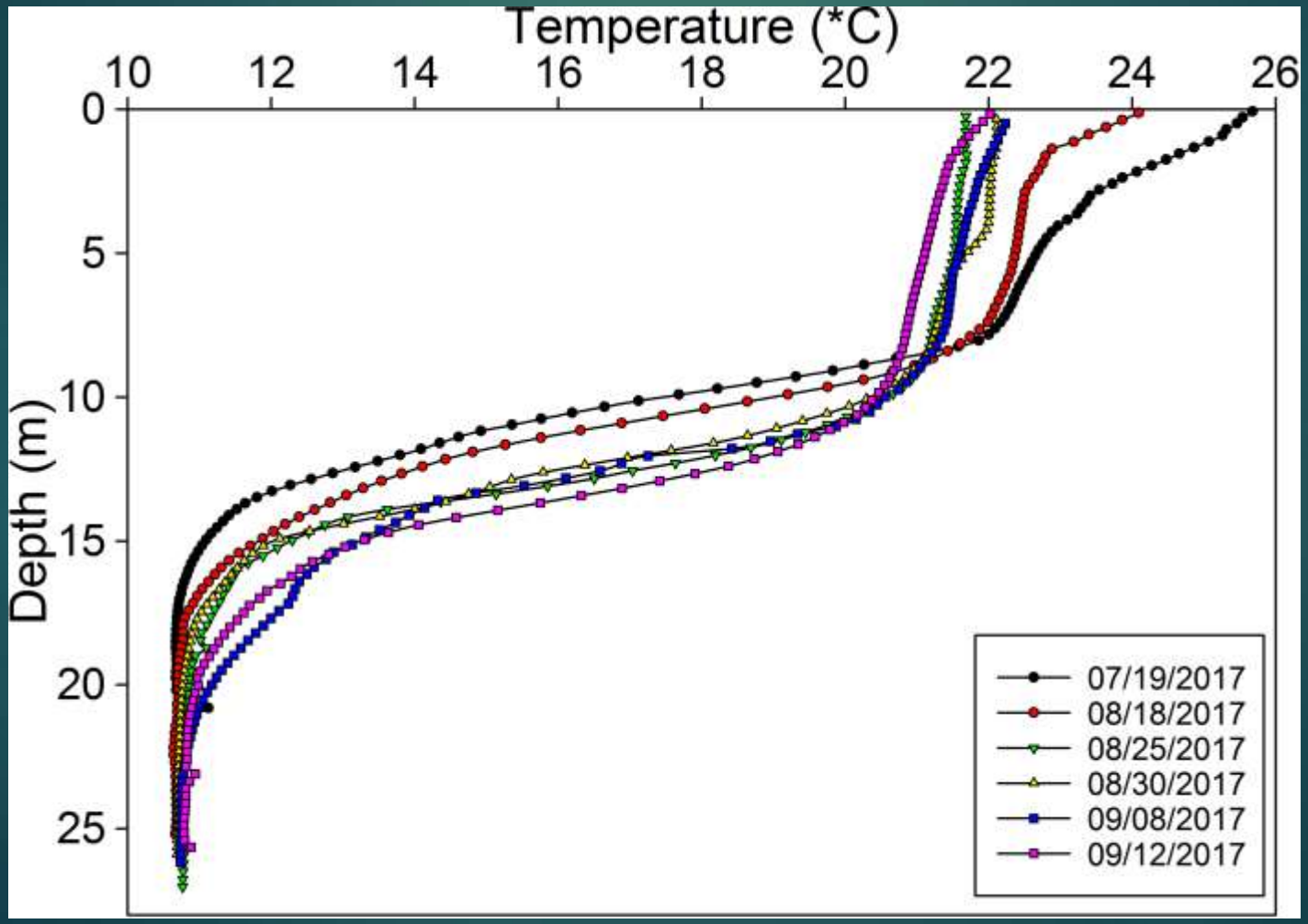
Turbidimetric growth profiling of *M. aeruginosa* from Carolina Biological Supply showing small ΔPcrit 0.5 during exponential growth





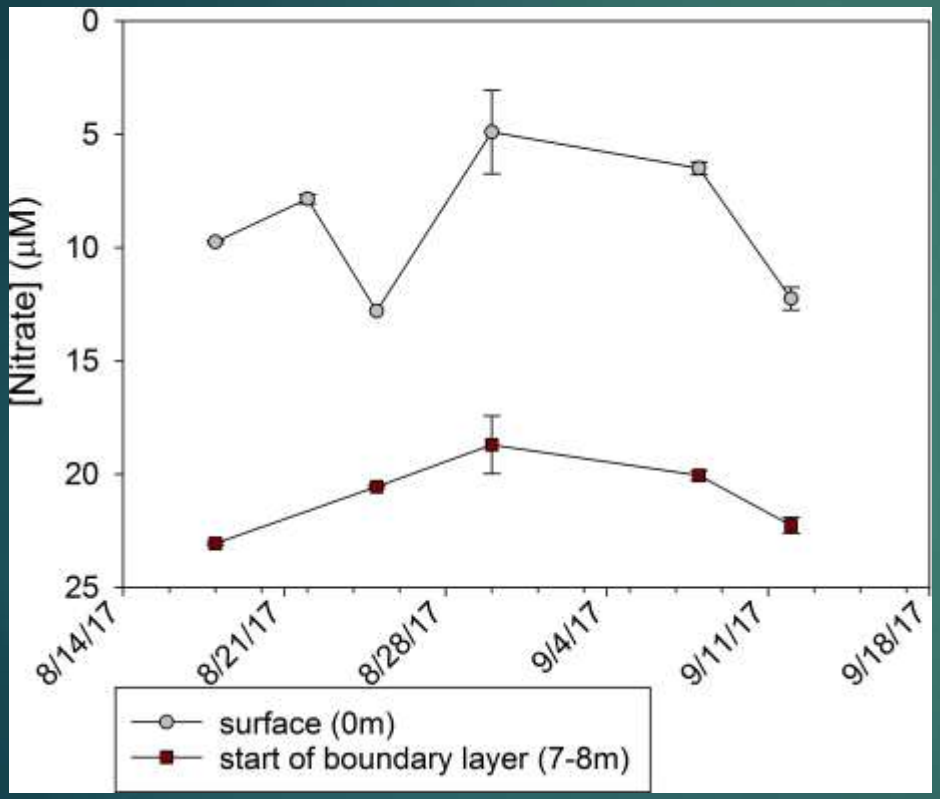

- $\Delta P_{TOT} = P_2 - P_1$
- $P(t) = 0.25 psig/s$
- $\Delta t_{TOT} = \Delta P_{tot}/(0.25 psig/s)$
- Rate of migration (ROM) = $FOV/\Delta t_{TOT}$
- $ROM_{peak1}$=0.33 m/hr

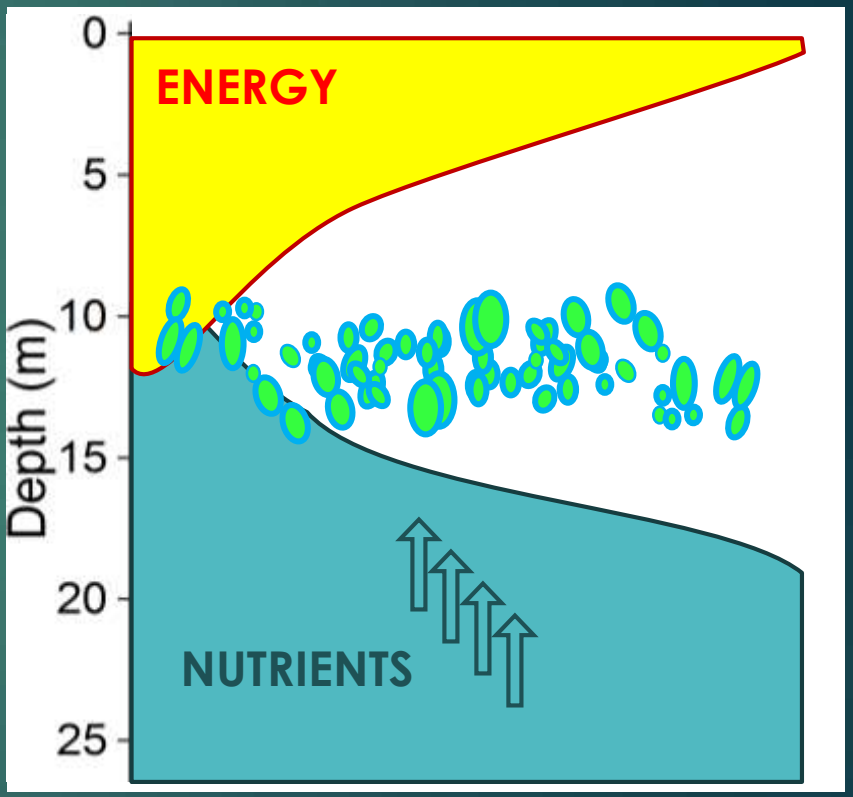
# Ross Island Lagoon Thermocline Progression (2017)




Dyer – unpublished results

# Thermocline progression leads to increased physical separation between access to nutrient and energy pools




Dyer – unpublished results

# LIGHT MATTERS!


Both *quality* and *quantity* of light have important effects on cyanobacterial metabolism

- Complementary chromatic adaptation
- Chlorosis
- MMA production
- Toxin production
- Buoyancy Regulation
- Etc.,
- Etc.,