Welcome to the
Benthic HABs Workgroup Webinar

January 29, 2020- 12:30 PM to 2:00 PM Pacific Daylight Time

Web Meeting Address: https://usace.webex.com/meet/jade.l.young
Meeting Number: 968 579 710
Phone Number: 1-888-363-4735
Access Code: 970 309 8

Have WebEx call your phone for best connection!
Otherwise, call-in using the phone number & access code




GUEST SPEAKER: ZACHARIAS SMITH

SPATIAL DISTRIBUTION AND METHODS FOR THE DETECTION OF PARALYTIC SHELLFISH TOXINS PRODUCED BY *MICROSEIRA (LYNGBYA) WOLLEI* IN NEW YORK STATE LAKES

ITEM I

Welcome, Introductions & Agenda Overview

Margaret Spoo-Chupka

AGENDA

I Welcome, Introductions & Agenda Overview
Margaret Spoo-Chupka

II Summary of CDC One Health HAB Surveillance Call
Margaret Spoo-Chupka

III Presentation: Spatial Distribution and Methods for the Detection of Paralytic Shellfish Toxins Produced by *Microseira (Lyngbya) wollei* in New York State Lake
Guest Speaker – Zacharias Smith

IV Follow up to Membership Survey Results – Setting up Networking Groups
Jade L. Young

V Open Discussion & Meetings
Christine Joab

ITEM II

Summary of CDC One Health HAB Surveillance Call

Margaret Spoo-Chupka

ITEM II
RESOURCES SHARED FROM CDC MEETING
MARGARET SPOO-CHUPKA

- California
  - Visual guide: https://mywaterquality.ca.gov/habs/what/visualguide_fs.pdf
  - Visual guide SOP: https://drive.google.com/file/d/0B40pxPC5g-D0R2QtUVZhYzNIaXc/view
  - FAQs for pets, livestock, and HABs: https://mywaterquality.ca.gov/habs/resources/domestic_animals.html
  - HAB Portal: https://mywaterquality.ca.gov/habs/index.html

- Wisconsin
  - Illness forms: https://www.dhs.wisconsin.gov/water/bg-algae/index.htm

ITEM III

GUEST PRESENTATION:

Spatial Distribution and Methods for the Detection of Paralytic Shellfish Toxins Produced by Microseira (Lyngbya) wollei in New York State Lakes

Zacharias Smith

Spatial Distribution and Methods for the Detection of Paralytic Shellfish Poisoning Toxins Produced by *Microseira* (*Lyngbya*) *wollei* in New York State Lakes

Zacharias J. Smith, Ph.D

Applied Sciences, Ramboll
State University of New York College of Environmental Science and Forestry
Syracuse, New York
Zach.Smith@ramboll.com

Benthic Cyanobacteria - *Phormidium*

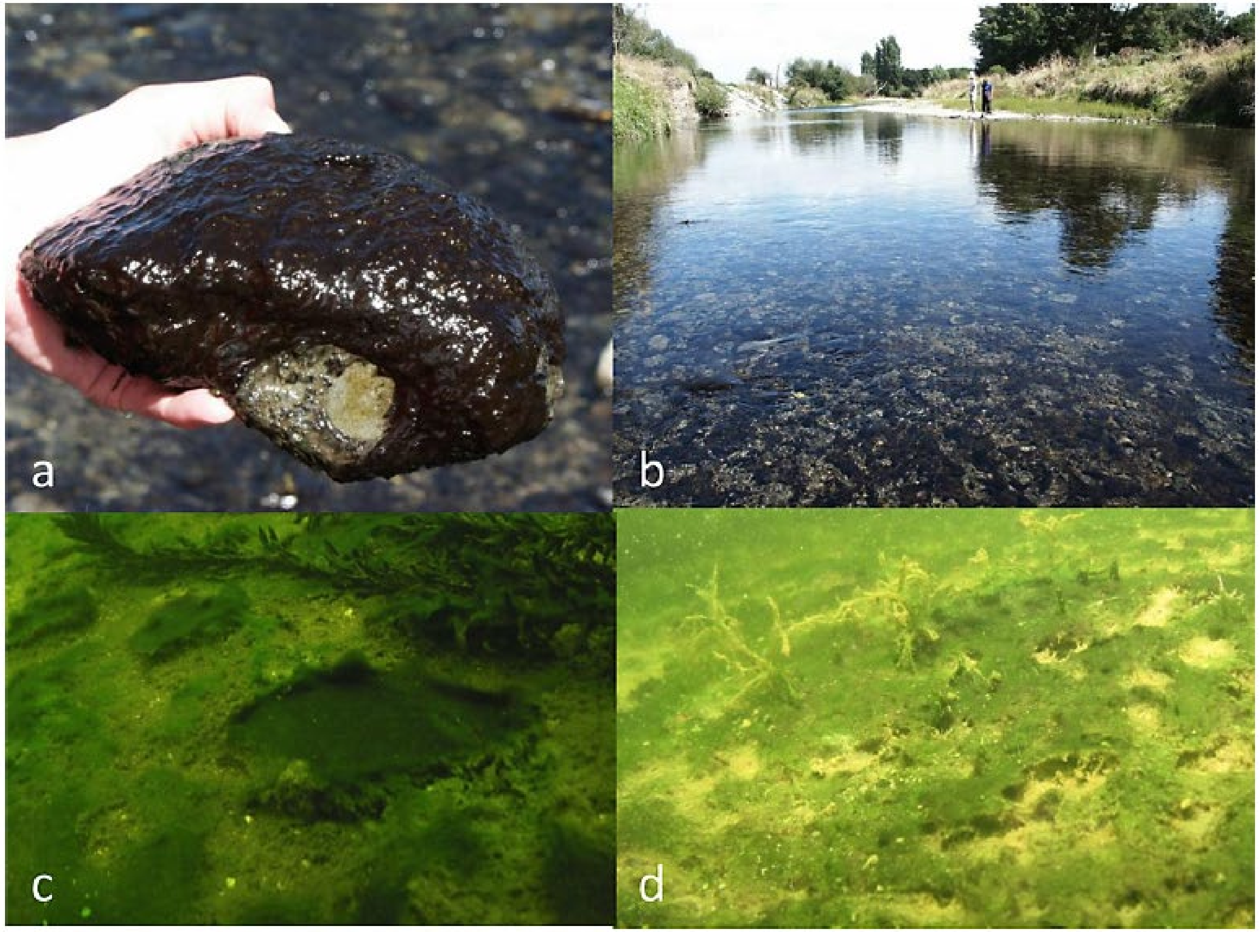
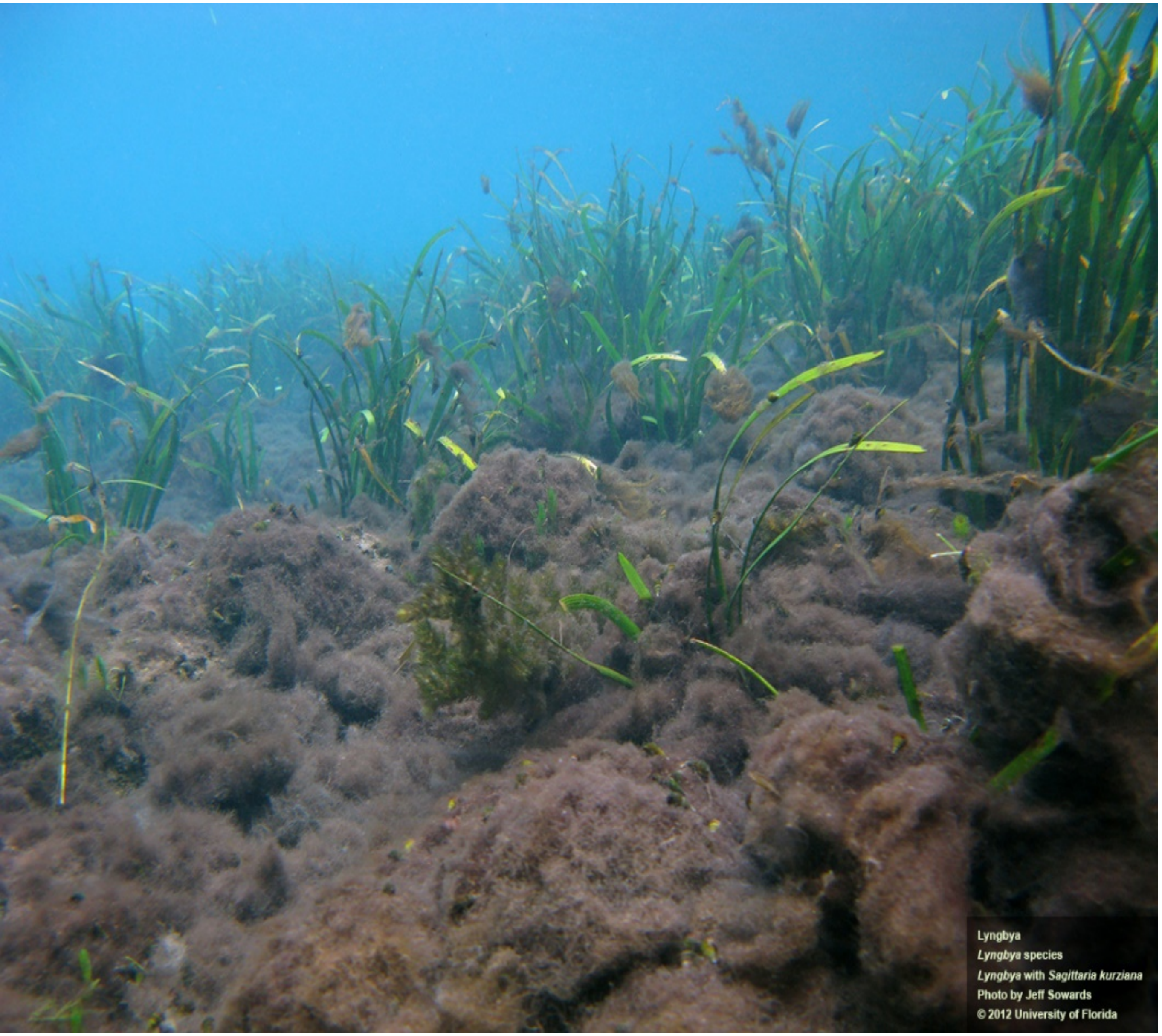
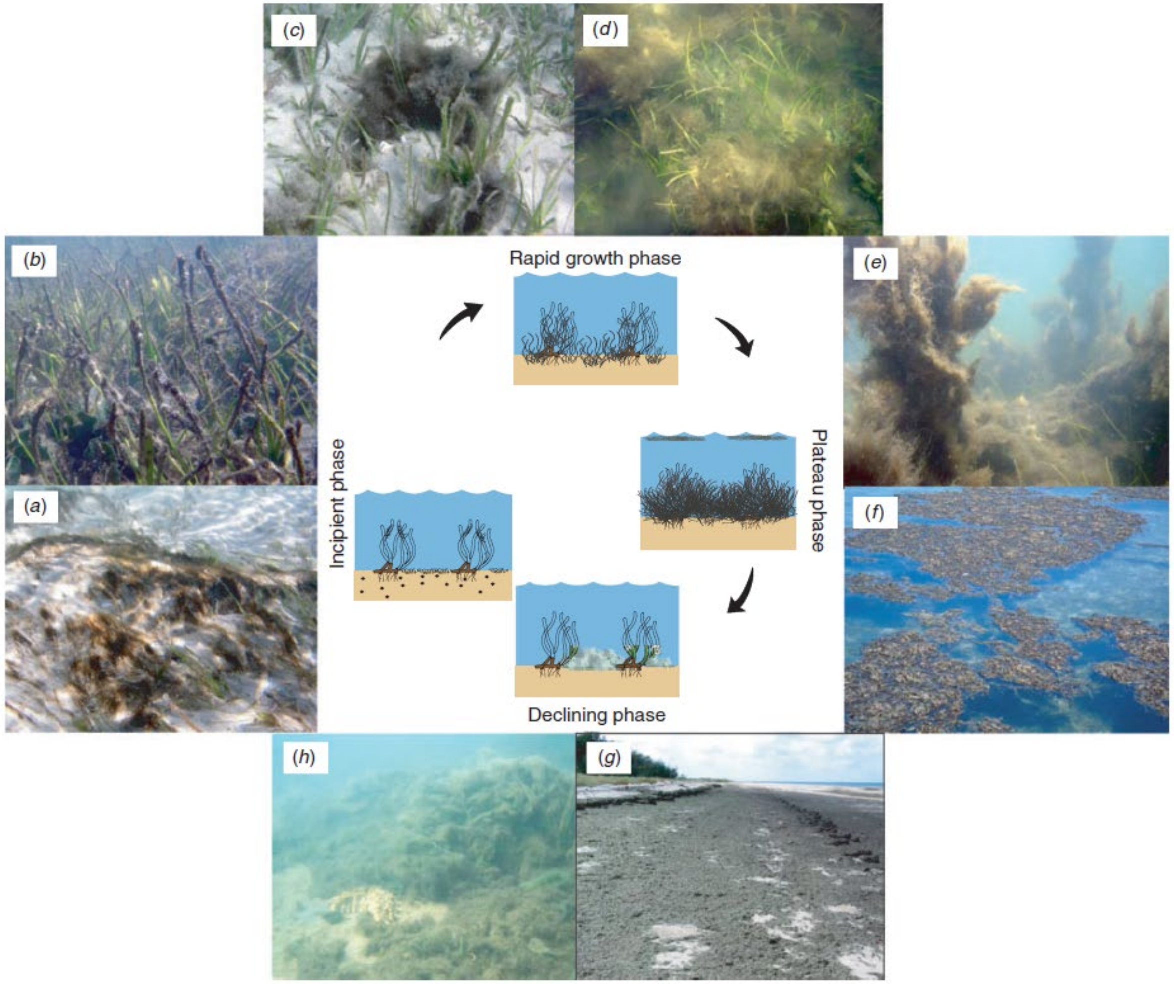


Fig. 1 — (a,b) *Phormidium* mats in the Waipoua River (North Island, New Zealand). Benthic mats in (c) Lake Rotoiti, (d) Lake Okareka (North Island, New Zealand). Photographers: a,b, Susie Wood (Cawthron, New Zealand); c,d, Rohan Wells (National Institute of Water and Atmospheric Research, New Zealand).

Microseira (*Lyngbya*)

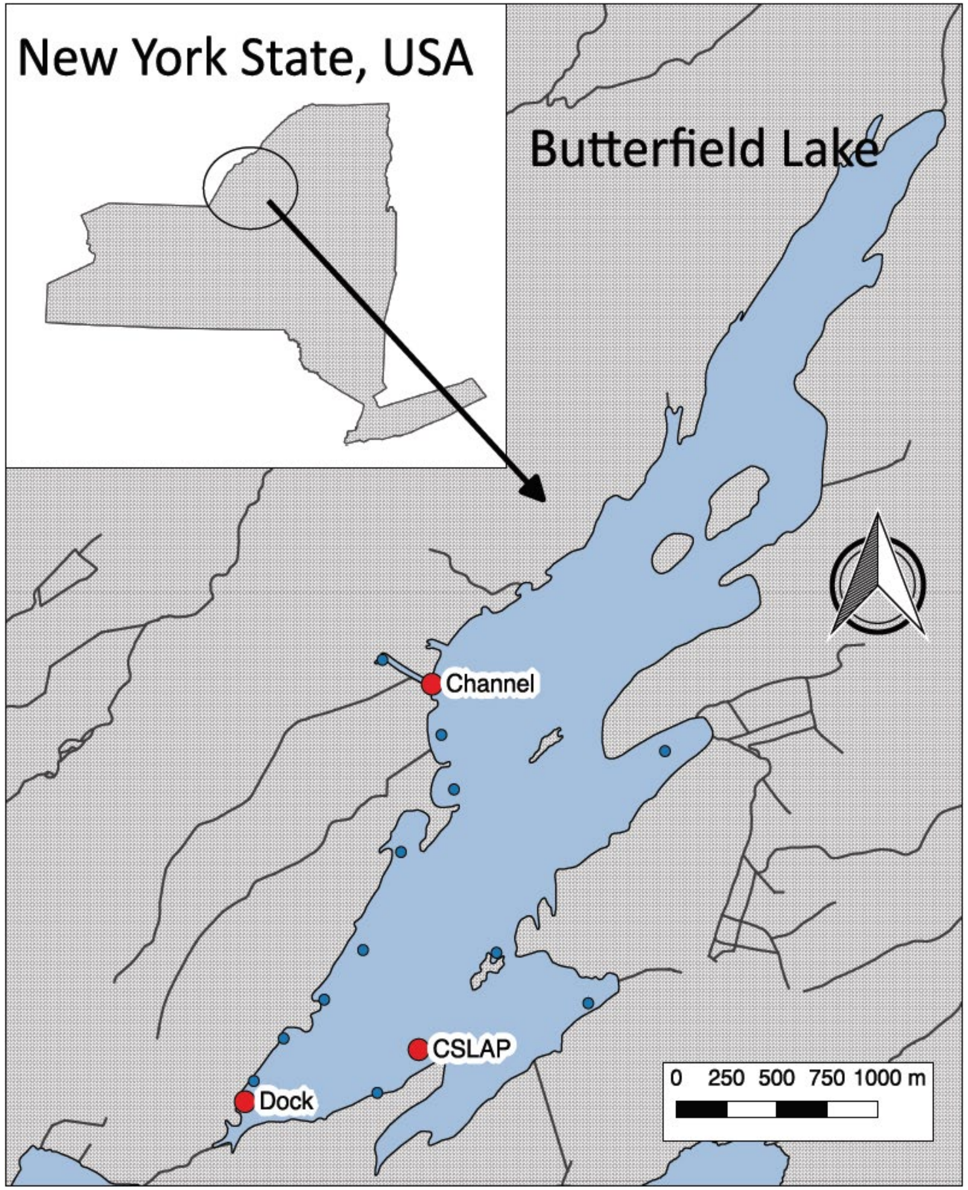




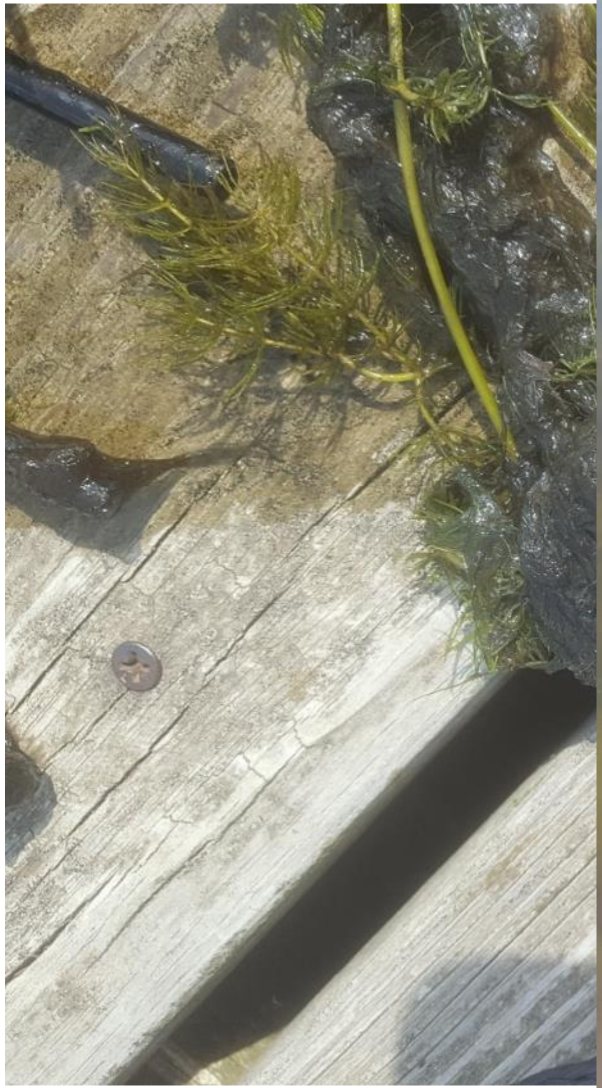
Marine *Microseira* off the coast of Queensland, Australia
Ahern et al., Mapping the distribution of, biomass, and tissue nutrient levels…, 2007

Butterfield Lake

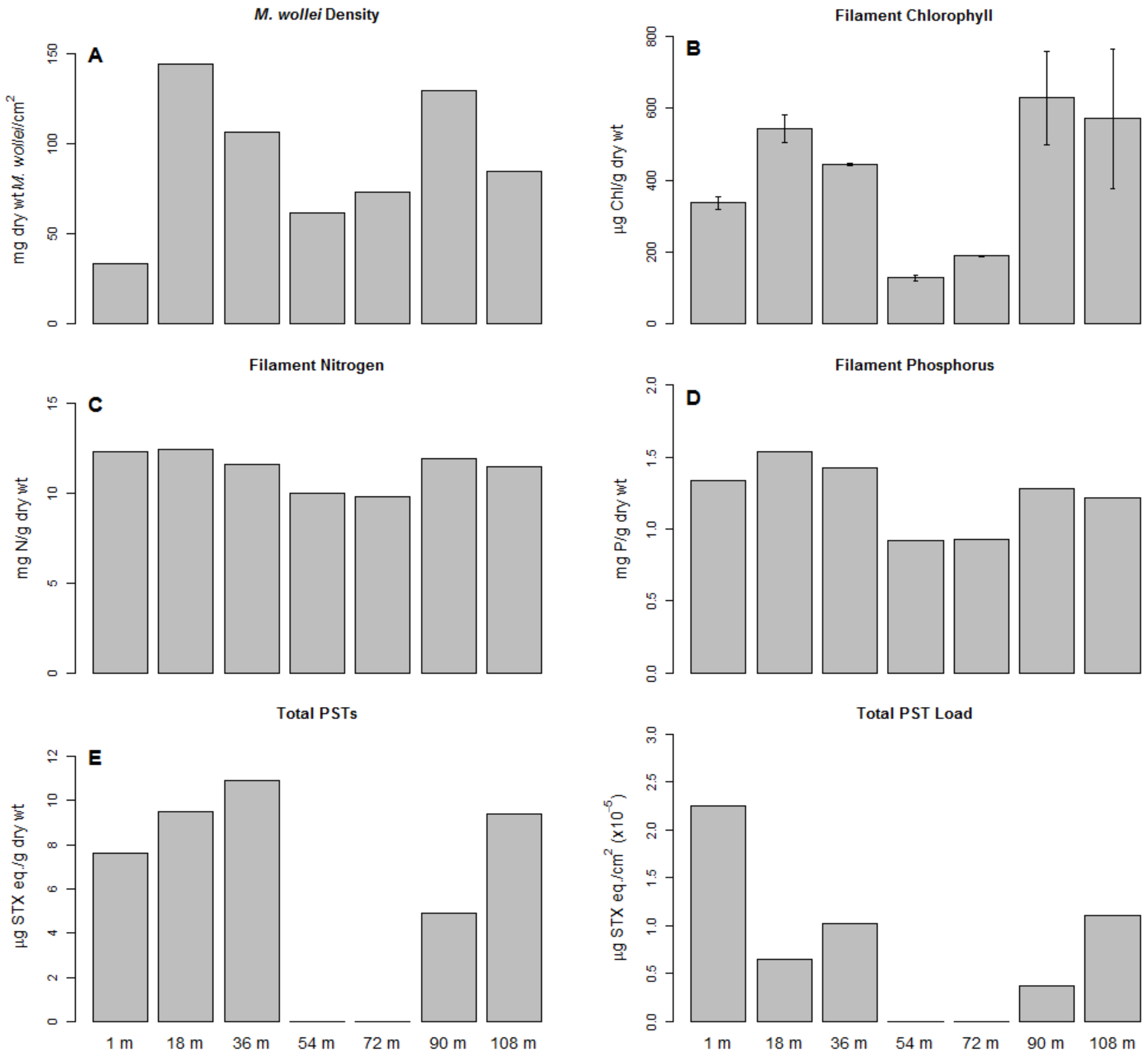
Sampling Sites in Butterfield Lake



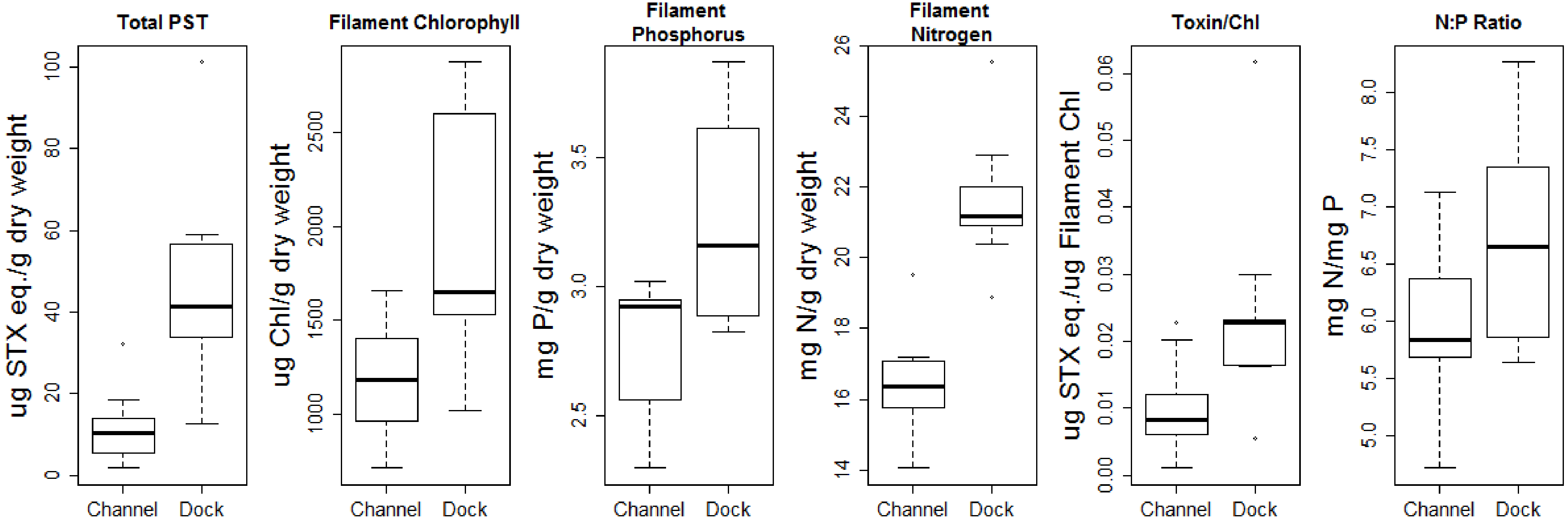
Microse *llei* at



Variability of *Microseira* Along the Channel



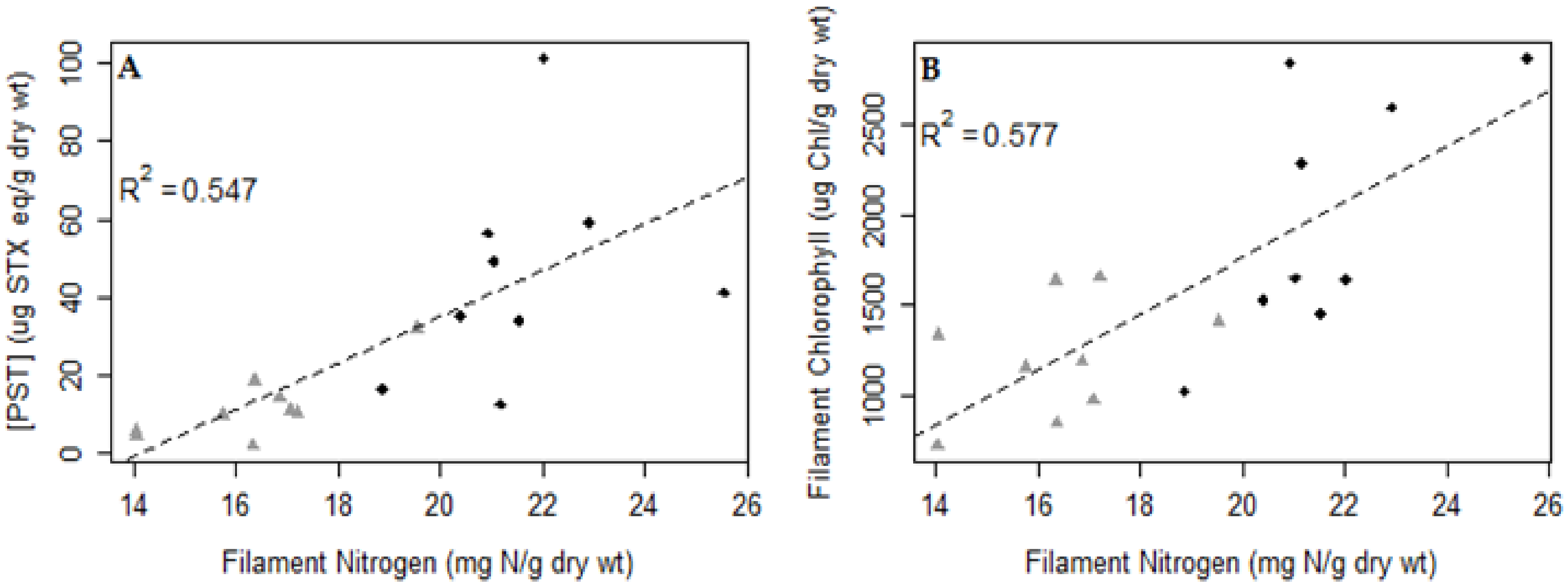
M. wollei at the Channel & Dock



Six parameters measured in *Microseira wollei* filaments collected biweekly at the Channel and Dock sites in 2017.

Chl & PSTs Relationships Varied between Sites

- Some relationships were simple, such as those between filament nitrogen and either chlorophyll or PST content

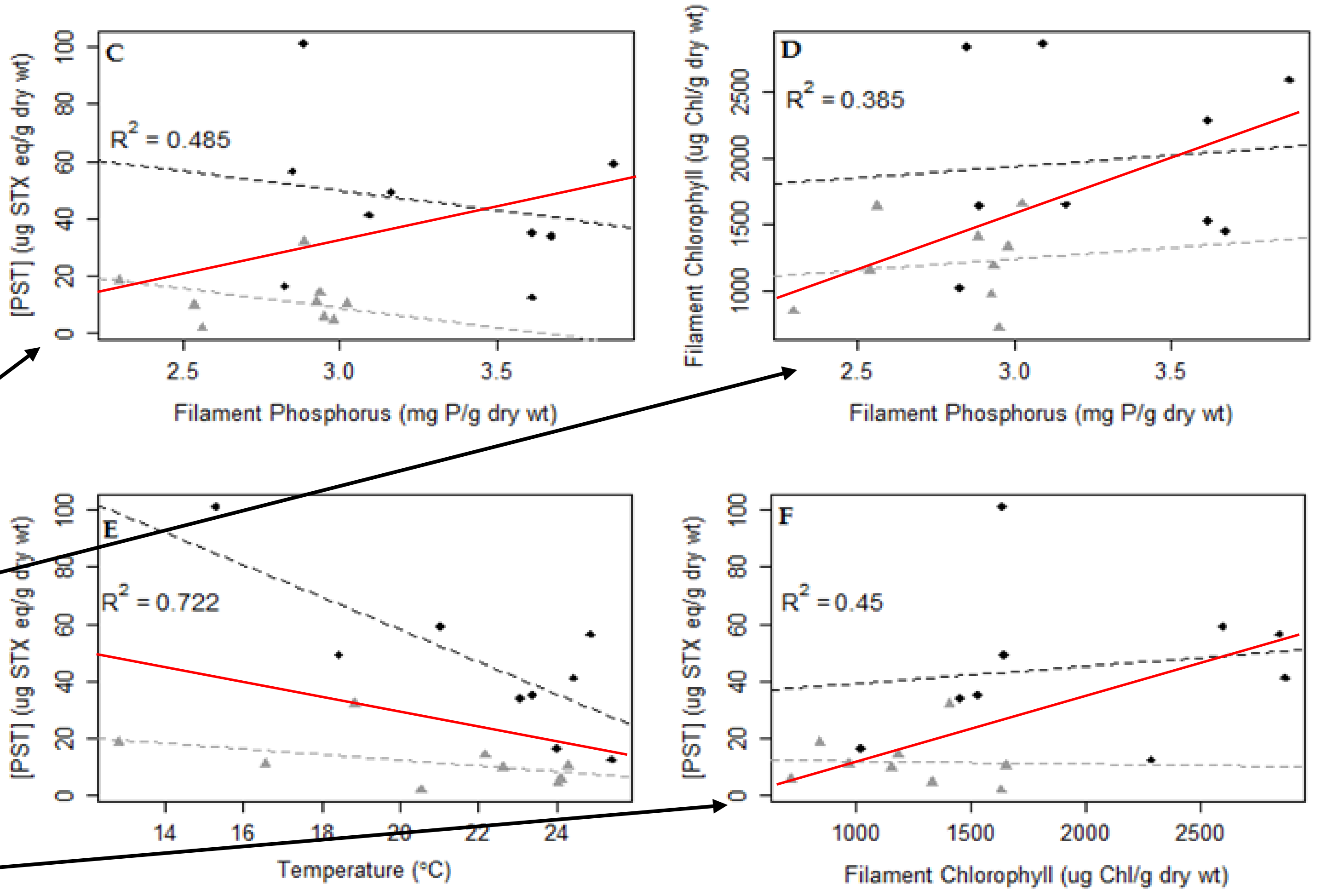


Analysis of Covariance (ANCOVA) or Dummy Variable Regression

- ANCOVA tests whether the slopes and intercepts between two lines are significantly different
- Site was important for both PSTs and Chl
  - But it was not the same in all cases
- Regression without evaluating site as a predictor could produce misleading conclusions

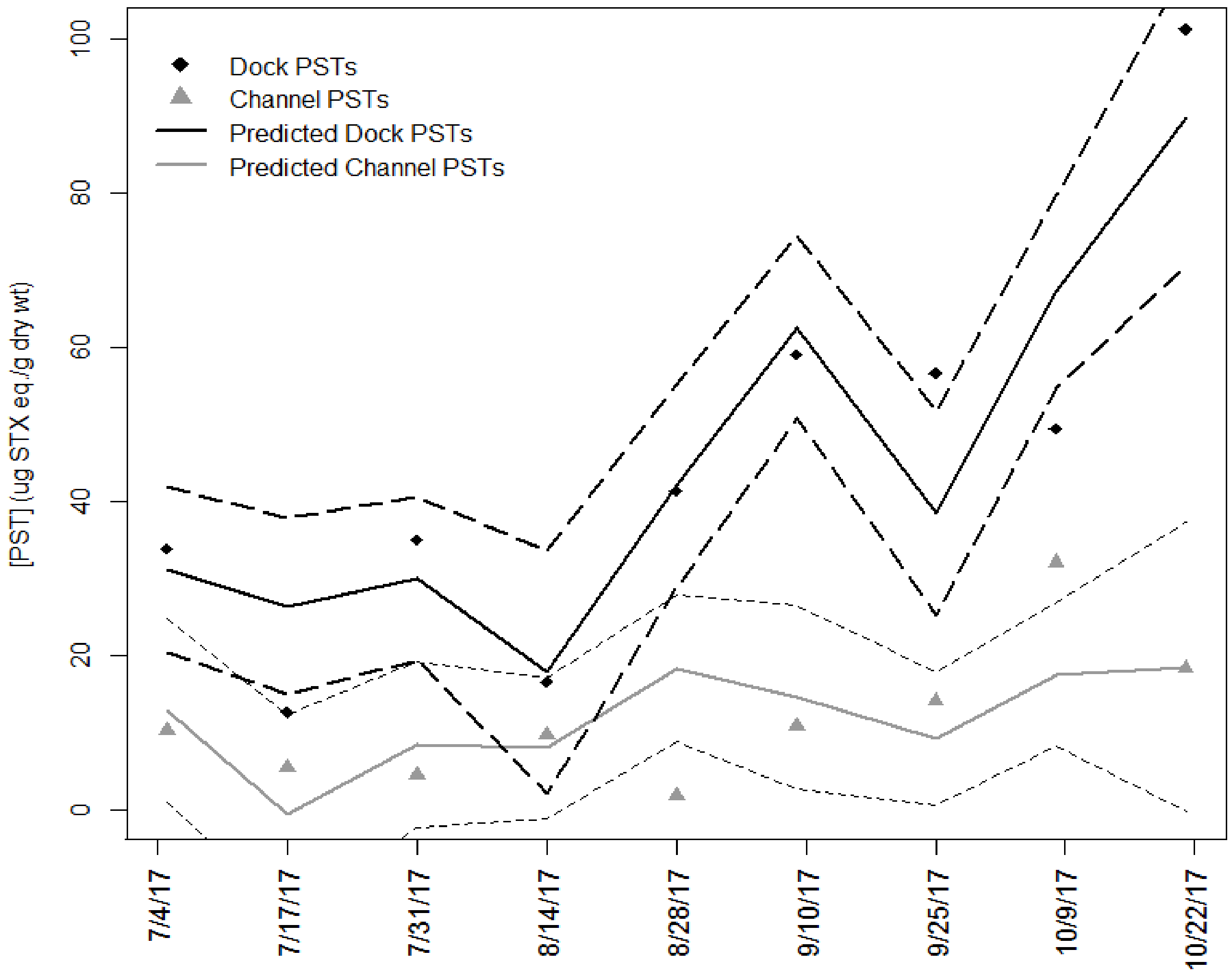
Different intercepts

Different Slopes and Intercepts



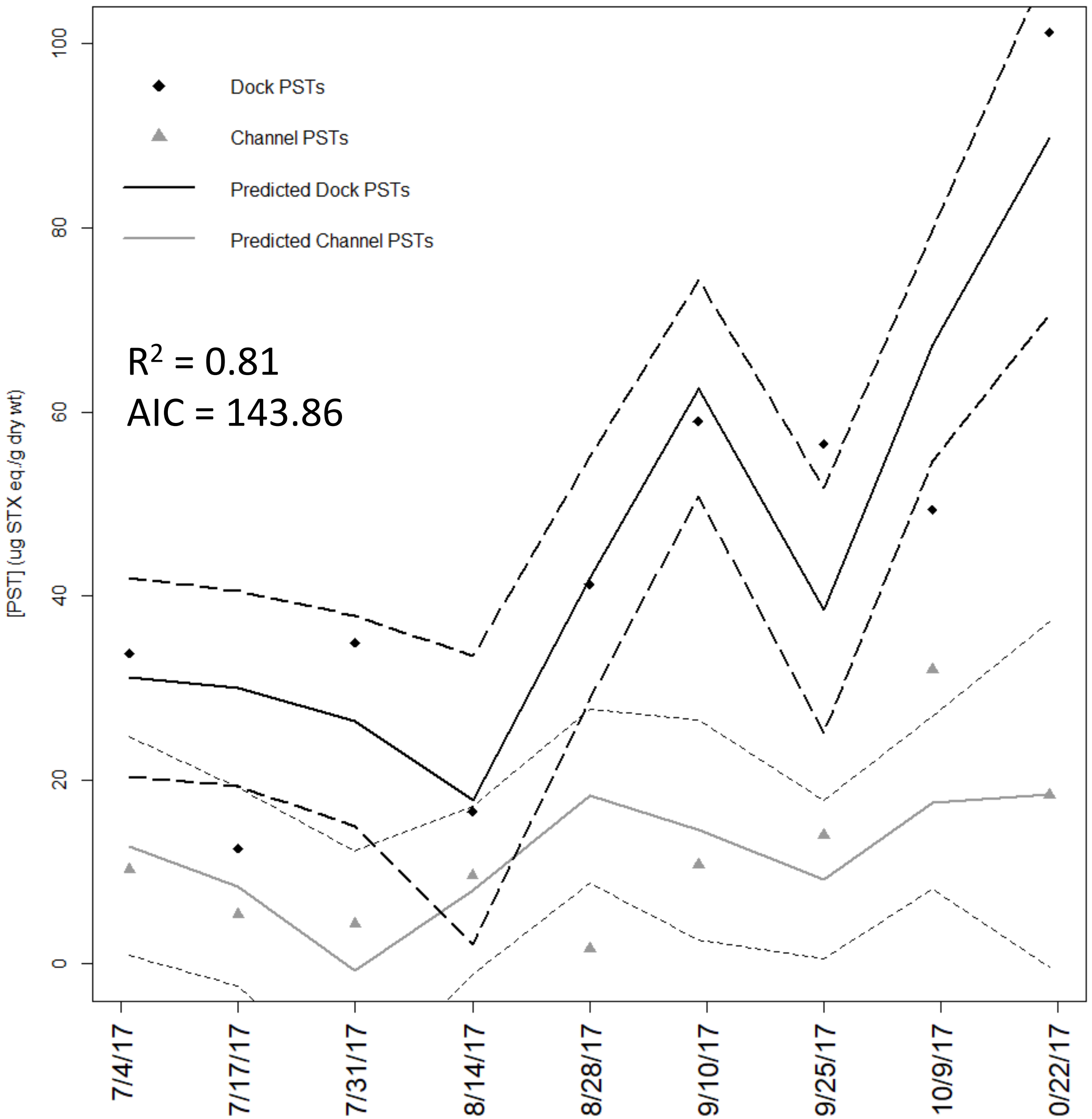
Multiple Regression for PSTs

- Temperature was crucial in explaining the differences in toxin between the sites
- Nutrient terms were not included in the model
- Some points fell outside the 95% CI, did not measure all parameters (PAR)

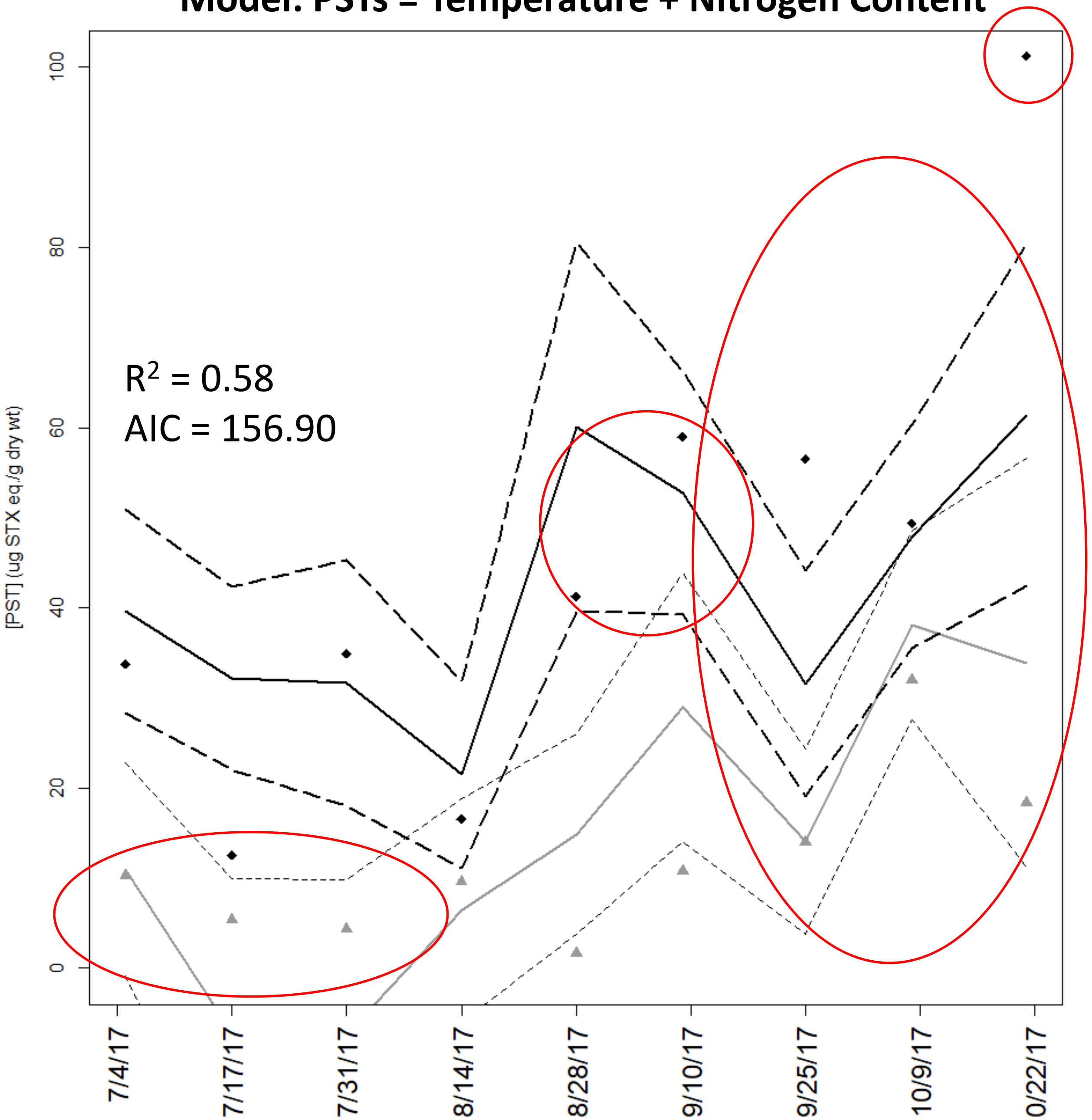


Model: [PSTs] = [chlorophyll] + (temperature*site)

Original Model (with Temperate/Site Interaction)



Example Model (No Site or Site Interaction)
Model: PSTs = Temperature + Nitrogen Content



Benthic PSTs in Butterfield Lake

- *M. wollei* were not distributed evenly, where mats were found at 2/15 sites, nor were the cyanobacteria mats distributed evenly at the two sites
  - Presence may have been related to competition with plants
  - Identifying sites containing *Microseira* in lakes with low water clarity is a significant challenge
- PST abundance was site specific and changed over time
- Temperature was not necessarily a driver, as it may correlate with other parameters (e.g. PAR)
- *M. wollei* were N deficient (N:P ~ 6-7) in a heavily P deficient lake (N:P ~ 40)
  - Likely source of P was from sediments

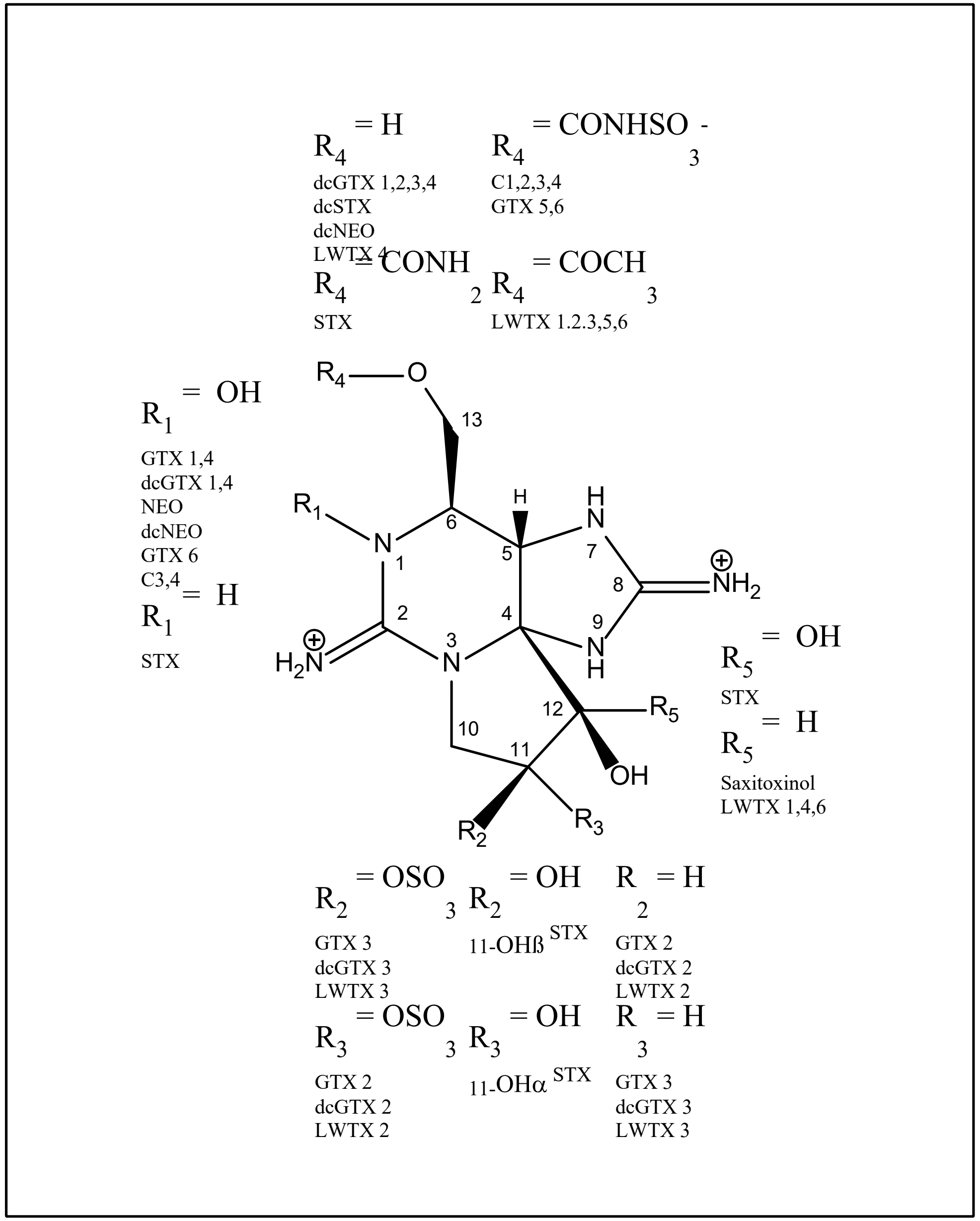
Paralytic Shellfish Toxins (PSTs)

- >60 different variants
- Wide range in toxicity
  - Saxitoxin LD-50
    10 ug/kg (i.p.)
  - C-toxins and LWTXs
    ~10-10,000x less toxic

- Multiple Detection Methods
  - Receptor Binding Assay
  - ELISA
  - Fluorescence
  - Mass Spectrometry

"Untargeted" (Receptor Binding Assay, ELISA, Fluorescence)

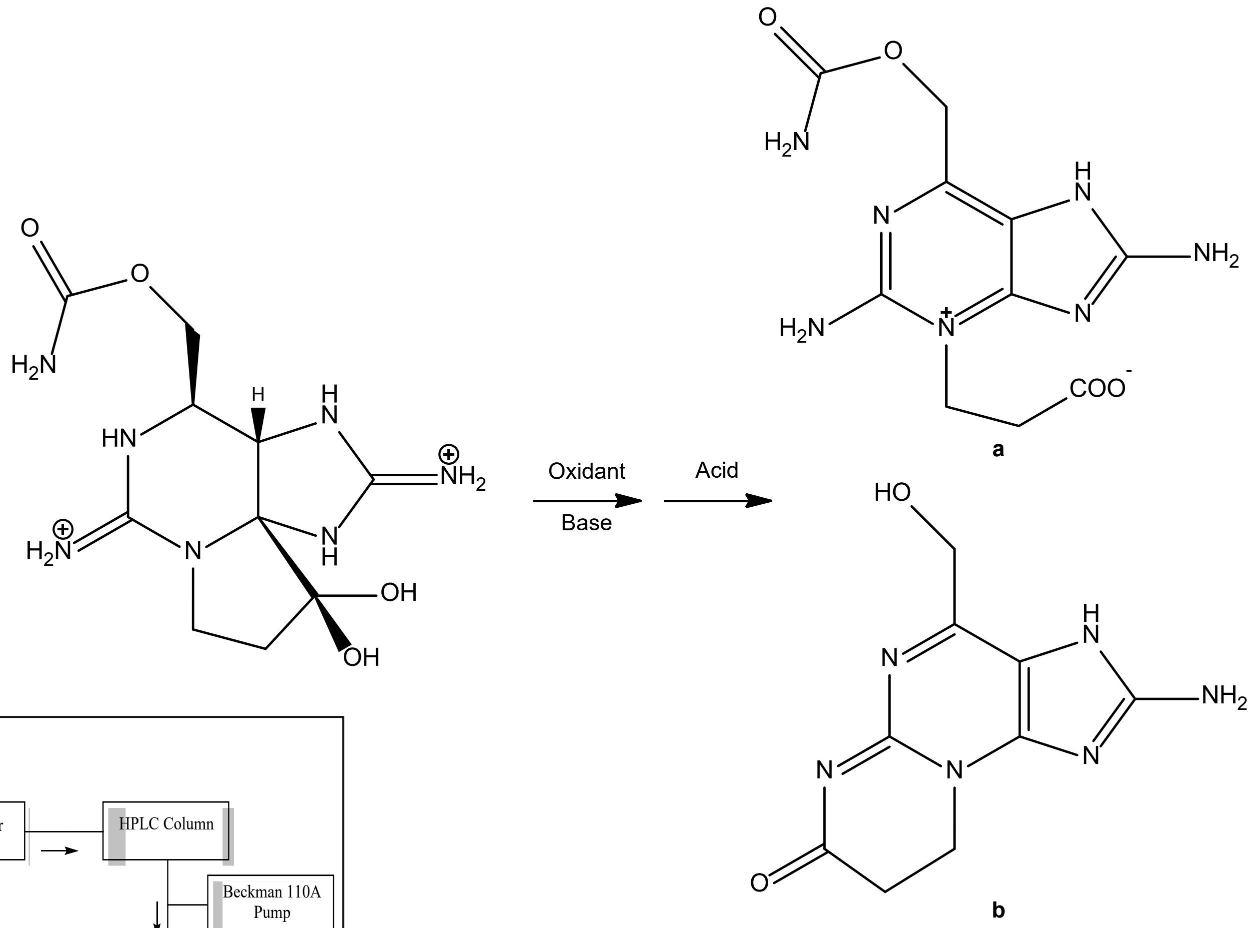
"Targeted" (Mass Spectrometry)

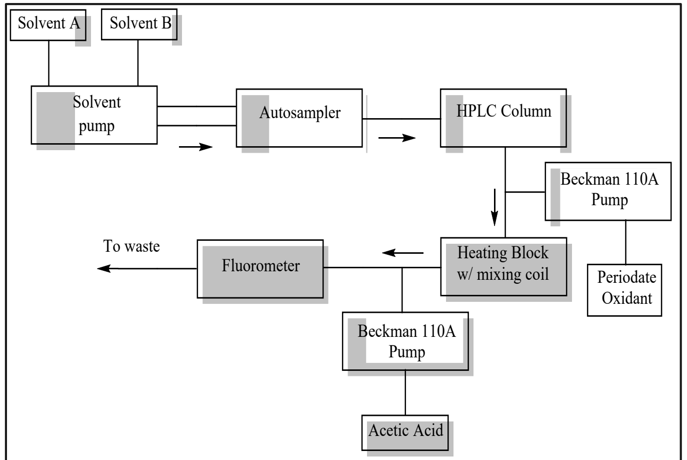


Relative Response to Saxitoxin for Different PST Methods

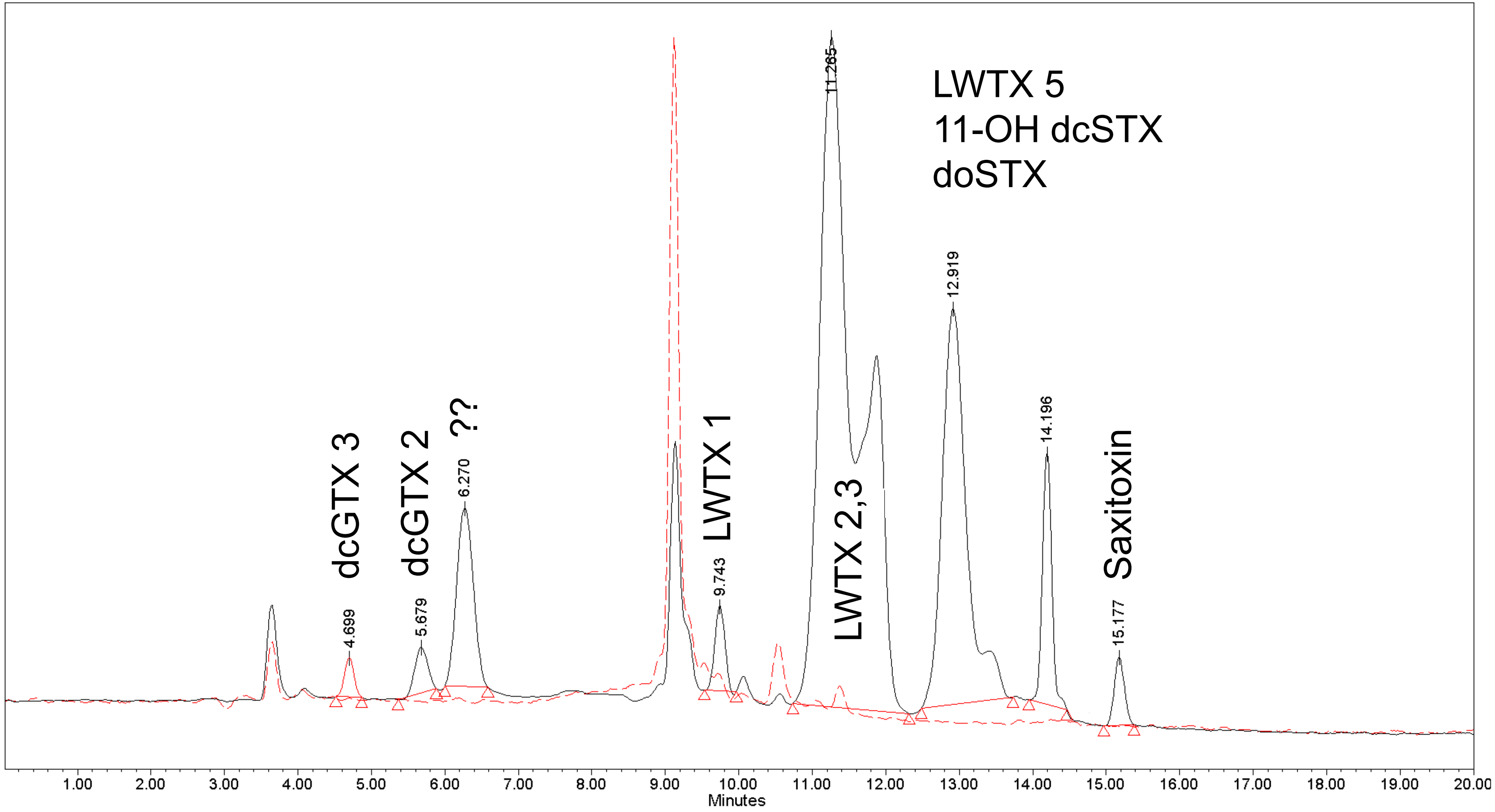
Toxin	PCOX Response Relative to STX	Mouse Bioassay Relative Toxicity [73]	Abraxis STX-ELISA Cross-reactivity[a] [65]	Receptor Binding Assay Cross-reactivity [91]
STX	1	1	1	1
NEO	0.41	0.5-1.2	0.013	0.73
GTX1	0.10	0.8-1	<0.02*	1.04**
GTX2	4.66	0.4	0.23*	0.34**
GTX3	3.52	0.6-1.1	0.23*	0.34**
GTX4	0.08	0.3-0.7	<0.02*	1.04**
GTX5	0.71	0.1-0.2	0.23	0.033
GTX6	0.44	0.1	-	-
dcSTX	1.13	0.4-1.02	0.29	0.10
dcNEO	0.30	0.02-0.4	0.06	-
dcGTX1	-	0.5	-	-
dcGTX2	2.71	0.2-0.3	0.014*	-
dcGTX3	2.46	0.2-0.5	0.014*	-
dcGTX4	-	0.5	-	-
LWTX1	0.09	0 [29]	0.13*	-
LWTX2	-	0.11 [29]	0.13*	-
LWTX3	-	0.06 [29]	0.13*	-
LWTX4	-	0 [29]	0.13*	-
LWTX5	-	0.14 [29]	0.13*	-
LWTX6	-	0 [29]	0.13*	-
C1+C2	1.20	0-0.2	-	-

Analytical Methods for Benthic PSTs

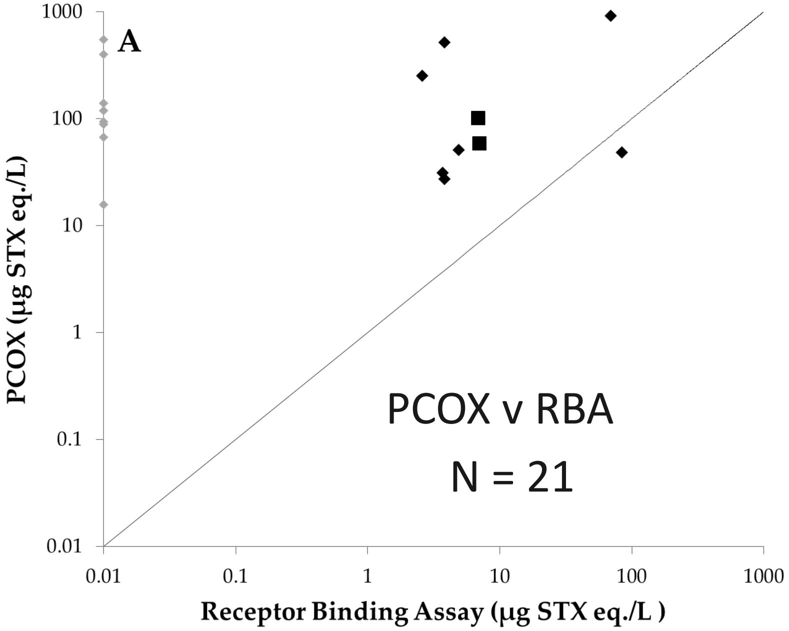


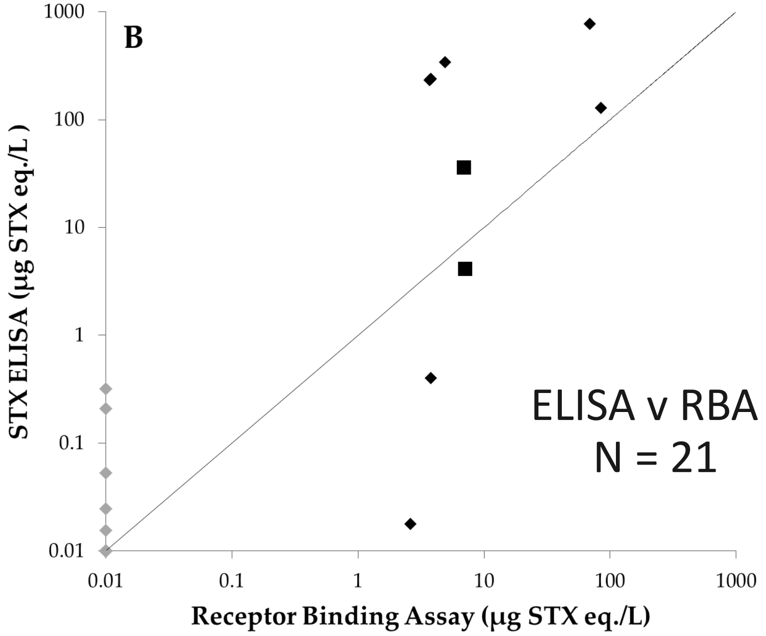


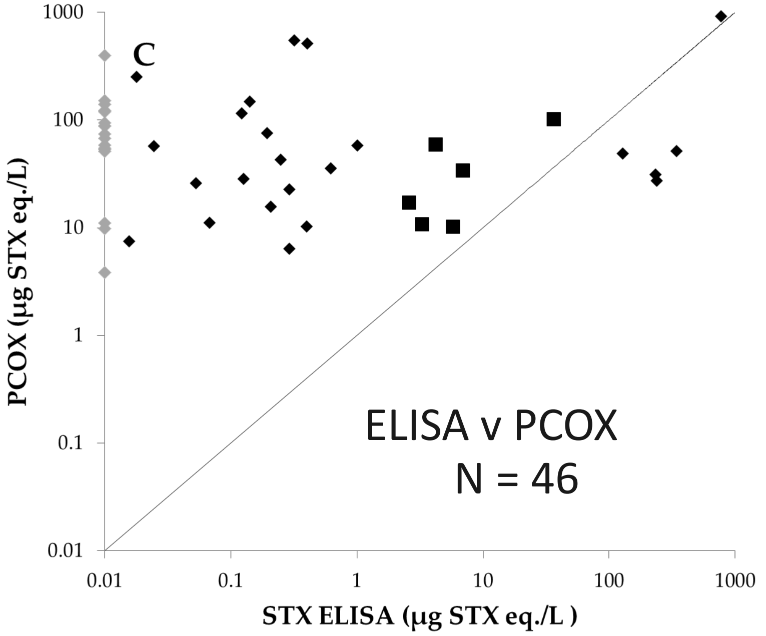
Paralytic Shellfish Toxins in *Microseira*



ELISA vs PCOX vs RBA







All toxic by PCOX
Top Right: 13/21 non-toxic
Top Left: 12/21 non-toxic
Bottom: 16/46 non-toxic

■ : Lyngbya (µg/g)
◆: Water Samples (µg/L)
◆: Non-toxic by RBA or ELISA

Are These Toxin Concentrations Dangerous?

Table 1. Paralytic shellfish poisoning toxin (PST) concentrations measured in six samples by three different analytical methods.

Sample Date	Dock			Channel		
	HPLC-FL [1]	ELISA [1]	LC-MS/MS [2]	HPLC-FL [1]	ELISA [1]	LC-MS/MS [2]
7/4/2017	33.77	6.94	22.33	10.23	5.75	3.63
9/10/2017	58.98	4.18	20.12	10.81	3.27	4.85
10/22/2017	101.25	36.24	37.95	16.00	2.58	9.56

[1] Total PSTs calculated in μg saxitoxin (STX) eq./g dry wt. [2] LC-MS/MS PSTs were quantified using 12 common marine PST standards. Standards were not available for the lyngbyatoxins (LTXs) so the contributions of these toxins to the total PST pool as measured by LC-MS/MS were not included.

	HPLC-FL	ELISA	Receptor Binding Assay
9/10/2017	58.9 ug/g	4.2 ug/g	7.0 ug/g
10/22/2017	101 ug/g	36.2 ug/g	6.9 ug/g
Indian Lake, NY	923 ug/g	51.4 ug/g	69 ug/g

Possibly, but it depends on the method used for detection

Are the PSTs a Danger to Humans? Dogs?

- Using an estimated no-observed-adverse-effect-level (NOAEL) of 0.5 ug STX eq./kg bodyweight
- 7 ug STX eq./g dry weight Butterfield Lake *Microseira*
- Highest dry weight percent *Microseira* of 15%
- Estimated with a 65 kg human

- Estimated dose for a lethal dose in humans – 300-800 ug

Low-level Exposure

$$\frac{\frac{0.5ug\ \text{STX eq.} \times 65\ \text{kg}}{kg\ bodyweight}\ \text{x}\ 65\ kg\ human}{Microseira\ PST\ Concentration * 15\%\ dry\ weight}$$

$$= \boldsymbol{\sim 31\ g\ wet\ weight\ Microseira}$$

Potentially Lethal Dose

$$\frac{\frac{300 - 800\ ug\ \text{STX eq.}}{Human}}{Microseira\ PST\ Concentration * 15\%\ dry\ weight}$$

$$= \boldsymbol{\sim 285 - 761\ g\ wet\ weight\ Microseira}$$

Assuming the same toxicity in dogs, Microseira mass needed to cause illness may be much lower

Analysis of PSTs Produced by *Microseira* and Potential Health Risks

- *M. wollei* produce high concentrations of total PSTs
  - Many variants are much less toxic than saxitoxin
  - Therefore potential toxicity as measured by the receptor binding assay may be lower than is suggested by some methods
- Humans are unlikely to consume the mass of material needed to cause illness, as the *Microseira* are not highly toxic
  - Acute die-off of cyanobacteria and release of toxins into the water column could cause illness
  - Animals with lower body weight, including dogs, are at higher risk than humans.
- Different analytical methods give different results for PSTs.
  - The lack of available standards for the majority of known PSTs (less than a third of known variants) further increases the difficulty of analysis relative to most other cyanobacterial toxins.

Acknowledgements

- Thank you to Dr. Gregory Boyer for his guidance during this and other projects
- Thank you to Juliette Smith, Pearse McCarron, Andrew Turner, Greg Doucette, Tod Leighfield, Dan Beach, and Marta Sanderson for all of their assistance with the multi-method comparisons
- Thank you to my new colleagues at Ramboll who provided valuable comments for the presentation

RAMBOLL

NOAA NCCOS

esf

National Research Council Canada / Conseil national de recherches Canada

VIMS | WILLIAM & MARY
VIRGINIA INSTITUTE OF MARINE SCIENCE

Cefas

toxins

Article

Spatial and Temporal Variation in Paralytic Shellfish Toxin Production by Benthic *Microseira (Lyngbya) wollei* in a Freshwater New York Lake

Zacharias J. Smith [1,*], Robbie M. Martin [2], Bofan Wei [1], Steven W. Wilhelm [2] and Gregory L. Boyer [1]

1 Department of Chemistry, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA; bwei101@syr.edu (B.W.); glboyer@esf.edu (G.L.B.)
2 Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA; rmarti49@vols.utk.edu (R.M.M.); wilhelm@utk.edu (S.W.W.)
* Correspondence: zjsmith@syr.edu

Received: 3 December 2018; Accepted: 9 January 2019; Published: 15 January 2019

Abstract: Butterfield Lake is a mesotrophic lake in New York State where residents and pets have experienced unexplained health issues. *Microseira wollei* (basionym *Lyngbya wollei*) was found at two of 15 sites in Butterfield Lake and analyzed for microcystins, anatoxins, cylindrospermopsins, and paralytic shellfish poisoning toxins (PSTs). Only PSTs and trace levels of anatoxin-a were detected in these samples. This is the first published report of PSTs within a New York State lake. To evaluate the environmental and temporal drivers leading to the observed toxicity, PST content at the two sites

FOOD BIOLOGICAL CONTAMINANTS

Ultrahigh-Performance Hydrophilic Interaction Liquid Chromatography with Tandem Mass Spectrometry Method for the Determination of Paralytic Shellfish Toxins and Tetrodotoxin in Mussels, Oysters, Clams, Cockles, and Scallops: Collaborative Study

Andrew D. Turner, Monika Dhanji-Rapkova, and Sum Y.T. Fong
Centre for Environment, Fisheries and Aquaculture Science, Barrack Rd, The Nothe, Weymouth, Dorset DT4 8UB, United Kingdom
James Hungerford
U.S. Food and Drug Administration, 22201 23rd Dr, S.E., Bothell, WA 98021
Paul S. McNabb
Private Consultancy, 7 Elliott St, Nelson 7010, New Zealand
Michael J. Boundy and D. Tim Harwood
Cawthron Institute, 98 Halifax St East, Nelson 7010, Private Bag 2, Nelson 7042, New Zealand

Collaborators: S. Aanrud; C. Alfonso; M. Alvarez; Z. Amzil; A. Antelo; F. Arévalo; M. Barrera; E. Braekevelt; J. Bunæs; E. Cagide; M. Cangini; D. Carrasco; M. Casey; P. Chan; D. Faulkner; A. Gago-Martínez; R. Gibbs; R. Hatfield; C. Hayman; O. Hiroshi; V. Hort; J. Hutchinson; T. Jordan; M. Mariappan; J. Leão; B. Maskrey; P. McCarron; S. McGrath; A. Milandri; S. Milandri; A. Moroño; S. Murray; M. Nicolas; A. Queijo; M. Pompei; D. Réveillon; W. Rourke; C. Salgado; V. Savar; B. Stokes; B. Suarez-Isla; T. Suzuki; K. Thomas; R. Watanabe

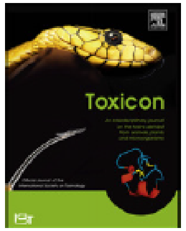
Background: **An ultrahigh-performance LC (UHPLC)–tandem MS (MS/MS) method for determination of paralytic shellfish poisoning toxins and tetrodotoxin (TTX) in bivalve molluscs was developed. To be used for regulatory testing, it needed to be validated through collaborative study.** ***Objective:*** **The aim was to conduct a collaborative study with 21 laboratories, using results to assess method performance.** ***Methods:*** **Study materials incorporated shellfish species mussels, oysters, cockles, scallops, and clams and were assessed to demonstrate stability and homogeneity. Mean concentrations determined by participants for blind duplicate samples were used to assess reproducibility, repeatability, and trueness.** ***Results:*** **Method performance characteristics were excellent following statistical assessment of participant method accuracy against expected values. No significant difference was found in the trueness results determined by different chromatographic column types. Acceptability of the between-laboratory reproducibility for individual analytes was evidenced by >99% of valid Horwitz ratio values being less than the 2.0 limit of acceptability. With excellent linearity and sensitivity fit-for-purpose over a range of mass spectrometer instruments, the UHPLC-MS/MS method compared well against other detection methods. It includes additional paralytic shellfish toxin (PST) analogues as well as TTX, which, to date, have not been incorporated into any other hydrophilic marine toxin official method of analysis.** ***Conclusions:*** **The results from this study demonstrate that the method is suitable for the analysis of PST analogues and TTX in shellfish**

Toxicon 60 (2012) 1148–1158

Contents lists available at SciVerse ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon



Investigation of extraction and analysis techniques for *Lyngbya wollei* derived Paralytic Shellfish Toxins

Amanda J. Foss [a,b,*], Edward J. Phlips [a], Mark T. Aubel [b], Nancy J. Szabo [c]

[a] *The Fisheries and Aquatic Sciences Program in the School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences (IFAS), University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, USA*
[b] *GreenWater Laboratories/CyanoLab, 205 Zeagler Drive, Palatka, FL 32177, USA*
[c] *Analytical Toxicology Core Laboratory, University of Florida, Building 471, Mowry Road, Gainesville, FL 32611, USA*

A R T I C L E I N F O

Article history:
Received 20 March 2012
Received in revised form 12 July 2012
Accepted 24 July 2012
Available online 14 August 2012

Keywords:
Cyanobacteria
Lyngbya wollei
Saxitoxin
Paralytic Shellfish Toxins (PSTs)
LC/MS
HPLC/fluorescence

A B S T R A C T

Paralytic Shellfish Toxins (PSTs) are highly toxic metabolic by-products of cyanobacteria and dinoflagellates. The filamentous cyanobacterium *Lyngbya wollei* produces a unique set of PSTs, including *L. wollei* toxins (LWT) 1–6. The accurate identification and quantification of PSTs from *Lyngbya* filaments is challenging, but critical for understanding toxin production and associated risk, as well as for providing baseline information regarding the potential for trophic transfer. This study evaluated several approaches for the extraction and analysis of PSTs from field-collected *L. wollei* dominated algal mats. Extraction of PSTs from lyophilized *Lyngbya* biomass was assessed utilizing hydrochloric acid and acetic acid at concentrations of 0.001–0.1 M. Toxin profiles were then compared utilizing two analysis techniques: pre-column oxidation (peroxide and periodate) High Performance Liquid Chromatography (HPLC) with Fluorescence (FL) detection and LC coupled with Mass Spectrometry (MS). While both acid approaches efficiently extracted PSTs, hydrochloric acid was found to convert the less toxic LWT into the more toxic decarbamoylgonyautoxins 2&3 (dcGTX2&3) and decarbamoylsaxitoxin (dcSTX). In comparison, extraction with 0.1 M acetic acid preserved the original toxin profile and limited the presence of interfering co-extractants. Although pre-chromatographic oxidation with HPLC/FL was relatively easy to setup and utilize, the method did not resolve the individual constituents of the *L. wollei* derived PST profile. The LC/MS method allowed characterization of the PSTs derived from *L. wollei*, but without commercially available LWT 1–6

ITEM IV

Follow up to Membership Survey Results – Setting up Networking Groups

Jade L. Young

Setting up networking subgroups

Presented for the Benthic HABs Discussion Group on 29 JAN 2020 by co-facilitator Jade Young
US Army Corps of Engineers, Louisville District
Jade.L.Young@usace.army.mil
502-315-7439

MISSION STATEMENT

"The mission of this international collaborative is to accelerate mutual understanding of benthic HABs in rivers and lake systems, by sharing data and monitoring protocols, experiences and lessons learned."

Facilitators

Christine Joab

Environmental Scientist

Central Valley Regional Water Quality Control Board, California

916-464-4655

Christine.Joab@waterboards.ca.gov

Margaret Spoo-Chupka

Biologist

Metropolitan Water district of Southern California

909-392-5127

MSpoo-Chupka@mwdh2o.com

Jade Young

Limnologist

US Army Corps of Engineers, Louisville District, Kentucky

502-315-7439

Jade.L.Young@usace.army.mil

Summary of previous affiliations and topics

UC Berkeley

Cawthron Institute, New Zealand

Metropolitan Water District of Southern CA

North Coast Regional WQ Control Board, CA

Southern CA Coastal Water Research Program

CA State University, San Marcos

University of CA, Davis

Northern KY University

US Army Corps of Engineers

- Temporal and spatial distribution
- SPATT samplers
- Toxic species
- Challenges
- Ecology & toxins
- Monitoring and management
- Research
- Monitoring tools
- Laboratory methods
- Taxonomic information
- Management techniques
- Results
- Downstream dispersal
- Toxin synergy
- Culturing methods
- Toxin measurement methods
- Invertebrate toxicity

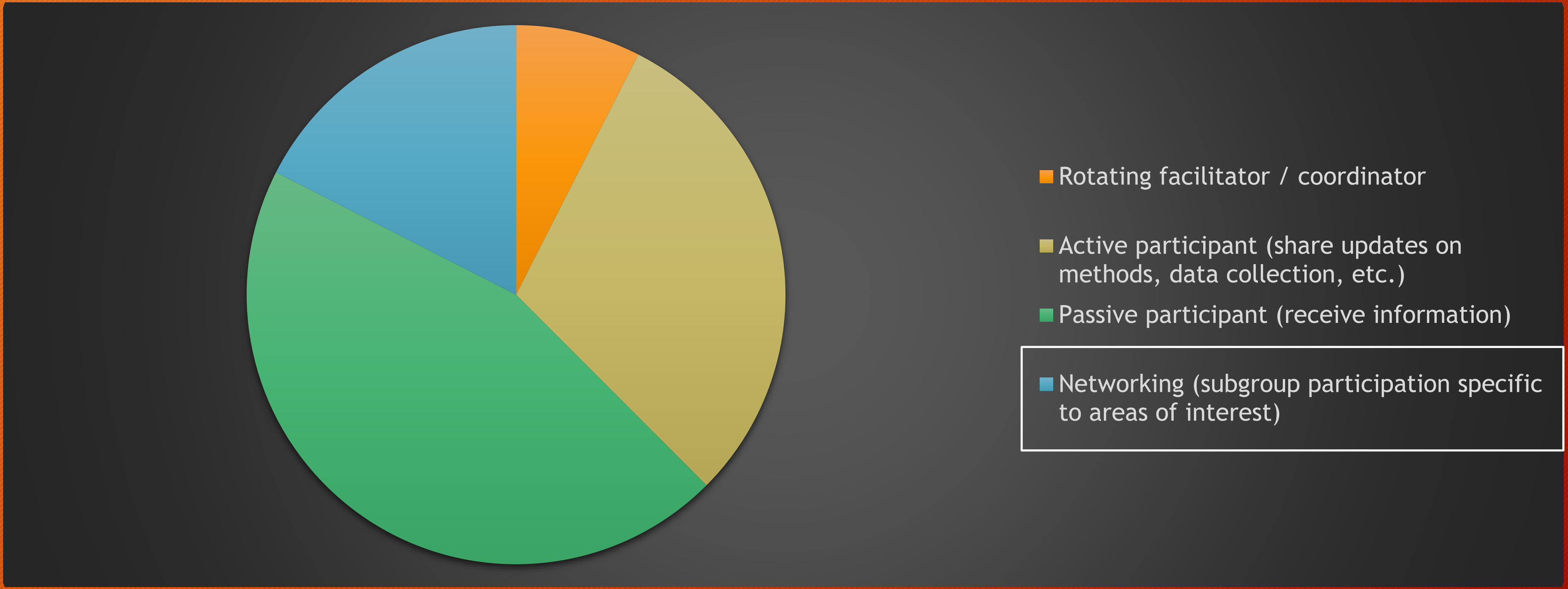
2019 Membership Re-survey

Summary of Responses

- 40 responses collected
- 17 new members
- New agencies represented

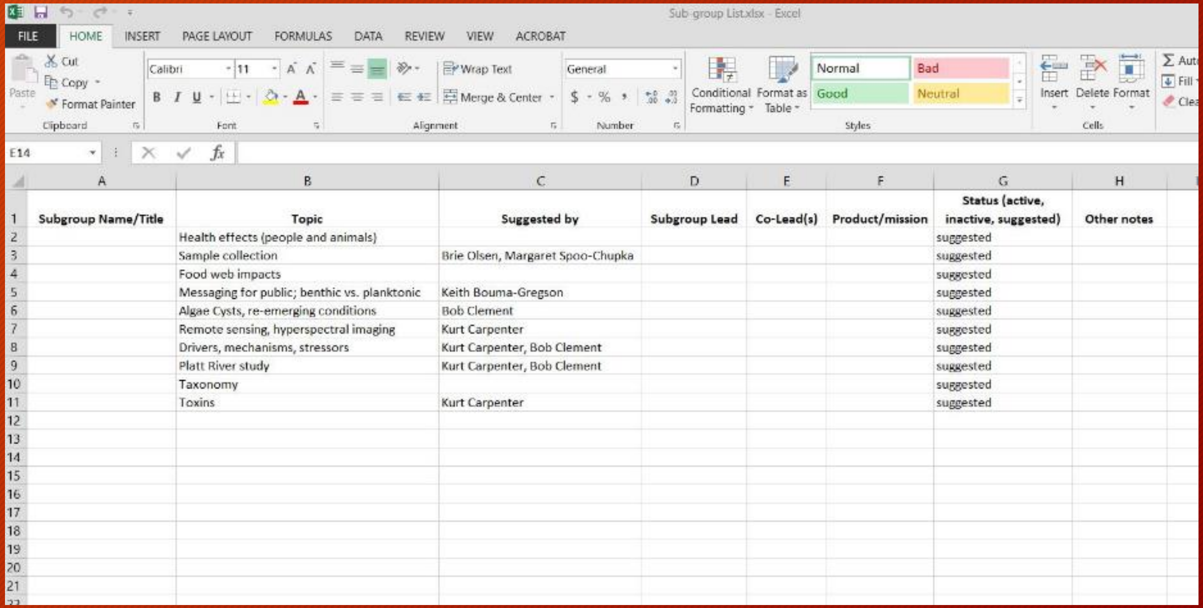
Anabaena sp. mat in the Russian River by Rich Fadness.

As a member of the Benthic HABs Discussion Group, which role is of most interest to you?



Subgroups component - suggestions

- Topic of interest
- Proposal/plan/mission/end product
- Lead, co-leads
- Membership



Subgroup Name/Title	Topic	Suggested by	Subgroup Lead	Co-Lead(s)	Product/mission	Status (active, inactive, suggested)	Other notes
	Health effects (people and animals)					suggested	
	Sample collection	Brie Olsen, Margaret Spoo-Chupka				suggested	
	Food web impacts					suggested	
	Messaging for public; benthic vs. planktonic	Keith Bouma-Gregson				suggested	
	Algae Cysts, re-emerging conditions	Bob Clement				suggested	
	Remote sensing, hyperspectral imaging	Kurt Carpenter				suggested	
	Drivers, mechanisms, stressors	Kurt Carpenter, Bob Clement				suggested	
	Platt River study	Kurt Carpenter, Bob Clement				suggested	
	Taxonomy					suggested	
	Toxins	Kurt Carpenter				suggested	

Subgroup obligations

- Notify co-facilitators for tracking
- Periodically check-in / report to the larger group

Future directions

This is a member driven group.

Jade will be tracking subgroup information.

ITEM V

Open Discussion & Upcoming Meetings

Christine Joab

UPCOMING HAB MEETINGS

- APRIL 21-23, 2020 TORONTO, ONTARIO
  4^{TH} ANNUAL INTERDISCIPLINARY FRESHWATER HABS WORKSHOP

- MAY 3-7, 2020 DUBLIN, IRELAND
  SETAC EUROPE 30^{TH} ANNUAL MEETING
  - Session under Environmental Policy, Risk Management, and Science Communication on Marine and Freshwater Pelagic and Benthic Harmful Algal Blooms: Toxin Production, Detection, Fate, Effects, monitoring and management by Triantafyllos Kaloudis and James Lazorchak

- MAY 18-21, 2020 GLASGOW, UK
  MODELING AND PREDICTION OF HARMFUL ALGAL BLOOMS

- JUNE 3-5, 2020 WOODS HOLE, MA
  NOAA'S SOCIOECONOMICS WORKSHOP

- OCTOBER 11-16, 2020 LA PAZ, BAJA CALIFORNIA SUR (MEXICO 2020)
  19^{TH} INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

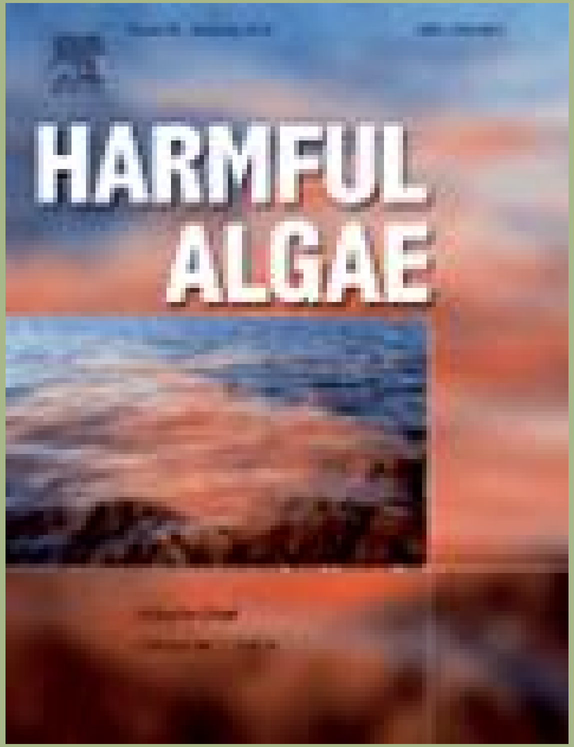
- ANY OTHERS TO SHARE?

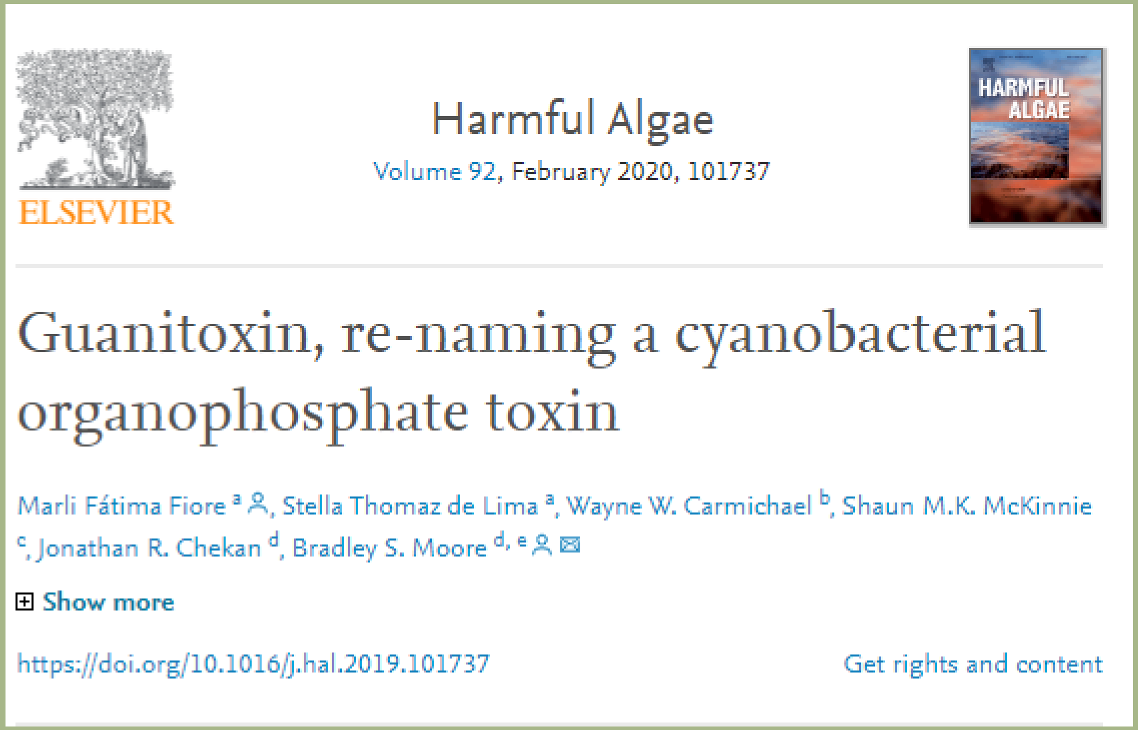
▶RECENT JOURNAL ARTICLE

Guanitoxin, re-naming a cyanobacterial organophosphate toxin

Harmful Algae, Volume 92, February 2020

https://www.sciencedirect.com/science/article/pii/S1568988319302124




ELSEVIER

Harmful Algae

Volume 92, February 2020, 101737

Guanitoxin, re-naming a cyanobacterial organophosphate toxin

Marli Fátima Fiore [a], Stella Thomaz de Lima [a], Wayne W. Carmichael [b], Shaun M.K. McKinnie [c], Jonathan R. Chekan [d], Bradley S. Moore [d, e]

⊞ Show more

https://doi.org/10.1016/j.hal.2019.101737

ITEM V

Wrap Up

Facilitators

WRAP UP

- PRESENTATION MATERIAL POSTED TO BENTHIC HABS WORKGROUP WEBPAGE HTTPS://WWW.EPA.GOV/CYANOHABS/EPA-NEWSLETTER-AND-COLLABORATION-AND-OUTREACH-HABS#BENTHIC
- SEND ADDITIONAL QUESTIONS ON PRESENTATION TO Zach.Smith@ramboll.com
- IF YOU'D LIKE TO BE ADDED TO THE BENTHIC HAB WORKGROUP DISTRIBUTION LIST, SEND AN EMAIL TO THE BENTHIC HAB FACILITATORS.
- WANT TO BE A PRESENTER OR A FACILITATOR? CONTACT US!
- **BENTHIC HAB FACILITATORS:**
  Christine Joab Christine.Joab@waterboards.ca.gov
  Margaret Spoo-Chupka Mspoo-Chupka@mwdh2o.com
  Jade Young Jade.L.Young@usace.army.mil
  Dr. Lesley D'Anglada Danglada.Lesley@epa.gov