Eighth Symposium on Harmful Algae in the U.S.

Long Beach, California

November 15 – 19, 2015

EIGHTH SYMPOSIUM ON HARMFUL ALGAE IN THE U.S.

SYMPOSIUM CHAIRS

Meredith Howard	Southern California Coastal Water Research Project
David Caron	University of Southern California

LOCAL ORGANIZING COMMITTEE

Clarissa Anderson	University of California, Santa Cruz
Holly Bowers	Monterey Bay Aquarium Research Institute
Lilian Busse	formerly at the San Diego Water Quality Control Board
Raphael Kudela	University of California, Santa Cruz
John Ryan	Monterey Bay Aquarium Research Institute

STEERING COMMITTEE

Lisa Campbell	Texas A&M University
Lesley D'Anglada	U.S. Environmental Protection Agency
Jonathan Deeds	U.S. Food and Drug Administration
Quay Dortch	NOAA National Ocean Service
Sonya Dyhrman	Lamont Doherty Earth Observatory
Kate Hubbard	Fish and Wildlife Research Institute
Barbara Kirkpatrick	Gulf of Mexico Coastal Ocean Observing System
Judy Kleindinst	Woods Hole Oceanographic Institution
Keith Loftin	U.S. Geological Survey
Timothy Otten	Oregon State University
Michael Parsons	Florida Gulf Coast University
Mindy Richlen	Woods Hole Oceanographic Institution
Kevin Sellner	Chesapeake Research Consortium

SESSION CHAIRS

Approaches to Physiology and Physiological Ecology:
Hans Dam, Christopher Gobler

Bloom Prediction, Forecasting and Modeling:
Dennis McGillicuddy, Richard Stumpf

Diarrheic Shellfish Poisoning:
Jonathan Deeds, Quay Dortch

Emerging HABs and Toxins:
Keith Loftin, Cheryl Greengrove

Emerging Recognition of Freshwater and Inland HABs:
Catherine Wazniak, Dave Hambright

Emerging Technologies, Instrumentation, and Methodologies:
Alan Wilson, Bryan Brooks

HABs and Toxins at the Land-Sea Interface:
Avery Tatters, Taylor Armstrong

Human and Animal Health Impacts:
Marc Suddleson, Leanne Flewelling

Monitoring and Management of HABs:
Kathryn Coyne, Greg Doucette

Population Genetics and Species Distributions:
Katherine Hubbard, Kendra Negrey

NOAA Lunchtime Session:
Quay Dortch, Allison Allen

HABs and Climate Change:
Kevin Sellner, Lesley D'Anglada

Hot Times in the California Current: Understanding the 2015 West Coast Domoic Acid Event:
Raphael Kudela Vera Trainer

Young Investigator Program:
Dave Caron, Clarissa Anderson

SYMPOSIUM SPONSORS

NOAA

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STUDENT AND MANAGER SUPPORT

NOAA

Sea Grant, University of Southern California

Fluid Imaging Technologies, Inc.

US Food and Drug Administration

MEAL INFORMATION:

Lunch: 12:00-1:30pm

Lunch will be provided for all registered participants and will be served in the Queens Salon (Promenade Deck). On Tuesday, November 17, 2015 boxed lunches will be available for all participants. The participants attending the NOAA special lunchtime session can eat in the Grand Salon during the session and those not attending can eat in the Queens Salon.

Breakfast and dinner is on your own. The Queen Mary has several dining options.

Midship marketplace: Starbucks coffee, homemade pastries, bagels, breakfast sandwiches, yogurt parfaits, fruit, oatmeal and much more. Open 6am - 6pm.

Promenade Café: Breakfast, quick lunch or casual dinner, the Promenade Café specializes in gourmet burgers, seasonal salads and comfort food classics. Open 6:30am - 10pm.

Chelsea Chowder House & Bar: A contemporary take on the traditional fish house, the menu focuses on seafood and steaks, including chowders, stews and brews. Open 5pm – 10pm.

There are also a list of restaurants in downtown Long Beach (http://www.downtownlongbeach.org/business-list?m=1&category=restaurants).

REGISTRATION CHECK IN:

Registration check in will be open on Sunday from 3:00pm to 6:00pm in the Hotel Registration Lobby and from 6:00pm to 8:00pm at the Welcome Mixer in the Britannia Salon. Registration check in will also be available Monday morning from 8:00am to 8:30am in the Grand Salon.

PRESENTATIONS:

Presentations may be loaded onto meeting computers during the registration check in on Sunday and Monday.

POSTERBOARDS:

Posterboards will be available for poster hanging beginning Monday morning at 7am in the Windsor Salon. Posters should be hung by Monday afternoon and will remain up for the duration of the meeting. Posters must be removed from the Windsor Salon on Thursday by 5:00 pm.

TRAVEL AWARDS COMMITTEE:

Deana Erdner	University of Texas
Michael Parsons	Florida Gulf Coast University
Mindy Richlen	Woods Hole Oceanographic Institution
Kate Hubbard	Fish and Wildlife Research Institute
Quay Dortch	NOAA National Ocean Service
Susanne Menden-Deuer	University of Rhode Island

STUDENT PRESENTATION AWARDS

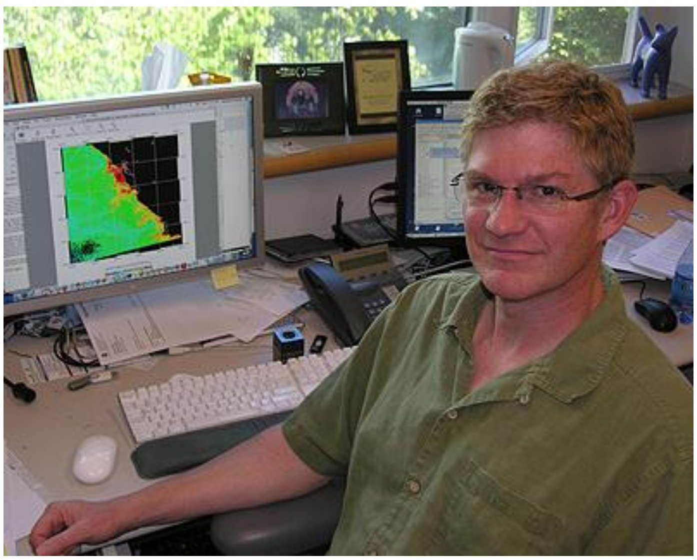
The student presentations awards will be announced at the banquet. Thank you to Deana Erdner for coordinating the process and to all of the judges!

PLENARY PRESENTATIONS

WELCOME TO THE CALIFORNIA CURRENT, 2014-2015: THE YEAR(S) OF CRAZY

Raphael Kudela

University of California, Santa Cruz, Department of Ocean Sciences, Santa Cruz, CA



The US west coast has been experiencing several years of anomalous conditions including a multi-year drought, warm anomalies in both 2014 and 2015, the emergence of freshwater HABs as a serious and growing threat, the imminent arrival of a large El Niño, and unusual bloom events and emergence of new HAB organisms such as *Akashiwo sanguinea* and *Gonyaulax spinifera,* massive blooms of *Pseudo-nitzschia*, and continued issues with *Dinophysis* and *Alexandrium.* These issues have caused many to re-evaluate the west coast as an upwelling-dominated system that is largely driven by bottom-up factors, and to explore the role of basin-scale oscillations and climate change, anthropogenic nutrient inputs, and expansion of HAB organisms through natural and human-mediated causes. Against this background we have also seen the development and maturation of ocean observing networks, increased use of technology for HAB research and management, and maturation of HAB prediction systems. A whirl-wind tour of California (and west coast) HAB status, trends, and impacts will highlight what we know about the “year(s) of crazy” and whether we should expect these conditions to become the new normal.

AN OUTSIDERS VIEW OF THE TOLEDO DRINKING WATER EVENT OF 2014: LESSONS LEARNED AND FUTURE PROSPECTS

Gregory L Boyer, (and Friends)

State University of New York, College of Environmental Science and Forestry, Syracuse, NY

Cyanobacteria blooms are established phenomena in the Western basin of Lake Erie. Blooms have been reported since the early 1950s with toxic blooms reported since 2000. The major variable in these blooms is their spatial extent and toxicity. Most toxic blooms are associated with the cyanobacterium *Microcystis*. Several metropolitan cities derive their drinking water from the central and western basin of Lake Erie. When toxic blooms occur at these drinking water intakes, this can impact the supply services by these facilities. In August of 2014, a major cyanobacteria bloom occurred in the western basin of Lake Erie. Past bloom events (2011) have had higher concentrations of cells, toxins and areal extent, however this particular bloom was pushed south by northerly winds and accumulated at the Collins Park Water Treatment intake for the City of Toledo. Initial readings of microcystin toxicity were >5.5 µg/L in the raw water and 0.6 µg/L in the finished water. These values climbed to 2.6 µg/L in the finished water, prompting the City of Toledo Heath Department to issue a “Do not drink” and “Do not boil” water advisory. Following this, Governor John Kasich declared a state of emergency for the region. Restaurants and food facilities were requested to temporarily suspend their operations. The local Brewery discarded nearly 50,000 gallons of product, and 9000 cases of bottled water were delivered to distribution centers throughout the city by the evening of August 3. Additional distributions continuing until the advisory was lifted on August 5th. Several hundred people report to area hospitals due to “suspected algal poisoning”. Such events provide real world insight into crisis management and the economic costs associated with such events. This event galvanized congress into recognizing that freshwater harmful algal blooms can impact human health and lead directly to US EPA issuing a revised guidance value for cyanobacteria toxins in drinking water. Despite the chaos, human health was protected in the Toledo Event. The event will likely lead to improved emergency management protocols for HAB events, routine sample monitoring and greater coordination in HAB response protocols.

SPECIAL SESSIONS and EVENING ACTIVITIES

TUESDAY NOVEMBER 17, 2015

12:30 – 1:45 pm, Grand Salon, "R" Deck

NOAA Session, Chairs: Quay Dortch and Allison Allen

At the NOAA special session there will be updates and an opportunity to ask questions on the Ecological Forecasting Roadmap in relation to HAB forecasting, the HAB Operational Forecast System, the Phytoplankton Monitoring Network, and the competitive HAB programs. A boxed lunch will be provided.

The following presentations will be followed by a discussion:

Allison Allen, Richard Stumpf, and Gregory Doucette. Ecological forecasting road map and HAB forecasting at NOAA

Karen Kavanaugh, Edward Davis and Katherine Derner. Improvements and expansion of NOAA's HAB Operational Forecast System

Steve L. Mortonand Shawn Gano. The use of technology to assist citizen scientists monitoring HABs and changes in environmental conditions

Quay Dortch, Marc Suddleson, Alan Lewitus, and Rob Magnien. Future plans for the NOAA HAB Competitive Programs

6:30 – 8:00 pm, Grand Salon, "R" Deck

HABs and Climate Change, Chairs: Kevin Sellner and Lesley D'Anglada

Changes in climate have been projected in the IPCC GCMs with substantial shifts in regional weather patterns, temperatures, and CO_2 in many aquatic systems. Changes in these factors could favor the growth of HABs, although substantial research remains to be done to better understand the association between phytoplankton, and particularly HAB taxa, and these altered conditions. Mark Wells, PISCES representative and co-convener of the recent HABs Climate Change meeting in Sweden, will provide a short overview on what we know and don't know about the relationship of climate change and HABs, and identify possible focus areas for the future. The presentation will be followed by an open community discussion to identify critical research to pursue in the next decade.

WEDNESDAY NOVEMBER 18, 2015

6:30 – 8:00pm, Grand Salon, "R" Deck

Hot Times in the California Current: Understanding the 2015 West Coast DA Event

Chairs: Raphael Kudela and Vera Trainer

During Spring-Summer 2015, the US West Coast experienced one of the largest contiguous HAB events ever described, coincident with several other anomalous events including unusually warm temperatures associated with "the warm blob(s)", a multi-year drought, and the imminent arrival of perhaps one of the largest El Niño events. *Pseudo-nitzschia* and domoic acid were observed from just south of Santa Barbara to as far North and West as Kodiak, Alaska. During this session we will briefly summarize the data/results available at the time of the meeting, and will discuss the desirability of organizing a west-coast synthesis of this unprecedented event. We will also discuss whether additional plans should be made for 2016 to document the impact of the potential El Niño event, since El Niños have historically been associated with more toxic blooms. We encourage interested participants to come with their own observations and data, and to discuss organization of both the retrospective synthesis and plans for the coming year.

THURSDAY NOVEMBER 19, 2015

7:00pm-10:00pm, Aquarium of the Pacific

Symposium Banquet and Award Announcements

The aquarium is located in downtown Long Beach. There are several options for transportation to the Aquarium, including The Passport Shuttle (free), taxicab or uber car.

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Symposium Schedule

SUNDAY NOVEMBER 15, 2015

Time		Event	Location
3:00-6:00		Registration check in	Hotel Registration Lobby, Located on "A" Deck
2:30-6:00		Young Investigators Program Chairs, David Caron, Clarissa Anderson	Victoria Room and Deck Located on Promenade Deck Beer and refreshments served
	2:30	Meet and greet, introductions	
	3:00-6:00	Program Overview 1) Short presentation: Harry Nelson, Vice President of Aquatic Markets, Fluid Imaging, Inc. (and sponsor for the YIP). 2) Short presentation: Alan E. Wilson, Division of Environmental Biology, NSF. "The NSF, from the inside." 3) Speed-Dating: One-on-one 'meet and greet' interactions with each of the attendees. This will be a fun and painless way to meet your peers at the Symposium, and exchange a bit of information in a very informal setting. 4) Discussion and Q/A with Donald M. Anderson, Woods Hole Oceanographic Institution, on the topic of navigating a career in HAB research. Please send an email to Dave Caron (dcaron@usc.edu) or Clarissa Anderson (clrander@ucsc.edu) if you plan to attend.	
4:00-6:00		NHC Committee Meeting	Board Room Located on Promenade Deck
6:00-8:00		Welcome Mixer Registration check in	Britannia Salon & Deck Located on "M" Deck Beer, wine, refreshments and hors d'oeuvres served

MONDAY NOVEMBER 16, 2015

All oral sessions will take place in the Grand Salon, located on the "R" Deck.

8:30	Opening Remarks Meredith Howard and David Caron, Symposium Co-Chairs
8:45-9:30	**Plenary, Raphael Kudela:** Welcome to the California Current, 2014-2015: The Year(s) of Crazy

ORALS SESSION 1: BLOOM PREDICTION, FORECASTING AND MODELING PART 1

Chairs: Dennis McGillicuddy, Richard Stumpf

Time	**Presenter**	**Title**
9:40	Richard Stumpf	Climatologic Analysis Of Cyanobacterial Blooms In Lake Erie: A Case Study For Developing Predictive Tools
9:55	Dennis McGillicuddy	A Red Tide Of *Alexandrium Fundyense* In The Gulf Of Maine
10:10	Pat Tester	Effects Of Ocean Warming On Growth And Distribution Of Dinoflagellates Associated With Ciguatera Fish Poisoning In The Caribbean
10:25	Gary Kirkpatrick	CHANS: Modelling Environmental Factors Influence On Human Respiratory Irritation From Natural Exposure To *Karenia Brevis* Aerosols

10:30-11:00	**Break**	

ORALS SESSION 2: BLOOM PREDICTION, FORECASTING AND MODELING PART 2

Chairs: Dennis McGillicuddy, Richard Stumpf

11:00	David Ralston	A Simple Model For Prediction And A Complex Model For Process Studies Of *Alexandrium Fundyense* In An Estuary
11:15	Darren Henrichs	Can Co-Occurring Species Reveal *Dinophysis* Bloom Origins?
11:30	Regina Radan	Determining Potential Environmental Factors Affecting The Trends Of Toxigenic *Alexandrium Catenella* Abundance In Monterey Bay, CA
11:45	Vince Lovko	Spatial And Temporal Development Of Phytoplankton Community Composition During A *Karenia Brevis* Bloom In The Northeastern Gulf Of Mexico
11:50	Katie Derner	Refining *Karenia Brevis* Detection: An Assessment Of Ensemble Imagery Products For Operational Forecasting
12:00-1:30	**Lunch**	**Queens Salon, Promenade Deck**

ORALS SESSION 3: APPROACHES TO PHYSIOLOGY AND PHYSIOLOGICAL ECOLOGY PART 1

Chairs: Hans Dam, Christopher Gobler

1:30	Timothy Davis	Investigating The Role Of Reactive Oxygen Species In Driving Bloom Toxicity In Western Lake Erie
1:45	Christopher Gobler	Niche differentiation among cyanobacterial populations revealed via eco-transcriptomic surveys of Lake Erie
2:00	Hans Dam	Fitness Cost Of Grazer-Induced Toxin Production In The Marine Dinoflagellate *Alexandrium Fundyense*
2:15	Michael Brosnahan	An examination of loss rates experienced by a localized, inshore population of *Alexandrium fundyense* through its complete life cycle
2:30	Allen Place	Characterization Of Pores In Black Lipid Membranes Made By Karlotoxin 2 (KmTx2) From *Karlodinium Veneficum* (Dinophyceae)
2:45	Tsvetan Bachvaroff	The War Between Harmful Dinoflagellates And *Amoebophrya* sp.
3:00	Robbie Martin	The Effects Of Microcystin-LR On *Escherichia Coli* Evaluated Through Transcriptomics, Metabolomics, And Lipidomics
3:05	Rosemary Jagus	Phylogeny And Function: Identification Of The Prototypical eIF4E Translation Initiation Factor In Dinoflagellates
3:10	Katherine Perri	Photosynthetic Yield (F_v/F_m) As An Indicator Of Nutrient Limitation In *Microcystis Aeruginosa*
3:15-3:45	**BREAK**	

ORALS SESSION 3: APPROACHES TO PHYSIOLOGY AND PHYSIOLOGICAL ECOLOGY PART 2

Hans Dam, Christopher Gobler

3:45	Pat Glibert	Nutrients And HABs: Beyond The Classic Eutrophication Perspective
4:00	Jayme Smith	Microbial Food Web Interactions And The Effect Of Nutrient Competition On Domoic Acid Production Of The Diatom *Pseudo-nitzschia*
4:15	Yoonja Kang	Discovery Of A Resting Stage In *Aureoumbra Lagunensis* (Pelagophyceae): A Mechanism Facilitating Its Recent Expansion?
4:30	Lauren Krausfeldt	Investigation of the Nitrogen Cycle within Harmful Algal Bloom Communities
4:35	Yanfei Wang	Expression And Activity Of Novel Nitrate Reductase Enzymes In *Chattonella subsalsa* And Implications For Competitive Dynamics In Marine Environments
4:40	Saddef Haq	One Pathway-Two Products: A Polyketide Synthase Pathway In Dinoflagellates Results In Both Toxin And Fatty Acid Production
4:45-5:00	Break	
5:00-6:30	**Poster Session 1:**	Windsor Salon, "R" Deck Wine, beer and refreshments available for purchase

Bloom Prediction, Forecasting And Modeling

Approaches To Physiology And Physiological Ecology

See poster schedule below for list of poster presentations

TUESDAY NOVEMBER 17, 2015

8:30-9:15	**Plenary, Greg Boyer:** An Outsiders View Of The Toledo Drinking Water Event Of 2014: Lessons Learned And Future Prospects	

ORALS SESSION 4: DIARRHEIC SHELLFISH POISONING

Chairs: Quay Dortch, Jonathan Deeds

9:20	Lisa Campbell	The Imaging Flowcytobot For Monitoring Harmful Algal Blooms In The Gulf Of Mexico
9:35	Vera Trainer	The Tipping Point For An Expanding Problem: Lipophilic Toxins In Washington State And Beyond
9:50	Theresa Hattenrath-Lehmann	Direct Nitrogen Use By The DSP-Producer, *Dinophysis Acuminata*: Experiments Using Cultures And Field Populations
10:05	Neil Harrington	Developing An Early Warning System For An Emerging Threat To Human Health: Diarrheic Shellfish Poisoning In Washington State
10:10	Catherine Wazniak	Occurrence Of *Dinophysis* And Associated DSP Toxins In Maryland
10:15	Ernesto Garcia-Mendoza	Diarrheic Shellfish Toxins In Mediterranean Mussels From The Northwest Coast Of Baja California, Mexico

10:20	Juliette Smith	Effects Of Prey Type And Food Quality On Toxin Dynamics Within An Isolate Of *Dinophysis acuminata*
10:25-10:50	Break	

ORALS SESSION 5: HABs AND TOXINS AT THE LAND-SEA INTERFACE

Chairs: Avery Tatters, Taylor Armstrong

10:50	Meredith Howard	Widespread Prevalence Of Microcystins From A Variety Of Southern California Waterbodies And Implications For Toxin Loading To Coastal Waters
11:05	Avery Tatters	Multiple Stressors at the Land-Sea Interface
11:20	Corinne Gibble	Depuration Of Microcystin Toxin In *Mytillus californianus* And Implications For Use As An Indicator Organism
11:35	Melissa Peacock	Does San Francisco Bay have a toxic algae problem?
11:40	Ellen Preece	Transfer of the Harmful Algal Toxin, Microcystin, From Freshwater Lakes To Puget Sound, Washington, and Toxin Accumulation in Marine Mussels *Mytilus trossulus*
11:45	Beth Stauffer	Growth Of Heterotrophic Protists On Red Tide-Forming Phytoplankton Species: Effects Of Toxin Production And Interspecific Prey Interactions

11:50	Wei Chen	The Interaction Between The Brevetoxin And Its Native Receptors
12:00-2:00	**Lunch**	**Queens Salon, Promenade Deck**

NOAA SPECIAL SESSION

Chairs: Quay Dortch, Allison Allen

12:30-1:45		Grand Salon, “R” Deck

ORALS SESSION 6: EMERGING TECHNOLOGIES, INSTRUMENTATION AND METHODOLOGIES PART 1

Chairs Alan Wilson, Bryan Brooks

2:00	John Ryan	Ecological Dynamics In California HAB Hotspots: Eddy Incubation, Retention, And Subsurface Refugia
2:15	John Berry	A Novel Matrix Assisted Laser Desorption Ionization/Time-Of-Flight (MALDI-TOF) Method For Analysis Of BMAA, And Application To The Bioaccumulation In South Florida Food Webs
2:30	Van McClendon	Movement Towards An Adaptive Systems Approach To Freshwater Management: Combining Waterbody Treatments With Watershed Management

2:45	Mark Van Asten	The Development Of Biotoxin Gene qPCR Assays For The Monitoring And Management Of Biotoxin Risk In Aquatic Environments
3:00	Amanda Foss	Using The MMPB Technique To Confirm Microcystins Measured By ELISA AND HPLC (UV, MS, MS/MS)
3:15	Raphael Kudela	Realized And Potential HAB Hotspots Along The California Coast: What Triggers A Bloom?
3:20	Clarissa Anderson	Domoic Acid In Marine Food Webs: A Novel Approach To Trace Nitrogen Sources And Transfer
3:25	Jordon Beckler	Spatial Heterogeneity in the surface expression of a *Karenia brevis* bloom in the eastern Gulf of Mexico.
3:30-3:45	Break	

ORALS SESSION 7: EMERGING TECHNOLOGIES, INSTRUMENTATION AND METHODOLOGIES PART 2

Chairs Alan Wilson, Bryan Brooks

3:45	Harry Nelson	Continuous Imaging Flow Cytometer For The Automated Detection, Identification, And Enumeration Of Cyanobacteria

4:00	Steven Steinberg	CellScope Aquatic: A Lab Quality, Portable Cellphone-Based Microscope For On-Site Collection Of Algae Images
4:15	Alan Wilson	Benchtop Fluorometry Of Phycocyanin As A Rapid Approach For Estimating Cyanobacterial Biovolume
4:30	Houshuo Jiang	Development And Testing Of A High-Speed Microscale Imaging System For Exploration Of Harmful Algal Behavior And Interactions
4:45	Gabriela Hannach	Use Of Flowcam Technology To Monitor And Characterize Marine Algal Blooms In Central Puget Sound

5:00-6:30 **Poster Session 2:** Windsor Salon, "R" Deck
Wine, beer and refreshments available for purchase

Diarrheic Shellfish Poisoning

HABs and Toxins at the Land-Sea Interface

Emerging Technologies, Instrumentation, and Methodologies

See poster schedule below for list of poster presentations

SPECIAL SESSION: HABs AND CLIMATE CHANGE

Chairs: Kevin Sellner, Lesley D'Anglada

6:30-8:00 Grand Salon, "R" Deck

Wine, beer and refreshments available for purchase

WEDNESDAY NOVEMBER 18, 2015

ORALS SESSION 8: MONITORING AND MANAGEMENT PART 1

Chairs Kathryn Coyne, Greg Doucette

8:30	Bryan Brooks	Are Harmful Algal Blooms Becoming The Greatest Inland Water Quality Threat To Public Health And Aquatic Ecosystems?
8:45	Susan Corum	Long-Term Public Health Monitoring for Toxic *Microcystis* blooms in the Klamath River of Northern California
9:00	Erin Stelzer	CyanoHAB Prediction And Microcystin Degraders In Lake Erie And Ohio Inland Waters
9:15	Joel Allen	High Frequency Monitoring Of CyanoHABs And Cyanotoxin Production To Characterize Periods Of Greatest Risk On An Inland Reservoir
9:30	Daniel Ayres	Successfully Converting A Federally Funded HAB Monitoring Project To A State-Funded Permanent Program; The Importance Of Telling The Story Well
9:35	Gregory Doucette	Development Of An Immunoassay For Autonomous, Subsurface Detection Of Particulate Microcystins In Lake Erie
9:40	Carey Nagoda	Cyanotoxins And Blooms Detected In Multiple Water Body Types Throughout The San Diego Region
9:45	Erin Stanfield	Monitoring Sediment-Bound Microcystins Across California's Stream Pollution Trends (SPoT) Program Watersheds

9:50	Karen Taberski	Statewide Strategy For Monitoring And Managing Freshwater HABs In California: A Water Quality Managers' Perspective
9:55	Amanda Ellsworth	*Gambierdiscus* On The Move: How Do Tidal Cycles And Moon Phases Affect Distribution?
10:00-10:30	Break	

ORALS SESSION 9: MONITORING AND MANAGEMENT PART 2

Chairs Kathryn Coyne, Greg Doucette

10:30	Chris Whitehead	Southeast Alaska Tribal Toxins (SEATT): A Partnership To Monitor Harmful Algal Blooms
10:45	R. Wayne Litaker	Identification Of The *Alexandrium* Species Responsible For Paralytic Shellfish Poisoning In Alaska
11:00	Alison Sirois	The Maine Biotoxin Program: Management And Monitoring Approaches For The Maine Shellfish Industry
11:15	Don Anderson	Estimating Shellfish Toxicity Using *Alexandrium* Cell Counts Obtained from Environmental Sample Processors (ESPs) in the Gulf Of Maine
11:30	Kathryn Coyne	Recurrent Blooms Of *Karenia Papilionacea* In Delaware Coastal Waters
11:35	Mary Carmen Ruiz-de la Torre	The Socio-Economic Effects Of *Gymnodium Catenatum* Bloom In The Northwest Region Of Gulf Of California, Mexico

11:40	Jennifer Runyan	Soundtoxins: Harmful Algal Bloom Monitoring In Puget Sound, Washington
11:45	Elizabeth Tobin	A Social-Ecological Systems Approach To Improve Understanding Of *Alexandrium* Blooms And Their Sociocultural Impacts In Southeast Alaska
11:50	Susan Launay	Studies Of Nutrient Contributions From Major Estuaries Of Southwest Florida To The Gulf Of Mexico And Their Effect On *Karenia Brevis* Blooms
11:55	Dianne Greenfield	Validation Of A Sandwich Hybridization Assay Approach To Rapidly Assess Multiple Harmful Algal Bloom Species Linked With Fish Kills And Public Health Concerns In The Southeastern United States
12:00-1:30	**Lunch**	**Queens Salon, Promenade Deck**

ORALS SESSION 10: POPULATION GENETICS AND SPECIES DISTRIBUTIONS

Chairs: Katherine Hubbard, Kendra Negrey

1:30	Katherine Hubbard	Complex Bloom Dynamics Revealed By Genetic Characterization Of US Atlantic And Gulf Of Mexico *Pseudo-nitzschia* Communities
1:45	Holly Bowers	Unraveling Diversity In *Pseudo-nitzschia* Species Throughout A Toxic Event In Monterey Bay, California
2:00	Francisco José Acosta Espinosa	Intra-Individual Heterogeneity In *Prymnesium parvum* ITS1 Regions And Its Implications For The Biogeography Of A Recent US Invasion

2:15	Kristen Feifel	An *Alexandrium* Cyst Record From The Hypoxic Fjord Effingham Inlet, British Columbia
2:20	Charles Tilney	Diversity, Dynamics And Biogeographic Distributions Of Pico- And Nano-Planktonic Pelagophytes And Chlorophytes
2:25	Kathleen Pitz	Development Of Fluorescence *In Situ* Hybridization (FISH) Probes To Detect And Enumerate *Gambierdiscus* Species As Applied To Wai 'Opae, Hawaii
2:30	Darcie Ryan	Assembling And Analyzing The Transcriptomes Of Three Toxin-Producing *Karenia* Species

2:35-3:00 Break

3:00-5:00 **HAB COMMUNITY TOWN HALL MEETING**

5:00-6:30 **Poster Session 3:** Windsor Salon, "R" Deck
Wine, beer and refreshments available for purchase

Monitoring and Management

Population Genetics and Species Distributions

See poster schedule below for list of poster presentations

SPECIAL SESSION: HOT TIMES IN THE CALIFORNIA CURRENT: UNDERSTANDING THE 2015 WEST COAST DOMOIC ACID EVENT

Chairs: Raphael Kudela, Vera Trainer

6:30-8:00 Grand Salon, "R" Deck

Wine, beer and refreshments available for purchase

THURSDAY NOVEMBER 19, 2015

ORALS SESSION 11: HUMAN AND ANIMAL HEALTH

Chairs: Marc Suddleson, Leanne Flewelling

8:30	Elizabeth Hamelin	Monitoring Human exposure to algal toxins from surveillance to clinical analysis
8:45	Bridget Ferriss	A Model For Human Consumption Levels Of Domoic Acid In The Pacific Northwest
9:00	Kathi Lefebvre	Affects Of Chronic Low-Level Seafood Toxin Exposure On Learning And Memory Into Old Age
9:15	Brian Klimek	Linking Ciguatoxicity To The Life History And Ecology Of Red Lionfish (*Pterois volitans*) In The Western Atlantic
9:30	Leanne Flewelling	Domoic Acid In Stranded Juvenile Green Sea Turtles, *Chelonia mydas*, In Southeast Florida
9:45	Porter Hoagland	CHANS: Modeling The Dynamics Of HABs, Human Communities, And Policy Choices Along The Florida Gulf Coast
10:00	Nicholas Hahlbeck	CHANS: The Spatial-Temporal Distribution Of *Karenia brevis* On The West Florida Coast And Its Relationship To Precipitation And River Discharge.
10:05	Lynn Grattan	Chronic, Low Level Domoic Acid Exposure And Memory In Adults: The Coastal Cohort Eight Years Later

10:10	Kerri Danil	Marine Algal Toxins And Their Vectors In Southern California Cetaceans
10:15-10:45	Break	

ORALS SESSION 12: EMERGING RECOGNITION OF FRESHWATER AND INLAND HABs PART 1

Chairs: Catherine Wazniak Dave Hambright

10:45	Keith Loftin	Cyanotoxins In Inland Lakes Of The Continental United States: Photic Zone Occurrence And Potential Recreational Health Risks In The EPA National Lakes Assessment 2007
11:00	Todd Miller	Global Distribution And High Resolution Temporal Monitoring Of Toxic Or Otherwise Bioactive Cyanobacterial Metabolite Mixtures In Lakes Associated With Drinking Water
11:15	David Caron	"Go West, Young Haptophyte": Fish Kills Attributable To *Prymnesium parvum* Spread To Southern California
11:30	Heather Raymond	Occurrence Of Cyanotoxins In Ohio Public Water System Source And Finished Drinking Waters And A Comparative Analysis Of Analytical Methods For Microcystin Detection
11:45	Shelly Tomlinson	High Resolution Ocean Color Products To Enhance Cyanobacteria Monitoring Efforts In Inland Lakes
11:50	John Berry	A Possible Contribution Of Teratogenic Carotenoids From Cyanobacteria To The Decline Of Amphibian Populations In The U.S. (And Globally)
11:55	Scott Blanco	Role Of Thermocline Dynamics In Regulating Cyanobacterial Bloom Density And Cyanotoxicity In A Hypereutrophic Coastal California Lake Over Two Years In A Mediterranean Climate

12:00-1:30	**Lunch**	**Queens Salon, Promenade Deck**

ORALS SESSION 13: EMERGING RECOGNITION OF FRESHWATER AND INLAND HABs PART 2

Chairs: Catherine Wazniak, Dave Hambright

1:30	Lucas Beversdorf	Cyanotoxins: from the lake to the faucet
1:45	Casan Scott	Understanding Influences Of Reservoir pH Variability On Fish Toxicity From Harmful Blooms Of *Prymnesium paruvm*
2:00	Bryan Brooks	Total Dissolved Solids Reflective Of Natural Resource Extraction Activities In The Northeastern United States Stimulate Growth And Toxicity Of The Harmful Alga *Prymnesium parvum*

ORALS SESSION 14: EMERGING HABs AND TOXINS

Chairs: Keith Loftin, Cheryl Greengrove

2:15	Nicolaus Adams	Detection Of *Azadinium* Spp. In Washington State Waters Using Quantitative Real-Time Polymerase Chain Reaction Analysis
2:30	Katherine Baltzer	Investigating Sub-Lethal Effects Of Caribbean Ciguatoxins On Zebrafish (*Danio Rerio*) Behavior
2:45	Wolfgang Vogelbein	Emergence Of The Toxic Alga *Alexandrium monilatum* In The Lower Chesapeake Bay: Toxigenicity In Aquatic Animals

3:00	Cheryl Greengrove	Emergency Response Mapping Of *Alexandrium* Cysts In The Surface Sediments Of Puget Sound WA
3:05	Christopher Loeffler	Ciguatera Fish Poisoning Management: Assessing Toxin Levels In The Invasive Species *Cephalopholis Argus* From Two Distinct Environmental Regions Of Hawaii
3:10	Kimberly Reece	Emerging Patterns And Biological Impacts Of Harmful Algal Blooms In Lower Chesapeake Bay

3:15-3:30 **CLOSING REMARKS**

3:30-5:00 **Poster Session 4:** Windsor Salon, "R" Deck
Wine, beer and refreshments available for purchase

Human and Animal Health

Emerging Recognition of Freshwater and Inland HABs

Emerging HABs and Toxins

See poster schedule below for list of poster presentations

6:30-10:30 Banquet and Awards — Aquarium of the Pacific

Poster Presentation Schedule

MONDAY NOVEMBER 16, 2015

POSTER SESSION 1 Windsor Salon, "R" Deck

BLOOM PREDICTION, FORECASTING AND MODELING

Name	**Title**	**Poster #** *indicates speedtalk
Craig Burnell	A Spatio-Temporal Analysis Of PSP Congener Profile Patterns In Shellfish Along The Coast Of Maine	1
Edward Davis	Building A Next Generation Geospatial Infrastructure For Operational Harmful Algal Bloom Forecasting	5
Katie Derner	Refining *Karenia brevis* Detection: An Assessment Of Ensemble Imagery Products For Operational Forecasting	9*
Danielle Dupuy	Implementing A GIS Application For Lake Erie Harmful Algal Bloom Forecasting	13
Analise Keeney	Assessment Of The Gulf Of Mexico Harmful Algal Bloom Operational Forecast System: A Comparative Analysis Of Forecast Skill And Utilization, 2004-2015	17
Gary Kirkpatrick	Chans: Modelling Environmental Factors Influence On Human Respiratory Irritation From Natural Exposure To *Karenia brevis* Aerosols	21*
Vince Lovko	Spatial And Temporal Development Of Phytoplankton Community Composition During A *Karenia brevis* Bloom In The Northeastern Gulf Of Mexico	25*

APPROACHES TO PHYSIOLOGY AND PHYSIOLOGICAL ECOLOGY

Name	Title	Poster # *indicates speedtalk
Alexis Fischer	Challenging The Endogenous Annual Clock Paradigm: Effects Of 'Cold-Conditioning' On The Germination Timing Of *Alexandrium* Cysts	29
Saddef Haq	One Pathway-Two Products: A Polyketide Synthase Pathway In Dinoflagellates Results In Both Toxin And Fatty Acid Production	33*
Rosemary Jagus	Phylogeny And Function: Identification Of The Prototypical eIF4E Translation Initiation Factor In Dinoflagellates	37*
Lauren Krausfeldt	Investigation of the Nitrogen Cycle within Harmful Algal Bloom Communities	41*
Alex Leynse	*Gambierdiscus*: Managing excess cellular carbon	45
Robbie Martin	The Effects Of Microcystin-LR On *Escherichia Coli* Evaluated Through Transcriptomics, Metabolomics, And Lipidomics.	49*
Rika Muhl	The Role Of pH In Facilitating Range Expansion In *Prymnesium parvum* Into Bays Of Texas	53
Gihong Park	A Novel Approach To Measuring The Cost Of Toxin Production In The Marine Dinoflagellate *Alexandrium Fundyense*	57

Katherine Perri	Photosynthetic Yield (F_v/F_m) As An Indicator Of Nutrient Limitation In *Microcystis Aeruginosa*	60*
José Ernesto Sampedro-Ávila	Effects Of Dissolved Organic Matter (Dom) Produced By Bivalves On *In Situ* Growth Rates Of *Lingulodium polyedrum.*	63
Kathy Van Alstyne	Eavesdropping Seaweeds: Reactive Oxygen Species (ROS) Induce Antioxidant And Chemical Defense Production In The Bloom-Forming Ulvoid Alga *Ulva lactuca*	66
Yanfei Wang	Expression And Activity Of Novel Nitrate Reductase Enzymes In *Chattonella Subsalsa* And Implications For Competitive Dynamics In Marine Environments	69*
April Woods	Reactive Oxygen Species And Domoic Acid: Role Of Unbalanced Growth And Light Energy In Toxin Production By *Pseudo-nitzschia australis*	72

TUESDAY NOVEMBER 17, 2015

POSTER SESSION 2 Windsor Salon, “R” Deck

DIARRHEIC SHELLFISH POISONING

Name	**Title**	**Poster #** ***indicates speedtalk**
Neil Harrington	Developing An Early Warning System For An Emerging Threat To Human Health: Diarrheic Shellfish Poisoning In Washington State	2*

Catherine Wazniak	Occurrence Of *Dinophysis* And Associated DSP Toxins In Maryland	6*
Ernesto Garcia-Mendoza	Diarrheic Shellfish Toxins In Mediterranean Mussels From The Northwest Coast Of Baja California, Mexico	10*
Juliette Smith	Effects Of Prey Type And Food Quality On Toxin Dynamics Within An Isolate Of *Dinophysis acuminata*	14*

HABS AND TOXINS AT THE LAND-SEA INTERFACE

Name	**Title**	**Poster #** *indicates speedtalk
Taylor Armstrong	Nitrogen Isotope Fractionation Of Saxitoxin In *Alexandrium Fundyense* When Grown On Nitrate, Ammonium, And Urea	18
Melissa Peacock	Does San Francisco Bay have a toxic algae problem?	22*
Ellen Preece	Transfer of the Harmful Algal Toxin, Microcystin, From Freshwater Lakes To Puget Sound, Washington, and Toxin Accumulation in Marine Mussels *Mytilus trossulus*	26*
Beth Stauffer	Growth Of Heterotrophic Protists On Red Tide-Forming Phytoplankton Species: Effects Of Toxin Production And Interspecific Prey Interactions	30*
Wei Chen	The Interaction Between The Brevetoxin And Its Native Receptors	34*

EMERGING TECHNOLOGIES, INSTRUMENTATION, AND METHODOLOGIES

Name	Title	Poster # *indicates speedtalk
Clarissa Anderson	Domoic Acid In Marine Food Webs: A Novel Approach To Trace Nitrogen Sources And Transfer	38*
Jordon Beckler	Spatial Heterogeneity in the surface expression of a *Karenia brevis* bloom in the eastern Gulf of Mexico.	42*
Raphael Kudela	Realized And Potential HAB Hotspots Along The California Coast: What Triggers A Bloom?	46*
Kevin O'Shea	Oxidative Treatments For The Degradation And Detoxicification Of Cyanotoxins, Microcystins And Cylindrospermopsin.	50
Kyoko Yarimizu	The Role Of Iron In Algal-Bacterial Interactions As Related To Harmful Algal Blooms	54

WEDNESDAY NOVEMBER 18, 2015

POSTER SESSION 3 Windsor Salon, “R” Deck

MONITORING AND MANAGEMENT

Name	**Title**	**Poster #** ***indicates speedtalk**
Ann Abraham	Multi-Laboratory Validation Of An LC-MS Method For The Determination Of Brevetoxin Contamination In Molluscan Shellfish	3
Daniel Ayres	Successfully Converting A Federally Funded HAB Monitoring Project To A State-Funded Permanent Program; The Importance Of Telling The Story Well	7*
Marie-Yasmine Bottein	Linking Nuclear Science And Seafood Safety: Reliability Of The Receptor Binding Assay For PSP And Ciguatera Toxins	11
Mary Carmen Ruiz-de la Torre	The Socio-Economic Effects Of *Gymnodium Catenatum* Bloom In The Northwest Region Of Gulf Of California, Mexico.	15*
Kathryn Coyne	Recurrent Blooms Of *Karenia Papilionacea* In Delaware Coastal Waters	19*
Gregory Doucette	Development Of An Immunoassay For Autonomous, Subsurface Detection Of Particulate Microcystins In Lake Erie	23*
Amanda Ellsworth	*Gambierdiscus* On The Move: How Do Tidal Cycles And Moon Phases Affect Distribution?	27*

Karen Taberski	Statewide Strategy For Monitoring And Managing Freshwater HABs In California: A Water Quality Managers' Perspective	64*
Elizabeth Tobin	A Social-Ecological Systems Approach To Improve Understanding Of *Alexandrium* Blooms And Their Sociocultural Impacts In Southeast Alaska	67*
Jennifer Wolny	Harmful Algal Blooms in Maryland's Coastal Bays	70

POPULATION GENETICS AND SPECIES DISTRIBUTIONS

Name	**Title**	**Poster #** ***indicates speedtalk**
Kristen Feifel	An *Alexandrium* Cyst Record From The Hypoxic Fjord Effingham Inlet, British Columbia	73*
Kendra Negrey	Regional And Global Phylogentic Relationships Of *Cochlodinium* spp. In Coastal Waters Of The Eastern Pacific	75
Kathleen Pitz	Development Of Fluorescence *In Situ* Hybridization (FISH) Probes To Detect And Enumerate *Gambierdiscus* Species As Applied To Wai 'Opae, Hawaii	77*
Darcie Ryan	Assembling And Analyzing The Transcriptomes Of Three Toxin-Producing *Karenia* Species	79*
Ivonne Santiago	Diversity And Toxicity Of The Genus *Pseudo-nitzschia* In Oaxaca Coastal Waters, Mexico	81

THURSDAY NOVEMBER 19, 2015

POSTER SESSION 4 Windsor Salon, "R" Deck

HUMAN AND ANIMAL HEALTH

Name	**Title**	**Poster #** ***indicates speedtalk**
Kerri Danil	Marine Algal Toxins And Their Vectors In Southern California Cetaceans	4*
Roberto Diaz	The Neurological Effects Of Florida Red Tide (FRT) Blooms	8
Lynn Grattan	Chronic, Low Level Domoic Acid Exposure And Memory In Adults: The Coastal Cohort Eight Years Later	12*
Nicholas Hahlbeck	CHANS: The Spatial-Temporal Distribution Of *Karenia brevis* On The West Florida Coast And Its Relationship To Precipitation And River Discharge.	16*
Gary Hitchcock	CHANS: A Study Of Significant Hot Spots Of *Karenia brevis* And Their Associated Socio-Economic Implications	20
Porter Hoagland	CHANS: The Characteristics of Cost-Effective Policy Responses for Harmful Algal Blooms	24
Tamecia Moore	CHANS*: Karenia brevis* And Its Effects On School Absenteeism (GRADES K-12th) In Sarasota County	28

EMERGING RECOGNITION OF FRESHWATER AND INLAND HABs

Name	Title	Poster # *indicates speedtalk
John Berry	A Possible Contribution Of Teratogenic Carotenoids From Cyanobacteria To The Decline Of Amphibian Populations In The U.S. (And Globally)	32*
Scott Blanco	Role Of Thermocline Dynamics In Regulating Cyanobacterial Bloom Density And Cyanotoxicity In A Hypereutrophic Coastal California Lake Over Two Years In A Mediterranean Climate	36*
Keith Bouma-Gregson	Toxic Benthic Cyanobacterial Mats In A California River Network	40
Bryan Brooks	Total Dissolved Solids Reflective Of Natural Resource Extraction Activities In The Northeastern United States Stimulate Growth And Toxicity Of The Harmful Alga *Prymnesium parvum*	44*
Rosalina Hristova	Wadeable Streams As Widespread Sources Of Benthic Cyanotoxins In California	48
Michelle Neitzey	A Lakewide Metatranscriptomic Survey Of The Eutrophic Shallow Lake Tai (China)	52
Morgan Steffen	Integration Of Authentic Monitoring For HAB Species Within An Undergraduate Laboratory Course	56

Shelly Tomlinson	High Resolution Ocean Color Products To Enhance Cyanobacteria Monitoring Efforts In Inland Lakes	59*
Catherine Wazniak	Cyanobacteria Booms In Maryland: A Re-emerging Threat	62
Marie Smutná	Phytoplankton As Producer Of Retinoid–Like Compounds	86
Hai Xu	Critical Nutrient Thresholds Needed To Control Harmful Cyanobacterial Blooms In Eutrophic Lake Taihu, China	82
Guangwei Zhu	The Spring Cyanobacterial Bloom And Its Influence On Drinking Water Supply In Taihu, China	84
Mengyuan Zhu	The Role Of Tropical Cyclones In Stimulating Cyanobacterial (*Microcystis* spp.) Blooms In Hypertrophic Lake Taihu, China	88
Xiao Xi	Allelopathic Inhibition of Cyanobacteria by Barley Straw – mechanism and application	89

EMERGING HABs AND TOXINS

Name	**Title**	**Poster #** ***indicates speedtalk**
Cheryl Greengrove	Emergency Response Mapping Of *Alexandrium* Cysts In The Surface Sediments Of Puget Sound WA	65*

Oral Presentation Abstracts

INTRA-INDIVIDUAL HETEROGENEITY IN *PRYMNESIUM PARVUM* ITS1 REGIONS AND ITS IMPLICATIONS FOR THE BIOGEOGRAPHY OF A RECENT US INVASION

Francisco Acosta[1], Boris Wawrik[1], K David Hambright[1]

[1]University of Oklahoma, Norman, OK

Classification of HAB-forming microorganisms is a critical step to management, since in several cases they differ in their capacity for toxicity, growth or other factors relevant to HAB-formation. Due to their high mutation rate, the Internal Transcribed Spacer (ITS) regions are among the preferred markers for strain classification in eukaryotic organisms. However, some studies suggest that the assumption that these regions are homogeneous within strains is incorrect, and that intra-individual polymorphism can be found in some groups. *Prymnesium parvum*, originally a marine protist, is a relatively recent invasive species in freshwater systems across the southern US, currently present in up to 20 states. It is toxigenic and can form large HABs which can cause massive mortalities of fish and other gill-breathing organisms. Previous studies have characterized a number of US *P. parvum* strains using the ITS1 gene and hypothesized a UK origin. We have found, after extensive sequencing of 15 novel strains and additional sequencing of previously characterized strains, that ITS1 heterogeneity is a common genomic feature for US strains. Furthermore, we identified two main phylogenetic groups that may be derived from different European strains, one from the UK and the other from Scandinavia. We also found that in some basins strains from these two groups probably coexist. Our results challenge known classification schemes, and suggest an additional level of complexity for ITS1-based taxonomies that needs to be considered for biogeographic studies of this and other protistan invasions.

DETECTION OF *AZADINIUM* SPP. IN WASHINGTON STATE WATERS USING QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION ANALYSIS

Nicolaus G. Adams[1],Jerry Borchert[2], James Garlant[3], and Vera L. Trainer[1]

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[3]University of Arizona, Tuscon, AZ, 85721

Azadinium are relatively small dinoflagellates, some species of which produce toxins known as azaspiracids which are an emerging threat to consumers of shellfish harvested in Washington State. Azaspiracids (AZAs) can cause a syndrome in humans called Azaspiracid Shellfish Poisoning (AZP) that includes symptoms such as nausea, vomiting, severe diarrhea, and stomach cramps. Azaspiracids are regulated in the European Union, but currently not regulated in the United States. In the summer of 2014, seawater samples were analyzed for the presence of *Azadinium* species at 28 sites in Washington State waters and Solid Phase Adsorption Toxin Tracking (SPATT) disks were deployed for AZA detection at 25 sites. Quantitative polymerase chain reaction (qPCR) analysis detected the presence of three *Azadinium* species (*A. spinosum, A. obesum*, and *A. poporum*) throughout Puget Sound but none were detected on the outer coast. Although the qPCR signal was below the lowest value on the standard curves this indicates that the assay is highly sensitive. Azaspiracids 1, 2, and 3 were not detected in the SPATT analyses but other unknown peaks were detected, suggesting that other classes of AZAs may be present in the region. The detection of *Azadinium* spp. in Washington State waters demonstrates the need for future work to develop molecular probes that can be used in sandwich hybridization assays onboard the Environmental Sample Processor, an autonomous water sampling and analysis robot. With further development, these assays have the potential for use by resource managers as tools for early detection of *Azadinium* and AZAs.

HIGH FREQUENCY MONITORING OF CYANOHABS AND CYANOTOXIN PRODUCTION TO CHARACTERIZE PERIODS OF GREATEST RISK ON AN INLAND RESERVOIR

Joel Allen[1], Jody Shoemaker[1], Mike Elovitz[1], Chris Nietch[1], Jingrang Lu[1], Armah De La Cruz[1], Mia Varner[1], Kit Daniels[1], Tim Neyer[2]

[1]USEPA Office of Research and Development, Cincinnati, OH
[2]Clermont County, OH Water Resources Department

A monitoring approach combining wet chemistry and high frequency (HF) water quality sensors has been employed to improve our understanding of the ecology of an inland reservoir with a history of cyanoHAB events. Lake Harsha is a multi-use reservoir managed by the USACE in southwest OH that has experienced an increase in cyanoHAB frequency and intensity. Nutrient, algal taxa, chlorophyll-a, and physico-chemical data have been collected on the lake since 2010 at three week intervals. Beginning in 2014 in cooperation with Clermont County Drinking Water Division, a high frequency monitoring program was implemented to complement the tri-weekly data and provide DWTP operators time-relevant information regarding source water quality. High frequency data included in-vivo fluorescence and physico-chemical parameters and were collected at two locations. These data, coupled with limited microcystin (MC) analyses demonstrated the utility of HF data for tracking the cyanoHAB status of the reservoir. It was also apparent that MC concentrations were potentially underestimated as MC sampling did not coincide with bloom peaks indicated by the HF data. To better characterize the cyanobacterial population and both intracellular and extracellular MC production, an intensive sampling regime was developed for 2015 including LC-MS/MS analysis of select MCs, cylindrospermopsin, and anatoxin-a, MC ELISA quantification, transcriptomic analyses, protein phosphatase inhibition assay, nutrients, chlorophyll-a, and total organic carbon. Samples were collected weekly except during the initial bloom in June, when samples were collected daily. This approach allowed for the characterization of the cyanobacterial population dynamic and greatest periods of MC production. Data and observations from both 2014 and 2015 will be presented.

TRANSITIONING HAB RESEARCH TO OPERATIONS: AN OVERVIEW OF NOAA'S ECOLOGICAL FORECASTING ROADMAP

Allison L. Allen[1], Richard Stumpf[1], Gregory Doucette[2]

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[2] National Oceanic and Atmospheric Administration, National Ocean Service, Charleston, SC

For more than a decade, NOAA has been a lead U.S. Federal agency in studying and facilitating research of ecological processes, as well as developing experimental forecasts for a variety of ecosystem stressors, including harmful algal blooms. In 2012 NOAA established an Ecological Forecasting Roadmap to formulate a coordinated and systematic approach to developing and delivering the ecological forecast products and services at a national scale, but with regional specificity and delivery. Such a broadly-supported NOAA strategy for ecological forecasting offers management solutions to assure protection, maintenance and restoration of the health and productivity of ocean, coastal and Great Lakes ecosystems, for both natural resources and human communities. This agency-wide effort also connects internal NOAA activities to related efforts in the external academic community and private sector. The Roadmap provides a mechanism for NOAA and its partners to prioritize and address forecast requirements. While great strides have been made in HAB research, detection, monitoring, and modeling, barriers still exist in transitioning forecasts to operations, and each region of the U.S. both has unique challenges and is currently in a different state of maturity with respect to HAB forecasting. NOAA has an evolving 5-year plan for developing and transitioning HAB forecasts to operations, with efforts actively underway in Lake Erie, the Gulf of Maine, Pacific Northwest, California, Chesapeake Bay, Alaska, and other regions. This presentation will provide more detailed information NOAA's plans and priorities for transitioning mature HAB research and experimental forecasts to operations.

ESTIMATING SHELLFISH TOXICITY USING *ALEXANDRIUM* CELL COUNTS OBTAINED FROM ENVIRONMENTAL SAMPLE PROCESSORS (ESPs) IN THE GULF OF MAINE

Donald M. Anderson[1], Bruce A. Keafer[1], Dennis J. McGillicuddy, Jr.[1], Andrew R. Solow[1], Alison Sirois[2]

[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]Maine Department of Marine Resources, West Boothbay Harbor, ME

Shellfish monitoring programs throughout the world rely on flesh testing to detect and quantify biotoxins in shellfish tissues. Here we describe a novel approach that utilizes estimates of *Alexandrium fundyense* cell abundance obtained using Environmental Sample Processors (ESPs) to generate estimates of shellfish toxicity at nearby monitoring stations. Three ESPs were deployed 6-25 km from shore along a 115km stretch of the Gulf of Maine coast in 2014. Cell abundance estimates from the ESPs were used with weekly measurements of shellfish toxicity at nearby monitoring stations to fit the parameters of a simple, one-compartment model of toxin uptake and depuration in mussels. In 2014, the ESP-based shellfish toxicity estimates at three mooring sites were in excellent agreement (highly significant, $p < 0.001$) with the flesh-testing monitoring results for the nearest Maine Department of Marine Resources monitoring stations. Furthermore, the daily ESP data provided valuable insights into toxin dynamics that were not evident in the weekly flesh tests. These promising results are now being evaluated under different environmental conditions and at different locations through another set of ESP deployments in 2015 (ongoing at this time). This talk will review the 2014 and 2015 data, and discuss the potential for semi-automated sensors like the ESP to provide high-frequency information on shellfish toxicity to monitoring programs.

THE WAR BETWEEN HARMFUL DINOFLAGELLATES AND *AMOEBOPHRYA* SP

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Amoebophrya is a parasitic dinoflagellate genus that enters the host cell, typically grows for two to three days, then destroys the host during release of infective dinospores. Several harmful dinoflagellates are impacted by the genus including *Alexandrium, Akashiwo, Dinophysis*, and *Karlodinium*. Transcriptomic surveys of two *Amoebophrya* strains (ex. *Karlodinium veneficum* and *Akashiwo sanguineum*) were conducted, revealing major metabolic pathways in the parasite. Rhodopsin transcripts were surprisingly diverse and abundant, suggesting a role either in energy production or as a photoreceptor. The likely rhodopsin pigment, retinal, is probably synthesized using a gene acquired from bacteria, but the precursor isoprenoids are likely synthesized by the host. Similarly, parasites may be unable to synthesize sterols from isoprenoids. Other pathways including those for tryptophan synthesis appear to be modified when compared the host dinoflagellates. Surprisingly, given the high DNA content of the host, both parasite strains have genes for nucleotide synthesis. The major difference between the parasite strains was in nucleotide content and codon preference, with the parasite of *K. veneficum* showing strong AT bias. Codon bias was most pronounced in genes involved in motility and DNA binding. These data demonstrate transcriptomic data can be a window into host parasite interactions in non-model systems.

INVESTIGATING SUB-LETHAL EFFECTS OF CARIBBEAN CIGUATOXINS ON ZEBRAFISH (*DANIO RERIO*) BEHAVIOR

Katherine Baltzer[1,2,3], Alison Robertson[2,3], Tyler B. Smith[1]

[1]University of the Virgin Islands, Charlotte Amalie, VI
[2]University of South Alabama, Mobile, AL
[3]Dauphin Island Sea Lab, Dauphin Island, AL

Ciguatera fish poisoning (CFP) is the most common seafood-borne illness affecting humans worldwide. This disease is caused by consumption of fish contaminated with ciguatoxins. While the human health effects of ciguatoxins are well documented, the effects of ciguatoxins on fish behavior remain unknown. To address this question, we performed a 42-day chronic sub-lethal oral exposure of Caribbean ciguatoxin (C-CTX) in zebrafish (*Danio rerio*). General behaviors and a startle response were video recorded on a weekly basis and quantified via digital tracking (EthoVision, Noldus Inc.). Fish treated with C-CTX exhibited significantly increased hyperactive behaviors including elevated velocity, distance travelled, and highly mobile duration after 14 days of testing. Significantly increased thigmotaxis and meander were observed in C-CTX treated fish while control fish exhibited decreased velocities and distance travelled over time, indicating a level of habituation to behavioral testing. This effect was significantly suppressed in C-CTX treated fish. No significant changes were observed in the ability to startle in either group. These observations suggest that CTX presence may negatively affect fitness, potentially facilitating toxin transfer through trophic levels in the marine environment by making fish exposed to toxins more vulnerable to predation. This study provides the first evidence of a quantifiable behavioral effect of C-CTX in fish and provides a baseline for future studies on marine species.

A NOVEL MATRIX ASSISTED LASER DESORPTION IONIZATION/TIME-OF-FLIGHT (MALDI-TOF) METHOD FOR ANALYSIS OF BMAA, AND APPLICATION TO THE BIOACCUMULATION IN SOUTH FLORIDA FOOD WEBS

John P. Berry[1]

[1]Department of Chemistry and Biochemistry, Florida International University

The unusual amino acid, β-methylamino-L-alanine (BMAA), from cyanobacteria has been purportedly linked to neurodegenerative diseases (e.g. amyotrophic lateral sclerosis, Alzheimer's disease), and transfer through food webs proposed as an important vector. Toward developing improved techniques for identifying BMAA occurrence, and understanding its role in neurodegeneration, a method of detection and quantitation, specifically based on matrix assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry was developed, and subsequently employed to evaluate relevant biological samples from South Florida waters where BMAA accumulation, and human transfer, has been previously reported. The MALDI-TOF technique was found to effectively measure BMAA in a range of biological matrices, and moreover, was notably able, specifically by means of MALDI-TOF/TOF (i.e. tandem mass spectrometry), to discriminate BMAA from diaminobutyric acid (DAB) as a structural isomer also produced by cyanobacteria suggested to potentially confound analytical results. The details of the techniques along with analytical results obtained from application to relevant environmental samples from South Florida systems, comparison to conventional (e.g. liquid chromatography/mass spectrometry) techniques and potential for understanding toxicity of BMAA will be discussed.

CYANOTOXINS: FROM THE LAKE TO THE FAUCET

Lucas Beversdorf[1], Kayla Rude[2], Chelsea Weirich[1], Sarah Bartlett[3], Mary Seaman[2], Joseph Piatt[2], Todd Miller[1]

[1]Joseph Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI 53211
[2]Department of Chemistry, Carroll University, Waukesha, WI 53186
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Freshwater cyanobacterial blooms are becoming increasingly problematic in regions that rely on surface waters as drinking water sources. A major challenge confronting drinking water treatment plants (DWTPs) is identifying and managing toxic blooms that can develop near drinking water intake sites and the removal of those toxins if they enter the system. In this study, we measured concentrations of nine cyanotoxins and six cyanopeptides at seven different locations in eutrophic Lake Winnebago, Wisconsin, three locations of which contain intakes for nearby DWTPs. We also monitored various chemical, physical, biological, and meteorological parameters to determine the strongest indicators of toxin presence in the raw water at each drinking water intake. All microcystins and five out of the six cyanopeptides were detected in lake and raw drinking water samples and were significantly correlated with turbidity and absorbance at 254 nm. Those results are being used to develop a predictive, nowcast model. In addition, we measured cyanotoxin/peptide concentrations at various stages throughout the drinking water treatment process. At one DWTP, microcystins were detected at multiple stages of the treatment process, including finished tap water. Interestingly, microcystins increased and decreased at different stages, sometimes appearing downstream when they were not detected upstream, suggesting different treatments may accumulate toxins that can break-through later on. While most tap water samples were below the World Health Organization level for safe drinking water, it is still unknown whether chronic, low-dose exposure will have adverse affects on human health. Additionally, better drinking water treatments will be needed in order to efficiently remove all toxins from drinking water.

UNRAVELING DIVERSITY IN *PSEUDO-NITZSCHIA* SPECIES THROUGHOUT A TOXIC EVENT IN MONTEREY BAY, CALIFORNIA

Holly Bowers[1], April Woods[2], John Ryan[1], Roman Marin III[1], G. Jason Smith[2], Christina Mikulski[3], Gregory Doucette[3], Katherine Hubbard[4], Kendra Negrey[5], Raphael Kudela[5], James Birch[1], and Christopher Scholin[1]

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During spring 2015, Monterey Bay experienced an intense bloom of toxic *Pseudo-nitzschia* (Pn). This event was sustained over several weeks, with *P. australis* cell concentrations persisting in the 10^5-10^6 cells/L range during the height of the bloom. Domoic acid (DA) levels set a record for this region, with values consistently exceeding 10^4 ng/L. Fortuitously, the bloom occurred during a scheduled ECOHAB campaign aimed at studying this HAB hotspot. As a result, a myriad of instruments were in place to spatially and temporally sample conditions. We utilized a host of *in situ* and laboratory-based molecular techniques to uncover Pn species diversity throughout the event. Prior to bloom initiation, whole cell and sandwich hybridization analyses of environmental samples, coupled with ELISA on cultured isolates, revealed a mixed Pn community dominated by non-toxic *P. multiseries* (~70%; this species has been nearly absent from the bay for the last several years). *P. australis*, *P. arenysensis*, *P. fraudulenta*, and *P. pungens* were also present. Isolates of *P. pungens* (n=16) exhibited high cellular DA quotas likely contributing to early bloom toxicity. During the bloom, two moored Environmental Sample Processors with onboard genetic probe and DA arrays revealed a persistent population of toxic *P. australis* in the north and south bay. SEM on frustules recovered from DA arrays confirmed species present. Cultures were used to understand an uncoupling of DA and cell concentrations after the peak of the bloom. Diversity within the Pn community was further revealed via cell counts, DNA sequencing, and ARISA, on samples collected by ship and an AUV.

ARE HARMFUL ALGAL BLOOMS BECOMING THE GREATEST INLAND WATER QUALITLY THREAT TO PUBLIC HEALTH AND AQUATIC ECOSYSTEMS?

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When harmful algal bloom (HAB) events require restrictions on fisheries, recreation and drinking water uses of inland water bodies significant economic consequences result. Unfortunately, the magnitude, frequency and duration of HABs in inland waters are poorly understood across spatiotemporal scales, and are differentially engaged among states, tribes and territories. HAB impacts are not as predictable as those from conventional chemical contaminants, for which water quality assessment and management programs were primarily developed, because interactions among multiple natural and anthropogenic factors determine the severity to which a HAB will occur in a specific waterbody. These forcing factors can also affect toxin production. Beyond site-specific water quality degradation caused directly by HABs, the presence of HAB toxins can influence routine surface water quality monitoring, assessment and management practices. HABs present significant challenges for achieving water quality protection and restoration goals when these toxins confound interpretation of monitoring results and environmental quality standards implementation efforts for other chemicals and stressors. Whether HABs presently represent the greatest threat to inland water quality is debatable, though HABs in inland waters of developed countries typically cause more severe acute impacts to environmental quality than conventional chemical contamination events. Herein, we identify several timely research needs. Environmental toxicology, environmental chemistry, and risk assessment expertise must interface with ecologists, engineers and public health practitioners to engage the complexities of HAB assessment and management, to address the forcing factors for HAB formation, and to reduce the threats posed to inland surface water quality.

AN EXAMINATION OF LOSS RATES EXPERIENCED BY A LOCALIZED, INSHORE POPULATION OF *ALEXANDRIUM FUNDYENSE* THROUGH ITS COMPLETE LIFE CYCLE

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In recent years, a small, localized population of the PSP toxin-producing dinoflagellate *Alexandrium fundyense* has been the subject of intensive yearlong study to better characterize the controls limiting its growth. This population is centered in Salt Pond, a tidal body within Nauset Marsh (Cape Cod, MA), and is isolated from other blooms by the interaction of the pond's bathymetry, the organism's vertical migration behavior, and temperature- and salinity-driven stratification during bloom periods. Planktonic stages have been monitored through weekly surveys and deployments of a unique observation raft that hosts an Imaging FlowCytobot and an autonomous chlorophyll profiler. Loss and recruitment of benthic cysts has been monitored through collection of sediment cores, tube trap deployments and periodic spatial surveys of cyst abundance. In 2013, total *A. fundyense* cyst abundance was estimated at three time points and at the bloom's peak when cells were present as a mixture of vegetative and sexual swimming stages. In spite of a concerted shift by >70% of the population to sexual stages, new cyst recruitment within the pond was less than 10% of the population's maximum. One mechanism limiting recruitment was a shift in vertical swimming behavior by sexual life cycle stages to shallower depths. This shoaling of migration relaxed retention within the pond during the bloom's termination. Also, unexpected was a two-fold reduction in cyst abundance after the bloom's termination and prior to initiation of the next year's bloom, indicating the importance of loss processes during dormant cyst periods for control of *A. fundyense* populations.

THE IMAGING FLOWCYTOBOT FOR MONITORING HARMFUL ALGAL BLOOMS IN THE GULF OF MEXICO

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The Imaging FlowCytobot (IFCB), which combines flow cytometry and video technology to capture images of individual cells, has successfully provided early warning for seven harmful algal bloom events on the Texas coast as part of the Texas Observatory for Algal Succession Time series (TOAST). Data from TOAST are available from the Gulf of Mexico Coastal Ocean Observing System (GCOOS) website (http://gcoos.org/products/index.php/bio/hab/daily/). A commercial version of the IFCB (McLane Research Laboratories, Inc.) is now available with a more robust design and utilizing a 4.5 mW laser. The system is available with a 512GB SSD (~400 GB are available for local data storage). Typically, ~2 GB are acquired each day, so the system can provide storage for at least several months of data. Additionally, a more detailed user manual is in development with feedback from a number of end-users. The newest analysis software includes improved and more efficient image processing. The latest version of the website interface (the "dashboard") features a number of improvements including expanded viewing access and new plotting features. In addition to early warning of *Karenia brevis* and *Dinophysis ovum* events, the long-term data set from the IFCB at TOAST has permitted mapping distributions of individual taxa, elucidating patterns of microplankton community composition, examining predator-prey relationships, and identifying interannual variability in relation to environmental forcing.

"GO WEST, YOUNG HAPTOPHYTE": FISH KILLS ATTRIBUTABLE TO *PRYMNESIUM PARVUM* SPREAD TO SOUTHERN CALIFORNIA

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Prymnesium parvum is a mixotrophic, toxic haptophyte alga that has caused fish kills worldwide for many years. Within the last three decades, the alga has formed fish-killing blooms throughout the south-central U.S. states, most notably in Texas. While fish kills have also occurred in Californian freshwater ecosystems for decades, they have typically been attributed to depletion of dissolved oxygen, often linked to warm summer water temperatures and excessive (but not necessarily toxic) algal/cyanobacterial growth. A direct connection to the haptophyte, *Prymnesium parvum*, has not been suspected or confirmed in the region until very recently, although its presence in Californian waterways has been known for some time.

Several lakes in southern California experienced blooms of *P. parvum* resulting in massive fish kills during the past year. The blooms did not take place concurrently in the lakes but occurred over a fairly broad seasonal range (April through November). Questions remain as to whether the outbreaks of *P. parvum* and resultant fish kills represent instances of recent invasions of the lakes by the alga, or a more gradual response to changing environmental conditions in lakes in the region. A possible connection to the current 3+ years of drought in the region will be discussed. The appearance of toxic *P. parvum* blooms adds to the rapidly expanding list of water quality issues in the region, and raises the urgency for monitoring Californian lakes for the 'golden alga', studies to understand the conditions promoting recent toxic events, and management approaches for dealing with blooms.

LONG-TERM PUBLIC HEALTH MONITORING FOR TOXIC *MICROCYSTIS* BLOOMS IN THE KLAMATH RIVER OF NORTHERN CALIFORNIA

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The Karuk Tribe has been monitoring toxic blooms of *Microcystis aeruginosa* in the mid-Klamath River since 2005. The toxin was first detected in Copco Reservoir, in 2004 at levels far exceeding guidelines for recreational use. A comprehensive monitoring program was initiated in 2005 and is still in place. Annual toxin producing blooms of the cyanobacterium *Microcystis aeruginosa* have been documented in both Copco and Iron Gate Reservoirs (lowermost projects of the Klamath Hydroelectric Project (KHP)), and levels of the hepatotoxin microcystin greatly exceed public health standards. The reservoirs are the source of the blooms to the downriver environment and annually export both the cyanobacteria and the cyanotoxin at levels that exceed public health standards. Microcystin bioaccumulation has been documented in yellow perch in the reservoirs, salmon juveniles reared in Irongate hatchery below the reservoirs, and adult Chinook salmon and steelhead in the downstream river environment. High levels of microcystin were found in Klamath River freshwater mussels even when ambient concentrations were very low indicating toxin biomagnification. Yellow perch and freshwater mussels showed tissue levels exceeding World Health Organization tolerable daily intake levels. Toxic algae-related water quality impacts stress freshwater biota in the Klamath River that are important Tribal Trust species and subsistence food sources and have a negative effect on cultural beneficial uses.

FITNESS COST OF GRAZER-INDUCED TOXIN PRODUCTION IN THE MARINE DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE*

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Recent work provides strong evidence for dramatic grazer-induced toxin production in the marine dinoflagellate *Alexandrium fundyense*. This response is elicited by several phyla of metazoan grazers. The fitness cost of induction of toxin production is not known or understood yet. Resource allocation theory posits that resources allocated to defense are at the expense of cell growth. Thus, one would expect that induced toxin production must come as a cost to growth (fitness) to the prey. Here, we show results of experiments designed to measure the fitness cost of grazer-induced toxin production. *Alexandrium fundyense* was exposed for three-days to the copepod *Acartia tonsa* during early and late exponential, and post stationary phase of a simulated laboratory bloom. Growth rate was measured after grazer exposure in cells that had been directly in contact with the grazers (direct exposure), exposed to grazer clues (indirect exposure) and not exposed to the grazers (control) in either nutrient-replete (f/4) or nutrient poor (filtered seawater). A trade-off between toxin production rate and cell growth was evident, demonstrating the fitness cost of inducible toxin production. The cost of toxin production was highest earlier in the bloom, and lowest during the post stationary phase. Investment in toxin production far exceeded requirements for defense. Cost of toxin production was also higher for nutrient-replete cells. These results have important implications for understanding and modeling bloom dynamics.

INVESTIGATING THE ROLE OF REACTIVE OXYGEN SPECIES IN DRIVING BLOOM TOXICITY IN WESTERN LAKE ERIE

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Despite decades of research, the physiological function of cyanobacterial toxins remains uncertain. A growing body of evidence suggests that reactive oxygen species (ROS) may in fact be an important driver of cyanobacteria community structure and toxicity. First, sources of ROS, such as hydrogen peroxide (H_2O_2) are more pervasive and dynamic than previously recognized. Second, ROS are highly toxic to cyanobacteria and other phytoplankton and have a profound effect on their physiology, but tolerance of ROS varies widely across taxa. Because ROS are produced through photochemical reactions with dissolved organic matter (DOM), there is a potential feedback between increasing DOM from blooms or terrestrial runoff, ROS, and cyanobacterial community composition and toxicity. Furthermore, a recent theory is that one ecological function of microcystins (MC) is to protect cells from oxidative stress. In this study, we monitored the concentrations of H_2O_2 across three sites in western Lake Erie on a weekly basis before during and after the bloom of 2014. These measurements were analyzed in conjunction with other water quality parameters including pigment and nutrient concentrations, ratios of MC and non-MC producing cyanobacteria, total phytoplankton community composition. We coupled the monitoring with microcosm experiments investigating the effect of added H_2O_2 as a function of nutrient (N+P) concentrations on community composition, particulate, dissolved and conjugated MC and ratios of non-MC producing and MC-producing cyanobacteria. Results from the monitoring and microcosm studies suggest that H_2O_2 may play a role in bloom toxicity if nutrient concentrations are sufficient for MC production.

A MODEL FOR HUMAN CONSUMPTION LEVELS OF DOMOIC ACID IN THE PACIFIC NORTHWEST

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Domoic acid (DA), a neurotoxic amino acid, is naturally produced by marine phytoplankton and presents a significant health threat to human populations via transfer of the toxin through the foodweb. Razor clams (*Siliqua patula*) are a commonly harvested shellfish that can retain DA up to one year, exposing human consumers to chronic low levels of DA (below the regulatory limit of 20 ppm). Chronic, low level exposure to DA has been connected to such toxic effects as renal toxicity, increased toxin susceptibility and impaired mitochondrial function in laboratory studies, placing coastal communities at risk due to their high levels of shellfish consumption. We are conducting a year-long survey of human, razor clam consumers to quantify DA exposure scenarios based on shellfish consumption rates and DA concentrations in razor clams. We will combine the survey data with archived razor clam DA monitoring data from 1991-2015 to (1) recreate actual DA exposure of WA coastal razor clam consumers in 2015, and (2) quantify DA exposure scenarios for human shellfish consumers based on shellfish consumption rates, ranges of DA concentrations in razor clams, and hypothetical razor clam harvest rates. Here, we will provide preliminary estimates of long term, human exposure to DA, via razor clam consumption, using partial survey returns. By quantifying chronic, low level, human exposure to DA we will create a vital connection between laboratory evidence of toxic effects of chronic low DA exposure and human health risks, and inform management policies protecting human shellfish consumers from environmental toxins.

DOMOIC ACID IN STRANDED JUVENILE GREEN SEA TURTLES, *CHELONIA MYDAS*, IN SOUTHEAST FLORIDA

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Domoic acid (DA), a neurotoxin produced by several species of *Pseudo-nitzschia*, causes illness and mortality in marine mammals and seabirds along the U.S. Pacific coast. DA exposure has been documented in the Gulf of Mexico and along the southeastern U.S. coast but has not been associated with mortality in these areas. Between November 2014 and April 2015, strandings of small juvenile green sea turtles, *Chelonia mydas*, increased across southeast Florida. Most affected individuals displayed neurological signs. As part of the investigation, more than 50 turtles were analyzed for HAB toxins. DA was detected in most turtles tested that had died and in some of the live turtles. Concentrations ranged from very low (<1 ng/ml plasma) to a maximum of 29 µg DA/g in intestinal contents. Postmortem examination also revealed heavy parasitic infection in most of the turtles, complicating interpretation of the DA results. Consistent findings included enteric coccidiosis (*Caryospora* sp.) with associated enteritis. Several turtles also had neurological infection by blood flukes. The degree to which DA contributed to this event remains uncertain. The neurological signs exhibited by some turtles were different from those expected from parasitism or physiological causes alone, suggesting an effect from DA. DA has not been associated with mass turtle mortalities, and little is known about its effects on reptiles. Our results demonstrate that sea turtles in the Atlantic can be exposed to significant levels of DA and highlight our lack of knowledge about the cumulative or synergistic health effects of HAB toxins and other stressors.

USING THE MMPB TECHNIQUE TO CONFIRM MICROCYSTINS MEASURED BY ELISA AND HPLC (UV, MS, MS/MS)

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Microcystins have been detected in both raw and finished drinking water using a variety of analysis techniques, including bioassays (ELISA, protein phosphatase inhibition) and HPLC (UV, MS, MS/MS). The principal challenge to microcystin analysis is accounting for the >150 congeners that have been described to date. Although there are ELISA antibodies that result in congener independent measurement, the analysis is suitable for screening and requires confirmatory analysis. Confirmatory individual variant HPLC analyses are prone to under reporting microcystins due to the method specificity. One method that does not require individual variant quantitation, but allows for LC-MS/MS confirmation, is the MMPB technique. Samples are chemically oxidized to cleave the Adda amino acid portion of microcystin, a common moiety to all variants. Analysis is then conducted on the subsequent MMPB molecule using LC-MS/MS. In this study, the MMPB technique was employed and calibrated for the analysis of water samples using a certified reference standard of microcystin-LR. Raw and finished water sources with native microcystins were screened using an Adda enzyme linked immunosorbent assay (ELISA) and results were compared to individual variant analyses (LC-UV, LC-MS, LC-MS/MS) and the MMPB method. Individual variant analysis did not account for all the microcystins present in the samples, as indicated by ELISA and MMPB data. Instrument detection limits for total microcystins using the MMPB method were 0.5 µg/L, with method detection limits (10x concentration) of 0.05 µg/L. Results demonstrated the MMPB technique is a simple and valuable technique to confirm ELISA data when analyzing for total microcystins.

DEPURATION OF MICROCSYTIN TOXIN IN *MYTILUS CALIFORNIANUS* AND IMPLICATIONS FOR USE AS AN INDICATOR ORGANISM

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Aquatic organisms are often used in management practices as indicator species, and information gleaned from these organisms can be applied to water quality assessment and detection of Harmful Algal Blooms (HABs). Marine bivalves, such as California mussels (*Mytilus californianus*), are particularly useful in assessing accumulation of HAB toxins because they are widespread, are common prey items, and often consumed by humans. We examined the depuration rates of California mussels for both particulate and dissolved microcystin toxin. Pilot work indicated that California mussels take up microcystin within 24 hours; based on that, mussels were collected from Monterey Bay and shocked with microcystin for 24 hours at varying concentrations, simulating a riverine flushing event to the coastal ocean. After the 24 hour shock period, mussels were placed in constantly flowing seawater, sampled through time, and analyzed for microcystin via LCMS. Our results indicate that mussels depurate particulate microcystin toxin slowly. Toxin was still detectable for more than two months following initial exposure. Dissolved toxin is also taken up by California mussels but depurates more rapidly. Mussels in dissolved trials were clear of toxin after 72 hours post exposure. This research highlights the need for monitoring of microcystin in water and in prey species. The identification of this potential sentinel species could be used to inform the Mussel Watch Program or aid in establishing an analogous monitoring program. These results also suggest that ephemeral discharge to the coastal ocean could have negative impacts to higher trophic levels for weeks to months following exposure.

NUTRIENTS AND HABS: BEYOND THE CLASSIC EUTROPHICATION PERSPECTIVE

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It has long been known that nutrient availability regulates algal growth. A classic kinetic perspective suggests that too few nutrients and growth is limited, but once nutrients are saturated growth does not increase. While excessive nutrients may lead to eutrophication, understanding responses of specific species or species groups to nutrient pollution is complicated. Eutrophication is often associated with HAB proliferation, but HABs are not always associated with eutrophication although their distribution may be related to relative nutrient availability. The complexity in species' responses arises from the fact that the rate of export of nitrogen (N) and phosphorus (P) is not evenly distributed in time or space leading to stoichiometric imbalances, and not all forms of N or P are used equally by all primary producers and, in the case of N, these different N forms may be used differently under different N:P stoichiometric conditions. Moreover, these nutrient differences affect algal nutrient physiology and community assembly at all concentration levels, from limiting to super-saturating with important implications for the increasing proliferation of HABs.
In this talk, an overview of physiological regulation by major functional groups will first be provided. Then, specific examples of effects of changes in stoichiometry will be shown, based on culture studies of *Prymnesium parvum* and *Karlodinium veneficum*. Finally, a synthesis of major HAB relationships and nutrient stoichiometry for Chesapeake Bay will be shown.

NICHE DIFFERENTIATION AMONG CYANOBACTERIAL POPULATIONS REVEALED VIA ECO-TRANSCRIPTOMIC SURVEYS OF LAKE ERIE

Christopher J. Gobler[1] and Matthew J. Harke[1]

While toxic cyanobacterial blooms in Lake Erie threaten drinking water supplies and are promoted by nutrient loading, the precise nutrient regime that selects specific cyanobacteria populations is poorly understood. Here, we assess shifts in cyanobacterial abundances and global gene expression patterns in response to natural and manipulated gradients in nitrogen (N) and phosphorus (P) in western Lake Erie to identify gene pathways that facilitate dominance by different genera. Gradients in orthophosphate concentrations played a key role in shaping cyanobacteria communities and had the largest effect on transcriptomic responses. Under high P conditions, *Anabaena* and *Planktothrix* were the dominant cyanobacterial populations and P promoted nifH expression in Anabaena. While additions of P elicited the upregulation of genes involved in phage infection defense, genomic rearrangement, and nitrogen acquisition in *Microcystis,* these conditions also led to lower *Microcystis* abundances. In the presence of low levels of P, however, *Microcystis* became the dominate cyanobacteria as it upregulated genes associated with P scavenging (pstSCAB, phoX) and storage (ppk1). Nitrogen did not alter *Microcystis* abundances but did increase the expression of protease inhibitors (aer, mcn gene sets) that may deter zooplankton grazing as well as microcystin synthetase genes (mcy) with urea enrichment yielding significant increases in microcystin concentrations. The expression of genes that facilitate the adaptation of *Anabaena* and *Planktothrix* to high P regions and *Microcystis* low P zones suggest that management schemes that reduce the delivery of P to western Lake Erie may alter the composition, but not the persistence of cyanobacterial blooms.

AN UPDATE ON INTERAGENCY HABHRCA PRIORITIES

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After the reauthorization of the Harmful Algal Bloom and Hypoxia Research and Control Amendments Act in 2014, an Interagency Working Group on HABHRCA (IWG-HABHRCA) was formed, co-chaired by NOAA and EPA. It is charged with developing a Comprehensive Research Plan and Action Strategy for HABs and hypoxia, the delivery to Congress of which is expected in fall 2015. A separate section of the report assesses HABs and Hypoxia in the Great Lakes. Further, HABHRCA calls for substantial stakeholder involvement in producing reports and has a much greater emphasis on freshwater HABs and hypoxia. In addition to completing the Research Plan and Action Strategy, the IWG-HABHRCA has begun working on its next report, a Great Lakes Plan, anticipated to be delivered Congress in summer 2016.

This town hall discussion will provide an overview of the efforts by the IWG-HABHRCA and an update on the products developed in response to the new legislation. It will summarize emerging stakeholder needs and concerns, the recommendations prescribed by the report, and the next steps for the IWG-HABHRCA, including information on the Great Lakes efforts. It also will allow for discussion on management needs, gaps, and successes.

MONITORING HUMAN EXPOSURES TO ALGAL TOXINS: FROM SURVEILLANCE TO CLINICAL ANALYSIS

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Harmful algal blooms (HABs) are an emerging public health issue. HAB-related illnesses are not well-characterized by current data on clinical presentation, and there are challenges to confirming the extent of HAB toxin exposure when people become ill. CDC has collaborated with state and Federal partners to develop HAB surveillance capacity and create an electronic public health reporting platform. This system, which is currently being tested by multiple states, will collect and link individual reports of human and animal illness to HAB exposures. To confirm suspected HAB exposures, clinical methods are essential as symptomology is often insufficient for specific diagnoses. Analytical methods, developed within the CDC, can identify and quantitate select algal toxins in human urine using liquid chromatography coupled with mass spectrometry for sensitive and selective detection. However, these capabilities are currently limited to a few toxins and additional methods are in development to address these continued public health concerns. With the availability of these assays, specimen collection and analysis has the potential to complement future response efforts to HABs. Health surveillance coupled with clinical testing will provide essential information for identifying, confirming, and documenting the public health impacts of HABS.

USE OF FLOWCAM TECHNOLOGY TO MONITOR AND CHARACTERIZE MARINE ALGAL BLOOMS IN CENTRAL PUGET SOUND

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While algal blooms are a natural phenomenon there has been a worldwide increase in the frequency and duration of blooms that can be linked to human impact, primarily in relation to changes in climate and water quality. King County's long running marine monitoring program is designed to assess water quality in the Central Puget Sound Basin. Routine data collection includes a suite of physical, chemical, and biological parameters, including a phytoplankton component that was added more recently with the purpose of addressing a serious deficit in information on the Sound's lower trophic levels. Subsequent acquisition of a FlowCAM imaging particle analysis system has made it possible to start building an extensive and robust long term dataset for Puget Sound plankton. An important program goal is to use this information to improve our understanding of the environmental controls underlying the dynamics of algal blooms in relation to anthropogenic impacts.

Surface samples from eight Puget Sound Central Basin locations were collected biweekly in 2014 and 2015. With the aid of FlowCAM we identified and characterized approximately 60 taxonomic categories using relevant descriptors of assemblage composition, such as particle size distribution, abundance and biovolume. The resulting comprehensive dataset was used to assess the climatic, physical, and nutrient conditions, as well as community attributes, that are associated with bloom events. We examined the conditions leading to seasonally predictable blooms as well as those associated with the more sporadic blooms that are typical of harmful flagellate taxa, such as *Alexandrium, Akashiwo* and *Heterosigma*.

DIRECT NITROGEN USE BY THE DSP-PRODUCER, *DINOPHYSIS ACUMINATA*: EXPERIMENTS USING CULTURES AND FIELD POPULATIONS

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In recent years, North America has witnessed an expansion of *Dinophysis* blooms yielding shellfish toxicity over the USFDA action level (160 ng g^{-1} of shellfish tissue) on the East, West and Gulf coasts of the US. While blooms of harmful algae have frequently been linked to excessive nutrient loading, *Dinophysis* is a mixotrophic alga whose growth is typically associated with prey availability and field studies of *Dinophysis* and nutrients have been rare. This presentation will describe a series of field and laboratory experiments assessing how blooms of *Dinophysis* are affected by nutrients. Using *Dinophysis* bloom water for experiments performed over a three year period the addition of inorganic and organic nitrogen significantly ($p<0.05$ for all) enhanced Dinophysis densities. However, given the presence of planktonic members that could potentially be direct (*Mesodinium*) or indirect (flagellates) prey of *Dinophysis* we could not distinguish between indirect (nutrient stimulation of prey) vs direct (nutrient stimulation of *Dinophysis*) use of these nutrients. Hence, we established a *Dinophysis* culture isolated from a NY estuary to assess the effects of nutrients on the growth of *Dinophysis* with and without its prey, *Mesodinium rubrum*. *Dinophysis* cultures grown with and without prey grew significantly faster when offered various nitrogenous nutrients compared to respective controls. Furthermore, we unequivocally demonstrated that *Dinophysis* is capable of direct assimilation of multiple ^{15}N-labeled nitrogen sources, albeit at varying rates for different compounds. Our results demonstrate that, like other HABs, blooms of the mixotrophic dinoflagellate, *Dinophysis*, can be directly stimulated by anthropogenic nutrient loading.

CAN CO-OCCURRING SPECIES REVEAL DINOPHYSIS BLOOM ORIGINS?

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The western Gulf of Mexico experiences periodic phytoplankton blooms caused by many different species. Blooms of the toxic dinoflagellate Dinophysis ovum have become a nearly annual occurrence off the coast of Texas and ingestion of shellfish that fed on *D. ovum* can lead to diarrhetic shellfish poisoning. Blooms of three toxic dinoflagellate species (*D. ovum, Prorocentrum minimum,* and *P. texanum*) occurred off the coast of Texas in close succession during a four month period from January – April 2010. The same three species also occurred, with varying abundances and timing, during blooms in four other years. A spatially-explicit, individual-based model of phytoplankton was used to identify potential origins of cells comprising these blooms. To identify potential source(s) of blooms, the model was run in reverse with cells inserted according to abundance estimates obtained from the Imaging FlowCytobot in Port Aransas, Texas. Results from the 2010 simulation showed the bloom of Prorocentrum texanum at Port Aransas, Texas originated from the northeast, near the coast of Louisiana. During a summer 2014 cruise off the coast of Louisiana, a bloom of *P. texanum* was identified along with *Mesodinium rubrum*, a prey item of *Dinophysis*, and elevated abundance of *D. caudata* (another species present during Dinophysis blooms in Texas). However, no *D. ovum* cells were identified in the cruise samples. The lack of *D. ovum* cells could be a result of unfavorable environmental conditions (e.g. warmer water temperature). For confirmation, field sampling in the Louisiana region should include the late fall/early winter time period.

CHANS: MODELING THE DYNAMICS OF HABS, HUMAN COMMUNITIES, AND POLICY CHOICES ALONG THE FLORIDA GULF COAST

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Coupled human-nature systems (CHANS) involve dynamic interactions between humans and nature, often influenced by and affecting the distinct dynamic characteristics of each component. We present an overview of an ongoing interdisciplinary research program focused on a specific type of systems that couple expanding and fluctuating human coastal populations to episodic blooms of toxic marine algae, drawing examples primarily from human interactions with blooms of the toxic dinoflagellate *Karenia brevis* from the eastern Gulf of Mexico ("Florida red tides"). We introduce a set of HAB Symposium "speed" presentations and associated posters based on multi-disciplinary research. Using extant, but extraordinary, data to specify empirical models, this program of research has focused on characterizing the influence of anthropogenic sources on *K. brevis* blooms, assessing the public health and economic impacts of these blooms in an exposure-response framework, and defining the choice of appropriate human policy responses to the hazard. We present examples of the generic aspects of CHANS systems in the context of Florida red tides, and we discuss also some of the challenges involved in compiling and analyzing the relevant data to support our positive and normative analytical efforts.

WIDESPREAD PREVALENCE OF MICROCYSTINS FROM A VARIETY OF SOUTHERN CALIFORNIA WATERBODIES AND IMPLICATIONS FOR TOXIN LOADING TO COASTAL WATERS

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Cyanobacterial blooms and associated toxins have become increasingly problematic globally and cause a variety of harmful effects that impair beneficial uses of waterbodies. Cyanotoxins can have far reaching effects downstream of their origin, creating issues in brackish and marine waters. Despite the health risks associated with cyanotoxins, there is no statewide monitoring program in California and relatively little is known about the prevalence of cyanotoxins in southern California waterbodies. Recent screening assessments revealed widespread prevalence of microcystins from a variety of waterbody types including streams (benthic algae), depressional wetlands, lakes, reservoirs, coastal lagoons and estuaries. Microcystins were commonly detected in most waterbodies in Southern California, and from benthic samples in one-third of wadeable stream reaches statewide. Multiple cyanotoxins (cylindrospermopsin, anatoxin-a, saxitoxin and microcystins) were detected at a subset of sites, indicating the potential for other cyanotoxins to be prevalent. These results suggest multiple terrestrial sources of cyanotoxins, including benthic cyanobacteria from wadeable streams, as potential loading sources to downstream coastal waters in Southern California.

Traditional sampling approaches have been shown to miss toxic events and to be poor indicators of the temporal variability of the ecosystem. In response to this challenge, several intensive studies in San Diego will be presented that successfully used passive samplers, Solid Phase Adsorption Toxin Tracking (SPATT), to capture the prevalence of microcystins in a diverse array of waterbodies. The results from these screening assessments indicated that microcystins are pervasive, and missed by traditional sampling approaches.

COMPLEX BLOOM DYNAMICS REVEALED BY GENETIC CHARACTERIZATION OF US ATLANTIC AND GULF OF MEXICO *PSEUDO-NITZSCHIA* COMMUNITIES

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The marine diatom genus *Pseudo-nitzschia* includes more than 40 species, approximately half of which occur in US Atlantic and/or Gulf of Mexico coastal waters. Here, genetic tools for *Pseudo-nitzschia* spp. detection were incorporated into state phytoplankton monitoring programs at two coastal locations (Bar Harbor, ME and St. Joseph Bay, FL), where *Pseudo-nitzschia* blooms have recently been linked with observations of the neurotoxin domoic acid (DA) in shellfish. Regional-scale oceanographic surveys in the Gulf of Maine and the Gulf of Mexico provided further opportunities to sample *Pseudo-nitzschia* bloom initiation, toxicity, and/or decline. More than 500 environmental samples were screened using DNA fingerprinting (ARISA) to target the variable internal transcribed spacer 1 (ITS1) region of the rRNA gene. Modified ARISA approaches that incorporated either nested PCR or degenerate primers allowed resolution of additional species and successfully amplified a subset of samples for which ARISA did not initially work. Nearly 40 unique ITS1 amplicon sizes were observed, many of which were identified by targeted environmental sequencing to represent either known or novel inter- and intra-specific *Pseudo-nitzschia* diversity. Genetic analyses revealed ecologically relevant, statistically significant structure in *Pseudo-nitzschia* communities, i.e., seasonally, during blooms, latitudinally, and across nearshore/offshore and vertical environmental gradients. These multi-regional observations of *Pseudo-nitzschia* spp. highlight complex relationships between taxonomic and ecological diversity and environmental processes that are being used to enhance region-specific monitoring, management, and predictive capabilities.

DEVELOPMENT AND TESTING OF A HIGH-SPEED MICROSCALE IMAGING SYSTEM FOR EXPLORATION OF HARMFUL ALGAL BEHAVIOR AND INTERACTIONS

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For many harmful algal bloom (HAB) species, behavior and interactions at individual level not only is crucial to their success in ecosystems but also can potentially affect bloom initiation, abundance, distribution and development. It is thus relevant to investigate and understand individual-level behaviors in the context of HAB dynamics and ecology. However, because individual-level behavior and interactions often occur at sub-millimeter spatial and millisecond temporal scales, to observe them poses a significant technological challenge. Traditionally, microscopes are used but with two major issues: (1) small sample volumes result in strong "wall" effects, and (2) the vertical optical axis constrains observations in a horizontal field-of-view and therefore gravitational influence is neglected. To address these issues, we have developed a high-speed microscale imaging system (HSMIS) that adopts a horizontal optical axis to achieve a vertically oriented sub-millimeter field-of-view. A long working distance objective lens allows observations of free-swimming cells held in a cell culture vessel that is large enough to eliminate wall effects. To register rapid movements of both cell body and flagella simultaneously, HSMIS uses a high-resolution, high-speed camera that records sub-micrometer cell features at frame rates up to 2000 frames per second. The illumination source for the HSMIS has also been designed to minimize convective flow within its observation vessel. Thus far, the system has been used to observe free-swimming behavior of the dinoflagellate *Alexandrium fundyense* in both laboratory cultures and field samples, and in laboratory experiments examining predator-prey interactions between the dinoflagellate *Dinophysis acuminata* and the ciliate *Mesodinium rubrum*.

DISCOVERY OF A RESTING STAGE IN *AUREOUMBRA LAGUNENSIS* (PELAGOPHYCEAE): A MECHANISM FACILITATING ITS RECENT EXPANSION?

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Despite the recent expansion of brown tides to the East Coast of Florida and to Cuba, a resting stage has not been identified in any pelagophyte. Here we present our evidence demonstrating the ability of *Aureoumbra lagunensis* to enter a resting stage in response to environmental stressors including high temperature, nutrient depletion, and darkness as well as their ability to revert from resting stages back to vegetative cells. Exposure of vegetative cells to high temperature (~35°C) or nutrient limitation resulted in the transformation of cells into resting stage cells that became round in shape, larger in size, filled with red accumulation bodies, contained a lower concentration of cellular chlorophyll, and displayed a dramatically reduced photosynthetic efficiency relative to vegetative cells. Analysis of vegetative and resting stage cells using Raman microspectroscopy indicated resting stage cells were enriched in lipids associated with their red accumulation bodies and were depleted in carotenoids relative to vegetative cells. Upon the introduction of resting stage cells into nutrient replete conditions at 21°C, resting stage cells reverted to vegetative cells over a ~one week period, increasing their chlorophyll content, photosynthetic efficiency, and growth rate, but losing their lipid accumulation bodies, getting smaller, and taking on a less symmetrical shape. The ability to form a resting stage is likely to facilitate the expansion of *Aureoumbra lagunensis* to new regions via anthropogenic transport processes. Results from the revival of resting stage cells stored for an extended period (months) in the dark will also be presented.

IMPROVEMENTS AND EXPANSION OF NOAA'S HARMFUL ALGAL BLOOM OPERATIONAL FORECAST SYSTEM

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Since 2004, NOAA has disseminated an operational harmful algal bloom (HAB) forecast for the Gulf of Mexico to serve as a decision support tool for coastal resource managers, public health officials, researchers, and the general public. Sustained operations for over ten years has provided systematic, reliable, and robust HAB forecast products to stakeholders in the region. Leveraging NOAA's Ecological Forecasting Roadmap, the HAB-OFS program intends to expand beyond the Gulf of Mexico as part of the national ecological forecasting plan. Work is ongoing to transition demonstration forecasts in Lake Erie and the Gulf of Maine to operations. Concurrently, improvements to maintain operational performance standards and enhance the efficiency and effectiveness of current operational products are being enacted based on the results of routine evaluations of forecast quality and feedback gathered from stakeholders. The improvements include 1) modifications to the product format, 2) development of more efficient forecast tools, 3) refinement of ocean color satellite imagery products, and 4) creation of a next-generation GIS-based infrastructure. Processes to improve the research to operations (R2O) cycle are also being established within NOAA to support product advancement and ensure that forecasts are consistently refined based on emerging research and evolving user needs. The HAB-OFS will continue to strongly rely on collaboration and coordination with its regional stakeholders. As these changes are planned and introduced, user feedback will be needed to ensure that the modifications have satisfactorily addressed these requirements and to identify future needs.

LINKING CIGUATOXICITY TO THE LIFE HISTORY AND ECOLOGY OF RED LIONFISH (*PTEROIS VOLITANS*) IN THE WESTERN ATLANTIC

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Red lionfish (*Pterois volitans*) are an invasive fish species native to the Indo-Pacific that was first introduced in the Atlantic Ocean off the east coast of Florida in the 1980s. Since their introduction, lionfish populations have spread throughout the western Atlantic. The proliferation of lionfish has come at the detriment of native reef species both through predation and competition. A suggested method for controlling lionfish numbers is to develop a fishery for the species. However, lionfish populations in certain areas of the western Atlantic overlap with areas known for ciguatera fish poisoning (CFP). Ciguatera fish poisoning is caused by the ingestion of fish containing ciguatoxins (CTX). The goal of this study is to examine lionfish life history and whether these factors influence ciguatoxicity. Lionfish were sampled from the Florida Keys (n=105) and U.S. Virgin Islands (n=96). All lionfish were measured, weighed, sexed, aged and had their stomach contents analyzed. White muscle tissue and livers were acetone extracted and tested for CTX using a mouse neuroblastoma cytotoxicity assay. From the Florida Keys, 19% of livers and 2% of muscle samples from lionfish showed signs of CTX. Samples of lionfish from the U.S. Virgin Islands were ciguatoxic in 44% of livers and 18% of muscle samples. This is the first reported presence of ciguatoxicity in lionfish from the Florida Keys while further corroborating previous studies from the U.S. Virgin Islands. This summer, quantification of CTX will be completed and the relationship between ciguatoxicity and lionfish ecology will be assessed.

AFFECTS OF CHRONIC LOW-LEVEL SEAFOOD TOXIN EXPOSURE ON LEARNING AND MEMORY INTO OLD AGE

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The seafood toxin domoic acid is a potent neurotoxin that is responsible for the human illness known as Amnesic Shellfish Poisoning (ASP). Neurotoxic symptoms of ASP are severe and occur when toxin levels are high in shellfish. Regulatory limits (20 ppm) have been put into place to prevent the acute high-level exposures that illicit overt neurotoxic symptoms (seizures, memory loss, confusion, death). However, the effects of low-level chronic exposure to domoic acid are still a concern due to lack of visible outward signs of toxicity. Many seafood-consuming populations exist that are exposed to low levels of domoic acid on a regular basis. In the present study, we used a mouse model to identify sub-clinical effects of low-level chronic domoic acid exposure on learning and memory. Learning and memory were tested at 1, 6 and 25 weeks of exposure as well as at 9 weeks of recovery (no exposure) to see if cognitive deficits exist and if so, if they persist after exposure ends. Additionally, a set of mice were exposed once a week for 9 months (age 3 to 12 months) to low-level asymptomatic doses of domoic acid, then tested at multiple old age time points to see if early life span exposure exacerbates cognitive decline with age. The results of cognitive tests under the above exposure scenarios will be discussed. Preliminary findings suggest that severe cognitive deficits occur with long-term chronic exposure.

IDENTIFICATION OF THE *ALEXANDRIUM* SPECIES RESPONSIBLE FOR PARALYTIC SHELLFISH POISONING IN ALASKA

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Paralytic shellfish poisoning (PSP) represents a major economic and health threat to coastal communities along the US west coast, particularly in Alaska where PSP is sufficiently prevalent that it is deemed a public health emergency with mandatory reporting to the State's Division of Epidemiology. PSP results from consumption of shellfish that have accumulated saxitoxins, potent neurotoxins most commonly produced by certain dinoflagellate species in the genus *Alexandrium*. Despite the persistent threat posed by PSP, little is known about which *Alexandrium* species occur in Alaskan coastal waters. The objective of this study was to develop species-specific PCR assays for the six *Alexandrium* species likely to occur in Alaskan coastal waters and to use those assays to screen phytoplankton, sediment, and single cell isolates collected from four locations in the Gulf of Alaska ranging from Southeast Alaska to Kodiak Island (>700 km) to establish which species were present. A molecular approach was adopted because *Alexandrium* species are difficult to distinguish using traditional microscopic methods. This inability to distinguish species is important from a monitoring standpoint because toxic and non-toxic species frequently co-occur, making it difficult to assess PSP risk from cell counts alone. Screening using the molecular assays revealed that both *Alexandrium fundyense* and *A. ostenfeldii* occurred over the entire study area, with *A. fundyense* being most abundant. *Alexandrium fundyense* is known to be the dominant saxitoxin producer in the Gulf of Main and elsewhere. This finding suggests that the *A. fundyense* research carried out over the past 20 years in the Bay of Fundy and Gulf of Maine may be directly applicable to managing this problem in Alaska. The assays developed in this study can be incorporated into monitoring systems to provide managers with early warning when the onset of toxic blooms pose a heightened risk of PSP.

CYANOTOXINS IN INLAND LAKES OF THE CONTINENTAL UNITED STATES: PHOTIC ZONE OCCURRENCE AND POTENTIAL RECREATIONAL HEALTH RISKS IN THE EPA NATIONAL LAKES ASSESSMENT 2007

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The largest spatial survey of cylindrospermosins, microcystins, and saxitoxins in the United States was conducted as part of the EPA National Lakes Assessment 2007. Integrated photic zone samples were collected from 1,161 lakes during May-September 2007. Cyanotoxin, cyanobacteria, and chlorophyll concentrations were compared with World Health Organization (WHO) guidance for public health protection. Cylindrospermopsins, microcystins, and saxitoxins were detected by ELISA in 4.0, 32, and 7.7 % of samples with mean concentrations (detections only) of 0.56, 3.0, and 0.061 µg/L, respectively. Co-occurrence of the three cyanotoxin classes was rare (0.32%). Cyanobacteria were present in 98% of samples and dominant in 76% of samples. Potential anatoxin-, cylindrospermopsin-, microcystin-, and saxitoxin-producing cyanobacteria occurred in 82, 66, 95, and 78% of samples, respectively. Anatoxin-a and nodularin-R were detected in 15 and 3.7% of samples (n=27) that were analyzed by LC/MS/MS. Both *Cylindrospermopsis sp.* and *Nodularia sp.* occurred rarely (3.9 and 0.24%). *Cylindrospermopsis sp.* was not associated with cylindrospermopsin occurrence. Microcystin concentrations exceeded the WHO guidance for moderate and high risk in 0.40 % and 0.72% of samples. In contrast, WHO guidelines based on cyanobacterial abundance and chlorophyll were exceeded in 27 and 44% of samples, respectively. Only 27% of samples were categorized in the same risk category by evaluating concurrence among all three WHO microcystin risk metrics. The lack of parity amongst WHO guidelines was expected given chlorophyll's lack of specificity to cyanobacteria and not all cyanobacteria produce microcystins.

MOVEMENT TOWARDS AN ADAPTIVE SYSTEMS APPROACH TO FRESHWATER MANAGEMENT: COMBINING WATERBODY TREATMENTS WITH WATERSHED MANAGEMENT

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Despite almost three decades and $billions applied to EPA's Nonpoint-Source Management Program (NSMP), nutrient and other pollutant inputs to freshwater are increasing, as are eutrophication, cyanobacterial harmful algal bloom, cyanotoxin, and noxious compound incidences. Although the Point-Source Management Program is successful, accounting for only 5-10% of inputs, the NSMP is limited by high cost, large-scale implementation difficulty, and marginal effectiveness of some best-management practices. EPA's policy shift eliminating the Waterbody Management (Clean Lakes) Program in the early 1990s compounded the problem. Sustainable physical, chemical, and biological waterbody treatments can quickly and cost-effectively suppress cyanobacteria, remove accessible and concentrated nutrients, and strengthen beneficial trophic cascades. The North American Lake Management Society recently enacted policy that calls for full Clean Water Act implementation using an Adaptive Systems Approach (ASA). An ASA uses rigorous scientific and cost-benefit analyses in selecting options from all three programs based on merit alone, and periodically evaluates outcomes and options for cost-effective improvements. Recent EPA actions may signal movement towards an ASA, including: 1) a long-term vision document allowing approaches alternative to Total Maximum Daily Loads (TMDLs); 2) hosting a webinar on waterbody treatments and; 3) posting a webpage describing waterbody treatments. North Carolina suspended TMDL-based nutrient management rules for 14,000-acre Jordan Lake reservoir. NC is testing cyanobacterial suppression by solar-powered circulation in Jordan, and evaluating ASA options for Jordan and other impaired reservoirs. This presentation reviews initial Jordan Lake results, EPA and state movements toward an ASA, and NALMS promotion of ASA policy.

A RED TIDE OF *ALEXANDRIUM FUNDYENSE* IN THE GULF OF MAINE

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In early July 2009, an unusually high concentration of the toxic dinoflagellate *Alexandrium fundyense* occurred in the western Gulf of Maine, causing surface waters to appear reddish brown to the human eye. The discolored water appeared to be the southern terminus of a large-scale event that caused shellfish toxicity along the entire coast of Maine to the Canadian border. Rapid-response shipboard sampling efforts together with satellite data suggest the water discoloration in the western Gulf of Maine was a highly ephemeral feature of less than two weeks in duration. Flow cytometric analysis of surface samples from the red water indicated the population was undergoing sexual reproduction. Cyst fluxes downstream of the discolored water were the highest ever measured in the Gulf of Maine, and a large deposit of new cysts was observed that fall. The timing of the red water event coincided with an anomalous period of downwelling-favorable winds, and model simulations illustrate that could have played a role in aggregating upward-swimming cells. Regardless of the underlying causes, this event highlights the importance of short-term episodic phenomena on regional population dynamics of *A. fundyense*.

GLOBAL DISTRIBUTION AND HIGH RESOLUTION TEMPORAL MONITORING OF TOXIC OR OTHERWISE BIOACTIVE CYANOBACTERIAL MATABOLITE MIXTURES IN LAKES AND ASSOCIATED DRINKING WATER

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Bloom- forming cyanobacteria and their toxins pose a significant threat to the long term sustainability of drinking water sources and lake recreational environments. While microcystins are some of the most wide- spread cyanotoxins, cyanobacteria are capable of producing a variety of toxic or otherwise bioactive metabolites (TBMs) that vary in their potency and effects in animals and humans. The ecological distribution of these TBM mixtures in drinking water and across aquatic systems is not well characterized. For the past four years we have characterized the spatial variability in TBM profiles from lakes distributed across continents globally and at high sub- daily temporal resolution in one lake and its associated drinking water systems. Sixteen TBMs were targeted by liquid chromatography tandem mass spectrometry, including microcystins (MCLR, MCLA, MCRR, MCYR, and dmMCLR), anabaenopeptins (A, B, and F), cyanopeptolins (1020, 1041, 1007), microginin-690, cylindrospermopsin, anatoxin-a, homoanatoxin-a, and saxitoxin. For the spatial study 34 lakes were sampled (0-2 m depth) at least 12 times over the cyanobacterial growth season in 10 different countries, seven states in the United States including west coast, east coast, and mid-west. In the temporal study Lake Winnebago, WI was sampled at high- resolution every 6 hours and drinking water produced from the lake was sampled weekly or daily at four drinking water treatment plants. In the spatial study the most frequently detected TBMs were Anabaenopeptin-A, Cylindrospermopsin, and Anatoxin-a while TBMs with the highest mean and max levels were Anabaenopeptin-B (2.1 and 149 ug/L), Anabaenopeptin-F (1.4 and 140 ug/L), and MCLR (1.9 and 138 ug/L). Lakes with highest and most frequently detected TBMs were in the U.S. upper mid-west. In the temporal study, sub- daily monitoring revealed diel cycling of TBMs at select periods. Most frequently detected TBMs were similar to the spatial study. In addition, highest levels of microcystins occurred during non- bloom periods or just prior to peak bloom conditions. All TBMs except dmMCLR were detected in finished drinking water at least once during the study period. On one date the sum of all microcystins in finished drinking water was seven times the maximum accepted concentration recommendation of 1 ug/L.

THE USE OF TECHNOLOGY TO ASSIST CITIZEN SCIENTISTS MONITORING HABS AND CHANGES IN ENVIRONMENTAL CONDITIONS

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The Phytoplankton Monitoring Network (PMN) is a NOAA research-based program utilizing volunteers to monitor phytoplankton species composition and environmental conditions. PMN volunteers are trained by NOAA staff to sample and identify phytoplankton from freshwater to marine environments. Volunteers are trained to identify general classes of phytoplantkon, including 14 known toxin producers. The network area includes coastal Atlantic, Gulf and Pacific waters as well as freshwater environments. Volunteers participate in hands-on research by collecting water samples, identifying species of interest, and entering data on a weekly or bi-weekly basis. The PMN currently has over 150 sampling sites covering marine, estuarine and freshwater environments. Volunteers monitoring these sites represent public and private schools, colleges and universities, Native American tribes, state and national parks, aquariums, 4-H centers, civic groups, and other non-governmental organizations. The PMN was able to grow into a national monitoring program by use of various web based tools such as an interactive web site and a geographic information system (GIS) tool for data visualization. Volunteer training sessions and workshops are given by NOAA scientists using internet teleconference capabilities and next generation digital microscopes. This presentation will outline the use of these technologies and highlight the second generation smart phone application, *Phyto*. The app has updated the index of common species including those typically associated with coastal HABs as well as freshwater species.

CONTINUOUS IMAGING FLOW CYTOMETER FOR THE AUTOMATED DETECTION, IDENTIFICATION, AND ENUMERATION OF CYNOBACTERIA

Harry Nelson[1], Bruce Dalton[1], Michael Cohrs[1]

[1]Fluid Imaging Technologies

Numerous technologies make use of fluorescence measurements to detect cyanobacteria and for the purpose of estimating biovolume or cell counts within water samples. While useful, results from these instruments can be significantly skewed by turbidity and the presence of other fluorescing pigments and particles. Fluid Imaging Technologies has recently adapted the technology of their imaging flow cytometer FlowCAM so that it can detect the presence of the phycocyanin pigment in cyanobacteria. By detecting the phycocyanin pigment of cyanobacteria cells with the use of an appropriate laser and optical filters, the instrument can now distinguish cyanobacteria from other organisms in the water system, relying on pigment analysis in addition to image analysis of morphological parameters for the purpose of identification. Here we present an overview of the technology along with data demonstrating the efficacy of the instrument.

CHARACTERIZATION OF PORES IN BLACK LIPID MEMBRANES MADE BY KARLOTOXIN 2 (KMTX2) FROM *KARLODINIUM VENEFICUM* (DINOPHYCEAE)

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This study demonstrates that the polyketide toxin karlotoxin 2 (KmTx-2) produced by *Karlodinium veneficum*, a dinoflagellate associated with fish kills in temperate estuaries world-wide, disrupts artificial lipid planar bilayers via pore formation. The functional properties (i.e. current amplitude and open probability) of the KmTx-2-ion channel exhibit steep voltage dependence. Different gating modes (open-closure patterns) are also exhibited by the ion channel formed by KmTx-2. Karlotoxin's membrane insertion (incorporation), channel formation, and/or channel activity depend on the type of sterols present in the lipid bilayer. Desmethyl sterols (i.e. cholesterol) were most permissive of channel formation consistent with prior studies on numerous cell types. Typically, ion channels are selective either for anions of for cations; however, KmTx-2 has a selectivity that varies dependent on the present ionic species. In experiments using monovalents ions (K+ and Cl-) KmTx-2 seems noticeably more selective for K+ over Cl-. However, when divalent cations (Ca2+) are tested vs monovalent anions (Cl-) the toxin seems more selective for Cl- over Ca2+. Thus, it looks like the pore has the ability to selectively pass monovalent cations over monovalent anions, but has more selectivity for monovalent anions over divalent cations. This study provides a more detailed mechanistic explanation for the association between *K. veneficum* blooms and fish kills that has long been observed in temperate estuaries worldwide.

DETERMINING POTENTIAL ENVIRONMENTAL FACTORS AFFECTING THE TRENDS OF TOXIGENIC *ALEXANDRIUM CATENELLA* ABUNDANCE IN MONTEREY BAY, CA

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Despite *Alexandrium catenella* being a small part of the community of phytoplankton in Monterey Bay, CA, there is evidence of an increase in abundance and paralytic shellfish toxins (PSTs) on the west coast potentially due to increases in sea surface temperatures. *A. catenella* causes detrimental effects to humans, marine mammals, fish and seabirds from ingestion of contaminated fish or shellfish, and is routinely monitored in California because of the potential for negative impacts at low cell densities. Besides the potential correlation between temperature and *A. catenella* abundance and toxicity, other ecological variables must be examined to identify potential trends. Preliminary data using a long-term data set from the Santa Cruz Wharf has shown that besides the spike previously reported in *A. catenella* abundance in 2004 through 2006, there was little *A. catenella* activity until around the fall/winter of 2011, where there continued to be increases in abundance during the spring and fall of 2012, 2013, and 2014. Though PSTs have remained below the regulatory limits except during the winter of 2013, the toxins are still present. We will present results for the relationships between PSTs and nutrients (NO_3^-, NH_4^+, urea, PO_4^-, and Si $(0H)_4$), chlorophyll a, water temperature, and salinity as well as the correlations between *A. catenella* and the above parameters and the predictability of these relationships. These results will ultimately lead to a better understanding of *A. catenella* growth dynamics and could potentially be used to forecast blooms and the presence of PSTs in Monterey Bay, CA.

A SIMPLE MODEL FOR PREDICTION AND A COMPLEX MODEL FOR PROCESS STUDIES OF *ALEXANDRIUM FUNDYENSE* IN AN ESTUARY

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Nauset estuary on Cape Cod (Massachusetts, USA) has recurrent toxic blooms of the dinoflagellate *Alexandrium fundyense*. An extensive set of observations of cell concentrations, nutrients, and physical conditions over 4 consecutive years (2009-2012) characterized the spatial and temporal variability within the estuary and interannually. A highly resolved, 3-d model of the hydrodynamics and *A. fundyense* population in Nauset was developed and quantitatively evaluated against the observations. Based on analysis of the model, the dominant factors controlling the bloom were the water temperature, which regulates organism growth rates, and the efficient retention of cells due to bathymetric constraints, stratification, and cell behavior (diel vertical migration). The strong dependence of the bloom on water temperature can be characterized with a simple analytical model that calculates growing degree days. The growing degree day approach collapsed significant variability in the timing of bloom onset, development, and termination across years and among ponds. This relatively simple metric could be used as an early-warning indicator in similar systems with localized, self-seeding HABs, and was effectively applied to the bloom in Nauset in the spring of 2015.

OCCURRENCE OF CYANOTOXINS IN OHIO PUBLIC WATER SYSTEM SOURCE AND FINISHED DRINKING WATERS AND A COMPARATIVE ANALYSIS OF ANALYTICAL METHODS FOR MICROCYSTIN DETECTION

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Ohio EPA has sampled Public Water Systems (PWS) for cyanotoxins since 2010, after results from the National Lakes Assessment indicated cyanotoxins were present in Ohio lakes. Sampling is guided by the State of Ohio PWS Harmful Algal Bloom Response Strategy and is primarily incident-response based, but includes some ambient sampling. Ohio EPA analyzed over 1800 samples from 54 PWSs for cyanotoxins and twelve PWSs voluntarily analyzed over 2500 samples. Microcystin was present in the majority of source waters sampled. Cylindrospermopsin, saxitoxin, and anatoxin-a were detected less frequently. Source water microcystin concentrations >1.0 ug/L occurred most often in August and September, but concentrations peaked in some source waters as early as May and as late as November. Elevated microcystin detections in finished drinking water resulted in drinking water advisories for Carroll Township and the City of Toledo. To better understand the spatial and temporal distribution of microcystin (MC) variants in Ohio and compare results from five different analytical methods, Ohio EPA submitted source water samples from 11 different locations with microcystin-producing blooms to Greenwater labs. MC-YR was the most commonly occurring variant, followed by [Dha^{7}] MC-LR and [DAsp^{3}] MC-RR. MC-LR was only detected at 5 of the 11 sites and always co-occurred with other variants. The dominant variant generally remained consistent within a water body. Overall, 16 MC variants were detected in Ohio's source waters, including 3 unknown MC variants. LC-MS based methods confirmed ELISA results (no ELISA false positives), but LC-UV methods were prone to false positives and negatives.

ECOLOGICAL DYNAMICS IN CALIFORNIA HAB HOTSPOTS: EDDY INCUBATION, RETENTION, AND SUBSURFACE REFUGIA

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ECOHAB field programs in Monterey Bay and San Pedro Bay, California, have studied HAB ecology in various seasonal and episodic conditions. We highlight some of the ecological processes revealed by these studies, focusing on toxigenic *Pseudo-nitzschia* (Pn) populations. During fall 2013 in Monterey Bay, the most abundant and toxic Pn populations were found in an eddy outside of the bay. Satellite remote sensing data indicate that the eddy was a retentive incubation feature enriched by coastal upwelling. During spring 2015 in Monterey Bay a bloom dominated by *P. australis* developed. Following a period of intense upwelling, weaker regional wind forcing was associated with formation of dense subsurface layers in which Pn populations remained healthy and dominant. Monitoring by Environmental Sample Processor (ESP) instruments showed persistence of Pn and high levels of domoic acid in the mixed layer; other observations showed the bulk of Pn populations were concentrated in the thermocline, below the depth of ESP sampling. Retention of cells in the mixed layer within the bay was indicated by drifter studies; over the same time a dense layer of Pn persisted along the thermocline. ESP monitoring in San Pedro Bay during springtime studies in 2013 and 2014 also captured analogous fluctuations in Pn populations associated with variability in upwelling. During those previous studies, AUV-targeted sampling revealed the formation of dense phytoplankton patches in deep shelf water associated with upwelling relaxation. Collectively, these observations point to a set of conditions that lead to Pn bloom initiation, regional retention, and subsurface concentration.

UNDERSTANDING INFLUENCES OF RESERVOIR pH VARIABILTY ON FISH TOXICITY FROM HARMFUL BLOOMS OF PRYMNESIUM PARUVM

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Since first identified in Texas in 1985, *Prymnesium parvum* has produced harmful algal blooms (HABs) responsible for devastating fish kills in inland waters across North America. Traditionally, *P. parvum* HABs primarily occurred in costal and marine systems but are now increasing in these inland waters. Building on previous studies by our research team, which investigated salinity, pH, light, inflows, and nutrient influences on *P. parvum* bloom initiation, termination and toxicity dynamics, here we examined spatiotemporal pH variability in coves of Lake Granbury, Texas, in which *P. parvum* HABs appear to initiate. We then examined toxicity to fish of the fatty acid amide linoleamide, recently proposed as a *P. parvum* toxin, at pH levels representative of conditions in affected reservoirs. 24 hour fathead minnow (*Pimephales promelas*) LC_{50} values for linoleamide were 51.5 mg/L at pH 7.5, but reduced by over 50% (22.6 mg/L) at pH 8.7, which is generally consistent with pH dependent observations reported by several research groups. However, the onset of mortality (LT_{50}, lethal time) at both pH 7.5 and 8.7 were 24.4 and 19.6 hrs, respectively, which is not consistent with our previous findings of the onset of *P. parvum* toxicity to fish (< 2 hours) in laboratory experiments and field studies. Such observations suggest that linoleamide is not the primary contributor to *P. parvum* toxicity in inland waters, and may not be the most biologically active molecule produced by this organism.

THE MAINE BIOTOXIN PROGRAM: MANAGEMENT AND MONITORING APPROACHES FOR THE MAINE SHELLFISH INDUSTRY

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The Maine Department of Marine Resources Bureau of Public Health (DMR BPH) is responsible for monitoring and managing marine biotoxins and associated contamination of bivalve shellfish to protect public health. The biotoxin program operates according to the guidelines of the National Shellfish Sanitation Program Model Ordinance. In the last decade, a combination of changing program needs and prioritization of industry driven shellfish resources required a re-evaluation of marine biotoxin management in Maine. In the last three years Maine has implemented a new management framework that has proven very successful. This presentation will describe management changes and ongoing challenges with maintaining an effective and relevant state regulatory program. Specifically this will include the costs and benefits of chemical analysis (High Performance Liquid Chromatography Post Column Oxidation HPLC PCOX) for for paralytic shellfish toxins analysis for regulatory shellfish monitoring and weekly routine regulatory testing for emerging toxins like domoic acid, a recent and reoccurring issue in Maine. Also a management approach for less predictable bloom driven events management will be described based on work completed in collaboration with Woods Hole Oceanographic Institute and *Pseudo-nitzschia* spp. determination of cell abundance thresholds that can be used to direct management actions. Finally, discussion will also include management rationale for utilizing region wide closures with exception areas during predictable bloom events to target those species most relevant and/or valuable to industry, aquaculture and recreational harvest.

COASTAL ANTHROPOGENIC NUTRIENT INPUT AND HARMFUL ALGAL BLOOMS: A 'NATURAL' EXPERIMENT IN SANTA MONICA BAY, CALIFORNIA

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Hyperion Wastewater Treatment Plant (WWTP) is located in Playa del Rey in Santa Monica Bay. It serves an estimated 4 million people and is one of the largest WWTPs on the west coast, discharging approximately 1.06×10^9 liters per day of nutrient rich, secondarily treated wastewater (effluent) into the bay. Under normal operations, Hyperion discharges its effluent from a pipe that is 8.1 km offshore at a depth of 57 m, well below the euphotic zone, where the effluent is diluted approximately 84:1. A secondary outflow pipe located 1.6 km offshore at a depth of 15 m (estimated dilution: 1:13) is used during emergencies and during repairs to the main outfall. Hyperion determined that its 8.1 km pipe required internal repairs which would take approximately 6 weeks, representing a significant input of anthropogenic nutrients into the bay through the emergency pipe and establishing the potential for a large scale HAB during the repairs. During the course of the effluent diversion, three large phytoplankton blooms occurred, each dominated by different taxa and each occurring in different regions of the bay. Maximum surface chlorophyll values ranging from 20 to >195 µg/L were observed. Potential HAB genera *Pseudo-nitzschia*, *Chattonella,* and *Heterosigma* were observed during the diversion, but no toxicity or ecosystem disruption was documented. A combination of sensor, drifter, shipboard observations, and incubation experiments documenting the variable community composition, spatial heterogeneity and temporal variability of the biological response to the diverted effluent will be presented.

CELLSCOPE AQUATIC: A LAB QUALITY, PORTABLE CELLPHONE-BASED MICROSCOPE FOR ON-SITE COLLECTION OF ALGAE IMAGES

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Collecting algae samples from the field presents issues of specimen damage or degradation caused by preservation methods, handling and transport to laboratory facilities for identification. Traditionally, in-field collection of high quality microscopic images has not been possible due to the size, weight and fragility of high quality instruments and training of field staff in species identification. Scientists at the Southern California Coastal Water Research Project (SCCWRP) in collaboration with the Fletcher Lab, University of California Berkeley, Department of Bioengineering, tested and translated Fletcher's original medical CellScope for use in environmental monitoring applications. Field tests conducted by SCCWRP in 2014 led to modifications of the clinical CellScope to one better suited to in-field microscopic imaging for aquatic organisms. SCCWRP subsequently developed a custom cell-phone application to acquire microscopic imagery using the "CellScope Aquatic "in combination with other cell-phone derived field data (e.g. GPS location, date, time and other field observations). Data and imagery collected in-field may be transmitted in real-time to a web-based data system for tele-taxonomy evaluation and assessment by experts in the office. These hardware and software tools was tested in field in a variety of conditions and settings by multiple algae experts during the spring and summer of 2015 to further test and refine the CellScope Aquatic platform. The CellScope Aquatic provides an easy-to-use, affordable, lightweight, professional quality, data collection platform for environmental monitoring. Our ongoing efforts will focus on development of real-time expert systems for data analysis and image processing, to provide onsite feedback to field scientists.

CYANOHAB PREDICTION AND MICROCYSTIN DEGRADERS IN LAKE ERIE AND OHIO INLAND WATERS

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The presence of cyanobacterial harmful algal blooms (cyanoHABs) that produce cyanotoxins, such as microcystins, have been identified in fresh water ecosystems worldwide, including Lake Erie which is used as a source of drinking water for eleven million people. Multiple approaches are needed to address this problem and include both predicting cyanoHABs before and when they occur and treating source waters that have microcystin concentrations above acceptable levels. The U.S. Geological Survey Ohio Water Science Center is working with state and local agencies and through the Great Lakes Restorative Initiative to develop methods to predict and treat cyanoHABs . Phycocyanin, nutrient concentrations, pH, lake-level change, and turbidity were significantly related to microcystin concentrations at four recreational lake sites; these factors could potentially be used in predictive models. Several species of heterotrophic bacteria have been found to have the ability to break down the microcystin toxin in natural environments. Samples were collected from Lake Erie source waters and sand filters to identify and characterize microcystin-degrading bacteria and to determine the presence of a known microcystin degrading gene (mlr) using quantitative polymerase chain reaction (qPCR). Predictive models and information on the natural populations of microcystin degraders in Lake Erie may one day help drinking water plants enhance their treatment processes for removal of microcystins.

CLIMATOLOGIC ANALYSIS OF CYANOBACTERIAL BLOOMS IN LAKE ERIE: A CASE STUDY FOR DEVELOPING PREDICTIVE TOOLS

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Lake Erie has seen recurring cyanobacterial blooms in this century, with problem blooms in nine of the last 14 years. Satellite products can provide standardized and objective quantification of bloom biomass, allowing for evaluation of timing, spatio-temporal distribution and frequency, and inter-annual variability. These analyses require more than evaluation of individual satellite scenes. Many cyanobacterial blooms float near the surface, but can be mixed subsurface if given strong wind conditions, thereby reducing the surface concentration. As a result, the development of a climatology must consider the variability in surface concentrations due to variations in the wind. One strategy is to composite multiple days of data (consecutive 10-day periods) by taking the maximum value at each mapped spatial unit (i.e. a satellite pixel). This approach captures the concentration when more of the biomass is near the surface. For Lake Erie, the resultant product has led to estimates of annual bloom severity that have been used with nutrient loading data to develop forecasts of the seasonal bloom intensity. These models also help in planning nutrient reduction strategies to reduce the blooms, in particular addressing the need to distinguish between dissolved and particulate phosphorus. The pattern of bloom development through the season may also guide planning for response to the blooms. These applications can be applied to other regions having similar issues.

MULTIPLE STRESSORS AT THE LAND-SEA INTERFACE

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Despite our heightened understanding and awareness of marine and freshwater HABs, there has been much less attention on the interactions between these species at the land-sea interface, and their toxins that may accumulate there. Freshwater flows that enter or mix with the marine realm (especially in estuaries and lagoons) may be responsible for transport of harmful organisms and their bioactive metabolites. Likewise, some cyanobacteria and eukaryotic algae have broad salinity tolerances that may permit their existence and growth across a range of salinities. When considering the presence of notorious marine HABs in the Southern California Bight, we have observed that 'estuarine/brackish' waters are able to support both freshwater and marine species in addition to chemicals that are stable across environmental gradients. Recent sampling of a variety of coastal sites yielded unexpected putative toxin hits. In addition to 'showcase' compounds such as paralytic shellfish poisoning toxins and domoic acid, our preliminary data reveals the presence of previously undocumented algal toxins in our area. Of particular interest is the presence of numerous harmful species and simultaneous detection of multiple toxins in discrete water samples. These findings highlight the need to expand monitoring efforts for additional organisms and 'under the radar' compounds at the land-sea interface.

EFFECTS OF OCEAN WARMING ON GROWTH AND DISTRIBUTION OF DINOFLAGELLATES ASSOCIATED WITH CIGUATERA FISH POISONING IN THE CARIBBEAN

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Projected water temperatures at six sites in the Gulf of Mexico and Caribbean Sea were used to forecast potential effects of climate change on the growth, abundance and distribution of *Gambierdiscus* and *Fukuyoa* species, dinoflagellates associated with ciguatera fish poisoning (CFP). Data from six sites in the Greater Caribbean were used to create statistically downscaled projections of water temperature using an ensemble of eleven global climate models. Growth rates of five dinoflagellate species were estimated through the end of the 21st century using experimentally derived temperature vs. growth relationships for multiple strains of each species. The projected growth rates suggest the distribution and abundance of CFP-associated dinoflagellate species will shift substantially through 2099. Rising water temperatures are projected to increase the abundance and diversity of *Gambierdiscus* and *Fukuyoa* species in the Gulf of Mexico and along the U.S. southeast Atlantic coast. In the Caribbean, where the highest average temperatures correlate with the highest rates of CFP, it is projected that *G. caribaeus*, *G. belizeanus* and *Fukuyoa ruetzleri* will become increasingly dominant. Conversely, the lower temperature-adapted species *G. carolinianus* and *Gambierdiscus* ribotype 2 are likely to become less prevalent in the Caribbean Sea and are expected to expand their ranges in the northern Gulf of Mexico and farther into the western Atlantic. The risks associated with CFP are also expected to change regionally, with higher incidence rates in the Gulf of Mexico and U.S. southeast Atlantic coast, with stable or slightly lower risks in the Caribbean Sea.

THE TIPPING POINT FOR AN EXPANDING PROBLEM: LIPOPHILIC TOXINS IN WASHINGTON STATE AND BEYOND

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In Washington State, there has been an explosion in the occurrence of various lipophilic algal toxins that have led to human illnesses and contributed to a complete ban since 2010 on the export of all shellfish to the European Union. Of greatest concern is the presence of algal toxins associated with diarrhetic shellfish poisoning (DSP) and azaspiracid shellfish poisoning (AZP). The first DSP cases to be reported in the USA were documented in 3 recreational harvesters who ate mussels from a Puget Sound dock during summer 2011. Over the last five years, the SoundToxins and Olympic Region Harmful Algal Bloom (ORHAB) collections of water samples from areas used for shellfish farming or recreational harvesting along the coastline and in Puget Sound have contained alarming concentrations of Dinophysis spp., consistently between 1,000 and 75,000 cells/L with an astonishing maximum of 298,000 cells/L in late July 2010. In addition to expanding issues associated with DSP toxins, there is definitive evidence that other lipophilic toxins, azaspiracids (AZAs), are present in Washington State. Collectively, these toxin classes have an enormous economic and health impact in Europe and are monitored closely; however, only DSP toxins, but not AZAs are monitored in Washington State. Detection of three Azadinium species by PCR and the occurrence of unexplained human illnesses in Puget Sound in the summer of 2014 highlight the urgent need for comprehensive monitoring of lipophilic toxins and the causative species, including the use of advanced technologies such as the Environmental Sample Processor.

THE DEVELOPMENT OF BIOTOXIN GENE QPCR ASSAYS FOR THE MONITORING AND MANAGEMENT OF BIOTOXIN RISK IN AQUATIC ENVIRONMENTS

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The past ten years has witnessed major advances in our understanding of the genetic basis for toxin production. The information gained from the discovery of these toxin biosynthetic pathways has also enabled the genetic screening of various environments for drinking water and seafood quality management.

The synthesis of saxitoxin in dinoflagellates occupying marine environments has been recently found to be catalysed by a group of enzymes encoded by sxt genes, beginning with the, sxtA. This gene exists in multiple genomic copies in the investigated strains of *Alexandrium* species. A quantitative PCR assay targeting domain 4 of the sxtA gene to detect saxitoxin-producing dinoflagellates in marine environmental samples has been developed for use in marine environment management. The presence of sxtA correlates with the abundance of the saxitoxin-producing species of *Alexandrium* species seen in the United States.

The development of this assay follows on from the recent commercialization of a multiplex quantitative PCR assay for the simultaneous detection, discrimination and quantitation of total cyanobacteria, microcystin and nodularin synthetases (mcy/nda), cylindrospermopsin synthetase (cyr) and saxitoxin synthase (sxt) genes. These genes being the critical components of the biosynthetic pathway and show good correlation to toxin production and risk.

The talk will discuss recent data showing how these gene based assay have performed in detecting the presence of toxin producing cyanobacteria and dinoflagellates in the aquatic environments of North America and how they may be used in the management of HAB events.

EMERGENCE OF THE TOXIC ALGA *ALEXANDRIUM MONILATUM* IN THE LOWER CHESAPEAKE BAY: TOXIGENICITY IN AQUATIC ANIMALS

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Alexandrium monilatum is a toxic dinoflagellate known to bloom along the US southern Atlantic and Gulf coasts where it has been associated with finfish and shellfish kills. In 2007, VIMS scientists identified *A. monilatum* to be the dominant species of a late summer bloom that persisted for several weeks in the York River, VA. This bloom was associated with impacts on experimental animals, including total mortality in *Rapana whelk* and cownose rays maintained in systems receiving raw and sand-filtered river water, respectively. Local blooms were also suspected to have caused chronic mortalities in commercially cultured larval oysters during the 2012 and 2013 bloom seasons. The primary toxin of *A. monilatum* is a lipophilic, secondary metabolite called goniodomin A (GDA). The mechanisms of toxicity are presently very poorly understood. We therefore conducted bioassay-based investigations to better understand potential health risks to local aquatic organisms and humans and to take first steps to investigate mechanisms of GDA toxicity. Bioassays exposing *Artemia nauplii*, oyster veligers and sheepshead minnow larvae to live cultured and lysed *A. monilatum* cells resulted in significant dose-dependent mortality profiles. Bioassays using oyster veligers and larval finfish exposed to purified GDA obtained from York River bloom material exhibited similar dose response mortality profiles. Histologic and ultrastructural evaluation of moribund sheepshead minnows exposed to 10 µg GDA/ml indicated pathology in the respiratory and neural tissues that may begin to identify the underlying mechanisms of toxigenicity for GDA.

SOUTHEAST ALASKA TRIBAL TOXINS (SEATT): A PARTNERSHIP TO MONITOR HARMFUL ALAGAL BLOOMS

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The Southeast Alaska Tribal Toxins (SEATT) partnership was formed in September 2013 to unify twelve southeast Alaska tribes in monitoring HAB events that pose a human health risk to the subsistence shellfish harvester. Monitoring includes weekly phytoplankton species identification and quantification, collection of filtered water and shellfish samples to analyze for toxins, cyst bed mapping, and recording environmental parameters. This is the first phytoplankton or PST monitoring program for subsistence user groups within the state of Alaska. With "eyes on the water" each week, SEATT will be able to advise their communities of the potential human health risks when harvesting subsistence shellfish. Each SEATT community uses the data collected to assess their vulnerability to human health risks associated with toxic shellfish. The SEATT partners recognize the increased frequency of harmful algal blooms (HABs) due to warming ocean temperatures, and how these blooms are putting tribal citizens at a greater risk when participating in ceremonial and subsistence harvest activities and are determined to use real time data to minimize those risks. This program brings together scientists from the National Oceanic and Atmospheric Administration (NOAA) Marine Biotoxin Program in Seattle WA and Charleston SC, the University of Alaska Fairbanks School of Fisheries and Ocean Science, and Washington State Department of Health Biotoxin Program to work with Tribal environmental staff from SEATT. The partnership is crucial in maintaining the accuracy and integrity of the data being shared by researchers, Tribal leaders, and resource managers associated with the SEATT program.

BENCHTOP FLUOROMETRY OF PHYCOCYANIN AS A RAPID APPROACH FOR ESTIMATING CYANOBACTERIAL BIOVOLUME

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Cultural eutrophication continues to threaten freshwater resources through, in part, the promotion of harmful algal blooms that are usually dominated by autotrophic prokaryotic cyanobacteria. Strains of several cyanobacterial taxa, including Anabaena and Microcystis, are capable of producing intracellular toxins or off-flavor compounds that can contaminate drinking water supplies, negatively affect associated organisms, and harm local economies. Traditionally, microscopy has been used to identify and quantify cyanobacteria, but this approach can be time consuming and challenging depending on the composition and abundance of phytoplankton taxa. Molecular technologies can provide fast turnaround times, however they can be costly and require extensive training and expertise for data collection and interpretation and their results are rarely confirmed with other techniques. Fluorometry, especially *in situ* probes, is gaining in popularity but few studies have developed strong correlations between cyanobacterial pigment concentrations and biovolume. Because of the need for a rapid and reliable way to quantify cyanobacterial biovolume from whole water samples, we conducted a series of laboratory experiments aimed at refining a protocol that uses benchtop fluorometry to measure the cyanobacterial pigment, phycocyanin, to accurately estimate cyanobacterial biovolume. In our study, we found strong correlations between phycocyanin concentration and cyanobacterial biovolume (but not for cell densities) both within and across ponds, which varied widely in productivity and algal diversity. Given that cyanobacterial biovolume can be efficiently quantified on the same day of sample collection, benchtop fluorometry of phycocyanin should be considered by water resource managers interested in understanding complex spatiotemporal dynamics of toxigenic cyanobacterial blooms.

Speedtalk Presentation Abstracts

DOMOIC ACID IN MARINE FOOD WEBS: A NOVEL APPROACH TO TRACE NITROGEN SOURCES AND TRANSFER

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The frequency of *Pseudo-nitzschia* blooms and high domoic acid (DA) events continues to increase in California coastal regions with detrimental effects across the food web. Understanding the macronutrient sources used by *Pseudo-nitzschia* populations during DA production is crucial to identifying the factors driving DA synthesis. This information is also critical to predicting future harmful *Pseudo-nitzschia* blooms and reducing the impact of DA on coastal marine food webs. Compound specific stable isotope analysis (CSIA) of amino acids (AAs) is a powerful, relatively new approach used for examining the marine distribution of nitrogen (N), determining the trophic level of consumers, and tracing sources and changes in N cycling at varying temporal and spatial scales. Using the naturally occurring distribution of stable isotopes in animal tissues, isotope ratios of N ($^{15}N/^{14}N$) are measured from individual amino acids. The 'trophic'-AAs are used to estimate animal trophic position, while 'source'-AAs preserve the values of primary producers and can be used to identify N sources. We are applying CSIA-AA for the first time on *Pseudo-nitzschia* cells and animal tissues to (1) identify the source of N used in the production of DA (e.g. discriminate between upwelling-derived N versus anthropogenic sources), (2) trace DA and N transfer in marine food webs, (3) determine the trophic position of animals affected by DA, (4) identify foraging habitat for top predators such as California Sea Lions, sea otters, and cetaceans, and (5) identify whether generalist or specialist consumers are more affected by DA to quantify impacts on food web dynamics.

SUCESSFULLY CONVERTING A FEDERALLY FUNDED HAB MONITORING PROJECT TO A STATE-FUNDED PERMANENT PROGRAM; THE IMPORTANCE OF TELLING THE STORY WELL

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Over the last dozen years, representatives of two state agencies and the University of Washington, with assistance from NOAA HAB scientists, have twice successfully navigated the state legislative process to add (in 2003) and later increase (in 2015) a surcharge to recreational shellfish harvest licenses. This surcharge generates funds to support the Olympic Region Harmful Algal Bloom (ORHAB) partnership, a monitoring program providing early warning of harmful algal blooms off the Washington State coast and the Washington State Department of Health Office of Shellfish Programs for marine bio-toxin testing.

In a policy climate that has been averse to increasing fees or taxes, success was found by focusing on "selling" the need for HAB monitoring. The path to success included using a series of individual meetings with key coastal legislators, making connections with local community leaders, educating the public, and using a collaborative team that included a HAB scientist, a public health expert and a shellfishery manager to tell a compelling story in a series of legislative hearings.

This presentation will detail these steps and share some specific thoughts on how to use these steps in similar legislative settings elsewhere.

SPATIAL HETEROGENEITY IN THE SURFACE EXPRESSION OF A *KARENIA BREVIS* BLOOM IN THE EASTERN GULF OF MEXICO.

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Little is known about the small-scale spatial distribution of *Karenia brevis* within bloom patches. Satellite remote-sensing of chlorophyll suggests there is small-scale heterogeneity in the surface expression of *K. brevis* bloom patches, but the taxonomic specificity of those results is an issue. Underway near-surface chlorophyll fluorescence also shows significant heterogeneity of chlorophyll distribution, but that method is also not taxonomically specific and suffers high variability due to the light history of the phytoplankton. Microscopic enumeration and chemotaxonomic analysis of taxa contained within discrete grab samples of water are taxonomically specific, but suffer from low spatial resolution due to the high labor and monetary costs involved in collecting and analyzing samples. A cruise in a *K. brevis* bloom patch northwest of Tampa, Florida in early August 2014 collected a suite of parameters that enabled a comparison of methods that provided various levels of spatial resolution and taxonomic specificity. The methods included: 1) grab water samples at fixed stations for microscopic enumeration and chemotaxonomic analysis; 2) underway chlorophyll fluorometer; 3) FlowCam (Fluid Imaging) image analyzer; and 4) Optical Phytoplankton Discriminator (OPD). The spatial distribution of the surface expression of the bloom patch matched at representative scales of sampling. The OPD and underway chlorophyll fluorometer provided the highest spatial resolution and showed there was heterogeneity not detected with discrete station sampling. Results from the observation methods of taxonomic composition showed systematic differences between the OPD and chemotaxonomic methods that were partially explained by the differences in taxonomic standards used by the two methods.

A POSSIBLE CONTRIBUTION OF TERATOGENIC CAROTENOIDS FROM CYANOBACTERIA TO THE DECLINE OF AMPHIBIAN POPULATIONS IN THE U.S. (AND GLOBALLY)

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Cyanobacteria are widely associated with degradation of freshwater and coastal habitats, particularly as part of episodic and seasonal blooms, and algal proliferation associated with eutrophication of these habitats. Moreover, growing evidence indicates recent and rapid increases in cyanobacterial blooms (i.e. frequency, duration) and proliferation, more generally, and specifically links these increases to global climate change (e.g. warming), increased eutrophication and other related environmental factors. Assessments of the potential impacts of cyanobacteria toxigenicity have, however, remained largely focused on a relatively small subset of taxa-specific toxic metabolites, and particularly, water-soluble hepatotoxic and neurotoxic alkaloids and peptides linked to human intoxications. As part of on-going studies focused on the identification and characterization of potentially novel "toxins" from cyanobacteria, relevant aquatic animal models, including teleost (i.e. zebrafish) and amphibian (i.e. "African clawed frog" *Xenopus* sp.) representatives, were employed. Accordingly, a series of carotenoids, and specifically otherwise well known xanthophyll glycosides were surprisingly found to be potently teratogenic (i.e. developmental toxins) in these vertebrate systems. Specifically, it is proposed, based on various lines of evidence, that these carotenoids act as so-called "pro-retinoids," leading to the production of retinoic acid analogs that are, in fact, known to be potent teratogens. The cumulative toxicological data, and the potential for contribution of these teratogenic pro-retinoids, as ubiquitous metabolites of cyanobacteria, to well documented declines in amphibian populations in the U.S. and, indeed, globally will be discussed. A model linking global climate change and eutrophication (via carotenoid teratogenicity) to these declines will be presented.

ROLE OF THERMOCLINE DYNAMICS IN REGULATING CYANOBACTERIAL BLOOM DENSITY AND CYANOTOXICITY IN A HYPEREUTROPHIC COASTAL CALIFORNIA LAKE OVER TWO YEARS IN A MEDITERRANEAN CLIMATE

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Toxic cyanobacterial blooms (CBs) are an emerging threat to freshwater resources globally. Research has historically emphasized CB dynamics in temperate over Mediterranean systems. Here, we examine effects of thermal dynamics, dissolved nutrients, and other physiochemical parameters in controlling CB and microcystin (MC) toxin levels in a Mediterranean-climate lake. Pinto Lake is a shallow, Mediterranean-climate Lake on California's Central Coast, experiencing intense seasonal CBs. Through weekly sampling, we demonstrate that thermocline strength (deltaT) was a strong bottom-up factor controlling surface dissolved nutrients, CB, and MCs at the Lake from 2012-2013. MCs strongly correlated with Microcystis in both years, having a strong negative association with DIN:DIP ratio. Microcystis had a moderate positive association with SRP, TOC, and specific conductivity in 2012, and with benthic oxygen and specific conductivity in 2013. In both years, Microcystis had a moderate negative association with DIN:DIP, while DIN:DIP was negatively associated with deltaT. Microcystis had a complex relationship with deltaT, not being linearly associated with thermocline strength in either year. In 2012, the Lake displayed warm polymixis, allowing hypolimnetic nutrients to fuel a highly dense, toxic bloom (mean toxicity >57 ppb). In 2013, however, the Lake exhibited warm dimixis, with highly stable stratification, producing only weak CB toxicity (mean toxicity <2 ppb) via physical separation of hypolimnetic nutrients from the photic zone. Our findings demonstrate the dynamic nature of mixis in a Mediterranean climate, with severe strong implications for water quality and reservoir management as Mediterranean climate types are projected to expand under future climate scenarios.

ARE HARMFUL ALGAL BLOOMS BECOMING THE GREATEST INLAND WATER QUALITLY THREAT TO PUBLIC HEALTH AND AQUATIC ECOSYSTEMS?

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When harmful algal bloom (HAB) events require restrictions on fisheries, recreation and drinking water uses of inland water bodies significant economic consequences result. Unfortunately, the magnitude, frequency and duration of HABs in inland waters are poorly understood across spatiotemporal scales, and are differentially engaged among states, tribes and territories. HAB impacts are not as predictable as those from conventional chemical contaminants, for which water quality assessment and management programs were primarily developed, because interactions among multiple natural and anthropogenic factors determine the severity to which a HAB will occur in a specific waterbody. These forcing factors can also affect toxin production. Beyond site-specific water quality degradation caused directly by HABs, the presence of HAB toxins can influence routine surface water quality monitoring, assessment and management practices. HABs present significant challenges for achi eving water quality protection and restoration goals when these toxins confound interpretation of monitoring results and environmental quality standards implementation efforts for other chemicals and stressors. Whether HABs presently represent the greatest threat to inland water quality is debatable, though HABs in inland waters of developed countries typically cause more severe acute impacts to environmental quality than conventional chemical contamination events. Herein, we identify several timely research needs. Environmental toxicology, environmental chemistry, and risk assessment expertise must interface with ecologists, engineers and public health practitioners to engage the complexities of HAB assessment and management, to address the forcing factors for HAB formation, and to reduce the threats posed to inland surface water quality.

THE INTERACTION BETWEEN THE BREVETOXIN AND ITS NATIVE RECEPTORS

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Karenia brevis, the major harmful algal bloom (HAB) dinoflagellate of the Gulf of Mexico, plays a destructive role in the region negatively impacting coastal economies, marine life and public health. These impacts have been attributed to the brevetoxins, potent neurotoxins produced by *Karenia brevis*. Brevetoxins are ladder-shaped polyether (LSP) compounds which bind to and activate voltage-sensitive sodium ion channels causing channel opening and depolarization of excitable cell membranes. However, the endogenous role of brevetoxins is still uncertain. A number of hypotheses have been put forward including roles in osmoregulation, chemical defense or a grazing deterrent. In an effort to shed light on the endogenous function of brevetoxins, we sought to localize brevetoxin to an organelle and to identify a native receptor. Using fluorescent and photolabile brevetoxin probes, brevetoxin has been shown to localize to the chloroplast of *K. brevis* where it binds to the light harvesting complex II (LHC II) and thioredoxin (Trx). The interaction of brevetoxin with these two chloroplast proteins and the consequences of this interaction will be presented. This work may contribute to understand the native role of ladder-shaped polyether compounds in the dinoflagellates.

RECURRENT BLOOMS OF *KARENIA PAPILIONACEA* IN DELAWARE COASTAL WATERS

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Since 2007, there have been recurrent blooms of the red tide species, *Karenia papilionacea*, along the coast of Delaware. Samples from four sites were collected each year from May through November, 2008-2014, and screened for the presence of *K. papilionacea* by microscopy. Sequence analysis indicated that the Delaware strain is distinct from a strain isolated from the Gulf of Mexico and also from a strain that originated in New Zealand. A quantitative PCR (qPCR) assay was developed that is specific to the Delaware strain and used to evaluate changes in cell density over the course of the season each year. Our results indicate that *K. papilionacea* was absent from Delaware coastal waters each year until mid-July or early August in 2008-2014 samples, after which it was consistently present until the end of the sampling season in October or November. The highest cell densities typically occurred in September or October each year. Overall, the results suggest several possible sources of inoculum for *K. papilionacea* blooms in coastal Delaware waters. The source may be from populations that overwinter south of Delaware, populations that overwinter in coastal Delaware sediments, and/or populations that originate from the Gulf Stream and are transported near-shore during the summer. We plan to examine these hypotheses during summer, 2015, to determine the source of inoculum for *K. papilionacea* blooms in coastal Delaware waters.

MARINE ALGAL TOXINS AND THEIR VECTORS IN SOUTHERN CALIFORNIA CETACEANS

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We present the first synthesis of domoic acid (DA) and saxitoxin detections in the feces and urine of stranded and bycaught southern California cetaceans, over a 13 year period (2001-2013), along with corresponding stomach content data. Not only does this synthesis provide the first record of saxitoxin in a North Pacific cetacean, but also the first records of DA in a West Coast bottlenose dolphin (*Tursiops truncatus*) and a fin whale (*Balaenoptera physalus*). DA was most often detected in long-beaked common dolphins (*Delphinus capensis*), followed by short-beaked common dolphins (*Delphinus delphis*), and *T. truncatus*. DA concentrations were higher in feces (median=33.6ng/g; max=39,640 ng/g) than in urine (median=8.3ng/g; max=360 ng/g). The most common prey items found in the stomachs of DA positive *D. capensis* were market squid (*Loligo opalescens*) and northern anchovy (*Engraulis mordax)*, indicating that they are the likely vectors of the observed DA. Detection of DA in 54% of *D. capensis* that died from trauma (e.g. fishery bycatch) suggests that the population is often exposed to low concentrations of DA. Saxitoxin does not appear to be as pervasive as DA. Out of 103 specimens collected from 7 different species, only 3 *D. capensis* tested positive for saxitoxin (maximum=5.2ng/g in feces, 17 ng/g in urine). The relative abundance of Pacific sardine (*Sardinops sagax*) (59%) and *E. mordax* (39%) in the stomachs of these specimens indicate that these fish are the likely vectors of the detected saxitoxin.

REFINING KARENIA BREVIS DETECTION: AN ASSESSMENT OF ENSEMBLE IMAGERY PRODUCTS FOR OPERATIONAL FORECASTING

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The Harmful Algal Bloom Operational Forecast System (HAB-OFS) employs a combination of automated processing and manual analyses of data to create decision support tools and products to mitigate HAB impacts. Satellite ocean color imagery is a key component of this analysis, used for early detection and monitoring of *Karenia brevis* bloom location, movement and intensification in the Gulf of Mexico. Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua imagery, provided by NOAA's CoastWatch Program, is processed using an algorithm that highlights areas of anomalously high chlorophyll. Although the chlorophyll anomaly product is effective, it is not *K. brevis*-specific and may highlight blooms of other algal species. To refine *K. brevis* detection, an ensemble approach was evaluated that combined the currently used chlorophyll anomaly with algorithms that target specific properties of *K. brevis* blooms - the relative particulate backscatter and the spectral shape characteristics in the blue-green portion of the spectrum, centered on 490 nm. A comparative analysis of the current chlorophyll anomaly product and the ensemble products was performed on a sample set of images from the southwest Florida coast between April 2010 and December 2013. Results from the evaluation indicated that the ensemble imagery products performed better than the chlorophyll anomaly alone 77.5% of the time, decreasing false positives, targeting the spatial extent of *K. brevis* blooms more specifically than the chlorophyll anomaly alone, and reducing the over-prediction of bloom presence. Based on these results, the ensemble product will be transitioned to operations and incorporated into the HAB-OFS bulletins.

DEVELOPMENT OF AN IMMUNOASSAY FOR AUTONOMOUS, SUBSURFACE DETECTION OF PARTICULATE MICROCYSTINS IN LAKE ERIE

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Timely detection and monitoring of harmful algal bloom (HAB) development and toxicity are of growing importance, especially for freshwater systems that supply drinking water to many municipalities. The urgent need to advance detection capabilities was highlighted by the recent 'do not drink' advisory issued for roughly 500,000 Toledo, OH residents in August 2014. The Environmental Sample Processor (ESP) is an autonomous, in-water instrument capable of assessing concentrations of potentially toxic HAB species and their toxins in near real-time. Although the ESP has been deployed extensively in marine coastal waters, this technology has not been utilized in freshwater systems to monitor potentially toxic cyanobacteria and cyanotoxins. Given the current regulatory focus on drinking water contamination by microcystins (MCs), and the need for advanced warning of increasing toxin levels, a collaborative effort was initiated to deploy the ESP technology in western Lake Erie. Emphasis was placed on developing an efficient toxin extraction method and a sensitive competitive ELISA (cELISA; ADDA-specific) for MCs compatible with the ESP functionality. Initial results using an aqueous methanol solvent showed toxin recoveries from *Microcystis* cultures comparable to the Abraxis QuikLyse™ kit, whereas an early iteration of the cELISA yielded 'in-assay' detection limits in the low nanogram per milliliter range. Progress on further optimization of extraction and assay protocols will be presented, along with plans for deploying the ESP as a component of the GLERL Real-time Coastal Observing Network (ReCON) to support NOAA's cyanoHAB forecasting efforts.

***GAMBIERDISCUS* ON THE MOVE: HOW DO TIDAL CYCLES AND MOON PHASES AFFECT DISTRIBUTION?**

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Ciguatera is the leading form of phycotoxin-related seafood poisoning in humans worldwide. Ciguatera is caused by toxins produced by members of the benthic, epiphytic dinoflagellate genus, *Gambierdiscus*. Bioaccumulation of these toxins in coral reef food chains ultimately affects human health and food seafood safety. Recently, efforts have been initiated to standardize monitoring techniques. One example is artificial substrate (screen) deployments, in which the number of *Gambierdiscus* cells that settle (or colonize) the screens in a 24-hour period is used as a proxy for cell densities on host macroalgae. Over the past two years, we have deployed screens and have observed a range of *Gambierdiscus* densities on the screens, from zero (e.g. September 2014) to >100 cells per cm2 (e.g. December 2013). Screen cell densities were correlated with algal cell densities in some, but not all cases, suggesting that other factors may influence *Gambierdiscus* colonization of screen surfaces. Previous studies have demonstrated that other dinoflagellates are influenced by tidal cycles and moon phases. Additionally, wave energy is suspected to play a role in dislodging *Gambierdiscus* from the benthos, which could ultimately influence cell settlement on the 24 hour screens. The purpose of this study, therefore, was to determine how *Gambierdiscus* distributions are influenced by tidal cycles, moon cycles, and wave energy.

AN *ALEXANDRIUM* CYST RECORD FROM THE HYPOXIC FJORD EFFINGHAM INLET, BRITISH COLUMBIA

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Alexandrium exhibits a dual-stage life cycle alternating between a mobile, vegetative cell in the water column and a sessile, benthic cyst stage in the sediments. The cyst stage represents a link between an individual cell and subsequent, potentially dense aggregations of dividing vegetative cells in the water column. The objective of this study was to assess the relationship between the concentrations of *Alexandrium* cysts in a sediment core and nearby paralytic shellfish toxin (PST) concentrations in shellfish collected along the shores of Effingham Inlet, British Columbia. The bottom waters of Effingham Inlet are seasonally hypoxic which is important to this study for two reasons. First, it reduces the likelihood that benthic organisms could survive and disturb the sediment. Thus, the temporal resolution of the sediment record in Effingham Inlet remains intact allowing for the development of relatively high resolution sediment core record. Second, the hypoxic waters present in Effingham Inlet prevent *Alexandrium* cysts from germinating, negating the need to account for loss due to germination. We developed a high resolution, historical record of *Alexandrium* cysts that was appropriate to compare with the available blue mussel (*Mytilus edilus*) PST record to better ascertain the relationship between cyst densities in the sediments and nearby toxicity and the ability of the cyst record to reflect historical toxicity. The data suggest that a smoothed, estimate of PST concentrations can weakly predict the number of cysts deposited on the nearby seafloor.

DIARRHEIC SHELLFISH TOXINS IN MEDITERRANEAN MUSSELS FROM THE NORTHWEST COAST OF BAJA CALIFORNIA, MEXICO

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Here, we report the presence of diarrheic shellfish toxins (DSPt) in Mediterranean mussels (*Mytilus galloprovincialis*) cultivated in Todos Santos Bay (Northwest coast of Baja California, Mexico). For the first time, the presence of okadaic acid (OA), dinophysistoxins and Pectenotoxins (PTX) is confirmed by LC-MS/MS in Mexico. The major exportation volumes of oysters and mussels of Mexico are produced in the region. The presence of marine biotoxins associated to microalgae blooms had affected this activity. In 2010, two sanitary bans due to positive response of the DSP mouse bioassay (MBA) were implemented for the first time in two coastal lagoons of the region. In 2012, shellfish extraction was closed in Todos Santos Bay for approximately 3 months and for one month in 2013 due to positive MBA results. Samples collected during the sanitary bans were analyzed by LC-MS/MS. During two periods in 2012 (July to August and October to November) the sum of DSPt were well above the regulation action level. Specifically, in October to November several samples with concentrations above 1000 µg OA eq kg^{-1} were detected and the maximum concentration of 2344 µg OA eq kg^{-1} was measured on this period. These toxins were associated with the accumulation of *Dinophysis fortii* and *D. acuminata*. PTX2 was produced mainly by this last species. The accumulation of *Dinophysis* was associated with a strong stratification of the water column after upwelling events near the Bay. Implications of the appearance of these emerging toxins for the monitoring of marine phycotoxins in Mexico are discussed.

CHRONIC, LOW LEVEL DOMOIC ACID EXPOSURE AND MEMORY IN ADULTS:

THE COASTAL COHORT EIGHT YEARS LATER

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The potential impact of human exposure to high levels of domoic acid (DA) through shellfish consumption has been well documented. The hallmark symptom is severe memory problems with concomitant abnormalities in the hippocampus, a cerebral region associated with memory processes. That is, "Amnesic Shellfish Poisoning" (ASP). Over the past decade, low levels of domoic acid have been found in razor clams from beaches in the Pacific Northwest. Thus, questions were raised regarding whether or not chronic exposure to low levels of DA through razor clam consumption would lead to memory difficulties or ASP in an attenuated form. To answer this question, the CoASTAL cohort, comprised of participants from three at-risk Native American communities, was formed. Early studies suggested that adults who consumed more than 15 razor clams per month performed more poorly on memory tasks than those who ate fewer or no razor clams. However, no association was found with DA exposure. Currently, data is reported for 515 adults who were followed for eight years with extensive dietary exposure and memory studies. Based upon multivariate statistical analyses, high consumers continued to perform more poorly on word recall measures ($p = .002$). However, after controlling for age, higher mean exposure (razor clams x DA level) was associated with lower memory scores (total recall $p = .055$; delayed recall $p = .027$). Findings suggest that chronic exposure to low levels of DA through razor clam consumption may contribute to reduced memory scores, albeit still within normal limits, in the highest consuming adults

VALIDATION OF A SANDWICH HYBRIDIZATION ASSAY APPROACH TO RAPIDLY ASSESS MULTIPLE HARMFUL ALGAL BLOOM SPECIES LINKED WITH FISH KILLS AND PUBLIC HEALTH CONCERNS IN THE SOUTHEASTERN UNITED STATES

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Knowledge of harmful algal bloom (HAB) abundances has important implications for assessing bloom severity, and rapid detection enables forecasting of bloom events. Specifically, technologies that expedite accurate monitoring of a bloom's progress will facilitate 'early warnings' of potentially toxic events and enable managers and health officials to take appropriate actions (shellfish or beach warnings/closures) that protect public safety. We are developing a sandwich hybridization assay (SHA) for southeastern US populations of 3 HAB species, two raphidophytes, *Fibrocapsa japonica* and *Chattonella subsalsa*, and the diatom *Pseudo-nitzschia pseudodelicatissima*. SHA uses two ribosomal RNA (rRNA)-targeted oligonucleotides (a species or group-specific capture probe combined with a group-specific signal probe) to directly detect (without amplification or purification) organism(s) of interest. *F. japonica* and *C. subsalsa* are responsible for declining water quality and fish kills throughout the southeast, particularly in South Carolina (SC), and evidence of domoic acid in tissues of stranded pigmy sperm whales, combined with toxic *P. pseudodelicatissima* isolates, indicate that these species pose regional health and environmental threats. Capture probes were designed for southeastern strains of each species and evaluated in tandem with existing signal probes for raphidophytes and *Pseudo-nitzschia* spp. Here we describe initial calibration results, evaluations of cross-reactivity, and assay verification using bloom and non-bloom environmental samples collected in SC during 2014 and 2015. Ongoing research on natural populations of each species continues to evaluate the applicability of SHA to accurately predict blooms under a range of environmental conditions.

EMERGENCY RESPONSE MAPPING OF *ALEXANDRIUM* CYSTS IN THE SURFACE SEDIMENTS OF PUGET SOUND WA

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In September and October 2014, an unprecedented bloom of *Alexandrium* occurred in Dabob and Quilcene Bays, Puget Sound, WA. The area where the bloom took place has historically been biotoxin free. At the peak of the event, toxin levels detected in shellfish reached 12,688 µg STX equiv. per 100 g shellfish tissue – more than 150 times greater than the regulatory limit for human consumption. *Alexandrium* species produce resting cysts that overwinter in sediments on the seafloor. The following season, the cysts germinate and can provide the inoculum for more blooms. Out of concern that the 2014 bloom may have formed a new "seed bed" that could increase the risk for future blooms, an emergency cyst mapping survey was conducted in this area in January 2015. The work was supported in part by the NOAA ECOHAB Program and Penn Cove Shellfish. Prior cyst mapping efforts in 2011, 2012 and 2013 found zero or very low (i.e., 5-10 cysts per cc wet sediment) concentrations of cysts in the area. In January 2015, an order of magnitude greater concentration of cysts was observed; up to 120 and 180 cysts per cc wet sediment in Quilcene Bay and Dabob Bay, respectively, indicating that a new *Alexandrium* seed bed formed in the area following the 2014 bloom. In response to this finding, monitoring efforts were enhanced for both cells and toxins in the area, and this increased vigilance provided managers with an early warning of a toxic event in April 2015.

CHANS: THE SPATIAL-TEMPORAL DISTRIBUTION OF *KARENIA BREVIS* ON THE WEST FLORIDA COAST AND ITS RELATIONSHIP TO PRECIPITATION AND RIVER DISCHARGE

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The Coupled Natural and Human Systems program of US National Science Foundation is studying relationships between harmful algal blooms, environmental factors, and the associated human health and economic impacts in coastal communities. Several studies have examined potential linkages between bloom dynamics and riverine inputs, although most have considered bloom events spanning intervals of three to several years. We evaluated spatial and temporal patterns of major bloom events over 20 years along the West Florida Coast. Approximately 63,000 cell count measurements from 1994-2013 were analyzed to define the time series of a 'center of mass' location for each bloom event. The center of mass analysis corroborated patterns of bloom movement and temporal development described in previous studies. Cell counts were evaluated with rainfall and river discharge data to define seasonal patterns of occurrence at the mouths of five West Florida estuaries. The analysis indicated weak positive relationships between *K. brevis* abundance and river discharge. Maximum discharge lagged seasonal watershed precipitation by one to several months. Correlations between discharge and cell counts within 10 km of the estuary mouths indicated weak, yet positive, relationship with lags of 1 to 4 months. An analysis of aggregated monthly cell counts and discharge rates indicated patterns of seasonal maxima with differences in median cell counts varying by as much as an order of magnitude. These relationships suggest a possible riverine effect on cell counts that is minor relative to peak abundances, but a potential important influence on bloom dynamics and worthy of further investigation.

ONE PATHWAY-TWO PRODUCTS: A POLYKETIDE SYNTHASE PATHWAY IN DINOFLAGELLATES RESULTS IN BOTH TOXIN AND FATTY ACID PRODUCTION

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PolyKetide Synthases (PKS) are multi-domain enzyme complexes, which catalyze two carbon condensations into a variety of complex compounds ranging from antibiotics, immunosuppressants, and toxins. It was previously believed that dinoflagellates contained only single Ketosynthase (KS) modules for the production polyketides and fatty acids. However, through Illumina RNA-seq analysis our laboratory has found extensive multi-domain PKSs, non-ribosomal peptide synthases (NRPS) and hybrid PKS/NRPSs conserved across three dinoflagellate species - the toxin producing *Amphidinium carterae* and *Karlodinium veneficum* and the non-toxic *Akashiwo sanguinea*. Upon comparison of the modules in a large PKS protein it was discovered that the acyl transferase (AT) module was present in nontoxic *A. sanguinea* gene but absent in genes from both toxin producing species. To test the functionality of this multimodule PKS in *A. carterae* we used a known fatty acid synthesis inhibitor, cerulenin, to examine 14C acetate incorporation into fatty acids and amphidinol. Cerulenin completely abolished two carbon incorporation *in vivo* into both fatty acids and toxin. We believe that the three KS domains in this protein are covalently inactivated by cerulenin. We plan to synthesize and utilize radiolabeled cerulenin to freeze the PKS machinery, followed by proteomics analysis of resulting labeled complexes, allowing us to elucidate the organization of the PKS complexes involved in toxin and fatty acid synthesis.

DEVELOPING AN EARLY WARNING SYSTEM FOR AN EMERGING THREAT TO HUMAN HEALTH: DIARRHETIC SHELLFISH POISONING IN WASHINGTON STATE

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The first confirmed DSP human illnesses in the United States occurred due to the consumption of blue mussels from Sequim Bay, Washington in 2011. The emergence of this new threat to public health had an immediate impact on the Jamestown S'Klallam Tribe, whose shellfish beds are located nearby. In 2012, a collaborative research project with NOAA and the Washington Department of Health was initiated together with the volunteer-based SoundToxins partnership, to determine whether routine monitoring of *Dinophysis* abundance could provide an early warning of concentrations of DSP toxins accumulated by shellfish. It was found in Sequim Bay that *D. acuminata* was the primary species present during toxic events and increases in subsurface cell density often predicted increases in shellfish toxicity. However, surface water samples analyzed from several other sites did not show elevated *Dinophysis* abundance as a precursor to DSP toxins in shellfish. In Sequim Bay tidal cycle and water depth influenced the abundance of *Dinophysis* sampled over time, demonstrating the need to optimize sampling protocols in order to successfully detect cells prior to toxic shellfish events. The synergy of research, management, and volunteer monitoring partnerships was invaluable in streamlining an early warning system for the protection of human health against the threats of DSP in the U.S. Pacific Northwest.

PHYLOGENY AND FUNCTION: IDENTIFICATION OF THE PROTOTYPICAL eIF4E TRANSLATION INITIATION FACTOR IN DINOFLAGELLATES

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The genetic organization and gene expression in dinoflagellates has been shown to be distinct from other eukaryotes. A wide range of studies has implicated mRNA recruitment as a major site of regulation of gene expression. mRNA in dinoflagellates is *trans*-spliced with a 22-nucleotide 5'-spliced-leader sequence bearing a multi-methylated cap. We have investigated the translational initiation factor, eIF4E, that recruits mRNA by binding to the 5'-cap. Like other eukaryotes, dinoflagellates encode multiple eIF4E family members anticipated to fulfill a range of functions. Three distinct and novel clades of eIF4E have been recognized in dinoflagellates that are distinct from the three metazoan classes of eIF4E. The dinoflagellate *Amphidinium carterae* encodes eight eIF4E family members. We assayed six of these for expression levels, m^7GTP binding, yeast knockout complementation and affinity for m^7GTP using surface plasmon resonance (SPR). Transcripts of each are expressed through a diel cycle, but only eIF4E-1 family members and eIF4E-2 are expressed at the level of protein. Recombinant eIF4E-1 family members and eIF4E-3a, but not eIF4E-2, are able to bind to m^7GTP-agarose beads. Of the clade 1 eIF4Es, only eIF4E-1a and -1d complement a *S. cerevisiae* strain deficient in functional eIF4E, consistent with function as translation initiation factors. However, only eIF4E-1a can be recovered from *A. carterae* extracts by m^7GTP affinity binding. Overall we show differences in expression and function among *A. carterae* eIF4E family members. Only eIF4E-1a emerges with characteristics consistent with the role of the prototypical translation initiation factor.

CHANS: MODELLING ENVIRONMENTAL FACTORS INFLUENCE ON HUMAN RESPIRATORY IRRITATION FROM NATURAL EXPOSURE TO *KARENIA BREVIS* AEROSOLS

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The Coupled Human and Natural Systems program of National Science Foundation is supporting our effort to elucidate linkages between harmful algal blooms and associated impacts on human health, society and economy of coastal communities. The human respiratory system is negatively impacted by inhaled toxic aerosols from the Florida red tide dinoflagellate, *Karenia brevis*. It has been hypothesized that surf height, wind speed and wind direction were the predominant factors determining the amount of toxin reaching exposed individuals.

Recent findings indicate that there are more environmental factors influencing the level of respiratory impacts from brevetoxins. We report on analyses that include additional factors such as air temperature, water temperature, dew point, barometric pressure, *K. brevis* cell counts, distance and direction from *K. brevis* cells to the individual. Factors contributing most to differences between no respiratory irritation and mild respiratory irritation were water temperature, air temperature, and dew point. Those factors contributing most to differences between mild respiratory irritation and moderate respiratory irritation were wind direction, surf conditions, and wind speed.

Data on respiratory impacts was provided by the Beach Conditions Reporting System (BCRS). This system provides twice daily reports from lifeguards and park rangers at public beaches. These subjective reports include observed levels of respiratory irritation among beach-goers. Other environmental data were provided by a variety of local sources including private and public weather stations and many monitoring and research efforts. The resultant model provides a means to forecast respiratory impacts from observations of HAB distribution, and meteorological and oceanographic conditions.

INVESTIGATION OF THE NITROGEN CYCLE WITHIN HARMFUL ALGAL BLOOM COMMUNITIES

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Recurring cyanobacterial blooms in freshwater Lake Tai (Taihu) in China have remained problematic for decades. Blooms have historically been linked to phosphorus loading, both here and around the globe. Recent research, however, shows both phosphorus and nitrogen are important in bloom formation in Lake Tai and that there may be a seasonality to this process. To date the relationship between the nitrogen cycle and these freshwater bloom communities is not well understood. To investigate this relationship, samples were collected during monthly (June – October) surveys at multiple stations within Lake Tai in order to capture an entire bloom event. Nucleic acids (RNA) were extracted and gene expression levels were determined using a targeted microarray approach for N-cycling genes, specifically nifH, amoA, hszA, nosZ, nrfA, and nxrB. Analyses demonstrated that gene expression levels clustered by function, not necessarily by location or season. Ammonia concentrations appeared to drive N-cycling gene expression at one station while other environmental factors, such as dissolved oxygen and water depth, were linked to expression at the other stations. Further analysis revealed statistically significant differences in the expression profiles of N-cycling genes between stations. Results suggest expression of genes related to the nitrogen cycle are controlled by different environmental factors depending on location and time of year, implying that a single gene-based model for Taihu may not be appropriate.

REALIZED AND POTENTIAL HAB HOTSPOTS ALONG THE CALIFORNIA COAST: WHAT TRIGGERS A BLOOM?

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Although several HAB organisms are present in California, *Pseudo-nitzschia* is rapidly becoming the single greatest threat and problem for human and ecosystem health. Our primary objective is to develop a better understanding of the ecophysiological conditions leading to bloom and toxin initiation for *Pseudo-nitzschia*, by comparing two “hot spots”, Monterey Bay and San Pedro Bay, California. We hypothesize that there is a unique set of environmental conditions leading from bloom initiation to toxicity that can be identified through a comparative approach, allowing us to contrast potential factors (such as stratification, nutrient load, nutrient type) between regions, and that blooms initiate both offshore and as subsurface layers. Contrary to expectations, several potential HAB events failed to develop in Monterey and San Pedro despite the presence of toxigenic *Pseudo-nitzschia* and environmental conditions conducive to bloom formation, while spring 2015 has evolved into one of the largest bloom events in 15 years in Monterey (and much of the west coast) but not in Southern California, at the time of abstract submission. By deploying a “sensor-network” of gliders, AUVs, drifters, Environmental Sampling Processors (ESPs), ship operations, numerical and statistical models, and remote sensing, we have compiled an unparalleled dataset of observational and ecophysiolgical experimental data during non-bloom and bloom periods. An overview and synthesis of the HAB Hotspots program will be presented emphasizing the comparative approach (regions, seasons, bloom versus non-bloom) highlighting the advantage of an adaptive sensor-network approach to identify the environmental conditions leading to toxic bloom formation.

STUDIES OF NUTRIENT CONTRIBUTIONS FROM MAJOR ESTUARIES OF SOUTHWEST FLORIDA TO THE GULF OF MEXICO AND THEIR EFFECT ON *KARENIA BREVIS* BLOOMS

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Characteristic nutrient regimes of Southwest Florida estuaries and the extent to which they persist in coastal waters were investigated. Bulk water samples were collected in 2012-2015 from the major estuaries (Tampa Bay, Charlotte Harbor, and San Carlos Bay fed by the Caloosahatchee River) during wet and dry seasons. Samples were also collected at stations near and off shore (10 m and 20 m isobaths) of the estuary inlets throughout the year. At each station, physical water quality parameters were collected, as well as dissolved inorganic and total nutrients, particulate nutrients, CDOM absorption, and cell counts of *Karenia brevis*. The nutrient parameters of coastal waters displayed numerous reproducible and significant differences by estuarine region, and were most often different by station during the wet season. For some parameters significant differences persisted in the offshore stations. If a characteristic nutrient regime from estuarine outflow was the dominant source of nutrient support for blooms, then one might expect to see a particular region where *Karenia* cell densities routinely achieved higher concentrations. Despite the unique nutrient regimes of the three major estuarine regions, cell densities when *Karenia* was present were not significantly different along the coastal study area, failing to support simplistic theories of bloom progression from estuarine outflow.

CIGUATERA FISH POISONING MANAGEMENT: ASSESSING TOXIN LEVELS IN THE INVASIVE SPECIES *CEPHALOPHOLIS ARGUS* FROM TWO DISTINCT ENVIRONMENTAL REGIONS OF HAWAII

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Ciguatera fish poisoning (CFP) is a foodborne illness resulting from the consumption of reef dwelling fish containing ciguatoxins (CTXs). CTX precursors originate in benthic dinoflagellates of the genus Gambierdiscus. These precursors are consumed by grazers, and are biotransformed, bioaccumulated, and biomagnified in the food chain, occurring mainly as lipid-soluble CTXs in predatory fish. CFP, a global seafood safety hazard, is endemic in Hawaii. Management of CFP currently relies upon avoidance of commonly toxic fish species, and/or CTX prevalent locations. However, additional information is critically needed, particularly for refinement of knowledge in the regional distribution of toxic fish. This study will assess the prevalence and levels of CTXs in the invasive species *Cephalopholis argus* (peacock grouper or roi) from two discrete environmental regions of Hawaii Island, Kohala (leeward side) and Hilo (windward side). Composite toxicity of CTXs in extracts of fish muscle will be assessed by N2a neuroblastoma cell assay, and CTXs confirmed (regional biomarker Pacific-CTX-1) by using liquid chromatography tandem mass spectrometry. Initial surveys, identified spatial differences in the toxicity of *C. argus*, with many containing CTXs at levels above FDA guidance (0.01 µg P-CTX-1 equiv./kg). From Kohala and Hilo, 80 and 10%, respectively, of samples collected were above guidance (n=20); indicating a potential regional variation in toxicity. Understanding the spatial and temporal variability of CTXs in fish will enhance the prediction and prevention of CFP in endemic regions. This study will provide valuable information on regional differences in CTX levels in fish that will be useful in future management efforts.

SPATIAL AND TEMPORAL DEVELOPMENT OF PHYTOPLANKTON COMMUNITY COMPOSITION DURING A *KARENIA BREVIS* BLOOM IN THE NORTHEASTERN GULF OF MEXICO

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Alterations in physical, chemical and biological factors affect successional patterns of phytoplankton communities and can lead to shifts that favor bloom-forming species. A major bloom of the dinoflagellate *Karenia brevis* occurred in the Big Bend area of the Florida Gulf Coast in July - October of 2014, resulting in widespread mortalities of reef fishes and other benthic organisms. In August and September 2014, we examined spatial and temporal variation of phytoplankton community structure inside and outside of the bloom region through a series of field surveys, including two one-day small boat surveys and three multiday ship-based surveys. Water samples collected from near-surface, near-bottom and mid-column depths were processed for phytopigment identification and quantification via HPLC and subsequent chemotaxonomic analysis (ChemTax) for community composition determination. Imaging particle analysis (FlowCam) and *K. brevis* enumeration via light microscopy supplemented pigment-based community data. Community data demonstrated a pattern of succession from a mixed community with low total biomass outside of the bloom area towards either a *Karenia*-dominated community, with *Prochlorophytes* and *Haptophytes* as sub-dominant groups, or a diatom-dominated community with low abundances of other groups, within the bloom area. Patterns of water column stratification appeared correlated with observed patterns of community structure. Diatom-dominated assemblages were sometimes observed at sampling locations with *Karenia*-dominated assemblages, but at different depths, typically separated by a prominent pycnocline. Results indicate that water column structure exerts a primary influence on the observed community structure. This work will be considered in the context of *K. brevis* bloom initiation, development, and decline.

THE EFFECTS OF MICROCYSTIN-LR ON *ESCHERICHIA COLI* EVALUATED THROUGH TRANSCRIPTOMICS, METABOLOMICS, AND LIPIDOMICS.

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Toxic blooms of *Microcystis aeruginosa* occur in freshwater ecosystems and have been increasing in frequency and severity over the last three decades worldwide. Research into the causes and dynamics of *M. aeruginosa* blooms has traditionally focused on abiotic factors, e.g., nutrient loading, light, and temperature. However, *M. aeruginosa* co-occurs within complex microbial communities and it is likely that biotic factors such as community interactions play a significant but poorly investigated role in bloom dynamics. We hypothesize that cyanotoxins, such as microcystins, influence heterotrophic bacteria community structure, which provides feedback on bloom formation and dynamics. We investigated the effects of microcystin-LR on the physiology of aquatic heterotrophic bacteria using *Escherichia coli* MG1655 as a model organism. *E. coli* was subjected to 1 and 10 mg/L microcystin and its response was determined by measuring changes in its transcriptome, metabolome, and lipidome every 15 minutes for one hour. 95, 104, 103, and 84 genes were differentially expressed at 15, 30, 45, and 60 minutes, respectively. This included genes for carbon metabolism, cofactor synthesis, and uncharacterized membrane- and periplasmic-associated proteins. Though previous reports have indicated microcystin exposure induces oxidative stress, we did not detect expression differences in genes classically associated with oxidative stress responses. In addition, significant differences were noted in about 20% of detected metabolites. The results point to subtle but significant effects of microcystin-LR on the physiology of *E. coli* and presumably, by extension, other aquatic Gram-negative bacteria, the biological significance of which is yet to be fully understood.

CYANOTOXINS AND BLOOMS DETECTED IN MULTIPLE WATER BODY TYPES THROUGHOUT THE SAN DIEGO REGION

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Cyanotoxins, toxins that can be released from many cyanobacteria (a.k.a., blue-green algae) species, are considered contaminants of emerging concern and potentially have far-reaching impacts on public health, natural resources management, water supply, and recreation. There is no statewide cyanotoxin monitoring program in California, therefore, the San Diego Regional Water Quality Control Board conducted screening studies in several water body types over multiple years. In conjunction with numerous water quality assessment programs, targeted and probabilistic surveys were conducted (years 2011-2014) for streams, depressional wetlands, lakes, reservoirs, and coastal wetlands. A decision tree framework for toxin analysis was developed for efficient use of limited resources. Passive samplers, SPATT (Solid Phase Adsorption Toxin Tracking), provided a valuable initial microcystin screening. Grab samples were analyzed for particulate and whole water fractions of microcystins (additional cyanotoxins for a small subset of samples), chlorophyll a, and at some sites, nutrients and species identification.

Microcystins were detected in all of the water body types and were extremely prevalent in lakes, reservoirs and coastal wetlands. Toxin concentrations detected from grab samples were above recommended recreational use action levels at some sites, but overall underestimated the prevalence of microcystins in most water bodies. Additionally, microcystins were detected in locations not visually indicative of having cyanobacteria blooms, and different water bodies illustrated different types of visual blooms. Our findings are important for development of an effective monitoring program and suggest that sampling based solely on visual observations of blooms may underestimate toxin prevalence and miss toxic events.

DOES SAN FRANCISCO BAY HAVE A TOXIC ALGAE PROBLEM?

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San Francisco Bay (SFB) is an urbanized, nutrient-enriched estuary, and considered the most anthropogenically-altered estuary in the United States. SFB has historically resisted many classic symptoms of eutrophication; though recently, increased primary productivity and potential impairment is trending upwards. One indication of this impairment are the many toxin producing species of harmful algae that have been documented by microscopy in quantities high enough to produce toxic blooms. Yet, bloom events have rarely been documented, likely due to the size and expense of monitoring SFB. A pilot program to identify toxin in SFB using Solid Phase Adsorption Tracking (SPATT) was conducted from 2011 - present on bi-monthly USGS cruises. Results indicated persistent presence of both domoic acid and microcystin toxins throughout, indicating consistent low-level effects from both toxins, with periodic concentrations of high concern. Separately, mussel samples taken throughout the Bay in September 2012, 2014 and April 2015 identified via LCMS that both DA and MCY are being transferred to the food web, along with a third toxin: saxitoxin. Because of our results from the mussel tissue analysis, grab samples from the USGS cruises were analyzed by LCMS for all three toxins. Our preliminary results indicate that toxins were measureable throughout SFB. The combination of grab, SPATT, and mussel samples help to provide a complete picture of the presence of both marine and freshwater toxins that could be potentially magnifying the impacts of HABs on this ecosystem.

PHOTOSYNTHETIC YIELD (F_v/F_m) AS AN INDICATOR OF NUTRIENT LIMITATION IN *MICROCYSTIS AERUGINOSA*

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Photosynthetic yield (F_v/F_m), based on measures of fluorescence, has been used as a measure of cellular health in both field populations and laboratory cultures of freshwater phytoplankton. It has been applied to assess the condition of Great Lakes plankton communities. The traditional value of F_v/F_m of ~0.6 as the benchmark for healthy cells was derived using eukaryotic organisms. As such, it may not be appropriate for cyanobacteria due to their different photosystem structure. We compared the Fv/Fm values obtained for a Lake Erie isolate of bloom forming *Microcystis aeruginosa* under nutrient replete and limited conditions. F_v/F_m was measured using the traditional DCMU method and different instrumentation of increasing complexity including the Genty parameters from a bbe Algal Online Analyzer, a Turner Designs PhytoFlash, and a Waltz PhytoPAM. Findings of this study and their implications for culture and fieldwork in the Great Lakes and other waterbodies will be presented.

DEVELOPMENT OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) PROBES TO DETECT AND ENUMERATE *GAMBIERDISCUS* SPECIES AS APPLIED TO WAI ‘OPAE, HAWAII

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Species within the *Gambierdiscus* genus produce lipophilic ciguatoxins that can bioaccumulate in coral reef fish, and if consumed by humans, can cause ciguatera fish poisoning (CFP). Globally, tens of thousands of individuals are likely afflicted with CFP on an annual basis, with up to 10% of the local population on some islands in endemic areas becoming ill. Prior studies have shown that multiple *Gambierdiscus* species co-occur, and have significant differences in toxicity. The ability to accurately distinguish *Gambierdiscus* species and determine community composition is therefore central to assessing CFP risk, but new tools for species identification are needed. Since most *Gambierdiscus* species are indistinguishable using light microscopy, we designed fluorescence in situ hybridization (FISH) probes to differentially label six species, thus permitting their identification and enumeration in field samples. This technology enables accurate cell-based assessment of community composition for the first time. Probes were tested on cultures of *Gambierdiscus* from St. Thomas, USVI; French Polynesia; and Hawaii, and applied during a field study on the Big Island of Hawaii. In culture testing, probes selectively detected their species of interest. Analysis of field samples using the probes showed that they were reliably able to label their target species, and enabled quantification of the *Gambierdiscus* community.

TRANSFER OF THE HARMFUL ALGAL TOXIN, MICROCYSTIN, FROM FRESHWATER LAKES TO PUGET SOUND, WASHINGTON AND TOXIN ACCUMULATION IN MARINE MUSSELS *MYTILUS TROSSULUS*

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Cyanobacteria toxins, such as the hepatotoxin microcystin (MC), are well known to contaminate freshwater ecosystems. However, monitoring for MC in marine environments has been minimal, despite evidence that MC can contaminate marine receiving waters. Freshwater organisms, such as finfish and shellfish, can accumulate MC, but due to minimal monitoring in marine environments, poisonings of marine biota have only occasionally been reported. Eutrophic inland waters produce annual toxic cyanobacteria blooms that drain into Puget Sound, an inlet of the Pacific Ocean. Puget Sound shellfish are important economic resources that are commercially distributed worldwide and are harvested by many residents as a locally available, inexpensive protein source. We confirm, for the first time, the freshwater to marine transfer of MCs and subsequent bioaccumulation of MC by Puget Sound mussels. ELISA analysis estimated maximum MC concentrations in source lakes of 2700 µg/L, up to 0.34 µg/L in marine waters and 6.5 µg/kg in mussels. Confirmatory analyses by LC-MS/MS on water and mussel samples identified MC-LA as the major toxin. Although relatively low levels of MC were found in mussels, toxin presence is indicative of an unhealthy ecosystem, and may impact food safety, especially for populations reliant on consuming Puget Sound shellfish.

EMERGING PATTERNS AND BIOLOGICAL IMPACTS OF HARMFUL ALGAL BLOOMS IN LOWER CHESAPEAKE BAY

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Recently several potentially toxic harmful algal bloom (HAB) species have been identified in Chesapeake Bay waters. Blooms of *Cochlodinium polykrikoides* and *Alexandrium monilatum* are increasing in intensity and duration throughout lower Chesapeake Bay. Until the 1990's, *C. polykrikoides* blooms were largely confined to the York River. In recent years, however, heavy blooms have occurred throughout the lower Bay. *Alexandrium monilatum* is a common HAB species that historically blooms along the US southern Atlantic and Gulf coasts. A significant bloom event in the York River, VA in 2007 marked the re-emergence of this toxic species in the mid-Atlantic since reports of it in the region during the 1940's and 1960's. There is a population progression in the York River with peak cell concentrations of *C. polykrikoides* followed two to three weeks later by peak concentrations of *A. monilatum*. Until 2011, *A. monilatum* blooms were localized in and near the York River, but in recent years cells were found in water samples throughout southern Chesapeake Bay and in mid-Atlantic coastal waters. Intensification of *C. polykrikoides* and *A. monilatum* bloom activity in Chesapeake Bay is cause for concern. These blooms have been associated with local mass mortalities of oyster larvae, as well as research organisms exposed to natural water. Laboratory bioassays exposing larval fish and shellfish to field-collected bloom samples and to isolate cultures from the region have demonstrated acute toxicity. Sediment surveys and bloom mapping are being done to identify cyst beds and to more accurately predict the spatial distribution of blooms.

THE SOCIO-ECONOMIC EFFECTS OF *GYMNODIUM CATENATUM* BLOOM IN THE NORTHWEST REGION OF GULF OF CALIFORNIA, MEXICO.

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Harmful algal blooms (HAB) represent a significant and expanding threat to human health and fisheries resources. Shellfish closures, wild or farmed fish mortalities, and tourism are some of the impacts of major HABs. In Mexico the threat from outbreaks of toxic algae are poorly understood since direct and indirect costs are poorly quantified. A massive bloom of *Gymnodinium catenatum* occurred during the period of January to April 2015 causing significant lost sales of shellfish products in the Northwest region of Gulf of California and unknown social impacts. Here we present a first approach about the socio-economic effects caused by this event in the region. The duration, the affected shoreline length, average toxicity levels and values of affected coastal resources were documented and analyzed. We consider socio-economic effects of four basic types: public health, commercial fisheries, recreation and tourism, and monitoring and management. Our analyses are based on surveys, a review of the literature and our own calculations. We define economic effects broadly to mean the cost of lost sales of commercial species for example Geoduck clams *Panopea globosa* (Dall 1998). The purpose of this study was to asses impacts at local scales and to assess the cost and benefits of mitigations strategies; the importance of measure the social cost of natural hazards, as an essential component of management programs in Mexico is also discussed.

SOUNDTOXINS: HARMFUL ALGAL BLOOM MONITORING IN PUGET SOUND, WASHINGTON

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SoundToxins, a diverse partnership of Washington state shellfish and finfish growers, environmental learning centers, Native American tribes, and Puget Sound volunteers, is a monitoring program designed to provide early warning of harmful algal bloom events in order to minimize both human health risks and economic losses to Puget Sound fisheries. The overall goal of this cooperative partnership is to establish a cost-effective monitoring program that will be led by state managers, environmental learning centers, tribal harvesters, and commercial fish and shellfish farmers. The objectives of the SoundToxins program are to determine which environmental conditions promote the onset and flourishing of HAB events or unusual bloom events, to determine which combination of environmental factors can be used for early warning of these events and to document unusual bloom events and new species entering the Salish Sea. To accomplish this, seawater samples are collected weekly by the participants at 31 different sites throughout Puget Sound. These are analyzed for salinity, temperature, nutrients, chlorophyll, phytoplankton species, and marine biotoxins. Phytoplankton species diversity is described and the four target HAB species are specifically identified and enumerated. These target species *are Pseudo-nitzschia* species, *Alexandrium catenella*, *Dinophysis species*, and *Heterosigma akashiwo*. The program is jointly administered by NOAA Fisheries and Washington Sea Grant in partnership with the Washington State Department of Health. For more information, go to www.soundtoxins.org.

ASSEMBLING AND ANALYZING THE TRANSCRIPTOMES OF THREE TOXIN-PRODUCING *KARENIA* SPECIES

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The marine dinoflagellate species *Karenia brevis*, *Karenia papilionacea*, and *Karenia mikimotoi* are close phylogenetic relatives. However, while *K. papilionacea* and *K. brevis* each produce brevetoxin (PbTx-2), *K. mikimotoi* instead synthesizes the cytotoxin gymnocin. To understand the genetic similarities and differences among these three species, the transcriptomes of *K.brevis*, *K. papilionacea*, and *K.mikimotoi* were assembled and compared. Using high-throughput sequencing technology, >100 million 150-bp paired-end RNA reads were sequenced from K. brevis Wilson, *K. papilionacea* C91, and *K. mikimotoi* C22 cultures. After reads were trimmed for quality and length, they were processed with two de novo transcriptome assembly programs, Trinity and Velvet-Oases. The assembler output files were combined into one nonredundant reference transcriptome per species. Orthologous *Karenia* transcripts were identified using the reciprocal BLAST method. Additionally, all putative, continuous open reading frames (ORFs) longer than 300 nucleotides were annotated against proteins in the NCBI nr database. Annotation results were assigned potential enzymatic pathways with the Blast2GO KEGG mappting function. The comparative transcriptomic analysis identified 6,561 transcripts with ORFs > 300 nucleotides that were expressed by *K. brevis* and *K. papilionacea* but not *K. mikimotoi*. Based on the annotation results, these "unique" transcripts belonged to diverse putative enzymatic pathways, including polyketide synthesis. In fact, twenty-one putative polyketide synthase (PKS) genes were characterized in the "unique" pool. Most of the PKS transcripts had just one catalytic domain, like previously characterized *K. brevis* PKS sequences. The "unique" orthologs provide valuable insight into the biology of brevetoxin-producing dinoflagellates.

EFFECTS OF PREY TYPE AND FOOD QUALITY ON TOXIN DYNAMICS WITHIN AN ISOLATE OF *DINOPHYSIS ACUMINATA*

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Previous field and culture studies indicate differences in toxin production between geographical populations and isolates of *Dinophysis acuminata*; however, the factors driving differences in toxigenicity have not yet been fully elucidated. We investigated the role of prey line (i.e., geographical isolates), food quality and quantity in the growth and production of toxins by *Dinophysis* as a possible explanation for regional variation in toxicity. As such, *D. acuminata* from the northwestern Atlantic, U.S. was offered a matrix of prey lines in a full factorial design, 1 x 2 x 3; one dinoflagellate strain was fed one of two ciliates, *Mesodinium rubrum*, isolated from coastal regions of Japan or Spain, which were grown on one of three cryptophytes isolated from Japan, Spain, or the northwestern Atlantic, U.S. Additionally, we manipulated predator:prey ratios to examine any effect of prey quantity on *Dinophysis* growth or toxin production, and we determined the nutritional potential of each prey line. These studies revealed the origin or strain of ciliate, and less so the cryptophyte, directly impacted the growth of *Dinophysis acuminata*. Inherent differences between the two ciliates, e.g., biovolume and food quality, appear to drive this strain-specific impact on growth, however, we cannot rule out other possible predator – prey dynamics that could contribute such as ease of capture or any allelopathic effects of phycotoxins. Ciliate origin also resulted in significant differences in toxin content and toxin profile in *D. acuminata*. This work provides links between predator – prey dynamics and toxin production and a possible explanation for regional variability in toxin production between distant populations.

MONITORING SEDIMENT-BOUND MICROCYSTINS ACROSS CALIFORNIA'S STREAM POLLUTION TRENDS (SPoT) PROGRAM WATERSHEDS

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Freshwater cyanobacterial harmful algal blooms (CHABs) are an emerging threat to drinking water resources and aquatic habitat through the production of potent cyanotoxins. Hepatotoxic microcystins, a class of cyanotoxins produced by several cyanobacterial taxa, have increasingly been identified in freshwater habitats worldwide. Microcystins, stable cyclic heptapeptides, may persist in the environment for weeks to months in water and sediments. An optimized method for extraction of microcystins from sediments was developed and employed to analyze streambed sediments from California's Stream Pollution Trends Program (SPoT). SPoT is part of the California's Surface Water Ambient Monitoring Program and assesses long-term trends in sediment contaminants. Sediment-bound microcystins were extracted and purified via a modified sodium pyrophosphate and solid-phase extraction columns method (Chen et al. 2006) and quantified with enzyme-linked immunosorbent assay (ELISA). A sub-sample was separately extracted and analyzed for method validation and was verified with an external lab by both ELISA and LC-MS. In 2014, microcystins were detected in 31% (n=87) of sites from watersheds in eight out of the nine California Water Quality Control regions. Microcystins were found in sediments from diverse habitat types and land uses. Monitoring microcystins bound to stream sediments may be an indicator of upstream toxic CHABs or in-stream toxin production. Ongoing research includes analysis of spatial and temporal patterns in toxicity and evaluating whether in situ or upstream processes are responsible for sediment-bound microcystins.

GROWTH OF HETEROTROPHIC PROTISTS ON RED TIDE-FORMING PHYTOPLANKTON SPECIES: EFFECTS OF TOXIN PRODUCTION AND INTERSPECIFIC PREY INTERACTIONS

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Heterotrophic protists are significant grazers of marine microalgae, though their ingestion of and ability to grow on bloom-forming and toxic species can be variable and highly species-specific. The heterotrophic dinoflagellate *Noctiluca scintillans* has been shown to ingest and grow on various species of potentially toxic microalgae. This study documents a wide range of growth rates for *N. scintillans* (-0.08 to 0.79 d^{-1}) on several species of dinoflagellates and raphidophytes considered to be harmful, including *Heterosigma akashiwo, Lingulodinium polyedrum, Chattonella marina, Akashiwo sanguinea*, and *Alexandrium catenella*. This study specifically demonstrates that saxitoxin-producing *A. catenella* was not supportive of *N. scintillans* growth, despite ingestion rates comparable to those obtained from positive growth of *N. scintillans* on *H. akashiwo* but lower than rates from previously published reports for *N. scintillans* feeding on the congener *Alexandrium minutum*. Growth of *N. scintillans* was negatively affected when *A. catenella* was present at concentrations ≥ 3.14 x 104 ng C ml^{-1} (1.55 x 10^4 cells ml^{-1}) and when it was the only prey available. *N. scintillans* growth was relatively unaffected by high concentrations (16.73 ng ml^{-1}) of purified dissolved saxitoxin, indicating that dissolved toxin alone did not impede growth of the grazer when preferred prey were available. However, growth of *H. akashiwo* exposed to *A. catenella* culture and cell-free filtrate was negatively affected, suggesting a potential indirect effect of *A. catenella* on *N. scintillans* growth through interspecific prey interactions and reduction in the availability of high-quality food.

STATEWIDE STRATEGY FOR MONITORING AND MANAGING FRESHWATER HABS IN CALIFORNA: A WATER QUALITY MANAGERS' PERSPECTIVE

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Cyanobacteria blooms and other nuisance freshwater HABs have increased globally in extent, frequency and duration due to various anthropogenic factors which include climate change, nutrient loading and water residence time. In recent years, freshwater HABs have gained public attention in California as a result of several well documented problematic areas that include the Klamath River, Pinto Lake, Clear Lake, San Francisco Bay and Delta, East San Francisco Bay Lakes and Monterey Bay. Current conditions in California are ideal for continued HAB issues due to the ongoing drought, low snowmelt, warm weather and urban and agricultural development (resulting in high nutrient loads). In the past year, four lakes in the Los Angeles region have experienced massive and costly fish kills (attributed to *Prynmesium parvum*) and several dog deaths have resulted from toxic cyanobacteria blooms in East San Francisco Bay lakes. In response to these conditions, California water quality managers and scientists have developed a Freshwater HABs Program that is guided by a coordinated statewide monitoring, assessment and reporting strategy. Technical elements that will support the strategy include: 1) field sampling and laboratory guidance documents and SOPs, 2) dedicated laboratory resources, 3) the use of satellite imagery to identify blooms, 4) newsletters and a website to report satellite imagery, lab data, bloom information and incident reports of illnesses or deaths in animals or humans, 5) trainings on sampling, health and safety, species identification, bloom management and how to access and report information, and 6) a report on status and trends of freshwater HABs in California.

DIVERSITY, DYNAMICS AND BIOGEOGRAPHIC DISTRIBUTIONS OF PICO- AND NANO-PLANKTONIC PELAGOPHYTES AND CHLOROPHYTES

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Between 2011-2013 vast blooms of pico- and nano-planktonic algae disrupted ecosystem structure and function in Florida's Indian River Lagoon (IRL). Little is known about the diversity of these algae, largely because of the difficulty in microscopic identification. Genetic approaches however, can rapidly identify morphologically similar species, and hold promise for revealing the true diversity and dynamics of these phytoplankton. We developed a variety of rRNA-targeted molecular tools to evaluate and monitor pico- and nano-phytoplankton diversity both biogeographically and through time. In particular, we developed a combined genetic fingerprinting assay specific to chlorophytes, and qPCR assays for *Aureococcus anophagefferens, Aureoumbra lagunensis*, and for a chlorophyte sp. dominant during the 2011 "superbloom" in the IRL. Furthermore, we improved the specificity of an existing qPCR assay for the phylum Chlorophyta by employing blocking primers for two non-target phyla. Additional primers were designed for *A. lagunensis* CCMP1503, after partial 18S rRNA sequencing (900bp) revealed that this strain had <97% similarity to other isolates of *A. lagunensis* and *A. anophagefferens*, which along with uncultured environmental sequences, revealed novel inter- and intra-specific diversity among the pelagophytes. We further tested these newly-designed assays on samples from the Gulf of Mexico and the US east coast (from FL to MA) and applied them for monitoring pico-/nano-plankton at three sites in the IRL (Jan-Aug 2015). These molecular tools are being further implemented for rapid screening (e.g. by flow cytometry) to improve bloom monitoring and management and to provide insight into the occurrence and drivers of specific bloom-forming taxa.

A SOCIAL-ECOLOGICAL SYSTEMS APPROACH TO IMPROVE UNDERSTANDING OF *ALEXANDRIUM* BLOOMS AND THEIR SOCIOCULTURAL IMPACTS IN SOUTHEAST ALASKA

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Paralytic Shellfish Poisoning (PSP) in Alaska is a persistent problem that significantly impacts human health and the availability of shellfish resources. Shellfish are an important traditional food source for many Alaskans, but regular outbreaks of PSP make recreational and subsistence shellfish harvest unsafe. Within the last 5 years alone, there have been 34 cases of PSP reported. Despite the recognized impacts of PSP in Alaska, little research has been done on the causative organism, *Alexandrium* sp. This three-year study aims to enhance sustainability of shellfish harvest in Southeast Alaska by addressing the ecology of *Alexandrium* harmful algal blooms (HABS) and their sociocultural impacts. Ecological research involves *Alexandrium* cyst bed mapping to identify bloom initiation sites and weekly year-round sampling to identify environmental conditions promoting bloom formation and toxin production in Northern Southeast Alaska. A "snow-ball sampling" approach is used to distribute surveys and conduct interviews with shellfish harvesters to obtain critical information about how local communities can reduce vulnerability to PSP and how local/traditional knowledge can support ecological research efforts. Preliminary findings from our first field season include cyst distributions and environmental conditions associated with toxic *Alexandrium* blooms in the Juneau region. The expansive Alaskan coastline imposes significant challenges for HAB monitoring. Use of local and traditional knowledge can improve understanding of historic patterns of PSP in rural areas and help direct future monitoring efforts.

HIGH RESOLUTION OCEAN COLOR PRODUCTS TO ENHANCE CYANOBACTERIA MONITORING EFFORTS IN INLAND LAKES

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As extensive blooms of toxic cyanobacteria are a recurrent problem in lakes and reservoirs throughout the U.S., a synoptic monitoring strategy is desired. An algorithm that considers the spectral shape centered on a wavelength of 681 nm (the fluorescence peak), has been used to estimate cyanobacteria biomass for the forecast of blooms in Lake Erie, and shows promise in detecting blooms in smaller inland lakes throughout the United States. A correlation to Chl a was developed for lakes in Florida and is now being investigated to improve management efforts in the state of California. We have developed image analysis tools to evaluate cyanobacteria biomass data from 300 m satellite imagery, collected by the Medium-spectral Resolution Imaging Spectrometer. A retrospective analysis of the available MERIS data (May 2002 to April 2012) will be presented on several lakes within California, to assist management efforts necessary in maintaining safe beaches, drinking water and protecting public health. Preparations are being made to apply these techniques to real-time imagery that will be provided by the Ocean and Land Colour Instrument on the Sentinel-3 satellite expected to be launched by the European Space Agency in late 2015. Training on the use of these image products and tools was provided to California managers, a step in developing a national capability to use this type of data.

EXPRESSION AND ACTIVITY OF NOVEL NITRATE REDUCTASE ENZYMES IN *CHATTONELLA SUBSALSA* AND IMPLICATIONS FOR COMPETITIVE DYNAMICS IN MARINE ENVIRONMENTS

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Chattonella subsalsa is a potentially harmful alga broadly distributed in coastal regions around the world. This species can be toxic to variety of marine organisms and has been associated with mass fish kills in many countries. *C. subsalsa* uses both nitrate and ammonium as nitrogen sources. For organisms using nitrate as a nitrogen source, nitrate reductase (NR) catalyzes the first enzymatic and also the rate-limiting step in nitrate assimilation. Previous research of our lab found a novel nitrate reductase, CsNR2-2/2HbN, in this alga, but its expression and regulation remain unclear. In this study, we found a second novel nitrate reductase, CsNR3, in this alga. It has a unique 14-3-3 binding domain at the hinge1 region. The 14-3-3 binding domain is commonly found in higher plants, and works to regulate NR activity. This is the first report of this binding domain in nitrate reductase of algae. Expression and regulation of CsNR2-2/2HbN and CsNR3 under different nitrogen sources, temperature, and light intensity will be examined. Our preliminary data show that nitrate reductase activity and growth of *C. subsalsa* was inhibited by high concentrations of ammonium, while nitrate reductase activity and growth of *H. akashiwo* was not. Small-scale microcosm experiments with environmental samples containing both *H. akashiwo* and *C. subsalsa* will be conducted with addition of nitrate, ammonium, or both nitrate and ammonium. The growth of each alga and the expression of CsNR2-2/2HbN and CsNR3 will be measured to investigate competitive dynamics of these two species under different nitrogen conditions.

OCCURRENCE OF *DINOPHYSIS* AND ASSOCIATED DSP TOXINS IN MARYLAND

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The harmful alga *Dinophysis acuminata* has bloomed periodically in Maryland's offshore waters and within the Maryland Coastal Bays. This species can produce a variety of toxins that may cause stomach and intestinal upset in humans if ingested via shellfish. Bloom concentrations of *D. acuminata* have been detected in routine monthly monitoring in the MD Coastal Bays in 2002, 2005, 2006, 2009, 2010 and 2012. Additionally, bloom concentrations were found offshore of MD in 2011. DSP toxins were found above the FDA threshold level in shellfish (16mg/100g) for the first time during 2012. Fortunately, these exceedances were in an area closed to shellfish harvesting. Results of a three year study that investigated the presence of *Dinophysis* spp. and diarrhetic shellfish poisoning toxins in the Maryland Coastal Bays will be reported. Data from the first year (2013) indicated that uptake of *Dinophysis* toxins differed among four shellfish species. Scallops had the highest toxin levels, followed by ribbed mussels, then hard clams and oysters. During the second year (2014), the cage study was repeated with only hard clams and ribbed mussels, and the number of sites were expanded to include more shellfish areas and south of the Ocean City Inlet. The presence of *Dinophysis* was confirmed south of the Ocean City Inlet indicating a possible management issue for the National Park at Assateague Island. In 2015 increased phytoplankton monitoring revealed the presence of a new species: *Dinophysis norvegica*.

Poster Presentation Abstracts

MULTI-LABORATORY VALIDATION OF AN LC-MS METHOD FOR THE DETERMINATION OF BREVETOXIN CONTAMINATION IN MOLLUSCAN SHELLFISH

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Structure-based methodologies of high specificity are being developed for monitoring brevetoxin (BTX) contamination in molluscan shellfish. BTXs are lipophilic polyether neurotoxins produced by the marine dinoflagellate *Karenia brevis* that accumulate in filter-feeding shellfish during blooms. Consumption of BTX-contaminated shellfish causes neurotoxic shellfish poisoning (NSP). Prevention of NSP in the U.S. currently relies upon environmental monitoring of *K. brevis* blooms and assessment of shellfish toxicity by mouse bioassay. Efficient methods are needed for improved monitoring and management programs. Recently, we identified biomarkers of BTX exposure and toxicity in the Eastern oyster and hard clam. The selected biomarkers are the metabolites BTX-B1, BTX-B2, and S-desoxy-BTX-B2. A liquid chromatography-mass spectrometry method was developed, and its performance assessed by single laboratory validation. We report here results of a multi-laboratory validation of the method following the FDA Foods Program Guidelines for Chemical Methods. Performance characteristics were evaluated in three laboratories for commercially important shellfish species using fortified and naturally incurred samples. Mean recovery in oysters ranged from 74-92%, within-laboratory variation (RSDr) of <11%, and between-laboratory variation (RSDR) <14%. In clams, recoveries ranged from 85-98%, RSDr <9%, and RSDR <11%. Between-laboratory precision using incurred samples (6 samples, 12 replicates each) ranged from 8-10%. Method limits of detection (LOD) ranged from 0.5-2 ppb and method detection limit (MDL) was established at 50 ppb. Overall, the method performed well, yielding reproducible and robust results, and brings the monitoring and management of BTXs in molluscan shellfish to a novel and dynamic level.

NITROGEN ISOTOPE FRACTIONATION EFFECTS ON SAXITOXIN IN *ALEXANDRIUM FUNDYENSE* WHEN GROWN ON NITRATE, AMMONIUM, AND UREA

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Excess nutrient input from associated wastewater treatment plants, septic leaching or groundwater contamination, and fertilizer runoff has been linked to an increase in harmful algal bloom (HAB) events in freshwater and estuarine environments. However, the relationship between HABs and anthropogenic nutrient sources is site and organism specific. Stable isotopes are routinely used to identify and track nitrogen (N) sources to a body of water, as sources can be differentiated based on isotopic signatures. By focusing on the isotopic signature of a dominant organism in a system, we can better control for variation in interspecies isotope fractionation and potentially provide more information about the source, chemical form, and N uptake processes that lead to cell growth and toxin production of a particular toxic species. Preliminary trials have shown that the $\delta^{15}N$ of saxitoxin, a nitrogen-rich neurotoxin produced by both freshwater cyanobacteria and marine dinoflagellates, can be used as an organism-specific tracer to identify nitrate sources. As natural systems contain several chemical forms of N, we investigated saxitoxins as a robust tracer in the presence of nitrate, ammonium and urea. Three isolates of *Alexandrium fundyense* (PW06, GTCA-28, NS-SP3) that vary in saxitoxin congener composition, were grown on these forms of N. We monitored cultures for growth, N uptake rates and recycling, and the $\delta^{15}N$ of saxitoxin, whole cells, and N source. *Alexandrium fundyense* utilized nitrate (0.22 d^{-1}), ammonium (0.17 d^{-1}), and urea (0.19 d^{-1}) for growth, and results will be presented on the isotopic fractionation effects on each N form. Beyond this project's application in nutrient management and bloom dynamics, these results provide additional information regarding saxitoxin synthesis in *Alexandrium fundyense.*

LINKING NUCLEAR SCIENCE AND SEAFOOD SAFETY:

RELIABILITY OF THE RECEPTOR BINDING ASSAY FOR PSP AND CIGUATERA TOXINS

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Compliance to national regulation for harmful algal toxins is, today, a greater obstruction in seafood commercialization (for national consumption or export) than the production itself. Risk of toxic seafood remains a reality, particularly in less developed coastal communities, including small islands, which rely on seafood as major source of protein. Hence, it is critical to pursue the development and implementation of simple and reliable detection methods, to support and assure safe seafood supplies, and maintain and develop sustainable social, economic and environmental balance. In this context the International Atomic Agency has served as a major international platform for scientific knowledge, methods development and technology transfer in harmful algal bloom management. This work reports progress in the reliability of the nuclear based receptor binding assay for paralytic shellfish poisoning toxins (PSP-RBA AOAC official method 2011.27) and ciguatera (CFP-RBA) in the context of activities implemented by the IAEA in cooperation with other national (NOAA, ILM, PNRI) and international organization (IOC-UNESCO) to assess HABs and mitigate their impacts.

TOXIC BENTHIC CYANOBACTERIAL MATS IN A CALIFORNIA RIVER NETWORK

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Most cyanobacterial harmful algal blooms occur in estuaries and lakes, but over the last decade in the Eel River in Northern California benthic cyanobacterial mats have killed 11 dogs. In rivers in Mediterranean climates, benthic algae fuel summer aquatic food webs. When algal assemblages tip towards toxic cyanobacteria water quality is degraded, negatively impacting food webs and public safety. During two drought summers, 2013 and 2014, we investigated the common cyanobacterial species and the spatial and temporal dynamics of cyanotoxin production in the watershed. Solid phase adsorption toxin tracking (SPATT) samplers were used to measure dissolved cyanotoxin (microcystins and anatoxin-a) levels at 10 sites throughout the watershed. In 2014, intracellular cyanotoxin concentrations of cyanobacterial mats were also measured. Species of *Anabaena* and *Phormidium* were the dominant cyanotoxin producers in the watershed. Anatoxin-a concentrations were higher than microcystin for both SPATT and intracellular samples. SPATT cyanotoxin levels peaked during mid-summer in warm middle-reaches of the watershed. Out of 123 intracellular samples, 50% detected anatoxin-a, while 24% detected microcystins. Both SPATT and intracellular cyanotoxin levels varied weekly, suggesting rapid turnover between toxin-producing and non-producing strains in the mats. We hypothesize that low river discharge and warm water temperatures associated with droughts increase the abundance of cyanobacteria in the Eel River. Knowledge of environmental conditions that promote cyanobacteria in rivers is needed for management to maintain productive food webs and reduce public health threats from cyanotoxins.

A SPATIO-TEMPORAL ANALYSIS OF PSP CONGENER PROFILE PATTERNS IN SHELLFISH ALONG THE COAST OF MAINE

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The Gulf of Maine is a hotspot for the outbreak of paralytic shellfish poisons. Recently adopted advances in technology and methodology have increased our ability to not only monitor these outbreaks as they progress throughout each season, but also to understand the patterns and magnitude of their occurrence. Bigelow Laboratory for Ocean Sciences in conjunction with the Maine Department of Marine Resources adopted the HPLC-PCOX method (AOAC Official Method 2011.02) for measuring saxitoxin and eleven of its congeners, which has been in use for two regulatory seasons, with FDA approval. The data collected over this time provides new insight into the spatio-temporal progression of PSP toxicity in shellfish along the Maine coast. Connections between the toxicity profiles of planktonic communities and those observed in the shellfish samples are explored. This has revealed distinct patterns in progression of PSP toxin accumulation along the coast of Maine that are related to what is understood of the source and development of the Alexandrium blooms in the Gulf of Maine.

BUILDING A NEXT GENERATION GEOSPATIAL INFRASTRUCTURE FOR OPERATIONAL HARMFUL ALGAL BLOOM FORECASTING

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Since 2004, NOAA has maintained the Gulf of Mexico Harmful Algal Bloom Operational Forecast System (HAB-OFS) to assist early *Karenia brevis* identification and response efforts. An in-house software platform designed specifically for the HAB-OFS is currently used for data ingestion, visualization, product generation and dissemination. However, as the HAB-OFS matures and expands nationally, a next-generation infrastructure is required to manage new datasets and handle increasingly complex data processing and analyses, incorporate new technologies, and display near-real time data. The HAB-OFS team has proposed a redesigned next-generation infrastructure based on GIS architecture to manage the end-to-end lifecycle of the HAB-OFS products. This new platform increases the efficiency and reliability of data ingestion and analysis to streamline the existing procedures. The HAB-OFS team envisions the system disseminating HAB bulletins as geo-referenced PDFs which enhances the user experience by enabling better visualization of GIS layers and access to metadata for easier interpretation. The use of an interactive web map is also being explored in order to enable data layers to update dynamically in near-real time, further supporting well-informed and timely response to HAB events. As these products are developed further, stakeholder input will be essential. With this new infrastructure, HAB-OFS will be better positioned to keep pace with the evolution of HAB science and technology and the national expansion of NOAA's HAB forecasting program.

THE NEUROLOGICAL EFFECTS OF FLORIDA RED TIDE (FRT) BLOOMS

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Karenia brevis is a marine dinoflagellate responsible for Florida Red Tide blooms off the west coast of Florida. *K. brevis* contains brevetoxins, a neurotoxin that is absorbed by shellfish as well as released into the air. Brevetoxins are known to cause disruptions in normal neurological functions and are associated with neurotoxic shellfish poisoning (NSP). Previous research has emphasized the effect of FRT blooms on human health, from gastrointestinal to respiratory illnesses. However, there has been little research examining the effect of FRT blooms on neurological illnesses. There is research highlighting the biochemical effects of brevetoxins on mammalian nervous systems, so these symptoms can be matched to hospital codes that describe a hospital patient's affliction. With these hospitalization codes, it is possible to study the relationship between FRT blooms and the occurrence of neurological illnesses in affected counties. The hospital data consists of inpatient data from 1988-2010 and emergency room data from 2005-2010. We will also use data containing *K. brevis* cells per liter as a measure of red tide occurrences.

IMPLEMENTING A GIS APPLICATION FOR LAKE ERIE HARMFUL ALGAL BLOOM FORECASTING

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Since 2009, the National Oceanic and Atmospheric Administration (NOAA) has been routinely delivering monitoring and forecasting products for the toxic cyanobacteria bloom in western Lake Erie. These forecasts provide managers and the public with the current and expected bloom position, allowing them time to avoid potential hazards of the blooms. Weekly forecasts are delivered as a georeferenced PDF to an email subscriber list of over 1,000 individuals, including people from local, state, and federal agencies. GIS tools have proved useful in enhancing and streamlining existing bulletin procedures enabling additional information to be incorporated, increasing the value to managers. Customized toolsets have been developed to calculate bloom statistics from satellite imagery, ingest field estimates of toxins and cells, as well as automate the bulletin creation. The use of the GIS tools has enabled the streamlining of daily updates which are disseminated to a small group of local water managers. These advancements will be discussed in conjunction with the outcome of the 2015 Lake Erie bloom season.

MONITORING ALGAL TOXINS USING IMMUNOASSAY - RESULTS FROM SPENT CULTURE MEDIA ANALYSIS FROM NCMA CULTURE COLLECTION

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The U.S Environmental Protection Agency recently issued health advisory values for algal toxins in drinking water of 0.3 micrograms per liter (ppb) microcystins and 0.7 ppb cylindrospermopsin for children younger than school age. For all other ages, the health advisory values for drinking water are 1.6 ppb microcystins and 3.0 ppb cylindrospermopsin. The NCMA facility maintains marine algal cultures for research and commercial purposes. The collection contains over 3000 cultures, a portion of which are similar or related to algal strains which have been shown to have the potential to produce toxins under certain conditions. Media samples from 237 of these were collected and tested on the Microcystin immunoassay test kit developed by Beacon Analytical Systems. All were shown to be negative or less than 0.1 ppb Microcystin LR. Eight media samples obtained from known microcystin producing strains of *Microcystis aeruginosa* including one implicated in recent drinking water contamination incidents, were also tested. Five samples were quantified as positive with Microcystin LR equivalents ranging from 32 to 152 ppb. Six samples from these eight screened with Beacon Analytical Systems immunoassay test kits for Saxitoxin and Cylindrospermopsin were negative or less than 0.01 ppb or 0.1 ppb respectively.

CHALLENGING THE ENDOGENOUS ANNUAL CLOCK PARADIGM: EFFECTS OF 'COLD CONDITIONING' ON THE GERMINATION TIMING OF *ALEXANDRIUM* CYSTS

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Alexandrium cysts in the Gulf of Maine have been shown to have an annual free-running oscillation in germination. This clock was thought to be completely endogenous, with a fixed timing pattern for germination that is unaffected by environmental conditions. However, new data suggests that this theory is overly simplistic, as it does not account for the effect of temperature. Older studies have shown how cold conditioning can both improve dinoflagellate cyst viability and synchronize germination, and seasonal temperature shifts can stimulate germination. Until now, we've held these ideas of endogenous and exogenous control of germination to be distinct because it seemed plausible that cysts in deep- (e.g. Gulf of Maine) and shallow-water systems (e.g. Nauset Marsh System) would have alternate methods of control. Here we present field and laboratory data, and a conceptual model of the complex interplay of internal and external factors regulating *Alexandrium* cyst germination. Cold-dark storage experiments at temperatures ranging from 2 to 8°C demonstrate that cold conditioning is capable of shortening the requisite dormancy period in *A. fundyense*, thereby advancing the timing of the annual clock by three months. The amount of cold conditioning needed for cysts to attain a given level of germination can be quantified using 'chilling units', an agricultural phenology concept that accounts for both temperature and time. These results suggest that an endogenous annual clock is common in both deep- and shallow-water *Alexandrium* cysts, but it is finely tuned to environmental temperature, thereby allowing for different germination timing in the two habitats.

EVALUATION OF A RAPID NSP ASSAY FOR USE IN SHELLFISH REGULATION AND AQUACULTURE

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In the Gulf of Mexico, annual blooms of the harmful dinoflagellate, *Karenia brevis,* threaten public health with the risk of Neurotoxic Shellfish Poisoning (NSP) and damage the shellfish industry through associated closures of shellfish harvesting areas. Currently, the only federally approved method of regulatory analysis for NSP toxins is the American Public Health Association Mouse Bioassay (MBA). The MBA is time-consuming, resource intensive, non-specific, and unethical. In contrast, the brevetoxin-specific Enzyme Linked Immunosorbent Assay (ELISA) is a rapid and cost effective method to measure NSP. However, it has not yet been evaluated as an alternative to the MBA as required by the Interstate Shellfish Sanitation Conference and the USFDA. The objectives of our Florida Sea Grant-funded project were to compare the ELISA to the MBA using actual regulatory shellfish samples collected during and after *K. brevis* blooms and to complete a single-lab validation of the MARBIONC brevetoxin ELISA. Here we present data and compare regulatory outcomes from over 300 naturally-incurred samples of Eastern oysters (*Crassostrea virginica*), hard clams (*Mercenaria mercenaria*), and sunray venus clams (*Macrocallista nimbosa*). In the subset of samples for which mouse units were obtained (>20 mouse units/100g), we found significant correlations between the MARBIONC ELISA and MBA results in hard clams and sunray venus clams; there was a weaker correlation between these metrics for oysters. These results highlight the potential for the MARBIONC brevetoxin ELISA to improve shellfish regulatory practices in Florida and other Gulf states.

CHANS: A STUDY OF SIGNIFICANT HOT SPOTS OF *KARENIA BREVIS* AND THEIR ASSOCIATED SOCIO-ECONOMIC IMPLICATIONS

Diana Moanga, Maria Estevanez, Steven Ullman, Gary Hitchcock

Within recent decades, much effort has been devoted to studying *K. brevis* bloom dynamics on the West Florida Shelf. However, in order to evaluate socio-economic and ecological impacts associated with these events, an interdisciplinary approach is required. The use of Geographic Information Systems (GIS) to explore the intricate linkages between biological and social factors and their associated spatial-temporal patters reveals insight into the effects of bloom maxima.

A critical factor in understanding *K. brevis* dynamics requires mapping and monitoring bloom development and transport, and identifying local maxima, or 'hot spots'. In the existing literature 'hot spots' are typically defined as either areas of high algal biomass, zones of chlorophyll a anomalies, or locations where bloom events most frequently occur. However, to our knowledge, a quantitative assessment of 'hot spots' for *K. brevis* is lacking. Furthermore, few existing studies provide any confidence levels for harmful algal hot spots.

A GIS analysis of the spatial distribution and characteristics of annual *K. brevis* blooms has been completed. Clusters of statistically significant hot spots are identified for several blooms, and site-specific conditions are defined. Potential correlations between the locations of hot spots, school absenteeism percentages and fluctuations in hotel occupancy rates have been examined. The goal of this research is to develop an index for evaluating the socio-economic impacts of *K. brevis* blooms.

CHANS: THE CHARACTERISTICS OF COST-EFFECTIVE POLICY RESPONSES FOR HARMFUL ALGAL BLOOMS

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A growing concern for coastal management is the choice of appropriate public or private responses to HABs as a natural hazard. Considerable efforts have been devoted to understanding the scientific aspects of HABs, including their distributions in space and time, their ecological roles, and the nature of their toxic effects, among others. Much energy also has been directed at exploring socio-economic impacts and identifying potential management actions, including actions to prevent, control, or mitigate blooms. Using blooms of Florida red tide (*Karenia brevis*) as a case study, we develop an approach to the choice of policy responses to *K. brevis* blooms. Importantly, several new types of public health, environmental, and socio-economic impacts now are beginning to be revealed, including human gastro-intestinal and potential neurological illnesses; morbidities and mortalities of protected species, including manatees, cetaceans, and sea turtles; increased numbers of hospital emergency room visits for the elderly; increased respiratory morbidities in workers, such as beach lifeguards; and potential reduced K-12 school attendance. Optimal policy responses to this hazard are likely to depend critically upon why and where a bloom occurs, its spatial and temporal scales and toxicity, and the nature of its impacts. In the face of significant ongoing scientific uncertainties, and given estimates of impacts, we find that policies to expand and stabilize scientific research programs and environmental monitoring efforts, to develop and implement education programs for both residents and tourists, and to communicate the physical aspects of blooms to the public in a timely fashion are likely optimal.

THE RELATIONSHIP BETWEEN MICROCYSTIS CELL DENSITY AND MICROCYSTIN TOXIN: DETERMINING CELL DENSITY THRESHOLDS FOR PUBLIC HEALTH GUIDELINES

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In general specific algal toxin measurements and comparison to subsequent public health thresholds provide the most accurate means of determining public health risk. For example, cyanobacterial cell density can overestimate the risk of cyanotoxin poisoning if cyanobacteria are present but not producing toxin (toxin producing genotypes can vary seasonally), and they can also underestimate the risk of cyanotoxin poisoning because cyanotoxins may persist in the water after a cyanobacterial bloom has subsided and is no longer visible. However, the presence of cyanobacteria, whether visually as scums, or microscopically identified, can also be utilized as public health criteria. Using the long-term public health monitoring dataset for the Klamath River we developed a non-parametric probability-based relationship that allows critical *Microcystis* cell density levels that minimize false negative scenarios (low cell density but microcystin levels that exceed public health guidelines) to be determined. These relationships are based on computation of the percent exceedances of a particular level of microcystin concentration at a given cell density, and by evaluating the inflection point in the probability curve, protective cell density numbers can be determined. In general, *Microcystis* cell densities lower than those recommended by the World Health Organization and California State Water Resources Control Board are necessary if they are intended to be protective of public health by reducing the risk of exceeding critical toxin levels.

ASSESSMENT OF THE GULF OF MEXICO HARMFUL ALGAL BLOOM OPERATIONAL FORECAST SYSTEM: A COMPARATIVE ANALYSIS OF FORECAST SKILL AND UTILIZATION, 2004-2015

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Since 2004, NOAA has maintained the Gulf of Mexico Harmful Algal Bloom Operational Forecast System (HAB-OFS) to issue weekly bulletins that aid in early identification of *Karenia brevis* and assist in response efforts. Forecast quality and bulletin utilization are evaluated regularly based on user feedback and observations. Since 2012, the HAB-OFS has issued 200+ bulletins for Florida with greater than 90% product utilization. Of the 200+ bulletins issued since 2010 for Texas, product utilization has grown to over 90%. Performance as measured by relative accuracy and bias have been consistently high for forecasts of bloom transport, intensification, and the associated level of respiratory irritation on a scale from “none” to “high”. The performance of the forecasts for “high” levels of respiratory irritation is especially important for protecting health because at that level the general public may experience noticeable discomfort, which can be more severe for those with pre-existing pulmonary conditions. Preliminary results indicate that “high” level forecasts issued for Florida continue to perform well with greater than 60% improvement over chance. Forecasts issued for Texas performed similarly during the 2011-2012 bloom year with greater than 90% improvement over chance, but respiratory irritation has been more difficult to assess in Texas because confirmation relies on observations. Data gaps over the coverage region result in areas where respiratory forecasts cannot be adequately confirmed. All results have been compared to assessments from previous operational years (2004 to 2012) and are being used as guidance to improve future forecasting protocols for the HAB-OFS program.

GAMBIERDISCUS: MANAGING EXCESS CELLULAR CARBON

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Members of the dinoflagellate genus *Gambierdiscus* are known for producing toxins that accumulate in fish and upon consumption, can ultimately lead to the contraction of ciguatera fish poisoning (CFP) in humans. Although we know that the toxins produced by *Gambierdiscus* can harm humans, it remains unclear as to what environmental conditions promote these dinoflagellates to produce such compounds.

Gambierdiscus spp. as well as many other dinoflagellate species, have been known to produce and excrete large quantities of organic molecules including toxins and mucus. It has been suggested that production of such compounds may be a result of exposure to high irradiances and a way to dispose of excess carbon. *Gambierdiscus spp*. have been shown to reach maximum growth rates at rather low light intensities (≈ 45 – 200 µmol photons m^{-2} s^{-1}) compared to those in which they may be exposed to in the field. Additionally, several species of *Gambierdiscus* can maintain near maximum growth rates at irradiances of up to 700 µmol photons m^{-2} s^{-1}. Comparing the kinetics of primary production and growth rate could possibly shed light on the reasoning behind production of compounds such as toxin, mucus, and other forms of DOC. The goal of this study was to 1) Determine if the rate of primary production remains proportional to that of growth rate over a range of irradiances 2) Assess light dependency of DOC and mucus production rates.

METABOLISM OF PARALYTIC SHELLFISH TOXINS IN GEODUCK CLAMS (*PANOPEA GLOBOSA*) (Dall 1898) FROM THE NORTHWEST REGION OF GULF OF CALIFORNIA, MEXICO.

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The geoduck clam *Panopea globosa* is an important commercial species grown at the Gulf of California. The extraction of this species and other bivalves from the Northern region of the Gulf of California was banned in January of 2015 due to the accumulation of paralytic shellfish poisoning toxins (PSPt). A massive bloom of *Gymnodinium catenatum* was the cause for the accumulation of PSPt in bivalves. This bloom was also associated with a massive death of marine mammals, marine birds and was related to the first recognized human intoxications in the region. Water samples and clams were collected for the measurement of PSPt concentration and *G. catenatum* cell abundance during and in the following months after the bloom. Maximum toxicity in viscera of *P. globosa* measured by mouse bioassays and liquid chromatography with post-column oxidation (HPLC-PCOX) was as high as 53,300 µg STX eq kg^{-1}. In contrast, maximum toxicity recorded in the siphon was below, but close to the regularity action level. Toxin profile of highly toxic samples of *P. globosa* was dominated by C1 and C2 toxins, followed by GTX5, GTX2 and low to undetectable values for GTX1 and 4, dcSTX and STX in the different tissue analyzed. The relative to highly toxic analogs such as dcSTX and STX increased during the PSPt depuration process. This characteristic maintained toxicities in viscera above de regulatory limit for more than 5 months after the bloom of *G. catenatum*. Implications on the regulation and metabolism of PSPt in geoduck clams is discussed.

CHANS: *KARENIA BREVIS* AND ITS EFFECTS ON SCHOOL ABSENTEEISM (GRADES K-12th) IN SARASOTA COUNTY

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To investigate the change in school absenteeism in Sarasota County, *Karenia Brevis* (KB) count data and Sarasota County school data have been analyzed and evaluated. Using Geographic Information Systems (GIS), we have designed a map to project the location of Sarasota County schools and the span of KB counts with a minimum of 10,000 cell counts per liter and above. In regards to school absenteeism, we assume that brevetoxins could possibly have a greater affect on the schools on the coast of Florida rather than the schools inland, due to the proximity of KB counts. We assume that schools on the coast will have a higher absentee percentage rate than schools inland. We hypothesize that the changes in school absenteeism is due to periods with extremely high KB counts and blooms. Brevetoxins could be reason for students experiencing increased respiratory or gastrointestinal illnesses, causing an increase in absent rates. Because individuals may experience the effects of brevetoxins days after being exposed, we expect to see a bloom occurrence/school absentee lag effect. A linear regression and non-parametric statistical analysis will be conducted, through a methodical approach, to confirm the statistical validity of the correlation between *Karenia Brevis* and changes in school absenteeism.

THE ROLE OF PH IN FACILITATING RANGE EXPANSION IN *PRYMNESIUM PARVUM* INTO BAYS OF TEXAS

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Prymnesium parvum is a recent invader into Texas waters and migrates with riverflow from system to system. This adaptable species is often associated with brackish and coastal systems with a documented history along coasts worldwide, and currently poses a threat to Texas bay systems in the Gulf of Mexico. *P. parvum* often dominates phytoplankton assemblages through allelopathic interaction, forming dense blooms that reduce phytoplankton diversity and kill zooplankton and fish. Although allelopathic interaction is an important component in understanding community dynamics, it is only one factor structuring phytoplankton assemblages. pH is known to strongly effect the efficacy of toxins produced by *P. parvum*, in which toxins are more potent at higher pHs. In this research, we conducted a comparative bay experiment using bottles and carboys with natural plankton assemblages using Galveston Bay and Matagorda Bay waters manipulated to different pH levels. Four week-long field experiments were deployed during fall 2014 and winter 2015, with cell count, biovolume and chl-a analyses currently underway. Early results of time series data indicate a difference among treatments between phytoplankton diversity and *P. parvum* density. How *P. parvum* responds at varying pH levels may suggest its success in expanding into different bay systems in Texas.

REGIONAL AND GLOBAL PHYLOGENTIC RELATIONSHIPS OF *COCHLODINIUM* SPP. IN COASTAL WATERS OF THE EASTERN PACIFIC

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In the past 30 years, blooms of the dinoflagellate *Cochlodinium* have rapidly expanded worldwide and often result in massive fish kills and economic loss. Two species, *C. polykrikoides and C. fulvescens* are considered to be harmful algal bloom (HAB) formers. *C. fulvescens* was first described from cultured cells isolated and in Japan and was documented in Monterey Bay, California in 2004. For the next few years, it was seen along the California coastline during bloom and non-bloom events. A bloom in Monterey Bay in 2007 resulted in the first documented commercial shellfish loss from this organism. Large subunit ribosomal DNA (LSU rDNA) sequences of *C. fulvescens* along the eastern Pacific revealed two ribotypes, suggesting ballast water transport as a means for their widespread distribution. Since 2007, *C. fulvescens* has been seen less frequently and in lower numbers until 2013, when a red tide in Monterey Bay was a mix of *Ceratium* and *Cochlodinium*. In 2014, characteristically seasonal red tides did not occur in Monterey Bay, but *Cochlodinium* was the most abundant dinoflagellate observed in the water during the autumn. Analysis of LSU rDNA sequences from *C. fulvescens* from the most recent blooms in Monterey Bay along with sequences of *Cochlodinium* from the Mediterranean Sea will help elucidate regional and global relationships of this poorly studied species.

A LAKEWIDE METATRANSCRIPTOMIC SURVEY OF THE EUTROPHIC SHALLOW LAKE TAI (CHINA)

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Worldwide, toxic cyanobacterial harmful algal blooms (cHABs) plague freshwater systems. Lake Tai (Taihu), China's third largest freshwater lake, experiences annual bloom events often dominated by toxic *Microcystis*, and is considered a model system for cHAB dominated lakes. An increasing number of studies suggest that loading of both nitrogen (N) and phosphorus (P) to the system has contributed to the hyper-eutrophic status of the lake. However, little is known about the physiological processes that precipitate *Microcystis* dominance each year. To better understand the biological mechanisms underlying bloom dynamics across Taihu, a lake-wide metatranscriptomic survey was completed in the summer of 2013. Samples were collected from six distinct sites across the lake and total mRNA was sequenced on the Illumina platform. Not surprisingly, *Microcystis* was a dominant member of the community mRNA profiles, representing close to 80% of all sequences at some sites. These data further contribute to the hypothesis that both N and P inputs control cHAB dynamics, as *Microcystis* transcript profiles show active scavenging for N occurring at the time of sampling. The results of this survey contribute new insight into the complex interactions that lead to the dominance of toxic cyanobacteria in freshwater lakes.

OXIDATIVE TREATMENTS FOR THE DEGRADATION AND DETOXICIFICATION OF CYANOTOXINS, MICROCYSTINS AND CYLINDROSPERMOPSIN

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Microcystins (MCs) and Cylindrospermopsin (CYN) are potent cyanotoxins produced during harmful algal blooms (HABs). MCs and CYN pose serious threats to human health because of their presence in drinking water sources. Conventional water treatment methods are often not practical or ineffective for the treatment of these cyanotoxins in drinking water sources. We have explored the oxidative degradation of these problematic compounds under a range of environmental conditions employing a number of oxidative processes, including ultrasonic irradiation, UV and visible light activated photocatalysis, radiolysis, and ferrate initiated degradation processes. The degradation pathways of these structurally diverse MCs varies significantly depending on the oxidant, but all treatments employed result in the extensive and predominant oxidation of the non-polar ADDA side chain. The hydrophobic ADDA chain is critical to the biological activity of the family of > 80 MC variants and number of nodularins and their powerful protein phosphatase inhibiting ability. The biological activities of the treated solutions are readily reduced and parallel the concentrations of the starting cyanotoxin. The rapid decrease in biological activities as a function of treatment indicates the product mixtures do not possess significant biological activity. Oxidative treatment of CYN also results in the production of numerous by-products, however the predominant pathways involve oxidation and destruction of the uracil ring critical for biological activity. Extended treatment can completely eliminate the specific biological activities associated with the toxins. The potential application, advantages, and disadvantages of these oxidative processes will be included in the presentation.

A NOVEL APPROACH TO MEASURING THE COST OF TOXIN PRODUCTION IN THE MARINE DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE*

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The goal of this study is to understand, at the molecular level, the relationship between toxin production and growth in the dinoflagellate *Alexandrium fundyense*. This species always bears saxitoxin (STX), but a portion is constitutive (always present) and another is inducible (increased in the presence of grazers). Because resources allocated to toxin production are at the expense of cell growth, grazer-induced toxin production must entail a cost to the prey. To measure the fitness cost of STX production, we have conducted a relative quantification of gene expression using qPCR. Our work indicates: 1) There is a negative relationship between STX transcritptome and abundance of the gene related to cell growth in *A. fundyense*; 2) In the absence of grazers, this relationship represents the constitutive cost of toxin production; 3) STX transcript abundance in *A. fundyense* increases in the presence of grazers. The change of transcriptome abundance represents the real-time cost of induced toxin production. These results can help us gain insights into the co-evolutionary arms race between prey and grazer by understanding the constraints on anti-grazing defenses. The work will also produce a novel, fast, and cost-effective means of quantifying the potential for STX production in marine samples and will be useful for biological oceanographic research and harmful algal bloom monitoring.

EMERGENCE OF *ALEXANDRIUM* MONILATUM BLOOMS IN CHESAPEAKE BAY: ASSESSING SEDIMENT CYST DISTRIBUTION AND HEALTH IMPACTS ON ADULT OYSTERS (*CRASSOSTREA VIRGINICA*)

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Effective management of harmful algal blooms (HABs) within a region requires an understanding of species-specific HAB spatial and temporal distributions, bloom dynamics, as well as potential health impacts. In 2007, the southern Chesapeake Bay witnessed its first blooms of the HAB species *Alexandrium monilatum*. Since then, *A. monilatum* has bloomed in the region almost annually. *A. monilatum* produces the toxin 'goniodomin A' and is suspected in local mass mortalities of oyster larvae (*Crassostrea virginica*) grown for aquaculture and restoration projects. Representatives from Virginia's multimillion dollar oyster aquaculture industry recently expressed great concern over *A. monilatum* impacts to their businesses; field and lab studies were designed to address these concerns. Sediment samples were collected from the southwest portion of the Chesapeake Bay in a systematic grid-sampling design to assess cyst (resting cell stage) distributions. Cysts were present in low densities at most sites, and cyst densities were high where blooms had been recorded in previous years. HAB toxicity bioassay methods developed at the Virginia Institute of Marine Science were modified to investigate adverse health impacts of five different *A. monilatum* cell density treatments on sub-adult oysters (~40-70 mm). Oysters delayed grazing when exposed to high densities (>1000 cells/mL) of *A. monilatum*. Data from the 2015 bloom season and additional bioassays will be presented. Results from these studies could aid in the prediction of *A. monilatum* bloom severity and health effects on wild and aquacultured oysters, enabling development of best management practices to minimize impacts to the Virginia oyster industry.

MONITORING, MITIGATION AND MANAGEMNET OF HARMFUL ALGAL BLOOMS IN COASTAL WATERS OF ABU DHABI EMIRATE.

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Harmful algal blooms (HAB) in the Arabian Gulf pose a compelling and growing threat to the region's coastal resources, economy, and public health. Abu Dhabi coastal waters are witnessing a steady rise in harmful algal blooms, peaking most recently at 28 recorded incidents in 2012. HAB studies initiated in 2002 by Environment Agency Abu Dhabi (EAD) indicate that heavy nutrient loads in the marine environment resulting from rapid economic development on the coast contribute to the development of harmful algal blooms. Management of harmful algal blooms in the Abu Dhabi coastal environment is all the more challenging due to the prevailing extreme environmental conditions, notably high temperature and salinity, as well as frequent sand storms.

EAD has spearheaded the development and implementation of a variety of approaches to prevention, control and mitigation of HABs. The present paper presents details on these measures to protect the environment and public health, including nutrient reduction; deployment of a HAB early warning network; pilot testing of a proprietary HAB removal system; HAB cyst distribution studies; formation of an Emirate marine water quality task force; enhancement of marine water quality monitoring; development of action plans to minimize discharges to the marine environment; improving water circulation; enhancing marine emergency response capability; and regulation of marine water quality through development of water quality standards, rules on dredging and disposal of dredging materials, and permitting of marine discharges.

DETECTING HARMFUL DINOFLAGELLATES IN THE MARYLAND COASTAL BAYS FILTER FEEDERS

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Harmful algae blooms can be a reoccurring problem in estuarine and coastal bay environments. *Karlodinium veneficum* and *Dinophysis* sp., are major contributors to toxic blooms all over the world and recent evidence has shown that toxic dinoflagellate abundances are increasing in the Maryland coastal bays (MCBs). Monitoring and detecting their presences can provide insight on the overall community structure and succession patterns of these toxic dinoflagellates. Of particular interest to us is the potential for these blooms to serve as prey for resident filter filters, and the overall impact on ecosystem health. Using PCR-based cloning methodologies, we are exploring the dinoflagellate community residing inside clams and mussels, and comparing these to environmental water samples. *Dinophysis* sp. was detected in both clams and mussels stomach content in July 2014. *Dinophysis* sp. has also been identified in our environmental samples along the MCBs in 2014 and 2015. *Karlodinium veneficum* is routinely sampled by the MD-DNR, and in regions where *K. venificum* and *Dinophysis* sp. is present, we will be performing ecosystem impact assessments. The MCBs are one of the most diverse estuaries on the east coast providing habitat for juvenile fish, osprey, black sea bass, and terrapins. Therefore it is imperative to monitor and identify management strategies to prevent the formation of toxic dinoflagellate blooms.

EFFECTS OF DISSOLVED ORGANIC MATTER (DOM) PRODUCED BY BIVALVES ON *IN SITU GROWTH* RATES OF *LINGULODINIUM POLYEDRUM*

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Harmful Algae Blooms (HABs) are events in which a phytoplankton population exhibits a massive growth that causes negative effects in aquatic ecosystems and/or society. In the last decades an increment tendency has been observed in the intensity, amplitude and frequency of these events. The combination of phototrophy and heterotrophy for the nutrition of an organism is defined as mixotrophy, and is an essential strategy for the initiation and maintenance of some FANs. Aquaculture generates important loads of organic matter with the potential to trigger HABs. We are currently involved in a project relating growth rate of the dinoflagellate *Lingulodinium polyedrum* to dissolved organic matter (DOM) produced by filter feeding bivalves and humic acids. We relate the culture kinetics of dinoflagellate, bacteria and virus like particle abundance with DOM concentrations. We use *L. polyedrum* as a model organism but hope to generalize our results to other mixotrophic dinoflagellates.

DIVERSITY AND TOXICITY OF THE GENUS *PSEUDO-NITZSCHIA* IN OAXACA COASTAL WATERS, MEXICO

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Toxic blooms of the diatom genus *Pseudo-nitzschia* (Pn) are widely recognized, as in other types of toxic algae blooms, Pn outbreaks are becoming more frequent, posing an increasing threat to wildlife, human health and seafood safety. The environmental conditions in Oaxaca coastal waters are consistent with those associated with bloom formation (upwelling events and pulses of nutrients inputs). In this study, various strains of *Pseudo-nitzschia* were isolated by micropipetting single cells or chains of cells, from coastal waters samples collected from Oaxaca, México. To gain further knowledge of the composition and toxicity of species in this region, the isolates were identified by transmission electron microscopy (TEM) and for molecular date of the internal transcript spacer region (ITS). The isolates were cultured in L1 medium at 24 ºC with irradiance levels of 100 mmol quanta seg-1 m-2 provided in a 12:12 light:dark cycle. In the stationary phase of growth, samples of cells were taken to analyze domoic acid content using HPLC with UV-Vis detection. Domoic acid content was observed in three of the seven strains found, in concentration of 3.46 to 475 fg DA cell-1. This is the first evidence of potentially toxigenic *Pseudo-nitzschia* species in the region.

***GAMBIERDISCUS* MIGRATION AND HABITAT COLONIZATION: ROLE OF SUBSTRATE PREFERENCE AND PREVALENCE IN THE WATER COLUMN**

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Gambierdiscus spp. are epibenthic dinoflagellate genera linked to ciguatera fish poisoning, the most common seafood-borne illness associated with toxic algae. Ciguatera largely affects communities in tropical and subtropical latitudes; however, the recent discovery of *Gambierdiscus* spp. in temperate waters suggests that their geographic range may be expanding. Migration of *Gambierdiscus* cells from the benthos to floating substrates can facilitate transport to new habitats, but there is a paucity of data regarding these migratory processes, including their prevalence in the water column and the role of substrate preferences. To investigate these questions, we carried out field sampling to quantify *Gambierdiscus* abundance on floating substrates and in the water column, and examined habitat colonization and substrate preferences through a series of laboratory experiments. Using test tubes with various suspended substrates, we characterized *Gambierdiscus* migration behavior, and compared substrate carrying capacities (plastic, screening material, and macroalgae) and density driven responses in the absence of passive transport variables (wind, waves, currents). *Gambierdiscus* spp. were also enumerated via fluorescent microscopy from floating substrates and water column samples collected during a Sea Education Association trans-Atlantic cruise on the SSV Corwith Cramer from the Canary Islands to St. Croix, USVI. These samples complimented our laboratory results and assessed the prevalence of *Gambierdiscus* in the water column at varying distances from the benthos, and on floating substrates. Our results provide a more comprehensive understanding of *Gambierdiscus* ecology and the potential for transport of Ciguatera-related, epibenthic dinoflagellates to new, favorable habitats.

APPLICATION OF ISSR PCR TO ASSESS INTRASPECIES GENETIC DIVERSITY IN LOCAL POPULATIONS OF *PSEUDO-NITZSCHIA* AND RELEVANCE TO TOXIGENICITY PATTERNS

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Domoic acid (DA) production can vary by orders of magnitude not only between *Pseudo-nitzschia* species but also among intraspecific isolates grown in common garden conditions. This suggests potential for cryptic intraspecific genetic variation not revealed by traditional markers used for molecular taxonomy (eg. SSU-, LSU-rDNA and ITS). Finer resolution genetic markers enabling broader genome scanning are needed to resolve this issue. Multilocus microsatellite analysis has identified significant intra-species variation in several *Pseudo-nitzschia* species. In contrast, Inter Simple Sequence Repeat (ISSR) assays, which amplify regions between known microsatellite repeat motifs, offer the advantage of broader genome coverage needed to reveal cryptic diversity. ISSR has been applied to develop species-specific markers for several harmful algal species, while their application to analysis of intraspecific variation in phytoplankton has been limited. Here we describe development of ISSR for assessment of population structure of the low/non toxic *P. fraudulenta* which was the dominant *Pseudo-nitzschia* species in Monterey Bay during the September 2013 CaHotSpots field surveys and *P. pungens* which was a major toxin producer preceding the May 2015 toxigenic bloom in Monterey Bay. We assess variation in ISSR length profiles with respect to spatio-temporal distributions of *Pseudo-nitzschia* isolates as well as their expression of DA toxicity in common garden growth conditions. We will also discuss use of this low cost method as a training module to provide students with hands on exposure to critical molecular biology skills.

PROPOSED ROLE OF METALS IN GONIODOMIN A BIOACTIVITY

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Alexandrium monilatum is a common HAB species that historically blooms along the US southern Atlantic and Gulf coasts, with recent expansion into the mid-Atlantic region. The primary toxin of *A. monilatum* is the lipophilic polyketide, goniodomin A (GDA). Extracts from cultured *A. monilatum* cells cause toxicity in a variety of eukaryotes, including mammals, teleosts, marine invertebrates, yeasts and fungi. The toxicity of GDA to eukaryotes has been ascribed to its interaction with actin and subsequent stabilization of the filamentous form (F-actin) relative to the monomeric G-actin, although the mechanism has only received minimal study. F-actin plays essential roles in cell migration and cytokinesis in animals, and in cell division, cell elongation, and organelle movement in plants. At physiological pH, each monomeric unit of actin has seven negative charges (isoelectric point 4.8), but GDA is a neutral species, leaving questions as to its mechanism of interaction with actin. Based on GDA's structural similarity to crown ethers, which readily bind metal ions to form inclusion complexes, we hypothesized that GDA forms metal ion complexes bearing a positive net charge. We utilized electrospray mass spectrometry to investigate the binding efficiency of physiologically relevant alkali metal ions, Na^+ and K^+, to GDA. Both metal ions bound well; the relative affinity of K^+ was significantly greater than Na^+. We propose that the positive charge causes electrostatic stabilization of GDA complexes with F-actin and changes the equilibrium position between G and F-actin to favor the latter, thereby disrupting crucial physiological processes.

PHYTOPLANKTON AS PRODUCER OF RETINOID–LIKE COMPOUNDS

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Water blooms dominated by cyanobacteria (photosynthesizing gram-negative bacteria), growing excessively in aquatic reservoirs have adverse impact on the quality of water and life of many aquatic and terrestrial organisms including humans. They can cause serious environmental and health problems in many surface waters. It is well documented that cyanobacteria produce wide range of metabolites - biologically active compounds. Our recent studies indicate, that one group of compounds production by water blooms have retinoid-like activity. The presence of bioactive compounds with retinoid-like activity was detectable in laboratory cultivated phytoplankton species including cyanobacteria (*Microcystis aeruginosa, Cylindrospermopsis raciborskii, Aphanizomenon gracile, Limnothrix redekei*) and algae (*Desmodesmus quadricauda*) and others. We have studied both extracts of biomass and extracellularly produced metabolites (exudates) of these species in repeated cultivations. Combination of *in vitro* bioassay with transgenic cell line and chemical analyses (HPLC – MS/MS) were used to characterize production of two potent retinoic acids (RAs) and total retinoid-like activity by selected phytoplankton species. The methods of sample processing were optimized for maximal recovery of retinoid-like activity. *In vitro* tests showed pronounced retinoid-like activity of all studied extracts of biomasses across species, while only exudates of cyanobacteria exhibited detectable activity. The two studied RAs (ATRA and 9 cis-RA) investigated by chemical analyses were detected more frequently in extracts than in exudates. Nevertheless, their contribution to observed *in vitro* effects seems to be relatively low for all tested samples. The work was supported by the Czech Science Foundation grant No. 14-29370P.

WADEABLE STREAMS AS WIDESPREAD SOURCES OF BENTHIC CYANOTOXINS IN CALIFORNIA

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Large rivers and lentic water bodies have long been recognized as being susceptible to toxin-producing cyanobacterial blooms, but little is known on cyanotoxin (e.g., microcystin) production in benthic cyanobacteria commonly inhabiting wadeable streams. "Probability"-based surveys of >1,200 wadeable stream reaches were conducted throughout California during the spring and summer of 2007-2013 as part of the Surface Water Ambient Monitoring Program of the State Water Control Board. 16 genera and 8 species of cyanobacteria, capable of producing microcystins according to the literature, were recorded frequently in studied streams. Cyanotoxins were detected in one-third of the tested stream reached (n=368), based primarily on one-time sampling from 2011 to 2013. Stream reaches where microcystins were present spanned a variety of surrounding land-use types, from open space to heavily urbanized/agricultural. In sites with detected microcystins (n=95), the potential microcystin-producing cyanobacteria species were: *Tolypothrix distorta* (17% of the sites), *Geitlerinema splendidum* (3%), *Nostoc carneum*, and *Dolichospermum flosaquae* (2% each), and *Hapalosiphon hibernicus* (1%), present in nearly a quarter of the sites (n=22). Other potentially microcystin-producing genera, such as *Anabaena, Cylindrospermum, Leptolyngbya, Microcystis, Oscillatoria, Phormidium, Rivularia*, and *Trichormus* were common. Lyngbyatoxin, saxitoxins, and anatoxin-a, toxins potentially produced by *Cylindrospermum stagnale*, *Geitlerinema amphibium*, *Lyngbya majuscula*, *Phormidium autumnale* and *Scytonema crispum*, were also detected at lower rates, at subsets of reaches (n=14 out of 99). Our results strongly suggests wadeable streams could be significant sources of cyanotoxin inputs to receiving waters, a finding with implications for water management within wadeable streams, downstream large rivers and lentic water bodies.

INTEGRATION OF AUTHENTIC MONITORING FOR HAB SPECIES WITHIN AN UNDERGRADUATE LABORATORY COURSE

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For decades, toxic harmful algal bloom (HAB) events have plagued the Chesapeake Bay Watershed. While a large network of both scientific and citizen-based monitoring groups exists across the watershed, current efforts are primarily focused on measuring environmental indicators such as nutrient concentrations, rather than screening for the presence of HAB species. To expand upon these efforts, students at James Madison University in Harrisonburg, VA completed a series of PCR-based assays to screen water samples for the presence of toxic cyanobacterial and algal bloom forming organisms. This endeavor was a component of a discovery-based learning strategy that was effectively employed to enhance student learning and engagement. In the Environmental Microbiology laboratory course, this strategy was used in a semester-long learning experience which involved collection and analysis of samples collected from both freshwater tributaries and the northern neck region of the Chesapeake Bay. The preliminary data collected by students indicate the presence of toxic cyanobacterial species in the Shenandoah River Watershed, the largest tributary of the Potomac River. Throughout the duration of the course, students were able to use legitimate molecular tools in microbial ecology to generate such data, which can be useful for the scientific community at large.

EAVESDROPPING SEAWEEDS: REACTIVE OXYGEN SPECIES (ROS) INDUCE ANTIOXIDANT AND CHEMICAL DEFENSE PRODUCTION IN THE BLOOM-FORMING ULVOID ALGA *ULVA LACTUCA*

Kathryn L. Van Alstyne, Sue-Ann Gifford, Lauren Sutton, Whitney Fleming

When exposed to environmental conditions that cause oxidative stress, such as desiccation, reduced salinities, and herbivory, the bloom-forming green seaweed *Ulva lactuca* releases ROS into the surrounding seawater. We conducted experiments to determine whether neighboring plants respond to dissolved ROS by increasing concentrations of DMSP, a natural product that functions as a precursor to antioxidants and antiherbivore defenses. To do this, we exposed pieces of algae to 0, 5, 20, or 80 µM hydrogen peroxide (H_2O_2). Two types of exposures were conducted: a single 3-hour long exposure and daily exposures for a week. Algae were harvested after 3 and 7 days and growth and DMSP concentrations were measured. Twenty and 80 µM H_2O_2 caused a significant decrease in DMSP after 3 days in the plants exposed to H_2O_2 every day, but there were no significant change in DMSP as a result of a single 3-hour exposure. After 7 days, DMSP significantly increased in all plants exposed to H_2O_2 on a daily basis and in plants experiencing a single exposure of 20 and 80 µM H_2O_2. Our results demonstrate that *Ulva* "eavesdrops" by detecting chemicals released by neighbors in response to physiological stresses and herbivory. It then increases concentrations of a natural product that will make the algae better able to respond to these types of stresses. The ability of *Ulva* to detect and respond to stresses experienced by neighboring plants may contribute to its ability to rapidly proliferate in intertidal areas where herbivores and physiological stresses are common.

CYANOBACTERIA BLOOMS IN MARYLAND: A RE-EMERGING THREAT

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Blooms of cyanobacteria have a long history in MD. In the 1930s *Microcystis* blooms on the Potomac were extensive and one of the first places to document human illness. Between the 1930s and 1970s, noxious cyanobacteria blooms increased on the Potomac River and northern Bay waters as nutrient loading increased and submerged aquatic vegetation populations declined. As nutrient levels decreased in the Potomac, blooms were less common until a decade ago when blooms began to reoccur and toxin analyses confirmed harmful levels. Current monitoring programs have detected numerous potentially toxin producing species from the freshwater to the marine in MD including: *Anabaena, Aphanocapsa, Aphanizomenon, Cyanobium, Cylindrospermopsis, Lyngbya, Microcystis aeruginosa, Oscillatoria/ Planktothrix isothrix, Planktothrix rubescens, Pseudoanabaena and Synechococcus*. Toxins that have been detected include microcystin, anatoxin, and saxitoxin. Of these, microcystin is the most commonly occurring algal toxin. Microcystin levels during blooms (>40,000 cells/ml) have ranged from below 10ppb (threshold used in MD for contact advisories) to >20,000 ppb. Dammed lakes and ponds are common bloom areas with high nutrients and slow moving water; however, several tidal tributaries also have reoccurring blooms. Bloom impacts include no contact advisories as well as dead birds, muskrats and dogs. "No contact advisories" placed on waterbodies have resulted in hundreds of days of limited use per year (no swimming, boating etc). Mitigation practices have included nutrient reduction strategies, dredging, potassium permanganate (in contained waterbody), barley straw, temporary draining of dammed lakes in winter, flocculation and hydrogen perioxide treatment. Downstream impacts have not been studied yet.

HARMFUL ALGAL BLOOMS IN MARYLAND'S COASTAL BAYS

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In 2015 the Maryland Department of Natural Resources and the University of Maryland Eastern Shore started an intensive phytoplankton monitoring program to examine the harmful algal bloom community in Maryland's coastal bays. Weekly sampling throughout the Assawoman Bay watershed began in March. Initiation of phytoplankton blooms began in late March with a bloom of a *Gymnodinium* species. Since the *Gymnodinium* bloom, there has been a cascade of bloom-forming species, including *Dinophysis* cf. *acuminata*, *Karlodinium veneficum*, *Prorocentrum minimum*, and *Alexandrium* sp. These blooms have occurred throughout the Assawoman Bay watershed but are associated mainly with the St. Martin River. Microscopy and DNA cloning methods are being used to identify the *Gymnodinium* sp. and *Alexandrium* sp. The phytoplankton community composition, including the succession of harmful algal bloom species, and potential environmental impacts will be examined. Possible explanations for the reported increase in the concentration and species diversity of HABs in the Maryland coastal bays since 2013 will also be discussed.

REACTIVE OXYGEN SPECIES AND DOMOIC ACID: ROLE OF UNBALANCED GROWTH AND LIGHT ENERGY IN TOXIN PRODUCTION BY PSEUDO-NITZSCHIA AUSTRALIS

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The contribution of photosynthetically derived energy allocation under induced stress conditions to DA production was studied in *Pseudo nitzschia australis*. The herbicide Methyl Viologen (MV), aka paraquat, which disrupts NAD(P)H production at photosystem I, was used to experimentally enhance cellular reactive oxygen species (ROS) production and limit growth of this diatom. Parameters measured include cellular ROS production, anti-oxidant capacity, DA production, cell growth and photosynthetic efficiency (via PAM). MV treated cultures were also amended with antioxidants. It was found that ROS quenching by addition of exogenous antioxidant was sufficient to inhibit toxin production, if not alleviate cell growth repression. Growth and toxin production by *P. australis* under nutrient replete, low irradiance (≤ 75 µmole photon m-2 s^{-1}) and high irradiance stress (≥400 µ umole photon m^{-2} s^{-1}) is being investigated to evaluated role of light stress induced ROS production.

These findings suggest that DA production, itself a complementary amino acid, is spurred by increased photon pressure and its consequences inside the cell. A decreased growth rate alone is not sufficient to prompt DA production levels characteristic of a given strain, instead, a shift in cellular DA quotient is induced as a generalized result to unbalanced growth under irrdiance stress. A biochemical model will be presented integrating these observations. This insight into *Pseudo nitzschia* cell physiology will help inform field observations on the variability of toxigenic *Pseudo-nitzschia* blooms.

Allelopathic Inhibition of Cyanobacteria by Barley Straw – mechanism and application

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Algae blooms, in particular cyanobacterial blooms as a result of eutrophication have been a worldwide phenomenon. Seeking for an environmental-friendly, low-cost and high-efficient emergency control method for cyanobacteria blooms is one of the key issues in the field of Environmental Science and Engineering. The allelopathic inhibition of algae by plants has showed enormous application potential in eutrophication and algae bloom control. Among them, the use of barley straw is by far the most successful application example. In this research, comparing to the widely reported validity of algal inhibition effects by occidental type of barley, effects and action modes of their oriental relative -- Tibetan barley were studied; algicidal effects of eight different kinds of barley straw decomposing prepared by different procedures were systematically compared, and by the use of non-linear mathematical model the general laws of this allelopathy were discussed; a estimation of the potential algicidal effects of using oriental barley straw in typical eutrophic Chinese waterbody was also performed. The natural chemicals in barley straw were screened, and the key allelochemical which effectively inhibits cyanobacteria growth was isolated and identified by multiple chemistry analytical tools, and its mechanism was also studied on the single cell level. The observation in this work will provide a theoretical gist and technology guide for the use of barley straw methods in algal control filed; meanwhile this work could also contribute to the recycle and reuse of agricultural wastes like straws.

CRITICAL NUTRIENT THRESHOLDS NEEDED TO CONTROL HARMFUL CYANOBACTERIAL BLOOMS IN EUTROPHIC LAKE TAIHU, CHINA

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Nutrient over-enrichment has led to dramatic increases in harmful cyanobacterial blooms, creating serious threats to drinking water supplies, ecological and economic sustainability of freshwater ecosystems. Nutrient-cyanobacterial bloom interactions were examined in eutrophic Lake Taihu, China. In situ microcosm nutrient dilution bioassays and mesocosm nutrient addition experiments were conducted to determine nitrogen (N) and phosphorus (P) concentration and load thresholds needed to control cyanobacterial bloom formation. Blooms were dominated by toxic, non-N_2 fixing *Microcystis* spp, from May to December. Dilution bioassays showed seasonality in nutrient limitation, with P-availability controlling pre-bloom spring conditions and N-availability controlling summer-fall blooms. Nutrient dilution and enrichment bioassays indicated that total nitrogen (TN) and total phosphorus (TP) concentration thresholds should be targeted at below 0.80 mg L^{-1} and 0.05 mg L^{-1}, respectively, to limit intrinsic growth rates of *Microcystis* dominated blooms. Management-wise, in-lake TN and TP concentrations in Taihu should be targeted to the thresholds of 1.26 mg L^{-1} and 0.082 mg L^{-1} respectively to bring Taihu's phytoplankton biomass to "acceptable" sub-bloom conditions of less than 20 µg L^{-1} chlorophyll *a*. Based on estimates of nutrient loading and observed stoichiometry of phytoplankton biomass, 61−71% TN and 20−46% TP reductions will be necessary for bloom control. These percentages will decrease over time as external and internal nutrient loads decrease following nutrient input restrictions.

THE ROLE OF IRON IN ALGAL-BACTERIAL INTERACTIONS AS RELATED TO HARMFUL ALGAL BLOOMS

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Evidence is increasing for a mutualistic relationship between bloom forming algae and some marine bacteria. However, how such algae-bacteria interactions might contribute to harmful algal blooms (HAB) has not been thoroughly investigated. In the phycosphere, the nutrient rich zone around phytoplankton where microbial activity is altered, bacterial-algal interactions are likely to be influenced by available nutrients. We hypothesize that bacteria may affect algal growth and bloom dynamics by their possible control of iron a trace element known to growth limiting to phytoplankton.

Iron is an essential nutrient for all living organisms, but it is extremely insoluble in the oceanic environment which restricts its bioavailability. To counteract this bacteria have evolved a group of high affinity iron chelating agents called siderophores but algae are not known to produce siderophores nor are they in general able to utilize them as a source of iron. However some bacterial siderophores can be degraded by light to produce soluble forms of Fe(II) and Fe(III) that could be utilized by mutualistic algal partners. Using quantitative RT-PCR, we detected the appropriate siderophore biosynthesis genes to estimate the quantity of photoactive siderophores in the marine water samples obtained from several research cruises and from Scripps pier during a bloom of the dinoflagellate *Lingulodinium polyedrum*. Our results present a strong case for the involvement of photoactive siderophores in potentially affecting iron speciation and hence its bioavailability to phytoplankton. Such data may aid us in gauging the importance of siderophore producers to bloom dynamics.

THE ROLE OF TROPICAL CYCLONES IN STIMULATING CYANOBACTERIAL (*MICROCYSTIS* SPP.) BLOOMS IN HYPERTROPHIC LAKE TAIHU, CHINA

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Weather-related disturbances, such as wind-generated waves, major rainfall events and large temperature shifts associated with frontal passages, are important drivers of ecological processes in shallow lakes. The influence of several Pacific tropical cyclones (typhoons) on cyanobacterial blooms in China's third largest lake, Taihu, was examined continuously, using an on-lake high-frequency recording platform, coupled to satellite-based remote sensing data. Short-term (on the order of hours) nutrient pulsing resulting from the passage of typhoons played a key role in bloom initiation and maintenance. Decreasing wind speeds and increasing air and water temperatures in the aftermath of cyclones were accompanied by increases in phytoplankton biomass. The synergistic effects of nutrient pulsing, elevated water temperatures and increased water column stratification after the passage of the cyclones stimulated blooms of the toxic cyanobacteria *Microcystis* spp. There were short-term successions of blooms following typhoons, and as blooms "crashed" they provided nutrient inocula for future blooms. Trends determined from historical in situ data indicated higher frequencies and intensities of blooms in "cyclone years". Typhoons are an important driver of biogeochemical and water quality perturbations at the ecosystem-level in this hypertrophic lake. These events play a key role in our ability to forecast blooms over both short (days) and longer-term (weeks) periods.

THE SPRING CYANOBACTERIAL BLOOM AND ITS INFLUENCE ON DRINKING WATER SUPPLY IN TAIHU, CHINA

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Lake Taihu, the third largest freshwater lake in China, has gained notoriety for its recurring and proliferating toxic cyanobacterial (*Microcystis* spp.) blooms. Because Taihu is the drinking water resource for more than 6 million people, the blooms influence the quality of water supply, especially in summer. Until recently, there have been few reports of spring bloom events influencing drinking water supplies. In March and April 2012, an unpleasant smell was reported in a drinking water plant of Suzhou, a major city (5+ million people) located near Taihu. We found that it was *Aphanizomenon*, not *Microcystis* that produced odor compounds in raw water. Excessive nutrient inputs stimulated *Aphanizomenon* growth in spring. Furthermore, suitable wind direction drove the odorous water from highly eutrophic region of the lake to the water intake site. The result suggested that the water quality can become quite unstable under eutrophic shallow lakes which are strongly influenced by water circulation patterns. Raw water treatment operations must adapt to expanding cyanobacterial blooms that can be rapidly transported to water intake sites.

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