3rd Symposium on Harmful Algae in the U.S.

Symposium Director:

Chris Scholin

Symposium Coordinators:

Judy Kleindinst, Annette Gough, Mary Arnold, Jeannette Fink

Steering Committee:

Greg Boyer	State University New York – Environmental Science and Forestry
Quay Dortch	NOAA, National Ocean Service, Silver Spring
Greg Doucette	Marine Biotoxins Program, NOAA/National Ocean Service
Pat Glibert	Horn Point Laboratory
Cindy Heil	MYFWC
Raphe Kudela	Ocean Sciences Department, University of California, Santa Cruz
Kevin Sellner	Chesapeake Research Consortium
Marc Suddleson	NOAA Ocean Service/CSCOR
Vera Trainer	NWFSC
Tracy Villareal	University of Texas at Austin

Session Chairs:

Bloom Ecology	Kevin Sellner, Raphe Kudela, Quay Dortch
Toxins:	Greg Boyer, Greg Doucette
Foodwebs:	Cindy Heil, Vera Trainer
Public Health:	Tracy Villareal, Pat Glibert
Outreach/Infrastructure:	Marc Suddleson, Chris Scholin

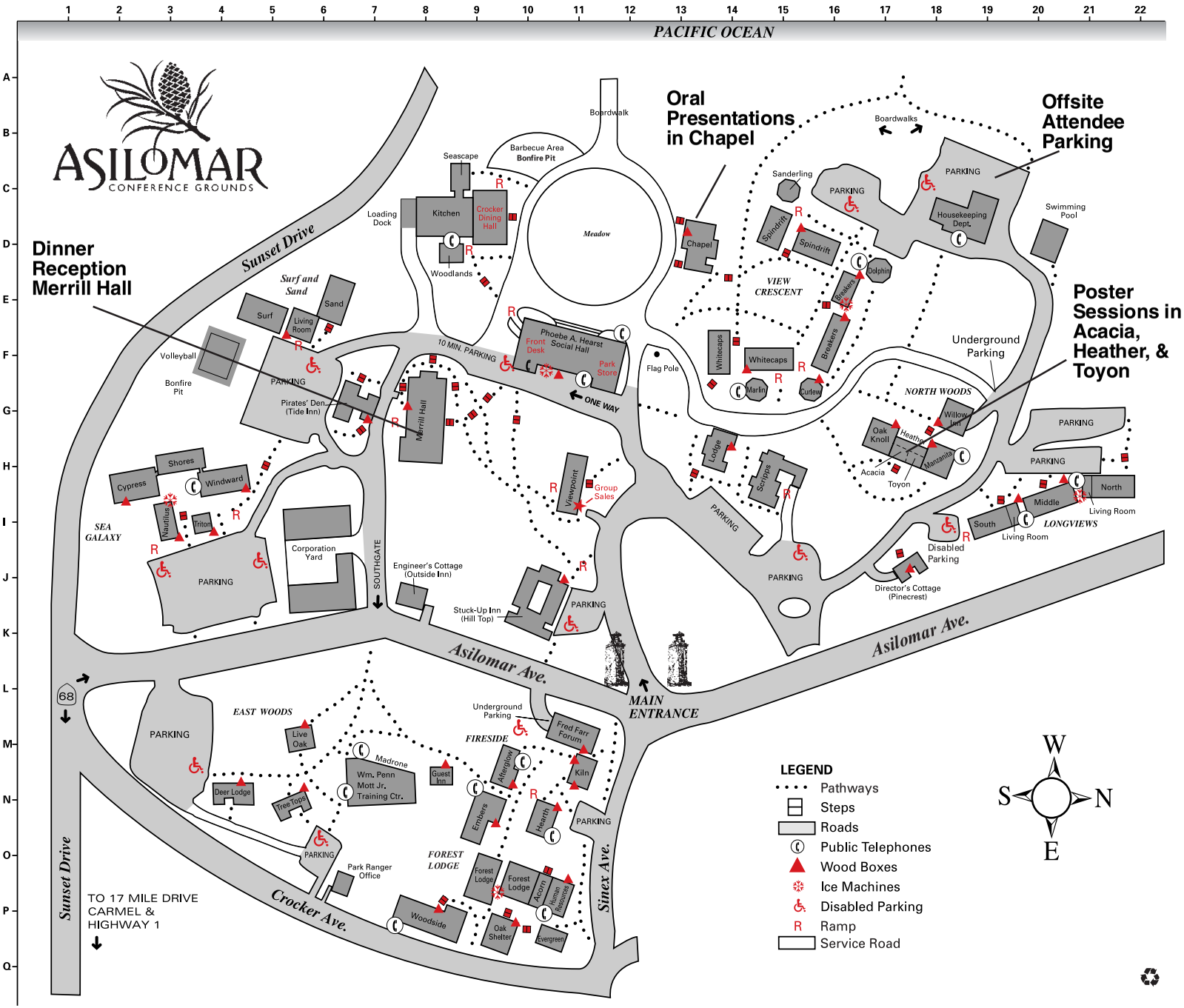
Sponsors:

Monterey Bay Aquarium Research Institute
NOAA/Center for Sponsored Coastal Ocean Research/Coastal Ocean Program
U.S. National Office for Marine Biotoxins and Harmful Algal Blooms

Student support:

NOAA/Center for Sponsored Coastal Ocean Research/Coastal Ocean Program
West Coast Center in Oceans and Human Health
Center of Excellence for Oceans and Human Health at the Hollings Marine Laboratory

Front Cover: A 3-D view of a phytoplankton layer (chlorophyll fluorescence) dispersed along the crest and concentrated in the trough of an internal wave (light blue isopycnal), observed at high resolution using an AUV (Ryan et al. 2005, Mar. Ecol. Prog. Ser. 287:23-32). The layer of phytoplankton contained Pseudo-nitzschia *australis, a toxigenic diatom linked to illness and mortality of marine wildlife (Scholin et al. 2000, Nature 403: 80-84). Source populations of organisms that ultimately give rise to HABs in coastal areas may occur offshore and be subsurface, sometimes in thin layers, and therefore are often difficult to detect using traditional ship surveys and even remote sensing. These blooms can be delivered to near shore areas by physical forcing, resulting in sudden increases in toxicity that are unrelated to local growth.*


ASILOMAR
CONFERENCE GROUNDS
PACIFIC OCEAN
Dinner Reception Merrill Hall
Oral Presentations in Chapel
Offsite Attendee Parking
Poster Sessions in Acacia, Heather, & Toyon
Sunset Drive
Surf and Sand
Surf
Sand
Living Room
Volleyball
Bonfire Pit
PARKING
Pirates' Den (Tide Inn)
Merrill Hall
Boardwalk
Seascape
Barbecue Area
Bonfire Pit
Loading Dock
Kitchen
Crocker Dining Hall
Woodlands
Meadow
Chapel
10 MIN. PARKING
Phoebe A. Hearst Social Hall
Front Desk
Park Store
ONE WAY
Flag Pole
Boardwalks
Sanderling
Spindrift
Housekeeping Dept.
Swimming Pool
VIEW CRESCENT
Dolphin
Breakers
Whitecaps
Marlin
Curlew
Underground Parking
NORTH WOODS
Willow
Oak Knoll
Heather
Manzanita
Acacia
Toyon
Lodge
Scripps
Shores
Cypress
Windward
Nautilus
Triton
SEA GALAXY
Corporation Yard
SOUTHGATE
Engineer's Cottage (Outside Inn)
Viewpoint
Group Sales
Stuck-Up Inn (Hill Top)
Disabled Parking
Director's Cottage (Pinecrest)
North
Middle
South
Living Room
LONGVIEWS
Asilomar Ave.
MAIN ENTRANCE
68
EAST WOODS
Live Oak
Madrone
Wm. Penn Mott Jr. Training Ctr.
Guest Inn
Deer Lodge
Tree Tops
Park Ranger Office
FIRESIDE
Underground Parking
Fred Farr Forum
Alterglow
Kiln
Embers
Hearth
FOREST LODGE
Forest Lodge
Acorn
Human Resources
Woodside
Oak Shelter
Evergreen
Sinex Ave.
Crocker Ave.
TO 17 MILE DRIVE
CARMEL &
HIGHWAY 1
LEGEND
Pathways
Steps
Roads
Public Telephones
Wood Boxes
Ice Machines
Disabled Parking
R Ramp
Service Road
W
S
N
E

Pre-registration on Sunday, October 2 from 3:00pm - 5:00pm at Asilomar, Acacia room. Refreshments will be served.

Schedule	Mon., Oct. 3	Tues., Oct. 4	Wed., Oct. 5	Thu., Oct. 6
7:30am-8:30am	Breakfast	Breakfast	Breakfast	Breakfast
8:30 am (First day only)	Welcome and Introduction			
8:40 am-10:00 am	Session 1 HAB plan overview Outreach and Infrastructure	Session 5 Toxins	Session 9 Bloom Ecology	Session 13 Bloom Ecology
Break 10:00 am - 10:30 am				
10:30am-11:50am	Session 2 Outreach and Instrastructure	Session 6 Toxins	Session 10 Bloom Ecology	Session 14 Bloom Ecology
Lunch 12:00pm - 1:30pm				
1:30pm - 2:30pm	Session 3 Outreach and Infrastructure	Session 7 Food Webs	Session 11 Bloom Ecology	Session 15 Bloom Ecology
Break 2:30pm - 3:00pm				
3:00pm - 4:00pm	Session 4 Toxins	Session 8 Public Health	Session 12 Bloom Ecology	Session 16 Bloom Ecology Business Mtg Site selection for next meeting
4:00pm-6:00pm Poster Session & Refreshments	Public Outreach & Toxins (Heather Meeting Room)	Fisheries and Food Web (Acacia Meeting Room)	Public Health & Bloom Ecology (Toyon Meeting Room)	
	Dinner 6:00pm			**Dinner Reception 6:00pm (Merrill Hall)**
8:00pm-10:00pm Special Evening Sessions		NOAA Event Response - M. Suddelson	West Coast Collaborations w/Emphasis on *Pseudo-nitzschia* - P. Miller & V. Trainer	

Monday, October 3, 2005

Session 1: Outreach and Infrastructure

Time	Presenter	Title
8:30	Scholin, Chris	Welcome and opening Remarks (8:30-8:40)
8:40	Glibert, Patricia	HAB plan overview
9:00	Marsh, Anne S.	A National Harmful Algae Indicator to Monitor the Condition of Coastal Waters in the United States
9:20	Kirkpatrick, Barbara	The START Story: Expansion From a Small Local Effort to Statewide Efforts and Benefits to the HAB Community
9:40	Morton, Steve	Utilization of Volunteers to Monitor Harmful Algal Blooms: The Southeastern Phytoplankton Monitoring Network

Break 10:00 am - 10:30 am

Session 2: Outreach and Infrastructure

10:30	Stumpf, Richard P.	Remote Sensing Detection of Red Tides: and Other Harmful Algal Blooms that Discolor the Water
10:50	Campbell, Lisa	Buoy-Based *in Situ* Imaging System for Real-Time Monitoring of *Karenia brevis* in the Gulf of Mexico
11:20	Haywood, Allison J.	Molecular Detection of *Karenia brevis* and Related Species Using Sandwich Hybridization Assays
11:30	Connell, Laurie	Filling the Bloom Monitoring and Research Resource needs of the HAB Community

Lunch 12:00pm - 1:30 pm

Session 3: Outreach and Infrastructure

1:30	Dalgleish, Fraser	Remote Imaging System for Monitoring Macroalgal HABs in Deep Reef Communities Off Southeast Florida
1:50	Donovan, Chelsea	New Solid-State Fluorescence Sensor Used to Monitor Photosynthetic Parameters
2:10	Fleming, Lora E.	An Epidemiologic Study of the Aerosolized Florida Red Tide Toxins on Asthmatics

Break 2:30 pm - 3:00 pm

Session 4: Toxins

3:00	Gulland, Francis	Environmental Exposures to Florida Red Tides: Effects on Emergency Room Respiratory Diagnoses Admissions
3:20	Landsberg,Jan	Saxitoxin Monitoring in Florida: One More Toxin to Deal With
3:40	Radwan, Faisal F.Y.	Identification of a Rapid Detoxification Mechanism for Brevetoxin in Rats

Tuesday, October 4, 2005

Session 5: Toxins

Time	Presenter	Title
8:40	Van Dolah, Frances	Functional Genomic Studies in *Karenia brevis:* Current Insight into Mechanisms Regulating Growth, Toxicity and Adaptation
9:00	Monroe, Emily	Brevetoxin and Polyketide Synthase Gene Expression Under Low-Nutrient Conditions in the Dinoflagellate, *Karenia brevis*
9:20	Bachvaroff, Tsvetan	Linking Genetic Differences Between *Karlodinium micrum* strains with differences in toxin type and abundance
9:40	Villareal, Tracy	Growth and Toxicity of the Dinoflagellate, *Gambierdiscus Toxicus*, Under Nitrogen and Phosphorus Limitation

Break 10:00 am - 10:30 am

Session 6: Toxins

10:30	Place, Allen R.	The Toxin from *Gymnodinium veneficum* Ballantine - Rediscovered: It's a Karlotoxin
10:50	Armstrong, Meredith	The Production of Yessotoxin in California Isolates of *Lingulodinium polyedrum*
11:20	Goldberg, Judah	Recurrent Presence of *Pseudo-nitzschia* and Domoic Acid in a Pacific Northwest Estuary
11:30	Vogelbein, Wolfgang	Determinants of Pathogenicity in *Pfiesteria piscicida* & *Pseudopfiesteria shumwayae*: Species and Strain Comparisons

Lunch 12:00pm - 1:30 pm

Session 7: Food Webs

1:30	Demir, Elif	Microzooplankton Grazing on *Heterosigma akashiwo* in Delaware Inland bays, and Application of QRT-PCR Technique
1:50	Flewelling, Leanne	Unexpected Vectors of Brevetoxins During Marine Mammal Mortalities
2:10	Foster, Vicki	Allelopathic Effects of *Karlodinium micrum* on Co-Occurring Dinoflagellates

Break 2:30pm - 3:00pm

Session 8: Public Health

3:00	Backer, Lorraine	Environmental Exposures to Florida Red Tides: Effects on Emergency Room Respiratory Diagnoses Admissions
3:20	Jellett, Joanne F.	Enhancing Public Health and Safety Through Distributed Testing: Models in The USA Using Jellett Rapid Tests
3:40	Reich, Andrew	Development of Public Health Response Plans for HABS: a "County Up" Approach

Wednesday, October 5, 2005

Session 9: Bloom Ecology

Time	Presenter	Title
8:40	Anderson, Don	Intrapopulation Variation of *Alexandrium fundyense* within the Gulf of Maine: Ribosomal DNA and Microsatellite Analyses
9:00	Erdner, Deana L.	Global Gene Expression Analysis of Nitrogen and Phosphorus Stress in the Toxic Dinoflagellate *Alexandrium fundyense*
9:20	Coyne Katherine J.	Nitrate Assimilation in *Heterosigma akashiwo*: Evaluation of Nitrate Reductase (NR) Gene Expression in Laboratory Cultures and *in Situ* Populations of *H. akashiwo* in the Delaware Inland Bays, DE.
9:40	Parrow, Matthew W.	Population DNA Distibution, Cellular DNA Content and the Diel NDA Cell Cycle of Cultured *Karlodinium* spp. (Dinophyceae)

Break 10:00 am - 10:30 am

Session 10: Bloom Ecology

10:30	Belas, Robert	Motility of a Dinoflagellate-Associated Bacterium, Silcibacter sp. TM1040, is Important in its Interaction with Pfiesteria Piscicida
10:50	Glibert, Patricia M.	Urea is a Good Predictor of Cyanobacteria in Florida Bay and on the Western Florida Shelf
11:20	Gobler, Christopher	The Impact of Nutrient Loading and Zooplankton Grazing on the Growth of, and Toxin Synthesis by, cyanobacteria Blooms in Lake Agawam, NY, USA
11:30	Haas, Leanoard W.	The Role of Dissolved Organic Matter in Heterotrophic Dinoflagellate Growth

Lunch 12:00pm - 1:30 pm

Session 11: Bloom Ecology

1:30	Twiner, Michael	Comparative Brevetoxin Dynamics During Lysis of *Karenia brevis* by Two Algicidal Bacteria
1:50	Sengco, Mario R.	Flow Effects on Interactions Between *Karenia brevis* and Clay Used in HAB Mitigation
2:10	Heil, Cynthia A.	Nutrient Quality Drives Differential Phytoplankton Community Composition on the West Florida Shelf During a *Karenia brevis* Bloom

Break 2:30pm - 3:00pm

Session 12: Bloom Ecology

3:00	Schaeffer, Blake	A Biochemical Investigation of *Karenia brevis* across a front off Sarasota, FL.
3:20	Anderson, Don	Bloom Development and Transport of Toxic *Alexandrium fundyense* Populations Within a Coastal Plume in the Gulf of Maine
3:40	Hutchins, David A.	Defining Ecological Niches Within a Multi-Species Raphidophyte HAB Consortium

Thursday, October 6, 2005

Session13: Bloom Ecology

Time	Presenter	Title
8:40	Trainer, Vera L.	Characteristics of the Juan de Fuca Eddy, a Source of Domoic Acid to the Washington Coast
9:00	Hickey, Barbara M.	A Lagrangian View of the Juan de Fuca Eddy: Macro-nutrients and Circulation
9:20	Lessard, Evelyn J.	Ups and Downs in the Life of a Toxic *Pseudo-nitzschia* Bloom in the Juan de Fuca Eddy off the Washington Coast
9:40	MacFadyen, Amy	Circulation and Biological Modeling in the ECOHAB PNW Region

Break 10:00 am - 10:30 am

Session 14: Bloom Ecology

10:30	Edwards, Kathleen A.	A Satellite View of Spatial and Temporal Variability of Chlorophyll a and SST in Coastal PNW Waters
10:50	Boyer, Gregory	MERHAB - Lower Great Lakes -Monitoring for Harmful Algal Blooms in Our Inland Seas
11:20	Mayali, Xavier	Ecdysis as a Defense Mechanism Against Bacterial Colonization: The Case of the Dinoflagellate *Lingulodinium polyedrum*
11:30	Lakeman, Michael B.	Towards Recognition of Cryptic Functional Diversity in Natural HAB Populations

Lunch 12:00pm - 1:30 pm

Session 15: Bloom Ecology

1:30	Tester, Patricia A.	Dinoflagellate Abundance in Mangrove Cay Embayments off the Coast of Belize
1:50	Parsons, Michael L.	The Autecology of *Gambierdiscus* in the coastal waters of Hawaii
2:10	Tomas, Carmelo	Growth, Nutrient Utilization and Evidence for Toxin Production by the New Toxic Flagellate *Chloromorum toxicum.*

Break 2:30pm - 3:00pm

Session 16: Bloom Ecology

3:00	Smayda, Ted	Multidecadal Changes in the Diatom:Flagellate Ratio and Si:N and Si:P Ratios in Narragansett Bay, and Influence of Si:N Supply Ratios on Diatom Species Competition
3:20	Dortch, Quay	Business meeting
3:40	TBD	Site selection for next meeting

Public Outreach and Infrastructure – Posters

PO1	Dalpra, Dana R.	Are All Those Outreach Materials We're Creating Doing Any Good?
PO2	Fisher, Kathleen, M.	Assessment of an operational Harmful Algal Bloom Forecast System for the Gulf of Mexico
PO3	Greenfield, Dianne I.	Application of the Environmental Sample Processor (ESP) for Remote Detection of Harmful Algae
PO4	Petrik, Kim	Molecular Detection of Karenia brevis and Related Species Using Fluorescent *in situ* Hybridization Assays
PO5	Pigg, Ryan	MERHAB 2002: Eastern GOMx Sentinel Program
PO6	Poulton, Nicole J.	Detection and Enumeration of Harmful Algal Bloom Species Using a Continuous Imaging Fluid Particle Analyzer (FlowCAM)
PO7	Sinigalliano, Christopher	Isolation of Toxic Algae from Marine Waters by High-Speed Flow Cytometric Single-Cell Sorting

Toxins – Posters

T1	Abbott, Jay P.	Statewide Distribution of Saxitoxins in Selected Florida Puffer Fish Species **(withdrawn)**
T2	Adolf, Jason	Ichthyotoxic *Karlocinium micrum* in the Swan River Estuary (Western Australia): An Emerging Threat in a Highly Eutrophic Estuarine System
T3	Bai, Xuemei	Effect of Host Toxicity on Success of the Parasitic Dinoflagellate *Amoebophrya,* with Preliminary Examination of Host and Parasite Membrane Sterol Composition
T4	Baugh, Keri	Stability of Domoic Acid Under Various Storage Conditions
T5	Bill, Brian D.	Domoic Acid in *Pseudo-nitzschia cuspidata* from Washington State Coastal Waters
T6	Bottein, Marie-Yasmine	Detection of Ciguatoxin in the Blood of Patients Diagnosed with Ciguatera Intoxication
T7	Bouillon, Rene-Christian	Photochemistry of Dissolved Domoic Acid in Natural Water Matrices
T8	Eberhart, Bich-Thuy	Application of a Polychlonal Antibody in the Development of Methods for Detecting Domoic Acid
T9	Faust, Maria A.	The Biodiversity of Harmful Dinoflagellates in the Belizean Coral Reef Mangrove Forest
T10	Ferry, John	Applying Combinatorial Photochemistry Techniques to Explore the Photodegratation of the Harmful Algal Bloom Toxin Domoic Acid

T11	Dyer, Brian	Susceptibility of Two Fishes (*O. Niloticus* and *C. Variegatus)* to *Pfiesteria shumwayae* and it's Associated Toxin: Influence of Salinity
T12	Grover, James P.	Growth, Toxicity and Composition of *Prymnesium parvum* in Relation to Temperature, Light and Salinity
T13	Henry, Michael S.	Aerosolized Brevetoxins: Compositional Changes from Water Bourne Brevetoxins to Aerosolized Brevetoxins Impacting Human Respiration
T14	Hotto, Amber, M.	Potential and Actual Microcystin Production in Lake Ontario Embayments
T15	Keltner, Karen	*Pseudo-nitzschia australis:* The Most Abundant Species of the Genus *Pseudo-nitzschia* at Two Central California Sites South and North of Pt. Conception, 2003-2005
T16	Litaker, R. Wayne	Development of a Simplified ELISA for Detecting Domoic Acid
T17	Lovko, Vincent J.	Factors Regulating Micropredation and Fish Pathogenicity in Heterotrophic *"Pfiesteria*-Like" Dinoflagellates
T18	Fahnenstiel, Gary	Assessment of Microcystins Using Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
T19	Neely, Tatum	A Modified Hemolytic Assay Suggests Toxin Activity Among *Karenia mikimotoi* Clones Isolated During Red Tide Events off the Texas Coast
T20	Pierce, Richard	Brevetoxins and Metabolites in NSP-Toxic Bivalve Molluscs: A Comparison of Methods
T21	Satchwell, Mike	Cyanobacterial Toxins in Lake Champlain - a Five Year Review
T22	Schnetzer, Astrid	*Pseudo-nitzschia* spp. and Domoic Acid in the San Pedro Channel and Los Angeles Harbor Areas of the Southern California Bight
T23	Silver, Mary	Toxic *Pseudo-nitzschia* from California: Some Emerging Patterns
T24	Smith, G. Jason	Molecular Physiology of Amino Acid Metabolism in *Pseudo-nitzschia australis*: Biomarkers for Growth Status or Domoic Acid Toxicity?
T25	Sutherland, Cristy M.	*Dinophysis* abundance, DSP Toxin Production, & Bioaccumulation in California Mussels, *Mytilus Californianus,* in Monterey Bay, CA, USA
T26	Terlizzi, Daniel E.	Membrane Sterols and Inhibition of Dinoflagellates by *Karlodinium micrum* Filtrate
T27	Wang, Zhihong	Analysis of Brevetoxin Metabolites in Bottlenose Dolphins Associated with Their Mortality in the Florida Panhandle During 2004

T28	Westrick, Judy	Preliminary Occurrence Study of Algal Toxin in Source and Finished Waters
T29	Wright, Jeffrey L.C.	Structure of Several New Hemolytic Toxins from Strains of *Prymnesium parvum* Isolated from Fish Ponds in North Carolina

Food Web – Posters

FW1	Antrobus, Rozalin	Oceanographic Conditions in Monterey Bay, CA as They Relate to *Alexandrium catenella* and PSP Toxins in Local Fisheries: A Two Year Time Series
FW2	Atwood, Karen	Brevetoxin Body Burdens in Seabirds from the Central West Florida Coast
FW3	Bargu, Sibel	An Overview of the Domoic Acid Contamination of Monterey Bay Food Webs
FW4	Borkman, David	Modification of *Heterosigma akashiwo* Annual Succession Patterns in Narragansett Bay: Influence of Long-Term (1959 - 1996) Habitat Changes on Interspecific Competition
FW5	Bretz, Carrie K.	Toxic Prey Can Alter Foraging Strategies of Key Marine Predators
FW6	Busse, Lilian B.	Did We Have Toxic Algal Blooms in California in the Past? - Some Insights from Historical Data
FW7	Casper, Erica T.	A Handheld Device for the Detection of *Karenia Brevis* via NASBA
FW8	Cheung, Itchung	Presence of Domoic Acid in California Rock Crabs, *Cancer antennarius* And *Cancer productus* in Monterey Bay, California
FW9	Deeds, Johnathan	Puffer Fish: An Emerging reservoir for Saxitoxins in Marine Food Webs in the US
FW10	Gobler, Christopher	Investigating the Role of Zooplankton Grazing in Controlling Harmful Brown Tide Blooms (A*ureococcus anophagefferens)* in Mid-Atlantic Estuaries
FW11	Doucette, Gregory	Exposure of North Atlantic Right Whales to Algal Biotoxins: The Proof is in the Poop
FW12	Postel, J.R.	Copepods and Diatoms: Paradigm or Paradox?
FW13	Gribble, Kristin E	Asexual and Sexual Reproduction in the Heterotrophic Dinoflagellate *Protoperidinium oblongum*
FW14	Bricelj, V. Monica	Transfer of Brevetoxins to The Benthos in the Context of Clay Mitigation of *Karenia brevis* Blooms
FW15	Hégaret, Hélène	No Apparent Effect of Two Species of the Toxic Dinoflagellate *Alexandrium* on Hemocyte Parameters of the Oysters *Crassostrea virginica* and *crassostrea gigas* **(withdrawn)**

FW16	Kudela, Raphael	Domoic Acid Concentrations in Blood Samples From Randomly Tagged California Sea Lions, *Zalophus californianus*, in California
FW17	Lefebvre, Kathi	Characterization of Dissolved and Particulate STX Levels in Both Field And Cultured *Alexandrium catenella* Samples
FW18	Maranda, Lucie	*Prorocentrum lima* (Dinophyceae) in Northeastern USA Coastal Waters: Abundance, Distribution and Toxin Transfer
FW19	Juhl, A.R.	Do Dinoflagellate PSP Toxins Affect Grazing by Gastropod Larvae?
FW20	Pate, S.E.	Effects of the Toxic Dinoflagellate, *Alexandrium Monilatom,* on Behavior and Survival of Four Shellfish Species
FW21	Shumway, Sandra E.	Bivalve Shellfish Can Serve as Vectors for Transport of Harmful Algae
FW22	Skelton, Hayley	Enzyme-Labeled Fluorescence Detection of Phosphatase Activity in the Heterotrophic Dinoflagellates *Pfiesteria Shumwayae* and *Cyrpthecodinium* sp.
FW23	Thomas, Kate	Movements, Dive Behavior and Survivability of California Sea Lion (*Zalophus Californianus*) Post-Rehabilitation for Domoic Acid Toxicity
FW24	Vigilant, Veronica	Domoic Acid in the Fat Innkeeper Worm, *Urechis caupo,* at Elkhorn Slough, CA
FW25	Zhao, Hui	Sterols of Harmful Marine Algae: Synthesis and Metabolism

Public Health Posters

PH1	Drummond, Allison K.	Microginin 690, a Novel Microginin-Type Metabolite from *Microcystis aeruginosa*
PH2	Hunter, Matthew	Oregon State Emergency Response to Recent Coast-Wide Closures Due to Domoic Acid Toxicity
PH3	Langlois, Gregg W.	Long-Term Monitoring of Marine Biotoxins in California: Why the Status Quo is Not
PH4	Lawrence, David	Efficacy Testing of Ballast Water Treatment Technologies: Preventing Non-Indigenous Phytoplankton Introductions
PH5	Miller, Peter E.	California Program for Regional Enhanced Monitoring of PhycoToxins (Cal PReEMPT)
PH6	Naar, Jerome P.	Brevetoxins, Like Ciguatoxins, Are Potent Ichthyotoxic Neurotoxins that Accumulate in Fish
PH7	Odell, Anthony	ORHAB: Early Warning and Rapid Response to Mitigate the Effects of Harmful Algal Blooms Along the Washington Coast
PH8	Rublee, Parke A.	Presence of *Pfiesteria piscicida* and *Pfiesteria shumwayae* in Coastal Water of Long Island, New York, USA

PH9	Scott, Brittany	Application of Historic Shellfish Data and Remote Sensing to Understand HAB Events on the Oregon Coast: Phase I
PH10	Venrick, Elizabeth L	Toxic *Pseudo-nitzschia*: What's in a number?
PH11	Villareal, Tracy A	Surveillance for Ciguatera Fish Poisoning in Recreational Fishers Utilizing Texas Gulf Coast Oil Rigs
PH12	Eleuterio, Lazaro	Removal of the Cyanobacterial Toxin Microcystin-LR by Biofiltration
		Bloom Ecology – Posters
B1	Bowers, Holly A.	Detecting Raphidophyte Species Throughout Chesapeake and Coastal Bays (Maryland) Using Real-time PCR Assays
B2	Orellana, M.V.	Flow Cytometric Analysis of Domoic Acid in Single Cells
B3	Brunelle, Stephanie, A.	Circadian Control of the Cell Cycle in the Dinoflagellate *Karenia brevis:* A Role for Blue Light and Characteristics of a Blue Light Receptor
B4	Cattolico, Rose Ann	Chloroplast Genomics of a Toxic Raphidophpyte
B5	Curtiss, Casey C.	The Presence and Persistence of a Potentially Harmful Dinoflagellate *Cochlodinium Catenatum*, in Monterey Bay, California
B6	Greengrove, Cheryl	*Alexandrium* Cysts in Puget Sound, Washington: Preliminary Results of a Survey
B7	Handy, Sara	A New Application of Quantitative Real-Time PCR: Simultaneous Enumeration of Multiple Raphidophyte Species by Multiprobing and Multiplexing
B8	Hayashi, Kendra	Applications of rDNA Its Sequence, Analysis to Assess Inter- and Intraspecific Diversity in *Pseudo-Nitzschia* Communities of Monterey Bay, CA
B9	Hoffer, Simon	Germination Experiments with *Alexandrium catenella* Cysts collected from Surface Sediments in Puget Sound
B10	Hubbard, Katherine	West Coast *Pseudo-nitzschia* Species Distinguished by Polymorphisms in the Internal Transcribed Spacer 1 (ITS1)
B11	Kamykowski, Daniel	Lagrangian Studies of *Karenia brevis* bloom initiation
B12	Kilpatrick, Gary	Detection of Karenia brevis in an Early Bloom Stage Using the Breve Buster
B13	Leblond, Jeffrey D.	Sterol Biomarker Families in Harmful Dinoflagellates: A Comparison of Sterol Composition to rDNA-Based Phylogeny
B14	McClintock, Liza	The Role of Copper for Iron Acquisition in the Juan de Fuca Eddy *Pseudonitzschia Bl*oom
B15	Portune, Kevin J.	Investigations of *Heterosigma akashiwo* (Raphidohyceae) Cyst Germination in Laboratory and Field Settings Using Molecular Techniques

B16	Tango, Peter J.	Forecasting *Microcystis* Bloom Characteristics on the Tidal Potomac River, Chesapeake Bay
B17	Vandersea, Mark W.	*Gambierdiscus*: Linking Taxonomy and Genetics
B18	Wolny, Jennifer L.	*Pseudo-nitzschia* species in Florida Coastal Waters
B19	Sellner, Kevin	Potential Role of Clay in Mitigating Chesapeake Bay Algal Blooms

Oral Presentations

INITIAL OBSERVATIONS OF THE 2005 *ALEXANDRIUM FUNDYENSE* BLOOM IN SOUTHERN NEW ENGLAND: GENERAL PATTERNS AND MECHANISMS

Donald M. Anderson[1], Bruce A. Keafer[1], Dennis J. McGillicuddy[2], Michael Mickelson[3], Kenneth E. Keay[3], P. Scott Libby[4], James P. Manning[5], Charles A. Mayo[6], David K. Whittaker[7], J. Michael Hickey[7], Ruoying He[2], Daniel R. Lynch[8] and Keston W. Smith[8]

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From May to July, 2005, an extensive bloom of *Alexandrium fundyense* occurred along the coast of southern New England. The outbreak eventually closed shellfish beds from central Maine to Massachusetts, including Nantucket Island and portions of Martha's Vineyard, and resulted in the closure of 40,000 km^2 of offshore federal waters. The bloom was exceptional in several ways: high toxin levels were measured farther south than ever before in New England; levels of toxicity in many locations were higher than previously observed at those stations; for the first time toxicity at some locations was above quarantine levels; cell concentrations far exceeded those observed in the coastal waters of southern New England in the past; and for the first time, the governors of Maine and Massachusetts officially declared the red tide to be a disaster, clearing the way for federal assistance.

Initial observations suggest that several factors contributed to this bloom. Abundant rainfall and heavy snowmelt substantially increased the amount of fresh water entering the Gulf of Maine. We hypothesize that this provided macro- and micro-nutrients, a stratified water column, and a transport mechanism that led to high cell abundances and a broad, region-wide dispersal of the organism. Warm temperatures in western waters also would have favored *A. fundyense* growth. In addition, several storms with strong winds out of the northeast occurred when cells were abundant and in locations where the winds could advect them into Massachusetts Bay and keep them there, leading to high cell concentrations and toxicity. Another contributing factor may have been the high abundance of newly deposited cysts in western Gulf of Maine sediments, as documented in a fall 2004 survey.

Here we evaluate this bloom and the patterns of toxicity in light of the conceptual models for *A. fundyense* dynamics developed during the ECOHAB - Gulf of Maine (GOM) program. Several features of the 2005 bloom conform to the mechanisms proposed in those models, including the alongshore transport of cells in major water masses and episodic intrusions of cells towards shore due to downwelling-favorable wind forcings. The models need to be refined and expanded, however, based on new data and observations. For example, it is now clear that cells and bloom patches can reach the outer side of Cape Cod and even Nantucket and Martha's Vineyard. Transport to the coastal waters of Rhode Island and even Connecticut/Long Island is also possible. A critical modification also may be necessary in terms of mechanisms through which *A. fundyense* cells occur in Massachusetts Bay. In the past, toxicity only developed when blooms were transported from the north and into the bay via the western segment of the Maine Coastal Current. Now, it is possible that the bay might serve as a source of cells through the germination of cysts deposited in those waters during the 2005 bloom. If proven in subsequent surveys, this potential for in situ bloom development could have major implications on the timing and extent of toxicity within Massachusetts Bay and southern New England waters in future years.

THE PRODUCTION OF YESSOTOXIN IN CALIFORNIA ISOLATES OF *LINGULODINIUM POLYEDRUM*

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Yessotoxin (YTX) is commonly produced by two dinoflagellates, *Protoceratium reticulatum* and *Lingulodinium polyedrum*. *P. reticulatum* has been confirmed to produce YTX and other analogs from isolates in New Zealand, Norway, Spain, Italy, Canada, the United Kingdom and recently, the United States. *P. reticulatum* isolated from Washington, California and Florida produced YTX in culture (Paz et al., 2004). *L. polyedrum* has also been determined to produce YTX in isolates from Italy, the United Kingdom, Ireland, Spain and the United States. Three cultures of *L. polyedrum* isolated from southern California coastal waters were tested for the presence of yessotoxin using Biosense Laboratory ELISA kits. Yessotoxin was detected in the particulate phase of two out of three cultures. Toxin was also detected in the dissolved phase. However, it is probably the result of salt matrix effects rather than measurable toxin. This is the first study to confirm yessotoxin production in California isolates of *L. polyedrum.* Additional isolates of *L. polyedrum* from California will be tested for yessotoxin production, using multiple batch culture experiments to establish per cell toxicity of California isolates compared to European strains, and the toxicity under different macronutrient, temperature and light regime conditions. Three forms of nitrogen (nitrate, ammonium and organic urea) will be used to determine the role of anthropogenic loading on toxin production. *L. polyedrum* has previously been shown to efficiently utilize ammonium and urea to meet the cell nitrogen requirement (utilization varied as a function of growth irradiance) (Kudela and Cochlan, 2000).

Both dinoflagellate species, *Protoceratium reticulatum* and *Lingulodinium polyedrum*, isolated from California coastal waters produce yessotoxin in culture. *L. polyedrum* is geographically dominant from central California southward, while *P. reticulatum* is more prevalent from central California northward. Therefore, yessotoxin is potentially present along much of the U.S. west coast.

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LINKING GENETIC DIFFERENCES BETWEEN *KARLODINIUM MICRUM* STRAINS WITH DIFFERENCES IN TOXIN TYPE AND ABUNDANCE

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Karlodinium micrum is a toxic dinoflagellate found in temperate waters worldwide. However, like other small athecate dinoflagellates the taxonomy and therefore distribution of this alga has been confused. Names that are now likely to be synonymous with *K. micrum* may include *Gymnodinium micrum*, *G. estuarale*, *G. galatheanum*, and *G. veneficum*. We have amassed a collection of 25 different *K. micrum* strains, mostly from the United States Atlantic coast, but with representatives from the English Channel, the North Sea, New Zealand and Australia. Most of these strains have a single ITS ribotype, although four European strains and one New Zealand strain diverge both by point mutations and an insertion. Multiple different toxin types were found, as measured with Liquid Chromatography coupled to a Mass Spectrometer (LC-MS). The mass of these toxins varies from 1402 daltons to 1210 daltons and multiple congeners are often found within a single strain. So far the same toxin, Karlotoxin 2, has been isolated from the U.S. East Coast south of the Chesapeake Bay, as well as from a Norweigan, and an Australian strain suggesting that this toxin could be a common precursor to the other toxin types. The specific activity of these toxins also varies when using a rainbow trout red blood cell assay. Even within strains that have the same ribotype and produce the same type of toxin there are large differences in the amount of toxin produced per cell when grown under the same conditions. Absolute toxicity of these different strains varies from none to approximately one picogram of toxin/cell in laboratory conditions.

Using the LC-MS technique will allow us to perform near real time analysis of environmental samples with similar throughput to PCR assays. This method has been tested in the laboratory and is able to quantify small quantities of different toxins from most of the strains tested. By directly testing the total quantity of toxin in a water sample we should be able to provide managers with a complementary dataset to cell counts and quantitative PCR results.

To determine whether these phenotypic differences can be correlated with specific genetic markers we have isolated Simple Sequence Repeats (SSR) from strains of the same ribotype. This allows for finer discrimination between strains. However, a simple analysis of SSR size does not correlate well with either toxin type or abundance, but does indicate the presence of genetically distinct *K. micrum* strains.

MOTILITY OF A DINOFLAGELLATE-ASSOCIATED BACTERIUM, *SILICIBACTER* SP. TM1040, IS IMPORTANT IN ITS INTERACTION WITH *PFIESTERIA PISCICIDA*

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Marine unicellular algae, especially dinoflagellates, co-occur with a diverse bacterial community that has the potential to dramatically affect algal physiology. In previous reports, we have shown that *Silicibacter* sp. TM1040, an á-Proteobacterium isolated from *Pfiesteria piscicida* cultures, forms an 'obligate' interaction and is required for normal growth of the dinoflagellate in laboratory cultures. *Silicibacter* sp. TM1040 metabolizes the organosulfur compound, dimethylsulfoniopropionate (DMSP), produced as a major secondary metabolite by *P. piscicida*, and senses and responds to the dinoflagellate via positive chemotaxis to DMSP and amino acids produced by *P. piscicida*. These data suggest that both chemotaxis to dinoflagellate products and bacterial motility are important in establishing the initial interaction between this bacterium and its dinoflagellate hosts. In the current report, the hypothesis that *Silicibacter* sp. TM1040 uses flagellar motility to initiate physical interaction with *P. piscicida* was tested. Utilizing the draft annotation of the genomic sequence of *Silicibacter* sp. TM1040, separate mutations in three genes encoding proteins that affect motility were constructed and phenotypically characterized. Two of the strains with motility defects (Mot mutants) do not produce flagella, while the third mutant produces flagella, but is poorly motile in broth and semi-solid agar media due to a defect in a gene encoding the major regulator of motility in this bacterium. When fluorescently-labeled wild-type *Silicibacter* sp. TM1040 cells are added to washed *P. piscicida* dinoflagellates, the bacterial cells readily attached to the dinoflagellate cells, and co-localized to both the cell surface and cytoplasmic interior of the dinoflagellate. In contrast, the attachment of the Mot^- strains to the dinoflagellate surface was significantly reduced, and all three Mot^- strains showed nearly complete loss of the ability to co-localize with the interior of their host. These data suggest that motility of *Silicibacter* sp. TM1040 is necessary for this bacterium to physically interact with *P. piscicida*. The wild-type and three Mot^- strains were used in add-back rate of growth experiments with axenic dinoflagellate zoospores. The growth rate of axenic *P. piscicida* was significantly reduced when in the presence of either of the two Mot^- mutants that show a complete loss of flagellar motility, but was unaffected when *P. piscicida* was incubated with either the wild-type strain or the Mot^- mutant with impaired motility. These results are in agreement with the attachment data, and support the hypothesis that a fully functioning flagellar motility mechanism is critical for *Silicibacter* sp. TM1040 in establishing its interaction with *P. piscicida*. Implications of these findings to HAB bloom dynamics and ecology will be discussed.

MERHAB – LOWER GREAT LAKES - MONITORING FOR HARMFUL ALGAL BLOOMS IN OUR INLAND SEAS

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The North American Great Lakes located between the United States and Canada collectively contain approximately 10% of the World's fresh water and provide drinking water for more than 22 million people. In recent years, these inland seas have suffered harmful algal blooms in the form of toxic cyanobacteria. Toxic blooms of *Microcystis* are well documented in the relatively shallow western basin of Lake Erie where concentrations of the hepatotoxic microcystin LR (MC-LR) have exceeded 20 μg L^{-1}. Similarly both microcystins and the neurotoxin anatoxin-a have led to animal fatalities in the Lake Champlain basin. Lake Ontario, with its numerous nutrient-impacted embayments along the New York Coastline and deep offshore waters, also a documented history of cyanobacterial blooms. MERHAB-LGL is specifically focused on developing monitoring strategies for these important waters using a combination of molecular, chemical and classical techniques combined with remote sensing, and hydrodynamic modeling to safe guard our drinking water supplies. In 2005, MERHAB-LGL has partnered with the NOAA's Great Lakes Environmental Research Laboratory (GLERL) to participate in their International Field Year on Lake Erie. This is an extensive field sampling program to look at distribution of harmful algal blooms in Lake Erie. The results of this recent effort, along with more than four years of field sampling on Lake Erie, Lake Champlain and Lake Ontario will be presented.

BUOY-BASED *IN SITU* IMAGING SYSTEM FOR REAL-TIME MONITORING OF *KARENIA BREVIS* IN THE GULF OF MEXICO

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The objective of this MERHAB project is to develop a buoy-based *in situ* continuous monitoring system capable of detecting increases in abundance of the toxic dinoflagellate *Karenia brevis.* A real-time early warning system for early detection of potential harmful algal blooms (HABs) and a rapid response to such events have been suggested as the most effective ways to mitigate the impact of HABs. We have tested a prototype submersible imaging flow cytometer system (FlowCAM; FluidImaging Technologies, Inc.) in laboratory and simulated field conditions. A number of modifications to the standard laboratory FlowCAM (optics, LED for videoimage capture, flow cell design) were necessary for submersible operation. Also, methods for in situ image analysis and compression were examined to improve efficiency of image capture and transmittal of image data to allow the FlowCAM to be used in conjunction with the existing Texas Automated Buoy System (TABS) data systems. Results from the system deployed on a new buoy located off the coast at Corpus Christi, TX, will be presented.

FILLING THE BLOOM MONITORING AND RESEARCH RESOURCE NEEDS OF THE HAB COMMUNITY

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Based on discussions at recent US National HAB planning workshops it appears that there is some agreement on the need for better coordination within the HAB community to make various types of standard reference material and protocols generally available. As this community looks to the future we must determine what form this coordination will take. To support ongoing studies in the HAB research and monitoring community what services, materials or information do we need to provide for bloom detection and abundance determination? What do we use as a validation standard for new assays?

Focusing on molecular assays, there is currently a wide diversity of methods available. In this session we will quickly review currently used molecular methods and present a format for discussion with all stakeholders in order to gauge the need or level of interest for sustaining a coordinated effort of material and standard dissemination.

Audience participation is requested during the discussion section.

NITRATE ASSIMILATION IN *HETEROSIGMA AKASHIWO*: EVALUATION OF NITRATE REDUCTASE (NR) GENE EXPRESSION IN LABORATORY CULTURES AND *IN SITU* POPULATIONS OF *H. AKASHIWO* IN THE DELAWARE INLAND BAYS, DE.

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The reduction of nitrate to nitrite is often considered to be the rate-limiting step in nitrate assimilation by plants and algae, and is catalyzed by nitrate reductase (NR). Regulation of NR gene expression has been studied extensively in vascular plants and a few species of green algae and marine diatoms, but little is known about induction and regulation of NR gene expression in other phytoplankton species. As part of a multi-disciplinary EPA STAR ECOHAB project, we are beginning to define the ecological niches for two raphidophyte species, *Heterosigma akashiwo* and *Chattonella subsalsa*, that form mixed blooms in the Delaware Inland Bays, DE. Laboratory culture experiments demonstrate that *H. akashiwo* has a higher maximum growth rate at saturating nitrate concentrations than *C. subsalsa* and is able to maintain equivalent growth rates at nitrate levels that are an order of magnitude lower than *C. subsalsa*. In an effort to understand competitive differences in nitrate utilization for the two species, we cloned and sequenced the NR gene from the Delaware Inland Bays isolates of *H. akashiwo* and *C. subsalsa*. Induction and regulation of NR mRNA expression levels were then evaluated in laboratory cultures using quantitative real-time PCR. Here, we present the effects of nutrient concentrations, light intensity, and diurnal cycle on expression of the NR gene in *H. akashiwo*. We also performed nutrient and light manipulations of natural field samples collected during blooms of *H. akashiwo* in the Delaware Inland Bays. Nucleic acids were extracted from these samples for evaluation of NR gene expression by *H. akashiwo* in control and manipulated samples. This work represents the first reported gene sequence and *in situ* gene expression analysis of nitrate reductase for a raphidophyte species.

REMOTE IMAGING SYSTEM FOR MONITORING MACROALGAL HABS IN DEEP REEF COMMUNITIES OFF SOUTHEAST FLORIDA

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An emerging invasion of macroalgal Harmful Algae Blooms (HABs) has prompted researchers at HBOI to survey and monitor several reef sites for bottom biota coverage along the southeast Florida coast. Several species of the green algae *Codium isthmocladum*, *Caulerpa brachypus*, and *Caulerpa racemosa* pose potential detrimental threat to coral reefs and their associated food webs. The HABs have been observed down to at least 150 ft and researchers currently believe that survey of even deeper reefs (up to 300 ft) is necessary for a comprehensive understanding of the bloom dynamics. One hypothesis is that nutrient pollution from Class I injection wells is escaping into the coastal ocean through porous rock before reaching the desired 3000 ft depths.

Past research and monitoring efforts of these HABs have been directed at coastal reefs in <130 ft depths, mainly due to physical limitations of accessibility by SCUBA divers. To date, the main tools for surveying the reef *in situ* were digital video equipment followed by time-consuming image post processing. In order to further investigate the causes and impacts of HABs on Florida's reefs, survey deeper reefs and cover larger areas than currently possible, we developed a simple, relatively inexpensive integrated acousto-optical imaging system, deployed on a remotely operated vehicle (ROV) for visually surveying deep reef sites (up to 300 ft) without the complexity associated with placing a human in an extreme environment. Furthermore, an ROV, deployed from a small boat that can handle its load and operation crew, is far more effective at performing large area reef surveys than a scuba diver. The new system offers the scientist active control, accuracy and repeatability to investigate specific areas of interest.

The deep reef monitoring system consists of two major components: a high-performance video camera mounted on a HBOI search and observation class ROV (max. rated depth 1000 ft) and a narrow beam depth sounder attached to the surface vessel. The video system utilizes a down looking 3 Chip CCD progressive scan video camera (Sony TRV-900), two variable intensity (0 – 150 Watt) halogen lights and two 18 Watt HID fill lights. The operational range of the system is 2 – 5 ft above the seabed. The actual range to the bottom is determined in real time by triangulation using a red 10mW laser range finder, and incorporated into the data stream for future processing. A vessel-deployed sonar (Airmar B256, 1kW transducer, 3° x 5° at 200kHz) and GPS receiver are used for crude mapping of the reef topography prior to launching the ROV. It is possible to generate low resolution geo-referenced seabed profiles, which provide real time information about the reef area to be surveyed and contribute to efficient utilization of the survey resources.

The visual signal is broadcasted to the topside to provide a real-time, high resolution video stream of the reef being surveyed at 15 frames per second. The 720 by 480 images acquired by the system cover a field of view of 882mm by 640mm at the ROV operational altitude of 3 ft, allowing the scientist to visually discriminate features in the sub-centimeter range. When later used with the PointCount' 99 software, this allows for accurate identification of the algae under study and differentiation between these species from natural, non-harmful reef members. The individual images are also used to create optical mosaics therefore allowing the scientist to view the entire transect area as a single image. During operation, a forward looking video camera with a pan/tilt capability and real-time returns from the forward looking scanning imaging sonar (both are standard feature of the ROV) are used to assist in the piloting of the vehicle, especially for obstacle avoidance. To geo-reference the optical

mosaics to an accuracy where it is possible to revisit particular features, the low resolution *a priori* sonar maps will be registered with the higher resolution laser range maps acquired during the transect.

In future work we plan to extend the HAB monitoring capabilities of this system by augmenting the current acousto-optical system with a sensors package to detect salinity and turbidity gradients which are typical of sites where groundwater discharge occurs. These multi-dimensional datasets would further consolidate the understanding of the mechanisms behind the macroalgal HABs in Florida and elsewhere.

MICROZOOPLANKTON GRAZING ON *HETEROSIGMA AKASHIWO* IN DELAWARE INLAND BAYS, AN APPLICATION OF QRT-PCR TECHNIQUE

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The Delaware Inland Bays (DIB) are subject to numerous mixed blooms of raphidophytes each year from mid May to the end of October. *Heterosigma akashiwo* is one of the consistently occurring raphidophytes. Due to its tolerance of a wide range of salinities and temperatures, *H. akashiwo* blooms occur throughout the bays at varying densities. During these blooms often one or two species of *Chattonella spp* are also observed, indicating a dynamic consortium of raphidophyte species. In this study, the effectiveness of microzooplankton grazing pressure is assessed as a top-down control mechanism on various densities of *H. akashiwo* blooms in mixed communities. We applied the dilution method looking at traditional parameters such as cell counts and extracted chlorophyll *a*. Additionally, we used the Quantitative Real-Time PCR (QRT-PCR) method to assess species-specific grazing pressure on *H. akashiwo*. *H. akashiwo* was subject to microzooplankton grazing pressure at rates ranging from $g = 0.02 - 1.86$ per day at various sites. Microzooplankton grazing also occurred on other phytoplankton species in the absence of *H. akashiwo* in the sampling sites. Grazing pressure on *H. akashiwo* may give an advantage to other raphidophytes such as *Chattonella* spp. that are too large to be consumed at high rates by microzooplankton, and thus could contribute to the dynamics of the consortium.

NEW SOLID-STATE FLUORESCENCE SENSOR USED TO MONITOR PHOTOSYNTHETIC PARAMETERS

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An *in situ* variable fluorescence system has been developed that will allow real-time measurement of the primary variable fluorescence variables; Fv, Fo and Fm. Advances in solid-state light detectors and the development of advanced signal processing circuitry have led to the development of a new generation of fluorescence instrumentation that can be used to measure photosynthetic parameters in a wider range of platforms and locations. Market pressures for smaller and more energy efficient sensors has been the primary motivation in the development of *in situ* variable fluorescence sensor and a line of small, filter fluorometers for algal and cyanobacterial biomass measurements. Variable fluorescence data is emerging as an important biological indicator and is being used for indicators of nutrient state, productivity, and algal bloom formation. The photosynthetic quantum efficiency (Fv/Fm) depicts initial physiological changes in phytoplankton that act as a precursor to algal blooms. The ability to measure this parameter in situ allows researchers and managers to detect real-time predictors of algal blooms (HABs). The variable fluorescence system is described and performance data presented.

A SATELLITE VIEW OF SPATIAL AND TEMPORAL VARIABILITY OF CHLOROPHYLL-A AND SST IN COASTAL PNW WATERS

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The razor clam fishery along the coast of the Pacific Northwest has been repeatedly closed by domoic acid poisoning. The production of the toxin by *Pseudo-nitzschia* species is currently under study by the ECOHAB PWN field program. Results to date suggest that an eddy off the mouth of Juan de Fuca Strait (Fig. 1a) is an initiation site for blooms of toxic *Pseudo-nitzschia* which are then advected onto the coastal beaches during fall storms. This talk will present results from satellite analysis performed in support of ECOHAB PNW. While the available satellite data (sea surface temperature and chlorophyll-a concentration) do not capture the biological processes behind the toxification of the clams, they provide information about the physical setting in which the toxification has occurred. Statistical analysis is performed on 7 years of SeaWiFS chlorophyll-a data and 18 years of AVHRR SST data in order to characterize the space and time variability of the coastal waters of the Pacific Northwest (Fig. 1a). When a seasonal cycle is fit to the satellite data, the eddy is distinguished from the coastal shelf region by several characteristics. In the eddy, the annual peak in productivity occurs earlier than on the shelves to the north or south (Fig. 1b). The range in SST values over the course of the year is lower in the eddy than on the coastal shelves to the north or south, suggesting that conditions in the eddy are relatively steady; in turn, the annual change in SST for both the eddy and the shelf regions is less than offshore (Fig. 1c). The relationship between variability in the eddy and the coastal shelves and candidate forcing mechanisms, such as wind-driven upwelling or outflow from the Juan de Fuca Strait, will be discussed.

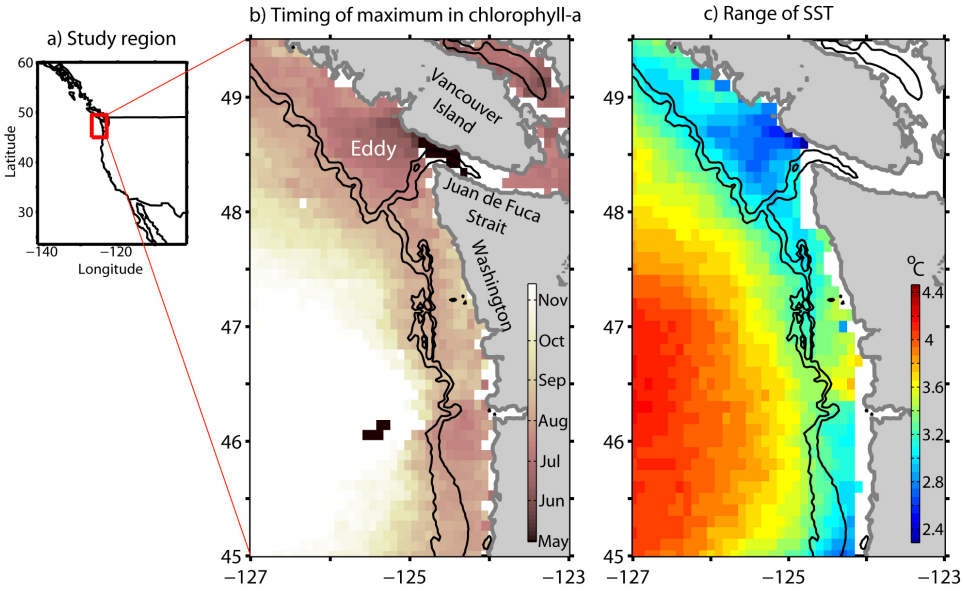

Figure 1: a) The study region (red square). b) Timing of the annual maximum in chlorophyll-a, based on the phase of the first annual harmonic. The typical location of the eddy off the mouth of the Juan de Fuca Strait is labeled. c) Range of SST values (°C) over the year, based on the amplitude of the first annual harmonic.

GLOBAL GENE EXPRESSION ANALYSIS OF NITROGEN AND PHOSPHORUS STRESS IN THE TOXIC DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE*

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Blooms of the toxic dinoflagellate *Alexandrium* are responsible for outbreaks of paralytic shellfish poisoning around the globe. To better understand the molecular and cellular aspects of toxic bloom formation in this organism, we have employed Massively Parallel Signature Sequencing (MPSS) to better understand nutrient physiology and toxin production in this organism. MPSS is a method of global expression profiling that generates a 17-nucleotide sequence 'tag' for one million individual gene transcripts in a cell. MPSS was performed using *Alexandrium* cells grown under nitrogen or phosphorus starvation, conditions that decrease and increase cellular toxin content respectively. Differentially regulated genes should thus include those involved in toxin production, N stress and P stress. The results reveal an unexpectedly large number of unique gene tags in *Alexandrium*, as compared to other eukaryotes that have been analyzed. The expression of several thousand tags is significantly different ($p<0.001$) between the two conditions. Sequence tags were mapped back to their corresponding gene transcripts through a combination of 3'RACE and EST sequencing. RACE and EST analyses identified several tags whose expression levels differed under N- or P-starvation. Quantitative reverse-transcription-PCR was performed to compare the expression of these potentially differentially regulated transcripts under -N, -P and replete conditions. Expression of five of the transcripts is regulated by N- and/or P-starvation: two of the transcripts are down-regulated under both conditions; two are up-regulated in -N cells; and one is highly up-regulated under -P conditions. Efforts to identify the transcripts and prospects for their use as specific indicators of nutrient stress will be discussed.

AN EPIDEMIOLOGIC STUDY OF THE EFFECTS OF THE AEROSOLIZED FLORIDA RED TIDE TOXINS ON ASTHMATICS

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Florida red tides annually occur in the Gulf of Mexico, resulting from blooms of the marine dinoflagellate, *Karenia brevis*. When the organism releases its brevetoxins into the water, the combination of wind and surf cause the toxins to enter the marine aerosol. When inhaled, these toxins cause itchy eyes, cough, and rhinorrea. When toxins are aerosolized in animal models, there is significant increase in airway resistance at picogram levels. A study of persons who visited the beach recreationally found a significant increase in self-reported respiratory symptoms after exposure to aerosolized Florida red tides. Anecdotal reports indicate that persons with underlying respiratory diseases may be particularly susceptible to adverse health effects from these aerosolized toxins.

An epidemiologic study is underway to identify the impacts of these aerosolized toxins in humans. A cohort of asthmatics $\geq$ 12 years with asthma is repeatedly evaluated with a brief symptom questionnaire, nose and throat swabs, and NIOSH-approved spirometry before and after going to the beach. Environmental monitoring, water and air (i.e., *K. brevis*, brevetoxins and particulate size distribution) sampling, and personal monitoring (for toxins) are performed. Brevetoxin concentrations are measured by LCMS, HPLC, and a newly developed brevetoxin ELISA. These field studies have been conducted repeatedly during a Florida red tide and with no red tide over the past 3 years. Participants are significantly more likely to report respiratory symptoms after Florida red tide exposure. Participants demonstrate small but statistically significant decreases in pulmonary function (FEV_1, FEF_{25-75}, and PEF) after only 1 hour of Florida red tide toxins exposure, particularly among the more severe asthmatics. Similar evaluations during non Florida red tide exposure periods do not significantly differ.

This is the first study to show objectively measurable acute adverse health effects from repeated exposure to aerosolized Florida red tide toxins in persons with asthma. The results of the study provide improved information to the public, especially those with lung disease, when on shore Florida red tides occur.

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UNEXPECTED VECTORS OF BREVETOXINS DURING MARINE MAMMAL MORTALITIES

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Brevetoxins produced by the Florida red tide dinoflagellate *Karenia brevis* are known to induce massive fish kills and to cause illness in humans who ingest toxic filter-feeding shellfish or inhale toxic aerosol. Although the involvement of brevetoxins has been suspected in several large-scale mortalities of manatees (*Trichechus manatus latirostris*) and dolphins (*Tursiops truncatus*), establishing brevetoxin poisoning as the cause of the mortalities has often been hindered by limited confirmation of toxin exposure in only a fraction of the animals, diagnoses of additional complicating pathologies, and unknown routes of exposure.

In the last decade, Florida's Gulf coast has witnessed six red tide-related marine mammal mortality events, with four occurring in the last four years. Two of these recent mortalities have occurred in the absence of detectable *K. brevis* red tides, shedding light on some previously understudied routes of exposure to brevetoxin for higher vertebrates. In spring of 2002, 34 endangered Florida manatees died in southwest Florida, and in spring of 2004, 107 bottlenose dolphins died in the Florida Panhandle. In both cases, exposure to brevetoxins was unambiguously confirmed by measurement of elevated concentrations in multiple tissues of all animals tested, while extremely elevated concentrations in stomach contents indicated exposure through ingestion. Field investigations performed while the events were ongoing resulted in the identification of high concentrations of brevetoxins in the manatees' and dolphins' food sources and documented for the first time the accumulation of brevetoxins associated with seagrass and in naturally-exposed fish.

Seagrasses (predominantly *Thalassia testudinum*) were collected from southwest Florida in areas where manatee carcasses were being recovered during mortality events in 2002, 2003, and 2005. Maximum brevetoxin concentrations measured in composite seagrass samples were 1.1 µg/g in 2002, 1.6 µg/g in 2003, and 2.6 µg/g in 2005. Toxicity was mainly (but not exclusively) associated with epiphytes and detritus on the surface of the blades. The persistence of brevetoxins in seagrass varied from year to year and may depend on the composition of the epiphytic community.

The dolphins in the 2004 mortality died with very full stomachs, and six undigested menhaden were found to contain extremely high brevetoxin concentrations (up to 33.2 µg/g in viscera and 1.5 µg/g in muscle). Subsequently, fish (n = 47) were collected from St. Joseph Bay, where the majority of the dolphins stranded. No *K. brevis* cells and no elevated concentrations of brevetoxins were observed in St. Joseph Bay in the two weeks prior to fish collection. However, all of the fish collected from St. Joseph Bay had significant levels of brevetoxins in muscle (up to 0.4 µg/g) and up to 5.0 µg/g in the viscera.

Historically, exposure of herbivorous manatees and piscivorous dolphins to dangerous levels of brevetoxins through ingestion of their routine diet has not been of great concern. Despite almost annual *K. brevis* blooms in the Gulf of Mexico, mass mortalities of marine mammals have been relatively rare. Brevetoxin-contamination of their typical food sources (seagrasses and fish) was previously undescribed or conceptually controversial. This documentation of brevetoxin accumulation in seagrass and in live fish reveals novel mechanisms for brevetoxin vectoring via food webs, demonstrates that brevetoxin-contaminated food webs pose a tangible threat to marine mammals, and illustrates the potential for delayed or remote animal exposure to brevetoxins in the absence of a concurrent *K. brevis* bloom.

ALLELOPATHIC EFFECTS OF *KARLODINIUM MICRUM* ON CO-OCCURRING DINOFLAGELLATES

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Karlodinium micrum is a toxic, bloom-forming, mixotrophic dinoflagellate associated with fish mortalities in coastal waters on the eastern seaboard, USA and co-occurs with the ichthyocidal dinoflagellates *Pfiesteria piscicida* and *Pfiesteria shumwayae. K. micrum* toxin, karlotoxin, acts by inducing pores in the cell membranes of susceptible species leading to osmotic disruption; toxin production varies among strains and with culture conditions. In these experiments, we examined the effects of cell-free filtrates from different *K. micrum* strains on the survival and predator-prey dynamics of co-occurring dinoflagellate species. We observed the allelopathic effect of five strains of *K. micrum* on multiple *P. shumwayae* strains and two strains each of Cryptoperidiniopsoid species and *P. piscicida*. The *K. micrum* examined included three strains from the CCMP (1974, 1975, and 2283) and two strains clonally isolated from water collected during a fish death incident in the Back River of Virginia in March 2005 (VIMS 2004 and 2006).

Cell-free filtrates of disrupted *K. micrum* cultures (disrupted by the addition of DOM) adversely affected *Pfiesteria piscicida*, *Pfiesteria shumwayae* and Cryptoperidiniopsoid species in 24-hour karlotoxin exposure studies. The VIMS 2004 culture isolated from a recent fish kill caused the most immediate disruption of the dinoflagellates tested. Cryptoperidiniopsoid species began to lyse 30 minutes following exposure to a cell-free filtrate derived from 1000 cells/ml. The cell-free filtrates of the other

K. micrum strains tested at 1000 cells/ml had no effect on dinoflagellates tested. Strain variability was observed in the capability of *K. micrum* to disrupt exposed cells. At greater cell concentrations (e.g. 15,000 cells/ml of CCMP 1974, 1975 and 2283) disruption of exposed cells was consistent with the reported variation in *K. micrum* toxicity. Cryptoperidiniopsoid species and *P. shumwayae* (CCMP 2358) were most susceptible to rapid osmotic lysis upon exposure to karlotoxin (occurring 30 min -3 hours following exposure to karlotoxin, kmtx1).

Predator-prey dynamics were examined by combining *K. micrum* and other dinoflagellate species/strains in the presence and absence of cell-free *K. micrum* filtrates. Previous experiments indicated that *K. micrum* could be either predator on or prey of other dinoflagellate species/strains. In the present experiment the effects of both *K. micrum* toxicity and cell-free extracts on predator-prey dynamics were examined. The results of this study will provide insight into the allelopathic effects of *Karlodinium micrum* on commonly co-occurring dinoflagellate species expressed as both a direct effect on cellular integrity and as a factor modulating the predator-prey dynamics among these species. The results of these studies emphasize the necessity of examining multiple strains of species when examining complex ecological interactions.

UREA IS A GOOD PREDICTOR OF CYANOBACTERIA IN FLORIDA BAY AND ON THE WESTERN FLORIDA SHELF

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Florida Bay has been the focus of recent scientific and management concern because of significant ecological changes that have been associated with ongoing eutrophication and land-use changes. The central region of Florida Bay has experienced frequent microalgal blooms in the past decade typically dominated by the cyanobacteria genus *Synechococcus*. Previous studies have characterized eastern Florida Bay region as phosphorus limited, and western Florida Bay as generally nitrogen limited, but this conclusion has largely been based on examination of inorganic nutrient forms and their availability. With increasing use of organic forms of nitrogen as agricultural nutrients, and with increasing evidence that organic nitrogen forms such as urea can be present in elevated concentrations in waters receiving agricultural runoff, the comparative ecological significance of organic nutrients can no longer be discounted. We have previously documented that during a large *Synechococcus* outbreak in Florida Bay November 2003, the abundance of cyanobacteria in the phytoplankton community (as estimated from zeaxanthin: chlorophyll *a* ratios) was positively related to the percent uptake of urea, and negatively related to the percent that inorganic nitrogen contributed to total nitrogen uptake. Here we extend these findings and compare results from November 2003 with three additional field efforts in Florida Bay and one field effort on the Western Florida Shelf. In all cases the ratio of zeaxanthin: chlorophyll *a* was a positive function of the percent that urea contributed to total nitrogen uptake and a negative function of the fraction of inorganic nitrogen uptake. These findings add to our growing body of evidence that urea is a significant form of anthropogenic nitrogen which is preferred by several harmful algal species and may contribute to their proliferation in some regions.

THE IMPACT OF NUTRIENT LOADING AND ZOOPLANKTON GRAZING ON THE GROWTH OF, AND TOXIN SYNTHESIS BY, CYANOBACTERIA BLOOMS IN LAKE AGAWAM, NY, USA

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During 2003 and 2004, we investigated the dynamics of toxic cyanobacteria populations in Lake Agawam, a eutrophic lake on Long Island, NY, USA. Concurrently, experiments were conducted to evaluate the contrasting effects of zooplankton grazing and nutrient loading on the abundance and toxin content of cyanobacteria populations. Lake Agawam hosted dense blooms of *Microcystis* spp. and *Anabaena* spp. with cell densities exceeding 10^5 cells ml^{-1} and chlorophyll *a* concentrations exceeding 200 µg L^{-1}. Microcystin was present in all samples collected during both years (up to 45 µg L^{-1}; May-Nov; n = 130) while anatoxin-a was detected during late summer only (~1 µg L^{-1}). Polymerase chain reaction (PCR) analysis targeting the microcystin synthesis gene (mcyE) indicated that *Microcystis* spp., but not *Anabaena* spp., was responsible for microcystin production in this system. Moreover, reverse transcriptase PCR indicated the *Microcystis* population strongly expressed the mcyE gene during summer months, but rarely expressed the gene during the fall when in situ populations and microcystin levels in the lake declined. During summer, when there was strong mcyE expression by the *Microcystis* population, experimental zooplankton (Daphnia sp.) enrichment had no impact on cyanobacteria biomass (100% of experiments conducted; n=6). In contrast, during fall months when the mcyE gene was not expressed, zooplankton enrichment resulted in significantly reduced ($p < 0.05$) cyanobacteria biomass relative to control treatments in most experiments (80%; n=5). Regarding nutrients, bloom populations transitioned from nutrient replete during spring and early summer to N-limited during late summer when in situ N levels were depleted. Specifically, experimental N loading significantly increased *Microcystis* sp. biomass and microcystin concentrations relative to unamended control treatments at this time. In sum, these results suggest that the dominance of *Microcystis* sp. blooms during the summer is linked to both nutrient loading and the suppression of zooplankton grazing via toxin synthesis.

RECURRENT PRESENCE OF *PSEUDO-NITZSCHIA* AND DOMOIC ACID IN A PACIFIC NORTHWEST ESTUARY

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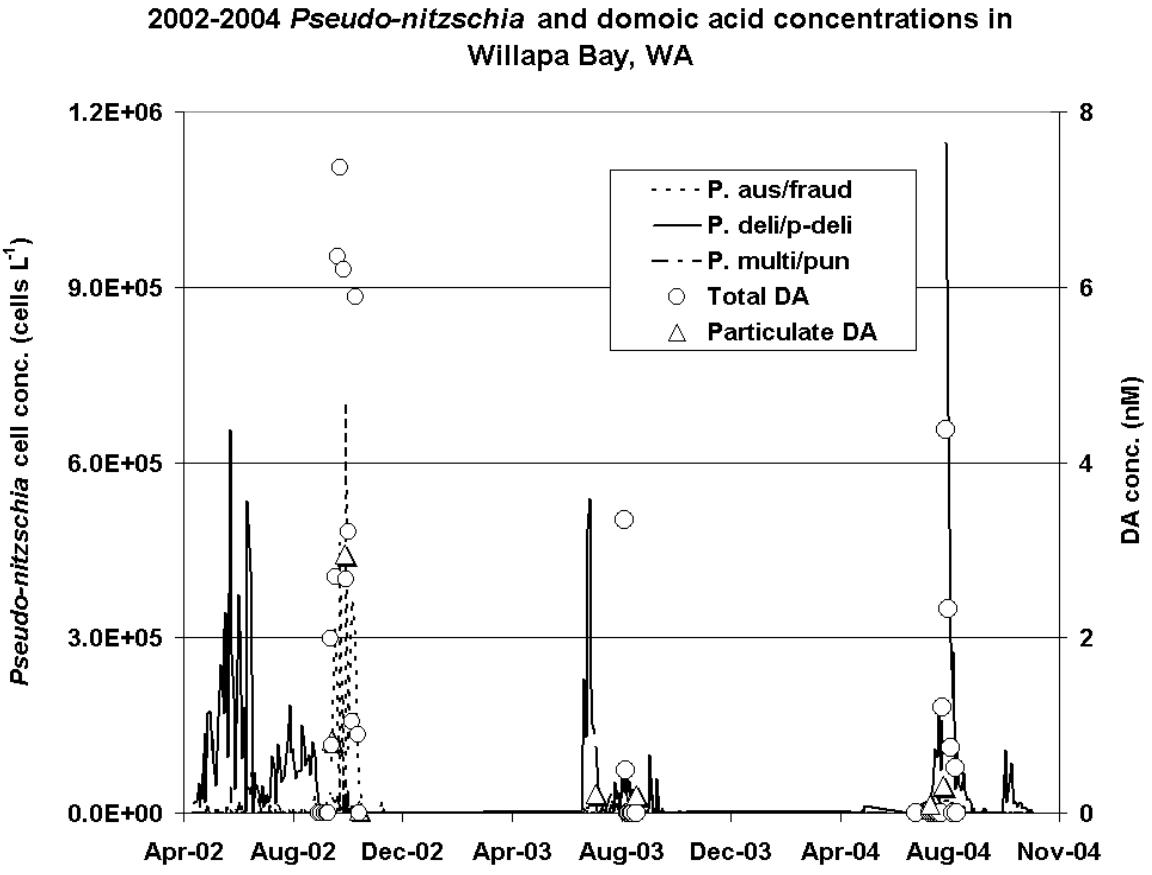
As members of the Olympic Region Harmful Algal Bloom (ORHAB) partnership we are tasked with monitoring for harmful algal species and assessing environmental conditions when these species occur in Willapa Bay; a coastal estuary in southwest Washington with an important commercial shellfish industry. Seasonal data obtained from a moored automated water sampler during summer 2002 through fall 2004 indicate the recurrent presence of toxic *Pseudo-nitzschia* species, the diatom responsible for production of the neurotoxin domoic acid (DA). We measured *Pseudo-nitzschia* species cell densities and corresponding particulate and total DA levels in preserved samples collected from an autosampler, and compared these values to basic oceanographic data (salinity, temperature, and fluorescence). Total DA concentrations peaked (7.36 nM) in 2002 during an extended period of high salinity (>30 psu) waters in the Bay. Preliminary analyses suggest an oceanic source for these species that were advected into the Bay by tidal and wind-driven forces. Our results indicate the continued need for HAB monitoring in coastal regions and within estuaries, including Puget Sound, which experienced the first shellfish closure in 2003 due to DA.



SUB-LETHAL AND LONG-TERM EFFECTS OF EXPOSURE TO DOMOIC ACID IN STRANDED CALIFORNIA SEA LIONS

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Acute domoic acid toxicosis resulting from the glutamate agonist action of domoic acid is well documented in California sea lions (*Zalophus californianus*) and is manifested as neurological signs, including ataxia, disorientation, seizures, and death. However, the long-term and sub-lethal effects of domoic acid toxicity have not been fully investigated. Reproductive failure as a result of abortion and premature parturition was observed in 149 of 442 intoxicated adult females admitted to rehabilitation centers in California between 1998 and 2002 that survived acute toxicosis. Domoic acid was detected by liquid chromatography with tandem mass spectrometry in amniotic fluid, fetal urine and gastric fluid samples tested up to 2 weeks after initial stranding. This suggests the fetus acts as a sink for domoic acid that is typically rapidly cleared from model mammalian species (half life in primates is 4 hours). Of a further 179 California sea lions that stranded since 2002 showing neurological signs typical of domoic acid exposure, 46% exhibited neurological effects longer than 2 weeks after initial stranding. Magnetic resonance imaging on live animals and histopathology from animals that either died or were euthanized revealed varying degrees of unilateral and bilateral hippocampal atrophy, neuronal necrosis and gliosis in the limbic system. Behavioral changes observed in these animals ante mortem included aggression, and stereotypical behaviors including flipper chewing and circling. These data suggest that exposure to domoic acid can have effects on sea lion reproduction and survival beyond acute mortality documented to date.

THE ROLE OF DISSOLVED ORGANIC MATTER IN HETEROTROPHIC DINOFLAGELLATE GROWTH

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Coastal eutrophication is recognized as an important factor affecting and sometimes regulating the dynamics of harmful algal blooms. Recent observations have emphasized the role of organic nitrogen to support the growth of autotrophic HAB's. *Pfiesteria spp.* and similar heterotrophic dinoflagellates are typically considered to be obligate predators, requiring live prey for growth. Recently, we reported the growth of the dinoflagellate *Pfiesteria shumwayae*, (VIMS 1049, CCMP 2089) on a lipid-rich dissolved organic media. Further aspects of the growth of heterotrophic dinoflagellates on dissolved media are reported here. Chemosensory capabilities of heterotrophic dinoflagellates are an important component of prey detection. In the present study, we compared the capability of multiple strains of *Pfiesteria shumwayae* and two strains each of *Pfiesteria piscicida* and Cryptoperidiniopsoid species for their chemosensory response to specific organic compounds and their capability for growth on those compounds. Further, we report on efforts to characterize specific components of the dissolved culture media with the goal of defining the essential nutrients required for *P. shumewayae* growth. Increased understanding of the nutritional requirements of *P. shumwayae* growth on dissolved organic media can be used to elucidate its response to organic enrichment in the environment and its role as a predator in the estuarine food web.

MOLECULAR DETECTION OF *KARENIA BREVIS* AND RELATED SPECIES USING SANDWICH HYBRIDIZATION ASSAYS

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Sandwich hybridization assays have proven useful for rapidly (6-8 samples/hr) detecting low cell concentrations of various toxic phytoplankton of public health significance. The brevetoxin (BTX) -producing dinoflagellate *Karenia brevis* is well known for many, episodic bloom events that have caused fishery closures and other economic impacts in the Eastern Gulf of Mexico. A semi-automated sandwich hybridization assay (SHA) was developed for *K. brevis* following the successful development of SHAs for other notable HAB taxa, including diatoms (genus *Pseudonitzschia*), raphidophytes (genera *Heterosigma* and *Fibrocapsa*) and thecate dinoflagellates (genus *Alexandrium*). The 5,000 cells/liter trigger level for fishery closures due to *K. brevis* is conservative for the species and accounts for possible bioaccumulation of BTXs in shellfish during the 24-hr period required for mouse testing. The required detection limit for *K. brevis* is consequently ~1,000 cells/liter, making detection by traditional methods problematic as the lower limit for microscopy is close to the required detection limit.

The SHA for *K. brevis* was designed to better manage brevetoxin risk by unequivocally identifying the species concerned from closely related species recently discovered in the Gulf of Mexico to a target cell concentration of 1,000 cells per liter or less. The standard curve for *K. brevis* (Wilson 1953 strain) shows that the required lower detection limit of is achievable (Fig. 1), and both laboratory and field testing have shown that the assay is specific to *K. brevis* (Fig. 2) even when closely related species are present. Assays have now also been developed for *Karenia mikimotoi, K. selliformis, K. papilionacea*, and *Karlodininium micrum* (Fig 3.) and are also target/species specific in the limited testing to date.

The advantage of these assays over traditional methods is that they can be incorporated into remotely deployed devices, such as the Environmental Sample Processor, to enable near real-time analysis on the basis of a user-defined sampling schedule. Challenges remain to be addressed, such as the issues of sampling handling, the reference strain providing the standard curve from which cell concentrations are back-calculated, and having a reliable method by which to verify cell counts, particularly when low cell concentrations are present.

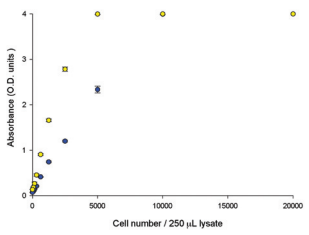

Fig. 1. Absorbance for the tri-dioxygenin (DIG) labeled K. brevis *probe in optical density units (blue=650nm, yellow=450 nm;* y *axis) for different cell concentrations per 250 mL lysate (*x*-axis).*

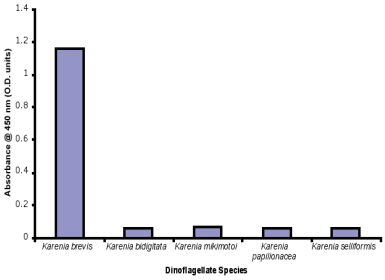

*Fig. 2. Absorbance in optical density units (*y *axis) for* Karenia brevis *(single fluor, 24,000 cells/liter) and four closely-related species at 30-50,000 cells/liter (*x*-axis).*

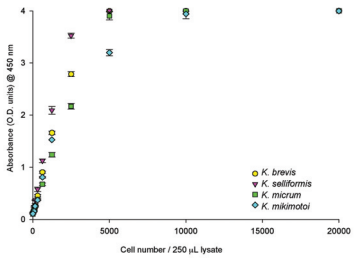

Fig. 3. Absorbance for the tri-dioxygenin (DIG) labeled probe in optical density units (450 nm; y *axis) for three species of* Karenia *in cell concentrations per 250 mL lysate (*x*-axis).*

NUTRIENT QUALITY DRIVES DIFFERENTIAL PHYTOPLANKTON COMMUNITY COMPOSITION ON THE WEST FLORIDA SHELF DURING A *KARENIA BREVIS* BLOOM

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Blooms of the toxic dinoflagellate *Karenia brevis* occur annually in the oligotrophic waters of the inner Florida shelf where low ambient nutrient concentrations make the detection of nutrient sources supporting these blooms problematic. Compounding the difficulties of detecting nutrient sources of these blooms are the inputs from multiple unique river systems (the Shark, Caloosahatchee, Peace and Manatee Rivers) to this region. In May 2003, a 5 day survey was conducted of over 65 stations in an area which encompassed both the coastal receiving waters of these rivers and a *K. brevis* bloom within this area. Designed to assess the influence of each riverine system on the regional patterns of phytoplankton community composition and nutrient utilization, the survey measured a variety of physical, chemical (dissolved inorganic and organic nitrogen and phosphorus) and biological (particulate carbon, nitrogen and phosphorus, phytoplankton community composition (HPLC pigments, direct microscopic counts), urease and phosphatase enzymes) parameters. Three chemically and biologically distinctive coastal regions were identified in the area from Tampa Bay south to Florida Bay, each of which was characterized by unique $N:P_{particulate}$ ratios, dominant nutrient forms, pigment ratios and urease and alkaline phosphatase activities. Between Tampa Bay and the Caloosahatchee River mouth, both PO_4 and DOP concentrations were elevated, a high percentage of total dissolved nitrogen was present as DON and $N:P_{particulates}$ were <5. A high peridinin: Chl *a* ratio occurred in this region, and alkaline phosphates activities of both dissolved, bacterial and phytoplankton were all elevated, signifying utilization of organic P within this region. This region received significant riverine dissolved P inputs which derived from the Hawthorn phosphate deposits, a regionalized phosphate rich area in central Florida. Further to the south in the area between the Caloosahatchee River mouth and the western Everglades, dissolved phosphorus concentrations decreased to <0.26 ?M while the relative contribution of NH_4 and urea to the total dissolved nitrogen pool increased. Urease activity was elevated and the region was generally characterized by elevated zeaxanthin: Chl *a* ratios. This region receives significant agriculturally derived nutrient inputs via the gated Caloosahatchee River. The southernmost region west of Florida Bay was characterized by elevated NO_3, with high fucoxanthin: Chl *a* ratios and undetectable PO_4. These coastal regions reflect the differing riverine inputs to each river, which in turn are influenced by the major landscape shifts that occur in southwest Florida. A high phosphorus input region to the north is superimposed upon a north-south gradient in dominant form of nitrogen inputs, from DON in the north, to NH_4 and urea in the central region to NO_3 in the southern region. A population of *Karenia brevis* (maximum concentration 2.15×10^5 cells L^{-1}) was located nearshore at the interface between the high PO_4 region to the north, and the high urea/NO_3 region in the central region. The $N:P_{particulate}$ of the bloom was between 16-18, suggesting that the interface between these two regions represented an optimal nutrient environment for *Karenia* growth and bloom maintenance.

A LAGRANGIAN VIEW OF THE JUAN DE FUCA EDDY: MACRONUTRIENTS AND CIRCULATION

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A number of surface drifters were deployed in the Juan de Fuca eddy during ECOHAB PNW cruises to study transport pathways and to allow shipboard measurements following a patch of water. In September 2004, three drifters were followed for periods of more than a week, with shipboard profiles of biological/chemical/physical water properties measured as frequently as 3 hr intervals. During this cruise, the bloom of *Pseudo-nitzschia* in the Juan de Fuca eddy was highly toxic, containing up to 15 million cells per liter of *P. cuspidata*. One of the drifters was followed as it entered, transited and exited the eddy region, and the water mass that it tracked was sampled for a period of ten days; another drifter remained in the eddy for 21 days, clearly illustrating the retentive nature of the eddy. Particulate domoic acid and chlorophyll increased and then decreased following the drifter pathways.

Comparisons between nitrate and temperature or salinity data distinguish the biological versus physical control of the nutrient supply. For example, differences in trends in temperature and nitrate in shallow layers versus deeper layers provide a clear illustration of biological utilization in surface layers versus re-supply at deeper layers during the first few days of the drift. The data demonstrate that the Juan de Fuca eddy has several physical mechanisms for providing nitrate to sustain offshore blooms—these processes distinguish the eddy region from the coast and help to sustain the toxic blooms observed in the eddy. Internal wave activity is accentuated in this region by the complex topography and the pycnocline is shoaled over the eddy throughout the summer season. Accordingly, vertical motion due to internal wave activity in the upper 50 m causes large variability in all data series at a fixed depth, with colder, more saline water associated with higher nitrate, chlorophyll-depleted water. The upward movement of the high nitrate waters, combined with wind mixing could provide a mechanism to re-supply nutrients to near surface layers in the eddy region. The time series also elucidated another important mechanism for re-supplying nutrients to the upper water column—cooling at the surface followed by vertical mixing, a process not usually considered important in coastal upwelling systems. This cooling occurs only during periods of downwelling favorable winds, generally associated with colder air temperatures in this region in summer.

DEFINING ECOLOGICAL NICHES WITHIN A MULTI-SPECIES RAPHIDOPHYTE HAB CONSORTIUM

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Mixed blooms of toxic raphidophytes with one or more co-dominant members have recently invaded Delaware's shallow, eutrophic Inland Bays ecosystems. HAB events worldwide are usually dominated by a single species, or more rarely by a group of species within the same genus. In contrast, these blooms are often formed by a consortium of at least four separate taxa of raphidophytes, including *Heterosigma akashiwo*, *Chattonella subsalsa*, *Chattonella* cf. *verruculosa*, and *Fibrocapsa japonica*. Several distinct strains of these individual species also appear to be present.

This unusual multi-species invasion suggests that a new HAB niche may have recently opened in these bays with selection occurring at the class rather than at the species level. It is unknown what factors may have favored raphidophytes over other algal groups, and inter-specific competitive dynamics within this consortium are also not understood. Determining how each species manages to avoid competitive exclusion by other members of the consortium poses a challenging question that has applications not only for understanding these damaging HAB occurrences, but also raises fundamental issues about the nature of competitive interactions in basic phytoplankton ecology.

As part of a multi-disciplinary EPA STAR ECOHAB project, we have investigated the dynamics of these blooms over the last two years. Here, we attempt to define the similarities and differences in the ecological niches of two of these sympatric raphidophyte species, *Heterosigma akashiwo* and *Chattonella subsalsa*. Using experiments with cultured isolates and concurrent observations of field blooms, we have been able to begin to define the preferred environmental conditions that characterize blooms of each species. *H. akashiwo* tolerates the entire range of salinities and temperatures encompassed by *C. subsalsa*, and also thrives at much lower values. Both species have similar growth responses to light. *H. akashiwo* has half saturation constants for growth on nitrate, ammonium, and phosphate that allow it to maintain equivalent growth rates at nutrient levels up to an order of magnitude lower than *C. subsalsa*, and also has a higher maximum growth rate at saturating nutrient concentrations. Finally, *H. akashiwo* has the ability to use organic nitrogen sources like urea that *C. subsalsa* cannot.

We use these results to define the preferred physical and chemical niches of each species. When combined with physio-chemical models of the estuarine systems where they occur, this approach has the potential to empirically define the areas where blooms of each species are likely to occur. Our results suggest that *Heterosigma akashiwo* is the superior competitor relative to *Chattonella subsalsa* under virtually all conditions of temperature, salinity, and light and nutrient availability. The persistence of *C. subsalsa* as a co-dominant species in the Inland Bays despite the ubiquitous presence of *H. akashiwo* suggests that other factors restrict the ecological dominance of the latter species. We suggest that top-down control by micro-grazing, which significantly impacts *H. akashiwo* abundance but not that of the much larger cells of *C. subsalsa*, may serve to offset the competitive advantage of *Heterosigma* for virtually all bottom-up control variables and allow both species to co-exist during mixed bloom events.

ENHANCING PUBLIC HEALTH AND SAFETY THROUGH DISTRIBUTED TESTING: MODELS IN THE USA USING JELLETT RAPID TESTS

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The advent of field testing using Rapid Tests for PSP, ASP and DSP toxins which operate much like the home pregnancy kit to detect the marine biotoxins has recently made distributed testing possible. Distributed testing can enhance public health and safety in many ways - by enabling pre- and post-harvest management in the aquaculture industry and wild fisheries, by providing access to remote areas, by increasing overall sampling frequency and, in the case of phytoplankton monitoring, by providing early warning of impending shellfish toxicity. Distributed testing can augment the information available to the centralized testing authority thereby widening the safety net. The economic case for significant budgetary savings for regulatory authorities that can be achieved through distributed testing will be presented. Three models of distributed testing being investigated in Washington, California and Alaska will be discussed, along with some of the advantages and challenges associated with these three distributed testing schemes.

THE START STORY: EXPANSION FROM A SMALL LOCAL EFFORT TO STATEWIDE EFFORTS AND THE BENEFITS TO THE HAB COMMUNTIY

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Solution to Avoid Red Tide (START) is a grassroots citizen's organization with the mission to control and mitigate red tides in an environmentally safe manner. In 1996, START was formed after a severe Florida red tide remained on the shores of western Florida for 15 months. The initial direction of START remains the same as it was in1996, to focus the science community on applied research for development of mitigation techniques. START has established many important relationships between the tourism and visitor's bureau's in Florida and the science community. Implementation of a travel and leisure program aimed at disseminating accurate information about red tide as a mitigation tool was lead by START. Focus groups of tourism industry leaders facilitated by START established community buy in to the program. Currently in Florida, new outreach materials are being developed and START will again introduce them to the tourism community and provide feedback so the materials are embraced and used by the community. In the Fall of 2004 when NOAA/NOS went operational with the HAB Bulletin, great concern was voiced individually by local tourism leaders about the Bulletin. START arranged for a meeting of scientists from the NOAA HAB Bulletin office, Florida Wildlife Research Institute, Mote Marine Laboratory, and community tourism and visitor's bureau leaders. After discussion about the Bulletin and community concerns, modifications to the dissemination of the Bulletin were made by NOAA. START has also lobbied at the Local, State, and Federal level to continue to fund research projects and minimize the impact yo-yo funding has on progress in HAB science.

START has grown from a small community based organization to a multi- county organization with chapters based in Longboat Key, Bonita Springs, Boca Grande, Naples, and soon to be added, Sanibel. The relationship START has with both the science community and the business community is unique and can greatly assist the HAB community. Discussions have already occurred suggesting it should be a national organization.

ENVIRONMENTAL EXPOSURES TO FLORIDA RED TIDES: EFFECTS ON EMERGENCY ROOM RESPIRATORY DIAGNOSES ADMISSIONS

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Human exposure to Florida red tides formed by *Karenia brevis*, occurs from eating contaminated shellfish and inhaling aerosolized brevetoxins. Recent studies have documented acute symptom changes and pulmonary function responses after inhalation of the toxic aerosols, particularly among asthmatics. These findings suggest that there are increases in medical care facility visits for respiratory complaints and for exacerbations of underlying respiratory diseases associated with the occurrence of Florida red tides.

This study examined whether the presence of a Florida red tide affected the rates of admission with a respiratory diagnosis to a hospital emergency room in Sarasota, FL. The rate of respiratory diagnoses admissions were compared for a 3-month time period when there was an onshore red tide in 2001 (red tide period) and during the same 3-month period in 2002 when no red tide bloom occurred (non red tide period). There was no significant increase in the total number of respiratory admissions between the two time periods. However, there was a 19% increase in the rate of pneumonia cases diagnosed during the red tide period compared with the non red tide period. We categorized home residence zip codes as coastal (within 1.6 km from the shore) or inland (greater than 1.6 km from shore). Compared with the non red tide period, the coastal residents had a significantly higher (54%) rate of respiratory diagnoses admissions than during the red tide period. We then divided the diagnoses into subcategories (i.e. pneumonia, bronchitis, asthma, and upper airway disease). When compared with the non red tide period, the coastal zip codes had increases in the rates of admission of each of the subcategories during the red tide period (i.e. 31%, 56%, 44%, and 64%, respectively). This increase was not observed in the inland zip codes. Finally, during the red tide period, the coastal residents had an increased risk of emergency room admission for all respiratory conditions when compared with the inland residents.

These results suggest that the healthcare community has a significant burden from patients, particularly those who live along the coast, needing emergency medical care for both acute and potentially chronic respiratory illnesses during red tide blooms.

TOWARDS RECOGNITION OF CRYPTIC FUNCTIONAL DIVERSITY IN NATURAL HAB POPULATIONS

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The molecular revolution has allowed the development of techniques to rapidly and accurately identify harmful bloom-forming species of algae in environmental samples. More recently, these techniques have allowed researchers to track the intra-specific population dynamics of phytoplankton in their environment. The most commonly used molecular markers in these studies are targeted to highly conserved rDNA sequences or rapidly evolving non-coding regions such as microsatellites. Neither of these molecular targets, however, is necessarily correlated with physiologically relevant functional traits.

Given the very large, rapidly growing populations typical of a harmful bloom, spontaneous mutants are certain to arise, at least some of which are likely to be adaptive. If these adaptive mutants out-compete the rest of the population to fixation, a new phenotype dominates. Under this "selective sweep" model, the genotype of the new population will differ only at the gene which determines the adaptive function. For this reason, the change in population structure will not be detected by molecular approaches targeted to anonymous or non-coding regions of the genome. It is our contention that current molecular approaches to studying HAB populations operationally exclude cryptic functional diversity of ecological relevance.

We will present data from laboratory studies which model selective sweeps in the toxic alga *Heterosigma akashiwo*. We have shown that selectable mutants spontaneously arise in batch culture and under altered environmental conditions (e.g. iron limitation, divalent ion stress, reduced salinity) these mutants become adaptive and sweep to fixation. Efforts to characterize the molecular mechanisms of these adaptations will be presented. The long term goal of this study is to develop functional markers for interrogating natural HAB populations in order to be able to track adaptive responses to changes in the environment.

SAXITOXIN MONITORING IN FLORIDA: ONE MORE TOXIN TO DEAL WITH

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With a wide range of habitats, climates, and biological diversity, Florida's coastal waters continue to experience new and sustained impacts from HABs. Florida has now documented more than fifty harmful algal species; all major groups with a potential to affect public health, cause economic losses, and impact ecological resources. In early 2002, with the advent of puffer fish poisoning (PFP) originating from the Indian River Lagoon (IRL), saxitoxin was discovered in Florida's marine waters (Quilliam et al., 2002) and associated with the dinoflagellate *Pyrodinium bahamense* for the first time in the United States (Landsberg et al., 2002). The sudden appearance of saxitoxins at potentially lethal concentrations in an area previously unknown to have such toxins, signals a new and unprecedented public health and natural resource problem for Florida. Elsewhere, saxitoxins are usually associated with Paralytic Shellfish Poisoning (PSP), an acute intoxication occurring in humans after the consumption of toxic shellfish. When filter-feeding shellfish consume and retain saxitoxins in their meats, and humans consume toxic shellfish in non-regulated areas, severe and potentially lethal cases of PSP can occur. As neurotoxins, saxitoxins block nerve transmission and cause tingling and numbness, paralysis, and loss of motor control. In extreme cases, death can result. Throughout many areas of the world, however, and particularly in the United States, the prevention of PSP is well regulated by state and federal agencies. In Florida's marine waters, prior to 2002, there was no risk from PSP. Since the detection of saxitoxins in Florida's puffer fish, the state initiated an intensive statewide monitoring program to determine concentrations and distribution of saxitoxins in various species, particularly puffer fish, and to monitor *Pyrodinium bahamense* in the IRL. Because they are immune to the effects of saxitoxins, puffer fish can accumulate high toxin concentrations in the muscle, making them an extreme threat to consumers. Following Food and Drug Administration (FDA) action levels (80 µg STX eq./100g meat) for acceptable limits of saxitoxins in seafood, the FWC has banned puffer fish harvesting in the IRL since 2002. The Florida Department of Agriculture and Consumer Services (FDACS) and FWC are also monitoring target shellfish species in the IRL. Thus far, apart from puffer fish that consistently exceed the action limit for saxitoxin levels, shellfish beds (*Mercenaria* sp.) in the northern IRL were closed as a precautionary measure for a six day period only in October 2003, just exceeding the acceptable limit. Trace or below action levels of saxitoxins have been confirmed in a wide variety of fish and invertebrates. The question of why *P. bahamense* blooms have suddenly become a problem in the IRL is part of a three-year collaborative study with the FDA and NOAA and funded by ECOHAB. Subject to characterization by HPLC and LCMS, trace saxitoxins have also been detected by rapid screening methods (ELISA Ridascreen – Flewelling et al., unpub. data) in a number of species in freshwater or low salinity habitats outside of the IRL where *Pyrodinium* is absent. Other potential microalgal sources for saxitoxins are being investigated.

Landsberg, J. H. et al. 2002. Pufferfish poisoning: widespread implications of saxitoxin in Florida. Xth International Conference on Harmful Algae, St. Petersburg Beach, Florida, October 2002, Abstr. p. 160.

Quilliam, M., Wechsler, D., Marcus, S., Ruck, B., Wekell, M. and Hawryluk, T. 2002. Detection and identification of paralytic shellfish poisoning toxins in Florida pufferfish responsible for incidents of neurologic illness. Xth International Conference on Harmful Algae, St. Petersburg Beach, Florida, October 2002, Abstr. p. 237.

GROWTH AND TOXICITY OF THE DINOFLAGELLATE, *GAMBIERDISCUS TOXICUS*, UNDER NITROGEN AND PHOSPHORUS LIMITATION

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Ciguatera fish poisoning is the most common seafood intoxication in humans and results from the consumption of tropical marine finfish contaminated with lipid-soluble toxins produced by the dinoflagellate *Gambierdiscus toxicus* Adachi et Fukuyo. Although *G. toxicus* was identified as the causative agent of ciguatera in 1977, the factors that determine its toxicity are still poorly understood. In order to better understand the role of nutrient limitation in the toxicity of *G. toxicus*, two Caribbean strains (CCMP 1651 and 1655) of the toxic dinoflagellate were grown in xenic batch cultures under either nitrogen (N) or phosphorus (P) limitation. The cultures were maintained at 28 °C, 35 psu, 100 µmol m^{-2} s^{-1} (12:12 light:dark cycle) in modified L2 media. N-limited cultures were grown on one of four N sources (nitrate, ammonium, a mix of free amino acids, or putrescine) at an initial concentration of 50 µM N. P-limited cultures were grown on one of three P sources (phosphate, µ-glycerophosphate, or a mix of nucleotides) at an initial concentration of 3 µM P. Both strains grew on all of the N and P sources with growth rates ranged from 0.11 d^{-1} to 0.18 d^{-1}. Inorganic and organic nutrient sources supported similar growth rates with the exception of strain 1655 under N-limited growth which exhibited significantly faster growth on ammonium versus the other N sources. During exponential growth under N-limitation, the growth rate remained constant. During exponential growth under P-limitation, the growth declined as N:P increased. Total N and P pools were uncoupled from conditions in the water and cell division continued after the limiting nutrient was below detectable limits. Overall N:P varied from 3 to 34 in strain 1651 and 2 to 37 in strain 1655. Chlorophyll per cell peaked during exponential growth under N-limited growth, but remained constant under P-limited growth. Under N-limited growth, strain 1655 was significantly more toxic in stationary phase (0.05 pg C-Ctx Eq $cell^{-1}$) compared to exponential phase (0.03 pg C-Ctx Eq $cell^{-1}$) with the N source having no effect on toxicity. Overall, the use of organic substrates and the variability in the internal N and P pools suggest that *G. toxicus* can take advantage of a variety of nutrient sources that are available on short time scales to support future growth. Instantaneous growth rate can be supported by nutrients acquired hours to days previously. This storage ability gives *G. toxicus* the ability to grow for significant periods in the absence of ambient nutrient inputs. As a result, *G. toxicus* abundance in the field may depend on past as much as present nutrient availability.

UPS AND DOWNS IN THE LIFE OF A TOXIC *PSEUDO-NITZSCHIA* BLOOM IN THE JUAN DE FUCA EDDY OFF THE WASHINGTON COAST

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In September 2004, we followed the population dynamics of a highly toxic *Pseudo-nitzschia cuspidata* bloom in the Juan de Fuca Eddy for ten days. This was an unusual bloom, as it was co-dominated by *P. cuspidata* and a vertically migrating small (ca. 15 µm) flagellate. Dilution growth and grazing experiments were performed daily at the depth of the 50% light level (2-4 m) while following a drifter. At the beginning of the drift, *P. cuspidata* abundance was already relatively high (3700 ml^{-1}) and cells were toxic (3 fM domoic acid $cell^{-1}$), indicating the bloom was already in progress. Over the course of the drift, *P. cuspidata* abundances oscillated up and down, but there was generally an increase followed by a decline. *P. cuspidata* abundance, as well as total chlorophyll, varied inversely with nitrate in the surface water, indicating nitrate utilization, and nitrate was depleted by the end of the drift as the drifter left the eddy. The up and downs of cell numbers and nutrients were in part due to physical processes in the eddy mixing nitrate into the surface waters, as well as growth. *P. cuspidata* growth rates were moderately high (0.5 - 0.94 d^{-1}) and did not appear to be nitrogen limited until the drifter left the eddy, where growth rate was very low (0.02 d^{-1}). Grazing rates on *P. cuspidata* were very low, even though microzooplankton biomass was relatively high, which contributed to the accumulation of *P. cuspidata*, but could not account for the declines. Particulate domoic acid (DA) reached >40 nM and followed cell numbers closely. Cellular DA therefore remained relatively constant throughout the bloom, but showed a modest increase (from 3 to 5 fM $cell^{-1}$) as nitrate fell below 2 µM. Towards the end of the bloom, as cell numbers dropped from their peak, dissolved DA increased dramatically, from ca. 2 to 15 nM; the release may have been related to micronutrient availability. Neither particulate nor dissolved DA was correlated with grazing rate, nor was grazing rate affected by additions of dissolved DA. Therefore, DA did not appear to be the cause of low grazing on *P. cuspidata*.

INTRAPOPULATION VARIATION OF *ALEXANDRIUM FUNDYENSE* WITHIN THE GULF OF MAINE: RIBOSOMAL DNA AND MICROSATELLITE ANALYSES

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Sequencing of the ribosomal DNA (rDNA) regions and analysis of polymorphic microsatellite markers were used to assess the population diversity and structure of the toxic dinoflagellate *Alexandrium fundyense* in the Gulf of Maine. The rDNA analysis examined nine cultured *A. fundyense* strains that were used to represent the extent of geographic diversity in the Gulf of Maine and adjacent areas. These nine strains were originally isolated from various locations in the northeastern U.S., from Long Island to the Bay of Fundy and the St. Lawrence estuary. In all, over 2700 bases of the ribosomal DNA operon were sequenced from each culture, including the D1-D6 and D8-D10 regions of the LSU rDNA and the ITS1/5.8S/ITS2 rDNA regions. Very little variability was observed in the ribosomal regions, with the nine strains exhibiting greater than 99.5% similarity. Phylogenetic trees produced from these sequenced regions provided no resolution.

To provide greater resolution of the genetic relationships between these closely related strains, we employed microsatellite markers developed by Nagai et al. (2004)[1] to genotype *A. fundyense* strains. We are currently characterizing four microsatellite loci in a collection of 54 clonal isolates of *A. fundyense* established from several sites within the Gulf of Maine. Results to date indicate that 1) multiple alleles of each locus are present within the Gulf of Maine strains i.e. the loci are polymorphic across the wider region; 2) polymorphism is observed within groups of strains isolated from the same location; and 3) microsatellite data are able to resolve differences within this population that are not evident at the rDNA level. Our analysis of inter-strain relatedness and population genetic structuring reveals significant intra-population genetic heterogeneity that may be indicative of different genotypic groups or clusters of *A. fundyense* within the Gulf of Maine.

[1] *Nagai S, Lian C, Hamaguchi M, Matsuyama Y, Itakura S, Hogetsu T (2004) Development of microsatellite markers in the toxic dinoflagellate* Alexandrium tamarense *(Dinophyceae).* Molecular Ecology Notes ***4****, 83-85.*

CIRCULATION AND BIOLOGICAL MODELING IN THE ECOHAB PNW REGION

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Recent studies suggest that the Juan de Fuca Eddy, a seasonal nutrient-rich retentive feature off the Washington and British Columbia coasts, may be an initiation site for the toxic *Pseudo-nitzschia* blooms that impact shellfish along the Washington coast. As part of ECOHAB PNW, models are being utilized i) to simulate the ocean circulation during and after field surveys, ii) to conduct process studies into the mechanisms underlying the generation and collapse of the eddy and iii) to investigate mechanisms of bloom formation in the Juan de Fuca Eddy and the regional scale transport of *Pseudo-nitzschia* to the coast. Both diagnostic and prognostic models (ROMS) are used. The prognostic circulation model is coupled to an NPZ model for studies of bloom formation.

Results to date from hindcasts of the field surveys show a more developed Juan de Fuca eddy later in the upwelling season contributing to a broader region of high nutrients off the mouth of the Juan de Fuca Strait. Under intermittent upwelling/downwelling conditions (September 2004), a much tighter recirculation is observed in the eddy and residence time increases dramatically, allowing for an extended bloom duration. The prognostic model runs examine the relative importance to eddy formation, duration and magnitude of a number of factors, including variable wind stress, strait outflow and the strength of the shelf edge coastal jet as well as interactions with the underlying topography. The export of water from the eddy to the adjacent shelves as well as onshore transport of eddy water is also investigated.

A NATIONAL HARMFUL ALGAE INDICATOR TO MONITOR THE CONDITION OF COASTAL WATERS IN THE UNITED STATES

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In 1992 the H. John Heinz III Center for Science, Economics and the Environment, published *The State of the Nations Ecosystems*, a report that serves as a blueprint for periodic reporting on the condition and use of ecosystems in the United States. The report identified harmful algae as one of sixteen indicators for coastal ecosystems in the U.S., but stopped short of identifying a specific metric to measure harmful algae.

During a recent workshop, we proposed that most harmful algae could be measured on a national scale using two complementary metrics that focus on event intensity. The first metric would report on the percentage of coastline monitored in state shellfish programs that exhibits harmful algal events of low, medium or high intensity. The level of intensity would be determined by the duration of the event and the maximum concentration of the toxin in shellfish. The definition of these intensity levels would be region- and toxin-specific and would be identified in consultation with state shellfish managers. The second metric, harmful algal events in nearshore areas (including bays and estuaries), would capture events in areas where toxins are not regularly monitored in shellfish programs. It would report on the percentage of observations at sentinel stations and coastal monitoring programs that describe harmful algal events of varying degrees of intensity. Intensity would be determined by cell count/toxin concentration and the duration of the event. As with the shellfish metric, intensity thresholds would be set separately for each region and each species or toxin, and would be scaled to a national level based on its intensity rating.

The two metrics were not designed around currently available data. With some additional monitoring, the two metrics could be feasibly measured in the near-term and would cover most harmful algal events in coastal ecosystems. In the long-term, if a more extensive remote monitoring system is in place, these metrics could be replaced by a single metric that quantifies the number of observations of harmful algal species within ~ 50 miles of the shore that are above a certain intensity threshold.

The indicator would not report on algal species that cause harm as a result of oxygen depletion, habitat loss, starvation, or respiratory or reproductive failure in animals because of their high abundance or biomass. These species are covered to some extent by other indicators in the Heinz Center report.

ECDYSIS AS A DEFENSE MECHANISM AGAINST BACTERIAL COLONIZATION: THE CASE OF THE DINOFLAGELLATE *LINGULODINIUM POLYEDRUM*

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Ecdysis, the asexual formation of temporary cysts by some species of thecate dinoflagellates, has previously been shown to occur in response to adverse conditions, including rapid changes in temperature, nutrient stress, and the addition of chemicals including allelopathic substances. One previous study (Nagasaki et al. 2000, Nippon Suisan Gakkaishi 66[4] 666-673) described cells from a dinoflagellate culture forming temporary cysts to resist attack by an algicidal bacterium, which kills by attachment. No other information about algicidal bacterial colonization of dinoflagellates or its influence on ecdysis currently exists. In this study, we tested hypotheses about bacterial colonization of the dinoflagellate *Lingulodinium polyedrum* and its influence on ecdysis. We hypothesize that bacterial colonization is detrimental to the dinoflagellates, and that ecdysis is an adaptation to remove unwanted bacterial colonizers from the dinoflagellate surface.

We have isolated several bacterial strains that cause previously axenic *L. polyedrum* cultures to lose their motility and undergo ecdysis within 2 to 4 days following bacterial inoculation. Using the technique of tyramide-signal amplification fluorescent *in-situ* hybridization (TSA-FISH) with DNA probes, we are able to quantify bacterial attachment to *L. polyedrum* cells over the course of co-incubation experiments (see Figure 1). Preliminary results indicate that colonization occurs before ecdysis, which is consistent with our hypothesis. Furthermore, removing the bacterial cells from the temporary cysts (by washing with sterile seawater) allows the cysts to regain their motility. In light of the knowledge that temporary cysts of *L. polyedrum* occur in the field, we will discuss our findings with respect to bloom ecology and bacterial interactions with dinoflagellates.

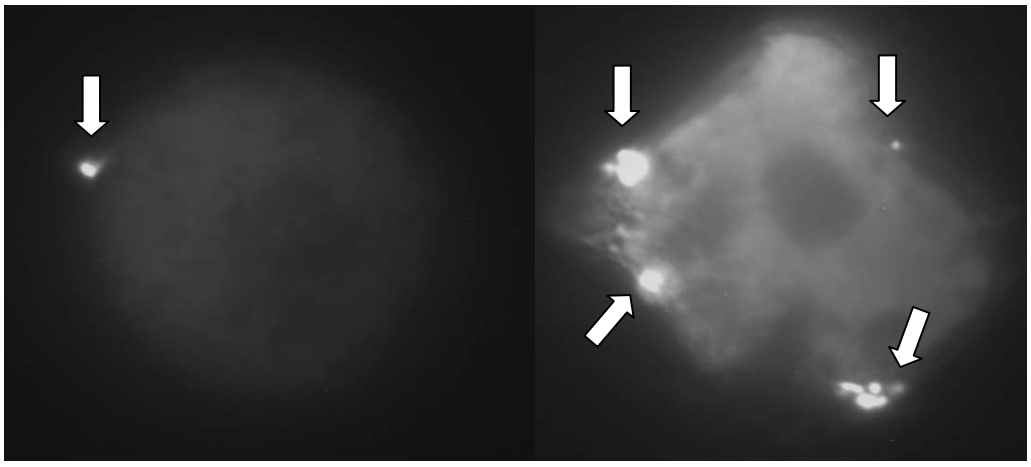

Figure 1: *Greyscale epifluorescent micrographs of TSA-FISH (eubacterial probe) treated* L. polyedrum *cells incubated with a bacterial culture. Arrows point to bright signal from bacteria. Left:* L. polyedrum *cell with one attached bacterial cell. Right:* L. polyedrum *cell with ~20 attached bacterial cells.*

BREVETOXIN AND POLYKETIDE SYNTHASE GENE EXPRESSION UNDER LOW-NUTRIENT CONDITIONS IN THE DINOFLAGELLATE, *KARENIA BREVIS*

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Karenia brevis is a dinoflagellate that forms blooms off the Gulf coast of Florida producing red tides that cause marine mammal mortalities, shellfish poisonings, and respiratory illness through the production of brevetoxins. Brevetoxins belong to a group of secondary metabolites called polyketides produced by a family of enzymes known as polyketide synthases (PKSs). PKS genes have been identified in fungi, bacteria, and several species of dinoflagellates; however, whether these genes are expressed by the dinoflagellates or co-occurring bacteria remains controversial. We have identified several genes through high throughput sequencing of a cDNA library to *K. brevis* with similarity to PKS genes in other organisms that also align at the protein level to various regions of a *Nostoc* PKS gene. Many of these ESTs have been successfully sequenced from the 3' end and contain a poly-A tail, suggesting these genes are of eukaryotic origin.

In an effort to assign a functional role of these probable PKS genes in brevetoxin biosynthesis, we next sought to determine if their transcript levels followed the same expression pattern as brevetoxin under differing experimental conditions. A number of factors, such as nutrient limitation (N and P), salinity, and antibiotic treatment, have been shown to alter brevetoxin expression in laboratory isolates of *K. brevis*. Here we grew cultures of *K. brevis* under low concentrations of nitrate (50µM, 10µM, and 1µM) and phosphate (5µM, 1µM, and 0.1µM). The exponential growth rates of the low nutrient cultures were similar to the growth rate seen in control cultures, but nutrient limited cultures progressed into stationary phase earlier than control cultures in a concentration dependent manner. Brevetoxins and RNA were extracted in parallel experiments from nutrient limited cultures and control cultures on days 12 (late log) and 17 (stationary phase) of growth. Brevetoxin concentrations, measured by receptor binding assay and HPLC-MS/MS, showed small differences in the low nutrient cultures from the controls at day 12, but by day 17, all low nutrient cultures expressed higher cellular concentrations of brevetoxin than the controls. Real-time PCR analysis of four of the probable PKS sequences examined indicated that there was little or no change in expression at day 12, but all of the PKS genes were upregulated by day 17 relative to control cultures. The correlation between PKS gene expression and brevetoxin expression levels provides the first evidence that these genes are involved in brevetoxin biosynthesis in *K. brevis*.

UTILIZATION OF VOLUNTEERS TO MONITOR HARMFUL ALGAL BLOOMS: THE SOUTHEASTERN PHYTOPLANKTON MONITORING NETWORK

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The SouthEast Phytoplankton Monitoring Network (SEPMN) has been in existence for four years. Starting with 3 groups localized around Charleston, SC, this program has grown to include 46 groups monitoring over 62 sites along the coast of North Carolina, South Carolina and Georgia. Volunteer groups are composed of school students, volunteer citizen groups, Aquariums and State park personnel. SEPMN is designed in cooperation with North Carolina, South Carolina and Georgia teachers and citizen groups to create a participatory educational exchange between students, volunteers and researchers and increased knowledge of harmful algae in the South Atlantic.

Each volunteer group is instructed on basic phytoplankton identification and sampling techniques. The groups are supplied with a 20µm plankton net, refractometer, thermometer, and an Olympus MIC-D digital microscope. These microscopes allow greater resolution for detailed examination of phytoplankton which is not typically available with microscopes in schools. Because of the digital format of the microscopes, pictures of potential harmful algae can be sent to NOAA's Marine Biotoxins Program via the internet for faster identification. Communication between researchers and volunteer groups is facilitated by an interactive web site (http://www.chbr.noaa.gov/CoastalResearch/SCPMN/). The web site also includes a photo library of all species examined by the monitoring groups.

Observation and identification of phytoplankton along the North Carolina, South Carolina and Georgia coast will be useful in developing a species list and record of distribution patterns, as well as alerting scientist to the presence of potentially harmful species. Potential and known HAB species observed by the monitoring network include *Dinophysis*, *Pseudo-nitzschia* and *Prorocentrum*.

POPULATION DNA DISTRIBUTION, CELLULAR DNA CONTENT, AND THE DIEL DNA CELL CYCLE OF CULTURED *KARLODINIUM* SPP. (DINOPHYCEAE)

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Seven strains of *Karlodinium veneficum* and one strain of *Karlodinium* cf. *armiger* were examined for DNA content by flow cytometric analysis of cells stained with the DNA fluorophore SYBR Green. Each strain exhibited distinct 1C, intermediate, and 2C DNA subpopulations indicative of respective eukaryotic cell cycle phases G1, S, and G2M. The measured 1C DNA content of *K. veneficum* was estimated using rainbow trout erythrocyte nuclei as an internal standard, and varied from 7.8 ± 0.65 pg cell^{-1} (mean ± 1 SD) in a strain from Norway (CCMP415) to 14.7 ± 1.5 pg cell^{-1} in a strain from Spain (CSIC-1). The five tested strains from the U.S. (Maryland and South Carolina) did not differ significantly in measured 1C DNA content (11.2 ± 0.6 pg cell^{-1}). The estimated 1C DNA content of *K. armiger* (GC-3) was 32.9 ± 2.4 pg cell^{-1}, a significantly higher amount than was found in *K. veneficum*. It was unknown whether chromosome number also varied with DNA content.

K. veneficum strains from Norway (CCMP415) and South Carolina (CCMP2282) were examined for cell cycle progression over 48 h. Both had a similar diel periodicity of DNA synthesis (S) and cell division (G2M) that were phased with the 12:12 (h) light:dark photocycle (Fig. 1). Cells with 1C DNA (G1) entered S phase late in the light period, and the maximum proportion of cells in S phase (15-22%) occurred near the light/dark transition. The G2M maximum (10-13%) occurred near the middle of the dark period, and the majority of G2M cells completed cell division by the end of the dark period. The duration of S + G2M was about 12 h.

Cells with two longitudinal flagella, a feature generally attributed to dinoflagellate planozygotes, were consistently observed in *Karlodinium* spp. cultures. Thus, some cells with 2C DNA may have formed by gamete fusion rather than DNA synthesis. Cells with 4C DNA, as would be expected in a conventional meiotic cycle, were not detected in appreciable numbers (< 0.3%). If persistent sexuality occurred, the observed lack of 4C DNA cells could have implications for the meiotic process in *Karlodinium* spp. Alternatively, *Karlodinium* vegetative cells may develop two longitudinal flagella prior to asexual division, as was originally reported for this dinoflagellate more than 40 years ago [1,2]. This possibility requires further study.

1. Ballantine D. 1956. J. Mar. Biol. Ass. U.K. *35:467-474.*
2. Leadbeater B. & Dodge J.D. 1967. Archiv fur Mikrobiol. *57:239-254.*

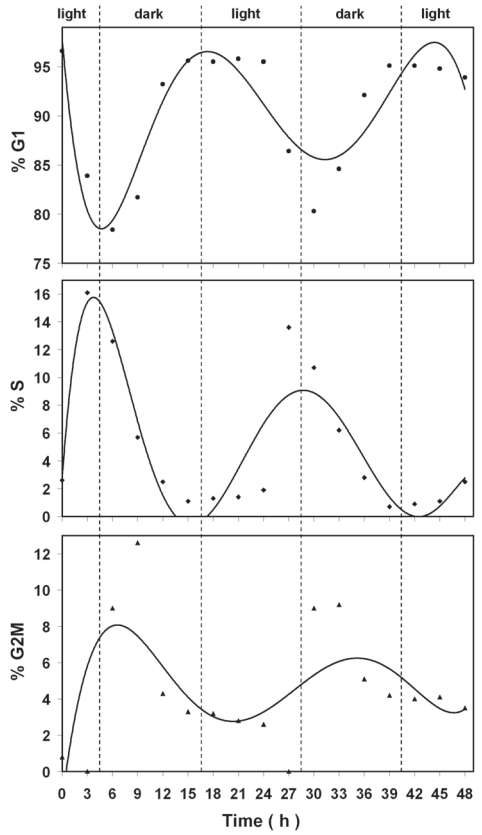

Fig. 1. K. veneficum *(CCMP415) cell cycle phase fractions G1, S, and G2M over 48 h. Curves are 5th degree polynomials fitted to the phase fraction data.*

THE AUTECOLOGY OF *GAMBIERDISCUS* IN THE COASTAL WATERS OF HAWAII

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Ciguatera is a reef fish-borne food poisoning caused by bioaccumulated and biomagnified toxins produced by the benthic dinoflagellate, *Gambierdiscus toxicus*. While the causal organism is known, the temporal and spatial patchiness of ciguatera outbreaks confounds our efforts to study the environmental factors leading up to a ciguatera outbreak. Ciguatera is a common threat in Hawaii, but locally-caught fish were not implicated in ciguatera cases until the mid-1970s, suggesting that some factor(s) of coral reef ecosystem dynamics changed at that time. Over 500 water and benthic samples were collected over the past four years at six sites around the Big Island of Hawaii to determine what factors appear to most influence the abundance of *Gambierdiscus* in Hawaii reef environments. Examples of such factors include the following findings. While univariate results suggest that nitrate concentrations play a significant role in *Gambierdiscus* abundance, multivariate results give more weight to ammonium concentrations. *Gambierdiscus* isolates from East Hawaii (leeward side of Hawaii) are more tolerant of lower salinity than West Hawaii isolates. *Gambierdiscus* appears to favor the red alga, *Ceramium*, as substrate, possibly suggesting an auxotrophic requirement. *Gambierdiscus* lives in close association with other epiphytic microalgae, some of which appear to produce compounds that stimulate *Gambierdiscus* growth, whereas others appear to stimulate *Gambierdiscus* growth. The results of this study demonstrate that *Gambierdiscus* growth is influenced by several abiotic and biotic factors, and the patchiness of ciguatera outbreaks may reflect the interaction of multiple variables rather than one single stimulatory factor.

THE TOXIN FROM *GYMNODINIUM VENEFICUM* BALLANTINE – REDISCOVERED: IT'S A KARLOTOXIN

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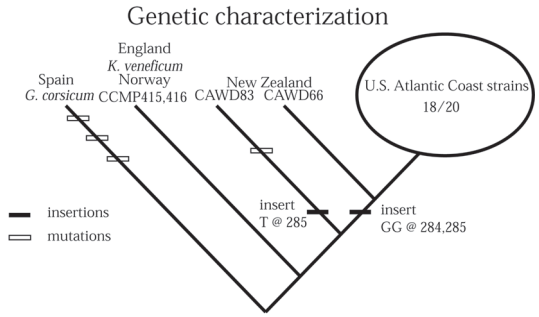
On June 8th, 1949 Dr. Mary Parke of the Plymouth Laboratory of the Marine Biological Association isolated a small dinoflagellate from seawater at 7.3 meters depth from the Knap Buoy region off Plymouth Sound (lat. N. 50° 19'30", long. W 04°10'). The following year on June 28th a second very similar dinoflagellate was isolated from a seawater sample from the Hamoaze, over Rubble Bank, off King William Point, South Yard, Devonport (lat. N. 50° 21'50", long. W 04°10'55"). Both were deposited in the Type Culture Collection (Plymouth collection no 102 and 103, respectively). Subsequently, Dorothy Ballantine (1956) described and named the two species, *Gymnodinium vitiligo* and *Gymnodinium veneficum.* The greatest difference between the two species was physiolgical, as *G. vitiligo* was harmless, whereas *G. veneficum* produced a very powerful toxin which was lethal to fish and nearly every other organism tested including mice. In 1957, B.C. Abbot and D. Ballantine described the partial purification and chararcterization of the toxin from *G. veneficum.* To paraphrase their findings, *"…The toxin molecule must be large, as it cannot penetrate a dialysis membrane; it is soluble in water and the lower alcohols, but insoluble in ether and chloroform. It is unstable in acids, … though in neutral solution is more or less thermostable. …With regard to mode of action it depolarizes nerve and muscle membranes. …This depolarization probably occurs by interference with the sodium exchange mechanism, allowing rapid entry of sodium into the cells."*



Using a Litaker et al. *K. micrum* specific primer (starts 124 b downstream of LSU) and a dino SSU reverse primer have a ~500bp 'ITS' pcr product

Unfortunately, *G. vitiligo* was lost in culture but *G. veneficum* remains in culture at the Plymouth Laboratory. In colloboration with Dr. Richard Pipe at the Plymouth Laboratory, we have grown 3 liters of *G. veneficum* and analyzed its toxic activity. Based on all of our analysis (cell size and volume, pigments, sterols, fatty acids and ITS sequence), *Gymnodinium veneficum* is a *Karlodinium species*. Moreover, like Abbot and Ballentine, we find that 85 to 90% of toxic activity is released upon filtration and can be purified exactly as we have previously described for karlotoxins. On a HPLC C8 reverse phase column two major toxic peaks (KvTx1 and KvTx2) are obtained upon methanol gradient elution. Both are hemolytic to rainbow trout erythrocytes and found in nearly equivalent cell quotas (0.93 pg/cell vs 1.25 pg/cell). The UV spectra of the two toxins differ with KvTx1 having a peak at 225nm while KvTx2 has a UV absorbtion maximum at 235nm. LC/MS analysis of the two peaks finds masses of 1208.8d and 1267.8d for KvTx1 vs 1242.7d and 1301.8d for KvTx2. Each of these species also has a +16 dalton congener. We have found that KvTx1 is identical to the toxin we have characterized in a Norwegian isolate of *K. micrum* (CCMP 415). KvTx2 appears similar but not identical to what we have observed with another Norwegian *K. micrum* isolate (CCMP 416). Using flurorescent polarization of dehydroergosterol, we are determining the sterol specificity of KvTx1 and KvTx2. Both toxins kill larval zebrafish with symptons identical to that described by Abbot and Ballantine for gobies (*Gobius virescens).* Based on these finding we are in full support of Bergholtz, Daugbjerg & Moestrup (2005) in changing the name of *Karlodinium micrum* to *Karlodinium veneficum* (Latin veneficum – poisonous, bearer of venom). Moreover, toxic *K. veneficum* can now be found globally including the English Channel.

IDENTIFICATION OF A RAPID DETOXIFICATION MECHANISM FOR BREVETOXIN IN RATS

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We examined detoxification of brevetoxin in rats through metabolic activities and key elimination routes by analyzing samples from individual rats exposed to two brevetoxin congeners (PbTx-2 and PbTx-3). Brevetoxins were detected by radioimmunoassay in methanolic extracts of blood within 1 h post intraperitoneal administration. The toxin assay response was about three times higher in PbTx-2-treated rats vs. the same dose (180 µg / kg) of PbTx-3. This difference persisted for up to 8 h post-exposure. When the blood samples were re-extracted with 20% methanol to enhance recovery of potential polar brevetoxin metabolites, 25-fold higher assay activity was present in the PbTx-2 treated rats. Analysis of urine from the same animals identified 7-fold more activity in the PbTx-2 treated rats that accumulated over the course of 24h. Radioimmunoassay-guided high performance liquid chromatographic analysis of urine from PbTx-2 treated rats yielded three major peaks of activity. The first peak was attributed to the two cysteine adducts, cysteine-PbTx sulfoxide and cysteine-PbTx (MH^+: m/z 1034 and 1018). The second peak was attributed to the oxidized form of PbTx-2 (MH^+: m/z 911) and its reduction product PbTx-3. The third peak remains unidentified. Brevetoxin cysteine conjugate and its sulfoxide product contributed nearly three quarters of the brevetoxin immunoactivity. Our findings indicate that the most commonly occurring PbTx-2 is rapidly transformed to a polar metabolite of a reduced biological activity that appears in blood and remains for up to 8 h, yet is cleared mostly to the urine within 24 h.

DEVELOPMENT OF PUBLIC HEALTH RESPONSE PLANS FOR HABS: A "COUNTY UP" APPROACH

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Florida is home to all major toxin-producing marine, estuarine and freshwater microalgae. The subtropical warm climate, 1200 miles of coastline, varied aquatic habitats, and human interaction through seafood consumption and from water activities create an environment where the presence of aquatic toxins can have a significant impact on public health. Recent high profile Harmful Algal Bloom (HAB) events have occurred in Florida including Florida Red Tide blooms in the Gulf of Mexico; poisonings from consumption of saxitoxin-contaminated puffer fish; ciguatera fish poisonings; and anecdotal reports of cyanobacteria-associated illnesses. During most of these events, there was no pre-planned response from local health departments, making it difficult to develop appropriate avenues for communication, disseminate information to the public, limit on-going exposures, and to identify opportunities for preventing human illness in the future.

In 1999, the Florida Legislature created the HAB Task Force and was assigned to develop recommendations that can be implemented by state and local governments to address HAB related events. In 2004, a Public Health Technical Panel was formed to develop broad recommendations for the composition of local HAB contingency plans. During the past year, representatives from selected Florida Department of Health County Health Departments (CHDs) were invited to participate in the development of a generic response plan for each of the HABs which occur in their jurisdiction. Representatives from various state agencies, Centers for Disease Control and Prevention, research institutes, universities, and consultants also participated. This county-based approach was initiated to gain insight into local experiences in addressing HABs including available expertise, identification of important local governmental entities and advocacy groups, and challenges such as staff limitations, competing issues, and data/information sharing obstacles.

This presentation will describe the development of a public health model for an integrated HAB response plan incorporating public health and environmental monitoring activities. Elements for a model plan were identified by the Technical Panel and include background documentation; purpose of plans; current state of response capabilities; agency responsibilities; and concept of operations such as preparedness activities, response actions, follow-up procedures, and a contact resource guide. The Technical Panel will provide recommendations on what should be included in each of these elements. A model plan will be developed for each type of HAB. Once these are completed, specific generic plans will be developed for each of the HABs in a modular format that will comprise a User's Manual for CHDs. The Manual will be used by CHDs to develop county-specific response plans reflecting local capabilities and limitations. These CHDs will be part of a public health team that recruits additional counties for the development of response plans in areas of Florida that experience HABs but have not responded with local efforts. This county-up approach will ensure the integration of local to state to federal resources and responses to HABs which occur in Florida.

A BIOCHEMICAL INVESTIGATION OF *KARENIA BREVIS* ACROSS A FRONT OFF SARASOTA, FL.

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Karenia brevis is a harmful algal bloom (HAB) dinoflagellate typically located in the Gulf of Mexico. Blooms of *K. brevis* usually develop offshore on the west Florida shelf and can eventually move shoreward causing fish kills. Though the development of these blooms is determined by a number of physical, chemical, and biological environmental factors, laboratory studies suggest that the internal biochemical status of the cell controls growth, reproduction, and possibly migratory behavior. The internal biochemical status of the cell is directly influenced by the cell's ability to utilize ambient light and nutrients for growth and reproduction. Understanding mechanisms that allow *K. brevis* to efficiently use light and nutrients are crucial in identifying the organism's ability to use local waters to proliferate. This project investigated a frontal bloom of *K. brevis* as it was advected onshore south of Sarasota, FL on January 27, 2005. Samples were collected east of the front beginning at 11:30 and continued through the front until 18:00 to ensure access to new surface aggregates over the sampling interval. Water was collected in 2L Niskin bottles at the surface in an eight meter water column. Physical parameters included salinity, temperature, and ADCP current measurements while biochemical measurements included yield, lipids, toxins, and cell carbon and nitrogen. On completion of analysis, field measurements will be compared with previous laboratory studies for future use in biophysical models of *K. brevis* population dynamics.

FLOW EFFECTS ON INTERACTIONS BETWEEN *KARENIA BREVIS* AND CLAY USED IN HAB MITIGATION

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Water column removal efficiency (RE) of *Karenia brevis* (a non-thecate dinoflagellate) by inert clay has previously been determined in experimental systems without flow, yielding RE values from 70 to 95%, after 2-4 hrs of flocculation and settling. Flow effects have only been considered for the thecate, non-toxic dinoflagellate, *Heterocapsa triquetra* (Archambault et al. 2003; Beaulieu et al. 2005) It remains to be determined whether the mechanism of dinoflagellate removal by clay is via flocculation, and/or the result of clay-induced changes in cell surface properties and swimming ability. Therefore, this study examined the removal efficiency of *K. br*evis in flow by phosphatic clay, aggregate formation and composition, and the effects of clay and flow on this red-tide organism.

Paired control-treatment experiments were conducted in two annular recirculating flumes (30-cm channel width, 20-cm water column height), in which two flow speeds, 3 and 13 cm s^{-1}, were maintained by a rotating half-lid. Flow characterization for this design has been provided by Porter (1999). *K. brevis* cultures were added to each flume (2,000 cells mL^{-1}) immediately followed by the clay slurry (0.25 g L^{-1}) or filtered seawater for the control. Water samples were taken at regular intervals for cell counts and microscopic observations, and for analysis of particle size and composition using a small-volume particle microsampler (Archambault et al. 2001). Three resuspension events, simulating a tidal regime, were done at 3, 6 and 9 hr by increasing flow to 20 cm s^{-1} for 30 min. The experiment was terminated after 24 hrs. For sequential replication, this entire procedure was repeated at each flow speed at least once.

K. brevis removal by clay was relatively effective at low flow: RE ranged from 62 to 87% after 3 h, and from 69 to 86% after 6 h at 3 cm s^{-1}. The formation of large aggregates was clearly observed over the first 3 hrs, and following each resuspension event. Aggregates captured within the first 10 min showed *K. brevis* cells within a clay matrix, suggesting entrapment. *K. brevis* removal occurred after each resuspension event as flow decreased; RE was 95-97% after 24 h. Cell motility in the clay treatment was lost completely after the first resuspension event, but not affected in controls. Non-motile cells also appeared to lose their characteristic morphology. In contrast, at 13 cm s^{-1}, removal of *K. brevis* was more variable between runs, and only attained RE values of 48 to 61% after 24 h. Large aggregates formed initially, but they appeared to be sheared apart before settling occurred. Cells lost their motility within 5 min of clay dispersal, and lost their characteristic morphology after 1 h.

This study determined that the effectiveness of water column *K. brevis* removal by clay is reduced at high flow. *K. brevis* was shown to be incorporated within flocs, which supports a flocculation/entrapment model for cell removal at low flows. This study clearly demonstrated the deleterious effect of suspended clay on *K. brevis* cells, especially at high flow, as evidenced by loss of motility and anomalous cell morphology, but not detected by a viability stain. The relative significance of these mechanisms of cell removal remains to be determined. Further studies will be needed to examine the impacts of clay exposure on the viability, capacity for cell division, and toxin leakage of *K. brevis*. Results of this study are relevant not only to evaluate the effectiveness of clay mitigation, but also to understand the interactions between *K. brevis* and resuspended sediment in estuaries.

Archambault, M-C., Grant, J. and Hatcher, A. (2001) Deep Sea Res. Part I 48: 2331-2346.
Archambault, M-C., Grant, J. and Bricelj, V.M. (2003) Mar. Ecol. Prog. Ser., 253: 97-109.

MULTIDECADAL CHANGES IN THE DIATOM: FLAGELLATE RATIO AND SI:N AND SI:P RATIOS IN NARRAGANSETT BAY, AND INFLUENCE OF SI:N SUPPLY RATIOS ON DIATOM SPECIES COMPETITION

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The ratio of the mean annual diatom to flagellate abundance (numerical) in Narragansett Bay based on weekly sampling from 1959 to 1996 exhibited a pronounced multidecadal, recurrent oscillation. This functional group ratio progressively decreased five-fold, from ca. 5:1 to 1:1, then progressively recovered to ca 5:1, followed thereafter by a similar decrease and recovery pattern. There were two distinct cycles during the 38-year time series exhibiting this pattern. Nutrient measurements begun in 1973, and detrended, also exhibited long-term patterns: mean annual inorganic phosphorus concentrations decreased and silicate concentrations increased, while inorganic nitrogen (NH4 and NO3) concentrations were cyclical. The trends in the mean annual ratios of Si:N (range ca. 4.5 to 0.45) and Si:P (range ca. 20 to < 10:1) and the diatom:flagellate ratio were strongly and positively correlated, indicative of the importance of Si in regulating the long-term pattern of functional group selection observed. The Spearman r (non parametric) correlation coefficient between the trends in the diatom:flagellate ratio and Si:P ratio was + 0.79, and +0.71 with the Si:N ratio, both statistically significant. The interannual patterns of selected flagellate species within this functional group oscillation will be shown. The results of chemostat experiments conducted with three major diatom species in Narragansett Bay to examine the influence of Si:N supply ratios on their interspecific competition and species selection will be presented, and discussed from the perspective of the strengths and limitation of the nutrient ratio theory to explain the selection and blooming of harmful algal species.

REMOTE SENSING DETECTION OF "RED TIDES" AND OTHER HARMFUL ALGAL BLOOMS THAT DISCOLOR THE WATER

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Use of ocean color for harmful algal bloom detection requires blooms that either dominate the biomass or co-vary consistently with phytoplankton that does. Species that rarely dominate the biomass, such as *Alexandrium* spp. require ecological characterization using temperature or salinity. Others such as *Pseudo-nitzschia* spp. rarely influence biomass along the Washington coast, yet may do so under certain conditions along the California coast. The blooms that most often influence water color are those of *Karenia brevis* in the Gulf of Mexico, and *Microcystis* spp, blooms in the Great Lakes. A key issue for routine monitoring of HAB species by satellite imagery is discriminating harmful blooms from background chlorophyll or other bloom-forming organisms.

The key components for optical detection are: (1) variations in absorption spectra owing to the presence of ancillary pigments; (2) variations in backscatter spectra owing to size differences; (3) relative variations in backscatter and chlorophyll absorption due to pigment packaging and scattering efficiency in the cells (the latter may be due to gas vacuoles, etc.); and (4) consistent variations in the characteristics of the water containing the bloom, owing to the detritus generated by the bloom or to precursor optical conditions. Some of these are particularly problematic in coastal waters with current satellites or optical algorithms. Backscatter analyses, for example, is strongly impacted by the presence of sediments. Total absorption is influenced by mixed blooms or by colored dissolved pigments. To further complicate the issue, theoretically based bio-optical algorithms used to obtain absorption and backscatter are dependent on the input of accurate remotely sensed water reflectances. The quality of atmospheric correction may lead to reflectances that are not necessarily accurate enough to apply the bio-optical algorithms.

While challenges exist, patterns and procedures can and do aid in identifying some of the blooms. As has been previously shown along the west coast of Florida, new blooms tend to be *Karenia.* These are identified by temporal anomalies in the chlorophyll field against the preceding two months (as we have previously described). However, the appearance of new chlorophyll due to resuspension, diatom and *Trichodesmium* blooms can confound this assessment. Spectral analysis techniques, including backscatter/chlorophyll ratios, and spectral curvature to identify pigment absorption variations, can provide relative patterns that help in identifying some of these other features. *Microcystis* blooms tend to have strong backscatter and some color shifts that can aid in identification. While fixed thresholds are not evident, relative patterns can provide guidance to aid in the identification of blooms of interest, or in eliminating blooms that are not of species of concern.

DINOFLAGELLATE ABUNDANCE IN MANGROVE CAY EMBAYMENTS OFF THE COAST OF BELIZE

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Ciguatera causing dinoflagellates are found in tropical waters world wide and the western Caribbean is no exception. In the oligotrophic waters of the central lagoon off Belize we found rich dinoflagellate communities in mangrove embayments with a high proportion of toxic species. We selected an embayment at Douglas Cay for intensive study during the dry seasons of 2002-2004. This system was highly productive and is characterized by a unique combination of island morphology, wind sheltering and low tidal amplitudes that limited flushing and allowed accumulation of organic detritus. Average bacterial biomass ($4x10^9$ cells L^{-1}) and production (7.4 mg C L^{-1} h^{-1}) were an order of magnitude higher than in the surrounding oligotrophic lagoon (Chrost unpub data). High rates of nutrient recycling by bacteria were indicated by the high residual concentrations of ammonia (0.4-10 µM), urea (2-5 µM), and phosphate (0.4 -1.3 µM). These high ambient nutrient concentrations supported 5 to 20-fold higher chl *a* biomass (3.4-7.0 mg/L) than observed in the surrounding oligotrophic lagoon.

The high chl *a* biomass in this system was also supported by a close coupling between the physical structure of the water column and oxygen produced by benthic algae. Daytime light intensities were high throughout the water column, exceeding ~ 1000 µEinstiens m^2 s^{-1} at the bottom of the embayment. This high irradiance allowed development of a mat community rich in dinoflagellates, diatoms and cyanobacteria. During the middle of the day the mat became supersaturated with oxygen as indicated by extensive bubble formation. Solar heating and evaporation caused stratification, which limited the diffusion of oxygen into the water column. Destratification, caused by cooling of the surface waters after sunset, resulted in a daily turnover of the water column within the embayment. This turnover mixed highly oxygenated water at the bottom of the embayment into the water column, resulting in a daily oxygen maximum in the early evening (~ 4 mg L^{-1}). Bacterial respiration reduced oxygen levels throughout the night and into the early morning (~ 1 mg L^{-1}). During the day, water column photosynthesis, and some limited diffusion from the benthos offset the bacterial respiration, causing the oxygen levels to rise slightly by mid day (~ 2 mg L^{-1}). Water column photosynthesis, however, was insufficient to raise the oxygen level further because phytoplankton could account for no more than 10% of total oxygen production in the system. By the late afternoon, bacterial respiration again exceeded water column productivity and oxygen levels steadily declined (~ 1 mg L^{-1}) until the nighttime turnover occurred again. The coupling between the physical turnover and benthic oxygen production prevented the system at Douglas Cay from becoming hypoxic or anoxic and sustained the high phytoplankton biomass.

High light intensities, high nutrients, and low flushing rates favored dinoflagellates, which comprised more than half of the chl *a* biomass (53-57%) in the Douglas Cay embayment. In contrast, over 80% of the biomass in the open lagoon was composed of cyanobacteria with only a minor dinoflagellate component. In all, twenty-two photosynthetic and three heterotrophic species were identified from water column and mat samples at Douglas Cay. Among the species present were the toxic dinoflagellates *Coolia monotis*, *Gambierdiscus toxicus*, *G. yasumotoi*, *Gambierdiscus* spp., *Dinophysis caudata* and *D. rotundatum*, *Ostereopsis labens, O. marina*, *O. siamensis*, *Prorocentrum hoffmannianum* and *P. rathymum*. These species are all implicated in ciguatera fish poisoning. The physical and biological processes operating at the Douglas Cay embayment have resulted in a system which is naturally eutrophic. This system has selected for an algal assemblage rich in dinoflagellates and may portend shifts in community structure expected from anthropogenic nutrient inputs to other shallow marine ecosystems.

GROWTH, NUTRIENT UTILIZATION AND EVIDENCE FOR TOXIN PRODUCTION BY THE NEW TOXIC FLAGELLATE *CHLOROMORUM TOXICUM*

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The notable mortality of menhaden (Brevortia tyrannus) in coastal inland bays of Delaware in 2000 was accompanied by the presence of 10^7 cells/L of an unusual flagellate and the presence of potent brevetoxins PbTx-2, -3, and -9. The organism, presently named *Chloromorum toxicum* was previously unknown from these coastal waters and presumed to be the agent of toxicity. Clonal cultures of this species were established from blooms in Delaware and grown under laboratory conditions to determine the growth characteristics under differing salinities, nitrogen sources (nitrate, ammonia and urea) and phosphorus (inorganic phosphorus vs. organic phosphorus). In addition, cultures of *C. toxicum* were extracted as log and stationary phase cultures and examined for the presence of brevetoxins using methods involving LCMSMS.

Chloromorum toxicum exhibited a salinity preference with best growth at salinities of 10 to 25 and maximum growth rates (k) varying from 0.4 to 1.3 divisions/day. Growth at extreme salinities (<10 and >25) was much reduced and no growth was observed at a salinity of 0. This species was found to grow on all nitrogenous substrates studied although it was particularly sensitive to elevated values (> 10 µM) of ammonia. Best growth was found with urea and when nitrogen was presented in combination as urea and ammonia, growth progressively declined with the increase in ammonia content. Growth on nitrate was intermediate to the other sources and no inhibition was observed at any levels of nitrate. As for phosphorus, both inorganic (phosphate) and organic (glycerol-phosphate) phosphorus were utilized equally well. There appeared to be no preference for growth with phosphorus source. Whole cultures in late log and stationary phase were harvested by extraction in chloroform. The chloroform component was dried using a rotovaporator. Dried toxins were redissolved in acetone. Known aliquots of the acetone extract were again dried, redissovled in methanol, filtered and examined by LCMSMS. Peaks corresponding in molecular weight of PbTx -2, -3, -1 and -6 were resolved and confirmed when compared to brevetoxin standards. *Chloromorum toxicum* is thus a mid estuarine species, sensitive to ammonia nitrogen but capable of utilizing both organic and inorganic substrates for nitrogen and phosphorus, able to maintain moderate to high growth and a confirmed brevetoxin producer. This new species is potentially able to deliver neurotoxins to temperate estuaries where it can readily form blooms.

CHARACTERISTICS OF THE JUAN DE FUCA EDDY, A SOURCE OF DOMOIC ACID TO THE WASHINGTON COAST

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ECOHAB Pacific Northwest cruises off the Washington coast show that the Juan de Fuca eddy, a topographically-trapped seasonal feature is a site where domoic acid can predictably be found during summer months. The highest toxin levels are generally located in the colder water in the eddy and along its edges. The location of toxin-producing *Pseudo-nitzschia* is similar during each cruise, whereas the specific cellular toxicities varies dramatically among cruises. Although *Pseudo-nitzschia* are found in the nearshore coastal upwelling zone as well as in the vicinity of the eddy, *Pseudo-nitzschia* have not been found to be toxic in blooms associated with actively upwelling water. The eddy region has a number of characteristics that differentiate it from the coastal upwelling zone. In particular, particle residence times in the eddy region are several times those in an active upwelling zone. Available iron supplies to phytoplankton in the eddy are limited and result in a specific composite of diatoms and flagellates. The relationship of iron availability to domoic acid production and release in *Pseudo-nitzschia* cells may, in part, explain the greater toxicity of cells in this region. Also, survey data suggest that the supply of macronutrients to the eddy is much more persistent than to the coastal upwelling zone—in the latter region nutrient supply is entirely cut off for several days following each reversal from upwelling (high salinity) to downwelling (low salinity) conditions. In contrast to the coastal upwelling zone, the eddy region is supplied by the comparatively constant, nutrient-rich near surface estuarine flow out of the strait.

COMPARATIVE BREVETOXIN DYNAMICS DURING LYSIS OF *KARENIA BREVIS* BY TWO ALGICIDAL BACTERIA

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Flavobacterium sp. (strain S03) and *Cytophaga* sp. (strain 41-DBG2) are algicidal bacteria active against the brevetoxin (PbTx)-producing dinoflagellate, *Karenia brevis*. Both algicidal bacteria cause lysis of *K. brevis*, but *Flavobacterium* sp. requires physical contact with the target algal cells (i.e., direct attack), whereas *Cytophaga* sp. releases a dissolved algicidal agent (i.e., indirect attack). However, little is known about the fate of PbTx associated with *K. brevis* cells following attack by algicidal bacteria. Experiments involving the co-culture of *K. brevis* (Charlotte Harbor isolate C2) with each algicidal strain were conducted, and a time course of algal and bacterial growth, as well as the production, loss, and size-fractioned distribution of PbTx, was followed over 15 days. Cultures were differentially filtered at each time point to yield two particulate size fractions (>5 µm, 5 - 0.22 µm) as well as a dissolved component (<0.22 µm) for PbTx analysis. Particulate samples were extracted in methanol, while dissolved PbTx was adsorbed onto C18 SPEC discs (Varian Chromatography) prior to elution with methanol, and toxin levels were determined by receptor binding assay. Co-culture with a non-algicidal bacterium *Cytophaga latercula* (10^3 cells/mL) and addition of sterile natural seawater served as negative controls.

Both control cultures showed expected exponential growth through Day 5. Particulate PbTx in the >5 mm size fraction (likely associated with intact cells) of the control cultures correlated positively with growth and peaked at *ca.* 246 ng/mL (max. cell quota about 15 pg/cell). Changes in the dissolved fraction also correlated with the >5 mm component, but the increase with cell concentration showed a lag time of about five days (Fig. 1), prior to reaching a maximum of about 70 to 80 ng/mL by Day 15. In marked contrast, exposure of *K. brevis* to either *Flavobacterium* sp. (10^2 cells/mL) or *Cytophaga* sp. (10^3 cells/mL) resulted in algal cell lysis between Days 1 and 2. Following lysis, the cell-associated PbTx (>5 mm) levels declined rapidly from 85 to <13 ng/mL by Day 3 and then to *ca.* 3 ng/mL by Day 15. However, dissolved toxin concentrations showed a clear spike to about 45 ng/mL on Days 2 and 3 in conjunction with cell lysis. The dissolved PbTx appeared labile and decreased to about 10 to 15 ng/mL by Day 15. Interestingly, PbTx levels in the 5 - 0.22 µm particulate size fraction, presumably organics from lysed algal cells and/or bacterial cells, remained relatively constant in both controls and treatments (3 to 20 ng/mL). To our knowledge, this is the first study to examine size fractioned PbTx dynamics in *K. brevis* cultures under either control conditions or during attack by algicidal bacteria. Our findings have implications for the fate and trophic transfer of PbTx during bloom events and should also be considered when assessing the potential of various HAB control and mitigation strategies that could lead to the lysis of *K. brevis* cells. Future experiments will examine gene expression patterns in *K. brevis* during attack by algicidal bacteria with the aim of better understanding the response of these algal cells to lytic agents.

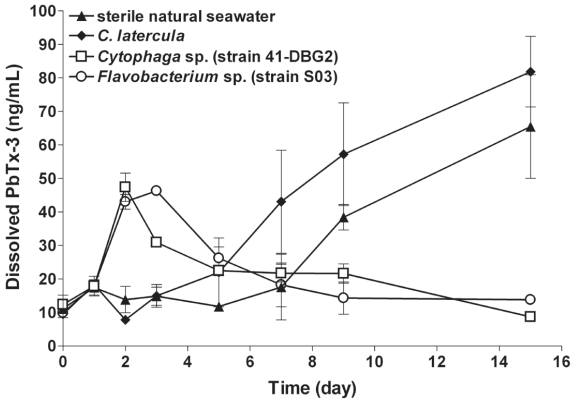

Figure 1. Changes in dissolved (<0.22 µm) PbTx from *K. brevis* cultures over time. Cultures were exposed to either sterile natural seawater, the non-algicidal bacterium *C. latercula* (10^3 cells/mL), or the algicidal bacteria *Flavobacterium* sp. (strain SO3) and *Cytophaga* sp. (strain 41-DBG2) at 10^2 and 10^3 cells/mL, respectively.

FUNCTIONAL GENOMIC STUDIES IN *KARENIA BREVIS*: CURRENT INSIGHT INTO MECHANISMS REGULATING GROWTH, TOXICITY, AND ADAPTATION

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Karenia brevis is the dinoflagellate responsible for Florida red tides that cause extensive marine animal mortalities and human illness through the production of neurotoxic brevetoxins. Since little is known about the molecular biology of *K. brevis* or dinoflagellates in general, we chose to take a functional genomic approach to investigate mechanisms regulating *K. brevis* bloom dynamics. Large scale screening of two cDNA libraries has been carried out to yield a total of 25,000 sequence reads. The first library is from cells under log phase growth in nutrient replete conditions (15,000 sequences). The second is generated from cells exposed to a variety of stresses (N or P limitation, heat, H_2O_2, $PbCl_2$, stationary phase; 10,000 sequences). The combined expressed sequence tags (ESTs) yielded approximately 12,000 unique gene clusters. Gene specific oligonucleotides designed for each unigene were used to develop a *K. brevis* DNA microarray. We are currently employing this microarray to gain insight into mechanisms controlling the cell cycle, toxicity, and stress responses in *K. brevis*. We will present an overview of current insights gained from microarray gene expression profiling experiments.

DETERMINANTS OF PATHOGENICITY IN *PFIESTERIA PISCICIDA* & *PSEUDOPFIESTERIA SHUMWAYAE:* SPECIES AND STRAIN COMPARISONS

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Dinoflagellates of the "Toxic *Pfiesteria* Complex" have been implicated in fish mortality and human illness in mid-Atlantic USA estuaries. Adverse fish and human health effects are still attributed by some investigators to the secretion of potent exotoxins by these organisms (Burkholder et al., 2005). However, we have demonstrated that *Pseudopfiesteria shumwayae* (formerly *Pfiesteria shumwayae*: strain CCMP-2089: Litaker et al., 2005) causes adverse fish health effects through micropredatory epidermal feeding rather than secretion of potent exotoxins. In our hands, *Pfiesteria piscicida* (strain CCMP-2091) similarly kills fish via micropredation without evidence of toxin secretion, but at vastly reduced rates compared to *P. shumwayae*. Micropredatory epidermal feeding was recently confirmed by others as the primary mechanism of fish pathogenicity in *P. piscicida* and *P. shumwayae* (Drgon et al., 2005; Gordon and Dyer, 2005). The biological and ecological determinants that modulate the population dynamics and pathogenicity of these organisms are not well understood. Thus, to clarify the underlying mechanisms responsible for the drastically different fish mortality rates observed between these two species, we conducted comparative dose response, membrane insert, life history and quantitative morphometric studies. Cell density (i.e., flagellated cells, reproductive cysts), life history dynamics, cell morphometrics, chemoattraction and feeding capacity appeared to be critical determinants accounting for the observed differential pathogenicity between *P. shumwayae* and *P. piscicida*. In contrast, exotoxin secretion did not appear to be a determinant of fish pathogenicity for either species. Further, comparisons of multiple strains of both species provide no evidence that a potent exotoxin is involved in fish morbidity and mortality. This work was funded in part by ECOHAB Grant NA-16OP1487, The Commonwealth of Virginia's *Pfiesteria* Initiative and the Centers for Disease Control and Prevention.

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BLOOM DEVELOPMENT AND TRANSPORT OF TOXIC *ALEXANDRIUM FUNDYENSE* POPULATIONS WITHIN A COASTAL PLUME IN THE GULF OF MAINE

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Toxic *Alexandrium fundyense* blooms in the Gulf of Maine (GOM) are a common occurrence causing Paralytic Shellfish Poisoning (PSP) along both the eastern and western sections of the Maine coastline. Our objective was to determine if the bloom dynamics of the two areas are linked early in the bloom season when initial outbreaks of toxicity are common in the western GOM. As part of the ECOHAB-GOM program, *A. fundyense* cell abundance and hydrographic data were acquired during two cruises in May and June, 2001 that spanned both areas. Surface drifters were also released into the nearshore coastal flow of the eastern GOM. The data provided a coherent view of the springtime evolution of toxic *A. fundyense* blooms within a less-saline (<32 psu) feature that extended along both the eastern and western Maine coastlines, which we term the GOM Coastal Plume (GOMCP). As part of a MERHAB study in 2003-04, the same region was re-sampled 3 successive times during a 10-day period. *A. fundyense* populations were always observed in less-saline waters (<32psu) that extended alongshore and offshore in agreement with the 2001 results. The most significant finding of these studies was that *A. fundyense* populations along the eastern Maine coast were delivered along an "inside track" relative to the core of the eastern Maine Coastal Current (EMCC). The density-driven transport pathway carried cells across the mouth of Penobscot Bay and into the western GOM coincident with outbreaks of nearshore PSP toxicity. This pattern was consistent with circulation models of the coastal GOM that unambiguously revealed a bifurcated flow with the branch nearest the coast directed alongshore to the western GOM. The transport is also strongly influenced by wind. In particular, some of the cells within the nearshore flow may be lost to the interior GOM when upwelling-favorable winds transport them offshore and into the large-scale circulation dominated by the cyclonic flow of the Jordan Basin Gyre. Downwelling-favorable winds keep the cells close to the coast and rapidly transport them into the western GOM. These studies unequivocally demonstrate the connectivity between early season *A. fundyense* bloom formation in the eastern GOM that resulted in blooms and toxicity in the western GOM. This long-distance pathway may have played a role in the unusual bloom event in southern New England during spring 2005.

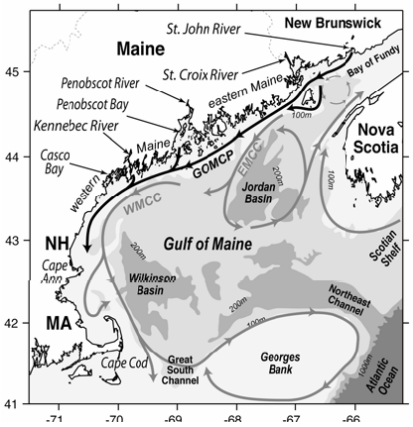

Fig 1. Surface circulation of the GOM showing the GOM coastal plume (GOMCP), a feature responsible for transport of A. fundyense *populations.*

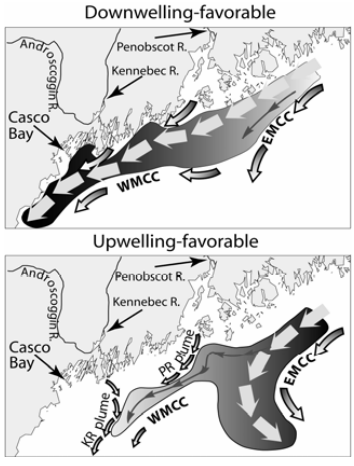

Fig 2. Conceptual diagram of the transport of A. fundyense *within the GOMCP (shaded) during downwelling and upwelling favorable winds.*

Poster Presentations

ICHTHYOTOXIC *KARLODINIUM MICRUM* IN THE SWAN RIVER ESTUARY (WESTERN AUSTRALIA): AN EMERGING THREAT IN A HIGHLY EUTROPHIC ESTUARINE SYSTEM

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Karlodinium micrum has formed persistent blooms associated with fish kills in the Swan River Estuary upstream of Perth, Australia (S 31° 57', E 115° 52') during the austral autumn for the last three years. These blooms have occurred in the upper, highly eutrophic reaches of the estuary during a time of year characterized by exceedingly dry conditions, high temperature and salinity (>25 ppt), relatively low surface nutrient concentrations, and generally low bottom water oxygen levels. Previous analyses of toxin levels and fish pathological specimens from the Swan River Estuary (2003, 2004) suggest that karlotoxins contribute to fish kills in this system. In March 2005, we began sampling *K. micrum* cell numbers and karlotoxin levels to better understand the role of *K. micrum* in Swan River Estuary fish mortalities that are anticipated to occur between April and June based on past years' observations. We hypothesize that increased karlotoxin concentrations, either through increased cell numbers or increases in cellular toxicity (pg toxin $cell^{-1}$) will precede or accompany major fish kills.

K. micrum is characterized by a high degree of variability in cellular toxicity both within and between genetically-distinct strains. Toxin isolated from a Swan River Estuary *K. micrum* bloom during March-April 2005 was dominated by KmTx2 based on HPLC retention time and mass (M = 1344.8 daltons) determined by LC-MS. An ion of M = 1402.8 daltons, not previously observed among the *K. micrum* strains we presently have in culture, co-eluted with KmTx2 in the Australian *K. micrum* bloom samples.

Sampling done on two dates (March 31 2005, April 4 2005) along a 5 km stretch of the upper Swan River Estuary where *K. micrum* was blooming showed spatially distinct peaks of cell density and cell toxicity. *K. micrum* density was greatest (1 – 1.5 $x10^5$ cells ml^{-1}) in the middle of this stretch of the estuary whereas cell toxicity was an order of magnitude higher at the furthest upstream site than at sites downstream. *K. micrum* density at the furthest upstream station was 4 $x10^4$ cells ml^{-1}. Cellular toxicity levels at the furthest upstream station (0.2 – 0.5 pg $cell^{-1}$) were not exceptionally high compared to toxin levels measured in cultured isolates. Peak karlotoxin concentration in the water (cell-associated + free toxin, ~20 ng ml^{-1}) was highest at the furthest upstream site. These toxin levels are not expected to be ichthyotoxic and only a few fish mortalities (~30 juvenile Sea Bream) were observed on these sampling trips.

Cultured *K. micrum* derived from the April 2005 bloom will be used to further examine the type and quantity of toxin produced, and conditions that modulate toxin production and/or release from the cell. *K. micrum* from the Swan River Estuary ingested a cultured cryptophyte, *Rhodomonas*, fed to it although few cryptophytes and no food-vacuole inclusions were observed within the near-monospecific *in situ* bloom. Cultured material will be used to study the relationship of toxicity, feeding and growth in the Australian *K. micrum* strain.

OCEANOGRAPHIC CONDITIONS IN MONTEREY BAY, CA. AS THEY RELATE TO *ALEXANDRIUM CATENELLA* AND PSP TOXINS IN LOCAL FISHERIES: A TWO YEAR TIME SERIES

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Monterey Bay is a highly productive and biodiverse region located along the central coast of California, which is frequently affected by the presence of harmful algal blooms (HABs). The richness of this ecosystem can be attributed to the influence of two major upwelling centers on the outskirts of the bay. In the present study we aim to understand the dynamics of cell abundance in *Alexandrium catenella*, the organism responsible for Paralytic Shellfish Poisoning (PSP) in this region, and its relationship, if one exists, to toxins in commercial fisheries. Qualitative observations of *A. catenella* in California over the last decade suggest that PSP events frequently occur in association with wind relaxation events, the periods between upwelling (Langlois 2002). To address this theory we will use a two year time series (2003-2005), emphasizing the relationships between *A. catenella*, particulate saxitoxin (pSTX) and oceanographic conditions. Data collected from weekly samples at two sites in Monterey Bay allow us to compare the physical (temperature, salinity and upwelling index), chemical (nutrient concentrations) and biological (*A. catenella* abundance, pSTX concentrations, chlorophyll a, and community species composition) conditions which occur during PSP events.

To date, the data suggest that during a toxic event *A. catenella* remains a small to moderate proportion of the phytoplankton community, yet cell densities reach relatively high levels. The results also indicate that non-upwelling conditions do provide a favorable environment for *A. catenella* growth. During periods when *A. catenella* was present, saxitoxin was detected in 25 of 96 samples of fish viscera collected in Monterey Bay. Of the 14 species examined for saxitoxin, Pacific sardines (*Sardinops sagax*) contained the highest concentration of toxin and the most frequent hits. The detection of saxitoxin in Pacific sardines at very low cell densities suggests that they are potential vectors for the transfer of PSP toxins to higher trophic levels. The data gathered over the last two years suggest that commercial fisheries targeting planktivorous fishes in central California may be threatened by PSP toxins in the event of toxic blooms.

BREVETOXIN BODY BURDENS IN SEABIRDS FROM THE CENTRAL WEST FLORIDA COAST

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Impacts of Florida's *Karenia brevis* red tides on seabird populations have been largely anecdotal. Reviews by Landsberg (2002) and Shumway et al. (2003) document avian mortalities in Florida waters but a direct correlation between neurological symptoms and the presence of brevetoxin in avian tissues was not documented until the study by Kreuder et al. (2002). They found elevated brevetoxin levels in Double-Crested Cormorants (*Phalacrocorax auritus*) displaying neurological symptoms later termed brevetoxicosis. However, body burdens in other sea and shore birds that feed at various levels in the food chain are largely unknown as are brevetoxin levels of phytophagous fish and other filter feeders that constitute the food supply of these bird populations. A cooperative study was initiated utilizing local rehabilitation centers combined with a beach survey (done in conjunction with the Tufts Center for Conservation Medicine SEANET program) to provide samples of birds, their tissues, and their food supply for toxin analyses. All brevetoxin analyses were done by ELISA at FWRI.

To date, levels of brevetoxin, as PbTx, in samples collected from avian fauna have highly variable concentrations of toxin. They range from extremely high levels in birds that died after exhibiting symptoms of brevetoxicosis to non-detectable in the majority of birds tested. Toxin levels were low but above background in several birds found dead on the beach during our SEANET beached bird survey transects in 2004. In 2002 hundreds of scaup died in conjunction with a *K. brevis* bloom. Extremely high PbTx levels were found in the GI tract (9,000 to 16,000 ng/g) of two representative birds indicating their food supply (small bivalves) was highly contaminated by brevetoxin (Landsberg et al. 2003). In 2004 stomachs and livers from two Royal Terns (*Sterna maxima*) collected on Shell Key contained low levels of PbTx (14 to 33 ng/g). Such levels are considered "positive" responses since the actual concentrations are not significantly different than zero. However, no *K. brevis* blooms occurred during 2004 so the fact that a "positive" toxin response was found suggests that some species maintain a body burden of toxin for long periods with only exposure to background levels of *K. brevis*. Toxin levels in the viscera of fish collected during the October 2003 red tide also contained high levels of toxin. Specimens of Thread Herring (*Opisthonema oglinum*), a phytophagous fish, obtained from a local commercial fish house which supplies fish to rehabilitation centers to feed birds being treated for various problems, including brevetoxicosis, contained > 2,100 ng/g PbTx. Therefore birds in rehabilitation may have continued exposure to toxin. These levels of brevetoxin in Thread Herring also provided further evidence for the claim that filter-feeding fish can be a major vector for transmittance of brevetoxin to higher trophic levels (Flewelling et al., in press). Additional samples from several species of seabirds that were exhibiting pronounced symptoms of brevetoxicosis and subsequently died are presently being processed along with additional representatives of the coastal food web.

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EFFECT OF HOST TOXICITY ON SUCCESS OF THE PARASITIC DINOFLAGELLATE *AMOEBOPHRYA*, WITH PRELIMINARY EXAMINATION OF HOST AND PARASITE MEMBRANE STEROL COMPOSITION

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Parasitic dinoflagellates of the genus *Amoebophrya* infect species of photosynthetic and heterotrophic dinoflagellates from coastal waters around the world. Once infected, host cells are unable to reproduce and are killed as the parasite completes its life cycle. These parasites thus have the potential to influence host population dynamics and have been considered as possible biological controls for harmful red-tides. While *Amoebophrya* species are known to infect toxic bloom-forming dinoflagellates, little is known about the effect of host toxins on parasite success. Here we tested two hypotheses (1) host toxicity has a negative influence on *Amoebophrya* performance and (2) growth of the parasite in none toxic hosts amplifies the effect of host toxin on parasite success. To test these hypotheses, we challenged six strains of *K. micrum* ranging from very low to very high toxin content with *Amoebophya* dinospores harvested from *K. micrum* of moderately high toxicity and from a related non-toxic host species, *Gyrodinium corsicum*. Following 24 h incubation, samples were preserved and stained for determination of parasite prevalence (% hosts infected) and parasite load (parasites/host). Counter to expectations, parasite prevalence and load both showed a positive correlation with toxicity of *K. micrum* strains. Furthermore, propagation of *Amoebophrya* in *G. corsicum* for over 50 generations had no apparent effect on parasite performance in *K. micrum* strains. Resistance of cells to *K. micrum* toxin has been linked to the membrane sterol gymnodinosterol. Results presented here suggest that gymnodinosterol present in *Amoebophrya* is retained when grown in *G. corsicum.* In an effort to explore that possibility, we are comparing the sterol composition of *Amoebophrya* to strains of *G. corsicum* and *K. micrum* known to be infected by the parasite.

AN OVERVIEW OF THE DOMOIC ACID CONTAMINATION OF MONTEREY BAY FOOD WEBS

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Interest in the movement and impact of algal toxins in marine food webs is increasing as dramatic poisoning events continue to be reported. One such toxin that can be transferred through the food web is a diatom-produced neurotoxin domoic acid (DA), responsible for a severe neurologic and gastrointestinal illness called Amnesic Shellfish Poisoning (ASP). Monterey Bay (MB), California has been a region impacted by toxic phytoplankton populations over the last decade. Sanctuary waters have been monitored longer for the presence of DA producing *Pseudo-nitzschia* than any other region in western North America, but the information on the presence of DA in MB marine food webs is scattered and limited to reports on distinct poisoning/mortality events or to data accumulated by regulatory agencies for monitoring purposes. Although data are still quite limited on the exposure of pelagic animal populations to DA toxins, the Monterey Bay Sanctuary region is probably the most studied system, due to its frequent exposure to toxic blooms, the presence of sentinel species (such as Sea Lions) that alert researchers and the public to toxic blooms, and the interest of local researchers in such phenomena.

The impact of toxic blooms on the ecosystem is still poorly understood. One of our goals in this presentation is to review the literature for extensive DA contamination through the MB food web and compile the known DA vectors and victims that occur in MB during the *Pseudo-nitzschia* blooms. Secondly, we will look at the food web dynamics and determine which intermediate organisms contain the highest toxin concentrations during the toxic blooms, how far the toxin can be carried in the food web via those potential vectors and the outcome of exposure to DA, if known. Exposure of predators to DA depends very much on the feeding behavior of their prey. Therefore, we also will discuss the types of feeding strategies that prey can use and how these different feeding strategies can affect the exposure of predators to this toxin.

STABILITY OF DOMOIC ACID UNDER VARIOUS STORAGE CONDITIONS

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Harmful algal bloom researchers often collect phytoplankton samples during multi-day research cruises and frequently need to store these samples for later toxin analysis. In order to obtain accurate measurements and avoid the confounding effects of toxin degradation, proper storage procedures must be defined and used. To this end, the present study examined the stability of the water soluble algal toxin, domoic acid (DA), under various storage conditions using both a certified reference standard prepared in filtered seawater and field-collected seawater samples. Filtered seawater (0.45µm) was spiked with a purified DA reference standard (DACs-1C) and 1 ml aliquots were stored in sealed glass HPLC vials in both the light and dark at room temperature and at 4°C. The same samples were stored at -20°C and -80°C in the dark. Samples preserved with 10% methanol were also examined using each storage condition. DA was quantified using HPLC-UV methods in aliquots from each treatment stored for various periods of time. Additionally, to determine appropriate storage conditions for field-collected phytoplankton samples, filtered particulate DA samples collected during a *Pseudo-nitzschia* bloom in September, 2004, were sub-sampled and stored at room temperature in the light and dark as well as at 4°C and -20°C, in the dark only. DA was measured at several time periods during the 6-month storage treatment. In general, DA was relatively stable during long-term storage with minor influences by light and temperature. However, DA levels in room temperature and –4°C treatments were significantly affected by evaporation, which caused an apparent increase in DA concentration over time. Although toxin increases due to evaporation can be easily corrected by accounting for volume loss, the prevention of evaporation using appropriate sized and tightly-sealed containers is preferable. Recommendations for proper storage of DA samples will be discussed.

DOMOIC ACID IN *PSEUDO-NITZSCHIA CUSPIDATA* FROM WASHINGTON STATE COASTAL WATERS

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During the September 2004 ECOHAB-Pacific Northwest cruise, a toxigenic bloom of *Pseudo-nitzschia cuspidata* (Hasle) was found to encompass a 30-mile wide area south of Vancouver Island and west of Washington State. Over 94% of the total *Pseudo-nitzschia* observed in samples from this Juan de Fuca eddy region were *P. cuspidata*. Particulate domoic acid (DA) levels reached 1.3 pg DA cell^{-1} with maximum cell densities of 13 x10^{6} cells L^{-1}. Cultures were established and grown on nutrient enriched seawater media to verify toxin production by *P. cuspidata*. Particulate DA levels in cultures reached 0.02 pg DA cell^{-1} and maximum cell numbers were 3 x10^{8} cells L^{-1}. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were both necessary to obtain information on species specific characteristics due to the morphological similarities of the species within the *P. pseudodelicatissima/ cuspidata* complex. Based on small subunit (SSU) ribosomal gene comparisons, *P. cuspidata* is most closely related to *P. delicatissima* and most distant from the *P. multiseries/ P. pungens/ P. australis* clade. No confirmed *P. pseudodelicatissima* isolates were available to include in this study. Additionally, whole cell hybridization assays were performed onboard using species specific large subunit (LSU) ribosomal directed probes. The *P. pseudodelicatissima* probe muD2 did not cross react with *P. cuspidata*. The development of a species-specific probe for *P. cuspidata* could lead to a more timely identification and assessment of the potential toxic threat of this species to marine resources.

MODIFICATION OF *HETEROSIGMA AKASHIWO* ANNUAL SUCCESSION PATTERNS IN NARRAGANSETT BAY: INFLUENCE OF LONG-TERM (1959-1996) HABITAT CHANGES ON INTERSPECIFIC COMPETITION

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In Narragansett Bay and other temperate estuaries, competition for the summer phytoplankton niche is usually between a diatom (typically *Skeletonema*) and one or more small flagellates (*Prorocentrum minimum, Heterosigma akashiwo*). A 38-year (1959 to 1996) time series of weekly observations of Narragansett Bay phytoplankton was analyzed to evaluate the interactions of climate, physical, chemical and biological variables on phytoplankton species selection and succession during the summer 'open niche' period. During late spring and early summer (weeks 22-30), all three of these species are present, with *H. akashiwo* blooms typically reaching ca. 1,000 cells ml^{-1} (maximum 32,000 cells ml^{-1}); *P. minimum* blooms are typically ca. 100 cells ml^{-1} (maximum 900 cells ml^{-1}) and *Skeletonema* is typically present at several hundred cells m^{-1}. Application of a competition index for these three species revealed that the flagellates and diatom dominated this summer niche period with approximately equal frequency. Of the 31 years evaluated, *Skeletonema* was dominant in 16 years. The two flagellate species shared dominance in the remaining years, with *H. akashiwo* dominant more frequently dominant (12 of 31 years) than *P. minimum* (3 of 31 years).

Within this trio, species selection was regulated by meteorological, chemical (=nutrient) and biological drivers. Controlling variables for *Skeletonema* versus flagellate selection included nutrient concentration and grazer abundance prior to the initiation of the summer bloom period. Pre-bloom silicon concentration was significantly greater in years with summer diatom blooms compared to years having flagellate blooms (mean = 7.4 µM vs. 5.8 µM). The summer copepod *Acartia tonsa* appeared later in the season and was reduced in abundance in years when *Skeletonema* was the dominant early-summer bloom species, suggesting that the summer diatom bloom success is partially dependent on release from grazing pressure. In years when a flagellate was selected, *P. minimum* blooms occurred in years that were dry (mean riverflow ca. 20% below mean), bright (in situ light was ca. 1/3 greater) and had increased DIN levels relative years having *H. akashiwo* blooms.

Once *Heterosigma* had been selected for , large (>100 cells ml^{-1}) *Heterosigma* blooms displayed a linear dependence on pre-bloom phosphorus (DIP) concentration. *Heterosigma* has relatively high P requirements, and some strains, including a Narragansett Bay strain, are unable to synthesize alkaline phosphatase. Large blooms did not occur if pre-bloom DIP concentration was below ca. 0.5 µM; blooms of > 1,000 cells ml^{-1} occurred only if pre-bloom DIP was >1.0 µM. Between the 1960s and the 1990s, *Heterosigma*'s dominance of the summer niche declined. Several habitat variables, especially declining phosphorus concentration and increasing *in situ* light, exhibited long-term trends that may have reduced *Heterosigma's* competitive ability during the early summer.

DETECTION OF CIGUATOXIN IN THE BLOOD OF PATIENTS DIAGNOSED WITH CIGUATERA INTOXICATION

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Blood collection cards have been widely used for screening purposes to assess errors of metabolism and, more recently, investigation have been made on their use to assess exposure to environmental pollutants or natural toxins. We have previously shown that marine toxins such as brevetoxin, domoic acid, as well as ciguatoxins could be detected in the blood of intoxicated laboratory mice. In the present study, we investigate the application of the blood collection card method to biomonitor ciguatoxin exposure in humans diagnosed with ciguatera intoxication. For this purposes, human blood samples were collected on blood cards during a large case-control study conducted by M-L. Chateau-Degat and her collaborators, in French Polynesia. Blood samples were taken at 3 different periods: at the onset, 15 days and 60 days later. After extraction, dried blood samples were analyzed for their ciguatoxin levels and compared to control human blood. Our results using the neuroblastoma cytotoxicity assay as specific ciguatoxin detection method revealed significant cytotoxic activity in patients' blood extracts whereas no activity was detected using control blood, indicating that this approach could be a useful procedure for clinical diagnosis of ciguatera.

PHOTOCHEMISTRY OF DISSOLVED DOMOIC ACID IN NATURAL WATER MATRICES

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Despite the significance of domoic acid to the biogeochemistry of aquatic systems, little is known regarding the fate of the toxin once it is released into the water column. This is a particularly significant question during bloom events because the majority of toxin produced may not be transferred to higher trophic levels but may instead be released into the water column where seawater extracellular concentrations can reach >100 nmol L^{-1} with cell concentrations over 10^6 cells L^{-1}. It has been suggested that photochemical reaction may represent a possible sink for dissolved domoic acid. In this study, we investigated the photochemical degradation of dissolved domoic acid in natural water matrices.

We have measured the photodegradation rate coefficient for domoic acid (100nM) in 0.2 ìm-filtered Wrightsville Beach, NC seawater (WBSW) and deionized water at 24°C, and found the rates to be similar. This suggests that domoic acid is mainly photodegraded through a direct photochemical pathway. The role of trace metals on the photochemical degradation rate of domoic acid was also investigated, by adding 100 nM of both Fe(III) and Cu(II) to WBSW samples. It was found that trace metals had no significant effect on domoic acid photodegradation, indicating that the formation of trace metal chelates did not enhance photodegradation of the toxin in seawater.

Monochromatic irradiation experiments were carried out to determine the wavelength dependency of the photodegradation of domoic acid. To our knowledge, this is the first time the wavelength dependence of the photochemical transformation of any algal toxin has been determined. We observed that the quantum yield of domoic acid photodegradation in both WBSW and deionized water decreased with increasing wavelength and decreasing energy of incoming radiation with the average value ranging from 0.03 to 0.20 in the ultraviolet wavelength range (280 – 400 nm).

We also estimated environmental turnover rate coefficients for domoic acid photodegradation in order to evaluate the relative importance of photochemical processes as an elimination mechanism for domoic in natural waters. Using determined quantum yields, modeled solar spectral irradiance, and seawater optical properties, *in situ* photochemical degradation rates of domoic acid have been estimated for three different coastal locations Monterey Bay, California, Washington State Coast, and coastal Prince Edward Island. Our results suggest that sunlight-mediated reactions are an important, yet previously unrecognized sink, of dissolved domoic acid in seawater.

Finally, adsorption behavior of the domoic acid in seawater solutions containing various particles was also assessed. For all treatments containing filtered WBSW and particles, only a few percent of dissolved domoic acid were lost. These results suggest that adsorption on particles is not a significant loss mechanism for domoic acid.

DETECTING RAPHIDOPHYTE SPECIES THROUGHOUT CHESAPEAKE AND COASTAL BAYS (MARYLAND) USING REAL-TIME PCR ASSAYS

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Blooms of Raphidophyte species have been associated with kills of captive and wild fish populations throughout the world. Although the exact killing mechanisms are somewhat unclear, it is known that several of these species produce brevetoxin or brevetoxin-like compounds. Also, the production of reactive oxygen species such as superoxide, hydroxide and hydrogen peroxide radicals along with production of hemolytic substances by some species of raphidophytes are postulated to cause gill damage leading to fish mortality. Toxic polyunsaturated fatty acids are yet another element associated with raphidophyte toxicity and in combination with reactive oxygen species may result in the lethal effects observed in some raphidophyte blooms. Many of these species have been identified in Chesapeake and Coastal Bay waters, however traditional identification in complex environmental samples by microscopy is tedious and difficult because these organisms do not preserve well. Previous work conducted by our laboratory and collaborators resulted in six validated real-time PCR assays to target various raphidophyte species. These assays were deployed on approximately 500 surface water samples collected in 2005 as part of Maryland Department of Natural Resources' ongoing monitoring efforts to detect HAB species throughout tidal waters of Maryland. Sampling stations were located throughout the main stem of the Bay, coastal bays, and from tributaries on the western and eastern shores. In the past, Raphidophyte detection was largely focused on environmental event response samples in Maryland tidewaters. The goal of this analysis was to get a broadscale assessment of the spatiotemporal distribution of these organisms throughout the Bays. Assays were run for *Chattonella verruculosa* and *C. cf. verruculosa*, both confirmed brevetoxin-producers. *C. subsalsa* and the *C. marina/C. antiqua/C. ovata* complex have all demonstrated toxicity and an assay was developed to distinguish *C. subsalsa* from these. This species complex, although distinguishable based on morphology, are not genetically distinct across three separate loci (18S; ITS1-5.8S-ITS2-partial LSU; 16S). Ongoing efforts by our laboratory focus on identifying an alternate locus to use for speciating these three organisms. Finally, assays detecting *Heterosigma akashiwo* and *Fibrocapsa japonica*, both known producers of brevetoxin-like compounds, were run on the 2005 samples. The broad range of stations sampled provides an extended assessment of the distribution of Raphidophyte species throughout the Chesapeake and Coastal Bays during 2005.

TOXIC PREY CAN ALTER FORAGING STRATEGIES OF KEY MARINE PREDATORS

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Harmful algal bloom (HAB) toxins have been shown to mediate the strength of consumer-prey interactions, and thus ecosystem patterns and processes, by altering the foraging behavior of principal predators in coastal marine systems. In a series of companion studies examining the role HABs play in structuring marine vertebrate/invertebrate predator-prey relationships, we compared the foraging behavior and diet of key marine mammal and avian predators with prey abundance and seasonal/spatial variation of paralytic shellfish poisoning toxins (PSPT) in selected invertebrate prey species. Results of these foraging studies suggest that some high-level marine predators are able to detect and avoid consumption of lethal concentrations of HAB toxins by altering their foraging strategies, as demonstrated by site avoidance, prey switching, and selective tissue rejection behaviors. Consequently, the ability of prey species to retain toxins may deter or exclude these ecologically important predators from areas affected by HABs, potentially altering ecosystem structure and function. The ecological implication of this shifting of predation pressure away from preferred prey has yet to be determined.

POTENTIAL ROLE OF CLAY IN MITIGATING CHESAPEAKE BAY ALGAL BLOOMS

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Because of increasing concern for algal blooms in Chesapeake Bay and proximal coastal bays, laboratory studies were undertaken to examine the removal of several bloom species through the addition of treated kaolin clay. *Prorocentrum minimum*, *Chattonella subsalsa,* and a small coccoid cyanobacterium were grown in the laboratory and exposed to 0.9 g clay L^{-1}. *In vivo* fluorescence (IVF) was measured on 4 replicates for each taxon (control and treated) before clay additions, 2.5 h after clay addition, and 4 d later. There was a significant decrease in IVF in all clay treatments with largest reductions in IVF noted for *Prorocentrum* and *Chattonella* (99% and 92%, respectively) within 2.5 h of the addition; there was no further decline in IVF over the next four days. For the cyanobacterium, clay was not as effective, removing only 61% and 38% of total cells over the 4 d when initial densities were 10^9 and 10^8 cells L^{-1}, respectively. These results suggest that treated kaolin may be an effective mitigation strategy for flagellates common to Bay blooms whereas coccoid cyanobacteria may persist following clay additions.

CIRCADIAN CONTROL OF THE CELL CYCLE IN THE DINOFLAGELLATE *KARENIA BREVIS*: A ROLE FOR BLUE LIGHT AND CHARACTERISTICS OF A BLUE LIGHT RECEPTOR

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The molecular mechanisms controlling the cell cycle in the Florida red tide dinoflagellate, *Karenia brevis*, are of interest because they ultimately regulate the rate of formation of toxic algal blooms. Previous work in our laboratory has shown that the cell cycle in *K. brevis* is phased to the diel cycle, such that cells enter the cell cycle at precise times relative to the onset of light. Here, we demonstrate that the cell cycle is under control of a circadian rhythm that is entrained by the dark/light transition. In a number of organisms, blue light serves to entrain circadian rhythms. Therefore, we next investigated the effect of red and blue light on cell cycle progression. In the presence of blue light, *K.brevis* appears to enter S-phase early, whereas in red light, cell cycle progression is delayed in S-phase entry. This suggests the presence of both blue and red light signaling pathways in *K. brevis*. Here, we characterize a blue-light receptor identified through EST (expressed sequence tag) screening of a *K. brevis* cDNA library. Cryptochromes are blue-light receptors found in bacteria, plants and animals. The *K. brevis* ESTs have highest homology to a newly identified class of cryptochrome called Cry DASH. Phylogenetic analysis of the photolyase/blue light receptor gene family shows that the *K .brevis* cryptochrome falls within the cryptochrome DASH clade and not the photolyase, cryptochrome 1 or cryptochrome 2 clades. Members of the Cry DASH class are generally localized to the mitochondria or chloroplast and have DNA binding activity suggestive of a transcriptional regulatory activity. Other classes of cryptochromes have been shown to be under circadian control, with rhythmic oscillations in gene expression. *K. brevis* Cry DASH did not display either diel or circadian changes in transcription as assessed using quantitative Real-Time PCR. This is the first blue light receptor to be identified in a dinoflagellate. Extensive high throughput sequencing (25,000 sequences) of two *K. brevis* cDNA libraries, one prepared from cells harvested during the dark-phase and one from the light-phase of the diel cycle, has failed to identify a sequence with homology to known red light receptors.

DID WE HAVE TOXIC ALGAL BLOOMS IN CALIFORNIA IN THE PAST? – SOME INSIGHTS FROM HISTORICAL DATA

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Reports of toxic phytoplankton, including the domoic acid producing marine diatom *Pseudo-nitzschia*, are increasing. An important question is whether these toxic *Pseudo-nitzschia* blooms occurred in the past or are increasing, perhaps due to anthropogenic impacts.

There are no historical data on concentrations of domoic acid available, and historical patterns of marine mammal stranding events in California are not well recorded. In this study we have accessed marine mammal stranding data from several historical archives in Southern California between 1947 and 1983. We have also examined old *Pseudo-nitzschia* data from 1917 and 1939. Data from these diverse sources may help us to determine whether toxic outbreaks of *Pseudo-nitzschia* have increased over the last century.

A HANDHELD DEVICE FOR THE DETECTION OF *KARENIA BREVIS* VIA NASBA

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The annual value of shellfish harvesting in Florida exceeds $20 million and employs over 2500 workers. Blooms of *Karenia brevis*, the red tide forming dinoflagellate in the Gulf of Mexico, often result in closures of shellfish harvesting beds and can have a disastrous impact on the industry. There is a need, therefore, for rapid and inexpensive monitoring of both water and shellfish meats to ensure the safety of shellfish harvested for human consumption. To address this issue, we have developed a protocol for easy field extraction of cellular RNA from water samples and coupled it with a handheld nucleic acid sequence based amplification (NASBA) sensor that amplifies and detects target mRNA specific to *K. brevis.* The extraction protocol is modified version of the RNeasy Mini Kit spin protocol and requires no specialized equipment or training. All components necessary for the extraction are supplied in kit form. Once extracted, the RNA is amplified and detected by NASBA in our handheld sensor. The detector is equipped with two LEDs and two filter sets allowing for the detection of the target RNA and an internal control molecule within a single reaction. While maintaining the 41°C required for the NASBA reaction, readings of the fluorescence intensity are captured using a 16-bit analog to digital converter, processed and routed to the handheld device's serial output. The numeric data is then processed using an in house developed software (Lab View 6.0 based) to provide a real time fluorescence plotting of the NASBA amplification. In duplicate reactions, the amplification curves generated with the handheld detector closely mirrored the curves generated with the bench top EasyQ detector (bioMérieux) and there was no difference in the sensitivity obtained using the handheld device versus the bench top models. This extraction protocol and detection sensor will be a valuable tool for rapidly monitoring of *K. brevis* in field environments, and form the basis for shellfish harvesting management decisions.

PRESENCE OF DOMOIC ACID IN CALIFORNIA ROCK CRABS, *CANCER ANTENNARIUS* AND *CANCER PRODUCTUS* IN MONTEREY BAY, CALIFORNIA

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Dungeness and rock crabs, a major fishery on the west coast have been found to contain domoic acid. The purposes of this study were (1) to determine the levels of domoic acid in raw crab hepatopancreas (i.e. midgut) or "crab butter", a valued food item for some ethnic communities; (2) to determine the animal vector of domoic acid for crabs; and (3) to determine whether the concentration of domoic acid in crabs correlates with domoic acid in *Pseudo-nitzschia* populations in the water. Domoic acid concentrations in the hepatopancreas of 6 crab species, *Cancer antennarius* (Brown Rock Crab), *Cancer magister* (Dungeness crab), *Cancer productus* (Red Rock Crab), *Carcinus maenas* (European Green Crab), *Epialtus productus* (Kelp Crab) and *Loxorhynchus grandis* (Sheep Crab) are reported with particulate domoic acid concentrations and abundance of toxic *Pseudo-nitzschia* sp. in waters along the central California coast.

We report significant domoic acid levels in *C. antennarius* and *C. productus* during periods when toxic *Pseudo-nitzschia australis* and *Pseudo-nitzschia multiseries* were absent from the water column and no detectable particulate domoic acid in the water. The highest concentration of domoic acid found in rock crabs at Elkhorn Slough and the Santa Cruz Municipal Wharf were 372 ppm and 246 ppm, respectively, exceeding the US FDA regulatory limit of 30 ppm (U. S. Food and Drug Administration, 1993). An important central and southern California commercial crab fishery, rock crabs may be a potential vector for domoic acid poisoning in higher trophic levels and Amnesiac Shellfish Poisoning (ASP) in humans.

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CHLOROPLAST GENOMICS OF A TOXIC RAPHIDOPHYTE

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Stramenopiles encompass a broad assemblage of algae, including representatives that cause recurring and destructive harmful algal blooms. Although a significant number of these eukaryotes are photosynthetic, little is known concerning the replication, genetic complement or function of their chloroplasts. To date, in the many thousands of stramenopile species that exist, only two chloroplast genomes have been sequenced. Both are diatoms (Bacilliariophyceae). In this study, the complete chloroplast genome sequence has been determined for the toxic raphidophyte *Heterosigma akashiwo* - an alga that has served as a model system for the analysis of stramenopile chloroplast biogenesis. Our data: (a) show that the *Heterosigma* chloroplast genome differs considerably from those of the diatoms *Odontella sinensis* and *Thalassiosira pseudonana* in genome architecture (Fig. 1); (b) reveal that *Heterosigma* cpDNA encodes genes that are commonly found in the nucleus of chlorophytes, as well as genes that do not exist in any chloroplast genome sequenced to date; (c) provide sequence for generating primers to analyze chloroplast gene expression in *Heterosigma* or to retrieve orthologues of *Heterosigma* genes in other stramenopiles; (d) allow the application of comparative proteomics. The practical use of chloroplast DNA sequences for monitoring HAB molecular processes will be discussed.

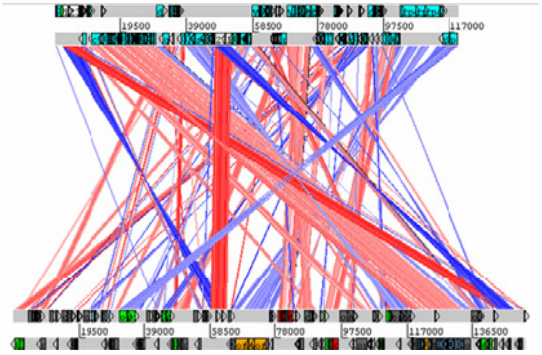

Fig.1 Comparative gene arrangement between complete chloroplast genomes of Heterosigma akashiwo *and* Odontella saneness. *Red and blue lines represent genes coded on different DNA strands.*

THE PRESENCE AND PERSISTENCE OF A POTENTIALLY HARMFUL DINOFLAGELLATE, *COCHLODINIUM CATENATUM*, IN MONTEREY BAY, CALIFORNIA (U.S.A)

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A potentially harmful alga, tentatively identified as the dinoflagellate *Cochlodinium catenatum*, appeared in the Monterey Bay in July 2004 and persisted well into the winter months of 2005. During this period of time, massive blooms were observed with cell densities of upwards of 50,000 cells per liter that discolored large expanses of surface waters. Though it has been observed on the Central Coast of California before, the abundance and persistence of *C. catenatum* during this blooming event has never been documented, which may indicate a possible shift in climatic conditions, or an invasion of a previously infrequent dinoflagellate in the Monterey Bay. Though a toxin has not been identified, there appears to be a very clear correlation between the blooming activity of *C. catenatum* and the extensive mussel mortality that was observed from the toxin monitoring station located at the Santa Cruz Wharf. In this study, we intend to decipher the causes behind the incursion of *C. catenatum* in the Monterey Bay and uncover any possible toxic mechanism that is yet to be established. Weekly water samples from the Santa Cruz Wharf and the M1 mooring site were collected during this event and data including *C. catenatum* abundance, temperature, salinity, nutrient concentration, and phytoplankton biomass and species composition was assembled. Another objective is to identify the potential harmful mechanism *C. catenatum* likely is inflicting on higher trophic levels, such as mussels. Understanding the cause of this appearance may elucidate the likelihood of climatic changes, while also providing us with insight into the ecology of this species, a species that may require monitoring.

ARE ALL THOSE OUTREACH MATERIALS WE'RE CREATING DOING ANY GOOD?

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The west coast of Florida has annual blooms of the toxic dinoflagellate, *Karenia brevis* and Sarasota, FL is considered the epi-center for these blooms. Numerous outreach materials, including Frequently Asked Question (FAQ) cards, exhibits for local museums and aquariums, and several websites have been developed to disseminate information to the public (i.e. www.mote.org, www.floridamarine.org, www.redtideonline). In addition, during intense onshore blooms, much media attention, primarily via newspaper and television become focused on red tide. However, the only measure of effectiveness of these outreach methods has been by numbers of exposure -- as in number of people to visit a website, number of visitors at a museum and/or aquarium, and number of FAQ cards distributed. Little or no assessment has been conducted to determine if these materials make a lasting difference. Also, the local residents frequently respond that they are very knowledgeable about Florida red tide. This study addresses these issues by creating an evaluation tool for the assessment of public knowledge about Florida red tide. A focus group of Florida red tide outreach developers is used to create the assessment tool. The location of the evaluation is on the west coast of Florida, in Sarasota and Manatee Counties, and also on the east coast of the state, an area that very infrequently has onshore *Karenia brevis* blooms. The hypothesis is that there is no difference in the knowledge base of the two different geographic areas and there is no difference in the knowledge between local residents and visitors. This assessment will aid the various agencies developing outreach materials to know what the gaps in public knowledge regarding red tides are, and also identifying the sources of information preferred by the public.

PUFFER FISH: AN EMERGING RESERVOIR FOR SAXITOXINS IN MARINE FOOD WEBS IN THE U.S.

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Recent investigations into several unexplained cases of puffer fish poisoning (PFP) that occurred in Florida, New Jersey, Virginia, and New York demonstrated that southern puffer fish (*Sphoeroides nephelus*) represent a new vector for the transfer of dinoflagellate-derived saxitoxins (STXs), responsible for paralytic shellfish poisoning (PSP), to human consumers in US waters. Tetrodotoxin (TTX), which is nearly indistinguishable pharmacologically from STXs, is the traditional cause of PFP in Japan and other Asian countries. TTX is observed mainly in puffer fish skin and viscera, but not typically in significant concentrations in muscle. STXs have been described previously in both marine and freshwater puffers in Asia, but these were the first reports of PSP from puffer fish consumption in the US. All of the above mentioned PFP cases have now been linked to puffers originating from the Indian River Lagoon (IRL) located in central Atlantic-coast Florida. Since monitoring began in 2002, STX eq. concentrations in the muscle of southern puffers from this area have routinely exceeded the action level of 80 µg STX eq./100g tissue. STX concentrations in four unconsumed filets from a 2004 poisoning event averaged 5768 +/- 1898 STX/100g tissue.

We are currently investigating the potential for the bioaccumulation of saxitoxins from a food chain source in Mid-Atlantic northern puffer fish (*Sphoeroides maculatus*), a traditionally non-toxic species that historically sustained a large commercial and recreational fishery. Hard clams (*Mercenaria mercenaria*), contaminated with a saxitoxin-producing isolate of the dinoflagellate *Alexandrium* sp., were fed to wild-caught northern puffer fish at a rate of 5% of their body weight per day. After 5 weeks, animals were euthanized and tissue compartments were analyzed for toxins by high-performance liquid chromatography. Significant accumulation of saxitoxins was observed in puffer fish fed with hard clams maintained on STX producing *Alexandrium* sp., whereas no STX was detected in puffer fish fed hard clams maintained on non-toxic feeder algae. Clam meat contained 300 µg STX/100g tissue. Rank order for STX concentration per tissue compartment in puffer fish was: ovary > mucous > muscle > skin > intestine > testis > liver > gall bladder. Ovary and mucous, the two tissue compartments with the highest toxin concentration, accumulated average levels of 2,500 and 1,500 µg STX/100g tissue, respectively, while liver only achieved levels ca. 20 µg STX/100g tissue. Muscle, accumulating average concentrations of 300 µg STX/100g tissue, accounted for over 40% of total toxin taken up in the tissues analyzed. Tissue accumulation patterns were similar to those observed for naturally contaminated southern puffer fish from the IRL.

Results indicate that the historically non-toxic northern puffer fish possess the same ability to accumulate PSP toxins from a food chain source as has been described for Florida southern puffer fish responsible for recent PFP events. While the directed fishery for northern puffers has been greatly reduced in recent decades, significant recreational harvesting still occurs. A permanent ban on all puffer fish harvesting is being enforced in five counties in central Atlantic-coast Florida surrounding the Indian River Lagoon. PSP monitoring programs need to be aware of this additional reservoir for PSP toxins in marine systems.

INVESTIGATING THE ROLE OF ZOOPLANKTON GRAZING IN CONTROLLING HARMFUL BROWN TIDE BLOOMS (AUREOCOCCUS ANOPHAGEFFERENS) IN MID-ATLANTIC ESTUARIES

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While harmful brown tide blooms, caused by the pelagophyte *Aureococcus anophagefferens,* have occurred in NY estuaries for 20 years, the frequency of bloom occurrence has diminished greatly in recent years. Blooms which occurred annually in the Peconic Estuary during the 1980s have not occurred in this system for over a decade. Similarly, blooms on Long Island's south shore have become infrequent, with only low *A. anophagefferens* cell densities detected in 2003 and 2004. Physical, chemical, and meteorological conditions in and around these bays have not changed noticeably in recent years. Since some zooplankton are capable of grazing on *A. anophagefferens*, we investigated potential role of zooplankton grazing as a cause of suppression of brown tides in several NY estuaries. Since intense *A. anophagefferens* blooms have occurred annually in Chincoteague Bay, MD since 1999, we concurrently executed a parallel study in that system. During an intense bloom in MD (> 10^6 cells ml^{-1}), we measured significant rates of microzooplankton grazing on multiple classes of picoplankton (heterotrophic bacteria, *Synechococcus* sp., picoeukaryotes) during all experiments, but detected grazing on *A. anophagefferens* during only one experiment when brown tide cell densities were diminished (~ 6 x 10^4 ml^{-1}). In NY, we found low grazing rates (0.34 ± 0.19 d^{-1}) during the last occurrence of a brown tide bloom (2002; 10^6 cells ml^{-1}), but these rates were significantly higher (0.80 ± 0.06 d^{-1}; $p < 0.05$) during years when blooms did not occur (2003 and 2004; ~10^4 cells ml^{-1}). Experimental enrichment of mesozooplankton concentrations during the intense brown tide bloom in MD significantly reduced cell densities of all picoplankton populations enumerated, except *A. anophagefferens* which was unaffected. In contrast, the same additions yielded significantly reduced *A. anophagefferens* densities during non-bloom years in NY. Differences in the impact of grazing between sites suggest that zooplankton may be controlling brown tides in NY and allowing them in MD. The significant impact of zooplankton in the NY location, which once experienced low grazing during blooms, suggests the resident zooplankton may have shifted toward a community which is unaffected by this noxious alga.

EXPOSURE OF NORTH ATLANTIC RIGHT WHALES TO ALGAL BIOTOXINS: THE PROOF IS IN THE POOP!

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Intensive study of the western North Atlantic right whale (*Eubalaena glacialis*) over the past 25 years has yielded evidence of reproductive dysfunction in this highly endangered cetacean population numbering about 300 individuals. Among the factors identified as potentially contributing to this phenomenon, exposure to marine biotoxins associated with harmful algal blooms (HABs) has received little consideration. HABs are generally considered to be an increasingly common phenomenon in the world's coastal ocean waters and the distribution of right whales shows considerable spatio-temporal overlap with certain harmful species. The potential coincidence with toxin producing algae covers a large portion of of the whales' northward migration through the Gulf of Maine and onto their summer feeding grounds in the Bay of Fundy. We thus initiated a study to evaluate the potential for right whale exposure to algal biotoxins.

Over the course of a four-year investigation (2001-2004) involving the analysis of right whale fecal samples for algal biotoxins, we have confirmed the presence of paralytic shellfish poisoning (PSP) toxins (saxitoxins) and domoic acid in numerous animals on virtually an annual basis. Maximum toxin levels obtained to date for fecal material were 1 ug STX equiv. g^{-1} and 16 µg DA g^{-1}. In many cases, both of these potent neurotoxins were present in the feces of a single animal, including lactating females and calves. However, it did not appear that the toxin concentrations measured in fecal samples reflected acutely toxic exposure levels for these actively feeding right whales. The principal vector for transfer of PSP toxins from their dinoflagellate producers (*Alexandrium* spp.) to right whales is the copepod, *Calanus finmarchicus*, a primary prey species containing as much as 0.7 µg STX equiv. g^{-1} in populations sampled from the Bay of Fundy while the whales were present. In the case of domoic acid, produced by diatoms of the genus *Pseudo-nitzschia*, the route of trophic transfer is also likely to be *C. finmarchicus*; however, data supporting this hypothesis are not yet available and DA levels in this grazer are currently under investigation. The repeated detection of saxitoxins and domoic acid in right whale feces over four years establishes the consistent exposure to these algal biotoxins and provides an impetus to examine more closely their potential effects on the health and reproduction of this population.

MICROGININ 690, A NOVEL MICROGININ-TYPE PEPTIDE METABOLITE FROM *MICROCYSTIS AERUGINOSA*

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Freshwater cyanobacteria are known to produce several classes of unique peptide metabolites. *Microcystis aeruginosa* in particular has been a rich source of many interesting peptides, most notably heptapeptides of the hepatotoxic microcystin family. During the isolation and purification of microcystin-LR, the most abundant and most toxic microcystin, some additional unrelated peptides of interest were found. ESI-MS analysis of these compounds revealed a fragmentation pattern suggesting the presence of a β-amino acid, 3-amino-2-hydroxy decanoic acid (Ahda) as well as fragments consistent with the presence of two C-terminus tyrosine residues, both indicative of microginin-type peptides. Microginins are linear peptide metabolites, and previously characterized members of this group have been found to possess angiotensin converting enzyme (ACE) inhibition as well as aminopeptidase M (APM) inhibition. Consequently, these compounds may be of interest as lead compounds in the discovery of novel anti-hypertensive agents as well as treatments for congestive heart failure. Structure activity relationships have shown that the amino and hydroxyl groups of the Ahda residue as well as the di-tyrosine C terminal structure are both important in the ACE inhibitory activity. Extensive NMR and mass spectral data were used to establish the structure of this microginin derivative.

APPLICATION OF A POLYCLONAL ANTIBODY IN THE DEVELOPMENT OF METHODS FOR DETECTING DOMOIC ACID

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Polyclonal antiserum raised in rabbits against ovalbumin (OVA)-domoic acid conjugate was employed in the development of methods to detect domoic acid (DA), a neurotoxin produced by diatoms of the genus *Pseudo-nitzschia*. DA molecule was coupled to the carrier protein (OVA) through one of its three carboxyl groups using a carbodiimide reaction. This immunogen produced an anti-DA serum that is sensitive and specific to free domoic acid. We have successfully used the resulting antibody in an indirect competitive enzyme-linked immunosorbent assay (cELISA) to determine DA levels in contaminated shellfish.

This antibody is also being tested in a new surface plasmon resonance (SPR) detector system developed at the University of Washington, which uses minute changes in surface refractive index to detect analytes. Recent advances in SPR sensor technology make it possible to use our anti-DA antibody to develop a small, compact, battery-operated system for real-time monitoring of DA in the field. The SPR detector system is a competition-based assay where a low concentration of antibody is exposed to the immobilized domoate conjugate attached on the gold sensor surface. A mixture of antibody plus sample or standard is then added to the flow cell over the sensor. Rates of antibody binding to the domoate immobilized on the sensor surface are determined in the absence and presence of varying concentrations of DA in the sample or standard. Since very dilute concentrations of antibody are needed for the competition assay, many assays may be carried out with small amount of antibody. We are presently testing the prototype unit for establishing DA standard curves, examining matrix effects and determining detection limits.

REMOVAL OF THE CYANOBACTERIAL TOXIN MICROCYSTIN-LR BY BIOFILTRATION

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Introduction
The occurrence and persistence of the cyanobacterial toxins, microcystin-LR, in natural waters have been reported worldwide and its risk to public health and animals has been associated with water consumption. The toxic effects of this toxin on humans and animals include total failure of respiratory system, hepatocyte necrosis and tumor promotion in the liver. Conventional water treatment processes such as coagulation, flocculation and filtration have failed to remove algal toxins to recommended levels required by the World Health Organization (WHO). However, there has been reported effective biological degradation of microcystin-LR in field and laboratory studies using water samples from lakes where cyanobacterial blooms have historically occurred.

Hypothesis
Given biological degradation of microcystin-LR is very effective and that filters in water treatment plants can successfully remove naturally organic matter (NOM) via biofiltration, it is expected that microcystin-LR can be degraded by biologically active filters. Biological filters are established in water treatment plants when ozonation is introduced as a disinfectant. Following the approval by the US Environmental Protection Agency legislation to reduce Disinfection By-Products (DBP's) in potable waters, biological filters have become an important water treatment unit to meet the newly established DBP's standards.

Methods
Bench-scale microcystin biodegradation tests were carried out using an enrichment bacterial culture from Lake Mead, Nevada. Three bioreactors were incubated at room temperature for 7 days in the dark to avoid phototrophic growth. In each reactor containing 100 ml of Errington & Powell's medium, 32 mg/L of microcistin-LR was added. In order to evaluate the biodegradability of microcystin in the presence of different amounts of carbon, the amounts of glucose, citric acid, L-glutamic acid and succinic acid from the aforementioned medium were varied to obtain bioreactors containing 100% , 50 % and 0% additional carbon. Sub-samples from the reactors were taken daily for microcystin analysis and evaluation of its degradation rates.

The microcystin degrading enrichment culture was then used to inoculate two bench-scale biofilters operating with typical design parameters of a drinking water treatment facility. The filters were packed with variable amounts of silica sand (effective size 0.51 mm) and anthracite (effective size 0.9 mm) to provide different empty bed contact times (EBCT). After a biofilm was established on the surface of the filter media, the filters were fed in continuous mode at hydraulic loading rate of 2.5 m/h. Dechlorinated tap water containing readily biodegradable organic matter (i.e. acetate and formate), bentonite and the toxin were added tothe filters. Acetate and formate are typical by-products of the ozonation of natural organic matter (NOM) and they are present in the influent water to the filtration units. Bentonite was used to simulate the particulate matter in surface water. Microcystin-LR concentrations varying from 10-130 g/L were added in the influent water. This range corresponds to dissolved microcystin-LR levels detected in lake waters during algal blooms. The concentration of microcystin-LR in the effluent were monited every 12 hours and determined by Enzyme Linked Immunosorbent Assay (ELISA).

Results
The results of the biodegradation tests revealed a reduction of approximately 67% in the bioreactor to which no additional carbon source was added. Lower reductions was obtained in

the experiments with carbon source addition. Therefore, it appears that microcystin itself can be used as a carbon source by the enrichment bacterial culture. These results are encouraging because concentrations of acetate and formate, byproducts of NOM ozonation, in drinking waters are low. Therefore the potential for removal of microcystin via biofiltration is high.

THE BIODIVERSITY OF HARMFUL DINOFLAGELLATES IN THE BELIZEAN CORAL REEF MANGROVE FOREST

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The ecology of marine dinoflagellates in coral reef-mangrove ecosystem is complex because dinoflagellates dwell in the plankton, patch reefs, sea grass beds, sand and on the surface of macroalgae. Significant variability has been observed among harmful dinoflagellate species from Belizean oceanic coral reef-mangrove forests with respect to their biodiversity and distribution within the microscopic food web. Dinoflagellates from two distinct locations, in oceanic Pelican Cays Archipelago waters of the western Caribbean Sea are reported, and compared with dinoflagellates from mangrove forests from the tropical Atlantic Barrier Coral Reef, Belize. There is limited information on the distribution of HAB species in the two areas. Our objective was to compare the biodiversity of benthic, epiphytic and thicoplanktonic dinoflagellates in water samples collected inside and outside sampling sites of these mangrove islands that serve habitats to diverse assemblages of HAB species including species forming red tides. Here, we present comparative information on the distribution of dinoflagellates from three oceanic cays at Cat Cay, Douglas Cay, Manatee Cay and from the detritus driven habitats Twin Cays, South Water Cay, Tobacco range and Carrie Bow Cay situated north of Pelican Cays. These sites represent great typological diversity. The biological communities vary markedly from one pond to another distinguish them as separate ecological community.

Previous work has established that dinoflagellates are the dominant component of microalgae in naturally enriched, protected embayments, maintaining high biomass, forming red tides in Douglas Cay, Twin Cays and Tobacco Range. Benthic photosynthetic dinoflagellates were most numerous: forty-five photosynthetic and three heterotrophic species, and seven red-tide forming species were identified. Species of HAB dinoflagellates indentified: *Coolia monotis*, *Dinophysis caudata* and *D. rotundata*, *Gambierdiscus toxicus*, *G. australes* and *G. polynesiensis*, *Ostreopsis labens*, *O. marina*, and *O. siamensis*, and *Prorocentrum hoffmannianum*, *P. rathymum* and, *P. lima*. Red-tide forming species: *Bysmatrum subsalsum*, *Coolia monotis*, *Gonyaulax reticulatum*, *Plagodinium belizeanum*, *Peridinium quinquecorne*, and *Prorocentrum rathymum*. Nine Prorocentrum species represented 60% of total cell assemblages in colored coral rubble. Dinoflagellates represented total of hundred-ten species in thirty-tree genera, eighty photosynthetic, thirty heterotrophic and twelve mixothrophic species at Pelican Cays. Sixteen HAB species were recorded that are potentially harmful to marine life or toxic to humans. The findings illustrate the richness and biodiversity of dinoflagellate assemblages a conservative estimate of the real total. Despite the high number of HAB species, rather few are known to cause problem. Caribbean coral reef-mangrove forests despite their ubiquity and prominent position between land and sea, these tropical ecosystems still hold countless surprises for researchers

APPLYING COMBINATORIAL PHOTOCHEMISTRY TECHNIQUES TO EXPLORE THE PHOTODEGRADATION OF THE HARMFUL ALGAL BLOOM TOXIN DOMOIC ACID

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The effects of Fe(III), NO_3^-, PO_4^{3-}, dissolved organic matter, and salinity on the photodegradation of the harmful algal bloom toxin domoic acid are reported. A multivariate, microscale, high throughput experimental approach is described for evaluating the contribution of these different species to the removal of domoic acid from the water column. Under the conditions in our study ([Fe(III)] 0.23-1.54 µM; [NO_3^-] 0-35 µM; [PO_4^{3-}] 0-4µM; DOM 0-10 mg/L; salinity 0-35 ppt) it was apparent that dissolved iron was a significant catalyst for domoate photooxidation. In contrast, dissolved organic matter acted to preserve it through a combination of competitive absorption and competitive complexation of Fe(III). No other variables had a statistically significant impact. At an incident light intensity of 750 W/m^2 and initial domoate concentration of 300 ppb, domoate half-lives ranged over 7-14 hours, depending on initial Fe(III) and dissolved organic matter loadings. A model for predicting domoate photodegradation rates under a wide variety of different environmental conditions is reported.

ASSESSMENT OF AN OPERATIONAL HARMFUL ALGAL BLOOM FORECAST SYSTEM FOR THE GULF OF MEXICO

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Blooms of a toxic dinoflagellate, *Karenia brevis*, occur nearly every year on the Gulf coast of Florida, typically between August and December, and are reportedly the most common harmful algal bloom (HAB) occurring in the eastern Gulf of Mexico. While numerous fish kills and various marine bird and mammal deaths have been linked to the blooms, very low levels (5,000µg/L) of *K. brevis* prompt the closure of shellfish beds to prevent Neurotoxic Shellfish Poisoning (NSP) in humans. Under favorable wind conditions, surfacing blooms near shore release a potent brevetoxin aerosol to further afflict coastal regions with respiratory illness and distress; thus prompting beach closures. In order to assist coastal managers in mitigating damages due to harmful algal blooms, a new ecological forecast system for the Gulf of Mexico was developed in a multi-office effort of NOAA. In September 2004 this ecological forecast system was transitioned from research and outreach to operational status, creating the Gulf of Mexico HAB Operational Forecast System (GOM HAB-FS).

The GOM HAB-FS involves a combination of automated processing and analysis using a Web-Based analysis tool. SeaWiFS imagery is processed and analyzed to determine the potential presence of a harmful bloom. Past and forecasted winds available through the National Data Buoy Center, North American Mesoscale model, and National Weather Service marine forecasts, as well as observations and sampling data from several organizations including the Florida Fish and Wildlife Research Institute and Mote Marine Laboratory are incorporated into bulletins for bloom confirmation. These resources are utilized in conjunction with imagery to analyze and forecast HAB location, spatial extent, intensification, and potential beach impacts. Key successes to becoming operational include training of multiple forecasters, technology transfer, standard operating procedures, consistency of analysis, and improved tools. The operational status enables the dissemination of bulletins twice weekly to coastal resource managers and State officials, a daily public conditions report identifying potential bloom impacts available through the Web (http://www.csc.noaa.gov/crs/habf/index.html), and on-call forecaster response to inquiries and special case scenarios.

Since GOM HAB-FS went operational, two substantial harmful blooms have been identified through satellite imagery, confirmed by in situ data, and tracked by forecasters. The operational system is developing methods for evaluating both usability and skill of the forecasts. Usability involves determining frequency of user response to information reported in the HAB-FS bulletins. Skill involves the accuracy of bloom identification, location, spatial extent, intensification, and potential impacts. The bulletin usability and skill assessment will be utilized in making improvements to the Forecast System.

COPEPODS AND DIATOMS: PARADIGM OR PARADOX?

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Copepod secondary production in temperate and high latitudes has traditionally been linked to the spring diatom bloom. Laboratory studies have recently challenged this view showing that either reduced fecundity or viability of the offspring occurred when copepods were fed high concentrations of diatoms. However, field evidence that diatoms affect copepod reproduction is still sparse. We examined vertical distribution, grazing rates, egg production rates, and naupliar viability for two dominant copepods, *Calanus pacificus* and *Pseudocalanus newmani*, in a semi-enclosed fjord, Dabob Bay, Puget Sound, WA. These species differ in size, reproductive strategy and vertical distribution, suggesting that their feeding behavior and physiological response to feeding will differ depending on prey abundance, distribution, and composition. Our results suggest that the reproductive output of *P. newmani* is more negatively affected by certain bloom conditions than that of *C. pacificus*. Both hatching success and naupliar survival of *P. newmani* were reduced following a peak of some *Thalassiosira* species that produced certain aldehydes. Further, the copepods fed selectively on available prey and the dominant taxa during diatom blooms were not necessarily those grazed upon most.

In our studies, diatoms of the genus *Thalassiosira*, especially *T. pacifica* and *T. aestivalis*, with the ability to produce anti-mitotic aldehydes were main contributors to the spring bloom in Dabob Bay. During most of the bloom period (February to April) no effect on copepod reproduction was detected. However, copepod egg hatching and naupliar survival were severely affected when toxic species represented the bulk of the available food and there were few prey alternatives. After such bloom conditions, recruitment of *P. newmani* nauplii declined to almost zero in the following weeks; *C. pacificus* was less, but also measurably, affected. The species-specific differences in the copepod response is attributed to different grazing behavior: *C. pacificus* switched between prey items and seemed to avoid the most toxic items when other food was available, while *P. newmani* did not select against the aldehyde producers.

Papers based on these studies have been submitted and will be published in a dedicated issue of Progress in Oceanography.

STEROLS OF HARMFUL MARINE ALGAE: SYNTHESIS AND METABOLISM

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The harmful algae that produce toxins such as saxitoxin and the brevetoxins also often contain sterols with unusual structures. We recently hypothesized that these sterols serve as chemical defenses by interfering with the nutrition and growth of marine invertebrates[1]. These sterols may be refractory to the normal bioconversion of dietary sterols to cholesterol, and may also interfere with the biosynthesis of steroid hormones by mechanism-based inhibition. To test this, methods for the synthesis of gram quantities of algal sterols were developed and used to prepare specifically ^{13}C-labeled material. The sterols were incorporated into the microalgal diet of juvenile bay scallops (*Argopecten irradiens*) and brine shrimp (*Artemia salina*). Analysis by ^{13}C-NMR spectrometry indicated the metabolic fates of the labeled sterols. Addition of a sterol that is incorporated and metabolized, and which is labeled in a different position was used as a positive internal control in cases where no bioconversion of the test sterol was detected.

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SUSCEPTIBILITY OF TWO FISHES (*O. NILOTICUS* AND *C. VARIEGATUS*) TO *PFIESTERIA SHUMWAYAE* AND ITS ASSOCIATED TOXIN: INFLUENCE OF SALINITY

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Researchers examining the mechanisms of icthyotoxicity of *P. shumwayae* have come to different conclusions about the role of toxin in this process. Some attribute fish mortality solely to direct attack by these pedunculate dinoflagellates on exposed fish tissue while others have provided evidence for a role of a soluble toxin. Detection of toxin, especially in low concentrations, is a function of the sensitivity of the selected bioassay methods and the various groups addressing this question have utilized different methods. One notable difference in fish bioassay methods utilized to detect *Pfiesteria* toxin is the type of fish utilized. Studies that have not detected *Pfiesteria*-associated toxin in bioassays generally have chosen *C. variegatus* as the test fish while those that have detected toxin generally used *Oreochromis* spp. In this study response of these two different types of fish were compared to determine their relative sensitivity to direct attack by *P. shumwayae* and to its associated toxin. The results indicate that *Oreochromis niloticus* is more susceptible to *Pfiesteria shumwayae* and its associated toxin than *C. variegatus* and implicate differences in the ability these species to osmoregulate as a contributing factor for this phenomenon.

APPLICATION OF THE ENVIRONMENTAL SAMPLE PROCESSOR (ESP) FOR REMOTE DETECTION OF HARMFUL ALGAE

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Molecular approaches for identifying harmful algal bloom (HAB) species and affiliated toxins are central to many research and resource management activities, but such methods historically have required the return of discrete samples to a laboratory for analysis. We are exploring development and application of the Environmental Sample Processor (ESP), a novel instrument designed to detect remotely, subsurface, and in near real-time, a wide range of waterborne microorganisms (Scholin et al. 2005; see also http://www.mbari.org/microbial/ESP). To demonstrate the feasibility of detecting of target organisms remotely in the ESP we devised custom DNA probe arrays that target rRNA sequences indicative of specific species or groups of species (Scholin et al. 2005). The entire automated process, from collection of a live sample to broadcast of an imaged array, currently takes ~2 hours and occurs subsurface. The reagents employed in these assays are stable for extended periods (none used in the ESP require refrigeration), and the chemical reactions themselves are amenable to microfluidic scaling. Different arrays can be tailored to specific groups of organisms such as 'planktonic microbes', 'harmful algae', or 'invertebrate larvae', etc. In this fashion we have demonstrated that the ESP can support detection of many different rRNA target sequences using a common methodology, suite of reagents and core sample processing instrumentation. Two-way communication with the instrument allows the user to receive data and instrument status reports, as well as alter the ESP's sampling schedule, modify analytical protocols, etc. Additionally, the ESP is equipped to archive discrete samples for microscopy, nucleic acid, and phycotoxin analyses following deployments.

To date, the ESP has successfully automated application of several different classes of DNA probe arrays in single deployments lasting over 20 days. Additionally, standard curves relating abundance of specific HAB species (*Pseudo-nitzschia australis* c and *Alexandrium tamarense*) to the intensity of corresponding probes spotted on arrays have been established using laboratory cultures. Dr. G. Doucette and co-workers at the Marine Biotoxins Laboratory in Charleston, SC, are also developing a competitive ELISA technique to detect and quantify domoic acid for deployment aboard the ESP. Our goal is to use that assay in concert with the DNA arrays to enable the remote, integrated assessment of algal cell abundance (*Pseudo-nitzschia spp*) and associated toxin levels. The ESP is scheduled for a number of deployments in Monterey Bay, CA, from June – August 2005. In this presentation we will review our progress to date and future plans.

Scholin, C.A., G.J. Doucette and A.D. Cembella. 2005. Prospects for developing automated systems for in situ *detection of harmful algae and their toxins. In:* Monographs on oceanographic methodology *(Babin, M., Roesler, C, and Cullen, J. eds). UNESCO (in press).*

ALEXANDRIUM CYSTS IN PUGET SOUND WASHINGTON: PRELIMINARY RESULTS OF A SURVEY

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Paralytic Shellfish Poisoning (PSP) has been a problem in Puget Sound and other western Washington waters for hundreds, possibly thousands, of years. Monitoring for toxin in commercial shellfish by the Washington Department of Health began in the early 1930s, but was infrequent. After three deaths occurred near Neah Bay in 1942, an annual closure for the harvest of all shellfish except razor clams has been imposed from Dungeness Spit along the Strait of Juan de Fuca and the Pacific coast to the mouth of the Columbia River from April 1 to October 31. Monitoring for recreational harvest elsewhere was sporadic until the 1970s when toxin was found in shellfish on beaches in the northern Sound. In 1978, record high levels of toxin near 30,000 µg/100 g of shellfish were found in the Whidbey Basin and toxin gradually spread into the southern Sound with the first closure there in 1988. Since that time PSP has occurred in all areas of Puget Sound (Trainer et al. 2003) with toxin levels sometimes in the 9,000-12,000 µg/100g shellfish range (Washington Department of Health records). However, little is known about the biology of the organism that produces the toxin. In the 1980s, Nishitani (Norris), Chew and their associates studied *Alexandrium catenella* primarily in Quartermaster Harbor, an area known to have recurrent toxin events (Nishitani and Chew 1984). They found a rapid increase in both *Alexandrium* cells and PSP in mussels following a five week period when the temperature was >14°C and the thermally stratified layer was 4 m deep. They also found that *Alexandrium* undergoes diel vertical migration with the spread of cells depending on the stability of the water column. *Alexandrium* cells were parasitized by *Amoebophyra ceratii*, leading to the decline of the population. Their survey for *Alexandrium* cysts in 1981 found motile cells, cysts, or low levels of PSP toxin in shellfish throughout southern Puget Sound (Nishitani, pers. comm.), but no one has apparently repeated the cyst survey. Here, we present preliminary results of our ECOHAB project to determine the relationship between PSP in shellfish based on historical records from the Washington Department of Health and the distribution of cysts and vegetative cells of *Alexandrium* spp. in Puget Sound.

Sediment and water samples have been collected from 32 sites distributed throughout Puget Sound. Here we present results of grain size and heavy metal concentrations that might affect cyst germination, and preliminary results of sediment analyses for cyst presence. On-going analyses include cyst germination studies (see poster by Hoffer et al. 2005) and the presence of *Alexandrium* motile cells in water samples. Future studies include analyzing cores for ^{210}Pb levels to determine possible age, bioturbation, and remixing of surface sediments and testing of motile cells obtained from germinated cysts for PSP production using Jellett Test Kits. Sites where cyst concentrations are high will be reexamined for sediment accumulation and mixing parameters during cruises in 2006. Sites at constrictions into bays and at sills will be sampled to determine where cyst accumulation, turbulence, and resuspension occur.

Hoffer, S., R.A. Horner, and C.L. Greengrove. 2005. Germination experiments with Alexandrium catenella *cysts collected from surface sediments in Puget Sound. Third Symposium on Harmful Algae in the U.S. Pacific Grove, CA.*

Nishitani, L. and K.K. Chew. 1984. Recent developments in paralytic shellfish poisoning research. Aquaculture 39:317-329.

Trainer, V.L., B-T.L. Eberhardt, J.C. Wekell, N.G. Adams, L. Hanson, F. Cox, and J. Dowell. 2003. Paralytic shellfish toxins in Puget Sound, Washington State. J. Shellfish Res. 22:213-223.

ASEXUAL AND SEXUAL REPRODUCTION IN THE HETEROTROPHIC DINOFLAGELLATE *PROTOPERIDINIUM OBLONGUM*

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Protoperidinium is a cosmopolitan genus of heterotrophic dinoflagellates, of which there are 200 described species. One species, *P. crassipes*, has been implicated as the source of azaspiracid shellfish toxin. Despite their prevalence in the plankton, nothing is known of the life histories of *Protoperidinium* spp., except that many species produce dormant cysts. In this study, life cycle stages of the heterotrophic dinoflagellate, *Protoperidinium oblongum,* were determined for a culture isolated from Vineyard Sound, Massachusetts. Division, sexuality, mandatory dormancy and germination rates of hypnozygotes, and identity of morphologically distinct life-history stages were revealed for the first time using a suite of morphological and molecular tools.

Asexual division occurred by eleutheroschisis within a temporary, immotile cyst, yielding two daughter cells. Morphological features, including apical and antapical horns, were not fully formed upon emergence from the division cyst, but developed rapidly after cell division. Daughter cells were half to two-thirds the size of parent cells and swam attached in an epitheca-to-hypotheca orientation before separating.

Stages indicating a completed sexual life cycle, including gametes, planozygotes, hypnozygotes, and presumptive planomeiocytes were observed. Sexual reproduction was constitutive in unialgal, non-clonal cultures. Although gametes formed in clonal cultures, no cysts were ever produced, indicating that this species may be heterothallic. Gametes were isogamous, approximately half the size and lacking the pink pigmentation of the vegetative cells, and were never observed to feed. Given their different morphology, gametes may have been mistaken as separate species. The LSU rDNA sequence (D1-D6 region) of small cells was the same as that of *P. oblongum* vegetative cells, indicating that these morphologically distinct stages were not a culture contaminant.

Gamete fusion resulted in a small planozygote with two longitudinal flagella. Hypnozygotes, or cysts, were dorso-ventrally compressed with a smooth, clear cell wall, and had apical and antapical horns of varying length. Cysts had a mandatory dormancy period of 40-50 days, and germination was positively correlated with temperature. The product of cyst germination was a large germling cell with two longitudinal flagella. Detailed studies are required to determine when meiosis occurs during this process, so the germling cell is considered a presumptive planomeiocyte. From observations made to date, the life cycle of *P. oblongum* appears similar to that of other dinoflagellates. Because of the difficulty in culturing heterotrophic dinoflagellates, all species identifications within *Protoperidinium* have been made from field samples. Given the variability in morphology of different life stages seen in culture, however, taxonomic revision may be in order.

GROWTH, TOXICITY AND COMPOSITION OF *PRYMNESIUM PARVUM* IN RELATION TO TEMPERATURE, LIGHT AND SALINITY

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We measured the growth rate, stationary density, toxicity, and nutrient element composition of *P. parvum* at a range of temperatures, salinities, and light intensities, using batch cultures of a strain of these algae originating from Texas waters. Exponential growth rate in nutrient-saturated media was a unimodal function of temperature through a range of 5-35°, with highest growth rates at about 29°C. Exponential growth rate in nutrient-saturated media increased approximately linearly with light and salinity for the ranges tested of 14-420 µE m^{-2} s^{-1} for irradiance and 0.5–35 psu for salinity. Interactions among temperature, light and salinity did not significantly affect exponential growth rate. Stationary cell densities were generally highest at moderate values of temperature, salinity and light. Toxicity of stationary-phase cells to fathead minnows was generally lower under conditions that permitted rapid exponential growth. Nutrient-sufficient, stationary-phase cells had elevated quotas of C, N, and P at lower temperatures, and deviated from Redfield stoichiometry at all temperatures due to P accumulation. Nutrient-deficient cells grown at 20° deviated from Redfield stoichiometry in the opposite direction, with reduced P quotas. Rapid growth at high temperatures has not been found in most strains of these algae originating from more temperate climates, suggesting that populations in Texas have adapted to warmer weather. Our experiments also imply that blooms in summer could occur in Texas. Generally they have not, suggesting that factors other than high temperature prevent them in these waters.

A NEW APPLICATION OF QUANTITATIVE REAL-TIME PCR: SIMULTANEOUS ENUMERATION OF MULTIPLE RAPHIDOPHYTE SPECIES BY MULTIPROBING AND MULTIPLEXING

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Quantitative real-time PCR (QRT-PCR) is a powerful method for species-specific detection and enumeration of HAB algae, but it can be time consuming and expensive for multi-species detection. We explored multiprobing as a way to save money and time. Multiprobing is the use of multiple species-specific fluorescent probes in one tube, allowing for the simultaneous detection and enumeration of more than one species using the same primer pair. This technology utilizes probes that fluoresce at different wavelengths and allows screening of multiple reporter dyes, limited only by the number of dyes that can be detected by the instrument. This approach can cut analysis time and costs at least in half, depending on the number of species that can be accurately detected and enumerated in the mixture. In addition, we compared multiprobing to multiplexing (using two different, more specific primer sets and species-specific probes). This method has been applied in biomedical research, but has not been tested for investigations of environmental populations of HAB species. We found that up to three raphidophyte species plus an internal standard can be detected and enumerated simultaneously in a single reaction, and we are investigating the sensitivity of this method for each species. In addition, different probe designs were explored for their potential advantages and disadvantages. This method is perfectly suited for investigation of HAB events such as the mixed blooms of harmful raphidophytes (*Chattonella* cf. *verruculosa*, *C. subsalsa*, *Heterosigma akashiwo*, and *Fibrocapsa japonica*) that co-occur in the Delaware Inland Bays (DIB), U.S.A.

TRANSFER OF BREVETOXINS TO THE BENTHOS IN THE CONTEXT OF CLAY MITIGATION OF *KARENIA BREVIS* BLOOMS

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Blooms of the brevetoxin-producing *Karenia brevis* in the Gulf of Mexico cause massive fish kills, and food poisoning and adverse respiratory effects in humans. Sedimentation of toxic cells following inert clay application could reduce toxin incorporation by commercially important suspension-feeding bivalves and direct public health impacts, but could potentially lead to brevetoxin (PbTx) accumulation by benthic deposit-feeders. The goal of this study was therefore to compare suspension- and deposit-feeding as pathways for brevetoxins. We investigated: i) the toxic effect of *Karenia brevis* (Wilson strain, CCMP718) on both feeding modes using a facultative deposit-suspension feeding tellinid bivalve, the clam *Macoma balthica*, as a model species (13 to 16 mm in shell length); ii) the relative effectiveness of brevetoxin transfer via suspension- and deposit-feeding over 24-h exposure and iii) the fate and dynamics of brevetoxins (Highman strain, CCMP2229) in the benthic compartment. Sedimentation of *K. brevis* (equivalent to removal of ~300 cells ml^{-1} from a 1m water column) was achieved by treatment with 0.25 g phosphatic clay l^{-1}. Brevetoxin concentrations in *K. brevis*, sediments and clam tissues were measured by ELISA and LC-MS. Brevetoxin metabolites in shellfish were determined by LC-MS.

Karenia brevis reduced both suspension- and deposit-feeding activity and toxin body burden attained comparable levels with both feeding modes after 24h-exposure to suspended vs. settled *Karenia brevis* [1.2-1.6 mg PbTx (g tissue wet weight)$^{-1}$]. Longer-term toxification experiments, in which clams were exposed to an initial pulse of settled *K. brevis* for 7 days, showed that they rapidly accumulated brevetoxins (exceeding the regulatory level of 0.8 mg PbTx g^{-1} within ~12h) and toxin body burden remained high and relatively constant [~4 mg PbTx (g wet weight)$^{-1}$] over time. When clams were transferred into clean sediment for 15 days after 48-exposure to settled *Karenia*, toxicities decreased from 5.2 ± 1.6 to 1.5 ± 0.4 mg PbTx (g wet weight)$^{-1}$. Toxins were mainly accumulated in the viscera such that the rate of detoxification of whole clams (10.9% toxin loss per day) was mainly driven by detoxification of this tissue compartment.

This study demonstrates that brevetoxins can be rapidly accumulated by a surface deposit-feeding bivalve from sedimented *K. brevis* cells and that comparable toxin levels can be attained by both suspension- and deposit-feeding modes. Detoxification of tissues following deposit-feeding occurred but toxicities remained above the regulatory level after 15 days. This relatively extended residence time in shellfish tissues may allow toxin transfer from deposit-feeders to their secondary consumers, e.g., crabs and fish.

APPLICATIONS OF rDNA, ITS SEQUENCE ANALYSIS TO ASSESS INTER- AND INTRASPECIFIC DIVERSITY IN *PSEUDO-NITZSCHIA* COMMUNITIES OF MONTEREY BAY, CA

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Monitoring of the harmful algal genus, *Pseudo-nitzschia*, is confounded by the occurrence of intraspecific variation in DA production, such that the presence of a potential toxic species does not always equate to the occurrence of a toxic bloom. DA production appears to be induced by specific environmental conditions (e.g. Si-limitation), and capacity for toxin synthesis can vary by over 2-orders of magnitude among lab isolates under common conditions in a given *Pseudo-nitzschia* species, suggesting an underlying genetic control of toxicity. Internal transcribed spacer (ITS) regions are variable rDNA segments flanked by conserved large and small ribosomal subunits (LSU and SSU) and have been used to dissect phylogenetic relationships among *Pseudo-nitzschia* species (Lundhom et al. 2003). A study in the Mediterranean reported up to 30% divergence among *Pseudo-nitzschia delicatissima* isolates in the ITS regions, suggesting that cryptic diversity may account for variability in DA toxicity (Orsini et al. 2004). Based on these results, the ITS regions were targeted as a starting point to characterize intraspecific diversity in *Pseudo-nitzschia australis*. Beginning in May 2003, water was collected weekly with a 5 m vertical net tow at the Monterey Wharf II (36.36.21N, 121.53.35W). Aliquots were archived for phytoplankton community composition, pigment analysis, total DA, particulate DA, and nucleic acids. If present in weekly samples, multiple isolates of *Pseudo-nitzschia* cells were isolated and subsequently purified to unialgal cultures by transferring chains of cells into 25 mL culture tubes with f/2 media.

Oligonucleotide primers targeting conserved regions in the SSU and LSU rDNA borders of the ITS regions were used to successfully amplify ITS sequences from our *Pseudo-nitzschia* isolate collection. Sequence analysis of *P. australis* isolates from differing temporal and geographical ranges revealed surprisingly low diversity (<0.56% divergence) among toxic and non-toxic *P. australis* cultures. Low genetic diversity in the *P. australis* ITS regions suggests that cryptic species are not contributing to DA toxicity patterns in this group. Furthermore, these results indicate that coarse-scale molecular markers routinely used for taxonomic discrimination are not informative as probes to identify strains with distinct physiological phenotypes. However, diversity in the ITS regions between species allowed us to design primers for the genus *Pseudo-nitzschia* as well as several common *Pseudo-nitzschia* species in Monterey Bay: *P. australis*, *P. fraudulenta*, *P. pungens*, *P. delicatissima*, and *P. multiseries*. Primer specificity was confirmed with tests on laboratory cultures. Genus-specific primers incorporated into quantitative PCR (Q-PCR) assays enable the enumeration of total *Pseudo-nitzschia* numbers, while species-specific primers allow the quantification of each species in the community. A multiplex PCR with species-specific primers is being developed to enable rapid assessment of community composition, complimenting other molecular assays on field derived samples. The ITS assays are being used to probe our archived field nucleic acid samples; the molecular species composition results will be calibrated by SEM analysis of the corresponding field voucher samples. Collectively, these efforts are enhancing our ability to study *Pseudo-nitzschia* community ecology in Monterey Bay. The search for genomic markers distinguishing between toxic and non-toxic strains of *P. australis* is continuing using alternate genomic mapping techniques.

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AEROSOLIZED BREVETOXINS: COMPOSITIONAL CHANGES FROM WATER BORNE BREVETOXINS TO AEROSOLIZED BREVETOXINS IMPACTING HUMAN RESPIRATORY FUNCTION

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The toxic dinoflagellate, *Karenia brevis*, produces a suite of polyether neurotoxins (brevetoxins) that cause massive fish kills and neurotoxic shellfish poisoning (NSP). A unique characteristic of *K. brevis* blooms is the associated airborne brevetoxin component that results in severe respiratory problems in exposed populations. This study was undertaken in collaboration with human exposure studies to determine the composition of aerosolized brevetoxins to which beach goers are exposed during a *K. brevis* bloom. Brevetoxins were extracted from water collected along the shore and from marine aerosol samples collected on the beach during and in the absence of a coastal *K. brevis* bloom. The most abundant brevetoxin associated with *K. brevis* cells in the water was PbTx-2, followed by PbTx-3, with a ratio of PbTx-2/-3 of approximately 17/1. Aerosol samples, however, exhibited a drastic change in PbTx composition, with PbTx-3 and PbTx-2 being nearly equivalent. The ratio of PbTx-2/-3 in aerosol was approximately 1/1. The carboxylic acid of PbTx-2 and various brevetoxin hydrolysis products were also observed in marine aerosols. Previously, these compounds were identified in *K. brevis* culture and bloom water, and metabolized in shellfish, but have not been described in marine aerosols to which people and other mammals are exposed. Knowledge of these compounds provides critical information regarding cause and effects relationships for toxin exposure.

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GERMINATION EXPERIMENTS WITH *ALEXANDRIUM CATENELLA* CYSTS COLLECTED FROM SURFACE SEDIMENTS IN PUGET SOUND

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Occurrences of Paralytic Shellfish Poisoning (PSP) have increased and moved southward through Puget Sound over the past 20 years (Trainer et al. 2003). This trend has been documented by the Washington State Department of Health through its shellfish monitoring program. However, there have been few data collected on the distribution of the organism (*Alexandrium catenella*) responsible for these outbreaks. We were recently funded by NOAA ECOHAB to do a regional survey of *Alexandrium* spp. cysts in the surface sediments of Puget Sound. The preliminary results of this survey appear in a separate presentation at this meeting (Greengrove et al. 2005).

Alexandrium catenella cysts collected from surface sediments in Puget Sound as part of this regional cyst survey are being used in a germination experiment designed to evaluate temperature and substrate effect on excystment and saxitoxin production. Excystment characteristics from two sites in Puget Sound will be compared in two substrate treatments at three different temperatures. Cysts will be incubated at 4, 14, and 20°C in culture medium and in situ surface sediment material. Duration of cyst dormancy and the number of motile *A. catenella* cells after excystment will be monitored. After excystment, motile *A. catenella* cells will be isolated and added to another culture container under identical environmental conditions to evaluate differences in saxitoxin production. Saxitoxin production will be examined by means of Jellett Rapid Test strips (Jellet et al. 2001). Environmental parameters of each study site will be used to evaluate the results of the analysis. Further germination experiments will be conducted after additional sediment collections in 2006.

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POTENTIAL AND ACTUAL MICROCYSTIN PRODUCTION IN LAKE ONTARIO EMBAYMENTS

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Outbreaks of toxic cyanobacteria have been increasing on Lakes Ontario, Champlain and Erie. Cyanobacteria in these toxic blooms can produce a family of hepatotoxic peptides, called microcystins. There are two hypotheses surrounding microcystin production in the Great Lakes: (1) the toxic blooms originate offshore and are circulated throughout the lake via water currents and (2) the blooms initiate in eutrophic embayments and are transported to the main lake. To investigate the relationship between the genetic potential for microcystin formation and the actual production of microcystins in Lake Ontario embayments and open water, samples were collected from along the southern and eastern shores of Lake Ontario during the summers of 2001 and 2003, and from open waters during the summer of 2003. DNA samples were amplified by PCR using primer sequences specific to the cyanobacterial 16S ribosomal gene (CYA), *Microcystis* spp. 16S rRNA (MIC), and the non-ribosomal microcystin biosynthetic pathway (*mcy*B and *mcy*D). These results will be compared to microcystin production, as measured by the protein phosphatase inhibition assay (PPIA). This information will be used to evaluate the relative contribution of embayments vs. offshore regions to microcystin production in Lake Ontario.

WEST COAST *PSEUDO-NITZSCHIA* SPECIES DISTINGUISHED BY POLYMORPHISMS IN THE INTERNAL TRANSCRIBED SPACER 1 (ITS1)

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The diatom *Pseudo-nitzschia* produces domoic acid, a neurotoxin that can cause illness and death in mammals. Along the Washington coast, domoic acid-based closures of razor clam harvests have occurred since the early 1990's. In contrast, the first domoic acid-based closure of shellfish harvests in the Puget Sound estuary occurred about 10 years later in September 2003. As part of the Pacific Northwest Center for Human Health and Ocean Studies, we are examining whether genetic and physiological differences among cells may help explain the distribution of shellfish closures in Washington waters. As a first step in developing genetic markers for *Pseudo-nitzschia spp.*, the internal transcribed spacer 1 (ITS1) sequence was determined for 12 isolates, including *P. multiseries*, *P. pungens*, *P. seriata*, and *P. australis*, collected from estuaries and waters along the Washington and California coasts. Species are easily distinguished from one another using ITS1 sequences. Seven cultured isolates of *P. pungens* share a common ITS1 haplotype, regardless of whether the cells were collected from inland or coastal waters. Based on these sequences and *Pseudo-nitzschia spp.* sequences in GenBank, genus-specific PCR primers were developed and used with field samples collected from different sites within the Pacific Northwest. In contrast to data from cultured isolates, at least five ITS1 haplotypes of *P. pungens*, including the "culture haplotype," were identified in ITS1 clone libraries of an environmental sample from Puget Sound. In addition, one ITS haplotype with no similarity to sequences in GenBank was identified multiple times in an environmental sample collected from the west coast of Vancouver Island. This suggests that the isolates currently in culture may not adequately reflect the full range of *Pseudo-nitzschia* diversity present in field populations. We are currently developing techniques to identify *Pseudo-nitzschia* species based on species-specific ITS length polymorphisms. This approach will complement morphology-based identifications and will allow us to rapidly determine species assemblages of *Pseudo-nitzschia* at different locations. Both species composition and intraspecific variability may be significant factors in the development and perpetuation of toxic events.

OREGON STATE EMERGENCY RESPONSE TO RECENT COAST-WIDE CLOSURES DUE TO DOMOIC ACID TOXICITY

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The Oregon Department of Fish and Wildlife (ODF&W) and Oregon Department of Agriculture (ODA) announced on Friday, May 13, 2005, the extension of an existing coastwide razor clam closure to include mussels and non-razor clams, on the entire Oregon coast. On April 26, 2005, the entire Oregon coast was closed to razor clamming; the south coast from Charleston to the California border was closed from previous bloom events. Both mussels and razor clams exceeded the safe regulatory level of 20 ppm toxin; domoic acid in mussels reached 128 ppm, razor clams had maximum levels of 213 ppm. These closures are estimated to cost local communities and the State of Oregon over 4 million in lost revenue. The recent closures of southern Washington beaches (Long Beach, April 29, 2005) due to elevated levels of domoic acid in razor clams (up to 20 ppm), illustrate the importance of communication between Washington and Oregon State managers in the effective mitigation of the effects of harmful algal blooms. In fact, the Oregon Dept of Agriculture shellfish program chose to increase razor clam sampling and hold commercial product for use as bait, based on reports of increasing cell counts from Washington phytoplankton monitoring. The effectiveness of phytoplankton sampling in providing early warning of shellfish toxicity was illustrated just prior to this coast-wide closure event in Oregon State. Several Oregon beaches were sampled during the first week of April and *Pseudo-nitzschia* cell numbers of over 600,000 cells/L were noted. These were later determined by scanning electron microscopy to be almost 100% *P. australis*. Cruises of opportunity were conducted to determine the expanse of the bloom that caused this event. Oregon's response to this current HAB event could be more comprehensive and cost-effective with coastwide phytoplankton monitoring in place. ODA could target domoic acid sampling of recreational and commercial shellfish where toxic plankton are found to be in greater abundance. Through MERHAB emergency response funding, a phytoplankton monitoring program is planned for the State of Oregon.

LAGRANGIAN STUDIES OF *KARENIA BREVIS* BLOOM INITIATION

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Karenia brevis occurs at background concentrations of 1-1000 cells L^{-1} in the Gulf of Mexico. Cells that eventually contribute to harmful algal bloom (HAB) events off the west Florida coast appear to stage 18-74 km offshore in oligotrophic water columns < 40 m thick. The rapid development of near shore HAB events in satellite-based observations may depend on offshore subsurface source populations below one optical depth, while cruise-based observations suggest that source populations may utilizes nutrient resources near the sediment interface. Though near-bottom upwelling from offshore is a possible nutrient source, evidence suggests that interstitial nutrient regeneration from decaying organic matter (diatom blooms, *Trichodesmium*) that fluxes into the water column is the more likely nutrient source. *K. brevis* is thought to act like an extension to the microphytobenthos in water columns where the sediment interface is below the euphotic zone but where the euphotic zone is within reach of cells capable of ascending at 1 m h^{-1}. These near-bottom *K. brevis'* populations are then available for transport and accumulation in coastal fronts formed in response to upwelling-favorable winds and outwellings from Florida bays. The roles of *K. brevis*' behavior and physiology in HAB formation are examined using expanded Eulerian numerical physical-biological models and behaving, environmentally responsive programmable Lagrangian drifters. Results based on numerical physical-biological models demonstrate that behavior is an integral part of *K. brevis'* aggregation. Deployments of behaving, environmentally responsive programmable drifters are underway to test alternate *K. brevis'* behaviors and physiological capabilities under the same physical forcing conditions on the west Florida shelf.

PSEUDO-NITZSCHIA AUSTRALIS: THE MOST ABUNDANT SPECIES OF THE GENUS *PSEUDO-NITZSCHIA* AT TWO CENTRAL CALIFORNIA SITES SOUTH AND NORTH OF PT. CONCEPTION, 2003-2005

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Our study was part of a multi-campus collaboration (HAB-TrAC) to obtain a long-shore perspective on the abundance of harmful algal blooms and their associated toxins at California coastal sites between Santa Cruz and San Diego. We report on the abundance of domoic acid (DA) producing species, *P. australis* and *P. multiseries*, and the total abundance of the genus Pseudo-nitzschia south and north of Pt. Conception in Santa Barbara and Avila. Extensive toxic blooms were observed from pier sampling for both locations during spring 2004. Increases in volumetric DA levels were closely associated with increased *Pseudo-nitzschia* abundance. *P. australis* was the dominant *Pseudo-nitzscha* species during all the observed bloom events for both sites. *P. multiseries* made up a greater portion of the total *Pseudo-nitzschia* population between bloom events than during blooms, and unlike other HAB-TrAC sampling locations, *P. australis*, *P. multiseries*, and other *Pseudo-nitzschia* species were observed in low concentrations almost year round in both locations. Toxin producing species, *P. australis* and *P. multiseries*, were present approximately 70% of the time in Santa Barbara and Avila, while other *Pseudo-nitzschia* species were present 80-90% of the time. These numbers strengthen the California Health Department's concern that Pt. Conception, a prominent west coast cape, may be a "hot spot" for harmful algal bloom (HAB) events.

DETECTION OF *KARENIA BREVIS* IN AN EARLY BLOOM STAGE USING THE BREVEBUSTER

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On October 20, 2004, during a monthly HAB survey of the southwest coast of Florida, a shipboard BreveBuster signaled the presence of *Karenia* sp at a single location just offshore of Naples Florida. Microscopic examination of a water sample collected near the surface by Niskin bottle confirmed the presence of a medium level of *Karenia brevis* (Figure 1).

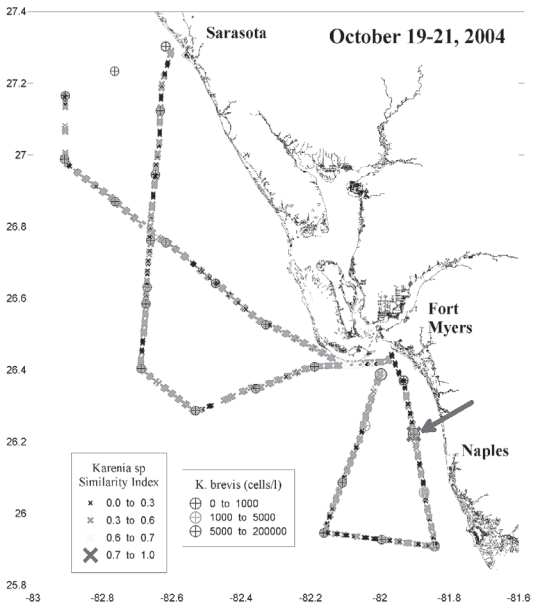

Figure 1. Florida red tide survey cruise track from October
19 - 21, 2004 depicting the *Karenia* sp Similarity Indexes determined by a shipboard BreveBuster. Red arrow points
to the site where the BreveBuster reported the presence of *Karenia* sp and the confirming microscope cell count.

A time series of NOAA HAB Bulletins documented the subsequent development of a bloom patch adjacent to the BreveBuster detection site over the following two weeks. This bloom patch was revisited during a MERHAB cruise three weeks after the first encounter as the patch moved southward toward the Florida Keys. During that cruise the BreveBuster, while running comparison studies with the sandwich hybridization molecular probe detection technology, was able to map the near-surface distribution of the bloom. Good comparisons were found between the BreveBuster-generated bloom distributions and those depicted in the NOAA HAB Bulletins for the time period of the cruise. That bloom continued to drift southward until it reached the Keys in late December 2004.

LONG-TERM MONITORING OF MARINE BIOTOXINS IN CALIFORNIA: WHY THE STATUS QUO IS NOT

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California has a long history of experience with the paralytic shellfish poisoning toxins, extending back to the customs of the coastal tribes. In 1927 California health officials recognized the serious potential of PSP in the wake of a major outbreak affecting 102 people. In response, the California PSP Prevention Program was established by what is now the California Department of Health Services (DHS), the first such monitoring effort in the country.

Sixty-four years later, with the advent of domoic acid in 1991, the state's PSP program was broadened to include domoic acid monitoring. As a direct result of support and technical guidance by the U.S. Food and Drug Administration, DHS was able to quickly begin routine screening of shellfish, finfish, and crab samples for this toxin. Equally important at this time was the development of a volunteer-based phytoplankton monitoring program, whose goal was to provide cost-effective, near real-time information on the distribution and abundance of any toxigenic species. The following provides a brief summary of this monitoring data.

A review of the past 23 years of data demonstrates strong seasonal patterns of PSP toxicity and tremendous variability in magnitude and distribution throughout the years. The greatest frequencies and magnitudes of PSP toxicity occur in central California, particularly Marin County north of the Golden Gate, and decline as you move both northward and southward along the coast. Analysis of this toxicity data, in conjunction with remote sensing information available from the National Oceanographic and Atmospheric Administration and phytoplankton distribution and abundance data, provides interesting insight into potential environmental cues for toxigenic blooms. Strong upwelling events, followed by uninterrupted periods of "relaxation" or downwelling, appear to provide an increased risk for the PSP toxins in shellish.

The first recognized occurrence of domoic acid in California was in the fall. However, a review of the past 13 years of shellfish, fish, and crab toxicity data, together with data on phytoplankton distribution and relative abundance, reveals a bimodal distribution with a strong spring component. As with the PSP toxins, domoic acid magnitude and distribution has varied greatly from year to year. During the first several years of monitoring, the relative lack of toxicity in mussels in the midst of observations and reports of high numbers of *Pseudo-nitzschia* led to concern that these bivalves were not adequate indicators for this toxin. The past several years have seen extremely high domoic acid levels in a variety of seafood species: 380 µg/g in mussels, 230 µg/g in sardines, 374 µg/g in pelagic red crab, and 75µg/g in rock crab. As a result of this heightened activity there have been record impacts to marine mammals. Fortunately there have been no recorded human health impacts. In 2002 a well-developed pattern emerged of sequential blooms "moving" down the coast of California from Santa Cruz to Los Angeles, a distance of hundreds of miles. Record levels of domoic acid were recorded in California shellfish, perhaps partially exonerating our venerable indicator bivalve.

DHS is continuing to investigate ways to improve the frequency and quality of field-based monitoring data as the most cost-effective means to protect public health. The time and effort contributed by volunteers and program participants from other agencies to assist this monitoring program is the key to maintaining a successful early warning system for potentially dangerous blooms that could impact a variety of seafood species and, consequently, the people that harvest and consume these resources.

EFFICACY TESTING OF BALLAST WATER TREATMENT TECHNOLOGIES: PREVENTING NON-INDIGENOUS PHYTOPLANKTON INTRODUCTIONS

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Ballast water discharge is recognized as a pathway of introduction for potentially harmful, non-indigenous species of phytoplankton into coastal waters. Various ballast water treatment options have been proposed to reduce the abundance of these organisms before discharge. Potential treatments include: ultraviolet light, filtration, ozone, hypochlorite, the biocide SeaKleen, deoxygenation, and heat.

We are testing the efficacy of these technologies at reducing the abundance of ballast water phytoplankton at microcosm, mesocosm, and shipboard scales. Here we present results from mesocosm trials with an electrolytic hypochlorite generating system, which combined filtration and hypochlorite to reduce phytoplankton abundance.

Treatment experiments were conducted over wide range of seasonal conditions and employed various hypochlorite doses, in addition to testing with and without 50µm filtration. Experiments were conducted in 280 L tanks filled with water from Puget Sound containing a natural assemblages of phytoplankton, zooplankton, and bacteria. Treatments and controls were typically replicated with four tanks per experimental group. To quantify the reduction of phytoplankton resulting from treatment we measured chl *a* concentration (µg/L), an indicator of phytoplankton biomass, and the Most Probable Number (MPN) of phytoplankton, a viability based culture method. To determine the MPN, phytoplankton samples from control and treatment groups were used to inoculate growth medium (f/2) over a dilution series. These samples were then transferred to incubators set to optimize growth. The pattern of growth over the dilution series allowed for a calculation of the MPN, an estimate of the number of viable cells per ml. The effectiveness of a particular treatment at reducing the number of viable phytoplankton was determined by comparing control and treatment abundance estimates. The MPN method confers a strong advantage compared to cell presence/absence methods of traditional microscopy, because a cell present after treatment may not be capable of growth. To our knowledge this may represent the first application of the MPN method to enumerate viability of phytoplankton in ballast water treatment trials.

Preliminary results indicated that when a suitable Total Residual Oxidant (TRO, measured in mg Cl_2/L) was achieved via hypochlorite generation both chl *a* and MPN of phytoplankton were significantly reduced (99.9%). Additionally, there appears to be differential susceptibility to treatment according to phytoplankton taxa, where dinoflagellates show better survival than diatoms at high TRO doses.

STEROL BIOMARKER FAMILIES IN HARMFUL DINOFLAGELLATES: A COMPARISON OF STEROL COMPOSITION TO RDNA-BASED PHYLOGENY

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Over the past few decades many dinoflagellates have been surveyed for the presence of unique sterols that can serve as diagnostic chemical tracers (biomarkers) for those species that are harmful. A collective examination of these surveys illustrates that many dinoflagellates, both harmful and non-harmful, produce the 4-methyl sterol, dinosterol, which is rarely found in other classes of algae. Although its common presence in dinoflagellates has led to its use as a general biomarker for the class Dinophyceae as a whole, its presence (or absence) alone is not specific enough to delineate harmful species from non-harmful ones. Rather, one must consider what other sterols are present along with, or in the absence of, dinosterol, and how genus- and/or species-specific these sterols may be. For example, Leblond and Chapman (2002) and Giner et al. (2003) have shown that the harmful genera, *Karenia* and *Karlodinium*, produce two primary sterols, (24*R*)-4α-methyl-5α-ergosta-8(14),22-dien-3β-ol (ED) and its 27-*nor* isomer (NED), that have a very limited distribution within the class Dinophyceae. Thus, these two sterols form a family that serves as a set of more specific biomarkers than dinosterol. Analogous sterol biomarker families also exist, respectively, for other harmful genera, such as *Amphidinium* and *Alexandrium*.

An examination of several of these sterol composition surveys also indicates that those genera which produce characteristic sterol biomarker families are often found as tight groups with high bootstrap support in both 18S and 23S rDNA-based studies of phylogeny. Yet to date, there has been no collaborative effort between the respective fields of dinoflagellate lipid biochemistry and dinoflagellate phylogeny to demonstrate how sterol composition may be used as a biochemical reinforcement of evolutionary relationships that are derived primarily from gene sequences. The aim of this presentation is thus to link the two fields to provide a more comprehensive and integrated overview of dinoflagellate phylogeny and sterol chemotaxonomy.

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Leblond, J.D. and Chapman, P.J. 2002. A survey of the sterol composition of the marine dinoflagellates Karenia brevis, Karenia mikimotoi, *and* Karlodinium micrum*: distribution of sterols within other members of the class Dinophyceae.* J. Phycol. *38:670-82.*

DOMOIC ACID CONCENTRATIONS IN BLOOD SAMPLES FROM RANDOMLY TAGGED CALIFORNIA SEA LIONS, *ZALOPHUS CALIFORNIANUS*, IN CALIFORNIA

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The toxigenic diatom *Pseudo-nitzschia* is a common bloom forming species in California that produces the neurotoxin domoic acid, responsible for Amnesiac Shellfish Poisoning (ASP). At high enough levels, domoic acid can cause sea lions and other marine mammals to become beached with symptoms such as seizuring and death. During major HAB events, these illnesses and/or mortalities are often the first warning signs of a major ASP outbreak.

Relatively little is known about the background concentration of domoic acid in the marine food web, since most studies have focused on seizuring animals. Because *Pseudo-nitzschia* is present year-round in central California, We suspect that many mammals may not show any symptoms of domoic acid poisoning, but could still have trace amounts of the toxin in their system. Conversely, seizuring animals are indicative of an ASP outbreak, but the precise location of the bloom may be poorly associated with the location of beached or stranded animals.

We determined the concentration of domoic acid in blood plasma from randomly tagged California sea lions, *Zalophus californianus* collected between July-November, 2003. Domoic acid concentrations were determined after clean-up with Millipore Centri-Free cartridges using reverse-phase high performance liquid chromatography (HPLC). There were detectable levels of domoic acid measured in most of these sea lions, with domoic acid concentrations ranging from 0-0.31µg/mL, comparable to levels reported during the 1998 ASP event which resulted in over 400 sea lion mortalities. Because domoic acid is rapidly depurated from blood in mammals (>95% in 4 hours), these levels indicate environmental exposure during the previous several days. Many of the animals were equipped with ARGOS satellite location/dive recording tags, providing information about where the sea lions traveled after the blood samples were collected. These ancillary data provide an idea of their pattern of foraging at this time of the year, and could help us determine where the encounters with toxic prey items are occurring.

At this time, we are verifying domoic acid concentrations using the cELISA assay kits from Biosense Laboratories, and are continuing to analyze additional samples. We have archived a total of more than 100 individual blood samples from sea lions, collected between 2003 and 2005. Results showing the background concentrations in non-seizuring animals in relation to foraging patterns and environmental conditions will be presented.

CHARACTERIZATION OF DISSOLVED AND PARTICULATE STX LEVELS IN BOTH FIELD AND CULTURED *ALEXANDRIUM CATENELLA* SAMPLES

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Traditionally, harmful algal bloom studies have primarily focused on quantifying toxin levels contained within the phytoplankton cells of interest. In the case of paralytic shellfish toxins (PSTs), particulate toxin levels and the effects of dietary consumption of toxic cells by planktivores have been well documented. However, little effort has been invested into quantifying the levels of dissolved PSTs that may be released into seawater from toxic cells during blooms. In order to fully evaluate the risks of harmful algal bloom toxins in the marine food web, it is necessary to understand all potential routes of exposure. Through collaboration with the Jamestown S'Klallam Tribe, we were able to obtain weekly phytoplankton samples collected from Sequim Bay, Washington during summer 2004. Cell densities as well as particulate (pSTX) and dissolved (dSTX) saxitoxin levels were quantified in field samples via manual cell counts and receptor binding assays, respectively. In addition, several *Alexandrium catenella* cultures were maintained in the laboratory and the same parameters were examined. Although dSTX was not detected in field samples, eight individual grow-out experiments yielded dSTX levels ranging from ca. 10 to 30 mg STX equiv./L. Maximum cell densities in field samples were much lower than culture cell densities (ca. 4×10^4 vs. 4×10^7 cells/L, respectively) and likely explain the lack of dSTX detected in Sequim Bay samples. We also performed a quality control study to insure that cell lysis (as measured by chlorophyll and toxin levels in samples processed under various levels of vacuum pressure) was not contributing to dSTX levels detected in our dense cultures. Results from our culture studies confirm that *A. catenella* cells do release toxins into seawater, that this release is highly variable, and that the dissolved route of exposure may pose a risk to larval planktonic organisms that overlap spatially and temporally with PSP blooms.

DEVELOPMENT OF A SIMPLIFIED ELISA FOR DETECTING DOMOIC ACID

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Domoic acid (DA), a toxin which bio-accumulates in the food chain, is produced by diatoms in the genus *Pseudo-nitzschia*. Humans are generally exposed to DA by consuming tainted shellfish or crabs. We are developing direct competition assays for detecting domoic acid using electrochemical sensors based on the enzyme-linked immunosorbent assay (ELISA) format. This format is amenable for use in remote sampling platforms or moored arrays because the sensors are easily miniaturized and do not require a spectrophotometer as in standard colorimetric ELISAs. Samples can also be run on small hand-held electrochemical devices amenable to field sampling. This specific assay uses a rabbit anti-domoic acid (anti-DA) polyclonal antibody that has been cross-linked with biotin. The biotinylated antibody can be readily adsorbed onto NeutrAvidin (avidin analogue) coated electrodes because the high binding affinity between the NeutrAvidin and biotin. This allows a uniform and stable coating of the electrode surface by the anti-DA antibody. In addition to the biotinylated antibody, a stable domoic acid-horseradish peroxidase (DA-HRP) conjugate was also synthesized and tested to ensure it was efficiently bound by the anti DA-antibody. Empirical studies were then conducted to optimize the amount of biotinylated anti-DA antibody needed to coat electrodes for maximum sensitivity. Standard curves were established by adding 25 ml of assay buffer containing concentrations of DA between 0.1 and 1000 ng mL^{-1}. The free DA in these standards was allowed to bind the anti-DA antibody for 20 minutes before an additional 25 ml of buffer containing the optimal amount of HRP-DA was added. Twenty minutes later, the electrodes were rinsed 3 times with wash buffer and 50 ml of K-Blue substrate was added. K-blue contains an electron transporter TMB and hydrogen peroxide (H_2O_2). HRP reduces H_2O_2, and in the process becomes oxidized to form (HRP_{ox}) as follows:

$$H_2O_2 + 2H^+ + HRP_{red} \rightarrow HRP_{ox} + 2H_2O \quad (1)$$

The enzyme is then regenerated by TMB, which becomes oxidized after donating an electron to the oxidized HRP:

$$2TMB + HRP_{ox} \rightarrow 2TMB^+ + HRP_{red} \quad (2)$$

When the oxidized TMB^+ contacts the surface of the electrode it picks up an electron.

$$TMB^+ + e^- \rightarrow TMB \quad (3)$$

The monitor then measures the catalytic reduction current as electrons pass from the surface of the electrode through TMB to HRP to H_2O_2. The higher the amount of DA-HRP bound, the greater the signal. Since this is a direct competition assay, the signal drops in direct proportion to the amount of free DA bound in the first incubation step. The assay was found to have a linear range from 1 to 250 ng mL^{-1}. This assay can also be adapted to the regular colorimetric format for those researchers who wanted to analyze samples in the laboratory. The only significant difference in the two assay formats would be that the anti-DA antibody would be bound to commercially prepared avidin coated 96-well plates, and that a colorimetric rather than the K blue substrate would be added in the last step of the assay.

Current work is focusing on production of anti-DA antibodies. Having a specific high-affinity monoclonal antibody would make it easier to produce consistent DA assay kits and to eliminate any need to affinity purify the polyclonal antibodies.

FACTORS REGULATING MICROPREDATION AND FISH PATHOGENICITY IN HETEROTROPHIC "*PFIESTERIA*-LIKE" DINOFLAGELLATES

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Members of the "Toxic *Pfiesteria* complex", *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae* (formerly *Pfiesteria shumwayae*, Litaker et al. 2005) are described as potentially harmful to fish and human health, yet the underlying determinants of pathogenicity have not been fully resolved. Previous work in our laboratory has demonstrated the ability of *P. shumwayae* to act as a micropredator on fish, resulting in fish mortality in laboratory assays with mortality rates, time to death and pathology identical to what has been reported for toxic *Pfiesteria*. Recently, micropredation has been confirmed as the most significant and consistent mechanism of fish mortality in laboratory bioassays with toxic strains of *P. shumwayae* (Gordon and Dyer 2005) and has also been identified as a causative mechanism of fish pathogenicity and mortality in *P. piscicida* (Drgon et al. 2005). We have previously reported that other small heterotrophic dinoflagellates that are morphologically and behaviorally similar to *Pfiesteria*, frequently referred to as *Pfiesteria*-like species, can also act as micropredators on fish and can cause fish mortality. Our current work demonstrates that the ability of these species to feed upon fish and to cause mortality by this mechanism is highly variable, even among strains within a species. Multiple strains of several "*Pfiesteria*-like" dinoflagellates (including *P. shumwayae*, *P. piscicida*, cryptoperidiniopsoid species, "Lucy-like" species and "Shepherd's Crook-like" species) were evaluated, in a dose-dependent fashion, for their ability to feed upon larval fish and to cause mortality. Although tested strains of *P. shumwayae* consistently caused fish mortality at initial cell densities <1000 cells/ml, one strain, CCMP 2359, demonstrated significantly lower fish mortality than the other tested strains. Even at densities of >10K cells/ml, some strains of "Lucy" and *P. piscicida* were unable to cause fish mortality whereas other strains of these species were able to cause significant mortality within 96 hrs. We are currently working to identify and quantify factors that influence the ability of these dinoflagellates to feed upon and, consequently, cause pathology and mortality to larval fish. Among the factors being tested are cell size/volume, chemoattraction, ingestion capacity, and mechanisms involved in attachment to and penetration of prey. The relationship of these factors to the micropredatory behavior of the tested cultures will be discussed. Additionally, although the ability of *Pfiesteria* and related organisms to cause fish mortality in laboratory bioassays via micropredation has been well established, the contribution of these organisms to natural fish mortality has not been examined. Thus, the environmental relevance of micropredatory feeing upon fish health will be considered. This work was supported in part by ECOHAB Grant NA-16OP1487.

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THE ROLE OF COPPER FOR IRON ACQUISITION IN THE JUAN DE FUCA EDDY *PSEUDONITZSCHIA* BLOOM

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Toxigenic, pennate diatoms of the *Pseudonitzschia* genus are consistently found in the Juan de Fuca eddy region off the coast of British Columbia and Washington State. The toxin produced by these bloom-forming diatoms is domoic acid, a neurotoxin responsible for Amnesic Shellfish Poisoning. The function of domoic acid by these cells is unresolved, although the structure of domoic acid is analagous to trace-metal chelators involved in acquisition or sequestration . The synergistic relationship between iron limitation and copper stress on *Pseudonitzschia* was examined during a drifter study following the development of a toxic *Pseudonitzschia* bloom in September 2004. Deckboard incubation experiments indicate that phytoplankton growth is enhanced by iron enrichments, while photosynthetic carbon uptake as a function of irradiance (P vs. E analysis) revealed that copper increases the photosynthetic efficiency of the *Pseudonitzschia* dominated community. These results suggest that domoic acid may alleviate iron stress by complexing copper, which is required for a high efficiency iron uptake pathway into the cell. The production of domoic acid appears to provide a competitive advantage for *Pseudonitzschia* when iron limiting conditions prevail within the natural environment.

PROROCENTRUM LIMA (DINOPHYCEAE) IN NORTHEASTERN USA COASTAL WATERS: ABUNDANCE, DISTRIBUTION AND TOXIN TRANSFER

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The seasonal distribution of *Prorocentrum lima* was studied within the epibiotic community associated with wild and cultured shellfish at eight sites in northeastern USA coastal waters during a two-year period. Four sites in semi-sheltered environments, thus with limited turbulence, provided sufficient data for an evaluation of population dynamics: two were in Maine and two in Rhode Island. The two southernmost *P. lima* populations displayed a double peak in abundance, one from March to June, with several thousands cells per g dry weight of <90-ìm epibiota (exceeding 10,000 in one instance), and a second one, sometimes minor, in autumn**.** At the two northern stations, *P. lima* populations also peaked twice but with a delay in timing. All four sites harbored significant populations of filamentous brown seaweeds (*Pilayella littoralis, Ectocarpus* spp. and occasionally *Hincksia* spp.) and aggregations of tube-forming or chains of diatoms attached to larger seaweeds or fouling aquaculture structures. These different epibiotic associations appear to provide, by their soft feathery filaments, a protective matrix for the epiphytic dinoflagellate somewhat similar to that provided by analogous three-dimensional macroalgae in tropical/subtropical regions where *P. lima* is most abundantly recorded. The fact that *P. lima* was rarely detected in the plankton confirms the fact that conventional techniques devised for the water column would be ineffective in this case, when monitoring for diarrhetic shellfish poisoning (DSP) potential is considered.

The <90-µm fraction of epibiota was analyzed for its content in okadaic acid (OA)-equivalent and generally yielded values in excess of 50 ng (g dry weight)$^{-1}$ of epibiota only when *P. lima* exceeded 500 cells per g dry weight, loosely matching the seasonality in *P. lima* density. The relationship did not however hold in all cases, as high cell concentrations did not always lead to a high content in OA-equivalent toxins. Of the shellfish hepatopancreas analyzed so far, none reached levels that would have required regulatory closures of harvesting.

The observed seasonality and habitat preference of *P. lima* provides some needed first steps toward the identification of the need and design of a DSP monitoring program for the waters surveyed, but the relationship between the abundance of the toxin producer and shellfish contamination with diarrhetic toxins remains elusive.

DO DINOFLAGELLATE PSP TOXINS AFFECT GRAZING BY GASTROPOD LARVAE?

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Two subclones of the dinoflagellate, *Alexandrium lusitanicum*, were used in laboratory grazing experiments. The original clonal isolate of *A. lusitanicum* produced PSP toxins (primarily gonyautoxins 1 – 4) and one subclone derived from this isolate retains the original PSP toxin content and profile. A second subclone, derived from the same isolate, no longer synthesizes PSP toxins. Nevertheless, the two subclones are morphologically and genetically identical (at all loci examined to date). These two subclones were separately presented as prey to gastropod veliger larvae. Ingestion of the two subclones was compared to determine whether the presence or absence of PSP toxins affected grazing.

Across a range of ecologically-relevant prey concentrations, ingestion rates first increased, then declined at higher prey concentrations. Ingestion rates on the two clones were not significantly different. Predator saturation was not responsible for the depression of ingestion at higher prey concentration. In addition, veliger mortality during incubations was linearly dependent on prey concentration, with no significant differences between the two clones. Depressed ingestion and increased predator mortality at higher prey concentrations indicated that *A. lusitanicum* produced a grazing deterrent. However, because both subclones gave the same response, the presence or absence of PSP toxins was not responsible for the effects. *A. lusitanicum* produced an unknown substance that functioned as a grazing deterrent.

Toxic algae likely produce many bioactive substances. When toxic algae are found to have alleopathic effects, those effects should not be attributed to the known toxins without substantiating evidence. Unknown substances could otherwise be responsible for the results. The characteristics of the unknown grazing deterrent produced by *A. lusitanicum* are similar to those predicted in earlier work for a deterrent from the congeneric dinoflagellate, *A. monilatum* (Juhl and Franks 2004). Dinoflagellates within the genus *Alexandrium* may produce a variety of anti-grazing substances that are unrelated to PSP toxins.

Juhl, A. R., and Franks, P. J. S. 2004. Cell-Concentration Dependence In Net Growth of Alexandrium monilatum. *Harmful Algae 3: 218-219.*

ASSESSMENT OF MICROCYSTINS USING SURFACE-ENHANCED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

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Microcystis aeruginosa, the dominant bloom-forming, toxic cyanobacterium occurring throughout the Laurentian Great Lakes, produces a suite of monocyclic heptapeptide hepatotoxins, known as microcystins - the most important of which is microcystin (M)-LR. As part of NOAA's *Center for Great Lakes & Human Health*, we are validating analytical approaches to quantify microcystins.

Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI TOF MS) is a novel, 'chip-based' technology - patented by Ciphergen Biosystems - for MS-based analysis of macromolecules within extremely small sample volumes (2 µL to 1 mL). This recently-developed technology binds protein/peptide compounds of interest (within complex mixtures) onto protein 'chips' incorporating a proprietary biological or chemical surface prior to ionization by laser and detection by MS. Accordingly, SELDI TOF MS provides the instrumental basis for the capture and analysis, in parallel, of microcystin variants, including M-LR, M-YR, and M-RR.

Using SELDI TOF MS, Yuan and Carmichael (2004; *Toxicon* 44:561-570) detected and analyzed microcystins at femtamolar sensitivity (ca. 1.25 pg μL^{-1}). They noted that SELDI TOF MS offered significant advantages over existing microcystin analyses (ELISA; liquid chromatography, LC; LC-MS) by allowing for limited sample preparation, shorter sample preparation and analysis times, and lower detection limits. Using an existing SELDI TOF MS (housed at USF-Center of Ocean Technology), we have duplicated Yuan and Carmichael's analyses of microcystins after capture onto both hydrophobic/reverse phase and normal phase surface-array 'chips'.

However, Yuan and Carmichael (2004) noted that the competitive binding of proteins not-of-interest with microcystins limited 'capture' of hepatotoxins onto hydrophobic protein chips. For SELDI TOF MS to emerge as a routine and reliable means to detect toxins at public health-relevant concentrations, protein chips possessing both preferential and optimal capture of microcystins must be developed and validated. Accordingly, we have validated the selective, enhanced capture (and subsequent detection) of microcystin variants on (from) protein chips incorporating a biologically-active surface.

In situ monitoring of cyanotoxins throughout the Great Lakes would allow for real-time human health observations, thereby providing for greater public security while simultaneously increasing our ability to perceive and predict (forecast) the physical world. Ultimately, we are seeking to advance the development and validation of a MS-based sensor to detect and quantify microcystins. Because of its 'chip-based' technology and its rapid and reliable MS-based quantitative determination of peptides, SELDI TOF MS is an attractive technology for integration into automated, laboratory, shipboard or *in situ* analysis of microcystins.

CALIFORNIA PROGRAM FOR REGIONAL ENHANCED MONITORING OF PHYCOTOXINS (CAL-PREEMPT)

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California's expansive coastline is threatened by blooms of a variety of harmful algal genera, including *Pseudo-nitzschia* and *Alexandrium*, and these pose a serious threat to public health, fisheries and aquaculture. Even while the threat from harmful algal blooms is on the rise, the costs associated with public monitoring in California continue to rise in an era of reduced budgets. Economic realities thus compel managers to find ever more efficient methods and strategies to keep up with their increased monitoring burden. These efforts are critical to ensuring public safety and also provide valuable environmental data for resource managers. Growing concern over harmful algal blooms has spurred research to develop efficient and cost-effective technologies for species and toxin detection. New detection methods are now available, as are remote sensing capabilities for bloom tracking, but a constraint to adoption of these new methods by the California Department of Health Service (CDHS) is the lack of state funds for ground-truthing them, a necessary step before full adoption and incorporation into the state's monitoring effort. For example, pre-screening plankton and shellfish samples for domoic acid and saxitoxin in the field, using simple test kits, could reduce the number of samples submitted to the regulatory laboratory by 80 – 90%, representing a significant potential savings in analytical costs. Before CDHS can adopt these kits, they must be assured of their efficacy. To bridge the gulf between availability of new tools and integration of those into monitoring efforts, NOAA, through its Monitoring and Event Response Program for Harmful Algal Blooms (MERHAB), is providing funding to perform necessary validation of new tools for incorporation of them into the CDHS monitoring program.

We have established pilot project sites where new technologies are incorporated into an intensive monitoring program, in combination with a tiered decision-making protocol that dictates specific steps to take in response to field observations. The power of this approach is that it paves the way for ultimately shifting much of the monitoring effort to the field, where a network of volunteers, with overall guidance from the CDHS, pre-screen samples using new technologies, thus ensuring early warning of impending blooms while avoiding un-necessary and expensive lab-based sample testing. Using the best available remote sensing data in conjunction with field data provided by the volunteer force will enable tracking the inception, proliferation, advection and decline of bloom events in real-time along the California coast. In turn, this provides managers necessary information to make informed decisions on when and where to increase field efforts.

This presentation will provide an overview of our MERHAB-funded program, detailing our goals and expected outcomes, and highlighting our accomplishments to date.

BREVETOXINS, LIKE CIGUATOXINS, ARE POTENT ICHTHYOTOXIC NEUROTOXINS THAT ACCUMULATE IN FISH

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Brevetoxins and ciguatoxins are potent neurotoxins produced by the red tide dinoflagellate *Karenia brevis* and the epibenthic dinoflagellate *Gambierdiscus toxicus*, respectively. Human ingestion of brevetoxin-contaminated shellfish and ciguatoxin-contaminated fish results in two severe forms of food poisoning called neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP). Both groups of toxins have similar chemical structures and are the only compounds known to activate voltage-sensitive sodium channels in mammals through a specific interaction with site 5 of the alpha sub-unit of the channel.

While ciguatoxins accumulate in fish by dietary transfer up the food chain, brevetoxins exposure during *K. brevis* blooms (Florida red tides) typically result in massive fish kills. Until we documented the involvement of brevetoxin-contaminated planktivorous fish in a large-scale mortality of bottlenose dolphins in 2004 (Flewelling *et al.* 2005), live fish were not considered a potential source for neurotoxin poisoning since ichthyotoxicity was believed to preclude accumulation in live fish. Here we show that ichthyotoxicity does not necessarily prevent accumulation in fish, as toxicity to fish can be modulated by the exposure route. By exposing fish to brevetoxin-contaminated prey (shellfish and *K. brevis* in culture) we demonstrate that fish can bioaccumulate, maintain, and slowly depurate elevated concentrations of brevetoxins in their tissues without obvious adverse effects. A preliminary assessment of the prevalence of brevetoxins in live fish from Florida Gulf coast waters revealed that brevetoxin contamination is more common than initially thought. Nearly 70% of the individual fish tested (n=315), from 47 different species (77%), contained measurable concentrations of brevetoxins in their tissues. Although brevetoxins in fish muscle did not exceed the shellfish regulatory limit (with the exception of the planktivorous menhaden implicated in the dolphin mortality), this study demonstrates that fish liver and digestive tract can contain very high toxin concentrations. Therefore, it appears that consumption of planktivorous fish during red tides or of whole or uncleaned fish could be hazardous to humans.

Leanne J. Flewelling, Jerome P. Naar, Jay P. Abbott, Daniel G. Baden, Nélio B. Barros et al., Red tides and marine mammal mortalities, Nature *(in press)*

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A MODIFIED HEMOLYTIC ASSAY SUGGESTS TOXIN ACTIVITY AMONG *KARENIA MIKIMOTOI* CLONES ISOLATED DURING RED TIDE EVENTS OFF THE TEXAS COAST

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Karenia brevis, a toxic marine dinoflagellate occurring primarily in the Gulf of Mexico and Caribbean waters, is the main causative agent in large scale fish kills, marine mammal mortality, and respiratory distress and Neurotoxic Shellfish Poisoning (NSP) in humans. *Karenia mikimotoi* is a morphologically similar, co-occurring species which is reportedly non-toxic when occurring within Gulf waters. In other regions of the world's oceans, *K.mikimotoi* is recognized as an extremely toxic species, causing large fish kills off the coasts of Japan, New Zealand, and South Africa.

Recently, there has been increasing evidence to suggest the production of a variety of bio-active compounds in addition to brevetoxin derivatives among various *Karenia* isolates. The goals of this study were (1) to determine if hemolytic toxins are present in either *K. brevis* or *K. mikimotoi* isolates from the Gulf, and (2) to determine if significant differences in production of hemolytic toxins exist among distinct *Karenia* isolates.

Using a modified version of the Erythrocyte Lysis Assay (ELA), developed by Eshbach et al. (2001), erythrocytes from Red Drum (*Sciaenops ocellatus*) were used to detect hemolytic activity in crude algal extracts of *Karenia* clones isolated from samples collected off the coast of Texas. Red Drum were selected as they are endemic to coastal areas throughout the Gulf, and are one of many finfish species affected by toxic algal blooms, making this species a valid ecological target.

Assay results show there is markedly higher hemolytic activity among *K.mikimotoi* clones versus *K.brevis*. *K.brevis* clones SP2 and SP3 (1999 bloom), showed some hemolytic activity at cell concentrations of 1-5x10^{6}cells-ml$^-$ 1 but averaged only 33.7% of standard Saponin (soap tree bark extract, Sigma) activity. While *K.mikimotoi* clones C5, C9 & B1 (2002 bloom), averaged 61.3% activity of standard, at identical cell concentrations. While *K. mikimotoi* has not yet been implicated in fish kills in the Gulf, these results provide further evidence in the production of hemolytic compounds within the *Karenia* genus. These compounds may not be revealed using screening methods designed for brevetoxin detection, and therefore research still needs to be conducted to determine the role of these toxin producers in Gulf of Mexico bloom events.

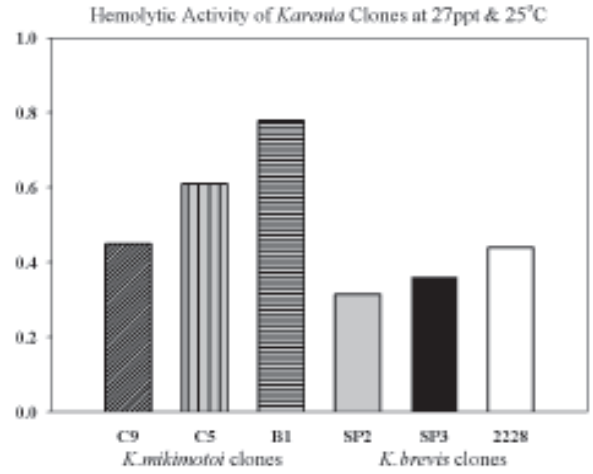

Figure 1. Hemolytic activities of Karenia *clones as percentage of standard (Saponin-Sigma) activity. Activities are averaged from three replicate samples in Red Drum assay. All cultures were grown at salinities of 27ppt and 25°C, and harvested in late log phase of growth. All samples reflect cell densitiesof 1-5x10^{6}cells mL^{-1}.*

ORHAB: EARLY WARNING AND RAPID RESPONSE TO MITIGATE THE EFFECTS OF HARMFUL ALGAL BLOOMS ALONG THE WASHINGTON COAST

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In 1999, academic, federal, tribal and state researchers, and managers in Washington State formed a partnership called Olympic Region Harmful Algal Blooms (ORHAB) that has established a monitoring program for harmful algal blooms. The objectives of ORHAB are to investigate the origins of blooms of toxic algae, monitor where and when the blooms occur, assess the environmental conditions conducive to blooms and toxification of intertidal shellfish such as razor clams (*Siliqua patula*), and to explore methods that can be used to reduce harmful algal bloom impacts on the Washington coastal economy. ORHAB monitoring data generated from the state, tribal, and private industry technicians are combined, analyzed, and e-mailed in regular updates to provide early warning of HAB threats to coastal managers. Distinguishing the seven species of *Pseudo-nitzschia* found along the Washington coast is impossible using standard light microscope techniques, so ORHAB technicians have developed a method of classifying them by size and morphological similarities; short and broad (*P. australis/heimii/fraudulenta.)*, small and narrow (*P.* cf. *pseudodelicatissima/delicatissima)*, and long and narrow (*P. multiseries/pungens*) Using the results of the last five years of monitoring as a guide, warning levels of *Pseudo-nitzschia* on the Washington coast have been established for the short and broad size group at 3×10^4 cells/L or for the small and narrow group at 1×10^6 cells/L for over 1 week. To date, *P. multiseries* has not been a problem species on the outer Washington coast, however a warning level of 1×10^5 cells/L has been suggested for the long, narrow cell type. When a bloom of *Pseudo-nitzschia* spp. reaches a given warning level, seawater particulates are tested for domoic acid (DA) content using Jellett rapid tests. If these test strips are positive for DA in seawater (the tests are calibrated to ~200ng/L), razor clams are analyzed for DA using test strips calibrated to ~10ppm. If positive, an “alert” is issued to managers and clam samples are delivered to the Washington Department of Health for quantification of DA content using high performance liquid chromatography. These actions have resulted in reduced costs, faster analysis of shellfish samples, and increased knowledge of current *Pseudo-nitzschia* cell count numbers and toxin levels through regular data updates; thereby leading to more beach openings for commercial, recreational, and subsistence harvest of shellfish.

FLOW CYTOMETRIC ANALYSIS OF DOMOIC ACID IN SINGLE CELLS

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Diatoms of the genus, *Pseudo-nitzschia,* produce the neurotoxin domoic acid under a variety of environmental conditions. Domoic acid is generally measured in bulk using a variety of methods, including ELISA assays, masking the variability in domoic acid produced by single cells. To examine the variations in domoic acid production by single cells of *Pseudo-nitzschia multiseries*, we have developed an immunofluorescent assay using a high affinity polyclonal antibody to domoic acid recently developed by the Northwest Fisheries Science Center Culturing and Analytic Facility, coupled with flow cytometric analysis. We suggest that domoic acid is stored within secretory vesicles and is attached to an acidic polymeric network prior to secretion, in a manner similar to other secretory cells. We have examined field samples as well as cultures and compared the single cell measurements with bulk ELISA measurements.

EFFECTS OF THE TOXIC DINOFLAGELLATE, *ALEXANDRIUM MONILATUM,* ON BEHAVIOR AND SURVIVAL OF FOUR SHELLFISH SPECIES

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The levels of toxins accumulated within shellfish are influenced by the shellfish clearance rates and behavioral responses. The most common characteristic behavior expressed by bivalves when exposed to toxic dinoflagellates is reduced filtration or an increase in valve closure, which effectively decreases exposure to the bloom (Gainey and Shumway 1988). Little is known regarding interactions between shellfish and *Alexandrium monilatum*, which is a toxigenic dinoflagellate species that forms blooms mostly in the Gulf of Mexico. Toxic strains have been linked to fish and invertebrate kills (Howell 1953, Williams and Ingle 1972, Perry et al. 1979, Norris 1983). In this study, grazing studies were conducted to determine the clearance rates of adult *Crassostrea virginica* (eastern oyster), *Mercenaria mercenaria* (northern quahog), *Argopectin irradians* (bay scallop), and *Perna viridis* (green mussel) when exposed to a toxic strain of *A. monilatum* (AMO3 isolate). Clearance rates of all four shellfish species were significantly depressed in comparison to clearance rates of control animals that were fed benign cryptophyte algal prey (Table 1). Exposure to *A. monilatum* also caused a significant decrease in the valve gape of these shellfish species, in comparison to the valve gape of control animals. In addition to these experiments, bioassays were conducted to determine whether *A. monilatum* can cause shellfish mortality. Eastern oysters and quahogs survived after 24 hr of exposure to *A. monilatum*, but there were high mortality of bay scallops and green mussels. Overall, these data suggest that *A. monilatum* blooms can affect shellfish survival by reducing clearance rate and valve gape, affecting their food intake, and inducing mortality in some shellfish species.

Table 1. Clearance rates (ml hr^{-1}, mean ± 1 SD) of shellfish exposed to A. monilatum *at 550 cells ml^{-1}, or to benign algal prey (cryptomonad) at 10^4 cells ml^{-1}.*

Shellfish Species (n=5)	**Benign *Cryptomonas***	**Toxic *A. monilatum***
Crassostrea virginica	1510 ± 400	170 ± 30
Mercenaria mercenaria	1230 ± 680	130 ± 60
Argopectin irradians	1060 ± 210	130 ± 60
Perna viridis	940 ± 600	150 ± 30

Gainey LF Jr, Shumway SE (1988) A compendium of the responses of bivalve mollusks to toxic dinoflagellates. Journal of Shellfish Research *7:623-628.*

Howell JF (1953) Gonyaulax monilata *sp. nov., the causive dinoflagellate of a red tide on the east coast of Florida in August-September, 1951.* Transactions of the American Microscopy Society *72:153-156.*

Norris DR (1983) The occurrence of a toxic dinoflagellate in the Indian River system. Florida Scientist *46:150-153.*

Perry HM, Stuck KC, Howse HD (1979) 1st record of a bloom of Gonyaulax monilata *in coastal waters of Mississippi.* Gulf Research Reports *6:313-316.*

Williams J, Ingle RM (1972) Ecological notes on Gonyaulax monilata *(Dinophyceae) blooms along the west coast of Florida.* Florida Department of Natural Resources Marine Laboratory Leaflet Series *1:12.*

MOLECULAR DETECTION OF *KARENIA BREVIS* AND RELATED SPECIES USING FLUORESCENT *IN SITU* HYBRIDIZATION ASSAYS

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Fluorescent *In Situ* Hybridization (FISH) assays are otherwise known as *whole cell* assays because they are used to label molecules of intact cells rather than the cell lysates used in PCR methods and Sandwich Hybridization Assays (SHAs). The usefulness of FISH assays is that not only can the location of the labeled probe be visualized, but the cell morphology can also often be retained, although some changes in cellular size and shape are inevitable upon fixation. If cell morphology is retained, confirmatory identification of the labeled cells may also be possible if sufficient cells are labeled to overcome any artefacts introduced by fixation or cell physiology prior to fixation. FISH assays designed to target various species of the dinoflagellate genus *Karenia* were tested in matrix format with fixed culture samples in the laboratory. The separate probes were then applied to fixed whole seawater samples from the Eastern Gulf of Mexico region. Previous results confirmed that species of *Karenia* other than *K. brevis* were present in the Gulf of Mexico, and current research aims to delineate the spatial scale and temporal variation in species composition to provide alternative methods of molecular identification of toxic or harmful dinoflagellates in addition to lysate-based methods, for both isolation purposes and better brevetoxin (BTX) risk management.

BREVETOXINS AND METABOLITES IN NSP-TOXIC BIVALVE MOLLUSCS: A COMPARISON OF METHODS

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Brevetoxins (BTX) produced by the marine dinoflagellate *Karenia brevis* are rapidly accumulated and metabolized by Gulf of Mexico oysters (*Crassostrea virginica*) and clams (M*ercinaria mercinaria*). Metabolites include abundant cysteine and oxidized cysteine conjugates of BTX-1 and BTX-2, and lesser quantities of BTX-peptides and parent redox and hydrolysis products. Circumstantial evidence suggests that these metabolites contribute to neurotoxic shellfish poisoning in mammals. Seawater and shellfish in Sarasota Bay, Florida were surveyed through an entire *K. brevis* bloom event. *K. brevis* counts were performed, and shellfish samples were extracted and analyzed using mouse bioassay, Na-channel receptor binding assay, in vitro cytotoxicity assay, BTX ELISA and LC/MS. Results were normalized to standard BTX-3 equivalent response. For in vivo and in vitro assays BTX-3 normalization yielded measures of composite toxicity. For LC/MS BTX-3 normalization produced measures of relative abundance for chosen parent BTX ions and BTX-metabolite ions. All of the methods utilized showed ppm-level sensitivity for BTX and metabolites and on graphic representation clearly showed the accumulation and depuration of BTX-related toxicity (assays) and toxins (LC/MS & ELISA) from the K. brevis bloom.

MERHAB 2002: EASTERN GOMX SENTINEL PROGRAM EARLY DETECTION OF *KARENIA BREVIS* IN THE EASTERN GULF OF MEXICO

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Blooms of the toxic dinoflagellate *Karenia brevis*, occur annually in coastal waters of the eastern Gulf of Mexico (GOMx) and can cause serious marine resource, public health, and economic impacts. Federal, state, academic and private partnerships have been created to minimize these impacts through early forecasting and subsequent mitigation of blooms events. Current difficulties in forecasting occurrence and impacts of *K. brevis* blooms can be traced to the absence of appropriate monitoring technologies. The successful operational advantage of any monitoring program will be dependent upon real-time sampling, regional-based data assimilation and modeling, and event-response for confirmation.

MERHAB 2002: Eastern GOMx Sentinel Program is a NOAA funded collaboration between Florida Fish and Wildlife Conservation Commission, University of South Florida and Mote Marine Laboratory to develop and assess the utility of a networked system of autonomous sampling platforms for early *K. brevis* bloom detection. These monitoring platforms incorporate physical, chemical and biological sensor packages and utilize existing and newly established buoys (The West Florida *Coastal Ocean Monitoring and Prediction System*) as well as autonomous underwater vehicles carrying modular sensor payloads. Validation of a bio-optical phytoplankton detector and a species-specific *Karenia* molecular-probe array has provided positive, consistent results in both laboratory and field trials during 3 different *K. brevis* blooms. Autonomous underwater sensors and water-column profilers have both been tested in the 2005 *K. brevis* bloom successfully.

The water column profiler used in this program is a Bottom Stationed Ocean Profiler (BSOP). The BSOP is a relatively inexpensive, autonomous profiling vehicle capable of carrying different sensor payloads for different scientific missions. The unit, easily deployable from a small boat, is kept at rest on the bottom until an internal alarm activates the sensor for a buoyancy-adjusted ascent. The sensor acquires data during the ascent, transmits data via satellite and e-mail, and then returns to the bottom to await the next programmed ascent. Repeated deployments of a BSOPs within a *K. brevis* bloom have been accomplished for autonomous sampling periods of up to 10 days.

This program demostrates that these technologies gives federal, state, academic, and private research groups a pro-active response to the detection and monitoring of *Karenia brevis* opposed to the traditional reactive response that was adopted in the past. The technologies for this program can be developed to detect other HABs around the world.

INVESTIGATIONS OF *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE) CYST GERMINATION IN LABORATORY AND FIELD SETTINGS USING MOLECULAR TECHNIQUES

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Phytoplankton germination is regarded as a potential initiator in algae blooms for several HAB species. Large scale germination events under optimal conditions could lead to a sudden increase in abundance of particular species, causing blooms to suddenly appear in areas where they are otherwise undetected. The cause and effect of germination by most phytoplankton species has been largely understudied in situ due to sampling difficulty or lack of detection sensitivity.

Physicochemical factors causing germination of the known cyst-forming Raphidophyte *Heterosigma akashiwo* were examined in both laboratory and field settings. In the laboratory settings, sediment collected from sites with known *H. akashiwo* cysts were covered with seawater and subjected to different light and temperature treatments over a time series to determine which key physical factors trigger germination. Presence and abundance of germinated vegetative *H. akashiwo* cells in the overlying water were examined using quantitative real-time PCR (Q-PCR) and microscopic techniques. In field settings, water contained in environmental mesocosms positioned over sediments known to harbor *H. akashiwo* cysts was collected over a period of several weeks and examined microscopically for vegetative *H. akashiwo* cells. Data on physicochemical parameters such as temperature, nutrients, salinity, and dissolved oxygen were also taken to correlate each of these factors to germination of *H. akashiwo*. Detection and quantification of both cysts in the sediment and germinated vegetative cells in the water column were performed using Q-PCR. These methods can be applied to other cyst-forming phytoplankton species to evaluate the effects of physical factors on germination in laboratory and in situ experiments, and also to determine the magnitude of germination. Each of these factors could have important implications on initiating future blooms.

DETECTION AND ENUMERATION OF HARMFUL ALGAL BLOOM SPECIES USING A CONTINUOUS IMAGING FLUID PARTICLE ANALYZER (FLOWCAM®)

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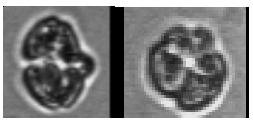
Phytoplankton detection, specifically of harmful algal species, has a requirement for continuous monitoring either at a stationary location (floating dock or laboratory) or aboard a ship. A major drawback of monitoring using standard microscopes for identification and enumeration in a laboratory or from field samples is the amount of time required for analysis. Fluid Imaging Technologies Inc. has developed an instrument for phytoplankton detection called a FlowCAM® (Flow Cytometer and Microscope). FlowCAM®, combines the capabilities of a flow cytometer with a digital-imaging microscope and automates phytoplankton detection and enumeration.

Flow cytometry has been used to study the physiology and ecology of phytoplankton species that have unique sizes and pigments. However, larger size classes of phytoplankton, which include many toxic or harmful algal species, often cannot be discriminated from other phytoplankton species based on size and autofluorescence alone. Every particle or phytoplankton image captured by the FlowCAM® is saved for further identification (Figure 1). In addition to autofluorescence collection the length and width of each particle is also determined. Using a combination of specific aspect ratios (ratio of maximum length to maximum width), images and autofluorescence, different species including harmful algal species can be identified and enumerated in the laboratory and possibly environmental samples depending on the cell concentration in the field.

The key benefits of this technology are the ability to analyze phytoplankton continuously, determine size (length and width), and most importantly the collection of digital images for further analysis in post-processing.

A. Photo credit Ed. Busky



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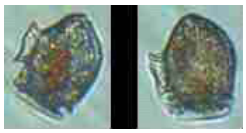

C.

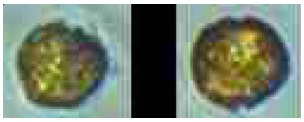

Figure 1. Representative images of potentially harmful algal species detected by FlowCAM®. (A. Karina brevis *B.* Dinophysis *spp. C.* Alexandrium *spp.)*

PRESENCE OF *PFIESTERIA PISCICIDA* AND *PFIESTERIA SHUMWAYAE* IN COASTAL WATER OF LONG ISLAND, NEW YORK, USA

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Water and sediment samples were collected during summer and early fall from 44 estuarine locations in Suffolk County, Long Island, NY from 1989 through 2004. Physical and chemical parameters were measured and real-time polymerase chain reaction assays were conducted for the presence of the toxic dinoflagellates *Pfiesteria piscicida* and *Pfiesteria shumwayae*. Both species were relatively common and at nearly every site at least once. To date, no strong correlations have been found relating the presence of either species to environmental parameters. Partial SSU rDNA sequences of several clonal isolates of each species were found to be identical to GenBank entries for *P. piscicida* and *P. shumwayae*. At least one isolate was found to kill fish in a bioassay. Despite widespread presence of both *Pfiesteria* species and demonstration of potential to harm fish, no blooms of these dinoflagellates have been observed, nor has there been any evidence of *Pfiesteria* related fish or human health problems in these waters

CYANOBACTERIAL TOXINS IN LAKE CHAMPLAIN – A FIVE YEAR REVIEW

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Cyanobacterial toxins have been detected annually in Lake Champlain for the past 5 years. Over this period several cyanotoxin related events have occurred including the deaths of several dogs and the closing of public recreation areas. To better understand the spatial and temporal distribution of cyanobacterial toxins on this lake we undertook a lake wide sampling program during the summers of 2000 to 2004. At each station, water samples were collected and analyzed for nutrients, algal abundance and the cyanobacterial toxins microcystin, anatoxin-a and saxitoxin/neosaxitoxin (PSP's).

PSEUDO-NITZSCHIA SPP. AND DOMOIC ACID IN THE SAN PEDRO CHANNEL AND LOS ANGELES HARBOR AREAS OF THE SOUTHERN CALIFORNIA BIGHT

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Multiple species of *Pseudo-nitzschia* spp. have been documented along the Californian coast, and these species include known producers of the neurological toxin domoic acid (DA). Some of the highest particulate DA concentrations ever recorded along the US west coast were measured within the Los Angeles harbor area during spring 2003 (12.7 ng DA per mL). During the last 3 years hundreds of fatalities within mammal and seabird populations in the Southern California Bight area have been attributed to domoic acid poisoning. SEM and molecular analyses (rRNA gene sequence) revealed that *P. pungens* (2002), *P. pseudodelicatissima* (2003), *P. australis* and *P. cf. cuspidata* (2004) were among the key players during these recent toxic events. We conducted yearly surveys during spring time (2003 – 2004) covering a 9 km-wide offshore stretch from the Palos Verdes Peninsula to Newport Beach, to determine *Pseudo-nitzschia* spp. abundances and/or DA concentrations. Our results indicate that toxicity levels were often highest close to shore or even within the breakwater of the harbor. Sediment trap samples recovered from the San Pedro Channel in spring 2004 were positive for DA, indicating that toxic blooms in the Southern Californian Bight might also have an impact on benthic communities. Within 2 weeks of measuring maximum abundances of *Pseudo-nitzschia* spp. in surface waters, record levels of particulate DA (up to 86 ng DA per mL) were measured in sediment trap material collected at 800 m depth. The appearance and persistence of toxic *Pseudo-nitzschia* blooms along one of the most populated coastal stretches in California (Los Angeles area) warrants further investigation on the environmental factors that trigger cell growth and toxin production by *Pseudo-nitzschia* spp.

APPLICATION OF HISTORIC SHELLFISH DATA AND REMOTE SENSING TO UNDERSTAND HAB EVENTS ON THE OREGON COAST: PHASE I

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Oregon currently has a shellfish monitoring program in place with the Oregon Department of Agriculture. However, this program is only able to randomly test shellfish along the coast to detect toxic events after they have occurred and the public is already in danger of poisoning. Using the archived data of shellfish toxin levels from the ODA, we have identified particular times and locations of large toxic events along the Oregon coast. We have created a working data base using the data from the last 25 years of saxitoxin monitoring and the last ten years of domoic acid monitoring. We will use this information to choose time periods during which satellite data can be used to understand oceanographic conditions in the sea immediately prior to, during, and after these toxic events. This will allow us to identify possible water conditions that lead to blooms of toxic species of phytoplankton. We will then test our predictive capability by sampling the water during times of predicted blooms. This will help to optimize future regulatory sampling along the coast by the ODA.

BIVALVE SHELLFISH CAN SERVE AS VECTORS FOR TRANSPORT OF HARMFUL ALGAE

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Commercially-exploited bivalve molluscs, including oysters, clams, scallops and mussels, are moved from one body of water to another for purposes of aquaculture, restoration and contaminant depuration. Our study is testing the hypothesis that harmful algae can be introduced into new environments by means of these shellfish relocations. To test this hypothesis, we first identified which managed shellfish species and which HABs co-occur geographically and then established an experimental protocol to screen shellfish-HAB pairs for consumption of the algae and release of living propagules after removal from the HAB exposure. Several cultured strains of harmful algae, such as *Pfiesteria piscicida, P. shumwayae, Prorocentrum minimum*, *Alexandrium fundyense, A. monilatum* and *Heterosigma akashiwo*, were fed to various species of bivalve molluscs, *Crassostrea virginica* (Eastern oyster), *Argopecten irradians* (bay scallop), *Mercenaria mercenaria* (northern quahog = hard clam), *Mytilus edulis* (blue mussel) and *Perna veridis* (green mussel) to assess the ability of the algal cells to pass intact through the digestive tract and subsequently grow. Shellfish were exposed to the algae at natural bloom concentrations, or to a control of a commonly used food source, *Rhodomonas* sp., for four hours. Clearance and feeding rates were measured, and feces and pseudofeces were collected and observed under the microscope for the presence or absence of intact, viable cells or temporary cysts of the algae. Ten bivalves of each species were also exposed for two days to a simulated harmful algal bloom at a natural bloom concentration. The algae were removed after two days of exposure, and the bivalves were kept for two more days in ultrafiltered seawater. Biodeposits were collected and observed under the microscope after 24 and 48 additional hours to evaluate again the occurrence and condition of any algal cells. Subsamples of biodeposits were transferred into algal culture medium and filtered seawater and monitored microscopically for algal growth. Intact algal cells of *Pfiesteria piscicida, P. shumwayae*, *Prorocentrum minimum*, *Alexandrium fundyense, A. monilatum* and *Heterosigma akashiwo* were seen in biodeposits; generally these re-established growing populations. These data show clear evidence that transplanted bivalves may serve as vectors transporting toxic or harmful algae.

TOXIC *PSEUDO-NITZSCHIA* FROM CALIFORNIA: SOME EMERGING PATTERNS

Mary W. Silver[1] and the Santa Cruz team (Rozalind Antrobus, Sibel Bargu, Itchung Cheung, Casey Curtiss, Veronica Vigilant)[1], Barbara Prezelin[2] and the Santa Barbara team (Steve McKagan, Claudia Mengelt, Karen Keltner)[2], Elizabeth Venerick[3], Lilian Busse[3] and Greg Langlois[4]

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As part of a three-campus research group (HABTRAK) from the University of California, we are presently completing a 2-year study of the patterns of toxic *Pseudo-nitzschia* species, together with its toxin, domoic acid (DA), along a 500-mile span of California coastline from San Diego, near the Mexican border, to Monterey Bay, south of San Francisco. Our study has focused on a possible synchronicity of toxic blooms along the coast, on understanding the locations where blooms are most frequent, where the cell densities and per-cell toxin quotas are highest and on linking these planktonic phenomena with DA levels in shellfish obtained by the state health department. Five sampling sites were visited weekly, 4 of them being piers and 1 an offshore site (in Monterey Bay), to obtain cell counts of the 2 local toxic species (*P. australis*, *P. multiseries*), hydrographic data, and DA concentrations in the cells

The data so far suggest that simple propagation models for blooms are not applicable over the study area, but, more likely, toxic events during the 2-year period represent more regional events. Furthermore, sites where toxic blooms are the densest or most frequent are not necessarily the regions of highest cell toxicities. The state monitoring program, which measures DA in mussels (*Mytilus californianus*) suspended from the 4 piers, obtained its material at the same time that we collected water samples, thus allowing us to calculate a "calibration" between DA in shellfish with DA in toxic cells in the water at the pier sites. We show here the first field-based calculation for the US West coast, showing the relationship between DA in mussels versus numbers of toxic cells in the water and the relationship DA in the mussels and DA in the water. DA in mussels will be correlated with DA data, time averaged over various intervals, to show the "best-fit" relationship, which could help indicate the retention time of DA in mussels in the field.

ISOLATION OF TOXIC ALGAE FROM MARINE WATERS BY HIGH-SPEED FLOW CYTOMETRIC SINGLE-CELL SORTING

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Toxic algae, particularly marine dinoflagellates responsible for a variety of Harmful Algal Blooms (HABs), can be very difficult to isolate and culture by traditional methods. This presents significant challenges when attempting to generate new toxic algal cultures from the environment for genetic characterization and toxin screening. Current traditional methods for the isolation and establishment of toxic uni-algal cultures typically involves either serial dilution to extinction or microscopic observation and handpicking of single cells of specific morphological type from natural samples containing complex microbial assemblages. Both of these traditional techniques also typically have very low success rates for the establishment of uni-algal cultures of toxic dinoflagellates. These approaches are very time-consuming, require a high level of taxonomic expertise and experience by the microscopist, and commonly have rates of successful culture establishment of less than 1%, especially for dinoflagellates. We demonstrate here the use of electronic cell sorting as a reliable high-throughput alternative to traditional hand-picking methods. The work presented here utilized high-speed multi-parameter flow cytometry cell sorting with a DakoCytomation MoFlo instrument to enrich-sort and single-cell-sort viable cells of a wide variety of dinoflagellate species, and other potentially toxic algae, from natural seawater samples, both from on-going Harmful Algal Blooms and from pre-bloom or non-bloom conditions. High-speed electronic single-cell-sorting demonstrated a successful culture rate as high or higher than traditional hand-picking techniques, while permitting orders of magnitude more uni-algal culture attempts than would be feasible by traditional hand-picking, over a wide range of culture conditions. Over fifty uni-algal cultures of various types have been established so far by environmental single-cell flow cytometry sorting from three sampling cruises alone. These new cultures are currently being characterized and screened for toxicity, and so far several new toxic algal cultures have already been identified by this process. Flow cytometric single-cell sorting provides a rapid and effective approach for the isolation and establishment of harmful algae cultures from a wide variety of habitats and will likely see a significant increase in application to HAB studies in the near future, especially with the advent of the new generation of smaller, more portable, and more versatile high-speed cell sorters that will soon be available.

ENZYME-LABELED FLUORESCENCE DETECTION OF PHOSPHATASE ACTIVITY IN THE HETEROTROPHIC DINOFLAGELLATES *PFIESTERIA SHUMWAYAE* AND *CRYPTHECODINIUM* SP.

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Phosphatases, enzymes that hydrolyze organic phosphorus, include both alkaline and acid varieties with pH-dependent optima. Alkaline phosphatases are typically found on the cell surface and function in phosphorus uptake from the extracellular environment. Expression of alkaline phosphatase has been linked to phosphorus deficiency in phytoplankton. Acid phosphatases are typically intracellular and function in digestion of food and in autophagy, the process by which cells degrade and recycle cytoplasm and organelles. Phytoplankton phosphatase research has focused primarily on alkaline phosphatase expression in photosynthetic species, including dinoflagellates. Acid phosphatases have been less studied in algae and have been examined in very few dinoflagellates (*Lingulodinium polyedrum*, Schmitter and Jurkiewicz 1981; *Crypthecodinium cohnii*, Barlow and Triemer 1986).

Traditionally, phosphatase activity has been measured using bulk colorimetric or fluorometric methods that cannot resolve variability within populations and among taxa in mixed assemblages. Recently, the molecular probe ELF-97® (Enzyme Labeled Fluorescence; Molecular Probes, Inc., Eugene, OR) was developed for *in situ* fluorescence measurements of both alkaline and acid phosphatases in individual cells. In this study, ELF-97® was used to examine phosphatase activity in the heterotrophic dinoflagellates *Pfiesteria shumwayae* and *Crypthecodinium* sp., both of which were cultivated on a fish cell line in the absence of bacteria (Parrow et al. 2005). This represents the first study to use ELF-97 to investigate phosphatase activity in heterotrophic dinoflagellates. Active phosphatases generally were localized in dense deposits near or surrounding the cell food vacuole(s) (Figure 1) of both species. This enzyme localization suggested a digestive role, indicating that these are likely acid phosphatases. Cell surface phosphatase activity, indicative of alkaline phosphatases, was not observed. Enzyme expression was also highly variable among individual cells, with some cells showing no activity.

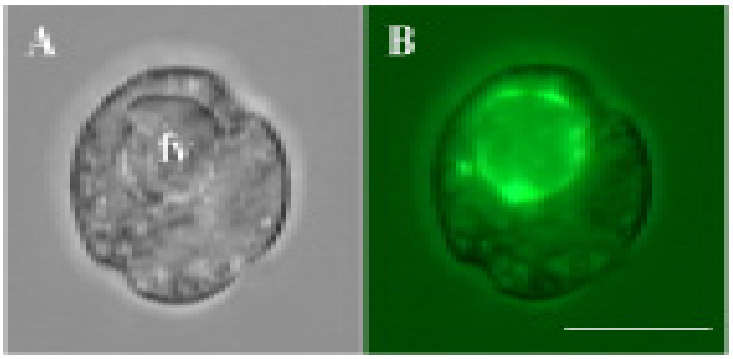

Fig. 1. Light micrographs of a Pfiesteria shumwayae *cell. A) brightfield illumination B) epifluorescence, showing ELF-97 phosphatase activity localized around the food vacuole (fv). Scale bar = 10µm.*

Barlow, S.B., and R.E. Triemer. 1986. J. Histochem. Cytochem. *34: 1021-1027.*
Parrow, M.W., J.M. Burkholder, N.J. Deamer, and J.S. Ramsdell. 2005. Aquat. Microb. Ecol. *39: 97-105*
Schmitter, R.E., and A.J. Jurkiewicz. 1981. J. Cell Sci. *51: 15-23.*

MOLECULAR PHYSIOLOGY OF AMINO ACID METABOLISM IN *PSEUDO-NITZSCHIA AUSTRALIS*: BIOMARKERS FOR GROWTH STATUS OR DOMOIC ACID TOXICITY?

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Domoic acid production capacity is known to vary widely between independent isolates of *Pseudo-nitzschia australis* and *P. multiseries*; it can also be induced in some isolates by a variety of environmental challenges (e.g. Si-limitation). The extent to which genotype or environmental stimuli control these toxicity patterns is poorly characterized in *Pseudo-nitzschia* and other HA species in general. Regardless, this phenotypic variation suggests that monitoring efforts could be enhanced by development of biomarkers for toxin biosynthesis complementary to existing molecular taxonomic probes. Previous efforts revealed that domoic acid (DA) accumulation was associated with unique free amino acid profiles in several *Pseudo-nitzschia* species, characteristically maintaining large ornithine (ORN), arginine (ARG) and low proline (PRO) pools relative to glutamate (GLU). Enzymatic studies also revealed higher activity along the ORN-dependent pathway for proline biosynthesis in toxic *P. australis* and *P. multiseries*, supporting the hypothesis that high ORN-cycle activity may be indicative of DA production capacity.

In an attempt to describe the gene expression profiles underlying active DA production, our group has developed and analyzed forward and reverse cDNA subtraction libraries for silicate limited *P. australis* sampled from low growth rate, high toxin production and high growth rate, low toxin production culture conditions, respectively. Over 1000 subtraction clones covering three independent forward libraries have been sequenced to date and ca. 5% of these clones exhibit significant homology to genes with known functions in amino acid metabolism. One of these expressed sequences exhibited strong deduced amino acid sequence homology to a putative acetyl-ornithine aminotransferase (*acOAT* or *argD*) gene in *Thalassiosira pseudonana* and cyanobacteria. This gene product plays a central role in the ORN and ARG biosynthetic pathways. RT-PCR based cloning from cDNA pools derived from toxic *P. australis* clones 03184 5D, 6D and 03199 1B isolated from Monterey Bay enabled further characterization of the *acOAT* transcript and revealed the presence of at least two transcript forms which differ in 3'-UTR length. Gel-based multiplex RT-PCR and genomic PCR assays, using nucleic acids isolated from the same cell sample along with primers specific to *P. australis* beta-actin (*bAct*) as an internal normalization control, revealed that relative to *bAct* expression, *acOAT* was non-detectable in log-phase cultures but highly expressed in stationary cultures, confirming the forward subtraction results. We have used this strategy to compare expression patterns in the toxic clones (5D, 6D, 1B) and cell lines with non-detectable DA isolated from Bodega Bay, CA (04063 3C and 04063 8F). Relative to *bAct* expression or *acOAT* gene dose, DA accumulating cells exhibit consistently higher expression levels (ca. 8x) of the longer *acOAT* transcript. Isolation of the full length *acOAT* cDNA(s) is underway to determine if the expression of different alleles correlates with DA toxicity phenotype. Additionally, we will test this molecular expression assay using nucleic acid samples covering non-bloom, early bloom and post bloom events at our Monterey Wharf II (36.36.21 N, 121.53.35 W) field monitoring site. At minimum *acOAT* expression analysis provides a powerful marker of physiological entry into growth-limiting conditions and may serve as a proxy for onset of enhanced DA production in this species.

DINOPHYSIS ABUNDANCE, DSP TOXIN PRODUCTION, & BIOACCUMULATION IN CALIFORNIA MUSSELS, *MYTILUS CALIFORNIANUS,* IN MONTEREY BAY, CA, USA

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In 2000, Robin Weber from our laboratory confirmed that toxins responsible for DSP (diarrhetic shellfish poisoning) were present in phytoplankton samples from Monterey Bay, California. Given that shellfish harvested from the west coast are not currently tested for DSP, the extent to which the toxin may be reaching human consumers is unknown, though the presence of potentially toxic species has been recognized for many decades in this region. When humans consume bivalves containing DSP toxins, severe gastrointestinal distress can occur within 30 minutes and chronic exposure to the toxins may lead to long-term problems. The primary goal of this project is to determine seasonal and hydrological patterns of *Dinophysis* species in Monterey Bay and elsewhere along the California coastline. A second objective is to determine whether DSP toxins are present in local shellfish. We will accomplish these objectives by collecting weekly water samples in Monterey Bay over an annual cycle, as well as obtaining quarterly water samples along a 700-mile transect of the California coast. Weekly samples of hepatopancreas from California mussels, *Mytilus californianus*, will be tested for DSP toxins by the Canadian Food Inspection Agency, a certified laboratory with established DSP regulatory limits. Our preliminary results of the time series study show a strong seasonal variation in total *Dinophysis* cell abundance between summer and fall months in Monterey Bay. In summer months *Dinophysis* cell densities can reach > 1000 cells L^{-1}. It has been reported that densities as low as 200 cells L^{-1} can result in toxic shellfish and affect human health. These results suggest that on several occasions *Dinophysis* cell abundances could potentially represent a human health hazard in Monterey Bay. Our initial results of the quarterly coastal survey indicate that *D. acuminata* dominates the north coast of the state, while *D. rotundata, D. fortii*, and *D. tripos* dominate the south coast. The observed species composition may correlate with oceanographic parameters, i.e. the north coast is colder and fresher whereas the south is warmer and saltier, but further analysis is needed to determine whether such environmental parameters are linked with toxin production. Lastly, we have obtained the first data on DSP toxins in California mussels. From the hepatopancreas samples tested, 62% contained 3 of the 5 toxins associated with DSP, 30% contained 4 of the 5 toxins, and 8% contained all 5 toxins. The DSP toxins tested included okadaic acid, dinophysistoxin-1, pectenotoxin-2, pectenotoxin-2-secoacid, and pectenotoxin-2-secoacid 7-epi. Currently two of these lipophilic toxins, okadaic acid and dinophysostoxin-1, are known to cause severe gastrointestinal distress and have been linked to mutagenic and tumor promoting activity. To date, no other data appear to have been obtained showing that DSP toxins accumulate in west coast shellfish. Furthermore, US west coast public health agencies have not yet recognized this syndrome as a public health threat, presumably because of lack of data on the phenomenon, and currently do not monitor for these toxins. At the completion of this project, we will have a better understanding of how *Dinophysis* abundance, toxin production and bioaccumulation of DSP toxins in shellfish are correlated and thus the extent to which health agencies may need to begin monitoring for this potential human health threat on the US west coast.

FORECASTING *MICROCYSTIS* BLOOM CHARACTERISTICS ON THE TIDAL POTOMAC RIVER, CHESAPEAKE BAY

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Cyanobacteria blooms have been a recurrent issue on the Potomac River since the 1960s. The level of variability in bloom expression is most evident in the example of the past three years; in 2002 no bloom samples (>10,000 cells/ml) were recorded while 2004 yielded the highest median densities (163,000 cells/ml) since the inception of the Chesapeake Bay Water Quality Monitoring Program in 1985. Timing of the bloom onset, duration, magnitude, and bloom extent were summarized from the 1985-2004 Maryland Department of Natural Resources phytoplankton data set and used to develop a conceptual prediction model of summer bloom conditions based on preseason river flow relationships. In spring 2005, a publicly available forecast for summer *Microcystis* bloom conditions was produced for the tidal Potomac River. The relationships supporting the model development and an evaluation of the 2005 forecast versus observed bloom patterns will be presented.

Potomac River *Microcystis* bloom forecast model:

Annual Flow for the previous year	Bloom levels first detected in the monitoring program	Bloom duration	Bloom Extent: Prediction modified by spring flow condition	
			Spring flow	Bloom extent
DRY	Early-mid summer or no bloom	Short (Median= 1.25 months)	DRY	Short (< 10 miles)
			MODERATE	Short
			WET	Moderate (10-20 miles)
MODERATE	Early summer or no bloom	Moderate (Median = 2 months)	DRY	Short
			MODERATE	Moderate
			WET	Long (>20 miles)
WET	Late spring to early summer,always a bloom detected	Long (Median = 2.38 months)	DRY	Moderate
			MODERATE	Moderate
			WET	Long (> 20 miles)

MEMBRANE STEROLS AND INHIBITION OF DINOFLAGELLATES BY *KARLODINIUM MICRUM* FILTRATE

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Karlodinium micrum toxicity was first observed in the Chesapeake Bay area in a series of fish kills at Hyrock farm (a hybrid striped bass aquaculture operation). *K. micrum* populations developed rapidly and quickly dominated the phytoplankton communities. However, other dinoflagellates co-occurred with *K. micrum* at lower cell densities. It has been suggested (Deeds and Place, in review) that the dominant membrane sterol of *K. micrum*, gymnodinosterol provides defense against it own toxin and that the presence of this sterol in other dinoflagellates would prevent inhibition by karlotoxin. This study was undertaken to determine the effects of toxin containing *K. micrum* filtrate on the growth of thecate and nonthecate dinoflagellates and to compare growth responses to the sterol composition of the dinoflagellates. *Gyrodinium uncatenum*, *Gymnodinium sanguineum* and *Amphidinium carterae* were not inhibited by filtrate. *G. uncatenum* contains Gymnodinosterol (the dominant and presumed protective sterols of *K. micrum*) as a minor sterol component. The primary sterol of *A. carterae*, amphisterol has an unusual D8(14) nuclear unsaturation also found in Gymnodinosterol (Leblond and Chapman, 2002) supporting the view (Place and Deeds, 2003) this nuclear unsaturation plus the 4- a methyl group is required for Karlotoxin resistance. The strongest inhibition by *K. micrum* filtrate was observed in the thecate dinoflagellate *Heterocapsa triquetra* which does not contain any of the protective sterols with the D 8(14) nuclear unsaturation. *H. triquetra* does posess dinosterol and several other 4- a methyl containing sterols suggesting the D 8(14) nuclear unsaturation is the structural feature most important in preventing inhibition. Only limited inhibition was observed in *Gyrodinium estuariale* and *Prorocentrum minimum*.

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Leblond JD and Chapman PJ. 2002. A survey of the sterol composition of the marine dinoflagellates Karenia brevis, Karenia mikimotoi *and* Karlodinium micrum*: Distribution of sterols within other members of the class Dinophyceae. Journal of Phycology 38 pp. 670-682.*

Place AR and Deeds JR 2003. Harmful algal toxins and sterols: not so strange bedfellows? Abstract. Second Symposium on Harmful Marine Algae in the U.S. Woods Hole, Mass.

MOVEMENTS, DIVE BEHAVIOR, AND SURVIVABILITY OF CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS*) POST-REHABILITATION FOR DOMOIC ACID TOXICITY

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Domoic acid is a neuroexcitatory toxin that can cause gastrointestinal and neurological problems if ingested. California sea lions *(Zalophus californianus)* were first noted displaying clinical symptoms of domoic acid toxicity in 1998 along the central California coast. Behavioral and physical symptoms included head weaving, unresponsiveness, ataxia, scratching, seizures, and lesions within the hippocampal region of the brain. In 1998, animals were admitted to The Marine Mammal Center (TMMC) for treatment and released back into the wild, however, behavior and survival of these animals was unknown. We used satellite telemetry to monitor dive behavior, migration, and survival of California sea lions after rehabilitation at TMMC in Sausalito, CA. Before the animals were released, satellite relayed data loggers (SRDLs) were attached between the scapulae with Devcon® 5 minute epoxy or Loctite® 422. To date, 9 animals have been released and tracked for up to 130 days. Two of the eight animals re-stranded and were euthanized, one animal's survivability is questionable due to the last transmitted location being half way between Monterey Bay and Hawaii, and five animal's survivability is unknown due to premature tag failure and the inability to re-sight the animals. Four females, however, migrated to San Miguel Island, CA as expected and the juvenile male remained in Carmel Bay, CA after release. The ninth animal is currently being tracked; therefore, it is too early to assess the success of this animal. Migration and dive behavior of these animals will be compared with a control group that to our knowledge has been unaffected by domoic acid toxicity. These data will allow us to determine if California sea lions can be successfully rehabilitated once exposed to domoic acid.

GAMBIERDISCUS: LINKING TAXONOMY AND GENETICS

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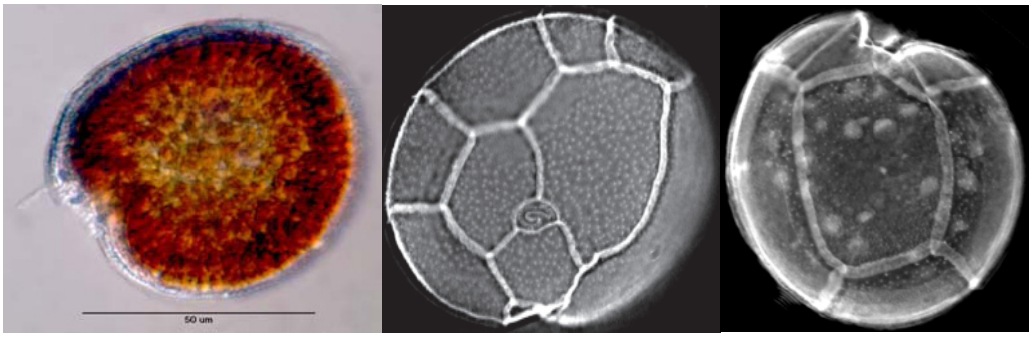
Gambierdiscus species produce a variety of ciguatoxins that bioaccumulate in the food chain. Human toxicity is generally associated with consuming tainted fish such as barracuda or groupers. Much effort has been devoted to characterizing the structure and toxicity of ciguatoxins. However, the taxonomy and ecology of the dinoflagellates that produce these toxins is relatively understudied, largely due to difficulties encountered when trying to discriminate species by light microscopy. SEM techniques can provide accurate identification and have been used to identify six *Gambierdiscus* species that exhibit pan tropical distributions. SEM techniques, however, are too expensive, time consuming and non-quantitative to use in analyzing routine field samples. Having accurate distribution and abundance data may be crucial to interpreting temporal and geographic differences in toxicity. Currently, there are reports that toxicity of reef fishes varies both temporally and regionally. A fundamental question that arises from these observations is whether the variations in toxicity are related to species distribution and abundance or to other factors such as environmental induction of toxin production. The former question can only be answered by having accurate abundance and distribution data for various species. Quantitative molecular assays afford an excellent means of obtaining this information.

Before these types of molecular assays can be developed, it is necessary to have genetically well defined cultures. Unfortunately, these cultures do not exist. To overcome this obstacle, we have established numerous ciguatera dinoflagellate cultures isolated from the barrier reef system in Belize, Central America and from Florida. In addition, a *Gambierdiscus* species from a deepwater reef system off the coast of North Carolina has also been isolated. We have employed electron and fluorescent microscopy to analyze cellular morphology. Currently we are developing molecular methods to sequence a ~ 3000 bp region of the ribosomal gene complex for each species. The sequence data will be used to develop quantitative PCR methods to rapidly identify and quantify specific *Gambierdiscus* species in future field studies. This poster will present the results from our taxonomic surveys.



A. Light micrograh of a Gambierdiscus *sp. collected from a deepwater reef, offshore of North Carolina. B and C. Epifluorescent micrographs of calcofluor stained cells showing the epithecal and hypothecal plates respectively.*

TOXIC *PSEUDO-NITZSCHIA*: WHAT'S IN A NUMBER?

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The HAB-TrAC program involves three campuses of the University of California (Santa Cruz, Santa Barbara and San Diego) in a study of the coherence of toxic phytoplankton outbreaks along the central and southern California coast. Together, we have collected a large amount of data on the distribution and abundance of the toxic *Pseudo-nitschia* species, *P. australis* and *P. multiseries*. Data were collected routinely from five locations and may include at least three populations. Data include cell counts and domoic acid measurements on sea water particulates. Cell counts, made on duplicate subsamples, were treated with species-specific, fluorescently labeled probes. They are similar to many standard cell counts in that they are based on microscopic enumeration of material retained on a 1.2ì filter, and thus the statistical problems discussed have a broad generality. Both the precision and sensitivity of the counts are expected to be related to the number of cells and the distribution of cells into chains. Unfortunately, the latter is poorly known.

We tried a number of different approaches to estimate precision and sensitivity, in spite of the complication of chain lengths. On this poster, I summarize our thinking ("conclusions" may be too strong a word) on three important questions, in the hopes that our results may benefit others.

1. Can we estimate the sensitivity of counts of chained species, such as *Pseudo-nitzschia* spp.?
2. Can we estimate the confidence interval for counts of such species, in the absence of a well-determined variance?
3. Does the relationship between the variance and the mean change between the two species, or among regions? Given the expected relationship between precision and chain-length, might changes in the variance to mean relationship tell us anything about chain length?

DOMOIC ACID IN THE FAT INNKEEPER WORM, *URECHIS CAUPO*, AT ELKHORN SLOUGH, CA

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Recent research has measured high levels of domoic acid (DA) in *Urechis caupo*, an echuiran worm inhabiting mudflats along the Californian coast. I have found *U. caupo* in Elkhorn Slough, Monterey Bay, CA to have DA concentrations as high as 1300 mg DA/g. However, the toxin producing diatom, *Pseudo-nitzschia spp.*, has never been monitored in the slough, an important estuarine national research reserve. Within the slough environment, *U. caupo* plays an important ecological role as both a prey item and in affecting the benthic environment by increasing the depth of oxygen penetration and providing a habitat for multiple commensal species. Data on the levels of DA in *U. caupo* in Elkhorn Slough from July 2004 to August 2005 will be presented, along with DA levels in the surface water and sediment. Laboratory data on the depuration rate and half-life of DA in *U. caupo* will be presented and used to determine the exposure window of DA to the animals sampled in the field.

SURVEILLANCE FOR CIGUATERA FISH POISONING IN RECREATIONAL FISHERS UTILIZING TEXAS GULF COAST OIL RIGS

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Ciguatera fish poisoning (CFP) is a form of fish poisoning caused by the consumption of a wide variety of subtropical and tropical marine finfish that have accumulated naturally-occurring ciguatoxins through their diet. It is the most common form of marine seafood poisoning worldwide. Ciguatoxin precursors are produced by *Gambierdiscus* spp. that are common to ciguatera endemic regions throughout the Caribbean and Pacific. While CFP has been identified in the NW Gulf of Mexico, the distribution of CFP in the northern and Gulf of Mexico is unknown.

The artificial reefs created by the approximately 4000 offshore oil drilling platforms (rigs) along the gulf coast of Texas and Louisiana provide ideal habitat for reef fish. Together with state-sponsored artificial reef programs, these artificial habitats have become popular destinations for Texas Gulf coast sportfishers (both hook and line and spearfishers) because of the diversity of fish as a result of the vertical habitat structure.

Coinciding with the increased popularity of fishing near oil rigs, the Texas Department of Health has received reports of cases of CFP among fishers and spearfishers who have eaten fish caught on the oil rigs off the coast of southeastern Texas. The first reported case of CFP in Texas occurred in August 1988 when a family of three became ill after eating barracuda that was caught while spearfishing near an oil rig. Researchers at the University of Texas at Austin, Marine Science Institute have received word-of-mouth reports of CFP among fishers who caught fish on Texas oil rigs. These reports suggest that CFP is more prevalent that previously thought.

Surveillance activity will identify the prevalence of CFP in the recreational fishing community of coastal Texas and determine if recreational fishing on offshore oil rigs represents an emerging source of exposure to ciguatoxins. Our study population will be comprised of recreational fishermen and spearfishers who harvest fish from Texas Gulf Coast oil rigs. We will conduct face-to-face or telephone interviews with persons 12 years and older who have ever consumed fish caught by hook and line or speargun on the oil rig platforms off the Gulf coast of Texas. By conducting active surveillance for cases, this study will aid efforts to understand the scope of ciguatera poisonings in the northwestern Gulf of Mexico, and determine if recreational fishing on offshore oil rigs represents an emerging source of exposure to ciguatoxins, and educate at-risk populations in preventative measures.

ANALYSIS OF BREVETOXIN METABOLITES IN BOTTLENOSE DOLPHINS ASSOCIATED WITH THEIR MORTALITY IN THE FLORIDA PANHANDLE DURING 2004

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The mortality event of bottlenose dolphins in the Florida panhandle durine 2004 occurred in the absence of a *Karenia brevis* bloom, a dinoflagellate species that produces neurotoxic polyether toxins - brevetoxins. Dietary exposure of brevetoxin by these panhandle dolphins was considered to be the cause of this mortality event based on the high concentrations of brevetoxin identified in their food source.[1] Unambiguous confirmation the source of brevetoxin is difficult. Analysis of the brevetoxin metabolites in the stomach fluid of the stranded animals may provide some information on the source of intoxication. Little is know about the metabolism of the brevetoxins in marine mammals. Analysis of the toxin metabolites in the tissues (stomach, liver, and kidney) may provide some information on the metabolism of the toxins in this marine mammal species. In this study, we examined the brevetoxin metabolite profile by LC/MS and radioimmunoassay in the tissues (stomach, liver, and kidney) of the stranded dolphins and in guts of fish collected from the area of the mortalities. In samples collected during this mortality event, brevetoxin-3 was predominately found in extracts prepared from stomach contents of dolphin. Cysteine, glutathione, and other amino acid related metabolites were detected in stomach contents of both dolphin and fish. Brevetoxin-3 was identified as the major toxin in all tissues analyzed, however, in liver and kidney cysteine and glutathione metabolites have not been found at comparable levels by LC/MS. Identification of these metabolites in these dolphins and fish may indicate exposure of brevetoxin through the food chain rather than direct exposure to a toxic algal bloom.

1. Leanne Flewelling, Jerome Naar et al., 2005, Fish and seagrass as vectors of brevetoxins during two marine mammals mortalities, Nature, in press.

PRELIMINARY OCCURRENCE STUDY OF ALGAL TOXIN IN SOURCE AND FINISHED WATERS

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The United States Environmental Protection Agency 1998 Contaminant Candidate List contained freshwater algae and their toxins. Contaminants on this list must be prioritized based on human health risk which includes occurrence information and health effects. In order to focus their resources, the USEPA created a priority list based on frequency in surface waters and toxicity which contains 5 heptotoxins, 4 adducts of microcystin (LR, RR, LA, YR) and cyclindrospermopsin, and a neurotoxin, anatoxin-a. This preliminary study focused on algal identification/enumeration and the determination of the presence of toxins in drinking water utilities that were at high risk for exposure to the algae and toxins on the priority list. In the summer 2001 study both source and finished water sample concentrations where determined by ELISA to be below the detection limit of 0.2 mg/L in all 76 samples. In the summer 2002 study 13 source water samples tested positive for microcystin, but no finished water samples tested positive. In summary, after 2 summers of algal monitoring 8% of the source water samples tested positive for microcystin, however only 1% of these samples were above 1mg/L. There were no instances where algal toxins made it through the drinking water treatment processes of any of the utilities observed. These samples were archived and are currently being analyzed for cyclindrospermopsin, anatoxin-a and microcystins by LC/MS. These results will be included.

PSEUDO-NITZSCHIA SPECIES IN FLORIDA COASTAL WATERS

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Domoic acid poisoning events have been documented in the United States since the early 1990s. However, there has never been a domoic acid poisoning event recorded in Florida waters, despite the presence of *Pseudo-nitzschia*. A March 2004 *Pseudo-nitzschia* bloom (maximum concentration of 4.64 x 10^5 cells/L) in St. Joseph Bay, Florida prompted an examination of the distribution of *Pseudo-nitzschia* species in Florida coastal waters. Both the FWRI HAB monitoring program and the Florida HAB historical database were utilized to provide samples for SEM identification and for analysis of distributional and seasonal trends, respectively. Blooms of *Pseudo-nitzschia* spp. are common in Florida coastal waters and cell concentrations can reach up to 12.3 x 10^6 cells/L (*P. pseudodelicatissima* in St. Joseph Bay, December 2004) in estuarine and coastal shelf environments. Seven species, including the known domoic acid producers *P. delicatissima* and *P. pseudodelicatissima* and two potentially toxic species, *P. pungens* and *P. turgidula*, have been identified from five different geographic locations in Florida. A clone of *P. pseudodelicatissima* from Cinco Bayou was isolated and had demonstrable domoic acid production under laboratory conditions. These data suggest that while *Pseudo-nitzschia* blooms are common in Florida waters and toxic species are present, the environmental factors required to elicit domoic acid associated intoxication events are not.

STRUCTURE OF SEVERAL NEW HEMOLYTIC TOXINS FROM STRAINS OF *PRYMNESIUM PARVUM* ISOLATED FROM FISH PONDS IN NORTH CAROLINA

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The Raphidophyte *Prymnesium parvum* is known to produce the prymnesins, extremely large (MW > 2K Daltons) non-proteinaceous compounds, which display potent hemolytic activity. A serious fish kill in artesian aquaculture ponds in North Carolina, led to the discovery of large concentrations of *P. parvum* in these ponds. Laboratory cultures of this organism were found to display hemolytic activity, and organic extracts of the cells displayed similar activity.

Following large-scale laboratory culturing, the crude organic extract of the cells was processed by a series of chromatography steps, using a hemolytic bioassay to guide the fractionation process. As the fractionation progressed, the activity as found to reside in the more lipophilic HPLC fractions, and a series of compounds were isolated that all displayed potent hemolytic activity. LC/MS analysis of these compounds indicated that these compounds fell within a molecular weight range of 650-800 Da, considerably smaller than the previously reported prymnesins. In a final HPLC purification step, six bioactive compounds were obtained. Analysis of the preliminary NMR data indicated these compounds were indeed all closely related, and were likely ceramide derivatives, containing novel fatty acyl chains. The structure of these compounds will be described, and preliminary structure-activity results reported.

STATEWIDE DISTRIBUTION OF SAXITOXINS IN SELECTED FLORIDA PUFFER FISH SPECIES

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As of April 2005, 28 cases of puffer fish poisoning (PFP) have been reported, primarily from Florida, but including New Jersey, New York and Virginia with the first incidents occurring in January 2002. Previously unknown in Florida's marine waters (Landsberg et al. 2002), all incidents were due to saxitoxin (Quilliam et al. 2002) present in the muscle of southern puffer fish (*Sphoeroides nephelus*) originating from the northern Indian River Lagoon (IRL) on Florida's Atlantic coast (Bodager 2002). Since these initial reports statewide surveys of the distribution of saxitoxins (STXs) in a wide variety of marine species have been conducted utilizing the Ridascreen® STX ELISA kit (Usleber et al. 1991) and receptor binding assay. To date the highest levels of STXs continue to be found in southern puffer fish from the IRL where they pose a significant threat to public health with maximum levels in the muscle just over 20,106 µg STXeq./100g tissue, n=353 (action level for shellfish is 80 µg STXeq./100g tissue). However, lower levels have also been found in the muscle of southern, checkered and bandtail pufferfish from other Florida waters (Table 1).

Ongoing research seeks to positively identify the major source of STXs within the IRL, with the dinoflagellate *Pyrodinium bahamense* being the most likely. In addition, vectors for STX transfer as well as toxin depuration studies are currently being conducted to better understand the differences in toxin levels between locales and among puffer species.

	Maximum STX in muscle (µg STXeq./100g.)		
	Southern Puffer	Checkered Puffer	Bandtail Puffer
Geographic Area	*S. nephelus*	*S. testudineus*	*S. dorsalis*
Indian River Lagoon (IRL)	20106 (n=353)	104 (n=51)	42 (n=3)
Tequesta, southern IRL, southeast FL	3133 (n=76)	41 (n=74)	1778 (n=13)
Tampa Bay, central west FL	332 (n=143)		
Jacksonville, northeast FL	180 (n=67)		
Charlotte Harbor, southwest FL	69 (n=37)		1 (n=1)
Florida Keys, south FL	11 (n=3)	17 (n=2)	268 (n=15)
Apalachicola, northwest FL	10 (n=17)		

Table 1. Maximum STX concentrations measured in muscle of selected puffer species from FL.

Bodager, D. 2002. Outbreak of saxitoxin illness following consumption of Florida pufferfish. Fl. J. Environ. Health 179: 9-13

Landsberg, J. H. et al. 2002. Pufferfish poisoning: widespread implications of saxitoxin in Florida. Xth International Conference on Harmful Algae, St. Petersburg Beach, Florida, October 2002, Abstr. p. 160.

Quilliam, M., Wechsler, D., Marcus, S., Ruck, B., Wekell, M. and Hawryluk, T. 2002. Detection and identification of paralytic shellfish poisoning toxins in Florida pufferfish responsible for incidents of neurologic illness. Xth International Conference on Harmful Algae, St. Petersburg Beach, Florida, October 2002, Abstr. p. 237.

Usleber, E., Schneider, E. and Terplan, G. 1991. Direct enzyme immunoassay in microtitration plate and test strip format for the detection of saxitoxin in shellfish. Letts. Appl. Microbiol., 13: 275-277.

NO APPARENT EFFECT OF TWO SPECIES OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM* ON HEMOCYTE PARAMETERS OF THE OYSTERS *CRASSOSTREA VIRGINICA* AND *CRASSOSTREA GIGAS*

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Alexandrium is a dinoflagellate genus that includes toxin-producing species responsible for paralytic shellfish poisoning. Toxic species can have physiological effects on bivalves feeding on them, but these effects are not fully understood. Thus, two experiments were conducted to determine possible effects *Alexandrium* on cellular immune-system status of oysters. Function of the immune system, attributed mainly to circulating cells called hemocytes, is the main defense mechanism in molluscan shellfish to noxious, toxic, or pathogenic agents. Eastern oysters, *Crassostrea virginica*, were exposed for 7 days to cultured *Alexandrium fundyense* (low and high concentration) and *Thalassiosira weissflogii*, individually or in a mixed diet. Pacific oysters, *C. gigas,* were exposed to a mix of *A. catenella* and *T. weissflogii* cultures for 1 to 4 days at 12°C and 18°C. The immune status of oysters was assessed by measuring the characteristics, concentrations and percentages of several hemocytes cell types, as well as hemocyte function: mortality, aggregation, phagocytosis and respiratory burst. Hemocyte characteristics of *Alexandrium*-exposed and control oysters were compared statistically to determine possible immuno-modulation by the toxic cells.

Despite physiological effects of *A. fundyense* on *C. virginica* (adductor-muscle paralysis), results showed no effect of the toxic culture on oyster immune status; all diets supported a "good" immune profile. Exposure of Pacific oysters to *A. catenella* had no apparent effect on immune status either. Moreover, no correlation was found between hemocyte parameters and neither time of exposure the oysters to *A. catenella* nor toxin accumulation in oyster tissues. In contrast, temperature affected the hemocyte parameters of the Pacific oysters regardless of feeding treatment; oysters held at 18°C had higher numbers of hemocytes (especially granulocytes), higher phagocytosis, and a higher basal level of oxidative burst. Physical displacement of the oysters also caused changes in hemocyte parameters. This study demonstrated no effect of two different *Alexandrium* spp. upon two oyster species, despite demonstration of physiological effects: paralysis or toxin accumulation. Our findings show no evidence that *Alexandrium* blooms increase the susceptibility of exposed oysters to pathogens or parasites.

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