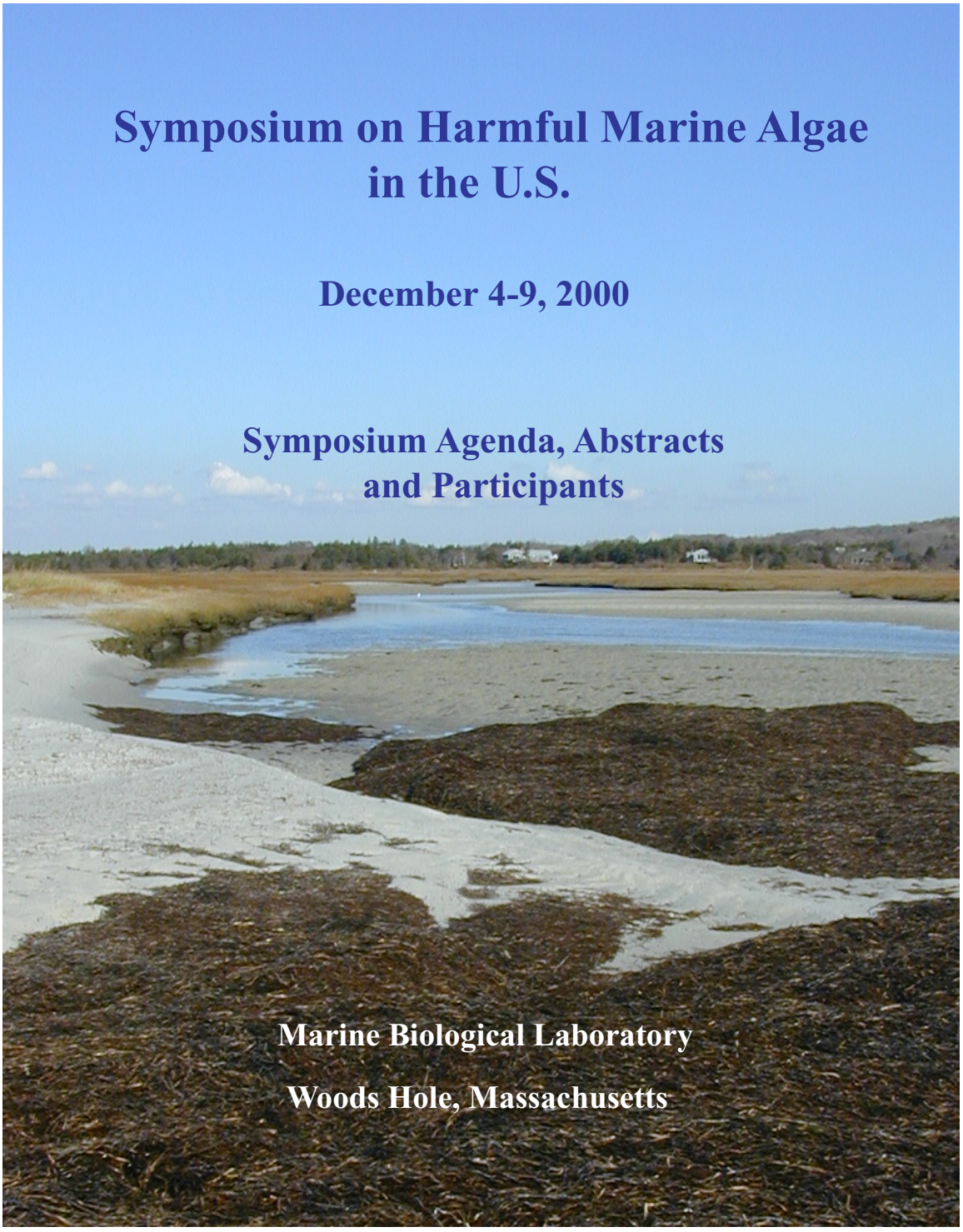
Symposium on Harmful Marine Algae in the U.S.

December 4-9, 2000

Symposium Agenda, Abstracts and Participants



Marine Biological Laboratory

Woods Hole, Massachusetts

Symposium Director:
Donald M. Anderson

Symposium Coordinator:
Judy Kleindinst

Steering Committee:

Don Anderson	Woods Hole Oceanographic Institution
Dan Baden	University of North Carolina, Wilmington
Sue Banahan	NOAA, National Ocean Service, Silver Spring
JoAnn Burkholder	North Carolina State University
Pat Glibert	University of Maryland Center for Environmental Science
John Heisler	EPA, Oceans and Coastal Protection Division
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Rick Stumpf	NOAA, National Ocean Service, Silver Spring
Pat Tester	NOAA, National Ocean Service, Beaufort
Fran VanDolah	NOAA, National Ocean Service, Charleston
Tracy Villareal	The University of Texas at Austin

Session Coordinators/Chairs:
(Note: Names of Session Chairs are underlined)

ECOHAB – Florida:	Karen Steidinger, Pat Tester, Fran VanDolah
Gulf of Mexico HABs	Quay Dortch, Tracy Villareal
Pfiesteria – NC, SC, FL	JoAnn Burkholder, Jan Landsberg, Alan Lewitus, Wayne Litaker
Pfiesteria – DE, MD, VA	Pat Glibert, Dave Oldach, Jeff Shields
West Coast HABs	Rita Horner, Chris Scholin, Vera Trainer
Non-regional HABs	Kevin Sellner, Tracy Villareal
ECOHAB – GOM	Don Anderson, Dave Townsend
Brown Tides	Sue Banahan, Greg Boyer, Cornelia Schlenk

Sponsors:

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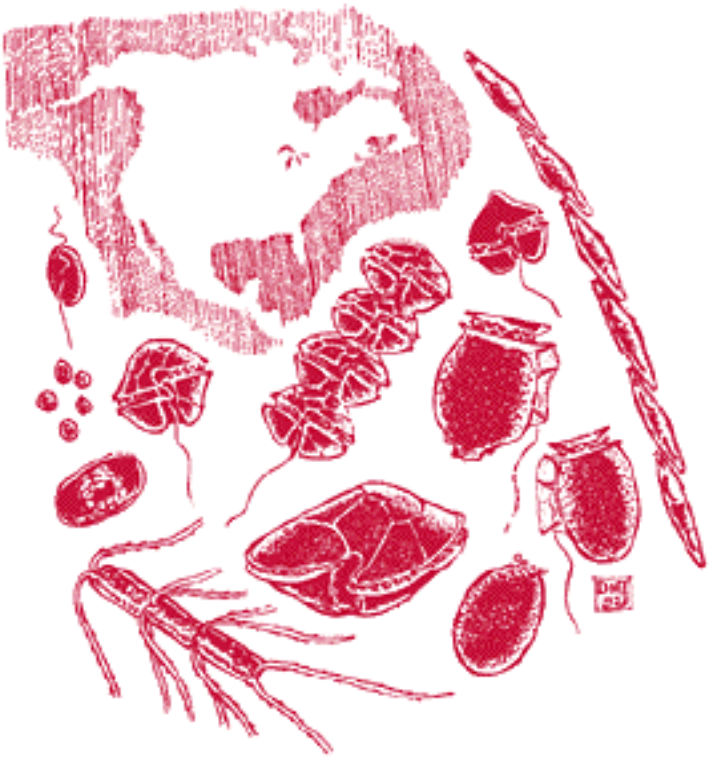

Symposium Agenda, Abstracts

and Participants

Symposium on Harmful Marine Algae in the U.S.
Program*
December 5-9, 2000

Schedule	Tues., Dec. 5	Wed., Dec. 6	Thurs., Dec. 7	Fri., Dec. 8	Sat., Dec. 9
8:30 – 8:50	Welcome and Introduction				
8:30 – 12:15	ECOHAB – Florida	*Pfiesteria* research – NC, SC & FL	West Coast HAB research	ECOHAB – GOM research	Brown Tides (NY, NJ, DE, MD, TX)
12:15 – 1:30	Lunch break, Swope Dining Hall	Lunch break, Swope Dining Hall	Lunch break, Swope Dining Hall	Lunch break, Swope Dining Hall	Lunch break, Swope Dining Hall
1:30 – 5:15	Gulf of Mexico regional HAB research	*Pfiesteria* research – DE, MD & VA	Non-regional HAB research	Technical workshops/ demos (TBA)	Plenary discussion
	Break	Break	Break	Break	Break
6:00 – 7:30	Dinner, Swope Dining Hall	Dinner, Swope Dining Hall	Dinner, Swope Dining Hall	Dinner, Swope Dining Hall	Lobster dinner, Swope Dining Hall
7:30 -	Discussion Sessions (TBA)	Poster Session (for Mon. & Tues. themes) Swope upstairs	Social Hour, Swope upstairs	Poster Session (for Thurs., Fri. & Sat. themes) Swope upstairs	Reception, Swope Meigs Room

*NOTE: All science presentations will be held in the Lillie Auditorium, MBL, MBL Street, Woods Hole. Meals will be held in the Swope Dining Hall, MBL.

Symposium on Harmful Marine Algae in the U.S.

December 4-9, 2000
Marine Biological Laboratory
Woods Hole, Massachusetts

Program Schedule:

Monday, December 4, 2000

3:00 – 6:00 p.m.	Registration, Swope Lobby
6:00 – 7:30 p.m.	Dinner, Swope Dining Hall
7:00 – 9:30 p.m.	Reception, Swope upstairs lobby

Tuesday, December 5, 2000

8:30 – 8:50 a.m. **Lillie Auditorium**	Welcome and Introduction **Don Anderson**, Woods Hole Oceanographic Institution
	Session I – ECOHAB – Florida (K. Steidinger, F. Van Dolah, P. Tester – Session Coordinators) (P. Tester – Session Chair)
8:50 – 9:10	ECOHAB: Florida overview – the environment **Karen Steidinger**, John Walsh, and Gary Kirkpatrick
9:10 – 9:30	Roles of endogenous cellular rhythms and life cycle stage recruitment in *Gymnodinium breve* bloom development **Frances M. Van Dolah**, Michele Barbier, Tod A. Leighfield, Karen A. Steidinger, Bill Richardson and Peter M. McGuire
9:30 – 9:50	ECOHAB Florida: fate and effects of brevetoxins in selected biota, water, and sediments along the west Florida shelf, USA **Jan Landsberg**, Pat Tester, Richard Pierce, Damian Shea, Fran Van Dolah, Emilio Sosa, Mike Henry, Jack Fournie, Leanne Flewelling, Sabrina Varnam, and Tod Leighfield
9:50 – 10:10	ECOHAB – Florida: bio-optics and physiology **Gary Kirkpatrick**, David Millie, Steve Lohrenz, Oscar Schofield, Gary Fahnenstiel, Donald Redalje and Terrance Evens
10:10 – 10:30	BREAK

10:30 – 10:50	Hydrography and nutrient characteristics within the ECOHAB: Florida control volume on the west Florida shelf **Gabriel A. Vargo**, Cynthia A. Heil, John J. Walsh, Kent Fanning, Carmelo R. Tomas, Karen A. Steidinger, Danylle Ault, Merrie Beth Neely, Kristen Lester, and Rachel Merkt
10:50 – 11:10	ECOHAB Florida, physical oceanography **Robert H. Weisberg**, Ruoying He, William Hemme, Zhenjiang Li, and Huijun Yang
11:10 – 11:30	The role of behavior in *Gymnodinium breve* bloom formation **Daniel Kamykowski**, Gerald S. Janowitz, Gang Liu, Edward J. Milligan, and Robert E. Reed
11:30 – 12:00	Coupled numerical models of Florida red tides of *Gymnodinium breve* **John J. Walsh**, W. Paul Bissett, Bradley Penta, Dwight A. Dieterle, Robert H. Weisberg, Zhenjiang Li, and Huijun Yang
12:00 – 12:15	Discussion session (if time allows)
12:15 – 1:30	LUNCH BREAK, Swope Dining Hall
Lillie Auditorium	**Session II – Gulf of Mexico Regional HAB Research** (T. Villareal, Q. Dortch, Session Coordinators) (Q. Dortch, Session Chair)
1:30 – 1:50	*Pseudo-nitzschia* spp. in the northern Gulf of Mexico: overview and response to increasing eutrophication **Q. Dortch**, M.L. Parsons, G.J. Doucette, G. A. Fryxell, A. Maier, A. Thessen and C.L. Powell
1:50 – 2:10	Algicidal bacteria active against *Gymnodinium breve*: use of molecular techniques to assess changes in microbial communities following the introduction of bacteria **Xavier Mayali** and Gregory J. Doucette
2:10 – 2:30	Variable brevetoxin production in *Gymnodinium breve* attributable to growth conditions and strain differences Richard M. Greene, **Janis C. Kurtz**, Roman S. Stanley, Cynthia A. Chancy, Michael C. Murrell, Fred J. Genthner, John E. Rogers, and Calvin C. Walker

2:30 – 2:50	*Gymnodinium breve* red tides in the Gulf of Mexico: analysis of brevetoxins and metabolites in shellfish **R.W. Dickey** and S.M. Plakas
2:50 – 3:10	*Gymnodinium breve* in the western Gulf of Mexico: two years of coastal sampling off the Texas coast **Tracy A. Villareal**, Mary A. Brainard, and H. Magaña and Lawrence W. McEachron
3:10 – 3:40	The effect of nitrogen source on the growth and toxicity of speices of the genus *Prorocentrum* Cary L. Burns and Jonathan R. Pennock
3:40 – 4:00	Discussion session (if time allows)
4:00 – 5:00	Informal discussion groups or free time – put up posters
5:00 – 6:00	INFORMAL SOCIAL HOUR, Swope upstairs Lobby
6:00 – 7:30 -	DINNER, Swope Dining Hall
7:30 –	DISCUSSION SESSIONS (TBA)

Wednesday, December 6, 2000

Lillie Auditorium	**Session III – *Pfiesteria* research – NC, SC, & FL** (J. Burkholder, J. Landsberg, W.Litaker, A. Lewitus – Session Coordinators) (J. Burkholder, Session Chair)
8:30 – 8:50	*Pfiesteria*, *Pfiesteria*-like species, and fish health in Florida: an update **Jan Landsberg**, Karen Steidinger, Susan Cook, Elizabeth Singh, Emilio Sosa, Ann Forstchen, Robin Wood, Parke Rublee, Paula Scott, Jennifer Wolny, and Brian Bendis
8:50 – 9:10	Preliminary characterization of "Cryptoperidiniopsoid" cultures isolated from Florida **Steve L. Morton**, Tina Mikulski, Elizabeth R. Fairey, Brad Mitchell, Peter D.R. Moeller, Bill Richardson, Karen Steidinger, and John Ramsdell
9:10 – 9:30	*Pfiesteria* spp. and "P*fiesteria*-like organisms" in South Carolina estuaries **Alan J. Lewitus**, J.M. Burkholder, C. Cary, H.B. Glasgow Jr., K.C. Hayes, A.F. Holland, J.M. Law, and P.A. Rublee

9:30 – 9:50	Nutrient enrichment and the toxic *Pfiesteria* complex: comparative stimulation by swine effluent, poultry manure leachate, human sewage, and other sources **J. Burkholder**, C. Zheng, H. Glasgow, N. Deamer-Melia and M. Parrow
9:50 – 10:10	Distribution of *Pfiesteria* species: comparison of results from water and sediment samples across multiple scales, 1998-2000 **Parke A. Rublee**, Eric F. Schaefer, Coy Allen, Janera Harris, Holly Bowers, Torstein Tengs, and D.W. Oldach
10:10 – 10:30	BREAK
10:30 – 10:50	*Pfiesteria* field ecology and toxic activity: trends from a decade of intensive study in North Carolina estuaries **R. Reed**, H. Glasgow, J. Burkholder, N. Deamer-Melia and M. Mallin
10:50 – 11:10	Toxic *Pfiesteria* promotes acute and chronic lesions in finfish, in controlled experimental trials **H. Glasgow, Jr.**, R. Smolowitz, N. Deamer-Melia and J. Burkholder
11:10 – 11:30	Interactions between *Pfiesteria* and representative species of commercially valuable shellfish **S. Shumway**, J. Springer, J. Burkholder and H. Glasgow
11:30 – 11:50	Characterization of a putative toxin produced by *Pfiesteria piscicida* **J.S. Ramsdell**, P.D.R. Moeller, E.R. Fairey, A.C. Melo, K.L. Kimm-Brinson, B. Mitchell, S.A. Morton, N. Deamer-Melia , H.B. Glasgow, and J.M. Burkholder
11:50 – 12:15	Discussion session (if time allows)
12:15 – 1:30	LUNCH BREAK, Swope Dining Hall
Lillie Auditorium	**Session IV – *Pfiesteria* research – DE, MD & VA** (P. Glibert, D. Oldach, J. Shields - Session Coordinators) (P. Glibert, Session Chair)
1:30 – 1:45	Approaches to the investigation and interpretation of possible *Pfiesteria*-related events in Maryland **Robert E. Magnien**, David M. Goshorn, David W. Oldach, Holly A. Bowers, and Torstein Tengs
1:45 – 2:00	Intensive monitoring for *Pfiesteria* and related HAB events

	B. Boicourt, L. Codispoti, M. Roman, V. Holliday, H. MacIntyre, P. Glibert, R. Magnien, and B. Michael
2:00 – 2:15	Assessing temporal and spatial variability in *Pfiesteria piscicida* distributions using molecular probing techniques **Kathryn J. Coyne**, David A. Hutchins, Clinton E. Hare and S. Craig Cary
2:15 – 2:30	Functional type (toxicity status) controls *Pfiesteria* response to nutrients and algal versus fish prey **J. Burkholder**, H. Glasgow, P. Glibert, A. Lewitus, M. Parrow, C. Zheng, P. Cancellieri and N. Deamer-Melia
2:30 – 2:45	Nitrogen uptake and nutrient relationships in laboratory cultures and field assemblages of *Pfiesteria* **P.M. Glibert**, A. Lewitus, J. Burkholder, H. Glasgow, M. Mulholland, and C. Lee
2:45 – 3:00	Potential grazing on *Pfiesteria piscicida* by microzooplankton in the Pocomoke River **Diane K. Stoecker** and Daniel E. Gustafson, Jr.
3:00 – 3:20	BREAK
3:20 – 3:35	Trophic relationships of phytoplankton and microzooplankton with *Pfiesteria*-like heterotrophic dinoflagellates in Pocomoke River and Transquaking/Chicamacomico Rivers, MD, USA **Richard V. Lacouture**, Jennifer Gronefeld, Ann Marie Hartsig, Stella Sellner and Amy Imirie
3:35 – 3:50	Results of a series of fish bioassays with the toxic dinoflagellate *Pfiesteria piscicida* **Harold G. Marshall**, Andrew S. Gordon, David W. Seaborn, Brian Dyer, William M. Dunstan, and A. Michelle Seaborn
3:50 – 4:05	Skin lesions in estuarine fishes: a comparative pathological evaluation of wild and laboratory-exposed fish **Wolfgang K. Vogelbein**, Kimberly S. Reece, Jeffrey D. Shields, David E. Zwerner, Patrice L. Mason, Larry W. Haas and Vicki Blazer
4:05 – 4:20	Development and testing of molecular diagnostics for *Pfiesteria*-like organisms in laboratory and environmental samples **Kimberly S. Reece**, Nancy A. Stokes, Wolfgang K. Vogelbein, Wayne L. Litaker, Jeffrey D. Shields, Larry W. Haas, Patrice L. Mason, Victoria M. Foster and Eugene M. Burreson

4:20 – 4:35	*Pfiesteria* or fungus? Induction of skin ulcers in menhaden with zoospores of *Aphanomyces* spp. **Yasunari Kiryu**, Jeffrey D. Shields, Wolfgang K. Vogelbein, David E. Zwerner, Howard Kator, and Vicki S. Blazer
4:35 – 4:50	The ambush predator *Pfiesteria piscicida*: fad or fallacy **Patrick Gillevet**, Thomas Nerad, Michael T. Peglar, Greg C. Garman, Stanley Webb, Bonnie Brown, and Charles J. O'Kelly
4:50 – 5:15	Discussion session (if time allows)
6:00 – 7:30	DINNER, Swope Dining Hall
7:30 –	POSTER SESSION, Swope upstairs and Meigs Room

Thursday, December 7, 2000

Lillie Auditorium	**Session V – West Coast HAB research** (C. Scholin, R.Horner, V. Trainer – Session Coordinators) (C. Scholin, Session Chair)
8:30 – 9:00	HABs-related physical oceanography off the U.S. west coast **Barbara M. Hickey**
9:00 – 9:30	The challenges of forecasting and managing toxic *Pseudo-nitzschia* blooms on the U.S. west coast **Vera L. Trainer**
9:30 – 10:00	Field studies of toxic phytoplankton in central California: 1999-2000 **Mary Silver**, Susan Coale, Shonna Dovel, Kathi Lefebvre, Greg Doucette, Ron Tjeerdema, and Rikk Kvitek
10:00 – 10:20	BREAK
10:20 – 10:50	Approaches to the detection of domoic acid in marine food webs **Gregory J. Doucette**
10:50 – 11:20	Trace metals and *Pseudo-nitzschia* blooms: a possible role for the toxin domoic acid **Eden Rue**, Maria Maldonado, Ken Bruland and Mark Wells
11:20 – 11:50	Summary **Rita A. Horner**
11:50 – 12:15	Discussion session (if time allows)

12:15 – 1:30	LUNCH BREAK, Swope Dining Hall
Lillie Auditorium	**Session IV – Non-regional HAB Research** (T. Villareal, K. Sellner, Session Coordinators and Chairs)
1:30 – 1:50	Long term occurrence patterns and dynamics of ichthyotoxic *Heterosigma akashiwo* in Narragansett Bay **Yaqin Li** and Theodore J. Smayda
1:50 – 2:10	An expansion of harmful raphidophyte blooms in U.S. coastal waters **Carmelo R. Tomas**
2:10 – 2:30	The potential for sediment-water column interactions to stimulate growth of the bloom-forming dinoflagellate *Prorocentrum minimum* **Hugh MacIntyre**, Jason Adolf and Angela Dubois
2:30 – 2:50	Use of cell specific PAM -fluorometry to characterize light acclimation responses of *Gambierdiscus toxicus* (Dinophyceae) **Tracy A. Villareal** and Steve Morton
2:50 – 3:10	Evidence for adaptation to toxins in populations of the softshell clam, *Mya arenaria,* subjected to recurrent toxic blooms **V. Monica Bricelj**, Scott P. MacQuarrie, Betty M. Twarog, and Vera L. Trainer
3:10 – 3:30	BREAK
3:30 – 3:50	Rapid testing using the MIST alert™ for paralytic shellfish poisoning (PSP), for trials within the United States **Joanne F. Jellett**
3:50 – 4:10	Antillatoxin, a novel neurotoxin from the marine cyanobacteria *Lyngbya majuscala,* is a potent activator of voltage-gated Na^+ channels **Thomas F. Murray**, W.I. Li, F.W. Berman, T. Okino and W.H. Gerwick
4:10 – 4:30	Unusual sterols from harmful algae: more than biomarkers? **José-L. Giner**, Gregory Boyer, Juan Faraldos, Xiaoyong Li and Hui Zhao
4:30 – 4:50	High evolutionary rates in *Gymnodinium galatheanum* chloroplast DNA sequences and development of a molecular detection assay **Torstein Tengs**, Holly A. Bowers, Andrew P. Ziman, Diane K. Stoecker and David W. Oldach

4:50 – 5:10	Harmful algal blooms in the United States: estimates of economic impacts and policy responses **Porter Hoagland**, Di Jin, Hauke Kite-Powell and Tracey Morin
5:10 – 5:40	Discussion session (if time allows)
6:00 – 7:00	DINNER, Swope Dining Hall
7:00 – 8:00	INFORMAL SOCIAL HOUR, Swope Upstairs Lobby

December 8, 2000

Lillie Auditorium	**Session VII – ECOHAB – Gulf of Maine** (D.Anderson, D. Townsend, D. McGillicuddy – Session Coordinators) (D. Townsend, Session Chair)
8:30 – 8:45	ECOHAB-GOM: the ecology and oceanography of toxic *Alexandrium* blooms in the Gulf of Maine **Donald M. Anderson**
8:45 – 9:30	Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp., in the Gulf of Maine **David W. Townsend**, Neal R. Pettigrew and Andrew C. Thomas
9:30 – 10:00	*Alexandrium fundyense* blooms in the western Gulf of Maine Donald M. Anderson, **Bruce A. Keafer**, James H. Churchill, Richard P. Signell and Wayne R. Geyer
10:00 – 10:20	BREAK
10:20 – 10:35	Physiological diagnostics and behavior of the toxic dinoflagellate *Alexandrium fundyense,* in Casco Bay, Maine – evidence of nitrogen limitation **Nicole J. Poulton**, J. Geoff MacIntyre, John J. Cullen, and Donald M. Anderson
10:35 – 10:50	Accumulation of PSP toxins in zooplankton assemblages in the Gulf of Maine **Jefferson T. Turner**, Christine L. Powell, David M. Kulis, Bruce A. Keafer, Donald M. Anderson, and Gregory J. Doucette
10:50 – 11:05	An overview of interactions between zooplankton grazers and *Alexandrium* sp., and effects of grazing on bloom dynamics in the near-shore environment of the Gulf of Maine **Gregory J. Teegarden**, Robert G. Campbell, Allan D. Cembella, and Edward G. Durbin

11:05 – 11:20	*Alexandrium* cyst dynamics in the Gulf of Maine **Donald M. Anderson**, Bruce A. Keafer, Patricia A. Matrai, Maureen Keller
11:20 – 11:50	Modeling *Alexandrium* spp. blooms in the Gulf of Maine **Dennis J. McGillicuddy, Jr.** , Richard Signell, Charles Stock, Daniel R. Lynch and Andrew Thompson
11:50 – 12:15	Discussion session (if time allows)
12:15 – 1:30	LUNCH BREAK, Swope Dining Hall
1:30 – 5:00 p.m.	**Technical Workshops/Demonstrations**
6:00 – 7:30 p.m. -	DINNER, Swope Dining Hall
7:30 -	POSTER SESSION, Swope upstairs & Meigs Room

Saturday, December 9, 2000

Lillie Auditorium	**Session VIII – Brown Tides** (S. Banahan, C. Schlenk, G. Boyer – Session Coordinators) (S. Banahan, Session Chair)
8:30 – 9:00	An overview of brown tide in the northeast U.S. **Gregory Boyer**
9:00 – 9:20	The impact of bottom-up and top-down processes on the abundance of *Aureococcus anophagefferens* during the 1999-2000 brown tide bloom in Great South Bay, NY, USA **Christopher J. Gobler**
9:20 – 9:40	Causes and prevention of brown tides in the northeastern United States: the importance of trophic links in the plankton and benthos **David A. Caron**, Darcy J. Lonsdale, Rebecca Schaffner, Robert Cerrato and Julie Rose
9:40 – 10:00	Benthic-pelagic coupling and LI brown tide **Michael W. Lomas**, Hugh L. MacIntyre, Jeffrey C. Cornwell and Todd M. Kana
10:00 – 10:20	BREAK
10:20 – 10:40	Growth of *Aureococcus anophagefferens* on complex sources of dissolved organic nitrogen in culture **Gry Mine Berg**, Julie LaRoche, and Dan Repeta

10:40 – 11:00	Amino acid oxidation and peptide hydrolysis in populations seasonally dominated by *Aureococcus anophagefferens* **Margaret R. Mulholland**, Christopher Gobler and Cindy Lee
11:00 – 11:20	Brown tide assessment project in Barnegat Bay, NJ and the presence of viral-like particles in natural populations of *Aureococcus anoohagefferens* **Mary Downes Gastrich**, O.R. Anderson, and Elizabeth M. Cosper
11:20 – 11:40	Role of long-term variation in freshwater input and dissolved organic nitrogen delivery in the initiation and maintenance of the 1985 Narragansett Bay brown tide **David Borkman** and Theodore J. Smayda
11:40 – 12:00	Physical, chemical and biological conditions associated with the Narragansett Bay brown tide **Theodore J. Smayda** and David Borkman
12:00 – 12:20	Discussion session (if time allows)
12:20 – 1:30 p.m. -	LUNCH BREAK, Swope Dining Hall
1:30 – 5:00 p.m.	**Plenary Discussion, Lillie Auditorium**
	Update on U.S. – European Union interactions on HABs (E. Lipiatou)
	Summaries of discussion sessions
	HABs in the U.S. – comparative discussion (common impediments, common mechanisms, identified needs for methods development, etc.)
	Status of National HAB program (congressional activities, future initiatives, workshops, etc.)
5:00 – 6:00 p.m. -	BREAK
6:00 – 7:30 p.m.	LOBSTER DINNER, Swope Dining Hall
7:30 – 11:00 p.m.	RECEPTION, Meigs Room, Swope

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ABSTRACTS OF ORAL PRESENTATIONS

ECOHAB-GOM: THE ECOLOGY AND OCEANOGRAPHY OF TOXIC *ALEXANDRIUM* BLOOMS IN THE GULF OF MAINE

Donald M. Anderson
Woods Hole Oceanographic Institution, Woods Hole MA 02543

A five-year program called ECOHAB-GOM was initiated to address several fundamental issues regarding *Alexandrium* blooms in the Gulf of Maine: 1) the source of the *Alexandrium* cells that appear in the fresh water plumes in the western Maine coastal current (WMCC); 2) *Alexandrium* cell distribution and dynamics in the eastern Maine coastal current (EMCC); and 3) linkages among blooms in the WMCC and the EMCC. Utilizing a combination of numerical modeling, hydrographic, chemical, and biological measurements, moored and drifting current measurements, and satellite imagery, the project will characterize the structure, variability and autoecology of the major *Alexandrium* habitats in the Gulf of Maine.

In the western Gulf, *Alexandrium* blooms and patterns of PSP have been linked to a coastal current or plume of low salinity river outflow (the WMCC). One major project goal is to investigate an area near Casco Bay implicated as the major "source region" for the toxic cells that populate that coastal current. Field surveys will elucidate the biological, chemical, and physical processes that control bloom initiation and development, the delivery of cells from that source region into the WMCC, and the manner in which late-season, localized blooms are retained there to re-seed future blooms with cysts. The second major set of objectives is to characterize the linkage between toxic blooms and the EMCC, to investigate the role of tidal mixing, frontal systems, and upwelling/downwelling in *Alexandrium* dynamics, and to define the linkage between EMCC *Alexandrium* populations and those in both the WMCC (downstream) and the Bay of Fundy (upstream). A series of "process" studies will focus on discrete blooms or patches of cells and quantify such parameters as in situ growth rates and grazing losses of *Alexandrium*, the nutritional physiology, vertical migration behavior and transport of this species, and the partitioning of toxins within the food web. A hierarchy of coupled physical-biological models are being used together with ECOHAB-GOM data for investigation of: 1) detailed structure within each habitat; 2) interconnections among habitats; and 3) the role of the larger Gulf-scale circulation in the long-term maintenance of *Alexandrium* populations in the region. ECOHAB-GOM is thus a combined modeling/observational program, utilizing the most current and innovative technologies in an approach commensurate with the multiple scales and oceanographic complexity of PSP phenomena in the Gulf of Maine. More details on this project, including an update of cruise activities and results can be found at the project web page at: http://crusty.er.usgs.gov/ecohab/

ECOHAB-GOM Principal Investigators and their affiliations are: David W. Townsend (University of Maine), James H. Churchill (Woods Hole Oceanographic Institution), John J. Cullen (Dalhousie University), Gregory J. Doucette (Medical Univ. of South Carolina), Wayne R. Geyer (Woods Hole Oceanographic Institution), John Hurst (State of Maine Dept. of Marine Resources), Maureen D. Keller (Bigelow Laboratory for Ocean Sciences), Theodore C. Loder III (University of New Hampshire), Daniel R. Lynch (Dartmouth College), Jennifer L. Martin (Canadian Dept. of Fisheries and Oceans), Dennis J. McGillicuddy (Woods Hole Oceanographic Institution), Neal R. Pettigrew (University of Maine), Richard P. Signell (U.S. Geological Survey), Andrew C. Thomas (University of Maine), Jefferson T. Turner (University of Massachusetts Dartmouth).

ALEXANDRIUM FUNDYENSE BLOOMS IN THE WESTERN GULF OF MAINE

Donald M. Anderson[1], Bruce A. Keafer[1], James H. Churchill[1], Wayne R. Geyer[1] and Richard P. Signell[2]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]US Geological Survey, Woods Hole, MA 02543

The first outbreaks of PSP in the Gulf of Maine are reported nearly every year along the Western Maine coastline, especially in the Casco Bay region. Previous research has shown that this region is strongly influenced by the Kennebec River, one of the major freshwater inputs into the western Gulf, and that surface populations of *Alexandrium fundyense* are generally associated with the river plume. An "initiation zone" has also been hypothesized in the Casco Bay area, though the specific distribution and source of those cells are not known. During the spring of 1998 and 2000, weekly hydrographic surveys in Casco Bay and the adjacent Kennebec River plume area collected *A. fundyense* samples at various depths to 20m to observe physical water properties associated with the blooms. Hydrographic moorings with current meters were deployed at several sites along with mussel bags hung near the surface. Further information on the current field was obtained from satellite-tracked drifters.

Prior to detection of shellfish toxicity (i.e., late April), low abundances (<100 cells liter^{-1}) of *A. fundyense* cells were observed within the inshore waters of Casco Bay as well as in offshore waters beyond the influence of the Kennebec River plume. During the initial onset of toxicity, cell abundances increased to ca. 200-300 cells liter^{-1} with populations found at both inshore and offshore stations. In 1998, the toxicity was first detected at an offshore mooring suggestive of an offshore origin of the bloom. However, in 2000, toxicity was first reported inshore. These observations are consistent with the view that there are two populations of *A. fundyense*; one located in Casco Bay and another in the adjacent offshore waters and to the east (i.e., "upstream").

During both years, the rapid rise in shellfish toxicity in mid-May was recorded at the inshore monitoring stations and at the mussel moorings following downwelling favorable conditions. Surface concentrations of toxic cells ranged from <100 to ca. 1000 cells liter^{-1} with no evidence that the higher concentrations were associated with any particular water mass. Within the plume waters, a 24 hour study indicated that the toxic cells predominantly occupied the stratified, surface waters (<10m) even during the night, but subsurface populations were occasionally observed below the pycnocline that were linked to advection from the "upstream" waters. Higher abundances of cells were sometimes found near a frontal boundary where the offshore waters converge with the Kennebec and Penobscot river plumes. Evidence from Acoustic Doppler Current Profiler (ADCP) and drifters suggested that toxic cells found near the convergence were transported in a coastal "jet" which drives the cells rapidly alongshore with the Western Maine Coastal Current.

These results indicate a wide distribution of *A. fundyense* in this highly dynamic region. Cells are present at approximately the same times in both inshore waters, offshore waters, and at convergences associated with river plumes. Therefore, it is not yet possible to determine whether there is an inshore "source", offshore "source", or multiple "sources" for *A. fundyense* cells in the western portion of the Gulf of Maine, versus a more broadly dispersed initial population. The development of the offshore bloom appears dependant on complex physical-biological processes (accumulation vs. growth) associated with the different water masses. Meanwhile, inshore populations within Casco Bay are generally retained in a slower flow leading to the higher toxicities observed in the bay. The Casco Bay populations may derive from the offshore populations, delivered to the bay with downwelling winds, or they could arise independently from cyst populations in the shallow nearshore waters. In either case, there is still an identifiable "initiation zone" in the general region where the relative abundance of *A. fundyense* and toxicity is consistently higher earlier in the year compared to any area along the Gulf of

Maine coastline. The study is ongoing with another field year remaining to elucidate the dominant processes responsible for initiation and development of *A. fundyense* blooms in the region.

ALEXANDRIUM CYST DYNAMICS IN THE GULF OF MAINE

Donald M. Anderson[1], Bruce A. Keafer[1], Patricia A. Matrai[2], Maureen Keller[2*]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME 04575

Alexandrium fundyense, the dinoflagellate responsible for Paralytic Shellfish Poisoning (PSP) in the Gulf of Maine (GOM), has a dormant cyst stage in its life history that plays a critical role in bloom dynamics. Given the highly seasonal nature of PSP outbreaks in the region, the cold winters, and the temperature requirements for *A. fundyense* growth (5-21 ˚C), blooms of this species presumably begin from germinated cysts. Surveys of cyst abundance in GOM sediments document high cyst concentrations in deeper, offshore basins, grading to much lower concentrations in shallow waters. It was hypothesized that the most significant input of *A. fundyense* cells would be from cysts in shallow waters that would be exposed to high light levels and more rapid temperature increases than the cysts in the darker and colder offshore waters, even though cyst concentrations are 1-2 orders of magnitude higher offshore. During the ECOHAB-GOM project, several parallel efforts were made to estimate the magnitude and timing of cell input via cyst germination. These efforts, and their present status, are summarized as follows:

1) Cyst germination traps: Several benthic chambers designed to capture germinated cells were collected. Thus far, none of these have provided reliable data.

2) Cyst fluorescence: Surface sediments were collected at a number of stations and *A. fundyense* cysts examined for signs of chlorophyll fluorescence, thought to be a sign of impending germination. Data thus far show no meaningful patterns or trends.

3) Modeled cyst germination: Laboratory experiments were conducted to characterize *A. fundyense* cyst germination rates as functions of temperature, light and time of year (the latter reflecting control of germination by an internal clock). In conjunction with a large-scale cyst map of the region, these data are being used in model simulations driven by environmental and hydrographic conditions observed and modeled in 1993. Results thus far provide a cell inoculum to the water column that varies temporally and spatially. In particular, it appears that the deep-water cyst seedbed dominates the inoculum process, but that seedbed is not in the proper location to account for observed blooms in the Casco Bay area. An eastward extension of this seedbed, lying outside the modeled domain could be the source of *A. fundyense* cells to the Casco Bay region. Model simulations have suggested a mechanism whereby upwelling-favorable winds move nearshore waters over the deep-water cyst seedbeds, capturing germinated cells that are then carried to shore when winds shift to downwelling-favorable. Details of this modeling effort will be provided by McGillicuddy et al. in a separate presentation. Armstrong et al. and Thompson et al. present posters with details of similar cyst germination rate experiments for eastern Gulf of Maine populations which will allow an extension of the modeling effort.

These observations on cyst distribution and dynamics will be discussed in the context of observed *Alexandrium* motile cell distribution in 1998 and 2000.

*Portions of this work were started under the leadership of the late Dr. Maureen D. Keller.

GROWTH OF AUREOCOCCUS ANOPHAGEFFERENS ON COMPLEX SOURCES OF DISSOLVED ORGANIC NITROGEN IN CULTURE

Gry Mine Berg[1], Julie LaRoche[1], Dan Repeta[2]
[1]Institut für Meereskunde, Kiel University, 24105 Kiel, Germany
[2]Woods Hole Oceanographic Institution, Woods Hole, MA 02540, USA

Aureococcus anophagefferens repeatedly blooms in several Long Island (New York, USA) embayments, forming "brown tides" that discolor the water. Surveys of the northeast coast of the USA have shown that *A. anophagefferens* exits in several places that have no records of brown tide. Therefore, the recurrence of the brown tide in Long Island is somewhat unusual. The coastal bays in Long Island are strongly influenced by groundwater, contributing the largest input of fixed nitrogen. In years of draught and low groundwater flow, the supply of NO_3^- is sharply reduced leaving dissolved organic nitrogen (DON) as the largest source of nitrogen available to the phytoplankton. The ability of *A. anophagefferens* to grow on DON has been hypothesized to be an important factor in sustaining the brown tide during periods of dissolved inorganic nitrogen depletion. In order to test this hypothesis we prepared an axenic culture of *A. anophagefferens* and followed growth with a number of DON substrates as the sole source of nitrogen in culture. In addition to commercially available substrates we used >1 kDa ultrafiltered DON isolated from West Neck Bay (WNB) pore waters, Long Island. Efforts to characterize components of the bulk DON pool were conducted in parallel with investigations of the bioavailability of these components.

For the preparation of an axenic, artificial seawater culture of strain CCMP 1784 we modified a protocol published by Cottrell and Suttle (1995 J. Phycol. 29: 385-387). Exponentially growing cultures in F/2 media were exposed sequentially to Penicillin G, Neomycin, Streptomycin, and Penicillin G. Of the antibiotics tested, Penicillin G was the most effective in eliminating bacterial contaminants. Bacterial strains isolated from the culture medium were identified through amplification of bacterial 16S rRNA gene sequences using PCR. Two bacterial strains isolated from the culture media, belonging to the Gamma Proteobacteria and to the Cytophaga-Flavobacteria, were of marine origin.

A. anophagefferens showed good growth on > IkDa WNB DON. To date, this is the first study demonstrating that an autotrophic phytoplankton can grow on bulk DON as the sole source of nitrogen, suggesting that autrotrophs have the capability to enzymatically degrade complex DON. Future research will investigate enzyme pathways involved with DON degradation, and on interactions between *A. anophagefferens* and heterotrophic bacteria.

INTENSIVE MONITORING FOR *PFIESTERIA* AND RELATED HAB EVENTS

B. Boicourt[1], L. Codispoti[1], M. Roman[1], V. Holliday[2], H. MacIntyre[1], P. Glibert[1], R. Magnien[3], and B. Michael[3]
[1]Horn Point Laboratory, University of Maryland Center for Environmental Science, PO Box 775, Cambridge, MD 21613
[2] BAE SYSTEMS, Integrated Defense Solutions, 6500 Tracor Lane, Austin, TX 78725
[3]Maryland Department of Natural Resources, Resource Assessment Service, 580 Taylor Ave., D2, Annapolis, MD 21401

Intensive monitoring efforts, a collaboration between the University of Maryland Center for Environmental Science, Maryland Department of Natural Resources, and NOAA, are ongoing for determining environmental conditions leading to the outbreaks of *Pfiesteria* and related HAB species. Our intent is to develop an adaptable, autonomous system measuring a key suite of variables and reporting in real time. Initial efforts have been brute-force, deploying a wide range of new and conventional sensors from both fixed and floating platforms, in an attempt to assemble a tractable and sensitive detection system. In addition to monitoring meteorological and physical variables, sensors are deployed to monitor chlorophyll, zooplankton, optics, oxygen, nutrients and presence of fish. Acoustic telemetry is employed for underwater communications, and spread-spectrum radio relays the data to shore stations where the data are placed on the internet. A modified Chesapeake Bay Observing System (CBOS) buoy and shallow water platforms were employed to mount the various sensors in the lower Pocomoke River, a tributary of Chesapeake Bay, during late summer/early fall 2000.

Nutrient enrichment has been shown to stimulate *Pfiesteria*, but the relationships between nutrient loading and *Pfiesteria* growth are not clear. For nutrient monitoring, WS Ocean System nutrient monitors are employed to autonomously determine nitrate+nitrite and phosphate concentrations approximately every two hours. During instrument deployments, discrete samples are collected at the same location and time every few days for calibration of nutrients and water column chemistry. We are working on developing a system that can autonomously collect ammonium data during future deployments.

A suite of bio-optical instruments (fluorometer, turbidimeter and AC-9) are used for detecting variations on the optical characteristics of the water. Absorption spectra are resolved into the contribution of CDOM, detritus, and plant pigments at 8 wavelengths, using empirically-derived relationships based on fluorescence and back-scatter. The approach may permit the development of species-specific spectral signatures, depending on the robustness of the algorithms for correcting the very high concentrations of CDOM and suspended sediment at the site. Although *Pfiesteria* cannot be detected with this approach directly, various spectral signatures may be correlated with its presence.

Two biological components which have the potential to affect *Pfiesteria* are zooplankton and fish. Zooplankton consume *Pfiesteria* and thus are a biological control of their abundance and potential to bloom. High concentrations of fish can potentially trigger the metamorphosis of toxic stages of *Pfiesteria*. An in situ acoustic sensor capable of monitoring zooplankton and fish is being used to detect zooplankton and fish. The instrument is moored on the sediment surface, and includes a side-looking low-frequency acoustic transducer for detection of fish schools, and a high-frequency sensor aimed at the surface for monitoring the zooplankton in the water column above the mooring.

ROLE OF LONG-TERM VARIATION IN FRESHWATER INPUT AND DISSOLVED ORGANIC NITROGEN DELIVERY IN THE INITIATION AND MAINTENANCE OF THE 1985 NARRAGANSETT BAY BROWN TIDE

David Borkman and Theodore J. Smayda
Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881

The 1985 brown tide bloom of *Aureococcus anophagefferens* in Narragansett Bay was the dominant HAB event in a nearly 40-year time series of weekly observations of Narragansett Bay phytoplankton. Mechanisms responsible for this summer-long bloom, which occurred simultaneously in several estuaries along the Northeastern US coast, are not fully known. Several features of *A. anopha*gefferens physiology and ecology indicate that freshwater input patterns with accompanying patterns in delivery of organic nutrients may play an important role in bloom initiation and maintenance. We present a time-series of estimated dissolved organic nitrogen (DON) loading and related physical data for Narragansett Bay, Rhode Island. Trends in Narragansett Bay riverine and groundwater DON input are analyzed, indicating a relative peak in riverine DON concentration accompanied by a decrease in the riverine nitrate:DON ratio in 1985. Levels of *A. anophagefferens* that may have been supported by this DON delivery are estimated and compared to observed abundance in Narragansett Bay. The spring of 1985 was marked by a departure from the usual relation between groundwater levels and salinity in Narragansett Bay, indicative of a change in Narragansett Bay estuarine circulation patterns. Changes in freshwater delivery into Narragansett Bay in 1985 and accompanying relative increases in DON delivery are implicated in the initiation and maintenance of the 1985 brown tide.

AN OVERVIEW OF BROWN TIDE IN THE NORTHEAST U.S.

Gregory L. Boyer
College of Environmental Science and Forestry, SUNY Syracuse, NY 13210

Brown tide blooms are regional, episodic phenomena. The first major occurrences of brown tide (*Aureococcus anophagefferens*) were reported in 1985 in the eastern and southern bays of Long Island (NY), Narragansett Bay (RI), and Barnegat Bay (NJ). Since then blooms of varying severity and duration continue to occur in Long Island waters and Barnegat Bay, and as of summer 1998, brown tide cells were reported in the eastern bays of Maryland and Delaware.

Brown tide blooms can have had serious impacts on shellfish fisheries. The massive bloom of 1985 resulted in the recruitment failure of scallops in the Peconic Bay (Long Island) system. While there have been some modest harvests since that failure, bay scallop populations have not recovered to their pre-1985 levels. In recognition of a need to focus more expertise into understanding this phenomenon, a research program to understand the causes of these blooms was developed. The Brown Tide Research Initiative (BTRI) began in 1996 with two objectives: 1) to isolate, develop, and maintain axenic cultures of *Aureococcus*, and 2) identify the environmental factors that contribute to the initiation, duration, and cessation of brown tide blooms.

Multiple isolates of *Aureococcus* have been established and are maintained in culture at CCMP, however problems with maintaining axenic cultures persist. To address the broader 2nd objective, investigators have been evaluating the relative importance of factors such as DIN, DON, dissolved iron, groundwater loading, and light in *Aureococcus* growth physiology. The environmental and ecological factors examined are water column stability and residence times, changes in the species composition of the microbial plankton community, microbial and bivalve grazing, and elucidating bio-geochemical processes at the sediment-water interface.

Initial assumptions were that *Aureococcus* blooms were, in part, the result of unique growth characteristics. However, research to date suggests that *Aureococcus* shares similar growth characteristics with other picoplankton. Blooms are more likely the result of a combination of ecological and environmental factors.

EVIDENCE FOR ADAPTATION TO TOXINS IN POPULATIONS OF THE SOFTSHELL CLAM, *MYA ARENARIA,* SUBJECTED TO RECURRENT TOXIC BLOOMS

V. Monica Bricelj[1], Scott P. MacQuarrie[1], Betty M. Twarog[2], and Vera L. Trainer[3]
[1]Inst. for Marine Biosciences, National Research Council, 1411 Oxford St., NS B3H 3Z1, Canada
[2]Darling Marine Center, University of Maine, Walpole, ME 04573
[3]Northwest Fisheries Science Center, Seattle, WA 98112-2097

Our ECOHAB study demonstrates that softshell clam, *Mya arenaria*, populations differ markedly in their sensitivity to PSP toxins depending on their history of exposure to paralytic shellfish poisoning (PSP) episodes in Atlantic coastal waters. Significant differences were found in burrowing capacity, feeding (clearance) rates, nerve resistance to saxitoxin (STX), metabolic rates, toxin accumulation and survival of juvenile clams during laboratory exposure to a highly toxic *Alexandrium tamarense* isolate. Marked phenotypic differences in these parameters were also documented among individual clams within a population. Sensitive clams predominate in populations not previously exposed to PSP toxins, whereas resistant phenotypes prevail in historically exposed populations. Toxicity of viscera could vary up to 52x among contrasting phenotypes exposed to common conditions of toxification. Our results strongly support the hypothesis that softshell clam populations recurrently exposed to toxic blooms undergo genetic or epigenetic adaptation to PSP toxins, via selective mortality or reduced fitness of sensitive individuals.

Repeated testing of the same individuals showed that clams with no history of exposure to PSP experienced significant (up to 4-fold) reduction in metabolic rate (rate of oxygen consumption, VO_2) during laboratory exposure to bloom levels of toxic *A. tamarense* cells, relative to controls exposed to non-toxic algae. In contrast, clams from a population recurrently affected by PSP showed only transient reduction of VO_2 after 9 days of toxification, and recovery of VO_2 to normal levels at 18 d of toxification. Laboratory exposure to PSP toxins also induced significant clam mortalities relative to non-toxic controls, starting within 7-11 days of toxification. Mortality rates were population-specific (higher in an unexposed population than in one historically affected by PSP), and phenotype-specific. Thus sensitive individuals within each population (characterized by loss of burrowing capacity during toxification) consistently suffered higher mortalities than resistant individuals. Considerable unexplained variation was observed, however, in cumulative mortality rates among three independent experiments. Neither starvation nor impaired ability to maintain a normal position in sediment due to paralysis, were responsible for mortalities in sensitive clams. However, our observations suggest that O_2 exchange is severely limited in sensitive clams exposed to toxins, and could eventually cause hypoxia/anoxia within the pallial cavity and in tissues, a potential cause of toxin-induced mortalities.

In vitro exposure of isolated cerebro-visceral nerve trunks to increasing concentrations of STX showed marked differences in sensitivity of individual clams to PSP toxin, both among and within clam test populations. Toxin concentrations required to block the nerve action potential ranged from $5x10^{-6}$ to $2x10^{-4}$ g STX ml^{-1}, with most clams from the historically exposed population at least 10-fold more resistant than the unexposed. Inter-population differences in nerve response were detectable prior to laboratory toxin exposure, and were thus not induced by toxin accumulation in tissues, but rather an intrinsic property of individual nerves. Gene sequencing of the sodium channel of resistant and sensitive clams has begun (L. Connell et al. poster, this conference) as part of future studies designed to elucidate the mechanism responsible for differences in toxin sensitivity, and thus the capacity for toxin accumulation, at the molecular level.

FUNCTIONAL TYPE (TOXICITY STATUS) CONTROLS *PFIESTERIA* RESPONSE TO NUTRIENTS AND ALGAL VERSUS FISH PREY

J. Burkholder,[1] H. Glasgow,[1] P. Glibert,[2] A. Lewitus,[3] M. Parrow,[1] C. Zheng,[1] P. Cancellieri[1] and N. Deamer-Melia[1]
[1]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606
[2]Horn Point Environmental Laboratory, University of Maryland, Cambridge, MD 21613
[3]Belle Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442

Many "toxic algae" (here, including heterotrophic dinoflagellates and blue-green algae or cyanobacteria, as well as photosynthetic taxa such as toxic *Alexandrium* spp.) have naturally occurring toxic as well as apparently benign (non-inducible) strains with undetectable toxin production. About 60% of 470 *Pfiesteria* clones isolated by JB/HG over the past decade from estuaries known for toxic *Pfiesteria* outbreaks have tested as ichthyotoxic. *Pfiesteria* zoospores may be 1of 3 functional types as actively toxic (TOX-A, in fish-killing mode), temporarily nontoxic (TOX-B, capable of toxin production but without recent access to live fish), or non-inducible (NON-IND, apparently unable to cause fish stress, disease, and death). As a separate phenomenon and an apparent artifact of culture conditions, toxic *Pfiesteria* strains (like many other toxic algae) gradually lose toxin-producing capability in culture. About 98% of all cultured toxic *Pfiesteria* strains have become NON-IND after 6-8 months even when maintained with live fish, and after a shorter duration when maintained with other prey).

We tested the hypothesis that the three functional types of *Pfiesteria* differ significantly in response to N and P enrichments, algal prey, fish prey, and other factors. The cultures of *Pfiesteria piscicida* and *P. shumwayae* used in this research were isolated from the Neuse Estuary. One isolate each of each species was confirmed by JB/HG as toxic to fish (toxicity cross-confirmed by H. Marshall, ODU; uni-dinoflagellate clonal quality – Heteroduplex mobility assay, D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by PCR probes from HG/JB and FISH probes of P. Rublee, UNC-G). A second isolate of each species was cloned and tested as NON-IND, with cross-confirmations as above. TOX-A *Pfiesteria* spp. were grown with live fish for 3-4 months prior to the experiments; TOX-B cultures were grown with live fish for 2-3 months, then were switched to a diet of cryptomonad prey for 3 weeks; and NON-IND cultures were similarly grown with cryptomonad prey. In the 1st experimental series, we tested *Pfiesteria* zoospore production in response to N_i or P_i enrichment + cryptomonad prey (500 µg NO_3^- or PO_4^{-3}/L). A 2nd experiment tested *Pfiesteria* response to crypto-monad prey (batch mode, 6 d, n=4). In a 3rd experiment, we examined *Pfiesteria* response to fresh fish mucus (minutes, microcapillary tube assay).

NON-IND *Pfiesteria* attained highest zoospore production on both N and P enrichments, and on cryptomonad algal prey. This functional type retained kleptochloroplasts for longer periods and exerted the lowest grazing pressure on algal prey, suggesting increased reliance on photosynthesis in a more 'plant-like' mode. TOX-B *Pfiesteria* was intermediate in stimulation by N and P, kleptochloroplast retention, and cell production with cryptomonad prey. TOX-A zoospores had lowest cell production on N and P enrichment, and on algal prey; and their kleptochloroplast retention was negligible. Although this functional type had the highest algal grazing rates and most tightly coupled Lotka-Volterra fluctuations with cryptomonad prey, the consumed prey supported negligible zoospore production relative to that of unfed controls. Trials with fresh fish mucus indicated strongest attraction by TOX-A *Pfiesteria*, with intermediate to low attraction by TOX-B zoospores and negligible attraction by NON-IND *Pfiesteria.* These data demonstrate that functional types of *Pfiesteria* spp. are strikingly different in response to nutrient enrichment, algal prey, and fish prey. The data raise a 'red flag' in warning against use of NON-IND strains in research to gain insights about environmental controls on toxic *Pfiesteria.*

NUTRIENT ENRICHMENT AND THE TOXIC *PFIESTERIA* COMPLEX: COMPARATIVE STIMULATION BY SWINE EFFLUENT, POULTRY MANURE LEACHATE, HUMAN SEWAGE, AND OTHER SOURCES

J. Burkholder, C. Zheng, H. Glasgow, N. Deamer-Melia and M. Parrow
Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

Toxic *Pfiesteria* outbreaks have been documented in poorly flushed eutrophic estuaries impacted by anthropogenic nutrient loading from poorly treated animal wastes, human sewage, and other sources. A frequently asked question in efforts to develop management strategies to reduce *Pfiesteria* activity is the relative importance of various nutrient forms and sources in stimulating *Pfiesteria* populations. Here we report a series of short-term (5-day), semi-continuous experiments designed to separately test the response of *Pfiesteria piscicida* and *P. shumwayae* (sp. nov.) zoospores to N versus P enrichment; and to nutrient sources including swine effluent, poultry wastes, and human sewage.

Pfiesteria piscicida and *P. shumwayae* were isolated from the Neuse Estuary, cloned, and confirmed as toxic to fish (uni-dinoflagellate clones confirmed by the Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by molecular probes from our lab. and the FISH probe of P. Rublee, UNC-G; toxicity cross-confirmed by H. Marshall, ODU). To eliminate the confounding influence of nutrient-rich fish excreta, we tested each *Pfiesteria* species in the absence of fish (TOX-B functional type), with/without nutrient-deplete cryptomonads (N,P-limited; cloned from multi-species material of the CCMP listed as *Rhodomonas* 757) as a prey source. Swine effluent was collected from a depth 0.5 m in a lagoon at a swine operation near NCSU; poultry waste leachate was collected from a waste pit at an NCSU research facility; and raw human sewage was supplied from the Raleigh municipal WWTP. These nutrient sources were sterile-filtered and then diluted in series (1:10, 1:50, 1:100, 1:1000) using 15-psu filtered Instant Ocean water. The nutrient content (N,P,C – organic, inorganic forms) of each source was characterized. Controls consisted of cryptomonad prey, *P. piscicida*, *P. shumwayae*. Treatments included [cryptomonads + nutrient source]; [each *Pfiesteria* species + nutrient source]; and [each *Pfiesteria* species + nutrient source + cryptomonads]. To gain additional insights about the stimulatory effects of nutrients, a similar experimental design was used to separately test effects of enrichment with P versus N on zoospore production of each *Pfiesteria* spp.

We documented significantly higher *Pfiesteria* zoospore abundance in all treatments with each nutrient source, relative to abundance in controls without nutrient additions. Cell production increased with increasing nutrient source concentration except at the lowest dilution (10:1) where there was a longer lag effect before zoospore production increased. *P. piscicida* showed higher stimulation by P than by N enrichment, and maximal cell production with swine wastes. In contrast, *P. shumwayae* cell production was higher with N than with P enrichments, and maximal with poultry wastes. Using fluorescent markers for each species (*P. piscicida* – Alexa Fluor 488; *P. shumwayae* – Alexa Flour 350), we are continuing this effort by examining the comparative response of *P. piscicida* and *P. shumwayae* to nutrient sources in mixed-species trials. These data have provided insights to explain observations about *Pfiesteria* abundance and toxic activity over the past decade in estuaries draining urbanized versus agricultural watersheds.

THE EFFECT OF NITROGEN SOURCE ON THE GROWTH AND TOXICITY OF SPECIES OF THE GENUS *PROROCENTRUM*

Cary L. Burns[1] and Jonathan R. Pennock[2]
[1]University of South Alabama and Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, Alabama 36528
[2]Dauphin Island Sea Lab and The University of Alabama, 101 Bienville Boulevard, Dauphin Island, Alabama 36528

The environmental factors that select for growth of harmful algal species is not well known, but some studies suggest that utilization of organic nutrients may be a way that harmful algae can out compete faster growing species. This study investigated the effect of nitrogen source on the growth and toxin production of four potentially harmful dinoflagellates of the genus *Prorocentrum*: two planktonic species, *P. minimum and P.* cf. *scutellum*, one semi-planktoninc/epibenthic species, *P. mexicanum* and one benthic species, *P. hoffimannianum*.

Experiments were carried out in triplicate batch cultures in modified L1-Si media (Guillard & Hargraves 1993) using five different sources of nitrogen (N) at an initial concentration of 50 µM. Nitrogen was delivered as: (1) nitrate, (2) ammonium, (3) urea, (4) l-glutamic acid, and (5) high molecular weight dissolved organic nitrogen (DON, concentrated from Mobile Bay waters using tangential flow ultra-filtration). Growth rates were monitored as cell numbers and *in vivo* fluorescence over periods of 10–28 days, depending on the growth rates of the individual species.

Results indicate that none of these species were capable of growing on the natural DON as a sole source of nitrogen. Although there were slight variations in some replicates, all species achieved equal growth on nitrate, ammonium and urea; growth on l-glutamic acid was generally lower. All species were also tested for okadaic acid (OA) production. *Prorocentrum hoffmannianum* was the only species to show significant OA production. OA production in this species was found to vary with nitrogen source and with growth stage/rate. Preliminary results (to be completed by the Symposium) suggest that OA production by *P. hoffnannianum* is greater in late log and stationary phase when grown on inorganic nutrients, and greater in log phase when grown on organic nutrients as the sole N source.

CAUSES AND PREVENTION OF BROWN TIDES IN THE NORTHEASTERN UNITED STATES: THE IMPORTANCE OF TROPHIC LINKS IN THE PLANKTON AND BENTHOS

David A. Caron[1], Darcy J. Lonsdale[2], Rebecca Schaffner[1], Robert Cerrato[2] & Julie Rose[1]
[1]Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway AHF 301, Los Angeles, CA 90089-0371 (dcaron@usc.edu)
[2]Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, NY 11794-5000

Numerous factors have been implicated in the outbreak of harmful algal blooms of the pelagophyte *Aureococcus anophagefferens*, including specific meteorological, chemical, physical and biological conditions. Few of these factors have been examined experimentally using natural assemblages. Our group has been performing studies in 300 liter mesocosms in an effort to test specific factors that might be involved in the initiation of brown tides, and to identify means of preventing or mitigating these events. We have devised an experimental system in which we have repeatedly induced brown tides, a situation which has allowed us to investigate some of the parameters that have been proposed as factors promoting (and preventing) HABs by *A. anophagefferens*. Our work in prior years demonstrated that additions of specific inorganic (NO_3^-, NH_4^-, PO_4^{-3}) and organic (urea) nutrients, or micronutrients (Fe), were not sufficient to stimulate significant net population growth of the alga, although other phytoplankton species were definitely stimulated. In contrast, physical disturbance to the microbial food web (via submersible pumps) resulted in increases in the absolute and relative abundances of *A. anophagefferens.* Experiments carried out this past summer were aimed (in part) at determining whether or not selective grazing by microbial consumers could explain the success of *A. anophagefferens* in natural, mixed phytoplankton assemblages. The brown tide alga in these experiments reached maximal abundances of >300,000 cells ml^{-1}. Dilution experiments were performed to examine grazing on *A. anophagefferens* (via an antibody assay) and on the total phytoplankton assemblage (via chlorophyll analysis). Interestingly, the results of these studies indicated that rates of mortality for *A. anophagefferens* were generally similar to rates for the whole phytoplankton assemblage. That is, we could not demonstrate that the rejection of the brown tide cells by microbial consumers was a major factor explaining increases in its population abundance. We conclude that both growth stimulation (studies in 2000) and reduced predation (previous work) remain viable explanations for blooms of *A. anophagefferens.* Alternatively, some factor(s) unrelated to grazing that were induced by physical agitation (e.g. altered nutrient availability resulting from the action of the submersible pumps) may explain our results of previous years.

In addition to demonstrating factors involved with bloom initiation, we have repeatedly demonstrated that the presence of hard clams, *Mercenaria mercenaria*, has a dramatic effect on the absolute and relative abundance of the brown tide alga within natural phytoplankton assemblages. Population growth of *A. anophagefferens* in the presence of clams was dramatically constrained under conditions that otherwise resulted in high abundances of the alga. In addition, the presence of hard clams prevented a shift in the phytoplankton assemblage to dominance by brown tide cells. An overview of our experimental results to date will be provided.

ASSESSING TEMPORAL AND SPATIAL VARIABILITY IN *PFIESTERIA PISCICIDA* DISTRIBUTIONS USING MOLECULAR PROBING TECHNIQUES

Kathryn J. Coyne, David A. Hutchins, Clinton E. Hare and S. Craig Cary
University of Delaware College of Marine Studies, 700 Pilottown Rd., Lewes, DE 19958

The toxic dinoflagellate, *Pfiesteria piscicida*, poses a significant threat to natural resources and to public health. Although often linked to increases in pollution and nutrient loading, the environmental factors that contribute to toxic *Pfiesteria* blooms are unclear. An understanding of these relationships is critical to the development of monitoring strategies for high risk areas and requires that the presence of *P. piscicida* be rapidly and accurately assessed. Routine monitoring by light microscopy lacks both the sensitivity and accuracy required for species-specific detection of low levels of *Pfiesteria*. In this study, we used molecular probes to identify and enumerate *Pfiesteria piscicida* and several closely related members of the *Pfiesteria* species complex in the Delaware Inland Bays and the Pocomoke River, Maryland. Low levels of *Pfiesteria* were detected in water and sediment samples confirming the presence of *Pfiesteria* as a minor but prevalent member of the phytoplankton community in mid-Atlantic estuaries. We also describe a novel technique, Polymerase Chain Reaction-Fluorescent Fragment Detection (PCR-FFD), for specific and quantitative detection of low levels of *Pfiesteria* in environmental samples. Using PCR-FFD, we conducted a diel study of *Pfiesteria* in the Broadkill River, Delaware. Preliminary data generated by this study indicates a possible tidal influence on the presence and concentration of *Pfiesteria* as well as possible correlations to biological variables and phosphate.

***GYMNODINIUM BREVE* RED TIDES IN THE GULF OF MEXICO: ANALYSIS OF BREVETOXINS AND METABOLITES IN SHELLFISH**

R.W. Dickey and S.M. Plakas
FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528

Red tides of the marine dinoflagellate *Gymnodinium breve* occur regularly in the lower latitudes of the Gulf of Mexico (southwest Florida and south Texas) and infrequently in northern Gulf waters. *G. breve* produces lipophilic neurotoxins (brevetoxins) which are responsible for marine animal mortalities, non-fatal human health effects, and localized economic recession. Human health effects include respiratory distress from exposure to seawater aerosols containing brevetoxins, and food poisoning (neurotoxic shellfish poisoning, NSP) from consumption of brevetoxin-contaminated shellfish. Analyses of shellfish from red tide events and from experimental exposures show that brevetoxins are readily accumulated and metabolized by shellfish. The metabolites identified by liquid chromatography/mass spectrometry (LC/MS) include diastereomers of cysteine-conjugated brevetoxin-3, their oxidation products and a thio-glycerol adduct. These molluscan metabolites of brevetoxins retain toxicity to mammalian test systems and are slowly eliminated from shellfish tissues in situ. The only officially recognized method for NSP shellfish analysis, mouse bioassay, underestimates the toxicity of brevetoxin-contaminated shellfish because the toxin metabolites are not recovered using the official protocol. More efficient extraction techniques and in vitro or instrumental methods (e.g. cytotoxicity, sodium channel competitive binding, radioimmunoassay, LC/MS) show ppb level sensitivity for the brevetoxins and their metabolites. These methods are currently used for a more complete characterization of brevetoxin metabolism in shellfish, and will be further refined for application in NSP HAB events.

PSEUDO-NITZSCHIA SPP IN THE NORTHERN GULF OF MEXICO: OVERVIEW AND RESPONSE TO INCREASING EUTROPHICATION

Q. Dortch[1], M. L. Parsons[2], G. J. Doucette[3], G. A. Fryxell[4], A. Maier[1], A. Thessen[1] and C. L. Powell[3]
[1]Louisiana Universities Marine Consortium, Chauvin, LA 70344
[2]Natural Sciences Division, Marine Science Department, University of Hawaii-Hilo, 200 W. Kawili Street, Hilo, HI 96720
[3]Marine Biotoxins Program, Center for Coastal Environmental Health & Biomolecular Research, National Ocean Service, 219 Fort Johnson Road, Charleston, SC 29412
[4]Integrative Biology A6700, University of Texas, Austin, TX 78712

Pseudo-nitzschia spp. are extremely abundant on the Louisiana shelf in the extended plume of the Mississippi River. Abundances often exceed 10^6 cells/liter and were sometimes greater than10^7 cells/liter, especially in the spring when river flow is high. In fact, it is frequently the most abundant diatom. *Pseudo-nitzschia* spp. do not occur as often or at such high abundances in lower salinity estuaries, but peak abundances can still exceed 10^6 cells/liter. *Pseudo-nitzschia* spp. have been observed at salinities from 0.5-36 ppt and growth can be sustained in cultures of some clones down to 6 ppt.

At least 6 species have been observed in this region, including *P. pseudodelicatissima*, *P. delicatissima*, *P. multiseries*, *P. pungens* (2 varieties), *P. cf. americana*, and *P subfraudulenta*, and there may be one or more unknown species. Five of the species observed in Louisiana waters have been shown to produce domoic acid (DA) in field samples or laboratory cultures elsewhere. Clones of *P. pseudodelicatissima,* but not clones of *P. delicatissima,* isolated from the Louisiana shelf, produce DA. In this area DA has been measured in plankton samples from the field, including samples taken directly over oyster beds. Despite the presence of abundant and toxic *Pseudo-nitzschia*, there have been no known incidents of Domoic Acid Poisoning (DAP) reported from the northern Gulf of Mexico.

Nonetheless, several lines of evidence provide strong proof that *Pseudo-nitzschia* spp. are stimulated by high nutrient inputs, thereby increasing the potential for DAP incidents in areas where eutrophication is increasing.

1. *Pseudo-nitzschia* spp. are more abundant in the spring when Mississippi River flow and nutrient inputs are highest and in the areas directly influenced by the Mississippi River. Univariate and multivariate statistical analyses of species, nutrient availability, and other environmental conditions show that individual *Pseudo-nitzschia* species are stimulated by different conditions.
2. There has been a large documented increase in nutrient inputs from the Mississippi River since the 1950's. Historical phytoplankton data (1950's, 1970's, 1990's) show a large increase in *Pseudo-nitzschia* abundance over that time. Further, the abundance of *Pseudo-nitzschia* in sediment cores taken from the Louisiana shelf has increased up core even more rapidly than other indicators of eutrophication.
3. Nutrient additions were made to microcosms (N, P, or Si and all combinations) of natural populations taken from the shelf at different seasons. In those microcosms where a response was observed, *Pseudo-nitzschia* spp. were either the only species to respond by rapid growth within 12-24 hours of nutrient addition (2 out of five microcosm experiments) or one of several species responding (2 out of five microcosms). The nutrient or nutrient combination stimulating growth was highly variable, including P alone (1 microcosm), N, P, and Si in combination (2 microcosms), or primarily N with P secondary (1 microcosm).

APPROACHES TO THE DETECTION OF DOMOIC ACID IN MARINE FOOD WEBS

Gregory J. Doucette
Marine Biotoxins Program, Center for Coastal Environmental Health & Biomolecular Research, National Ocean Service, 219 Fort Johnson Rd., Charleston, SC 29412

Just over a decade ago in eastern Canada, the neurotoxin domoic acid was identified as the causative agent of a human intoxication syndrome known as amnesic shellfish poisoning. Since that event in 1987, domoic acid and the diatoms that produce it (i.e., *Pseudo-nitzschia* spp.) have been reported from many U.S. coastal regions, and are now recognized as a public health concern through their contamination of seafood resources. However, it has become increasingly clear that toxic *Pseudo-nitzschia* species also pose a wider threat to coastal ecosystems based on their association with unusual mortality events involving marine birds and mammals. In order to describe the process by which domoic acid is moved through marine food webs, it is imperative that robust, reliable methods of toxin detection be established. We have adopted a tiered approach to domoic acid detection in diverse sample types (e.g., plankton, seawater, invertebrates, fish, mammals) involving a high throughput receptor binding assay and tandem mass spectrometry, which provides information on toxic activity as well as the unambiguous confirmation of toxin presence. Moreover, innovative techniques for sample collection and extraction of domoic acid from specific matrices have been established that yield high quality samples and optimize our toxin detection capabilities. Finally, methods for the automated, *in-situ* collection of plankton samples for toxin analysis are under development, with the ultimate aim being remote detection of domoic acid. These various approaches to toxin detection will be discussed in the context of their application to studies of domoic acid in marine food webs.

BROWN TIDE ASSESSMENT PROJECT IN BARNEGAT BAY, NJ AND THE PRESENCE OF VIRAL-LIKE PARTICLES IN NATURAL POPULATIONS OF *AUREOCOCCUS ANOPHAGEFFERENS*

Mary Downes Gastrich[1,2] , O.R. Anderson [2], and Elizabeth M. Cosper[3]
[1]New Jersey Department of Environmental Protection, Division of Science, Research and Technology, P.O. Box 409, Trenton, NJ 08625
[2]Lamont Doherty Earth Observatory of Columbia University, Palisades, NY 10964
[3]Coastal Environmental Studies, Inc., 83 Carlough Road, Bohemia, NY 11716

Brown tide blooms, caused by *Aureococcus anophagefferens*, were documented in Barnegat Bay in 1995 and were associated with a reduction in growth of juvenile hard clams at a commercial aquaculture facility. In 1999, a significant and extensive bloom was reported in Little Egg Harbor. There are environmental factors present in Barnegat Bay which appear to be similar to other bays (e.g., south shore bays of NY) that have experienced blooms (e.g., shallow bay, elevated salinity, poor flushing time and long residence times). Because of limited data, particularly related to the 1999 brown tide bloom, the New Jersey Dept. of Environmental Protection, in cooperation from the U.S. EPA, established a Brown Tide Assessment Project in 2000 to determine the spatial and temporal extent of these blooms and ultimately to develop a predictive model leading to control strategies. Water samples were collected from up to 44 stations from Raritan Bay to areas south of Barnegat Bay and Great South Bay from April through November 2000. The brown tide organism was enumerated using a newly developed monoclonal antibody (ELIZA) technique. Selected water quality parameters were also measured (e.g., salinity, temperature, nutrients). Water samples from 1999 and 2000 were also collected and viewed, using transmission electron microscopy, to quantify the presence of viral-like particles (VLPs) in natural populations of *A. anophagefferens.*

The results of monoclonal analysis confirmed that several sites in Little Egg Harbor, NJ including Ship Bottom and Tuckerton, had a substantial brown tide bloom with the highest concentrations of *A. anophagefferens* over a million cells per mL representing full bloom conditions in early June. The highest cell counts were observed in the vicinity of Little Egg Harbor, below the Barnegat Inlet, with cell counts up to 2.2 X 10^6 cells per mL on June 8 which decreased to 3.0 X 10^4 cells per mL in early July. At Tuckerton, the counts reached two million per mL on June 15 and decreased to a low of 3.5 X 10^4 cells per mL on July 12. At Ship Bottom, the cell numbers reached 1.8 X 10^6 cells per mL on June 23 and decreased to 4.1 X 10^5 cells per mL on July 12. While concentrations of *A. anophagefferens* exceeded 10^5 cells/mL (representing smaller blooms) in areas near and just north of the Barnegat Inlet and south of Little Egg Harbor in Great Bay, representing an extended geographic occurrence of these blooms, full bloom concentrations were not observed in these areas. The severe brown tide bloom appeared to be concentrated in Little Egg Harbor and the southern part of Barnegat Bay between Barnegat Inlet and Little Egg Inlet.

For the first time, intracellular viral-like particles (VLPs) were quantified in the brown tide organism, *Aureococcus anophagefferens* during the 1999 brown tide bloom in Barnegat Bay and Little Egg Harbor, NJ. Up to 8% of the total individual *A. anophagefferens* cells examined (Total = 4,380) from natural populations contained VLPs (ca. 140 nm in diameter). The intracellular VLPs were similar in size and morphology to viruses reported in natural populations of *A. anophagefferens* from Narragansett Bay over a decade earlier and were also similar to observations of intracellular viruses that were inoculated previously into laboratory cultures of *A. anophagefferens.* Preliminary data also confirms the presence of VLPs in natural populations of *A. anophagefferens* sampled during the brown tide bloom in 2000 in Barnegat Bay. The presence of VLPs in natural populations of *A. anophagefferens* is significant because they have not been previously quantified in field blooms. The role of viral infection needs further study in relation to the bloom dynamics. Further sampling is needed in 2001 to continue the spatial and temporal analysis including an assessment of environmental factors that may be associated with the promotion and sustenance of brown tide blooms in Barnegat Bay.

THE AMBUSH PREDATOR *PFIESTERIA PISCICIDA*: FAD OR FALLACY

Patrick Gillevet[1], Thomas Nerad[2], Michael T. Peglar[2], Greg C. Garman[3], Stanley Webb[3], Bonnie Brown[3], and Charles J. O'Kelly[4]
[1]George Mason University, Manassas, VA 20110 USA
[2]American Type Culture Collection, Manassas, VA 20110 USA
[3]Virginia Commonwealth University, Richmond, VA USA
[4] Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575

The ambush predator hypothesis is based on three principal tenets: (1) in the presence of fish, *Pfiesteria piscicida* undergoes sexual reproduction and transforms into an amoeba that feeds on lesioned fish, (2) the dinoflagellate releases exotoxins that narcotize and cause lesions in fish, and (3) the toxic ambush predator behavior is induced in aquaria if fish are present during an extended incubation period.

We have isolated and characterized the most prevalent amoebae with stellate floating forms as well as *Pfiesteria* complex (PCOs) dinoflagellates from rivers in Maryland and VA where epizootic events involving large numbers of dead or moribund fish have occurred. Based upon morphological and molecular data the amoebae were shown not to be life stages of the co-isolated dinoflagellates. These findings suggest that the reported amoeboid life stages of *Pfiesteria piscicida* and related dinoflagellates are likely phylogenetically distinct taxa. After nearly two years of continual observation, clonal strains of *Pfiesteria*-like dinoflagellates and *P. piscicida* reference strains (CCMP 1830,1831,1834) have never yielded an amoeboid form. Further, clonal cultures of *P. piscicida* have been observed to form planozygotes indicating that *P. piscicida* is capable of undergoing sexual reproduction in the absence of fish or fish derived materials without converting to amoeboid stages.

During a fish survey (Oct 1999) in the lower James River near Richmond, VA, penetrating lesions similar to those reported for *P. piscicida* were found in approximately 75% of Atlantic menhaden sampled. *Kudoa clupeidae* was found in histological sections from fish without evident lesions and from fish with pre-lesions (raised unruptured regions of the epidermis) as well as from fish with open lesions. No evidence of fungal, bacterial, or amoeba involvement were found in fish without lesions or in fish with pre-lesions. This study indicates that *Kudoa clupeidae* may be a significant agent in initiating lesion development in recent outbreaks of fish mortality in eastern Atlantic coastal waters and calls into doubt the role of *P. piscicida* and related dinoflagellates (PCOs) as the causative agents of these epizootics.

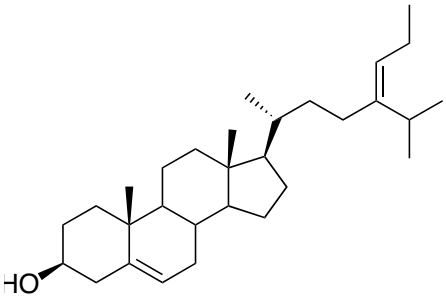
We have characterized the complex microbial consortia from fish culture experiments using Amplicon Length Heterogeneity Fingerprinting. We observed the presence of several protist and bacterial taxa that may be correlated with fish mortality. The results indicate the existence of other putative microbial organisms besides *Pfiesteria* that may be the causative agent of fish mortality in these fish culture experiments.

Thus, the three basic tenets of the ambush-predator hypothesis are called into question by our studies casting further doubt that *Pfiesteria piscicida* plays any role as the causative agent of epizootic events involving penetrating fish lesions in the Chesapeake Bay and its tributaries.

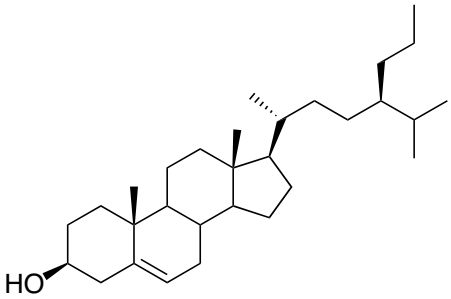
UNUSUAL STEROLS FROM HARMFUL ALGAE: MORE THAN BIOMARKERS?

José-L. Giner, Gregory Boyer, Juan Faraldos, Xiaoyong Li and Hui Zhao
Department of Chemistry, SUNY-ESF, Syracuse, NY 13210, USA.

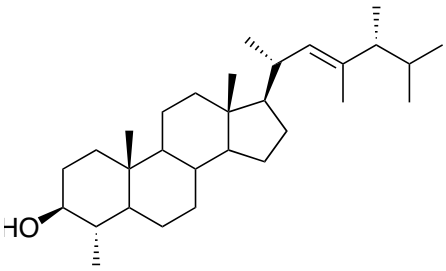
Unusual sterols found in marine algae are useful biomarkers. Dinosterol is found only in dinoflagellates and 24-propylidenecholesterol is found only in pelagophyte algae. We have found useful sterol biomarkers for *Aureococcus anophagefferens*, *Aureoumbra lagunensis*, and *Gymnodinium breve.* The biomarker for *Aureoumbra lagunensis* is an extremely rare sterol, while the sterols characteristic of *Aureococcus anophagefferens* and *Gymnodinium breve* are unique to these organisms. These biomarkers allow us to probe the sediment record for evidence of past harmful algal blooms and offer an alternative method of detection for harmful algae.



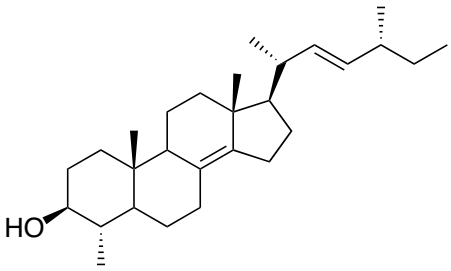
From Aureococcus anophagefferens



From Aureoumbra lagunensis



From dinoflagellates



From Gymnodinium breve

Why do these algae produce these unusual sterols? What benefit is it to them? Many important grazing organisms rely on dietary sterols. Arthropods and molluscs lack the capability of *de novo* sterol biosynthesis and fulfill their sterol requirement by modifying dietary sterols. It is likely that unusual algal sterols interfere with this process. The inability for grazers to meet their sterol requirements may be important to bloom formation and maintenance.

TOXIC *PFIESTERIA* PROMOTES ACUTE AND CHRONIC LESIONS IN FINFISH, IN CONTROLLED EXPERIMENTAL TRIALS

H. Glasgow, Jr., [1] R. Smolowitz,[2] N. Deamer-Melia[1] and J. Burkholder[1]
[1] Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606
[2] Marine Biological Laboratory, Woods Hole, MA 02543

Bioactive substances from the toxic *Pfiesteria* complex have been shown to destroy fish epidermis, and to render fish susceptible to opportunistic bacterial and fungal pathogens in lesion formation. Here we report the findings from repeat trial experiments (n=12) in which we (i) characterized acute lesion development and other pathology in tilapia (juveniles, t.l. 5-8 cm) ex-posed to toxic clonal *Pfiesteria piscicida* (TOX-A functional type); and (ii) tracked chronic lesion development in tilapia following 'recovery' from exposure to toxic clonal *P. piscicida*.

Pfiesteria piscicida was isolated from the Neuse Estuary (isolate ND-PP990708), cloned, and confirmed as toxic to fish (uni-dinoflagellate clones -- Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by molecular probes from our lab. and the fluorescent *in situ* hybridization probe of P. Rublee, UNC-G; fish bioassay process, following Koch's postulates for modified for toxic rather than infectious agents; toxicity cross-confirmed by H. Marshall, ODU).

Toxic zoospore densities $\geq$ 100 cells/mL induced epithelial destruction and lesions. In repeat trials, acute lesions formed within $\leq$ 12 hr (sometimes in $\leq$ 2 hr, typically in < 8 hr), generally with hemorrhaging (sometimes within minutes) and often culminating in rupture of the peritoneal sack with exposure of the viscera. Dermatological lesion formation involved intra- and extracellular edema and necrosis of epithelium (with pyknotic and eosinophilic cytoplasm), progressing to erosions that extended through the basement membrane (50-80% loss of epidermis, depending on exposure duration). Epidermal and skeletal muscle tissues were characterized with mild to severe multifocal granulocytic and lymphocytic epdermatitis; moderate dermal edema; marked diffuse lymphocytic epidermatitis; and/or mild to marked necrotizing lymphocytic epidermatitis. Other pathology was documented in the gill (cytomegalic bacterial inclusions and mild to severe edema); cornea (mild to severe erosion); pharynx (mild to severe edema); hepatopancreas (mild multifocal lymphoplasmacytic, granulcytic, hepatopancreatitis [sometimes necrotizing]); kidney (mild multifocal tubular mineralization [$\pm$ granuloma formation] and minimal multi-focal lymphohematopoietic necrosis); and brain (moderate subacute to chronic multifocal meningitis, mild to acute granulocytic optic neuritis, and encephalitis). Control fish, maintained similarly except without exposure to toxic *Pfiesteria*, remained healthy, and did not show pathologies.

Recovery from sublethal exposure to toxic *Pfiesteria* was tested by removing fish with mild to moderate lesion development from additional exposure to toxic *Pfiesteria* (basis: no detection of *Pfiesteria* from daily analysis of water samples in culture vessels, using light microscopy at 600x) and tracking fish health for 6 wk. Control fish were treated similarly except for no prior exposure to toxic *Pfiesteria*, the test fish sustained 'easy infections' from bacteria and fungi. About 80% of the fish developed ulcers with moderate to severe, acute myonecrosis and mixed gram-negative bacterial infections (predominantly *Aeromonas hydrophila*). Ulcers were not observed in the control fish. These findings are consistent with Noga et al. (1996, *Marine Pollution Bulletin* vol. 32), and indicate that exposure to toxic *Pfiesteria* can promote chronic as well as acute lesion development in finfish.

NITROGEN UPTAKE AND NUTRIENT RELATIONSHIPS IN LABORATORY CULTURES AND FIELD ASSEMBLAGES OF *PFIESTERIA*

P.M. Glibert[1], A. Lewitus[2], J. Burkholder[3], H. Glasgow[3], M. Mulholland[4], and C. Lee[4]
[1] Horn Point Laboratory, University of Maryland Center for Environmental Science, PO Box 775, Cambridge, MD 21613
[2] Belle Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442
[3] Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606
[4] Marine Sciences Research Center, SUNY, Stony Brook, NY 11794

Due to the largely heterotrophic nutrition of *Pfiesteria*, the degree to which nutrients are directly or indirectly taken up by this organism is of considerable interest. *Pfiesteria* is typically associated with nutrient rich conditions in the field, but the extent to which eutrophication may stimulate this organism is yet unclear. Laboratory studies have demonstrated the direct uptake of both inorganic and organic nutrients by *Pfiesteria*. Rates of nitrogen uptake, as determined using ^{15}N tracer techniques, compare with rates of nitrogen acquisition by phagotrophy under certain growth conditions. Direct comparisons of patterns in nitrogen uptake were made using actively toxic ("TOX A"), previously toxic ("TOX B") and non-toxic (algal-fed, "NON –IND") *Pfiesteria piscicida*, and rates varied with feeding history and culture incubation conditions.

Direct uptake of nitrogen may not be the only pathway by which this organism obtains dissolved nitrogen. Recently toxic cultures of *Pfiesteria* have been shown to have mechanisms for obtaining nitrogen in the form of amino acids via extracellular oxidation. High rates of extracellular amino acid oxidation have also been observed in the field when *Pfiesteria* has been confirmed to be present. This pathway of nitrogen acquisition has previously been observed for some other phytoplankton species, and in particular, dinoflagellates.

In Maryland's tributaries, outbreaks of toxic *Pfiesteria* have consistently been associated with ratios of organic carbon: organic nitrogen that are elevated above levels that are observed during periods of non-*Pfiesteria* activity. With the ability of *Pfiesteria* to access nutrients by both heterotrophic or autotrophic pathways, it may be able to grow successfully on available particulate or dissolved, inorganic or organic, nutrients when other necessary conditions, such as reduced grazing pressure and appropriate temperatures are present.

THE IMPACT OF BOTTOM-UP AND TOP-DOWN PROCESSESS ON THE ABUNDANCE OF *AUREOCOCCUS ANOPHAGEFFERENS* DURING THE 1999-2000 BROWN TIDE BLOOM IN GREAT SOUTH BAY, NY, USA

Christopher J. Gobler, Natural Science Division, Southampton College of Long Island University, Southampton, NY 11968

Beginning in the fall of 1999, the most intense Brown Tide (*Aureococcus anophagefferens*) bloom in NY waters since the 1980's occurred throughout Great South Bay (GSB). The bloom persisted through the summer of 2000, with peak, monospecific cell densities exceeding 1 x 10^6 cells per mL. To identify factors which contributed to the initiation and persistence of this bloom, a 1-yr observational and experimental field campaign was established in October 1999 at stations in the eastern (Patchogue Bay) and western (Bay Shore Cove) portions of GSB. Nutrient bioassays were conducted in parallel with dilution-style microzooplankton grazing experiments to allow the importance of bottom-up and top-down factors to be simultaneously evaluated. During the study, dissolved organic nitrogen (DON) concentrations present in GSB were high (30 – 40 μM), while dissolved inorganic nitrogen (DIN) levels were relatively low (1 – 4 μM). Although the addition of nitrogen (nitrate or urea) during short-term (24 - 48 h) nutrient bioassays typically enhanced the growth rates of the total phytoplankton community, such additions often had no impact on or caused a decrease in growth rates of *Aureococcus* relative to unamended control treatments. These observations suggest *Aureococcus* was able to subsist on the copious DON pool in GSB, while growth of non-Brown Tide phytoplankton depended on ambient N supply rates. Dilution experiments indicated that grazing rates on *Aureococcus* were significantly lower ($P<0.05$) than those on the total phytoplankton community, suggesting that microzooplankton selectively avoided *Aureococcus* during this Brown Tide event. Significantly higher microzooplankton grazing rates ($P<0.05$) on the picoplankter, *Synechococcus sp.* compared to *Aureococcus* during this bloom event indicated that reduced grazing on the Brown Tide was likely not a function of cell size. The sum of these results demonstrates, for the first time, that both top-down (low grazing rates) and bottom-up (a high DON, low DIN nutrient regime) factors can contribute to the proliferation of Brown Tide blooms on Long Island.

VARIABLE BREVETOXIN PRODUCTION IN *GYMNODINIUM BREVE* ATTRIBUTABLE TO GROWTH CONDITIONS AND STRAIN DIFFERENCES

Richard M. Greene, Janis C. Kurtz, Roman S. Stanley, Cynthia A. Chancy, Michael C. Murrell, Fred J. Genthner, John E. Rogers, and Calvin C. Walker
U.S. EPA, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL 32561

Blooms of the dinoflagellate *Gymnodinium breve* can produce sufficient concentrations of brevetoxin to negatively impact coastal ecosystems, human health, and local economies. Assessment of risks associated with *G. breve* blooms and subsequent brevetoxin exposure requires better understanding of factors governing bloom development and toxin production. Although the complete biosynthetic pathway for brevetoxin has not been elucidated, reports in the literature suggest some degree of geographic or strain-specific variability in total cellular brevetoxin concentration, ranging from 7 to 17 pg $cell^{-1}$ (Baden and Tomas, 1988; Hua et al., 1996).

During Fall 1999, a *G. breve* bloom impacted a 150 mile stretch of the Florida panhandle shoreline. In samples collected from16 locations on 3 fall sampling dates, *G. breve* abundance ranged from 0.1 - 20 x 10^6 L^{-1}. Using HPLC methods, we measured total brevetoxin (PbTx 1, 2, 3, 6) concentrations that greatly exceeded those reported in the literature. In shelf waters off Pensacola and Navarre beaches and in estuarine waters of Cinco Bayou and Santa Rosa Sound, total brevetoxin ranged from 47 to 67 pg $cell^{-1}$ (n=5), 59 to 126 pg $cell^{-1}$ (n=3), and 12 to 63 pg $cell^{-1}$ (n=8), respectively.

Brevetoxin production and accumulation in response to nitrogen, phosphorus, and light availability were examined in laboratory cultures using three *G breve* strains; the Piney Island (courtesy of FMRI) and Charlotte Harbor (courtesy of NOAA) strains isolated from the central west Florida coast, and the Pensacola Beach strain (EPAJR1) isolated from the Fall 1999 panhandle bloom. When grown under standard conditions with f/2 nutrient concentrations, total brevetoxin concentrations in Piney Island and Charlotte Harbor strains ranged from 10 to 20 pg $cell^{-1}$, whereas the Pensacola Beach strain produced about 30 pg $cell^{-1}$ five months following isolation. However, by reducing nitrate (<10 µM) or phosphate (<0.6 µM), by substituting urea (<5 µM), or by lowering irradiance (30 µmol quanta m^{-2} s^{-1}), total brevetoxin content increased to >100 pg $cell^{-1}$ in the Piney Island and Charlotte Harbor strains.

These results suggest that physiological responses to growth limiting conditions may enhance brevetoxin production, and that there may be genetic differences between strains that result in variable toxin production. Understanding and modeling stressor-response relationships, such as the effects of environmental conditions on growth and toxin production, will be important to predicting bloom toxin concentrations, toxicity, and ecological effects.

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HABS-RELATED PHYSICAL OCEANOGRAPHY OF THE U.S. WEST COAST

Barbara M. Hickey
School of Oceanography, University of Washington, Seattle, WA 98195

The oceanography of the U.S. West coast is dominated by the California Current System, a system of currents with strong interannual, seasonal and several day scale variability. In contrast to most east coast environments, the shelf is relatively narrow, so that nutrient-rich deeper water can be effectively brought to the surface by the upwelling that occurs in the growing season along the entire coastal boundary. It is the wind-driven upwelling of nutrients from deeper layers that fuels coastal productivity, resulting in both a strong seasonal cycle and several day fluctuations that mimic changes in the wind direction and, hence, upwelling. During an upwelling event, phytoplankton respond to the infusion of nutrients near the coast and this "bloom" is moved offshore, continuing to grow while depleting the nutrient supply. When winds reverse, the bloom moves back toward shore where it can contact the coast or enter coastal estuaries. In the mean, coastal currents in near surface layers at most latitudes are northward in winter and southward in spring and summer, although direction reversals occur frequently in every season.

The majority of current and nutrient fluctuations over the shelves along the coast are determined by wind forcing--sometimes by the local wind stress, but also by "remote" wind stress, which sends signals northward along the coast in the form of propagating waves. In contrast to the east coast, alongshore topography of the coastline is relatively straight and wind systems are large scale. Thus, currents and water properties (e.g., temperature, stratification etc.) are similar over relatively large (> 500 km) distances along the coast. This similarity in ocean conditions may have strong implications with respect to the generation and movement of HABs.

In the several regions where large coastal promontories occur, phytoplankton are swept offshore and southward by the meandering jets and/or eddies that form where the coastal jets detach from the shelf. At both the north and south extremities of the U.S. west coast, topography is more complex and planktonic retention areas are more likely to occur. For example, off the Washington coast a semi-permanent eddy develops seaward of the Strait of Juan de Fuca and this eddy has been shown to contain high levels of domoic acid. During some years domoic acid from this eddy appears to move onshore and also into coastal estuaries during the first major storm of the fall season.

HARMFUL ALGAL BLOOMS IN THE UNITED STATES: ESTIMATES OF ECONOMIC IMPACTS AND POLICY RESPONSES

Porter Hoagland, Di Jin, Hauke Kite-Powell and Tracey Morin
Woods Hole Oceanographic Institution, Woods Hole, MA 02543

During the last several decades, harmful algal bloom (HAB) events have occurred in more locations than ever before throughout the United States. The number of algal species involved in such events has increased, there are more known toxins, and more fisheries resources are affected. Anderson et al. (2000)† estimate the economic impacts of HAB events in the United States where such impacts were measurable with a fair degree of confidence during 1987-92. They examine impacts of four basic types: (1) public health; (2) commercial fisheries; (3) recreation and tourism; and (4) monitoring and management. Their conservative estimate of total economic impacts in the United States due to HABs is on the order of $50 million per year. They define "economic impacts" broadly to mean either lost gross revenues in the relevant product or factor markets, expenditures for medical treatments, environmental monitoring and management, or other costs that would not have been incurred in the absence of HABs. Although estimates of lost economic surpluses are more useful for normative analyses, economic impact analysis still may help in the identification and implementation of appropriate policy responses. We present the preliminary results of national level scenarios that estimate the direct, indirect, and induced economic impacts of HABs in the United States. These scenarios consider annual average impacts at the state level during 1987-98 and hypothetical impacts associated with historic extreme HAB events. We relate these estimates to the results of the Anderson et al. study. We discuss the usefulness of these estimates for developing appropriate policy responses.

Anderson, D.L., P. Hoagland, Y. Kaoru and A. White. 2000. Estimated annual economic impacts resulting from harmful algal blooms (HABs) in the United States. Woods Hole Oceanographic Inst. Tech. Rept., WHOI 2000-11. (99 pp)

RAPID TESTING USING THE MIST ALERT™ FOR PARALYTIC SHELLFISH POISONING (PSP), FOR TRIALS WITHIN THE UNITED STATES

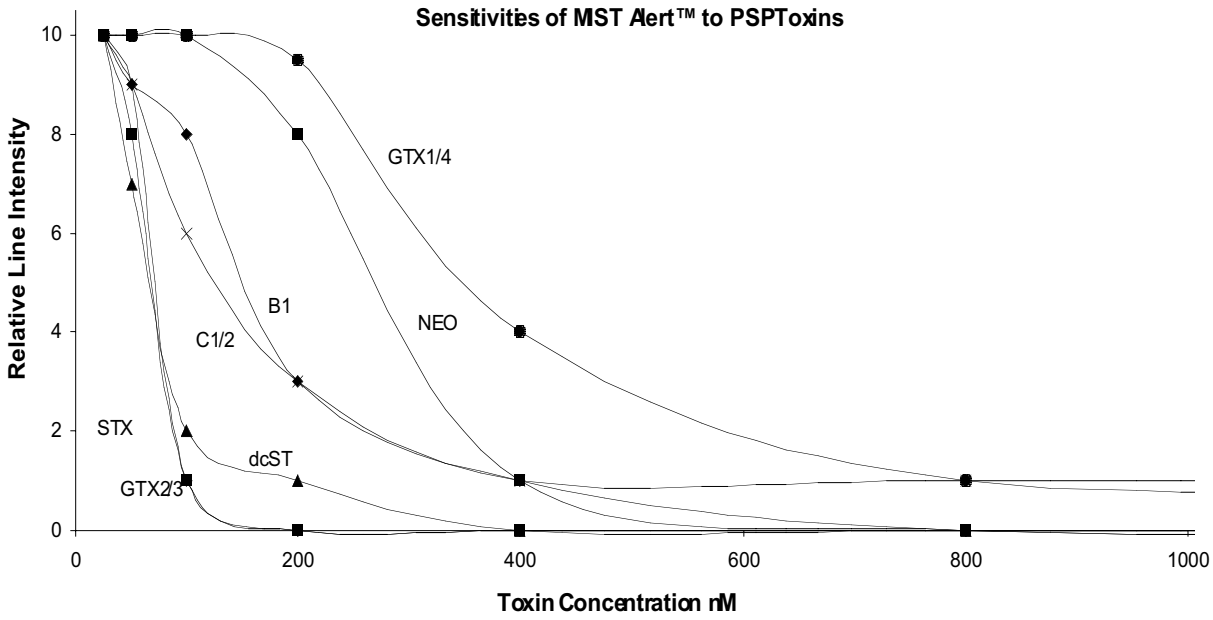
Joanne F. Jellett
Jellett Biotek Limited, PO Box 790, Dartmouth, Nova Scotia, CANADA, B2Y 3Z7

Jellett Biotek Limited, a Canadian biotechnology company has developed, in collaboration with the National Research Council of Canada, Institute of Marine Biosciences, a rapid test to screen for paralytic shellfish poisoning. The MIST Alert™ for PSP, is a simple, single sample test that provides a qualitative (yes/no) visual indication of the presence of toxicity in approximately 15 minutes. The development of a single color line on the test indicates a positive sample, which is not safe to eat; two lines appearing means the sample is safe.

The MIST Alert™ can be used for various applications such as a quality control tool in a shellfish processing plant, in the field as a harvest management tool and in the regulatory lab to screen out negative samples. The tests are currently being tested in parallel with the mouse bioassay in validation trials using shellfish samples from two US states, Maine and Alaska.

The trial results to date show an excellent agreement with the mouse bioassay. The trial in Alaska with the Department of Environmental Conservation (DEC) halfway through the trial period has an agreement level of 95.24% on 786 MIST Alert™ tests performed. Early results from Maine (89 tests performed) indicate an agreement level around 90% when compared to the mouse bioassay. The main PSP toxin analogues used in the development, and quality control of production of the MIST Alert™ tests are saxitoxin (STX) and neosaxitoxin (NEO). A number of other analogues have also been tested on the MIST Alert™ in detail, including gonyautoxin (GTX) 2/3 and 1/4, B1 and decarbamoylsaxitoxin (dcST).

The false positive and negative results of the trials will be discussed as well as the application of the MIST Alert™ for PSP to the regulatory environment and aquaculture industry.



THE ROLE OF BEHAVIOR IN *GYMNODINIUM BREVE* BLOOM FORMATION

Daniel Kamykowski[1], Gerald S. Janowitz[1], Gang Liu[2], Edward J. Milligan[1], and Robert E. Reed[1]
[1]Department of Marine, Earth & Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695
[2]NOAA/NESDIS/ORA/ORAD E/RA3, NOAA Science Center RM 711, 5200 Auth Road, Camp Spring, MD 20746

The prediction of bloom formation by *Gymnodinum breve*, an autotrophic dinoflagellate capable of swimming at a rate of 1 m/h, requires a consideration of cellular motion in the context of ambient water motion. Unfortunately, the rules that *G. breve* follows in choosing its swimming direction are poorly known. Field observations of the distribution patterns of *G. breve* demonstrate that cell aggregations can occur throughout the euphotic zone, but diel vertical migration generally is difficult to discern. Laboratory experiments (Kamykowski et al., 1998 [MEPS 167:105]) in 225 L columns (45 x 150 cm) under nutrient replete conditions demonstrate that the diel vertical migration of *G. breve* is characterized by a surface aggregation during daylight that diffuses through the available water column during the night. The strength of the surface aggregation depends on the time since last division and the biochemical composition of the cells. Based on an experiment with a quantized culture, parent cells apparently divide into unequal daughter cells. Daughter cells that contain smaller lipid reserves preferentially aggregate at the surface during the day, while those with larger lipid reserves tend to remain distributed throughout the available water column during the day. As both daughter cells age, the strength of the surface aggregation decreases until the sequence is re-initialized by another cell division (Kamykowski et al., 1998 [JPR 20:1781]). A numerical model of *G. breve* biology tuned to its known physiology and behavior provides output that most closely resembles the patterns of laboratory diel vertical migration when behavior is a based on positive phototaxis during the day and weakened negative geotaxis (that is, an increased tendency to descend compared to other times) at dusk, a cell division that yields unequal daughter cells, and a swimming orientation that is influenced by inhibitory light intensity (descent) and by the state of the cellular carbon and nitrogen pools (Liu et al., 2000 [MEPS, In Press]). This biological numerical model is modified for field application (deeper water columns, but with cell behavior as the sole component of vertical motion) by allowing cells to sense the nutrient gradient in the water column. When this modified model is applied to different scenarios of vertical nutrient sources representative of the West Florida Shelf including near-surface *Trichodesmium* blooms, near bottom upwelling plumes, and/or near-surface outwelling from terrestrial sources, cell aggregations occur at different locations in the euphotic zone depending on nutrient availability. The vertical distribution patterns are reminiscent of the field observations. The transition zones where different nutrient sources compete for cells generate model output characterized by complex vertical distribution patterns of cell number and of cell quality in terms of carbon and nitrogen reserves.

These different studies support a working hypothesis that *G. breve* applies its motility in response to a variety of environmental and internal cellular cues that can interact in complex patterns. Under conditions where cell motility is effective in determining vertical location, field aggregations may occur where the balance of these conditions provide an optimal water column location for cell survival and/or growth. Under conditions where vertical water motion is more significant, a gradient of disruption can be envisioned as cells strive for optimal locations that they may never attain. In both instances, the vertical location of the cells determines exposure to horizontal currents and subsequent movement across the shelf and along the coast.

ECOHAB – FLORIDA: BIO-OPTICS AND PHYSIOLOGY

Gary Kirkpatrick[1], David Millie[2], Steve Lohrenz[3], Oscar Schofield[4], Gary Fahnenstiel[5], Donald Redalje[3] and Terrance Evens[2]
[1]Mote Marine Laboratory, Sarasota, FL, 34236, USA
[2]US Department of Agriculture, Agricultural Research Service, New Orleans, LA, 70179, USA
[3]Center for Marine Sciences, University of Southern Mississippi, Stennis Space Center, MS, 39529, USA
[4]Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ, 08901, USA
[5]National Oceanic and Atmospheric Administration, Great Lakes Ecological Research Laboratory, Muskegon, MI, 49441, USA

The Florida red tide organism, *Gymnodinium breve,* blooms in shallow continental shelf waters principally along the coast of the Gulf of Mexico. Dense blooms are often found at the water surface where irradiance exposure, including ultraviolet, is very intense. Laboratory cultures of *G. breve* are slow to acclimated to high irradiance, seeming to do best at low irradiance. The acclimation capability of this dinoflagellate is critical to modeling its presence at high cell concentrations in surface waters where it is transported by wind forcing.

A field study with laboratory culture, acclimated to moderate irradiance levels, showed high absorption cross sections in the early part of the diurnal cycle followed by a dramatic reduction at midday. Quantum yield was strongly depressed at midday and recovery was not evident until near sunset. A subsequent field experiment with laboratory culture that had been more extensively acclimated to moderately high irradiance exhibited similar diurnal patterns to those just described. The extent of midday depression of the quantum yield was directly correlated to peak irradiance for each of the three experimental days. Elimination of ultraviolet from the irradiance spectrum did not produce a detectable change in response. Photoprotective pigments were elevated in the higher-light acclimated cultures. Additionally, on days of high peak irradiance the ratios of the photoprotective pigments diadinoxanthin and diatoxanthin indicated strong protective activity.

These findings will be detailed and available results from cruises currently underway will be incorporated as appropriate. This characterization of the photoacclimation capability of *Gymnodinium breve* will provide guidelines for the Florida red tide system modelers addressing this issue in their comprehensive model.

PFIESTERIA OR FUNGUS? INDUCTION OF SKIN ULCERS IN MENHADEN WITH ZOOSPORES OF *APHANOMYCES* SPP.

Yasunari Kiryu[1], Jeffrey D. Shields[1], Wolfgang K. Vogelbein[1], David E. Zwerner[1], Howard Kator[1], and Vicki S. Blazer[2]
[1]Virginia Institute of Marine Science, Gloucester Point, Virginia 23062
[2]National Fish Health Research Laboratory, USGS, Kearneysville, West Virginia 25430

Menhaden, *Brevoortia tyrannus*, develop skin ulcers that have been attributed to exposure to *Pfiesteria piscicida* toxins. However, the consistent presence of a fungal agent in the ulcers suggests a different etiology. We have completed three challenge studies to determine the infectivity of *Aphanomyces invadans* in relation to its role as a primary or secondary etiologic agent. (1) Hyphal injection: macerated hyphae of two strains of *A. invadans* (INV, an Asian strain, and WIC, an endemic strain in menhaden from Maryland) were injected subcutaneously into 10-11 menhaden. Fish were kept in static aquaria (6 ‰, 76 L, preconditioned whisper filters) at 23.0°C, and monitored daily for 26 d. Hyphal injections caused characteristic lesions. Small, incipient skin lesions developed after 4-5 d. Large necrotic lesions were present on 60% of fish injected with WIC after 26 d. No lesions were detected in fish injected with INV or the saline control. (2) Zoospore injection: secondary zoospores of WIC and INV and an additional strain, ATCC #62427 (isolated from menhaden from North Carolina) were injected subcutaneously into menhaden. Fish were inoculated with 1.9×10^3 zoospores (WIC-low), 1.9×10^4 (WIC-high), 6.5×10^3 zoospores (INV strain), or 7.5×10^3 zoospores (ATCC). Both low and high doses of WIC caused incipient, mildly granulomatous lesions in fish after 4-5 d. Fish injected with the high dose WIC died within 7 d. After 10 d, all of the fish inoculated with the low dose WIC had died. Fish treated with INV developed lesions after 9 d; i.e., later than those injected with WIC. Fish injected with ATCC or saline did not develop lesions after 21 d. (3) Zoospore bath challenges: fish were exposed in baths containing zoospores of WIC. Treatments consisted of net-stressed fish (Net stress, exposed for 2 h to either 7.0×10^2 and 7.0×10^3 zoospores/ml), fish with a few scales removed (Trauma, exposed for 1 h to 7.0×10^3 zoospores/ml), fish with no handling (No trauma, exposed for 5.5 h to 1.4×10^3 zoospores/ml), and unexposed controls. Mortality and prevalence of the lesions were high (88-100%) for fish in stress treatments (net, trauma), while they were lower (20-23%) for fish in the "No trauma" treatment, a more environmentally realistic exposure.

Lesions from fish inoculated with hyphae or zoospores or bath exposed with zoospores were histologically identical to those observed in wild menhaden collected from several estuaries and rivers along the middle North Atlantic coast. The deeply penetrating ulcers were characterized by dermatitis, myofibrillar degeneration, and a deep necrotizing granulomatous myositis. Experimentally induced lesions, however, exhibited more invasiveness, often involving the liver or kidney. Incipient granulomas were apparent after 5 d with inoculation or bath exposure to zoospores, and became more abundant with time. Infections developed into frank lesions over a relatively short time frame of 7-9 d. We demonstrated that "typical" skin ulcers attributed to exposure to *Pfiesteria piscicida* can be experimentally induced following inoculation or bath exposure with fungal zoospores of an endemic strain of *A. invadans*.

TROPHIC RELATIONSHIPS OF PHYTOPLANKTON AND MICROZOOPLANKTON WITH *PFIESTERIA*-LIKE HETEROTROPHIC DINOFLAGELLATES IN POCOMOKE RIVER AND TRANSQUAKING/CHICAMACOMICO RIVERS, MD, USA

Richard V. Lacouture, Jennifer Gronefeld, Ann Marie Hartsig, Stella Sellner and Amy Imirie
Academy of Natural Sciences Estuarine Research Center, St. Leonard, MD, USA 20685

In laboratory experiments, reproduction and stage transformations of *P. piscicida* have been linked to abiotic (inorganic nutrients, temperature and salinity) and biotic factors (organic nutrients and algal and fish prey density) (Burkholder and Glasgow 1997). In these experiments, it has been demonstrated that *P. piscicida* has specific algal prey preferences. Highest zoospore production took place in treatments that were fed *Cryptomonas sp.*. Treatments fed flagellates from other taxonomic groups (Chlorophyceae and Prymnesiophyceae) were characterized by significantly lower abundances of *P. piscicida* zoospores. A correlation between non-toxic vegetative cells and chlorophyll *a* and specific algal prey has also been documented in field studies in the Pamlico and Neuse River estuaries, North Carolina (Fensin 1997). In this study, spring chlorophyll *a* and densities of the dinoflagellate *Prorocentrum minimum* were correlated positively with zoospore abundance. This laboratory and field data indicate links between nutrient enrichment, algal prey types and densities and *P. piscicida* zoospore densities.

The population dynamics of *Pfiesteria*-like heterotrophic dinoflagellates are likely controlled by bottom-up factors (food) as well as top-down forces (grazing). Oligotrichous ciliates and tintinnids > 20 µm have been shown to prey on *P. piscicida* zoospores in laboratory experiments (Stoecker et al. in prep.). In contrast, other forms of metazoa and non-ciliate grazers were relatively ineffective in reducing the densities of zoospores. Grazing rates calculated in this laboratory study (0-0.46 zoospores/h) indicate that protistan grazing may be able to regulate the densities of *P. piscicida* zoospores.

In the Pocomoke and Transquaking/Chicamacomico Rivers, the presumptive numbers of *Pfiesteria*-complex organisms closely tracks the density of cryptophytes. The few occasions when there is a disconnect between PCOs and this favorite prey group seem to occur as a result of fluctuating grazing pressure. For instance, during the 9/3/99 sampling in the Chicamacomico River (CCM0069) there was a peak density of cryptophytes but a relatively low number of *Pfiesteria*-like heterotrophic dinoflagellates. This disconnect between food and grazer was likely the result of peak densities of PCO consumers, oligotrichous ciliates and tintinnids > 20 µm. Bi-weekly field data from the Pocomoke River and Transquaking/Chicamacomico Rivers will be analyzed for the summer of 2000 and compared to the results generated from 1998 and 1999 in further attempts to establish trophic linkages between the population dynamics of *P. piscicida* – like heterotrophic dinoflagellates and specific algal prey and microzooplankton predators.

ECOHAB FLORIDA: FATE AND EFFECTS OF BREVETOXINS IN SELECTED BIOTA, WATER, AND SEDIMENTS ALONG THE WEST FLORIDA SHELF, USA

Jan Landsberg[1], Pat Tester[2], Richard Pierce[3], Damian Shea[4], Fran Van Dolah[5], Emilio Sosa[1], Mike Henry[3], Jack Fournie[6], Leanne Flewelling[1], Sabrina Varnam[2], and Tod Leighfield[5]
[1]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL 33701
[2]National Ocean Service, NOAA, Beaufort, NC 28516
[3]Mote Marine Laboratory, Sarasota, FL 34236
[4] Department of Toxicology, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27607
[5]National Ocean Service, NOAA, Charleston, SC 29412
[6]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL 32561

Gymnodinium breve red tides have been historically associated with mass mortalities of aquatic organisms. Organisms are potentially exposed to brevetoxins either through ingestion of *G. breve* cells, toxin bioaccumulation, aerosolized transport, water-borne toxin after cell lysis, and sediment sinks. In parallel with other components of the Florida: ECOHAB program, one of the objectives of this study was to determine the fate and effects of brevetoxins in water, sediments, and in selected biota during an intensive bloom.

Samples were collected off the Florida west coast during a non-bloom period and also during three bloom periods. Water and phytoplankton samples were collected at surface, mid, and bottom depths. Benthic pinfish *Lagodon rhomboides*, planktonic thread herring *Opisthonema oglinum*, zooplankton, sediment, and representative benthic invertebrate samples were collected at selected stations. Samples were processed for brevetoxins (PBTx-2 and PbTx-3) by several methods including MEKC-LIF, receptor-binding assay, and HPLC. Selected fish tissues were also evaluated by histopathology and immunocytochemistry. Brevetoxins in the water column were processed by two different methods; one to distinguish toxins associated with suspended particles from dissolved toxins and the other to distinguish intracellular from extracellular toxins. All sediment, water, and biota samples collected during the non-bloom period were negative for brevetoxins.

During a bloom, most of the brevetoxins in the water column were associated with particles (and cells) with very little in a true "dissolved" state. Early stages of the bloom indicated that most of the toxins were intracellular. The extracellular toxins increased relative to intracellular toxins as the bloom progressed. Low concentrations of PbTx2 (<0.5-11.8 ng/g) and PbTx-3 (<0.5-2.9 ng/g) were detected in sediment samples. There was no positive correlation of brevetoxins in sediments and *G. breve* cell numbers in the overlying water column. The persistence of a bloom may have a greater effect on sediment brevetoxin concentrations than transient high *G. breve* concentrations. From a range of benthic animals tested only shrimp, clams, and anemones were positive for brevetoxins. PbTx-2 (7.6-282 ng/g) and PbTx-3 (1.7-71ng/g) were detected in mixed zooplankton collected when *G. breve* cell numbers ranged between 0.74-2.85 x 10^6 cells/L. PbTx-2 (0.62-320 ng/g) and PbTx-3 (0.08-85 ng/g) were detected in both fish species, but only in specific tissues. Planktonic fish had higher brevetoxin concentrations than benthic fish. Brevetoxin-induced pathology was not detected. PbTx-2 and PbTx-3 concentrations in fish tissues and whole zooplankton samples were not correlated with *G. breve* counts, but this does not take into account prior history of exposure or the presence of extracellular toxins in water. Potential trophic linkages between *G. breve*, extracellular brevetoxins, zooplankton, benthic invertebrates, and fish are confirmed.

PFIESTERIA, *PFIESTERIA*-LIKE SPECIES, AND FISH HEALTH IN FLORIDA: AN UPDATE

Jan Landsberg[1], Karen Steidinger[1], Susan Cook[1], Elizabeth Singh[1], Emilio Sosa[1], Ann Forstchen[1], Robin Wood[1], Parke Rublee[2], Paula Scott[1], Jennifer Wolny[1], and Brian Bendis[1]
[1]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL 33701
[2]Department of Biology, University of North Carolina at Greensboro, Greensboro, NC 27402

Since the late 1970s, numerous fish species from several Florida freshwater and estuarine systems - primarily striped mullet (*Mugil cephalus*), silver mullet (*Mugil curema*), and sheepshead (*Archosargus probatocephalus*) - have been affected by lesions, principally ulcerative mycosis (UM). The potential role of *Pfiesteria* as a causative agent in the development of UM has been discussed (e.g. Burkholder et al. 1998). In conjunction with studies on the distribution and etiology of lesions in fish from Florida, we are conducting an intensive statewide survey of the distribution and identification of *Pfiesteria* and *Pfiesteria*-like species (PLS, also known as *Pfiesteria*-like organisms, PLOs). Our most recent surveys have determined that fish affected by UM are found predominantly in low salinity or freshwater habitats where *Pfiesteria* does not usually occur in Florida. Thus far, we have confirmed the presence of *P. piscicida* by molecular probe at only one site in southeast Florida in an area with no historical records of fish kills or lesioned fish. However, repeated surveys (> 35 samples) in this area have failed to reconfirm the presence of *P. piscicida*. *Pfiesteria shumwayae* (proposed nov. sp. Glasgow and Burkholder) has been positively identified by molecular probe at two additional east coast sites in areas that have traditionally had very few or no lesioned fish. Repeated surveys (>40 samples) have also failed to reconfirm the presence of *P. shumwayae*, although samples are still being analyzed. Additional temporal samples in hot spot areas are warranted. The PLS referred to by us as "Lucy" has been confirmed by microalgal assay in the same area that was confirmed positive for *P. piscicida* and in one area confirmed positive for *P. shumwayae*. "Lucy" has been confirmed in the St. Lucie River, but appears to have a limited distribution thus far in Florida. Cryptoperidiniopsoids are the most widely distributed PLS around the state and occur in known fish lesion areas such as the St. Johns and St. Lucie rivers. Water quality measurements, sediment profiles, and other environmental data have not yet indicated any significant correlation with PLS distribution. An intensive sampling program has been established in the St. Johns River at seven sites to evaluate environmental variables and occurrence of PLS events. One site includes a floating, automated platform configured with continually recording sensors (NO_3, PO_4, relative fluorescence, salinity, temperature, DO, turbidity, currents, meteorological measurements, and other variables).

Although areas containing fish with UM do not appear to be correlated with areas where *Pfiesteria* has been found, we are investigating possible links between lesioned fish and other potentially toxic PLS. The potential role of bioactive compounds produced by PLS in the initiation of fish lesions, and particularly of UM, cannot yet be ruled out. However, the role of the fungus *Aphanomyces invadans* as a primary pathogen in the etiology of UM is almost conclusive (Kiryu et al. 2000). Although lesioned fish in Florida are often associated with a low incidence of myxosporean (primarily *Myxobolus* or *Kudoa*) or microsporean infestations in deeper-lying musculature, these parasites are not considered a primary cause of skin lesions. Studies are underway to determine if *Aphanomyces*, which are associated with lesions in fish in other areas of the United States and in the Far East, are also associated with UM in Florida's fish.

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PFIESTERIA SPP. AND "*PFIESTERIA*-LIKE ORGANISMS" IN SOUTH CAROLINA ESTUARIES

Alan J. Lewitus[1,2], J.M. Burkholder[3], C. Cary[4], H.B. Glasgow Jr.[3], K.C. Hayes[1], A.F. Holland[2], J.M. Law[5], and P.A. Rublee[6]
[1]Belle W. Baruch Institute, University of South Carolina, Georgetown, SC 29442
[2]Marine Resources Research Institute, SC DNR, Charleston, SC 29412-2559
[3]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27695
[4]College of Marine Studies, University of Delaware, Lewes, DE 19958-1298
[5]College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
[6]Biology Department, University of North Carolina at Greensboro, Greensboro, NC 27402-6174.

Pfiesteria piscicida, *P. shumwayae*, and *Cryptoperidiniopsis* spp. are present and predictably widespread in South Carolina estuaries. However, results from routine monitoring and fish kill or lesion event sampling have consistently indicated low abundances compared to estimates from similar programs in North Carolina and Maryland that sample areas with a history of *Pfiesteria* toxic activity. One of the areas targeted in the SC event response efforts is Bushy Park (upper Cooper River, Charleston), a site of annually recurrent menhaden lesions (peak of 25-30% frequency on captured fish in September or October). The finding that "*Pfiesteria*-like organism" (PLO) abundances were always low in samples collected during lesion events in Bushy Park suggested that other causative factors were responsible for lesion development. However, the involvement of *Pfiesteria* spp. in at least lesion initiation could not be discounted if a) the association between toxic *Pfiesteria* blooms and fish populations was short-lived, localized in space, or even intermittent, or b) toxic *Pfiesteria* amoebae were involved. In summer 2000, we expanded our efforts in Bushy Park to include tests of these hypotheses and other hypothetical causes of lesions (e.g. fungi, *Kudoa*). Results will be presented from samples collected prior to and during the lesion event, and analyzed for PLO abundance by automated sampling, and molecular probe identification and quantification of water column and sediment samples. These data will be correlated with findings from gross and histopathological examinations of fish collected at this site.

Although, based on the above conservative interpretation of 1998-1999 results, there is uncertainty regarding *Pfiesteria*'s potential involvement in SC fish events, no evidence supporting toxic activity of the organism in SC estuaries currently exists. Even if *Pfiesteria*-related problems in SC estuaries have occurred, it is clear that the dinoflagellate's impact on fish in SC is historically extremely minor compared to the situation in NC. We hypothesize that the reason why *Pfiesteria* is not abundant in SC estuaries is due to the low phytoplankton biomass that characterize these systems. For example, in a statewide assessment of SC estuaries, nearly 90% of chlorophyll *a* values in samples collected during the spring and summer were < 20 $\mu g\ l^{-1}$, indicative of oligotrophic-to-mesotrophic conditions, and only 2% exceeded 40 $\mu g\ l^{-1}$. In comparison, the Neuse and Pamlico Rivers, areas most commonly linked to *Pfiesteria* outbreaks, are characterized by annual chlorophyll *a* maxima typically > 40 $\mu g\ l^{-1}$, and spring mean values > 20 $\mu g\ l^{-1}$. Correspondingly, NO_3 concentrations also were generally much lower in SC estuaries. Based on the demonstrated positive relationship between PLO abundance, chlorophyll *a*, and inorganic nutrient concentrations (in laboratory experiments and Neuse River field correlations), we hypothesize that relatively low phytoplankton prey abundance in SC estuaries restricts PLO population growth.

Reports of HABs in SC estuaries are rare, contrasting strikingly with the situation in NC, where dinoflagellate red tides, cyanobacterial blooms, and outbreaks of *Pfiesteria,* have been frequently reported over the last decade, and are thought to be linked to high nutrient loading. If the lack of HAB problems such as *Pfiesteria* in SC is related to the relatively low impact of anthropogenically-related nutrient loading along the SC coast, then it follows that the threat of HABs to SC waters may increase

as nutrient inputs from coastal development continue to escalate (the SC coast is among the nation's fastest growing areas). To date, our general assessment is that, whereas *Pfiesteria* spp. are present and potentially widespread in SC estuaries, they typically are in low abundance. Although we cannot discount *Pfiesteria* as a cause of recurrent menhaden lesions in the Cooper River, there is no evidence to suggest their involvement in this or other SC fish kill or lesion events. We therefore consider SC estuaries as reference sites for comparison with more anthropogenically impacted estuaries where higher *Pfiesteria* abundances are typically found, and/or where toxic events have been documented.

LONG TERM OCCURRENCE PATTERNS AND DYNAMICS OF ICHTHYOTOXIC *HETEROSIGMA AKASHIWO* IN NARRAGANSETT BAY

Yaqin Li and Theodore J. Smayda
Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882

Long term patterns and trends in abundance of *Heterosigma akashiwo* in Narragansett Bay based on weekly observations from 1959 to 1996 are described. Large interannual variations characterized annual peaks and mean abundance levels. Since the high abundance years between 1959 to 1978, a significant decrease in mean annual abundance has occurred during 1979 to 1996. Despite these long term changes in abundance, its annual bloom cycle has remained characterized by two seasonal pulses: the first (usually larger) pulse usually lasts from late May to early July, and the second, lower pulse from October to November. Temperature appears to be a key factor in initiation of the first pulse. Seasonal and interannual variations in its abundance and bloom dynamics are not correlated with river flow, unlike elsewhere within its distributional range. Correlations between local abundance and the North Atlantic Oscillation Index suggest that *Heterosigma akashiwo* bloom dynamics in Narragansett Bay are influenced by large scale atmospheric patterns. This association and accompanying variations in irradiance and nutrient concentrations and in abundance of the competing diatom species, *Skeletonema costatum*, are described.

BENTHIC-PELAGIC COUPLING AND LI BROWN TIDE

Michael W. Lomas[1], Hugh L. MacIntyre[1], Jeffrey C. Cornwell[1] and Todd M. Kana[1]
[1]UMCES, Horn Point Laboratory, Cambridge, MD 21613, USA

Blooms of the Brown Tide organism *Aureococcus anophagefferens* have been intermittent in the coastal bays of Long Island during the past 15 years. Several hypotheses have been proposed to explain these bloom events, but no single unifying hypothesis has emerged that is widely supported. There are two general working hypotheses, one relating to 'top-down' control involving grazer avoidance and one relating to 'bottom-up' control involving regulation by nutrients. Our prior work on the Brown Tide phenomenon has been focused on bottom-up regulation of *Aureococcus*' photosynthetic physiology and its ability to utilize dissolved organic nitrogen (DON).

The standing stock of inorganic nutrients in the water column is low relative to the standing stock of particulate-bound phytoplankton nitrogen during *Aureococcus* bloom events. Nutrient inputs from the shallow sediments are likely to be important although little is known about sediment fluxes in brown tide waters, particularly with regard to the organic nutrient fluxes. This research program focuses on benthic-pelagic coupling in eastern Long Island bays. Specifically, we have hypothesized that the release of DON from sediments is a significant factor in selecting for the growth and dominance of *Aureococcus* in Long Island Bays. We have developed a conceptual benthic-pelagic model in which the dominance of system level primary production can switch between benthic primary producers (microphytobenthos, macroalgae, or submerged aquatic vegetation) and pelagic primary producers depending upon the distribution of energy (i.e. light) and nutrients in the water column and sediments. These two "states" are connected by feedback mechanisms that are driven by fluctuations in the physical nature of the system.

This model is being studied in selected embayments in eastern Long Island (Quantuck and Flanders Bays). Several sites within these ecosystems were compared in May, July, and September of 2000. Both Bays served as a nutrient trap as dissolved nitrogen concentrations increased 2-fold from May to July, driven solely by increases in organic nitrogen. In accordance with this observation, the planktonic community in both bays shifted to a more heterotrophic state in July associated with increased bacterial activity.

These bays differed substantially in terms of the underwater light environment. Quantuck Bay showed substantial increases in total underwater light attenuation from May to July, whereas in Flanders Bay, total light attenuation didn't change with season although the importance of various components of light attenuation varied. This disparity in seasonal water column light attenuation between Quantuck and Flanders Bays may well have a significant impact on the balance between water column and benthic primary production.

Only Quantuck Bay in July was found to have significant populations of *Aureococcus* (>72,000 cells/ml) coincident with a substantial increase in organic nitrogen and a shift to a pelagic dominated production system. Although no conclusions can be drawn as yet, differences in ecosystem functioning between Long Island Bays are consistent with our conceptual model and the blooming of *Aureococcus*.

THE POTENTIAL FOR SEDIMENT-WATER COLUMN INTERACTIONS TO STIMULATE GROWTH OF THE BLOOM-FORMING DINOFLAGELLATE *PROROCENTRUM MINIMUM*

Hugh MacIntyre[1], Jason Adolf[1] and Angela Dubois[2]
[1]Horn Point Laboratory, University of Maryland, PO Box 775, Cambridge, MD 21613, USA
[2]Bowdoin College, Brunswick, ME 04011, USA

Prorocentrum minimum is a dinoflagellate that regularly forms blooms in Chesapeake Bay. The factors that allow very dense blooms to last have not been fully elucidated. Observations of the 1998 bloom in the Choptank River suggest that it persisted in spite of a daily demand for nitrogen that was 30 - 795 times the size of the DIN pool and a demand for phosphorus that was 1 - 6 times the size of the DIP pool. A principal components analysis of the observations suggests that the environmental parameters varied in response to three dominant factors. These were interpreted as meteorologically-driven forcing of river flow; the density of the bloom itself and its effect on the light environment; and benthic resuspension. Corresponding physiological measurements indicated that diverse aspects of the photosynthetic apparatus responded to each of these factors. The high degree of correlation between physiological performance and presumed nutrient supply suggests that coupling between the water column and benthos may be important in supplying nutrients during periods when inputs from the river's source waters are low. Time-series measurements of water column characteristics at the same site showed a repeated pattern of resuspension associated with night-time shifts in wind direction and were consistent with the idea that benthic coupling may play a role in supplying nutrients.

We also present direct evidence that sediment-derived nutrients can enhance the growth rate of *P. minimum*. Sediment pore-water collected from 18 sites on the Delmarva peninsula increased nutrient-replete growth rates in axenic cultures of *P. minimum* by 33 - 93% over a control in mineral growth medium (f/10). The degree of enhancement varied with the concentrations of DOC and DON (which showed a high degree of covariation) and less strongly, with concentrations of NH_4^+.

These observations cannot demonstrate directly the use of benthic-derived nutrients by *P. minimum*. However, when taken together, they demonstrate that transport of particles (and presumably solutes) across the sediment-water interface occurs regularly; that benthic porewater can strongly stimulate growth of *P. minimum*; and that field observations of a bloom population's physiological responses are consistent with benthic coupling. Sediment-water column interactions may therefore play a role in bottom-up regulation of *P. minimum* blooms in shallow waters.

APPROACHES TO THE INVESTIGATION AND INTERPRETATION OF POSSIBLE *PFIESTERIA*-RELATED EVENTS IN MARYLAND

Robert E. Magnien[1], David M. Goshorn[1], David W. Oldach[2], Holly A. Bowers[2], and Torstein Tengs[2]
[1]Maryland Department of Natural Resources, 580 Taylor Ave., D-2, Annapolis, MD 21401
[2]University of Maryland School of Medicine, Baltimore, MD 21201

Pfiesteria was first documented in Maryland in 1992 (Lewitus et al., 1995. Estuaries 18: 373-378), but it was not until 1997 that it drew much public attention when several fish kills on Maryland's Lower Eastern Shore associated the dinoflagellate with fish health, fish mortality, and human illness (Gratten et al., 1998. Lancet 353: 532-539, MD DNR 1998). In consultation with external scientific panels and numerous local, state and federal agencies, Maryland responded with a conservative public policy that placed a priority on protecting human health, while recognizing that a number of scientific uncertainties still existed. Using multiple lines of evidence, the State temporarily closed several miles of tidal rivers in 1997 until the suspect conditions were not evident for a period of two weeks. This public policy continues and has remained flexible since the beginning to utilize the latest scientific understanding, measurement methods, and decision-making processes to assess the possibility that a toxic outbreak of *Pfiesteria* is occurring.

Since 1998, a system of intensive environmental and fish community monitoring has been established in Maryland. These "comprehensive assessments" are used as a surveillance system for possible outbreaks and to refine our understanding of the factors that may lead to the occurrences of toxic or non toxic *Pfiesteria* in association with fish health/mortality. Additionally, a "rapid response" capability has been established to draw upon a pool of trained biologists that can be deployed within hours to investigate suspicious findings. In most cases, these findings involve fish kills or the presence of diseased fish, primarily Atlantic menhaden with ulcerative lesions. These two monitoring programs are being coordinated with a number of *Pfiesteria*-related research studies, including those investigating links to environmental conditions, fish health, and human health, and those pursuing more precise and rapid techniques for the identification of *Pfiesteria* and its toxin(s).

Maryland has employed the presence of unexplained fish lesions as one of the indicators to pursue more targeted investigations of *Pfiesteria* and associated environmental factors. Evidence of active, toxic *Pfiesteria* was documented at the sites of the 1997 fish lesion / mortality events, and the presence of non toxic *Pfiesteria* has been documented at the site of almost every major menhaden lesion outbreak that has been investigated from 1998 to 2000. Although the relationships between lesioned menhaden and *Pfiesteria* remain areas of active research, in the absence of rapid and definitive screening methodologies for potential toxic outbreaks, fish health remains a viable, albeit imperfect, indicator of possible *Pfiesteria* activity. Human health concerns can also be used to trigger an investigation.

The interpretation of data collected during a rapid response investigation is challenging because it is currently impossible to measure *Pfiesteria*-related toxin(s) directly, and river closure decisions must be made quickly (24 -72 hrs.). Thus, multiple lines of evidence are used in a "weight of evidence" approach using measurements in the field and results from multiple laboratories. One of the most valuable additions to our measurement suite is the rapid (less than 24 hrs) molecular probe technology that has recently been developed (Oldach et al., 2000. PNAS 97(8): 4303-4308) and has been incorporated into our monitoring program starting in 1999. If no *Pfiesteria* genetic material is detected with these molecular techniques, we assume that the probability of toxicity due to *Pfiesteria* is very low. An actively toxic event is also ruled out if there are no obvious signs of distressed behavior in resident fish or a significant fish kill in progress. Finally, an actively toxic event is ruled out if the counts of *Pfiesteria*-like cells are below those reported in the literature to cause toxic impacts to fish.

Thus, all three conditions described above - presence of *Pfiesteria* genetic material, fish in distress or fish kill, and high cell counts - are necessary to consider any event as potentially toxic.

RESULTS OF A SERIES OF FISH BIOASSAYS WITH THE TOXIC DINOFLAGELLATE *PFIESTERIA PISCICIDA*

Harold G. Marshall[1], Andrew S. Gordon[1], David W. Seaborn[1], Brian Dyer[1], William M. Dunstan[2], and A. Michelle Seaborn[2]
[1]Dept. Biological Sciences, [2]Dept. of Ocean, Earth, and Atmospheric Science, Old Dominion University, Norfolk, VA 23529-0266

A series of fish bioassays were conducted to test the ichthyotoxic activity of a toxic strain of the dinoflagellate *Pfiesteria piscicida* using tilapia, with *P. piscicida* producing fish deaths in 9 of 10 of these experimental bioassays. Once toxicity was established, *P. piscicida* maintained this toxicity causing fish deaths in the culture vessels as long as the dead fish were replaced with live fish. In addition, this toxicity was perpetuated following a series of inoculations to other culture vessels. Among these bioassays, there were differences in the period between inoculation of *P. piscicida* cells and the onset of fish deaths. Using *P. piscicida* from a culture that had previously been maintained on algal cells (*Cryptomonas* sp.) it took 16 days before fish deaths occurred. In contrast, fish deaths occurred within hours when using a culture that had recently (previous day) killed fish. Initial inoculations of live *P. piscicida* into these bioassays was ca. 50-60 zoospores mL^{-1}, with approximately a 10-fold increase in numbers within the culture vessels before the first fish death occurred, with later concentrations at >5,000 zoospores mL^{-1} during periods of highly active toxicity. Throughout these bioassays the control fish remained healthy, with only one death occurring among 90 fish. In contrast, 100's of fish in the culture vessels of the bioassays that were exposed to the toxic *Pfiesteria* died during the study.

There were no discernable differences in the microflora/fauna and bacterial populations in the control versus the bioassay vessels, with protozoan ciliates rarely detected, and autopsies of moribund fish from the bioassay facility indicated a general lack of bacterial infection. Neither oxygen or ammonia levels were determined to be factors in the fish deaths.

Fish bioassays (using tilapia) were also conducted to determine toxicity of the Pfiesteria-like organisms *Cryptoperidiniopsis* sp. and *Gyrodinium galatheanum*. There were no fish deaths associated with these species over periods of 10-14 weeks, but at concentrations that did not exceed 800 zoospores mL^{-1}. These may have been non-toxic strains of these species, or higher cell concentrations may be needed to produce fish toxicity (e.g. 115,000 mL^{-1}, Nielsen, 1993).

The toxic *Pfiesteria piscicida* cultures were provided by Dr. J. Burkholder and Dr. H. Glasgow (NCSU) for these studies. Clonal cultures of *Cryptoperidiniopsis* sp. and *G. galatheanum* were developed from sediment samples taken in Virginia estuaries. Prior to and after this series of bioassays took place, cells from these cultures were identified by SEM plate analysis and cross-confirmed by two outside laboratories using gene sequencing protocols.

ALGICIDAL BACTERIA ACTIVE AGAINST *GYMNODINIUM BREVE*: USE OF MOLECULAR TECHNIQUES TO ASSESS CHANGES IN MICROBIAL COMMUNITIES FOLLOWING THE INTRODUCTION OF BACTERIA

Xavier Mayali[1,2] and Gregory J. Doucette[2,3]
[1]Grice Marine Laboratory, University of Charleston, Charleston, SC 29412
[2]Marine Biotoxins Program, NOAA/NOS/CCEHBR, Charleston, SC 29412
[3]Marine Biomedical and Environmental Sciences, Medical University of South Carolina, Charleston, SC 29412

A growing number of studies have suggested that algicidal bacteria may play a role in naturally regulating the development and termination of harmful algal blooms (HABs). Interest in such microbes has been further enhanced by their potential use as part of a HAB management strategy. Our laboratory has isolated two bacterial strains from the west Florida shelf that are lethal to *Gymnodinium breve*, a bloom-forming, toxic dinoflagellate responsible for severe economic losses in this region through its impacts on fisheries and tourism.

Phylogenetic analysis of the entire 16S rDNA sequence for both algicidal bacteria indicates that one strain (41-DBG2) is most closely related to *Cytophaga latercula* (92% similar) within the Cytophaga-Flavobacterium-Bacteroides (CFB) phylum, while the other strain (ANSW2-2) is most closely related to *Alteromonas macleodii* (99% similar) of the γ-Proteobacteria. We have analyzed available sequences for algicidal bacteria isolated from different locations and found that most cluster together within these two taxa. Our results indicate that algicidal bacteria from each of these two phylogenetic groups may be descendents of an ancestor that could have evolved the means to exploit algal-derived organic matter.

Fluorescent in-situ hybridization (FISH) with strain-specific oligonucleotide probes is currently being used to enumerate algicidal bacteria in laboratory time-course experiments, as well as for their detection in field populations. In addition, denaturing gradient gel electrophoresis (DGGE) is being used to assess changes in microbial communities associated with *G. breve* following the introduction of algicidal bacteria to algal cultures. Use of these two complementary techniques have indicated the following in experiments employing algicidal bacterium strain 41-DBG2:

1. Following inoculation into bacteria-containing *G. breve* cultures at 10^3 cells/mL, 41-DBG2 reaches densities of over 10^6 cells/mL within 2-3 days, after which its growth rate declines markedly. Little decrease in numbers occurs within 10 days of inoculation, suggesting that conditions remain favorable for the subsistence of 41-DBG2 over the short-term.
2. Total bacterial numbers increase as *G. breve* cells die and lyse, due likely to the release of organic nutrients from the algal cells into the medium.
3. The microbial assemblage associated with *G. breve* changes following the addition of 41-DBG2, with some phylotypes disappearing and others appearing throughout the killing event.
4. Algicidal activity of introduced bacteria seems to vary according to the target alga's physiological status, with resistance to attack decreasing with declining algal growth rate.

Our data suggest that algicidal bacterium 41-DBG2 may be able to fill a vacant niche (or displace another organism from its niche) within a *G. breve* culture, quickly reaching a high density and eventually causing death of the culture. Use of the above molecular approaches will aid in determining whether a similar process occurs over the course of a natural *G. breve* bloom.

MODELING *ALEXANDRIUM* SPP. BLOOMS IN THE GULF OF MAINE

Dennis J. McGillicuddy, Jr.[1] , Richard Signell[2], Charles Stock[1], Daniel R. Lynch[3] and Andrew Thompson[3]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]U.S. Geological Survey, Woods Hole, MA 02543
[3]Thayer School of Engineering, Dartmouth College, Hanover, NH 03755

Convolution of coastal circulation with the population dynamics of *Alexandrium* spp. creates enormously complex patterns in the abundance of this organism. Herein we attempt to diagnose the physical-biological interactions that control *Alexandrium* blooms using coupled models together with observations from ECOHAB-GOM and prior studies. One of the main gaps in knowledge identified in earlier work was the lack of information about the source function for the input of new cells into the water column. Two new data sets relevant to this issue have emerged from early ECOHAB-GOM results: (a) surveys of the cyst distribution, and (b) laboratory work which has documented the functional dependence of germination on environmental parameters such as light and temperature. Use of this information has led to an improvement in our ability to model the observed distributions of *Alexandrium* spp. observed in 1993, as compared with earlier simulations based on a riverine input (Franks and Signell, 1997). In particular, cell concentrations in the vicinity of Casco Bay are much more realistic. Further downstream, the differences are less pronounced. From these simulations, it appears that the non-linear response of the river plume to wind forcing provides a mechanism for cross-isobath transport of *Alexandrium* cells. Under upwelling conditions, the plume thins and extends far offshore where it is inoculated by upward-swimming cells that germinated from the offshore cyst bed. When the winds shift to favor downwelling, the plume thickens and moves onshore, thereby exposing the coast to high concentrations of *Alexandrium*.

Aspects of the large scale ECOHAB-GOM surveys have been examined using finite-element particle tracking simulations based on the climatological circulation fields for the region. The synopticity of the 1998 surveys was evaluated by adjusting the station positions for advection by the mean flow and then comparing distribution maps with those created using the original station locations. Although some differences are visible, the main features of the observed *Alexandrium* distribution appear to be robust with respect to the mean advective transport. Additional particle tracking simulations examine the potential source regions for the peaks in distribution observed in the interior of the Gulf of Maine. These suggest that Penobscot Bay and its estuaries to its west made little contribution to the observed peaks. Areas surrounding Mt. Desert Island and Grand Manan Island were possible sources of cells, while the Bay of Fundy appears to provide a source to the western Gulf. Inflow from the Scotian Shelf is another potentially important pathway.

Work is underway to test and expand upon the pathways identified by the climatological modeling using time dependent simulations for the 1998 and 2000 field years over the entire Gulf of Maine. Of particular interest is the linkage between *Alexandrium* populations in the eastern and western gulf. Initial simulations show clear transport events that bridge the two regions, and future simulations will test the conditions under which the eastern population might seed the western gulf blooms.

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PRELIMINARY CHARACTERIZATION OF "CRYPTOPERIDINIOPSOID" CULTURES ISOLATED FROM FLORIDA

Steve L. Morton[1], Tina Mikulski[1], Elizabeth R. Fairey[1], Brad Mitchell[1], Peter D.R. Moeller[1], Bill Richardson[2], Karen Steidinger[2], and John Ramsdell[1]
[1]Marine Biotoxin Program, NOAA/NOS, Center for Coastal Environmental Health and Biomolecular Research, 219 Ft. Johnson Rd. Charleston, SC 29412
[2]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Ave. SE, St. Petersburg, FL 33712

Cultures of different species of "Cryptoperidiniopsoid" dinoflagellates were grown under controlled conditions. Each culture was identified via scanning electron microscopy at the Florida Marine Research Institute before shipment to the Marine Biotoxin Program. Each strain was re-identified after mass culture and toxin analysis. Strains were grown in 100 L batches cultures and harvested at late-log growth phase. Production of biological active substances by each culture was examined from both resulting cell mass and spent culture medium. Both cell mass and spent culture medium were passed though a silica column and eluted with an elutropic solvent series. Totals of 5 samples were collected for both the cell mass extract and spent culture medium. Each of the 10 extracts was tested for the possibility of bioactivity using both live assays and cell based assays. Live bioassays included brine shrimp and sheepshead minnows while cell based assay included the GH4C1 cytotoxicity assay. Solvent fractionation yielded several fractions that were active. A non-polar fraction was active on the shrimp bioassay and the sheepshead minnow bioassay. Subsequent structural analysis of this fraction showed this activity in part was due to DEHP, a man-made phthalate ester. This and other fractions are still under pharmacological characterization. A polar fraction was active on the brine shrimp bioassay and the cytotoxicity assay but was inactive on the sheepshead minnow assay. This data provides initial evidence of bioactive substances from cultures of Cryptoperidiniopsoid. Whether this organism produces a toxic substance is presently unknown and will require future pharmacological and chemical investigations.

AMINO ACID OXIDATION AND PEPTIDE HYDROLYSIS IN POPULATIONS SEASONALLY DOMINATED BY *AUREOCOCCUS ANOPHAGEFFERENS*

Margaret R. Mulholland[1], Christopher Gobler[2] and Cindy Lee[1]
[1]Marine Sciences Research Center, SUNY Stony Brook, Stony Brook, NY 11794-5000
[2]Southampton College, Long Island University, Southampton, NY 11968

Previous studies have demonstrated that the Brown Tide species *Aureococcus anophagefferens* can use dissolved organic nitrogen (DON) to meet its N demand when growing under bloom conditions. Further, elevated levels of DON relative to DIN may create conditions favorable for bloom initiation. Recent results suggest that dissolved organic material (DOM) can be used not only as an N source but as a C source by *A. anophagefferens*; cells can thereby augment autotrophic metabolism with heterotrophy. To evaluate the relative importance of organic and inorganic nutrients to the growth of *A. anophagefferens* and associated picoplankton relative to co-occurring phytoplankton, we are conducted a seasonal study in which we measured inorganic and organic N uptake, organic C uptake, *A. anophagefferens* abundance, and rates of peptide hydrolysis and amino acid oxidation in size-fractionated samples from Quantuck Bay, Long Island. We found that rates of amino acid oxidation and peptide hydrolysis increased between April and June as Brown Tide populations developed and inorganic N sources were depleted. However rates decreased in July when Brown Tide populations collapsed. Much of the amino acid oxidase activity in June, when brown tide was present at about 350,000 cells ml^{-1}, was in the bacterial size fraction (< 1.2 μm) while the bulk of the peptide hydrolysis was in the < 5.0 μm size fraction. As seasonal Brown Tide populations developed, N uptake rates also increased; the < 5.0 μm size fraction accounted for most of the N uptake in May and June.

When dissolved inorganic N (NH_4^+ or NO_3^-) and organic compounds with different N contents (urea, glutamate and glucose) were added to incubations of natural populations, rates of extracellular enzyme activity and N and C uptake were differentially affected among size-fractions, probably as a result of relative differences in the growth stimulation among bacteria, picoplankton, and larger phytoplankton. Virtually all of the peptide hydrolysis was always accounted for in the bacterial (< 1.2 μm) and Brown Tide (< 5.0 μm) size fractions. The effect of N and C additions among size fractions shifted seasonally as did population structure and the availability of combined N sources. Our results suggest that seasonal changes in extracellular enzyme activity and N and C uptake in response to nutrient additions may reflect, 1) the degree to which C or N limits growth in various size-fractions and 2) competition among organisms for limiting nutrients.

We conclude that the relative availability of DIN, DON and DOC may be important in determining the dominant metabolism (autotrophy vs. heterotrophy) of *A. anophagefferens*. In addition, seasonal shifts in population structure affect dominant pathways through which organic material is cycled.

ANTILLATOXIN, A NOVEL NEUROTOXIN FROM THE MARINE CYANOBACTERIA *LYNGBYA MAJUSCALA,* IS A POTENT ACTIVATOR OF VOLTAGE-GATED Na^+ CHANNELS

Thomas F. Murray[1], W.I. Li[1], F.W. Berman[1], T. Okino[2] and W.H. Gerwick[2]
[1]Department of Physiology and Pharmacology, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602
[2]College of Pharmacy, Oregon State University, Corvallis, OR 97331

Lyngbya majuscala is a pantropical marine cyanobacteria whose blooms are associated with adverse impacts on human health. These acute adverse effects include severe contact dermatitis, eye irritation and asthma-like respiratory distress. Antillatoxin is a novel lipopeptide derived from *Lyngbya majuscula* that produces acute neuronal injury and death in primary cultures of rat cerebellar granule neurons. This neurotoxic response is prevented by coapplication of either antagonists of the NMDA subtype of glutamate receptor or the blocker of voltage-dependent Na^+ channels, tetrodotoxin (TTX). Moreover, TTX also antagonized antillatoxin-induced Ca^{2+} influx in cerebellar granule neurons. To further assess the interaction of antillatoxin we used [^{3}H]batrachotoxin A 20-α-benzoate (BTX-B) as a probe for neurotoxin site 2 on Na^+ channel alpha subunits expressed in intact cerebellar granule neurons. [^{3}H]BTX-B specific binding to Na^+ channels was enhanced by 100 nM brevetoxin (PbTx-1) and 10 μM deltamethrin. Similar to the influence of PbTx-1 and deltamethrin antillatoxin stimulated [^{3}H]BTX-B binding to intact neurons. When combined with PbTx-1, antillatoxin produced a more robust stimulation of [^{3}H]BTX-B binding than did deltamethrin and PbTx-1. The [^{3}H]BTX-B affinity for Na^+ channels in cerebellar granule neurons was 38.5 nM in the presence of PbTx-1 and deltamethrin, while in the presence of PbTx-1 and antillatoxin the [^{3}H]BTX-B affinity increased to a Kd value of 8.9 nM. Antillatoxin caused a 4-fold increase in $^{22}Na^+$ influx in intact neurons which was completely blocked by TTX. These data suggest that antillatoxin is a novel activator of voltage-gated Na^+ channels.

PHYSIOLOGICAL DIAGNOSTICS AND BEHAVIOR OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE,* IN CASCO BAY, MAINE – EVIDENCE OF NITROGEN LIMITATION

Nicole J. Poulton[1], J. Geoff MacIntyre[2], John J. Cullen[2], and Donald M. Anderson[1]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02540, USA
[2]Department of Oceanography, Dalhousie University, Halifax, NS B3H 4JI, Canada

One challenge in phytoplankton ecology is to measure species-specific physiological responses to changes in environmental conditions. Of particular importance are harmful algal bloom (HAB) species such as the toxic dinoflagellate *Alexandrium fundyense* which typically inhabit coastal regions where they are usually not dominant. Within the Gulf of Maine, environmental factors, specifically nitrogen, are likely to be a controlling factor for *A. fundyense* blooms. Therefore, the ability to ascertain the nutritional status of this species in field assemblages in critical to understanding its bloom dynamics.

Since *A. fundyense* usually inhabits coastal areas that are frequently limited by nitrogen, behavioral adaptations and intracellular responses to nitrogen availability are a primary consideration. It was therefore desirable to identify diagnostic indicators and behavioral adaptations of *A. fundyense* to nitrogen stress. Using laboratory water columns, nitrogen (N)-starved batch cultures, and N-limited, semi-continuous cultures, indicators of different N-nutritional states were identified. It was determined that low N concentrations in the surface of a mesocosm did not induce a Casco Bay *A. fundyense* isolate to vertically migrate to deep nutrient pools. Prolonged N-stress caused dramatic changes intracellular biochemistry, specifically chlorophyll a, carbohydrate, and protein content, as well as C:N, toxin content and composition. Ratios of different toxin derivatives were identified that increased with increasing N-stress and appear to be sensitive and robust indicators of N-status.

Once indicators were developed for N-stress, variability in toxin content and composition were examined in the coastal waters of Casco Bay, Maine during an *A. fundyense* bloom in the spring of 1998. Over the course of the field season, toxin compositional changes occurred that were generally consistent with increasing levels of N-stress as the bloom progressed and N levels decreased. As shown in N-limited culture, large increases in some toxin ratios (e.g., GTX1,4:STX and NEO:STX) were observed during the latter portion of the field season, coinciding with low N:P ratios and undetectable levels of dissolved inorganic nitrogen in ambient waters. Overall, the toxin compositional trends were quite remarkable and suggest that this approach may provide valuable species-specific physiological information without the need for elaborate cell separation schemes such as flow cytometry or immunomagnetic bead sorting. Further laboratory studies are underway to better characterize the toxigenic response of *A. fundyense* isolates to environmental stresses before this suite of toxin indicators can be considered robust.

CHARACTERIZATION OF A PUTATIVE TOXIN PRODUCED BY *PFIESTERIA PISCICIDA*

J.S. Ramsdell,[1] P.D.R. Moeller,[1] E.R. Fairey,[1] A.C. Melo,[1] K.L. Kimm-Brinson,[1] B. Mitchell,[1] S.A. Morton,[1] N. Deamer-Melia[2] , H.B. Glasgow[2], and J.M. Burkholder[2]
[1]Marine Biotoxins Program, NOAA-National Ocean Service, Charleston, NC 29442
[2]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

The health hazards attributed to *Pfiesteria piscicida* point to the need to characterize bioactive substances capable of causing adverse effects produced by this organism. Extracts of *Pfiesteria* cell mass as well as filtered culture water have been chromatographically partitioned in reproducible fashion, yielding fractions that demonstrate biological activity. These active fractions contain substance(s) that induce cytotoxicity in GH_4C_1 rat pituitary cells and at non-toxic concentrations induce a c-fos luciferase reporter-gene. The pharmacologic activity of a putative toxin (pPfTx) produced by *P. piscicida* has been examined by characterization of the signaling pathways that induce the c-fos luciferase construct in GH_4C_1 rat pituitary cells. A class of purinegic receptors mediates this c-fos pathway with analog selectivity and functional ionic conductances including elevated cytosolic free calcium and enhanced YOPRO-membrane permeability, consistent with a purinergic receptor of the P2X7 class. The irreversible P2X7 antagonist, adenosine 5'-triphosphate-2',3'-dialdehyde, was used to demonstrate that the pPfTx requires this pathway for activation. P2X7 receptors are found predominantly on myeloic cells including mature macrophages, mast cells and microglial cells. A role of P2X7 receptors in the action of pPfTx is of interest, in consideration of the fact that this toxic dinoflagellate has been reported to cause a range of health impacts in both finfish and humans. The effects linked to *Pfiesteria* toxicity may be related to an inflammatory response, either in macrophages in the periphery or microglia in brain tissue. Implication of P2X7 receptors as a potential target for the bioactive substance produced by toxic *P. piscicida* provides a common basis for the investigation of symptoms that previously have been regarded as unrelated, such as ulcers in menhaden and cognitive dysfunction in humans.

DEVELOPMENT AND TESTING OF MOLECULAR DIAGNOSTICS FOR *PFIESTERIA*-LIKE ORGANISMS IN LABORATORY AND ENVIRONMENTAL SAMPLES

Kimberly S. Reece[1], Nancy A. Stokes[1], Wolfgang K. Vogelbein[1], Wayne L. Litaker[2], Jeffrey D. Shields[1], Larry W. Haas[1], Patrice L. Mason[1], Victoria M. Foster[1] and Eugene M. Burreson[1]
[1]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062
[2]Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, NC 27599

Currently the only reliable method to accurately identify and distinguish *Pfiesteria piscicida* and other *Pfiesteria*-like organisms (PLOs) is through 3-D reconstructions of the thecal plate structures by scanning electron microscopy (SEM). We are doing a comprehensive survey of DNA sequences for the internal transcribed spacer (ITS) region and portions of both the small (SSU) and large (LSU) subunit genes of the ribosomal DNA complex for available PLO clonal cultures. Sequence information is being used for phylogenetic studies and to develop DNA-based diagnostics. Sequence comparisons among the PLO cultures and to other dinoflagellates and protozoans allowed design of species–specific and genus–specific PCR primers and DNA probes. Clonal cultures of *Pfiesteria piscicida*, *Pfiesteria* sp. "B", *Cryptoperidiniopsis* spp., "Shepherd's crook" and "Lucy-like" PLOs were first analyzed and identified by SEM and then obtained for molecular analysis. DNA was isolated from a total of 18 clonal PLO cultures and 3 food source cultures. DNA sequences were obtained from the food sources to assure that PLO, rather than food source DNA clones, were selected for analysis. SSU, ITS and LSU DNA sequences from the PLOs were aligned and subjected to phylogenetic analyses. The SSU gene was highly conserved while the ITS region (excluding the 5.8S portion) and portions of the LSU gene fragment demonstrated considerable sequence variation. Comparison of DNA clone sequences obtained from clonal cultures demonstrated degenerate sites in the ITS region and the LSU gene and even a few in the SSU gene. Overall the molecular data strongly supported identifications made by SEM. Phylogenetic analyses grouped together all the cultures identified as *P. piscicida* and placed *Pfiesteria* sp. "B" between the *Pfiesteria piscicida* clade and *Cryptoperidiniopsis* spp. In addition, cultures identified by SEM as "Lucy" or "Lucy-like", grouped together in the DNA-based phylogenies.

Sequence alignments and comparisons were used to design four sets of PCR primers to specifically amplify *P. piscicida*, *Pfiesteria* sp. "B", *Cryptoperidiniopsis* spp. or the "Lucy" group clonal culture DNAs. PCR primers and reaction conditions were optimized for specificity with clonal culture DNAs and then tested for their ability to amplify these DNAs from water and sediment samples. In addition, specific DNA probes were designed for in situ hybridization. Samples from fish bioassay tanks and the environment have been tested with the DNA probes and PCR primers. Results of molecular diagnostic assays agreed with SEM identifications and PCR assays confirmed the presence of PLO species detected by in situ hybridization. For example, the *Pfiesteria* sp. "B" probe hybridized to dinoflagellate cells found in the lateral line canal and alimentary tract of fish from an experimental tank with high cell counts of *Pfiesteria* sp. "B". DNA probe analyses indicated that dinoflagellates found in the alimentary tracts of menhaden collected from two sites in North Carolina were "Lucy-like" cells. PCR amplification of DNA from water samples collected at the same sites as these menhaden indicated the presence of "Lucy-like" DNA.

PFIESTERIA FIELD ECOLOGY AND TOXIC ACTIVITY: TRENDS FROM A DECADE OF INTENSIVE STUDY IN NORTH CAROLINA ESTUARIES

R. Reed,[1] H. Glasgow[1], J. Burkholder,[1] N. Deamer-Melia[1] and M. Mallin[2]
[1]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606
[2]Center for Marine Sciences Research, UNC-Wilmington, Wilmington, NC 28409

Our research team has amassed a decade of data on toxic *Pfiesteria* outbreaks, extending back to those first documented in 1991. During that time, we have tracked 88 toxic *Pfiesteria* outbreaks in North Carolina waters, most of which affected the Albemarle-Pamlico. This system is the second largest estuary on the U.S. mainland, and is regarded as the most important fish nursery ground on the U.S. Atlantic Coast. Toxic *Pfiesteria* outbreaks have been implicated as the primary cause in the death of well over 1 billion fish, 90% as Atlantic menhaden but also including southern flounder, spot, croaker, striped bass, American eel, and other species.

The ongoing, long-term study has included special focus on the mesohaline Neuse Estuary as the most active system for toxic *Pfiesteria* outbreaks. For the past eight years, the sampling program has included 8 stations weekly and 16 biweekly, with additional sampling during major storm events. Through use of boat-mounted ADCP to obtain improved flow data, and high-frequency sampling to obtain improved water quality data, we have determined that P loading to the mesohaline Neuse has decreased while N loading has significantly increased (especially TN_i, by ca. 40%). We also recently installed a series of seven automated platform stations in the mesohaline Neuse, with maintenance of the stations at $\leq$ 3-day intervals. These stations can measure physical, chemical, and biological conditions hourly in depth profiles automated samplers, with real-time data transmitted to a freely accessible website. The stations have been strategically positioned in 'hot spots' for major fish kills (related to *Pfiesteria*, low oxygen stress, and other factors) so that we can strengthen acquisition of 'before' and 'during' data needed to improve diagnosis of the causative factors leading to fish kills.

The extended period encompassed by this dataset has enabled us to construct a conceptual model of *Pfiesteria* seasonal dynamics in relation to various environmental factors, based on statistically significant interactions from trend analysis. For example, in the Pamlico Estuaries where P loading has decreased by ca. 40%, toxic *Pfiesteria* outbreaks have significantly declined in frequency and duration. On the basis of archived sample analysis with molecular probes that recently have become available, P decline with concomitant N increase has coincided with an apparent shift at *Pfiesteria*-related fish kills from clear dominance by *P. piscicida* to occasional co-dominance by *P. piscicida* and *P. shumwayae* sp. nov. These data support laboratory experiments that have shown comparatively higher P stimulation of *P. piscicida* zoospores, and higher N stimulation of *P. shumwayae*. The conceptual model is guiding collaborative research in progress to construct a quantitative, predictive model of *Pfiesteria* abundance and toxic activity.

This dataset has also enabled detection of a significant effect of high-intensity-storm years on subsequent toxic *Pfiesteria* activity. For 1-2 years following a year with 2-3 hurricanes or severe tropical storms that have passed through North Carolina, toxic *Pfiesteria* outbreaks affect relatively few fish, in comparison to the number of fish affected in the year preceding the high-intensity storms. This trend apparently is related to flooding displacement of resident *Pfiesteria* populations down-estuary to less conducive areas for toxic activity. In addition and with laboratory findings in support, the history of recent toxicity apparently is an important factor influencing subsequent toxicity. Populations that have been engaged in fish-killing activity in the previous season likely are more prone to become actively toxic in the next growing season than populations that have not been in fish-killing mode.

DISTRIBUTION OF *PFIESTERIA* SPECIES: COMPARISON OF RESULTS FROM WATER AND SEDIMENT SAMPLES ACROSS MULTIPLE SCALES, 1998-2000

Parke A. Rublee[1], Eric F. Schaefer[1], Coy Allen[1], Janera Harris[1], Holly Bowers[2], Torstein Tengs[2], and D.W. Oldach[2]
[1]Biology Department, Univ. North Carolina at Greensboro, Greensboro, NC, 27412
[2]Inst. Human Virology, Univ. Maryland, Baltimore, MD 21201

We have used PCR methods for the detection of *Pfiesteria* species from states along the US East and Gulf coasts since 1988. During the first year we tested only for *P. piscicida.* In 1999, we began testing for *P. shumwayae* as well as *P. piscicida*, and we began testing sediments as well as water column samples. During summer and fall of 2000, we tested water and sediment samples collected simultaneously from multiple East and Gulf coast sites. The distribution of *P. piscicida* detected in our studies ranged from New York to Texas. The distribution of *P. shumwayae* appears to be similar. During the summer and fall of 2000 we found a much higher incidence of *P. shumwayae* than *P. piscicida*.

On a fine scale, water and sediment samples are differential indicators of *Pfiesteria* spp. activity. Positive water samples indicate active, though not necessarily toxic, populations of zoospores and/or amoebae at a site. In contrast, sediment samples are likely a better indicator of endemic populations which can serve as the inoculum for planktonic populations, or they may represent residual populations from an earlier event. For example, in one case, a water sample collected from a reported fish kill site, but collected after extensive rainfall, was found to be negative, while a sediment sample collected within a week from the same site tested positive. In many cases we found positive indications of *Pfiesteria* spp. in sediment samples when there was no signal detected in the overlying water. This was further confirmed when we studied fine scale distribution of *Pfiesteri*a spp. at sites in the Neuse River, NC where frequent fish kill and lesion events have occurred.

On a regional scale, a major factor affecting the distribution of *Pfiesteria* sp. appears to be tidal flushing of estuarine areas, consistent with previous observations that most fish kill or lesion events occur in poorly mixed waters and that rainfall or storms can dissipate such events rapidly. Overall, results continue to suggest that *Pfiesteria* spp. are a widespread and probably common member of estuarine benthic and planktonic communities.

TRACE METALS AND *PSEUDO-NITZSCHIA* BLOOMS: A POSSIBLE ROLE FOR THE TOXIN DOMOIC ACID

Eden Rue[1], Maria Maldonado[2], Ken Bruland[1] and Mark Wells[2]
[1]Institute of Marine Sciences, 1156 High Street, University of Ca, Santa Cruz, CA 95064
[2]School of Marine Sciences, U. of Maine, Orono, ME 04469

Toxigenic species of the pennate diatom *Pseudo-nitzschia* can produce domoic acid, an analog of the excitatory neurotransmitter glutamate and a known causative agent of the human illness amnesic shellfish poisoning (ASP). Although the trophic transfer of this phycotoxin has resulted in mass marine bird and mammal mortality, the physiological role of domoic acid to the causative organism is still unknown. The similarity in chemical structure of domoic acid to other phytosiderophores suggests a role for domoic acid as a trace metal chelator. Using a highly sensitive adsorptive cathodic stripping voltammetric technique, we found that domoic acid forms strong chelates with iron and copper, having conditional stability constants of $K^{cond}_{FeDA,Fe(II)'} = 10^{8.7 \pm 0.5} M^{-1}$ and $K^{cond}_{CuDA,Cu(II)'} = 10^{9.0 \pm 0.2} M^{-1}$. Certain species may therefore produce domoic acid to selectively bind trace metals in order to either increase the availability of an essential micronutrient such as in the case of iron, or to decrease the availability of a potentially toxic trace metal, such as in the case of copper. The combination of binding strength and the potential production/release rates of domoic acid raises the possibility that domoic acid significantly alters the chemical speciation, and thus the availability, of both iron and copper in seawater. In addition, domoic acid may be particularly important in solubilizing particulate iron suspended in these coastal waters, where *Pseudo-nitzschia* blooms tend to occur.

To further investigate why *Pseudo-nitzschia* species produce domoic acid and the possible role of trace metals in this production, *Pseudo- nitzschia* sp. isolated from Monterey Bay were grown in a chemically well defined artificial seawater media, under trace metal clean conditions. This allowed us to reproducibly induce iron-limitation (μ = 50% μmax) and copper-toxicity (μ = 30–50% μmax) in the cultures. We found that under iron and copper stressed conditions (but macronutrient replete conditions) the rates of domoic acid production/release by the cells were significantly increased. However, the particulate (non-released domoic acid) production rate of domoic acid remained essentially unchanged between these differing metal-containing culture conditions during log phase growth, resulting in the ratio of extracellular to intracellular domoic acid production rates to be 20 x higher under iron-limited conditions! These data show that domoic acid is being released to the surrounding medium under iron stress. To determine if domoic acid release affected metal acquisition by the cells, we measured iron uptake rates with and without domoic acid in solution. Iron uptake rates for *Pseudo-nizschia multiseries* (domoic acid producer) and *Pseudo-nizschia pungens* (non-domoic acid producer) were consistently faster (3 x) when domoic acid was present in solution. Our findings indicate that the physiological role of domoic acid for toxigenic *Pseudo-nizschia* species is involved with bioactive metals, and that the domoic acid production is tied closely to the acquisition (iron) or detoxification (copper) of metals in coastal waters.

INTERACTIONS BETWEEN *PFIESTERIA* AND REPRESENTATIVE SPECIES OF COMMERCIALLY VALUABLE SHELLFISH

S. Shumway,[1] J. Springer,[2] J. Burkholder[2] and H. Glasgow[2]
[1] Natural Sciences Division, Southampton College – LIU, Southampton, NY 11968
[2] Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

In response to substances in finfish excreta/secreta, species of the toxic *Pfiesteria* complex pro-duce bioactive substances that can cause finfish death and disease. As a unique trait among toxic dinoflagellates, they are also known to exhibit direct attack behavior toward live finfish, but interactions between *Pfiesteria* and shellfish have not been intensively examined. In a series of con-trolled laboratory trials, we assessed the response of representative adult and pediveliger shellfish to zoospores of *Pfiesteria piscicida*. In addition, we examined attraction of *Pfiesteria* zoospores and amoebae to shellfish tissues, behavioral responses of *Pfiesteria* to larval shellfish, and survival of *Pfiesteria* zoospores consumed by adult eastern oysters. *Pfiesteria piscicida* was isolated from the Neuse Estuary, cloned, and confirmed as toxic to finfish (JB/HG laboratory; uni-dinoflagellate clones, Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by PCR probes from HG/JB and FISH probes of P. Rublee, UNC-G; fish bioassay process, toxicity cross-confirmed by H. Marshall, ODU).

Acute challenges of toxic *P. piscicida* (actively toxic or TOX-A zoospores, 2-5 x 10^3/mL) were completed with adult shellfish including bay scallops (*Argopecten irradians,* shell width 5 cm, 3/replicate, n=3), northern quahogs (*Mercenaria mercenaria*, shell width 6-8 cm, n=3), and eastern oysters (*Crassostrea virginica,* shell width 10-12 cm, n=5). Control animals were maintained similarly with benign algae (diatom *Thalassiosira*). Bay scallops showed an extreme escape response (seconds) followed by shell gaping and death (minutes to hours). Quahogs were intermediate in response (shell closure in minutes to hours, death in 1-2 days), whereas adult oysters were narcotized with reduced filtering but were alive at 21 days. *P. piscicida* zoospores and amoebae showed strong attraction to most scallop tissues tested with exception of gonad; strong attraction to quahog siphon (zoospores) and stomach tissues (zoospores, amoebae); and low response to oysters except for gill tissues.

Two other *P. piscicida* clones from the Neuse Estuary were used for other experiments on *C. virginica* and *A. irradians* (1st isolate yielding two functional types as TOX-A and TOX-B [previously tested as capable of toxin production in the presence of live fish, but maintained for 6 wk without fish]; 2nd isolate for NON-IND functional type, benign, i.e., previously tested as incapable of toxicity in the presence of live fish). Both TOX-A and TOX-B zoospores sometimes attacked and consumed oyster and scallop pediveliger larvae that had discarded their vela (only adductor muscle tissue remained; minutes). During 1-hr trials when *P. piscicida* was maintained within dialysis membrane to prevent direct contact with pediveligers, there was high (TOX-A, 90-100%) to moderate (TOX-B, 40-50% larval mortality, but negligible mortality when pediveligers were exposed to benign prey. In another experiment, oyster pediveligers appeared to detect residual toxicity from TOX-B zoospores; their grazing activity was highest on NON-IND zoo-spores, with intermediate and lowest grazing on TOX-B and TOX-A zoospores, respectively. In contrast, adult oysters grazed significantly less TOX-A *Pfiesteria*, but grazing was comparable on TOX-B and NON-IND zoospores. Examination of adult oyster faeces indicated that zoospores had formed temporary cysts in the digestive tract. Within 20 hr after gut tract passage, 90% of the previously TOX-A zoospores had excysted and regained motility, with lower (ca. 40-70%) survival shown by TOX-B and NON-IND zoospores. These data indicate that toxic *Pfiesteria* zoospores could potentially affect recruitment and survival of commercially important shellfish species. The demonstrated ability of adult oysters to remove toxic zoospores from the water column indicates a potential, as well, for trophic mitigation/control of toxic *Pfiesteria* outbreaks.

FIELD STUDIES OF TOXIC PHYTOPLANKTON IN CENTRAL CALIFORNIA: 1999-2000

Mary Silver[1,2], Susan Coale[1], Shonna Dovel[1], Kathi Lefebvre[1], Greg Doucette[3], Ron Tjeerdema[4], and Rikk Kvitek[5]
[1]University of California, Santa Cruz, CA 95060
[2]Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039
[3]Marine Biotoxins Lab, NOAA/NOS Charleston, SC 29412
[4]University of California, Davis, CA 95616
[5]California State University at Monterey Bay, Seaside, CA 93955

Since 1991, toxic species of *Pseudo-nitzschia* have been known to be sources of domoic acid on the U.S. west coast, responsible for mortality events involving marine birds and mammals. Longer term records in the Central California region, the site of most of the mortality events, show a typical seasonal pattern of occurrence of *Nitzschia*-like species, the taxon formerly used to enumerate the morphologically similar species that include *Pseudo-nitzschia*. However, a full annual record for the toxic species has not yet been presented, nor has the spread of the toxin through the food chains been followed through the cycle. The purpose of our ECOHAB-funded research is to document the cycle in a coastal site near the epicenter of past toxic events, determining simultaneously the pattern of toxic *Pseudo-nitzschia* species abundance, domoic acid (DA) concentrations in the phytoplankton, and the spread of the toxin into benthic and pelagic populations over the cycle.

This presentation will review our findings on the occurrence of toxic *Pseudo-nitzschia* species and DA levels at a coastal station in northern Monterey Bay, a concentration site for phytoplankton in the region. A 14 month sequence of *Pseudo-nitzschia australis* and *P. multiseries* abundance will be presented, along with associated DA concentrations, and the pattern of DA levels in schooling planktivorous fish (sardines and anchovies) from the Bay. Additional data for an approximately 5 month period will show the co-occurring abundance cycles of toxic *Alexandrium* and *Dinophysis* species at the same site. The results suggest that episodic blooms of toxic microalgal species are not uncommon in this relatively "pristine" coastal system, that a wider array of species than previously suspected may be sequentially present, and that at least one of the toxins (DA) is transmitted into pelagic foodwebs by schooling fish abundantly consumed by marine birds and mammals in the region.

PHYSICAL, CHEMICAL AND BIOLOGICAL CONDITIONS ASSOCIATED WITH THE NARRAGANSETT BAY BROWN TIDE

Theodore J. Smayda and David Borkman
Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881

A retrospective analysis of the 1985-1986 *Aureococcus anophagefferens* brown tide in Narragansett Bay was carried out under the auspices of the Brown Tide Research Initiative. Regional climatic events appear to have been important in triggering this event. Evidence for this includes: regional synchroneity and correlations with the North Atlantic Oscillation Index (NAO) and proxies for atmospheric/weather parameters, including wind direction, strength, rainfall, cloudiness, temperature and groundwater levels. Correlations occurred between the NAO and Groundwater Index (GW), similar to that reported for Long Island brown tide bloom sites. There is no strong evidence to suggest reduced flushing was the basic cause of the 1985 brown tide outbreak, contrary to previous views and unlike that proposed for Long Island embayments. The issue of wether Narragansett Bay was environmentally different in 1985 relative to long term patterns was addressed applying Principal Components Analysis, and revealed that 1985 was a unique year within the 32-year time series analyzed: it clusters with drought years 1956, 1966 and is among the three years of highest irradiance and lowest river flow. The role of nutrients and grazing control in this bloom event, and the commonalities and divergences in brown tide dynamics in Narragansett Bay, Long Island embayments and Laguna madre are also considered. Analysis of the 38-year time series for Narragansett Bay suggests brown tide events there will occur twice per century.

ECOHAB: FLORIDA OVERVIEW – THE ENVIRONMENT

Karen Steidinger[1], John Walsh[2], and Gary Kirkpatrick[3]
[1]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL 33701
[2]College of Marine Science, University of South Florida, St. Petersburg, Florida 33701
[3] Mote Marine Laboratory, Sarasota, FL 34236

ECOHAB: Florida is a five year federally funded (NOAA and EPA jointly) and state-supplemented program to study *Gymnodinium breve* blooms on the west Florida shelf (WFS). The goal is to develop a coupled physical-biological model to predict the development and landfall of this type of harmful algal bloom or red tide. Marine animal mortality, human respiratory irritation, and toxic shellfish (Neurotoxic Shellfish Poisoning) can occur in coastal and nearshore waters once the bloom has developed and intensified as it moves shoreward. Not all blooms are transported inshore. The circulation patterns that cause upwelling can move a bloom further offshore whereas coastal downwelling can drive it onshore. Blooms typically last several months but can last up to 18 months with offshore waters reinoculating inshore areas. A large bloom can occupy thousands of km^2. In 1999, there were three simultaneous *G. breve* blooms between Pensacola and Jacksonville. These phytoplankton blooms start offshore on the mid-shelf and can develop to fish-killing proportions in about two to four weeks. Seventy percent of historical red tides have initiated between September and December. There are four sequential phases of red tide development on the WFS, 1) initiation in oligotrophic waters from resting or vegetative cells, 2) growth (vegetative growth exceeds losses), 3) concentration (physical mechanisms), and 4) dissipation or termination (where red tide can be entrained and advected by a current or a prevailing wind system). In other areas of the Gulf of Mexico where *G. breve* blooms occur, the development and progression of blooms may not follow the same pattern.

There are 23 ECOHAB: Florida investigators representing 13 institutions. The goal is being pursued by 1) characterizing and monitoring (monthly) a control volume (11,000 km^2) of water on the WFS between Tampa Bay and Charlotte Harbor using three cross shelf, two along shelf, and one diagonal transect, 2) repeating a portion of the central cross shelf transect monthly, and 3) conducting an annual three week process cruise in and outside of *G. breve* blooms for physiological, behavioral, and toxin distribution studies. The objectives are to 1) model each phase of the bloom and forecast landfall, 2) describe the physical environment, cross shelf transport, and concentrating mechanisms, 3) determine the interactions of cellular, behavioral, life cycle, and community regulation processes with environmental forcing factors during stages of bloom development, 4) determine the source of nutrients that provide growth inshore and offshore and allow persistence, and 5) determine the fate and effect of *G. breve* toxins in the marine environment (including the food web).

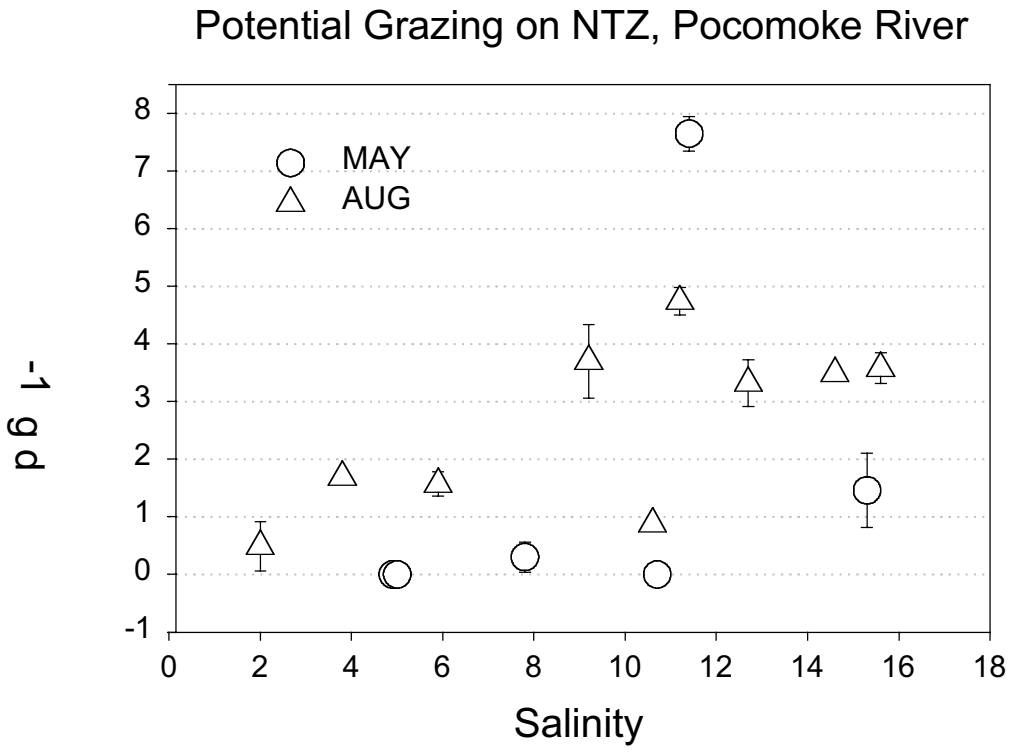
Other micro-, meso- and macroscale studies (e.g., HyCODE) on the WFS (funded by the state of Florida, NOAA, NASA, MMS, ONR, USGS, and EPA) are able to use data from the ECOHAB: Florida cruises and moored buoy arrays, and federally available satellite imagery to characterize the control volume area, as well as areas that may influence it. Some of these data are real-time, or near real-time, and will be used for interpretation of remotely sensed data and for modeling shelf processes. Hopefully remote sensing (water and/or satellite sensors) will be used in the future to monitor systems and subsystems to feed biophysical prediction models for harmful algal blooms and their effects. Long-term monitoring commitments for calibration and validation of prediction models or for status and trend analyses require a dedicated regional or system data and metadata management network and core group, particularly for common use data such as basic environmental data.

POTENTIAL GRAZING ON *PFIESTERIA PISCICIDA* BY MICROZOOPLANKTON IN THE POCOMOKE RIVER

Diane K. Stoecker and Daniel E. Gustafson, Jr.
UMCES, Horn Point Laboratory, PO Box 775, Cambridge, MD 21613

Our objectives were to determine if microzooplankton grazing has the potential to prevent net growth of non-toxic zoospores (NTZ) and to determine if grazing pressure is related to salinity. In a previous study at a mesohaline site on the Chicamacomico River, the instantaneous rate of grazing mortality, *g*, varied from 0 to 10 d^{-1} and in 6 out of 10 incubations was > 2 d^{-1}. The maximum growth rate of NTZ in culture is < 2 d^{-1}. We wanted to determine if potential grazing pressure in the lower Pocomoke River varied with season or salinity. Although *Pfiesteria* cultures grow best at ~ 15 psu, most fish kills associated with *Pfiesteria*-like dinoflagellates have occurred at salinity <10. We hypothesized that this may be due to less grazing pressure on *Pfiesteria*-sized dinoflagellates at lower salinity sites.

The grazing pressure (*g* d^{-1}) of natural assemblages of microzooplankton on cultured non-toxic zoospores (NTZ) was measured in water samples collected from sites of different salinity on the lower Pocomoke River in May and August 2000. NTZ of a non-inducible strain (FDEPMDR23) of *P. piscicida* were stained with a vital green fluorescent dye, 5-chloromethylfluorescein diacetate, and added to treatments with (<200 µm) and without (<1.2 µm) the natural microzooplankton assemblage. Grazing coefficient, *g*, varied from 0 to 8 d^{-1} in May and from 0 to 5 d^{-1} in August (Fig.). In 6 of 9 incubations with samples of >9 psu, *g* was > 2 d^{-1}. In the 6 incubations with < 9 psu samples, *g* was < 2 d^{-1}, and in 3 cases was zero. Potential grazing pressure on NTZ varied with date and site, but in incubations with > 9 psu water, *g* was usually greater than the maximum potential growth rate of NTZ. Microzooplankton grazing is an important factor that may regulate net growth of *Pfiesteria piscicida* populations in the plankton.



AN OVERVIEW OF INTERACTIONS BETWEEN ZOOPLANKTON GRAZERS AND *ALEXANDRIUM* SP., AND EFFECTS OF GRAZING ON BLOOM DYNAMICS IN THE NEAR-SHORE ENVIRONMENT OF THE GULF OF MAINE

Gregory J. Teegarden[1], Robert G. Campbell[3], Allan D. Cembella[2], Edward G. Durbin[3]
[1]Bowdoin College, 6500 College Station, Brunswick, Maine 04011
[2]Institute for Marine Biosciences, NRC, 1411 Oxford St., Halifax, Nova Scotia, Canada
[3]Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Laboratory and field studies were conducted during an ECOHAB targeted individual project, to investigate the mechanisms and biology of zooplankton feeding and potential impacts of feeding during blooms of *Alexandrium fundyense* in the near-shore Gulf of Maine. Laboratory experiments established that copepods are capable of discriminating toxic cells from non-toxic cells, and that non-toxic *Alexandrium* sp. cells are preferred to many similar algal types while toxic cells are frequently avoided. Selective feeding was not however universal, and also appeared to depend on the concentration of toxic *Alexandrium* sp. cells as well as the species composition of the prey field experienced by zooplankton. Selective feeding by zooplankton in *Alexandrium* sp. blooms is possible or likely, but not easily predicted, since dominant grazers, prey species composition, and *Alexandrium* sp. concentration must be known to predict response with any confidence. When zooplankton consume toxic *Alexandrium* sp. cells, some toxins are accumulated, but laboratory experiments demonstrate that retention of toxins in body tissues is very inefficient (<5% of ingested toxin). Zooplankton may act as vectors of toxin to higher trophic levels, but are not efficient vectors (see poster by Teegarden et al.).

The impact of zooplankton grazing on *Alexandrium* sp. was investigated in field studies during the spring of 1998 and 1999 in coastal waters of the Gulf of Maine. Samples were collected at weekly intervals from several stations for zooplankton and phytoplankton abundance, biomass, species composition, and toxin content. Grazing rates of the dominant zooplankton species were determined using natural water samples. Dominant zooplankton included copepods (primarily *Acartia hudsonica* and *Calanus finmarchicus*) and barnacle nauplii (*Semibalanus* sp.). The feeding behavior of the zooplankton on *Alexandrium* sp. was species specific: *Acartia* was non-selective, *Calanus* was somewhat selective, and *Semibalanus* avoided ingesting *Alexandrium* sp. During 1998, there was a moderate bloom of *Alexandrium* with peak concentrations reaching 3000 cells/L in late May at one of the inshore stations. In contrast, in 1999 concentrations were very low throughout the study period. During both years zooplankton biomass was low during the early spring but increased exponentially over the study period. In 1998, grazing impacts increased from 0 to 80% day^{-1} in concert with the increase in zooplankton biomass and appeared to contribute to the bloom's demise. Our findings suggest that grazing can be an important source of mortality and will depend on zooplankton filtration rates, degree of selective feeding, and the biomass and species composition of both the phytoplankton and zooplankton communities (see poster by Campbell et al.).

HIGH EVOLUTIONARY RATES IN *GYMNODINIUM GALATHEANUM* CHLOROPLAST DNA SEQUENCES AND DEVELOPMENT OF A MOLECULAR DETECTION ASSAY

Torstein Tengs[1], Holly A. Bowers[1], Andrew P. Ziman[1], Diane K. Stoecker[2] and David W. Oldach[1]
[1]Institute of Human Virology, University of Maryland at Baltimore, 725 West Lombard Street, Baltimore, MD 21201
[2]University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, MD 21613

Nuclear and chloroplast-encoded small subunit ribosmal RNA sequences were obtained from several strains of the toxic dinoflagellate *Gymnodinium galatheanum.* Phylogenetic analyses and comparison of sequences indicate that the chloroplast sequences have a higher evolutionary rate than the nuclear homolog. The chloroplast sequences were chosen as targets for the development of a 5'-3' exonuclease assay for detection of the organism. The assay has a very high degree of specificity and has been used to screen environmental water samples from a fish farm where the presence of this dinoflagellate species has previously been associated with fish kills. Various hypotheses for the derived nature of the chloroplast sequences are discussed, as well as what is known about the toxicity of the species.

AN EXPANSION OF HARMFUL RAPHIDOPHYTE BLOOMS IN U.S. COASTAL WATERS

Carmelo R. Tomas
Center for Marine Science, University of North Carolina at Wilmington, 1 Marvin K. Moss Lane, Wilmington, NC 28409

Historically Raphidophyte blooms have been a common feature of some coastal areas of the U.S. Most notable were the recurrent blooms of *Heterosigma akashiwo* (formerly called *Olisthodiscus luteus*) in Narrangasett Bay and Long Island Sound. Data spanning over 20 years indicated that these blooms were seasonal, periodic but varied in intensity from year to year. While causing massive discoloration *H. akashiwo* blooms were rarely accompanied by mortality of finfish and shellfish. In recent years, blooms of *H. akashiwo* have become common in the Pacific Northwest region where salmon, shellfish and crabs were found dead after exposure to waters containing large concentrations of this species. These reports agree with the observations from nearby British Columbian waters where *H. akashiwo* blooms have commonly killed salmonoid fish since 1986 to the present. During the last two years, Texas has experienced frequent blooms of *H. akashiwo* in the Corpus Christi/Padre Island area where blooms were found accompanying mortality of finfish.

Another genus of the Raphidophytes, *Chattonella* has also become a common bloom species within the last 10 years. *Chattonella subsalsa* was noted as a seasonal bloom forming species in Bayboro Harbor and greater Tampa Bay and Florida Bay. While initially co-occurring with *H. akashiwo, C. subsal*sa normally dominated in the later phases of the bloom during the seasonal warmer periods of mid-late summer. During the 2000 summer season, (June-September) blooms of *Chattonella* were identified from Corpus Christi, Texas, Perdido Bay, Bayboro Harbor and St. Johns River Estuary, Florida, New and Neuse Rivers, North Carolina, Ayre Creek, Maryland and Bald Eagle Creek, Delaware. For the past five years, blooms of *Chattonella* have also been reported for the Salton Sea (California) where extensive fish and bird deaths were observed. While the exact species of *Chattonella* is not clearly defined for all cases, with the exception of Tampa Bay, *Chattonella* blooms occurred in regions where fish kills were reported. The blooms often accompanied or followed the fish mortalities and rarely were noted as being associated as a causative agent.

Japanese investigators have reported toxins from three of the four Raphidophyte genera, namely, *Heterosigma, Chattonella* and *Fibrocapsa*. Each of these species was demonstrated to be associated with fish kills in the Japanese Inland Sea and more recently in New Zealand. *Chattonella marina* was identified as killing cultured fish in Tasmania. Blooms of *Chattonella verruculosa* have for the second year in a row caused extensive blooms in the North Sea visible by satellite imagery. Mortality of fish was associated with the blooms. Considering these reports and increase in occurrence, closer scrutiny is required to define the significance of the Raphidophyte blooms. Do they represent a new threat? Is the increased frequency a function of evolving environments? Do they represent a viable vector for new toxins in coastal waters? Are we to expect a continuation of more frequent blooms in the near future? Are there new Raphidophyte species involved in this apparent increase?

A greater effort in the ability to identify these species is required in routine monitoring efforts. Fish kill events in lower salinity estuarine waters need to be considered in light of the presence of toxic Raphidophytes. Greater efforts are needed towards the detection of their toxins by a rapid, accurate and easy field method. And an understanding of not only the acute toxicity of large blooms but the role of chronic low levels of biotoxins in estuarine waters critically needs to be better understood to fully appreciate the risks associated with inshore Raphidophyte blooms.

OFFSHORE BLOOMS OF THE RED TIDE DINOFLAGELLATE, *ALEXANDRIUM* SP., IN THE GULF OF MAINE

David W. Townsend, Neal R. Pettigrew and Andrew C. Thomas
5741 Libby Hall, School of Marine Science, University of Maine, Orono, ME 04469

We conducted three large-scale surveys of the coastal and offshore waters of Gulf of Maine during the summer of 1998, and two similar surveys in spring and summer of 2000. Hydrographic data were collected and concentrations of phytoplankton chlorophyll, inorganic nutrients and densities of *Alexandrium* cells were measured in discrete water samples. Moorings, measuring Doppler current profiles and temperature and conductivity at several depths, were deployed within the Eastern Maine Coastal Current (EMCC) system during each of the field seasons. During 1998 the moored program focused on the alongshelf coherence within the EMCC, while in 2000 the emphasis was on flow structures in the frontal region near Penobscot Bay and the linkages to the Western Maine Coastal Current (WMCC). Daily NOAA AVHRR and NASA SeaWiFS satellite data were downloaded and processed to provide coincident spatial patterns of sea surface temperature and chlorophyll over each cruise period.

Our specific objectives were to:

(1) Investigate the physical oceanography, nutrient chemistry, and abundances and distributions of *Alexandrium* in the coastal and offshore waters of the northern Gulf of Maine and to identify the factors that regulate *Alexandrium* population dynamics.

(2) Determine the linkages between the major Gulf-wide current systems, in particular the Eastern Maine Coastal Current and western Maine coastal waters, and between the Gulf of Maine and the Bay of Fundy with respect to freshwater, nutrients, and *Alexandrium* cells.

(3) Identify environmental factors that result in the initiation of *Alexandrium* blooms in Gulf of Maine waters, and how those blooms are controlled with respect to their spatial and temporal distributions.

Our most complete results, as of this writing, are for the 1998 field season. Both surface and subsurface concentrations of *Alexandrium* displayed maximum cell densities in the offshore waters of the Gulf on all cruises. Highest cell densities in surface waters (ca. 5.5 x 10^3 cells L^{-1}) were observed in two broad patches: one in the Bay of Fundy and another in shelf and offshore waters of the central and eastern Gulf of Maine in association with the Eastern Maine Coastal Current. Highest subsurface densities of cells appeared to be associated with the frontal edges beyond the cold surface waters associated with the Eastern Maine Coastal Current. As the summer progressed, the highest surface densities of *Alexandrium* receded toward the eastern portions of the Gulf and the Bay of Fundy. Locations of high cell densities were described and interpreted using a non-dimensional light-nutrient parameter, computed as the ratio of the depth of the 10% surface irradiance to the depth of 4 μM NO_3 concentration.

We suggest that the offshore distributions of relatively high densities of *Alexandrium* are naturally-occurring and can be related to seasonal and vertical patterns of inorganic nutrient concentrations/ratios and the ambient light field. Possible mechanisms responsible for periodic development of PSP outbreaks in nearshore shellfish beds are discussed in light of our recent findings, and placed in the context of an historical overview of past observations in the Gulf of Maine region.

THE CHALLENGES OF FORECASTING AND MANAGING TOXIC *PSEUDO-NITZSCHIA* BLOOMS ON THE U.S. WEST COAST

Vera L. Trainer
NOAA/National Marine Fisheries Service/Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112

The food web transfer of domoic acid to shellfish, crustaceans, seabirds, finfish, and marine mammals has been recently documented on the U.S. West coast. Sub-regions of the U.S. West Coast face unique challenges during toxic episodes, including choosing the appropriate sentinel organism and understanding which *Pseudo-nitzschia* species are responsible for toxicity. Data collected during West Coast cruises in the years 1997-2000 indicate that often the highest toxin levels and greatest numbers of toxic cells are positioned in water masses associated with offshore eddies or in upwelling zones near coastal promontories. Such cruise data are essential in the characterization of offshore initiation sites that will lead to the effective placement of automated sensors, such as moored arrays. In addition, beach monitoring is a necessary component of regional species characterization, resulting in the development of specific molecular and biochemical tools needed to assist managers in each coastal area. Indeed, beach samples collected in 1998 indicated that a *P. pseudodelicatissima* bloom was responsible for razor clam toxicity on the Washington coast, whereas toxin produced by *P. australis* resulted in sea lion mortalities in central California. The challenges faced on the West Coast due to HAB-related mammal mortalities, widespread closures of shellfish harvest, and human illness can only be met by sustained beach monitoring programs such as the Olympic Region Harmful Algal Bloom (ORHAB) project and dedicated research cruises. These onshore and offshore efforts will give us a comprehensive picture of the oceanography influencing the location and intensity of domoic acid-producing HABs. Complete characterization of physical, biological and chemical conditions that favor harmful *Pseudo-nitzschia* blooms, only possible through large-scale, synergistic collaboration, is a prerequisite for forecasting of these events. A forecasting capability will substantially improve the management of valuable coastal resources and the protection of human health, both of which are affected by these toxins.

ACCUMULATION OF PSP TOXINS IN ZOOPLANKTON ASSEMBLAGES IN THE GULF OF MAINE

Jefferson T. Turner[1], Christine L. Powell[2], David M. Kulis[3], Bruce A. Keafer[3], Donald M. Anderson[3], and Gregory J. Doucette[2,4]
[1]Biology Dept., U Mass Dartmouth, North Dartmouth, MA 02747-2300
[2]Marine Biotoxins Program, NOAA/National Ocean Service, Charleston, SC 29412
[3]Biology Dept., Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[4]Marine Biomedical & Environmental Sciences, Medical Univ. SC, Charleston, SC 29412

The transfer of algal toxins through marine food webs can affect the health of both humans and wildlife, as well as negatively impact the trophic structure of ecosystems. In the case of PSP toxins produced by dinoflagellates, the most common route of entry into the food web is via direct consumption of toxic algae by filter-feeding bivalves as a component of their natural diet. Another potentially important, although poorly understood, vector for the transfer of these toxins into higher trophic levels is the zooplankton grazer community, which represents the prey of certain marine mammals as well as various planktivorous fish species. Such fish can, in turn, experience reductions in fecundity and recruitment with exposure to PSP toxins, and may also transfer toxins to their predators. PSP toxins contained in mackerel were, in fact, implicated in an unusual mortality event several years ago involving humpback whales. Moreover, a recent study by our group in Massachusetts Bay demonstrated not only the accumulation of PSP toxins in zooplankton, but also the preferential movement of toxin into larger size fractions although these animals did not numerically dominate the grazer assemblage. Some larger zooplankton species comprise a majority of the diet for the North Atlantic right whale and thus pose a potential health threat to these endangered marine mammals.

Given the paucity of detailed information on the role of zooplankton grazers as vectors for PSP toxins, we initiated a study of zooplankton mediated trophic transfer of these toxins as a component of the ECOHAB Gulf of Maine regional program. We will be reporting the results of detailed survey cruises in the Casco Bay region of the Gulf of Maine conducted in 1998, which describe the PSP toxin distribution among several plankton size fractions (20-64, 64-100, 100-200, 200-500, >500 µm) as well as the taxonomic composition of these size classes. As expected, the predominant PSP toxin signal was detected in the 20-64 µm size class containing toxic *Alexandrium* spp., and changed according to fluctuations in the cell concentrations of these dinoflagellates. Nonetheless, toxicity was also associated with all other size classes examined at some point during the study, with the distribution pattern varying markedly among stations within a cruise and also between cruises. Unlike our previous work in Massachusetts Bay, toxin signals were occasionally detected in the 64-100 µm size class. Preliminary analyses indicated that these samples did not contain *Alexandrium* cells, but rather were dominated by tintinnid ciliates as well as larger, non-toxic dinoflagellates. The overall pattern of toxin accumulation in the remaining three size fractions was similar to that observed previously, with toxicity occurring consistently in the 200-500 and/or >500 µm size fractions, which were generally dominated by copepods of the genera *Calanus*, *Centropages*, and *Pseudocalanu*s - all potential grazers of *Alexandrium* cells. Although toxicity was not associated with the 100-200 µm fraction as frequently, there were several cases in which this fraction, comprised mostly of copepod nauplii, adults of small copepods (e.g., *Oithona similis*), and rotifers, accumulated as much or more toxin than the larger size classes. More detailed analyses of zooplankton taxonomic composition, *Alexandrium* cellular toxicity, and implications for PSP toxin transfer to higher trophic levels will be discussed.

ROLES OF ENDOGENOUS CELLULAR RHYTHMS AND LIFE CYCLE STAGE RECRUITMENT IN *GYMNODINIUM BREVE* BLOOM DEVELOPMENT

Frances M. Van Dolah[1], Michele Barbier[1], Tod A. Leighfield[1], Karen A. Steidinger[2], Bill Richardson[2] and Peter M. McGuire[3]
[1]NOAA/NOS Center for Coastal Environmental Health and Biomolecular Research, 219 Ft. Johnson Rd., Charleston, SC 29412
[2]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Ave, SE, St. Petersburg, FL 33712
[3]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610

Blooms of the Florida red tide dinoflagellate, *Gymnodinium breve*, occur almost every year off the west coast of Florida. As a component of the Florida ECHOAB program, this work investigates cellular level controls involved in *G. breve* bloom dynamics. *Gymnodinium breve* has both asexual and sexual life cycle phases, both of which play roles in the formation of Florida red tides. The sexual cycle has been only partially elucidated through the planozygote stage. Current laboratory studies include experiments to evaluate planozygote longevity and hypnozygote (resting cyst) formation. The repeated occurrence of early stage blooms (e.g., 5000 - 20,000 cells per liter) in isolated spots in the mid-shelf region suggests that bloom initiation may result from a benthic life cycle stage. The existence of resting cysts of *G. breve* has not yet been confirmed; however, laboratory observations have identified the existence of a benthic palmelloid stage that may alternatively serve as a seed population for *G. breve* blooms. Confirmation of the presence of cysts, palmelloid cells, or other benthic stages of *G. breve* in bloom initiation zones is the focus of current studies. Identification of unusual life cycle stages is problematic in field samples; therefore, we have developed an antibody probe to cell surface proteins of *G. breve* to assist in these identifications.

Once initiated, the development of a *G. breve* bloom proceeds through asexual cell division. In both laboratory and field populations, *G. breve* growth rates are consistently found to average 0.2-0.5 divisions per day. Like most phototrophic dinoflagellates, the cell cycle in *G. breve* appears to be under the control of a circadian rhythm. Consequently, the cell cycle is phased to the diel cycle, such that the portion of the population destined to divide on a given day enters S- phase (DNA synthesis) 6-8 h into the light phase, and enters mitosis 12-15 h later, during the dark. Naturally occurring blooms, observed over four years of ECOHAB cruises, exhibit similar diel cell cycle phasing. In laboratory cultures, the dark/light or "dawn" transition was shown to provide the diel cue that serves to entrain the *G. breve* cell cycle: a forward or backward shift in the timing of this cue results in a concomitant shift in the timing of S-phase entry. We have identified by western blotting and immunolocalization two key components of the cell cycle regulatory complex that are known in higher eukaryotes to control entry into both S-phase and mitosis: cyclin and cyclin dependent kinase (CDK). Inhibition of the activity of this complex with a specific CDK inhibitor, olomoucine, blocks the *G. breve* cell cycle both prior to S-phase and prior to mitosis, and demonstrates a functional role for this complex in cell cycle regulation. Our current work focuses on the mechanisms by which the diel entraining signal acts on these cell cycle regulators to control cell cycle progression.

HYDROGRAPHY AND NUTRIENT CHARACTERISTICS WITHIN THE ECOHAB: FLORIDA CONTROL VOLUME ON THE WEST FLORIDA SHELF

Gabriel A. Vargo[1], Cynthia A. Heil[1], John J. Walsh[1], Kent Fanning[1], Carmelo R. Tomas[2], Karen A. Steidinger[3], Danylle Ault[1], Merrie Beth Neely[1], Kristen Lester[1], and Rachel Merkt[1]
[1]College of Marine Science, Univ. of South Florida, St. Petersburg, FL 33701
[2]Center for Marine Science, Univ. of North Carolina at Wilmington, Wilmington, NC 28403
[3]Florida Marine Research Institute, Florida Wildlife Conservation Commission, St. Petersburg, FL 33701

Blooms of *Gymnodinium breve* may re-occur annually in coastal waters of the West Florida Shelf primarily within the area bounded by Tampa Bay on the north and Charlotte Harbor on the south (i.e. the Ecohab: Florida control volume). This region is oligotrophic with typical inorganic nitrate and phosphate concentrations of <0.5 and 0.2 μM, respectively, within 2 to 4 km of the shoreline. *G. breve* is well adapted for life in this oligotrophic environment with a K_s value for nitrate uptake of 0.42, and for growth utilizing phosphate, ammonia and urea on the order of 0.18, 0.47 and 1.07, respectively. However, when blooms with chlorophyll concentrations >10 μg/l persist within limited geographic areas for 2 to 4 months, additional nutrient sources are required for long-term maintenance.

Ongoing monthly quasi-synoptic cruises collect standard hydrographic measurements at approximately 65 locations along three cross-shelf and two along shelf transects to determine the hydrographic features and potential nutrient sources for bloom inception, growth, and maintenance. Several hydrographic features can be related to bloom formation and persistence. Steep cross shelf thermal and salinity gradients occur in nearshore waters during the winter and rainy season, respectively. Rainy season outwelling of estuarine waters have a characteristic signal of elevated inorganic and organic nutrients with inorganic molar N:P ratios of ~1.0, and leads to the formation of fronts in coastal waters and chlorophyll signatures at the mouth of each estuary. Vertical thermal stratification of shelf waters from May through October leads to the formation of near bottom chlorophyll maxima, dominated by diatom populations, which are potentially fueled by nitrate originating from offshore intrusions of the Loop Current. Fall and winter months are typified by vertically well-mixed water columns which resuspend PON and DON from decaying summer populations.

Phosphate flux from estuarine and/or sediment sources is sufficient to meet bloom requirements. Although inorganic nitrogen levels are low, DON concentrations within the estuaries and in nearshore waters range from 5.0 to 15.0 μM and may provide support for nearshore blooms. Mid to late summer blooms of the diazotrophic cyanobacterium *Trichodesmium erythraeum* , which often precede *G. breve* blooms, appear to be stimulated by iron input from Saharan dust events, and may also act as a source of organic N via excretion of amino acids or inorganic N via bacterial breakdown of excretory material and particulate matter. Elevated DON levels coincide with *Trichodesmium* cell counts in offshore and mid-shelf areas. Other potential DON sources include the degradation of POM such as sea grass blades from near-shore and estuarine sources.

Measurements of particulate δ^{15}PON from various locations within the control volume strongly suggest that DON from several potential sources is used by *G. breve*. The δ^{15}PON values for surface phytoplankton populations during May-June, 1998 ranged from -1.6 to +2.8 ^{0}/oo with a mean of 0.3 ^{0}/oo suggesting *Trichodesmium* accounted for most of the phytoplankton biomass. The δ^{15}PON of estuarine derived and sea grass derived material has a similar but slightly heavier range; +1.1 to +2.9 ^{0}/oo while the diatom dominated near bottom chlorophyll maxima found during September, 1998 after nitrate depletion had the δ^{15}N signature of Gulf of Mexico sub-thermocline nitrate; e.g. +6.7 to 8.3^{0}/oo.

The December, 1998 red tide with approximately 5 μg chl/l in nearshore waters had a δ^{15}PON of $+4.8^{0}$/oo which reflects use of a ^{15}N-enriched DON substrate which has been modified by bacterial or herbivore fractionation or mixing with DON derived from near bottom nitrate enriched diatom stocks. Several potential N sources are therefore available in support of *G. breve* blooms on the West Florida Shelf. Ongoing studies are aimed at distinguishing between offshore and coastal sources of utilizable DON.

USE OF CELL SPECIFIC PAM-FLUOROMETRY TO CHARACTERIZE LIGHT ACCLIMATION RESPONSES OF *GAMBIERDISCUS TOXICUS* (DINOPHYCEAE)

Tracy A. Villareal[1] and Steve Morton[2]
[1]Marine Science Institute, The University of Texas at Austin, 750 Channel View Dr., Pt. Aransas, Texas 78373
[2]NOS/NOAA; 219 Fort Johnson Rd.; Charleston, SC 29412

The fluorescence parameter F_v:F_m was determined on single *Gambierdiscus toxicus* over a diurnal cycle using a microscopy-PAM system. Cells collected from foliose red algae growing in the subtidal margin of Southwater Cay, Belize were examined at intervals during the day (May, 2000). An experimental treatment of rinsed cells free of the host algae was incubated in-situ under both sunny and cloudy conditions, and the results were compared to natural samples.

The dark adapted yield parameter F_v:F_m reached 0.7-0.8 in pre-dawn and early morning natural samples (maximum = 0.81). Pre-dawn samples were dominated by high yield cells, but a significant number of cells exhibited lower yields (Fig. 1). High yield cells were present throughout the day, but the proportion of low yield cells increased over the course of the day resulting in a decrease in the average F_v:F_m. Yield decreased during the day at a similar rate under both cloudy and sunny conditions to a daily average minimum of 0.4-0.5. However, the maximum values for individual cells still exceeded 0.7. Incubated samples on cloudy days showed a similar response to natural populations; however, incubated samples under sunny conditions showed a significantly greater depression of Fv:Fm and reached a minimum of 0.15 (Fig. 2). Cells in this treatment started to recover in late afternoon, and fully recovered after one dark period to near maximum yields (Fig. 2).

The maximum dark adapted yield in *G. toxicus* approaches the theoretical maximum for PAM fluorometry and does not suggest physiological limitation of photosynthesis by either N or Fe. The down regulation of F_v:F_m appears to be a response to solar radiation, and indicates the existence of active non-photochemical quenching to protect the photosystem. However, the depression of the yield in host-free incubation bottles under sunny conditions as well as the persistence of high yields at mid-day suggests that behavior mechanisms may be at work as well. *G. toxicus* may be actively moving to shaded regions of the algae to reduce its exposure to solar radiation.

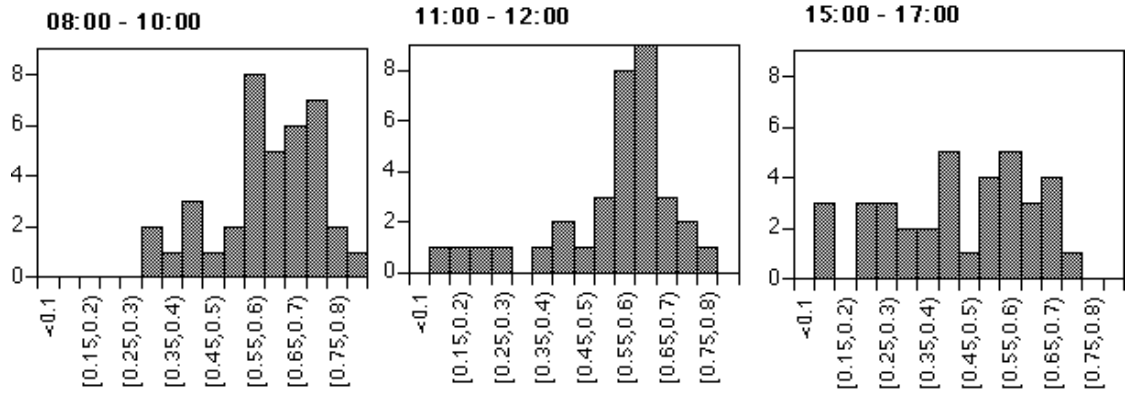


Fig. 1. Frequency histogram of yield in *G. toxicus.* Results are binned into 2 hour time periods.

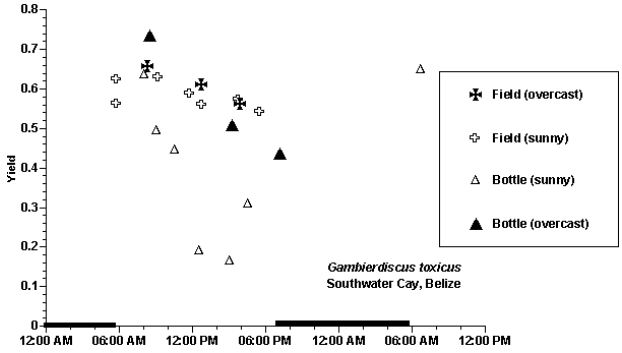


Fig. 2. Diurnal variability in average yield in natural and experimental samples of *G. toxicus*.

GYMNODINIUM BREVE IN THE WESTERN GULF OF MEXICO: TWO YEARS OF COASTAL SAMPLING OFF THE TEXAS COAST

Tracy A. Villareal[1], Mary A. Brainard[1], and H. Magaña[1] and Lawrence W. McEachron[2]
[1]Marine Science Institute, The University of Texas at Austin, 750 Channel View Dr., Port Aransas, Texas 78373
[2]Coastal Fisheries Division, Texas Parks and Wildlife Department, 702 Navigation Circle, Rockport, Texas 78382

We established a sampling program in Texas coastal waters to examine near shore waters (<15 km) for the presence of *G. breve* and related species. The first two years of sampling noted *G. breve* from the waters inside 15 km in <10% of the samples. During this period, significant red tide events occurred along the Texas coast near Brownsville and the Louisiana border. In all cases, the routine sampling (twice per month) did not provide advance warning of the bloom. The first event in Oct. 1999 off of Brownsville, TX may have been related to introduction of offshore populations (>15 km) during strong northeasterly winds. This bloom advected to the south over a two week period. An offshore transect that found nutrient-rich water advecting onto the shelf in association with an anticyclonic feature. This mesoscale feature resembled Loop Current intrusions and could have provided the offshore source waters for the bloom. There was no surface manifestation of this intrusion on the shelf.

A July, 2000 bloom moved north up the coast from Brownsville, TX and may have been transported in a coastal upwelling plume that extended from Tamulipas, Mexico to near Corpus Christi, Texas. However, this upwelling event was associated only with temperature reduction at the surface (T>26 ° C) and had no apparent nutrient enrichment (Fig. 1). A subsequent red tide off Sabine, Texas occurred near the Louisiana border (Aug-Sept. 2000). This bloom was primarily offshore and impacted the coastal zone only for a brief period in late August south of Galveston. A brief analysis of the historical circulation data suggests the seasonal migration of the coastal current convergence could be responsible for maintaining this bloom in a gyre like feature in the northern shelf. The historical record of *G. breve* blooms notes similar complex dynamics and suggests the seasonal coastal current patterns may play a fundamental role on determining whether blooms propagate onshore and in which direction they are transported.

Initial culture studies on G. *breve* isolated in Oct. 99 showed a lower salinity tolerance between 23 and 25 PSU with a maximum growth rate of 0.3 –0.4 div d^{-1}. Ongoing experiments are evaluating the growth response over a wider range of light, salinity and temperature conditions.

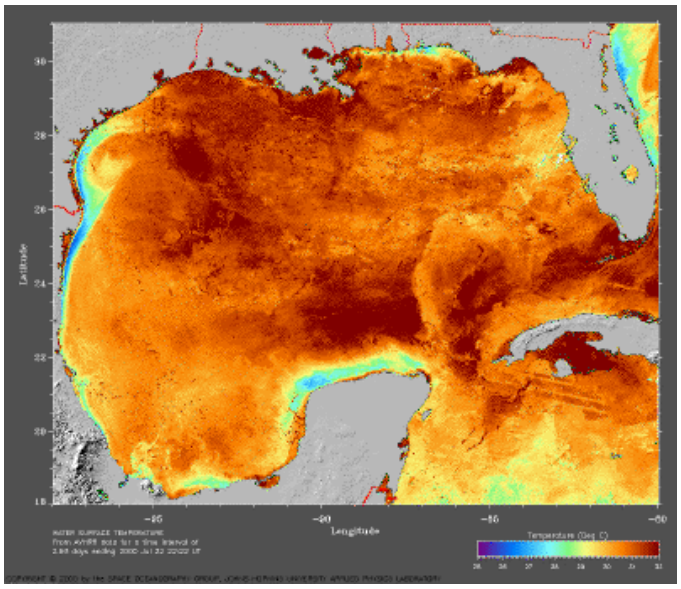


Fig. 1. Sea surface temperature image showing coastal upwelling along northern Mexico and

SKIN LESIONS IN ESTUARINE FISHES: A COMPARATIVE PATHOLOGICAL EVALUATION OF WILD AND LABORATORY-EXPOSED FISH

Wolfgang K. Vogelbein[1], Kimberly S. Reece[1], Jeffrey D. Shields[1], David E. Zwerner[1], Patrice L. Mason[1], Larry W. Haas[1] and Vicki Blazer[2]
[1]Department of Environmental Science, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062
[2]National Fish Health Research Laboratory, Leetown Science Center, USGS, Kearneysville, WV 25430

The toxic dinoflagellate, *Pfiesteria piscicida*, is widely blamed for acute mortalities and skin ulcers in menhaden, *Brevoortia tyrannus*, from Mid-Atlantic U.S. estuaries. However, the underlying causes of these acute fish kills and the so-called "*Pfiesteria*-specific" skin ulcers of menhaden remain poorly understood and controversial. We initiated field and laboratory studies to clarify the role, if any, of *Pfiesteria* spp. and other environmental factors in menhaden lesion development. Histopathologically, skin ulcers in > 250 wild menhaden from Chesapeake Bay and the Pamlico Estuary, NC all harbored a deeply invasive, pathogenic fungus eliciting severe tissue necrosis and intense granulomatous inflammation. One of us (VB) recently determined this fungus to be *Aphanomyces invadans*, the causative agent of epizootic ulcerative syndrome (EUS) in fishes from the Indo-Pacific Region. The consistent presence of this fungus and the granulomatous nature and severity of the resulting inflammatory response suggest these lesions are chronic (age > one week). Their use as an indicator of current, localized *Pfiesteria* activity may therefore not be valid. However, these field data do not allow us to rule out a possible early role for *Pfiesteria* spp. as initiator of lesion development. It is therefore important to better understand the pathology of *Pfiesteria* exposure and to investigate potential environmental factors that might modulate lesion pathogenesis in exposed fishes. To this end, we conducted a series of preliminary laboratory challenges of tilapia (*Oreochromis niloticus*) and mummichog (*Fundulus heteroclitus*) with high concentrations (5000 - 30,000 cells/ml) of *Pfiesteria* sp. "B" (characterization based on SEM and molecular analyses). Results indicate *Pfiesteria*-associated pathology is consistent with acute toxin exposure and differs significantly from pathology observed in wild menhaden with ulcerative mycosis caused by *A. invadans.* Fish mortalities in the assays were directly associated with abundance of *Pfiesteria* "B" cells. Grossly, fish exhibited loss of scales, mucus coat and occasional mild petechial hemorrhage. Histologically, fish exhibited widespread loss of epidermis with bacterial colonization but minimal associated inflammation. Epidermal erosion extended into the oral and branchial cavities. Gills exhibited epithelial lifting, loss of secondary lamellar structure and infiltration by lymphoid cells. Interestingly, dinoflagellates were observed within skin folds, scale pockets and the lateral line canal system of the head region. Epithelial lining of the lateral line canal and associated sensory structures exhibited degeneration and necrosis. However, bacterial colonization of tissues harboring dinoflagellates was a consistent observation. In situ hybridization analyses using specific DNA probes to *P. piscicida, Pfiesteria* sp. "B" and the "Lucy" group confirmed that dinoflagellates associated with fish tissues were *Pfiesteria* sp. "B". We consider this association to be an artifact of the very high dinoflagellate concentrations in our exposures. However, in situ hybridization analyses of wild menhaden intestine with these probes may serve as a sensitive, specific and quantifiable bioindicator of local *Pfiesteria* activity in east coast estuaries.

COUPLED NUMERICAL MODELS OF FLORIDA RED TIDES OF *GYMNODINIUM BREVE*

John J. Walsh[1] , W. Paul Bissett[2] , Bradley Penta[1], Dwight A. Dieterle[1], Robert H. Weisberg[1], Zhenjiang Li[1], and Huijun Yang[1]
[1]College of Marine Science, University of South Florida, St. Petersburg, FL 33701
[2]Florida Environmental Research Institute, 4807 Bayshore Boulevard, Tampa, FL 33611

Successful ecological models are data-driven, distilling qualitative hypotheses and aliased field observations into simple analogues of the real world in a continuing cycle of model testing and revision. Prediction of the origin, transport and fate of *Gymnodinium breve* blooms on the West Florida shelf is the goal of the ECOHAB: Florida project - based on 1) shipboard and remote sensing surveys of hydrography, nutrients, DOM, species composition, pigments, and zooplankton 2) experimental cruises and laboratory studies describing their physiology, life cycles, optical properties, and toxin transfer, 3) arrays of current meters, 4) circulation submodels, 5) cell metabolism and migration submodels, and 6) coupled bio-optical models. The last component utilizes these submodels and assimilated observations in a complex, numerical food web to describe the consequences of phytoplankton competition in terms of signals seen by satellite, aircraft, and moored sensors. We have used one-dimensional models to specify the rules of engagement between *G. breve* and other functional groups of phytoplankton, two-dimensional models to explore the consequences of their interaction with the microbial food web, and three-dimensional models to predict their transport, landfall, and residence time at the surface of the sea.

Following competition theory, our present models of the limiting resources of light, nitrate, ammonium, DON, phosphate, DOP, iron, and silicate should allow the coexistence of eight functional groups of phytoplankton, without differential grazing pressure on chromatically-adapted phytoplankton. In our analogue of the West Florida shelf, CO_2 and N_2 are state variables, but they are considered to be in excess of algal needs. From our simulation analyses thus far, we find that 1) diatoms win when estuarine and shelf-break supplies of nitrate are made available to a model community of small and large diatoms, coccoid cyanophytes and *Trichodesmium*, non-toxic and red-tide dinoflagellates, microflagellates, and coccolithophores, 2) a numerical recipe for large red tides of G. breve instead requires DON supplies, mediated by iron-starved, nitrogen-fixers in response to Saharan dust events, while their small blooms may persist on sediment sources of DON, 3) selective grazing must still be exerted on the other non-toxic dinoflagellates by copepods, 4) bacteria drive the outer shelf food web into P-limitation, until coastal supplies of low N/P ratio of ~1 favor nitrogen-fixers, 5) light-cued vertical migration of *G. breve* in relation to seasonal changes of summer downwelling and fall/winter upwelling flow fields determines both their duration within the first optical depth as a remotely-sensed signal and the intensity of red tide landfalls along the barrier islands and beaches of West Florida, and 6) termination of G. breve blooms is likely to result from cumulative, biomass-dependent losses in the form of UV-B irradiation, microbial-induced lysis, and unselective grazing pressure from protozoans and heterotrophic dinoflagellates.

ECOHAB FLORIDA, PHYSICAL OCEANOGRAPHY

Robert H. Weisberg, Ruoying He, William Hemme, Zhenjiang Li, and Huijun Yang
College of Marine Science, University of South Florida, St. Petersburg, FL. 33701

The circulation of west Florida continental shelf exhibits seasonal variability in both its background currents and the responses of these currents to synoptic scale weather forcing. The seasonal circulation is primarily forced by local momentum and buoyancy inputs. However, it is also modulated by mass and heat exchanges with the adjacent Gulf of Mexico. In situ measurements show summer and winter seasons of predominantly downwelling and upwelling circulations, respectively. This occurs regardless of adjacent offshore Loop Current influence, although such influence does affect the seasonal behavior by modifying the across-shelf density field. The seasonally varying density field, in turn, presents a critical control on the response of the shelf to local weather forcing. Under stratified conditions we find that the inner-shelf responses to upwelling and downwelling favorable winds are rectified such that the upwelling responses are disproportionately larger than the downwelling responses. This behavior is attributed to the relative slopes of the isopycnals and the bottom. Buoyancy torque adds constructively with planetary vorticity tilting by the sheared coastal jet under upwelling favorable winds, whereas it adds destructively under downwelling favorable winds. The end result is that the bottom Ekman layer response is enhanced (suppressed) for upwelling (downwelling), and this bottom Ekman layer asymmetry causes the asymmetry in the inner-shelf responses. Since the biology of the west Florida shelf is strongly influenced by bottom Ekman layer processes, these physical oceanographic findings are potentially very important for ECOHAB.

Along with its in-situ measurements, ECOHAB Florida has a numerical circulation modeling component aimed at simulating the seasonal and synoptic scale variability and supplying three-dimensional circulation fields for use in ecological models. For reasons given above the numerical model results are critically tied to the in-situ density field and to the fluxes of momentum and buoyancy, both locally and at the shelf break. Model simulations are therefore limited by model initializations and forcing functions. Without adequate in-situ information, modeling is of limited use. Nowcast or hindcast model runs tends to be useful for integration times of order one month. Improving upon this requires improving upon both the surface and the offshore boundary condition data and providing adequate interior density data for model assimilation. Initial attempts at combining the physical and ecological models are encouraging.

ABSTRACTS OF POSTERS

DEVELOPMENT OF THE VOLUNTEER OFFSHORE RED TIDE MONITORING PROGRAM FOR THE GULF COAST OF FLORIDA

Jay P. Abbott, Earnest W. Truby, and Karen A. Steidinger
Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Avenue S.E., St. Petersburg, FL 33701

Gymnodinium breve red tides originate offshore on the west Florida shelf and then can be transported to inshore areas by currents and winds. In the past, detection of blooms before they reached inshore waters was mostly by research cruises or the occasional report of discolored water or dead fish by boaters, commercial fishermen or airplane pilots. The rationale for this program was to have regular sampling in areas offshore of areas where red tides have traditionally come inshore, Tampa Bay to Charlotte Harbor, and where shellfish harvesting is economically important.

Developing a plan for the Volunteer Offshore Monitoring Program involved determining where, when and how the sampling should take place as well as who should be targeted for involvement.
Since red tides may affect any portion of Florida's Gulf coast, a few key areas were selected for volunteer recruitment. These areas included Pensacola, Panama City, Apalachicola, Cedar Key, St. Petersburg, Charlotte Harbor, Naples and the Lower Keys. The coastal nature of these areas, with easy access to the Gulf of Mexico, make them ideal commercial charter fishing operations.

Utilizing FMRI contacts, Internet web-sites, and general networking, charter captains in these areas were contacted about participating in the program. Most commercial charter captains understand that monitoring for red tide is in their own best interest, however, sampling may differ from scheduled times based on weather, business, and other reasons. It was important for us to understand that these volunteer captains were sampling on their paying customer's time. The sampling procedure and return shipping process needed to be quick and user friendly. All the sampling bottles and shipping materials were pre-labeled and any instructions were made as concise and unambiguous as possible. The volunteer captains were asked to sample at offshore distances of 1,5,10,20, and 30 miles approximately twice a month. When possible, additional volunteers in the same area were recruited. This reduced the possibility of sampler "burnout" by any one captain as well as providing backup coverage during mechanical troubles, vacations, etc.

Possibly the most important key to obtaining sampling stability is regular contact with the volunteer captains and providing them with some type of visual feedback on their work. Maps displaying the red tide cell counts from each sample are relayed to the volunteers, allowing them to serve as monitors of their local waters. It is crucial that the volunteers feel as though they are a part of a meaningful program and that their efforts are recognized. Current activities involve developing appropriate rewards and enlisting captains to collect bottom water samples. At present only surface water samples are collected.

ENVIRONMENTAL CONDITIONS ASSOCIATED WITH DOMOIC ACID IN RAZOR CLAMS ON THE WASHINGTON COAST

Nicolaus G. Adams[1], Mitch Lesoing[2], and Vera L. Trainer[1]
[1]National Marine Fisheries Service, Northwest Fisheries Science Center, Environmental Conservation Division, Seattle, Washington 98112
[2]Quileute Natural Resources, Quileute Indian Tribe, La Push, Washington 98350

In October 1998, record levels of the neurotoxin domoic acid were detected in razor clams (*Siliqua patula*, Dixon) resulting in the closure of shellfish harvesting areas along the Washington coast. This toxin was measured in seawater samples collected at Kalaloch Beach and Second Beach on the central Washington coast using a receptor binding assay and liquid chromatography-tandem mass spectroscopy. Domoic acid levels ranging from 0-2700 ng/L were measured in seawater samples containing from 70-100% *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle at concentrations of 1.0-15 x 10^6 cells/L, resulting in maximum levels of cellular toxin of approximately 500 fg/cell. A cultured isolate of this species collected from Kalaloch Beach also produced DA, as determined by the receptor binding assay, during late exponential and stationary stages of growth. The toxic *P. pseudodelicatissima* bloom in the late summer and autumn of 1998 occurred 2-3 weeks after strong coastal upwelling during a period of anomalously low rainfall, typical in post-El Niño years. Higher toxin levels in seawater at Kalaloch Beach compared to Second Beach were attributed to the periodic nature of upwelling at Kalaloch Beach, demonstrated by a 175-fold increase in nitrate in seawater coincident with a 5^{o}C decrease in sea surface temperature on September 1. The upwelling event in September was followed by wind relaxation and reversal at the end of that month, resulting in the transport of toxic cells toward the coast where nutrients were already present to fuel the algal bloom. A pulse of nutrients, either from rainfall or upwelling, to coastal regions that have experienced several weeks of low nutrients, followed by wind relaxation or reversal events that transport cells to inshore regions, are suggested to be important factors in the initiation of the most toxic *Pseudo-nitzschia* spp. blooms on the Washington coast.

IMPACT OF DINOFLAGELLATE-COEXISTING BACTERIA ON THE PHYSIOLOGY OF *PFIESTERIA*-LIKE DINOFLAGELLATES

Mohammad R. Alavi and Robert M. Belas
Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt Street, Baltimore MD 21202

In nature, dinoflagellates exist alongside a myriad of other microorganisms. In numbers alone, the largest percentage of this microbial population is made up of the bacteria. Some of these bacteria may directly and indirectly influence the dinoflagellates physiology and metabolism. For this reason, we are interested in understanding how the bacterial community specifically affects the physiology and toxin production of *Pfiesteria*-like dinoflagellates. We have taxonomically classified the bacterial species co-existing in four laboratory cultures of *Pfiesteria* and *Cryptoperidiniopsis* spp. originally obtained from sites of Chesapeake Bay fish kills. Our approach was based on the use of oligonucleotide primers and polymerase chain reaction to amplify a portion of the small ribosomal subunit gene (16S rDNA) from both the culturable and nonculturable bacteria in these cultures. The nucleotide sequence of each unique clone was determined and the homology between the dinoflagellate bacteria and known species was used to construct phylogenetic trees of taxonomic relatedness. We then produced axenic dinoflagellate cultures and studied the influence of culturable bacterial isolates on the growth and feeding behavior of these cultures by add-back experiments. Our results indicate that there are substantial differences between axenic dinoflagellate cultures and their bacterized counterparts. These differences include, but may not be limited to, reduction in growth rate and lowered cell density, as well as changes in feeding behavior.

SALINITY TOLERANCE FOR 62 STRAINS OF *PFIESTERIA* AND *PFIESTERIA*-LIKE STRAINS

Robert A. Andersen and Barbara E. Sullivan
Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 USA

Based upon preliminary studies, experimental cultures were prepared at salinities of 0.5, 1, 2, 3, 4, 5 and 6 ppt and 35, 40, 45, 50, 55 and 60 ppt for lower upper tolerance limits, respectively. Cells were grown at 22^{o} C under a 12 hr light/dark cycle. Cultures (at 12 ppt salinity) were fed immediately before beginning the experiment with *Rhodomonas* (CCMP768); no food was added to the cultures during the experiment. For each salinity treatment, the water was prepared as a batch and then dispensed. After 5 days, each test tube was examined with a dissecting microscope to determine the presence of swimming dinoflagellate cells. After this examination, the salinity of each test tube was measured again using a refractometer to verify that the experimental salinity had not changed due to evaporation. If no swimming cells were observed in all three tubes, the contents of two tubes were transferred into a clean Petri dish so that a more thorough examination was possible. If no swimming cells were observed in the Petri dish, then the third tube was adjusted to 12 ppt and the culture was maintained for two additional days. At the end of the two-day period, the test tube was examined again for swimming cells as described above. When swimming cells were observed at the end of the two-day period, we assumed that viable cysts were the source of the swimming cells; when no swimming cells were observed at the end of the two day period, we assumed that all cells and cysts died. After five days, most Chesapeake Bay and Neuse River system cells were still swimming at 1 ppt and 40 ppt; one strain had swimming cells at 0.5 ppt and six strains had swimming cells at 50 ppt. After 5 days, most Wilmington River cells were still swimming at 4 ppt and 50 ppt; several strains had swimming cells at 3 ppt and two had swimming cells at 60 ppt. Putative cysts allowed for even greater salinity tolerance because many cultures without swimming cells at extremely low or high salinities produced swimming cells when the salinity was adjusted back to 12 ppt. There was a statistically significant ($p < 0.001$) difference between the mean lower and mean upper salinity tolerances of the Wilmington River system and the Chesapeake Bay system or Neuse River system. The original Wilmington River water samples were more saline, suggesting that salinity tolerance is under genetic control.

THE EFFECTS OF CLAY, USED IN THE CONTROL OF HARMFUL ALGAL BLOOMS, ON THE GROWTH RATE OF JUVENILE HARD CLAMS, *MERCENARIA MERCENARIA*

Marie-Claude Archambault[1], Monica Bricelj[1,2], Jon Grant[1] and Donald M. Anderson[3]
[1] Department of Oceanography, Dalhousie University, 1411 Oxford Street, Halifax, NS B3H 4J1, Canada
[2]Institute for Marine Biosciences, National Research Council, Halifax, NS B3H 3Z1, Canada
[3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1049

In recent years there has been increasing interest in using clays as a means of mitigating toxic or Harmful Algal Blooms (HABs) at relatively shallow, enclosed mariculture sites. This control method, which causes flocculation and accelerated sedimentation of large algal cells (dinoflagellates), has been used at fish aquaculture sites in Korea and Japan. It is presently being evaluated as a potential method for controlling HABs in the United States (ECOHAB, Woods Hole Oceanographic Institution).

The sedimenting flux of flocculated clay-algal particles stripped from the water column may have implications for the health of benthic suspension feeders, e.g. commercially important bivalves. This is the first of a series of experiments to evaluate these effects by re-creating the field application of a Phosphatic Clay (IMCP, 2 μm mean equivalent spherical diameter, ESD) to a HAB condition using a laboratory re-circulating flume, and *Prorocentrum micans*, as a test, non-toxic dinoflagellate species. It was hypothesized that survival and growth rate of juvenile *Mercenaria mercenaria* would be adversely affected by the bottom deposition of a relatively thick clay and dinoflagellate layer (~ 7 mm in depth, at a loading rate of 155 g dry weight m^{-2}) in a low flow environment. Initial results indicate that clams subject to the deposition of clay and dinoflagellates over a coarse sand bottom, and fed *Isochrysis galbana* for two weeks, suffered a 50% decrease in shell growth rate compared to clams in a zero clay/dinoflagellate control. No significant mortality occurred in either treatment. Ongoing analysis of these experiments will consider clam response in terms of tissue growth and condition measures. Particle size and clay-algal interactions in different flow conditions were quantified using a Small Volume Particle Microsampler (SVPM), which captures intact flocculated particles on a filter, and is subject to image analysis. Floc characteristics were quantified in order to determine their potential effects on clam feeding behavior. Results indicate that the mean ESD of flocs ranges from 5 to 15 μm regardless of the tested flow conditions. This floc size range is maintained after the resuspension of clay-dinoflagellate deposits allowed to settle over 24 hrs. Dinoflagellates were stripped from the flume water column at a removal efficiency of 90% in 4.5 hours by sinking clay aggregates. The flume water column was completely expunged of particles within 48 hours. Continuing experiments involve varying flow/deposition conditions, e.g. near-bottom clay resuspension at higher flows, and further particle imaging as a measure of floc dynamics.

TEMPERATURE-CONTROLLED GERMINATION OF CYSTS OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE*, FROM BAY OF FUNDY, CANADA

Tamieka Armstrong[1], Matthew Harris[1], Brian Thompson[2], Patricia Matrai[2], and Maureen Keller[2*]
[1]University of New England, Biddeford, ME 04005, USA
[2]Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME 04575, USA

The physiology of the eastern Gulf of Maine *Alexandrium* populations is unknown and laboratory work is required to develop an understanding of *Alexandrium* dynamics in this area. To this effect, an experiment was conducted to determine the rates of cyst germination as a function of temperature (2, 4, 6, 8 and 15°C) and light (light vs. dark), following the experimental protocol used previously by Bronzino et al. for cysts of the Casco Bay region. Preliminary observations of the percentage of cyst germination in individual sediment samples kept at 8°C over time show that the germination rate increased exponentially after 12 days. On the other hand, excystment at this temperature under dark conditions shows the same pattern as under light conditions but with lower abundance. Results from the various incubation temperatures will be compared. Germination functions will be developed and compared with those derived for the Casco Bay *Alexandrium* isolates.

*This work was started under the leadership of the late Dr. Maureen D. Keller.

IDENTIFICATION OF CELL CYCLE REGULATORS IN THE FLORIDA RED TIDE DINOFLAGELLATE, *GYMNODINIUM BREVE*

Michele Barbier, Tod Leighfield and Frances Van Dolah
Marine Biotoxins Program, Center for Coastal Environmental Health and Biomolecular Research, National Ocean Service, Charleston, SC 29412

The diel cycle is a key regulator of the cell cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*, and may play a rate limiting role in bloom formation. Both laboratory cultures and field populations of *G. breve* exhibit phased cell division in which approximately one-third of the population divides each day. The dark/light "dawn" transition provides the diel cue that serves to entrain the *G. breve* cell cycle, with S-phase beginning 6-8 h into the light phase, and mitosis following 12-14 h later. This research is aimed at identifying the molecular targets of this diel cue. Here we identify in *G. breve* the two components of cell cycle regulatory complex, cyclin and cyclin dependent kinase (CDK), which are the molecular basis of cell cycle regulation in eukaryotes. The presence of CDK was identified by western blotting and by cell cycle inhibition with the specific CDK inhibitor, olomoucine. Cyclin was identified in *G. breve* by western blotting using two antibodies: the first one raised against cyclin B of the yeast *Schizosaccharomyces pombe*, the second one raised against the cyclin box of the sea-urchin cyclin. Both antibodies recognize a ~64 kD antigen in *G. breve,* and cross- react specifically with the control peptide cyclin B1, but not with cyclin A. This suggests that the *G. breve* cyclin identified is a cyclin B homologue.

The expression of the cyclin B homologue in *G. bre*ve was followed by western blotting at different time points during cell cycle. Unlike cyclin B in higher eukaryotes, which is expressed only late in the cell cycle, the cyclin B homologue in *G. breve* was expressed at similar levels throughout cell cycle. This unusual behavior was confirmed by immunohistochemistry. Preliminary results show that cyclin B was present in the cytoplasm throughout the cell cycle, where it appeared to be localized to the centrosomes. Unlike higher eukaryotes, cyclin B did not appear to be translocated to the nucleus prior to mitosis; this may reflect the fact that the nucleus remains intact during mitosis in dinoflagellates. However, it was localized to the mitotic spindles in mitotic telophase, similar to observations in mammalian cells. The unusual constitutive expression of dinoflagellate cyclin has been previously demonstrated in *Crypthecodinium cohnii* (Barbier et al., 1995), and has also been shown in the myxomycete, *Physarium polycephalum*.

CAN KRILL TRANSFER DOMOIC ACID TO HIGHER TROPHIC LEVELS SUCH AS SQUID AND WHALES?

Sibel Bargu[1], Christine L. Powell[2], Gregory J. Doucette[2] and Mary Silver[1]
[1]Institute of Marine Sciences, University of California, Santa Cruz, CA 95064
[2]Marine Biotoxins Program, NOAA/National Ocean Service, Charleston, SC 29412

Euphausiids (krill) are a ubiquitous prey for many consumers. The principal predators of Pacific euphausiids are squid, baleen whales, and certain seabirds (e.g., ashy storm petrel). Krill are potential vectors for domoic acid (DA) transfer from toxic *Pseudo-nitzschia* to higher trophic levels in the Monterey Bay food web. An understanding of the quantitative trophic interactions and body burden of DA in krill is required to predict whether krill can effectively vector the toxin. Nonetheless, laboratory experiments on euphausiids together with their larger predators are not currently feasible.

This poster presentation will describe the *Pseudo-nitzschia* link to whale and squid diets in Monterey Bay based on results from the examination of blue and humpback whale fecal material and squid stomach contents collected from 1993 to 2000. Scanning electron microscopy was used to identify *Pseudo-nitzschia frustules* in fecal material or stomach contents of whale and squid. Our aim was to determine whether these diatoms were transmitted to the predators through their food source, as was reported previously for a sea lion mortality event in 1998. Samples are also being tested for domoic acid activity using a receptor binding assay. Detection of *Pseudo-nitzschia frustules* in feces and stomach contents demonstrated that both baleen whales and squid obtained this diatom as part of their diet. Results of toxin analyses currently underway will be discussed in the context of potential transfer of DA via krill vectors from *Pseudo-nitzschia* to higher trophic levels.

VERTICAL DISTRIBUTION OF *ALEXANDRIUM* SP. IN THE WATER COLUMN

Stephanie L. Bennett[1], David W. Townsend[1]
[1]5741 Libby Hall, School of Marine Sciences, University of Maine, Orono, ME 04469

Much research has been done on the species of *Alexandrium* that are the cause of PSP in the Gulf of Maine, trying to establish under what conditions dinoflagellate blooms are likely to occur. Past surveys of *Alexandrium* in the GOM have focussed on the phytoplankton populations in the surface layers. With evidence published by McIntyre et al in 1997 that *Alexandrium* were capable of diel vertical migration under laboratory conditions of nutrient stress, the question of whether this occurs in natural populations arises. My research is an investigation into the vertical distribution of *Alexandrium* in the Gulf of Maine, and how that distribution changes over time.

In June of 2000, two sites in the Gulf of Maine were chosen for 24-hour sampling of the vertical profile. Both sites were well offshore and chosen because of the presence of *Alexandrium* in the net tow and the absence of the dense diatom population that we saw near shore. Water samples were taken at 5-meter intervals between the surface and 55 meters, a total of 12 depths collected each hour for 25 hours. All seawater was collected in 5 L Niskin bottles attached to the CTD rosette. The ship followed a 25 m drogue during each experiment. Hydrographic data was collected and water was drawn from each sample for measurements of chlorophyll, inorganic and organic nutrients and *Alexandrium* counts. Cell counts were done using a stain comprised of an antibody, specific to the genus *Alexandrium*, attached to a fluorescent tag. All counts were done using an epifluorescence microscope.

Results will be presented, showing the depth distribution of *Alexandrium* populations in relation to total chlorophyll, light and nutrients.

DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR RAPID DETECTION OF *PFIESTERIA PISCICIDA* AND RELATED DINOFLAGELLATES

Holly A. Bowers[1], Torstein Tengs[1], Howard B. Glasgow, Jr.[2], JoAnn M. Burkholder[2], Parke A. Rublee[3] and David W. Oldach[1]
[1]Institute of Human Virology and University of Maryland School of Medicine, Baltimore, MD 21201;
[2]Department of Botany, North Carolina State University, Raleigh, NC 27695
[3]Biology Department, University of North Carolina at Greensboro, Greensboro, NC 27402

Pfiesteria complex species are heterotrophic and mixotrophic dinoflagellates that have been recognized as harmful algal bloom species (HAB) associated with adverse fish and human health effects along the East Coast of North America, particularly in its' largest (Chesapeake Bay, Maryland) and second largest (Albemarle-Pamlico, North Carolina) estuaries. In response to impacts on human health and the economy, monitoring programs to detect the organism have been implemented in affected areas. However, until recently, specific identification of the two toxic species known thus far, *Pfiesteria piscicida* and *Pfiesteria shumwayae* (sp. nov.), required scanning electron microscopy (SEM). SEM is a labor-intensive process in which a small number of cells can be analyzed, posing limitations when the method is applied to environmental estuarine water samples. To overcome these problems, we developed a real-time PCR-based assay that permits rapid and specific identification of these organisms in culture and heterogeneous environmental water samples. Various factors likely to be encountered when assessing environmental samples were addressed and assay specificity was validated through screening of a comprehensive panel of cultures, including the two recognized *Pfiesteria* species, morphologically similar species, and a wide range of other estuarine dinoflagellates. Assay sensitivity and sample stability were established for both unpreserved and fixative-preserved (acidic Lugol's solution) samples. The effects of background DNA on organism detection and enumeration were also explored and, based on these results we conclude that the assay may be utilized to derive quantitative data. This real-time PCR-based method will be useful for many other applications, including adaptation for field-based technology.

TEMPORAL AND SPATIAL DISTRIBUTION OF *PFIESTERIA PISCICIDA* IN CHESAPEAKE BAY WATERS, 1999-2000

Holly Bowers[1], Torstein Tengs[1], Andrew Ziman[1], Peter Tango[2], David Goshorn[2], Robert Magnien[2], Renee Karrh[2] and David Oldach[1]
[1]Institute of Human Virology and University of Maryland School of Medicine, Baltimore, MD 21201;
[2]Maryland Department of Natural Resources, Annapolis, MD 21401.

Pfiesteria piscicida, a heterotrophic dinoflagellate, has been associated with adverse fish and human health effects in estuarine environments extending from Delaware to Florida. Due to public concern over the risk of exposure to putative toxins and the economic loss related to fish kills in the Chesapeake Bay in Maryland, the Maryland Department of Natural Resources and collaborating laboratories began monitoring various tributaries of the Bay in 1997 and developed those efforts into an extensive and comprehensive program for 1998 - 2000. Targeted rivers include those in which *P. piscicida* has been previously detected (through molecular methods, scanning electron microscopy and fish kill bioassays), rivers having a possible predisposition to *Pfiesteria* activity (chemical and physical characteristics similar to affected systems), and rivers with no history or apparent tendency for *Pfiesteria*-related problems. A real-time PCR-based assay for detection of the organism developed by our laboratory was utilized in the comprehensive monitoring program. In 1999, *P. piscicida* was not detectable in surface waters through the spring and early summer, with the organism being detected at only one station by mid-July. More intensive sampling during 2000 has detected *P. piscicida* in April at one site and at seven other sites by mid-July. In general, nutrient concentrations and phytoplankton biomass were higher at stations where *P. piscicida* was detectable in the water column, in comparison to sites repeatedly testing negative. Detection of *P. piscicida* in the water column has been repeatedly associated with the occurrence of summer/fall populations of Atlantic menhaden with ulcerative lesions that have been found to harbor a number of pathogens (e.g. fungi, bacteria, parasites). Further analysis of biological and physicochemical parameters associated with the detection of *P. piscicida* and assessment of those parameters in waters consistently negative for the organism may enable identification of conditions conducive to *Pfiesteria* blooms. Hypotheses generated with these data will be tested through ongoing longitudinal analyses of samples collected at multiple stations throughout the Chesapeake Bay region.

AN EVALUATION OF HPLC COUPLED WITH ELECTROCHEMICAL OXIDATION (ECOS) FOR THE ANALYSIS OF PSP TOXINS IN SHELLFISH

Gregory L. Boyer and Gregory G. Goddard
Faculty of Chemistry, State University of New York, College of Environmental Science and Forestry, Syracuse, New York, 13210

High performance liquid chromatography (HPLC) is a powerful tool for the analysis of PSP toxins found in shellfish. PSP toxins must be oxidized to form a fluorescent derivative prior to detection. This oxidation can be done either by a post-column chemical reaction (PCRS) or by an electrochemical reaction (ECOS). Electrochemical oxidation has both advantages and limitation when compared to the traditional approach. Both techniques give similar results when compared to the mouse bioassay. An examination of 40 toxic samples of giant scallop (*Placopectin magellanicus*) and 25 toxic samples of geoduck clam (*Panopea generosa*), was conducted using the mouse bioassay, HPLC with PCRS and HPLC with ECOS. Both shellfish types contained mostly gonyautoxins, with GTX-2,3 accounting for the bulk of the total toxicity. Distinct matrix effects were observed using the two shellfish types. Scallop samples presented a much dirtier matrix and required clean up on a solid phase extraction cartridge prior to analysis using the ECOS system. Both HPLC-PCRS (slope of the regression line = 1.01; R-squared = 0.94) and HPLC-ECOS (slope = 0.93; R-squared = 0.96) accurately predicted the mouse bioassay results using scallop samples. The geoduck samples were assayed without prior clean up on a solid phase extraction cartridge. For these samples, the PCRS accurately predicted the mouse bioassay results (slope = 0.92; R-squared = 0.98) whereas ECOS tended to underestimate the total toxicity as determined by the mouse bioassay (slope = 0.70; R-squared = 0.95). Most of the variation was contributed by samples whose total toxin content was less than 100 µg STX eq. per 100 g FW. With proper sample preparation, both HPLC-PCRS and HPLC-ECOS provided a viable alternative to the mouse bioassay with the ECOS system simpler to purchase, maintain and operate. Detailed conditions for the set-up, use and operation of the HPLC-ECOS system with shellfish will be provided.

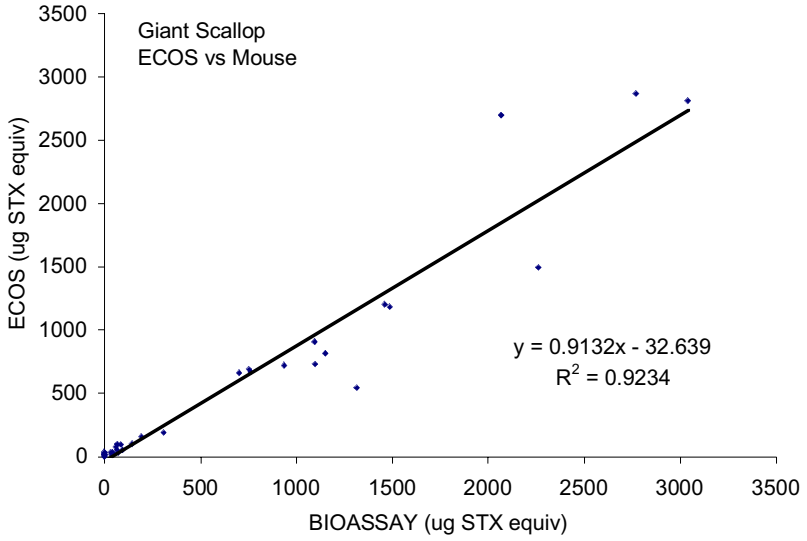


Figure. The relationship between total toxin as determined using HPLC with the electrochemical oxidation system (ECOS) versus the mouse bioassay for 40 giant scallop samples: slope = 0.913 ± 0.04, y-intercept = -32.639 ± 40.6, n=40)

THE ROLE OF ZOOPLANKTON GRAZERS IN *ALEXANDRIUM* SP. BLOOM DYNAMICS IN THE NEAR-SHORE ENVIRONMENT OF THE GULF OF MAINE

Robert G. Campbell, [1] Gregory J. Teegarden,[1,2] Allan D. Cembella,[3] Edward G. Durbin[1]
[1]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882 USA
[2]Environmental Studies, Bowdoin College, 6500 College Station, Brunswick, Maine 04011 USA
[3]Institute for Marine Biosciences, NRC, 1411 Oxford St., Halifax, Nova Scotia B3H 3Z1 CANADA

The role of zooplankton grazers in *Alexandrium* sp. bloom dynamics was investigated in coastal waters of the Gulf of Maine during the spring of 1998 and 1999. We collected samples at weekly intervals from several stations for zooplankton and phytoplankton abundance, biomass, species composition, and toxin content. In addition, grazing rates of zooplankton were determined using natural water samples. Dominant zooplankton included copepods (primarily *Acartia hudsonica* and *Calanus finmarchicus*) and barnacle nauplii (*Semibalanus* sp.). The feeding behavior of the zooplankton on *Alexandrium* sp. in moderate bloom conditions (≤3000 cells/L) was species specific; *Acartia* was non-selective, *Calanus* was somewhat selective, and *Semibalanus* avoided them. Toxin was measured in zooplankton tissues collected from the field stations and was present for several weeks after the decline of the bloom. Despite our laboratory findings that toxin retention was inefficient (see abstract by Teegarden), it appears that retention is sufficient to pose risks to higher trophic levels. *Acartia* was by far the most abundant zooplankton species at the near-shore stations and its filtration rates on *Alexandrium* sp. was positively related to temperature but independent of ambient chlorophyll concentrations. The filtration rates did not saturate over the range of food concentrations encountered during the study, and consequently, measured grazing impacts were in good agreement with estimates from temperature and biomass measurements alone. During 1998, there was a moderate bloom of *Alexandrium*; peak concentrations reached 3000 cells/L in late May at one of the inshore stations. In contrast, in 1999 concentrations were very low throughout the study period. During both years zooplankton biomass was low during the early spring but increased exponentially over the study period. In 1998, grazing impacts during the peak and subsequent decline of the bloom increased from 25 to 80% day^{-1} in concert with the increase in zooplankton biomass and appeared to contribute to the bloom's demise. Our findings suggest that grazing can be an important source of mortality and will depend on zooplankton filtration rates, degree of selective feeding, and the biomass and species composition of both the phytoplankton and zooplankton communities.

INVOLVEMENT OF DOMOIC ACID IN MARINE MAMMAL MORBIDITIES AND MORTALITIES ON THE WEST COAST OF THE U.S. DURING THE YEAR 2000

Mei Mei Ch'ng[1,2], Tod A. Leighfield[1], Mark A. Busman[1], Frances Gulland[3], Keith Matassa[4], Melissa Chechowitz[5], Teri Rowles[6] and Frances M. Van Dolah[1]
[1]NOAA, NOS, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC
[2]National University of Malaysia (UKM), Selangor, Malaysi
[3]The Marine Mammal Center, Marin Headlands, Sausalito, CA
[4]Marine Mammal Care Center, Fort MacArthur, San Pedro, CA
[5]California Department of Fish and Game, Santa Cruz, CA
[6]NOAA, NMFS, Office of Protected Species, Silver Spring, MD

Domoic acid (DA) is an neuroexcitatory amino acid that has been the causative agent for amnesic shellfish poisoning in humans (Prince Edward Is., 1987), seabird mortalities (Monterey Bay, CA, 1991), and sea lion mortalities (Monterey Bay, 1998). This study summarizes DA analyses of body fluids from marine mammal stranding events that occurred along the California coast in the year 2000. For the first time, DA poisoning has had widespread impacts on several different marine mammal species with diverse feeding habits and geographic distributions. Year 2000 west coast marine mammal mortality events began early in the spring, with an abnormally high number of gray whale mortalities occurring during their northward migration from the Baja calving grounds to the Alaska feeding grounds. Over 350 gray whale mortalities were documented on the west coast this year, compared with 273 in 1999 and less than 50 in previous years. Of these, 25 mortalities were reported in the San Francisco Bay area (San Mateo to Marin counties). Unlike many of the stranded gray whales elsewhere on the west coast, most of the animals that stranded in the Bay area in during April and May were in good body condition. Their stranding followed, by approximately two weeks, a bloom of the DA producing diatom *Pseudonitzschia australis* in Monterey Bay, an area the whales would have migrated through. Body fluid samples (urine, feces) were positive for DA in two of the 11 animals tested (for the other 9 animals, only blood was available for analysis). Gray whales feed on benthic invertebrates by disturbing the sediment and then sieving disrupted sediments through their baleen. Therefore, it was reasoned that toxin exposure might be through a benthic food web that became exposed to DA as the planktonic diatom bloom declined. Urine from 4 of 7 benthic-feeding sea otters that died during the same time frame (February – April) in this region also tested positive for DA.

In June - July, more than 90 sea lions stranded on the central coast of California, primarily in San Luis Obispo County. Most of the stranded animals were in good body condition, but displayed seizure and scratching activities documented previously in the 1998 sea lion mortality event. All animals displaying DA symptoms tested positive for DA in urine or feces. Blood samples from the same animals rarely had measurable levels of DA; thus, analysis of blood in future mortality events may be regarded as non-informative. Concurrent *Pseudo-nitzschia* bloom activity was documented in this region. Subsequent to sea lion strandings in the San Luis Obispo area, seizuring sea lions began to occur approximately 100 miles to the south, in Ventura and Los Angeles counties (mid-late July), where all symptomatic animals tested were positive for DA in urine. Sea lions most likely acquired DA toxicity through the planktonic food web, as previously described (Scholin et al. 2000 Nature 403: 80-83).

In addition to these species, two humpback whale strandings occurred during this time frame, the first in May and the second in late July. The second stranding co-occurred with a transition from *Pseudo-nitzschia* dominated algal blooms to *Alexandrium* dominated blooms. Because *Alexandrium* is a saxitoxin (STX) producer (paralytic shellfish poisoning), serum and stomach contents were analyzed for both DA and STX. Humpback whale samples were negative for both algal toxins.

ANNUAL BLOOM CYCLES OF TOXIC *PSEUDO-NITZSCHIA*, CELLULAR DOMOIC ACID AND MACRONUTRIENT DYNAMICS IN MONTEREY BAY, CALIFORNIA

Susan Coale[1], Mary Silver[1,2], Shonna Dovel[1], Raphael Kudela[1,2] and Ron Tjeerdema[3]
[1] University of California, Santa Cruz, CA 95064
[2] Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039
[3]University of California, Davis, CA 95616

Much of our understanding of the physiology and toxin production in species of *Pseudo-nitzschia* is inferred from laboratory based studies conducted on cultures of *P. multiseries*. These studies have done much to advance our knowledge but are limited since they were conducted on clonal isolates instead of natural populations.

Here we present, for the first time, a 12 month sequence of field observations documenting several bloom cycles of *Pseudo-nitzschia australis* and *P. multiseries.* Abundances of toxic *Pseudo-nitzschia* species, together with cellular domoic acid (DA) concentrations and corresponding levels of water column macronutrients were measured. We compare these data with previously reported HABs and results of current laboratory research on DA production in cultures of *Pseudo-nitzschia sp*. to better understand bloom dynamics and DA production in natural populations. Preliminary results indicate particulate DA concentrations in the water column are strongly correlated with the abundances of the known toxin producers *Pseudo-nitzschia australis* and *P. multiseries.* In addition, cellular concentrations of DA from these field samples corroborate published values for *Pseudo-nitzschia* cultures. Peak abundances of *Pseudo-nitzschia* do not necessarily correspond to maxima in chlorophyll a concentrations but likely reflect rapid response to changing nutrient levels or other meso scale features. Likewise, cellular concentrations of DA in *P. australis* may vary due to changing ratios of macronutrients. The results and preliminary interpretations from this ongoing study will be presented.

DIFFERENTIAL EFFECTS OF TOXIC *ALEXANDRIUM* SP. ON NORTHERN VERSUS SOUTHERN POPULATIONS OF THE COPEPOD *ACARTIA HUDSONICA*

Sean P. Colin[1] and Hans G. Dam[1]
[1]Department of Marine Sciences, University of Connecticut, Groton, CT

The frequency and duration of harmful algal blooms have been increasing worldwide with detrimental effects including fish kills and human illness. Locally, along the east coast of North America, the toxic dinoflagellate *Alexandrium* sp. appears to have spread in its geographical distribution. The consequences of this spreading to grazers are not understood. Our goal is to examine the effects of *Alexandrium* on the copepod *Acartia hudsonica* (=*clausi*) from five different regions along the coast of North America (New Brunswick, Maine, Cape Cod, Connecticut, and New Jersey) which have differences in the frequency and toxicity of *Alexandrium* blooms. We hypothesize that, in the presence of local adaptation, copepod populations from regions which experience regular and highly toxic *Alexandrium* blooms will exhibit enhanced fitness when feeding on toxic *Alexandrium* compared to copepod populations from regions where the blooms rarely occur and are less toxic. To test this hypothesis, we performed laboratory experiments examining the ingestion, egg production and hatching rates of *A. hudsonica* feeding on a toxic strain of *Alexandrium*. For all of the copepod populations, ingestion and egg production rates increased with concentration of *Alexandrium* (ranging from 25-500 µgC/l). However, consistent with our hypothesis, the northern populations (Cape Cod, Maine and New Brunswick) exhibited significantly higher ingestion and egg production rates than the southern populations. In another experiment, the five copepod populations were given toxic and non-toxic *Alexandrium*, and non-toxic *Tetraselmis* sp. (a green flagellate) at a high (500 µgC/l) and low concentration (100 µgC/l). Again, we found that the populations from Connecticut and New Jersey exhibited significantly lower ingestion and egg production rates when fed the toxic *Alexandrium* strain than the other two diets. Thus is appears that local adaptation has occurred in northern *A. hudsonica* populations enabling them to resist the toxic effects of *Alexandrium* better than the southern populations. Possible causes for the observed decrease in rate processes of the southern copepod populations will be discussed in light of experiments examining the possibility of physiological incapacitation.

CLONING AND IDENTIFICATION OF A SODIUM CHANNEL GENE FROM THE CLAM *MYA ARENARIA*

Laurie Connell[1], V. Monica Bricelj[2], Betty M. Twarog[3], Scott P. MacQuarrie[2], William T. Roubal[1], Vera L. Trainer[1]
[1]Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA, 9811
[2]Institute for Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia, B3H 3Z1 Canada
[3]University of Maine, Darling Marine Center, Walpole, ME, 04573

Paralytic shellfish poisoning (PSP), caused by consumption of bivalve molluscs that have been suspension-feeding on toxigenic dinoflagellates (species of *Alexandrium* in North America), constitutes a public health hazard and causes severe economic losses on both coasts of the U.S. Paralytic shellfish toxins (PST) inhibit nerve impulse action potential via specific blocks of the voltage-gated sodium channel. It is known that bivalve species vary greatly in their sensitivity to PSTs and thus in their capacity for toxin accumulation. However, the molecular basis of this variability is unknown. Specific mutations in the pore region of the sodium channel can reduce or eliminate binding to the nerve fiber of the potent PST, saxitoxin (STX) (Noda et al. 1989; Terlau et al. 1991). It is hypothesized that mutations in the STX-binding regions of the sodium channel protein are able to confer resistance to shellfish exposed to PSTs, analogous mutations are found in insects with pyrethroid resistance (Martinez-Torres et al. 1998; He et al. 1999).

Previous work has established that the clam, *Mya arenaria*, can indeed be physiologically impaired and lose their burrowing capacity by exposure to *Alexandrium* and that their nerve action potential is blocked by STX (Bricelj et al. this meeting). However, these responses were shown to vary among individuals. For example, most clams from populations previously exposed to toxic *Alexandrium* blooms are able to burrow during toxin exposure, yet those from previously unexposed populations were not. Less is known about what mechanism(s) clams utilize to resist the effects of PST exposure. In order to characterize a potential mechanism for clam resistance to STX the sodium channel gene will be sequenced and compared among populations with varying resistance to *Alexandrium* exposure.

Because STX is known to bind specifically to sequences at the sodium channel pore, a first step in this study involves the identification of the sodium channel gene from the target clam species and is followed by a comparison of the pore regions among populations. *Mya arenaria* nerve and ganglion tissues were dissected from populations with no history of exposure to PSTs for use in both biochemical and molecular biological assays. Using western blot techniques, nerve tissue was probed with a polyclonal sodium channel antibody resulting in the detection of a ~250Kd protein, a size consistent with sodium channels from other organisms. Total RNA was extracted from the pooled tissues for cDNA synthesis. Degenerate sodium channel PCR primers were designed by consensus sequence comparisons compiled from mammalian, insect, and molluscan genera. The DNA sequence of PCR amplicons derived from cDNA using degenerate primers was determined. The resulting predicted amino acid sequence revealed a high degree of homology with voltage-gated *para*-type sodium channel genes. As expected, the putative amino acid sequence of the sodium channel domain IV pore regions SS1 and SS2 from STX-sensitive clam populations did not have a mutation at amino acid D1717. This *M. arenaria* sodium channel gene sequence was then used as a probe to retrieve sodium channel genes from *M. arenaria* nerve tissue cDNA libraries. cDNA library derived sodium channel gene isolation and sequencing is currently underway. Future work will involve comparison of the pore regions between individual clams previously tested for STX susceptibility/resistance and determination of the relative regulation of those genes.

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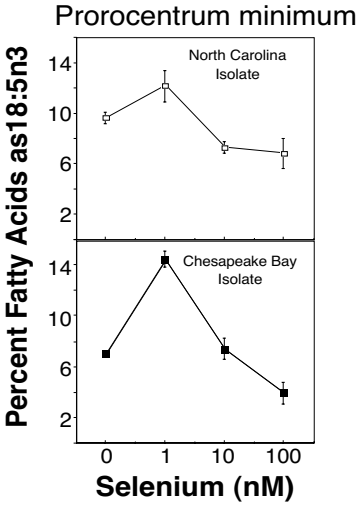
RELATIONSHIP BETWEEN OCTADECAPENTAENOIC ACID, ALGICIDAL TREATMENTS, AND TOXICITY IN TWO BLOOM FORMING DINOFLAGELLATES, *GYRODINIUM GALATHEANUM* AND *PROROCENTRUM MINIMUM,* FOUND IN THE CHESAPEAKE BAY, MD.

Jonathan R. Deeds[1], Daniel E. Terlizzi[2,1], Diane Stoecker[3], Robin Way[1], and Allen R. Place[1]
[1]University of Maryland Biotechnology Institute, Center of Marine Biotechnology, Baltimore, MD 21202 [2]University of Maryland Sea Grant Program, College Park, MD, 20742
[3]University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD, 21613

Since 1996, fish kill events associated with blooms of *Gyrodinium galatheanum*, a small (10-15 μm) mixotrophic, non-thecate dinoflagellate, have occurred periodically at an estuarine pond production facility, raising hybrid striped bass on the Chesapeake Bay. It has been observed at this facility that *G. galatheanum* blooms treated with potassium permanganate ($KMnO_4$) result in fewer, or no, fish mortalities, compared with treatments of copper sulfate ($CuSO_4$). One bloom in 1996, treated with $CuSO_4$, resulted in the loss of 20,000 fish. We have shown Chesapeake Bay isolates of both *Gyrodinium galatheanum* and *Prorocentrum minimum* to contain the hemolytic endotoxin octadecapentaenoic acid (18:5n3). The fatty acid 18:5n3 has been suggested as the toxic agent responsible for aquaculture related fish kills due to the dinoflagellates *Gyrodinium aureolum* and *Gymnodinium* cf. *mikimotoi*. This study was undertaken to determine if differences exist between algicidal treatments of $CuSO_4$ and $KMnO_4$ on two bloom forming dinoflagellates, *G. galatheanum* and *P. minimum*, and if these differences influence ichthyotoxicity.

We have shown that, depending upon the addition or deletion of soil extract and/or chicken manure extract to culture media, *G. galatheanum* and *P. minimum* cells can contain from 4.88% - 15.43% and from 5.81% -19.44%, respectively, of their total fatty acids as 18:5n3. The addition of 1 nM selenium to selenium replete culture media was shown to stimulate a similar effect in two different isolates of *P. minimum*. Further, we have shown that for *G. galatheanum* cells, the major pools for 18:5n3 are the monogalactosyl diacylgylcerols (MGDG) and diagalactosyl diacylglycerols (DGDG). This is also in accordance with other toxic dinoflagellates containing 18:5n3. *Pfiesteria piscicida*, another dinoflagellate implicated in MD fish kills, was shown to be present at the facility and its role in the kills cannot be ruled out. Several isolates of *P. piscicida,* cultured on cryptomonads, were found to contain no 18:5n3. This study was part of a continuing research effort to determine if poly-unsaturated fatty acids (PUFAs) contribute to ichthyotoxicity in selected Chesapeake Bay dinoflagellates.

Cell lysis (70-90% max.) occurred in both clonal cultures tested upon exposure to varying levels of $KMnO_4$, while cell lysis due to $CuSO_4$ only occurred in *G. galatheanum* cultures (approx. 75% max.). In a related experiment at the above mentioned facility, samples of a bloom of *Katodinium rotandatum* treated with either $CuSO_4$ or $KMnO_4$, showed increased aqueous inorganic N and P levels in both treatments, suggesting cell lysis or leakage. However, chlorophyll *a* concentrations dropped only in $KMnO_4$ treated samples suggesting oxidation of certain cellular components. The results obtained thus far suggest that: 1. Both *G. galatheanum* and *P. minimum* contain a potentially toxic hemolytic lipid fraction, and 2. Although both $CuSO_4$ and $KMnO_4$ treatments were algicidal, $CuSO_4$ treatments may allow the intact release of cellular contents while $KMnO_4$ treatments may not. Further experimentation will be required to validate these



hypotheses and to determine their respective involvement in ichthyotoxicity. Results will be discussed in relation to algicidal treatment effects on PUFA content and release.

OCCURRENCE OF *ALEXANDRIUM OSTENFELDII* IN THE GULF OF MAINE

Abigail Deitz[1] and David W. Townsend[1]
[1]5741 Libby Hall, School of Marine Sciences, University of Maine, Orono, ME 04469

Phytoplankton samples were collected in the Gulf of Maine during April-May and June of 2000 as part of the broadscale survey cruises of the GOM-ECOHAB Program. Samples were examined for the presence of *Alexandrium* sp. based on an immunofluorescent technique that involves a genus-specific antibody-antigen assay. In this procedure, a secondary antibody is conjugated to a fluorescent compound, fluorescein isothiocyanate (FITC), which allows visual detection using an epifluorescence microscope. Enumeration of the samples revealed two distinct categories of size ranges, those cells of diameter 30-35µm, indicative of *A. tamarense* and/or *A. fundyense*, and larger cells, greater than 40µm diameter, with some reaching a maximum of 55µm. These larger cells exhibited unique intracellular characteristics in the form of golden-colored inclusions when viewed under epifluorescence. Inclusions found in these large cells numbered from one to three per cell and usually appeared round to elongate in shape and lacked internal structure. The presence of these inclusions may indicate mixotrophy. We suspect that these larger cells are *Alexandrium ostenfeldii* (Jacobson and Anderson, 1996; Balech and Tangen, 1985).

Highest surface (2m depth) densities of *A. ostenfeldii* were observed in the June 2000 samples, although maximum densities were <100 cells L^{-1}; cell densities were <50 cells L^{-1} in April-May. The distributions of *A. ostenfeldii* in April-May showed no single center of abundance but were generally widespread. Concentrations on the order of 50-100 cells L^{-1} occurred offshore of Penobscot Bay, in the Casco Bay region and at the mouth of the Bay of Fundy. In June, the greatest concentration of cells occurred at the mouth of the Bay of Fundy and along the axis of the Eastern Maine Coastal Current. Distributions of *A. ostenfeldii* were similar to those of total *Alexandrium* sp., except that *A. ostenfeldii* was absent from Jordan Basin in June, while other *Alexandrium* spp. were abundant. Possible correlations with nutrients and hydrography will be presented.

ASSESSMENT OF HEAT TREATMENT TO REDUCE RISK OF MICROBIAL INTRODUCTIONS VIA BALLAST WATER

Elif Demir, Martina Doblin, Lisa Drake, Fred Dobbs
Ocean Earth and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Avenue, Norfolk, VA 23529-0276

International shipping has great potential to transport harmful organisms via ballast water. The invading capacities of these non-indigenous organisms were recognized after several prominent invasions in different locations around the world (e.g., zebra mussels in the North American Great Lakes and toxic dinoflagellates in Australia). Scientists are now evaluating different methods for treating ballast water to reduce the risks of introducing harmful and non-harmful organisms to coastal environments.

A recent study (Rigby et al., 1999) tested the effects of heating ballast water of tropical origin, using heat diverted from the ship's engine during a 10 day transoceanic voyage. A temperature increase of 10^{o}C over 2 days killed most of the phytoplankton cells and dinoflagellate cysts, but the fate of other microbes was not determined. We conducted a similar study in the laboratory, using water from the lower Chesapeake Bay to determine the effectiveness of heat treatment in temperate climes. Water was collected, filtered (53 μm), dispensed into dark bottles to simulate conditions in ballast tanks, and seeded with *Gymnodinium catenatum* cysts (produced from strains HU11 and DE08). The water was heated from 25°C, its temperature on collection, to 35°C over 2 days, mimicking the thermal regime used by Rigby et al. (1999). Controls were not heated but otherwise treated the same. Throughout the 10-day incubation, samples were collected for chlorophyll (fluorescence), bacteria (direct counts), and virus-like particles (direct counts). After 10 days, cysts of *G. catenatum* were enumerated. Findings from this study will help assess the efficacy of heat treatment of ballast water from temperate sources.

GROWTH CHARACTERISTICS OF CLONAL *PFIESTERIA PISCICIDA* IN CULTURE

Tomas Drgon, Danara Krupatkina, Jana Drgonova, and Gerardo R. Vasta
Center of Marine Biotechnology, University of Maryland Biotechnology Institute
701 East Pratt Street, Baltimore MD 21202

Pfiesteria piscicida is a heterotrophic dinoflagellate that exhibits predatory behavior towards live algae and other marine microorganisms. Due to its association with fish kills along the mid-Atlantic coast of North America and possible impact on human health it has become the subject of intense research on its biological and molecular aspects. This required the development and optimization of reliable culture methodologies that would yield a reproducible biomass. Published reports on the culture and life cycle of *P. piscicida* have described multiple life stages present in aquaria containing fish and environmental water or sediments. We examined selected growth characteristics of clonal *P. piscicida* cultured in the presence of cryptomonads, and attempted to produce high density cultures to be used as a source for isolation of DNA, RNA, proteins or other molecules of interest. Cultures of *Rhodomonas* sp. (50 ml; $3x10^5$ cells/ml) in 75 ml flasks were inoculated with 5 ml of a fast-growing culture of clonal *P. piscicida* to a final density of 10^3 cells/ml, and grown under standard conditions (85 $\mu M.m^{-2}sec^{-1}$ light intensity; 14h/10h light/dark; 23° C temp). Samples were collected daily, and relative cell numbers of *P. piscicida* and *Rhodomonas* sp., *P. piscicida* cells containing two nuclei, and cells with ingested *Rhodomonas* sp. were determined by microscopy. *Rhodomonas* sp. actin by assessed by PCR. Size distribution of *P. piscicida* cells was determined microscopically by measuring the cells' long axis (n= 50-100) with a micrometer eyepiece. Reproducible high-density *P. piscicida* cultures ($2x10^5$ cells/ml) were obtained by the optimized protocol described above. One day after inoculation there was a substantial increase in numbers of round-shaped binucleated *P. piscicida* cells. This was followed by increases in *P. piscicida* cell numbers and median cell size, and a sharp decline in *Rhodomonas* sp. numbers, which was followed by a decrease in *P. piscicida* cells with visible ingested *Rhodomonas* sp. This was correlated with the disappearance of the actin signal for *Rhodomonas* sp. After the disappearance of *Rhodomonas* sp. cells from the culture, *P. piscicida* cells with visible ingested *Rhodomonas* sp. were only observed during the following 1 - 2 days. After the culture was depleted of *Rhodomonas* sp., there was a sharp drop in *P. piscicida* cell numbers, with a concomitant decrease in median cell size. An increase in the proportion of *P. piscicida* cells with two or more nuclei was observed at the end of the 10-day culture period. These cells, however, differed morphologically from those observed immediately after inoculation.

CHARACTERIZATION OF ACTINS AND TUBULINS FROM THE HETEROTROPHIC DINOFLAGELLATE *PFIESTERIA PISCICIDA*. SUBCELLULAR LOCALIZATION BY IMMUNOFLUORESCENCE

Jana Drgonova, Eric J. Schott, Cathleen A. Coss, Danara Krupatkina, Tomas Drgon and Gerardo R. Vasta
Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St. Baltimore, MD 21202

The phagotrophic dinoflagellate *Pfiesteria piscicida*, a species associated with fish kills in mid-Atlantic estuaries, requires algal prey as a source of nutrition. *P. piscicida* biflagellated zoospores actively swim towards their prey, attach to it, and feed on its contents in a process known as myzocytosis. When the prey is abundant, and ambient conditions favorable, *P. piscicida* multiplies vegetatively by cell division. Mitosis in dinoflagellates differs from nuclear division in other eukaryotes by the permanent presence of a nuclear envelope and an extranuclearly localized spindle, the apparatus responsible for separation of chromosomes. Swimming, feeding and cell division rely on functional actin and tubulin cytoskeletons, and their characterization is critical for understanding the molecular aspects of these processes. Furthermore, actins and tubulins are well conserved throughout the eukaryotic lineages, and their primary structures have proven useful tools for phylogenetic analysis. Thus, *P. piscicida* actins and tubulins may provide valuable information, in addition to that from rRNA genes, for revealing its taxonomic relationships to other Dinozoa. DNA from *P. piscicida* was isolated and used as a template for PCR amplification with "universal" actin primers (kindly provided by Dr. G.W. Warr, Medical University of SC, USA). Two PCR products, 0.7 kb and 1.9 kb were cloned and sequenced. A new set of primers designed on the 0.7 kb amplicon was used to confirm its origin in *P. piscicida* DNA. This *P. piscicida* actin fragment had no introns and was about 70 % identical to the corresponding actin fragments from the dinoflagellates *Prorocentrum minimum, Gyrodinium galatheanum,* and *Amphidinium carterae.* Three partial β-tubulin sequences from *P. piscicida* were obtained using degenerate primers designed by Keeling et al. (J. Euk. Microbiol. 45: 561-570, 1998). Two of the sequences were 97 % identical to each other, 70 % identical to the third one, and all three contained introns. Alpha-tubulin was localized by indirect immunofluorescence to the longitudinal flagellum.

MOLECULAR IDENTIFICATION AND DETECTION OF *CYLINDROSPERMOPSIS RACIBORSKII* (CYANOBACTERIA) USING NIFH AND CPCBA-IGS

Julianne Dyble, Pia H. Moisander and Hans W. Paerl
Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell St, Morehead City, NC, 28557, USA

Cylindrospermopsis raciborskii is a cosmopolitan cyanobacterial species, found worldwide in temperate and tropical habitats. It is an invasive species that is increasingly common in eutrophying freshwater and estuarine systems and has been a significant threat to water quality for many years in L. Balaton (Hungary), Paranoá Reservoir (Brazil), and the Murray-Darling River System (Australia). Since it forms toxic blooms and is often found in water used for drinking and recreation, sickness and death in both animals and humans has been attributed to *C. raciborskii* toxins. *C. raciborskii* is a dominant member of the phytoplankton community in the St. Johns River System, FL and Falls Lake, NC at certain times of the year. Not only are these water bodies used as drinking water sources, but they are also in the drainage basins of larger estuarine systems. Establishment of *C. raciborskii* in these systems, and other US estuaries, could be detrimental to human health as well as provide a mechanism of transport for expansion.

Molecular approaches are particularly useful in characterizing differences in populations, especially those that are morphologically identical, to track their sources and dispersion. *C. raciborskii* populations from NC and FL were characterized based on two environmentally relevant genes: nifH and cpcBA-IGS. NifH is a highly conserved gene that encodes one of the protein subunits of nitrogenase, the enzyme involved in N_2 fixation. CpcBA-IGS is the highly variable phycocyanin intergenic spacer region, which is part of the phycocyanin operon that gives cyanobacteria their characteristic blue-green color. We developed primers specific to *C. raciborskii* for both nifH and cpcBA-IGS based upon DNA sequences from these gene fragments obtained from multiple *C. raciborskii* cultures. These primer sets have been used to detect *C. raciborskii* in environmental samples, even at low cell densities. Differentiating populations is critical to determining population sources and predicting expansion. Molecular tools are useful to obtain a quantitative measure of population similarity as well as detect those target strains in a mixed phytoplankton community. Molecular approaches are also valuable in determining how many different strains exist and need to be considered by managers.

TIME SERIES OF OPTICAL PROPERTIES AND BLOOM ECOLOGY FROM A BROWN TIDE AND AN ADJACENT CONTROL SITE IN LONG ISLAND

Stacey M. Etheridge[1,2] and Collin S. Roesler[1,2]
[1]Univ. of Connecticut, Dept. of Marine Sciences, Groton, CT
[2]Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME

Since 1985 Long Island, New York embayments have been plagued with recurrent blooms of the 2.5 μm chrysophyte *Aureococcus anophagefferens*. Several hypotheses abound regarding the ecological controls on these blooms, including the ability of this species to out compete others due to its unique capacity to utilize organic nitrogen and carbon. These blooms, referred to as brown tides due to the color they impart to the water, were the focus of this study. From 17 May-8 June 2000, a time series of ocean color, particulate and dissolved absorption, dissolved fluorescence, particulate scattering, phytoplankton pigments, and particle size distributions were collected from two Long Island embayments. A brown tide developed in Quantuck Bay, whereas in West Neck Bay *A. anophagefferens* cells were in low concentrations and represented an insignificant contribution to the algal community.

During the brown tide in Quantuck Bay, spectral radiance reflectance changed in both magnitude (brightness) and shape (Fig. 1a), while phytoplankton and colored particulate organic material (CPOM, non-phytoplankton) contributed significantly to total absorption of blue photons (Fig. 1b,c). Phytoplankton size-fractionated absorption demonstrated that most of the cells were between 1-3 μm, consistent with *A. anophagefferens*. Spectral shape indicated that after the first day of the study, algal community structure remained constant. Absorption by the <0.2 μm CDOM in Quantuck Bay approximately equaled that by phytoplankton (Fig. 1d). Near the end of the time series the contribution by the 0.2-0.7 μm size fraction increased, suggesting new CDOM release or colloidal aggregation.

The control site exhibited a different suite of optical properties and size contributions. The relatively constant shape and slight magnitude fluctuations detected in radiance reflectance in West Neck Bay suggested minor community structure alterations (Fig. 1e). The <0.2 μm CDOM dominated the absorption coefficient (Fig. 1h). The concentration and spectral shape of this component remained invariant during the study. Phytoplankton and CPOM absorption were comparable (Fig. 1f,g). CPOM displayed no variation in shape; however, phytoplankton absorption, due mainly to cells between 1-3 μm and some <1 μm, changed in spectral shape indicating that the algal community varied slightly.

Further investigation of the optical properties separated into size-fractionated components provides characteristics of bloom ecophysiology. It is feasible that modeling these parameters from remotely sensed ocean color will provide a breadth of knowledge about bloom dynamics.

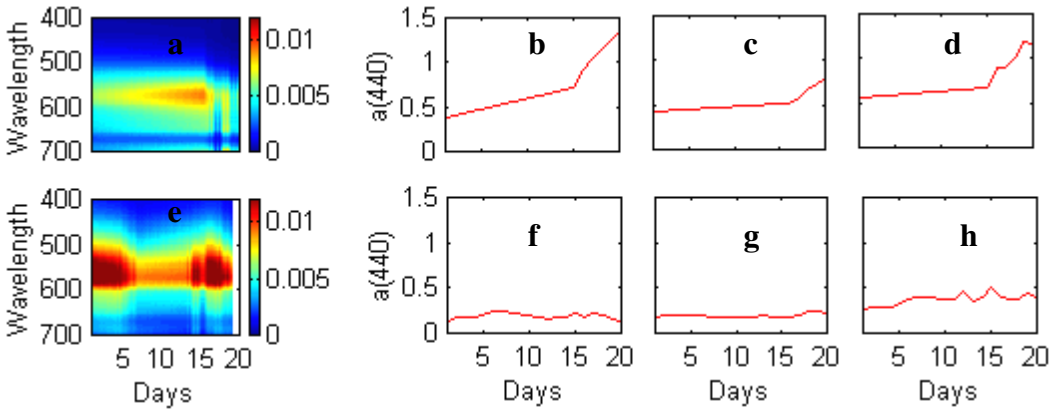


Figure 1 a) Spectral radiance reflectance, b) phytoplankton, c) CPOM, and d) CDOM absorption at 440 nm at Quantuck Bay. Plots e-h display those measurements for West Neck Bay.

PHOTOPHYSIOLOGICAL RESPONSES OF THE RED-TIDE DINOFLAGELLATE *GYMNODINIUM BREVE* (DINOPHYCEAE) UNDER NATURAL SUNLIGHT

Terence J. Evens[1], Gary J. Kirkpatrick[2], David F. Millie[1,2] and David J. Chapman[3]
[1]Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70179
[2]Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236
[3]Department of Ecology, Evolution and Marine Biology, and the Marine Science Institute, University of California at Santa Barbara, Santa Barbara, California 93106

Little is known concerning the physiological mechanisms by which microalgae, particularly bloom-forming taxa, tolerate large and variable amounts of photosynthetically available radiation (400-700 nm) and ultraviolet radiation (295-400 nm). Because *Gymnodinium breve* Davis often accumulates at or near the air-water interface, the diurnal, photophysiological responses of this red-tide dinoflagellate were investigated. Laboratory cultures of *G. breve* were incubated outdoors, and allowed to acclimate to attenuated natural irradiance. Aliquots of photoacclimated cultures were exposed to PAR+UV or PAR-only irradiances, incubated within Sarasota Bay, Florida (USA), and assessed for diurnal responses of *in vivo* fluorescence and *in vitro* pigmentation, lipid, carbohydrate, and protein contents over three distinct photoperiods varying from overcast to partly cloudy to extremely sunny conditions. The maximum quantum yield for stable charge separation at photosystem II exhibited midday depressions (roughly) symmetric about solar noon on the overcast and partly cloudy days, but exhibited a pronounced hysteresis on the sunny day. The induction and relaxation of the xanthophyll cycle over the course of the photoperiod during the partly cloudy and sunny days resulted in stoichiometrically inverse cellular accumulation of the photoprotective pigments, diadinoxanthin and diatoxanthin. Only minor adjustments in the cellular chlorophyll *a* and fucoxanthin contents occurred during any photoperiod. No differences in the epoxidation state of the xanthophyll-cycle pigments or in the maximum quantum yields occurred between cultures exposed to PAR-only or PAR+UV treatments. However, the observed differences in the oxygen production rates and other biochemical parameters between cultures exposed to PAR-only or PAR+UV treatments were not directly attributable to UV-induced effects. These findings indicate that *G. breve* possesses an inherent UV resistance and a robust photosynthetic capability, thereby allowing cells to acclimate to variable irradiance regimes over relatively short time scales.

EFFECT OF CLAY SUSPENSIONS ON CLEARANCE RATE IN SIX SPECIES OF BENTHIC INVERTEBRATES

Lisa Ewert[1], Dane Frank[2], Sandra E. Shumway[1], J. Evan Ward[2]
[1]Southampton College, Long Island University, Southampton, NY 11968
[2]Department of Marine Sciences, University of Connecticut, Groton, CT 06340

Harmful algal blooms pose a threat to areas where fisheries and aquaculture products are a vital part of the economy. Recent attempts have been made, especially in Asia, to displace harmful algal blooms by spraying fine particulate mineral suspensions (e.g. "china clay") over the surface of affected coastal waters. In practice, the particles adsorb onto the surface of the algal cells, promoting coagulation and displacement to the bottom. Very little is known, however, about the impact of this technique on benthic communities and processes.

To examine the effects of china clay on filter feeding invertebrates, short-term feeding experiments were performed in the laboratory on six benthic species: the bay scallop (*Argopecten irradians*), Eastern and Pacific oysters, *Crassostrea virginica* and *C. gigas*, blue and foolish mussels, *Mytilus edulis* and *M. trossulus*, respectively. The percent time that hydroids, *Obelia* sp. remained open in the presence of clay was also analyzed at different concentrations. China clay used as a means of harmful algal bloom mitigation in Korea was supplied by colleagues. Measurements of depletion rates of the invertebrates were performed using solutions of 10, 100, 1000, or 10,000 $mg \cdot L^{-1}$ of clay suspended with the unicellular alga *Rhodomonas lens* ($2.5x10^4$ $cells \cdot ml^{-1}$) in 0.4µm filtered seawater. Size distribution and concentration of particles were determined before and after experiments using a Coulter Multisizer. The effects of clay on clearance rates and behavior of the invertebrates were species specific. The clearance rate of *C. virginica* was not impacted until clay concentrations reached 10,000 $mg \cdot L^{-1}$. *Argopecten irradians* showed a statistically significant difference between clearance rates of *Rhodomonas* and all clay concentrations. *Mytilus edulis* showed a decrease in clearance rates at the 1000 and 10,000 $mg \cdot L^{-1}$ concentrations. Clearance rates of *M. trossulus* and *C. gigas* were affected at clay concentrations of 100, 1000, and 10,000 $mg \cdot L^{-1}$. The percent-time-open of hydroids was statistically lower than the control at the 10, 100, and 10,000 $mg \cdot L^{-1}$ concentrations.

These results clearly demonstrate that clay has a significant and negative impact on filter feeding invertebrates and that some species are more sensitive than others to the suspended particulates. The use of clay as a strategy for the mitigation of harmful algal blooms should be approached with extreme caution.

DEVELOPMENT AND APPLICATION OF NEW CELL-SURFACE ANTIBODIES IN THE STUDY OF *PFIESTERIA PISCICIDA*

Tim Feinstein, Keri Costa and Senjie Lin
University of Connecticut Department of Marine Sciences, Groton, CT 06340, USA

We developed cell-surface antibodies against *Pfiesteria piscicida* and a major prey species, *Rhodomonas* sp., in an attempt to develop specific new tools in the study of this toxic dinoflagellate. Well-designed antibodies allow the quantitative measurement of field concentrations and have additional uses when coupled with immunomagnetic beads. We tested the antibodies' affinity and specificity on cultures *Pfiesteria* and related species and field samples. Some nonspecific binding by crude anti-*Pfiesteria* sera was encountered and was significantly mitigated through pre-adsorption of the serum onto other species and through blocking procedures. We also tested immunomagnetic protocols using mixtures of *Pfiesteria* with *Rhodomonas* as well as field samples, and negative separation. Direct and indirect methods with different sample fixation and blocking protocols were compared. We achieved a good negative separation (use of anti-*Rhodomonas*-coated beads to remove *Rhodomonas* and leave behind *Pfiesteria*) using anti-*Rhodomonas* but efforts continue to improve the specificity of the positive separation procedure (use of anti-*Pfiesteria*-coated beads to isolate *Pfiesteria*) that may be more useful for in situ applications.

EMERITA ANALOGA (STIMPSON)- POSSIBLE NEW INDICATOR SPECIES FOR THE PHYCOTOXIN DOMOIC ACID IN CALIFORNIA COASTAL WATERS

M. E. Ferdin[1], Rikk G. Kvitek[1], Carolyn Bretz[1], Mary W. Silver[2], Gregory J. Doucette[3], Christine L. Powell[3], Christopher A. Scholin[4]
[1]California State University, Monterey Bay (CSUMB), CA 93955
[2]University of California, Santa Cruz (UCSC), CA 95064
[3]Marine Biotoxin Program, NOAA/National Ocean Service, Charleston, SC 29412
[4]Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039

Documented occurrences of harmful algal blooms (HAB's) producing the neurotoxin, domoic acid (DA), has increased dramatically along the west coast of North America over the last decade. Blooms of DA synthesizing diatoms (Pseudo-nitzschia spp.) have been associated with the death and injury of hundreds of marine birds and mammals, posed serious health risks to humans, and threatened to significantly impact coastal fisheries and economies dependent on marine resources. Unlike the paralytic shellfish poisoning (PSP) toxins, also common on the west coast, a reliable intertidal indicator species for monitoring the presence of DA in the environment remains to be identified. Here we evaluate and confirm the utility of the common sand crab (*Emerita analoga*) as a DA indicator species in comparison with sea mussels (*Mytilus californianus*), the species currently used by the California Department of Health Services Shellfish Program for HAB toxin monitoring. Mussels and *Emerita* sampled from natural populations at two state beaches in Santa Cruz, California (Apr. 1999 - Feb. 2000) were tested for DA by the HPLC-UV method. Determinations of DA in mussels were below instrument detection. However, detectable levels of the toxin in *Emerita* ranged from 0.07 to 10.4 ug DA g-1 and coincided with observed trends in densities of DA producing *Pseudo-nitzschia* species nearshore. The rise and fall of DA in *Emerita* in synchrony with *Pseudo-nitzschia* abundance, combined with this common intertidal species' accessibility and ease of DA extraction, recommends *Emerita* as a reliable, cost effective monitoring tool for DA.

CANNIBALISM IN *PFIESTERIA PISCICIDA, PFIESTERIA* "B" AND PFIESTERIA-LIKE DINOFLAGELLATES

V. Foster, L. W. Haas, L. A. Ott
Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia, P.O. Box 1346, 23062

Under laboratory culture conditions, the fate of heterotrophic dinoflagellates, including *Pfiesteria piscicida*, *Pfiesteria* "B", "Lucy", *Cryptoperidiniopsis sp*., and "Shepherd's crook" is directly related to the abundance of available cryptophyte prey. We observed that as prey abundance decreased, heterotrophic dinoflagellate cultures cannibalized each other resulting in decreased cell concentrations. All observations were made from clonal, aseptically maintained stock cultures of heterotrophic dinoflagellates. Cannibalism is evident by the appearance of two nuclei visible inside a single dinoflagellate cell using epifluorescent microscopy. Depending on the time elapsed since the prey dinoflagellate was ingested, various-sized remnants of ingested nuclei are visible within a food vacuole inside the cannibalizing dinoflagellate. In the presence of abundant cryptophyte prey, cannibalism was observed at a low rate in all of the heterotrophic dinoflagellate cultures. In cultures where cryptophyte prey availability was greatly reduced, an increased rate of cannibalism was observed (ten-fold increase of dinoflagellates containing two nuclei). Our observations provide direct visual evidence of heterotrophic dinoflagellate cannibalism. These results suggest that in experiments where heterotrophic dinoflagellate concentration plays a role, cannibalism must be considered as a factor affecting experimental population concentrations as well as the overall experimental dynamics.

FINE-SCALE VERTICAL DISTRIBUTIONS AND POTENTIAL MICROZOOPLANKTON GRAZING IMPACT ON TOXIC DINOFLAGELLATES IN EAST SOUND, WA

Dian J. Gifford
Graduate School of Oceanography, University of Rhode Island

Many microplankton taxa occur in <0.5 m thick layers in the water column of East Sound, WA. Vertical profiles of this highly structured water column were collected during June in 1997 and 1998 using a high resolution profiling package. In both years the toxic dinoflagellate *Dinophysis acuminata* was located in a layer near the surface. The toxic dinoflagellate *Alexandrium catenella* was located in a layer centered at ~5 m depth. *A. catenella* cells were plainly visible in the food vacuoles of a variety of heterotrophic protist taxa distributed within and around the *A. catanella* layer, including *Lacrymaria* sp., *Polykrikos schwartzii*, and various aloricate choreotrich ciliates. These protozoan predators exhibited maxima around, but not within, the *A. catenella* layer. The role of predation by protozooplankton in cropping and termination of HABs is not well understood. These preliminary observations demonstrate (1) that the spatial distributions of HAB organisms can be highly localized in the water column and (2) that protozooplankton are potentially important predators on HAB organisms.

GEOHAB: GLOBAL ECOLOGY AND OCEANOGRAPHY OF HARMFUL ALGAL BLOOMS

The GEOHAB Scientific Steering Committee. P. Gentien, Chair. The U.S. representatives are P.M. Glibert[1] and D. Anderson[2]
[1]Horn Point Laboratory, University of Maryland Center for Environmental Science, PO Box 775, Cambridge, MD 21613
[2]Woods Hole Oceanographic Institution, Woods Hole, MA

GEOHAB is a new international programme sponsored by The Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) that will foster international collaborative research on harmful algal blooms (HABs). The aim is to compare ecosystem types sharing common features, key species involved and the oceanographic processes that influence their population dynamics. The overall goal of GEOHAB is to improve the prediction of HABs by determining the ecological and oceanographic mechanisms underlying the population dynamics of HABs by integrating biological, chemical, and physical studies supported by enhanced observation and modeling systems. The approach of GEOHAB is comparative, from the cellular to the ecosystem level. GEOHAB will address such research questions as:

1. What are the environmental factors that determine the changing distribution of HAB species, their genetic variability and the biodiversity of the associated communities?
2. What are the effects of increasing human activities (such as eutrophication and the translocation of species) on the occurrence of HABs?
3. What are the unique adaptations of HAB species that determine when and where they occur and the extent to which they produce harmful effects?
4. How do HAB species, their population dynamics and community interactions respond to changes in their environment?

GEOHAB projects will be funded by a variety of national and international sources; the programme therefore is a coalescence of projects at many levels – from large, multi-investigator, multi-national field investigations, to co-ordinated laboratory studies on specific processes or methods. The GEOHAB Programme will assist in bringing together investigators from different disciplines and countries to exchange technologies, concepts, and findings. This may take the form of formal or informal workshops, working groups, or collaborating teams of investigators.

NUTOX: an EUROHAB project
EFFECT OF NUTRIENT RATIOS ON HARMFUL MARINE PHYTOPLANKTON AND THEIR TOXIN PRODUCTION

Coordinator **:** Edna Granéli[1], *Partners* Egil Sakshaug[2], Jan Pallon[3], Rosa Martinez[4], Bernd Luckas[5], and Serge Maestrini[6]
[1]Marine Sciences Dept., University of Kalmar, S-391 82 Kalmar, Sweden
[2]Biological Station, Trondheim, Norway
[3]Dept. Nuclear Physics, Lund, Sweden
[4]Dept. of Aquatic and Environmental Sciences & Techniques, Santander. Spain, [5] Inst. of Nutrition & Environment, Jena, Germany, [6]CREMA, L'Houmeau, France

The NUTOX project initiates long-term research to understand the interactions between HAB and the changes of environmental conditions in the European coastal ecosystems related to human activities (EUROHAB initiative). Since 1998, the NUTOX team is focusing its research to determine if the increase of N and P in relation to Si, in the Baltic Sea and the Norwegian Sea, has favoured the occurrence of blooms of non-siliceous HAB species (i.e. dinoflagellates, haptophytes, cyanobacteria), and to understand how the ratios between N and P affect toxin production in some of these species. Making general recommendations for minimizing HAB toxin production is the ultimate goal of NUTOX, bringing together biologists, chemists and physicists of Northern, Western and Southern Europe. More specifically, the project addresses the following questions: Are potentially toxic flagellates and cyanobacteria outcompeting diatoms at high ratios and concentrations of N. P in relation to Si? Is toxin production directly connected to external nutrient (N, P) conditions? Is toxin production connected to cellular nutrient status? Is toxin production regulated at the gene level? Can toxin production be reduced by nutrient manipulation?

The NUTOX approach was to conduct studies with natural phytoplankton communities in mesocosms, and with strains isolated from European waters in semi-continuous and batch cultures. These studies were combined with algal ecophysiology investigations, the creation of molecular probes, the use of a nuclear microprobe for C, N and P analyses in single cells (elemental mapping) and bio-optical tools, the development of new analytical methods for algal toxins.

Among the achievements already made by NUTOX the following can be mentioned:

5. Potentially toxic flagellates and cyanobacteria can outcompete diatoms at high ratios and concentrations of N: P in relation to Si. However, silicon is not controlling the algal succession in summer phytoplankton communities in the Baltic Sea. Lowest algal biomass was obtained under N-deficient conditions compared to P-deficient or N, P, Si sufficient conditions. *H. triquetra* was most abundant in N-deficient compared to nutrient sufficient conditions. This result confirms the dominance of this species in the Baltic Sea from July to September where inorganic N concentrations are below detection

6. Toxin production is directly connected to cell nutrient quotas and therefore to external nutrient (N, P) concentrations. Toxin content is usually high when the algae are under nutrient stress for the ichthyotoxic species (*Chrysochromulina polylepis, Prymnesium* spp., *, Gymnodinium mikimotoi,*. However, for the species producing N-rich toxins such as saxitoxin and nodularin (*Alexandrium tamarense, A. minutum, Nodularia spumigena)* production decreased in N-limited cultures.

7. Toxin production can be reduced on a short time scale by nutrient manipulation in controlled conditions, and this is achieved by restoring the nutrient balance between

nitrogen and phosphorus. However, this approach implies the supply of the limiting nutrient in excess which would lead to increasing eutrophication. Therefore, decreasing both nitrogen and phosphorus loading to coastal systems is the ultimate solution to reduce algal toxin production.

8. Analyses of *D. norvegica* single cells chemical composition (originated from the Baltic Sea), showed that the C, N, P quotas showed a large diversity, indicating that all the cells were lightly to N- deficient (C:N = 20 to 55).

9. Probes for detecting the presence of stress proteins in *A. tamarense* cells have been designed.

THE ECONOMIC EFFECTS OF *PFIESTERIA* IN THE MID-ATLANTIC REGION

Timothy Haab[1], John Whitehead[2], Douglas Lipton[3], James Kirkley[4], George Parsons[5]
[1]Department of Agricultural, Environmental and Development Economics, Ohio State University, Columbus, OH 43210
[2]Department of Economics, East Carolina University, Greenville, NC 27858
[3]Department of Agricultural and Resource Economics, University of Maryland, College Park, MD 20742
[4]Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062-1346
[5]Departments of Economics and Marine Studies, University of Delaware, Newark, DE 19716

While significant amounts of research are currently being conducted to assess the biological, ecological and environmental effects of *Pfiesteria piscicida* and other harmful algal blooms (HABs), very little work has been conducted to look at the economic impacts or lost benefits due to *Pfiesteria* outbreaks or HABs. We report on the results from a 2-year Mid-Atlantic (North Carolina, Virginia, Maryland and Delaware) study of the economic impacts of *Pfiesteria* and HABs, on seafood consumption. The study consisted of a phone-mail survey of 2,000 Mid-Atlantic residents focusing on current seafood consumption patterns and reactions to harmful algal blooms, *Pfiesteria* outbreaks, and various education materials. Questions addressed include: What are the current seafood consumption patterns in the Mid-Atlantic region? What is the effect of a HAB/*Pfiesteria* outbreak on seafood consumption in the Mid-Atlantic region? What are the potential economic impacts of an outbreak (as opposed to welfare losses)? What are the regional similarities or differences in response to outbreaks (Albemarle/Pamlico Sound estuary system versus Chesapeake system)?

Preliminary results suggest that localized *Pfiesteria* associated fish kills significantly decrease the demand for seafood over large geographic regions creating large short-term economic losses. Reducing consumer uncertainty in relation to the risks from a *Pfiesteria* outbreak significantly mitigates the negative consumption effects of an outbreak. Demand models of seafood consumption demonstrate that perceived reductions in risk associated with an outbreak can significantly lessen the economic losses during and after an outbreak. Public distribution of scientific findings regarding the risks associated with *Pfiesteria* can reduce demand uncertainty and consequently reduce the economic losses.

GYRODINIUM GALATHEANUM GRAZING ON *PFIESTERIA PISCICIDA* AND *PFIESTERIA*-LIKE DINOFLAGELLATES

L. W. Haas, V. Foster, L. Ott and A. Rocha
Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia, P.O. Box 1346, 23062

Gyrodinium galatheanum is a mixotrophic dinoflagellate commonly observed in mid-Atlantic estuarine waters. Past laboratory and field observations revealed a range of *G. galatheanum* algal prey including flagellates and cyanobacteria (Li et al., 1999, 2000). We report here the results of grazing studies of *G. galatheanum* on *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates including *Pfiesteria* "B", "Lucy" and *Cryptoperidiniopsis sp*. All grazing experiments were conducted using clonal cultures. Under laboratory conditions (24 C and 12:12 light:dark cycle), *G. galatheanum* was capable of grazing *P. piscicida* and other similar dinoflagellates to near extinction within several days. The effect of the nutritional status of both predator and prey on grazing dynamics was examined. Pre-conditioning of *G. galatheanum* at various levels of inorganic nutrients appeared to have no effect on its grazing rate. The nutritional status of the prey appears to influence their susceptibility to being grazed, as grazing rates increased as food scarcity induced a reduction in prey cell size presumably related to starvation. Our results suggest that the mixotrophic nature of *G. galatheanum* allows this organism to persist under a wide range of environmental conditions and effectively graze other dinoflagellates as the food supply is reduced.

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SCRIPPSIELLA CAROLINIUM, A NEWLY IDENTIFIED DINOFLAGELLATE CAUSING WIDESPREAD RED TIDES IN SOUTH CAROLINA ESTUARIES

Kenneth C. Hayes[1,3] and Alan J. Lewitus[1,2]

[1]Belle W. Baruch Institute, University of South Carolina, Georgetown, SC 29442
[2]Marine Resources Research Institute, SC DNR, Charleston, SC 29412-2559
[3]Aquatic Biology Section, Bureau of Water, SC DHEC, Columbia, SC 29201

Reports of harmful algal blooms (HABs) in South Carolina estuaries or coastal waters are rare. Prior to 1998, the only published record of a HAB in South Carolina marine waters was a 1988 *Gymnodinium breve* red tide that originated in the Gulf of Mexico and was transported with the Gulf Stream to continental shelf waters off North Carolina and then southward to South Carolina nearshore waters. It is unknown whether the lack of HAB reports reflects the character of the state's estuaries (e.g., generally shallow and well-flushed, low-to-moderate nutrient levels) or a historical lack of effort in detecting HABs. In the spring of 1998, a new species of dinoflagellate, *Scrippsiella carolinium* (Fig. 1, formal description in prep) formed a red tide in Bulls Bay near McClellanville, South Carolina, the first documentation of a red tide localized to South Carolina estuarine waters. In the spring of 1999, the dinoflagellate formed red tides at several sites in South Carolina estuaries ranging > 100 miles apart (Georgetown to Hilton Head), commonly comprising > 99% of the total phytoplankton biomass, and at times reaching > 10^5 cell ml^{-1}. Anecdotal information from fishermen and scientists involved in long-term monitoring projects suggested that these red tides were a new phenomenon in SC estuaries. Studies in one of the affected estuaries (North Inlet near Georgetown) revealed that bloom initiation followed rain events, which resulted in low salinity water containing high concentrations of forest-derived dissolved organic material; e.g. dissolved organic carbon, DOC. Over the course of the bloom periods, *S. carolinium* abundance varied inversely with DOC, DON, and DOP concentrations, and positively with dissolved inorganic carbon. These patterns likely reflect high respiratory rates during the blooms, and may indicate that *S. carolinium* has a high ability to use organic nutrients loaded into estuaries. The causal link of organic carbon-rich loads to blooms of this species, *Prorocentrum minimum*, and some other red tide dinoflagellates suggests commonality in nutrient patterns that select for some red tides. Therefore, the potential influence of nutrient loading patterns on HAB stimulation should include consideration of both organic and inorganic nutrient types.

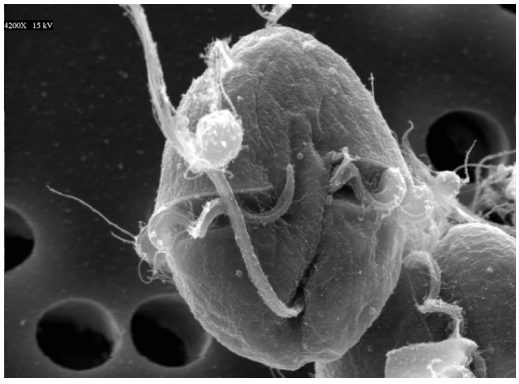


Fig. 1. *Scrippsiella carolinium*. SEM courtesy of Howard Glasgow, Jr., NCSU's Center for Applied Aquatic Ecology.

EFFECTS OF CLAY FLOCCULATION ON BREVETOXIN IN SEAWATER

Michael S. Henry, Richard H. Pierce, and Patricia C. Blum
Mote Marine Laboratory, Sarasota, Florida

The Florida red tide has been a recurrent and serious problem along the West Coast of Florida. It is caused by the dinoflagellate, *Gymnodinium breve*, a single celled alga that can grow to extremely high cell densities sufficient to discolor the water. *G. breve* produces an array of highly potent neurotoxins commonly called brevetoxins. Episodes of red tide cause shellfish toxicity, mortalities of fish and other marine organisms, as well as respiratory irritation in humans and marine mammals. Studies at Woods Hole Oceanographic Institution (WHOI) have shown clay to be a promising approach to control red tide by the use of clay as a flocculent to aggregate cells and other particles into a floc that settles to the bottom. Poly-aluminum chloride (PAC) is a compound commonly added with clay to facilitate the production of a floc. Studies were conducted at Mote Marine Laboratory to determine the effects of clay flocculation on brevetoxin in seawater. Two concentrations of clay were used with and without the addition of PAC. Two concentrations of *G. breve* were also used, 5 and 10 million cells per Liter. These red tide levels represent naturally occurring blooms. The higher loading of clay tested represented the amount used in the field pilot study (500 g/M^2) conducted in cooperation with WHOI in March 1999. The lower loading reflected the amount of IMCP clay currently being used in studies conducted at WHOI (0.25 mg/L).

These experiments were conducted in 4-L beakers with 3-L of 5×10^6 cells/L or 10×10^6 cells/L of diluted *G. breve* culture. The clay was added to each beaker as a wet slurry at the rate of 10g (high level; dry weight) or 0.75 g (low level; dry weight) IMCP clay in 100ml salt water. Each treatment was conducted in triplicate. For the experiments using PAC, it was added immediately after the addition of the clay in 10 ml of deionized water. After settling for 2.5 hours, a 10 ml sub-sample was collected for post flocculation cell counts and the rest of the samples was processed as described below for brevetoxin analysis.

The clarified culture above the settled clay (ca 2.8-L) was collected by siphon. The settled clay was then transferred to a 500 ml beaker. The *G breve* controls (no clay) were processed by extraction of the whole 3-L. The culture was extracted by using a C-18 disc (Ansys Diagnostics) and the toxin was eluted from the disc with methanol.

After allowing the clay to settle for 24 hours, the clay was transferred to centrifuge tubes and dewatered by centrifugation at 2800 rpms for 4 minutes. The clay pellet was extracted with an ultrasonic probe in acetone. After sonication the clay was centrifuged and the acetone collected. The acetone was evaporated to dryness and transferred to a vial in 3 ml of methanol for HPLC analysis.

Seven different treatments are presented. Clay, with and without PAC, at a high (Hi) and Low (Lo) loading as well as 5 and 10 million cells per liter cultures were tested. The treatments were as follows: 1) High clay loading with *G. breve* at 5 mc/L, 2) Same as 1 with the addition of PAC, 3) Low clay loading with *G. breve* at 5mc/L, 4) Same as 3 with the addition of PAC, 5) Low clay loading with lysed *G. breve* cells at 5mc/L, 6) Low clay loading with *G. breve* at 10 mc/L, and 7) Same as 6 with PAC. Figures 1 and 2 show cultures immediately after the addition of clay, and two and a half hours later, respectively.

For all treatments of live *G. breve* cells, the percent reduction of brevetoxin was greater than 94%. When used with lysed cells, the clay was only able to remove 80 % of the toxin from the lysed culture. In the absence of cells, this represents a situation where the clay is binding extracellular toxin,

suggesting that both the physical flocculation of cells as well as the binding of toxin occurs during the clay settling process.

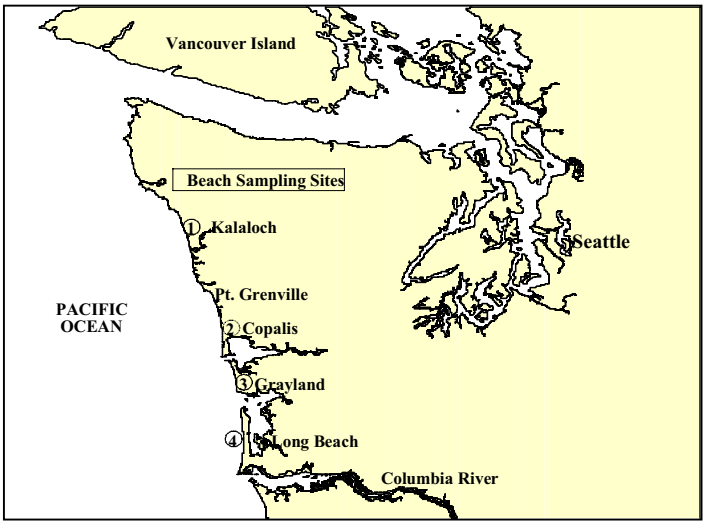
Low loading of clay worked as well, if not better, than high loading. The addition of PAC was not significant. These data show that clay is a highly effective method for removal of brevetoxin from culture in the laboratory. Future studies will involve natural blooms in enclosed mesocosms.

OLYMPIC REGION HARMFUL ALGAL BLOOMS (ORHAB): WASHINGTON STATE HARMFUL ALGAL BLOOM MONITORING PROJECT

Rita A. Horner[1] and Vera L. Trainer[2]
[1]School of Oceanography, University of Washington, Seattle, WA 98195-7940
[2]National Marine Fisheries Service, Northwest Fisheries Science Center, Seattle, WA 98112

Harmful algal blooms (HABs) are a common occurrence on the Washington coast, but our knowledge of the processes that govern their timing and spatial distributions, subsequent advection to the coast, and dispersal are not sufficient to predict the possibility of shellfish contamination in the area. In response to this need, the Olympic Region Harmful Algal Bloom (ORHAB) group was organized as a forum for collaboration and cooperation among federal, state, and local government agencies, academic institutions, coastal Native tribes, marine resource-based businesses, and public interest groups. Its mission is to support applied and basic research on HABs and to build a greater local capacity to monitor and mitigate the effects of such events. The ultimate goal is to sustain a long-term monitoring program into the future without reliance on federal support.

A multi-agency, multi-disciplinary project funded by NOAA's Coastal Ocean Program is investigating the origins of open-coast toxic blooms, monitoring where and when the toxic species are present on the coast, assessing the environmental conditions under which blooms occur and are transported to intertidal shellfish populations, and exploring methods that can be used to forecast HABs. The primary focus will be on the presence of *Pseudo-nitzschia* spp. and domoic acid in razor clams (*Siliqua patula* Dixon). The monitoring sites were chosen because they have harvestable razor clam populations and several have historical phytoplankton data.



The working hypothesis is that phytoplankton blooms in water over the Washington continental shelf are the source of toxins in razor clams on coastal beaches. The precise timing of physical processes (currents, winds, upwelling, and downwelling) off the coast determines whether a toxic bloom will be advected into the nearshore region and be sustained there long enough for razor clams to become toxic. The project will determine the temporal and spatial distributions of *Pseudo-nitzschia* spp. and relate these to hydrological and meteorological parameters using standard oceanographic and biological methods. New techniques for the rapid detection of toxins and toxigenic species will be tested in the field as they become available.

The major partners/collaborators and contact persons are: Northwest Fisheries Science Center (Vera Trainer, project PI); Battelle Marine Laboratory (Dana Woodruff); Olympic Coast National Marine Sanctuary (Ed Bowlby); Pacific Shellfish Institute (Dan Cheney, Ralph Elston); Quinault Indian Nation (Joe Schumacker); Saigene Corporation (Paul Haydock); University of Washington Olympic Natural Resources Center (Miranda Wecker); University of Washington School of Oceanography (Barbara Hickey, Rita Horner); Washington Department of Ecology (Jan Newton); Washington Department of Health (Judy Dowell); Washington Department of Fish and Wildlife (Doug Simon, Dan Ayres).

RELATIONSHIPS BETWEEN SEDIMENT TYPE AND *ALEXANDRIUM TAMARENSE* CYST MEASUREMENTS IN THE WESTERN GULF OF MAINE AND CASCO BAY REGIONS

Jason Hyatt[1,2], Richard P. Signell[2], Bruce Keafer[1], and Donald M. Anderson[1]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]United States Geological Survey, Woods Hole, MA 02543

To date, the source of the harmful algae *Alexandrium tamarense* in the Western Gulf of Maine and Casco Bay has not been well quantified and is a topic of debate. One possible source of cells lies in the sediment in the form of dormant cysts. Sediment samples analyzed in the Anderson Laboratory at the Woods Hole Oceanographic Institution (WHOI) show a patchy distribution, with neighboring stations having very different values. As a result, the cyst map is very sensitive to interpolation techniques. In an effort to improve the map, we analyzed the sediment samples for sediment type, with the goal being to use well established sediment type (Poppe et al.) and sediment environment maps (Kelley et al.) to improve the cyst map. These sediment maps express the patchy nature of sediment in this energetic and bathymetrically complex region. The cyst map is an important input into a physical biological model of *Alexandrium tamarense*, which will yield insights toward predicting blooms.

If *Alexandrium* cells are forming cysts due to environmental stresses (e.g. seasonal changes in water temperature), these cysts might settle in a fashion similar to some sediment type, and even be transported along with the sediment. Cyst counts were mapped onto a ternary diagram to show that most of the high cyst counts were found in silty/clayey regions, but not all such regions contained cysts. Then the sediment types were compared with those predicted by the sediment type map. A spatially higher resolved map of sedimentary environment (accumulation, erosion, or reworking) was also used for comparison.

The analyses did not yield a great improvement of the original cyst map due to the relatively larger spatial scales of the current model grid, potential aliasing of the cyst data due to selectively coring in 'soft' areas (where coring is possible), and a lack of agreement with the sediment type map itself. However, the future possibility of a higher resolution model grid and good agreement with the highly resolved sedimentary environment map keep the analysis viably useful.

References:

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PHYSIOLOGY AND CELL CYCLE PROGRESSION OF *GYMNODINIUM BREVE* UNDER LIGHT AND NITRATE LIMITATION

Christopher S. Johnson[1] and Richard M. Greene[2]
[1]Florida State University, Department of Oceanography, Tallahassee, FL 32306
[2]US EPA, Gulf Ecology Division, Gulf Breeze, FL 32561

Reports of blooms of *Gymnodinium breve*, a brevetoxin-producing dinoflagellate, date back centuries, yet information on the physiology of this organism is lacking in the published literature. The primary objective of this study is to describe the physiological properties of laboratory cultures of *G. breve* in response to conditions of light and nitrate limitation. Additionally, the intraspecific variability in these physiological responses is addressed using several geographic isolates of this species.

Laboratory cultures of *G. breve* (Pensacola, Piney Island and Charlotte Harbor isolates) are grown over a range of irradiances from 16 - 360 μE m^{-2} s^{-1} and two nitrate concentrations, ~5 and ~50μM. Measured physiological parameters include population growth rates, photosynthesis-irradiance (P-I) curves, cellular carbon and nitrogen, and photopigment content and composition.

We are also investigating the regulation of cell cycle progression by growth-limiting factors in cultures of *G. breve* and its relationship to toxin production. Olson and Chisholm (1986) have demonstrated that both nitrogen and light limitation resulted in an extension of the G1 phase of the cell cycle in the dinoflagellate *Amphidinium carteri*. Toxin production in two other toxic dinoflagellates has been shown to be coupled directly to specific phases of the cell cycle (Taroncher-Oldenburg et al. 1997; Pan et al. 1999). However, to our knowledge, the relationship between limiting conditions, cell cycle progression, and toxin production has not been previously addressed for any brevetoxin-producing microalgae.

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BIOMONITORING FOR TOXICITY CAUSED BY HARMFUL ALGAL BLOOMS AND OTHER WATER QUALITY PERTURBATIONS

Andrew S. Kane[1], Tommy R. Shedd[2], Geoffrey T. Gipson[1], Mark W. Widder[2], Jennifer Choich[3], Ellen K. Silbergeld[3], Nora J. Deamer[4], JoAnn M. Burkholder[4], Mark Poli[5], Renate Reimschuessel[6] and William H. van der Schalie[7]

[1]University of Maryland Aquatic Pathobiology Center, Department of Veterinary Medicine, 8075 Greenmead Drive, College Park, MD 20742, USA
[2]U.S. Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, Frederick, Maryland 21702-5010, USA
[3]University of Maryland Program on Human Health and the Environment, 10 South Pine Street, Baltimore, Maryland, 21201-1192, USA
[4]North Carolina State University, Center for Applied Aquatic Ecology, 620 Hutton Street, Suite 104, Raleigh, North Carolina 27606, USA
[5]U.S. Army Medical Research Institute for Infectious Disease, 1425 Porter Street, Fort Detrick, MD 21702-5010, USA
[6]U.S. Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708, USA
[7]U.S. Environmental Protection Agency, National Center for Environmental Assessment, 401 M Street SW, Washington, DC, 20460, USA

Harmful algal blooms, including those associated with toxicity, hypoxia events and other water quality perturbations, have increased in frequency and severity worldwide. An automated, remote biomonitoring system is being developed to continuously monitor waterways that are susceptible to these water quality perturbations. The system consists of a series of flow-through chambers that expose sentinel fish. Changes in ventilatory and movement patterns, as they relate to altered or toxic water quality, were monitored. Paired electrodes in each chamber non-invasively transmit an amplified electrical signal from each fish to a computer. These signals and water quality data can be transmitted to remote locations for further analysis and potential management response. Freshwater laboratory trials with bluegill indicated that exposure to sublethal hypoxia produced an increase in ventilatory rate with minor associated depressions in ventilatory depth. For the purpose of validating the system with HAB toxins, bluegill were also exposed to brevetoxin (PbTx2) and *Pfiesteria* culture water. One hour exposure to 40 ppb PbTx2 at 25˚C, but not 19˚C, produced minor elevations in ventilatory rate with strong cough and movement responses. Brackish (15 ppt) *Pfiesteria* culture water exposures caused a different response pattern: strong elevations in cough rate and % movement. Initial efforts to link biomonitoring signals with stress mechanisms were initiated with PbTx2-exposed bluegill; these animals showed altered patterns of glucose metabolism in their brains based on incorporation of 2-deoxyglucose (2-DG) *in vivo*. These laboratory biomonitoring studies indicate different response patterns under different stress conditions, and demonstrate utility of the biomonitoring system and the 2-DG method.

PFIESTERIA AND OTHER STRESS FACTORS ASSOCIATED WITH KILLS AND ULCERATIVE LESIONS OF ESTUARINE FINFISH

Andrew S. Kane[1], Ana M. Baya[1], Cindy P. Driscoll[2] and Renate Reimschuessel[3]
[1]University of Maryland Aquatic Pathobiology Center, Department of Veterinary Medicine, 8075 Greenmead Drive, College Park, MD 20742, USA
[2]Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 South Morris Street, Oxford, MD 21654, USA
[3]U.S. Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708, USA

Ulcerative lesions in mid-Atlantic estuarine finfish are associated with bacterial, fungal, viral and parasitic infectious agents, as well as suboptimal water quality. Atlantic menhaden are a relatively susceptible species to such stressors. In recent years, ulcerative lesions on field collected menhaden have been associated with exposure to *Pfiesteria*-like dinoflagellates and their toxins. These lesions are typically solitary, focal, deep, often perianal, and granulomatous with oomycete hyphae. Although *Pfiesteria*-like dinoflagellates have not been recovered from fish ulcers, and there is little evidence that supports a single causal relationship between toxic *Pfiesteria* and ulcerative lesions, much emphasis has been placed on *Pfiesteria* and fish lesions in the scientific and news media. A group of fish health experts examined ulcerated fish from estuarine waters in Maryland and North Carolina. Ulcerative menhaden lesions histologically demonstrated a marked chronic inflammatory infiltrate in large areas of exposed necrotic muscle. The ulcers contained granulomas with fungal hyphae in the necrotic tissue. Gram negative rod-shaped bacteria were also observed in the lesions, a common finding in ulcers of aquatic organisms. Other menhaden specimens contained *Kudoa*-like protozoan parasites, but lacked granulomas. Menhaden with solitary ulcers sampled from Delaware also lacked granulomas but were associated with Pennellid copepods. Bluegill, channel catfish, yellow perch, striped bass and carp sampled from Maryland waters with *Pfiesteria* were externally non-remarkable. These findings suggest that "typical" ulcerative lesions observed on menhaden from areas with *Pfiesteria*-like dinoflagellate blooms are reflective of dermatosis which may be related to a variety of individual or combined environmental stressors. Exposure to dinoflagellate toxin(s) potentially represents one such stressor. This presentation reviews the multifactorial nature of ulcerative lesions on finfish, and the role of Atlantic menhaden in monitoring the health of estuarine systems.

AN INTEGRATED FIELD STUDY TO IDENTIFY FACTORS RESPONSIBLE FOR ULCERATIVE LESIONS IN JUVENILE MENHADEN IN THE GREAT WICOMICO RIVER, VIRGINIA

Kator, H., L.W. Haas, W. Vogelbein, D. Zwerner, D. Hata, and J. Shields
Addresses: Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The occurrence of deeply-penetrating ulcerative lesions in young-of-the-year (YOY) Atlantic menhaden (*Brevoortia tyrannus*) was investigated in the Great Wicomico River (GWR) estuary, VA, USA in 1998-2000. In 1998, 6,874 caught YOY menhaden exhibited 211 ulcerative lesions (3% lesion rate). Lesion prevalence increased substantially in menhaden caught at salinities below 12-14 psu with the highest prevalence (11.5 %, n=823) at 10-12 psu. A similar relationship was observed in 13 separate estuaries throughout Virginia in September-October, 1998, where YOY menhaden captured (n= 5,077, lesion rate 5.6%) exhibited a marked increase in lesion rate at salinities of 12-14 psu and lower. In 1999 (through 9/9/99), a total of 4,005 YOY menhaden caught in the GWR exhibited a total of only 7 lesions (0.2% lesion rate). In 1998 a wet spring resulted in water with salinities associated with high lesion-prevalence (i. e., ≤12 psu) occupying the entire upper half of the estuary. Dry conditions in the remainder of 1998 and in the warmer months of 1999 through August reduced the area of salinities of 6-12 psu to only a short segment (ca. 100 m) in the shallow, narrow headwaters of the estuary. The greatly reduced area of ≤12 psu water in the GWR in 1999 is postulated to be the primary cause of reduced lesion prevalence in this year. Comprehensive data sets collected to examine aspects of the biological, chemical and physical characteristics of the GWR did not reveal obvious differences between 1998 and 1999 that could account for the low lesion prevalence in 1999. Low dissolved oxygen, a predisposing factor to fish disease, occurred in bottom waters of the upper reaches of the estuary in all years. Comparative analyses of heterotrophic dinoflagellates, other components of the microbial community, and chlorophyll *a* for both years were similarly unremarkable. Menhaden with typical ulcerative lesions examined in 1998-9 and 2000 confirmed the presence of an aseptate fungus (water mold) belonging to the genus *Aphanomyces*. Studies in 1999 to detect and quantify water molds as an index of *Aphanomyces* presence, suggested runoff was an important factor controlling its occurrence. Although field work and data analysis in 2000 are not yet complete, ulcerative lesion prevalences in GWR YOY menhaden increased from none in May to a maximum of 14% in late August. July, August and early September experienced periods of intense, episodic thunderstorms which produced large reductions in surface salinities in the upper reaches of the GWR. These observations and other data imply lesion occurrence is related to the presence of freshwater runoff which favors the introduction of infective *Aphanomyces* spp. and/or provides very low salinity (ca. 1 psu) conditions required for zoospore formation and release.

COMPARISON OF MOLECULAR PROBES FOR THE IDENTIFICATION AND ENUMERATION OF *ALEXANDRIUM* SP. FROM THE GULF OF MAINE

Bruce A. Keafer[1], David M. Kulis[1], Kristin Gribble[1], Chris Scholin[2], Roman Marin[2] and Donald M. Anderson[1]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]Monterey Bay Aquarium Research Institute, Moss Landing CA 95039

Over the last several years, molecular probes for the identification and enumeration of HAB species have become more readily available, but a comparative evaluation of natural samples has not been well documented. Three different methods that target *Alexandrium* sp. were tested on samples collected during the ECOHAB-Gulf of Maine (GOM) cruises in the spring of 1998 and 2000. Two of these, an oligonucleotide probe (NA-1) that binds to large-subunit ribosomal RNA (LSU rRNA) of the North American *Alexandrium* ribotype and a monoclonal antibody probe (M8751-1) that recognizes the outer cell surface antigens of several *Alexandrium* species were used in fluorescent whole cell microscopic assays. In addition, analyses were performed using the NA-1 probe on sample lysates empolying a semi-quantitative sandwich hybridization (SH) assay where the resulting color intensity is proportional to the *Alexandrium* cell density.

During 1998, about 70 samples were analyzed using the 3 methods. Comparison of the two whole cell assays indicated that estimates of *Alexandrium* sp. were consistently higher with the immunofluorescent method vs. the oligonucleotide probe method. Re-examination of the samples revealed that the NA-1 probe recognized only the smaller *Alexandrium* cells that did not contain autofluorescent inclusion bodies, while the M8751-1 probe labeled both larger and smaller *Alexandrium* sp., some of which contained inclusion bodies. The SH method detected cells when densities were high enough to cause toxicity (~200 cells liter^{-1}), but did not detect very low abundances of *Alexandrium* prior to the bloom.

Based on the observed differences in the 1998 counts, the 2000 immunofluorescent counts were improved by discriminating the counts into 4 categories: <50 µm cells with inclusion bodies, <50 µm cells without inclusion bodies, > 50 µm cells with inclusion bodies and >50 m cells without inclusion bodies. The cells <50µm without inclusion bodies were identified as the *Alexandrium tamarensis* complex (i.e., *fundyense*, *tamarense*), while the cells >50um with inclusion bodies were identified as *A. ostenfeldii*. Sensitivity of the SH assay was improved by collecting more volume per sample and by enhancing the detection of the target LSU rRNA. As a result of these modifications, better agreement of the 3 methods was achieved. These data suggest that different probes recognize *Alexandrium* species differently, and that at least two *Alexandrium* species co-occur in GOM waters during the spring months. Thus, the choice of probe is not always clear. A researcher must consider these issues along with other factors such as ease of preservation and long-term sample integrity when choosing an enumeration tool. Ultimately, a suite of probes must be developed to accurately distinguish all the potential toxic species of *Alexandrium* that are present in the sample.

THE RELATIONSHIP BETWEEN THE OCCUPATIONAL EXPOSURE TO *GYMNODINIUM BREVE* (DINOPHYCEAE) TOXIN AND PULMONARY FUNCTION

Barbara Kirkpatrick[1], Terrance Kane[2], and Michael Henry[1]
[1]Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL, U.S, 34236 and [2]Lung Associates of Sarasota,1895 Floyd Street, Sarasota, FL, U.S. 34239.

The Western Coast of Florida frequently experiences a harmful algal bloom caused by the dinoflagellate, *Gymnodinium breve*, Davis (*G. breve*). *G. breve* releases a toxin when the cells lyse, and this toxin becomes part of the marine aerosol. When humans are exposed to *G. breve* toxin in marine aerosol, upper respiratory symptoms such as runny nose, nasal congestion, cough, and sore throat are commonly reported. Since the estimated size of the particle is 7 - 10 μm most toxin should be filtered by the upper airway. If the red tide toxin impacts the lower airway, bronchoconstriction of the smooth muscle may occur. The common method to detect bronchoconstriction is the measurement of the Forced Vital Capacity (FVC) and the forced vital capacity exhaled in 1 second (FeV_1). A pilot study in 1999, conducted on the Florida ECOHAB cruise revealed that most scientists studied did not have a significant change in pulmonary function when conducting research in a *G. breve* bloom. However, 2 of the 17 did have a change and these two scientists were nonsmokers, young, with no known pulmonary history. Further investigation was indicated.

For the September 2000 Florida ECOHAB cruise, similar procedures from 1999 were followed. The primary purpose of this cruise was to conduct experiments in a red tide bloom in the Gulf of Mexico. Volunteer scientists were instructed on the correct method to perform the FVC maneuver. They were also asked to complete a Health History Questionnaire (©Hollister Inc, 1980). The volunteers then performed the forced vital capacity maneuver at varying times of the cruises and also documented any respiratory symptoms. Variation in FVC and FeV_1 in correlation to surface cell counts, wind speed, and scientist subjective symptoms were analyzed. Findings from the cruise will be reported.

ALEXANDRIUM SPP. HYPNOZYGOTE CYSTS IN THE WATER COLUMN IN THE GULF OF MAINE

Sarah L. Kirn and David W. Townsend
5741 Libby Hall, School of Marine Sciences, University of Maine, Orono, ME 04469

Alexandrium spp. in the Gulf of Maine are known to produce hypnozygote resting cysts as part of their life cycle. These cysts are thought to form near the end of the growing season and fall to the bottom where they undergo mandatory quiescence on the order of a few months. Excystment of these benthic cysts in response to some environmental cue is thought to initiate the annual spring and summer blooms of *Alexandrium*. Light and temperature have been determined to affect excystment in the laboratory, but photoperiod, physical, or chemical cues have not been eliminated as possible mechanisms. After excystment, the newly germinated planomeiocite cells undergo division, establishing the spring *Alexandrium* vegetative cell population.

Assuming that the excystment cue is light or temperature related, this conceptual model is adequate for shallow water environments where light and vernal warming reach the benthos where the cysts lie. In deeper waters such as near coastal Gulf of Maine and moderately deep waters with extensive tidal mixing and subsequent turbidity such as in the mouth of the Bay of Fundy, the conceptual model falls short. Evidence exists, however, for offshore bloom initiation (see Townsend et al. abstract, this volume), which suggests some other source of cysts.

One possible source is the water column, and especially the bottom nepheloid layer. Physical processes (winter convection, tidal mixing) may resuspend cysts or slow the descent of newly formed cysts such that the entire dormancy period passes while the cysts remain in the water column. Either scenario would result in cysts near enough to the surface to receive seasonal environmental cues to trigger excystment.

To begin researching this possibility, samples were collected during a cruise in the Gulf of Maine in February of 2000. Samples were taken in 30 L Niskin bottles from three depths: 5 m above the bottom, the top of the nepheloid layer as located by transmissometer, and 2 m below the surface. In the lab, samples were sonified and stained with the fluorochrome stain primulin, then examined for cysts using an epiflourescence microscope.

Cysts are indeed present in the water in the Gulf of Maine in February. Fourteen of fifteen samples taken from 5 sample sites around Grand Manan Island and the mouth of the Bay of Fundy contained cysts. Concentrations in those fourteen samples ranged from 90 to >2500 cysts per cubic meter. The near bottom sample from southern Jordan Basin had the highest concentration of cysts recorded, at around 9000 cysts per cubic meter.

The presence of cysts in the water column makes offshore bloom initiation a possibility. Evaluating the efficacy of cysts in the water column initiating blooms hinges on knowing how many cysts are needed to inoculate a bloom, which in turn depends on vegetative growth rate which is at present not known with any certainty. These early results also raise questions regarding previous assumptions concerning bloom dynamics, such as the importance of benthic cyst beds and the physical origin of bloom initiation.

NUTRIENT REGULATION OF PHOTOSYNTHETIC PERFORMANCE AND VARIABLE FLUORESCENCE IN *PSEUDO-NITZSCHIA MULTISERIES*

Raphael Kudela[1] , Alice Roberts[1], Peter Miller[2], Joel Goldman[1], and Chris Scholin[2]
[1] Ocean Sciences Department, University of California, Santa Cruz, CA 95064
[2]Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039

Pseudo-nitzschia spp. are known to vary domoic acid production as a function of multiple nutrients, including nitrogen, phosphorous, silicate, iron, copper, and possibly lithium. Although there has been a great deal of work done on several of these nutrients (in particular N, P, and Si), the interactions between macro- and micronutrients are further complicated by the role of light in controlling diatom production. Silicic acid transport requires ATP, a byproduct of photosynthesis. Previous studies, however, have reported that it takes > 6h for transport to degrade, and that it does so fairly slowly. The implication is that silicic acid utilization is only weakly controlled by the availability of photosynthate and is essentially uncoupled from ambient irradiance, unlike C and N assimilation, which are strongly dependent on light and can become uncoupled from one another under non-steady-state growth. Recent evidence from Monterey Bay, California demonstrates that contrary to expectations, Si assimilation demonstrates a positive uptake vs. irradiance response. Work done with non-*Pseudo-nitzschia* diatom cultures also demonstrates that Si limitation can directly affect photosynthetic performance, and acts similarly to iron and nitrogen (nitrate) limitation. This response includes variability in photosynthesis versus irradiance parameters, and more interestingly, in reversible changes in the quantum efficiency of Photosystem II as measured by variable fluorescence.

Here we present results from continuous culture experiments using *Pseudo-nitzschia multiseries* (Hasle) grown under constant temperature and irradiance with either silicic acid or nitrate as the limiting nutrient. At the termination of the experiments photosynthetic performance was assessed using ^{14}C photosynthesis versus irradiance (P vs E) curves and by off-line assessment of Photosystem II efficiency using a Walz Pulsed Amplitude Modulation (PAM) fluorometer. As expected, DA production was significantly enhanced under Si limitation. We also observed a significant decrease in photosynthetic performance under both nitrate and silicate limitation, suggesting that silicic acid availability directly affects photosynthesis in this diatom with diagnostic symptoms similar to iron or nitrogen limitation. Results and preliminary analyses of these data will be presented, emphasizing the potential role of silicic acid in controlling the photosynthetic competency of this toxigenic species.

INFLUENCE OF HARMFUL ALGAL BLOOM TOXINS ON THE FORAGING BEHAVIOR OF SEA OTTERS AND SHOREBIRDS

R. G. Kvitek, C. Bretz, and K. Thomas
California State University Monterey Bay, 100 Campus Center, Seaside, CA 93955

We tested the general hypothesis that the foraging behavior and distribution of high level marine predators (sea otters and shorebirds) under natural conditions are mediated by benthic prey toxicity due to harmful algal blooms (HAB's). Sea otters in southeast Alaska did change their foraging behavior at sites were *Saxidomus giganteus* (Butter Clams) were found to contain paralytic shellfish poisoning toxins (PSPT) in high concentrations. At the most toxic sites (>500 µg STX/100g prey tissue weight) sea otters shifted their diet away from their primary butter clam prey to smaller and less abundant non-toxic species. At sites of intermediate prey toxicity (200 to 400 µg STX/100g) some sea otters continued to eat butter clams while discarding the most toxic body parts of these clams. In California, changes in shorebird (Oyster Catchers and Willets) feeding behavior was correlated with seasonal changes in PSPT concentrations in their primary prey (mussels and mole crabs respectively). We conclude that predators are not excluded from areas where their primary prey become toxic due to HABs, but they do alter their foraging behavior and diet in a graded response to toxin levels. These behavioral shifts in turn, can result in spatial and temporal refuge for preferred prey due to the reduction in predation pressure.

A DESCRIPTIVE ACCOUNT OF POTENTIALLY HARMFUL ALGAE IN INLAND BAYS AND NANTICOKE RIVER, DE

Richard V. Lacouture[1], Jennifer Gronefeld[1], Edythe Humphries[2], and Harold G. Marshall[3]
[1]Academy of Natural Sciences Estuarine Research Center, 10545 Mackall Road, St. Leonard, MD 20685
[2]Delaware Department of Natural Resources and Environmental Control, Division of Water Resources, Environmental Laboratory Section, 89 Kings Highway, Dover, DE 19901
[3]Old Dominion University, Dept. of Biological Sciences, Norfolk, VA 23529-0266

Beginning in 1998, Delaware Department of Natural Resources and Environmental Control (DNREC) initiated a routine monitoring program for phytoplankton species composition is Rehoboth, Indian River and Little Assawoman Bays and Nanticoke River, DE. This effort was largely precipitated from the discovery of several potentially harmful algae,. *Pfiesteria piscicida* and *Aureococcus anophagefferens*. Upon completing two years of taxonomic enumerations of samples from these waters, a list of taxa revealed a number of potentially harmful forms of microalgae. These taxa were compiled in a data base which included information on their physiology and ecology, toxin or alternate harmful mechanism, target species, threshold densities, possible environmental triggers, human health risks and trophic interactions. The database will also list references which will enable the user to access more detailed information on these various topics. The ultimate product will be an interactive spreadsheet-type database which can facilitate selected queries.

ACCUMULATION OF DOMOIC ACID BY THE COASTAL DIATOM *PSEUDO-NITZSCHIA MULTISERIES*: A POSSBLE COPPER COMPLEXATION STRATEGY

N. L. Ladizinsky and G. J. Smith
Moss Landing Marine Labs, 8272 Moss Landing Road, Moss Landing, CA 95039, USA

Domoic acid (DA) is a neurotoxic amino acid produced by several members of the diatom genus *Pseudo-nitzschia*. Trophic transfer of DA up the food chain has been implicated in the deaths of 100's of marine birds and marine mammals along the central California Coast. The physiological function of DA in *Pseudo-nitzschia* spp. has not been defined, although some evidence indicates that elevated metal concentrations can induce DA accumulation (Subba Rao and others 1998 P.S.Z.N. Mar. Ecol. 19:31). Although California coastal waters have experienced a decline in several heavy metals from 1977-1990, copper concentrations have increased by as much as 25% (Stephhenson, M. D. and Leonard, G. H. 1994 Mar. Poll. Bull. 28: 148). Many algae produce chelators, including amino acids, in response to toxic [Cu^{2+}] (Wu and others 1998 J. Phycol. 34: 113). Domoic acid, a tricarboxylic acid, has 4 functional groups that may readily form chelation complexes with transition metals like copper. Copper enrichment experiments indicate that while Cu^{2+} is toxic to *P. multiseries* at total [Cu] > 16.1μM (pCu ≈ 6.0), intracellular DA accumulation increases up to this point with no decline in growth rates relative to cultures grown in standard enriched seawater. These data suggest that intracellular accumulation of DA by *P. multiseries* may serve to mitigate the toxicity of elevated [Cu^{2+}]. Direct measurement of the impact of DA on cupric ion activity using sensitive chemiluminescent assays indicate that DA exhibits significant chelation capacity for cupric ion with a conditional stability constant, $K_{c,DACu(II)} = 10^{12.4}$ at pH 8.0. These data indicate that 1) accumulation of DA can be induced by increases in total dissolved copper and 2) that DA functions as a strong intracellular chelator. Field data from the ongoing *Pseudo-nitzschia* spp. Bloom in Monterey Bay will be presented examining the association of cellular DA content and cupric ion activity. Defining the Cu-DA dose response relationship in *Pseudo-nitzschia* can facilitate prediction of future toxic bloom events.

BIOMARKER LIPIDS IN RED TIDE (*GYMNODINIUM BREVE*) BLOOMS ALONG THE NORTHWEST FLORIDA COAST

Jeffrey D. Leblond[2] and Peter J. Chapman[1]
[1]United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561

The ability to characterize phytoplankton communities and algal blooms using lipids as biomarkers requires knowledge of their distribution and taxonomic significance. Such an approach would have application, for example, in distinguishing and tracking certain dinoflagellates such as the toxic species *Gymnodinium breve*, which is responsible for red tide events in the Gulf of Mexico. To explore this possibility, the lipids of over forty laboratory-cultured dinoflagellates, including three different isolates of *G. breve*, and a number of representatives of other eukaryotic algal classes such as the Bacillariophyceae, Haptophyceae, and Raphidophyceae, have been examined for the presence of chemotaxonomically useful fatty acids and sterols. A dense bloom (over 20 million cells/L) of *G. breve* in the fall of 1999 in the near shore waters of the Gulf of Mexico from Destin to Pensacola in northwest Florida provided an opportunity to compare the lipids of the field-collected samples with those found in laboratory cultures.

Extracted lipids were fractionated into different classes (neutral, glyco-, and phospho-lipids) prior to conversion to gas chromatography/mass spectrometry (GC/MS)-amenable fatty acid methyl esters (FAMEs) and sterol-trimethylsilyl (TMS)/acetate derivatives. The bloom of *G. breve* was found to contain two principal 4-methyl sterols, (24*S*)-4α-methylergosta-8(14),22-dien-3β-ol (ED) and its 27-*nor* derivative (NED), recently described by Faraldos and Giner (1998). The bloom sample was also found to contain the highly unsaturated long chain fatty acid, octacosaoctaenoic acid (28:8), recently discovered in dinoflagellates by Mansour et al. (1999).

Characterization of free and esterified sterols from laboratory cultures of *G. breve* has confirmed the predominance of these two sterols. ED and NED were shown also to be the primary sterols of the closely related dinoflagellates, *Gymnodinium mikimotoi* and *Gymnodinium galatheanum.* The wider distribution of this sterol pattern is consistent with the known close relationship between *G. breve* and *G. mikimotoi* (Haywood et al. 1996, Leblond et al. 2000). However, these sterols were also found as components of more complex sterol profiles in other members of the *Gymnodinium/Peridinium/Prorocentrum* (GPP) taxonomic group, thus limiting their biomarker potential.

References:

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[2]Current address: Biology Department, Middle Tennessee State University, Murfreesboro, TN 37123

THE PHYLOGENETIC RELATIONSHIP OF THE RED TIDE DINOFLAGELLATE *GYMNODINIUM BREVE* TO OTHER MEMBERS OF THE GENERA *GYMNODINIUM* AND *GYRODINIUM*

Jeffrey D. Leblond[1,3], John E. Rogers[2], and Richard Devereux[2]
[1]National Research Council Postdoctoral Associate
[2]United States Environmental protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561

Phylogenetic relationships between the red-tide dinoflagellate *Gymnodinium breve* and other members of the genera *Gymnodinium* and *Gyrodinium* have not been studied at the molecular level. *G. breve* is most noted for its production of brevetoxin, which has been linked to extensive fish kills, marine mammal mortalities, neurotoxic shellfish poisoning, and respiratory irritation caused by aerosolized toxin (Steidinger et al. 1997). The phylogeny of four isolates of *G. breve* and of seventeen other members of the genera *Gymnodinium* and *Gyrodinium* was determined by comparison of small subunit (SSU) rRNA genes. The sequences of the *G. breve* isolates differed from each other by less than 0.3%. In addition, sequences were obtained from twenty-three other members of the class Dinophyceae, including several members of the *Gymnodinium/Peridinium/Prorocentrum* (GPP) complex, and *Coolia* and *Fragilidium* species isolated in this laboratory. The rRNA sequences of the *G. breve* isolates were aligned with these and other previously published full-length dinoflagellate sequences. Phylogenetic analyses indicate that *G. breve* is closely related to the morphologically similar dinoflagellates *Gymnodinium mikimotoi* and *Gymnodinium galatheanum*. The phylogenetic positions of *G. breve*, *G. mikimotoi*, and *G. galatheanum* are further supported by data that show these three species possess sterols unlike other dinoflagellates (Leblond and Chapman, 2000).

References:

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[3]Current address: Biology Department, Middle Tennessee State University, Murfreesboro, TN 37123

SMALL PELAGIC FISH; DANGEROUS VECTORS OF DOMOIC ACID

Kathi Lefebvre[1], Shonna Dovel, Susan Coale[1], Frances Gulland[2], Greg Doucette[3], Ron Tjeerdema[4], and Mary Silver[1]
[1]University of California, Santa Cruz, CA 95064
[2]Marine Mammal Center, Sausolito, CA
[3]Marine Biotoxins Lab, NOAA/NOS Charleston, SC 29412
[4]University of California, Davis, CA 95616

Domoic acid producing *Pseudo-nitzschia* species have been responsible for mass mortality events involving seabirds and marine mammals in Monterey Bay California on several occasions since 1991. In at least three major DA-poisoning events, the northern anchovy (*Engraulis mordax*) was identified as the vector for toxin transfer from diatoms to pelicans (*Pelecanus occidentalis*), cormorants (*Phalacrocorax*), or sea lions (*Zalophus californianus*). Because of its prominent role as a DA vector in Monterey Bay, we began an ongoing study documenting DA levels in anchovies (as well as sardines) on a regular basis from freshly collected field samples. Here we present data on gut contents and DA levels detected in anchovies and/or sardines collected by fishermen from various sectors of Monterey Bay over a one year period. Over 70 fish samples were analyzed with the highest DA levels reaching 588 ppm in viscera preparations. DA levels and the presence of *Pseudo-nitzschia* in the digestive tracts of fish were correlated with diatom densities calculated from whole water samples taken from Monterey Bay at regular time intervals during the same one year period.

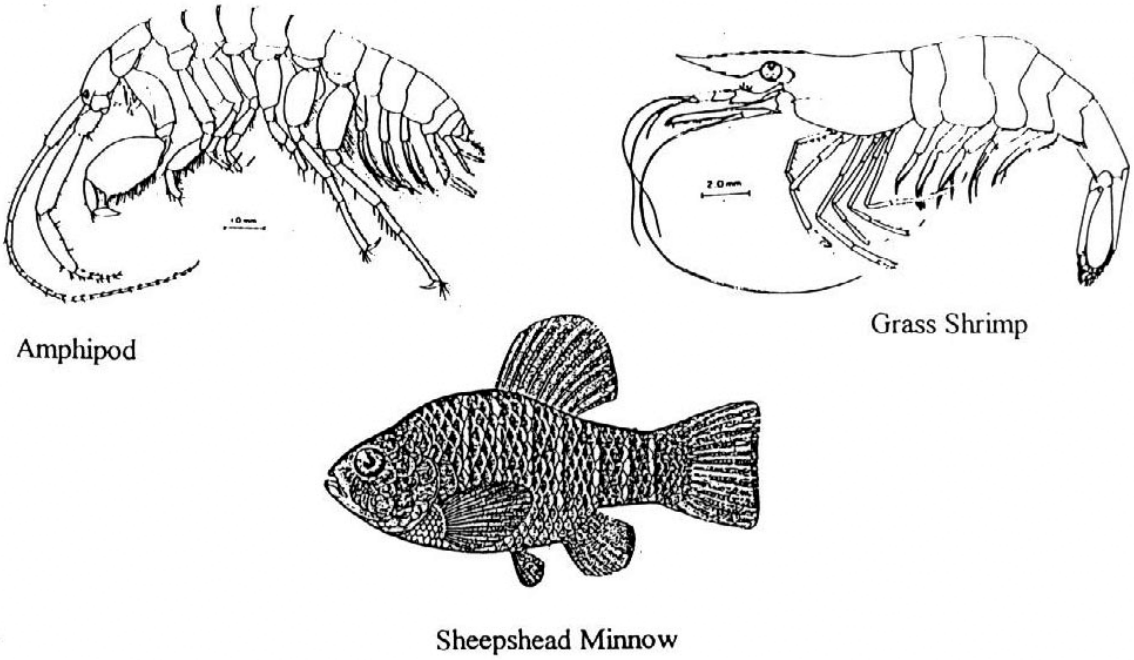
In the most recent west coast DA poisoning event, over 100 California sea lions were observed exhibiting seizures in the Morro Bay, California area from approximately July 23- August 6, 2000. Of eight fecal samples collected from sea lions exhibiting seizures, one contained detectable levels of DA (8.7 ug/g). Anchovies collected from Morro Bay waters during July, contained low levels of DA (23 ppm in viscera). *Pseudo-nitzschia* densities and DA levels measured in Morro Bay waters during July will also be presented.

EFFECTS OF CLAY FLOCCULATION OF THE FLORIDA RED TIDE DINOFLAGELLATE (*GYMNODINIUM BREVE*) ON BENTHIC ORGANISMS

Michael A. Lewis[1], Richard M. Greene[1], Aishao Li[2], Donald M. Anderson[2]
[1]U. S. Environmental Protection Agency, Gulf Breeze, Florida 32561
[2]Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Evaluating the feasibility of controlling red tide using clay flocculation is part of an ECOHAB- funded project. One aspect for the feasibility and future application of clays is the determination of potential negative environmental impacts. The removal of toxin-containing dinoflagellates from the water column may result in acute or chronic toxicity to benthic organisms. Using EPA-approved standard toxicity test methods, we designed a study to determine the acute and chronic toxicities of settled *G. breve* cells, clay and coagulant to several species of marine life (see below). The laboratory-conducted solid phase and pore water bioassays were of 4 to 28 days duration. The test species included *Cyprinodon variegatus* (sheepshead minnow), *Palaemonetes pugio* (grass shrimp), *Leptocheirus plumulosus* (amphipod), and *Ampelisca abdita* (amphipod). These species were exposed to the clay, coagulant and *G. breve* alone and in binary and ternary combinations at three treatment levels. Effects on organism survival and reproduction were determined and reported as LC50 values, no effect concentrations, and the lowest effect concentration. In addition to the laboratory bioassays, we will determine benthic quality below a natural red tide event by analyzing sediment toxicity, chemical quality and benthic community composition. The results of the acute and chronic toxicity evaluations will be discussed in this presentation as well as their relevance to the *in-situ* remediation of red tide events.

TEST SPECIES



Amphipod

Grass Shrimp

Sheepshead Minnow

REMOVAL OF *GYMNODINIUM BREVE* AND *PFIESTERIA PISCICIDA* USING UNTREATED AND FLOCCULANT-TREATED CLAYS AND SOME EFFECTS ON WATER CHEMISTRY

Aishao Li, Mario R. Sengco, and Donald M. Anderson
Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

During the past ten years, researchers in several countries have investigated the use of clays to physically remove red-tide organisms from the water column. These efforts have demonstrated that clay dispersal is a promising strategy to control harmful algal blooms (HABs) because it is highly effective and practicable, while the clay itself is generally inexpensive, available in large quantities, and likely to be environmentally benign. This poster presents results of several studies focusing on variations in removal efficiency among twenty-four different clays, with and without the addition of different flocculants, against two HAB species: *Gymnodinium breve* and *Pfiesteria piscicida*. In addition, experiments were also conducted (1) to determine the adsorption or release of inorganic nutrients into seawater by three selected clays and one flocculant, and (2) to determine biological oxygen consumption due to clay treatments.

Among the clays tested, one kaolinite (HDP) was the most effective in removing *Pfiesteria* cells (>95%) at a loading of 0.24 g $\bullet L^{-1}$. The removal efficiency of this clay increased after 7 ppm of polyaluminum chloride (PAC) was added. Similarly, Florida phosphatic clay (IMC-P), consisting mostly of montmorillonite, also displayed removal efficiencies greater than 90% against *Gymnodinium* cells at a loading of 0.25 g$\bullet L^{-1}$. The addition of 5 ppm PAC lowered the amount of clay needed by one order of magnitude at low clay concentrations (>80% removal efficiency at 0.02 g $\bullet L^{-1}$). Both clays showed high adsorption of ammonia (>50%) and nitrate (>50%) from nutrient-enriched seawater. HDP clay also removed a significant amount of phosphate (>80%). By contrast, IMC-P released phosphate into seawater – a result which was expected since the clay is a by-product of phosphatic mining and can retain a high phosphorus content (ca 30%). However, clays treated with PAC further enhanced the adsorption of ammonia and nitrate, and moderated the release of phosphate from Florida phosphatic clays. Finally, the dispersal of IMC-P led to an increase in dissolved oxygen concentration in seawater based on mesocosm experiments. The biological oxygen demand (BOD_5) was lower in the clay treatment than in the cell-alone treatment during a 5-day laboratory experiment.

These results indicated that using PAC-treated clays not only enhances its removal efficiency, resulting in less clay needed to treat a bloom, but it also has the potential to better control the ambient inorganic nutrients or inhibit the release of adsorbed nutrients from the clay. Moreover, the use of clay may also be environmentally benign in terms of reducing biological oxygen demand according of these results.

THE GLOBAL BIOGEOGRAPHY OF THE GENUS *ALEXANDRIUM*

Emily L. Lilly[1], Gaspar Taroncher-Oldenburg[2] and Donald M. Anderson[1]
[1]The Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2]Princeton University, Princeton, NJ 08544-1003

In the past few decades, the incidence of Paralytic Shellfish Poisoning (PSP) and the geographic range of the toxin-producing dinoflagellate genus *Alexandrium* have both increased worldwide. Genetic analysis of populations of *Alexandrium* can help to explain their origin, and thus the mechanisms for dispersal (both natural and human-assisted). Prior work has shown that analysis of LSU rDNA is an effective tool for revealing genetic diversity and phylogenetic relationships in Alexandrium that are not evident from morphology.

This study used an LSU rDNA-based RFLP assay with a broad set of isolates, representing the coastal areas of South America, Africa, New Zealand, Europe and the Mediterranean, in addition to new isolates from previously studied areas. Several novel ribotypes were found within both the tamarensis and the minutum/lusitancum groups. Isolates of *A. margalefii*, *A. pseudogonyaulax* and *A. ostenfeldii* all displayed unique ribotypes. In accordance with prior work, ribotypes do not reflect morphospecies; geographic origin is a better indicator of phylogeny than morphology. For all species, each ribotype contains either toxic or non-toxic isolates but not both. For isolates displaying new ribotypes with this RFLP assay, a portion of the LSU rDNA was sequenced. These were placed into phylogenetic context with previously sequenced ribotypes.

These results offer additional insights into the global distribution and dispersal of *Alexandrium*. Within the broadly distributed ribotypes, patterns are revealed that are suggestive of both natural and human-assisted dispersal. Other ribotypes thus far have been seen in single regions, likely representing isolated discrete populations.

MOLECULAR CLONING AND ANTISERUM DEVELOPMENT OF CYCLIN BOX IN THE BROWN TIDE ALGA *AUREOCOCCUS ANOPHAGEFFERENS*

Senjie Lin[1], Erika Magaletti[2] and Edward J. Carpenter[2]
[1]Department of Marine Sciences, University of Connecticut, Groton, CT 06340.
[2]Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794

Cyclins can be useful cell cycle markers for growth rate studies on harmful algal blooms. In this study, a gene fragment corresponding to cyclin box was cloned for the brown tide alga, *Aureococcus anophagefferens*. This algal gene fragment, designated as *Btcycl1*, was most similar to cyclin B. Based on the deduced amino acid sequence, oligopeptides were synthesized and used to raise an antiserum which reacted on western blots with a protein of about 63 kDa, the same size as cyclin B in other organisms. The cyclin B-like protein recognized by this antiserum, and the mRNA amplified using the primers, were more abundant in exponential cultures and decreased markedly in stationary cultures. This protein also appeared to be cell cycle-dependent. Immunofluorescence labeling showed that this antiserum specifically stained a protein in *Aureococcus* cells and had no cross-reaction with bacteria that were present in the algal culture. The *Btcycl1* sequence and the antiserum will provide a useful tool for studies on regulation of in situ growth rate for this brown tide alga.

cDNA SCREENING FOR *PFIESTERIA PISCICIDA*: SEARCH FOR SPECIES-SPECIFIC DNA MARKERS AND GROWTH-REGULATING GENES

Senjie Lin and Huan Zhang
Department of Marine Sciences, University of Connecticut, Groton, CT 06340

Due to the unusual life cycle and growth characteristics of *Pfiesteria piscicida* reported previously and observed recently in our laboratory, it is highly desirable to extensively screen cDNA libraries and identify genes that are related to growth regulation. In addition, because of presence of similar genera and species, genes or other DNA markers that contain species-specific signatures will be useful. To identify these genes or DNA markers, *Pfiesteria* cultures were grown under different conditions and RNA/DNA were extracted. Different behaviors of DNA suggested that *Pfiesteria* chromosomal DNA is probably bound by histone proteins as in typical eukaryotes in contrast to DNA in *Prorocentrum minimum*, another dinoflagellate, which appeared not to contain histones. mRNA was isolated from the crude RNA extract, and cDNA was prepared using a SMART technique. With modified poly (dT) primers, a number of genes were cloned, and sequencing effort is underway. Some of the sequences identified thus far include 3'-end of the mitogen-associated protein kinase (MAPK), tubulins, cytochrome b oxidase I, and some others with no homology to known genes. Search for cell cycle dependent genes are still underway. By using a mRNA differential display technique, we are also trying to isolate genes differentially expressed under different growth conditions. A comparative analysis of some of these genes will be presented.

EUROHAB-EUROPEAN INITIATIVE ON HARMFUL ALGAL BLOOMS

Elisabeth Lipiatou
European Commission, Research Directorate-General, Environment and Sustainable Development Programme, Marine Ecosystems Key Action, 200 Rue de Loi, B-1049 Brussels, Belgium.

Harmful algal blooms (HABs) show their negative impact along the European coasts in several different ways: the accumulation of algal toxins of different nature impinge upon shellfish aquaculture industries and cause prolonged closure and trade banning (France, Spain, Italy, Ireland, UK etc.); ichthyotoxic outburst may determine the death of hundreds ton of caged fish (Skagerrak, Kattegat); visual discoloration exerts a very negative impact on tourism (Spanish Mediterranean coasts); mucilages of different origin have the same impact on tourism, and in addition interfere with fisheries (Adriatic Sea, North Sea).

Many European countries share marine waters and even if certain countries are not sharing the same sea, currents are continuously transporting water masses from one country to the next. Thus, a HAB population in one country might be triggered by nutrients originated in another country. Nutrients are also transported by winds. Another process possibly triggering HAB is the transport of merchandise by shipping. The amount of ballast water in large modern ships potentially contains seed populations of harmful algae enough to initiate a bloom in another marine area when discharged in exchange for cargo.

All the factors stated above point to the advantage of an integrated European research effort to document the reasons for the different HAB occurring in our marine waters, as a basis for designing responsible strategies for better management of marine resources.

EUROHAB[1], the European Initiative on Harmful Algal Blooms started in 1999, is formulated to generate and co-ordinate the required research to manage better the effects of toxic/harmful marine microalgae in the marine and brackish waters of the EU.

The European Commission/DG Research is promoting today, through EUROHAB, both the high-level research and networking on HAB issues needed at European level in co-ordination with the national relevant activities.

EUROHAB is an "umbrella" which includes the environmental research projects sponsored by DG Research on research and infrastructures for harmful algae and harmful introductions to the sea. Those projects are today: BIOHAB (Biological control of harmful algal blooms in European waters), NUTOX (Effect of nutrient ratios on harmful phytoplankton and their toxin production), Harmful introductions by ships to European waters, DOMTOX (Importance of dissolved organic matter from terrestrial sources for the production, community structure and toxicity of phytoplankton European Atlantic and Baltic coastal waters) and HABES (Harmful Algal Bloom Expert system). The number of EUROHAB projects is expected to increase following the forthcoming call for proposals of the EC Environment Programme where research on HABs is highlighted.

[1] EUROHAB Science Plan- European Commission, Research in Enclosed Seas-5, EUR 18592, ISBN 92-828-6612-2, 1999.

POLYMORPHISMS IN THE ITS REGION OF DINOFLAGELLATES: IMPLICATIONS FOR PHYLOGENY AND PROBE DEVELOPMENT

Wayne Litaker[1,4], Kimberly S. Reece[2], Nancy A. Stokes[2], Karen Steidinger[3], and Pat Tester[1]
[1]National Ocean Service, NOAA, Beaufort, NC 28516
[2]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062
[3]Florida Fish & Wildlife Conservation Commission, Florida Marine Research Institute, St. Petersburg, FL 33701-5020
[4]Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, NC 27599

Numerous research efforts are underway to develop RNA or DNA based assays for identifying Harmful Algal Bloom Species (HABs). One of the fundamental questions that must be answered in developing reliable molecular assays is whether the target DNA or RNA sequences are species-specific. To answer this question requires sequence information from the target organisms and a suite of related species, as well as a panel of appropriate cultures for cross-reactivity testing. In this study we investigated whether the internal transcribed spacers regions, ITS1 & ITS2, or the 5.8S gene of dinoflagellates is an appropriate target for the species-specific PCR assays. The ITS1 and ITS2 regions of the ribosomal gene complex are more variable than the structural 5.8S, or large and small subunit genes, and have proved useful in phylogenetic studies of closely related species and as targets for primer development in other organisms. Specifically, we amplified, cloned, and sequenced the ITS/5.8S region from *Pfiesteria piscicida* and the following *Pfiesteria*-like organisms: *Pfiesteria* species "B", 3 Cryptoperidiniopsoid species, a Florida "Lucy" species, and "Cell J" - an unidentified PLO that co-occurs in the same environment as *P. piscicida*. Other more distantly related species sequenced for comparison included *Amyloodinium ocellatum*, *Prorocentrum minimum*, and *Heterocapsa triquetra*. Five independent isolates of *Pfiesteria piscicida* and 2 isolates of the *Pfiesteria* sp. B were sequenced to estimate the amount of ITS/5.8S variation between isolates of the same species. Within genome variation was assessed by sequencing 2 - 12 independent ITS/5.8S clones from each isolate. The sequencing results showed the ITS regions of closely related species were sufficiently divergent to provide unique targets for species-specific PCR assay development. In contrast, the 5.8S gene was less divergent and did not yield as many unique primer sites. Sequencing of clones from the same species revealed the existence of species-specific polymorphisms. Hence, each ITS region must be fully characterized to ensure the probes will bind a conserved site present in every genotype. The extensive ITS sequence variability between species means that sequences from closely related species could be aligned reliably, but that more distantly related species could not. Hence, ITS sequences will likely prove useful in determining the phylogenetic relationships among members of the *Pfiesteria* complex, or other closely related groups, but may not prove useful in determining overall phylogenetic relationships among dinoflagellates.

ALEXANDRIUM POPULATION AND NUTRIENT DYNAMICS IN CASCO BAY, MAINE

Theodore C. Loder III and Rebecca C. Clauss
University of New Hampshire, Durham, NH 03824

Since 1972, blooms of the toxic dinoflagellate, *Alexandrium tamarense*, have occurred almost every spring in the coastal region of Casco Bay, Maine. Seven cruises from April to June, 1998 were undertaken in this region to elucidate the physical, chemical, and biological environment of waters containing these harmful blooms. Samples were combined ("pooled") from depths of 1, 3.5, and 7 m in an attempt to obtain a homogenous water sample representative of the mixed surface layer. Consistent with previous work, blooms of *Alexandrium* in this region were concurrent with low inorganic nutrient conditions. Because these blooms typically occur soon after the spring diatom bloom, as the surface waters warm, dissolved inorganic nutrient concentrations are generally low. The highest *Alexandrium* cell densities (~1000 cells/L) were observed during mid May near shore (within the Kennebec River plume) and in an area approximately 25 miles offshore. Near shore aggregations of *Alexandrium* cells were likely the result of downwelling favorable winds (from the northeast), which commenced in early May. Areas marked by high cell densities (>400 cells/L) were associated with dissolved inorganic nitrogen concentrations of less than 2.5 µM and N:P ratios lower than 6:1. In areas where *Alexandrium* cell counts exceeded 200 cells/L, 82-99% of the total dissolved nitrogen (TDN) was in the organic form. Average DON concentrations in areas where blooms exceeded 200 cells/L were 8.5 µM, while in bloom areas of 100 cells/L or less DON concentrations averaged approximately 6.5 µM. It is hypothesized that *Alexandrium* may be able to obtain some of its nutrient requirements from the dissolved organic nitrogen pool, but further work is needed to determine this.

AUREOCOCCUS AND UREA METABOLISM IN LONG ISLAND BAYS

Michael W. Lomas[1]
UMCES, Horn Point Laboratory, Cambridge, MD 21613, USA

Nutrients have been, and will continue to be, targeted for research and management in the coastal zone, due to the links with increased phytoplankton production, and the associated shifts in ecosystem functioning. Such research, until recently, has focused predominantly on dissolved inorganic nitrogen, even though dissolved organic nitrogen (DON) can be a quantitatively larger pool. The inability to characterize and quantify significant fractions of the DON pool has been a major hindrance to this research. Although poorly characterized, this DON pool can consist of low molecular weight, labile components such as urea, amino acids, and proteins. Dogma suggests that bacteria are better competitors of organic substrates than phytoplankton simply due to their smaller size, and their heterotrophic nature.

Although marine bacterial hydrolysis of urea, through the activity of the enzyme urease, has been known for nearly 70 years, more recent studies have suggested that the hydrolysis of urea by phytoplankton can be substantially more important in coastal systems. In particular, it has been suggested that an enhanced ability to hydrolyze urea and other simple organic molecules may be one physiological advantage to the formation of blooms of the Long Island brown tide organism, *Aureococcus anophagefferens*.

As part of a larger ecosystem level sampling effort to understand the bloom dynamics of *Aureococcus,* urease activity of the water column biota was measured in two Long Island bays, Quantuck and Flanders Bay, in May and July of 2000. In May, both bays were characterized by similar mean urease activities ~0.3 µmoles NH_4^+ produced /liter seawater/h. Although phytoplankton biomass (estimated as chl *a*) was twice as high in Quantuck Bay as in Flanders Bay, it was not correlated to urease activity and neither bay had significant populations of *Aureococcus.* The seasonal transition from spring, May, to summer, July, resulted in substantial changes in both the rates and patterns of urease activity, as did a significant (>4") rain event during our period of sampling. July urease activities in Flanders Bay were significantly related to chl a, and suggested that bacterial urease activity was less important as shown by the non-significant y-intercept. Additionally, *Aureococcus* was not detected in the phytoplankton assemblage. To the contrary, in Quantuck Bay, urease activities were not related to chl a and were 3-4 times greater than would have been expected based on the chl a- urease relationship observed in Flanders Bay. Although the phase of the *Aureococcus* population in Quantuck Bay in July could not be assessed, *Aureococcus* was present at substantial numbers (>72,000 cells/ml and ~14% of chl a biomass). The rainfall event in July did not appear to alter the relationship between chl a and urease as measured in Flanders Bay, but in Quantuck Bay, urease activities were reduced 4-fold, without a change in chl a, to values that concurred with the measured chl a-urease relationship in Flanders Bay.

These observations suggest that under similar nutrient and phytoplankton biomass conditions (i.e. bulk chl a), these two bays differ substantially in the metabolism of urea during the summer. The functional relationship between components of the planktonic assemblage (e.g. *Aureococcus*) and urea remain to be fully understood.

MOLECULAR CHARACTERIZATION OF *GYMNODINIUM BREVE* STRAINS FROM THE TEXAS SHORE (GULF OF MEXICO) USING RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACERS AND 18S REGIONS

Pascale Loret and Lisa Campbell
Department of Oceanography, Texas A&M University, 3614 TAMU, College Station, TX 77843

The toxic dinoflagellate *Gymnodinium breve* is known to be a major species involved in bloom events in the Gulf of Mexico (Steidinger & Penta, 1999). It produces potent toxins that may result in human illness or death due to shellfish poisoning (Saunders et al., 1997). The occurrence of bloom events has significantly increased along the Texas shore during the last decade (T. Villareal, pers. comm.) but their origin remains poorly understood. For these reasons, the characterization of *G. breve* populations from Texas contributes to a better knowledge of the major species involved in bloom events in this region.

Three strains of *G. breve* were collected from the South Texas coast during the 1999 red tide event and the 18S ribosomal RNA gene (rDNA) and internal transcribed spacer (ITS) regions have been targeted to identify these isolates at the intra-specific level. The sequences we obtained for the ITS and 18s regions were compared with known sequences of *G. breve* strains isolated from the Florida coast.

The internal transcribed spacers and the 18S rDNA regions were amplified by polymerase chain reaction (PCR) and sequenced. Aligned nucleotide sequences of the 3 isolates of *G. breve* including the complete ITS and the 18S rDNA region showed that the size of both regions were conserved in the 3 isolates and were 600 and 1700 bp respectively. The 18S and ITS nucleotide sequences obtained for the 3 strains of *G. breve* from the Texas coast were aligned. A very high homogeneity was observed between the 3 isolates and for both genes. Similarly, the consensus sequences obtained for the 18S and ITS regions were aligned with the corresponding regions for a *G. breve* strain isolated from the Florida coast. The aligned sequences showed homogeneity of both regions among these different isolates from the Gulf of Mexico.

The present study based on the ITS and 18S regions has shown a high homogeneity between different strains of *G. breve* isolated from the Gulf of Mexico. The usefulness of both ITS and 18S regions as genetic markers for *G. breve* will be discussed.

A RAPID LARVAL FISH BIOASSAY FOR TESTING *PFIESTERIA* TOXICITY

Vincent J. Lovko, Wolfgang K. Vogelbein, Jeffrey D. Shields, Howard Kator, David E. Zwerner, Yasunari Kiryu
Department of Environmental Sciences, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062

The cause of fish mortality in large-scale static biotoxicity assays with *Pfiesteria* is often difficult to determine. Suboptimal water quality, microbial enrichment, and prior fish health issues have been major impediments to assay interpretation. We have developed a sensitive and rapid (96 hr) "fractionation assay" using larval mummichogs, *Fundulus heteroclitus,* and toxic cultures of *Pfiesteria* sp. “B”. This assay tested fish mortality in relation to the different constituents found in the complex biotic community of our large-scale *Pfiesteria* bioassay systems. Seven day old lab-reared mummichog and juvenile tilapia (25-35 mm), *Oreochromis niloticus*, were exposed to "dinoflagellate fractions", "bacterial fractions", "supernatant (soluble *Pfiesteria* toxin) fractions" and appropriate water quality controls. The source of the fractionation medium was raw water from "toxic" cultures of *Pfiesteria* sp. “B” causing 20-50% daily mortality in tilapia. Water was fractionated by centrifugation and filtration to obtain media enriched in dinoflagellates, bacterial contaminants, and toxin (supernatant fraction). The dinoflagellate fraction (*Pfiesteria* sp. “B” and other protozoa) was collected on a 5 µm filter and rinsed with sterile-filtered artificial seawater (ASW) under vacuum, floated in ASW, examined for viability, and suspended in an identical volume of ASW as that originally filtered. The bacterial fraction (and small bicoecid flagellates) was pelleted by centrifugation (9000 rpm, 45 min, 10E C, Sorvall RC-5B), resuspended in small aliquots of ASW, passed through a 5 µm filter, and resuspended in ASW in an identical volume as that originally centrifuged. The "soluble toxin" fraction was the supernatant from the centrifugation, that was passed through 5 µm and 0.45 µm filters. Controls consisted of (1) artificial seawater (ASW), (2) ASW with ammonia (as NH_4Cl) and pH adjusted to that of raw toxic culture water, and (3) raw toxic culture water. Mortality and water quality (NH_4, NO_2, DO, pH) were assessed every 24 hrs for 96 hrs. Tilapia were exposed in jars with 500 ml medium (treatment: 3 replicates of 4 fish). Mummichogs were exposed in micro-titer well plates with 15 ml medium/well (treatment: 9 replicates of 5 fish/well). Results indicate that tilapia are marginally sensitive to *Pfiesteria* "B", as extremely high cell densities are required to kill fish. However, larval mummichogs exhibited 100% mortality in raw water and "dinoflagellate fractions” yet negligible mortality in controls. Histopathology confirmed toxicity/mortality findings. The mummichog assay appears effective for rapid verification of dinoflagellate toxigenicity and provides a quantifiable approach to study *Pfiesteria* biology.

RELATIONSHIPS BETWEEN OCEANOGRAPHIC SATELLITE DATA AND *ALEXANDRIUM* DISTRIBUTIONS IN THE GULF OF MAINE

Remy M. Luerssen, Andrew Thomas, and Ryan Weatherbee
5741 Libby Hall, School of Marine Sciences, University of Maine, Orono, Maine 04469

Satellite image time-series of sea surface temperature and ocean color coincident with comprehensive field measurements of both *Alexandrium* and associated oceanographic parameters from 1998 and 2000 were collected and processed. Here we present the preliminary results of relationships between satellite measured patterns and field data from the three 1998 cruise periods. Sea surface temperature images from NOAA AVHRR were pre-filtered with a custom cloudmasking routine that was applied individually for each cruise period and for each satellite (NOAA 12, 14, and 15). In addition, within each cruise, sea surface temperatures were normalized to reduce the effects of anomalous atmospheric attenuation and diurnal variability. SeaWiFS data were processed using the latest (2000) NASA global coefficients. Both individual and composite scenes formed over each cruise period were examined. Qualitative relationships between the patterns seen in the satellite data and the in situ distributions of *Alexandrium* are shown using contours overlaid on the composite images. Initial results show that in June and July the distributions of *Alexandrium* generally followed the cold plume of the eastern Maine coastal current. In August, this association was weaker. In each month, the contours show that a recirculation feature, evident as a warm temperature pool at the mouth of the Bay of Fundy, is also coincident with a large population of *Alexandrium*. Distributions of *Alexandrium* suggest that, in the June, elevated concentrations are most closely associated with advective patterns evident in the SeaWiFS data, but in July and August concentrations are highest in association with boundaries between elevated coastal chlorophyll and more oligotrophic offshore waters. Preliminary results from regression analyses between satellite and ship-measured parameters will be shown to demonstrate quantitative relationships.

PROROCENTRUM LIMA IN THE GULF OF MAINE: SHOULD WE CARE?

Lucie Maranda[1], John Hurst[2], Laurie Bean[2], Jay McGowan[3], and Paul E. Hargraves[1]
[1]Graduate School of Oceanography, University of Rhode Island, Narragansett RI 02882
[2]Maine Department of Marine Resources, West Boothbay Harbor ME 04575
[3]Maine Department of Marine Resources, Lamoine ME 04605

The dinoflagellate *Prorocentrum lima* has been found at several sites along the coast of Maine during the summer in 1998 and 1999, some in areas where shellfish are harvested commercially. Identity was confirmed by scanning electron microscopy (SEM). Although *P. lima* is known to produce toxins (okadaic acid and derivative compounds), no incidence of diarrhetic shellfish poisoning (DSP) has been conclusively documented so far in the Gulf of Maine, despite toxicity events in the early 1990's in Nova Scotia, Canada. *Prorocentrum lima* was first observed near Georges Bank in 1994 in an offshore plankton tow. In coastal waters, samples containing the dinoflagellate came from wild mussel populations collected at low tide, while others originated from aquaculture sites. Many of the cells were isolated from water samples and net tows, and on a few occasions were found in association with epiphytic algae.

Our survey suggests that *P. lima* is relatively rare at most stations. However the widespread distribution of this toxin producer, its recurrence two years in a row and over several months, and presence close to mussels and in the plankton warrant increased monitoring to address public health concerns and foster a better understanding of its ecology, especially in light of anticipated shifts in shellfish cultivation methods.

DOES *ALEXANDRIUM FUNDYENSE* IN THE BAY OF FUNDY MIGRATE VERTICALLY?

Jennifer L. Martin and Murielle M. LeGresley
Fisheries and Oceans Canada, Biological Station, St. Andrews, NB. E5B 2L9

A number of dinoflagellates have demonstrated a nocturnal downward migration in order to utilize nutrients and growth-promoting substances. The organism, *Alexandrium fundyense*, is responsible for producing paralytic shellfish (PS) toxins in the Bay of Fundy resulting in many shellfish areas being closed to harvesting for a period of time each year.

A study of *A. fundyense* in the Bay of Fundy was conducted east of Grand Manan Island over a 54 hr period (1000 AM, July 27-1600 PM, July 29) where samples were collected at 3 h intervals from the surface and depths of 5 m, 10 m, 20 m, 30 m, 50 m, and 90 m. Analyses included dominant phytoplankton species with particular emphasis on total *A. fundyense* concentrations which included its various life cycle stages – fusing cells, duplets, planozygotes and newly formed resting cysts. Highest concentrations of total *A. fundyense* cells were detected in 15 of the 19 surface samplings with the highest numbers detected at 1000 PM. The remaining four samplings had highest concentrations detected at 5 m and all were between the hours of 1000 AM and 1600 PM. Surface concentrations ranged from 8.88×10^4 to 1.65×10^5 cells l^{-1}. Although cells were observed throughout the water column, *A. fundyense* numbers decreased with depth throughout the study and numbers at the 90 m depth ranged from 100-1900 cells l^{-1}. Similarly, planozygotes were observed throughout the water column with concentrations greatest in surface samples with the highest number 2.6×10^5 cells l^{-1} observed at 1300 PM. Duplets were observed to a depth of 20 m but the majority were detected in the surface waters with no duplets detected at depths of 50 and 90 m. Highest numbers of duplets were 9.46×10^5 cells l^{-1} at 700 AM. Numbers of fusing cells were significantly lower that those for duplets although the maximum density 5.11×10^3 cells l^{-1} was also detected at the surface. Newly formed resting cysts were also observed during the study, but only at depths of 50 and 90 m. Concentrations ranged from 20-300 cells l^{-1}.

Mean surface and 90 m temperatures were 12.1°C and 7.9 °C, respectively. Salinities ranged from 31.0-32.9 psu. Other species observed during the study included *Scrippsiella trochoidea*, *Mesodinium rubrum*, *Ptychocylis* sp., *Helicostomella* sp., *Favella* sp., and various other tintinnid species.

Further research is required to determine why the Bay of Fundy *A. fundyense* strain does not migrate vertically. Attempts to reconstruct the study and do further analyses have been unsuccessful as a result of the extremely low concentrations in recent years.

THE FLUORESCENCE EMISSION CONTRIBUTION TO THE COLOR OF RED TIDES

Helmut Maske*, James L. Mueller, Charles C. Trees
Center for Hydro-Optics & Remote Sensing, San Diego State University, 6505 Alvarado Road, Suite 206, San Diego, CA 92120

*permanent address: Ecología, CICESE,
Apartado Postal 2732
Ensenada, Baja California, Mexico, CP 22800

According to our working hypothesis the red color of dinoflagellate red tides is produced by the fluorescence of chlorophyll *a* emitted by very high concentrations of dinoflagellates near the sea surface. Parameters that change the relative contribution of fluorescence emission to the reflectance spectrum are: the depth distribution of chlorophyll, the quantum efficiency of fluorescence emission, the Stokes shift that determines the spectral overlap between chlorophyll absorption and fluorescence emission spectra, and the ratio of blue to red photosynthetic absorption that determines the energy pumped to the red emission, thus defining the balance between reduced reflectance in the blue and emission in the red. We used the Hydrolight radiative transfer model to confirm earlier Monte-Carlo results demonstrating the importance of high chlorophyll concentrations very close to the sea surface and to study the sensitivity of the red upwelling red radiance peak to the wavelength maxima in absorption spectra and emission, the red to blue absorption ratio and the quantum efficiency of emission. Within a *Gymnodinium sanguineum* bloom in Monterrey Bay pigment and particle absorption samples near the surface were taken with high vertical resolution, and hyperspectral L_u at 0.6m depth and E_d in air were measured. The radiance spectra are compared with model results that were based on measured pigment distribution.

FEEDING BY CILIATES ON HARMFUL MICROALGAE

George B. McManus and Carol H. Rosetta
Department of Marine Sciences, University of Connecticut, Groton CT 06340

Although blooms are sometimes an exception, the fate of most phytoplankton in the sea is to be eaten, and most of the eating is done by microzooplankton. These smallest of herbivores consist mostly of ciliates, heterotrophic dinoflagellates, and other protists. We have focused on ciliates as potential grazers of HAB species, using laboratory observations of feeding and growth to infer the potential role of these organisms in preventing or terminating blooms. The HAB species we have examined to date include the dinoflagellate *Prorocentrum minimum* (strain EXUV), the raphidophyte *Heterosigma carterae* (strain OL), and two prymnesiophyte strains, *Prymnesium parvum* (strain PRYM) and *Prymnesium parvum* (strain 97-20-1). All strains were obtained from the NMFS Milford Laboratory (the latter had obtained 97-20-1 from Bigelow Lab, Guillard Center for Culture of Marine Phytoplankton). All observations were performed with late log or stationary phase cultures of phytoplankton fed to ciliates isolated into culture from Long Island Sound.

P. minimum supported good growth in all of the ciliates that were large enough to eat it, including both tintinnids and non-loricate choreotrichs, even at concentrations well in excess of those found in typical blooms in Long Island Sound ($>3x10^4$ cells/ml). In contrast, *H. carterae* was not a suitable food for any of the ciliates. Thus far, we have fed the two prymnesiophytes to only one ciliate, the tintinnid *Favella sp*. Neither strain supported growth in this tintinnid, and comparisons with the non-toxic prymnesiophyte *Isochrysis galbana* (strain TISO) and with *P. minimum* suggest that they are actually harmful to this grazer, rather than simply being non-nutritious.

Additional observations using fluorescently-labelled algae to evaluate selective feeding on HAB species will also be discussed.

VISUALIZATION AND IDENTIFICATION OF ATTACHED AND INTRACELLULAR BACTERIA WITHIN SEVERAL STRAINS OF *PFIESTERIA PISCICIDA*

T.R. Miller and R. Belas
Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt Street Suite 236, Baltimore MD 21202

Bacteria co-occurring with strains of the toxic dinoflagellate *Pfiesteria piscicida* may affect the physiology and/or toxigenesis of the host dinoflagellate. In efforts to understand the role played by these bacteria, we have identified and localized the bacteria in and on *P. piscicida,* using oligonucleotide probes to regions of the bacterial 16S ribosomal RNA gene (rDNA). Polymerase chain reaction (PCR) amplification of bacterial 16S rDNA from DNA templates derived from the total bacterial population associated with the dinoflagellate was performed. The nucleotide sequence of each unique clone was obtained and this information was used to generate the species-specific probes, while group-specific probes were generated based on published data. The population of bacteria that was unattached or loosely attached to the dinoflagellate was removed by gentle washing of the eukaryotic cells, leaving behind only those bacteria that were firmly attached or internal to the host cell. The washed dinoflagellates and associated bacteria were then hybridized to each fluorescently-labeled oligonucleotide probe and the bacteria on and in the dinoflagellate cells were visualized by confocal scanning laser microscopy. These images were used to establish the cellular location of each bacterial species. Taxonomic identification of the attached and intracellular bacteria was accomplished by determining the relatedness of their 16S rDNA nucleotide sequence to known bacterial sequences using computer-based homology and phylogeny software. From these analyses, phylogenetic trees have been constructed showing the taxonomic inter-relatedness of the dinoflagellate-associated bacteria and the niches on and in the dinoflagellate cells that these bacteria occupy. The possible significance of these bacteria to the physiology and toxigenesis of the dinoflagellate is discussed.

SALINITY TOLERANCE OF ESTUARINE CYANOBACTERIA FROM NORTH CAROLINA AND FLORIDA

Pia H. Moisander[1], Ernest McClinton[2] and Hans W. Paerl[1]
[1]University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557
[2]University of Arkansas, Pine Bluff, AR 71611, USA

Salinity is thought to restrict growth and expansion of filamentous nitrogen fixing cyanobacteria into estuaries. Many species in this group produce hepato- or neurotoxins, placing additional importance on the issue of salinity constraints of these potentially harmful species. Because it is often impossible to tell if a phytoplankton community exists at a specific site as a result of advection, or actual growth, culture experiments are needed.

To examine the effects of salinity on growth and nitrogen fixation, filamentous nitrogen fixing cyanobacteria were isolated from the Neuse River Estuary (NRE), North Carolina, and St. Johns River (SJR) system in Florida. The isolates showed different salinity tolerances. For *Cylindospermopsis raciborskii* (SJR) isolates, the salinity limit for growth was between 4 and 5 ppt. The salinity tolerance was similar for two distinct morphotypes (straight/coiled) of *C. raciborskii*. Varying light level did not have an additional effect on *C. raciborskii* salinity tolerance. In contrast, *Anabaenopsis* sp. and *Anabaena aphanizomenoides*, isolated from the NRE (Marker 15) grew at salinities of up to 20 and 15 ppt, respectively.

For the harmful cyanobacterium *C. raciborskii*, limited salinity tolerance allows it to grow in low salinity estuarine waters. However, since *C. raciborskii* has lower salinity tolerance than the NRE isolates it may be a weaker competitor in the mesohaline segments of the Neuse River Estuary. However, *C. raciborskii* is found periodically in the upper reaches of the estuary. These results suggested that *Anabaena aphanizomenoides* and *Anabaenopsis* sp. can maintain growth in the estuarine water at relatively high salinities (15-20 ppt). Therefore, salinity barriers cannot explain the low frequency and magnitude of blooms of nitrogen fixing cyanobacteria in the nitrogen limited Neuse River Estuary. Further studies on the combination effects of salinity and other chemical and biological factors, such as pH are warranted.

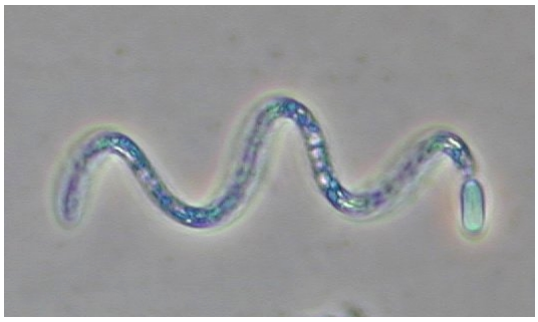


Figure 1. Coiled morphotype of *Cylindrospermopsis raciborskii*.

BREVETOXIN ANALYSIS IN SEAWATER, MAMMALIAN BODY-FLUID AND SHELLFISH HOMOGENATE BY COMPETITIVE ELISA

Jerome Naar, Andrea Bourdelais, Carmelo Tomas, Johnny Lancaster and Daniel G. Baden
Center for Marine Science, University of North Carolina at Wilmington, Wilmington NC 28409

A competitive Enzyme Linked Immuno-Sorbent Assay (competitive ELISA) was developed for analyzing brevetoxin (PbTxs). This assay is based on the activity of goat anti-brevetoxin antibodies obtained following immunization with KLH-PbTxs conjugate. The antibodies were used in combination with a multi-step signal amplification procedure for the detection of toxin. This procedure minimizes non-specific signal and background usually observed in complex matrices. Therefore, analysis can be performed in seawater, mammalian body fluid and shellfish homogenate without any extraction and/or purification steps. PbTxs analysis in liquid samples like seawater, urine and serum are performed without pretreatment, dilution or purification. The limit of quantification of PbTxs is $5x10^{-9}$ M in all the liquid samples. For shellfish monitoring, analysis are performed after homogenization of shellfish meat (5gr) with brevetoxin-ELISA buffer (200ml) and can be run on a single mollusk as well as on a pool of shellfish meat. Comparative quantification of PbTxs achieved in buffer, seawater, mammalian body fluid and shellfish homogenate spiked with the same amount of toxin (10ng/ml sample) varies no more than 5%. It is concluded that the matrix composition of the sample does not affect the performance of the assay. The limit of quantification of PbTxs was 10 micrograms by 100 grams of shellfish meat, that is 8 times more sensitive than the mouse bioassay. Because this assay is not affected by the matrix composition and can be performed in shellfish homogenate, it measures the real concentration of the brevetoxin present in the shellfish meat and not only the toxin fraction extracted by solvent in conventional assays. Therefore, this procedure can be used to prevent and diagnose human exposure to PbTxs and has the potential to replace the currently used mouse bioassay for monitoring PbTxs in shellfish.

INVESTIGATION OF THE CAUSALITY OF DERMAL LESIONS IN MENHADEN FROM THE JAMES RIVER, VA (OCTOBER 1999)

Thomas A. Nerad[1], Greg C. Garman[2], Stanley Webb[2], Bonnie Brown[2], Michael T. Peglar[1,3], and Patrick Gillevet[3]
[1]American Type Culture Collection, Manassas, VA
[2]Virginia Commonwealth University, Richmond, VA
[3]George Mason University, Manassas, VA

The dinoflagellate *Pfiesteria piscicida* has been reported as causing massive episodes of menhaden (*Brevoortia tyrannus*) mortalities in North Carolina and several other locations along eastern coastal watersheds of the United States. Focal ulcers primarily localized to the vent region of the fish have been observed in these episodes. These lesions resemble those also illustrated for *Aphanomyces, Saprolegnia* and various species of bacteria. Our recent observations have shown that the myxosporean *Kudoa clupeidae* is also associated with similar lesions. The myxosporean genus *Kudoa* was described as a parasite of Atlantic menhaden in North Carolina waters as early as 1947. The genus now comprises 45 species and has been of interest to parasitologists ever since. Some species of *Kudoa* liquefy muscle tissue making some fish unsuitable for commercial use and *Kudoa clupeidae* is the only species of the genus that has been linked to fish mortalities.

In the recent James River episode, the Atlantic menhaden sampled had lesions that were primarily localized to the vent region. Among several hundred Atlantic menhaden sampled, 75% had penetrating lesions. Twenty-five (25) percent had either no lesions or sub-dermal lesions (swollen areas on the body without any visible breaks in the epidermis) that quickly erupted when the fish were handled. Histological sections from 14 fish exhibiting lesion development ranging from non-evident to advanced, were hematoxylin and silver stained. Sterile swab samples were also taken from the same areas of the fish and examined for bacteria, fungi, free-living amoebae, and other protists. Molecular assays of sediment water and fish tissues were also conducted to determine whether PCOs (*P. piscicida* and related dinoflagellates) were present. No PCOs were detected. However, in fish without evident lesions and with sub-dermal lesions, only *Kudoa clupeidae* was found. On the other hand, various bacteria, fungi and amphizoic amoebae, as well as *Kudoa* were found in fish with all stages of open dermal lesions.

We developed *Kudoa* specific oligonucleotide probes based on published SSU rRNA gene sequences for four species of *Kudoa*. Using these probes we amplified and sequenced a 1395 bp segment of the SSU rRNA gene of *K. clupeidae.* A phylogenetic analysis of the sequence data indicated that *K. clupeidae* was distinct from the four other sequenced taxa and was most closely related to *K. miniauriculata*.

In nature *Kudoa* may initiate epidermal lesions that allow opportunistic pathogens such as bacteria, fungi and amoebae to further compromise fish health. This study suggests that *Kudoa clupeidae* may have been a significant agent in initiating lesion development in other recent outbreaks of fish mortality in eastern Atlantic coastal waters.

SEARCHING FOR VIRUSES INFECTIVE FOR *GYMNODINIUM BREVE*

John H. Paul, Lee Houchin, Dale Griffin, Theresa Slifko, and Mike Guo
College of Marine Science, University of South Florida, St. Petersburg, FL 33701

A project has been initiated to find lytic and temperate viruses infective for *Gymnodinium breve*. During a research cruise into the Gulf of Mexico in early 1999 a *G. breve* bloom near Ft. Myers was sampled for viruses infective for this red tide organism. A viral concentrate was made by vortex flow ultrafiltration of 20 l of the water to yield a 50 ml retentate. One ml aliquots of this concentrate caused 25 ml *G. breve* Davis Piney Island clone cultures to lyse ("crash") within 3 to 5 days. The "lytic agent" was propagated by inoculation into fresh cultures of *G. breve* and "serially passaged" over 12 times before lytic activity was lost. The lytic agent passed a 0.2 µm filter but was retained on a 0.02 µm filter and was inactivated by heating to 60^{o} for 10 min. Viral particles increased in cultures exposed to the lytic agent as detected by epifluorescence microscopy of SYBR Gold-stained culture filtrates. A dominant tailed Siphoviridae was observed by TEM in such preparations. However, no viral particles were observed in thin-sectioned preparations of bursting *G. breve* cells. It is hypothesized that the virus observed was infective for a bacterium that either was necessary for *G. breve* growth or that the lysis of bacteria in the culture cause *G. breve* mortality. Attempts to induce prophage by mitomycin C resulted in certain instances in *G. breve* lysis and viral production, yet this may have been the result of induction of bacterial lysogens in these cultures.

MOLECULAR DIFFERENTIATION OF STELLATE AMOEBAE, *PFIESTERIA PISCICIDA* AND RELATED DINOFLAGELLATES RECOVERED FROM THE CHESAPEAKE BAY REGION OF THE EASTERN UNITED STATES

Michael T. Peglar[1,2], Patrick Gillevet[2], Thomas A. Nerad[1], Charles J.O'Kelly[3], And Thomas K. Sawyer[4]
[1]American Type Culture Collection, Manassas, VA 20110
[2]George Mason University, Manassas, VA 20110
[3]Bigelow Laboratories for Ocean Studies, Booth Bay Harbor, ME
[4]Rescon Associates, Inc., Royal Oak, MD 21662

Commonly occurring amoebae and *Pfiesteria*-like dinoflagellates from major tributaries of the Chesapeake Bay were established in pure culture. Representative taxa of six genera of amoebae capable of forming temporary stellate or rayed stages were studied. Following two years of observation during continuous cultivation with algal prey, clonal cultures of *Pfiesteria*-like dinoflagellates (Pocomoke River isolates 1-8, Patuxent River isolate A) and clonal reference strains of *Pfiesteria piscicida* (CCMP 1830,1831,1834) and *Pfiesteria*-like dinoflagellates (CCMP 1828, 1835, 1838, 1839, 1845) have never yielded amoeboid life-cycle stages. In addition, monoprotist cultures of amoebae have never produced any dinozoite stages. Small subunit ribosomal RNA (SSU rRNA) genes were sequenced from monoprotist cultures of amoebae and several clonal cultures of dinoflagellates. Parsimony analysis of this data demonstrated that six genera of commonly occurring amoebae including *Korotnevella*, *Neoparamoeba*, *Paraflabellula*, *Platyamoeba*, *Rhizamoeba* and *Vannella* cluster in several independent clades that are all distinct from dinoflagellates. In addition, *P. piscicida* reference strain (CCMP 1834), two *Pfiesteria*-like isolates from the Pocomoke River, MD (isolates 6 and 8) and a tentative *Gyrodinium* sp. from the Patuxent River, MD cluster tightly within the dinoflagellate clade, separate from all groups of amoebae for which SSU rRNA gene data exists. The results of this study support the conclusion that representative taxa from six genera of the most commonly occurring amoebae of the Chesapeake Bay are not related to dinoflagellates.

HAB DISTRIBUTIONS IN ALABAMA COASTAL WATERS: 1998-1999

Jonathan R. Pennock[1] and Cary L. Burns[2]
[1]Dauphin Island Sea Lab and The University of Alabama, 101 Bienville Boulevard, Dauphin Island, Alabama 36528
[2] University of South Alabama and Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, Alabama 36528

In the late fall of 1996, a toxic bloom of *Gymnodinium breve* resulted in the closure of Alabama oyster reefs for the first time in history. As a result of this bloom, we initiated monthly monitoring of Mobile Bay and nearshore coastal water of the Gulf of Mexico to investigate the distributions of *G. breve* and other harmful algal species in the region. Our objectives were to monitor for a recurrence of *G. breve* and assess for the presence and distribution patterns of potentially harmful algal species.

During 21 months of sampling, there was no recurrence of *Gymnodinium breve* in Alabama coastal waters (Although, there was a *G. breve* bloom that moved across the Florida panhandle into the eastern most portion of Alabama coastal water during the fall/winter of 1999-2000, after our project had ended.). However, numerous potentially harmful algal species were observed. *Pseudonitzschia* sp., a potential domoic acid producing diatom, was observed year-round, over a wide range of salinities. Other potentially harmful dinoflagellates (e.g. *Ceratium* spp., *Prorocentrum* spp. and *Dinophysis caudata)* were also present in low numbers. We also observed high numbers of *Ceratium hircus* during the summer in waters of moderate salinity. A near unialgal bloom of *Prorocentrum minimum* reached concentrations of 2500 cells ml^{-1} in February 1999 and was associated with the Mobile Bay plume front.

The observed year-round presence of harmful algal species in Alabama coastal waters indicates a potential for harmful algal blooms to occur in this region.

HARMFUL ALGAL BLOOM OUTREACH

Heather Penta, Karen Steidinger, and Teresa Steely
Florida Marine Research Institute**,** Fish and Wildlife Conservation Commission, 100 8th Avenue SE, St. Petersburg, FL 33701

The purpose of harmful algal bloom (HAB) outreach is to distribute consistently accurate information about red tides, especially those in progress, to the public. When citizens believe that a HAB threatens them, they want immediate information about what, where, when, and how. What occurred? Where and when did it occur? How are humans affected? How is the environment affected? What is being done to fix it? At Florida Marine Research Institute (FMRI), we have a multidisciplinary approach to disseminate accurate information: the Internet, printed material, and public presentations.

Our web page [http://www.fmri.usf.edu] has two target audiences -the general public and fellow scientists. The web page is divided into four subsections: Red Tide; Florida's Red Tide Status; Research Projects; and More Information. The basic format follows:

1) Red Tide
   a) What is Red Tide? What is a HAB?
   b) HAB Species
   c) Red Tide and Marine Animals
   d) Shellfish Poisoning
2) Florida's Red Tide Status
   a) Current status
   b) Historical Red Tide
3) Current Projects
   a) ECOHAB: Florida
   b) Estuarine Monitoring
4) More Information about Red Tides
   a) Photo Gallery
   b) HAB Species of the Month
   c) Links
   d) Select Publications and Glossary

The printed material that we produce includes color brochures, Frequently Asked Questions (FAQs), Fact Sheets (both general and technical), press releases, and a training manual (Steidinger and Penta 1999). The training manual was produced for resource and regulatory personnel investigating HABs. All printed information is freely given to the public. Most of it is available through our web site.

Presentations are done in person and on-line (via Power Point). We have presented at scientific conferences, high schools, college classes, and local business groups (e.g. charter boat captains, dive clubs, and anglers). We also give presentations to tour groups visiting FMRI (school groups, concerned citizens, and legislators). The Power Point presentations include slides and text and can be seen on the Internet or down loaded as a PDF file.

Steidinger, K.A. and H.L. Melton Penta. 1999. *Harmful Microalgae and Associated Public Health Risks in the Gulf of Mexico.*

INTRA-CELLULAR, PARTICULATE AND DISSOLVED BREVETOXIN DISTRIBUTION DURING G*YMNODINIUM BREVE* BLOOMS IN THE GULF OF MEXICO, USA

Richard Pierce[1], Michael Henry[1], Patricia Blum[1], Patricia Tester[2] and Damian Shea[3]
[1] Mote Marine Laboratory, Sarasota, FL
[2] NOAA-NOS Center for Coastal Fisheries and Habitat, Beaufort, NC
[3] Department of Toxicology, North Carolina State University, Raleigh, NC

The dinoflagellate, *Gymnodinium breve*, produces several neurotoxins causing neurotoxic shellfish poisoning (NSP), massive fish kills and respiratory irritation in marine mammals and humans. The common method for public health advisories is enumeration of live cells in the water. Evidence for neurotoxins outside of cells indicates that contamination could result from water masses carrying suspended/dissolved neurotoxins in the absence of viable *G. breve* cells. Therefore, reliance on cell counts only for public health protection may be insufficient.

This study was undertaken as part of the EOCHAB-FL program to assess the concentration of toxins associated with *G. breve* cells (intra-cellular) relative to the amount of brevetoxins outside the cells (extra-cellular) either dissolved or associated with suspended particulate matter. These samples were collected during 1998 and 1999 ECOHAB-FL cruises in the Gulf of Mexico, USA. The water samples were processed by two different methods, one to distinguish toxins associated with suspended particles from dissolved toxins and the other to distinguish intra-cellular from extra-cellular toxins. The dissolved vs particulate method used gentle filtering under vacuum (<100 mm Hg) through a series of filters, a 0.7 µm porosity GF/F glass fiber filter followed by a 0.2 µm polycarbonate membrane. Toxins were separated into three fractions including those associated with cells and particles (> 0.7 µm), toxins associated with small particles (0.7>0.2 µm), and dissolved toxins (<0.2 µm). The intra- and extra-cellular toxin method used a stirred ultra-filtration cell concentrator for separating viable *G. breve* cells from seawater. Water samples were filtered through a 0.8 µm porosity polycarbonate membrane in the stirred cell at 5 psi to gently collect the viable *G. breve* cells on the membrane, allowing the "extra-cellular" toxins to pass through with the filtrate.

Dissolved toxins in the filtrate from the 0.2 µm polycarbonate filters were recovered by filtering through a C-18 bonded phase disc with subsequent elution in acetonitrile. The particulate toxins remaining on the GF/F and 0.2 µm filters were extracted by sonication in methanol. These samples were prepared for analysis by capillary electrophoresis with laser-induced fluorescence detection according to the method of Shea, 1997. Extra-cellular brevetoxins were recovered from the cell concentrator filtrate by passing through a C-18 bonded-phase extraction disc with subsequent elution of the toxins in methanol. The toxins associated with the concentrated *G. breve* cells were released by osmotic shock with distilled water, recovered on a C-18 bonded phase disc and eluted with methanol as above. These samples were prepared for HPLC-UV analysis according to the procedure of Pierce et al, 1992.

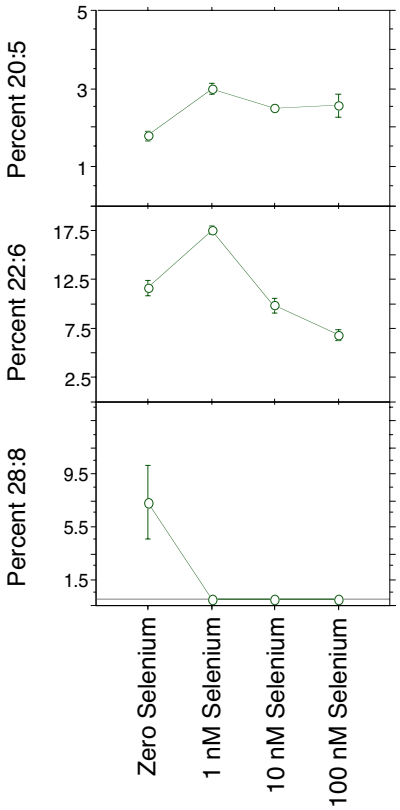
Results showed that most of the toxins in the water column were associated with particles (and cells) with very little in a true "dissolved" state. Early stages of the bloom indicated that most of the toxins were intra-cellular. The extra-cellular toxins increased relative to intra-cellular as the bloom progressed, indicating cell lysis and toxin retention in the water column. Further studies are underway to collect fractions more closely representing toxins with in *G. breve* cells relative to toxins in association with suspended particulates and in the dissolved fraction. Consideration also is given to the persistence of brevetoxins in the different fractions within the water column.

MODULATION OF VERY-LONG-CHAIN (C_{28}) HIGHLY UNSATURATED FATTY ACIDS BY SELENIUM IN TWO BLOOM FORMING DINOFLAGELLATES, *GYRODINIUM GALATHEANUM* AND *PROROCENTRUM MINIMUM,* FOUND IN THE CHESAPEAKE BAY, MD.

Allen R. Place[1], Jason Adolf[2], Jonathan R. Deeds[1], Robin Way[1], and Eric Lund[1]
[1]University of Maryland Biotechnology Institute, Center of Marine Biotechnology, Baltimore, MD 21202 [2]University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD, 21613

In recent years, harmful algal blooms, in particular those of the ichthyotoxic dinoflagellates *Gyrodinium galatheanum* and *Pfiesteria piscicida*, have been associated with massive mortality of fish in natural riverine systems along the east coast of the United States and in cultured fish ponds. We have shown Chesapeake Bay isolates of *Gyrodinium galatheanum* and *Prorocentrum minimum* both contain the hemolytic endotoxin octadecapentaenoic acid (18:5n3). This unique fatty acid has been suggested to be the toxic agent of *Gyrodinium aureolum* and *Gymnodinium* cf. *mikimotoi* responsible for aquaculture related fish kills. Furthermore, we have shown that for *G. galatheanum* and *P. minimum* cells, the major pools for 18:5n3 are the monogalactosyl diacylgylcerols (MGDG) and diagalactosyl diacylglycerols (DGDG) found in chloroplasts. This finding is also in accordance with other toxic dinoflagellates containing 18:5n3. Several isolates of *Pfiesteria piscicida,* as well as other related heterotrophs, when cultured on cryptomonads, were found to contain no 18:5n3.

As part of our work investigating the modulation of the hemolytic fatty acid 18:5n3 by alteration in culture conditions, cultures of *Prorocentrum mininum* and *Gyrodinium galaltheanum*, were grown in defined media with and without soil extracts and/or chicken manure extracts. Addition of either extract elevated the level of 18:5n3 in both species. When we analyzed the trace elemental composition of the extracts, the level of selenium was found to be high. Accordingly when we grew these species in defined artificial seawater with varying molarities of sodium selenite (0, 1, 10, and 100 nM) the level of 18:5n3 was modulated by the selenium in the culture medium (Deeds et al., 2001), with the highest percentage found at 1 nM for both species. Recently, very-long-chain (C_{28}) highly unsaturated fatty acids (VLC-HUFA) were identified in seven marine dinoflagellate species (Manour et al., Phytochemistry, 50 (1999 541-548). In general, the proportion of these fatty acids accounted for less than 2.3% of the total fatty acids in these species. Unexpectedly the level of VLC-HUFA (28:8n3) increased at 0 nM sodium selenite, while at all other selenite concentrations the VLC-HUFA was less than 1%. The VLC-HUFA (28:8n3) was not found in the MGDG and/or DGDG lipids but instead accumulated in phospholipids. Both 28:8n3 and 22:6n3 (DHA) contain the maximal number of methylene-interrupted double bonds in a straight-chain fatty acid and share a double bond across the 4-5 positions numbered from the carboxyl group. The synthesis of 28:8n3 appears to involve an additional elongation/desaturation cycle proposed for the synthesis of 22:6n3 in mammals from 20:5n3 (EPA). Selenium appears to be involved in restricting this pathway to a 22 carbon length fatty acid. These findings pose new questions as to whether VLC-PUFAs might contribute to ichthyotoxicity and whether environmental selenium levels influence ichthyotoxicity in selected Chesapeake Bay dinoflagellates.



PARALYTIC SHELLFISH POISONING IN ALASKA: A FIELD PROGRAM FOR DETECTION OF TOXIC ALGAE AND SHELLFISH

F. Gerald Plumley[1], Julie Matweyou[1], Chris Scholin[2], Vera L. Trainer[3], Dean L. Stockwell[1], Terry Whitledge[1], and Sherwood Hall[4]
[1]Institute of Marine Science, University of Alaska, Fairbanks, AK 99775
[2]Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039
[3]National Marine Fisheries Service, Seattle, WA 98113
[4]U.S. Food and Drug Administration, Washington, DC 20204

Shellfish are one of Alaska's most abundant natural resources. Paralytic shellfish poisoning (PSP) severely limits recreational, subsistence, and commercial harvesting. To optimize shellfish utilization, the Alaska Science and Technology Foundation recommended implementation of novel monitoring procedures to detect *Alexandrium* and provided funding for the state's first beach monitoring program for this toxic alga. Kodiak Island was chosen as the study site due to its insidious history with PSP events (shellfish frequently reach 20,000 µg toxin/100 gm). The focus of our work revolves around determining the abundance of *Alexandrium* using DNA probe methodologies. PSP toxin levels are being monitored with receptor binding assays and by HPLC. Ribbed mussels (*Mytilus edulis*), known for their rapid depuration rates, were chosen as sentinel shellfish species. A primary goal is to determine if there is a correlation (both short and long term) between *Alexandrium* abundance and shellfish toxicity. Water chemistry data are also being collected to determine if the seasonal blooms of *Alexandrium* are triggered by predictable environmental/weather patterns. Cultures of *Alexandrium* are being established for detailed laboratory analysis.

Shellfish samples collected during the winter of 1998/1999 had PSP toxins at levels too low for detection. No shellfish samples were collected in April/May but by early June, PSP toxins were reaching unsafe levels. Toxin levels peaked in late June/early July at 300 - 1100 µg/100 gm, and slowly declined to very low levels by mid August. Mussels from one of the nine test site maintained PSP toxin levels below the cutoff limit (80 µg/100 gm) during the entire summer of 1999. This site was situated geographically between two of the toxic sites. We are unable to explain the differences in shellfish toxicity between sites at this time. *Alexandrium* was present at high cell densities, 200 - 1000 cells/L, from early June through mid July at most sites. Technical problems prevented collection of a robust data set using the DNA probe methodologies in 1999, however, it was clear that the decreasing levels of PSP toxins observed in shellfish during late summer were correlated with declining numbers of *Alexandrium*.

Preliminary data from summer 2000 indicate that there were two small blooms of *Alexandrium*, both of approximately two weeks duration with moderate cell densities (generally less than 500 cells/L). The first bloom was in early June, the second in late August. We are optimistic that water chemistry data will help explain why there was one large bloom in 1999 and two smaller ones in 2000. We are also anxious to determine if shellfish toxicity will show two smaller peaks in 2000, each coinciding with one of the two small *Alexandrium* blooms. Shellfish and water column toxin levels, more detailed analysis of Alexandrium abundance data, and water column chemistry data should all be available within the next few months for discussion at the December HAB meeting.

THE POTENTIAL FOR ANTHROPOGENIC TRANSPORT OF THE BROWN TIDE ORGANISM, *AUREOCOCCUS ANOPHAGEFFERENS*

Linda C. Popels and David A. Hutchins
College of Marine Studies, University of Delaware, Lewes, DE 19958

Aureococcus anophagefferens is a pelagophyte that is responsible for the harmful brown tides that have affected New Jersey, Rhode Island and New York. The known range of *A. anophagefferens* has increased since the 1990 survey (Anderson et al.), with the organisms now found as far south as Maryland and Delaware. *A. anophagefferens* has also caused blooms in Saldanha Bay in South Africa beginning in 1997 (South African Marine and Coastal Management, 1998/1999). The geographical distribution of *A. anophagefferens* appears to be increasing. Two possible ways that the brown tide could be introduced to new areas are anthropogenic transport of the organism in ballast water or water retained in recreational boats.

Experiments were conducted to determine the potential for *A. anophagefferens* to survive conditions similar to those that may be experienced in ship ballast tanks. Cultured brown tide was able to survive for at least 30 days in the dark when stored at 12°C. Temperature may play a role in survival of brown tide in the dark, as cultures recovered fastest when stored at 12 C. We are investigating the influence of environmental factors like temperature, salinity and presence of inorganic or organic nutrients on how long *A. anophagefferens* can survive in the dark.

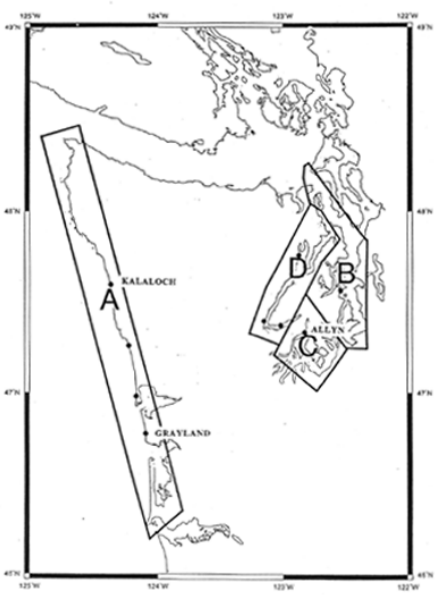
PRELIMINARY RESULTS FROM PHYTOPLANKTON AND ENVIRONMENTAL MONITORING, WESTERN WASHINGTON, USA

James R. Postel and Rita A. Horner
School of Oceanography, University of Washington, Seattle, WA 98195-7940

Washington state has a long history of harmful algal blooms as indicated by the presence of toxin in shellfish and mortalities of pen-reared salmonids. However, little is known about the temporal and spatial distribution of the causative algal species. Consequently, selected sites on the Washington Pacific Ocean coast and on inland waters of Puget Sound were monitored with varying frequency since 1990. Emphasis was on determining the presence and distribution of potentially harmful species: *Pseudo-nitzschia* spp., *Alexandrium* spp., *Dinophysis* spp., *Chaetoceros* spp., *and Heterosigma akashiwo*. Here we present preliminary results from five coast and five Puget Sound sites that were sampled twice monthly (weather permitting) from May 1997-January 2000(Fig. 1).

Water was collected with buckets and poured through a 20 µm mesh net to concentrate cells, directly with a net, or with water bottles. Temperature was measured with a laboratory thermometer, salinity was determined with a Gildline Autosal 8400A salinometer, and nutrients were measured with a Technicon Autoanalyzer II. Phytoplankton samples were analyzed using light and scanning electron microscopy

Our samples come from four distinct hydrographic regimes with conditions ranging from tidally-mixed estuaries and protected bays to open ocean beaches (Fig. 1). The coast beaches (A) are gently sloping with fine sand and are influenced by ocean currents and seasonal upwelling; they are prime locations for razor clams. Blooms of surf zone diatoms are common at these beaches. The Puget Sound Main Basin (B) is a fjord influenced by tidal and wind mixing and with a seasonal pycnocline that allows development of phytoplankton blooms; fish farms are present in some areas. Southern Puget Sound (C) has restricted circulation, low dissolved oxygen and the potential for non-point source pollution; shellfish farms are common. Hood Canal (D) is a fjord, with oxygen depletion throughout the year, but especially in late summer/fall; shellfish beds are common and there is a seasonal shrimp fishery.



Potentially harmful species were present at one or more sites in all months except March 1999. Further, all potentially harmful genera occurred at all sites except that *Alexandrium* was not positively identified in Hood Canal. *Alexandrium* was found sporadically and never in high numbers at open coast beaches. In the fall of 1997, it was present at Allyn in southern Puget Sound when record high levels (to nearly 7,000 µg/100 g) of PSP toxin were reported. *Pseudo-nitzschia* spp. were common on the open coast with blooms at Grayland in July 1997 (no domoic acid reported) and at Kalaloch in September 1998 when record high levels of domoic acid (287 µg/g) occurred in razor clams. *Pseudo-nitzschia* spp. were also common at Puget Sound and Hood Canal sites, but rarely occurring in high concentrations. *Dinophysis* spp. were present primarily in spring to summer and at all sites. *Heterosigma akashiwo* was rarely present, but a small bloom occurred at Allyn in October 1999.

Environmental conditions included extended periods of < 0.5 μM nitrate on the ocean beaches with periodic replenishment by upwelling events or seasonal changes in the coastal currents. The Puget Sound Main Basin seldom had < 5 μM nitrate due to strong tidal mixing and weak stratification; Hood Canal and southern Puget Sound generally had < 0.5 μM nitrate concentrations throughout the summer periods.

DYNAMICS OF *ALEXANDRIUM CATENELLA* BLOOMS IN QUARTERMASTER HARBOR, WA

James R. Postel, Paul N. Rudell, and Rita A. Horner
School of Oceanography, Box 357940,
University of Washington, Seattle, WA 98195-7940

Alexandrium catenella, that causes paralytic shellfish poison (PSP) in Puget Sound, WA, was studied in Quartermaster Harbor (Fig. 1), a small, semi-enclosed, seasonally stratified bay in central Puget Sound to determine the effects of biological, chemical, and physical factors on the formation, maintenance, and decline of blooms. A shore station was sampled near mid-bay 1-3 times per week from May to September 1998 to determine the environmental conditions of the surface layer in the bay during the optimal growing season for this species. Additionally, three cruises occupied a station at 20 m depth in 1997 and 1998 to study the diel migration pattern of the *Alexandrium* population and to estimate growth and mortality (from grazing) rates of the total dinoflagellate population from dilution experiments. Estimates of tidal flushing were made by calculating the volumes of the bay at various tide heights and the volumes of typical tidal prisms for mean, neap, and spring tide ranges.

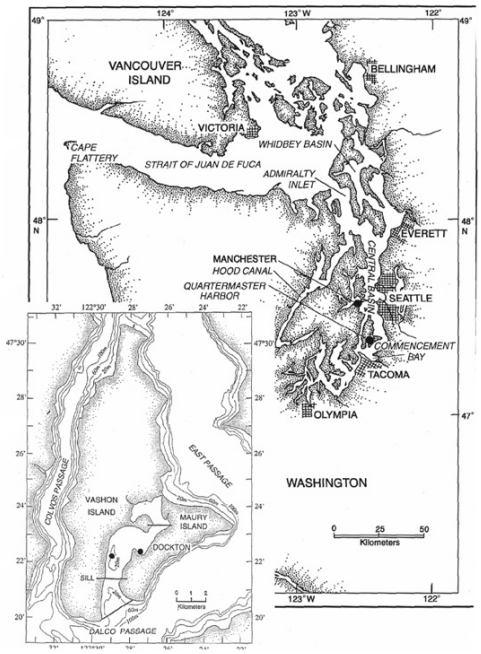


Figure 1. Quartermaster Harbor, Vashon Island, WA

PSP concentrations in caged mussels at 1 m depth exceeded 80 µg 100 g^{-1} mussel meat twice during 1997 and 1998, both times following the occurrence of *A. catenella* concentrations > 10,000 cells L^{-1}. The species was present continuously from late May through September 1998, blooming in July in surface water. Over this time interval surface temperatures varied from 14-20 °C; nitrate levels remained near-zero; phosphate and silicate were low, but measurable; and salinity ranged from 25-30 psu. Thermal stratification beginning in late spring led to the development of a shallow, low-nutrient surface layer (upper 1-3 m), a distinct pycnocline (3-5 m depth), and a deeper, nutrient-replete layer (5-20 m). During the July 1998 cruise strong spring tidal cycles eroded the pycnocline and mixed the water column within 24 h. This disrupted the distribution of *A. catenella* and contributed to bloom decline.

The diel vertical migration pattern of *A. catenella*, studied in 1997 and 1998, was limited to the upper 10 m of the water column with migration rates varying from 0.4 - 2.0 m h^{-1}. Maximal *A. catenella* cell numbers varied from < 10,000 - > 60,000 cells L^{-1}, but the phytoplankton assemblage was dominated by large diatoms which were present at > 500,000 cells L^{-1}. Dilution experiments in 1998 indicated that the total dinoflagellate population grew at about the same rate as the diatom population but was grazed more heavily by microzooplankton. If estimated loss due to tidal flushing of the population is added to grazing rate, the dinoflagellate population growth rate was less than the combined loss rate, but growth still exceeded loss for the diatoms during spring tidal exchange periods. However, during mean or neap tidal periods, population growth rate was higher than loss rate for both dinoflagellates and diatoms. Assuming that the results from the overall dinoflagellate population are applicable to *A. catenella*, it appears that when water temperature exceeds 13 °C, permitting active *A. catenella* growth, blooms are rarely generated and then only for a limited period each summer. A combination of biological, chemical, and physical factors can lead to decline of the bloom within the bay and prevent an accumulation of PSP in the shellfish.

DEVELOPMENT OF A PROTOCOL FOR DETERMINATION OF DOMOIC ACID IN MOLE CRABS (*EMERITA ANALOGA*): A POSSIBLE NEW INDICATOR SPECIES

Christine L. Powell[1], M.E. Ferdin[2], Carolyn Bretz[2], Mark Busman[1], Rikk G. Kvitek[2], and Gregory J. Doucette[1]
[1]Marine Biotoxins Program, NOAA/NOS/CCEHBR, Charleston, SC 29412
[2]California State University, Monterey Bay (CSUMB), CA 93955

The transfer of algal toxins through marine food webs has important implications for not only public health, but also the health of ecosystems and their trophic structure. Impacts of algal toxin trophic transfer are manifested as contaminated commercial and recreational fishery resources, as well as mortality events involving a variety of birds and mammals. Protection of public health requires identification of an indicator or sentinel species that rapidly accumulates toxin upon its appearance in local waters. Monitoring the movement of an algal toxin through food webs requires identification of potential points of entry and thus vectors for toxin transfer. In the case of domoic acid (DA), mole crabs (*Emerita analoga*) have the potential to serve both as a sentinel species and as a vector for trophic transfer of this potent neurotoxin produced by members of the diatom genus, *Pseudo-nitzschia*.

Monterey Bay, California is a site of recurrent, toxic and non-toxic *Pseudo-nitzschia* blooms, as well as extensive mole crab populations. This location was therefore selected to begin evaluating the utility of *E. analoga* as an indicator for DA presence in local waters. Moreover, one of the current sentinel organisms, the sea mussel (*Mytilus californianus*), shows minimal or undetectable toxicity during some bloom events in Monterey Bay. It is also anticipated that data collected during this project will provide information on the efficacy of mole crabs as a vector for DA transfer to higher trophic levels.

The efficiency of extracting algal toxins from diverse sample types is highly variable and depends on the individual sample matrix. Using a conventional aqueous methanol extraction method for shellfish as a starting point, we have developed and validated a highly efficient protocol for extracting DA from whole mole crabs that yields toxin recoveries of 96.5 $\pm$ 2.9 percent. We also confirmed by LC-MS/MS that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* bloom events (0.5-10 micrograms DA/g tissue), while the blue mussel showed no detectable toxin. In addition, an extensive comparison (n = 60) of inter-animal variability in DA content revealed values ranging over an order of magnitude (*ca*. 0.5 to 5 micrograms DA/g tissue) and no consistent trend with size class, based on either animal weight or length. These data on the toxicity of individual animals will be useful in designing an appropriate sampling strategy and, importantly, indicate that mole crabs do not appear to progressively bioaccumulate DA with age. In fact, initial observations by Ferdin et al. (this conference) revealed a rise and fall of DA toxicity in mole crabs, coinciding with similar changes in *Pseudo-nitzschia* cell concentrations. The extraction protocol developed herein will be implemented during upcoming field trials to further compare the DA toxicity of mole crabs vs. blue mussels and its correlation to the concentrations of toxic *Pseudo-nitzschia* cells in local waters. Mole crab toxicity values will also be available for reference, in the event of suspected DA-associated mortality events involving consumers of *E. analoga*.

FISH BIOASSAYS AND PILOT-SCALE APPLICATION OF CLAY TO CONTROL BLOOMS OF *HETEROSIGMA AKASHIWO* AT PACIFIC NORTHWEST SALMON NET PENS

J.E. Jack Rensel[1], Donald M. Anderson[2], Laurie B. Connell[3]
[1]Rensel Associates Aquatic Science Consultants, Arlington WA 98223
[2]Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[3]National Marine Fisheries Service, Seattle, WA 98112

Fish killing microalgae are a major impediment to the increasing fish mariculture activities in many locations around the world. Fish farmers use a variety of physical methods to mitigate effects of harmful algal blooms (HABs) including towing of pens, perimeter skirts, upwelling systems and other means. But these are not without risks, such as ripping of pens when towing and inefficient displacement of blooms by upwelling due to unpredictable vertical distribution of algae. Certain types of clay flocculation appears to be a promising means of treating some fish killing HABs in or around fish farms, based on laboratory tests and reports from large-scale applications in Korea and Japan. Recurring wide-scale blooms of *Heterosigma akashiwo* have caused major fish losses in the Pacific Northwest Coast in Washington State, but more so in British Columbia, where the industry is much larger and broadly distributed. Since *Heterosigma* does not produce a persistent toxin, but apparently kills fish through the action of reactive oxygen species that quickly dissipate in seawater, the alga is a good candidate for the use of clay mitigation. (Note there is a possibility that *Heterosigma* may produce a weak brevetoxin-like compound, based on one documented case). This work reports initial laboratory results of clay bioassays with Atlantic salmon (*Salmo salar*) and plans to test the mitigation method at a commercial fish farm using perimeter skirts to retain the clay in a small area and reduce reintroduction of the alga.

Replicated laboratory bioassays were conducted with clay concentrations ranging from 30 to 100 mg/L clay, held in suspension by air lift pumps for five hours, much longer than would occur in the field. Turbidity and total suspended solids were used as indices of clay loading, and were positively correlated to a high degree. Turbidity is the preferred measurement for practical and water quality standard reasons. Fish began "coughing" immediately upon introduction of the clay in the aquaria, a natural reaction to many types of irritants, and cough frequency occurred in a dose-related fashion up to 10 times per minute. The nature of the coughing was different from that previously seen with fish exposed to harmful *Chaetoceros concavicornis*; it was shallower and less pronounced. Coughing ceased immediately upon transfer of the fish to clean seawater at the conclusion of the bioassay. Gill tissue samples of other treated fish were collected for histological analysis. There was some mucus present on the affected gills, but not an usually large amount. These results suggest that there will not be any significant or permanent effects of clay on treated fish, as high levels of turbidity occur naturally during the freshwater phase of salmonid life. Prior studies have shown that juveniles of several salmon species tolerate much higher concentrations of silts or clays than were used in our experiments, and for much longer periods.

Our null hypothesis for the sediment chemistry and infauna fieldwork is that there will be no measurable impact on the seabottom or benthos of clay application. Pacific Northwest fish farms are required to locate where there are no other significant aquatic resources, including shellfish beds. Moreover, there is little permanent deposition of the wastes from these farms, as they are without exception located in current swept areas with erosional bottoms. Although *Heterosigma* occurs frequently each spring and fall at many farm sites, concentrations are usually less than that which will cause fish mortality in about 9 out 10 years at a given farm site. A typical farm producing several million pounds of fish annually will therefore only require an annual clay application (pro rata over 10 years) of about 40 kg per farm [(0.4 ha per farm x 0.1 kg m^2)/10]. There are only 9 large, separate commercial pens in the 0.8 million hectares of Puget Sound, so cumulatively the required clay amount

is trivial. The volume of treatment clay needed pales in comparison to the estimated 6 million metric tons of sediment including clay entering Puget Sound each year from riverine and shoreline erosion (Dexter et al. 1981 NOAA Tech. Memo. OMPA-13).

ESTABLISHING AND MAINTAINING CLONAL CULTURES OF *GYMNODINIUM BREVE*

Bill Richardson
Florida Fish & Wildlife Conservation Commission, Florida Marine Research Institute
100 Eighth Ave SE, St. Petersburg, FL 33701

The toxic dinoflagellate *Gymnodinium breve* has been grown and studied in the laboratory for almost five decades. Most of the early physiological studies were performed using the Wilson clone (isolated by W.B. Wilson from John Pass, Florida in 1953, CCMP718). Renewed interest in the physiology and genetics of *G. breve* has emphasized the need for the establishment and long term maintenance of clonal cultures from a broad geographic range. Until recently, the former successes in establishing cultures for study had not been widely reproduced. One of the primary reasons is that *G. breve* cells are fragile and are easily stressed. The method used in our laboratory is presented as a simple combination of established isolation and culture methodologies. The techniques have been used to establish and maintain 20 different clonal isolates of *G. breve* from the Gulf of Mexico and Atlantic waters for as long as 4 years.

Growth medium is prepared using oligotrophic seawater collected by lowering a submersible pump (all plastic components) over the side of a research vessel. The water is stored, prior to use, in the dark at 5°C in 20 liter polycarbonate carboys. The seawater is 0.45 µm filtered and then sterilized by autoclaving in 1.5 liter teflon bottles. Established cultures are maintained in growth medium prepared by adding sterile filtered nutrients as in "L1" (Guillard and Hargraves, 1993) except for the vitamin additions, which are added as in NH-15 (Gates and Wilson, 1960).

Samples for isolation are usually received as whole water samples shipped to the laboratory in plastic bottles or bags. Individual cells are drawn up into sterile micro-capillary borosilicate glass pipets (~300 µm diameter). Individual cells are then repeatedly washed by transferring them by micro-pipet to drops of sterile filtered oligotrophic seawater on a glass slide spot plate. All mouth pipeting is done on an inverted microscope (40X) under a laminar flow hood. After several washes, individual cells are placed in polystyrene tissue culture well plates containing 1 mL of growth media. The growth media used to establish the single cells in culture uses the medium described above at $1/10^{th}$ concentration in sterile filtered oligotrophic water. This media may be diluted 50% with GF/F filtered water from an established culture in an effort to decrease the shock that newly isolated single cells might experience when placed in water not "conditioned" by *G. breve* cells and their associated bacteria. The isolate cultures are allowed to reach densities of at least 25 cells/mL before additional media is added to the well. Additional media is added 2-3 times per week in small amounts, not exceeding 10% of the existing culture volume. This minimizes the stress to the growing cells while still meeting their needs. The isolates are transferred to successively larger flasks until they are finally moved to 13 liter Pyrex glass carboys. The isolates are maintained in these carboys in volumes of 3-8 liters at S=34, 23°C, and under a 12:12 L:D cycle at 60 $\mu Em^{-2}sec^{-1}$. Transfers of established cultures in carboys are made every 3-4 weeks using cultures with densities of approximately 10^7 cells/L to establish new cultures with densities of approximately 10^5 cells/L. Isolate cultures are maintained at growth rates between 0.2 and 0.3 divisions da^{-1}. Both "grandparent" and "parent" cultures are kept as a safeguard against unexpected culture mortality. The combination of basic culture methods described above, could be easily replicated and improved upon, resulting in the establishment and maintenance of additional *G. breve* or other *Gymnodinium* clonal isolates for use in taxonomic, physiological, and genetic investigations.

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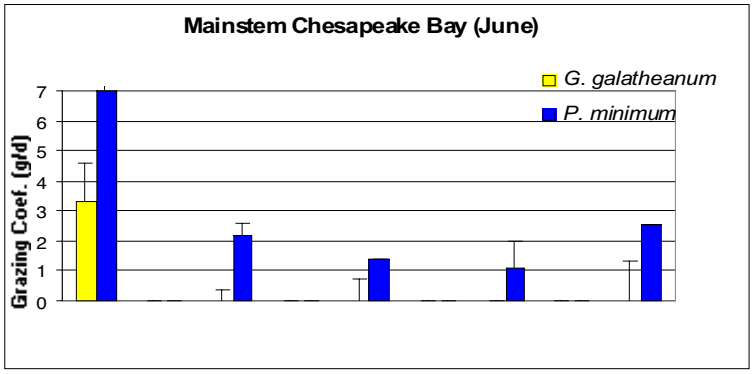
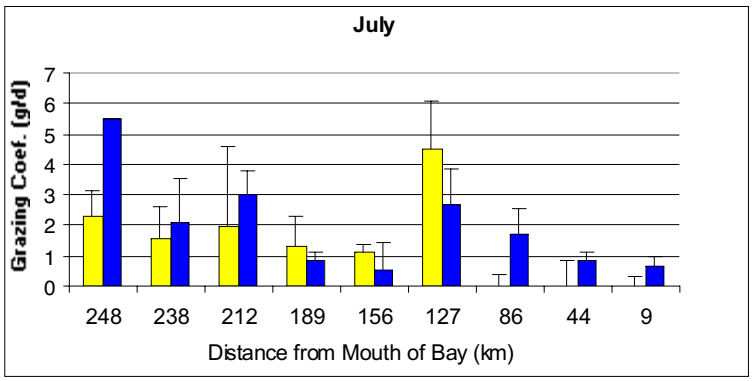
GRAZING ON *GYRODINIUM GALATHEANUM* AND *PROROCENTRUM MINIMUM* BY MICROZOOPLANKTON IN CHESAPEAKE BAY

Michelle S. Rome, Diane K. Stoecker, Matthew Johnson
Horn Point Laboratory, University of Maryland Center of Environmental Science, P.0. Box 775, Cambridge, Maryland 21613

G. galatheanum and *P. minimum* are two small, bloom-forming dinoflagellates that have been associated with red tides in Chesapeake Bay. Algal blooms are the consequence of net growth, from increased division rates, or decreased mortality rates. In the past, most attention and research has been directed towards variables that increase growth rates, however, little is understood about the impact of predation, or zooplankton grazing, in terms of development and termination of toxic blooms. This study examines the distribution of potential grazing pressure by microzooplankton on *G. galatheanum* and *P. minimum* in the Chesapeake Bay and its tributaries. Grazing experiments were conducted in June and July 2000 on separate cruises. Dinoflagellate cultures were stained with CMFDA, a vital green fluorescent stain, and added to treatments with (<200µm) and without (<1.2µm) the natural microzooplankton assemblages collected at each station. In >50% of the experiments, the grazing coefficient was higher than the maximum potential growth rate of *G. galatheanum* and *P. minimum* in culture, 0.94 d^{-1} (Li et al., 1999) and 1.15 d^{-1} (Grzebyk & Berland, 1996), respectively. Thus, grazing by microzooplankton has the potential to prevent net growth of these dinoflagellates. In June, there was a significant correlation between ciliate abundance (>20µm) and potential grazing on *P. minimum*. Grazing pressure appeared to be higher in June than in July, but the difference was not statistically significant. In conclusion, microzooplankton have a high potential to control dinoflagellate growth, although the grazing pressure was variable throughout Chesapeake Bay and its tributaries.





INHIBITION OF *ALEXANDRIUM FUNDYENSE* STR. CB301-A BY MARINE BACTERIA

Juliette N. Rooney-Varga, Mary Savin, Michael Ferrier, Andrew B. Golay
Biological Sciences Department, University of Massachusetts Lowell, 1 University Avenue, Lowell, MA 01854

Bacterial influences on toxic algal blooms have been studied extensively in several regions of the world but remain poorly understood in the Gulf of Maine, where *Alexandrium* spp. blooms have become a chronic problem. In the current study, we describe a bacterial assemblage and two bacterial isolates that either inhibited or killed *Alexandrium fundyense*. A bacterial assemblage from a non-axenic, late-exponential phase *A. fundyense* culture was inoculated into axenic *A. fundyense* str. CB301-A, which has been maintained as an axenic culture for about one year. This bacterial assemblage was found to cause the decline of *A. fundyense* CB301-A cells within 6 days of inoculation. Three members of this assemblage were isolated on agar plates containing f/2 medium amended with 0.5% peptone, 0.5% glucose, and 0.5% yeast extract. Of the three bacteria isolated, two strains (B1 and B2) inhibited CB301-A growth, while the third (B3) had no effect. 16S rRNA phylogenetic analysis revealed that isolate B1 was most closely related to strain S34, an uncharacterized member of the alpha-*Proteobacteria* that was isolated from the Sargasso Sea. Strain B2 was extremely closely related to *Marinobacter* sp. PCOB-2, a member of the family *Alteromonodaceae* isolated from a marine algal culture. We are currently continuing our characterization of these bacterial isolates and plan to determine their effects on other *Alexandrium* strains as well as non-toxic marine algae found in the Gulf of Maine.

DEVELOPMENT AND VALIDATION OF A PCR-BASED DETECTION ASSAY FOR *PFIESTERIA PISCICIDA* IN WATER AND SEDIMENT ENVIRONMENTAL SAMPLES

Keiko Saito, José- Antonio F. Robledo, and Gerardo R. Vasta
Center of Marine Biotechnology, Maryland Biotechnology Institute, 701 E. Pratt St. Baltimore, MD 21202

Pfiesteria piscicida is a heterotrophic dinoflagellate that has been associated with massive fish mortalities, and, in some cases, associated with health problems in watermen working along the lower Eastern Shore of the Chesapeake Bay. Similar episodes, although of lesser scale, have occurred in Chesapeake Bay tributaries. Plate tabulation has been the routine method for the identification of dinoflagellate species. Groups of species are characterized by a particular formula, and differences in shape or size of some of the plates can define distinct species within the group. This technique is time-consuming and requires special training. We have developed a rapid, sensitive, specific, and easy to implement in any laboratory PCR-based assay for the diagnostics of *P. piscicida*. Based on genetic information, the PCR assay uses primers from *P. piscicida* NTS and SSU regions yielding an amplicon of 429 bp. We have been routinely applying this assay in the core facility to identify *P. piscicida* in environmental water and sediment samples. Although the PCR assay is a very sensitive technique for the detection of specific sequences in complex DNA mixtures, the amplification reaction can be easily inhibited by compounds present in the sample. Indeed it is fundamental to develop controls that guarantee the detection of *P. piscicida* in a sample as a consequence of the absence and not the result of PCR inhibition. Here we present data on the effect of inhibitors from water and sediment samples in the PCR assay and we developed an internal standard that uses the same priming sites that the PCR-based assay for *P. piscicida*. DNA from environmental samples is spiked with the internal control and an aliquot of the mixture used PCR. If the internal control is detected, samples are subsequently tested for *P. piscicida*. When the internal control is not detected by PCR, serial dilution of the sample are prepared and used to establish the dilution of the sample at which there is no inhibition of the PCR. This sample dilution is then used for detection of *P. piscicida*. The use of internal standards would guarantee the quality of the sample and avoid false negatives in environmental samples and, based on data, we suggest its use for routine diagnostics of *P. piscicida*.

PFIESTERIA PISCICIDA IN SEDIMENT SAMPLES FROM FISH-FARMING PONDS: *IN VITRO* EFFECT(S) OF LIVE FISH ON THE EARLY PCR-BASED DETECTION OF ZOOSPORES IN THE SUPERNATANT WATER

Keiko Saito, José-Antonio F. Robledo, and Gerardo R. Vasta
Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202

Pfiesteria piscicida is a heterotrophic dinoflagellate that has been associated with massive fish kills during recent years in the mid-Atlantic estuaries of North America. Thus, the effects of live fish, and their secretions, excretions or metabolic products on the life cycle, proliferation, and toxicity of *P. piscicida* has become a research area of great interest. In this preliminary study, we examined the possible effect(s) of live fish on the presence and proliferation of *P. piscicida* in water or culture medium that has been incubated with sediments potentially containing putative life stages of this dinoflagellate species. Sediments (10 ml) from 10 active or drained ponds from a fish farming facility were sieved through a 60 _m mesh, and cultured in 750 ml flasks with 500 ml of artificial seawater (7 ppt; pH 8) containing one Sheepshead Minnow (*Cyprinodon variegatus*). (Controls were flasks with water containing no fish, or with culture medium (F/2) containing no fish.) Flasks were maintained at 23 ºC and 14 h light/10 h dark cycle. Enumeration of dinoflagellate zoospores in the supernatant and PCR-based specific detection of *P. piscicida* were carried out daily for 21 days, and the health of the fish was monitored throughout the experiment. Numbers of *P. piscicida*-like zoospores increased progressively, and positive PCR results for *P. piscicida* were obtained in flasks corresponding to all ten ponds. In flasks containing fish, however, *P. piscicida* could be detected as early as the second day, and flasks remained PCR-positive throughout the duration of the experiment. No fish deaths were recorded.

EFFECTS OF *ALEXANDRIUM FUNDYENSE*, ON NEWLY SETTLED JUVENILE WINTER FLOUNDER, *PLEURONECTES AMERICANUS*

Jennifer C. Samson[1], Judith S. Weis[1], and Sandra E. Shumway[2]
[1]Rutgers University, Newark, NJ 07102
[2]Southampton Campus, Long Island University, Southampton, Long Island, NY 11968

Alexandrium fundyense, a toxic dinoflagellate that produces paralytic shellfish toxins (PST; saxitoxin and its derivatives) has been implicated in fish kills during bloom conditions. *Alexandrium* is at the base of the aquatic food web, and is readily consumed by a variety of organisms, which can consequently accumulate the toxin. *Alexandrium* occurs temporally and spatially in estuaries with many species of larval and juvenile fish during the summer and fall. Larval winter flounder, *Pleuronectes americanus*, settle in their natal estuaries and shallow embayments during spring, where the newly settled juveniles remain for up to two years. Fidelity to specific spawning areas makes this commercially important species particularly sensitive to annual blooms of *Alexandrium*. We investigated the lethal and sublethal (behavioral) effects of trophic transfer of PST on newly settled juvenile winter flounder, using *Alexandrium*→copepod, *Coullana canadensis*→flounder, food chain.

The harpacticoid copepod, *C. canadensis*, was exposed to a toxic strain of *A. fundyense* for 4 consecutive days, as a mixture of 50:50 by volume, green algae and *A. fundyense* (500 cells/ml). Control copepods were fed a non-toxic strain of *A. tamerense*. Copepods actively consumed the dinoflagellates. Following exposure, toxic copepods were fed to newly settled fish (8-10mm TL).

In mortality experiments fish were fed 1 toxic copepod every 15 minutes. Feeding was halted when fish began swimming erratically in somersaults, eventually ending upside down on bottom, where they remained until the heart stopped beating. Erratic swimming occurred immediately after consuming the final copepod (6 ± 2.5 copepods per fish). Fish fed 6 control (non-toxic) copepods never exhibited this behavior.

Observation during preliminary experiments indicated that fish fed only 5 toxic copepods (sublethal dose) could be fed an additional 5 toxic copepods 24 hours later with no mortality. To elucidate further this apparent recovery, a time to death experiment was run. Fish were individually fed 5 toxic copepods, and then fed a second set of 5 toxic copepods, either 1, 2, 4 or 8 hours after completion of the first feeding. Survival analysis indicated a significant difference in the mean survival rate of each group (chi-square=10.964, df=3, n=33, $p \leq 0.01$) with high mortality in fish given only 1 and 2 hours between feedings and little or no mortality in the 4 and 8 hour groups. These data indicate a recovery with time. Observations during data collection suggest a loss of toxin in the feces, as some fish revive after defecation.

Experiments were also conducted to evaluate sublethal effects on behavior. Fish were fed either 5 toxic or control, copepods. Two hours after feeding, individuals were placed in watch glasses and given 5 minutes to eat 5 unexposed copepods. Number of attempts and captures, time spent in motion, and distance traveled, were recorded. There was no difference in attempts or captures between exposed and unexposed juvenile flounder (n=20/treatment); however exposed fish were not as active as control fish, resulting in a significant difference between the two treatments for time spent in motion ($t=2.539$, $p \leq 0.01$) and distance traveled ($t=2.067$, $p \leq 0.05$). Results indicate some effects of saxitoxin on behavior but not enough to impair feeding ability. Thus, newly settled winter flounder exposed to PST via trophic transfer exhibit both lethal and sublethal (behavioral) reactions to exposure, dependent upon number of toxic copepods eaten and rate of feeding.

LABORATORY ADVANCES AND THE EPIDEMIOLOGY OF MARINE TOXIN DISEASES

Helen Schurz-Rogers[1], Lorraine C. Backer[1], and Lora E. Fleming[2]
[1]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30329 USA
[2]University of Miami School of Medicine, Miami, FL 33136 USA

Advances in marine toxin laboratory science form the scientific basis for understanding the biology and epidemiology of the human illnesses caused by these toxins. We will review how both the advances in laboratory science and the lack of such advances have affected our understanding of the epidemiology of human illnesses caused by exposure to two dinoflagellate organisms, *Gymnodinium breve* and *Pfiesteria piscicida.*

Gymnodinium breve is a marine dinoflagellate that produces polyether toxins, called brevetoxins. Fish kills associated with blooms of *G. Breve*, called red tides, have been reported since 1844. Food poisonings (neurotoxic shellfish poisoning [NSP]) from consuming shellfish from Florida have been reported since the early 1900s, but the causative organism was not identified until the 1960s (after the discovery of *G. breve* in 1947). Work conducted in the 1980s identified and characterized the brevetoxins, allowing researchers to determine their biochemical activity (induction of channel-medicated Na^+ ion influx), pharmacology, biological activity (depolarization of muscle fiber membranes), and physiological effect (neurotoxicity). Brevetoxins can be detected and quantified in shellfish as well as in water and air samples and human exposure to brevetoxins occurs either from consuming contaminated shellfish or from inhaling sea spray containing fragments of the cells or the toxin. Therefore, because we have considerable information about routes of exposure, biochemical activity, and human symptoms, it is possible to conduct the appropriate epidemiologic studies to evaluate the public health importance of *G. breve*-associated diseases in human populations.

By contrast, despite the availability of molecular biology and analytical chemistry tools, the toxins associated with *Pfiesteria piscicida,* as well as their specific biochemical, pharmacological, biological, and physiologic activities, remain elusive. *Pfiesteria piscicida's* fish killing ability was only identified 10 years ago, and the first human health effects, associated with concentrated aerosol exposures in the laboratory, were reported seven years ago. In addition, these organisms are difficult to identify, appear to form fleeting blooms, and can be resistant to culturing. Thus, at this time, we cannot accurately quantify environmental exposure to the organism or to any toxin(s) it may produce. New molecular biology techniques may allow at least a rapid identification and enumeration of cells during a bloom, which could provide a relative estimate of exposure. However, without knowing the biochemical activity of the toxin(s) or an accurate assessment of exposure, it is difficult to develop a case definition for human illness. Because we have very little information about exposure and human symptoms, epidemiologic studies of human health effects from exposure to *P. piscicida* will produce limited information in evaluating the public health importance of this organism.

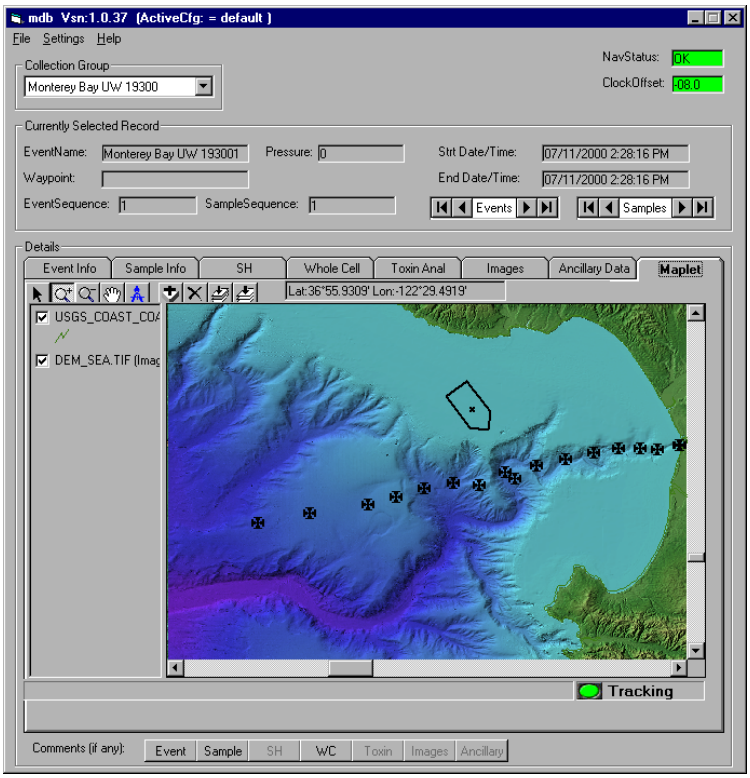
A SYSTEM FOR DATA ACQUISITION, MANAGEMENT AND ANALYSIS FOR MARINE MICROBIAL SYSTEMS RESEARCH – A TECHNICAL DEMONSTRATION

Richard Schramm, Chris Scholin, Gerry Hatcher
Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039, USA.

One of the primary goals of the microbiology research group at the Monterey Bay Aquarium Research Institute (MBARI) has been to develop and test the effectiveness of new methods for the determination of toxic and non-toxic phytoplankton species. Parallel to this effort, MBARI has developed a data management system to more efficiently acquire, process and visualize the sample results.

At the core of the system are a custom data entry and visualization application with a supporting relational database for storage and retrieval. The application is used to enter, edit and maintain all information about a sample and it's analyses while also providing a quick-look visualization capability through a 'maplet' built using MapObjects tools from Environmental Systems Research Institute (ESRI).

Sample data can be organized into either time-series (e.g. point), spatial (underway mapping) or profile (e.g. bottle hydrocast) collections. The system also provides the capability to track many types of analysis, including sandwich hybridization and whole-cell analysis, images, ancillary data retrieved from other databases (e.g. underway temperature, salinity, and fluorometer data) and analysis 'meta-data' (e.g. standardization curves, reagent batch numbers etc.). In addition the system's design allows for simple access to the database for high-level visualization and distribution from a variety of commercial tools such as GIS (ArcView/ArcInfo), spreadsheets (Excel) or via the World Wide Web.



At sea, the application can be used to capture sampling event information (time, latitude, longitude etc.) directly into a field-copy of the database in real-time using a shipboard or hand-held GPS receiver. Detailed phytoplankton analysis results and ancillary information about the sample are then electronically added to the database as soon as they become available, typically within one hour of the initial collection.

The entire system can run stand-alone on a single 'laptop' computer at sea. The field-copy and on-shore database modifications can be reconciled as soon as reliable network connections are established, ensuring consistency across databases. On-shore, multiple copies of the application may be run against the shared primary database allowing the science team to have full simultaneous access in their lab and at their desktops.

ECOHAB, THE U.S. INTERAGENCY PROGRAM TO SUPPORT RESEARCH ON THE ECOLOGY AND OCEANOGRAPHY OF HARMFUL ALGAL BLOOMS

Kevin G. Sellner
ECOHAB Coordinator, NOAA/NOS/CSCOR, N/SCI2, 1315 E-W Highway, Silver Spring, MD 20910

The study of harmful algal blooms (HABs) in the U.S. has recently received major commitments from five U.S. Federal agencies (National Oceanic and Atmospheric Administration, Environmental Protection Agency, National Science Foundation, Office of Naval Research, National Aeronautics Space Administration) for long-term funding through the interagency ECOHAB Program (Ecology and Oceanography of Harmful Algal Blooms). Arising from the community-driven 1995 report of the same name, ECOHAB now supports >30 research projects at over $40M U.S. The purpose of the program is to support research to increase general knowledge and understanding of HAB ecology, physiology, behavior, and toxin synthesis and links to environmental conditions including trophic complexity, water quality, local and regional circulation, bathymetry, and meteorology, as well as economic assessments and outreach. Projects range from targeted studies of one to several investigators for 1-3 years up to large multi-investigator, multi-institution, and multi-disciplinary regional studies for the development of biophysical models of bloom expression and distribution. Tools developed in these research projects, such as molecular probes, toxin assays, techniques for possible use in the prevention, control, and mitigation of HABs and their impacts, and biophysical models for 'forecasting' bloom distributions, are then considered for inclusion in standard monitoring programs fostered through several agencies and non-Federal public departments. In the future, it is hoped that research results from ECOHAB-supported research can become part of the international HAB research, monitoring, and event response programs now being initiated in the new EUROHAB and GEOHAB programs.

DETERMINING THE CAUSES OF MORTALITY IN BIOTOXICITY ASSAYS WITH *PFIESTERIA*: THE DEVELOPMENT OF FRACTIONATION STUDIES

Jeffrey D. Shields, Wolfgang K. Vogelbein, Yasunari Kiryu, Alynda Miller, Christopher M. Squyars, Larry W. Haas, Kimberly S. Reece, Nancy A. Stokes, Vincent J. Lovko, and Howard Kator
Virginia Institute of Marine Science, Gloucester Point, Virginia 23062, USA.

Aquarium-scale biotoxicity assays with *Pfiesteria* pose inherently difficult problems. Fish health must be carefully balanced with issues such as frequency of water changes, degradation of water quality, the presence of pathogens, and the density of the background community of micro-organisms and protozoa. Add to the equation, long-term exposures of fish to a variably toxic species such as *Pfiesteria piscicida*, or the potential for contamination with related species (*Pfiesteria* sp. B), and the problems quickly appear insurmountable. We undertook long-term (80+ d) bioassays of laboratory and field isolates (Neuse and Pamlico rivers) of several *Pfiesteria*-like dinoflagellates (*P. piscicida, Pfiesteria* sp. B., "Lucy," and "Shepherds Crook"). For field isolates, fish were immediately placed in water collected for biotoxicity assays. Subsamples were processed for counts of heterotrophic dinoflagellates, DNA analysis, and SEM verification. Exposures consisted of 25-50 fish in each aquarium (38 L, 12‰ artificial seawater in de-ionized water, preconditioned Whisper filters, 20° C) inoculated with isolates from cultures, sediments or freshly collected water samples. Fish were fed three times per week and monitored for signs of morbidity, lesions, and mortality. Controls consisted of fish in replicate treatments without dinoflagellates (unexposed). Thirty long-term toxicity bioassays (17 field isolates, 3 subcultures, and 10 clonal cultures, with controls) were conducted. Two aquaria showed moderate mortalities, and four aquaria showed high mortalities in relation to the density of *Pfiesteria* sp. B. Mortality events typically lasted 2-3 weeks with 1-2 sequelae shortly thereafter. In four cultures, heavy bacterial loads (*Vibrio* spp. and *Shewanella putrefasciens*) were present, and water quality was degraded due to low dissolved oxygen (high BOD), high ammonia and nitrite, and low pH in relation to organic wastes. High bacterial loads and low pH (5.8-6.0) occurred in the other two aquaria, but other conditions were ideal to support fish health. All aquaria possessed numerous ciliates (holotrichs, and hymenotrichs), rotifers, amoebae, microflagellates (bicoecids), and other taxa. Thus, even though densities of *Pfiesteria* were high (2.0-20.0 x 10^3 cells ml^{-1}), it was difficult to rule out other causal or contributory factors in fish deaths.

Water quality studies were undertaken with an appraisal of ammonia toxicity in tilapia, *Oreochromis niloticus*, at varying pH. Tilapia were resistant to high levels of reactive ammonia (LC_{50} = 9.72 ppm), and low pH (5.0, 6.5). Thus, ammonia was not a significant factor in the observed mortalities. We then developed a "fractionation" assay where small volumes (1-2 l) of "toxic" aquarium culture were filtered and centrifuged into different components: dinoflagellates (and other protozoa), bacteria (and microflagellates), and the "soluble toxin" fractions. Controls consisted of raw culture water, and sterile artificial seawater. Fractionation assays with tilapia in 500 ml volumes were inconclusive. However, fractionation assays with mummichogs in 15 ml volumes indicated conclusively that the dinoflagellate fraction caused significant mortality compared to different controls. Bacterial and "soluble" fractions did not cause appreciable mortality. Fractionation studies with a sensitive species or sensitive life-history stage, such as larval mummichogs, provide a reliable tool for assessing fish mortality in toxic exposures with *Pfiesteria*.

In conclusion, during our biotoxicity assays, several inherent issues arose regarding culture conditions to support optimal fish health, yet provide suitable conditions for the growth and assessment of *Pfiesteria*. We developed a protocol that accounts for water quality, the possibility of contamination, and an assessment of toxicity, but from our standpoint questions remain regarding the toxigenic nature of *Pfiesteria* sp. B.

FREE AMINO ACID (FAA) POOLS IN SPECIES AND STRAINS OF *Pseudo-nitzschia* FROM MONTEREY BAY, CA: DYNAMICS OF FAA PROFILES DURING A *PSEUDO-NITZSCHIA* BLOOM

G. Jason Smith and Nicolas Ladizinsky
Moss Landing Marine Laboratories, 8272 Moss Landing Rd., Moss Landing, CA 95039

The phenomenology of domoic acid (DA) production by strains of the *Pseudo-nitzschia* species complex has received considerable attention, leading to general observations that DA accumulation is stimulated by growth limiting or stress conditions. Our efforts have been guided by the hypothesis that DA, in addition to its obvious structural similarity, also serves as a functional analogue of the amino acid proline and is derived from proline catabolism. Ongoing studies have used HPLC-UV profiling of phenylthiocarbamyl amino acid (PTC-AA) derivatives to characterize the FAA composition and FAA pool dynamics in clonal isolates of several *Pseudo-nitzschia* species from Monterey Bay, CA. DA accumulation varied by 2-orders of magnitude among independent isolates of *P. multiseries* and *P. australis*, with the isolates of the latter species exhibiting consistently higher cellular yields of DA. Proline content was lower in cells accumulating high levels of DA (>1 fmole/cell) consistent with the hypothesis that DA is derived in part from proline catabolism. All *Pseudo-nitzschia* species accumulated large pools of taurine (≥ 50% of total FAAs) when grown in Monterey Bay seawater (34 ppt). This amino acid was not detected in other diatom species when grown under equivalent conditions. As taurine content covaried with DA accumulation in *Pseudo-nitzschia*, it may provide a useful biomarker for potentially toxic bloom events. Monterey Bay is presently experiencing a large and persistent summer bloom initially dominated by *P. australis*. This event provides a novel opportunity to compare FAA compositions in natural populations and laboratory cultures to determine whether the unique FAA profiles characterizing *Pseudo-nitzschia* are evident in heterogenous samples. At a nearshore sampling station (Monterey Warf 2, 36^{o} 36' 18"N, 121^{o} 53' 23"W), *Pseudo-nitzschia* cell abundance has remained near 10^{5} cells L^{-1} in the surface waters (0-2 m) through mid-September. DA content of the particulate fraction (≥ 20 μ net fraction) varied between 0.7 and 3.5 nM with estimates of cell specific content ranging between 6 and 30 fmole $cell^{-1}$. FAA pool compositions will be determined for samples encompassing transitions from high to low and low to high DA content and compared to datasets from previous laboratory cultures as well as *Pseudo-nitzschia* isolates from the current bloom. It is anticipated that taurine content in the field samples will not only track *Pseudonitzschia* spp. abundance but will also covary with cellular DA content. Possible interactions between DA, proline and taurine metabolism will be discussed.

INTRASPECIFIC VARIATION AMONG CULTURES AND BLOOM SAMPLES OF *AUREOCOCCUS ANOPHAGEFFERENS*

Joseph Stabile[1,2], Grace Montemarano[2], Frank Fazio[1] and Isaac Wirgin[2]
[1]Iona College, Department of Biology
[2]New York University School of Medicine, Department of Environmental Medicine

During the past decade blooms of the brown tide microalga, *Aureococcus anophagefferens*, have occurred sporadically in Peconic Bay and Great South Bay of Long Island (LI), NY. Blooms vary annually in the timing of their onset, duration and intensity. We hypothesize that temporal and spatial variability in bloom characteristics is due to underlying genetic variation among populations. This hypothesis was tested by sequence analysis of the internal transcribed spacer regions (ITS1 and ITS2) of rDNA. Brown tide homologous PCR primers were developed and used to amplify ITS sequences directly from water samples and cultured isolates. PCR products were cloned into pCR2.1 vector and 15 to 25 recombinant clones per sample were sequenced. Sequence data were obtained from 1995 summer bloom samples from two sites in Peconic Bay and one locale in Great South Bay and a 1999 winter bloom sample from Great South Bay. Sequence data were also obtained for cultured isolates CCMP 1784, 1785 and 1790 from LI and CCMP 1794 from Barnegat Bay, NJ. *A. anophagefferens* is unique among eukaryotes in that it has extremely high numbers of polymorphic ITS sequences within individuals. Cloned PCR fragments were assigned composite "types" on the basis of having unique combinations of polymorphic nucleotides. A total of 46 and 43 composite types were observed among samples for ITS1 and ITS2, respectively. Monte Carlo based chi-square analysis was performed to determine if there were significant differences in the frequencies of ITS types within and among bloom samples and cultured isolates. Statistically significant differences in the frequency of ITS types were observed between cultured isolates CCMP 1785 (LI) and CCMP 1794 (NJ) in comparison to all other isolates and LI bloom samples. This indicates that not all cultured isolates are representative of *A. anophagefferens* blooms and that there is geographic differentiation between some coastal sites. In addition, restriction fragment length polymorphism analysis of chloroplast DNA confirmed that there are genetic differences among cultured isolates. Interestingly, there were no significant differences in the frequency of ITS types between the 1995 summer and 1999 winter bloom samples from LI. This suggests variability at the peak of a bloom is low or that the resolution of the PCR-cloning technique is insufficient to distinguish closely related populations. Currently, Single Stranded Conformation Polymorphism analysis is being used to re-evaluate the data obtained from the rRNA analyses.

OBSERVATIONS OF SEA SURFACE TEMPERATURE AND WINDS ASSOCIATED WITH FLORIDA, USA, RED TIDES (*GYMNODINIUM BREVE* BLOOMS)

Richard P. Stumpf[1], Varis Ransibrahmanakul[1], Karen A. Steidinger[2] and Patricia A. Tester[3]
[1] National Ocean Service, NOAA 1315 East-West Highway, Silver Spring MD 20910
[2] Florida Marine Research Institute, FL DEP, 100 8th Ave. SE, St. Petersburg, FL 33701
[3] National Ocean Service, NOAA, 101 Pivers Island Rd. Beaufort, NC 28516

Blooms of *Gymnodinium breve* on the west coast of Florida USA are commonly initiated during the summer but rarely in the winter, with greatest frequency of occurrence at the coast in the fall. The seasonal changes in bloom occurrence correlates to seasonal variations in the wind and sea surface temperature. Blooms typically initiate offshore in the summer, when the winds are weakest. However, they are maintained at the coast during the fall, a period of strong winds blowing offshore. During January and February, satellite imagery shows cool water spreading from the shore towards offshore, raising the possibility that conditions may be less favorable for bloom initiation and maintenance. Also, the winds weaken during the same time. While providing these climatological relationships, our data set cannot resolve the circumstances around particular bloom events, including major winter blooms, probably because of lack of data on the offshore initiation and presence of the bloom.

PSP TOXIN RETENTION BY ZOOPLANKTON FEEDING ON *ALEXANDRIUM FUNDYENSE* – VECTOR OR SINK?

Gregory J. Teegarden[1], Allan D. Cembella[2], and Edward G. Durbin[3]
[1]Bowdoin College, 6500 College Station, Brunswick, Maine 04011
[2]Institute for Marine Biosciences, NRC, 1411 Oxford St., Halifax, Nova Scotia, Canada
[3]Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island

Zooplankton can consume toxic *Alexandrium* spp. dinoflagellates in the Gulf of Maine and retain PSP toxins, potentially acting as vectors of toxin. Experiments were designed to determine toxin budgets for common species of coastal copepods feeding on toxic *Alexandrium fundyense*, offered as monocultures or in mixtures of algal prey, by comparing calculated toxin ingestion rates and toxin content of copepod body tissue and fecal pellets. Both copepod tissue and fecal pellet fractions accounted for $\leq$5% each of the calculated ingested toxin, and thus $\geq$90% was lost as dissolved fraction toxin. The presence of alternate food did not significantly alter the efficiency of toxin retention. Experiments using varying concentrations of *A. fundyense* and alternate non-toxic species show no effect of cell concentration on toxin retention efficiency. Sloppy feeding is the probable mechanism for release of dissolved toxin.

Selective feeding was evident at high, but not low, concentrations of *A. fundyense*, suggesting that PSP toxins deter grazers more effectively when higher *Alexandrium* spp. cell concentrations are achieved. Total toxin retained and efficiency of retention varied among copepod species. Toxin profiles (% molar composition) of dinoflagellates and copepod tissues differed, indicating some metabolic transformation, while fecal pellets had toxin profiles that were intermediate in composition between copepods and dinoflagellates. Because of their low toxin retention efficiency, copepod grazers are probably a net sink for PSP toxins produced by *Alexandrium* spp., especially when cell concentrations are low ($<10^4$ cells L^{-1}) and relatively high zooplankton clearance rates are attained. Nevertheless, zooplankton can attain toxin body burdens sufficient to contribute to propagation of PSP toxins to other trophic levels.

THE EFFECT OF BARLEY STRAW EXTRACT ON THE GROWTH OF ESTUARINE DINOFLAGELLATES

Daniel E. Terlizzi[1], M. Drew Ferrier[2], Erin Armbrester[2], and Kara Anlauf[2]
[1]Maryland Sea Grant Extension Program, Baltimore, MD 21202
[2]Hood College, Frederick, MD 21701

Harmful algal blooms occur frequently in nutrient-enriched estuarine systems. They can be particularly problematic in impoundments used for the culture of fish or shellfish. There is a growing body of evidence that decomposing barley straw can exert an algistatic effect on certain nuisance freshwater algae; however, this method of control has not been explored for estuarine dinoflagellates. We assayed extract from decomposing barley straw for its ability to affect the growth rates of unialgal batch cultures in a number of dinoflagellate genera (including *Gymnodinium, Gyrodinium, Prorocentrum, Heterocapsa*, and *Peridinium*). After three weeks, growth of *Heterocapsa pygmaea* and *H. triquetra* was significantly inhibited ($p < 0.01$) in the presence of the extract (Fig. 1). Growth of *Prorocentrum minimum* was significantly enhanced (Fig. 2). The growth of other species was not significantly affected. Thus, pending further assays at larger scales, we conclude that barley straw shows promise as a component of integrated algal control for some dinoflagellate blooms.

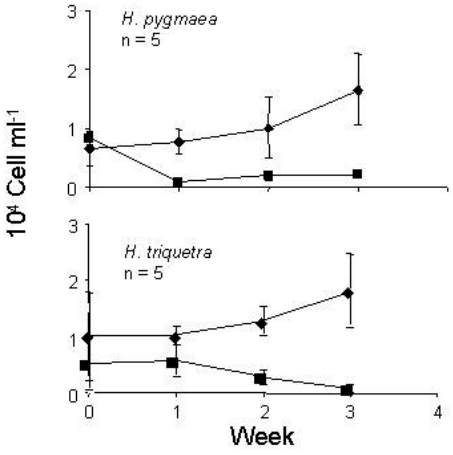


Figure 1. Mean growth response of *Heterocapsa* sp. to straw extract. Squares depict treatment with extract; diamonds are controls. Error bars represent +/- 1 standard deviation.

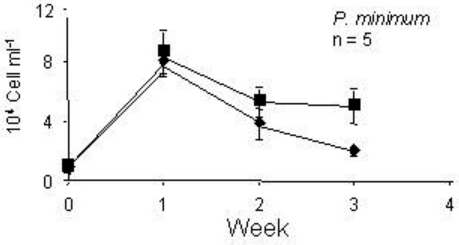


Figure 2. Growth response of *Prorocentrum minimum* to straw extract. Squares depict treatment with extract; diamonds are controls. Error bars represent +/- 1 standard deviation.

ANNUAL EXCYSTMENT OF *ALEXANDRIUM* HYPNOZYGOTES FROM EASTERN GULF OF MAINE POPULATIONS

Brian Thompson, Patricia Matrai, and Maureen Keller*
Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME 04575, USA

A germination experiment was begun last year in order to explore the possibility that an annual endogenous clock may help determine excystment of the *Alexandrium* hypnozygote from eastern Gulf of Maine populations, as has been shown for populations from Casco Bay, in the western side of the Gulf. Sediment samples were collected from three stations in the eastern Gulf of Maine (off Monhegan and Matinicus Islands and in the Bay of Fundy); subsamples have been kept refrigerated, under nitrogen, in the dark. Subsamples are incubated in f/2 growth medium biweekly, under constant temperature (15°C) and light conditions, and excystment determined over a 6-week period. Preliminary results show that germination started in February, continued through May-June, and decreased to a minimum by the end of July in all 3 stations, except for off Matinicus Island where it ended in mid-September. This information is significant in bloom prediction models.

*This work was started under the leadership of the late Dr. Maureen D. Keller.

BACTERIA AND THE SEXUAL REPRODUCTION OF THE DIATOM *PSEUDO-NITZSCHIA MULTISERIES* (HASLE) HASLE

Susan Thompson[1], Stephen S. Bates[2], Irena Kaczmarska[1] and Claude Léger[2]
[1]Department of Biology, Mount Allison University, Sackville, NB E4L 1G7, Canada
[2]Fisheries & Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB E1C 9B6, Canada

It is becoming increasingly clear that bacterial-phytoplankton interactions are an intrinsic component of harmful algal bloom (HAB) ecology and physiology (Doucette et al. 1998). Bacteria have been shown to directly or indirectly take part in biotoxin production, promote or inhibit the growth of HAB species, and stimulate or inhibit phytoplankton sexual reproduction. Here, we investigated the possible role of bacteria in mediating the sexual reproduction of the domoic-acid-producing diatom *Pseudo-nitzschia multiseries* (Bates et al. 1998), by mating pairs of axenic or non-axenic clones: CLN-8 x CLN-14 and CLN-7 x CLN-11.

Clones were rendered axenic by treatment with antibiotics and were tested for the absence of bacteria using BactoPeptone broth. The control non-axenic clones retained their natural composition of bacteria. Sexually compatible clones of either axenic or non-axenic cultures were mated in six-well petri dish clusters containing sterile f/2 medium, according to Davidovich and Bates (1998). A mixture of bacteria, isolated from each pair of non-axenic clones, was reintroduced into the corresponding mixture of axenic clones. Evidence of sexual reproduction was quantified under a light microscope by determining the percentage of gametes and auxospores, relative to the number of vegetative cells.

There was no sexual reproduction (i.e., no gametes or auxospores) in mixtures of axenic clones CLN-8 and CLN-14. Moreover, the reintroduction of bacteria to axenic mixtures restored sexual activity. In the mixtures to which bacteria were added, both gametes and auxospores were observed at a maximum percentage of 24.1% and 3.5%, respectively. In contrast, sexual reproduction did occur in axenic mixtures of clones CLN-7 and CLN-11. Both gametes and auxospores were observed in the axenic mixtures of this pair and in the mixtures to which bacteria were added. In the axenic mixtures, the maximum percentage of gametes and auxospores was 32.0% and 5.0%, respectively. In the mixtures to which bacteria were added, the maximum percentage of gametes and auxospores was 39.8% and 18.5%, respectively. It is possible that viable extracellular or intracellular bacteria remained in the antibiotic-treated clones CLN-7 and CLN-11; this could account for the observed sexual activity in these "axenic" clones. Experiments are continuing to resolve the discrepancy between the results of the axenic mixtures of these two pairs of clones, in order to unambiguously determine whether or not bacteria are required for the sexual reproduction of *P. multiseries*.

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EXTRACELLULAR COMPOUNDS THAT UNBALANCE CALCIUM-MEDIATED METABOLISM AS POSSIBLE TOXINS FROM *HETEROSIGMA AKASHIWO*

Michael J. Twiner[1], S. Jeff Dixon[2], Peter Chidiac[3], and Charlie G. Trick[1]
[1]Department of Plant Sciences, The University of Western Ontario, London, ON, N6A 5B7, Canada
[2]Department of Physiology, and Division of Oral Biology, School of Dentistry, The University of Western Ontario, London, ON, N6A 5C1, Canada
[3]Department of Pharmacology and Toxicology, The University of Western Ontario, London, ON, N6A 5C1, Canada

Toxin(s) from the ichthyotoxic alga *Heterosigma akashiwo* have been responsible for millions of dollars of lost aquaculture stocks around the globe. Suggested mechanisms of toxicity include the production of reactive oxygen species (ROS), the release of large quantities of mucus from mucocysts, and/or the production of an organic toxin that may be brevetoxin-like in structure (Khan et al. 1997). However, to date, none of these postulates has been confirmed and recent evidence suggests that an alternate proposal is needed.

We have taken unique steps in order to collect and test the bioactivity of a mixture of extracellular organics from *H. akashiwo* cultures on a variety of cultured carcinoma cell lines. Chronic exposure (12 to 24 hrs) of these extracellular organics to mammalian cell lines (UMR and HEK) have been shown to cause extraordinary effects on these cell lines, specifically by elevating cellular metabolism up to 10 fold basal. This was determined by monitoring mitochondrial succinate dehydrogenase activity via a tetrazolium-dye assay. With continued exposure (>36 hrs), cell death became evident which was preceded by detachment of cells from an original, confluent monolayer.

Acute exposure of *H. akashiwo* extracellular organics to the model insect cell line SF9, elevates intracellular calcium quickly and transiently by over 100 nM, followed by maintenance of elevated intracellular calcium concentrations for prolonged periods of time. However, this response can only be significantly observed by SF9 cultures that have been transfected with membrane-bound receptors that couple to G-protein specific pathways. Control cells and cells transfected with the vector alone (baculovirus) only elicit a minimal response suggesting pathway-specific effects of the bioactive compounds collected from *H. akashiwo* cultures.

Cell-assay-mediated determination of bioactive compounds from cultures of *H. akashiwo* have thus far shown that extracellular compounds from this alga can elicit biological effects towards multiple, cultured cell lines and may serve to isolate and identify a potential ichthyotoxic compound from this alga.

A FIELD TRIAL MONITORING *HETEROSIGMA AKASHIWO* IN PUGET SOUND, WASHINGTON USING THE SANDWICH HYBRIDIZATION ASSAY

John V. Tyrrell[1], Laurie Connell[2] and Chris A. Scholin[1]
[1]Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039
[2]NOAA, Environmental Conservation Division, Seattle, WA 98112

Sandwich hybridization technology has been applied to detecting *Heterosigma akashiwo*, *Fibrocapsa japonica* and *Chattonella spp*. (Tyrrell et al. in review, Scholin et al. 1999). Preliminary field testing of the *H. akashiwo* and *F. japonica* sandwich hybridization assays (SHA) in New Zealand has shown they detect target species at levels below that of concern (Rhodes et al. in review). Field testing of the SHA for *H. akashiwo* is also currently underway in Puget Sound, Washington. The objectives of that study are: 1) to determine the specificity and sensitivity of the SHA in the context of field samples; 2) to establish methodology for archiving field samples for future examination, and; 3) to determine the potential of the assay as a monitoring tool. In addition, the utility of the SHA is being examined for the detection *H. akashiwo* in clay mitigation experiments/trials.

All field samples from Puget Sound shown to contain *H. akashiwo* by light microscopy were also detected by the SHA. To date, no false positives have been returned using the SHA against a wide panel of organisms, including detritus and flocculent material. Preliminary data suggests that field samples can be preserved with acid Lugol's, or stored as frozen filters or frozen lysates for up to 30 days with no loss of signal. Also it appears that samples preserved with acid Lugol's may enhance the signal of the assay. This enhancement is currently under study. The results from preliminary trials in the laboratory show no interference to the SHA from clay at the maximum proposed application rates for clay mitigation. Results to date are extremely encouraging and together with the results from earlier trials in New Zealand indicate that the assay has potential for monitoring purposes. *H. akashiwo* is detected at levels well below the level of concern, thus allowing ample time to map the geographic boundaries of a bloom and whether it is growing or in decline. The SHA may be improved for monitoring purposes by integrating the plate reader with the robotic processor, making it more simplistic and easier to use. The SHA microtiter format can be transferred onto a solid support (filter membrane) and used on a remote *in situ* processor, which ultimately may be mounted onto a buoy, drifting arrays, ROVs, on-board ship, etc (Scholin et al. 1998). The SHA could also be applied to a dipstick format, which in comparison to the microtiter format would be cheaper, easier to use, but less quantitative.

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TROPHIC EFFECTS OF ESTUARINE BLOOM SPECIES UPON BENTHIC AND PLANKTONIC GRAZERS

G.H. Wikfors[1], H.G. Dam[2], G.B. McManus[2], S.E. Shumway[3], and R.M. Smolowitz[4]
[1]NOAA Fisheries, NEFSC, Milford, CT 06460
[2]Department of Marine Sciences, University of Connecticut, Groton, CT 06340
[3]Southampton College of Long Island University, Southampton, NY 11968 and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575
[4]Marine Biological Laboratory, Woods Hole, MA 02543

Harmful algal blooms (HAB's) may result from higher growth rates, lower mortality, physical aggregation, or some combination of these factors. We have been focusing on grazing mortality as a mechanism that may be involved in both bloom initiation and demise. In laboratory experiments, we are examining the effects of several estuarine HAB species on common benthic and planktonic grazers, including larval and juvenile bay scallops (*Argopecten irradians*), the copepod *Acartia tonsa*, and a variety of ciliate microzooplankton. We are measuring feeding and growth rates and examining sublethal effects using histological methods. To date, most of our efforts have been concentrated on the dinoflagellate *Prorocentrum minimum* (strain EXUV), the raphidophyte *Heterosigma carterae* (strain OL), and the prymnesiophyte *Prymnesium parvum* (several isolates). In temperate estuaries of the US Atlantic coast, these species frequently bloom in summer, and blooms appear to originate in bays and subestuaries, subsequently proliferating into open estuarine waters. Our preliminary results are consistent with the idea that inability of benthic grazers to control these species in shallow nearshore waters results in high populations that are exported to deeper waters of the estuary, where planktonic grazers gradually reduce their numbers. *P. minimum*, for example, is readily eaten by a variety of ciliates, as well as the copepod, but rejected in pseudofeces by the scallop. In addition, this HAB species causes pathological changes in the digestive systems of juvenile scallops when it is ingested. Some isolates of *Prymnesium parvum* are highly toxic to some stages of the bay scallop, affecting multiple organ systems, as well as to several ciliates tested (see McManus abstract). The project, which received funding from both ECOHAB and Connecticut Sea Grant, will result in a compendium of effects of several HAB species upon potential grazers and working hypotheses concerning the role of grazing suppression in population dynamics of the HAB species.

AUTHOR INDEX

LIST OF PARTICIPANTS

Nicolaus Adams
NOAA-NMFS
2725 Montlake Blvd. E.
Seattle, WA 98112
Tel: (206) 860-6787
Fax: (206) 860-3335
Nicolaus.Adams@noaa.gov

Mohammad Alavi
Center of Marine Biotechnology
University of Maryland Biotechnology Institute
University of Maryland
701 East Pratt Street
Baltimore, MD 21202
Tel: (410) 234-8877
alavi@umbi.umd.edu

Robert Andersen
Bigelow Laboratory for Ocean Sciences
180 McKown Point Road
W. Boothbay Harbor, ME 04575
Tel: (207) 633-9632
Fax: (207) 633-9715
randersen@bigelow.org

Donald M. Anderson
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2351
Fax: (508) 457-2027
danderson@whoi.edu

Marie-Claude Archambault
Oceanography Department
1355 Oxford Street
Life Science Center
Dalhousie University
Halifax, Nova Scotia B3H 4J1
Tel: (902) 494-3675
Fax: (902) 494-3877
marchamb@is2.dal.ca

Tamieka Armstrong
Bigelow Labs, c/o P. Matrai
180 McKown Pt.
W. Boothbay Harbor, ME 04575
Tel: (207) 633-9600
Fax: (207) 633-9641
tamieka.armstrong@webmail.une.edu

Carol Auer
NOAA Coastal Ocean Office
1315 East-West Highway
SSMC3, Room 9700
Silver Spring, MD 20910
Tel: (301) 713-3338 x123
Fax: (301) 713-4404
Carol.Auer@noaa.gov

Daniel Baden
University of North Carolina at Wilmington
One Marvin K. Moss Lane
Wilmington, NC 28409
Tel: (910) 962-2300
Fax: (910) 962-2405
badend@uncwil.edu

Susan Banahan
NOAA Coastal Ocean Program, N/SCI2
1315 East-West Highway, Room 9700
Silver Spring, MD 20910-3282
Tel: (301) 713-3338 x115
Fax: (301) 713-4404
Susan.Banahan@noaa.gov

Christina Band Schmidt
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-3608
Fax: (508) 457-2027
cschmidt@whoi.edu

Michele Barbier
NOAA – National Ocean Service
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8649
Fax: (843) 762-8700
Michele.Barbier@noaa.gov

Sibel Bargu
Institute of Marine Sciences
University of California, Santa Cruz
Santa Cruz, CA 95064
Tel: (831) 459-2948
Fax: (831) 459-4882
sbargu@cats.ucsc.edu

Stephen S. Bates
Fisheries and Oceans Canada
Gulf Fisheries Centre
P.O. Box 5030
Moncton, NB CANADA E1C 9B6
Tel: (506) 851-3982
Fax: (506) 851-2079
BatesS@mar.dfo-mpo.gc.ca

Laurie Bean
Department of Marine Resources
P.O. Box 8
West Boothbay Harbor, ME 04575
Tel: (207) 633-9555
Fax: (207) 633-9579
laurie.bean@state.me.us

Robert Belas
Center of Marine Biotechnology
University of Maryland
701 East Pratt Street
Baltimore, MD 21202
Tel: (410) 234-8876
Fax: (410) 234-8896
Belas@umbi.umd.edu

Stephanie Bennett
5741 Libby Hall
School of Marine Sciences
University of Maine
Orono, ME 04469
Tel: (207) 581-4348
Fax: (207) 581-4388
Stephanie.bennett@umit.maine.edu

Gry Mine Berg
Institut für Meereskunde
Kiel University
Düsternbrookerweg 20
24105 Kiel, Germany
Tel: 49 431 597 3816
Fax: 49 431 565876
gberg@ifm.uni-kiel.de

Paul Bissett
Florida Environmental Research Institute
4807 Bayshore Blvd., Suite 101
Tampa, FL 33611
Tel: (813) 837-3374 x102
Fax: (813) 902-9758
pbissett@flenvironmental.org

Bill Boicourt
University o Maryland Center for
Environmental Science
Horn Point Laboratory
P.O. Box 775
Cambridge, MD 21613
Tel: (410) 221-8426
Fax: (410) 221-8490
boicourt@hpl.umces.edu

Katie Rose Boissoneault Cellinari
Berwick Academy
6 Darby Field Common
Lee, NH 03824
Tel: (603) 659-7194
KCELLINE@berwickacademy.org

H. Suzanne Bolton
Office of Science and Technology
NMFS
1315 East-West Highway
Silver Spring, MD 20910
Tel: (301) 713-2363
Fax: (301) 713-1875
Suzanne.bolton@noaa.gov

David Borkman
Graduate School of Oceanography
University of Rhode Island
Kingston, RI 02881
Tel: (401) 874-6686
Fax: (401) 874-6682
dborkman@gsosun1.gso.uri.edu

Holly Bowers
Institute of Human Virology
Room N557
725 W. Lombard Street
Baltimore, MD 21201
Tel: (410) 706-4654
Fax: (410) 706-1992
bowers@umbi.umd.edu

Greg Boyer
320 Jahn Lab
State University of New York
CESF
Syracuse, NY 13210
Tel: (315) 470-6825
Fax: (315) 470-6856
glboyer@esf.edu

V. Monica Bricelj
Institute for Marine Biosciences
National Research Council
1411 Oxford Street
Halifax, N.S. B3H 3Z1 CANADA
Tel: (902) 426-8005
Fax: (902) 426-9413
monica.bricelj@nrc.ca

JoAnn M. Burkholder
Center for Applied Aquatic Ecology
North Carolina State University
620 Hutton St. – Suite 104
Raleigh, NC 27606
Tel: (919) 515-2726
Fax: (919) 513-3194
joann_burkholder@ncsu.edu

Lisa Campbell
Department of Oceanography
3146 TAMU
Texas A&M University
College Station, TX 77843
Tel: (979) 845-5706
Fax: (979) 845-6331
Lcampbell@ocean.tamu.edu

Robert Campbell
Graduate School of Oceanography
University of Rhode Island
South Ferry Road
Narragansett, RI 02882-1197
Tel: (401) 874-6692
Fax: (401) 874-6853
Campbell@gso.uri.edu

David A. Caron
Dept. of Biological Sciences
3616 Trousdale Parkway AHF 301
University of Southern California
Los Angeles, CA 90089-0371
Tel: (213) 740-0203
Fax: (213) 740-6720
dcaron@usc.edu

Craig Cary
College of Marine Studies
University of Delaware
700 Pilottown Road
Lewes, DE 19958
Tel: (302)645-4078
Fax: (302) 645-4007
caryc@udel.edu

Peter J. Chapman
U.S. EPA
Gulf Ecology Division
1 Sabine Island Drive
Gulf Breeze, FL 32561
Tel: (850) 934-9261/9200
Fax: (850) 934-9201
chapman.peter@epa.gov

James Churchill
Physical Oceanography Dept., MS #21
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2807
Fax: (508) 457-2165
jchurchill@whoi.edu

Susan Coale
Institute of Marine Sciences
University of California, Santa Cruz
Santa Cruz, CA 95064
Tel: (831) 459-2948
Fax: (831) 459-4882
slcoale@cats.ucsc.edu

Christel Cothran
Irvine Nature Center
8400 Greenspring Avenue
Stevenson, MD 21120
Tel: (410) 484-2413
Fax: (410) 484-3573
hemeton@bellatlantic.net

Sean Colin
Dept. of Marine Sciences
University of CT
1084 Shennecossett Rd.
Groton, CT 06340
Tel: (860) 405-9097
Fax: (860) 449-8085
sean.colin@uconn.edu

Laurie Connell
University of Washington
School of Fisheries
NWFSC
2725 Montlake Blvd. East
Seattle, WA 98112
Tel: (206) 860-3464
Fax: (206) 860-3467
laurie.connell@noaa.gov

Kathryn Coyne
College of Marine Studies
University of Delaware
700 Pilottown Road
Lewes, DE 19958
Tel: (302) 645-4288
Fax: (302) 645-4007
kcoyne@udel.edu

John Cullen
Department of Oceanography
Dalhousie University
Halifax, NS CANADA B3H 4J1
Tel: (902) 494-6667
Fax: (902) 494-2039
John.Cullen@dal.ca

Mary Culver
NOAA Coastal Services Center
2234 South Hobson Avenue
Charleston, SC 29405-2413
Tel: (843) 740-1250
Fax: (843) 740-1290
mary.culver@noaa.gov

Jonathan Deeds
Center of Marine Biotechnology
701 East Pratt St., Suite 236
Baltimore, MD 21202
Tel: (410) 234-8830
Fax: (410) 234-8896
deeds@umbi.umd.edu

Abigail Deitz
5741 Libby Hall
University of Maine
Orono, ME 04469
Tel: (207) 581-4314
Fax: (207) 581-4388
Abigail.deitz@umit.maine.edu

Elif Demir
OEAS, Old Dominion University
4600 Elkhorn Avenue
Norfolk, VA 23529-0276
Tel: (757) 683-5976
Fax: (757) 683-5903
edemir@odu.edu

Robert Dickey
FDA Gulf Coast Seafood Laboratory
1 Iberville Street
P.O. Box 158
Dauphin Island, AL 36528
Tel: (334) 694-4480 x249
Fax: (334) 694-4477
rdickey@cfsan.fda.gov

Paul DiGiacomo
Jet Propulsion Laboratory
MS 300-323
4800 Oak Grove Drive
Pasadena, CA 91109-8099
Tel: (818) 354-0697
Fax: (818) 393-6720
pmd@pacific.jpl.nasa.gov

Megan DiPirro
5741 Libby Hall, Room 204
University of Maine
Orono, ME 04469
Tel: (207) 581-4348
Fax: (207) 581-4388
megan.dipirro@umit.maine.edu

Martina Doblin
Dept. of Ocean, Earth & Atmospheric Sciences
Old Dominion University
4600 Elkhorn Avenue
Norfolk, VA 23503
Tel: (757) 683-5980
Fax: (757) 683-5303
mdoblin@odu.edu

Patrick Dooley
121 Discovery Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5001
Tel: (631) 632-6906
Fax: (631) 632-6917
pdooley@notes.cc.sunysb.edu

Quay Dortch
Louisiana Universities Marine Consortium
8124 Highway 56
Chauvin, LA 70344
Tel: (504) 851-2821
Fax: (504) 851-2876
qdortch@LUMCON.edu

Gregory Doucette
Marine Biotoxins Program
National Ocean Service
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8528
Fax: (843) 762-8700
greg.doucette@noaa.gov

Shonna Dovel
Institute of Marine Sciences
University of California
Santa Cruz, CA 95064
Tel: (831) 459-2948
Fax: (831) 459-4882
sdovel@cats.ucsc.edu

Mary Downes-Gastrich
NJ Dept. of Environmental Protection
Division of Science, Research and Technology
P.O. 409
401 E. State St.
Trenton, NJ 08625
Tel: (609) 292-1895
Fax: (609) 292-7340
mdownesg@dep.state.nj.us

Jana Drgonova
Center of Marine Biotechnology
University of Maryland
701 E. Pratt Street
Baltimore, MD 21202
Tel: (410) 234-8827
Fax: (410) 234-8896
drgonova@umbi.umd.edu

Julianne Dyble
University of North Carolina at Chapel Hill
Institute of Marine Sciences
3431 Arendell Street
Morehead City, NC 28557
Tel: (252) 726-6841
Fax: (252) 726-2426
dyble@email.unc.edu

Sonya Dyhrman
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-3608
Fax: (508) 457-2027
sdyhrman@whoi.edu

Stacey Etheridge
Bigelow Laboratory
180 McKown Point
W. Boothbay Harbor, ME 04575
Tel: (207) 633-9669
Fax: (207) 633-9641
setheridge@bigelow.org

Terence Evens
USDA – Agricultural Research Service
SRRC 1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4492
Fax: (504) 286-4367
tjevens@nola.srrc.usda.gov

Betty June Farkas
18032 Chalet Drive #301
Germantown, MD 20874
Tel: (202) 514-5226
Fax: (202) 514-1867
VTBJ@aol.com

Timothy Feinstein
UCONN
Department of Marine Sciences
1084 Shennecossett Drive
Groton, CT 06340
Tel: (860) 405-9233
Fax: (860) 405-9153
Tnf00001@uconn.edu

Maria Ferdin
California State University, Monterey Bay
P.O. Box 658
Marina, CA 93933
Tel: (831) 238-0387
maria_ferdin@monterey.edu

Drew Ferrier
Hood College
401 Rosemont Avenue
Frederick, MD 21701
Tel: (301) 696-3660
Fax: (301) 696-3667
dferrier@hood.edu

Michael Ferrier
Dept. of Biological Sciences
University of Mass at Lowell
1 University Avenue
Lowell, MA 01854
Tel: (978) 934-2872
Fax: (978) 934-3044
unklmiky@aol.com

Jim Fitzpatrick
HydroQual, Inc.
1 Lethbridge Plaza
Mahwah, NJ 07430
Tel: (201) 529-5151
Fax: (201) 529-5728
jfitzpatrick@hydroqual.com

Vicki Foster
Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
P.O. Box 1346
Gloucester Point, VA 23062-1346
Tel: (804) 684-7732
Fax: (804) 684-7293
foster@vims.edu

Sylvia Galloway
NOAA/NOS/CCEHBR
219 Ft. Johnson Rd.
Charleston, SC 29412
Tel: (843) 762-8525
Fax: (843) 762-8700
Sylvia.Galloway@noaa.gov

David L. Garrison
National Science Foundation
Room 725
4201 Wilson Boulevard
Arlington, VA 22230
Tel: (703) 292-8582
Fax: (703) 292-9085
dgarriso@nsf.gov

Dian Gifford
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882-1197
Tel: (401) 874-6690
Fax: (401) 874-6420
dgifford@gso.uri.edu

Patrick Gillevet
George Mason University
10900 University Blvd., MSN 4E3
Manassas, VA 20110
Tel: (703) 993-1057
Fax: (703) 993-8447
gillevet@ib3.gmu.edu

José-L. Giner
Department of Chemistry
SUNY-ESF
Syracuse, NY 13210
Tel: (315) 470-6895
Fax: (315) 470-6856
Jlginer@syr.edu

Howard Glasgow
Center for Applied Aquatic Ecology
North Carolina State University
620 Hutton St. – Suite 104
Raleigh, NC 27606
Tel: (919) 515-3421
Fax: (919) 513-3194
howard_glasgow@ncsu.edu

Pat Glibert
Horn Point Laboratory
P.O. Box 775
Cambridge, MD 21613
Tel: (410) 221-8422
Fax: (410) 221-8490
glibert@hpl.umces.edu

Christopher Gobler
Natural Science Division
Southampton College
Long Island University
Southampton, NY 11968
Tel: (631) 287-8397
Fax: (631) 287-8419
cgobler@southampton.liu.edu

David Goshorn
Maryland Department of Natural Resources
580 Taylor Avenue, D-2
Annapolis, MD 21401
Tel: (410) 260-8639
Fax: (410) 260-8640
dgoshorn@dnr.state.md.us

Edna Graneli
Marine Sciences Department
Kalmar University
391 82 Kalmar, SWEDEN
Tel: +46-480-447307
Fax: +46-480-447305
edna.graneli@hik.se

Kristin Gribble
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2569
Fax: (508) 457-2027
kgribble@whoi.edu

Larry Haas
Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
P.O. Box 1346
Gloucester Point, VA 23062-1346
Tel: (804) 684-7248
Fax: (804) 684-7293
lhaas@vims.edu

Sheean Haley
University of Connecticut
1084 Shennecossett Road
Groton, CT 06340
Tel: (860) 405-9099
Sheean.haley@uconn.edu

Sherwood Hall
FDA HFS-426
260 C Street, SW
Washington, DC 20204
Tel: (202) 205-4818
Fax: (202) 205-4881
shall@cfsan.fda.gov

Clinton Hare
College of Marine Studies
University of Delaware
700 Pilottown Road
Lewes, DE 19958
Tel: (302) 645-4008
Fax: (302) 645-4007
schroff@udel.edu

Paul Hargraves
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882-1197
Tel: (401) 874-6241
Fax: (401) 874-6240
pharg@gso.uri.edu

Matthew Harris
Bigelow Labs, c/o P. Matrai
180 McKown Pt.
W. Boothbay Harbor, ME 04575
Tel: (207) 633-9600
Fax: (207) 633-9641
matthew.harris@webmail.une.edu

Kenneth Hayes
Baruch Marine Laboratory
P.O. Box 1630
Georgetown, SC 29442
Tel: (843) 546-3623
Fax: (843) 546-1632
khayes@belle.baruch.sc.edu

Cynthia Heil
University of South Florida
Department of Marine Science
140 7th Avenue, S.
St. Petersburg, FL 33701
Tel: (727) 553-1667
Fax: (727) 553-1189
cheil@seas.marine.usf.edu

John Heisler
EPA
1200 Pennsylvania Ave., NW (4504F)
Washington, DC 20460
Tel: (202) 260-8632
Fax: (202) 260-9920
John.Heisler@epa.gov

Robert Hetland
3146 Texas A&M University
Department of Oceanography
College Station, TX 77843-3146
Tel: (979) 458-0096
Fax: (979) 845-6331
rhetland@ocean.tamu.edu

Barbara Hickey
University of Washington
School of Oceanography
Box 357940
Seattle, WA
Tel: (360) 825-3911
bhickey@u.washington.edu

Porter Hoagland
Marine Policy Center, MS #41
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2867
Fax: (508) 457-2184
phoagland@whoi.edu

Rita Horner
School of Oceanography
Box 357940
University of Washington
Seattle, WA 98195-7940
Tel: (206) 543-8599
Fax: (206) 543-0275
rita@ocean.washington.edu

Edythe Humphries
DNRED-DWR Environmental Lab.
89 Kings Highway
Dover, DE 19901
Tel: (302) 739-4771
Fax: (302) 739-3491
Ehumphries@dnrec.state.de.us

John Hurst
Department of Marine Resources
P.O. Box 8
West Boothbay Harbor, ME 04575
Tel: (207) 633-9555
Fax: (207) 633-9579
john.hurst@state.me.us

David Hutchins
Assistant Professor of Oceanography
College of Marine Studies
University of Delaware
700 Pilottown Road
Lewes, DE 19958
Tel: (302) 645-4079
Fax: (302) 645-4007
dahutch@udel.edu
Heisler.John@epa.gov

Jason Hyatt
Physical Oceanography Dept.
Clark 317, MS #21
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2814
Jhyatt@whoi.edu

Joanne Jellett
Jellett Biotek Limited
P.O. Box 790
Dartmouth NS
B2Y 3Z7 CANADA
Tel: (902) 424-8670 x147
Fax: (902) 424-4679
jjellett@innovacorp.ns.ca

David Johnson
NOS, CSCOR
1315 East-West Highway
Silver Spring, MD 20910
(301) 713-3338 x134
(301) 713-4044
david.johnson@noaa.gov

Christopher Johnson
Florida State University
3500 Creighton Road, Apt. #S-1
Pensacola, FL 32504
Tel: (850) 934-2453
Fax: (850) 934-9201
johnson.christopher@epamail.epa.gov

Daniel Kamykowski
1125 Jordan Hall
Dept. of Marine, Earth & Atmos. Sci.
North Carolina State University
Raleigh, NC 27695-8208
Tel: (919) 515-7894
Fax: (919) 515-7802
dan_kamykowski@ncsu.edu

Howard Kator
Virginia Institute of Marine Science
SMS
Gloucester Point, VA 23062
Tel: (804) 684-7341
Fax: (804) 684-7186
kator@vims.edu

Bruce A. Keafer
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2509
Fax: (508) 457-2027
bkeafer@whoi.edu

Ruth Kelty
National Center for Coastal Ocean Science
NOS/NOAA
1305 East-West Hwy, SSMC4, Room 9224
Silver Spring, MD 29010-3281
(301) 713-3020 x124
(301) 713-4353
ruth.kelty@noaa.gov

Steve Kibler
National Ocean Service, NOAA
101 Pivers Island Road
Beaufort, NC 28516
Tel: (252) 728-8735
Fax: (252) 728-8784
Steve.Kibler@noaa.gov

John Kieser
4980 N. Main Street
Bldg. 6, Apt. 5
Fall River, MA 02720
Tel: (508) 999-8484
Fax: (508) 999-8196
g_jkieser@umassd.edu

Barbara Kirkpatrick
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236
Tel: (941) 388-4441 x226
Fax: (941) 388-4312
bkirkpat@mote.org

Gary Kirkpatrick
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236
Tel: (941) 388-4441 x389
Fax: (941) 388-4312
gkirkpat@mote.org

Sarah Kirn
5741 Libby Hall
School of Marine Sciences
University of Maine
Orono, ME 04469
Tel: (207) 581-4348
Fax: (207) 581-4388
Sarah.Kirn@umit.maine.edu

Yasunari Kiryu
Department of Environmental Sciences
Virginia Institute of Marine Science
Gloucester Point, VA 23062
Tel: (804) 684-7771
Fax: (804) 684-7186
yasu@vims.edu

Judy Kleindinst
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2745
Fax: (508) 457-2027
jkleindinst@whoi.edu

Danara Krupatkina
Center of Marine Biotechnology
701 East Pratt Street, Suite 236
Baltimore, MD 21203
Tel: (410) 234-8884
Fax: (410) 234-8896
krupatki@umbi.umd.edu

Julia Kubanek
Center for Marine Science
Univ. of N. Carolina at Wilmington
1 Marvin Moss Lane
Wilmington, NC 28409
Tel: (910) 962-2365
Fax: (910) 962-2410
kubanekj@uncwil.edu

Raphael Kudela
Ocean Sciences
UCSC
1156 High Street
Santa Cruz, CA 95064
Tel: (831) 459-3290
Fax: (831) 459-4882
kudela@cats.ucsc.edu

David Kulis
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2859
Fax: (508) 457-2027
dkulis@whoi.edu

Jan Kurtz
U.S. EPA, Gulf Ecology Division
1 Sabine Island Drive
Gulf Breeze, FL 32561
Tel: (850) 934-9212
Fax: (850) 934-9201
Kurtz.jan@epamail.epa.gov

Rikk Kvitek
California State University
Monterey Bay
100 Campus Center
Seaside, CA 93955
Tel: (831) 582-3529
Fax: (831) 582-3073
rikk_kvitek@monterey.edu

Richard Lacouture
Academy of Natural Sciences
10545 Mackall Road
St. Leonard, MD 20685
Tel: (410) 586-9721
Fax: (410) 586-9705
lacouture@acnatsci.org

Nicolas Ladizinsky
Moss Landing Marine Labs.
8272 Moss Landing Road
Moss Landing, CA 95039-9647
Tel: (831) 633-7270 x*12
Fax: (831) 633-7263
nicolas_ladizinsky@monterey.edu

Jan Landsberg
Florida Marine Research Institute
Florida Fish and Wildlife Commission
100 Eighth Avenue, S.E.
St. Petersburg, FL 33701
Tel: (727) 896-8626
Fax: (727) 823-0166
jan.landsberg@fwc.state.fl.us

Julie LaRoche
Institut für Meereskunde
Düstenbrooker Weg 20
Kiel 24105 GERMANY
Tel: 0049-431-597-4030
Fax: 0049-431-565876
jlaroche@ifm.uni-kiel.de

Jeffrey Leblond
Department of Biology
P.O. Box 60
Middle Tennessee State University
Murfreesboro, TN 37132
Tel: (615) 898-2847
jleblond@mtsu.edu

Kathi Lefebvre
Institute of Marine Sciences
University of California, Santa Cruz
Santa Cruz, CA 95064
Tel: (831) 459-2948
lefebvre@biology.ucsc.edu

Tod Leighfield
NOAA – National Ocean Service
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8631
Fax: (843) 762-8700
Tod.Leighfield@noaa.gov

Mitch Lesoing
Quileute Tribe
P.O. Box 187
La Push, WA 98350
Tel: (360) 374-5695
Fax: (360) 374-9250
mlesoing@olypen.com

Alan Lewitus
Marine Resources Research Institute
P.O. Box 12559
Charleston, SC 29422-2559
Tel: (843) 762-5415
Fax: (843) 762-5110
Lewitus@belle.baruch.sc.edu

Aishao Li
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2688
Fax: (508) 457-2027
ali@whoi.edu

Yaqin Li
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882
Tel: (401) 874-6686
Fax: (401) 874-6161
yaqin@gsosun1.gso.uri.edu

Emily Lilly
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-3633
Fax: (508) 457-2027
elilly@whoi.edu

Senjie Lin
Department of Marine Sciences
University of Connecticut
Groton, CT 06340
Tel: (860) 405-9168
Fax: (860) 405-9153
senjie.lin@uconn.edu

Elisabeth Lipiatou
European Commission
200 Rue de la Loi
B-1049 Brussels, BELGIUM
Tel: 322-2966286
Fax: 322-2963024
Elisabeth.Lipiatou@cec.eu.int

R. Wayne Litaker
Medical School
University of North Carolina
at Chapel Hill
CB #7100, 442 Taylor Hall
Chapel Hill, NC 27599-7100
Tel: (919) 966-1730
Fax: (919) 966-6821
Wayne_Litaker@med.unc.edu

Theodore Loder
EOS, 348 Morse Hall
The University of New Hampshire
Durham, NH 03824
Tel: (603) 862-3151
Fax: (603) 862-1915
ted.loder@unh.edu

Michael Lomas
Horn Point Laboratory
P.O. Box 775
Cambridge, MD 21613
Tel: (410) 221-8230
Fax: (410) 221-8490
lomas@hpl.umces.edu

Darcy Lonsdale
Marine Sciences Research Center
State University of New York
Stony Brook, NY 11794-5000
Tel: (631) 632-8712
Fax: (631) 632-8820
dlonsdale@notes.cc.sunysb.edu

Pascale Loret
Texas A&M University
Department of Oceanography
Eller O & M Bldg.
College Station, TX 77843-3146
Tel: (979) 845-5706
ploret@ocean.tamu.edu

Remy Luerssen
21 Centre Drive, Apt. 7D
Orono, ME 04473
Tel: (207) 866-2522
Remy.Luerssen@maine.edu

Danielle Luttenberg
NOAA
National Centers for Coastal Ocean Science
1305 East-West Highway (N/SC1)
Washington, DC 20910
(301) 713-3020 x120
(301) 713-4353
Danielle.Luttenberg@noaa.gov

Daniel Lynch
Dartmouth College
HB 8000
Hanover, NH 03755
Tel: (603) 646-2308
Fax: (603) 646-3856
D.R.Lynch@dartmouth.edu

Hugh MacIntyre
Horn Point Laboratory
P.O. Box 775
Cambridge, MD 21613
Tel: (410) 221-8430
Fax: (410) 221-8440
macintyr@hpl.umces.edu

Scott MacQuarrie
National Research Council of Canada
1411 Oxford St.
Halifax, Nova Scotia
Canada B3H 3Z1
Tel: (902) 426-2239
Fax: (902) 426-9413
Scott.MacQuarrie@NRC.ca

Hugo Magana
University of Texas Marine Science
750 Channel View Drive
Port Aransas, TX 78373
Tel: (361) 749-6736
hugo20@flash.net

Robert Magnien
Maryland Department of Natural Resources
580 Taylor Avenue, D-2
Annapolis, MD 21401
Tel: (410) 260-8630
Fax: (410) 260-8640
rmagnien@dnr.state.md.us

Lucie Maranda
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882
Tel: (401) 874-6216
Fax: (401) 874-6240
lmaranda@gso.uri.edu

Roman Marin III
Monterey Bay Aquarium Research Inst.
7700 Sandholdt Road
Moss Landing, CA 95039
Tel: (831) 775-1859
Fax: (831) 775-1645
maro@mbari.org

Harold Marshall
Department of Biological Sciences
Old Dominion University
Norfolk, VA 23529-0266
Tel: (757) 683-4204
Fax: (757) 683-5283
hmarshal@odu.edu

Jennifer Martin
Fisheries & Oceans Canada
531 Brandy Cove Rd.
St. Andrews, NB ESB 2L9
Tel: (506) 529-5921
Fax: (506) 529-5862
martinjl@mar.dfo-mpo.gc.ca

Helmut Maske
CHORS/SDSU
6505 Alvarado Road, Suite 206
San Diego, CA 92120-5005
Tel: (617) 594-2272
Fax: (617) 594-8670
Hmaske@cicese.mx

Gene Massion
MBARI
7700 Sandholdt Road
Moss Landing, CA 95039
Tel: (831) 775-1922
Fax: (831) 775-1646
Magene@mbari.org

Patricia Matrai
Bigelow Laboratory for Ocean Sciences
180 McKown Pt.
W. Boothbay Harbor, ME 04575
Tel: (207) 633-9614
Fax: (207) 633-9641
pmatrai@bigelow.org

Xavier Mayali
NOS/CCEHBR
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8511
Fax: (843) 762-8700
xmayali@edisto.cofc.edu

Dennis McGillicuddy
AOPE Department, MS #11
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2683
Fax: (508) 457-2194
dmcgillicuddy@whoi.edu

Jay McGowan
Department of Marine Resources
P.O. Box 8
West Boothbay Harbor, ME 04575
jay.mcgowan@state.me.us

Gene Massion
MBARI
7700 Sandholdt Road
Moss landing, CA 95039
Tel: (831) 775-1922
Fax: (831) 775-1646
Mag ene@mbari.org

Todd Miller
Univ. of MD Biotechnology Institute
Center of Marine Biotechnology
701 E. Pratt St., Suite 236
Baltimore, MD 21202
Tel: (410) 234-8877
Fax: (410) 234-8896
millert@umbi.umd.edu

Michael Mitchell
Unity College
HC-78, Box 593
Unity, ME 04988
Tel: (207) 948-6178
mmitchel@unity.unity.edu

Pia Moisander
Institute of Marine Sciences
UNC-Chapel Hill
3431 Arendell Street
Morehead City, NC 28557
Tel: (252) 726-6841 x135
Fax: (252) 726-2426
moisande@email.unc.edu

Steve Morton
NOAA/NOS
219 Ft. Johnson Rd.
Charleston, SC 29412
Tel: (843) 762-8501
Fax: (843) 762-8700
Steve.Morton@noaa.gov

Margaret Mulholland
Marine Sciences Research Center
SUNY Stony Brook
Stony Brook, NY 11794-5000
Tel: (631) 632-3163
Fax: (631) 632-8820
mmulholland@notes.cc.sunysb.edu

Thomas Murray
Dept. of Physiology & Pharmacology
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602
Tel: (706) 542-3014
Fax: (706) 542-3015
tmurray@vet.uga.edu

Jerome Naar
Center for Marine Science
UNC at Wilmington
1 Marvin K. Moss Lane
Wilmington, NC 28409
Tel: (910) 962-2367
jeromenaar@yahoo.com

Kerry Norton
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-29215
Fax: (508) 457-2027
kerrynorton@hotmail.com

Thomas Nerad
American Type Culture Collection
10801 University Boulevard
Manassas, VA 20110--2209
(703) 365-2722
(703) 365-2730
tnerad@atcc.org

Shane O'Boyle
Martin Ryan Institute
NUI
Galway, IRELAND
Tel: 353 91 524411, x 3188
Fax: 353 91 525005
Shane.OBoyle@nuigalway.ie

Mr. Micheal O'Cinneide
Marine Environment & Health Services Div.
Abbotstown Laboratory Complex
Snugbord Road, Castleknock
Dublin 15, Ireland
Tel: 353 1 8210111
Fax: 353 1 8205078
micheal.ocinneide@marine.ie.

Chris O'Halloran
224 Baldwin Street
Santa Cruz, CA 95060
(831) 421-0887
Tel: (831) 421-0887
Fax: (831) 466-7859 (pager)
cohallo@chemistry.ucsc.edu

John Paul
University of South Florida
140 Seventh Avenue, S.
St. Petersburg, FL 33701
Tel: (727) 553-1168
Fax: (727) 553-1189
jpaul@seas.marine.usf.edu

Michael T. Peglar
American Type Culture Collection
Protistology Department
10801 University Boulevard
Manassass, VA 20110-2209
Tel: (703) 365-2700 x291
mpeglar@atcc.org

Jonathan Pennock
Dauphin Island Sea Lab
101 Bienville Blvd.
Dauphin Island, AL 36528
Tel: (334) 861-7531
Fax: (334) 861-7540
jpennock@disl.org

Heather Penta
Florida Marine Research Institute
100 8th Ave., SE
St. Petersburg, FL 33701
Tel: (727) 896-8626
Fax: (727) 550-4222
heather.penta@fwc.state.fl.us

Neal Pettigrew
School of Marine Sciences
Libby Hall
University of Maine
Orono, ME 04469
Tel: (207) 581-4384
Fax: (207) 581-4388
nealp@maine.edu

Richard Pierce
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236
Tel: (941) 388-4441
Fax: (941) 388-4312
rich@mote.org

Allen Place
Center of Marine Biotechnology
Columbus Center, Suite 236
701 East Pratt Street
Baltimore, MD 21202
Tel: (410) 234-8828
Fax: (410) 234-8896
place@umbi.umd.edu

Steven Plakas
U.S. FDA
1 Iberville Drive
Dauphin Island, AL 36528
Tel: (334) 694-4480, x.251
Fax: (334) 694-4477
splakas@cfsan.fda.gov

F. Gerald (Gerry) Plumley
Institute of Marine Science
University of Alaska
Fairbanks, AK 99755
Tel: (907) 474-6786
Fax: (907) 474-7204
fffgp@uaf.edu

Linda Popels
College of Marine Studies
University of Delaware
700 Pilottown Road
Lewes, DE 19958
Tel: (302) 645-4257
Fax: (302) 645-4007
lcpope@udel.edu

James Postel
University of Washington
School of Oceanography
Box 357940
Seattle, WA 98195-7940
Tel: (206) 543-4485
Fax: (206) 543-0275
postel@ocean.washington.edu

Nicole Poulton
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2584
Fax: (508) 457-2027
npoulton@whoi.edu

Christine Powell
Marine Biotoxins Program
NOAA/NOS
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8627
Fax: (843) 762-8700
christine.powell@noaa.gov

Frances Pustizzi
University of Delaware
College of Marine Studies
700 Pilottown Road
Lewes, DE 19958
Tel: (302) 645-4257
Fax: (302) 645-4007
franp@udel.edu

Robin Raine
Martin Ryan Institute
National University of Ireland
Galway, Ireland
Tel: 353 91 52 4411
Fax: 353 91 52 5005
robin.raine@nuigalway.ie

John Ramsdell
Coastal Research Branch
NOAA – National Ocean Service
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8510
Fax: (843) 762-8700
john.ramsdell@noaa.gov

Kimberly Reece
Virginia Institute of Marine Science
The College of William and Mary
P.O. Box 1346
Gloucester Point, VA 23062
Tel: (804) 684-7407
Fax: (804) 684-7796
kreece@vims.edu

Robert Reed
Center for Applied Aquatic Ecology
North Carolina State University
620 Hutton St. – Suite 104
Raleigh, NC 27606
Tel: (919) 515-3421
Fax: (919) 513-3194
rereed@unity.ncsu.edu

Karin Rengefors
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2948
Fax: (508) 457-2027
krengefors@whoi.edu

J.E. Jack Rensel
Rensel Associates Aquatic Science Consultants
4209 234th Street, N.E.
Arlington, WA 98223
Tel: (360) 435-3285
Fax: (360) 435-7409
jackrensel@msn.com

Daniel Repeta
Chemistry Dept., MS #4
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2635
Fax: (508) 457-2164
drepeta@whoi.edu

Lesley Rhodes
Senior Research Scientist
Cawthron Institute
Private Bag 2
Nelson, New Zealand
Tel: +64 3 5482319
Fax: +64 3 5469464
lesley@cawthron.org.nz

Bill Richardson
Florida Fish & Wildlife Cons. Comm.
Florida Marine Research Institute
100 Eighth Ave., S.E.
St. Petersburg, FL 33701
Tel: (727) 896-8626
Fax: (727) 550-4222
bill.richardson@fwc.state.fl.us

Raymond Roberts
Jellett Biotek Ltd.
101 Research Drive
Dartmouth, Nova Scotia
B2Y 4T6, CANADA
Tel: (902) 424-8670, x118
Fax: (902) 424-4679
rroberts@innovacorp.ns.ca

Helen Rogers
CDC-NCEH
1600 Clifton Road, MS E-23
Atlanta, GA 330333
(404) 639-2561
(404) 639-2565
hhs0@cdc.gov

John Rogers
U.S. EPA
Gulf Ecology Division
1 Sabine Island Drive
Gulf Breeze, FL 32561
Tel: (850) 934-9326
Fax: (850) 934-2401
Rogers.Johne@epa.gov

Michelle Rome
Brown University
Box 2740
Providence, RI 02912
Tel: (401) 867-4243
Fax: (401) 221-8490
Michelle_Rome@Brown.edu

Juliette Rooney-Varga
Biological Sciences Department
1 University Avenue
University of Massachusetts, Lowell
Lowell, MA 01854
Tel: (978) 934-4715
Fax: (978) 934-3044
Juliette_RooneyVarga@uml.edu

Carol Rosetta
The University of Connecticut
Department of Marine Sciences
1084 Shennecossett Road
Avery Point, CT 06340-6097
Tel: (860) 405-9090
Fax: (860) 405-9153
chrosetta@hotmail.com

Parke Rublee
University of North Carolina
at Greensboro
P.O. Box 26174
Greensboro, NC 27402-6174
Tel: (336) 256-0067
Fax: (336) 334-5839
rublee@uncg.edu

Eden Rue
1156 High Street
Earth & Marine Sciences Building
University of California, Santa Cruz
Santa Cruz, CA 95064
Tel: (831) 459-5152
Fax: (831) 459-4882
elrue@cats.ucsc.edu

Keiko Saito
Center of Marine Biotechnology
University of Maryland Biotech. Inst.
701 E. Pratt Street, #3061
Baltimore, MD 21202
Tel: (410) 234-8827
Fax: (410) 234-8897
saito@umbi.umd.edu

Amalia Salditos
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2921
Fax: (508) 457-2027
amalia_sal@hotmail.com

Jennifer Samson
Rutgers University, Newark
Department of Biological Sciences
101 Warren Street – Smith Hall
Newark, NJ 07102
Tel: (973) 353-5387
Fax: (973) 353-5518
jsamson@pegasus.rutgers.edu

Mary Savin
UMASS, Lowell
23 Wm. Fairfield Drive
Wenham, MA 01984
Tel: (978) 934-2872
Fax: (978) 934-3044
Mary_Savin@uml.edu

Rebecca Schaffner
Dept. of Biological Sciences
3616 Trousdale Pkwy AHF 301
University of Southern California
Los Angeles, CA 90089-0371
Tel: (213) 821-1800
Fax: (213) 740-6720
rschaffn@usc.edu

Cornelia Schlenk
121 Discovery Hall
SUNY at Stony Brook
Stony Brook, NY 11794-5001
Tel: (631) 632-6906
Fax: (631) 632-6917
cschlenk@notes.cc.sunysb.edu

Chris Scholin
Monterey Bay Aquarium Research Inst.
7700 Sandholdt Road
Moss Landing, CA 95039
Tel: (831) 775-1779
Fax: (831) 775-1645
scholin@mbari.org

Richard Schramm
Monterey Bay Aquarium Research Inst.
7700 Sandholdt Road
Moss Landing, CA 95039
Tel: (831) 775-1712
Fax: (831) 775-1620
rich@mbari.org

Kevin Sellner
NOAA N/SC12
Room 9752, Bldg. 3
1315 East-West Highway
Silver Spring, MD 20910
Tel: (301) 713-3338
Fax: (301) 713-4044
kevin.sellner@cop.noaa.gov

Stella Sellner
Academy of Natural Sciences
Estuarine Research Center
St. Leonard, MD 220685
sellner@acnatsci.org

Mario Sengco
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2749
Fax: (508) 457-2027
msengco@whoi.edu

Jeffrey Shields
Virginia Institute of Marine Science
P.O. Box 1345
Gloucester Point, VA
Tel: (804) 684-7128
Fax: (804) 684-7186
jeff@vims.edu

Sandra Shumway
Natural Science Division
Southampton College
Long Island University
Southampton, NY 11968
Tel: (631) 287-8407
Fax: (631) 287-8419
SShumway@southampton.liunet.edu

Richard P. Signell
U.S. Geological Survey
384 Woods Hole Road
Woods Hole, MA 02543
Tel: (508) 457-2229
Fax: (508) 457-2310
rsignell@usgs.gov

Mary Silver
Institute of Marine Sciences
University of California, Santa Cruz
Santa Cruz, CA 95064
Tel: (831) 459-2908
Fax: (831) 459-4882
msilver@cats.ucsc.edu

Ted Smayda
Prof. Oceanography
Graduate School of Oceanography
University of Rhode Island
Kingston, RI 02881
Tel: (401) 874-6171
Fax: (401) 874-6682
tsmayda@gsosun1.gso.uri.edu

G. Jason Smith
Moss Landing Marine Laboratories
8272 Moss Landing Road
Moss Landing, CA 95039
Tel: (831) 633-7270
Fax: (831) 633-7263
symbios@aol.com

Joseph Stabile
Iona College
NYU Medical Center
715 North Avenue
New Rochelle, NY 10801
Tel: (914) 633-2253
Fax: (914) 633-2240
jstabile@iona.edu

Karen Steidinger
Florida Marine Research Institute
100 Eighth Avenue, S.E.
St. Petersburg, FL 33701
Tel: (727) 896-8626
Fax: (727) 550-4222
Karen.Steidinger@fwc.state.fl.us

Charles Stock
AOP&E Department, MS #9
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
Tel: (508) 289-2611
Fax: (508) 457-2194
cstock@whoi.edu

Andrew Stoddard
Andrew Stoddard & Associates
112 Orchard circle
Hamilton, VA 20158-9734
Tel: (540) 338-3649
Fax: (540) 338-3649
studywq@aol.com

Diane Stoecker
Horn Point Laboratory
UMCES
P.O. Box 775
Cambridge, MD 21664
Tel: (410) 221-8407
Fax: (410) 221-8490
stoecker@hpl.umces.edu

Nancy Stokes
Virginia Institute of Marine Science
P.O. Box 1346
Gloucester Point, VA 23062
Tel: (804) 684-7410
Fax: (804) 684-7796
stokes@vims.edu

Gregory Teegarden
Bowdoin College
6500 College Station
Brunswick, ME 04011
Tel: (207) 725-3213
Fax: (207) 725-3405
gteegard@bowdoin.edu

Torstein Tengs
Institute of Human Virology
University of Maryland
725 West Lombard St., Room N557
Baltimore, MD 21201
Tel: (410) 706-4654
Fax: (410) 706-1992
tengst@umbi.umd.edu

Dan Terlizzi
Center of Marine Biotechnology
701 E. Pratt Street
Baltimore, MD 21201
Tel: (410) 234-8837
Fax: (410) 234-8896
dt37@umail.umd.edu

Patricia A. Tester
National Ocean Service, NOAA
101 Pivers Island Road
Beaufort, NC 28516
Tel: (252) 728-8792
Fax: (252) 728-8784
pat.tester@noaa.gov

Andrew Thomas
School of Marine Sciences
University of Maine
Libby Hall
Orono, ME 04469
Tel: (207) 581-4335
Fax: (207) 581-4388
thomas@maine.edu

Brian Thompson
Bigelow Laboratory for Ocean Sciences
P.O. Box 475
McKown Point
West Boothbay Harbor, ME 04575-0475
Tel: (207) 633-9600
Fax: (207) 633-9641
bthompson@bigelow.org

Carmelo Tomas
University of North Carolina at Wilmington
Center for Marine Sciences
1 Marvin K. Moss Lane
Wilmington, NC 28409
Tel: (910) 962-2385
Fax: (910) 962-2410
tomasc@uncwil.edu

Shelly Tomlinson
NODC/NOAA/NESDIS
F10C1
1315 East-West Highway #4660
Silver Spring, MD 20910
Tel: (301) 713-3272 x122
Fax: (301) 713-3302
Michelle.Tomlinson@noaa.gov

David W. Townsend
University of Maine
5741 Libby Hall
Orono, ME 04469
Tel: (207) 581-4367
Fax: (207) 581-4388
davidt@maine.edu

Vera L. Trainer
Marine Biotoxins
NWFSC-NMFS
2725 Montlake Blvd. E.
Seattle, WA 98105
Tel: (206) 860-6788
Fax: (206) 860-3335
Vera.L.Trainer@noaa.gov

Megan Treml
NOAA Coastal Services Center
2234 South Hobson Ave.
Charleston, SC 29405
Tel: (843) 740-1212
Fax: (843) 740-1315
Megan.Treml@noaa.gov

Charlie Trick
University of Western Ontario
Department of Plant Sciences
Biology and Geology Building
London, Ontario, Canada N6A 5B7
Tel: (519) 661-3899
Fax: (519) 661-3935
cyano@julian.uwo.ca

Jefferson T. Turner
Univ. of Massachusetts, Dartmouth
285 Old Westport Road
No. Dartmouth, MA 02747-2300
Tel: (508) 910-6332
Fax: (508) 999-8901
Email: jturner@umassd.edu

Betty Twarog
Darling Marine Center
University of Maine
193 Clark's Cove road
Walpole, ME 04573
Tel: (207) 563-3146 x290
Fax: (207) 563-3119
dmc-bt@maine.edu

Mike Twiner
University of Western Ontario
Department of Plant Sciences
Biology and Geology Building
London, Ontario, Canada N6A 5B7
Tel: (519) 661-2111 x86470
Fax: (519) 661-3935
mtwiner@julian.uwo.ca

John Tyrell
Monterey Bay Aquarium Research Inst.
7700 Sandholdt Road
Moss Landing, CA 95039
Tel: (831) 775-1903
Fax: (831) 775-1620
jtyrrell@mbari.org

Mark Vandersea
National Ocean Service, NOAA
101 Pivers Island Road
Beaufort, NC 28516
Tel: (252) 728-877?
Fax: (252) 728-8784
Mark.W.Vandersea@noaa.gov

Frances Van Dolah
NOAA – National Ocean Service
219 Fort Johnson Road
Charleston, SC 29412
Tel: (843) 762-8529
Fax: (843) 762-8700
Fran.Vandolah@noaa.gov

Gabriel Vargo
College of Marine Science
University of South Florida
140 7th Avenue, South
St. Petersburg, FL 33701
Tel: (727) 553-1167
Fax: (727) 553-1189
vargo@seas.marine.usf.edu

Sabrina Varnam
National Ocean Service, NOAA
North Carolina State University
101 Pivers Island Road
Beaufort, NC 28516
Tel: (252) 728-8735
Fax: (252) 728-8784
Sabrina.Varnam@noaa.gov

Tracy Villareal
Marine Science Institute
University of Texas – Austin
750 Channel View Drive
Port Aransas, TX 78373
Tel: (361) 749-6732
Fax: (361) 749-6777
tracy@utmsi.texas.edu

Wolfgang Vogelbein
Department of Environmental Sciences
Virginia Institute of Marine Science
Route 1208
Gloucester Point, VA 23061
Tel: (804) 684-7261
Fax: (804) 684-7186
wolf@vims.edu

Calvin C. Walker
U.S. EPA
Gulf Ecology Division
1 Sabine Island Drive
Pensacola, FL 32503
Tel: (850) 934-9245
Fax: (850) 934-2402
walker.calvin@epa.gov

John J. Walsh
College of Marine Science
University of South Florida
140 7th Avenue, South
St. Petersburg, FL 33701
Tel: (727) 553-1164
Fax: (727) 553-1189
jwalsh@seas.marine.usf.edu

Qian Wang
Center of Marine Biotechnology
University of Maryland Biotech. Inst.
701 East Pratt Street, Suite 236
Baltimore, MD 21202
Tel: (410) 234-8877
Fax: (410) 234-8896
wangq@umbi.umd.edu

Ryan Weatherbee
School of Marine Sciences
University of Maine
Room 214, Libby Hall
Orono, ME 04468
Tel: (207) 581-4334
Fax: (207) 581-4388
ryan.weatherbee@umit.maine.edu

Robert Weisberg
College of Marine Science
University of South Florida
140 7th Avenue S.
St. Petersburg, FL 33701
Tel: (727) 553-1568
Fax: (727) 553-1189
weisberg@marine.usf.edu

Gary H. Wikfors
NOAA/NEFSC
212 Rogers Avenue
Milford, CT 06460
Tel: (203) 579-7025
Fax: (203) 579-7017
Gary.Wikfors@noaa.gov

Jennifer Wolny
South Carolina Department of Natural Resources
Marine Resources Division
Belle Baruch Marine Field Laboratory
Hobcaw Barony Highway 17 North
Georgetown, SC 29440
(843) 546-3623
(843) 546-1632
jwolny@belle.baruch.sc.edu

Bryan Yonish
The Program in Molecular Biology and Biotechnology
University of North Carolina Medical School
CB#7100, 101 MBBRL
Chapel Hill, NC 27599
Tel: (919) 966-1730
Fax: (919) 966-6821
bayonish@med.unc.edu

Huan Zhang
Department of Marine Sciences
University of Connecticut
Groton, CT 06340
Tel: (860) 405-9233
Fax: (860) 405-9153
hzhang@uconnvm.uconn.edu