FLORIDA STATE UNIVERSITY: GAUGING THE EFFECT OF THE BP OIL SPILL ON DIATOMS, CALCAREOUS NANNOPLANKTON, AND RELATED PROTISTS AT OR NEAR THE BASE OF THE FOOD CHAIN IN THE NE GULF OF MEXICO

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SCIENCE ACTIVITIES

1) General Summary

The primary objects of this study are calcareous nannoplankton and benthic and planktonic (or pelagic) diatoms. These are major photosynthetic protist constituents at the base of the food chain in marine waters around the globe. Major impacts on their populations by spill oil and dispersants will be felt throughout the trophic system up to the vertebrate level. Both groups leave a fossil record, therefore, major changes in living populations can be assessed against previous baseline studies as well as their record through geologic time. Their skeletal constituents are different, however, as those of diatoms are composed of silica (SiO_2) and have a consistency of window glass spun biologically in intricate patterns that can seen in both light and electron microscopes. Skeletons of the calcareous nannoplankton (coccolithophorids) are composed of the mineral calcite ($CaCO_3$), also secreted in intricate and diagnostic patterns when examined at the ultrastructural level; these form the world's great chalk deposits (such as the White Cliffs of Dover).

Goals/Specific Aims

To assess the impact of the Deepwater Horizon oil spill and BP dispersants on the base of the food chain by:

***** Conducting baseline studies of the protist taxa in question from the coastal bays and estuaries to the continental slope along the Florida Panhandle, with particular emphasis Florida Panhandle bays previously studied by members of our team: Perdido, Escambia Bay, Blackwater Bay, Choctawhatchee Bay, Apalachicola Bay, and Apalachee Bay;

***** Testing in laboratory cultures the effects of Deepwater Horizon oils and dispersants on diatom and calcareous nannoplankton, particularly effects revealed by abnormalities in their skeletons;

* Comparing compositional changes in our extant protist populations with those preserved in the fossil record as revealed by our sediment samples;

* Examining and describing, as applicable, other taxonomic groups at or near the base of the food chain that appear in our samples to assess any disruptions in microzooplankton grazing on the phytoplankton;

* Conducting censuses of the protists collected in the field as applicable in both the light microscope (LM) and the scanning electron microscope (SEM).

First Year's Study Followed by a One-Year, No-Cost Extension of the Project

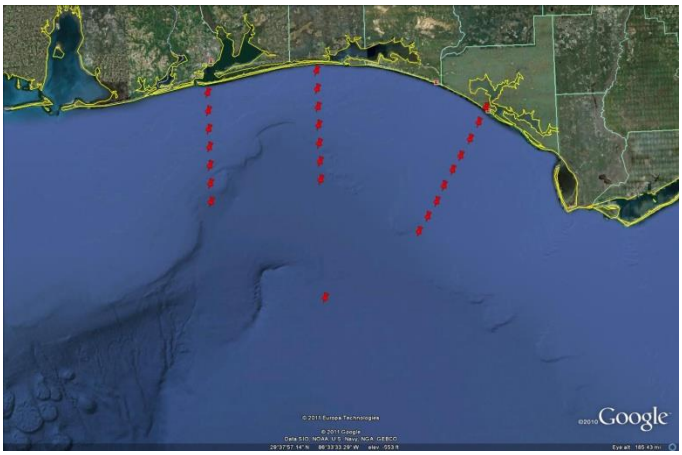
During the first year of this study 19 sampling expeditions (see Cruises and Field Expeditions", below) were conducted in the Florida Panhandle estuaries and bays as well as in the Gulf of Mexico. At that point a one-year, no-cost extension of the project was requested and granted so that our collecting expeditions could continue in the GOM where an additional three *R/V Bellows* and *R/V Weatherbird II* cruises were to be conducted through May, 2012. This also allowed more time to process samples collected via the SEM, a highly time-consuming task (about 2 hours per sample).

2) Results and scientific highlights

The majority of our resources were concentrated on taking plankton samples (diatoms, calcareous nannoplankton, and dinoflagellates) for our studies. Although the Period of Performance for this project was set by the Grantee to begin on August 13th, 2010, the funds were not received at FSU on October 14th of that year.

Nevertheless, field work for the project began on September 24, 2010, in the Florida Panhandle Bays, first by Dr. Nienow and his students taking net samples from land and piers in Choctawhatchee and Pensacola Bays. This was followed by Drs. Prasad and Nienow and students/postdoctorals sampling in five different bays (including Perdido, Apalachicola, St. Joseph Bays) and from boats kindly provided and crewed by members of the Florida Wildlife Refuges, the Apalachicola National Estuarine Research Reserve (NERR), and Florida Department of Environmental Protection. A total of 13 bays were sampled during the first year. As a result of that work, two of Dr. Nienow's students, Arjun Adhikari and Heera Malik, successfully completed and defended their master's degrees in August, 2011, using our samples collected from Choctawhatchee and Perdido/Pensacola Bays.

The initial attempt to sample in the Gulf of Mexico (GOM), scheduled for the third week in December, 2011, aboard the *R/V Bellows*, was cancelled due to strong winds and high seas. The next scheduled *R/V Bellows* cruise, led by Dr. Nienow with a crew of three VSU and FSU students/postdoctorals from January 22-25, 2011, however, was highly successful in obtaining samples despite very cold, windy weather and choppy seas. During the cruise net plankton (vertical tows of up to 75 meters, 25 µm mesh) and whole water samples (1 L, collected at 20 meter intervals from the bottom to the surface via a CTD Rosette) were taken at 23 of the possible 25 stations along three transects that extended from the coast across the Florida Panhandle Shelf to De Soto Canyon (see figure below). Whole water samples were filtered through nitrocellulose filters and air dried; net plankton samples were fixed with Lugol's iodine.



Dr. Richard Snyder and his colleagues and students from the University of West Florida aboard that initial cruise ran water chemistry analyses on the samples collected, which further enhanced the value of our phytoplankton samples. This set the template for all subsequent 3- to 4-day GOM expeditions on the *R/V Bellows* and *Weatherbird II* for the remainder of the project, each staffed by a 4-person team from VSU and FSU. In all, a total of 25 sampling expeditions were conducted in the Florida Panhandle estuaries and bays as well as in the Gulf of Mexico during this study.

Back in the laboratory, microscopic analyses began on the hundreds of samples collected, including scanning electron microscope (SEM) imaging of the diatoms and calcareous nannoplankton. Some SEM micrographs taken by graduate student Susan Foley at FSU and by Professor Nienow's group are attached at the end to this report (see Topic 12). The calcareous nannoplankton turned out to be far more diverse than expected based on the few (non-quantitative) studies done previously on this group in the GOM, particularly adjacent to or along the continental shelves. SEM facilities have been utilized at VSU and FSU (Biology and Physics); use of the former are free of charge, but charges for the latter initially ranged from $45 to $75/hour, more than our budget could possibly accommodate. Fortunately, that situation was alleviated considerably after the first year through negotiations with the FSU Physics Department and the Florida Geological Survey in Tallahassee.

As mentioned above, we were surprised by the wide variety of calcareous nannoplankton taxa along the shelf transects, more varied than reported previously in the literature and more than knowledgeable experts had lead us to believe possible. This indicates influxes of open GOM waters into De Soto Canyon and high up onto the shelf. By the time of our May, 2011 sampling, however, the abundance and diversity of the nannoplankton dropped considerably due to the influx of Mississippi River flood waters into De Soto Canyon, an unusual occurrence due to the record spring floods in the upper Mississippi River Valley that caused the opening of the emergency spillways upriver from New Orleans.

For quantitative work (the ultimate goal of this project), we began conducting census counts on the diatom and calcareous nannoplankton samples collected from the GOM. Because of their minute size, these counts must be done in the SEM, the use of which, as mentioned above, was quite expensive at FSU during the first year of the study. Therefore, those of us at

FSU sought to mitigate this problem initially by traveling to VSU for free use of Dr. Nienow's machine, a 90-minute drive one way. In addition, such census work is quite time consuming, taking up to two hours to make the 200 counts needed for a single sample. By the end of the grant period, we had completed counts of calcareous nannoplankton on 150 of the 1300 samples we have taken in the GOM. These studies will be continued, however, via the 3-year GRI "Consortium Project" that will allow us to carry on our work at VSU and FSU through end of the year 2014 (see below).

On another front, Dr. Laura Keller cultured calcareous phytoplankton in the laboratory and subjected them to exposure to Macondo crude oil and dispersant to see if either affected their skeletal structure or ability to reproduce. Surprisingly, to date, neither of these agents have shown any detrimental effects on these organisms.

Although our funding for this grant was for only one year, vessel support provided by FIO in the GOM was available for an additional nine months beyond that. For that reason we stretched our grant funds to allow us to take advantage of this unique sampling opportunity, which is a major reason we requested the one-year time extension of our FIO grant. To further aid us in this endeavor, we submitted a supplementary proposal to Gulf of Mexico Research Initiative (GRI RFP III) to fill a possible 5-month "gap" in funding after August 12, 2011, but unfortunately that proposal was declined. We also submitted, however, a 3-year GRI "Consortium Proposal" (GRI RFP III) through FSU for further GOM sampling, and that proposal via the "Deep-C Consortium" was funded. Thus, this new grant will allow us continue our on-going sampling and quantitative SEM studies on our GOM samples through 2014.

3) Cruises & field expeditions

Ship or Platform Name/Locality	**Chief Scientist(s)**	**Objectives**	**Dates**
Choctawhatchee & Pensacola Bays	James Nienow	Sample bay stations for benthic and planktonic diatoms	9-24-10
Apalachicola Bay	Akshintala Prasad, Nienow	Sample bay stations for diatoms	10-18-10
St. Joseph Bay	Prasad, Nienow, Sherwood Wise	Sample bay stations for diatoms	10-22-10
Choctawhatchee, Perdido and Pensacola Bays	Nienow	Sample bay stations for benthic and planktonic diatoms	11-6-10
Apalachicola Bay	Akshintala Prasad, Nienow	Sample bay stations for diatoms	12-3-10
Choctawhatchee, Perdido and Pensacola Bays	Nienow	Sample bay stations for benthic and planktonic diatoms	1-4-11
RV BELLOWS: Gulf of Mexico (GOM)	Richard Snyder (UWF), Nienow	Sample off-shore transects--diatoms & coccolithophorids	1-22-11 to 1-25-11
St. Joseph Bay	Prasad, Nienow	Sample bay stations for diatoms	1-27-11
Apalachicola Bay	Prasad, Nienow	Sample bay stations for diatoms	2-3-11
RV BELLOWS: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	2-21-11 to 2- 23-11
Choctawhatchee, Perdido and Pensacola Bays	Nienow	Sample bay stations for benthic and planktonic diatoms	3-15-11
RV WEATHERBIRD II, GOM	Snyder, Nienow, Anatoliy Shumnyk	Sample off-shore transects--diatoms & coccolithophorids	3-21-11 to 3-24-11
St. Joseph Bay	Prasad	Sample bay stations for diatoms	3-28-11

Choctawhatchee, Perdido and Pensacola Bays	Nienow	Sample bay stations for benthic and planktonic diatoms	5-10-12
Apalachicola Bay	Prasad	Sample bay stations for diatoms	5-12-11
St. Joseph Bay	Prasad, Nienow	Sample bay stations for diatoms	5-19-11
RV WEATHERBIRD II, GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	5-20-11 to 5- 24-11
RV WEATHERBIRD II, GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	6-12-11 to 6-15-11
Apalachicola Bay	Prasad, Wise	Sample bay stations for diatoms and coccolithophorids	6-22-11
RV BELLOWS: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	9-28-11 to 10-1-11
RV BELLOWS: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	10-23-11 to 10-26-11
RV WEATHERBIRD: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	12-13-11 to 12-16-11
RV WEATHERBIRD: GOM	Snyder, Nienow, Wise	Sample off-shore transects--diatoms & coccolithophorids	2-27-12 to 2-29-12
RV BELLOWS: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	3-17-12 to 3-20-12
RV BELLOWS: GOM	Snyder, Nienow	Sample off-shore transects--diatoms & coccolithophorids	5-5-12 to 5-7-12

4) Peer-reviewed publications (Note: a special section will focus on student and post-doctoral publications)
   a. Published, peer-reviewed bibliography (Copies of the papers are requested): None yet.
   b. Manuscripts submitted or in preparation :

J. N. Putland, B. Mortazavi, R. L. Iverson, and S. W. Wise. Phytoplankton Biomass and Composition along an Estuarine Salinity Gradient. 39 pp plus 7 figures.

This manuscript on the phytoplankton biomass of the Apalachicola Bay was submitted as Ms. No. 201112094 to the Marine Ecology Progress Series. On March 4, 2012, the editors informed us that it was not sufficiently within the scope of their journal for them to publish. We, therefore, submitted this manuscript to a different journal, "Estuaries and Coasts" (estuariesandcoasts@erf.org), under the same authorship but with a modified title of:

"Distribution of Phytoplankton in a River-dominated Estuary during two Summers of Contrasting River Discharge".

As of 1-21-13, we are waiting to hear whether this paper has been accepted for publication.

5) Presentations and posters (Note: a special section below will focus on student presentations).

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Gauging the effect of the BP Oil Spill on diatoms, calcareous nannoplankton, and related protists at or near the base of the food chain in the NE Gulf of Mexico.	S. Wise	S. Wise, J. Nienow, A. Prasad, L. Keller, J. Putland, A. Shumnyk, C. Shunneskog, S. Blair, M. Sullivan, S. Foley.,	PI workshop on the BP Oil-Spill Project, Orlando, FL.	N	May 23, 2011

6) Other products or deliverables: None

7) Data/Metadata: No data/metadata have been filed because the format for that has yet to be decided; these and subsequent data, however, will be filed via our ongoing Deep-C Consortium grant.

PARTICIPANTS AND COLLABORATORS

8) Project participants

First Name	Last Name	Role in Project	Institution	Email	Gender	Race	Citizenship
Sherwood	Wise	PI	FSU	swise@fsu.edu	M	W	USA
James	Nienow	Co-PI	Valdosta State Univ. (VSU)	jnienow@valdosta.edu	M	W	USA
Akshintala	Prasad	Co-PI	FSU	prasad@bio.fsu.edu	M	W	USA
Laura	Keller	Scientist	FSU	lkeller@bio.fsu.edu	F	W	USA
Charlotte	Sjunneskog	Scientist	FSU	csjunneskog@fsu.edu	F	W	Sweden
Michael	Sullivan	Scientist	St. Andrews School, Jackson, MS.	mjsulliv@fsu.edu	M	W	USA

MENTORING AND TRAINING

9) Student and post-doctoral participants

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion year	Gender	Race	Citizenship
Jennifer	Putland	Post-doc	Microzooplankton grazing on the phytoplankton	FSU	S. Wise	2012	F	W	USA
Anatoliy	Shumnyk	Post-doc	Water Sampling & Sediments	FSU	S. Wise	2012	M	W	Ukraine
Juan	Panera	Post-doc	Nannofossils	FSU	S. Wise	2012	M	W	Argentina
Stacie	Blair	PhD	Project Start-up and prep of the 1997 V. Pariente Dissertation for publication	FSU	S. Wise	2011	F	W	USA
Susan	Foley	PhD	Nannoplannkton of the De Soto Canyon area of the GOM	FSU	S. Wise	2014	F	W	USA
Mohammed	Aljahdali	MS	Pleistocene-Recent Nannofossils	FSU	S. Wise	2013	M	W	USA
Arjun	Adhikari	MS	Diatoms of Perdido/Pensacola Bays	VSU	J. Nienow	2012	M	Asian-India	India
Heera	Malik	MS	Diatoms of Choctawhatchee Bay	VSU	J. Nienow	2012	F	Asian-India	India
Nicholas	Myers	MS	Pleistocene nannofossils	FSU	S. Wise	2013	M	Af-Am	USA
Courtney	Bryller	BS	Modern FL Diatoms	VSU	J. Nienow	2012	F	W	USA
James	Tillman	BS	Modern FL Diatoms	VSU	J. Nienow	2012	M	Af-Am	USA
Adrian	Herbert	BS	Modern FL Diatoms	FSU	S. Wise	2012	M	W	USA
Erin	Wall	BS	N/A	FSU	S. Wise	2012	F	W	USA
Doug	Reinert	BS	N/A	FSU	S. Wise	2011	M	W	USA

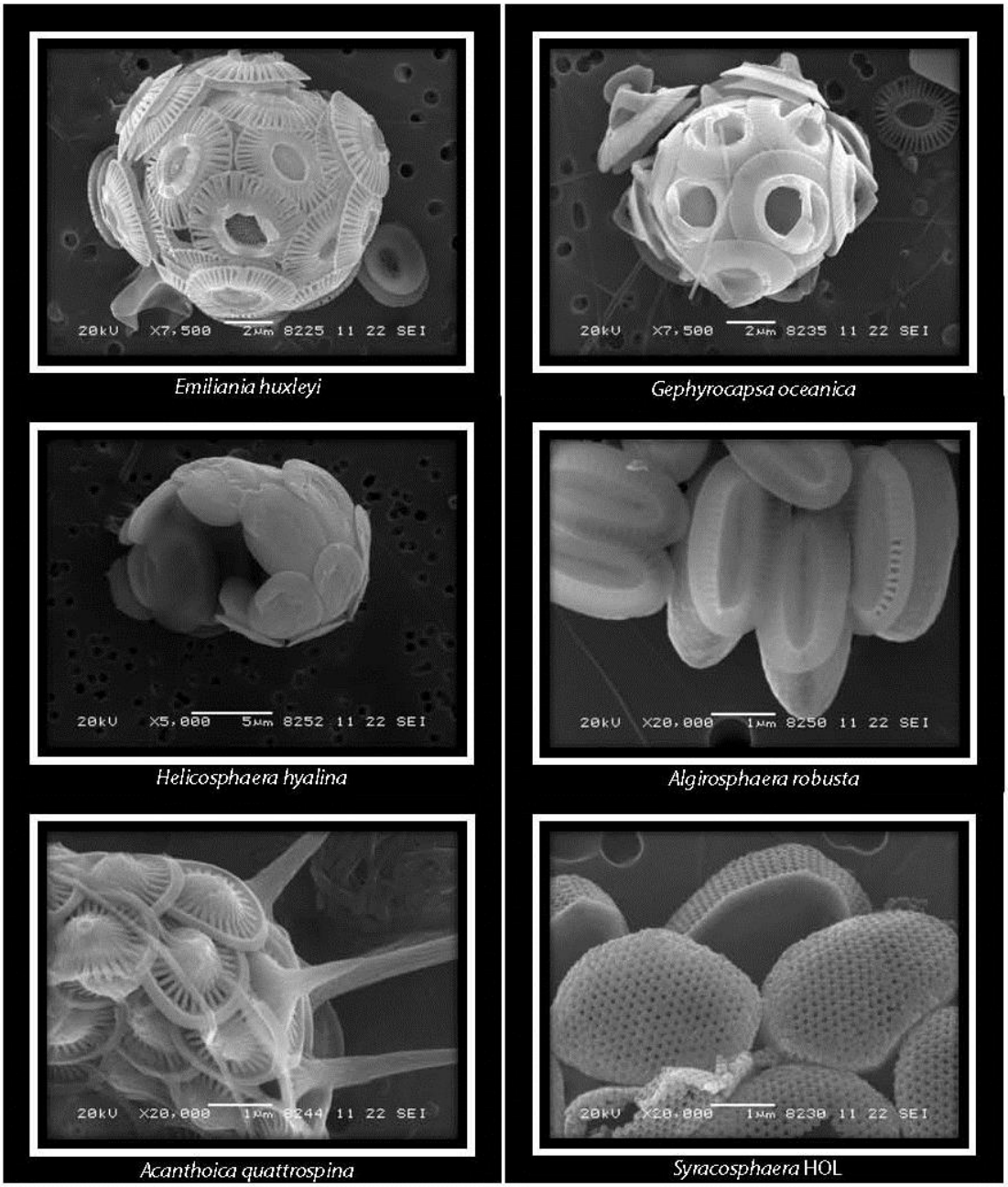
10) Student and post-doctoral publications: None.

11) Student and post-doctoral presentations and posters.

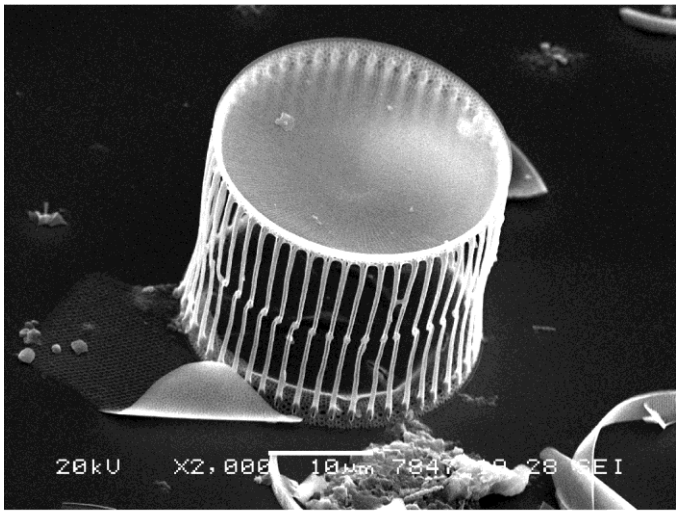
Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Observations of the Genera *Licmophora* Agardh and *Podocystis* Bailey in the Northeastern Gulf of Mexico	A. Adhikari	A. Adhikari, H. Malik, J. A. Nienow & A. K. S. K. Prasad	Annual Meeting of the Phycological Society of America, Seattle, Washington	Y	July 13-16, 2011
Light and scanning electron microscope observations of the diatom species *Coscinodiscus alboranii* and *Haslea wawrikae* in the northeastern Gulf of Mexico	H. Malik	H. Malik, A. Adhikari, J. A. Nienow & A. K. S. K. Prasad	Annual Meeting of the Phycological Society of America, Seattle, Washington	Y	July 13-16, 2011
Observations of diatom communities in northwestern Florida bays after the BP oil spill	A. Adhikari & H. Malik	A. Adhikari, H. Malik, & J. A. Nienow	33rd Annual Southeastern Phycological Colloquy, Florida International University, Miami, Florida	N	October 14-15, 2011
Observations of diatom communities in northwestern Florida bays after the BP oil spill	A. Adhikari	A. Adhikari, H. Malik, & J. A. Nienow	Annual Meeting of the Phycological Society of America, Charleston, South Carolina	Y	June 20-23, 2012

12) Images (see the next two pages below)

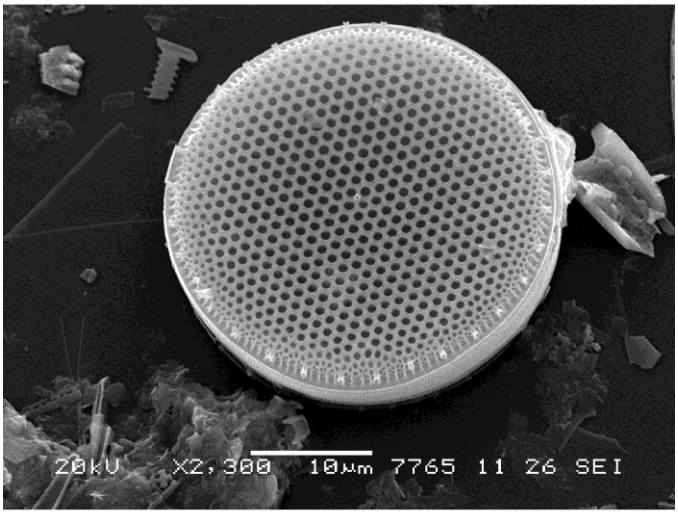
Calcareous Nannoplankton (taken by Susan Foley at the FSU Physics SEM Lab, 11-22-11; names of each taxon are given below the micrographs).



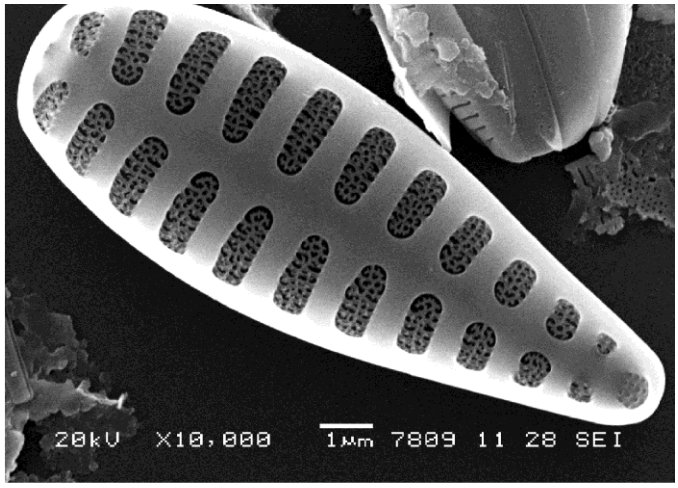
Planktonic Diatoms (taken by James Nienow and his students at the VSU Biology SEM Lab, 11-22-11; generic names of each taxon are given below the micrographs).



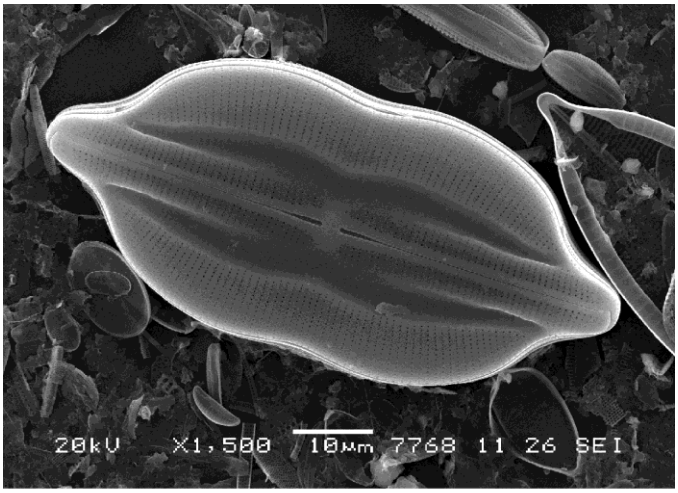
Skeletonema



Thalassiosira



Opephora



Lyrella